Risk Assessment for Controlling Mosquito Vectors with Engineered Nucleases: Sterile Male Construct

Record of expert elicitation

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Prepared for Stephanie James and elicited experts
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EXECUTIVE SUMMARY

In February 2014, CSIRO was engaged by the Foundation for the National Institutes of Health (FNIH) to conduct an independent assessment of the risks associated with an escape of mosquitoes genetically modified to be male-sterile by a construct that incorporates the I-Ppol Homing Endonuclease Gene (HEG).

The risk assessment was completed by using direct elicitation with domain experts to develop subjective prior probability distributions for key malaria transmission parameters, and basic events within four fault trees. The elicitation was performed with 8 members of the Target Malaria consortium, and 16 external experts approached for independent opinions. In total 1068 subjective probability density functions and 67 constants were retained for subsequent analysis, together with 1588 comments, covering 352 and 544 basic events (and vectoral capacity parameters) respectively.

After each elicitation the experts were given individual reports that provided a formal record of their elicitation. The reports documented the central credible intervals elicited from the experts, the 5th, 10th, 50th, 90th and 95th percentiles of the fitted distribution, together with the original basic event question and any comments made by the expert when addressing that basic event. Each expert was given an opportunity to amend either the central credible interval or their comments.

This report provides a final complete record of the entire elicitation. It identifies and discusses basic events that exhibit substantial disagreement between experts, and provides a summary of all the recommendations that the experts made regarding additional analysis or experiments that could be completed to help calculate the probability of the basic event in question.
1 INTRODUCTION

In February 2014 CSIRO was engaged by the Foundation for the National Institutes of Health (FNIH) to conduct a risk assessment of the proposal to control mosquito vectors by genetic engineering with the male-sterilizing I-PpoI Homing Endonuclease Gene (Windbichler et al., 2008). The scope of work for the risk assessment (see also Hayes et al., 2015) includes a structured hazard identification and a Fault Tree Analysis (FTA) supported by the direct elicitation of the probabilities of basic events and other parameters.

The hazard analysis completed for this risk assessment suggested that the assessment could be made tractable by focusing on 5 assessment endpoints:

1. An increase in the vectorial capacity of genetically modified mosquitoes.
2. Transmission of a novel (i.e. not previously known to be vectored by An. gambiae) blood-borne pathogen to human or vertebrate host.

The construction of the risk assessment fault trees, and the direct elicitation, was completed in three phases. During the first phase (30th April 2014 to 2nd May 2014), the CSIRO project team and members of the Target Malaria Consortium (TMC), developed a first draft of the fault trees for each endpoint. These were subsequently refined following review by CSIRO and the TMC during which time the first fault tree – increased incidence of malaria following a catastrophic release of all mosquitoes from an African insectary – was discarded in favour of a direct elicitation of the parameters that determine the vectorial capacity of arthropod pests (Hosack, 2014). These parameters form the basis of an assessment by CSIRO of the risk associated with the first endpoint (see Section 2).

The second phase of the fault tree analysis (FTA) was conducted from the 1st to the 25th July 2014 and from the 29th August 2014 to 20th September 2014. During these periods the CSIRO team completed direct elicitations around the vectorial capacity parameters, and probabilities of the basic events in the fault trees, with 16 independent experts at locations throughout the United States and the United Kingdom respectively (Table 1.1). Several amendments and additions to the structure of the fault trees were recommended by individual experts during this phase.

The third and final phase of the FTA was completed between the 16th and 24th October 2014. During this phase members of the CSIRO project risk team conducted direct elicitations on the basic events within the four fault trees, together with the vectorial capacity and dispersal parameters, with 8 members of the TMC. The vertical gene transfer section of fault tree 3 was excised during this period, and added to the structure of fault tree 5, to extend the scope of the last endpoint to the Anopheles gambiae complex (Sections 3.2 and 3.4).

In total the project team retained 1068 probability density functions and 67 constants for analysis, covering 352 basic events, and collated 1550 comments, covering 544 basic events (Figure 1.1). The objectives of this report are: (i) to provide a complete and final summary of the elicitation, (ii) examine events that exhibit significant disagreement between experts; and, (iii) to capture any recommendations made by the experts with respect to additional analysis or experiments.
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<td>School of Veterinary Medicine, UC Davis</td>
<td>2nd – 3rd July</td>
</tr>
<tr>
<td>1</td>
<td>Antony James</td>
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<td>Kent Golic</td>
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</tr>
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<td>1</td>
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</tr>
<tr>
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<td>1</td>
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<td>1</td>
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<td>2</td>
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<td>3</td>
<td>Roberto Galizi</td>
<td>Imperial College London</td>
<td>22nd – 23rd October</td>
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</table>

Table 1.1: Summary of the dates and locations of the elicitations completed with the US- and UK-based independent experts (Phases 1 and 2), and members of the Target Malaria Consortium (Phase 3).
Figure 1.1: Summary of the distribution functions and constants elicited with members of the Target Malaria Consortium and independent experts. FT1 refers to the vectorial capacity parameters. Some of the parameters elicited under FT3 were moved to FT5 following changes to the scope of FT5 made during the third phase of the elicitation process. Furthermore, many of the parameters in FT4 are dependent (same events) as events in FT3.
2 VECTORIAL CAPACITY

The primary objective of the vectorial capacity analysis is to assess the probability that modified and unmodified (with the I-Ppol construct) G3 laboratory stain mosquitoes have a higher vectorial capacity than their wild type equivalents. The rationale for this comparison is to identify what effect the construct per se might have on the vectorial capacity of the genetically modified mosquitoes, in order to separate it from the effect of laboratory rearing practices.

During Phase 1 of the elicitation process the CSIRO risk team and members of the Target Malaria Consortium agreed on a set of parameters that influence the vectorial capacity and dispersal of wild type *A. gambiae* (Table 2.1).

The vectorial capacity parameters are based on a generalisation of the Garrett-Jones and Shidrawi (1969)'s equation:

\[
\text{Vectorial capacity} = \frac{ma^2 p^n b}{-\log(p)},
\]

where \( m \) = vector density, \( a \) = probability of feeding on a particular host, \( p \) = daily survival rate, \( n \) = the intrinsic incubation period of malaria, \( b \) = the proportion of vectors that become infective and \( -\frac{p^n}{\log(p)} \) is the mean number of days of life in the infective condition per mosquito receiving the infection (the expected infective life). A number of additional parameters were also included in the elicitation at the request of the TMC.

In all cases the elicitation was repeated for female wild type, G3 and I-Ppol modified mosquitoes, and in the cases of dispersal distance and mortality rate, for male populations of these three groups (Table 2.1).
<table>
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<tr>
<th>Issue addressed</th>
<th>Parameter</th>
<th>Population</th>
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</thead>
<tbody>
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<td>Same biting capacity?</td>
<td>Number of bites per day on humans by female mosquitoes</td>
<td>Wild type (female)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G3 strain (female)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I-PpoI (female)</td>
</tr>
<tr>
<td>Same transmission capacity per bite?</td>
<td>Proportion of bites from infectious human that transmit infectious agent to mosquito</td>
<td>Wild type (female)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G3 strain (female)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I-PpoI (female)</td>
</tr>
<tr>
<td>Same transmission capacity per bite?</td>
<td>Proportion of bites from infectious mosquitoes that transmit infectious agent to human</td>
<td>Wild type (female)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G3 strain (female)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I-PpoI (female)</td>
</tr>
<tr>
<td>Same survival potential in the wild?</td>
<td>Mortality rate of mosquitoes once outside of insectary</td>
<td>Wild type (male)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wild type (female)</td>
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<td></td>
<td></td>
<td>G3 strain (male)</td>
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<tr>
<td></td>
<td></td>
<td>G3 strain (female)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I-PpoI (male)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I-PpoI (female)</td>
</tr>
<tr>
<td>Same fecundity?</td>
<td>Number of fertile eggs laid by blood fed female (following WT male mating?)</td>
<td>Wild type (female)</td>
</tr>
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<td></td>
<td></td>
<td>G3 strain (female)</td>
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<td></td>
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<td>I-PpoI (female)</td>
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<tr>
<td>Same dispersal capacity?</td>
<td>Dispersal distance (month/week/day)</td>
<td>Wild type (male)</td>
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<td>Wild type (female)</td>
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<td>G3 strain (male)</td>
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<td></td>
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<td>G3 strain (female)</td>
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<td>I-PpoI (female)</td>
</tr>
<tr>
<td>Likelihood of biting for two blood meals after release?</td>
<td>Probability of biting for two blood meals after release</td>
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<td></td>
<td></td>
<td>G3 strain (female)</td>
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<td>I-PpoI (female)</td>
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<tr>
<td>Same extrinsic incubation?</td>
<td>Extrinsic incubation period (number of days from mosquito acquiring infection to becoming infectious)</td>
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<td>G3 strain (female)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I-PpoI (female)</td>
</tr>
</tbody>
</table>

Table 2.1: Vectorial capacity parameters and additional malaria-risk parameters, together with their population targets, elicited in Phase 1 and 2 of the CSIRO risk assessment
3 SIGNIFICANTLY UNCERTAIN EVENTS

Figure 3.1 provides a summary of the number of elicitations (the vast majority of which were independent) per basic event or gate in the fault tree analysis, together with a measure of the disagreement between the expert’s beliefs, expressed as the difference (on a logarithmic scale) between the highest and lowest expected value.

Of the 352 distribution functions and constants retained for analysis, approximately 18% (63) exhibited “significant uncertainty” between experts, defined here as a difference between the highest and lowest expected value of greater than or equal to 5 orders of magnitude. The following sub-sections of this section of the report examine each of these events in more detail.

3.1 Vector novel blood-based pathogen

Table 3.1 summarizes the events in FT2 that the experts displayed significant disagreement over. The first three of these events – FT2011, FT2010 and FT2020 – refer to the probability that a novel pathogen survives the mosquito’s immune system, replicates in the mosquito and then travels to the salivary gland. In the case of FT2010, the expert’s comments suggest that the difference of opinion centers around: (i) the role of the immune system in preventing infection by novel pathogen; and (ii) the uncertainty created by the large number of possible novel pathogens.

<table>
<thead>
<tr>
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<th>N</th>
<th>q50w</th>
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<td>4.792</td>
<td>5.017</td>
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</table>

Table 3.1: Summary of the basic events in FT2 that display significant disagreement between experts’ beliefs. Here we identify ‘significant disagreement’ to be a difference between the highest expected value and lowest expected value (denoted EXw) greater than or equal to five orders of magnitude, i.e. $\log(\max(E[X_i])) - \log(\min(E[X_i])) \geq 5$. The difference between the highest and lower median (denoted q50w) is also shown for comparative purposes.

In the first case two of the experts suggested that the immune system does not play an important role in preventing infections by novel pathogens and hence the probability of FT2010 is high. In the second case two different experts suggest that across the different types of possible novel pathogens, the probability of one of them surviving the immune system will also be high. The other experts, however, suggest that this step must have a low probability otherwise mosquitoes would already be vectors for the pathogens concerned.

In the case of FT2011 (replication in the mosquito) two experts identify this as the most important rate limiting step with a very low probability, whereas others again suggest it could be high.
Figure 3.1: Summary of the disagreement between experts expressed as the difference between the highest and lowest expected value (denoted EXw) of each basic event (or gate) in the fault tree analysis. \( N \) refers to the number of (largely independent) elicitations for each event (or gate). We define events as significantly uncertain if the disagreement between experts is greater than or equal to 5 orders of magnitude.
because of the large number of possible pathogens. Some of the experts comments in this regard suggest that the analysis should distinguish between viruses, non-human plasmodium, human pathogenic bacteria and filaria.

For FT2020 (travels to the salivary gland) the comments that accompany the elicitations do not provide much insight into the causes of the disagreement between experts. Some simply see this as very likely given that the pathogen has survived the mosquitoes immune system and replicated within the mosquito, and others see it as requiring an evolutionary-like event.

The remaining events in Table 3.1 – FT21020, FT2112120, FT2112100 and FT2112101 – are events associated with mechanical transmission of novel pathogens. FT212020 refers to the probability that a novel pathogen adhered to the proboscis remains viable between bites. Here some of the uncertainty again appears to be linked to the large number of potential novel pathogens. Some experts, for example, pointed to the durability of the Hepatitis B virus, or documented cases of mechanical transmission of trypanosomes. Others, however, only acknowledge this as possible if the mosquito is disturbed whilst feeding and therefore bites again within a few minutes, otherwise the two to three days between bites significantly mitigates against the probability of this event.

FT2112100 and FT2112101 are the requisite events for mechanical transmission via blood transfer with infection occurring because the mosquito is ingested. The large disagreement around the probability of ingestion (FT2112100) is due to a single expert who might considered an outlier in this context. All other experts suggest that this is not an extremely rare event, whereas expert E16 (Figure 9.86) suggests there is 95% chance that this is less $1 \times 10^{-6}$. The disagreement around FT2112101 (pathogen survives the gut) again appears to be due to the large variety of potential pathogens with two out of five experts believing it is very rare, and the others suggesting it is quite possible, with one again pointing to oral transmission of trypanosomes as an example.

The reasons behind the uncertainty associated with FT2112120 (the probability that the pathogen is passed through the mosquito faeces) are not clear from the expert's comments. Two experts believed this to be quite plausible, presumably because they had a particular pathogen in mind. A third expert, however, saw this as highly unlikely.

3.2 Spread of the construct in non-target eukaryotes

Over half (16 out of 25) of the fault tree 3 events that exhibit significant disagreement between experts (Table 3.2) are associated with spread of the construct by homing and transposon-mediated acquisition of the construct. Seven of these events relate to the probability that the construct becomes integrated into the genome of a non-target eukaryote with its original integrase bound and active (FT303320 and FT30341).

The uncertainty induced by the disagreement over FT3033203 (probability that the construct enters the nuclease of a germline cell with the integrase bound – Figure 9.171) is due to two outliers: the first believed this probability was zero, whilst the second suggested it could be $\geq 0.5$ since it might be in a piggyBac. This could be due to misinterpreting the question since the equivalent piggyBac mediated event is treated separately in FT3034102. The uncertainty associated with this event (Figure 9.181) is due to the first outlier above who again suggested it has probability zero.

The uncertainty surrounding FT30341041 – the probability that the construct becomes integrated into the eukaryote germline using transposase/reverse transcriptase or integrase (T/RT/I) that is
already present in the cell – appears to be genuine in the sense that there is no evidence that the question was misinterpreted: two experts believed this probability to be low whereas a third thought it would be negligible (Figure 9.186).

The significant disagreement over the probability that the construct enters the germline cell of a non-target eukaryote (FT3033202 – Figure 9.168) is due to the same expert outlier as FT3033203 and FT3034102. This expert again believes that the event has probability zero whereas other experts acknowledged it is very unlikely but nonetheless possible. This same expert contributed to the uncertainty associated with the probability that the construct remains intact in the environment with the integrase bound (FT3033201 – Figure 9.167) by suggesting that this event has probability 1, although in this instance at least one other expert did acknowledge that it could have a high probability.

The reasons for the uncertainty associated with FT30332040 (probability that the construct integrates because the T/RT/I remains bound and active to the construct – Figure 9.173) are not clear because the two experts who believed this to be quite likely did not provide additional comments.

The significant uncertainty associated with the probability that there is a source of T/RT/I that is able to act on the piggyBac transposons flanking the construct in the mosquito is mainly due to single expert who acknowledges that the event is not "completely unlikely" but nonetheless assigns an extremely low probability to its occurrence, whereas the other experts suggest it is much more likely. In this context one expert suggests that an analysis of the similarity between the sequences at the ends of the construct and known An. gambiae piggyBacs would be helpful.

All of the basic events under FT30330 – the probability that (in the mosquito) the construct has flanking transposons of the same family (as the non-target eukaryote) also exhibit significant disagreement between experts. The fault tree analysis identifies three alternative mechanisms for this event. The first, the probability that the construct is flanked by transposons of the same family (FT303300 – Figure 9.162) may be uncertain due to different interpretations of the question. It is also worth noting that almost all of the experts recommended additional experimentation/analysis in this context, e.g. sequence wild type strains to identify transposons that are “known to move”.

The uncertainty associated with the probability that transposable elements of the same family insert either side of the construct (FT303301 – Figure 9.163) is due to one expert suggesting this has a negligible probability (but did not provide a comment) and one expert who was very unsure and gave an extremely large central credible interval. Again, many of the experts recommend additional experimentation/analysis in this context to ascertain the number of transposable element movements with the An. gambiae genome.

The third possibility – the construct inserts between two existing transposable elements (FT303302 – Figure 9.164) appears to be uncertain because the experts simply disagree on this as a plausible mechanism, with one expert questioning how this was possible at all. It appears possible that some experts may have misinterpreted this question.

Six of the events under spread by homing (FT3120) exhibit high levels of uncertainty between experts. Two of these events pertain to the probability that the construct maintains its germline activity, either when homing at the ribosomal repeat (FT312021) or when homing at the same target sequence (i.e. HEG does not mutate) at a different unexpected locus (FT312002). The uncertainty associated with both of these events (Figure 9.202 and Figure 9.210) appears to
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<th>q50w</th>
<th>EXw</th>
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Table 3.2: Summary of the basic events in FT3 that display significant disagreement between experts’ beliefs. Here we identify ‘significant disagreement’ to be a difference between the highest expected value and lowest expected value (denoted EXw) greater than or equal to five orders of magnitude, i.e. $\log(\max(E[X_i])) - \log(\min(E[X_i])) \geq 5$. The difference between the highest and lower median (denoted q50w) is also shown for comparative purposes.
be due to different opinions on the specificity of the construct promoters. Almost all of the experts acknowledged that this probability could vary tremendously depending on the non-target eukaryote, suggesting it could be quite high for other insects, particularly Dipterans, because of similarity between promoters, but would be extremely low for non-insects. One expert, however, indicated that the promoter is conserved across eukaryotes. The uncertainty stems from differences in opinion on the upper and lower bound of this probability in this context.

Two more of the uncertain events under homing refer to the probability that the (unmutated) construct moves into a recognition site on a different unexpected locus (FT312001) or moves into a recognition site on the ribosomal repeat (FT312020). The uncertainty associated with both of these events (Figure 9.201 and 9.209) appears to be genuine disagreement between the experts. Virtually all of the experts suggested that the probability of FT312001 would be the same in target (i.e. *An. gambiae* complex) and non-target eukaryotes but their individual opinions were simply very different on this issue.

The last two significantly uncertain events under spread by homing are FT312010 (construct mutates to recognize alternative recognition site) and FT3120130 (mutated construct does not cut ribosomal repeats). The significant uncertainty associated with FT312010 appears to be genuine differences of opinion between the experts (Figure 9.207), compounded by their own individual uncertainty when estimating this probability for all non-target eukaryotes. One of the experts suggested that the construct design team may be able to provide more accurate estimates of this probability. The uncertainty associated with FT3120130 again appears genuine with experts divided into two camps – those that think this probability is very high for a mutated construct and those who think it is still quite unlikely (Figure 9.208).

The remaining nine events in Table 3.2 refer to steps in the unmediated acquisition of the construct, acquisition via viral- and prokaryote-mediated processes, and spread of the construct by active drive. The uncertainty associated with FT301002 (the probability that there is direct contact in soil between the construct and competent bacteria – Figure 9.112) stems from one expert who suggested that the probability would be negligibly small, whereas three other experts thought this to be quite likely. Two of the four experts here also suggested that this probability may be informed or calculated from studies in the scientific literature.

The uncertainty associated with FT300011 and FT301011 (the probability that the construct DNA will remain intact within an aqueous environment – Figures 9.101 and 9.116) also stems from a similar difference opinion between experts with one (the same expert noted above) indicating that the probability of this is negligible, declining exponentially as the length of the DNA fragment increases, whilst others suggesting this is quite likely. Some experts believe the construct DNA will be more stable in a water than in soil, whilst others believe the reverse is true.

The two events under active drive that exhibit significant disagreement between experts are FT31211 and FT31215. These events refer to the probability that the construct moves to a sex determining chromosome (Y or W), and given that is has moved to this chromosome, the probability that it maintains germline specificity. Both of these events exhibit an even spread of responses across all experts (Figures 9.215 and 9.129) and do not point to any obvious outliers. One expert again notes that the Beta-tubulin promoter is conserved across eukaryotes and for this reason he believes that the probability of FT31215 is likely to be much higher than indicated by some of the other experts.

The last two events under unmediated acquisition are FT300020 and FT300012 – the probability
that the construct will enter the gut of a non-target eukaryote (Figure 9.106) and the probability intact construct DNA in an aquatic environment will enter a eukaryote (Figure 9.102). From the comments it appears that it is possible that these questions have been interpreted differently by different experts, and this may explain their significant disagreement. For FT300020, one outlier expert appears to confound this event with FT300021 (DNA remains intact in the gut) despite answering both questions. For FT300012, the comments suggest a different outlier expert re-interpreted the question.

The last two events in FT3 that exhibit significant disagreement between experts are the probability of virion-mediated acquisition (FT3020011 – Figure 9.136) and the probability that a product (RNA/protein) of the construct improved fitness (FT310020-9 – Figure 9.190). The disagreement associated with FT3020011 stems from an outlier expert who believes this has probability zero, whilst a second believes it is possible albeit with negligible probability. For FT310020-9 the reasons behind the disagreement are unclear – one expert suggests this has a 50/50 chance whilst the other suggests it will be extremely unlikely.

### 3.3 Spread of the construct in non-eukaryotes

Four of the five events in FT4 that exhibit significant disagreement between experts (Table 3.3) are associated with spread of the construct in prokaryotes via selection (FT4010). The events in descending order of uncertainty are: (i) the probability that Homing Endonuclease protein or RNA when expressed will improve a prokaryote’s fitness (FT401001 – Figure 9.226); (ii) the probability that flanking regions of mosquito DNA stay linked to the construct and these improve its fitness (FT401011 – Figure 9.228); (iii) the probability that the prokaryote’s fitness is improved because the integration of the construct in its genome disrupts or affects nearby genes (FT401010 – Figure 9.227); and, (iv) the probability that the construct becomes linked to one of the prokaryotes mobile genetic elements (FT401020 – Figure 9.230).

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Table 3.3: Summary of the basic events in FT4 that display significant disagreement between experts’ beliefs. Here we identify ‘significant disagreement’ to be a difference between the highest expected value and lowest expected value (denoted EXw) greater than or equal to five orders of magnitude, i.e.\(\log(\max(E[X_i])) - \log(\min(E[X_i])) \geq 5\). The difference between the highest and lower median (denoted q50w) is also shown for comparative purposes.

The comments associated with FT401011, FT401001 and FT401010 show an extremely divergent opinions, with one expert suggesting that FT401011 and FT401001 have probability zero, whilst another suggests that (for FT401011 at least) it could be as high as 50/50. These same two experts are consistently at odds over these issues, with the remaining experts somewhere
in between. Such divergence of opinion may warrant additional analysis and discussion to identify why two of the experts here have such divergent views. The reasons for the disagreement over FT401020 are unclear because one of the two experts who responded to this event did not provide additional comments.

The last event associated with significant disagreement in FT4 is FT41101 (the probability that integration of the construct into a virus improves it fitness by disrupting or affecting nearby gene – Figure 9.241). The disagreement here appears to be simply due to the expert’s different numerical expression of events that they both believe to be close to zero.

### 3.4 Spread of the construct in the An. gambiae complex

FT5 is the largest fault tree in this analysis with a total of 163 events and gates retained for analysis following the elicitation, of which 25 (approximately 15%) exhibit significant disagreement between experts (Table 3.4). There are, however, many dependent events within FT5 and these 25 events or gates represent 17 unique events/gates.

An important event that occurs in FT5 is the probability that the construct fails to sterilize all male *An. gambiae* mosquitoes (FT500000 – Figure 9.250 and FT50103 – Figure 9.258). The expert’s beliefs about the probability of FT500000 and FT 50103 show a fairly even spread, with the possible exception of expert 17 for FT500000. The significant disagreement here appears to reflect a genuine diversity of views. Importantly, several of the experts suggest that this probability should be calculated from data collected by the Target Malaria consortium. Initial calculations presented in (Hayes et al., 2015) suggest that the data collected to date indicate with 95% confidence that the probability of FT500000 is less than $9 \times 10^{-3}$.

A related event is the probability that hybrid males that carry the construct are not sterilized by it (FT51010000 – Figure 9.288). The expert opinion on this event are very divergent, with one expert being 95% confident that this probability is greater than 0.9, whilst another suggests that the data collected to date indicates that the probability is five orders of magnitude lower than this. This level of disagreement, and the potential availability of data, suggests that this question should be re-examined by the project team.

Another related event in this context is the probability that F1 hybrid males do not show hybrid sterility (FT51010002 – Figure 9.294 and FT510100021-9 – Figure 9.296). The significant disagreement associated with FT51010002 and FT510100021-9 should be re-examined by the project team and resolved. In the latter case, it is clear from the comments that one of the experts misinterpreted the question. In the former case, the same expert (from above) is again extremely confident that F1 hybrid males will be fertile if the construct fails to sterilize them, whereas others (see also Figure 9.295) indicate that the probability will be substantially lower.

Some of the significant uncertainty associated with the probability that the I-PpoI construct will insert into a recognition site in a ribosomal repeat (FT5011001 – Figure 9.262 and FT510111001 – Figure 9.329) is due to a single expert outlier who strongly believes that this probability is close to one, but who may have misinterpreted the question. Nonetheless, the uncertainty associated with this event would remain very high even after excluding this expert.

The disagreement associated with the probability that the I-Ppol construct would move into an alternative recognition site, given that such a site exists, (FT5011001 – Figure 9.262 and FT510111001 – Figure 9.310) appears to reflect genuine difference of opinion between the experts on this issue, with no obvious outliers or evidence that the question has been misinter-
Similarly, the disagreement associated with the probability that the HEG construct mutates to recognise an alternative site in the mosquito genome (FT5011020 – Figure 9.269 and FT510111020 – Figure 9.324) appears to reflect genuine differences of opinion between the experts although in this instance several experts suggest that this could be determined experimentally by the HEG construct design team.

Four of the events listed in Table 3.4 are associated with the probability that the negative fitness effects of the HEG construct are less than the driving effect because the construct inserts into an intron (FT5011121 – Figure 9.275 and FT510111131 – Figure 9.335), either a self-splicing intron (FT5101111310 – Figure 9.336) or a spliceosomal intron (FT5101111311 – Figure 9.337.)

In the case of FT5011121 and FT510111131, the significant disagreement between experts appears to be due to one (the same) expert misinterpreting the question and assigning a very high probability to this gate because the construct target site is in an intron. In the case of FT5101111310, the disagreement seems to stem from confusion over whether or not this is correctly part of the generating event chain, with one expert stating that self-splicing introns do not exist in the An. gambiae complex, whilst another suggesting this has probability one because the current site is already a self-splicing intron. In the case of FT5101111311, two experts agree that this event has a “vanishingly” or “extremely” small probability, but their numerical definitions of this differ, whilst the third expert believes this has probability zero.

The disagreement around FT50010 and FT500011 contrast with this. Here the expert responses are much more evenly spread and point to what appears to be simple differences of opinion ranging from the probability could be as high as 50/50 to negligible probability. One expert also notes that both of these probabilities should be calculated, although in the case of FT500001 this would currently be impossible because the construct to date has never been observed to fail.

The disagreement surrounding FT51110 – the probability that mitochondrial DNA in the mosquito acquires the construct (Figure 9.345) is due to one expert who believes that this has probability zero, whereas another thinks it is very unlikely but nonetheless plausible. A similar outlier effect is responsible for the disagreement around FT501111 – the probability that the construct maintains its germline expression if it inserted into a recognition site on a ribosomal repeat (Figure 9.272). Here all but one expert believed this would be a likely event.

The expert responses for FT510111000, the probability the genome of species in the An. gambiae complex contains an I-PpoI recognition site outside the X-linked ribosomal repeats (Figure 9.309), and FT50100, the probability that the construct moves to the Y chromosome (Figure
Table 3.4: Summary of the basic events in FT5 that display significant disagreement between experts’ beliefs. Here we identify ‘significant disagreement’ to be a difference between the highest expected value and lowest expected value (denoted EXw) greater than or equal to five orders of magnitude, i.e. \(\log (\max (E[X_i])) - \log (\min (E[X_i])) \geq 5\). The difference between the highest and lower median (denoted q50w) is also shown for comparative purposes.
9.255) are evenly spread across a large range of values, hence the high uncertainty, with no obvious outliers, and in both cases experts recommend that the consortium calculate this by searching for the target site in the genome.

The last four events listed in Table 3.4 are associated with the cleavage rate at the construct’s rDNA locus. The uncertainty associated with FT50110030 and FT510110030 – the probability that if (unmutated) I-PpoI moves into an alternative recognition and maintains germline expression it does not cleave ribosomal repeats (Figure 9.265 and Figure 9.312) appears to due to simple disagreements over whether or not moving into a new recognition site affects the cleavage efficiency with some experts maintaining that it will and others suggesting that it will not. The uncertainty associated with the probability of a low cleavage rate for a construct inserted on a ribosomal repeat (FT5011120 – Figure 9.274 and FT510111130 – Figure 9.334) is largely due to one (and two) expert outliers who believe this is very likely whereas all other experts think this has a negligible or almost negligible probability.
4 DATA AND EXPERIMENT RECOMMENDATIONS

Occasionally during the elicitations experts recommended that the Target Malaria consortium calculate the probability of an event or conduct additional analysis/experimentation in order to empirically estimate this probability. Some of these recommendations have already been noted in the previous sections of this report. This section provides a complete list of these recommendations to assist the consortium.

4.1 Change in vectorial capacity

A number of experiments, additional analysis or existing published data were identified by the experts when considering the vectorial capacity parameters:

- **FT1-1a and FT1b-factor.** Several experts suggested that the number of bites per days by female G3 and I-Ppol mosquitoes could be investigated experimentally.

- **FT1-2b-factor, FT1-2c and FT1-3b-factor.** Two experts suggested that the effect of the construct on the proportion of bites that transmit infectious agents (from human to mosquito and from mosquito to human) could be determined experimentally, for example by feeding I-Ppol mosquitoes on infected blood and measuring the frequency of plasmodium infection.

- **FT1-4f.** Similarly, one expert suggested that the mortality rate of mosquitoes outside the insectary should be comparatively easy to determine experimentally.

- **FT1-5a and FT1-5b.** The number of fertile eggs laid by blood fed females is reasonably well documented in the literature (see for example Govoetchan et al., 2013; Lyimo et al., 2013) and information on this is also available from the experiments already conducted by the Target Malaria consortium.

- **FT1-6a.** Similarly there are some estimates on the average dispersal distance of mosquitoes available from the literature, such as Service (1997) and Thomas et al. (2013).

- **FT1-8a.** One expert noted that the existence of a standard assay to determine the extrinsic incubation period – i.e. time taken for the infectious agent to show up in the mosquito saliva following a blood meal, and hence the effect (if any) of the construct on this parameter could be determined with standard experimental methods.

- **FT1-9b.** One expert also noted that it should be possible to safely determine experimentally the percentage of blood meals taken humans, for the G3 strain at least. The expert did not, however, elaborate further on how these experiments might be conducted.

4.2 FT3 Spread of the construct in non-target eukaryotes

In the context of FT3 experts recommended the following analysis and calculations:

- **FT303.** The probability of transposon mediated acquisition of the construct might be informed by the frequency of mariner elements in insects and the experiments conducted by Robertson (1993).

- **FT3010.** One expert suggested that the probability of the construct moving from the mosquito into a prokaryote is most likely to occur within the mosquito’s intestinal flora and hence the consortium should consider screening these for the presence of the construct. The expert cautions, however, that in all similar experiments that he is aware of evidence
for prokaryote transformation can only be observed under optimised conditions, and even then it may occur below detection rates – e.g. less than $1 \times 10^{-9}$.

- **FT300011.** A different expert suggested that the degradation rate of DNA in aquatic environments is known but did not elaborate further. A search of Google Scholar suggests that most information on DNA degradation refers to DNA samples under laboratory conditions, rather than environmental conditions relevant to this analysis.

- **FT303301.** Two experts suggested that estimates of the probability that transposable elements of the same family insert either side of the construct (in the mosquito) could be informed by the frequency of transposon movements within mosquito genomes, and in this context identified the work done by David O’Brochota (see for example O’Brochta et al., 2003).

- **FT30331 and FT30330.** Several experts suggested that the Target Malaria consortium sequence the entire genome of the wild type target mosquitoes (or at least 100kbps either side of the construct of modified mosquitoes), to identify if there is a source of transposase / reverse transcriptase / integrase (T/RT/I) that may act on nearby transposons that are flanking the construct, and to ascertain if the construct is flanked by transposons of the same family in the modified mosquitoes. For both events, experts suggested the consortium approach Zhijian Jake Tu for further opinion and information. In this context it is worth noting that Professor Tu recently contributed to an analysis that used next generation sequencing to discover evidence for Horizontal Gene Transfer of the MJ1 transposable elements between *Anopheles sinensis* and *Aedes aegypti* (Diao et al., 2011).

- **FT30340.** Similarly, one expert noted that the probability that there is a source of T/RT/I that acts on piggyBac in the modified mosquitoes would depend on the sequences at the ends of the construct and how close these are to the known *A. gambiae* piggyBacs. Other experts, however, disagreed on the likelihood of this event, some noting that piggyBacs are not endogenous to *An. gambiae*, whilst others noted that there is evidence of piggyBac elements (perhaps degenerate) in its genome. Sarkar et al. (2003), however, reports that the draft sequence of the genome of the *An. gambiae* mosquito contains five copies of piggyBac relatives.

- **FT31210.** Two experts suggested that the proportion of species in the eukaryote kingdom with heterogametic sex determination (e.g., XY sex determination) is known or could be calculated.

- **FT302121.** Finally, one expert recommended that the consortium consider deep genome sequencing of wild type target mosquitoes in order to identify the types of viruses that they have been, or currently are, infected with. This information may help to identify or eliminate infections with specific types of viruses that are very efficient at transovarial transmission (such as La-Crosse virus and Dengue virus). This may help inform the probability that a virus infects a later stage of the eukaryote’s life-cycle but then moves to the ovaries or testes.
4.3 FT4 Spread of the construct in non-eukaryotes

Expert recommendations regarding additional calculations, analysis or experiments in relation to the events in FT4 were as follows:


- **FT411150 and FT411151.** Similarly, it was suggested that the probability that the I-PpoI recognition site lies within an intron or within a non-essential region of the genome could be calculated by searching GenBank for viral family genomes estimating the average proportion of intronic or non-essential sequences.

4.4 FT5 Spread of the construct in the *An. gambiae* complex

In the context of FT5 the following calculations, experiments or other relevant analysis were suggested:

- **FT51001 and FT510011-9.** The probability that hybrids will be formed in the vicinity of the insectaries following a catastrophic release could be calculated or at least informed by the documented frequency of hybridisation between different members of the *An. gambiae* complex.

- **FT50100100-9.** The probability that F1 hybrid Arabiensis females do not show hybrid sterility could be informed by the experiments conducted by Slotman et al. (2005).

- **FT50100001-9.** Similarly an expert recommended that the consortium cross I-PpoI modified males with *An. coluzzi* to test the performance of the construct in the F1 hybrids.

- **FT5011000 and FT50111000.** Several experts independently recommended that the consortium search the published genome sequences of Anopheles species for sites that are similar to the I-PpoI target sequence to calculate the probability that an alternative site exists within the genome of species in the *An. gambiae* complex.

- **FT5011001 and FT50111001.** Whilst searching these published sequences one expert also noted that the consortium might seek to identify the presence of a transposase that might facilitate the construct to move into an alternative site in the genome.

- **FT501100310, FT5011100310 and FT501100311** The proportion of intronic or non-essential DNA within the genome of species in the *An. gambiae* complex could be used to provide a simple estimate of the probability that if an additional recognition site existed it would occur within an intron or within a region of non-essential DNA.

- **FT50111210 and FT50111211.** Similarly two experts suggested that the probability that the construct inserts into a self-splicing intron or spliceosomal intron, within a ribosomal repeat, could be calculated based on published genome sequences. Another expert, however, expressed that he was fairly sure that self splicing introns do not exist within mosquito rDNA.

- **FT5011020.** Several experts suggested that the probability that the construct mutates to recognise an alternative site in the genome could be calculated based on the information gathered during the whole of genome searches discussed above and/or informed by the
HEG mutation experiments being conducted by the HEG design team.

- **FT50100** One expert suggested that the probability that the I-Ppol construct moves to the Y chromosome could be calculated from the proximate size of THE Y chromosome and frequency of I-Ppol recognition site

- **FT510100020-9**. Several experts suggested there was experimental evidence that could inform the probability that F1 Arabiensis hybrid males do not show hybrid sterility and two publications were identified within these comments: Davidson (1967) and della Torre et al. (2002).

- **FT510021-9**. One expert recommended that the consortium conduct experiments within the insectaries to calculate the probability that (once hybrids between the GM insectary mosquitoes and compatible species have been formed) the construct is transmitted to the F1 offspring.

- **FT501110**. Finally, the high inter-expert uncertainty associated with the probability that the construct inserts into a recognition site in a ribosomal repeat (and the possible misinterpretation of this question) has been noted above. One expert, however, suggested that if this question referred to the non-homologous repair with the construct inserted then the frequency of non-homologous integration in Drosophila may provide some evidence to support an this estimate. Another expert suggested that question refers to ectopic homing and this could be tested (presumably in the laboratory).
5 REFERENCES


22 | CSIRO HEG RA record of expert elicitation

Figure 6.1: Number of bites per day on humans by female mosquitoes (WT)
E1: Mali site is unique with no larval habitat within 3 km. Should be experimental data to augment his answer. No difference or change in biting rate for WT and G3, but possibly for I-Ppol. Question is presumed to be bites per day “per” female mosquito. Normal attempts are 1-3 days per bite. Parameter is considered to be in dry season. Under ideal conditions, there will two blood meals for first egg laying event and then after that there will be one blood meal 3.5-4 days later, meals are interrupted about 10% of the time. For WT median = 0.4

E2: No knowledge on A. gambiae

E3: The environment in which they were raised will influence every question here. Mortality rate of healthy/well fed mosquitoes grown up on nice diet will be important to risk analysis. A. aegypti is a larger mosquito when fed a good diet and live longer but don’t bite as much. A. aegypti is smaller with shorter life if raised on a poor diet but bite more. Experiments could be done to determine biting rate for A. gambiae from the field cages. Compare WT mosquito raised under stressed v nice conditions - what is it’s fitness and what could we deduce about WT v G3 depending on strain. (0.3,0.7).0.7. A. aegypti in Colorado worked, but in Mexico did not because of different environment. Relative fitness of transgenics in Mexico on order of 5% of wild type. Construct with RNAi from KenOlson's group tested for fitness after being backcrossed for 5 generations, concluded that the fitness cost before backcross was huge. New elicitation completed (0.3, 0.7)0.7

E4: Data here

E14: Predicated on interventions already in place. i.e. bed net usage. This would drastically affect feeding to completion or getting disturbed mid-feed. Assume biting every three days. Biting to repletion will differ between sites. Doug Norris has a paper from Zambia documenting how many blood feeds a female takes per gonotrophic cycle. Related to ITN use. In Kenya might be higher rate of bites per female per day than in Mali. The expert or The expert would know better.

E15: Based on gonotrophic cycle, every 2-4 days, except if interrupted (0.25,1)0.9

E16: Need to talk to David Smith, mathematical modeller. Visiting lecturer at Oxford, but based in Washington DC. (0.25,0.6)0.9 lognormal
All experts: 2 of 352 EXw = 0.301

Figure 6.2: Number of bites per day on humans by female mosquitoes (G3)
E1: Probably same as WT or slightly lower, but longevity is perhaps shorter. They take blood meal slower in lab, but this could be just in lab environment (i.e., lab is typically cooler temperature than village house, the latter is closer to mosquitoes optimal temperature for growth). No reason to think that they would be different than WT once released from lab. G3  WT

E3: New elicitation completed (0.1, 0.4)0.8. Old comment: Bites per day relative to WT. Should be tested in Austrian facility (Vienna) Atomic Energy Facility. Bourtzis. May have permission to bring WT from Africa.

E4: Inclined to think they will bite less due to several generations in laboratory. HeatherFerguson would be a good contact for this question

E15: Based on G3 papers on male fitness, survival and mating success. Laboratory strains are usually lower fitness, but females usually higher energy reserves for wild type. G3 about 20% less than wild type (0.2,0.8)0.9

E16: 10% slower than WT. (0.225,0.54)0.9 lognormal
Figure 6.3: Number of bites per day on humans by female mosquitoes (G3)
E2: My response was a comparison of G3 laboratory-reared mosquitoes versus conspecifics from natural populations. In general, I expect well-tended laboratory-reared mosquitoes to be in better nutritional condition than wild-caught (WT) individuals. However, I also expect them to have been domesticated by adaptation to laboratory conditions. I know from my work with several Drosophila species that the domestication process can be quite rapid e.g. a few generations in the lab are sufficient to significantly change behaviour. I expect that the physiological condition of laboratory-reared individuals will increase their performance, whereas their domestication will decrease their performance relative to wild caught individuals (especially with respect to host-seeking and biting behaviour, I conjecture). I expect the latter (deleterious) effect to be greater. This logic is central to several of the answers I provided. However, it is important to realize that these conjectures can easily be tested with simple experiments.
Figure 6.4: Number of bites per day on humans by female mosquitoes (I-Ppol)
E1: Lower fitness and longevity. Possible higher biting rate attempts. Envision that they would be more frequent because they are less efficient feeders, more interruptions in feeding, and they compensate with higher biting rate. Tubulins also involved with other aspects of body function, not just reproduction.

E3: New elicitation completed (0.2, 0.5). Old comment: Bites per day of l-Ppol relative to WT

E15: Papers only on male fitness - large field cage and survival, and mating success - no information on females. Papers show reduced fitness but not sure on the contribution from males/females. Approximate fitness costs, same in females. Klein paper - not sure from the results if the GM is not competitive with G3 or if the construct is working. Think that GM are less competitive than G3. Suspect lower fitness - but hard to put number on it. Instinct that this is lower than for G3, but this is from pre-existing beliefs that GM is lower, not based on this particular construct. (0.1,1)0.7

E16: Should be 10% lower than G3. (0.2025,0.486)0.9
All experts: 5 of 352 EXw = 0

Figure 6.5: Number of bites per day on humans by female mosquitoes (I-Ppol)
E2: Bites per day of I-Ppol relative to WT
Figure 6.6: Proportion of bites from infectious human that transmit infectious agent to mosquito (WT)
E1: Typically related to competence to support infection. Some would put it lower than that, not sure if it is an artefact of membrane type experiments which have rates around 0.6. I think about it as slightly higher on real human skin, therefore median of 0.8.

E3: No number


E15: For wild-type females, standard models use 40%, but have seen less than 5% in practice, so 40% would be high part of range. (0.05,0.4)0.8. CSIRO: Has the expert interpreted the question over the whole of the parasite life-cycle of an infected (not infectious) person?

E16: About 0.6 known. Feeding effective mosquitoes on people, know the proportion of bites which infect. Done when malaria given to people with Neuro syphilis to induce a fever. There are several papers on this. Tom Smith or Nakul Chitnis worked on vector side of this (mathematical modellers both at Swiss TPH). David Smith who reworked this. At least four groups who’ve mathematically modelled this and tested against data including Azra Ghani at Imperial. Density dependent on level of infection in human population. Individuals with very low levels of infection but can still transmit. (0.4,0.8) 0.9
All experts: 7 of 352 EXw = 0.24

Figure 6.7: Proportion of bites from infectious human that transmit infectious agent to mosquito (G3)
E1: Usually G3 is slightly smaller bodied, and more homogenous (so mismatch to particular di-
versity of plasmodium which are adapted to A. gambiae), so same or slightly lower rate; therefore
median around 0.65 to 0.7.

E15: This is the vectorial capacity of laboratory strain and if it’s different than wild; baseline
should not be any different, but some work in West Africa has shown co-evolution of resistance
genes (Ken Vernick paper, first author Niare in Science) which would probably be lost in labora-
tory. G3 is not under selection for resistance. A sensible starting point is that WT and G3 are
either the same or G3 has higher susceptibility because they are not under selection for resis-
tance. Have done experiments (not with G3) with another strain, with parasites and get up to
80% infection. Could be the parasite strain not mosquito strain. With other laboratory strains of
mosquitoes can get quite high, but uncertain about G3. (0.05,0.75)0.8

E16: G3 may well be 10% less than WT . As in wild you assume local adaptation of parasite to
vector strains that are more transmittable. (0.36,0.72)0.9
Figure 6.8: Proportion of bites from infectious human that transmit infectious agent to mosquito (G3)
E2: G3 v WT: Relative transmission to WT is 0.5 (i.e. half as good). Experiments could be done with artificial blood feeding. Take different mosquitoes and feed them on infected blood and ask what is the probability that they’ll absorb plasmodium. After meal do a test to see if they absorb the plasmodium or not. We know mosquitoes are in different condition, so could do a simple lab experiment where ask what is probability.

E3: G3 v WT: Relative transmission to WT is 0.5 (i.e. half as good). Many factors. Could take larger blood meal so more plasmodia, but if more fit would be harder for parasite to get through.
All experts: 9 of 352 $EX_w = 0.136$

Figure 6.9: Proportion of bites from infectious human that transmit infectious agent to mosquito (I-Ppol)
E1: I would imagine that maybe they are somewhat deficient in performance or immune response to infection lower, but this could be damped by backcross. Thus I-Ppol might have lower or similar rate of infection.

E15: Could give an answer, but think this is so important that an experiment needs to be done here.

E16: Same as G3
Figure 6.10: Proportion of bites from infectious human that transmit infectious agent to mosquito (I-Ppol)
E2: I expect laboratory-reared and genetically engineered material will be similar to WT, conditional on having taken a blood meal. However, they may be somewhat better or somewhat worse at transmitting infectious agents.

E3: Answer is for I-Ppol relative to WT. Answer is assuming 5 or more backcross generations already.
Figure 6.11: Proportion of bites from infectious mosquitoes that transmit infectious agent to humans (WT)
E1: Number of sporizoids, infectious load is around 10. Number of salivary glands is in the thousands, so delivery of infectious dose is very likely. In normal bite, normal salivation is continuous and profuse, so easy to deliver infectious amount. In the WT around 0.9

E3: No number

E14: Quite high assuming salivary gland infections. Close to 1. Assuming heavy sporozoite infections. Would think (0.9,1)0.8

E15: (0.2,0.8)0.8

E16: This is 0.9. (0.8,0.95)0.8
Figure 6.12: Proportion of bites from infectious mosquitoes that transmit infectious agent to humans (G3)
E1: Same as WT

E14: No change, active transfer of saliva. Same as FT1-3a (now FT1-3a)

E15: Can't do an experiment on this one. Think influences of infectivity to humans is a mixture of host resistance/susceptibility (which won't change WT or G3). One thing that could influence this is the number of infectious spores in the salivary glands, but this is not necessarily limiting (need only 20 and there can be thousands). Guess would be that G3 has spores in salivary glands. Assume this would be the same; it could potentially be lower if less likely to develop sporozoites, but this answer assumes they already have them in the salivary glands. It could be lower if they are bad biters - i.e. can't probe very well and fly off more easily. Assume about same as WT. (0.2,0.8)0.8

E16: Same as WT(0.8,0.95)0.8
All experts: 13 of 352 EXw = 0.002

Figure 6.13: Proportion of bites from infectious mosquitoes that transmit infectious agent to humans (G3)
E2: Answer is for G3 relative to WT. Keep same response as above (0.4 to 1.2) 0.8

E3: Answer is for G3 relative to WT. Could do comparable experiment as above with rodent malaria or bird malaria. Keep same response as 2b. (0.5 to 1.5) 0.7 truncated normal
Figure 6.14: Proportion of bites from infectious mosquitoes that transmit infectious agent to humans (I-Ppol)
E1: With I-Ppol, biting may be slower, and increased probing (therefore salivation higher) so rate of infection could be higher.

E14: No change, active transfer of saliva. Same as FT1-3a (now FT1-3a)

E15: Same comments as above for G3. Can’t see why the HEG would make sporozoite more infectious. If they had influence on biting persistence then they would. Can’t do experiment on this. Can’t think of reason it would be different. (0.2,0.8)0.8

E16: Same as WT(0.8,0.95)0.8
Figure 6.15: Proportion of bites from infectious mosquitoes that transmit infectious agent to humans (I-Ppol)
E2: Answer is for I-Ppol relative to WT. No reason to change from above (0.8 to 1.2) 0.8
E3: Answer is for I-Ppol relative to WT. No reason to change from above (0.8 to 1.2) 0.8
Figure 6.16: Mortality rate of mosquitoes once outside of insectary (WT male)
E1: Comfortable with longevity in days as a parameter. WT males are comparable with females but not for laboratory habituated. Males in laboratory have about half of life expectancy as laboratory reared females, perhaps due to selection advantage in laboratory to mate early as possible with virgin female. For males around 10 days. Overall, predators (spiders) would reduce all slightly (minus 20%).
Figure 6.17: Mortality rate of mosquitoes once outside of insectary (WT male)
E2: Not this absolute question. Following responses relative to Fred’s absolute mortality for WT Male (?)

E3: Mortality rate about 12% per day (0.1 to 0.20) 0.8 but this should be somewhere in the literature for different environments.

E14: Should be able to get data from 1954 Monograph by Holstein in Burkina Faso, with survivorship curves for females. Not sure about males. For males survival much lower. There is a paper by Doug Patton with comparative data between wild-type and colony strains.

E16: Think that male mortality is going to be higher for males than females. Rough rule of thumb is they tend to be shorter lived. 10% increased mortality compared to females. So this goes for G3 and GM as well. (0.055,0.165)0.9
Figure 6.18: Mortality rate of mosquitoes once outside of insectary (WT male)
E15: Usually daily survival around 90% for females, with reserves. Males lower than females, about 80% day. (0.5,0.75)0.7
Figure 6.19: Mortality rate of mosquitoes once outside of insectary (WT female)
E1: The rows for FT1-4 were elicited originally elicited without predation effects, but were then multiplied by 0.80 to account for predation effects. Thus original elicitation (i.e., without predation effects) can be recovered by increasing by 1.25.
Figure 6.20: Mortality rate of mosquitoes once outside of insectary (WT female)
E2: Not this absolute question. Following responses relative to Fred’s absolute mortality for WT Male (?)

E3: Typically females live longer than males. Mortality rate about 10% per day (0.08 to 0.15) 0.8. Again, better to rely on data than on off the cuff opinions.

E14: See FT1-4a-mortality (now FT1-4a-mortality)

E16: Daily mortality rate. Rough rule of thumb 10% mortality per day. Dependent on temperature, relative humidity, and rainfall. (Assume 60% parity in mosquito populations.) (0.05,0.15)0.9. Really need to talk to the three modelling groups for these.
Figure 6.21: Mortality rate of mosquitoes once outside of insectary (WT female)
E15: Often use value of 90% average daily survival (0.6,0.95)0.9
Figure 6.22: Mortality rate of mosquitoes once outside of insectary (G3 male)
E2: Same as relative difference for G3 to WT females. No reason to expect different effect on mortality rate for females v males for G3 v WT.

E3: Same relative difference for G3 to WT females. No reason to expect different effect on mortality rate for females v males for G3 v WT.
Figure 6.23: Mortality rate of mosquitoes once outside of insectary (G3 male)
There were no comments for this question.
Figure 6.24: Mortality rate of mosquitoes once outside of insectary (G3 male)
E3: Leave same as for G3 females. No reason to believe this to be different (0.2, 0.9)
E14: See FT1-4a-mortality (now FT1-4a-mortality)
E16: 10% higher mortality than G3 females (0.066,0.198)
Figure 6.25: Mortality rate of mosquitoes once outside of insectary (G3 male)
E15: Males in laboratory had about 50% lipid content of males in the wild, and lipid concentration is one of the main predictors of survival. So up to 50% survival to wild type (0.3,0.6)0.7
Figure 6.26: Mortality rate of mosquitoes once outside of insectary (G3 female)
E2: G3 F v WT F. (1.2 to 4) 0.8
E3: G3 F v WT F. Once outside (0.8 to 5) 0.8 where '5' indicates 5 time mortality of (0.08 to 0.15) and 0.8 implies better survival hence (0.8 to 5) 0.8
Figure 6.27: Mortality rate of mosquitoes once outside of insectary (G3 female)
E1: Original quantiles were 14 and 21, then minus 20% for predation, giving 11.2 and 16.8, but then was adjusted to 8 and 16.8 in an attempt to address low likelihood of mortality for less than 1-2 days. Then an attempt was made to elicit more than two quantiles, but in the end this was abandoned, and we stayed with 11.2 and 16.8.
Figure 6.28: Mortality rate of mosquitoes once outside of insectary (G3 female)
E3: Good chance of mortality of 100% on first day as not adapted to the wild. So close to one. Lowest mortality about 0.1. (0.2, 0.9)

E14: Should be able to get data from 1954 Monograph by Holstein in Burkina Faso, with survivorship curves for females. Not sure about males. For males survival much lower. There is a paper by Doug Patton with comparative data between wild-type and colony strains.

E16: Think these are not so tough. So would expect 20% higher daily mortality rate. (0.06,0.18)
Figure 6.29: Mortality rate of mosquitoes once outside of insectary (G3 female)
E15: Laboratory females are getting blood which is a good resource for survival. It is also dependent on larval rearing condition leading to lipid content. Possibly least important is G3 or GM but more so density of rearing. If reared at low density might compensate for fitness costs. All things being equal, it would be less, but larval rearing overrides any lab-strain effects and adds considerable uncertainty, as would seasonal effects, which would determine post release survival. Reduction in values with respect to wild type could be 10 to 50 percent reduction. (0.1,0.95)0.9
Figure 6.30: Mortality rate of mosquitoes once outside of insectary (I-Ppol male)
E1: Assuming backcross but still in general area of G3 longevity
Figure 6.31: Mortality rate of mosquitoes once outside of insectary (I-Ppol male)
E3: Males die faster. Distribution similar to females but shifted slightly. (0.25, 0.6)0.8.
E14: See FT1-4d-mortality (now FT1-4d-mortality)
E16: 10% Higher mortality than GM females (0.0726, 0.2178)0.9
Figure 6.32: Mortality rate of mosquitoes once outside of insectary (I-Ppol male)
E15: At best about same as G3 males. Given Klein paper probably worse compared to G3 and
G3 with construct. But hard to specifically quantify this from paper as a mix within the paper.
Largest decrease possibly around 50% compared to G3, but at high end possibly around same
as G3. (0.15,0.5)0.7
Figure 6.33: Mortality rate of mosquitoes once outside of insectary (I-Ppol female)
E2: I Ppol F v WT F. (1.05 to 1.7) 0.8 log-normal
E3: I Ppol F v WT F. (0.7 to 1.1) 0.8 log-normal
Figure 6.34: Mortality rate of mosquitoes once outside of insectary (I-Ppol female)
E1: Construct effects are not well expected (understood?), but should be similar for both males and females.
Figure 6.35: Mortality rate of mosquitoes once outside of insectary (I-Ppol female)
E3: These will be better than G3 as back crossed to WT, so more like WT. (0.15, 0.5)0.8. Ok with tails

E14: See FT1-4d-mortality (now FT1-4d-mortality)

E16: 10% higher mortality rate than G3. (0.066, 0.198)0.9
Figure 6.36: Mortality rate of mosquitoes once outside of insectary (I-Ppol female)
E15: Same as G3. Probably some reduction in females, but hard to quantify as no detail available yet. Easy experiment to do. Same value as G3 but deliberately broad as should do experiment here. (0.1,0.95)0.9
Figure 6.37: Number of fertile eggs laid by blood fed female (following WT male mating) (WT female)
E1: Have good data on WT, and have tried to establish lines by laying wild caught eggs in lab. For I-Ppol (Mark Benedict experiments) there has been some work done. Fecundity median around 160 for first and second laying (range 120-200). Some differences between species, but within the above range. Yaro et al. (Year?) might have some data for comparison. CSIRO: Has The expert interpreted the question has number of eggs over mosquito life-time or per batch (per laying event)?

E3: No number

E14: Colony strains will feed every three days, but won’t be kept alive beyond 3-4 blood feeds. So may select for females laying larger egg batches. So laboratory strains may lay proportionately more. See Holstein.

E15: Lots of variation between local populations. 50-80 eggs with 95% CI interval. Lyimo et al 2013, Proceedings of Royal Society. (50,80)0.8

E16: This is known with some precision. Ask Ulrike Fillinger (Mbita). (35,65)0.9: Comment amended after feedback report: This is wrong, egg production is not normal. Median is about 50, but one can get 150 eggs per female. Many mosquitoes lay less than 10 eggs.
All experts: 38 of 352 EXw = 0.085

Figure 6.38: Number of fertile eggs laid by blood fed female (following WT male mating) (G3 female)
E1: The range for G3 is about 45-80, with median 60 for first and second laying. Typically in lab they only get one blood meal per laying event. We know that some fraction (around 10%) in G3 are not fertile, while WT it is near 100% fertility. Yaro et al. (Year?) might have some data for comparison.

E14: Wild F1 egg batch is around 50, with egg laying every three days, and need to account for mortality. In lab, only allow first few egg laying events, so early high-fecundity would be selected for. Unlikely to get lifetime reproductive success. Holstein might have some figures for number of fertile eggs laid. Paul Howell from CDC, MR4 laboratories. They do stock maintenance and might have the records.

E15: Number of eggs linked to female body size and blood meal size. And these two are linked as bigger means they can take more blood, i.e. a positive association. Laboratory size might be smaller and therefore less fecund. Rearing going to have big effect. Potential affect of G3 on fecundity. All else being equal, probably around same as WT, possibly some reduction, but need to do an experiment to quantify this. Fitness cost paper 2003 by Catteruccia. Quite a few papers looked at effect of GM constructs. If same blood meal as WT and grown up in same environment and about same size then might be similar. But the blood meal that G3 is maintained on will have big affect here, as the number of eggs can vary between human and animal blood. Host blood types influence the production then specialise to producing eggs on that type. At best the same as WT but probably lower. No answer here. Just comments.

E16: Same as WT(35,65)0.9
All experts: 39 of 352 \( EX_w = 0.131 \)

Figure 6.39: Number of fertile eggs laid by blood fed female (following WT male mating) (G3 female)
E2: Environmentally in great shape, maybe not genetically in great shape. Problem knowing size relative to WT. If systematically bigger maybe more fecund. Expect somewhat inbred laboratory culture and for that reason they'll be less fecund. These two factors account for uncertainty +/- 1 (?) relative to WT.

E3: Think refer to others answers about how they are affected. May be larger. 0.7 = 70% as many eggs as a wild type female?
Figure 6.40: Number of fertile eggs laid by blood fed female (following WT male mating) (l-Ppol female)
E1: For I-Ppol a guess would be that it falls around 40, with range of 25 to 65. Probably less fecundity, with minus 15% for being infertile.

E14: Big unknown. Equivalent to G3 probably.

E15: More confident in saying a reduction here. Going to derive from papers. Follow up day 2. Up to half as many eggs is the lower end, in some cases papers have reported no difference or a slight enhancement at the upper end. (25,80)0.9

E16: Some cost to fertility for GM, so 10% reduction to G3 versus WT. (31.5,58.5)0.9
Figure 6.41: Number of fertile eggs laid by blood fed female (following WT male mating) (I-Ppol female)
There were no comments for this question.
Figure 6.42: Average (over a month) daily dispersal distance (WT male)
E1: In the context of realistic movement pattern, a week is a good time value. In village sampled, the distance sampled between houses varied between more the a 1km and up to 6 km. Other data from Chuck Taylor and Greg Lanzaro, which showed great underestimate of maximal travel distance. Nominal value is about 200m in a week in a village setting, there are records of 1 to 2 kms when ovipositing. In a peri-urban environment the dispersal distance would be less, unless the availability of egg-laying habitat is scarce, which would increase the distance. If there are abundant larval habitats, then dispersal may be less than 200m around 100m. If habitat is lacking, then the distance will be more than 200m to a few kms. Males seek sugar source and mating swarms, which are more available than egg laying sites. Variance would be high if we consider both wet and dry season. Elicited values are for net total meters displacement over a week. Historic outbreak in Brazil noted 50kms per year travel during outbreak.

E3: Can’t give answer for males

E14: The expert is the only one with detailed studies. Mike Service has a 1993 paper "Mosquito migration the long and short of it". Anecdotal evidence.

E15: Poorly understood, depends on habitat. If available blood, then will not travel far. Only a handful of studies have looked at this. Males travel less as limited by survival. Generally there is big uncertainty here. Paper by Thomas and Lindsay 2000 (Transactions of the Royal Society of Tropical Medicine and Hygiene) has an upper limit of 8km (total) in Gambia. 1961 paper by Gillies Bulletin of Entomological Research, found a maximum of 5-8 km of total distance travelled. Most found within 2 miles, very few dispersed further (do a citation search on this one for other data papers). Studies on dispersion and survival of A. gambiae in East Africa, by means of marking and release experiments. Look at genetic connectedness between populations to get indication of dispersal distance. Labelled male mosquitoes with isotopes and looked at distance recaptured. Only about 10% males went further than about 2 miles.

E16: Not relevant questions as mosquito will fly as far as it needs to. If water directly outside the house it will go that far. Very related to ecology. Males will not go as far as females. For males 90% won’t go further than a 1km. Answer given as km (0.01,1)0.9 Lognormal. Comment amended following feedback report: Change this since 90% won’t go further than 1.6km, see Thomas et al (2013) PLOS ONE Vol 8, e68679.
All experts: 43 of 352 EXw = 0.284

Figure 6.43: Average (over a month) daily dispersal distance (WT females)
E1: See above notes for males.

E3: Over a week. Do not think capacity means anything (see table in handout). Giving average dispersal distance per week. Every week have to go from house to water where they are going to oviposit. If pond 0.1km away that’s where they’ll go. In country can move over 1 mile in a week, they have to move to where they will lay egg, sometimes lake or pond away from village. In city they could lay in mud puddle. Range from (0.1km to 1km) 0.8. Value elicited is for a week.

E14: There are some mark recapture studies, such as Philip McCall in Tanzania and Charles Taylor in 1993 in Mali.

E16: This has been published recently by C Bogh and CJ Thomas, mosquito flight distance, in 2013. (0.01,2)0.9 Lognormal
Figure 6.44: Average (over a month) daily dispersal distance (G3 male)
E1: Probably reduced, 70 years in captivity and are very docile, smaller, weaker. All effort goes into swarming effort and location of sugar source, with no selection for longevity and vigour.

E2: Very unlikely will compare comparably to WT.

E3: Compared to others previous questions here, behaviour of being raised in laboratory will win out.

E14: No idea

E16: Conservatively reduction in 20% of flight ability compared to very healthy WT. (0.08,0.8)0.9 Lognormal
Figure 6.45: Average (over a month) daily dispersal distance (G3 female)
E14: No idea

E16: Conservatively reduction in 20% of flight ability compared to very healthy WT (0.008, 1.6)0.9 Lognormal
All experts: 46 of 352 EXw = 0.511

Figure 6.46: Average (over a month) daily dispersal distance (I-Ppol male)
E1: Probably less active than G3.

E14: No idea

E16: Assume I-Ppol are 10% of G3. Construct is doing something which has a cost to the system. May well be neutral, but safe to assume as asking to do something additional there will be a cost to that. (0.0072,0.72)0.9 Lognormal
Figure 6.47: Average (over a month) daily dispersal distance (I-Ppol female)
E14: No idea

E16: Same again here, which is 10% less than G3 females (0.0072, 1.44) 0.9 lognormal
All experts: 48 of 352 EXw = 0.103

Figure 6.48: Average (over a month) daily dispersal distance (I-PpoI female)
There were no comments for this question.
All experts: 49 of 352 EXw = 0.171

Figure 6.49: Probability of biting for two blood meals after release (WT female)
E1: If just two meals on a human over any time span with interruption: median of 0.7 and 50% CI range of 0.5 to 0.85.

E2: No answer

E3: Very low. Somewhere between (0.01 to 0.1) 0.8. Tail down to zero. Change to (0.2 to 0.7) 0.8 beta distribution. Again, I wish I had had the data in front of me.

E14: Needs refining as to whether estimate of survivorship for 6 days. If not could be compounded by avoidance of humans population. 12 to 15 days for incubation, so should be 3 to 4 blood meals. 2 blood meals relevant for ONNV. 88-90% per day. So for 6 days = 0.9E6.

E15: Function of daily survival and gonotrophic cycle. Function of survival and number of bites per day. Two successive bites at maximum four days apart. So probability of taking a bite one day, by the survival over the next four days and getting a bite that day. If anything else additional here, behaviour wise (i.e. if bad at finding a host) for G3 and GM then will be more uncertainty. Potentially widen the uncertainty here from simple function of mortality times bites per day. Would be better to know probability of becoming infected and surviving extrinsic incubation period. What is the probability of getting a blood meal on two successive gonotrophic cycles. Multiply daily survival rate by length of gonotrophic cycle (approximate number of bites per day). Followed up on day 2: this should be focused on the probability of two blood meals with appropriate time interval between them (i.e. Extrinsic incubation period).

E16: Determined by ecology and control efforts. Three areas which are interested in doing releases. There are large scale insecticide treated nets everywhere. Assume 80% bed net coverage as that is current best practice. Derived mathematically from survival rates and feeding frequency. Should be able to calculate this from parameters already elicited. Given 1000 3-day old mosquitoes released, how many become infective if they were (1) WT (2) G3 (3) GM, (1) 100 (2) 60 (3) 20. Important thing here is survival, tweaking that just a bit changes the infective rate a lot. So therefore G3 could be less efficient. In R0 model the daily survival is most important. Assuming here 15% mortality per day, about 60% will take two blood meals. (0.4,0.8)0.9
Figure 6.50: Probability of biting for two blood meals after release (G3 female)
E1: For three day span with less interruption: 50% CI range of 0.4 to 0.7.
E2: Not confident answering this.
E3: Most going to die so no change of two blood meals (0.05, 0.2)0.8
E14: Not sure
E15: Potentially widen the uncertainty here from simple function of mortality times bites per day. Would be better to know probability of becoming infected and surviving extrinsic incubation period.
E16: Shift this to the left compared to WT (0.3,0.7)0.9
Figure 6.51: Probability of biting for two blood meals after release (I-Ppol female)
E1: They behave like G3 if not backcrossed and have reduced survivorship, which would reduce their chances. But may be slower to get blood meal, but overall range of 0.3 to 0.8.

E3: I-Ppol part way between G3 and WT (0.1, 0.5)0.8

E14: Not sure

E16: Shift this to the left compared to G3 (0.2,0.6)0.9
Figure 6.52: Extrinsic incubation period (number of days from mosquito acquiring infection to becoming infectious) (WT female)
E1: Cannot imagine why it would differ between target groups. Temperature conditions in the field (more than 35 degrees C) are higher than the laboratory where most data is from. It takes 8 days for first sporocites to appear after first meal, with 9 to 10 days as a nominal range.

E2: AAe standard assay to show after blood meal how long until it shows up in saliva. Can get this from data or experiments.

E3: On initial discussion at start of day gave these values between (7 to 14) 0.9 days, but on reflection didn’t want to give answer to this question.

E14: Temperature dependent. Approximately 12-15 days, faster incubation period at higher temperatures. The expert Collins at CDC did a lot of work on this. Also need to consider percentage of blood meals on humans. In Western Kenya there can meals on non-humans (bovids and dogs).

E15: Highly temperature dependent. Fastest time probably about 10 days, often about 12. Longer if colder. Depends on what type of malaria parasite it is. Speculative, parasite under huge pressure to go faster, so if there was a way it would have found this as this is the biggest constraint on transmission, so hard to imagine how it would go faster to infectious stage in a different type of mosquito. Can’t think why it would be different WT to G3 to GM. WT information available in papers.

E16: 10 days approximately (8,12)0.9 . Comment amended following feedback report: Draw in left hand size so mosquitoes become infective at day 9, with the peak at day 10.
Figure 6.53: Extrinsic incubation period (number of days from mosquito acquiring infection to becoming infectious) (G3 female)
E1: Same as WT, although immunological differences may affect competence it would not attenuate extrinsic incubation period.

E14: Not sure

E16: Should be same for both G3 and GM as property of parasite not the vector. (8,12)0.9
Figure 6.54: Extrinsic incubation period (number of days from mosquito acquiring infection to becoming infectious) (I-Ppol female)
E1: Same as WT, although immunological differences may affect competence it would not attenuate extrinsic incubation period.

E14: Not sure

E16: Should be same for both G3 and GM as property of parasite not the vector. (8,12)0.9
Figure 6.55: Percentage of blood meals taken on humans (WT female)
E14: Should included proportion of blood meals taken on humans. Can differ between east and west Kenya. In Kenya high proportion of meals taken on bovids and dogs. It is location dependent, see Gerry Collen, Transactions of Royal Society of Tropical medicine for East/West comparison.

E15: AG SS is Anopheles gambiae sensu strictu. Talking about old school AG SS before split into Coluzzi. Highly anthropophilic species. Day 2: this question gives guidance on potential for any behavioural changes. Lower bites on humans could be for any reason but this gives indication of host preference. (0.8,0.99)0.9

E16: This is going to vary according to location. With A. gambiae in East Africa being more anthropophilic than in West Africa. In East Africa going to be (0.8,0.99)0.9 but in East (West?) Africa may drop by 10% (0.7,0.95)0.9
All experts: 56 of 352 EXw = 0

Figure 6.56: Percentage of blood meals taken on humans in East Africa (WT female)
Figure 6.57: Percentage of blood meals taken on humans in West Africa (WT female)
E16: Split this questions by East and West Africa, then answered for whole of region.
Figure 6.58: Percentage of blood meals taken on humans (G3 female)

All experts: 58 of 352 EXw = 0
E14: Not sure
E15: Might be different if colonization has induced behaviour shift or if they have been fed on rodents for 30 years. Needs an experiment. Transmission dependent on human host preference. Could do fairly safely.
E16: G3 will feed on people. In laboratory they feed them on horse blood. But they will feed on people quite well. Use same as WT. (0.7,0.95)0.9
Figure 6.59: Percentage of blood meals taken on humans (I-Ppol female)
E14: Not sure
E15: Same as G3 for GM here.
E16: Same again, no reason to be different, so same as WT/G3. Difficult when speculating what the transgene might do to different systems in the mosquito. (0.7,0.95)0.9
Figure 6.60: What is the probability that a mosquito from the escaped population, in a year, will contact a human or other vertebrate infected with a pathogen not previously known to be vectored by Anopheles gambiae?
E1: Probably not very low, would be in excess of 80%, as there are so many escapees and many descendents.

E3: Probability high that mosquitoes will contact an infected human or vertebrate (i.e. HIV, dengue).

E6: The limiting factor is whether we are in an endemic or epidemic situation for a novel pathogen (i.e. what is the prevalence of the pathogen in the human population - which is what they mainly feed on). The expert thinks a broad range of 1e-2 (epidemic) to 1e-4 (endemic). Probably smaller for a GM because GM won’t live as long. It’s difficult to extrapolate between constructs but if a mosquito in a laboratory typically lives for 20 days, a GM typically lives for about 17 days. Takes about 5 to 10 generations to adapt WT to survive well in the laboratory, so it could take this long for laboratory crosses to adapt to the wild. Say a 5% reduction to all of the time dependent events.

E11: Quite possible. At least one or two will encounter infected vertebrate. This is possible. The probability is high with 10K mosquitoes assuming 5K females, at least one or two could encounter a vertebrate with a novel pathogen. Various kinds of viruses circulating in vertebrate populations.

E13: Contact with AIDS infected person very likely.

E14: Data on this and location specific. Very localised distributions. Children will have about 12 fevers per year, so chance of contact per year very high.

E15: This really depends on its biting behaviour. There are two issues, if it bites same as wild type and if it contacts same pool of hosts. (0.7, 0.9)0.8. GM v non-GM are the same.

E16: High, at worst time of year, lots of other things happening. Worst site Mbita, lots of HIV. 90% chance at least one mosquito will come into contact with an infected human or vertebrate in worst case scenario. (0.8, 0.99)0.9. GM v non-GM: Drop 10%

E17: This is very high, 10,000 mosquitoes or their descendents, have to contact a person and some of these people will have pathogens.

E18: Reasonably likely. (0.05, 0.1)0.7

E20: (0.7, 1)0.99 Beta didn’t fit, but want these values, so need to find a way to fit similar with upper bound at 1. I suppose nothing has a pdf at 1 so we can put this at (0.7, 0.99)0.99.

E22: Mosquitoes will definitely be infected by novel pathogens. Probability is high because there are lots of pathogens, also depends on the density of the vector and the density of infected people. Sick people with fever will have higher CO2 emission and will be more attractive to mosquitoes. Sick people tend to stay indoors, they have higher temperature and these are reasons why sick people are probably more likely to be bitten. Novel pathogens include viruses but most bacteria in the blood will kill you quickly so probably won’t be these, despite the fact that some bacteria (such as Leptospiroa) that are vector borne, and also some Rickets. Highly likely 10,000 mosquitoes over a mile radius, 3.14miles squared, assuming say 20,000 people. What fraction of people are infected with something that is not Plasmodium. Lets say 10% are ill with non-Plasmodium disease, mainly viral disease. One mosquito for every three people, so say 3% chance, high probability (0.0005, 0.05)0.75.

E24: Very low because the mosquitoes are being kept in disease endemic countries. All the things they would encounter would be things that A. gambiae in wild would already be in contact
with. Something very close to zero.(1E-6, 1E-5)0.9
Figure 6.61: Given that a mosquito has contacted an infected human or other vertebrate, what is the probability that it acquires a novel pathogen through a blood meal?
E1: Likely to acquire through blood or skin, near 0.5. Not sure how rare it would be (speculative answer here), if you have some virus infection, how much is detected in blood I am not sure. Also spread by A. gambiae, are O’nyong-nyong (minimal), and Wuchereria bancrofti (more common).

E6: The expert thinks this is high. If it has contacted an infected person it is likely to feed on it. Could be 50% chance or higher of feeding on it and therefore acquiring the pathogen. No difference with GM.

E11: Less than encounter rate but non-trivial.

E13: Essentially probability that it ingests a pathogen with blood meal; likely high probability.

E14: Ok

E15: Two reasons mosquitoes don’t get infected, (a) pathogen cannot infect the mosquito; and, (b) ecological barrier to infection (which under laboratory conditions can probably infect it, but in wild can not contact a host). Important here to consider potential behavioural change in host preference. One example was the change of biting habit of mosquitoes in Malaysian Borneo. Monkey malaria is now higher in people possibly due to mosquitoes changing host preference (perhaps due to forestry increasing contact, or monkeys being brought into cities). Most of the time it would not be a problem, but it could happen and would be big impact if it did. Ecological barrier removed. (1E-6,0.01)0.6. GM v non-GM: Possibly higher.

E16: Responses to questions are site specific. Burkina Faso (Bobo), Kenya (Mbita), Mali (Bamako). Quite low, very unlikely mean about 5% unlikely to be above 0.1. (1E-3,0.1)0.9. GM v non-GM: Same.

E17: Novel blood based pathogens include HIV but The expert doesn’t really know what all the diseases are in the blood. Thinking that this could also be up near one but not everybody has a blood borne pathogen. The expert is less certain about this than before. 10k escape, 5k are females, 50% of these contact a person, that’s 2.5k, what’s the chance that one of these people have a novel blood based pathogen. AG don’t bite things that eat them like birds or bats and this influences The expert’s response to FT2-112100 (now FT2112100) (ingestion question)

E18: Slightly less than the probability of contacting the individual. (0.01,0.1)0.6

E20: Given that the pathogen is not described and could be anything. The probability that any one pathogen is contained within a blood meal is very high (0.7,0.99)0.9.

E22: We should exclude all bacteria because usually bacteria do not infect the blood, so we are left with parasites, viruses and worms (see comments about the few blood borne bacteria). Arboviruses have evolved to be transmitted by a vector, yellow fever, dengue, rift valley fever, encephalitis, etc. These viruses have a very specific interaction with their vector. If they are infected they will pick it up. If you have a viremia then probably not. The expert calls this stage biological contamination. This is not infection (0.2, 0.8)0.90.

E24: Not qualified to answer
Figure 6.62: Pathogen reaches infectious load

All experts: 62 of 352 EXw = 0

FT201

F(x)

E14

CSIRO HEG RA record of expert elicitation
E14: Join FT2-010 (now FT2010) and FT2-011 (now FT2011). These are not independent. What is probability of a pathogen reaching infectious load. Fungal control methods trialled in Tanzania. Fungi can cause infections in immunocompromised patients. So if escaped mosquitoes have lower immune systems then they might be more likely to carry fungi. So increased risk with immunocompromised patients. i.e. Western Kenya where HIV rates are relatively high.

E15: No comments here

E17: The expert knows very little about pathogen mosquito interactions so he can’t comment on the rate limiting step here so skip these questions
Figure 6.63: Given that a mosquito has acquired a novel pathogen through a blood meal, what is the probability that the pathogen survives all of the mosquito’s immune systems (cellular, humoral and RNA interference)?
E1: Not sufficient knowledge to answer.

E6: The expert thinks that novel pathogens have 99% chance of surviving the immune system. Believes that the immune response shown to date are laboratory artefacts of artificially high doses (e.g. Malaria immune response). In nature doses are much lower and The expert doesn’t think there will be much of an immune response. Components of the immune system (e.g. RNAi) exist but function in nature is unknown. No difference with GM.

E11: I would suggest they are being too specific in distinguishing pathogen survival, and then to replication; pathogen survival also is mediated by physiologically hospitable environment of mosquitoes’ internal environment. Likely non-trivial, as there is evidence Hepatitis C in bedbugs persists despite non-transmission. Evidence of transmission via oral route (i.e. eating mosquito). Especially human pathogenic bacteria, which is what distribution is based on.

E13: Ok

E16: Very remote. HIV relatively common, likelihood of transmission is remote. Can’t see chance of contact with wild animal viruses and there is little if any evidence to support the idea that HIV can be transmitted by mosquitoes. (1E-7,1E-6) 0.8. GM v non-GM: Reduced by 10%

E18: Very low. Otherwise mosquitoes would be vectoring already. (1E-6,1E-5)0.7

E20: That any one individual of any of the various types of pathogen survives is high. (0.2,0.8)0.7

E22: Immune system step not important until after infection. The experts doesn’t think the immune system will eliminate the novel pathogen, he thinks it will "blunt" the pathogen. The mosquito has a 20 day reproduction cycle and it won’t put too much energy in clearing a pathogen. Its enough for the mosquito to simply slow it down. But it is true that A. gambiae does not transmit any viruses to humans but The expert is tempted to think this is because of the incompatibility of recognition virus receptor with mosquito lygens. Doesn’t think this is because of the immune system. The expert thinks most of the immune system is geared to controlling bacteria proliferation in the gut following blood meal. Can’t give a number here.
Figure 6.64: Given that a mosquito has acquired a novel pathogen through a blood meal (biological contamination), what is the probability that it survives the digestive enzymes.
E22: Very low probability, the gut is a very challenging environment, and those that do survive there have developed specific strategies to do so. This is a huge bottle-neck even for Plasmodium. Most die in the gut, only a small fraction can survive. This has nothing to do with the immune system (1e-3, 0.01)0.90.
Figure 6.65: Given that a mosquito has acquired a novel pathogen through a blood meal (biological contamination), and has survived the digestive enzymes, what is the probability that the novel pathogen infects (or enters) a gut cell (which is the first barrier)
E22: Same range probability as FT2-010a (now FT20100-9) but less certain. This could be looked at in the laboratory. The expert thinks that entering the cell is the more unlikely step, because once you enter you may get replication (1e-6, 1e-3)0.95.
Figure 6.66: How would you change your probabilities for FT2-010 if the 10,000 insectary mosquitoes were all genetically modified with the I-Ppol construct?
E20: Think that the probability that there could be some effects on immunity, there will be more uncertainty. Could go up or down. Which is relevant to FT2-021 (now FT2021), FT2-010 (now FT2010), FT2-1120 (now FT21120), FT2-245-GM (now FT22245GM). The probability that the GM weakens the immune system is reasonable. So I would say $p(\text{GM weakens immune system})$ is $(0.01, 0.5)0.8$. Note this is the $p(\text{GM})$ only i.e. not multiplied yet by $p(\text{pathogen survives immune system in non-GM insect})$. 
Figure 6.67: Given that the novel pathogen has survived the mosquito’s immune system, what is the probability that the pathogen replicates in the mosquito?
E1: Not sufficient knowledge to answer.

E6: This is the rate limiting step - highly unlikely. Nature is doing this experiment all the time. These mosquitoes are being exposed to these pathogens all the time and we only see a limited number of viable relationships. No difference with GM.

E11: Also constrained by mosquitoes internal physiological environment. This presumes that novel pathogen can make a critical adaptation. While most pathogens (viral, protozoan) novel to mosquito would be unlikely to replicate, many potentially human pathogenic bacteria might be able to transiently replicate in mosquitoes' blood meal, which is what elicited distribution is based on.

E14: Not replication rather it reaches infectious stage. For Filarial worm it takes up to 10 microfilaria. Small subset developed through infectious stage. Split by viruses and non-human plasmodium which have development and replication cycle and filaria, for example, which just have developmental cycle, no replication required.

E16: Pathogen chatter. These events are happening all the time but rarely spark, but occasionally they do and you get a bush fire. If managed to get this far quite smart, so likely 50/50 chance. (0.25,0.75)0.9. GM v non-GM: Same

E18: Same as above, low. (1E-6,1E-5)0.7

E20: The fact that it should be able to replicate, not only survive, makes this less likely than FT2-010 (now FT2010), but still likely because this could be any pathogen (0.1,0.5)0.6

E22: This is a rate limiting step, the mosquito does not act like a bag. Viral replication can also be studied in the laboratory, using live A. gambiae cells. But more likely then entering the cell. Once a virus has entered a cell they are able to subvert the cell machinery for its own benefit so it probably will be able to multiply. Here we enter a new scenario. How many virions can it produce, a good infection is 20k virion per cell but The expert doubts it will reach this level because there has been no co-evolution with the novel pathogen, the cell will probably die early because of this. Other questions are the virions able to infect other cells. Virus packaging is a very co-ordinated process, if the host cell is not able to produce package protein in the right proportion packaging will not be successful, so you could have some virion production but these will probably be low and less infective (1e-4, 0.1)0.75
Figure 6.68: Given that the pathogen has replicated in the mosquito, what is the probability that it travels to the salivary gland? (point of inoculation)
E1: Not sufficient knowledge to answer. Diversity of interactions are immense, and capacity to enter into glands may or may not be trivial. Model we have for malaria may not apply to novel pathogen. We could extrapolate from experiments with surrogate vectors, which demonstrates physiological capacity, but no one study has demonstrated how it could complete entire life cycle of pathogen.

E6: There is a 50% chance that it will get to the salivary gland because there are relatively few examples of a pathogen that is able to replicate but not disseminate, the exception is live vaccine viruses. This would be a lower probability for the GM because if the mosquito life span is condensed there is less time for it to get to the salivary gland before the mosquito dies.

E11: Very remote. Presumes we are witnessing an evolutionary event, and it ends up in salivary ducts in a manner that allows injection from blood feeding. Very remote, assuming an evolutionary event. Going to give ballpark for a rare event of 0.5 probability central credible interval bounded by 1 in billion to 1 in a million. Not only travels to salivary gland but ends up in salivary ducts in a manner that allows injection on blood-fed host by feeding insect.

E13: Ok

E14: Reword to" Given that the pathogen has replicated in the mosquito, what is the probability that it travels to point of inoculation?" Probability likely quite high for viruses, but low for dia-filaria, thus bimodal, with more probability mass to the higher probabilities. (0.2,0.8)0.3

E15: Probably high. Passive dispersion. (0.1,0.3)0.7. GM v non-GM: About the same.

E16: 50/50 chance. If it gets in it should be able to disseminate throughout the mosquito. Hesitate to answer as don’t know literature. If there’s evidence that viruses do not disseminate then this value should not be accepted. Need to speak to mosquito virologist. Most of these pathogens are going to be RNA viruses. (0.75,0.9)0.8. Caveat that going into unknown territory. Needs to be confirmed by virologist. GM v non-GM: Same

E18: No idea.

E20: This is much less likely because it requires tropism and traversal of membranes (0.001,0.1)0.6. Less likely than I previously stated. Put (0.0001, 0.01) 0.6

E22: This is similar concept to invading the gut cells. Very low probability, given the fact that also this time the novel pathogen has replicated in a non-natural host cell they are less infective. Would assume that the probability of reaching salivary gland is one order of magnitude less than invading the gut cell (1e-7, 1e-4)0.80.
Figure 6.69: Given that a mosquito has acquired a novel replicating pathogen what is the probability that the mosquito survives the pathogen’s incubation period?
E1: Not sufficient knowledge to answer.

E6: We should already know what the average life-expectancy of a mosquito is, and added to this the incubation period of viruses, pathogens and parasites that we know about. Don’t elicit this go figure it out. 3 to 6 new human pathogens a year but rarely associated with mosquitoes, hence the set of pathogens we need to look across is not intractable. Also lower for the GM because time dependent.

E11: Presuming 10 day incubation period, probability that mosquito would survive long enough to transmit parasite is roughly 5% to 0.5%. Survival slightly reduced because insectary population. Assume 10 day incubation period, survival at probably 0.8 per day. Some data available for A gambiae.

E13: Ok

E14: Daily survivorship 0.9, and for 15 days is 0.9 raised to power 15, for four days 0.9 raised to power 4; again bimodal due to filarial and viruses.

E15: Depends on how long it takes the parasite to develop, and if that changes the mosquito mortality. Fastest is a virus which disseminates within 6 to 7 days. Given daily survival is 6 days, use 0.9 raised to power 6 for an upper limit. (0.0001,0.5)0.9. GM v non-GM: Lower in GM.

E16: 1 in 1000. (1E-6, 0.01)0.9 Lognormal truncated to (0,1). GM v non-GM: Reduced by 10%

E17: About one in ten mosquitoes are likely to survive the pathogens incubation period

E18: Entirely dependent on the pathogen. This could range from 1 to 1E-6. (1E-6,0.99)0.95 - Need to convey this in figure, but too wide range to plot.

E20: This is quite high because some pathogens will have short incubation times (0.01,0.6)0.6. or indeed, may not need incubation at all if they can be directly transferred e.g. virus on proboscis.

E22: Assuming an incubation period of about a week, and adult mosquitoes mortality rate is about 10% (0.01, 0.1)0.95.
Figure 6.70: How would you change your probabilities for FT2-021 if the 10,000 insectary mosquitoes were all genetically modified with the I-Ppol construct?
E20: The question is not given here. Looking around I think it was "probability that the mosquito survives incubation period" and whether GM affects (increases) this? Also, the probability that the GM increases lifespan of mosquito is pretty low in my view, but not negligible. So I would say \( p(\text{GM causes increase in lifespan}) \) is \((0.01, 0.2)0.8\).
Figure 6.71: Given that a mosquito has survived the pathogen's incubation period what is the probability that it bites a subsequent human or vertebrate?
E1: Not sufficient knowledge to answer.
E6: Again data are available on biting rates and life expectancy. Thinks this will be lower for the GM mosquito.
E11: Given A. gambiae, it is very likely. This includes possibility of unsuccessful bite (i.e. via slapping). Assuming human-preferential A. gambiae. Chances are very high given survival but could get slapped.
E13: Could acquire pathogen in first blood meal, and it would replicate before final blood meal.
E14: Pr=1
E15: If lived this long probability of biting is very high. (0.7,0.9)0.8. GM v non-GM: About the same.
E16: Old mosquito now, so slowing down. (0.2,0.5)0.9. GM v non-GM: Reduced by 10%
E17: This is high. They bite every three days, it could be about 90%
E18: This is known. The probability of a mosquito taking a second blood meal.
E20: (0.2, 0.9)0.8. This is effectively the probability that a mosquito takes a second bloodmeal
E22: Essentially the rate of second biting, The expert is not sure but would guess around 30% (0.1, 0.3)0.95.
Figure 6.72: Given that a mosquito with a novel replicating pathogen has bitten a second human or vertebrate what is the probability that it transmits an infectious load to this individual?
E1: Not sufficient knowledge to answer.

E6: Already assumed that it has most likely picked it up from a person, so The expert thinks this one is potentially high. No difference with the GM.

E11: Assumes FT2-020 (now FT2020) where infectious agent is in salivary ducts and is transmittable via blood meal. However, not all transmitted loads in A. gambiae initiate subsequent infection in all humans (variation). This presumes that infectious agent is in the salivary ducts in a way that would permit injection with next blood meal. There is some data for A gambiae that not all bites result in infection. Have to cross threshold to achieve infectious load that would depend on individual. A very many possible variables to consider, very rough guess, e.g., effectiveness of pathogen.

E13: Ok

E14: Again a split between viruses and filaria.

E15: Pathogens so different some would never infect, some would. Consider the natural experiment of HIV and it has never happened. But we are seeing this happen with monkey malaria, so it is maybe possible. Assuming inoculation into new host, not infection of new host. For infection of new host a whole additional set of steps would be required here. So assuming ‘transmits’ is not infection. Pathogen got put in, whether infection would establish or not we don’t know. (0.3,0.8)0.9. GM v non-GM: About the same.

E16: (0.2,0.8)0.9. GM v non-GM: Same

E18: (1E-4,1E-3)0.6

E20: (0.01, 0.5)0.6

E22: This depends on the infectivity (0.2, 0.5)0.80.
All experts: 73 of 352 $EXw = 0$

Figure 6.73: Via proboscis (mechanical)
E22: Thinks this is impossible. For vector borne disease this is impossible because of limited survival in the environment. What about nonvectored disease such as measles. Contamination possible but very few particles probably, that will stay exposed for several days before the next person is bitten. Very low probability. This is the reason why they don’t transmit HIV and Hepatitis (which is a very stable virus in the environment). A good estimate would to calculate the surface of the proboscis. The proboscis is only a couple of mm long. Pr = 0, can use 1e-9 as an effective zero.
Figure 6.74: What is the probability that a mosquito escaped from an insectary, in a year, will contact a human or vertebrate infected with a pathogen not previously known to be vectored by Anopheles gambiae?
E15: Dependent
E16: Dependent
E17: See FT2-000 (now FT2000)
E18: Dependent
E20: Dependent
E22: See above
Figure 6.75: Given that an infected human or vertebrate is contacted, what is the probability that a novel pathogen sticks to the proboscis?
E6: It seems to be very rare based on data available. The expert doesn’t know why but he thinks it is because the actual part of the mosquito proboscis that penetrates during feeding is a very narrow tube and the other parts of the proboscis (the sheath) folds back. It was demonstrated in the laboratory years ago that it is probably dose dependent - so one reason why HIV isn’t transmitted mechanically is because typically the HIV virus load in the blood is low. One of the indicators that suggests it doesn’t happen is that mosquitoes bite indiscriminately but HIV infections are localised to particular social groups. Mechanical transmission of pathogens by arthropods is typically caused by “pool feeding” or where the disease itself is associated open legions, this is not how mosquitoes feed. No difference with GM.

E11: This is very remote. A gambiae not known to transmit anything mechanically.

E13: Ok

E15: Don’t know about this path of transmission, can’t answer.

E16: Don’t know

E18: (1E-3,1E-2)0.5

E20: (0.01,0.5)0.7

E22: Passive contamination is possible but not transmission. No numbers.
Figure 6.76: Given that the novel pathogen has adhered to the proboscis, what is the probability that the pathogen remains viable between bites?
E6: It could be high if the mosquito was disrupted during feeding and seeks another meal in a short period of time, and that could be 15 minutes to an hour. The typical course of events, however, is that A. gambiae won’t feed for another 4 days and most pathogens are going to die and lose viability during that time. No difference with GM.

E11: Very remote, at least 2 to 3 day survival requirement.

E13: Ok

E14: Given levels of UV exposure over a 3 day window, probably very slim. Again bimodal. High probability for when mosquitoes are disturbed during feeding, and thus a second bite within few minutes, otherwise will bite every three days. So probability of surviving 30 minutes (for example) is high. 0.3(0.1,0.6)- q0.95@0.9 lognormal truncated 0.1

E15: Don’t know enough to answer

E16: Genuinely going to be low. Very dependent on the pathogen. HIV dries up and dies, in relatively short time, where as HepB can remain infective for a year. More likely going to be HIV or Ebola rather than something long living (such as HepB). So think this is really low probability.(1E-6,5E-6)0.8 . GM v non-GM: Same

E18: Wide range. For some pathogens incredibly unlikely. But for some it is entirely plausible. No number

E20: (0.001,0.1)0.5. I would increase the upper bound here, mechanical transmission is documented for trypanosomes for example (something I subsequently found out) and other articles (www.ncbi.nlm.nih.gov/pubmed/3970308) (www.ncbi.nlm.nih.gov/pubmed/14990316)

E22: See comments in FT2-101 (now FT2101) here as well. The viability of a pathogen decreases exponentially in time. Looking at figures for survival in the environment of a pathogen which is pretty tough. The Hepatitis A virus is extremely resistant but there is not a single case of transmission via mosquito. The desiccation step is tremendous, for the mechanical contamination of the proboscis. No numbers.
Figure 6.77: Given that the pathogen has survived on the proboscis, what is the probability that the mosquito bites a second human or vertebrate?
E14: Dependent
E15: Dependent
E16: Dependent
E17: See FT2-022 (now FT2022)
E18: Dependent
E20: Dependent
E22: See above
Figure 6.78: Given that a mosquito with a novel pathogen on its proboscis has bitten a second individual, what is the probability that it transmits an infectious load from its proboscis to this individual's bloodstream?
E6: This depends on the infectious dose and on the immune status of the individual, for some viruses one virus particle is enough, for some it takes a 1000 particles. It could be high. Worst case scenario it could be a 50% chance, best case scenario it takes 1000 pfu to infect and there are only 5 pfu on the proboscis, and in reality this is probably the realistic situation. No difference with GM.

E11: Low number because structure of proboscis and surrounding sheath does not provide much space so that very small numbers of pathogens could be harboured in that environment. Furthermore absolutely no evidence of mechanical transmission by mosquitoes via this route of transmission. Seems vanishingly small probability.

E13: Ok

E14: Vanishingly small. Calculations from HIV. 1-2% 0.9CI.

E15: No bottleneck here so quite high probability. This information is known or similar to infection from a needle, which is mechanical transmission - has right amount on infectious dose on it.

E16: Low here. Use probabilities exactly same as FT21020. Much less chance of transmitting via a needle stick. Could look at literature from infection of needle stick injuries to get some idea. (1E-6,5E-6)0.8. GM v non-GM: Same

E18: Pathogen dependent. Wide range, pathogen dependent. No number.

E20: (1E-4,0.1)0.8

E22: Very low probability, can’t carry a lot of pathogen on the proboscis. No numbers.
Figure 6.79: Via blood transfer

All experts: 79 of 352 EXw = 0
E15: What is the probability that a mosquito escaped from an insectary, in a year, will transmit a novel pathogen via blood transfer? (1E-6, 1E-7) 0.6. GM v non-GM: If G3 and GM behave the same, cannot think of a reason this would be different.
Figure 6.80: What is the probability that a mosquito escaped from an insectary, in a year, will contact an individual infected with a pathogen not previously known to be vectored by Anopheles gambiae?
E14: Dependent
E16: Dependent
E17: See FT2-000 (now FT2000)
E18: Dependent
E20: Dependent
E22: See above
Figure 6.81: Given that a mosquito has contacted an infected human or other vertebrate, what is the probability that it acquires a novel pathogen through a blood meal?
E14: Dependent
E16: Dependent
E17: See FT2-001 (now FT2001)
E18: Dependent
E20: Dependent
E22: See above
Figure 6.82: Transmits infectious dose to second human or vertebrate
E13: The expert wants to elicit at this higher level. This has been tested at this level and found to be improbable.
Figure 6.83: Given a mosquito that has acquired a novel pathogen through a blood meal, what is the probability that the pathogen remains viable between infection and contact with a subsequent individual?
E6: The likelihood of it acquiring the pathogen is optimised under a full blood meal, and then typically it rests, lays eggs and then seeks another blood meal. The most likely way the pathogen would stay stable between infection and the typical four days between blood meals is for the pathogen to replicate, because it has to survive digestion. For most pathogens 4 days is not long enough for it to infect the mosquito (the extrinsic incubation period is usually longer than 4 days). Hence the probability that it remains viable between infection and second contact is relatively low. No difference with GM.

E11: Same as for FT2-010 (now FT2010)

E14: Bimodal here. Some can survive for a few months in faeces, where as in bacteria die quickly. (0.01,0.1)0.6

E15: Blood borne risk, such as in a hospital. The best people to talk to would be public health experts or clinicians.

E16: Uncertainty in this prediction is huge. Don’t know.

E20: Happy with high tail given so many potential pathogens (0.01,0.8)0.6

E22: No comments
Figure 6.84: How would you change your probabilities for FT2-1120 if the 10,000 insectary mosquitoes were all genetically modified with the I-Ppol construct?
E20: (0.01, 0.8)0.5
Figure 6.85: Exposure through ingestion
E22: If you ingest a fly much more dangerous because you are exposed to the oral-faecal pathogens that are resistant in the environment and contaminate the fly. So The expert would be much more concerned about swallowing a fly. Blood based pathogen have not evolved the mechanisms to survive the digestive processes. \( Pr = 0 \), can use \( 1e-9 \) as an effective zero.
Figure 6.86: Given that a mosquito has acquired a novel pathogen through a blood meal that remains viable between contacts, what is the probability that it is ingested by a second human or vertebrate?
E6: The expert has been around a lot of mosquitoes and he hasn’t eaten many! Would think relatively rare for human but not so for other vertebrates. Bats, birds, etc feed on mosquitoes. The expert doesn’t know how the major causes of mosquito mortality are distributed between normal mortality, predation by insects and predation by vertebrates. Could be a 1 in 5 chance that mosquito is eaten by a vertebrate in its normal daily life. If GM die quicker then there are less available for predation. If they fly slower they are easier to catch. Not sure if GM would have any difference.

E11: Not a rare event.

E14: Quite slight 0.7(0.01,0.02)

E16: Very remote. 1 in a million to be eaten by a bat (for example). The expert should have a better idea here. (1E-8,1E-6)0.9. GM v non-GM: Same

E17: On average, and having grown up with lots of mosquitoes, The expert would imagine less than one a year. Birds or bats are by far the most likely but see comment above them unlikely to have their blood borne pathogens, so answering here for human ingestion. Certainly less than 1 in 1000.

E18: (1E-3,1E-2)0.5

E20: Probability that human ingests, given FT2-112101 (now FT2112101) is survives human gut. (1E-6,1E-2)0.7
Figure 6.87: Given the mosquito has been ingested, what is the probability that the pathogen survives the human or vertebrate digestion system and infects the subsequent individual?
E6: Most of the pathogens that The expert is thinking the mosquito would come in contact with do not have the vertebrate digestive system as their infection route. Through the gut is therefore highly unlikely, but is there chance that prior to swallowing that the virus gets through the epithelium of the mouth. The highest chance is through the epithelium but still this is very rare. No difference with GM.

E11: Some evidence of oral transmission via trypanosome etc. Some examples of animals getting infected by parasites through ingestion. This is an area that could occur.

E14: Same as FT2-112100 (now FT2112100)

E16: Very remote. (1E-6,2E-8)0.75. GM v non-GM: Same

E18: Pathogen dependent, no number.

E20: (1E-4,0.1)0.7
Figure 6.88: Exposure through wound

All experts: 88 of 352 $\text{EXw} = 0$
E14: Exposure through wound / mucosa. Include through mucosa for bacteria.
E22: Mosquitoes do not land on wounds, they land on skin. Pr = 0, can use 1e-9 as an effective zero.
Figure 6.89: Given that a mosquito has acquired a novel pathogen through a blood meal that has survived between contacts, what is the probability that the mosquito lands on an open wound of a subsequent human or vertebrate?
E6: The expert thinks this is rare. Open wounds in humans are rare, outside of serious accidents, plus the area of a wound is small relative to the skin surface area even in exposed surface. No difference with GM.

E11: Rare

E14: Fractions of 1%. 0.8(0.001,0.01)

E16: Don’t know answer, but do know that flies are attracted to open wounds, so possible that mosquitoes are as well. Don’t know that this has been looked at before. This is the mode of transmission for Chagas disease. So might be possible to envisage scenario that mosquito feeds on a person, releases virus from the anus into the wound, and then this gets rubbed in. Remote (1E-6,1E-3)0.8. GM v non-GM: Same

E17: So the context here is immediately after blood feeding (because otherwise the blood meal will be digested and this is not a viable exposure pathway) the mosquito lands on an open wound of a human or vertebrate. This is still rare. The expert has never heard of mosquito being attracted to sores so this is going to be really rare.

E20: Fix wording in fault tree. Should read "Lands on open wound" (1E-6,0.5)0.8
Figure 6.90: Given that the mosquito has landed on the open wound, what is the probability that an infectious dose is delivered to the subsequent individual's bloodstream (e.g., the mosquito is squashed releasing the blood in its gut onto the wound).
E6: A. gambiae excretes whilst it feeds (unlike other mosquito species) and this is how T. cruczi is spread by bugs with the same behaviour. This unique biology of A. gambiae may therefore increases this likelihood. What percentage of mosquitoes are swatted (none for some vertebrates) possible for some humans. Unlikely but possible. No difference with GM.

E11: Rare. Also unlikely. 0.5 central CI with bounds between 1 in 100 and 1 in 1000.

E14: Same as per FT2-112110 (now FT2112110)

E16: Remote here as well, (1E-6,1E-3)0.8. GM v non-GM: Same

E17: The expert thinks this is a small number but wants to pass on this question

E20: (1E-6,0.01)0.8
Figure 6.91: Exposure through inhalation
E14: Two steps, pathogen transferred via faeces and the human inhales, both same as wound exposure values.

E22: First up this mechanism requires the pathogen to survive the full digestion system of the mosquito and still the maintain to the ability to survive in the environment and be infectious - impossible. Acknowledge that there are a very small number of micro-organisms that may survive the digestion process in the gut (e.g. Micro bacteria) but after that it is not possible. Pr = 0, can use 1e-9 as an effective zero.
Figure 6.92: Given that a mosquito has acquired a novel pathogen through a blood meal that has survived between contacts, what is the probability that the pathogen is passed through mosquito faeces?
E14: Same as per FT2-112110 (now FT2112110)
E16: Very remote (1E-6, 5E-7)0.8. GM v non-GM: Same
E17: The expert knows nothing about this as a potential pathway
E20: (0.01, 0.8)0.8
Figure 6.93: Given that the pathogen has been passed through mosquito faeces, what is the probability that an infectious dose is delivered to the subsequent individual's bloodstream through inhalation or self inoculation through mucosa.
E14: Same as per FT2-112110 (now FT2112110)

E16: Think this is so remote it is just not going to happen. Remoter than very remote. Very remote, \((1E-6, 5E-7)0.8\) because you need a certain amount of inoculum to infect, so this is not going to happen. GM v non-GM: Same

E20: \((0.001, 0.2)0.8\)
All experts: 94 of 352 EXw = 0.301

Figure 6.94: Given a catastrophic release of all 10,000 GM insectary mosquitoes, what is the probability that the I-Ppol construct leaves a mosquito and enters the soil environment?
E10: When any organism dies in the soil environment, its DNA enters the soil, this will be true for the mosquitoes DNA. The question is what happens to this DNA. Most of it is hydrolysed by DNAases, and then the nucleotides become a carbon source for microbial growth. This is part of the normal carbon cycle. This event has probability = 1

E13: Will be a high probability (he wants it bounded by 1). Assumes that the mosquito dies and construct is found in the soil (wanted this clarified as not comfortable with current wording 'leaves'). This assumption same for aqueous and gut environments.

E18: Doesn't leave. What is the probability that there is mosquito DNA in the soil. Quite high. (0.8, 0.99)0.9

E19: Mosquitoes will die, and the DNA will go into the environment Pr=1

E23: As said before, DNA is a pretty stable molecule, so it is likely that it will be present in the outside after the mosquito dies. But, depends on the environment. DNA will degrade faster in more moist environments (0.1, 0.999)0.8.
Figure 6.95: Given that the I-Ppol construct has left the mosquito what is the probability that the construct DNA remains intact in the soil?
E9: See row 126 and 127 for Jeff’s comment on a related (same?) issue

E10: First thing that will happen to DNA in soil is that it will be hydrolysed. The construct can remain intact if it is bound to clay which protects it from hydrolysis, or if it enters some other environment (like a very arid environment) where there is no enzymatic activity (e.g. recovery of ancient DNA), same set of environmental conditions can protect it (clay, very dry). The probability of conditions that preserve ancient DNA is low because mosquitoes are not going to end up in these kind of conditions, and binding to clay depends on soil characteristics, and even here the issue is how long will it be protected, as it may not be protected for a very long period. Assuming a 5000 bp construct, the longer the construct the less likely it is to remain intact. The expert once did experiments looks at G+C bias for binding to clays, the higher the content the higher it binds to clay, and the higher the cation content (e.g. Calcium better than Sodium for binding) results in better binding. One construct per cell but how many cells are there - i.e. how many constructs are there. The expert thinks 1 in 1e6 will survive a year as reasonable, a reasonable upper bound might be 1e-4, but would like to know how many copies of the construct will there be in 10,000 mosquitoes, before providing a number.

E13: Likely a low probability that it will remain intact. But this is time dependent: within a few minutes, probability intact is high; within a week, less; within a month, slim.

E18: Doesn't leave. Given there is mosquito DNA in the soil. Low. (1E-4, 1E-3)0.4

E19: DNA will remain intact for hours, probably days. But very unlikely that it will stay intact longer than several days. The range reflects that it remains intact in the soil long enough that it could be picked up (1E-5, 0.5)0.7

E23: Will depend on the environment (if the soil is dry or humid), we know that water and bacteria, for instance, can degrade DNA. In a dry situation, the DNA can be stable for a long time (0.1, 0.99)0.9.
Figure 6.96: Given that there is intact I-Ppol construct in the soil environment, what is the probability that it will enter a eukaryote?
E13: These two events (a) and (b) were the above event split into two. Potential that previous experts were considering both these steps in their answers to FT3-1005002 (now FT300003). If there is a mosquito that is there from it dying a short time ago and a spider eats it, then the probability is high, but the probability that it gets into the germline cell is very slim. Suggests possibly breaking this into separate events. Consumption/ingestion most likely mechanism, but also via contact with skin, etc.

E18: Median of about 1E-3. (1E-3, 1E-2)0.6

E19: Not very likely (1E-9, 1E-5)0.8
Figure 6.97: Given that there is intact l-Ppol construct in the soil environment, what is the probability that it will enter the germline cell of a eukaryote?
E23: We are talking about something that is in the soil, but we need a mechanism to transport the DNA into a Eukaryotes germline. Quite unlikely. Most of the known mechanisms are mediated by prokaryotes (1E-6, 0.001)0.7.
Figure 6.98: Given that the I-Ppol construct has entered a non-target eukaryote, what is the probability that it will enter the germline cell of a multi-cellular eukaryote?
E18: Depends if it’s single cell eukaryote, or multi-cell eukaryote. With single cell eukaryote, the probability of it entering the germline is similar to the probability of it entering the organism itself. For a multicellular organism the probability is considerably lower. So assuming it has entered the non-target, then the probability that it enters the germline is about 1 in 10000. This is for all non-single celled non-target eukaryotes. (1E-5, 1E-3)0.7

E19: Also very unlikely (1E-9, 1E-5)0.8
Figure 6.99: Given that the I-Ppol construct has entered a non-target eukaryote, what is the probability that it will enter the germline cell of a single-celled eukaryote?
E18: Pr=1. If single cell eukaryote, then once it has entered the organism, it will by definition be in the germline.
Figure 6.100: Given a catastrophic release of all 10,000 GM insectary mosquitoes, what is the probability that the I-Ppol construct leaves a mosquito and enters an aquatic environment?
E10: Females laying eggs would constitute this, with probability 1

E18: Also quite high. (0.8, 0.99)0.9. Depends on environmental conditions and how much water is there.

E19: Pr=1. Because there will be some mosquitoes that will die in the water. Out of 10000 at least 1 will die in water.

E23: That will be related to where the mosquito dies. Which should be more or less known. If the case is there is a 50:50 chance of dying in soil or in water, the probability would be the same as FT3-100500 (now FT300000).
Figure 6.101: Given that the I-Ppol construct has left the mosquito what is the probability that the construct DNA remains intact in an aquatic environment?
E9: See row 126 and 127 for Jeff’s comment on a related (same?) issue

E10: The big difference here is that there is less protection than in the soil environment. From the microbial point of view DNA is a tasty polymer, so to most of the microbial world this is going to be a good food source, which means they are going to consume it as a monomer carbon source. So now what is the probability of taking up 5k bps intact, the length now becomes the issue, there is an exponential decline in probability of being intact as size increases, 500 bps maybe be 5k? Looking at 1e-6 as a reasonable upper bound on this.

E18: By and large the degradation rate of organisms in aquatic and soil environments is broadly similar. So same as soil. (1E-4, 1E-3)0.4

E19: In the water DNA is even more likely to be intact than in the soil (1E-4, 0.5)0.75

E23: DNA is much less stable in aquatic environments, as far as I am aware. It could get in contact with DNAses coming from different organisms. The stability of DNA is much lower in water than in dry conditions. The rate of degradation of DNA in water is known. More concerned about soil than aquatic environment, if we are not talking about mechanisms that involve microorganisms. We should be able to calculate this.
Figure 6.102: Given that there is intact I-Ppol construct in an aquatic environment, what is the probability that it will enter a eukaryote?
E13: These two events (a) and (b) were the above event split in to two. Potential that previous experts were considering both these steps in their answers to FT3-100512 (now FT300013).
Definition changed by George

E18: Same as soil (1E-3, 1E-2)0.6

E19: Not very likely (1E-9,1E-5)0.8

E23: The transport of DNA could be facilitated in an aqueous environment. But, not confident in giving a number.
Figure 6.103: Given that there is intact I-Ppol construct in an aquatic environment, what is the probability that it will enter the germline cell of a eukaryote?
E23: The transport of DNA could be facilitated in an aqueous environment. But, not confident in giving a number.
Figure 6.104: Given that there is intact I-Ppol construct in a eukaryote, what is the probability that it will enter the germline cell of a multi-cellular eukaryote?
E18: Same as soil (1E-5, 1E-3)0.7

E19: Dependent
Figure 6.105: Given that there is intact I-PpoI construct in a eukaryote, what is the probability that it will enter the germline cell of a single-celled eukaryote?
E18: Pr = 1
Figure 6.106: Given a catastrophic release of all 10,000 GM insectary mosquitoes, what is the probability that the I-Ppol construct enters the gut of a eukaryote organism?
E10: Something will eat some mosquitoes with probability 1

E13: Combining across aquatic and soil environments. In non-aquatic environments it is likely to be spiders, monkeys from ingestion, etc. Enters gut, remains intact, then enters germline. Not comfortable with ‘leaves’ - won’t ‘leave’, will be within the eukaryote.

E18: Probability of a released mosquito getting eaten is high, about 1 in 100. (1E-3, 0.1)0.55

E19: It is ingested. Some are on the grass or leaves that can end up in gut of eukaryote. Quite likely (0.1, 0.99)0.9

E23: The mosquito is going to be digested first. There is no other way that DNA can enter the gut (1E-12, 1E-6)0.8
Figure 6.107: Given that the I-Ppol construct has entered the gut of a eukaryote organisms what is the probability that the construct DNA remains intact?
E10: There is probably data on this. People who study digestion probably know what happens to DNA segments, and could comment on the probability or provide data to show what happens to a 5000k bp construct. In the stomach the low pH is going to hydrolyse it (in humans though pH goes up as you get older). The expert is not an expert in this area.

E13: Remains intact so that it can enter germline cell.

E18: Virtually zero. About 1E-5. (1E-6, 1E-4)0.8

E19: Not likely (1E-7,1E-4)0.8

E23: In most eukaryotes the DNA will be digested. So the chance that it will remain intact will be pretty low (1E-3, 0.4)0.5
Figure 6.108: Given that there is intact I-Ppol construct in the gut of a eukaryote organisms, what is the probability that it will enter the germline cell of the eukaryote?
E18: Even lower than virtually zero. About 1E-6. (1E-7, 1E-5)0.8
E19: Same as previously for soil and aquatic environment (1E-9, 1E-5)0.8
E23: Same as FT3-1005002 (now FT300003)
Figure 6.109: Given that the I-Ppol construct has entered the germline cell of a eukaryote what is the probability that it gets into the nucleus?
E18: Probably 50%. (0.45, 0.55)

E19: No more than 1 in 10,000 (1E-7, 1E-4) 0.8. Comment amended following feedback report: I misinterpreted the question. The construct contains a NLS (nuclear localisation signal) which is almost universally recognised, therefore the probability it enters the nucleus is rather high.

E23: Same as FT3-10314 (now FT3034103)
Figure 6.110: Given the I-Ppol construct has entered the nucleus of a germline cell of a eukaryote what is the probability it recombines into the eukaryote's genome?
E13: Range of probability here given will capture that recombination is more likely if mosquito than other eukaryote due to Beta2 tubulin promoter. And therefore more likely through the aquatic environment.

E18: (1E-4, 1E-3)0.7

E19: (1E-7, 1E-5)0.8

E23: A random fragment of A. gambiae that contains the construct. So the probability will be related to the size of the fragment. The bigger the size the more likely the presence of other transposons that would mediate the recombination of the construct into the genome, and source of recombinases in the new non-target eukaryote. Strictly associated with the presence of transposase/reverse-transcriptase/integrase, so the same as FT3-103150 (now FT30341041) (no probabilities were elicited for that event).
Figure 6.111: Given that the I-PpoI construct has left the mosquito and entered a soil environment, what is the probability that the construct DNA remains intact?
E10: G+C ratio less important here. 1 in a million is a reasonable reference point. If one construct, then fraction of that, if a million constructs, then 1. At least 10 are going to survive a year given 1E-8, 1E-4. Median 1E-6 (implies 10000 going to survive per year). Assuming load in environment is 1 billion. Considering a single construct in elicitation. After Austin’s email 1e11 constructs in the soil but happy with numbers.

E13: Dependent. Same as with soil environment under unmediated acquisition.

E18: Dependent

E19: Dependent

E23: Dependent. Same as FT3-1005001 (now FT300001)
Figure 6.112: Given that there is intact I-PpoI construct in the soil environment, what is the probability that there is direct contact with competent bacteria?
E10: Detection limit when one tries to do transformation in the soil is about 1 in 1e-9. This would be an extremely low number, less than 1e-9 because that is the limit of detection in other gene transfer studies, and in this it would be less than that, because there is lots of surface area in soil and only a small proportion of it is occupied by bacteria cells of which a small proportion again are competent. Follow up with The expert on journal articles for this.

E13: Presence of "competent" bacteria will be limiting, so will likely be low probability.

E18: Likely. About 1 in 100. (0.05, 0.2)0.75

E19: (0.5E-4, 0.1)0.8

E23: It would be useful to look in the literature and figure out what type and number of competent bacteria are in soil, aquatic, gut, and mosquito environments, and from that get to a number that would answer this question. Soil and aquatic environments are likely to be more diverse, and therefore this event would be more likely to happen, than in gut and mosquito. On the other hand, in the gut of a mosquito the possibility of contact might be higher than in soil/aquatic environments. But, these are all parameters that could be estimated from the literature.
Figure 6.113: Given that there is direct contact between the I-Ppol construct and competent bacteria, what is the probability that it is taken up and survives the restriction enzymes?
E10: There are experiments that can be referred to in this category but most of the data is under optimised conditions. Under optimised conditions and optimised recipient cells this number would typically be 1e-3. The other important thing is that the competent bacteria includes bacteria that are compatible with up taking DNA (e.g. some Bacillus are known to be better at uptake, but others such as the phylum Acidobacteria, which are one of the top 3 phyla in soil, of which maybe less than 15 members are now in culture (and this group is thought to be very large due to ribosomal sequence variation - Peter Janssen maybe able to help here), and nothing is known about HGT. Hence if they represent a third of the bacteria for which we know nothing, how to do we evaluate this? Part of this is a competent bacterium, but a big factor is the number of bacteria in soil that may never be competent. The expert doesn’t think this will be a high probability. You can see evidence for HGT over evolutionary time scales in bacterial genomes. 1e-3 might be a reasonable upper bound.

E13: Two things that must happen it that it is taken up and then survives. Likely pretty low.

E18: Very low. About 1E-4. (1E-4, 5E-4)0.8

E19: (1E-6, 1E-3)0.8

E23: No idea. I believe that it is possible to estimate from the literature or consulting bacteriologists.
Figure 6.114: Given that the I-Ppol construct has been taken up and survived the restriction enzymes, what is the probability that it is recombined into the DNA and the microbe has sufficient energy to replicate?
E10: The issues here are: a) homologous recombination. Most successful gene exchange involves homologous recombination, so what is the chance of this construct being homologous to some proportion of the recipient genome. This is going to be very low, and this is influence by the G + C content, the more divergent the G+C ratios between construct and recipient genome the less likely homologous recombination is. In soil the dominant populations have a G+C content of about 60%. What is the G + C content of the construct? Later we learned that the construct content was 46.

E18: The microbe will replicate. The probability that an intact I-Ppol is recombined into the DNA is vanishingly small. About 1E-6. (1E-6, 1E-5)0.8

E19: (1E-8, 1E-5)0.7

E23: No idea. I believe that it is possible to estimate from the literature or consulting bacteriologists.
Figure 6.115: Given a catastrophic release of all 10,000 GM insectary mosquitoes, what is the probability that the I-Ppol construct leaves a mosquito and enters an aqueous environment?
E13: Same as with soil environment FT3-100500 (now FT300000)
E18: Dependent
E19: Dependent
E23: Same as FT3-100510 (now FT300010)
Figure 6.116: Given that the I-Ppol construct has left the mosquito and entered an aqueous environment, what is the probability that the construct DNA remains intact?
E10: The aquatic environment doesn’t have the protection of clay, so the probability of it surviving is less. So smaller than in soil. Wider range justified in soil as more heterogeneous environment, in two ways - basic soil nature but also chances of construct getting particular locations, whereas in aquatic environment more homogenous.

E13: Same as with soil environment

E18: Dependent

E19: Dependent

E23: Same as FT3-100511 (now FT300011)
Figure 6.117: Given that there is intact I-Ppol construct in an aqueous environment, what is the probability that there is direct contact with competent bacteria?
E10: Much higher in aquatic environment, compared to soil. The expert is thinking here that there is more of a biofilm and microbes are going to be enriched around eggs and dead larvae, and there are no physical barriers. Direct contact with bacteria high but competent bacteria lower so maybe 1e-3 is the upper bound.

E13: Same as with soil environment. See FT3-1010002 (now FT301002)

E18: Same as FT3-1010002 (now FT301002)

E19: (1E-4,0.1)0.8

E23: It would be useful to look in the literature and figure out what type and number of competent bacteria are in soil, aquatic, gut, and mosquito environments, and from that get to a number that would answer this question. Soil and aquatic environments are likely to be more diverse, and therefore this event would be more likely to happen, than in gut and mosquito. On the other hand, in the gut of a mosquito the possibility of contact might be higher than in soil/aquatic environments. But, these are all parameters that could be estimated from the literature.
Figure 6.118: Given that there is direct contact between the I-Ppol construct and competent bacteria, what is the probability that it is taken up and survives the restriction enzymes?
E10: Same as soil, see FT3-1010003 (now FT301003)
E13: Same as with soil environment
E18: Same as FT3-1010003 (now FT301003)
E19: Same as FT3-1010003 (now FT301003)
E23: No idea. I believe that it is possible to estimate from the literature or consulting bacteriologists.
Figure 6.119: Given that the l-Ppol construct has been taken up and survived the restriction enzymes, what is the probability that it is recombined into the DNA and the microbe has sufficient energy to replicate?
E10: Order of magnitude up from soil. The only difference here between soil, is that there could be more energy for replication. The issue of the homologous recombination is the same but because there is more available local carbon, this could be a little more favourable for the construct to be replicated. A reasonable upper bound may be 1e-5.

E13: Same as with soil environment - as per FT3-1010004 (now FT301004)

E18: Same as FT3-1010004 (now FT301004)

E19: Same as FT3-1010004 (now FT301004)

E23: No idea. I believe that it is possible to estimate from the literature or consulting bacteriologists.
Figure 6.120: Given a catastrophic release of all 10,000 GM insectary mosquitoes, what is the probability that the I-Ppol construct leaves a mosquito and enters the gut of a eukaryote?
E10: Something will eat the mosquitoes with probability 1
E13: Same as with soil environment
E18: Dependent
E19: Dependent. Same as FT3-100520 (now FT300020)
E23: Dependent. Same as FT3-100520 (now FT300020)
Figure 6.121: Given that the I-Ppol construct entered the gut of a eukaryote what is the probability that the construct DNA remains intact?
E10: Don’t know. There is probably data on this. People who study digestion probably knows what happens to DNA segments, and could comment on the probability or provide data to show what happens to a 5000k bp construct. In the stomach the low pH is going to hydrolyse it (in humans though pH goes up as you get older). The expert is not an expert in this area.

E13: Same as with soil environment

E18: Dependent

E19: Dependent. Same as FT3-100521 (now FT300021)

E23: Dependent. Same as FT3-100521 (now FT300021)
Figure 6.122: Given that there is intact I-Ppol construct in the gut, what is the probability that there is direct contact with competent bacteria?
E10: In mammals, if intact in large intestine, the probability is high that it will contact a microbe. Whether microbe is competent or not is major factor. The difference in intestinal tracts is whether the construct survives to reach the large intestine. In mosquito, or fish, its different. Relative to aquatic, here would say that number in general should be lower than aquatic. In contact with bacteria high, with competent bacteria The expert doesn’t know. We need to speak to someone who knows more about transformation in a range of different bacteria including gut bacteria. Relevant gut bacteria groups are: Insects, fish, amphibians, birds and bats. The pH will also be different in these different organisms but the gut bacteria will generally be anaerobes or facultative anaerobes. Maybe chat to Ned Walker about this or Jo Handelsman (did alot of work on ecology on caterpillar guts in Costa Rica, now at the Whitehouse).

E13: Same as with soil environment. See FT3-1010002 (now FT301002)

E18: Same as FT3-1010002 (now FT301002)

E19: Higher than soil because of the bacteria composition of the gut (1E-3,0.2) 0.8

E23: It would be useful to look in the literature and figure out what type and number of competent bacteria are in soil, aquatic, gut, and mosquito environments, and from that get to a number that would answer this question. Soil and aquatic environments are likely to be more diverse, and therefore this event would be more likely to happen, than in gut and mosquito. On the other hand, in the gut of a mosquito the possibility of contact might be higher than in soil/aquatic environments. But, these are all parameters that could be estimated from the literature.
Figure 6.123: Given that there is direct contact between the I-Ppol construct and competent bacteria, what is the probability that it is taken up and survives the restriction enzymes?
E10: Same as soil, see FT3-1010003 (now FT301003). The restriction enzyme strategy is pretty broad across all microbes, so whilst the taxa may be different in the gut, the strategy is probably general, so The expert suspects this is the same probability as soil and aquatic microbes but we should check with someone knowledgeable in this group. Sir Richard Roberts (New England Biolab, 1993 Nobel laureate) would be a good person to follow up with on this.

E13: Same as with soil environment

E18: Same as FT3-1010003 (now FT301003)

E19: Same as FT3-1010003 (now FT301003)

E23: No idea. I believe that it is possible to estimate from the literature or consulting bacteriologists.
Figure 6.124: Given that the I-PpoI construct has been taken up and survived the restriction enzymes, what is the probability that it is recombined into the DNA and the microbe has sufficient energy to replicate?
E10: Gut is the most favourable environment because it has energy to replicate and the homologous recombination is the same as soil and aquatic environment microbes, hence overall this would be an order of magnitude more than those habitats - i.e. 1e-4 might be a reasonable upper bounds for this.

E13: Same as with soil environment- as per FT3-1010004 (now FT301004)

E18: Same as FT3-1010004 (now FT301004)

E19: Same as FT3-1010004 (now FT301004)

E23: No idea. I believe that it is possible to estimate from the literature or consulting bacteriologists.
Figure 6.125: Given that there is intact I-PpoI construct within the mosquito, what is the probability that there is direct contact with competent bacteria?
E13: ok

E18: Pr=1.

E19: There are bacteria within the mosquito (1E-4, 0.1)0.85. Comment amended following feedback report: Lower bound increased by 10.

E23: It would be useful to look in the literature and figure out what type and number of competent bacteria are in soil, aquatic, gut, and mosquito environments, and from that get to a number that would answer this question. Soil and aquatic environments are likely to be more diverse, and therefore this event would be more likely to happen, than in gut and mosquito. On the other hand, in the gut of a mosquito the possibility of contact might be higher than in soil/aquatic environments. But, these are all parameters that could be estimated from the literature.
Figure 6.126: Given that there is direct contact between the I-Ppol construct and competent bacteria, what is the probability that it is taken up and survives the restriction enzymes?
E13: same as per soil -see FT3-1010003 (now FT301003)
E18: Same as FT3-1010003 (now FT301003)
E19: Same as FT3-1010003 (now FT301003)
E23: No idea. I believe that it is possible to estimate from the literature or consulting bacteriologists.
Figure 6.127: Given that the I-PpoI construct has been taken up and survived the restriction enzymes, what is the probability that it is recombined into the DNA and the microbe has sufficient energy to replicate?
E13: same as per soil - as per FT3-1010004 (now FT301004)
E18: Same as FT3-1010004 (now FT301004)
E19: Same as FT3-1010004 (now FT301004)

E23: No idea. I believe that it is possible to estimate from the literature or consulting bacteriologists.
Figure 6.128: Given a viable I-Ppol transformed prokaryote organism has been produced, what is the probability that it comes into contact with a eukaryote organism?
E10: This is probably going to be probability 1 for all three environments (soil, aqueous and gut) but we should check with other experts on this.

E13: Giving a range as there are different routes of entrance.

E18: Pretty likely (0.01,0.1) 0.9

E19: Quite likely (0.1,0.99) 0.8
Figure 6.129: Given that the I-Ppol construct has entered a non-target eukaryote, what is the probability that it will enter the germline cell of the eukaryote?
E13: Same as value given for FT3-1005002b (now FT300003)
E18: Dependent
E19: (1E-9, 1E-5)0.8
Figure 6.130: Given that the I-Ppol construct has entered the germline cell of a eukaryote what is the probability that it gets into the nucleus?
E13: Same as value given for FT3-1003 (now FT3001)
E18: Dependent
E19: No more than 1 in 10000 (1E-7, 1E-4)\textsuperscript{0.8}. Comment amended following feedback report:
Same as FT3-1003 (now FT3001).
Figure 6.131: Given the I-Ppol construct has entered the nucleus of a germline cell of a eukaryote what is the probability it recombines into the eukaryote's genome? (Given that contact between a transformed I-Ppol prokaryote and a eukaryote has been established, what is the probability it recombines into the eukaryote’s genome?)
E13: Same as value given for FT3-1004 (now FT3002)
E18: Dependent
E19: (1E-7, 1E-5)0.8
Figure 6.132: Given a catastrophic release of all 10,000 GM insectary mosquitoes, how many already are or will be infected with a mosquito virus?
E6: ONNV. Alpha virus. More of a problem in flaviviruses because relatively easy to engineer. Replication is sufficiently different that less tolerant to change. Put GFP (fluoro-protein) into ONNV and get great expression and it is stable for multiple rounds of replication. Possible in right temporal/spatial circumstances. In an area where virus is circulating and feeding on virion human/animal. Come into contact with infected people. Depending on location. Realistically ONNV is 1 in a million. Have to feed, have to be infected. Zero probability within containment. Worst case is escape when (for example) active ONNV epidemic. Virus from human, less than 10 in 10000. Suggest simple PCR to ensure no virus sequence already in the mosquitoes. Larvae have flaviviruses transmitted transovarially. Test subsample of local WT mosquitoes, suspect not infected either. Mosquitoes outside interbreeding with WT.

E8: Analyzing for known pathogens are those pathogens worked with in the site-specific laboratory or known to exist near the laboratory site. For instance, a risk mitigation strategy would be to routinely screen WTs using PCR for the major viruses of A. gambiae such as O nyong nyong. Viral nucleic acid or antigen can be identified by PCR-based assays or antibody assays, respectively. Pathogen nucleic acid can also be characterized with deep sequencing and bioinformatics approaches of the vector population if environmental exposures of vector populations are a concern, and repeated periodically, so the introduction of a pathogen in a laboratory is of concern. There are limited viruses that infect humans known to infect A. gambiae, so The expert is less concerned than if the target population was A. aegypti for example. This would be a risk mitigation strategy but probably too expensive to do regularly. Problem with this question is that we don't know what the baseline infection status is for mosquitoes in the area of collection and release. There are also insect-only viruses that won't be vectors to humans but possibly infectious to other insect populations. This probability of risk to humans depends on whether or not they are collecting adults in the wild or eggs or larvae in the wild. This elicitation is for adults and but it would be much less of a concern for eggs or larvae collections. Another potential risk mitigation strategy would be for them to collect eggs or larvae because collecting eggs/larvae would eliminate a lot of virus since virus in the adults would need to be transmitted transovarially or between insect stages and would be less risk for the insectary workers. Not sure of the feasibility of egg collection for A. gambiae.

E13: Probability that they are infected is high especially because each insectary performing own colonies so won't be testing for viruses. Big unknown what viruses are in colonies. The expert added additional value of q0.5=0.2.

E22: About 1% of the mosquitoes will be infected with a virus. On second thoughts happy with a lower value (1e-4, 1e-3)0.75
All experts: 133 of 352 EXw = 1.79

Figure 6.133: Direct acquisition
E22: There is a missing step here: the construct needs to be mobilised out of the mosquito genome. This requires presence of an active transposase. The probability of excision is very low because there is no transposase in the mosquito genome.
Figure 6.134: Given a mosquito modified with the I-Ppol construct is infected with a mosquito virus, what is the probability that the construct is excised from the mosquito genome by a transposase?
E22: This is a big limiting step (1e-12, 1e-10)0.80
Figure 6.135: Given a mosquito modified with the I-Ppol construct is infected with a mosquito virus, and the construct is excised from the genome via a transposase, what is the probability that the virus incorporates the construct into its own genome?
E22: This can happen once excised (1e-5, 1e-3)0.75
Figure 6.136: Virion mediated acquisition
E8: This is not unheard of in the sense that the mature virus is the virion, and it is possible for transgenes to be incorporated into envelope genes of the virion, but when we do this deliberately the viruses are usually attenuated (we have amended them so they have very low infectious status).

E13: Could be too much detail for what is known to elicit lower events in fault tree. Will consider it same as for direct acquisition- FT3-10200200 (now FT3020010). Virion incorporating slightly higher but once multiplied with infecting another cell and virus then same as above value.

E22: The expert wants to re-label this as “Cross packaging”. This is not going to happen for two reasons. Again you have to excision from the genome which is incredibly rare (see elicitation for FT3-10200200a (now FT30200100)) and then you have to have re-packaging which is also an incredibly rare event. This is one of the most negligible things The expert has seen in all the fault trees. Probability zero for 10k mosquitoes in a year Pr = 0.
Figure 6.137: Given that the virion has acquired the construct what is the probability that it infects another cell simultaneously infected with an unmodified virus?
E6: Possible and likely. In mosquito mid-gut when you look where viruses are they are accumulated down the posterior end of the gut. Maybe certain cells in a mosquito that are more susceptible than others. Can take equal quantities of virus labelled with different fluorescent proteins (red/green) which are put in simultaneously. If certain cells are more susceptible we should see different concentration of colours. But you don’t see cells which are especially susceptible, they are infected with both. Also time dependent. If, for example, a mosquito feeds on person X then picks up a virus and feeds on person Y a day later and who has the same virus it will get infected with both - coinfection - which drives virus evolution. However, if the feeds are 4 days apart, person X’s mosquito cells are resistant to getting a second infection.

E8: Simultaneous infection is possible but it is a fairly low probability, and it depends the virus titre (do you have enough virus to infect cell simultaneously). Upper bound of 1 in 100, Lower bound 1 in 10,000, 70% confident that the truth is in there.

E13: Very low. 1E-3
Figure 6.138: Given simultaneous infection, what is the probability that the unmodified virus incorporates the construct into its own genome?
E6: Viruses live/die evolve by mutation. They don’t swap genetic material between each other with the exception of one group.

E8: Similar probability as for direct acquisition. Use FT3-10200200 (now FT3020010)
Figure 6.139: Given that a mosquito virus has acquired the construct into its genome, what is the probability that it is able to replicate (autonomously or non-autonomously)?
E6: Depending on where in genome, depending on what virus. Assuming here inserts 5kbp then highly unlikely (not split RNA/DNA as per current column).

E13: Median of 1e-5
Figure 6.140: Given that a mosquito RNA virus has acquired the construct into its genome, what is the probability that it is able to replicate (autonomously or non-autonomously)?
E8: This probability would be an order magnitude less than for a DNA virus. The expert has only heard of one example, Bovine Viral Diarrhoea Virus, this picks up a ubiquiton gene from the host and this increases its virulence. Golic et al 2013 (?) is also an example of him hearing about this happening in the context of RNA mosquito. Use the quantiles for FT3-102003-DNA (now FT302002) and divide them by 1 - i.e. leave it as is - especially if you include retroviruses.
Figure 6.141: Given that a mosquito DNA virus has acquired the construct into its genome, what is the probability that it is able to replicate (autonomously or non-autonomously)?
E8: For an RNA virus usually the addition of any genetic material is going to attenuate it, particularly positive stranded RNA viruses with an icosahedral capsid. Additional synthetic nucleic acid material is usually attenuating. Negative strand RNA viruses have helical structures so they can accommodate it a bit more. Some DNA viruses are small, non-enveloped, and probably constrained by how much genetic material they can accommodate. Some envelope DNA viruses are big and hence more likely to accommodate a new construct.
All experts: 142 of 352 EXw = 0

Figure 6.142: Stable construct in the environment
E13: Very low. 1E-3
Figure 6.143: Transformed virus created
E13: Highly unlikely (1e-6). Happy that this incorporates all the events and structure below.
Figure 6.144: Via Bacteria/Bacteriophage combination
E10: Bacteriophage are host specific so they won’t spread it. If it is already infected with one type of phage, it can’t be reinfected with same type. Not a bacteriophage expert. Are different kinds of phage (other than what The expert previously mentioned), gets to be minor in overall picture. Minor because bacteriophage won’t spread it very far. Bacteriophage that would spread to broader host range - would become pervasive in environment. Could be that construct exists in environment. For Bacteriophage to transmit one to another it must find another host - density dependent relationship - once below a certain number - chance for a Bacteriophage to hit another host doesn’t happen. In oceans the dominant biology is viruses. Viral loop is huge. Generating productivity in ocean. Transfer of photosynthesis genes. Dependent on environment (soil v aquatic) in terms of dynamics. Also density dependent. Eric Wolmac at Delaware.

E22: This should be labelled as Lysophage (these don’t kill their host). Bacteriophages kill their host so The expert does not see this group as a viable mechanism. For 10,000 mosquitoes thinks this will be in the range of (1e-10, 1e-6). Use the number elicited at the gate here for the final calculation (1e-10, 1e-6)0.75
Figure 6.145: Given that there is intact I-Ppol construct in either soil, aqueous or gut environment, what is the probability that a competent bacteria is transformed with the construct?
E10: Confusing saying competent. Should be transformed bacteria, bacteria with construct. Comment only here. This probability available from previous calculations

E19: \((1E-6, 1E-4)0.75\)

E22: When a mosquito dies the mosquitoes gut bacteria will flourish and the use the mosquito DNA as an energy source. If the construct is not cut up in the process but remains intact then it is possible that a bacteria becomes transiently transformed with the construct. If we assume worst case scenario that all copies of the construct remain intact then transformation rate could be in the region of 1e-3. \((0.05, 0.3)0.75\) Subsequently reviewed this and suggested higher rate.
Figure 6.146: Given that a competent bacteria has been transformed with the construct, what is the probability that it is infected with a bacteriophage?
E10: Virtually every bacteria has a phage so this is probability 1
E22: Lysophages are quite rare compared to Bacteriophages generally. Only a small proportion of the bacteria will be infected with a Lysophage (1e-5, 1e-3)^0.6
Figure 6.147: Given that transformed bacteria is infected with a bacteriophage, what is the probability that the bacteriophage incorporates the construct into its genome?
E22: Based on relative size of bacterial genome to Lysophage genome, and transformation efficiency which are respectively 1e-2 and 1e-3 respectively hence looking at something with an upper end of 1e-5. This is on a per mosquito basis so needs to be multiplied by 10e3. Use the elicitation FT3-1022140 (now FT3020110) for the fault tree calculations (1e-7, 1e-5)0.80
Figure 6.148: Virion mediated acquisition
E22: The expert has re-labelled this as "via Lysophage re-activation". This is his Scenario B whereby the Lysophage is replicating and there are multiple copies of phage genome in the bacterial cell. In this stage it is likely to pick foreign sequences but re-activation is quite rare. The scenarios are: (i) DNA enters and goes directly into cell. This is FT3-1022140300 (now FT3020110200). (ii) gets into cell 1e-3, and then frequency of phage re-activation is 1e-5 but note there is allot of literature and a single number cannot capture the range across phage (1e-10, 1e-8)0.70
Figure 6.149: Given that there is intact I-Ppol construct in the soil, aqueous or gut environment, what is the probability that a cellular organism is transformed with construct?
E6: No chance. Don’t pick up DNA and get it into genomes.
E19: (1E-7, 1E-4)0.7
Figure 6.150: Given that a cellular organism has been transformed with the construct, what is the probability that it is infected with a virus?
E6: Time and space and organism dependent. 50% over a year.

E19: (1E-6, 1E-2)^0.65
Figure 6.151: Given that transformed cellular organism is infected with a virus, what is the probability that the virus incorporates the construct into its genome?
E6: Higher than within mosquito. Number of viruses that replicate in AG is extremely small - circa 2 to 4. In other organisms more viruses, and viruses more amenable to integration and replication. More opportunity than in mosquito. Value is twice as likely as within mosquito (see FT3-10200200 (now FT3020010))
Figure 6.152: Given that the virion has acquired the construct what is the probability that it infects another cell simultaneously infected with an unmodified virus?
E6: Viruses only live in other organisms. Virus could get construct through replication which means they are in the presence of other virus particles in cells, so therefore same probability as mosquito. Value same as FT3-10200211 (now FT30200111).
Figure 6.153: Given simultaneous infection, what is the probability that the unmodified virus incorporates the construct into its own genome?
E6: Value same as FT3-10200212 (now FT30200112). No obvious reason for why this would be different.
Figure 6.154: Given that a virus has acquired the construct into its genome, what is the probability that it is able to replicate (autonomously or non-autonomously)?
E6: More than in mosquito because more virus options. Mosquito virus side has restricted number of virus opportunities. Here within environment more opportunities so potentially higher. Don’t think debilitating so yes probably could replicate. Viruses with larger genomes could likely tolerate insertions more than those with smaller genomes. With alpha virus (1000bp to 1500bp can squeeze in but destabilising). Flaviviruses can’t get GFP (238 amino acids 350bp) in reliably. Might be marginally higher since perhaps dealing with some DNA rather than the RNA viruses that infect mosquitoes. Value twice the probability elicited for FT3-102003 (now FT302002).
Figure 6.155: Construct moves from virus into eukaryote germline
E6: Has to be a retrovirus (i.e. HIV) in order to get into host Eukaryote genome.

E13: There is known transmission of viruses between insects, thus HGT possible via viruses. We have some viruses that can be transmitted from insects to higher organisms (such as Arboviruses), but in A. gambiae, only virus known is ONNV. This does not recognize that mosquitoes can also receive viruses back from higher vertebrates. Thus it does not need to get into germline to be transmitted. This will add additional gates to the fault tree. Could transmit to higher eu-karyotes and then back into mosquito. Don’t know virus to know if can infect virus or integrate into host genome. This is unknown to George. If expert to mosquito viruses then they may be able to quantify further down. Can give value to this gate. But given value on general knowledge and known viruses.
Figure 6.156: Given that a transformed viable virus has been created, what is the probability that there is a eukaryote organism that is a suitable host for this virus?
E6: Need to discuss further re. retro viruses.

E8: Major issue here is horizontal spread to other unintended insects because it's harder to get from insect to other vertebrate hosts for most viruses in the environment. The expert thinks it would be an order of magnitude more likely for spread between insects than between insects and vertebrates.

E19: This is likely (0.001,0.5)0.7
Figure 6.157: Given that there is a suitable eukaryote host for the transformed virus, what is the probability that there is a suitable transmission mechanism to transmit the virus to the new host?
E8: If the suitable organism were another mosquito species, the virus needs to replicate above a certain threshold (typically 10e6-10e7 plaque forming units) for infection and transmission to occur. In a mosquito an arbovirus has to infect the midgut, escape the midgut, infect the salivary gland and then escape it. If we are talking about other viruses then there are other transmission mechanisms. If it is was a DNA virus that was transmitted via the environment (through contact between infected mosquito and the other eukaryote) then typically this requires a very high viral titre which most likely damages the mosquito host. There has been at least one example of Baculovirus (DNA virus) infecting mosquitoes. There are polydnavirus (not iridovirus, sorry, I had the wrong virus here) DNA packaged into virions is not viral genomic DNA per se, but rather wasp genomic DNA consisting primarily of wasp genes and non-coding DNA. The virus may be a way for wasps to shuttle genes and proteins into different hosts to suppress their immune response. So its a complicated world, with high variability between 100% chance of transmission (measles) to a low less than 5-10% chance. Y drive concern because of increased exposure time.
E19: (1E-7, 1E-4)0.6
Figure 6.158: Given that there is suitable host and transmission mechanism, what is the probability that the transformed virus infects the early zygote of the eukaryote (first few cell division before cell differentiation)?
E8: This is less likely than FT3-102121 (now FT302121)

E19: Very unlikely (1E-10, 1E-6)0.8
Figure 6.159: Given that there is suitable host and transmission mechanism, what is the probability that the transformed virus infects a later stage of the eukaryote’s life-cycle but then moves to the ovaries or testes?
E8: This depends on the virus. Some viruses are very efficient at transovarial transmission (e.g. the La-Crosse virus which you find in mosquito larvae - example of a bunya virus) and other flaviviruses do this. Insect only flaviviruses also do this. To narrow the game down its really important that they do a deep genome sequence of the WT to identify the types of viruses they have been or are infected with. Small RNA sequencing is a good way to do this, and the miRNA will also show up this way. A transcriptome analysis (taking the total RNA in the system) and then screening for bacteria and bacteriophages to try and shed some light on the bacteriophage pathway.

E19: Also very unlikely (1E-10, 1E-6)0.8
Figure 6.160: Given that a transformed virus has infected the germline of the eukaryote, what is the probability of integration of the I-Ppol construct into the host's genome?
E19: (1E-8, 1E-5)0.75
Figure 6.161: Mediated by other nearby flanking transposons
E11: This is very remote with the limiting rate given by the elicitation for FT3-1031 (now FT3034) (piggyBac Mediated acquisition). This probability is same as FT3-1031 (now FT3034).
Figure 6.162: What is the probability that the existing I-Ppol construct is flanked by transposons of the same family?
E4: Need to know what is the abundance of piggyBac family members in A. gambiae? If it is low then the probability of being flanked is low and vice versa. Jake Tu at Virginia Tech would have this data.

E6: See experimental data.

E7: We shouldn’t need to elicit this, the project should sequence the entire genome of the wild type mosquitoes that they are planning to do the backcross with and then search for transposons that are known to move.

E9: What they really need to know is the sequence of the WT strains, in particular if transposons of the same family are on the same chromosome because movement caused by transposons from different chromosomes are likely to result in a big fitness (cost?). For the Y drive construct seek a site that is not flanked by nearby transposons of the same family.

E18: Very low. Median 5E-4. (1E-5, 1E-4)0.7

E19: (1E-4, 0.1)0.8

E20: A priori this is the same as FT3-103002 (now FT303302), not knowing anything about the locus. Because the locus is known, we can say with more certainty if there are transposons nearby. So until we get this knowledge, this is left as the same probability as FT3-103002 (now FT303302) (1E-3, 0.5)0.9

E21: A bit of a weird question, implicit is that the whole block fits within a window that makes transposition possible. Refers to initial insertion.

E23: I have personally not looked into whether this is happening, but it is something that could be assessed empirically, and potentially be avoided. So, my answer is in regards to the possibility that you would miss the presence of the same transposon flanking the construct before releasing (1E-6, 1E-3)0.7.
Figure 6.163: What is the probability that transposable elements of the same family insert either side of the I-Ppol construct (in the mosquito)?
E4: David Obrochta published on different Gambia populations showing the number of transposable element movements

E9: The expert doesn’t think we can estimate this without a population survey on the rate at which transposons are moving in the natural populations around each insectary.

E18: Similarly low. (1E-5, 1E-4)0.7

E19: Probability of this is lower as it requires two independent events. (1E-6, 1E-3)0.8

E20: Much lower than FT3-103002 (now FT303302), because at least one denovo transposon insertion (if there is already an existing nearby transposon from the same family) event has to happen nearby, or two denovo events if there are none (1E-5, 0.01)0.8

E23: We know that transposons can jump in genomes. So, it can happen. Not sure what frequency it would occur. The probability would depend on the frequency of transposon mobilization and the size of the genome. The frequency of mobilization could be calculated, and the thus the probability of having the transposons of the same family flanking the construct. I don’t know the value. I have no idea, however, I think it is quite unlikely but wide range (1E-9,0.5)0.8.
Figure 6.164: What is the probability that the I-Ppol construct moves within the mosquito genome into another transposable element (or between two elements of the same family)?
E4: This is a similar event to the homology independent integration. The movement is the same, the probability of moving into transposable elements depends on their frequency in the genome from data above.

E6: Probability would depend on distance between existing transposable elements - i.e. further apart they are then more likely to insert between them.

E9: If there are active piggyBacs in the wild populations and they can hybridise then this is one of the higher probability events, much higher than homing. The probability depends on the local populations around the insectaries, but the only information we have is that there are some populations with piggyBac transposons (reference 33 in the background document). Again they could do a population survey in the populations around the insectaries and see if there was piggyBac in different locations which would suggest that they are moving.

E18: Similarly low. (1E-5, 1E-4)0.7

E19: (1E-8, 1E-4)0.8

E20: This would require two transposons within reasonable vicinity of each other, say less than 20kb, in order that they excise as one fragment. Guess that transposons about 10% of the genome, each transposon is about 3kb on average, so any insertion is likely to be within 10kb of a transposon, say about 30% of the time. So this would have to happen twice for two transposons. Top estimate 0.3*0.3. The probability that it moves into another element is about 10%. (1E-3,0.5)0.9

E21: How would it move?

E23: Similar to FT3-103000 (now FT303300). But, with a full understanding of the whole genome of the mosquito it is possible to estimate this probability. A higher possibility than in FT3-103000 (now FT303300) of missing transposable elements that could flank the construct in the new position (1E-6, 1E-2)0.7
Figure 6.165: Given a catastrophic release of all 10,000 GM insectary mosquitoes, what is the probability that there is a source of transposase / reverse transcriptase / integrase (T/RT/I) that acts on nearby transposons that are flanking the construct?
E4: David Obrochta’s numbers will give some idea of frequency of presumed active transposons. The probability of acting on nearby transposons depends on frequency of transposons in the genome (Jake Tu would give some idea).

E6: Should be able to calculate this.

E7: See Jess’ notes, the notes in my book and for FT3-103000 (now FT303300)

E9: If there are no nearby transposons then this is probability zero. As the distance between the construct and the L1R1 and L2R2 (see diagram by Jeff) increases then this probability diminishes rapidly. So first the probability of taking L1R1 is small, and the probability of taking both L1R1 and L2R2 is small again. This is something they should get data on - at least surveying 100kbs either side of the construct. They could do one lane of Illumina sequencing and that should be enough to reconstruct their genome structure.

E18: Steve’s view is that such enzymes are highly regulated for endogenous transposable elements. The likelihood that there are active enzymes is relatively low. (1E-3, 1E-2)0.7

E19: There could be some mosquitoes which have any of these elements. So that the chance that this will occur is quite high. Probably more than 1%. (0.001,0.99)0.8

E20: Mosquitoes have lots of different sources of transposase, hence the probability that these are expressed to some degree, and can act on the non-defined transposon family is very high (0.01, 0.8)0.8

E21: There should already be a source of transposons. Reasonably likely.

E23: No idea how many different transposons are available. It could be known if we know the transposon families in the Anopheles genomes.
Figure 6.166: Given that there is a source of T/RT/I that acts on nearby flanking transposons, what is the probability that the excised I-Ppol construct will leave the mosquito?
E4: \( \text{Pr} = 1 \)

E6: If transposase is present then there is a reasonable possibility that it could be excised. Not comfortable giving bounds.

E9: In a sense this is a high probability and many will get eaten by something - the event above has had to have happened before or at the same time that the mosquito dies but this can happen in anyone of the cells, the half-life of the construct in the cell is also low, so worst case scenario is that this could be as high as 0.9

E18: Not happening. Assuming naked DNA. \((1E-5,1E-4)0.8\) Reviewed on Day 2: This was for naked DNA, here talking about potential to get into the environment. Same as FT3-10311 (now FT3034100). \((0.8, 0.99)0.9\)

E19: \( \text{Pr}=1 \). But unsure on interpretation of question. Eventually this will be 1 as the mosquito will die and it will leave the mosquito.

E20: Since it can leave the mosquito in any way (e.g. leaching), then this likely to be high \((0.001, 0.8)0.8\)

E21: Same as FT3-10311 (now FT3034100)

E23: Same as FT3-10311 (now FT3034100)
Figure 6.167: Given that the excised I-Ppol construct has left the mosquito, what is the probability that it remains intact?
E4: $Pr = 1$

E6: Likely it would remain intact (not split as per current rows).

E18: Same as above, not going to happen. Even lower than FT3-10302 (now FT3033200). $(1E-6, 1E-5)0.8$

E19: This is low. $(1E-8, 1E-4)0.8$

E20: Going to be naked dsDNA which is reasonably resistant. It will be initially intact and survive for a significant amount of time (easy to imagine for several days) $(0.001, 0.8)0.8$

E21: Same as FT3-10312 (now FT3034101)

E23: Same as FT3-10312 (now FT3034101)
Figure 6.168: Given that there is intact excised I-Ppol construct in the environment, what is the probability that it gets into the germline cell of a eukaryote?
E4: Pr = 0. In the absence of any active or passive uptake just can’t see how this can happen.

E6: Very low. Not comfortable giving a number.

E9: This is hard, 1e- something in single or double digits - The expert thinks this is the key rate limiting step. But The expert says this would be an uneducated guess. Having the integrase associated with the construct or not is not going affect this probability.

E19: This is very low (1E-8, 1E-5)0.8

E20: Given that it is naked DNA, most likely, it is difficult to imagine a means that it could penetrate through the soma to a separate germline. However, it is more likely in single-celled eukaryotes that don’t have a separate germline. Very low (1E-10, 1E-5)0.7

E21: Same as FT3-10313 (now FT3034102)

E23: Same as FT3-10313 (now FT3034102)
Figure 6.169: Given that there is intact excised I-Ppol construct in the environment, what is the probability that it gets into the germline cell of a multi-cellular eukaryote?
E18: (1e-5, 1e-4)0.7
Figure 6.170: Given that there is intact excised I-Ppol construct in the environment, what is the probability that it gets into the germline cell of a single-celled eukaryote?
E18: About 1 in 100 (1e-3, 1e-2)^0.7
Figure 6.171: Given that the excised I-Ppol construct enters the germline cell of a eukaryote, what is the probability that it gets into the nucleus?
E4: Concentration dependent, with only small copy number would say this is Pr = 0.

E9: Thinks this may be around the 1-10% range but this may be an overestimate. The presence of the integrase doesn't influence this.

E18: Same as FT3-1003 (now FT3001)

E19: (1E-7, 1E-4)0.8. Comment amended following feedback report: Same as FT3-1003 (now FT3001)

E20: Given that it might be in a piggyBac construct, its DNA or RNA produced from it might be adapted to get to the nucleus. Could be quite high (1E-4, 0.5)0.8

E21: Same as FT3-10314 (now FT3034103)

E23: Same as FT3-10314 (now FT3034103)
Figure 6.172: Integrated into eukaryote germline
E6: Highly unlikely.
Figure 6.173: Given that the excised I-Ppol construct has inserted into the nucleus of a germline cell with flanking transposons of the same family, what is the probability that it integrates into the genome because the T/RT/I remains bound and active to the free transposon?
E4: Enzymes denature far more quickly than DNA in the environment

E18: No answer.

E19: (1E-5, 1E-3)0.6

E20: The probability that the protein remains intact and active while bound to the DNA is lower than the probability of the DNA itself remaining intact (FT3-10303 (now FT3033201)). (1E-7, 1E-3)0.7

E21: Same as FT3-103151 (now FT30341040)

E23: Same as FT3-103151 (now FT30341040)
Figure 6.174: Given that the excised I-PpoI construct has inserted with flanking transposons of the same family, what is the probability that there is a source of T/RT/I in the eukaryote germline cell?
E4: This is transposable element family dependent, would say that this is close to zero, because you have to have transposons of the same family and they have to be active and there are mechanisms that target this activity and shut it down (e.g. piwi)

E6: Thinking about 10%

E18: Assuming it's an intact transposon it will have an intact basal promoter. That may or may not be expressed in that environment depending on what other transposons are in that host organisms genome. So, if the same transposon family is endogenous, it is likely to be regulated, and unlikely to be expressed. Therefore event split into two below

E19: This is low, no more than 1E-5. (1E-8, 1E-4)0.85. Comment amended following feedback report: The probability to have T/RT/I is higher given that we are assuming any possible eukaryote. Upper bound can be 10%.

E20: Suggested wording change here. Instead of "flanking transposons" read "flanking transposon sequence". Related mosquitoes and other insects have lots of different sources of transposase, hence the probability that these are expressed to some degree, and can act on the non-defined transposon family is very high. Top end high (same as FT3-10301 (now FT30331)), in broader eukaryotes less likely to have the same family of transposase (especially outside of insects). (0.0001, 0.8)0.8

E21: Same as FT3-103150 (now FT30341041)

E23: Same as FT3-103150 (now FT30341041)
Figure 6.175: Given that the excised I-Ppol construct with flanking transposons of the same family has inserted into the nucleus of a germline cell that does not contain endogenous transposons of the same family, what is the probability that it integrates into the eukaryote germline because transposon encoded T/RT/I is expressed in the eukaryote germline cell?
E18: If a transposon enters a naive environment, without endogenous transposons of the same family, transposon encoded enzymes (T/RT/I) may be expressed. About 0.1. (0.1,0.5)0.7
Figure 6.176: Given that the excised I-Ppol construct has left the mosquito without the integrase still bound to the construct, what is the probability that it remains intact?
E9: Not sure how long it will be intact for. It may be still bound to an integrase that will be more sensitive. Worst case scenario for integration down the track is that the integrase is still bound to the construct but the probability of this remaining intact is much lower - the protein is much more likely to be destroyed. Don’t think it will have a long half life - maybe something in the 10% range for the scenario without integrase.
Figure 6.177: piggyBac mediated acquisition
E4: PiggyBac is the only human constructed transposon which works ubiquitously - i.e. doesn’t show the host specificity of other transposons

E11: On first day, not willing to venture opinion on individual steps due to lack of specific expertise, overall opinion, based on genome studies and literature, is that this has very small probability (virtually zero), but could remove likely further with non-functional terminal repeats. For example there is evidence of gene transfer into eukaryotes, but only a slight amount relative to total genome). Since the construct is a relatively small size and availability for transfer is short (about a 1yr), then this means that the overall chance is exceedingly remote. Eliciting probability only for top event.

E19: FT3-10310 (now FT30340) and FT3-103151 (now FT30341040) are different, otherwise the same probability as the BEs under FT3-1030 (now FT3033).
Figure 6.178: Given a catastrophic release of all 10,000 GM insectary mosquitoes, what is the probability that there is a source of T/RT/I that acts on piggyBac in the GM mosquitoes?
E9: The piggyBac was sourced from a moth so even if there was T/RT/I of the same family doubt this could act on the piggyBac (knowing something about the natural history of piggyBacs would help). The expert doesn’t know the sequence fidelity for the T/RT/I sources. To estimate this we would need to know the sequences at the ends of the construct and how close these are to the known A. gambiae piggyBacs. If there were similar end sequences then The expert would raise the probability. also need to know something about piggyBac in the local populations (as discussed for FT3-10300 (now FT30330)).

E11: No source of T/RT/I in mosquito. Transposase is relevant, but RT and I more of an issue for flanking transposons.

E18: piggyBac is not endogenous to mosquitoes. But, there is a possibility that it may be acquired via Baculovirus infection, but the probability of that is low. (5E-5, 5E-4)0.8

E19: This is the same, as the probability that there is one of these enzymes is the same. Same as FT3-10301 (now FT30331)

E20: High. There is evidence of piggyBac elements (perhaps degenerate) in the genome of A. gambiae. Some of these or related elements could provide a source of transposase enzyme. The probability that these could produce sufficient enzyme to catalyse piggyBac excision in some mosquitoes is reasonable. (1E-3, 0.2)0.7

E21: Talking about something that is already in the mosquito. Unlikely, but not completely.

E23: There is no evidence of immobilization in A. gambiae from piggyBac, but this has been shown in other species. So, it is possible (1E-5, 1E-3)0.8
Figure 6.179: Given that there is a source of T/RT/I that acts on piggyBac in the GM mosquitoes, what is the probability that the excised I-Ppol construct will leave the mosquito?
E9: Use same probability as FT3-10302 (now FT3033200)

E18: The probability that the construct will be mobilised is high. But, the likelihood of excised transposon directly accessing a non-target eukaryote via this route is low. The probability that an excised transposon leaves the mosquito and enters the environment is as before for soil/aqueous. (0.8, 0.99)0.9

E19: Same as FT3-10302 (now FT3033200)

E20: This is the same as FT3-10302 (now FT3033200). (1E-3, 0.5)0.9

E23: Could be quite likely to happen. If the transposase is acting, it could be quite likely that the DNA gets excised from the original site. But, leave the mosquito is not very likely, it would remain inside the organism. There is no mechanism that this DNA could leave the mosquito in an active way. The only thing is if the mosquito dies, and then the process is mediated by some prokaryote or other decomposing organism (1E-5, 1E-4)0.8.
Figure 6.180: Given that the excised I-Ppol construct has left the mosquito, what is the probability that the DNA remains intact?
E6: Same as for FT3-10303 (now FT3033201). Not split as per current rows.

E18: Given there is mosquito DNA in the environment, but the probability of maintaining an intact transposon is low. (1E-5, 1E-4)0.7

E19: Same as FT3-10303 (now FT3033201)

E20: This is the same as FT3-10303 (now FT3033201). (0.001, 0.8)0.8

E21: How many constructs that have left the mosquito would you have to look at before you find an intact DNA?

E23: DNA is a pretty stable molecule. So, it is actually possible that the cassette could remain intact. The lower bound refers to the DNA remaining intact for 1 year, while the upper bound is remaining intact for a few seconds before coming into contact with germline of another eukaryote (1E-2, 0.999)0.9.
Figure 6.181: Given that there is intact excised I-Ppol construct in the environment, what is the probability that it gets into the germline cell of a eukaryote?
E4: Still Pr = 0
E9: Same notes as per FT3-10304 (now FT3033202)
E19: Same as FT3-10304 (now FT3033202)
E20: This is the same as FT3-10304 (now FT3033202). (1E-10, 1E-5)0.7
E21: When you say the germline of an eukaryote, you think of the testicles of an elephant, but unicellular eukaryotes the cell is the germline, and they take up DNA frequently. So, it has to be a broad range.
E23: Pretty unlikely, as far as I know there are no mechanisms of transport in Eukaryotes for foreign DNA into germ cells (1E-5, 2E-4)0.8
Figure 6.182: Given that there is intact excised I-Ppol construct in the environment, what is the probability that it gets into the germline cell of a multi-cellular eukaryote?
E18: The expert has answered this for multi-cellular organisms. Same as FT3-10304a (now FT30332020-9)
Figure 6.183: Given that there is intact excised l-Ppol construct in the environment, what is the probability that it gets into the germline cell of a single-celled eukaryote?
E18: The expert has answered this for single celled organisms. Same as FT3-10304b (now FT30332021-9)
Figure 6.184: Given that the excised I-Ppol construct enters the germline cell of a eukaryote, what is the probability that it gets into the nucleus?
E9: Use the same probability as FT3-10305 (now FT3033203)
E19: Same as FT3-10305 (now FT3033203)
E20: This is the same as FT3-10305 (now FT3033203). (1E-4, 0.5)0.8
E21: Not very likely.
E23: I don’t think there is a known mechanism of transport of DNA from the cytoplasm to nucleus in Eukaryotes (at least I am not aware of a mechanism) (1E-4, 1E-2)0.6.
Figure 6.185: Given that the excised I-Ppol construct has inserted with flanking transposons of the same family, what is the probability that the T/RT/I remains bound and active to the free transposon?
E19: Same as FT3-103061 (now FT30332040)

E20: This will be less likely than FT3-103061 (now FT30332040) because this is a narrower class. (1E-8, 1E-3)0.7

E21: Similar range than FT3-103150 (now FT30341041), but slightly more likely, or longer right-hand tail. Because transposases remain bound to DNA.

E23: As far as I know there are no particular mechanism that involve bounding between DNA and transpose, for that reason it would be unlikely that the transposase would remain bound throughout the whole process of leaving the mosquito and entering the new organism's germline and nucleus in an active way (1E-6, 1E-3)0.7.
Figure 6.186: Given that the excised l-PpoI construct has inserted with flanking transposons of the same family, what is the probability that there is a source of T/RT/I in the eukaryote germline cell?
E19: Same as FT3-103060 (now FT30332041)

E20: Shift down slightly from FT3-103060 (now FT30332041) as this is one transposable element class which is a subset of the non-defined transposable elements in question FT3-103060 (now FT30332041). (1E-5, 0.5)0.8

E21: Has to be the right type of enzyme that will recognize the repeats. Many eukaryotes will have an RT/T/I, but not enough. It must be the right one. From Elephants to fungi, how many times would this even have to occur before you get the right match of transposase, and that the transpose is expressed.

E23: The source of transposases are in prokaryotes, but I am not sure if there are in Eukaryotes. No idea.
Figure 6.187: Given that the excised I-PpoI construct without the integrase still bound to it, has left the mosquito, what is the probability that the DNA remains intact?
E9: Use the same probability as FT3-10303-wolnt (now FT3033211)
Figure 6.188: Given that eukaryote males carrying the construct are viable, what is the probability that the construct fails to sterilise them?
E11: Low probability event. Relevant target site is common across eukaryotes, but highly conserved across many taxa. There should be some data on rDNA target site among eukaryote taxa, but cannot elicit an estimate.

E18: Entirely dependent on which eukaryote species it has entered. And it entirely depends where the target sites are, whether it is cutting up one of the sex chromosomes. (0.05,0.9)0.7 Relatively high chance that it won’t sterilise another organism, and a low chance that it will.

E19: This can be quite high. If the I-Ppol construct does not shred the X chromosome (ribosomal repeats), the construct will fail to sterilise them. (0.01, 0.99)0.9

E20: Might fail to sterilise simply because I-Ppol is not expressed. This might be due to a number of reasons, non recognition of promoter is the most likely and easy to imagine. Lower in closely related species, higher in others. (1E-7, 0.8)0.8
Figure 6.189: Given that the construct has introgressed into a wild type population, what is the probability that GM eukaryote male has higher fitness than a wild type male?
E7: Assuming that it is very unlikely to be expressed

E11: This probability is small but not vanishingly small. Somewhere between 1 in a million and a billion with 50% probability.

E20: Wording comment, replace "introgressed" with "carrying the construct" to match FT3-11000 (now FT31000). Very hard to imagine males carrying I-Ppol or GFP should be fitter than WT. GFP is at best neutral. I-Ppol at best neutral if not expressed, and damaging if it is. (1E-9, 1E-5)0.8
Figure 6.190: Given that the construct has introgressed into a wild type population, what is the probability that a product (RNA/protein) of the construct improves fitness?
E18: 50/50. (0.45, 0.55)0.95

E20: This is the same as what the expert gave for FT3-11001 (now FT31002).
Figure 6.191: Given that the construct has introgressed into a wild type population, what is the probability that the insertion has a beneficial effect by disrupting or affecting nearby genes?
E18: This is less likely. 1 in a 100. (1E-3, 1E-2)0.7

E20: Unlikely that disruptions cause beneficial fitness effects. But male sterile mutants are more easily recovered than female sterile mutants. The probability is lower for males than females that fitness will be improved by insertions. (1E-6, 1E-3)0.6
Figure 6.192: Given that the construct has introgressed into a wild type population, what is the probability that any flanking regions are beneficial and stay linked to the transgene?
E18: This is less likely. 1 in a 100. (1E-3, 1E-2)0.7

E20: Flanking regions could be beneficial and therefore the construct could 'hitchhike' but in a sexually reproducing organism the construct and the beneficial regions should be split up by recombination. How long they stay together will depend on how close they are to each other and the rate of recombination in the organism. In some organisms, recombination in males is less frequent. (1E-6, 1E-2)0.7
Figure 6.193: Given that the construct has introgressed into a wild type population, what is the probability that a GM eukaryote female has higher fitness than a wild type female?
E7: Same probability as FT3-11001 (now FT31002)

E11: When expressed in mosquito germline, neither male or females are produced. Believes that it would not be different for males. Similar to male, but not the same because female can be heterozygous and could receive increase in fitness in heterozygous state. But no basis for assigning different distribution relative to males, effectively same.

E20: Similar reasoning to FT3-11001 (now FT31002), but less likely that I-Ppol should be expressed. (1E-7, 1E-3)0.8
Figure 6.194: Given that the construct has introgressed into a wild type population, what is the probability that a product (RNA/protein) of the construct improves fitness?
E18: Same as FT3-1110M (now FT310020-9)

E20: This is the same as what The expert gave for FT3-11010 (now FT31011). (1E-7,1E-3)0.8
Figure 6.195: Given that the construct has introgressed into a wild type population, what is the probability that the insertion has a beneficial effect by disrupting or affecting nearby genes?
E18: Same as FT3-1111M (now FT310021-9)

E20: Unlikely that disruptions cause beneficial fitness effects. But male sterile mutants are more easily recovered than female sterile mutants. The probability is lower for males than females that fitness will be improved by insertions. (1E-5,1E-2)0.6
Figure 6.196: Given that the construct has introgressed into a wild type population, what is the probability that any flanking regions are beneficial and stay linked to the transgene?
E18: Same as FT3-1112M (now FT310022-9)
E20: See comments for males. (1E-5, 1E-2)0.7
Figure 6.197: Given that the I-PpoI construct is successfully inserted into the genome of a eukaryote’s germline, what is the probability that a product (RNA/protein) of the construct improves fitness?
E11: Analogous to boxes for male and female selection. Assumed this means improved fitness of GM over the wild type. This is analogous to FT3-1100 (now FT3100) and FT3-1101 (now FT3101) where it could be a function of either insertion site or product of construct, whereas here it is about product of construct only.

E18: Same as FT3-1110M (now FT310020-9)

E19: \((1E-6, 1E-4)^0.8\)

E20: Same as FT3-11010 (now FT31011) \((1E-7, 1E-3)^0.8\)
Figure 6.198: Given that the I-Ppol construct is successfully inserted into the genome of a eukaryote’s germline, what is the probability that the insertion has a beneficial effect by disrupting or affecting nearby genes?
E11: Analogous to boxes for male and female selection.
E18: Same as FT3-1111M (now FT310021-9)
E19: (1E-6, 1E-3)0.7
E20: The same as FT3-1111F (now FT310111-9) (1E-5, 1E-2)0.6
Figure 6.199: Given that the I-Ppol construct is successfully inserted into the genome of a eukaryote’s germline, what is the probability that any flanking regions are beneficial and stay linked to the transgene?
E7: The expert can't answer this question

E11: Analogous to boxes for male and female selection.

E18: Same as FT3-1112M (now FT310022-9)

E19: (1E-6, 1E-3)0.7

E20: See FT3-1112M (now FT310022-9). The probability that beneficial flanking regions are nearby is the same. But because there is no sexual reproduction, there is no recombination, so they will stay together much longer. (1E-4, 1E-1)0.7
Figure 6.200: Given that eukaryotes carrying the construct are viable, what is the probability that their genome contains an additional site outside the X-linked ribosomal repeat array that is recognised by I-PpoI?
E7: Same issue as FT3-0210000 (now FT510111000) but now we need to account for different genome sizes, bearing in mind that the human genome is an order of magnitude greater than mosquitoes, and plants is bigger again.

E9: The probability of randomly finding a 15bp recognition site in a genome is 1 in 4E15, approx. 1 in 1.1E9. The human genome is 3.1e9 bp so this could happen in the human genome, but an insect genome is 1e8 bp so this is one tenth, and in a fungae the unique genome size is approximately 1e7bps so that one hundredth, but the slop in the recognition site is going to increase the probability. So this is low probability but not the killer step. The expert can’t give a number here because what’s missing is the relative proportion of the different genome sizes in the eukaryote kingdom, the estimate will be heavily weighted to the smaller genome sizes.

E18: In Steve’s view, this is as likely as in a mosquito. So same as FT3-0210000 (now FT510111000).

E19: This can be in any eukaryote. This depends on the genome size of the eukaryote. The bigger the genome size, the higher the chance there is a site recognised. If we have some eukaryotes with genome of 10 billion, then this is possible. (1E-5, 2.5E-2)0.7

E20: (1E-3, 0.5)0.5. OK HGT

E23: It is possible that is happening, and is something that is currently being measured.
All experts: 201 of 352 EXw = 10.196

Figure 6.201: Given that I-Ppol recognises an additional site what is the probability that it moves into this site?
E7: Can’t put a number on this because genome size varies too much across all non-target eu-
karyotes. Would be a range broader than the answer given for FT3-021001 (now FT510111001) assuming that movement rates are the same which is a big assumption

E9: Use the same probability as FT3-021001 (now FT510111001)

E18: In Steve’s view, this is as likely as in a mosquito. So same as FT3-021001 (now FT510111001).

E19: Same as VGT FT3-021001 (now FT510111001)

E20: (1E-8, 1E-5)0.5. OK HGT same as VGT

E23: To move into a new site, it must not only cut, but non-homologous recombination must also happen. The construct must have flanking sequences homologous or very similar to those surrounding the new site. So, the possibility of integration in the new site will be strictly related to that new recognition site. If the new recognition site is in a highly repetitive region the chance of moving into the new target site will be high (1E-6, 0.1)0.6
Figure 6.202: Given that I-Ppol has moved into an additional site what is the probability that it maintains its germline expression?
E7: The expert doesn’t know. Might be able to make a better informed estimate with research into the similarity between promoters, if this was just Dipterans then maybe this is quite a high probability.

E9: There would be a huge range on this. For another insect maybe be a little bit (0.9) less than the probability that The expert gave for FT3-021011 (now FT51011111), but for other eukaryotes (non-insects) this probability drops off precipitously, The expert can give an upper bound but the lower bound can’t really specify.

E18: (0.05, 0.9)0.7 Same as previously with sterilising (FT3-11000 (now FT31000)) - i.e. Very broad.

E19: This is lower than in VGT, because the probability that the same promoter will be active and specific in non-target eukaryotes is very low. (1E-8, 1E-4)0.7

E20: (0.8, 0.99)0.9 VGT. High probability that it will maintain germline expression in related mosquitoes. Less so but still significant risk in other insects. Unlikely outside of insects. (1E-6, 0.99)0.9 This range reflects across non-target eukaryotes. This was given more for HGT, where as here VGT. HGT will be much lower because promoter not likely to be active in distant eukaryotes.

E21: Complete different species, with different regulatory factors. So, very unlikely. But, some other related insects could be more phylogenetically close.

E23: If the above has happened, then it will be likely given the data we have in the laboratory. This is because the promoter is conserved across eukaryotes (0.5, 0.99)0.8
Figure 6.203: Given that unmutated I-Ppol has moved into an additional recognition site and maintains germline expression, what is the probability that it does not cleave ribosomal repeats?
E18: Given the conservation of the I-Ppol target site, this is as likely as in a mosquito. So same FT3-0210040b (now FT5101110030).

E19: All spectrum of eukaryotes, so many of those will not have ribosomal repeats. (1E-5, 1E-2)0.6. The expert not very confident on how conserved the ribosomal repeats are among non-target eukaryotes.

E20: (1E-9, 1E-6)0.8 OK HGT

E21: Here you have to imagine some mechanism that would somehow protect the ribosomal cluster differently from this additional site. Seems unlikely. It is hard to think of such a mechanism. (Added by AGS: we need to take into account uncertainty about ribosomal DNA in other species).

E23: This will be mainly related to the presence/absence of the recognition site in the new organism. The target site for I-Ppol is highly conserved across Eukaryotes, the probability that it won’t cleave rDNA repeat will be low (1E-3, 0.5)0.8
Figure 6.204: Given that I-Ppol has moved into an additional recognition site and maintains its germline expression, what is the probability that the recognition site is in an Intron?
E7: Same issue as FT3-02100410 (now FT51011100310) again bearing in mind change in genome size

E9: As the genome size goes down the probability of the recognition site being in an intron goes down by a couple of orders of magnitude, because there are so many fewer introns and they tend to be small.

E18: As per FT3-02100410 (now FT51011100310), you should be able to calculate this.

E19: This is quite low. Median 1 in 10000. (1E-6, 1E-3)0.6. Comment changed following feedback report: Given the big range of all possible non-target organisms, the probability to hit an intron can be either low (for very big genome size ) to very small (for small genomes).

E20: (0.001, 0.9)0.8 OK HGT. Intronic content in other eukaryotes and genome size might be significantly different, hence wider bounds compared to VGT.

E21: Reworded as in FT3-02100410 (now FT51011100310). But, broaden to accommodate variation in intron number and size across organisms.

E23: This mainly based on laboratory experience. Most of the time the integration happens in some non-essential part of the genome (0.01, 0.2)0.8.
Figure 6.205: Given that I-Ppol has moved into an additional recognition site and maintains its germline expression, what is the probability that the recognition site is in a non-essential region of the genome?
E7: Same issue as FT3-02100411 (now FT51011100311) again bearing in mind change in genome size.

E9: As the genome size goes down the probability of the recognition site being in a non-essential region goes down by a couple of orders of magnitude, because there are so many fewer introns and they tend to be small.

E18: Same as FT3-02100411 (now FT51011100311)

E19: (1E-4, 1E-2)0.6. Comment amended following feedback report: Same rationale as FT3-11200310 (now FT31200310).

E20: (0.005, 0.9)0.7. Widened for similar reasons as FT3-11200310 (now FT31200310), i.e. proportion of non-essential content.

E21: Have to broaden to accommodate variation in the percentage of the genome that is coding vs non-essential across organisms.

E23: This mainly based on laboratory experience. Most of the time the integration happens in some non-essential part of the genome (0.5, 0.95)0.8.
Figure 6.206: Given that I-PpoI has moved into an alternative recognition site and maintains its germline expression, what is the probability that the negative fitness effects are recessive?
E18: Same as FT3-02100412 (now FT51011100312)

E19: (1E-5, 1E-2)0.7. Comment amended following feedback report: It's quite likely to have recessive fitness.

E20: (0.1, 0.99)0.8. Answered this regarding fitness effects due to the insertion at the additional site. Lowered the lower bound with respect to VGT to reflect possibility of invading haploid genomes. Please change to (0.1, 0.99)0.9.

E21: Many possibilities across the eukaryote range (different ploidy, for instance). It is more likely that dominant effects might appear across the broad range of organisms.

E23: In 50 lines generated in the laboratory, found one that had this effect. Considering this the mean of the distribution (0.001, 0.1)0.7.
Figure 6.207: Given that eukaryotes carrying the construct are viable, what is the probability that I-PpoI mutates and recognises an additional site outside the X-linked ribosomal repeat array?
E7: Same issue as FT3-0210001 (now FT510111020), but again bearing in mind change in genome size
E9: Use the same probability as FT3-0210001 (now FT510111020)
E18: In Steve’s view, this is as likely as in a mosquito. Same as FT3-0210001 (now FT510111020).
E19: (1e-6, 1E-3) 0.75
E20: (1E-11, 1E-6) 0.5 OK HGT. Lower bound lower than VGT. Because eukaryotes includes many organisms with much smaller genomes, so the probability that there is an additional site in the genome is much lower.
E23: A mutation that can cause an amino acid change in the functional site of the enzyme. The guys in Seattle, involved in protein engineering, could estimate this quite accurately.
Figure 6.208: Given that mutated I-Ppol has moved into an additional recognition site and maintains germline expression, what is the probability that it does not cleave ribosomal repeats?
E18: Given the conservation of the I-Ppol target site, this is as likely as in a mosquito. So same as FT3-0210040a (now FT5101110230).

E19: (1E-4, 0.05)0.75

E20: (1E-5, 0.1)0.8 OK HGT

E21: If I-Ppol has mutated to recognise a different locus (site) then it is very plausible that it would no longer recognise its original recognition site. (Added by AGS: more variation across Eukaryotes)

E23: Because the enzyme has mutated, it is recognizing a different target sequence, the possibility of that target sequence not being in the rDNA would quite high (0.5, 0.90)0.8
Figure 6.209: Given that eukaryotes carrying the construct are viable, what is the probability that the I-Ppol construct inserts into a recognition site on a ribosomal repeat?
E9: Use the same probability FT3-021010 (now FT51011110)

E18: This is high, given that the I-Ppol target site in ribosomal repeats is highly conserved. But need to reflect on the possibility that there may be eukaryotes where the target site is diverged. (0.3, 0.8)0.8

E19: (1E-8, 1E-4)0.8

E20: (1E-8, 1E-3)0.6 Same as VGT.

E23: The possibility of homing into the rDNA is really low because to do that the construct must be flanked by a homologous region (or highly similar) to the region flanking the rDNA repeat. The construct does not have that. Assume the probability is really low, but considering we are talking about an unknown organism (i.e. a new integration) (1E-3, 0.2)0.8.
Figure 6.210: Given that I-Ppol inserted into a recognition site on a ribosomal repeat, what is the probability that it maintains its germline expression.
E7: The expert can’t answer this one, depends on similarity of the promoters between the organisms.

E9: There would be a huge range on this. For another insect maybe be a little bit (0.9) less than the probability that The expert gave for FT3-021011 (now FT51011111), but for other eukaryotes (non-insects) this probability drops off precipitously, The expert can give an upper bound but the lower bound can’t really specify.

E18: (0.05, 0.9)0.7. Same as previously with sterilising (FT3-11000 (now FT31000)) - i.e. Very broad.

E19: Also low (1E-9, 1E-4)0.75

E20: High probability that it will maintain germline expression in related mosquitoes. Less so but still significant risk in other insects. Unlikely outside of insects. (1E-6, 0.9)0.9. This range reflects across non-target eukaryotes.

E23: Same as FT3-112002 (now FT312002)
Figure 6.211: Given that I-Ppol inserted into a ribosomal repeat and maintains its germline expression, what is the probability that it has low cleavage rate at the rDNA locus?
E7: Use the same probability as FT3-0210130 (now FT510111130)

E9: Hard for The expert to make an estimate on this for the same reasons outlined in FT3-0210130 (now FT510111130)

E18: Same as FT3-0210130 (now FT510111130) in VGT.

E19: (1E-6, 1E-4)0.7

E20: (1E-7, 1E-2)0.7. Same as for VGT.

E21: Slightly more likely then in VGT because we don’t know everything about all the organisms.

E23: Considering we are talking about a new organism, it is possible that polymorphisms exist in the recognition site that could lead to lower cleavage activity in the new organism (0.2, 0.8)0.8.
All experts: 212 of 352 EXw = 4.259

Figure 6.212: HEG inserted in intron
E21: All sorts of Eukaryotes, but this includes Fungi, so it must be more likely than in VGT.

E23: Talking just about rDNA repeat, the chance of homing in a rDNA intron is definitely lower than the chance of insertion of introns in coding regions across the genome. It can’t be calculated, because it would depend on the organism. Picturing the average size of the rDNA repeat compared to the rest of the genome (1E-2, 0.2)0.7.
Figure 6.213: Given that I-Ppol inserted into a ribosomal repeat and maintains its germline expression, what is the probability that it inserted in a self-splicing intron?
E7: Not a question The expert can answer

E9: Same comment as for FT3-02101310 (now FT510111310)

E18: If the HEG is acquired by a lower single celled eukaryote, there is certainly a possibility that rRNA transcribed spacers are self-splicing. But not likely in any higher eukaryotes.

E19: \((1E^{-7}, 1E^{-5})^{0.6}\)

E20: This is going to be more likely in HGT vs. VGT, because self-splicing introns are known to exist and work in lower eukaryotes.
Figure 6.214: Given that the I-Ppol construct is successfully inserted into the genome of a eukaryote's germline, what is the probability that the eukaryote has heterogametic sex determination (e.g., XY sex determination)?
E7: Not a question The expert can answer
E18: These numbers should be known. So calculate them
E19: This is a good proportion. More than 10%. (0.1, 0.6)0.7. Assume 50% of eukaryotes have heterogametic sex determination.
E20: Do not know this but a good estimate of this can be found. Could be as high as 60%, and as low as 1%. (0.01, 0.8)0.8
E21: More likely than not, but with a broad range to account for variability
E23: Given that the construct has inserted itself, it is more likely to happen in an Eukaryote with heterogametic sex determination (0.1, 0.8)0.5.
Figure 6.215: Given that the I-Ppol construct is successfully inserted into the germline genome of a heterogametic eukaryote, what is the probability that the construct moves to the sex-determining chromosome (Y or W)?
E19: (1E-6, 0.01)0.7

E20: Heterogametic sex chromosome could make up a varying proportion of the whole genome, which could conceivably be up to one third and as low as 1%. (0.005, 0.5)0.7

E21: Same as FT3-02110 (now FT5101100). Different species will have sex chromosomes of different size, but on average it should be the same as in VGT.

E23: Take as reference the A. gambiae transgenic line, we have never had a situation where the construct moved from an autosome to the sex determining chromosome (1E-12, 1E-4)0.8.
All experts: 216 of 352 $EX_w = 2.199$

Figure 6.216: Given that the I-Ppol construct inserted on the Y or W chromosome, what is the probability that it produces an enzyme that is able to cleave rDNA?
E19: Depends on the host. (1E-5, 1E-2)0.7
E20: The lower bound needs to be widened with respect to VGT. (1E-6, 0.9)0.9
E21: The expert suggested rewording. So, the question then becomes In how many Eukaryotes
the rDNA is conserved?
E23: The site of I-Ppol is really conserved across Eukaryotes, so it is quite likely (0.01, 0.2)0.5
Figure 6.217: Given that the I-PpoI construct inserted on the Y or W chromosome and produces an enzyme that is able to cleave rDNA, what is the probability that this rDNA is predominantly on the X or Z chromosome?
E7: Not a question The expert can answer
E19: (1E-6, 1E-2)0.7
E20: (1E-5, 0.9)0.8. OK HGT.
E21: Likely to be very low, as in most organisms it is not sex-linked.
E23: The ribosomal DNA position is only known for a few mosquito species. Not sure about it, but assume that 0.1 is the average (0.01, 0.2)0.5.
Figure 6.218: Given that the I-Ppol construct inserted on the Y or W chromosome and produces an enzyme that is able to cleave rDNA that is predominantly on the X or Z chromosome, what is the probability that it does not cause sterility?
E7: The expert doesn't believe that the construct will be expressed that well because we are in an organism that is quite far removed from the mosquito - we have assumed that it will be expressed but we haven't talked about how much expression will occur

E19: (1E-4, 0.01)0.6

E20: (1E-9, 1E-3)0.8. OK HGT.

E21: The expert says to delete the following: and produces an enzyme that is able to cleave rDNA that is predominantly on the X or Z chromosome. As sterility would not depend on that. High uncertainty because of physiological uncertainty associated with the construct induced sterility.

E23: If the conditions are pretty similar to that of A. gambiae transgene, the likelihood of not causing sterility is small. (Thinks the question about germline specific expression should be answered first, and that is the order in which they were elicited. So, assumes that germline expression occurs) (1E-2, 0.5)0.7.
Figure 6.219: Given that the I-Ppol construct inserted on the Y or W chromosome, what is the probability that it maintain germline specificity?
E7: Needs to be clarified as before to mean restricted to germline activity
E19: (1E-8, 1E-4)0.7
E20: (1E-7, 0.05)0.7 OK HGT
E21: The expert suggested re-wording of the question. And, suggests a change in order with this event preceding event FT3-11212 (now FT31212), with that event being re-worded to delete the word "produces".
E23: It is likely because the promoter is beta-tubulin which has a sequence that is quite conserved across Eukaryotes (0.2, 0.8)0.8.
Figure 6.220: Given an I-Ppol construct inserted on the Y or W chromosome that is able to cleave rDNA exclusively on the X or Z chromosome without causing sterility, what is the probability that the driving forces are higher than the fitness costs?
E19: (1E-3, 0.1)0.7

E20: (0.01, 0.9)0.8 OK HGT

E21: Again, variability across Eukaryotes.

E23: Could be possible, because in the case of not being sterile, it could potentially be a sex-ratio distortion mechanism in which only Y-sperm mature, which would cause strong drive (0.5, 0.9)0.8.
Figure 6.221: Acquisition of construct
E19: These are dependent so can use the gates in FT3 or these values here. Given to check internal consistency.

E21: The expert decided to elicit at this level (2E-6, 0.1)0.75
Figure 6.222: Given a catastrophic release of all 10,000 GM insectary mosquitoes, what is the probability that the I-PpoI construct is stably acquired by a prokaryote organism by transformation in the soil?
E10: Available from previous calculations

E18: Dependent

E19: \((1E-6, 1E-3)^{0.7}\). This depends on the environment. Different locations can have a big impact on the probability that this occurs. Also seasonally dependent. If it is very hot DNA breaks down. In Africa, don’t expect frozen areas where DNA can be preserved.

E21: Assume that stably means that it will stay in the organism over at least a couple of generations.
Figure 6.223: Given a catastrophic release of all 10,000 GM insectary mosquitoes, what is the probability that the I-Ppol construct is stably acquired by a prokaryote organism by transformation in an aquatic environment?
E10: Available from previous calculations
E18: Dependent
E19: (1E-6, 1E-3)^0.7
E21: Same as FT4-002 (now FT4000)
Figure 6.224: Given a catastrophic release of all 10,000 GM insectary mosquitoes, what is the probability that the I-Ppol construct is stably acquired by a prokaryote organism by transformation in the gut of a eukaryote organism?
E10: Available from previous calculations
E18: Dependent
E19: One order of magnitude lower, for both upper and lower limits, than soil and aquatic. (1E-7, 1E-4)0.7
E21: Would be more likely than above, as the ingestion would put together bacteria and mosquito.
Figure 6.225: Given that a viable transformed prokaryote organism has been created, what is the probability that the HE protein or RNA is expressed in the prokaryote?
E10: This is same as probability FT4-010021 (now NA)
E17: Don’t know enough about this to answer
E18: Low but not impossible. (1E-3, 1E-2)0.7

E19: Unlikely it is expressed. The promoter is a mosquito specific promoter. It shouldn’t be
expressed but there could be some situation (through some mechanism) that it is expressed.
The probability that it is expressed is very low. (1E-6, 1E-3)0.6

E21: This is not so unlikely, because the promoter might be there. How many times do you
transform it until you find one with expression? (1E-5, 0.1)0.9

E22: Low probability (1e-4, 1e-6)0.75.
Figure 6.226: Given that the HE protein is expressed in the prokaryote, what is the probability that the product (RNA or protein) of the construct per se improves the prokaryote’s fitness?
E6: Very low. Ok

E9: By looking at the “nature experiment”, or by doing a search on bacterial genomes, The expert can’t see how the construct could improve fitness.

E17: I-PpoI is a member of a family of nucleases that are found in a variety of microbes for which over evolutionary time there would have been abundant HGT events from one microbe to prokaryote and prokaryote to prokaryote, and as far The expert is aware there is no evidence that the gene has spread by natural selection. The same applies for the fluorescent proteins

E18: Not likely (1E-4, 1E-2)0.6

E19: HE protein is fused to GFP. Which in prokaryotes/bacteria could provide some fitness. So there is a probability that there is improved fitness, low but still possible. Unlikely that it provides fitness, but in some situations it might. (1E-5, 0.01)0.8

E21: How many prokaryotes do you have to transform to find an improvement of fitness, given the gene plus environment combination? (1E-12, 1E-6)0.8

E22: This is very low probability (1e-9, 1e-7)0.80.
Figure 6.227: Given that a viable transformed prokaryote organism has been created, what is the probability that the insertion of the construct in the genome improves the prokaryote’s fitness by disrupting or affecting nearby genes?
E6: Very low chance of improving fitness. Genes and nearby genes being tested already, can't disrupt gene and improve fitness, unless the gene has a negative feedback function. Could disrupt gene which means virus always kills host. Which technically might make it more fit.

E9: Most of the genome of a prokaryote is "stripped down" - they don’t have much in the way of non-essential regions of the genome, so it’s hard to see how affecting or disrupting nearby genes is going to improve fitness.

E10: Richard Lenski, curious how he would answer here. 80% of the prokaryote genome consists of Open Reading Frames (ORFs), so how can disrupting or affecting this improve fitness. The other 20% is now thought to be miRNA and DNA that plays other regulatory roles, so again very little scope here to improve fitness. The expert thinks 1e-6 is a reasonable upper bound.

E17: Prokaryotes are continually exposed to all types of nucleic acids including I-Ppol and there is no reason to think that there is anything unique in the construct DNA which would make it special compared to all the other sequences from Eukaryotes that they would be exposed to. As far as The expert is aware HGT from Eukaryotes to Prokaryotes is exceedingly rare. Furthermore, any bit of DNA can disrupt.

E18: About 1 in 100. (1E-3, 1E-2)0.5
E19: (1E-4, 1E-2)0.8
E21: (1E-12, 1E-6)0.9
E22: This is very low probability
Figure 6.228: Given that a viable transformed prokaryote organism has been created, what is the probability that regions of the mosquito genome flanking the construct stay linked to the transgene and improve the prokaryote’s fitness?
E6: Extremely low, would completely destabilise the virus. Viruses encodes just what is needed for virus and its DNA must fit in virus shell. Even if we force extra material in, it is rarely tolerated, and invariably eliminated during successive rounds of replication. Construct too big and not going to package. Inconceivable.

E10: This has to be less than FT4-0101 (now FT401010) because the exogenous DNA sequence is now larger and this will more likely decrease fitness than improve it. The expert thinks a reasonable upper bound may be 1e-7.

E17: Same as above The expert can’t see this happen for the same reason, tempted to make both Pr = 0

E18: 50/50. (0.3, 0.7)0.5

E19: (1E-4, 0.05)0.8

E21: (1E-10, 1E-5)0.85

E22: The expert does not think this can happen. It may happen but it won’t be stable so it will be biologically irrelevant.
Figure 6.229: Given that a viable transformed prokaryote organism has been created, what is the probability that the construct is increasing in frequency because of selection (i.e. hitch hiking)
E17: The expert is not sure why this should be restricted to MGEs, in principle it could hitch-hike with a non-mobile gene that is increasing in frequency, e.g. chromosomal antibiotic resistance. As far as The expert is aware there is no record of this ever been recorded from prokaryote gene sequences. Note you would have to get into a positively selected gene or MGE right at the beginning of the spread when that gene or MGE is very rare in the population for this to be important. So again vanishingly rare

E22: The expert thinks this is the most likely of the three because main environmental bacteria have their own transposons (1e-10, 1e-8)0.70.
Figure 6.230: Given that a viable transformed prokaryote organism has been created, what is the probability that the construct is linked to one of the prokaryotes Mobile Genetic Elements (MGE)?
E6: Viruses don’t have duplicated sequences. This assumes multiple copies of gene. Viruses ok with just one copy of gene. Need to clarify

E9: Usually a population of prokaryotes are clonally derived (hence identical) so not sure how to estimate this

E10: Depends on what gene and the nature of the selection that goes with it. If rare antibiotic then its not going to be selected for. If commonly used antibiotic then different. Or if a metal or detergent, then chance for selection is much greater and gene would go with it. In mobile element so chance of transfer to other organisms is very high. Chance of spread would depend on how other organisms are selected. Highly evolved ones are primarily in human impacted environments which use a lot of detergents etc. In rural Africa these are probably not so common. If it goes to New Delhi, going to be everywhere - MGE going to be in human modified environments. Frequency of MGE - what type of selective pressure. Probability of MGE picking up something non selective for random DNA incorporation has 3 components: (i) probability of a MGE, particularly a highly mobile MGE; (ii) probability of it to pick up a random DNA segment with no selective component; and, (iii) probability of other selective genes on MGE being selected for in local environment. The expert Gillings could answer these components. FT4-0103a (now FT401020) is for component 2 probability of picking it up. Assuming 1e11 constructs in 10k mosquitoes. If any of these mobile elements have other selections (such as metal resistance, antibiotic resistance). Canonical case here are mobile genetic elements that have mechanisms to acquire novel exogenous genes, examples are integrons (site specific regions that have mechanisms to accumulate other genes - The expert Gillings is the expert on this). This is the mechanism for multiple antibiotic, metals, detergent resistance (i.e. anything in the environment with a strong selective pressure). The rate limiting step is the probability that it happens to integrate into one of these highly mobile genetic elements (MGEs). If it does this would be worrisome (if you were trying to come up with a worst case scenario then this would probably be it). Ask The expert Gillans what is the chance that a 5000 bp construct could get into a proportion of a MGE for a transformed prokaryote (with no selective advantage attributable to the construct).

E17: The answer above covers this question and the next

E21: (2E-5, 0.01)0.85
Figure 6.231: Given that the construct has integrated into one of the prokaryote’s Mobiles Genetic Elements, what is the probability that it increases in frequency by selection (i.e., hitchhiking)?
E10: Assuming FT4-103a has occurred, then consider component 3 from above. Which is the probability of selection in the local environment. Could be quite variable.

E21: (1E-6, 1E-3)^0.8
Figure 6.232: Homing
E17: Prokaryote with the appropriate genetic systems for homing are rare. The construct landing in the recognition site is very unlikely. Prokaryotes tend not to have non-essential regions in their genome, and also landing in a self-splicing intron is very unlikely. Happy to give a number at the gate which is the same as the \((1e^{-11}, 1e^{-9})^{0.9}\)
Figure 6.233: Given that a viable transformed prokaryote organism has been created, what is the probability that the prokaryote genome contains an I-Ppol recognition site?
E9: See notes on bacterial genome size

E18: The random probability is very low. About 1 in 1E9 base pairs. The presence of I-Ppol sites in bacteria rDNA is known.

E19: Probability is very low, but considering massive number of prokaryotes available, there could be some which have the recognition site. (1E-8, 1E-4) 0.65. Comment amended following feedback report: It depends on the genome sites. It can be as high as 5%

E21: Very unlikely (1E-9, 1E-6) 0.85.
Figure 6.234: Given that a viable transformed prokaryote organism has been created, what is the probability that the I-PpoI construct mutates to recognise a site in the prokaryote genome?
E9: Very small fractions of mutations will change the recognition site, and this has to occur before an inactivating mutation. The expert thinks this is around the $1\times10^{-4}$ to $1\times10^{-6}$ type event.

E18: Certainly possible. ($1\times10^{-4}$, $1\times10^{-3}$)0.7

E19: ($1\times10^{-6}$, $1\times10^{-3}$)0.6

E21: A smaller prokaryote genome means there are fewer combinations possible, so lower specificity is more viable than in a Eukaryotic genome ($1\times10^{-10}$, $1\times10^{-7}$)0.9
Figure 6.235: Given that the viable transformed prokaryote’s genome contains an I-Ppol recognition site or I-Ppol mutates to recognise a site, what is the probability that the construct inserts into this site?
E9: Bacterial genomes tend to be 1e6 to 1e7 bps, which is about an order magnitude less than what we have been talking about in FT3 - so probability is accordingly reduced.

E18: This is low due to a lack of homology. (1E-6, 1E-5)0.7

E19: This is lower. (1E-8, 1E-5)0.7

E21: Very low (1E-9, 1E-7)0.85
Figure 6.236: Given that the I-PpoI construct inserts into a recognition site in the prokaryote’s genome, what is the probability that the I-PpoI protein is expressed in the prokaryote?
E9: It may insert in a place where the RNA gets expressed as part of a host operon, but then it would require translation into protein which The expert thinks is unlikely unless its in just the right position to make a fusion protein with the bacterial protein. This would be a really rare insertion (in the range 1e-6 to 1e-8).

E18: (5E-4, 5E-3)0.7
E19: (1E-8, 1E-5)0.6
E21: (1E-5, 0.01)0.9
Figure 6.237: Given that the I-Ppol protein is expressed, what is the probability the prokaryote's genetic system allows for HEG + and HEG - alleles to be present in the same cell to allow homing?
E9: The few bacteria that The expert is aware of don't have repetitive arrays, and so it's hard to see how this could occur.

E18: No idea.

E21: Very unsure about this. There should be a literature search done to understand a bit more of what are the possibilities, and get a more confident answer (1E-6, 0.01)0.6
Figure 6.238: Given that the viable transformed prokaryote’s genome contains an I-Ppol recognition site or I-Ppol mutates to recognise a site, what is the probability that this site is in a self-splicing intron?
E9: The expert is not even sure if introns exist at all in prokaryote genomes.
E21: Talking about the site itself being in the intron (1E-6, 0.001)0.7
Figure 6.239: Given that the viable transformed prokaryote’s genome contains an I-Ppol recognition site or it mutates to recognise a site, what is the probability that this recognition sequence is in a non-essential region of the genome?
E9: Again the bacterial genomes are “compact” and it’s very unlikely that any one bit of it is non-essential.

E18: The expert does not think there are many non-essential regions in bacterial genomes.

E19: (1E-5, 1E-3)0.65. Comment amended following feedback report: Prokaryotes genome is rather small (1-10Mb) and most of it is coding region. The non-essential part of it can be 1% to 10%

E21: (1E-2, 0.2)0.8
Figure 6.240: Given that a replicating transformed virus has been created, what is the probability that a product (RNA or protein) of the construct improves the virus' fitness?
E8: Just can't see this happening. Probability close to zero (1e-7 to 1e-6). Y drive concern is somewhat increased because the exposure time is increased.

E17: The expert cannot think why this would be different from prokaryotes to viruses, so copy answers all the way down and use same numbers.
Figure 6.241: Given that a replicating transformed virus has been created, what is the probability that the insertion of the construct in the genome improves the virus' fitness by disrupting or affecting nearby genes?
E8: P35 affects replication fitness but increases transmission by reducing the probability of killing the host before the virus is transmitted. What The expert is saying is that they have evolved to be about as efficient as they can be. The only thing that could change this would be if there was a mutation in the genes that control the susceptible hosts of the virus. - e.g. Chikungunya infecting Aedes Albopictus. Again this probability would be close to zero (1e-6 to 1e-7). Y drive concern is increased again because of the increased exposure time.

E17: Same as FT4-0101 (now FT401010)
Figure 6.242: Given that a replicating transformed virus has been created, what is the probability that regions of the mosquito genome flanking the construct stay linked to the transgene and improve the virus’ fitness?
E8: The expert can’t answer this question but see Jess’ notes on microRNA as one of three type of RNA interference mechanisms, and the need to scan for micro (mi)RNA signatures in flanking regions of the construct. Jake Tu at Virginia Tech has been cataloguing A. gambiae and Drosophila

E17: Same as FT4-0102 (now FT401011)
Figure 6.243: Given that a replicating transformed virus has been created, what is the probability that the construct spreads because it is linked to a region of the viral genome that is increasing in frequency by selection (i.e., hitch-hiking)?
E8: Can’t see how this would happen, would say that this probability is close to zero (1e-6 to 1e-7).

E17: Same as FT4-0103 (now FT40102)
All experts: 244 of 352 EXw = 0

Figure 6.244: Homing
E17: Same number and same logic as for prokaryotes
Figure 6.245: Given that a replicating transformed virus has been created, what is the probability that the I-PpoI construct mutates to recognise a site in the viral genome?
E6: Enzymatic activity of I-Ppol and site.

E8: This is pertinent only for DNA viruses and RNA viruses with DNA intermediates. The expert can't answer this at the moment. Y drive concern increased because exposure time increased.
Figure 6.246: Given that the I-Ppol protein is being expressed, what is the probability that the virus is a DNA virus?
E8: The expert thinks this would be a high probability because expression is much more likely in DNA viruses and you have much more opportunity for places where the gene could go and function.
Figure 6.247: Given that the viral-borne I-PpoI protein is being expressed in the virus’ host cell, what is the probability that HEG+ and HEG- viral genomes co-infect the same host cell?
E6: It depends on relative abundance of two virus types. And time of exposure. If for example HEG- virus is present infection by HEG+ virus may be prevented due to superinfection resistance. Mechanism uncertain.

E8: There are papers showing co-infection in the same cell but this probability is much lower than co-infection in the organism. Co-infection is relatively hard in cell culture, you have to ensure high multiplicity of infection for it to occur. Normally we dont see too many instances of this in nature, plus it has to happen immediately otherwise you might get resistance via super-infection. Y drives concern because there would be more opportunity for co-infection to occur.
Figure 6.248: Given that the I-PpoI construct has inserted into a recognition site in the viral genome, what is the probability that I-PpoI protein is expressed in the virus' host cell?
E8: (Note: DNA viruses include RNA viruses with DNA intermediate) The expert thinks there is a high chance of this because this depends on promoters that are associated with the virus and these could override the beta-tubulin promoter, same for retroviruses. For RNA virus the gene would have to be integrated in just the right way that it continues its open reading frame. Y drives concern because of increased exposure time.
Figure 6.249: Given that the I-PpoI construct has inserted into a recognition site in the viral genome, what is the probability that I-PpoI protein is expressed in the virus' host cell?
E8: The expert thinks there is a high chance of this because this depends on promoters that are associated with the virus and these could override the beta-tubulin promoter, same for retroviruses. For RNA virus the gene would have to be integrated in just the right way that it continues its open reading frame.
Figure 6.250: Given that GM males carrying the construct are viable, what is the probability that the construct allows some fertile males (not all are sterilised)?
E1: When you begin to move the construct into natural populations, some selection of mutations in target site cannot be ruled out. Males with some fertility would be highly favoured. The prospect of some variance on the target site is reasonable to consider and partial or full fertility is not to be discounted. There may be some advantages for dismantling some of the DNA via mutation which diminishes effect of construct. The chance of a single mutation may be on the order of 1e-5, and given scope of insectary production, it is a reasonable event to consider. There are different process for repair that retain homogeneity within DNA repeats. Could be altered if copies that are resistant to HEG protein appeared through mutation. This could possibly lead to replacement of susceptible rDNA target sites with the resistant mutation. If copies are available on the Y chromosome and accumulate there, they might jump start the replacement of susceptible rDNA with resistance on the X chromosome. Target of mutation about 100bp that could result in partial or complete blockage of sterility. This possibility has to be evaluated in large scale trials, or theoretically if these rates are already known. Given above, order of magnitude of around 1e-7 individuals that might get such a mutation, which is within scope of insectary production, and probability is non-trivial around 1e-5. The likelihood that an insectary population produces one such individual over, say a 6 month period, is relatively high, and this could be extended by mixing with WT post release.

E4: There are various mechanisms here under the heading of failure modes that lead to complete penetrance and failure modes that lead to incomplete penetrance

E5: There are various mechanisms here under the heading of failure modes that lead to complete penetrance and failure modes that lead to incomplete penetrance

E9: The expert thinks that 1-20% is possible here

E11: Should be some data on this from Austin’s group. They cross WT males with colony heterozygous females. The construct does not affect female heterozygotes. Construct expressed during spermatogenesis because of beta-tubulin promoter. If X carrying sperm, X chromosome gets chopped up by HEG protein.

E13: Can not be estimated by expert opinion; should be able to get these numbers from the two published research papers.

E14: Ok

E17: See comments under FT3-02000 (now FT51010000). Same as FT3-02000 (now FT51010000).

E18: Low (1E-5, 1E-4)0.8.

E19: (1E-5, 1E-2)0.75

E21: Same as FT5-2010 (now NA) (1E-8, 0.01)0.9.

E23: Same as FT5-2000 (now NA)
Figure 6.251: Given that some GM males carrying the construct are viable and fertile, what is the probability that the GM male mosquito has higher fitness than a wild type male?
E1: Presumption here is that the construct is now non-functional, it would have small or no effect on fitness and hence be neutral. If it happens, enzyme selection could be near neutral or weakly positive or negative in selection, giving downwardly convexed probability distribution (as in FT5-000 (now FT50000)). If it happens at target, then rDNA gene might come under selection and in next generation become separated from autosomal genes, which would have a positive benefit to carrier. Homogeneity across taxa indicates cost of mutation, but homogeneity could also derive from processes that simply reduce heterogeneity within a gene pool. Likely GM less fit than WT, so we can imagine distribution is skewed. As a heuristic we will 1) first estimate relative fitness curve normalized to WT genotype, and then 2) consider right tail, where GM might exceed 1.

E4: Answers here depend on the assay used to measure fitness (not defined here) and also assumes that offspring from GM males are also fertile (failure of construct complete penetrance) - i.e. assumes that the construct failure is permanent (not active in subsequent generations)

E5: Answers here depend on the assay used to measure fitness (not defined here) and also assumes that offspring from GM males are also fertile (failure of construct complete penetrance) - i.e. assumes that the construct failure is permanent (not active in subsequent generations)

E9: The expert thinks this is going to be pretty rare 1e-7 to 1e-8, not having very much offspring is pretty high fitness cost so it would have to something else that was driving the selection and its hard to see what this is.

E11: The whole notion of the construct is that it shreds all X-chromosome bearing sperm, unless whole system is failing, but even then it would not be more fit, thus its one of those remote possibilities. Would be similar to non-target eukaryote FT3-11001 (now FT31002). This is a very remote possibility. Same response as to eukaryotic selection FT3-11001 (now FT31002). No basis for a different response, no basis to expect different response of construct in non-target eukaryotic vs mosquito.

E13: Can not be estimated by expert opinion; should be able to get these numbers from the two published research papers.

E14: Ok

E17: See comments under FT3-02002 (now FT51010001). Same as FT3-02002 (now FT51010001)

E18: 50/50. (0.4, 0.6)0.8

E19: (1E-9, 1E-6)0.8

E21: Not only the same fitness but larger, so different from observed in Drift (1E-12, 2E-7)0.9.

E23: There is no molecular mechanism that will result in such improvement in fitness. Assume it is quite unlikely to happen (1E-6, 1E-2)0.7.
Figure 6.252: Given that GM males carrying the construct are viable, what is the probability that the construct allows some fertile males (not all are sterilised)?
E13: Can not be estimated by expert opinion; should be able to get these numbers from the two published research papers.

E14: Dependent

E17: See comments under FT3-02000 (now FT51010000). Same as FT3-02000 (now FT51010000)

E18: Dependent

E19: Dependent

E21: Same as FT5-2010 (now NA) (1E-8, 0.01)0.9.

E23: Same as FT5-2000 (now NA)
Figure 6.253: Given that GM females carrying the construct are viable and fertile, what is the probability that the GM female mosquito has higher fitness than wild type female?
E1: Presumes that female has suffered 0.2 to 0.3 reduction in relative fitness, when actually there was no expression of construct as we understand it (i.e., unplanned or unexpected). Starting with this reduction, the question is if mutation renders the enzyme inactive this might restore some of fitness loss and thus have greater chance of shifting/recovering fitness, because we are not starting at zero fitness as in males, but only about 0.7 for females. Final elicited values are very speculative regarding future fate of mutated construct.

E9: The expert thinks this would be rare but not as rare as the males, 1e-5 to 1e-6 because there isn’t the automatic fitness cost that occurs in the males.

E11: Would be same as for male in FT5-0001 (now FT500001). This is a very remote possibility. Same response as to eukaryotic selection FT5-0001 (now FT500001). No basis for a different response, no basis to expect different response of construct in sexes.

E13: Can not be estimated by expert opinion; should be able to get these numbers from the two published research papers.

E14: Same answer as for males. (Same as FT5-0001 (now FT500001))

E17: Same for males and females. Use same probability. Same as FT3-02002 (now FT51010001)

E18: 50/50. (0.4, 0.6)0.8

E19: (1E-10, 1E-6)0.8

E21: (1E-12, 2E-7)0.8

E23: There is no molecular mechanism that will result in such improvement in fitness. Assume it is quite unlikely to happen. Same as FT5-0001 (now FT500001)
Figure 6.254: Given that all GM males carrying the construct are sterile, what is the probability that the construct mutates to increases female fitness sufficiently to compensate for the male sterility?
E1: I think it is exceedingly low. A female has to be as much as twice as fit to compensate, because males are zero, 1/2 or 1/4 of her fitness will be reduced and the mutation is differentially not affecting males but favouring females. Must be rare; in order of less than 1e-7. Might be a way to do lab trials for single mutations and see if there is any improvement in fitness in some assay.

E4: Has to mutate (10E-4 to 10E-11 per generation per gene depending on mechanism) and then increase female fitness (defined as the number of F2 from parental female), bottleneck of WT in the laboratory occurs quickly so fitness depends to some extent on when release occurs, but there is still a "hybrid-vigour" chance at the first cross in the wild.

E5: Has to mutate (10E-4 to 10E-11 per generation per gene depending on mechanism) and then increase female fitness (defined as the number of F2 from parental female), bottleneck of WT in the laboratory occurs quickly so fitness depends to some extent on when release occurs, but there is still a "hybrid-vigour" chance at the first cross in the wild.

E9: Very rare The expert thinks 1e-8 and 1e-9 - would take an enormous selective advantage to compensate for the males.

E11: Very remote possibility, would be same as FT5-0011 (now FT500011). 0.5 chance of being between 1 in a million and 1 in a billion, same as for GM male fitness greater in FT5 other branch.

E14: ok

E17: Would need to virtually double female fitness to compensate for all males being sterile - e.g. double survival or fecundity. Very hard to see how this can happen. Pr = 0.

E18: Vanishingly small. (1E-6, 1E-5)0.9

E19: Low. (1E-10, 1E-6)0.8

E21: Same as FT5-210 (now NA) (1E-8, 0.01)0.9

E23: There is no molecular mechanism that will result in such improvement in fitness. Assume it is quite unlikely to happen (1E-9,1E-5)0.7.
Figure 6.255: Given that offspring carrying the autosomal construct are viable, what is the probability that I-PpoI construct moves to the Y chromosome?
E1: For some members of the population of A. gambiae complex, they have rDNA on Y chromosome and their presence will increase probability of jump from autosomal chromosome to Y. The Y-rDNA frequencies near the insectaries would affect level of this risk. Does not think he has sufficient knowledge to answer this question properly.

E9: Y drive probability here is obviously 1.

E11: In the evolutionary history of Anopheles, there are examples of single genes moving around, reordered gene places. Moving onto Y chromosome however less common. Rare event. One in a thousand to million.

E14: Should be able to calculate this from proximate size of Y chromosome and frequency of I-Ppol recognition site. Would be partially modified by the presence of ribosomal gene. There has been indication that the ribosomal gene can carry on the Y by Mark Benedict's group, who found preliminary evidence for this.

E18: Same as VGT FT3-02110 (now FT5101100)

E19: Same FT3-02110 (now FT5101100)

E20: Same as FT3-02110 (now FT5101100) (1E-6, 1E-2)0.8

E21: Same as FT3 VGT (1E-9, 1E-7)0.8.

E23: We know that recombination between autosomal chromosome and the Y chromosome has not been observed. Therefore, the probability is very low (1E-5,1E-3)0.7.
Figure 6.256: Given that the I-Ppol construct inserted on the Y chromosome, what is the probability that it produces sufficient enzyme that is able to cleave rDNA?
E1: Does not feel that he can answer this question.

E11: No reason to think that there is a very low probability. Construct has all machinery for producing active enzyme. Could be more difficult on Y chromosome because not a lot of expressed genes but examples exist. Tendency to lose functionality or activity after moving from native site, but don’t necessarily lose all activity.

E14: Not sure.

E18: Same as VGT FT3-02111 (now FT5101101)

E19: Same as FT3-02111 (now FT5101101)

E20: Same as FT3-02111 (now FT5101101) (0.001, 0.9)0.9. In response to NW comment about BE FT5-124 (now FT50104) being redundant: It can produce sufficient enzyme to cut rDNA, but if its not germline specific then it is going to have problems in the adult - it will produce it in all cells (cleaving its rDNA locus). It needs to produce sufficient, and in a restrictive fashion so that this only happens in the germline. So keep both enzyme and maintains germline expression.

E21: (1E-7, 1E-4)0.9

E23: Given that the construct has remained intact, and the promoter is still expressed (i.e. ignoring meiotic silencing of sexual chromosomes) (0.01, 0.5)0.7.
Figure 6.257: Given that the I-Ppol construct inserted on the Y chromosome and produces an enzyme that is able to cleave rDNA, what is the probability that this rDNA is predominantly on the X chromosome?
E1: Does not feel that he can answer this question.

E9: $Pr = 1$

E11: In A. gambiae the rDNA is known to reside on X chromosome. The expert did research 20 yrs ago that all or most of the rDNA is on the X chromosome. Usually repeated 500 copies in genome. Could be a few copies elsewhere.

E14: Close to 1. Some evidence that ribosomal on Y, so close to 1 rather than 1. For purpose here $Pr = 1$.

E18: Same as VGT FT3-02112 (now FT5101102)

E19: $Pr = 1$

E20: This is very high as in A. gambiae generally it is $(0.2, 0.95)0.9$

E21: $(0.8, 0.999)0.95$

E23: Assuming the spread is in the same form of release, this probability would be high $(0.6, 0.99)0.8$. 
Figure 6.258: Given that the I-PpoI construct inserted on the Y chromosome and produces an enzyme that is able to cleave rDNA that is predominantly on the X chromosome, what is the probability that it does not cause male sterility?
E1: Does not feel that he can answer this question.

E11: Low probability. It’s moved to Y chromosome and can cleave rDNA on X chromosome, which is the recipe for creating male sterility. It’s possible that there’s a lower level of function being delivered because the HEG protein doesn’t last as long.

E14: Same as leakage effect before. Same as FT5-0000 (now FT500000)

E18: Same as VGT FT3-02113 (now FT5101103)

E19: Same as FT3-02113 (now FT5101103). It causes sterility, but there could be some escapers.

E21: (1E-9, 1E-5)0.9

E23: We know that male sterility is related to the stability and activity of the enzyme in the fertilized embryo, so not related to the factors mentioned here (except the presence of rDNA on the X). So, these factors should not affect the sterility of the male if the level of expression is maintained (if meiotic silencing is not an issue) (0.6,0.99)0.8.
Figure 6.259: Given that the I-Ppol construct inserted on the Y chromosome, what is the probability that it maintain germline expression
E1: Does not feel that he can answer this question.

E9: Y drive probability here is obviously 1.

E11: Probably less than 1, because Y chromosome is not always reliable in expressing constructs, because it is a heterochromatically rich region.

E14: All dependent on before FT5-102 (now FT5011002)

E18: Same as VGT FT3-02114 (now FT5101104)

E19: Only specificity of expression (0.01, 0.8)0.8

E21: The expert thinks this is redundant

E23: Considering meiotic silencing of sex chromosomes, and the Beta-tubulin promoter is predominantly activated in male meiosis (0.01,0.4)0.8.
Figure 6.260: Given an I-Ppol construct inserted on the Y chromosome that is able to cleave rDNA predominantly on the X chromosome without causing sterility, what is the probability that the driving forces are higher than the fitness costs?
E1: The fitness cost depends on the sex ratio, the lower the female ratio the greater the cost of male-only offspring. We are interested in interruption of transmission and not elimination of species. Not very clear here if this strategy would collapse species to extinction (local or global) or if at a minimum equilibrium disease burden would be reduced. Driving force could be twice WT, and if there is cost around 20%, then advantage would be around 80%. But if there was a distorted sex ratio, with most being males, virtually all of the males would not mate. The ratio of WT to GM males is also critical, and if its is swamped by this, then once the fraction of females is below 20% then you would have equal selection for and against them. However, above is perhaps out of context of question, which is simply spread. This could be actually quite high in short term. Given cost of about 30% and 50% increase in transmissibility, the actual probability has a median of about 25%, with quantiles of 10% and 40%. Question becomes what is the probability that the fitness curve is above 1.0 (break even point).

E11: If all of the above is satisfied, then probability that drive is greater than fitness cost, has probability greater than 50%. If the enzyme is functioning on the Y site and is cleaving rDNA on X chromosome, even if it doesn't cleave all rDNA by definition creating a burden (fitness cost) on the mosquito that bears the construct. Given all benefits of a stage 3-like driving mechanism then this will be a non-negligible probability.

E14: Not sure.

E19: Same as FT3-02115 (now FT5101105)

E21: (2E-4, 0.25)0.90

E23: If all these factors are happening, then I would imagine the Y-driving system working. Probably result in male-biased GM progeny, which are going to have a really high driving force (0.5, 0.99)0.8.
Figure 6.261: Given that GM mosquitoes carrying the construct are viable, what is the probability that their genome contains an alternative site that is recognised by I-PpoI?
E1: We know that this is already observed without mutation. Not sure how common these entities are where they are found in the A. gambiae complex in the three locations in Africa. Does not want to answer this question without relevant information which is specific to local populations of A. gambiae. Empirical studies that scan DNA could focus on permutation of variants and allowing 1 or 2 bp to vary.

E4: You can ask what is the probability in a random genome sequence that the HEG recognition site will occur. But we actually know what the genome sequence is (we will soon have 14 Anopheles sp genomes sequenced, we currently have 100s of A. gambiae genomes done) so we can quantify the probability of the existence of an alternative site, and then we can allow for slight mismatches such as 13 out of 15. Would be better to calculate this number this way rather than elicit it. The mosquito genome is very polymorphic though. Would be better to data mine than elicit this

E5: You can ask what is the probability in a random genome sequence that the HEG recognition site will occur. But we actually know what the genome sequence is (we will soon have 14 Anopheles sp genomes sequenced, we currently have 100s of A. gambiae genomes done) so we can quantify the probability of the existence of an alternative site, and then we can allow for slight mismatches such as 13 out of 15. Would be better to calculate this number this way rather than elicit it. The mosquito genome is very polymorphic though. Would be better to data mine than elicit this

E9: They should predict this computationally and experimentally confirm if it is cutting somewhere else.

E11: There should be data for this. Mosquito genome is 260 million nucleotides long and the target site 15 base pairs long. For each base pair there is a 1 in 4 chance of having the right nucleotide so around 0.25 raised to the power 15 for the target site sequence. But target site is not exact (Figure 3 in background material). Somewhere in laboratory data there must be information on degree of degeneracy. Since target site is degenerate, this suggests that a target site(s) other than that in the rDNA could well be present elsewhere in genome. Reluctant to assign a number to this, but reasonably confident that it is not extremely remote, and should be addressed.

E14: Should be able to calculate this.

E18: Very low (1E-6, 1E-5)0.8.

E19: Know there are some other sites recognised by I-Ppol by cleavage at those sites compared to proper target site. Pr=1 that there are other sites, but this doesn’t reflect that that they will not be cleaved as efficiently, so lower probability (1E-4, 1E-2)0.75

E20: Same as FT3-0210000 (now FT510111000)

E21: We know the Anopheles genome a bit better than in VGT. So, it needs to be less likely (1E-9, 1E-4)0.9.

E23: This is something that can be calculated. So, there will be an actual number.
Figure 6.262: Given that I-PpoI recognises an alternative site in the genome, what is the probability that it moves into this site?
E1: Does not feel that he can answer this question.

E4: Can be done empirically as before

E5: Can be done empirically as before

E9: The expert thinks this will be a low probability. Enzyme can cut and make the break at the recognition site but why would it home? The breaks would typically repair via non-homologous end-joining (this could cycle until you get an error in this repair and then the site is no longer a recognition site). Homing via homologous repair (should not occur because? ) the construct should not be a template for homologous repair. This is the killer step 1e-11 to 1e-13 - The expert can’t see how it would just copy the whole thing back in perfectly. Use the same probability FT3-021001 (now FT510111001)

E11: Constitutes accidental homing or homing not guided by homologous recombination by new target site flanking sequence and sequence in construct. Likely a remote possibility.

E14: In piggyBac - no remobilisation of pBac vectors. Struggle to put a value, not zero as such. Team could screen whole AG datasets to identify likely transposase. Should be able to infer from that. Calculate this.

E18: \((1E-4, 1E-2)^{0.5}\)

E19: Same as FT3-021001 (now FT510111001)

E20: Same as FT3-021001 (now FT510111001)

E21: \((1E-12, 1E-8)^{0.9}\)

E23: Same as FT3-112001 (now FT312001)
Figure 6.263: Given that I-Ppol has moved into an alternative site, what is the probability that it maintains its germline expression?
E1: Does not feel that he can answer this question.
E4: Can be done empirically as before
E5: Can be done empirically as before
E9: Use the same probability as FT3-021002 (now FT51011002)
E11: If it actually moves intact, it should maintain its germline expression with high probability; effect of new location could compromise it however.
E14: If takes promoter with it relatively high if don’t get dissociation of beta tubulin promoter.
E18: Likelihood that I-Ppol is intact and functional is an order of magnitude lower than probability that it moves into the site (1E-5, 1E-3)0.7.
E19: Very high in A. Gambiae (0.5, 0.99)0.9
E20: (0.9, 0.99)0.9
E21: (0.1, 0.9)0.86
E23: Since there are two autosomal and two sex chromosomes, we assume there would be a 50:50 chance of being active depending on which type of chromosome it integrates in to. Remembering that sex chromosomes are silenced during meiosis (0.3, 0.7)0.8.
Figure 6.264: Negative fitness effects less than driving
E4: Not necessary to do this because events above would be very rare: 1e-double digits. Sufficient to say Pr = 1

E11: Overall, would guess that this is very small, and small values in FT5-100 (now FT501100), FT5-101 (now FT5011001) and FT5-102 (now FT5011002) would also be small thus FT5-10 (now FT50110) will be minor.
Figure 6.265: Given that I-Ppol has moved into an alternative recognition site and maintains germline expression, what is the probability that it does not cleave ribosomal repeats?
E1: Does not feel that he can answer this question.

E9: The expert is not sure where to go with this one because he thinks its very likely to decrease the efficiency of cutting the site but not sure by how much. Structural biologists might know the answer to this question. The expert knows people in UNC who might be able to answer this.

E11: Probability is low to zero. Target site cleavage is not dependent on the HEG insertion site therefore cleavage of ribosomal target should be retained even in HEG construct that moves to a new location. This question seems irrelevant. It should maintain activity in this situation. Not sure why this question is being asked. It seems like this question is a statement that is redundant to FT5-100 (now FT501100) and FT5-101 (now FT5011001) and FT5-102 (now FT5011002). It acknowledges that these events have happened.

E14: Should be able to calculate this from FT5-1000 (now FT5011000). If you know size of ribosomal repeat, you should be able to infer how likely it is not in a ribosomal repeat. Calculate this.

E20: The two boxes below replace this as it was split for mutated and unmutated I-Ppol. The rest of this sub branch will be replicated, but same values, so only this box needs splitting.
Figure 6.266: Given that I-Ppol has moved into an alternative recognition site and maintains its germline expression, what is the probability that the recognition site is in an Intron?
E1: Does not feel that he can answer this question.

E9: Again ICL should be able to calculate based on what proportion of the genome is intronic.

E11: Has to do with frequency of introns with respect to the rest of the genome, should be data on this. Best (crude) estimate would be to add up all the introns in a genome and divide it by genome size, assuming random likelihood of insertion.

E14: Calculate this. Same concept as FT5-1031 (now FT50110030)

E19: (1E-5, 1E-2)0.75. Comment amended following feedback report: Up to 10-20% of the mosquito genome is made of introns.

E20: See workings for FT3-02100410 (now FT51011100310) (0.05, 0.6)0.8

E21: (1E-6, 0.001)0.7

E23: Would depend on the proportion of intronic genomic DNA in the genome, and the negative fitness effect this insertion might cause (disruption of an essential gene). Same as FT3-11200310 (now FT312000310).
Figure 6.267: Given that I-PpoI has moved into an alternative recognition site and maintains its germline expression, what is the probability that the recognition site is in a non-essential region of the genome?
E1: Does not feel that he can answer this question.

E9: Use the same probability as FT3-02100411 (now FT51011100311)

E11: Essentially same as FT5-10300 (now FT501100310). Add up an estimate of non-essential inter-genic or heterochromatic regions and divide by genome length. The genome is annotated such that functional parts and coding regions are generally identifiable. Regulatory sequences that regulate transcription can also be identified to some extent. A lot of DNA is just a graveyard for transposons. Some with low-level function that tolerates mutations.

E14: Calculate this. Same concept as FT5-1031 (now FT50110030)

E18: Same as FT3-02100411 (now FT51011100311)

E19: (1E-3, 0.3)0.7

E20: See response to FT3-02100411 (now FT51011100311) (0.2, 0.8)0.7.

E21: (0.5, 0.9)0.851

E23: Same as FT3-11200311 (now FT31200311)
Figure 6.268: Given that I-PpoI has moved into an alternative recognition site and maintains its germline expression, what is the probability that the negative fitness effects are recessive?
E1: Does not feel that he can answer this question.

E9: The expert can’t answer this one but its probably quite high

E11: This is what happens when a random bit of DNA is inserted into organism. Maybe data from Drosophila would be relevant here to get a crude estimate. It’d likely be a modest probability. Most regions of the genome have some function and an insertion of a novel piece of DNA would have some possibility of detrimental impact on function but most regions of DNA have two (parental maternal) copies that can assume some functionality, and insertion would only affect one of two, so generally outcome would be recessive. This middle pathway is likely investigating something that is very unlikely given flanking sub trees (FT5-100 (now FT501100), -101, -102). But does not want to put a number on it.

E14: Not sure they can be recessive. Not dealing with an allelic system. Here we have insertion of completely novel section into genome. If there are fitness effects they will not be recessive. The question is not whether it is recessive, but whether it does have any fitness effect or effects are negligible (i.e., it could insert in junk DNA region with no likely effect). Does not think this should be one of the options. Really what we after here is - don’t disrupt normal function - this is captured in the two previous boxes, FT5-10300 (now FT501100310) and FT5-10301 (now FT501100311). Probability of inserting into gene, and then probability of inserting phase within that gene. Inserting into gene - disrupts product from one allele but not the other. Recessively and dominance not answer to fixed rates so not sure how to quantify this.

E18: Same as FT3-02100412 (now FT51011100312)

E19: \((1E-3, 0.1)0.6\)

E20: Same as FT3-02100412 (now FT51011100312) \((0.6, 0.99)0.8\)

E21: \((0.6, 0.9)0.8\)

E23: Same as FT3-11200312 (now FT31200312)
Figure 6.269: Given that GM mosquitoes carrying the construct are viable, what is the probability that I-Ppol mutates and then recognises an alternative site?
E1: Not sure how recognition is brought about; i.e. is it a matching template? Any mutation that is not leading to degeneracy and leads to recognition is required. Starting with assumption that 15 bp match, and mutation occurs in this location (single hit in this location) puts it in a probability of 1e-6. Not sure if every bp hit would change, or if adjacent sites would bind it, but likely not far off from 1e-6. This is not trivial given FT5 scenario of 10,000 mosquitoes, there 1) could be change in target site and 2) that it is also found in existing genome. Could use data from DNA scan mentioned in FT5-1000 (now FT5011000) comment to assess this likelihood. But if we assume that this alternative site exists in the genome, then we will elicit the chance of a mutation leading to change in target site being recognized by HEG protein. Given that there are 15 potential locations, and if any are hit in 1 out of three types, then it is roughly 15 times 1e-6 divided by 3 = 5e-6

E4: Andy Scharenberg’ group are mutating the HEG to see if it recognises an alternative site. Again it would be better to use their results to establish this probability. The experimental results are conservative because they are done under ideal conditions and we don’t expect mutations in nature to occur in this manner.

E5: Andy Scharenberg’ group are mutating the HEG to see if it recognises an alternative site. Again it would be better to use their results to establish this probability. The experimental results are conservative because they are done under ideal conditions and we don’t expect mutations in nature to occur in this manner.

E9: Use the same probability as FT3-0210001 (now FT510111020)

E11: Likely very remote, mutations at a particular site are in order of 1e-8 per generation, then it has to be recognized as an alternative site.

E14: Given no constraints as not active HEG - this could be calculated - no constraint function within AG - not in essential protein. So no mutations in I-Ppol will cause fitness effects. So given whole genome sequence, assume one polymorphism. Mutation is quite high. Can work out probability of mutation in target recognition sequence. Calculate this.

E18: Very low (1E-6, 1E-5)0.8

E19: Same as FT3-0210001 (now FT510111020)

E20: Same as FT3-0210001 (now FT510111020)

E21: (1E-12, 1E-8)0.9

E23: Same as FT3-1120001 (now FT312010), which was to speak to the Seattle crowd. No probability elicited.
Figure 6.270: Given that mutated I-Ppol has moved into an alternative recognition site and maintains germline expression, what is the probability that it does not cleave ribosomal repeats?
E18: Sam as FT3-0210040a (now FT5101110230)
E19: Upper bound 10%. Depends if it mutates completely. Lower bound 1E-4 (1E-4, 0.1)0.75
E20: Same as FT3-0210040a (now FT5101110230)
E21: (0.3, 0.7)0.8
E23: Because it has mutated to recognize a new site, in most of the cases, it would loose or partially lose its ability to continue to recognize the rDNA (0.4, 0.8)0.7.
Figure 6.271: Given that GM mosquitoes carrying the construct are viable, what is the probability that the I-PpoI construct inserts into a recognition site in a ribosomal repeat?
E1: Cannot speculate how likely it is, as there are many missing pieces of information. What are the cascade of events that lead to this? Requires more knowledge. A question here is that these are selfish elements that insert into a host, and initially need something that aids recognition and insertion. Does not feel that he can answer this question.

E4: This is non-homologous repair with the site inserted? There is data available for this as well, for Drosophila. Non-homology integration frequencies are known to be very low in Drosophila. The expert Golic would have this data. The expert has 10 years of experiments with mosquitoes and has never seen it.

E5: This is non-homologous repair with the site inserted? There is data available for this as well, for Drosophila. Non-homology integration frequencies are known to be very low in Drosophila. The expert Golic would have this data. The expert has 10 years of experiments with mosquitoes and has never seen it.

E9: Use the same probability FT3-021001 (now FT510111001)

E11: Literature to date shows that I-PpoI inserted into genome produces endonuclease that cuts rDNA but always described as a shredding process. If the construct is inserted into the rDNA and produces endonucleases that cleaves rDNA, the genome will try to repair via homologous recombination patterned after the homologous chromosome. This leads to propagation at a greater rate than half gametic ratio (non-Medellian ratio driving mechanism). This should be rare because there are 500 copies of the recognition site and anything that cuts any of them probably will shred all of them. No motivation for construct moving into rDNA. Also no rDNA target included within the I-PpoI construct that would promote homologous recombination and insertion into rDNA site.

E14: I-PpoI is inserting into 28s, and there are several hundred copies of 28s in genome. So assuming mobilised, then there is a high probability of moving into it. Assuming retaining same recognition site. 0.9(0.9,0.95)

E18: Same as VGT FT3-021010 (now FT51011110)

E19: Same as FT3-021010 (now FT51011110)

E20: Same as FT3-021010 (now FT51011110) (1E-8, 1E-3)0.6

E21: (1E-12, 1E-8)0.9

E23: For this event to happen, it would have to be through ectopic homing, which to happen the enzyme needs to cleave (but we assume that), and the construct must have a homologous sequence to that flanking the target site. This is something that can be tested. So, the accidental probability of something like this happening is quite low (1E-3, 1E-2)0.8.
Figure 6.272: Given that I-Ppol inserted into a recognition site on a ribosomal repeat, what is the probability that it maintains its germline expression.
E1: As long as it is connected to the specific tubulin promoter (and mediated by piggyBac), it is likely that it will maintain germline expression. Does not feel that he can answer this question.

E4: KentGolic for data?

E5: KentGolic for data?

E9: Use the same probability as FT3-021002 (now FT510111002)

E11: While it could conceivably maintain germline expression if stable at insertion site, the HEG expression from construct site would favour cleavage at all rDNA sites and excise construct and probably be lethal to construct-carrying gametes. Different processes at work from above question but would give similar probabilities to this rare event.

E14: Same as FT5-102 (now FT5011002)

E18: Same as VGT FT3-021011 (now FT51011111)

E19: Very high in A. gambiae (0.5, 0.99)0.9

E20: Incredibly high (0.8, 0.99)0.95

E21: (0.1, 0.9)0.86

E23: Same as FT5-124 (now FT50104). There is evidence that integration of similar constructs into the X chromosomes were completely silenced (Galizi et al. 2014. Nature Communications).
Figure 6.273: Negative fitness effects less than driving
E4: Not necessary to do this because events above would be very rare: 1e-double digits. Sufficient to say Pr = 1

E9: Copy all basic events under from FT3-021013 (now FT51011113)
Figure 6.274: Given that I-Ppol inserted into a ribosomal repeat and maintains its germline expression, what is the probability that its cleavage rate at the rDNA locus is sufficiently low that reduced male fitness or reduced X chromosome transmission do not preclude spread through the population, but still is active enough for homing to occur?
E1: This is what the I-Ppol is designed to do, once it is in rDNA it will be involved in homing and will not affect much the integrity of the rDNA and thus move through population, as per phase three (Y-drive) of project. No indication here that it would degrade. To not occur would require mutation in recognition or target site of HEG that would diminish homing or render enzymes inactive. Similar to “Selection” branch of FT5-0 (now FT500). Does not feel that he can answer this question specifically.

E9: Same comment as FT3-0210130 (now FT510111130). The expert thinks this will be the same probability but did not offer a number.

E11: About 500 I-Ppol target cleavage sites on the typical X chromosome. Given that all of these sites contain potential cleavage targets and that cleavage of multiple targets leads to X chromosome shredding, it seems highly unlikely that the homing endonuclease cleavage activity would occur at a consistent and sufficiently low level to both promote homologous insertion/propagation and avoid chromosome shredding. Must cleave at a low enough rate to allow for chromosome repair. 50% chance of being between 1 billion and 1 million.

E14: Cannot venture a guess on this one.

E18: Same as VGT FT3-0210130 (now FT510111130)
E19: Same as FT3-0210130 (now FT510111130)
E20: Same as FT3-0210130 (now FT510111130) (1E-7, 1E-2)^0.7
E21: (1E-8, 1E-5)^0.85

E23: The rDNA is multicopy, therefore the target site occurs in multicopy, so it is possible to have homing of the construct without having a big effect on the fitness of the transgenic male. If the construct only homes to one or a few of the rDNA copies, there will still be other functional ones (0.3,0.8)^0.7.
Figure 6.275: HEG inserted in intron
E21: Elicited at the gate (1E-10, 1E-6)0.95.

E23: Assuming the construct is homing on the rDNA region, I would assume it is more likely that the construct would be inserted in the target site region, where the cleavage happened. Because the target site is in an intron (0.6, 0.9)0.7.
Figure 6.276: Given that I-Ppol inserted into a ribosomal repeat and maintains its germline expression, what is the probability that it inserted in a self-splicing intron?
E1: Does not feel that he can answer this question.

E9: Same comment as FT3-02101310 (now FT5101111310)

E11: I don’t think there are any self-splicing introns in mosquitoes. This suggests the probability is likely equal to zero. Some organisms have organelle genomes that have self-splicing introns, usually non-complex eukaryotic organisms (e.g., yeast) without machinery found in more complex organisms (the rDNA folds back on itself in some manner that splices itself out). Pretty sure they are not in mosquito rDNA.

E14: Should be able to calculate this.

E18: Same as VGT FT3-02101310 (now FT5101111310)

E19: Same as FT3-02101310 (now FT5101111310)
Figure 6.277: Given that I-Ppol inserted into a ribosomal repeat and maintains its germline expression, what is the probability that it inserted in a spliceosomal intron?
E1: Does not feel that he can answer this question.
E9: Same comment as FT3-02101311 (now FT5101111311)
E11: There may be spliceosomal introns in insects, but no knowledge in this area for mosquitoes.
E14: Should be able to calculate this.
E18: Same as VGT FT3-02101311 (now FT5101111311)
Figure 6.278: What is the probability that there are compatible species (i.e. species that could mate with GM insectary mosquitoes) in the vicinity of the insectary?
E4: Pr = 1
E5: Pr = 1
E13: A. Gambiae target is both M and S, and so non-target would be other spp. such as Arabiensis.
E14: Assuming these locations Pr=1.
E15: Geographic variation in West Africa, quite a lot of A. Arabiensis and A. coluzzi, so probably quite high. Whereas HF has worked in Tanzania there is only one other compatible species. (0.1,0.7)0.8
E17: Same as FT3-2123 (now FT51113)
E18: Pr=1. Indigenous species in the locales where the insectaries are.
E19: (0.4, 0.99)0.9
E20: Very high (0.2, 0.99)0.9
E21: Uncertainly reflects the variability between the insectaries and over the wet/dry season
Figure 6.279: Given that there are compatible species in the vicinity, what is the probability that hybrids will be formed, following a catastrophic release of all 10,000 mosquitoes?
E13: Suggest talking to another expert on this (e.g. The expert Lanzaro). Assume Coluzzi and Gambiae are one and thus both target species, so George’s answer here considers only Arabiensis. Have assumed target is both Gambiae and Coluzzi here, need to confirm with project what the target is- is it one or both? Inclination should be both AG and Coluzzi or risk assessment will be high as high chance of transmission to other species.


E17: Same as FT3-2124 (now FT51114)

E19: In nature less than 0.1% hybrid formation between compatible species. That is the median range. Upper bound, 0.005, lower bound 1E-4, 0.75

E21: Assuming 50% females and 50% males are escaping
Figure 6.280: Given that there are compatible species in the vicinity, what is the probability that Arabiensis hybrids will be formed with these species, following a catastrophic release of all 10,000 mosquitoes?
E4: Probability is very small for Arabiensis which is the only other species that The expert is aware of which forms hybrids with Gambiae in nature. There is evidence for hybrids with Arabiensis over evolutionary time scales (10,000 of years), people rarely record collections but The expert has never seen any. (Elicitation for here taken from FT3-02001 (now FT51010002)-Ara?)

E13: See above comments. The expert answered FT3-011 (now FT51001) on Arabiensis

E14: A. gambiae cross A. arabiensis hybrid males are not viable.

E15: 1% or less. The expert Tripet in a ASTMH 2003. Probability that one will form, not how many (0.2, 0.7)0.8.

E17: Answered for all species above

E20: (0.01, 0.5)0.8
Figure 6.281: Given that there are compatible species in the vicinity, what is the probability that Coluzzi hybrids will be formed, following a catastrophic release of all 10,000 mosquitoes?
E4: There are 6 or 7 species in the complex, and there is rarely any hybridisation with any of these species other than Coluzzi. With Coluzzi there is a temporal effect between years (not a seasonal effect) and it varies. 0% to 12% of the population are F1 hybrids in Mali and Burkina. In Kenya however there is no hybridisation because Coluzzi are not in Kenya.

E13: Presumes this is certain, i.e., Pr essentially equate to 1, but this distinction is confusing, given what is the target species. If A. gambiae it will be same species across Africa, but if Coluzzi then only in West Africa as it is not in East Africa. In either case, the chance is very high, but increased uncertainty as there is a difference in rate of hybridization from east to west Africa. In West Africa have to rear Coluzzi as better vector. If have AG what is chance that transmitted to Coluzzi; if Coluzzi what is chance transmitted to AG. Both high probs. Hybridisation is more from east to west Africa so reflected in range of answer.

E14: Can calculate this also.

E15: Data out there, loads of experiments.

E17: Answered for all species above

E18: If release Coluzzi, the probability of forming a hybrid with Coluzzi is high, about 90%, but not sure on rates of hybridisation with Coluzzi and Arabiensis (if Coluzzi released). Left unanswered.

E20: The probability that we will see hybrids between Coluzzi and Gambiae are about 1 in 1000, or less than 1 in a 100. For at least one hybrid we have 1-(99/100) raised to the power 10000 which is close to 1. (0.2, 0.99)0.8
Figure 6.282: Given that hybrids between the GM insectary mosquitoes and compatible species have been formed, what is the probability that the construct will be transmitted to the F1 offspring?
E4: Pr = 1
E5: Pr = 1

E13: Likely high probability, but suggests taking it to a specialist, i.e. Maureen Coetzee, South Africa

E14: This will be biased by Coluzzi / Arabiensis. There are no post-zygotic mating barriers between A. gambiae and A. coluzzi, so transmission should be the same. With A. gambiae cross A. arabiensis hybrids males are non-viable so potential bias here.

E17: Same as FT3-2125 (now FT51115)

E18: Given male sterilising construct, probability is extremely low. (1E-3, 1E-5)0.99. The expert wants only 0.5% above 1 in 1000 for upper bound.

E19: If they mate, the probability that the construct will be transmitted. Once the hybrid is formed. Upper bound 50%, lower 1E-3, 0.75

E20: What is the probability that the hybrids have the construct. (Confusing use here of F1 and hybrids here, but they are they same thing.)
Figure 6.283: Given that Arabiensis hybrids between the GM insectary mosquitoes and compatible species have been formed, what is the probability that the construct will be transmitted to the F1 offspring?
E14: See comment for FT3-012 (now FT51002)-Col above
E17: Answered for all species above
E20: (0.3, 0.99)0.9
Figure 6.284: Given that Coluzzi hybrids between the GM insectary mosquitoes and compatible species have been formed, what is the probability that the construct will be transmitted to the F1 offspring?
E14: This can be worked out empirically. If you know transmission in colony can do this for Coluzzi and then for Arabiensis.

E17: Answered for all species above

E20: Very high probability that the hybrids are fertile. (0.5, 0.99)0.9
Figure 6.285: Given that the construct has been transmitted to the F1 offspring, what is the probability that they are viable?
E17: Same as FT3-2126 (now FT51116)

E18: Probability that they are viable is about 80%. Fairly confident that they will be viable. (0.8, 0.95)0.8

E19: Very low. Because in general hybrids are sterile, so very unlikely that hybrids are fertile. (1E-8, 1E-3)0.7
Figure 6.286: Given that the construct has been transmitted to the Arabiensis F1 offspring, what is the probability that they are viable?
E14: 50% would be non-viable. So 0.5*FT3-013 (now FT51003)-Col

E15: Much less viable. (0.001, 0.05)0.9

E17: Answered for all species above

E20: (0.3, 0.99)0.9
Figure 6.287: Given that the construct has been transmitted to the Coluzzi F1 offspring, what is the probability that they are viable?
E4: Introgression rates of chromosomes are very different in Coluzzi - e.g. chromosome 2 very high, X chromosome very low, a likely explanation for chromosome 2 is because this is where the insecticide resistance gene lies. I-Ppol is on 2L and this will be selected for due to insecticide, but recombination is suppressed around the centromere. Viability and introgression rates with other species is very low.

E14: This should be same as in colony - any viability from construct. But no mating barriers.

E15: Experiments out there but given an answer. (0.2,0.8)0.9

E17: Answered for all species above

E20: Very high (0.5, 0.99)0.9
Figure 6.288: Given that hybrid males carrying the construct are viable, what is the probability that the construct fails to sterilise them?
E17: This has been tested by The expert’s team in the laboratory for Coluzzi and Arabiensis, and sterility is complete in a sample size of thousands. So a typical knock-out rate of mutations in Drosophila is 1e-5 to 1e-6, so The expert thinks lower bound 1e-6.

E18: The reverse of FT3-012 (now FT51002). (0.9, 0.99) 0.9

E19: Upper bound 50%, lower bound 1E-7. It is likely that the construct sterilises them, so the probability that it fails to sterilise is quite low. Median 1E-3. 0.7

E20: (0.001, 0.2) 0.7. All sorts of reasons why it might fail to sterilise.

E21: The construct is being driven with promoters that are coming from A. gambiae so this should work with another species that A. gambiae can hybridize with but there is a chance that it doesn’t work. The expert says there are more than two species it can hybridise with so wants to keep species general. The real issue in his opinion is the promoter. Two possibilities: a) promoter works in other species so virtually all will be sterile; or b) the promoter fails to work then it fails for all and none will be sterile. FT3-0200a is assuming the promoter works in other species. Probability of sterility is less than one in a million. If the promoter is inactive virtually all will be fertile. So this question boils down what is the probability that the promoter is active in hybrids. Beta-tubulin is conserved so it is likely to work. FT3-0200b if the promoter doesn’t work then all will be fertile. Probability that the promoter doesn’t work in closely related species that they can hybridise is less than 1% so take FT3-0200a and multiply by 0.99
Figure 6.289: Given that Arabiensis hybrid males carrying the construct are viable, what is the probability that the construct fails to sterilise them?
E17: The expert has tested for both and would happy to use the same range above for both.
Same as FT3-02000 (now FT51010000)

E20: (0.001,0.2)0.7
Figure 6.290: Given that Coluzzi hybrid males carrying the construct are viable, what is the probability that the construct fails to sterilise them?
E4: Use same pdf as FT5-0000 (now FT500000). Coluzzi is distinguished from A. gambiae by polymorphism in the rDNA repeat hence there is a fleeting small possibility for I-PpoI construct to fail more often but happy to use the same probability. Coluzzi is the main (only) species that hybridises with A. gambiae (The expert has looked at over 10,000 of field collected mosquitoes and has never seen hybrids). The expert suggests running a simple experiment involving GM gambiae with Coluzzi females and see if the construct works in the F1 hybrids (Coluzzi lab colonies can be ordered from MR4).

E17: The expert has tested for both and would happy to use the same range above for both. Same as FT3-02000 (now FT51010000)

E20: Less likely to fail to sterilise. (0.005,0.1)0.7
Figure 6.291: Given that the F1 hybrid males are fertile, what is the probability that the F2 and beyond GM hybrid males have higher fitness than wild type males?
E14: If inserted close to WT/resistant allele it would affect the likelihood of spread. Need to know where inserted and likelihood of linkage with other known genes known to be subject to selection.

E17: The expert thinks this is low

E18: Probability relatively low, but not vanishingly so, about 1 in 1000. Working off 0.9. Upper bound of 1 in 100, lower bound of 1 in 10000. (1E-4, 1E-2)0.9

E19: Unlikely, no more than 5%. (1E-3, 0.05)0.75

E21: In a certain configuration this is possible, say the local population is inbred because of vector control and now you are introducing new traits that were not there before, but in general thinks this is pretty unlikely. The expert is more certain of the upper bound than the lower bound.
Figure 6.292: Given that the Arabiensis F1 hybrid males are fertile, what is the probability that the F2 and beyond GM Arabiensis hybrid males have higher fitness than wild type males?
E4: For all other hybrids this probability is almost certainly zero because of the hybrid sterility (see the previous event). 1 in 10,000 F2 eggs are produced. See Davidson (1964) Estratto dalla Rivista di Malariologia, Vol. XLIII N04-6. Use the 1:10,000 for the probability of this event.

E17: Answered above for each species

E20: \((1E-6, 1E-3)^{0.7}\)
Figure 6.293: Given that the F1 Coluzzi hybrid males are fertile, what is the probability that the F2 and beyond GM Coluzzi hybrid males have higher fitness than wild type males?
E4: Coluzzi and Gambiae have only been recognised as separate species since last year, prior to that they were described as M and S "forms". (Coetzee. M. 2013 Zootaxa). In nature hybridisation is asymmetric, A. gambiae chromosomes go into Coluzzi but not the other way round, due to the insecticide resistance. In the absence of insecticide resistance hybrids are typically less fit, but with insecticide resistance they are more fit. Coluzzi and Gambiae recognise themselves and in most years they don’t mate but then in some years they "just party" (Lee et al 2013 PNAS) and we see large numbers of hybrids. Prior to 2006 (the date of the introduction of bed nets) the hybrids only persisted for a few years but then died out because they were less fit. Post 2006 Coluzzi x Gambiae hybrids gained insecticide resistance. It looks like the hybrids are now replacing the pure Coluzzi and Gambiae so could be a small probability that they are more fit than either of the WT (Norris L. et al in prep PNAS). The elicitation is for C x G hybrids and the uncertainty reflects whether or not the background WT used in the insectary have the insecticide resistance gene and the associated region of Chromosome 2 introgressed on them or not. The expert recommends using the methods described in Lee et al (2014) Molecular Ecology Resources to look at the pattern of introgression in the WT populations around the insectaries, assuming these are the populations that they are going to use in the cage.

E17: Answered above for each species

E20: Hybrid male is likely to show lingering sterility and therefore will be less fit. Question on wording and the use of the term "fertile" here. Have answered (understood) it as: "Given that they can produce some offspring (rather than fully fertile)". Likelihood that males should be more fit is really low. (1E-6, 1E-3)0.9
Figure 6.294: Given that hybrid males carrying the construct are viable and the construct fails to sterilise them, what is the probability that the F1 hybrid males do not show hybrid sterility?
E17: Answered below for each species
E18: That they are fertile. Probability = 0.95. (0.9, 0.97)0.9
E19: Expect hybrid to be sterile. So probability that they show hybrid sterility is high, so proba-
   bility that they do not show hybrid sterility is low. (1E-7, 0.05)0.65
E21: The expert thinks that all species that can hybridise with A. gambiae show male F1 hybrid
   sterility. Variability reflects different species. The expert wants to keep it general and not focus
   on Coluzzi
Figure 6.295: Given that Arabiensis hybrid males carrying the construct are viable and the construct fails to sterilise them, what is the probability that the F1 Arabiensis hybrid males do not show hybrid sterility?
E4: Male AA x female AG leads to male sterility, but male AG x female AA leads to mostly fertile males, (see Table 1, page 212, Davidson, G. (1967), Genetics of Insect Vectors of Disease, Edited by Wright and Pal, Elsevier. NB: In this table ‘A’ is Gambia and ‘B’ is Arabiensis (see also Davidson, G. (1964) The Bulletin of the World Health Organisation). Use Pr = 0.5 here. Elicitation for this moved to FT3-011 (now FT51001)-Ara.

E14: For Arabiensis and Merus approximately 1. Pr=1. Check with Alexander Della Torre’s paper.

E17: There is experimental data on this, and no-one has discovered fertile male F1 hybrids out of a sample size of 1000s.

E20: In the laboratory they are not normally fertile. Not more than 1%. (1E-4, 0.1)0.8
All experts: 296 of 352 EXw = 12

Figure 6.296: Given that Coluzzi hybrid males carrying the construct are viable and the construct fails to sterilise them, what is the probability that the F1 Coluzzi hybrid males do not show hybrid sterility?
E4: There is no hybrid sterility with AG and AC crosses. Hence \( Pr = 1 \) for Coluzzi.

E14: Approximately zero. No evidence of post-zygotic mating barriers between Coluzzi and Gambiae. \( Pr = 0 \).

E17: Here the hybrid F1 males are fertile so the probability is 1.

E20: They're fertile. Probability that show hybrid sterility is low, so the probability that they do not show hybrid sterility is high. \((0.3, 0.99)0.9\).
Figure 6.297: Given that hybrid females carrying the construct are viable, what is the probability that the F1 hybrid females do not show hybrid sterility?
E17: Answered below for the separate species

E18: What is the probability that F1 hybrid females are fertile. This is high 0.9. (0.8, 0.95)0.8

E19: Even lower than equivalent for males. Same upper bound, lower bound one order magnitude lower. (1E-7, 0.02)0.65

E21: This is different from males. Most F1 hybrid females are fertile. This varies between species. But note that sometimes in hybrid one sex is absent (maybe males or females)
Figure 6.298: Given that hybrid Arabiensis females carrying the construct are viable, what is the probability that the F1 hybrid Arabiensis females do not show hybrid sterility?
E4: For A x G crosses there is reduced fecundity but a "significant" number do survive, reduced
 FECUNDITY but not sterile.

E14: Female hybrids should be fully fertile. Della Torre paper have experimental evidence.
Michelle Slotman in 2004. Calculate this

E17: This is the same as FT3-02001-Col (now FT510100021-9)

E20: (0.9, 0.99)0.9
Figure 6.299: Given that hybrid Coluzzi females carrying the construct are viable, what is the probability that the F1 hybrid Coluzzi females do not show hybrid sterility?
E4: For G x C crosses this is the same as the male probability.
E17: This is the same as FT3-02001-Col (now FT510100021-9)
E20: (0.7, 0.99)0.9
Figure 6.300: Given that the F1 GM hybrid female is fertile, what is the probability that F2 and beyond GM hybrid females have higher fitness than wild type females?
E14: Same issue as FT3-02002 (now FT51010001)
E17: This is the same as FT3-02002 (now FT51010001)
E18: Similar to answer for hybrid males, except slightly less likely. (1E-4, 1E-3)0.9
E19: Lower than for males. (1E-5, 0.05)0.75
E21: The expert doesn’t see why this should be any different from the males, but acknowledges that it can be. Use the same probability as the males.
Figure 6.301: Given that the F1 GM Arabiensis hybrid female is fertile, what is the probability that F2 and beyond GM Arabiensis hybrid females have higher fitness than wild type females?
E4: Elicitation is for A x G cross and it is lower but highly variable.
E17: Answered above for both species
E20: Some vigour could come from the fact that they are hybrids. But the GM trait will not be linked to this. (1E-4, 0.1)0.7
Figure 6.302: Given that the F1 GM Coluzzi hybrid female is fertile, what is the probability that F2 and beyond GM Coluzzi hybrid females have higher fitness than wild type females?
E4: For G x C cross these probabilities are the same as for the males because the main insecticide resistance is on chromosome 2 which is autosomal, maybe in year. The expert will know about any differences on the X chromosome that may affect fitness and whether or not this is additive, hence the XX females may respond differently. There are P450 genes that are related to insecticide resistance and there are some of these on the X chromosome, and this could make a difference but otherwise The expert thinks the probabilities would be the same.

E17: Answered above for both species.

E20: (1E-4, 0.1)0.7
Figure 6.303: Given that offspring carrying the autosomal construct are viable, what is the probability that I-Ppol construct moves to the Y chromosome?
E7: Partly depends on where the construct is. If the transgene is located near the tip of the chromosome it is easier to imagine it moving than if it were located more basally. We understand its closer to the centremere. It could move to the Y chromosome via a classic translocation event OR via transposition. This elicitation is for a classic translocation event. Spontaneous chromosome mutations in Drosophila occur 1 in 10,000 and only a fraction of these are translocations, but this typically incurs a fitness costs. Assuming 120,000 individuals

E9: For Y Drive its already on the Y chromosome

E17: Movement could occur via transposons or by translocation. The expert thinks that they should check if there are any transposons near to the construct insertion site. Spontaneous translocation rates are probably available for Drosophila

E18: About 1 in 5000. (1E-4, 1E-3)0.7

E19: This is very unlikely. (1E-9, 1E-6)0.8

E20: There is movement between the X and Y chromosome, although rare. Would need to look at frequency of Y linked translocations. Have not seen this in the laboratory, but then it is not easy to see. Not inconceivable to imagine translocations to the Y happening in the order of 1 in 1000 individuals. (1E-6, 1E-2)0.8

E21: Not that likely. If we look at the number of individuals that need to be screened before you see a translocation to the Y is 1 in 10E7. Number is low because translocations can mean many things, and for it to be viable, only the construct or a small portion of the chromosome containing the construct can be translocated.

E23: Same as FT5-120 (now FT50100)
Given that the I-Ppol construct inserted on the Y chromosome, what is the probability that it produces sufficient enzyme that is able to cleave rDNA?
E17: Much of the Y chromosome is silenced at meiosis.

E18: Relatively high, about 1 in 10. (0.01, 0.5)0.8

E19: Lower than 5%. (1E-4, 0.05)0.6

E20: rDNA should still be susceptible to cleavage because the site is so well conserved. But the Y chromosome is largely heterochromatic and not conducive to expression of genes. Correction: discard first answer (0.01, 0.9)0.9. Second response: two hundred transgenic lines generated and only two have ever been on the Y chromosome. Y 10% of genome. Only recover transgenes on the Y with a frequency of 1 in 100 that tells you that you only recover 1 every 10. So if you're on the Y there is a 90% chance that you will be silent or not expressed. (0.001, 0.9)0.9

E21: Male chromosomes are inactivated during meiosis, so there is a relatively low chance of this happening. How many random translocations to the Y need to happen for you to observe this event. Inactivation happens because of non-pairing during meiosis. The question might need rewording.

E23: Same as FT5-121 (now FT50101)
Figure 6.305: Given that the I-Ppol construct inserted on the Y chromosome and produces an enzyme that is able to cleave rDNA, what is the probability that this rDNA is predominantly on the X chromosome?
E7: The expert is not the right person to ask this. The expert Ashburner (Steve’s PHD advisor) would be better to answer this question.

E9: Don’t elicit this, we should know which species A. gambiae can hybridize with, and where rDNA is in these species. The expert suspects this is a high probability because it is only going to hybridize with species that are genetically close.

E17: This is true for all AG complex member as far as The expert knows

E18: About 99%. (0.95, 0.99)0.99

E19: Talking about hybrids, so compatible species or other mosquitoes. Quite common that rDNA is predominantly on the X in compatible mosquito species. Quite likely. But not confident answering this.

E20: Don’t know enough about other mosquitoes but it is easy to imagine cases where they are all on the X. (0.001, 0.9)0.8

E21: Some of the Anopheles have it, others don’t. (Basically, 50:50 chance)

E23: Same as FT5-122 (now FT50102)
Figure 6.306: Given that the I-Ppol construct inserted on the Y chromosome and produces an enzyme that is able to cleave rDNA that is predominantly on the X chromosome, what is the probability that it does not cause male sterility?
E7: Expression at a lower level may allow fertility, and it is likely that expression may occur at a lower level.

E9: The expert thinks that the probability of male sterility will be higher with the hybrids as compared to the original GM strain, but still not very low because (as The expert mentions) environmental effects (e.g. temperature) may make the protein less stable and hence reduce sterility, or more weakly expressed. But The expert doesn’t feel confident enough to put a probability on this. Corbin Jones (UNC) might be good for this.

E17: This requires either very finely balanced expression levels (this is possible), or destabilising mutation in the enzyme (very low probability).

E18: Very low, about 1E-4. (1E-5, 5E-4)0.8

E19: This is low (1E-7, 1E-3)0.75

E20: Barry Stoddard from Seattle has mutated I-Ppol to reduce its half-life and this requires three or four mutations. This activity still shreds the X chromosome fully but its conceivable that a similar number of mutations could reduce activity sufficient for this scenario. Conceivable that one point mutation could be enough to do this. Estimate mutation rate at 1E-6. (1E-9, 1E-3)0.8

E21: Given the above the chance of not causing male sterility is low. But, because it is on the Y, there is some uncertainty about when and how much is expressed. How many of these rare translocation events are viable, how many do I have to look to find a non-sterile male? Quite high, but based on observation.

E23: Same as FT5-123 (now FT50103)
Figure 6.307: Given an I-PpoI construct inserted on the Y chromosome, is able to cleave rDNA predominantly on the X chromosome without causing sterility, what is the probability that it maintains germline expression?
E7: Is this question: a) about specificity; or b) is it expressed. We have already assumed that it is being expressed. We interpret this as a question about specificity. We would rephrase: What is the probability that it has maintained germline restricted expression? (not broader).

E9: The expert thinks this is a high probability.

E17: Same as FT3-021002-Expert1 (now FT510111002)

E18: No reason for it not to be expressed in germline if it got this far. Probability is relatively high, about 0.9 (0.85, 0.95)0.8

E19: (1E-4, 1E-2)0.6

E20: Same as FT3-021011 (now FT51011111). Intrinsic property of the construct is the same, but because it is on the Y which is an unpaired chromosome you get meiotic silencing of unpaired DNA and inactivation of sex chromosomes in germline tissue and the beta2 tubulin promoter in this construct is subject to this silencing. So this construct would need to escape that silencing. Could escape that silencing by inserting into a paired region of the Y chromosome that pairs with the X chromosome. This is likely to be a small percentage, less than 5%. (1E-5, 0.05)0.7

E21: Given the spirit of the question: same FT3-021002 (now FT510111002)

E23: Same as FT5-124 (now FT50104)
Figure 6.308: Given an I-Ppol construct inserted on the Y chromosome, is able to cleave rDNA predominantly on the X chromosome without causing sterility and maintains germline expression, what is the probability that the driving forces are higher than fitness costs?
E9: This is the killer question. The expert thinks this is 1e-double digits. The expert can’t give a number here. Corbin Jones might be able to give a number here.

E17: Fitness costs should be low if germline specificity is maintained

E18: No idea.

E19: Keep high range. (1E-5, 0.1)0.7

E20: Negative fitness costs are likely to be due to reduced hatching rate caused by the rDNA cleavage with the X chromosome. If X gametes are made and still fertilise eggs leading to arrested embryos then the transgenic Y chromosome will not drive. It will remain at its initial frequency. But if only or predominance of Y gametes are transferred during fertilisation then there should be some drive. Because the native I-Ppol was shown in A. gambiae to produce a predominance of Y gametes, given the other conditions, i.e. that it does not cause sterility, it could be expected to do the same in a related non-target mosquito. (0.01, 0.9)0.8

E21: The question is at what point along the gradient of fertility (or sterility), will it be enough to allow drive. To find the right balance: requires it to hit the right spot of expression level

E23: Same as FT5-125 (now FT50105)
Figure 6.309: Given that hybrids carrying the construct are viable, what is the probability that their genome contains an additional site outside the X-linked ribosomal repeat array that is recognised by I-Ppol?
E7: This could be calculated as we know the preference for the recognition site. We should calculate this. See Figure 3 in the background document. Search for degenerate sites in genetic databases and make an estimate for this. The ICL team could do that. The expert suggests speaking to Ray Monnat (with Andy S team?) or Barry Stoddard.

E9: This could be experimentally derived by looking in the genome for sites that are sufficiently similar that the HEG could recognise. CRISPR is an example of where people have been doing the same thing - i.e. looking for off target cleavage sites. Another way of doing this is via SURVEYOR nuclease.

E14: Given genome size probability similar with FT5. Should remain same as with FT5-1000 (now FT5011000)

E18: Very low, about 1E-4 is the lower bound. Upper bound about 1E-3. 0.6 (1E-4, 1E-3)0.6. Reviewed again on day 2 and changed to match with elicitation in FT5. (1E-6, 1E-5)0.8

E19: Probability that I-Ppol recognises an additional site already present in the genome. Two mismatches allowed in the recognition site. Probability that it can find a random additional site is roughly (4 raised to power 13)/(10 raised to the power 8). Decrease this by an order of magnitude as this considered two mismatches. (1E-4, 0.03) 0.75

E20: Quite high. Certainly range of uncertainty high. (1E-3, 0.5)0.5. Trying to convey that it could very well be the case that they could be there.

E21: We know that there are certain strains or species of mosquitoes where the rDNA is not exclusively on the X chromosome. Also given that it is a high copy number of the ribosomal genes. The expert would not be surprised if you found one copy on another chromosome and it doesn’t need to be a functional copy either, just have the recognition site. There are even some A. gambiae strains (e.g. the Asembo strain) where the rDNA is not exclusively on the X chromosome. One other source of uncertainty is that we can have polymorphism in a species and also to detect a single copy we would have to sequence the entire genome of many individuals because of the polymorphism.

E23: To be measured. Same as FT5-1000 (now FT5011000)
Figure 6.310: Given that I-PpoI recognises an additional site what is the probability that it moves into this site?
E9: Use the same probability as FT3-021010 (now FT51011110)
E14: Calculate this as per FT5-101 (now FT5011001)
E18: About 1 in 1000. (1E-4, 1E-2)0.5
E19: General wording comment, replace ‘recognise’ with ‘cleave’ the target site. Very unlikely. Upper bound 1E-5, Lower bound 1E-9, 0.7
E20: At some point in its life the HEG must get within its recognition site without any homology on the first occasion that it invades the genome of a new species. So this event is not impossible. (1E-8, 1E-5)0.5
E21: The expert also thinks this is extremely low because I-Ppol is not mobile, the actual locus that actually the protein is not different from any other locus (?). Why should this move more than any other gene? It would be different if it was flanked by regions of homology but this is not how the ICL team are doing it.
E23: Same as FT3-112001 (now FT312001)
Figure 6.311: Given that I-Ppol has moved into an additional site what is the probability that it maintains its germline expression?
E7: Should re-phrase: Probability that it maintains restricted germline expression (expression is not broader).

E14: Same as per FT5-102 (now FT5011002)

E18: Likelihood that I-Ppol is intact and functional is an order of magnitude lower than probability that it moves into the site. (1E-5, 1E-3)0.7

E19: Upper bound could be quite high because it is compatible species. Upper bound 80%, lower bound 0.1%, 0.75. Day 2 amended to be (0.1, 0.8)0.75. Amended again following feedback report.

E20: High, already has germline expression so it’s just maintenance. (0.8, 0.99)0.9

E21: Given that we have a germline specific promoter and it has had germline activity in the past there it is reasonably certain that it would maintain germline activity. It could be silenced

E23: Same as FT5-102 (now FT5011002)
Figure 6.312: Given that l-Ppol has moved into an additional recognition site and maintains germline expression, what is the probability that it does not cleave ribosomal repeats?
E17: This is a low probability because the enzyme is still the same, it has not mutated. Plausible that expression is low so that cleaving of ribosomal repeats is low. Note elicitation is fitted to the log-normal distribution
Figure 6.313: Low fitness effects at target site
There were no comments for this question.
Figure 6.314: Given that I-Ppol has moved into an additional recognition site and maintains its germline expression, what is the probability that the recognition site is in an Intron?
E7: Suspect that the estimate won't be too different from Drosophila

E9: This cannot just be calculated as the proportion of intronic sequence in the hybrid genome because introns tend to be AT rich and the recognition site is relatively GC and AT even. So to calculate this you would need to look for intronic proportion and the extent to which this matches the AT bias. You should calculate this for A. gambiae and this will be the same number for the hybrids.

E14: As per FT5-10300 (now FT501100310)

E18: What fraction of the mosquito genome is represent by introns in coding genes. You should be able to calculate this. No answer.

E19: Less than 1%. (1E-4, 0.01)0.7. Comment amended following feedback: The probability to hit an intron is relatively high, assuming that 25% of genomes is genes, of which half have introns, and those make maybe two thirds of the gene. This means about 10% of the genome is introns.

E20: Assuming 15,000 genes at 6kb each, makes 90,000kb. Genome size is about 250mb. So one third is made up of genes and guess that of each gene two-thirds is intronic, so 60mb is intronic, which is about one quarter of the genome. (0.01, 0.6)0.8

E21: Note The expert has reworded this question! Check with Tony

E23: Would depend on the proportion of intronic genomic DNA in the genome, and the negative fitness effect this insertion might cause (disruption of an essential gene). Same as FT3-11200310 (now FT31200310).
Figure 6.315: Given that I-Ppol has moved into an additional recognition site and maintains its germline expression, what is the probability that the recognition site is in a non-essential region of the genome?
E14: As per FT5-10301 (now FT501100311)
E18: Probably relatively high about 80%. (0.75, 0.85)0.6
E19: Upper bound, 30%. Lower bound 1E-3. 0.75. Comment amended following feedback: For the same reason as FT3-02100411 [sic: FT3-02100410 (now FT51011100310)?], this makes the probability to hit a non-essential region quite high, more than 75%
E20: Use same figures as for FT3-02100410 (now FT51011100310), two-thirds of the genome is non-genic. (0.05, 0.8)0.7
E21: It doesn’t matter whether or not I-Ppol has mutated. Same probability. This is a high probability because most regions of the genome are non-essential.
E23: Same as FT3-11200311 (now FT31200311)
Figure 6.316: Given that I-PpoI has moved into an alternative recognition site and maintains its germline expression, what is the probability that the negative fitness effects are recessive?
E14: Basic event added by Martin, should have a step here for recessive fitness costs, which will be the same value as for FT5-10302 (now FT501100312).

E18: (0.25, 0.75)0.8 and 50% chance that fitness is recessive.

E19: (1E-4, 0.05)0.5

E20: High. Most mutations are recessive. (0.6, 0.99)0.8 hence very likely to be recessive.

E21: Haploid insufficiency is rare, so that the probability of recessive because of this (?), but the modified gene product (due to the HEG) could have a dominant negative fitness effect. The expert thinks that it is more likely than not that the fitness costs are recessive.

E23: Same as FT5-10302 (now FT501100312)
Figure 6.317: Given that hybrids carrying the construct are viable, what is the probability that they do not show hybrid sterility?
E7: Not a question The expert can answer
E9: Use the same probability as FT3-021012 (now FT51011112)
E14: Coluzzi no evidence of hybrid sterility, Arabiensis 0.5*Coluzzi. As per previous answer.
E18: Dependent
E19: Dependent
E20: Question needs rewording and need to check dependency and also need to check lead in from previous two questions 'Given that...'.
E21: See comments above
E23: Not sure.
Given that GM Arabiensis hybrids carrying the construct are viable, what is the probability that they do not show hybrid sterility?
E20: Dependent
Figure 6.319: Given that GM Coluzzi hybrids carrying the construct are viable, what is the probability that they do not show hybrid sterility?
E20: Dependent
Figure 6.320: Given that hybrids carrying the construct are viable, what is the probability that I-PpoI mutates and recognises the site where it is already located?
E17: This needs multiple changes to recognise completely different sequence
Figure 6.321: Given that I-Ppol has mutated to recognise the site that it is in, what is the probability that it maintains its germline expression?
E17: This will be a bit higher than FT3-021002-Expert1 (now FT510111002) because it doesn’t have to move
Figure 6.322: Given that I-Ppol has mutated to recognise the site that it is in and maintains germline expression, what is the probability that its cleavage rate at the rDNA repeat does not preclude spread through the population?
E17: Either it recognises the new sequence and it is relatively specific to that sequence (this is a tiny number); if it recognises both new sequence and ribosomal repeat it recognise almost all DNA and it would kill the organism. We don't have to consider this scenario because clearly this imposes a huge fitness cost.
All experts: 323 of 352 EXw = 0

Figure 6.323: Low fitness effects at original target site
E17: It has not moved from its original location and we know that there are low fitness effects at this location
Figure 6.324: Given that hybrids carrying the construct are viable, what is the probability that I-PpoI mutates and recognises an additional site outside the X-linked ribosomal repeat array?
E7: About 500 bp and a per nucleotide mutation rate of about 1e-8 per generation, assuming 120,000 mosquitoes (10k x 12 generations). What is the probability that this is going to be enough to recognise another site?

E9: Most mutations will be detrimental or neutral, a small fraction would change the recognition sequence and in a small subset of these that recognition sequence would exist in the hybrid.

E14: Same as per FT5-1001 (now FT5011020)

E18: Likelihood of this happening probably an order of magnitude lower than FT3-0210000 (now FT510111000). (1E-5, 1E-4)0.7. Day 2 changed this to match above, similar probability. (1E-6, 1E-5)0.8

E19: (1e-5, 1E-3)0.75

E20: Assume mutation rate of 1E6, the chance that mutation should change specificity rather than abolish activity is very low. Most mutations will destroy, where as here require the mutation to change specificity. (1E-9, 1E-6)0.5

E21: Note The expert’s change of wording. The expert thinks this is super low, the mutation has to hit that specific gene and one mutation does not easily change the specificity of a (homing) endonuclease.

E23: Same as FT3-1120001 (now FT312010), which was to speak to the Seattle crowd. No probability elicited.
Figure 6.325: Given that the mutated I-PpoI recognises an additional site what is the probability that it moves into this site?
E17: Use same probability as "Moves into additional site above" i.e. FT3-021001-Expert1 (now FT51011001)
Figure 6.326: Given that the mutated I-Ppol has moved into an additional site what is the probability that it maintains its germline expression?
E17: Use same probability as FT3-021002-Expert1 (now FT510111002)
Figure 6.327: Given that the mutated l-Ppol has moved into an additional recognition site and maintains germline expression, what is the probability that its cleavage rate at the rDNA repeat does not preclude spread through the population?
E17: For this to happen the enzyme needs to change specificity not merely widen specificity
All experts: 328 of 352 EXw = 0

Figure 6.328: Low fitness effects at target site
E17: Same as FT3-0210041-Expert1 (now FT5101110031)
Figure 6.329: Given that hybrids carrying the construct are viable, what is the probability that the I-PpoI construct inserts into its recognition site on a ribosomal repeat?
E7: Take the numbers from FT3-021001 (now FT510111001) and multiply them by 5 because ribosomal repeats get cut a lot by this nuclease and since DNA breaks tend to promote recombination then this increases the probability of recombination.

E9: The expert thinks this is one of the killer steps, this should be the same probability as that for any random piece of DNA inserting into the ribosomal repeat, because their construct should have cut off the pieces it would need to do this. 1e-single to double digits. If someone wanted to do this experimentally they would have to do it in something like yeast where you can get the sample size up to 10e8, but even there The expert thinks you may never observe it.

E14: As per FT5-110 (now FT501110)

E17: Somewhat higher than Scenarios Austin1 and Austin3 where insertion is at a different locus because there are many ribosomal repeats that it could move into

E18: It's going to be high as that's what it's built to do, about 95%. (0.85, 0.99)0.8

E19: This is the same as question about the probability that it moves into recognition site. (1E-9, 1E-5)0.7

E20: There is a possibility that this could be an order of magnitude higher than FT3-021001 (now FT510111001) because although similar in process the ribosomal repeats are in an array and so there could be several more cleavage and recombination events that might increase the likelihood of spurious insertion of I-Ppol. Plus also that I-Ppol is inserted in the rDNA normally is proof that it can somehow get there in the first place without homology. There might also be some adaptation to preferentially inserting here, although I don't know of this. (1E-8, 1E-3)0.6

E21: A bit better than observed for FT3-021001 (now FT510111001) 

E23: Same as FT5-110 (now FT501110)
All experts: 330 of 352 EXw = 0.758

Figure 6.330: Given that I-Ppol inserted into a recognition site on a ribosomal repeat, what is the probability that it maintains its germline expression
E7: This will be the same as FT3-021002 (now FT510111002)

E9: Assuming the construct includes the beta Tubulin promoter then this probability is quite high

E14: As per FT5-111 (now FT501111)

E17: Somewhat lower than scenarios Austin1 and Austin3 because rDNA repeat is constitutively expressed (always expressed in all tissues).

E18: High, about 95%. (0.85, 0.99)0.8

E19: Also the same as earlier. (0.001, 0.8)0.75

E20: High probability that it will maintain germline expression in related mosquitoes. Less so but still significant risk in other insects. Unlikely outside of insects. (1E-3, 0.9)0.9 This range reflects across non-target eukaryotes. Correction: this was given more for HGT, where as here VGT, so discard first response. Given that it has hybridised, then this is more likely. This was changed on Day 2 from (1E-6, 0.9)0.9 to (1E-3, 0.9)0.9

E21: Same as FT3-021002 (now FT510111002)

E23: Same as FT5-124 (now FT50104)
Figure 6.331: Given that hybrids carrying the construct are viable, what is the probability that they do not show hybrid sterility?
E7: This is not a question for the expert
E9: The expert thinks this is pretty similar to the Y drive issue - say about 10%
E14: As per above.
E17: Use same probability as FT3-021003-Expert1 (now FT5101110041-9)
E18: Dependent
E19: Dependent
E20: Dependent
E21: Same as FT3-021003 (now FT510111004)
E23: Don’t know.
All experts: 332 of 352 EXw = 0

Figure 6.332: NA
There were no comments for this question.
All experts: 333 of 352 \( EX_w = 0 \)

Figure 6.333: NA
There were no comments for this question.
Figure 6.334: Given that I-Ppol inserted into a ribosomal repeat and maintains its germline expression, what is the probability that its cleavage rate at the rDNA locus is sufficiently low that reduced male fitness or reduced X chromosome transmission do not preclude spread through the population, but still is active enough for homing to occur?
E7: Question not very clear

E9: Expression may be dampened if the construct is inserted into a different part of the genome, but the window that it has to work in is not large - i.e. the level of expression - if it is too low it won’t home and if it is too high then it will be detrimental to the organism. The expert is really not sure where to start with this probability - if its a larger sequence it will be protected from the local genomic environment but this in turn reduced the probability that a homing reaction will copy the whole thing back in.

E14: As per FT5-1120 (now FT5011120)

E17: Same as FT3-0210040-Expert1 (now FT5101110030)

E18: This is low about 1 in 1000. (1E-4, 0.002)0.7

E19: This is low. Given that there are hundreds of target sequences, the X will be shredded (1E-7, 1E-2)0.6

E20: Not unimaginable. It wouldn’t require any mutation of target site specificity, rather a decreased activity. Barry Stoddard from Seattle has mutated I-Ppol to reduce its half-life and this requires three or four mutations. This activity still shreds the X chromosome fully but its conceivable that a similar number of mutations could reduce activity sufficient for this scenario. Similar to FT3-0210001 (now FT510111020) but likely to be more probable (at least a couple of orders of magnitude) because mutations can be slightly more deleterious and could also happen in the promoter to lower activity. (1E-7, 1E-2)0.7

E21: Would have to imagine truncation or mutation that reduces expression or activity but not specificity, just enough to home but not to shred. Low but not impossible thing to happen.

E23: Same as FT5-1120 (now FT5011120)
All experts: 335 of 352 EXw = 6.232

Figure 6.335: HEG inserted in intron
E21: Very unlikely for that rDNA in animals have introns. Elicited for both here. Could be a bit too high, but there is considerable uncertainty.

E23: Same as FT5-1121 (now FT5011121)
Figure 6.336: Given that I-Ppol inserted into a ribosomal repeat and maintains its germline expression, what is the probability that it inserted in a self-splicing intron?
E7: This is not a question for the expert
E9: Isn’t the current site already a self-splicing intron? The way The expert is interpreting this whole part of the tree is that this would be probability = 1.
E14: As per FT5-11210 (now FT50111210)
E17: Self-splicing introns do not exist in AG complex as far as The expert knows
E18: Vanishingly small, about 1E-4. (1E-5, 5E-4)0.95. Steve’s understanding is that self-splicing introns are only present in lower eukaryotes.
E19: This is very low. Upper 1E-5. Lower bound 1E-8. 0.7
Figure 6.337: Given that I-Ppol inserted into a ribosomal repeat and maintains its germline expression, what is the probability that it inserted in a spliceosomal intron?
E7: This is not a question for the expert

E9: The way The expert is interpreting this whole part of the tree is that this would be probability = 0, but either way the sum of these two probabilities would have to be 1. Check with ICL on this.

E14: As per FT5-11211 (now FT50111211)

E17: The expert believes the spliceosomal introns do not work in rDNA and this would require two re-arrangements (I-PpoI into intron and then intron into rDNA)

E18: Steve’s understanding is that rRNA introns are not spliceosomal, so the probability is vanishingly small. (1E-5, 5E-4)0.95

E19: Not sure what a spliceosomal intron is so leave this.

E20: When homing at locus different from expected ribosomal repeat the probability of inserting into an intron anywhere in the genome is higher because there are so many more introns available. In this case of the ribosomal repeat, there would need to be one intron already in the ribosomal DNA locus and the recognition site would need to be contained within this intron. This would mean that the highly conserved site happened to find itself in a region that is not present in the final rRNA. This is extremely unlikely.
Figure 6.338: Given a catastrophic release of all 10,000 GM insectary mosquitoes, what is the probability that Wolbachia is present in the mosquito?
E17: Same as FT3-2121 (now FT51111)

E18: This is low, according to Steve’s understanding of Wolbachia in mosquitoes. Which may change over time. (1E-4, 1E-2)0.5
Figure 6.339: Given that Wolbachia has acquired the construct, what is the probability that there are compatible species (i.e., species that could mate with GM insectary mosquitoes) in the vicinity of the insectary?
E17: Same as FT3-2123 (now FT51113)
Figure 6.340: Given that there are compatible species in the vicinity, what is the probability that hybrids will be formed, following a catastrophic release of all 10,000 mosquitoes?
E17: Same as FT3-2124 (now FT51114)
Figure 6.341: Given that hybrids between the GM insectary mosquitoes and compatible species have been formed, what is the probability that the construct will be transmitted to the F1 offspring?
E17: Same as FT3-2125 (now FT51115)
Figure 6.342: Given that the construct has been transmitted to the F1 offspring, what is the probability that they are viable?
E17: Same as FT3-2126 (now FT51116)
Figure 6.343: Given that the construct has been transmitted to F1 offspring and they are viable, what is the probability that the F1 females are fertile?
E17: Same as FT3-2127 (now FT51117)
Figure 6.344: Given that the F1 females carrying the modified Wolbachia are fertile, what is the probability that the modified Wolbachia will spread in the recipient species?
E17: Same as FT3-2128 (now FT51118)
Figure 6.345: Given a catastrophic release of all 10,000 GM insectary mosquitoes, what is the probability that mitochondrial DNA acquires the construct?
E14: Talk to The expert Sinkins. Scott O'Neil, Charles Godfray.

E17: This is very low because it is difficult to transform mitochondria. It is very difficult to get DNA into mitochondria. The expert is not even sure that if you try you could do it in the laboratory. The expert does not know of any record of nuclear DNA transferring into mitochondria. So this is like surveying millions of evolutionary years of millions of organisms. So it is a very low number. So the uncertainty here is The expert’s knowledge.

E18: This is a question for Scott O’Neil.

E21: A lot of mitochondria in mosquitoes and they have DNA so it can happen. Acquisition is open to interpretation. DNA would have to leave the nucleus, mitochondria would have to be present. This can happen but the key issue is what next is this stable, will they increase. Possible the mitochondria are constructed in a way that make it very unlikely that the construct can get in there. Nikki is very uncertain how mitochondria pick up DNA.
Figure 6.346: Given that mitochondrial DNA has acquired the construct, what is the probability that Wolbachia is present in the mosquito?
E17: After reading the paper The expert thinks 10%. But this question should refer to the prevalence of Wolbachia in the same species outside the laboratory.

E18: Same FT3-2105 (now FT51100)

E21: Amended question. Wolbachia presence independent of Mitochondrial DNA. As written this has probability zero because all WT that are brought into the insectary will be screened for Wolbachia and maybe other "things", it's not clear yet. But The expert acknowledges Wolbachia maybe present in Hybrids so the question needs to be reworded for this to be a valid. Rewording. Need to clear this whole section of the tree up.
Figure 6.347: Given that Wolbachia has become associated with the transformed mitochondrial DNA, what is the probability that there are compatible species (i.e. species that could mate with GM insectary mosquitoes) in the vicinity of the insectary?
E17: This should be reworded to read "Given that Wolbachia and construct bearing mitochondria are in the same female, what is the probability...". This will be high and it depends on where the insectaries are located.
Figure 6.348: Given that there are compatible species in the vicinity, what is the probability that hybrids will be formed, following a catastrophic release of all 10,000 mosquitoes?
E17: The expert thinks the following: a) Coluzzi-Gambiae is about 1%. b) Either one with Arabiensis will be about 0.1% hybridisation rate
Figure 6.349: Given that hybrids between the GM insectary mosquitoes and compatible species have been formed, what is the probability that the construct will be transmitted to the F1 offspring?
E17: 100% if the cross is with GM female (plus construct) by WT male of different species, and 0% if it is the other way round, hence 50% overall.
Figure 6.350: Given that the construct has been transmitted to the F1 offspring, what is the probability that they are viable?
E17: 100% regardless if its Coluzzi or Arabiensis
Figure 6.351: Given that the construct has been transmitted to F1 offspring and they are viable, what is the probability that the F1 females are fertile?
E17: This will happen, F1 females are fertile irrespective of male species or male Wolbachia infection status
Figure 6.352: Given that the modified F1 females carrying the Wolbachia and the transformed mitochondria are fertile, what is the probability that the particular combination of Wolbachia and construct bearing mitochondria will spread in the recipient species?
E17: The Wolbachia recently described from Gambiae appears to have low vertical transmission rate. Cytoplasmic incompatibility has not yet been investigated (apparently). Wolbachia is already found in M and S - i.e. AC and AG. There is no reason to think construct will give selective advantage.
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