The use of the negative selection marker HSV-TK or DTA

We did not include a negative selection marker in the general targeting offer for several reasons. In the past the majority of the constructs contained the HSV-TK to perform +/- selection. However gancyclovir treatment of ES cells is quite toxic and it negatively affects the ability of ES cells to go germline. The situation with DTA is similar. In addition the negative selection is not only dependent on the promoter but also on the pA signal. Some people use it without pA, but in this case the message will only be stable in case a pA sequence is located in the vicinity of the integrated "unspecific" locus. Using a DTA with pA imposes the risk of killing the cells with the message generated transiently from the DNA that entered the cell after electroporation (before recombination takes place or in addition to the copy of DNA that finally is used in the recombination event).

In our opinion it is much better to accept a lower rate of homologous recombination in exchange for a better rate of germline transmission. In addition, by using RedET recombination the size of your homology arms are in general much larger than in the majority of the constructs made conventionally in the past (the long arm can be 7 or 8 kb without any problem) so the overall recombination rate is already increased. Since you can add restriction sites of your choice adjacent to the loxP and the floxed cassette screening is also simplified.

Less than 20% of our clients have requested TK/DTA in the past so we have decided to go with the preferences of the majority of our clients. The cloning of TK or DTA in the construct requires a lot of additional paperwork (IP issue) and would have of course influence on the time line and pricing of the construct. I hope you find my explanations helpful. Please cross-check again, whether you really need a TK/DTA. If you have any further questions please don't hesitate to write another email.

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