Court lab protocol
3/22/11

Protocol for oligo recombineering to make a point mutation

This protocol is intended to be a "crib sheet" for those that already are recombineering savy. For further details on all steps, see the "Step-by-step ssDNA protocol".

1. Design construction such that the MMR system can be avoided.

2. Electroporate ~100ng of salt-free oligo into 50μl of freshly prepared electocompetent, recombineering-proficient cells. For optimal results, be sure that the cells and oligo were properly mixed.

3. After the 1ml L broth is added and the mix is transferred to a sterile culture tube, outgrow the cell for 30 min – 2 hours at 32° with rolling or shaking. The proper length of outgrowth depends on the details of the construction.

3. After outgrowth, make 10-fold serial dilutions in TMG, minimal salts or similar osmotically balanced medium. Plate 0.1ml of dilutions on L plates (normally: 10^{-4}, 10^{-5}) to determine viable cells (~10^8/ml are expected although in some genetic backgrounds the viability can be lower) and on the appropriate plate for recombinants. For example if the recombinants are non-selective, you need only the L plates. If MMR is avoided and all other factors are optimized, you can expect as high as 50% of the colonies will be recombinant. Plates should be incubated at 32° to prevent further expression of the Red functions.

4. Screen for recombinants via colony PCR, colony size, or whatever appropriate means. An illustration of how PCR can be used to find the recombinant can be found in the "Step-by-step ssDNA protocol" as well as in the FAQ on the website.

5. Once a candidate colony is found, go back to the master plate, streak the colony out for single colonies and repeat the PCR screen on pure individual colonies.

6. Always sequence final constructs to be certain the oligo did not introduce any extraneous mutations.