Court Lab protocol
Updated 3/14/11

Checklist for recombineering targeted to plasmids

1. The recombineering host containing the Red functions should be a *recA* mutant. Strains containing the λ prophage with Red under *c*I*857* control must be propagated at 30-32°C.

2. Use with a freshly isolated monomeric plasmid preparation and determine the DNA concentration.

3. For creation of all mutations made with ss-oligos, use lagging strand oligos in order to obtain high efficiency recombineering. For point mutations, use a strain defective for methyl-directed mismatch repair or an oligo that creates a C-C mispair or other means to avoid the MMR system.

4. Ideally, do a transformation efficiency curve with your plasmid to determine the optimal DNA concentration. We suggest a 0.01-100ng range for a standard curve.

5. Based on the transformation efficiency, choose a plasmid DNA concentration that gives a high but not saturating transformation efficiency.

6. If possible, design the recombineering to remove a unique restriction site or add a restriction site by making several adjacent base alterations; although not essential, these steps help in isolating and identifying recombinants.

7. Co-electroporate the plasmid with the linear DNA.

8. Outgrow the electroporated cultures in at least 1ml LB with aeration for at least 2hrs at 30-32°C before applying selection.

9. After outgrowth, add 9 ml broth and drug for plasmid selection to electroporation mix and grow overnight at 30-32°C with aeration.

10. Isolate plasmid DNA using a standard mini-prep procedure.

11. Eliminate parental plasmid with restriction enzyme if possible (see step 5). Note that this will also eliminate recombinant hetero-multimers.

12. Retransform into a *recA* strain (e.g. DH5α or XL2-Blue) at a low DNA concentration (less than one plasmid per cell). Generally, this will be around 0.1ng.

13. Select or screen for desired phenotype. Possible methods of screening include restriction digestion, identification of plasmids with altered size as assayed by migration on agarose gels, sequencing, and PCR analysis i.e. MAMA PCR.

14. Among plasmids obtained from step 11, isolate plasmid DNA and screen for a monomer species.

15. Confirm the desired change by sequencing, since oligos can introduce unwanted secondary mutations due to errors during their synthesis.