Court lab protocol
Updated 3/14/11

Protocol for dsDNA recombineering to insert a drug cassette into the chromosome or a BAC

1. Electroporate 100-300ng of purified drug cassette-containing PCR product into freshly prepared electocompetent, recombineering-proficient cells.

2. After the 1ml L broth is added and the mix is transferred to a sterile culture tube, outgrow the cell for 2-3 hours at 32° with rolling or shaking. This allows for expression of the drug cassette as well as segregation of chromosomes (or BACs) without the cassette.

3. After outgrowth, make 10-fold serial dilutions in TMG (recipe below), minimal salts or similar osmotically balanced medium. Plate 0.1ml of dilutions on L plates containing the appropriate drug (see FAQ for drug concentrations to use). As you expect approximately 10^3-10^4 recombinants per 10^8 surviving cells, then either the undiluted, 10^{-1} or 10^{-2} dilutions should yield a good number of drug resistant recombinants. Plates should be incubated at 32° to prevent further expression of the Red functions.

Note: Total cells that survive electroporation can be determined by plating dilutions on L plates. Expect approximately 10^8/ml although we have seen up to 10-fold fewer with some recombineering strains.

4. Several recombinants should be struck out for single colonies on the appropriate L+drug plates.

5. The drug cassette insertion should be confirmed by PCR across both new junctions (see FAQ). Make certain there is not also bands for the WT gene, indicating a duplication.

TMG

10 mM Tris base
10 mM $MgSO_4$
0.01% gelatin