## 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 electrocompetent, 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  $32^\circ$  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<sup>8</sup>/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<sub>4</sub> 0.01% gelatin