Targeting Vector Preparation for Electroporation

1) Plasmid DNA should be prepared using EndoFree Plasmid Maxi protocol.

2) Digest 100 ug of targeting vector with a restriction digestion enzyme that cuts the vector outside arms of homology overnight (take ~1/50 of sample for gel analysis later).

3) Do ethanol precipitation for a couple of hours with 2.5 volume of 100% ethanol on ice (or -20 degree).

4) Centrifuge at maximum speed for 10 minutes to pellet DNA.

5) Wash DNA with 70% ethanol. Centrifuge at maximum speed for 5 minutes. Repeat wash step and allow pellet to air-dry.

6) Resuspend pellet in sterile PBS to a concentration of 1ug/ul.

7) Run a 0.8% gel with 500 ng samples of uncut, digested & unpurified, and digested & purified along with 1ug **λ-HindIII Digest Marker** (NEB cat. #N3012) to check for linearization and verify DNA concentration.

IMPORTANT! Project will not be accepted without λ-HindIII Digest Marker gel. Nanodrop readings can be misleading. Comparison to the marker allows the most accurate measurement of vector concentration. This will maximize the targeting efficiency.

8) Provide gel picture and 50ug of linearized DNA for electroporation.