Updated 2/18/11

Transformation by electroporation
(Conditions are for *E. coli* or *Salmonella enterica*)

Electroporator settings: 1.8kV
Cuvettes: 0.1 cm – chilled on ice in advance

LINEAR DNA recombineering substrate

dsDNA: 100-300 ng of salt free PCR product. We normally clean PCR products with a commercial PCR cleanup kit.

ssDNA: order oligos as salt free (we use IDT). No further purification is needed, even when suggested by the manufacture. We suspend oligos to 1 nmole/μl in TE for the stock solution. Avoid excessive freeze/thaw cycles. The working stock is diluted 100-fold to 10 pmoles/μl in sterile distilled H_2O. 0.5 μl of this stock is sufficient per 50μl transformation.

Once the cells are electrocompetent and recombineering-proficient (see previous protocol), the DNA substrate is introduced by electroporation. We use a Bio-Rad MicroPulser™ with the conditions noted above. For optimal results, proper mixing is essential and should be done as follows. Pipette 50μl of the electro-competent cells into a chilled cuvette. Add the linear substrate (volumes above). Using a 200 μl disposable pipette tip, pipette up and down several times to mix. At no time should electro-competent cells be vortexed. Use care to keep the cells in the electotransformation chamber of the cuvette before electrotransformation. Once electrotransformation is complete, quickly add the 1 ml of LB for recovery, pipetting up and down several times before finally transferring the entire volume immediately to a recovery tube. Subsequent steps (e.g. length of outgrowth) depend on the specifics of the desired recombination event. Outgrowth (recovery) times are discussed in:

1. Sawitzke, J. A., Costantino, N., Li, X. T., Thomason, L. C., Bubunenko, M., Court, C. & Court, D. L. (2011). Probing Cellular Processes with Oligo-Mediated Recombination and Using the Knowledge Gained to Optimize Recombineering. *J. Mol. Biol.*

2. Sharan, S. K., Thomason, L. C., Kuznetsov, S. G. & Court, D. L. (2009). Recombineering: a homologous recombination-based method of genetic engineering. *Nat. Prot.* **4**, 206-223.

Important controls for transformation include induced cells with no DNA and un-induced cells with DNA. Optimal electroporations give a time constant of >5.0 mSec. Lower time constants may produce recombinants but at a lower efficiency and may reduce total cell viability. Immediately after electroporation, 1 ml of L broth is added to the electroporation cuvette and cells are transferred to a sterile culture tube