Cre Electroporation Protocol

April 2015 Revision

Transient conditions:

Preparation of circular plasmid: pCAGGs-CRE

- Grow culture overnight.
- Perform a maxi prep on the overnight culture.
- Take a small aliquot of DNA from the prep and digest with enzyme that cuts more than once to confirm integrity of DNA.
- Ethanol precipitate at least 50ug circular plasmid DNA at -80^{o}C. for at least one hour (can go up to overnight.
- Perform 2 washes; one with 70% ETOH, and another with 100% ETOH.
- Air-dry pellet in hood for 30 minutes or longer until dry.
- Resuspend DNA overnight in 100ul sterile PBS.
- Run aliquot (at least 100ng/ul) on a gel to test.

Electroporation

- Grow one 10 cm dish of ES cells (approximately 5 x 10^{7} cells) for 2 days post-passage without selection drugs. (**Note: For media preparations for growth of various ES cells, please see KOMP Protocols above.)**
- Feed dish ~4 hours before electroporation.
- Trypsinize above confluent 10 cm dish of ES cells.
- Add 10 ml of ES cell media, resuspend cells and count. Use 10^{7} cells for each electroporation.
- Centrifuge, wash pellet once with PBS, centrifuge again and remove supernatant.
- Resuspend 10^{7} cells in 900 ul of room temp PBS.
- Gently mix with 100 ul (50 ug) circular plasmid and transfer to 4 mm electrode gap cuvette.
- Quickly electroporate cells at 250V and 500uF on high capacitance setting (BioRad Gene Pulser.)
- Allow cells to recover at room temp for 20 minutes in cuvette.
- Gelatinize sufficient number of 10 cm dishes.
- Dilute cells with ES cell media and plate at 3 x 10^{6} cells/plate on 10 cm gelatinized dishes. It may be necessary to pre-determine the optimum range of cells necessary to allow for ease of picking individual colonies. Plate 2-3 replicates per electroporation.
- Change media the next day with ES cell media; no selection drugs are required. Continue culturing cells with daily media changes if necessary for 7-10 days or until colonies become large enough to pick.
- Colonies are picked, trypsinized into individual 96 wells and allowed to grow until about 80% confluent before splitting for freezing, genotyping, etc. We routinely prepare at Least 2 backup frozen plates in addition to a plate for genotyping; one plate will be used to retrieve and expand the positive clones following genotyping and one is frozen as backup. Although they are fairly expensive, Thermo Scientific #3725 Matrix 96 well plates with tube inserts are useful for the frozen plates since they allow for thawing single samples.