Flp-e Electroporation Protocol

April 2015 Revision

Transient conditions:

Preparation of circular plasmid pCAGGS-Flp-e

- 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 0.4 cm 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. (If required, plates of puromycin resistant feeder cells, e.g., DR4 feeders, may be prepared ahead.)
- Dilute cells with ES cell media and plate at 3 x 10^{6} cells /plate on 10 cm gelatinized dishes. We suggest 3 replicates per electroporation if have enough cells.
- Change media the next day with ES cell media with no selection drugs.
- Begin selection 36-48 hours post-electroporation (we typically use 0.6 - 0.8 ug/ml puromycin* in ES cell media.) Maintain selection for 3-5 days. After this time, switch back to ES cell media without puromycin and continue culturing 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.

Notes:

*One source of Puromycin is Invitrogen/Gibco (A11138-02) which may be prepared as 10 mg/ml stock solution in sterile tissue culture grade water and stored in aliquots at -20^{o}C.

For each particular ES cell line, you may need to test the optimum selection concentration of puromycin. In our hands, this concentration was determined to be 0.6 - 0.8 ug/ml for JM8 ES cell lines.