

Cre Electroporation Protocol

Transient conditions:

Preparation of circular plasmid (all steps performed in TC hood)

- Ethanol precipitate 50ug circular plasmid DNA overnight at -80 Celsius. (They use either PGK-CreNLS or CAGGs-CreNLS, I will find out.)
- Perform 2 washes with 75% EtOH
- Air-dry pellet in TC hood for 30 minutes
- Resuspended DNA overnight in 100uL sterile PBS.
- Confirm quality of plasmid prep first by test cutting a small aliquot with ScaI to linearize the 7.7 kb vector.

Electroporation

- Grow 1 T75 flask of confluent ES cells (approx 5×10^7 cells) for 2 days post-passage without any selection drugs.
- Feed flask 4 hours before electroporation.
- Trypsinize confluent T75 flask of ES cells
- Add 20 mL M10G media, resuspend cells, and count
- Spin, wash pellet once with PBS, respin
- Resuspend cells in 700 ul room temp PBS
- Mix with 100 uL (50 ug) circular plasmid gently and transfer to 0.4cm (electrode gap) cuvette
- Quickly electroporate cells at 250V and 500 uF on high capacitance setting
- Allow cells to recover (sit at room temp) for 20 minutes in cuvette.
- Meanwhile gelatinize sufficient number of 10cm dishes
- Dilute cells with M10G media and plate at 5×10^6 cells/plate on 10cm gelatinized dishes. Plate at least 2 replicates per electroporation experiment.
- Change media the next day with M10G, no drugs