Flp-e Electroporation Protocol

For transient conditions:

Preparation of circular plasmid pCAGGS-Flp-e (all steps performed in TC hood)

- Ethanol precipitate 50ug circular plasmid DNA overnight at -80 Celsius.
- 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 5x10^7 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 5x10^6 cells/plate on 10cm gelatinized dishes. Plate at least 2 replicates per electroporation experiment.
- Change media the next day with M10G, no drugs
- Begin puromycin selection 36-48 hours post-electroporation (use 0.8 ug/ml puro* in M10G media). Maintain selection for 3 days.
- After 3 days switch back to M10G no drugs, and continue culturing until colonies become visible.

NOTES:

*Puromycin is from Sigma (P8833), prepared to 1mg/ml stock in sterile tissue culture grade water, 20 um-filter-sterilized, stored in aliquots at -20oC.

You may need to test the optimum concentration of puro for a particular cell line. In our hands the optimum for transient conditions was between 0.6-0.8 ug/mL for JM8-based lines. 1.0 ug/mL was too high as we recovered few or no colonies.

For subsequent manipulation of cell lines involving puromycin containing vectors, test for puro sensitivity of recovered clones upon expansion.

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For stable conditions:

Preparation of linearized plasmid pCAGGS-Flp-e (all steps performed in TC hood)

- Digest at least 60ug of plasmid with ScaI (minimum 4 hours)
- Ethanol precipitate digested DNA overnight at -80 Celsius.
- Perform 2 washes with 75% EtOH
- Air-dry pellet in TC hood for 30 minutes
- Resuspended DNA overnight in 100uL sterile PBS.
- Confirm linearization on a gel (expected band is 7.7 kb).
- 30ug of linearized plasmid is used for electoporation

Electroporation

- Grow 1 T75 flask of confluent ES cells (approx 5x10^7 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 800V and 30 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 5x10^6 cells/plate on 10cm gelatinized dishes. Plate at least 2 replicates per electroporation experiment.
- Change media the next day with M10G, no drugs
- Begin puromycin selection 48 hours post-electroporation (use 0.8 ug/ml puro* in M10G media). Maintain selection until colonies are viscible.
- After 3 days switch back to M10G no drugs, and continue culturing until colonies become visible.

NOTES:

*Puromycin is from Sigma (P8833), prepared to 1mg/ml stock in sterile tissue culture grade water, 20 um-filter-sterilized, stored in aliquots at -20oC.

You may need to test the optimum concentration of puro for a particular cell line. In our hands the optimum for transient conditions was between 0.6-0.8 ug/mL for JM8-based lines. 1.0 ug/mL was too high as we recovered few or no colonies.

For subsequent manipulation of cell lines involving puromycin containing vectors, test for puro sensitivity of recovered clones upon expansion.

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