JM8.F6 cells electroporation protocol

Before you start

- Plate SNL feeder cells
- Culture JM8.F6 C57Bl6/N ES cells (see separate protocol)
- Prepare DNA (see separate protocol)
- For all medium and reagents see separate protocol

- Change media on ES cells night before electroporation.
  Cells almost confluent
- Change media on ES cells 4 hours before electroporation
  Media yellow. Check confluency and differentiation of cells
- Pre-warm M15G media, PBS and trypsin in 37°C water bath
- Prepare cuvette for electroporation and place in hood

Things you'll need

Glass pipettes, pipette boy and plastic pipettes.
Universal tubes or 50ml falcon tubes
Electroporation cuvette
Multichannel pipette & filtered tips
Centrifuge
Haemocytometer
2x Trypsin + Glucose (see reagents protocol)
G15M media (see reagents protocol)
PBS

Electroporation:

- Remove media from dishes containing ES cells
  1x10cm dish of JM8.F6 cells yields sufficient cells for 3 electroporations
- Wash each dish twice with pre-warmed PBS (~7ml per dish).
  Work quickly adding PBS to the sides of all dishes, drain all dishes and repeat.
- Add 1.5ml of '2 x Trypsin + glucose' per dish. Incubate for 15 minutes
  Do not stack dishes in incubator. Agitate plate mid-way through incubation
- Add 8 ml G15M media to each dish and disperse clumps by mixing 3x.
- Place cell suspension in a falcon or universal tube. Spin for 3 minutes at 1300 rpm
- Carefully remove media with a pipette ensuring pellet is not disturbed.
- Gently add 10ml room temperature PBS, disperse only once, look for clumps, if clumpy disperse once more. If trypsinising multiple plates, combine cell suspensions into fewer tubes at this point.
- Use 100µl from one tube for cell count using the haemocytometer. Add 900ul PBS (1:10 dilution) and count.

Ignore large feeder cells, count only small ES cells. Remember to include dilution factor and total cell volume in cell count calculation.

- Spin tubes again at 1300rpm for 3 minutes
- Calculate amount PBS required for final volume :

Estimate that $1x10^7$ cells = 25µl volume
Cell count required for EP = $1x10^7$ cells in a final volume of 70ul
Therefore; PBS to be added to $1x10^7$ cells for a single EP = 70µl - 25µl = **45µl**
Adjust calculations for the number of EP's being performed.

- Carefully remove PBS wash from the cell pellet and add most of the final volume of PBS required, gently resuspend the cells and check the actual volume, make up to final volume if required.
- Set electroporator (700V, 400Ω, 25µF)
- Add 70µl cell suspension to the DNA.
- Mix cells and DNA *gently*.
- Transfer the cell/DNA suspension to the cuvette
- Check for air bubbles
  Tapping the cuvette on the bench helps remove bubbles.
- Electroporate.
  The time constant should be between 100-150µsecs.
- Leave cells to rest for 20 min at room temperature
  Keep the cells covered and in the hood.
- Label feeder plates with the electroporation number (EP) number, well location & gene name.
  Always double check labelling.
- Remove media from plates.
- Add 10ml of G15M media to each plate
- Transfer DNA/cell suspension to the correctly labelled plate
- Evenly distribute cells
  swirl clockwise, then anticlockwise, forward and back
- Incubate
- Next day, check for growth and media change to M15G + 100ug/ml G418