JN8.N4 cells electroporation protocol

Before you start

- Culture JM8.N4 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 M10G medium, 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)
M10G medium (see reagents protocol)
PBS

Electroporation:

- Remove medium from flasks containing ES cells
  4xT150 flasks of JM8.N4 cells yields sufficient cells for ~20 electroporations
- Wash each flask twice with pre-warmed PBS (~20ml per flask).
  Work quickly adding PBS to the flask, drain and repeat.
- Add 5ml of '2 x Trypsin + glucose' per flask. Incubate for 10 minutes
  Do not stack flasks in incubator. Agitate flask mid-way through incubation
- Add 10 ml M10G medium to each flask and disperse clumps by mixing 3x using a 10ml pipette (*the bigger ones don't work as well*)
- Add a further 10ml M10G to each flask to neutralise trypsin fully.
- Place cell suspension in a falcon or universal tube. Spin for 3 minutes at 1300 rpm
- Carefully remove media ensuring pellet is not disturbed.
- Gently add 10ml room temperature PBS, disperse only once *(be extremely gentle)*, look for clumps, if clumpy - disperse once more. If trypsinising multiple flasks, 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.

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 = $5x10^6$ to $1.5x10^7$ cells in a final volume of 70ul
(*we use $1.5x10^7$ most of the time, but less if we don't get enough cells from the expansion)*
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*.
- Quickly 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 gelatin from plates.
- Add 10ml of M10G 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 M10G + 100ug/ml G418