Mouse ES/iPS cell culture

Reagents

- Irradiated feeder MEFs (GlobalStem cat# 6001G)
- 0.05% Trypsin/EDTA: Invitrogen cat# 25300
- DMSO: Sigma cat# D2650-100ML
- Characterized FBS: Hyclone cat# SH30070.01
- FBS: Invitrogen cat# 16000-044
- LIF: Millipore cat# ESG1107
- 0.1% geletin: Millipore cat# ES-006-B
- 1x PBS: Invitrogen cat# 14190-136
- DMEM: Invitrogen cat# 11965-118
- Pen/strep: Invitrogen cat# 15140-155
- L-glutamine: Invitrogen cat# 25030-156
- Non essential amino acid (NEAA): Invitrogen cat# 11140-050
- Knockout Serum Replacement (KOSR): Invitrogen cat#10828
- Beta-Mercaptoethanol (BME): Sigma cat# M7522 -100ml

Fibroblast media (500 mls):

DMEM (450 ml)
10% Invitrogen FBS (50 ml)
Pen/strep (5ml)
L-glutamine (5ml)
NEAA (5 ml)

Mouse ES media (500 mls):

Component	Stock Conc	Final Conc	amount
DMEM			425 ml
KOSR		15%	75 ml
L-glutamine	100 mM	1 mM	5 ml
BME	14.3 M	0. 1 mM	3.5 ul
NEAA	10 mM	0.1 mM	5 ml
Pen/strep	100x	1x	5 ml
LIF	10^7 U/ml	1000U/ml	50 ul

Cell Freezing Media:

90% Hyclone FBS
10% DMSO

Thawing ES/iPS cells

1. Coat cell culture plate with 0.1% gelatin for 30 min and aspirate. Seed $1x10^6$ feeders/plate in fibroblast media on 10-cm plates and culture overnight.

2. Thaw 1 vial of ES/iPS cells quickly in 37C water bath and dilute into 9 mls of warm ES media.

3. Spin at 0.2 rcf for 5 min; aspirate media and resuspend in 10 ml of ES media.

4. Aspirate fibroblast medium from feeder plate and transfer the ES/iPS cell suspension onto the feeders.

5. Incubate overnight and change media the following day.

Passaging ES/iPS cells

1. Refresh media on newly thawed cells EVERYDAY until ready to be split (This may take 2-5 days)

2. Day before splitting cells, seed feeders as described above.

3. When cells are confluent, aspirate media, wash 1x with 1xPBS, add 2 ml 0.05%Trypsin-EDTA and incubate at 37C for 2-5 min.

4. Dislodge and resuspend trypsinized cells with 8 mls ES media; spin cells at 0.2 rcf for 5 min and aspirate media.

5. Resuspend cells in fresh ES media to achieve a split ratio of 1:5-1:10 onto plates with new feeders. Using this split ratio, mouse ES/iPS cells are usually passaged every 2-3 days.

If a large number of cells is not needed for experiments, 6-well or 12-well plates are usually used for maintaining ES/iPS cells.

Freezing ES/iPS cells

1. When cells are healthy and subconfluent, dislodge cells using trypsin as described above.

2. Resuspend cells in 10 mls ES media and spin at 0.2 rcf for 5 min and aspirate media.

3. Resuspend cell pellet with Freezing Media at 2mls per 1 well of a 6-well plate of cells.   Transfer 1 ml of cell suspension to each cryovial and store immediately in cell freezing container at -80C.

4. The next day, move vials to liquid nitrogen for long term storage.