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% gelatin: 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):

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock Conc</th>
<th>Final Conc</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM</td>
<td></td>
<td></td>
<td>425 ml</td>
</tr>
<tr>
<td>KOSR</td>
<td></td>
<td>15%</td>
<td>75 ml</td>
</tr>
<tr>
<td>L-glutamine</td>
<td>100 mM</td>
<td>1 mM</td>
<td>5 ml</td>
</tr>
<tr>
<td>BME</td>
<td>14.3 M</td>
<td>0.1 mM</td>
<td>3.5 ul</td>
</tr>
<tr>
<td>NEAA</td>
<td>10 mM</td>
<td>0.1 mM</td>
<td>5 ml</td>
</tr>
<tr>
<td>Pen/strep</td>
<td>100 x</td>
<td>1x</td>
<td>5 ml</td>
</tr>
<tr>
<td>LIF</td>
<td>$10^7$ U/ml</td>
<td>1000U/ml</td>
<td>50 ul</td>
</tr>
</tbody>
</table>

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 37°C 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 37°C for 2-5 min.

4. Dislodge and resuspend trypsini zed 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.