C57BL/6N-PRX-B6N #1 mES cells

Description
C57BL/6N-PRX-B6N #1 mES cells were derived from day 3.5 blastocysts of strain C57BL/6N and expanded on primary mouse embryonic fibroblasts (MEFs) in medium containing 1000U/mL LIF and cultured at 7.5% CO2 in humidified air at 37°C. Pluripotency was confirmed by microinjection of 12-14 cells into BALB/cJ, B6(Cg)-Tyrc-2J, and C57BL/6J blastocysts, as well as immunocytochemistry using antibodies reactive with SSEA-1 and Oct3/4 (see Figures 1 and 2).

Quality Assurance
In the absence of antibiotics, the cells were thawed and found to be free of bacterial and fungal contaminants. They tested negative for the presence of mouse pathogens and Mycoplasma species using the Infectious Microbe PCR AmplifiCation Test (IMPACT) system. The cells double in number every 10-12 hours. Random thawing of cryopreserved vials consistently yields greater than 80% viable cells. Giemsa banding with Trypsin and Wright (GTW) stain of 20 cells shows 19 having a normal male karyotype. For additional information on the ES cell line, go to www.primogenix.com.
Culturing PRX-B6N mES Cells

**Culture medium, final volume: 200 mL**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Final concentration</th>
<th>Vendor</th>
<th>Catalogue number</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMDM (Iscove’s DMEM)</td>
<td>154 mL</td>
<td>80%</td>
<td>HyClone</td>
<td>SH30259.02</td>
</tr>
<tr>
<td>FBS - ESC qualified</td>
<td>40 mL</td>
<td>20%</td>
<td>HyClone</td>
<td>SH30070.03 E</td>
</tr>
<tr>
<td>NEAA (MEM) 100x</td>
<td>2 mL</td>
<td>1x</td>
<td>HyClone</td>
<td>SH30238.01</td>
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<tr>
<td>L-glutamine [200mM]</td>
<td>2 mL</td>
<td>2 mM</td>
<td>HyClone</td>
<td>SH30034.01</td>
</tr>
<tr>
<td>Penicillin/Streptomycin</td>
<td>2 mL</td>
<td>1x</td>
<td>HyClone</td>
<td>SV30010</td>
</tr>
<tr>
<td>β-Mercaptoethanol</td>
<td>3 µL</td>
<td>0.2 mM</td>
<td>Sigma</td>
<td>M-7522</td>
</tr>
<tr>
<td>ESGRO- mouse LIF</td>
<td>20 µL</td>
<td>1000U/mL</td>
<td>Millipore</td>
<td>ESG1107</td>
</tr>
</tbody>
</table>

Mix all ingredients in the top of a 2µm PES filter unit and filter-sterilize. Store at 4°C. Discard any unused medium after 10 days.

**Cryopreservation Medium**

80% culture medium with 10% FBS and 10% DMSO, prepare in quantities that can be used the same day.

**Thaw and culture of mES cells**

1. **Day 0.** Plate irradiated MEF feeder cells one day in advance of thawing and plating the mES cells. When plating MEFs from a vial containing 5 x 106 cells, they will cover 1 x T75 tissue culture flask, 2 x T25 tissue culture flasks or 1 x 6-well plate.
2. **Day 1.** Thaw one vial of mES cells rapidly in a 37°C water bath. Spray vial with 70% EtOH and wipe with Kimwipe. Transfer contents to a the T25 flask containing MEF monolayer equilibrated 1-2 hours with 5mL ES cell culture medium prior to thawing the ES cells.
3. **Day 2:** Examine the cells under phase-contrast microscopy. Colonies should be visible and nicely distributed on the MEF feeder-cell layer. Depending on the size and density of the colonies, either replace the culture medium or passage the cells to a prepared T75 tissue culture flask.
4. **Day 3:** Examine cells under the microscope. If they were not passaged on Day 2, passage them today. If they were passaged on Day 2, depending on the size and density of the colonies, either replace the medium or trypsinize the culture for cryopreservation, electroporation, or expansion.
5. **Subculturing mES cells at a ratio of 1:2 to 1:3:** Remove and discard the ES cell culture medium. Rinse the adherent mES cells with small amount of 0.05% Trypsin/0.02% EDTA to remove traces of medium and serum. Add a quantity of 0.05% Trypsin/0.02% EDTA to cover the cell layer, usually 1/3 the amount used to culture the cells. Incubate cells with 0.05% Trypsin/0.02% EDTA for 5-7 minutes, watching for cell lift using the inverted microscope. Collect the cells in 0.05% Trypsin/0.02% EDTA and then rinse the culture dish with an equal amount of ES cell medium. Collect both aliquots (trypsinized cells and the rinse) in one tube. Centrifuge and suspend the pellet in either freezing medium for cryopreservation or in culture medium and transfer to a new culture vessel that contains a monolayer of MEFs. For best results, limit the time that the cells are in culture and change the medium daily and subculture every other day.

**Germline Transmission Results**

Following the injection of 12-14 ES cells into B6-albino, B6-black, or BALB/c blastocysts:

- ~50% of injected blasts yield live pups.
- ~50% of the pups are chimeric.
- ~90% of chimeras are male.
- ~75% of high color-coat chimeras go germline.
- Germline transmission was confirmed for this line ES cell line even after a targeted clone was subjected to a second electroporation to remove the selectable marker.
- Germline chimeras have been generated by injection into 4-cell embryos.