Colony Picking

Depending upon the cell line and the media used, ES cell colonies are generally ready for picking 5–10 days after electroporation. The most suitable colonies to select are those that appear rounded or oval in shape, with a phase contrast bright edge and often a dark necrotic center. Differentiated colonies are flat and often surrounded by fibroblast like cells that form cobblestone-like structures. These cells should be avoided when selecting cells. ES cells can be picked onto either gelatinized plates or a PMEF feeder cell layer depending upon the ES cell line used. If gelatinized plates are preferred, please disregard the use of feeder cells as described in the procedure below.

Materials & Reagents required:

- 96-well plate(s)
- ES Cell Medium:
  - DMEM (Cat. No. SLM-220-B)
  - 15-20% Fetal Bovine Serum (Cat. No. ES-009-B or ES-011-B)
  - 1% Nucleosides,100x (Cat. No. ES-008-D)
  - 1% Penicillin-Streptomycin, 100x (Cat. No. TMS-AB2-C)
  - 1% Non-Essential Amino Acids, 100x (Cat. No. TMS-001-C)
  - 1% L-Glutamine Solution, 100x (Cat. No. TMS-002-C)
  - 1% 2-Mercaptoethanol, 100x (Cat. No. ES-007-E)
  - 1000 units/mL ESGRO mLIF Supplement (Cat. No. ESG1106 or ESG1107)
- ES Cell Medium supplemented with Neomycin (150 µg/mL) or Hygromycin B (50 µg/mL)
- Incubator, 37 °C/5% CO_2
- Pipette
- PMEF Feeder cell coated 24-well plates
- 0.05% Trypsin-0.53mM EDTA (Cat. No. SM-2002-C)
- Microscope

Procedure:

1. The day before picking ES cell colonies, coat an appropriate number of 24-well plates with PMEF Feeder Cells or gelatin.
2. Prior to selecting ES cell colonies, ensure that you are wearing gloves, a gown and face mask. All surfaces including the microscope, bench, tip boxes and pipette should be wiped with ethanol prior to use.
3. Inspect the ES cell cultures at 4x magnification. Colonies selected for picking should be spaced well enough apart to ensure no contamination from surrounding colonies. When a desired colony is found, circle the colony with a pipette tip to loosen the surrounding fibroblast layer. With the pipette set to a volume of 15 µL, scrape the colony with the pipette tip to dislodge the colony, then aspirate (the colony is often visible inside the tip). Transfer the picked colony to an empty well in a 96-well plate.
4. Continue picking and transferring ES cell colonies to fresh wells using a new tip each time, until a suitable number is picked. Clones are often picked in batches of 48 cells to prevent fatigue.
5. Add a single drop of Trypsin to each well and incubate at 37 °C for 2 minutes. During this period, replace the PMEF Feeder Cell Media in the 24-well plates with 500 µL of ES cell medium.
6. Disperse each ES cell colony in the 96-well plate by using a pipette to break up each colony, avoiding excessive foaming. Transfer the suspension to the 24-well plate (including foam) containing 500 µL of ES cell medium, using a fresh tip for each well.
7. When all the colonies are transferred, mix each well using a clean pipette tip set to 400 µL. Incubate at 37 °C and 5% CO_2. Feed every day with medium supplemented with Neomycin (150 µg/mL) or Hygromycin (50 µg/mL) antibiotics.
8. New colonies should be evident within a few days. If the colonies are too close in proximity to each other, disperse them using a 1 mL pipette tip to break up the colonies and spread the cells (Trypsin is not required as colonies break up very easily). Each well should be evenly covered with colonies before harvesting.
9. Continue changing the ES cell medium every day until a good coverage of colonies in each well is achieved (typically 7–10 days).