

DERIVATION of TROPHOBLAST STEM CELL

The protocols and reagents described here are for the maintenance and derivation of trophoblast (TS) cell lines.

Original reference with some modifications:

Robertson, E.J. (1987) Embryo-derived stem cell lines. In Teratocarcinoma and Embryonic Stem Cells: A Practical Approach E.J. Robertson Oxford, UK: IRL Press

Hogan, B, Beddington, R, Constantini, F, Lacy, E. (1994) Manipulating the Mouse Embryo Cold Spring Harbor, NY: Cold Spring Harbor Press

Tanaka, S, Kunath, T, Hadjantonakis, A-K, Nagy, A, Rossant, J. (1998) Promotion of Trophoblast Stem Cell Proliferation by FGF4 Science 282:2072-2075.

Collection of embryos

TS cell lines described here are derived from 3.5dpc mouse blastocysts. Mice of interest are mated either through natural mating or super-ovulated. At the required time point (E3.5) the uterus is flushed and 3.5dpc blastocysts are collected and transferred to KSOM until plating.

TS derivations

Preparation of MEFs: one day before flushing prepare several 4-well plates of mitotically-inactivated MEFs (E12.5) plated at 5×10^4 cells per well of a 4-well plate in TS medium. This is the same density used to culture TS cells. [TS cell lines have also been derived from blastocysts in the absence of EMFI cells, but with 70%EMFI-CM].

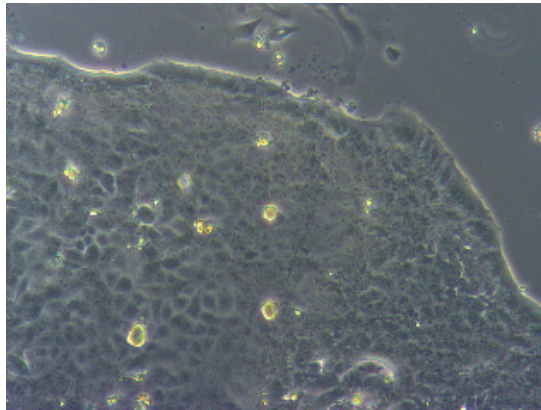
Plating blastocysts: Replace the TS medium on the MEFs with TS medium supplemented with FGF and Heparin as described in the trophoblast stem cell protocol (500µl per well). Using sterile conditions, place one blastocyst per well in the 4-well plates containing TS + F4H medium and culture at 37°C, 5% CO₂.

Time line of hatching:

- The blastocysts should hatch and attach to the wells in 24 to 36 hours (day 2).
- On day 3, a small outgrowth is formed from each embryo. Feed each culture with 500µl fresh medium.
- Day 4 is the day that the outgrowth is usually disaggregated. However, this will depend on its size; it should be smaller than the size of the outgrowth disaggregated for ES cell line derivation. The ideal size for TS cell derivation is illustrated on p. 87, Figure 4b of Robertson reference above, or on p.267 Figure 26 in Hogan et al reference. Larger outgrowths will also work, but with less efficiency.

Disaggregation of hatching: Once suitable outgrowths have been chosen embryos are ready to be passed. Remove the medium and wash the cells with PBS. Aspirate the PBS, add 0.1% trypsin/EDTA (100µl), and incubate for 5 minutes at 37°C, 5% CO₂. Using a P2 pipette or a drawn Pasteur pipette disaggregate the clump by pipetting up and down vigorously until the outgrowth is reduced to a small clump of cells. Immediately stop the trypsinization by adding 100µl TS media supplemented with FGF and HEPARIN and transfer the entire volume to a new well of a 4-well plate containing MEFs. Each well should be treated clonally and passed into an individual new well. Return cells to incubator and change medium every 2 days until TS colonies appear.

Passing TS cells: Between days 6 and 10 (highly variable) TS cell colonies will begin to appear. They look like flat, epithelial sheets with a distinctive colony boundary (Tanaka et al).



Some differentiation will be observed at the edges of the colonies. This is normal; they are most often giant cells and other unidentified cell-types that may be between a stem cell and giant cell phenotype.

When colonies reach approximately 50% confluence cells are ready to passage. To passage cells aspirate the medium and wash TS cells with PBS (500 μ l). Aspirate the PBS, add trypsin/EDTA (100 μ l), and incubate for 5 minutes at 37°C/5% CO₂. Stop trypsinization by adding TS + FGF and Heprin (400 μ l) and pipetting up and down to get a near single-cell suspension. Transfer cells to one well of a 6-well plate. This first passage is crucial; this is the most likely time for the culture to differentiate.

Maintenance: Feed the cells every two days with 3 ml TS media supplemented with FGF and Heprin. Follow the Trophoblast Stem Cell protocol provided on our website to expand cells up for freezing. After one or two more passages cells can be frozen and stored.

Updated: Aug 2015