SOP SCB 015_v1	Title: **Culture and maintenance of hPSC lines on Matrigel.**

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OBJECTIVE

Describe the maintenance and culture of hPSC (Human Pluripotent Stem Cells) lines on Matrigel including changing medium and splits.

MATERIALS

- Phosphate buffered saline without magnesium and calcium (PBS Cultek, Ref. 17-516F)
- Ultrapure EDTA 0.5M (Invitrogen, Ref. 15575-020)
- mTeSR1 complete kit (StemCell Technologies, Ref. 85850)

PROCEDURES

NOTE: All steps must be performed under aseptic conditions within a class II safety cabinet.

Medium change:

1. Prepare the volume of mTeSR1 complete **(**see **SOP SCB 003_v1)** required and let it warm at room temperature. Calculate 2mL per well of a 6-well plate or 10 mL per 100mm plate.
2. Remove the plate or plates corresponding to one line and place it inside the hood previously conditioned **(**see **SOP SCB 001_v1)**.
3. Place a Pasteur pipette in the vacuum pump hose and continue to remove the medium of each well or plate.
4. Using a pipette add fresh medium to each well or plate.
5. Return the plate or plates to the incubator at 37ºC and 5% CO2 once the medium is changed.

Split:

1. When the plate or well is about 80-90% of confluency, separate 4ml or 15ml of medium depending on if it is a well or a plate.
2. Transfer the plate to the previously conditioned cabin (see **SOP SCB 001_v1**). and remove the medium.

3. Wash with 1-2 mL of PBS if it is a well of a 6-well plate or 4-5 mL if it is a 100mm plate.
4. Add 1 mL of EDTA 0.5mM solution (Diluted in PBS and Filter sterilized) if it is a well of a 6-well plate or 3 mL if it is a 100mm plate and incubate at 37ºC for 2 minutes.
5. Remove the EDTA 0.5mM solution.
6. Lift the iPSC colonies using a 1000µl micropipette. For a well set a 500 µl volume approximately and for the 100mm plate set a 1000 µl volume approximately. To avoid excessive disaggregation of the colonies, if preferable do the lifting in several steps. Use 2mL or 5 mL of the medium separated for the well or plate respectively. To lift and recover colonies start flashing from the proximal part of the plate or well to the distal part until is all empty of colonies (This could be checked in the microscope). (Figure 1).

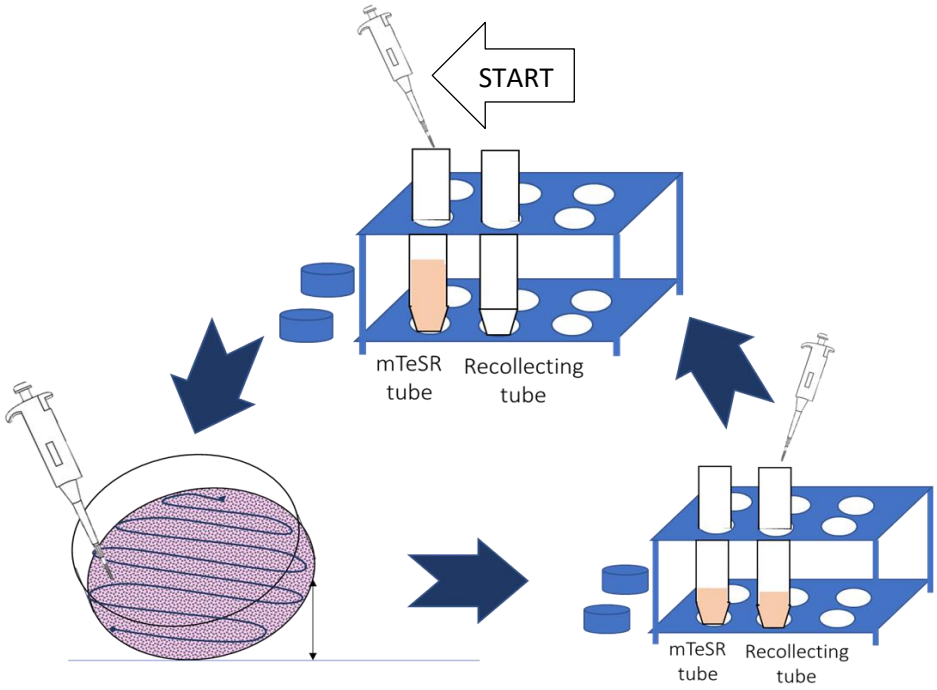

Figure 1. Schematic representation of the splitting process.

7. Once the colonies are recollected, remove the excess Matrigel of a 6-well plate or 100mm plate previously prepared with a Matrigel coating (see **SOP SCB 004_v1**) and transfer the cell's suspension obtained to it. Usually, the hPSC colonies are lifted from a well of a 6-well plate, in that case, transfer 1/6 of the cellular suspension to a 6-well plate and add 2mL of the medium. If it is to a 100 mm plate, add all the cellular suspension and complete with the medium until reaching a final volume of 10mL.
6. Place the plate in the incubator at 37ºC and 5% CO_2 and move it in a crossway several times to homogenously distribute the clumps in the well or plate.

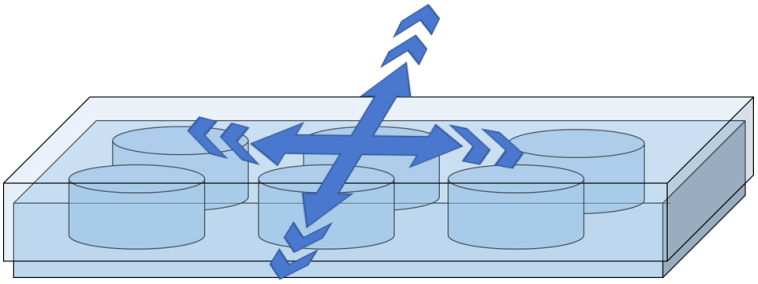

Figure 2. Crossway movement to homogenous distributions of the clumps.