[Protocol 1.01.0] Maintenance of iPSC

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Protocol 1.01.0 Maintenance of iPSC cells
Version: 1.0 (20.12.2021)

1. Introduction and Purpose

This protocol describes the culture and maintenance of established iPSC cell lines in divers media on Geltrex or Matrigel coated culture vessels.

Note: To avoid cross contamination for each line the following rules should be applied:
• Medium bottles or medium aliquots should be separated and appropriately labeled with the respective cell line name.
• Different cell lines must be handled separately while feeding/splitting etc. and placed indistinct locations within the incubator.

2. Culture monitoring
1. iPSC cultures should be checked frequently under the microscope regarding their morphology, presence of spontaneous differentiated cells and confluence. Based these observations, it has to be determined if the cultures require further action (e.g. removal of differentiated cells, passaging). Refer to reference images below
2. If cultures require passaging, refer to protocol 1.2 or 1.3.
3. If cultures require removal of differentiated cells, please check the next session or in case of sorting, refer to protocol 1.4.

3. Removal of spontaneous differentiated cells
Materials and Equipment:
• Stereo microscope build in a biological safety cabinet
• Scraping tool (self-made *see appendix for images below*)

1. Use a stereo microscope in a biological safety cabinet to visually identify regions of differentiation and remove these regions by scraping with a scraping tool.
Note: This selection should not exceed 20 % of the well if the culture is of high quality.
2. Perform a medium change described next to wash way floating differentiated cells. Before changing medium, an optional washing step using pre-warmed PBS or KO-DMEM F12 can be performed. This step helps especially if many differentiating cells have to be removed from cultures.

4. Medium change
Media and Reagents: Medium according to the species (*see protocol 1.7, 1.8, or 1.9*)
Materials and Equipment: Coated culture vessels (*protocol 1.5 or 1.6*), 5 ml, 10 ml pipettes, Aspiration pump
Pre-warm the medium at 37°C in a water bath for 5 min or equilibrate the medium to room temperature (22° ± 2°C) by placing outside the fridge for at least 10 min.
Note: only pre-warm the amount of medium needed to prevent degradation of the growth factors

Feeding Regime:
iPSCs grown in optimal media generally require daily medium change for optimal growth. Some iPSC lines will tolerate occasional double feeding (adding twice the required volume of medium). For instance, it is possible to perform a double feed on a Friday, with the next medium change on Sunday (meaning the doubled volume of required medium should be enough to change the medium two days later). However, it is not recommended to either go longer than 1 day without a medium change, or to feed the cultures every other day continuously.

The volume of media is tissue culture vessel dependent. Recommended volumes of maintenance media per tissue culture vessel are shown in the table below.

Culture Vessel		Surface Area [cm2]*	Total volume [ml]
6 well plate	1 well	~9,6	2
	6 wells	-	12
12 well plate	1 well	~4	1
	12 wells	-	12
24 well plate	1 well	~2	0,5
	24 wells	-	12
96 well plate	1 well	~0,35	150 µl
	96 wells	-	14,4
T25 flask	-	25	5
T75 flask	-	75	25

*Exact sizes are manufacturer/type dependent

1. Remove the medium from the wells using an aspirator pipette.
2. Aseptically add fresh medium by gently adding to the side of the well or flask using a 5 ml or 10 ml pipette.
3. Place cells in the incubator at 37°C and 5% CO2 or hypoxic conditions (5% O2).

Note: Don't let the cells dry out when changing more than one well in parallel.

Relevant applicable documents:
Protocol 1.02.0 Chemical passaging of iPSC using EDTA
Protocol 1.03 Passaging of iPSC into single cells using TrypLE/Accutase
Protocol 1.04.0 Sorting of differentiated cells
Protocol 1.05 Geltrex coating of culture vessels
Protocol 1.06 Matrigel coating of culture vessels
Protocol 1.07 Preparation of E8 medium
Protocol 1.08 Preparation of mTeSR1 medium
Protocol 1.09 Preparation of stemMACS/UPPS medium

Screenshot_2021-12-20_at_10.29.40.png

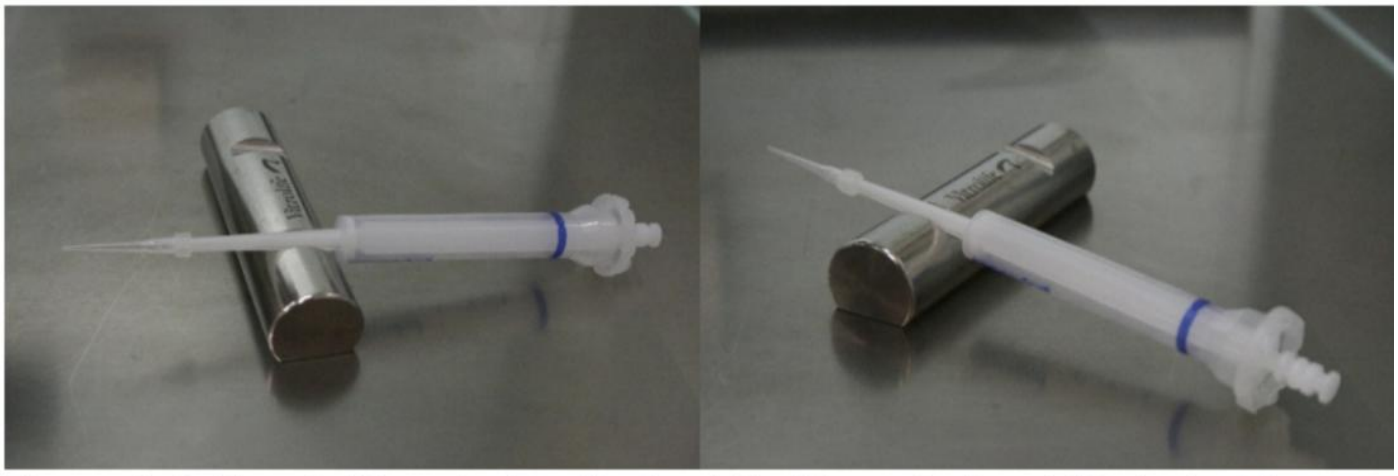

Figure 1: Self-made "Scraping Tool" - Combitip (Eppendorf) and 10 µL pipet tip

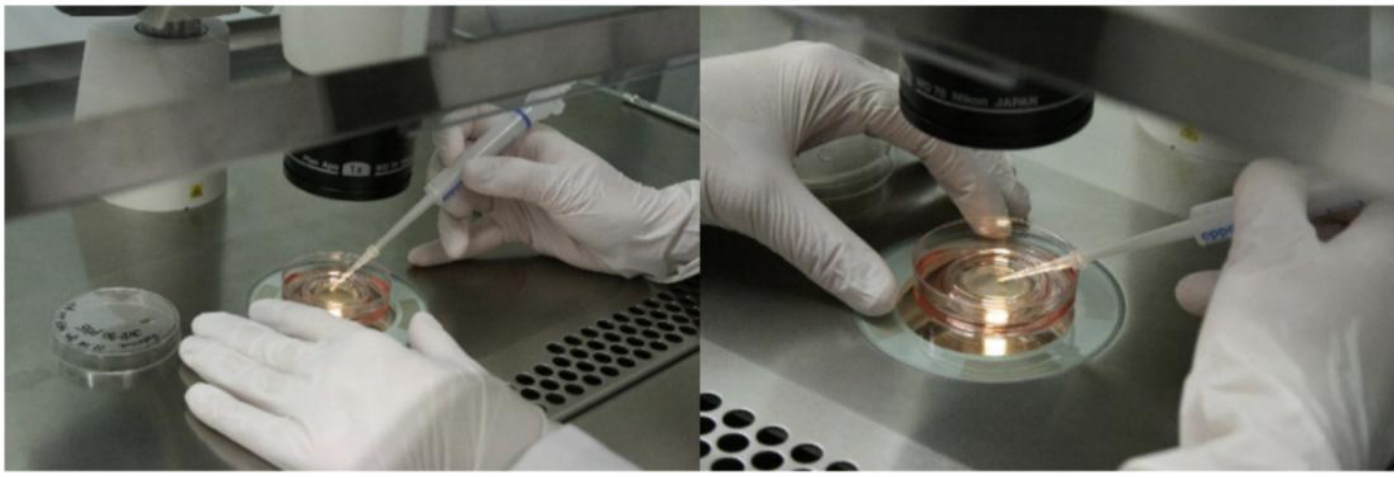

Figure 2: Scraping Tool in use.