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Manual picking and passaging of hPSC colonies

In 1 collection

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CorEuStem

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We use this protocol and it's working

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Abstract

This protocol describes picking and expansion of many colonies at once (manual clump passaging) and the picking of single colony (positive selection).

Guidelines

Depending on the growth characteristics of specific hPSC line, cells must be passaged every 5-7 days in order to maintain log phase of growth and avoid induction of differentiation.

Recommended split ratio for established cultures is around 5 colonies per 10 cm^2 surface area (i.e. 5 colonies per well of a 6-well plate (or 35 mm dish) or 10 colonies per 60 mm dish.

Materials

LABORATORY EQUIPMENT AND CONSUMABLES

Use sterile material

- 1/5/10 mL serological pipettes
- 15/50 mL conical tubes
- 200/1000µL tips and micropipettes
- Cell culture treated plastic vessels of choice e.g. 24, 12 or 6-well plates, 10cm dishes)
- Syringes (1mL) and needles (23 to 25G) or stem cell knife (see examples in pictures below)

1 mL syringe and needles (23G and 25G)

1 mL syringe and needles (23G and 25G)

Stem Cell Knife

- Aspirator pump with disposable pipette
- Class II Biosafety Cabinet
- Microscope, if available Stereo Microscope

Lynx (vision engineering)

Makrolite 4K (Vision engineering)

Different models of Stereo Microscopes

MEDIA AND REAGENTS

hPSC culture conditions and survival factors choice depend on hPSC line and individual lab practices. For options refer to protocols:

Maintenance of hPSC

Coating of tissue Culture Vessels for hPSC

Safety warnings

Manual clump passaging is recommended for colonies grown on vitronectin* or on feeder cells since their surface adhesion is less strong than when cultured on matrigel, geltrex or laminin.

1 Prepare vitronectin-coated vessels according to how many clones are to be picked, refer to protocol **Coating of tissue Culture Vessels for hPSC**

STEP CASE

Manual clump passaging 12 steps

Picking and passaging of many colonies at once

2 Pre-warm the required volume medium (refer to **Table 1**) at Room temperature for 00:30:00 in 50 mL sterile Falcon tubes. 30m

	A	B	C	D
	Culture Vessel		**Surface Area** [cm2]*	**Total volume**[ml]
	6 well plate	1 well	~9,6	2
	35 mm dish	1 dish	~9.6	2
	60 mm dish	1 dish	~20	4
	12 well plate	1 well	~4	1
	24 well plate	1 well	~2	0,5
	24 well plate	24 wells		12

Table 1. volume of medium to be prepared according to culture format

3 Remove the culture vessel containing the cells from the incubator.

4 Visually inspect the quality of the hiPSC culture under a stereomicroscope. For assesment of culture quality see **Reference pictures of hiPSC cultured in defined conditions**. If needed remove the differentiated regions as described in protocol: **Maintenance of hPSC** 15m

5 Aspirate the medium containing floating and/or differentiated cells. 1m

6 Add the appropriate volume of hPSC medium, refer to **Table 1** for recommended volumes. 2m

7 Using a syringe with a needle or a stem cell knife cut about 5 colonies/10cm^2 surface area into small pieces (*i.e. 5 colonies per well of a 6-well plate (or 35 mm dish) or 10 colonies per 60 mm dish*). 15m

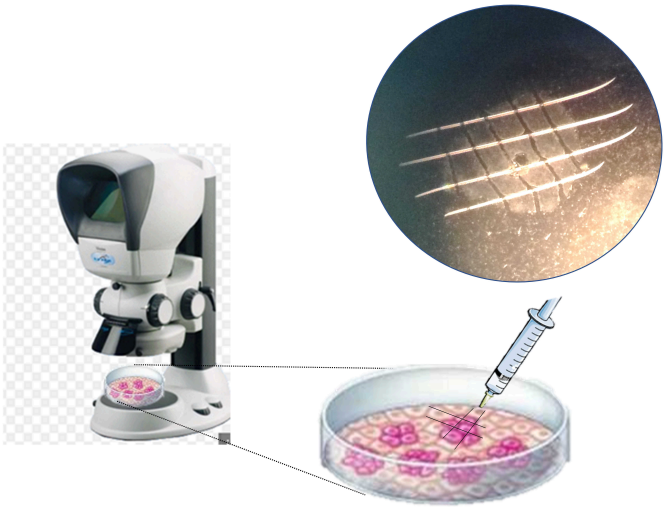

Figure 1. Schematic of the mechanical cutting of a colony from IMAGINi004-A iPSC line

8 Use a 200µL pipette with volume adjusted to 100µL to gently dislodge the pieces of colonies with the liquid stream from the pipette. 5m

9 Aspirate the residual coating solution from the destination culture vessel. 1m

10 Transfer the medium containing the pieces of colonies to the destination culture vessel using 1000µL pipette with volume adjusted to 1000µL. 2m

11 If needed adjust the volume of medium in the destination dish to the total volume. Refer to **Table 1** for recommended volumes. 2m

12 If necessary to repeat, add additional media to the source culture vessel and repeat from step 7 to 14 as many times as necessary, depending on the number of new dishes required.

13 Incubate the vessels at 37 °C and at [M] 5 % volume CO_2.

Protocol references

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