Huntington Disease iPSC Submitter Lines:

Protocol for Freezing/Thawing iPSC Lines

Thawing hESC/iPSC

• Remove hESC/iPSCs from liquid nitrogen storage tank.

• Thaw cryovial by gently swirling in waterbath until only a small ice pellet remains, being careful not to completely submerge the cryovial under water.

• Spray with 70% IPA (or 70% EtOH)

• Very gently, pipet cells from the vial into a 15ml conical centrifuge tube.

• Slowly, add 9.5ml media dropwise to reduce osmotic shock. While adding media, gently mix the cells in the tube (by gently tapping the tube with a finger).

• Centrifuge 1000rpm for 5 minutes.

• Wash cells by resuspending with 3ml media.

• Centrifuge 1000rpm for 5 minutes.

• Resuspend in 2ml and add 0.5ml per well of a 4 well plate that has MEFs already plated on it.

• Change media daily, however, it may take 2 weeks before cells are ready to be expanded.

iPS/hES Medium

KO DMEM-F12	Invitrogen 12660-012	400 ml
KOSR (aliquots at -20°C)	Invitrogen 10828-028	100 ml
NEAA (up to 6 months at 4°C)	Invitrogen 11140-050	5 ml
Glutamax (aliquots at -20°C) 200 mM L-glutamine in 5 ml aliquots	Invitrogen 35050-061	2.5 ml
bFGF	Invitrogen 13256-029	100ng/ml final concentration
βME (Stored at 4°C)	Invitrogen 21985-023	7 μl of βME to 5 ml of 200 mM l-glutamine – use stock for 1 wk post thaw and store at 4°C

- resuspend FGF in stock vial using KOSR, then transfer to DMEM
- filter sterilize
- media good for 2 weeks

Freezing hESC/iPSC

Cell Freezing Media: Cryopreservative Medium

Final Concentration	***Amount for 10ml Stock Solution***
60% DMEM-F12	6ml
20% defined FBS	2ml
20% DMSO (do not filter sterilize)	2ml

Resuspension Medium

Final Concentration	***Amount for 10ml Stock Solution***
80% DMEM-F12	8ml
20% defined FBS	2ml
10 μM Rock Inhibitor	10μl of 10mM Rock Inhibitor

(20 μl of 10 mM Rock inhibitor in a 10 ml stock solution. Combination with the cell freezing media results in a final concentration of 10 μM.)

FBS (Hyclone) – Fisher SH30070 03

DMSO (5ml ampoules) – Sigma D2650

ROCK Inhibitor- Cayman 10005583

• One hour before freezing add Rock inhibitor to the conditioned medium to a final concentration of 10 μM.

• Collagenase cells for approximately 7 minutes at 37 °C (until edges of colonies are curling up).

• With a 5 ml pipet, gently pipet and scrape colonies from plate. Add cell suspension to a 15 ml centrifuge tube and GENTLY break up colonies. It is important to be gentle in this step as “chunkier” colonies will thaw out better than single cells. Ideally, colonies meant for freezing are left slightly larger than they would be for splitting.

• Spin 5 minutes at 1000 rpm.

• Resuspend pellet (gently) in 3 ml ES media to wash away collagenase.

• Spin 5 minutes at 1000 rpm.

• Resuspend pellet (again, gently!) in 0.25 ml Resuspension Medium per vial. (This is one half the final volume required for freezing.)

• Dropwise, add an equivalent volume (0.25 ml per vial) of Cryopreservative Medium and mix. Your DMSO concentration is now 10%.

• Place 0.5 ml of cells in each freezing vial.

• Rapidly transfer the cells to a freezing container and place at -70 °C overnight. (Cells don’t like to be in DMSO at room temperature for long periods of time.)

• Transfer cells to liquid nitrogen the next day for long-term storage.