**Passage of epiblast stem cells and differentiation of ESCs into epiSCs**

Protocol copied from “epiSC\_culture\_surani” pdf doc, references below.

Culture medium (500ml total volume):

DMEM/F12 (1:1 / Cellgro, Cat 10-092-CV) 480ml

N2 supplement (Invitrogen, Cat 17502-048) 2.5ml

B27 supplement (Invitrogen, Cat 10889-038) 5ml

BSA (Sigma, Cat A3311) 25mg

MEM NEAA (100x / Gibco, Cat 11140) 5ml

L-glutamine (100x / Gibco, Cat 25030) 5ml

2-ME (Gibco, Cat 31350-10) 1ml

Pen/Strep (100x Gibco) 5ml

Culture medium (50ml total volume):

100X BSA stock:

5mg/ml in DMEM/F12 media

DMEM/F12 (1:1 / Gibco, Cat 21331-020) 47.2 ml

N2 supplement (Invitrogen, Cat 17502-048) 0.250ml

B27 supplement (Invitrogen, Cat 10889-038) 0.500ml

100x BSA (Sigma, Cat A3311) 0.500ml

MEM NEAA (100x / Gibco, Cat 11140) 0.500

L-glutamine (100x / Gibco, Cat 25030) 0.500

1000x 2-ME 0.05

Pen/Strep (100x Gibco) 0.500

Before use, add:

20ng/ml Activin A

(R+D Systems 338-AC – stock 20ug/ml in sterile 0.1% aka 1mg/ml BSA PBS.)

12ng/ml bFGF

(R+D Systems 233 FB– stock 12ug/ml in sterile 0.1% aka 1mg/ml BSA PBS)

EpiSC Culture:

EpiSCs are usually split every day (sometimes every second) at ratios of 1:2-1:4, with 1:3 being optimal. Split every day when culturing true epiblast stem cell lines.

1. pre-splitting:

Dilute 1mg/ml fibronectin (Millipore, Cat FC010) solution 1:60 in PBS, DIRECTLY IN PLATE TO BE USED. This is 18ul of Fibronectin per 1ml of PBS. Coat plates with Fibronectin for 1hr to ON at 37 degrees. Use 3ml per 10cm dish, 1.5 per 60mm and 1 per 35mm. Always dilute fresh.

2) Wash EpiSCs 1x with PBS and add pre-warmed Accutase (PAA, Cat. L11-007). Use 1ml per 35mm plate.

3) Put cells in 37C incubator for 1 to 3 minutes, until they readily detach when accutase is passed over them.

4) Blow cells off plate and add them to 2mls of culture medium with no growth factors.

5) Transfer to a conical and pellet for 3 min at 500g.

6) Resuspend cells in 2ml of culture medium with growth factors added. Media plus growth factors is good at 4C for about one week.

7) Aspirate fibronectin from new plate. Resuspend the cell pellet and transfer.

Freeze epiblast cells in 90% KSR and 10% DMSO.

Differentiating ESCs into EpiSCs:

Pass ESCs as above for 8 to 10 days.

Original references:

"Defined Conditions for Neural Commitment and Differentiation", Methods in Enzymology, Vol. 365 By Q Ling and Austin G. Smith, 2003

"Klf4 reverts developmentally programmed restriction of ground state pluripotency"

Development 136, 1063-1069(2009)