Newcastle University	

Standard Operating Procedure	Number: SOP19
Title: **Culture of iPSC from cryopreserved samples using the Matrigel / mTeSR system**	

Approval		Date Implemented:	
	Name	Signature	Date
Author:			
Principal Investigator:	Majlinda Lako		
QA			

Change History	**Reason for Issue/Change Summary**
Version No/Date Issued	

1.0 Purpose

Culture and expansion of iPSCs from cryovials delivered to the end user

2.0 Scope

Culture and expansion of iPSCs from cryovials delivered to the end user

3.0 Related Documents

BD Matrigel hESC-qualified Matrix guidelines for use

4.0 Responsibility

All STEMBANCC staff handling iPSCS

5.0 Health Safety & Environment

6.0 Materials & Equipment

Product name	Supplier	Catalogue number
EDTA (Versene, 0.02%)	**Lonza**	**17-711E**
PBS	**Gibco**	**2014-05**
mTeSR™1 Medium	**Stem Cell Technologies**	**05850**
FBS	**Gibco**	**10270106**
Dimethyl sulphoxide (DMSO)	**Sigma**	**D2650**
BD Matrigel™ hESC-qualified Matrix	**BD Biosciences**	**354277**
DMEM/F-12 Medium (for matrigel preparation)	**Invitrogen**	**11330-057**

7.0 'Procedure'

- The Matrigel vial is to be thawed on ice
- Once thawed, the vial is swirled to ensure the Matrigel is evenly dispersed. The top of the vial is sprayed with 70% ethanol and then air dried. Keep the product on ice and handle using sterile technique
- The material is then dispensed into appropriate aliquots (the volume of the aliquots is determined by the dilution factor provided on the certificate of Analysis- see example below), using precooled tubes and refrozen immediately. Multiple freeze thaws should be avoided.

The product is stable for a minimum of three months from the day of shipment when stored at -20°C. Stable for a minimum of six months when stored in aliquots at -70°C.

Color variations may occur in frozen or thawed vials of BD Matrigel, ranging from straw yellow to dark red due to the interaction of carbon dioxide with the bicarbonate buffer and phenol red. This is normal, does not affect product efficacy, and will disappear upon equilibration with 5% carbon dioxide.

Matrigel will gel rapidly at 22°C to 35°C. Thaw overnight at 4°C on ice. Gelled Matrigel may be re-liquified if placed at 4°C on ice for 24-48hours.

- Dilution Factor:
  The dilution factor for eact lot is based on the protein concentration. To use with STEMCELL Technologies mTeSR medium, the aliquots are prepared according to the dilution factor provided on the certificate of Analysis. The volume of the aliquots is typically between 270-350µl.

- Example calculation:
  Total Matrigel volume= 5ml
  Dilution factor= 310µl

  To use: add one aliquot of Matrigel (310µl) to 25ml of Knock-out DMEM to coat four 6-well plates (1ml/well).

- Matrigel coated plates can be stored for a maximum of one week but are normally used immediately

Procedure for establishment of iPSC cultures from frozen stocks

- Remove the cryovial of induced pluripotent stem cells from liquid nitrogen storage
- Warm the vial for 10-15 seconds in gloved hands to remove frost
- Immerse the vial in a water bath at 37oC without submerging the cap
- Swirl the vial gently to ensure an even temperature throughout the liquid but do not shake vigorously
- When a small piece of ice is still visible, remove the vial from the water bath
- Ensure the cap is tight then spray with 70% ethanol to sterilise the outside of the tube
- Transfer the cells into a sterile 15 ml conical tube using a 1 ml pipette
- Slowly add 11 ml of mTeSR medium while moving the tube back and forth to mix medium and cells evenly (this reduced the osmotic shock to the cells)
- Centrifuge at 300xg for 5 minutes
- Aspirate the supernatant liquid and discard
- Resuspend the cell pellet in 3.0ml mTeSR medium and gently pipette up and down
- Label a 6-well plate coated with matrigel with the cell line name, unique identification number, the passage number written on the cryovial, the date and your initials
- Remove the Matrigel plating medium and replace with 1.5 ml mTeSR medium which is supplemented with 10 µM ROCK inhibitor

- Slowly add 1.5 ml of the induced pluripotent stem cell suspension drop-wise into one well of the Matrigel coated six well plate
- Transfer the plate to the incubator and monitor the development of colonies until it becomes necessary to passage them.

8.0 Glossary

Definitions and abbreviations, as used in the document, both those that are common to the unit and the sector.

9.0 Appendices

Extracts from other documents (e.g. manufacturers instructions) that maybe useful to know of or reference, but are not essential to following the method described.
Templates (e.g. for recording results, recording operator use of equipment, length of operation) used in conjunction with the process.