Standard Operating Procedures for MS Stem

The following document is a list of SOPs to be followed in MS Stem (The iPSC facility) at Menzies Institute for Medical Research. The following SOPs have been read, reviewed, agreed, and signed by the following team members.

S. No.	Name	Signature with date
1	A/Prof. Kaylene Young	
2	Prof Bruce Taylor	
3	A/Prof Tony Cook	
4	Prof Alex Hewitt	
5	Dr Jac Charlesworth	
6	Prof Kathryn Burdon	
7	Ms Natasha Stevens	

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Contents

SOP: MS_Stem001- Procedure for Obtaining Participant Consent

Procedure for Obtaining Participant Consent			
SOP Number:	MS_Stem001	**Version Number:**	**V1**
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	See the list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of the SOP is to outline the procedure that should be followed to obtain participant consent for a research study and ensure the informed consent process is carried out in accordance with Good Clinical Practice Guidelines (2016), The Australian Code for the Responsible Conduct of Research (2018), UTAS Responsible Conduct of Research Framework. This SOP is based on the SWP046 (Interviewing) and SWP016 (Consent forms) from the Clinical Research Facility, Menzies Institute for Medical Research.
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS

- The research study must be approved by the Human Research Ethics Committee (HREC) before starting the study.

- Research Staff must be authorized by the Program Manager or Principle Investigator to conduct participant consent.
- You must be properly trained in the procedure and be observed by a senior staff member and deemed proficient before undertaking the procedure independently.

Items required:

1. Quiet, private area with auditory privacy
2. Informed Consent form
3. Pen and stationery
4. Video link

Procedure (Process time: variable)

1. The first interaction with the participant can be via the telephone or face-to-face. If face-to-face, have the participant be seated comfortably in an area that provides auditory privacy.
2. Introduce yourself and inform the participant that they have been referred to us by their clinician, as they showed interest in the research study, OR are being contacted by us because they or a family member expressed interest in our study.
3. Ensure you are using the correct version of the participant information sheet and consent form (PISCF), approved by ethics. Give participants an adequate explanation of the study and provide them with the HREC approved PISCF and link to the video. The consent process must include a discussion about:
    a. The research study
    b. Purpose of the research study
    c. Participants responsibility (one-time blood donation for the study)
    d. Reasonably foreseeable risks or inconveniences to the participants
    e. Short term and/or long-term benefits
    f. Participant involvement is voluntary, and the participant can refuse or withdraw at any time
    g. What happens when the participant withdraws?
    h. Expected time the participant may need to spend for the study
    i. How many participants are involved in the study?
4. Reassure the participant that all information they provide is confidential.
5. Ask the participant to consider the information provided for as long as they need and speak with family, friends, or their GP if they want to. Indicate that they could contact us to go through the informed consent process within the next 2 weeks and that you are available to answer any questions. If you do not hear from the participant, follow-up with them after 2 weeks.
6. If there are questions, address the queries as and when required.
7. Before signing the consent, ask the participant to explain the study in their own words and ask them some basic questions to test their comprehension of the research study and ensure they have sufficient capacity to consent.
8. Once capacity is confirmed and if the participant agrees, ask them to sign the participant informed consent form in your presence and you counter-sign it on the same date. Research Staff must not complete the participant section of the participant

informed consent form. All personal particulars must be filled by the participant (Name, signature etc).

9. Where the person giving consent is unable to read, is physically unable to sign or mark the document, or where a translator is being used for non-English speaking participants, they may give their consent in the presence of an impartial witness (i.e. someone not involved in the conduct of the trial).The witness signs and personally dates the consent forma to attest that the information was read and explained to the participant and that consent was freely given.
10. When the participant is out stationed and not physically present to sign the informed consent form, the signature of the informed consent form would be performed over Zoom or any other video call-based service. The signing part could be recorded (if possible). If this is not possible, the consent could be signed in the presence of the Phlebotomist (as a witness) and the informed consent forms mailed to us (originals, not scanned).
11. For counter-signing such informed consent, a note stating, “Signed over Zoom” or “Signed in presence of Phlebotomist”, would be required.
12. If an error is made, simply strike out with a single line through, initial and date the correction. Do not use white out or obliterate entries.
13. Once signed, take a photocopy of the informed consent form, and provide that copy to the participant. Keep the original filed in the study records.
14. If the participant does not wish to join the study, or lacks capacity to consent, thank the participant for their time and end the consultation politely.
15. Once informed consent is signed, ask the participant when they would like to visit the pathology lab for sample collection.
16. Provide the participant with the pathology referral form (for pre-screening and sample collection) and schedule their appointment with the pathology laboratory.
17. Once the sample is collected and received, thank the participant for their participation via a call or email.

Additional Note:

Version	Reason for Change	Author of change	Date Approved
1.0	N/A	N/A	

SOP: MS_Stem002- Procedure for Performing Phlebotomy

Procedure for performing Phlebotomy to collect donor blood samples			
SOP Number:	MS_Stem002	**Version Number:**	V1
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	See list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of the SOP is to collect blood from a donor to reprogram PBMCs to induced pluripotent stem cells. This process is an adaption of SWP056 performed in the Clinical Research Facility at the Menzies Institute for Medical Research. For more details please refer to SWP056. Blood collection will most often be performed at a pathology clinic, however, when it is performed by us, it is to be conducted in the Phlebotomy room in the level 3 clinic of MSP.
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS

- Participants do not have a blood phobia
- Participants are not exposed to potentially contaminated equipment or contaminated sharps

EQUIPMENT AND REAGENTS

Equipment

1. Sharps disposal container

Reagents

1. 70% Ethanol spray bottle for surface cleaning or Viraclean or Isowipes

Disposables

1. Needles (various gauge)
2. Vacutainer holder
3. Gauze pad
4. Band-Aid or IV pressure pads
5. EDTA containing blood collection tubes with labels
6. Pen and/or labels

Personal Protective Equipment

1. Laboratory coat/Lab-gowns
2. Non-powdered, nitrile or equivalent gloves
3. Covered shoes

Procedure (Process time: 5-10 min)

1. Introduce yourself and obtain the identification of the participant (verify name and DOB from the request application form).
2. Ask if the participant has any allergies or a blood phobia. Ask the participant if they have any concerns and determine whether they have previously undergone blood collection.
3. Prepare the essential items for the blood collection.
4. Position the arm of the participant at a level that is comfortable for the participant and the phlebotomist. Adjust the chair height or use a pillow or chair arm adjustment to achieve a comfortable position.
5. Apply a tourniquet to find a suitable vein. Once found, select the appropriate vein and needle gauge, depending on the vein.
6. Wipe the area with an alcohol swab and allow it to air dry.
7. Inform the participant that you are going to insert the needle.
8. Ask the participant to close their fist and gently insert the needle at a 30° angle with the bevel of the needle facing up.
9. Push the blood tube onto the tube holder and hold it steady until the desired amount of blood is collected. Remove the filled tube and continue with additional tubes if necessary.
10. Loosen the tourniquet.
11. When all samples are collected and removed from the tube holder, place a gauze pad over the needle insertion site and remove the needle.
12. Apply gentle but firm pressure to the site.

13. Discard the sharp directly into the sharps container and confirm with the participant that they are feeling alright.
14. Remove the gauze and inspect the site for bleeding. Apply band-aid on the insertion site.
15. Inform the participant to avoid lifting weights for about 1 hour after the procedure.
16. Gently invert the blood tubes containing EDTA to mix properly and avoid clotting of blood samples.
17. Label the tubes with patient ID and complete the paperwork as per the study protocol.
18. Clean blood spill, if any.
19. Disinfect reusable items e.g. chair before the next participant.

Additional Note:

Version	Reason for Change	Author of change	Date Approved
1.0	N/A	N/A	

SOP: MS_Stem003- Isolation of PBMC from Whole Blood

Isolation of PBMC (Peripheral Blood Mononuclear Cells) from Whole Blood			
SOP Number:	MS_Stem003	**Version Number:**	V1
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	See list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of this SOP is to isolate PBMCs from whole blood. The isolated PBMCs can be stored and/or reprogrammed to generate induced pluripotent stem cells
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS

- Always keep the blood at room temperature (RT)
- Perform the isolation and freezing of PBMCs less than 8 hours after the blood collection, if collected onsite. Best results are obtained if the procedure is performed less than 2 hours after blood collection.
- For out-stationed samples, PBMCs should be isolated and frozen within 48 hours of collection. Significantly reduced PBMC yields may be noted if this is delayed.
- Minimize the time that cells are in contact with Ficoll.
- Wash the cells thoroughly after the Ficoll step.
- Keep **brakes OFF** during centrifugation

EQUIPMENT AND REAGENTS

Equipment

1. Class II Biosafety Cabinet (BSC) set up in the PC2 laboratory
2. Refrigerated centrifuge with swing-out bucket rotor, capable of low speeds (300 to 1000 g) with 15- or 50-mL tube holders
3. Micropipettes range from 20 to 1000 µL
4. Pipet-Aid (cordless) with disposable serological pipettes (5- and 10- mL)
5. Refrigerator (2-8°C)
6. Bucket/beaker with disinfectant (Bleach/Trigene) for rinsing pipettes
7. Automated cell counter/Hemocytometer with a light-field microscope
8. Trypan blue
9. Test-tube stands to hold 15-, 50-mL falcon and blood tubes.

Reagents

1. HI-FBS (Heat-inactivated FBS) aliquot thawed from -20°C to room-temperature (as described in SOP # MS_Stem010).
2. Sterile 1X PBS without Ca^{2+} and Mg^{2+} (Thermofisher Cat # 14190144) or 1X HBSS without Ca^{2+} and Mg^{2+} (Thermofisher Cat # 14170112). You can also buy 10X and dilute the same with sterile water.
3. RPMI-1640 (Thermofisher Cat # 12633012)
4. Ficoll (GE Cat # 17144002) or Lymphoprep (StemCell Technologies Cat # 07801)
5. 70% Ethanol spray bottle for surface cleaning
6. 1:100 dilution of 5% sodium hypochlorite or 1:10 dilution of Trigene as a disinfectant.
7. Penicillin – Streptomycin (PS) Solution 10,000 IU (Thermofisher, Cat # 15140122)

Disposable

1. Labelled vacutainer containing 5- to 8-mL whole blood stored at RT (as described in SOP # MS_Stem002).
2. Falcon tubes (15-, 50-mL) or Sepmate (StemCell Technologies)
3. Serological Pipets (5-, 10-mL), sterile disposable. For larger blood volumes 25- and 50-mL may be required.
4. Micropipette tips, sterile and filtered 200 – 1000 µL
5. Tissue wipes

Personal Protective Equipment

1. Laboratory coat/Lab-gowns in PC2 lab
2. Non-powdered, nitrile or equivalent gloves
3. Covered shoes
4. Eye protection, not mandatory while working in the BSC.

Blood Handling conditions

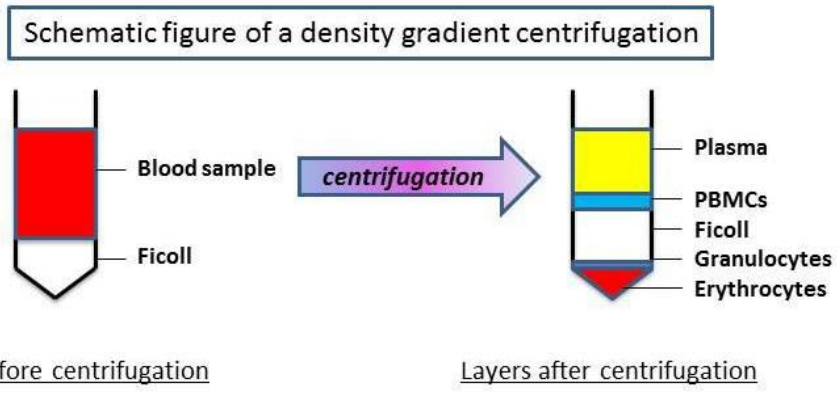
1. Vacutainer contains anti-coagulated whole blood
2. The required volume of blood (5- to 8-mL) has been collected

3. Fresh, anticoagulated whole blood specimens should be stored at room temperature (15 to 30°C) from the time of collection and delivered to the laboratory/processing unit as soon as possible.
4. The anticoagulated whole blood specimens should only be processed if they are received within 48 hours of blood collection.
5. Clotted specimens: Remove the clot before processing the sample. Inform the clinic or PI for possible sample replacement if cell yield is insufficient
6. Haemolysis may affect PBMC quality during reprogramming. Process the samples as usual and inform the clinic or PI for a possible replacement if cell yield is insufficient.
7. Unacceptable specimens: Unlabelled or mislabelled samples must be rejected. Leaking samples must be rejected. Notify the clinic and PI immediately.

Procedure (Process time: 1.5-2 hours)

1. Prepare 2% FBS-1X PBS solution (combine 48.5-mL 1X PBS with 1.0-mL of HI-FBS, 0.5-mL of PS, invert the falcon tube gently, 4-5 times). Store at RT. This solution can be stored overnight at 4°C but must be at RT before using.
2. Prepare 10-mL of complete RPMI-1640 medium by mixing RPMI basal medium with 5% HI-FBS or normal FBS (0.5-mL HI-FBS + 9.0-mL RPMI-1640 + 0.5 mL PS)
3. Spray all required items with 70% ethanol, wipe with a tissue paper and place them in the BSC.
4. In the BSC, mix the vacutainer containing blood 2-3 times by gentle inverting and return to the test-tube stand.
5. Open the vacutainer and gently mix with a 5-mL serological pipette. Take care to avoid hemolyzing the blood. Measure the volume of blood in the tube (± 0.5-mL variation acceptable).
6. Transfer 4-mL of whole blood to a fresh 15-mL falcon tube. Discard the pipette after rinsing in disinfectant, twice.
7. Add 1X volume (4-mL) of 1XFBS-PBS solution and mix well with a 10-mL serological pipette. Discard the pipette after rinsing in the disinfectant, twice.
8. In a 2nd fresh 15-mL falcon tube, add 4-mL of Ficoll or Lymphoprep. Gently layer the diluted blood (8-mL) over the Ficoll or Lymphoprep without mixing the 2 layers. Close the cap tightly.
9. Depending on the blood volume the following ratios are recommended:

Blood (mL)	PBS+ 2%FBS (mL)	Ficoll/Lymphoprep (mL)	Tube size (mL)
1	1	1.5	5
2	2	3	15
3	3	3	15
4	4	4	15
5	5	10	50
10	10	15	50
15	15	15	50



10. Place the 15-mL falcon tube, along with a proper balance, in the swing-out rotors of the centrifuge and spin at 400 g for 40 min at RT **with BREAKS OFF**
11. Carefully remove the tube from the centrifuge, wipe with 70% ethanol and place in the test-tube rack within the BSC.
12. Using a 5-mL serological pipette, remove and discard most of the plasma (top-most, yellowish layer). Do not disturb the interface (buffy coat) or the bottom RBC layer.
13. Using a 1000-uL tip gently collect the interface (cloudy buffy coat), which contains the cells, into a fresh 15-mL falcon tube.
14. Wash the collected buffy coat with 5-mL 1%FBS-PBS solution twice. Centrifuge at 300 g x 5 min x 4°C.
15. Resuspend the cells in 1-mL RPMI-1640 complete medium and perform the cell count.
16. Perform a cell count using trypan blue or automatic cell counter. Each cell suspension must have more than 90% viability and should, on average, have PBMC counts within the range detailed in the table below, unless the number of cells has been affected by medication, infection or any other medical reason.

Population	Mononuclear Yields (10^6cells/mL)
Adult	0.8 to 3.2
Paediatric (Less than 6m)	3.0 to 10.0
Paediatric (6m to 2y)	2.0 to 9.0
Paediatric (2y to 5y)	1.0 to 6.0
Paediatric (More than 5y)	0.8 to 4.0
Paediatric (unknown age)	1.0 to 10.0

17. The PBMCs are now isolated and can be cryopreserved or cultured before reprogramming.
18. Please refer to SOP# MS_Stem004 for cryopreservation or SOP# MS_Stem006 for culturing.

Variation using Sepmate (Process time: 45-50 min)

If you use Sepmate (StemCell Technologies), the above protocol must be modified.

1. Follow steps 1-6 then layer Lymphoprep in the Sepmate tube (follow manufacturer's instructions).
2. Gently layer the blood in the Sepmate tube.
3. Centrifuge in swing-out rotor centrifuge at 1200 g X10 min X RT with break ON.
4. Collect the Sepmate tube and simply pour the cells in a fresh 50-mL falcon (see manufacturer's instructions).
5. Spin the falcon at 300 g x10 min x 4°C to collect the PBMC pellet.
6. Follow steps 15 onwards.

Note: Since this process involves directly pouring the PBMC along with plasma, there may be a higher rate of RBC contamination in the final PBMC preparation. Although low-level RBC contamination does not hamper down-stream experiments, higher levels of RBCs may hamper down-stream applications.

Additional Note:

Version	Reason for Change	Author of change	Date Approved
1.0	N/A	N/A	

SOP: MS_Stem004- Cryopreservation of isolated PBMC

Cryopreservation of isolated PBMC (Peripheral Blood Mononuclear Cells)			
SOP Number:	MS_Stem004	**Version Number:**	V1
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	See the list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of the SOP is to cryopreserve PBMC
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS

- Perform the isolation and freezing of PBMC less than 8 hours after the blood collection, if collected onsite. Best results are obtained if the procedure is performed less than 2 hours after blood collection.
- For out-stationed samples, PBMC should be isolated and frozen with 48 hours of blood collection. Significantly lower yields of PBMC are expected as compared to fresh blood.
- While aliquoting cells into cryovials, gently mix the stock tube with a 5-mL serological pipette to obtain a uniform distribution of cells across all vials.
- Replace 2-isopropanol after 5 freeze-thaw cycles

EQUIPMENT AND REAGENTS

Equipment

1. Class II Biosafety Cabinet (BSC) set up in the PC2 laboratory
2. Refrigerated Centrifuge with swing-out bucket rotor, capable of low speeds (300 to 1000 g) with 15- or 50-mL tube holders
3. Micropipettes range from 20 to 1000 µL along with sterile-filtered tips
4. Pipet-Aid (cordless), with disposable serological pipettes (5- and 10-mL)
5. Refrigerator (2-8°C)
6. -80°C freezer for short-term storage of PBMCs
7. LN_2 for long-term storage of PBMCs
8. Bucket/beaker with disinfectant (Bleach/Trigene) for rinsing pipettes
9. Automated cell counter/Hemocytometer with a light-field microscope
10. Trypan blue
11. Nalgene Mr Frosty (1°C/min cryo-freezing container)
12. Stand for holding cryotubes

Reagents

1. HI-FBS (Heat-inactivated FBS) aliquot thawed from -20°C to room-temperature (as described in SOP # MS_Stem010) or normal FBS aliquot thawed from -20°C to room temperature.
2. RPMI-1640 (Thermofisher, Cat # 12633012)
3. 70% Ethanol spray bottle for surface cleaning
4. 1:100 dilution of 5% sodium hypochlorite or 1:10 dilution of Trigene as a disinfectant.
5. Cell culture or hybridoma grade DMSO (Sigma Cat # D2650-100mL) stored at RT and sealed with parafilm once opened.
6. CryoStor CS10 (StemCell Technologies, Cat # 07930)

Disposable

1. Labelled 15-mL falcon tube containing PBMCs in 1-mL RPMI-1640 complete medium, as described in SOP # MS_Stem003
2. Serological pipets (5- and 10-mL, sterile and disposable). For larger blood volumes 25- and 50-mL may be required.
3. 2D bar-coded cryogenic vials (Thermofisher)
4. Tissue wipes

Personal Protective Equipment

1. Laboratory coat/Lab-gowns in PC2 lab
2. Non-powdered, nitrile or equivalent gloves
3. Covered shoes
4. Cryogloves and face shields when using LN_2
5. Eye protection, not mandatory while working in BSC

Procedure (Process time: 30-45 min)

1. Prepare freezing solution by mixing 50-mL RPMI-1640, 40-mL of HI-FBS or normal FBS and 10-mL DMSO (5:4:1 ratio). Adjust the volume according to your requirements.

Mix thoroughly by gentle inversion. This solution can be made a day in advance and stored at 4°C and should be at 4°C before use.
2. Prepare 10-mL of complete RPMI-1640 medium by mixing RPMI basal medium with 5% HI-FBS or normal FBS (0.5-mL FBS + 9.5-mL RPMI-1640)
3. Spray all required items with 70% ethanol, wipe with a tissue paper and place them in the BSC.
4. Place the 15-mL falcon tube with PBMCs in RPMI complete medium (from SOP# MS_Stem003) in the BSC.
5. Based on the cell count, calculate the number of cryotubes required. Each vial should contain 1 x 10^6 cells/mL.
6. Centrifuge the cells at 300 g x 5 min x 4°C.
7. Scan the bar-code of the cryotube and record all necessary information in the MS biobank storage database. The information recorded should contain, Type of cells with patient code, viability, number, date of freezing, and name/initials of the researcher.
8. Aspirate/discard the RPMI-1640 with a 1-mL micropipette. Gently tap the pellet at the bottom of the falcon to dislodge it.
9. Take the cold freezing media from the fridge, mix once by inverting, spray with 70% ethanol, wipe and open inside the BSC.
10. Add the desired volume of freezing media to the PBMC pellet, gently mix with a 5-mL serological pipette to get a concentration of 1 x 10^6 cells/mL.
11. Quickly aliquot 1-mL of cell suspension into each cryovial and tightly screw the caps. Transfer the cryovials into the pre-chilled Mr Frosty (each holds 18 tubes).
12. Place the Mr Frosty in the designated -20°C for temporary storage.
13. Remove all items and clean the BSC and working area with 70% ethanol and UV the BSC.
14. Transfer the Mr Frosty to the -80°C freezer for overnight storage.
15. The next day, transfer the cryovials to the vapour phase of LN_2 and record the storage details for each cryovial (number, position, and place in the LN_2 container).

Variation (Process time: 30-45 min)

This variation is for a xeno-free freezing system using CryoStor CS10. The protocol is similar as above, except replace the in-house freezing media with CryoStor CS10. CryoStor CS10 has better thaw viability for sensitive cells, like human induced pluripotent stem cells.

Additional Note:

Version	Reason for Change	Author of change	Date Approved
1.0	N/A	N/A	

SOP: MS_Stem005- Thawing of Cryopreserved PBMC

Thawing of cryopreserved PBMC			
SOP Number:	MS_Stem005	**Version Number:**	**V1**
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	See list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of the SOP is to thaw cryopreserved PBMC for reprogramming to generate induced pluripotent stem cells (iPSC cells).
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS

- All media used in the protocol should be at room temperature.
- ~~Do not keep any complete media in the 37°C water bath after taking out from the refrigerator~~ Never use 37°C water bath to warm complete medium, once taken out from the refrigerator.

EQUIPMENT AND REAGENTS

Equipment

1. Class II Biosafety Cabinet (BSC) set up in the PC2 laboratory
2. Humified CO_2 Incubator set at 5% CO_2 and 37°C
3. Microscope

4. Refrigerated centrifuge with a swing-out bucket rotor, capable of low speeds (300 to 1000 g).
5. Table-top microfuge
6. Micropipettes range from 0.5 to 1000 µL along with sterile-filtered tips
7. Pipet-Aid (cordless), with disposable serological pipettes (5- and 10-mL)
8. Refrigerator (2-8°C)
9. Liquid N_2 container
10. Bucket/beaker with disinfectant (Bleach/Trigene) for tip discard
11. Test-tube stands to hold 15-, 50-ml falcon tubes.
12. Esky with ice

Reagents

1. StemSpan II (StemCell Technologies, Cat # 09655)
2. Erythroid Expansion Supplement 100X (StemCell Technologies, Cat # 02692)
3. RPMI-1640 (Thermofisher, Cat # 12633012)
4. Heat inactivated FBS or normal FBS
5. 70% Ethanol spray bottle for surface cleaning
6. 1:100 dilution of 5% sodium hypochlorite or 1:10 dilution of Trigene as a disinfectant.
7. Penicillin - Streptomycin (PS) Solution 10,000 IU (Thermofisher, Cat # 15140122)

Disposable

1. Falcon tubes (15-, 50-mL)
2. Serological Pipets (2-, 5- and 10-mL), sterile disposable. Large 25- and 50-mL may be required
3. Tissue wipes
4. 48W or 24W Tissue Culture Plate

Personal Protective Equipment

1. Laboratory coat/Lab-gowns in PC2 lab
2. Non-powdered, nitrile or equivalent gloves
3. Covered shoes
4. Cryoprotective shield and gloves mandatory while handing LN_2
5. Eye protection, not mandatory while working in BSC

Procedure (Process time: 15-30 minutes)

1. StemSpan II complete medium can be prepared the day before thawing PBMCs. Mix StemSpan II basal media with erythroid expansion supplement 100X (98 mL of StemSpan II and 1 mL of erythroid expansion supplement and 1 mL PS). Calculate the volume required. The complete medium has a 1-week shelf-life and must be discarded after a week. Store at 4°C.
2. Complete RPMI-1640 medium: Mix RPMI-1640 with 5% HI-FBS or normal FBS to make complete medium (94-mL RPMI + 5-mL FBS + 1-mL PS), mix by inverting. Calculate the volume required.

3. In the BSC, add 9-mL of RPMI complete media to a 15-mL falcon and place this tube in the 37°C water bath before going to collect the PBMC from LN_2 storage.
4. Take a 24W or 48W tissue culture plate and add 0.5-mL (48W) or 1-mL (24W) of Complete StemSpan II media to a single well. Place the plate in the CO_2 incubator before going to collect the PBMCs.
5. Fill an Esky with ice before going to the LN_2 storage room to collect the cryovial of PBMCs.
6. Find the location of the PBMCs to be thawed in the LN_2 storage database.
7. Wear a cryoprotective shield and gloves before taking out the desired PBMC vial from LN_2 storage and cross-check the vial with the box position. Scan the bar-code of the cryovial to reconfirm. Immerse the cryovial completely in ice and transport it to the lab.
8. In a beaker, collect 37°C water from the water-bath and immerse the PBMC vial. After 30 seconds gently stir the vial in the water bath.
9. While the cryovial is thawing, transfer the 15-mL falcon tube from the water bath to the BSC after cleaning with 70% ethanol.
10. When the cryovial content is ~80% thawed (a small frozen pellet remains), dry the vial, clean with 70% ethanol and transfer to the BSC.
11. Collect the content of the cryovial (should be 1-mL) with a 2-mL serological pipette and add the cells dropwise to 9-mL of RPMI-1640 complete medium.
12. Cap the falcon and mix the content by gentle turning and inversion.
13. Place the falcon tube in the swing-out rotor centrifuge and spin at 300 g x 3 min x RT.
14. Bring the 24W or 48W plate from the CO_2 incubator to the BSC.
15. After centrifugation is complete, carefully remove and discard the supernatant from the 15-mL falcon into the bleach-containing discard container.
16. Gently finger-tap the base of the falcon to dislodge the PBMC pellet.
17. Transfer 0.4-mL (48W) or 0.5-mL (24W) of StemSpan II complete media from the tissue culture plate and resuspend the PBMC pellet in the falcon tube.
18. Resuspend the pellet in StemSpan II media by gentle pipetting and transfer the cell suspension back to the same well (48W or 24W), ensuring uniformly distribution within the well.
19. Replace the lid and transfer the tissue culture plate to the CO_2 incubator.
20. For culturing PBMCs refer to SOP # MS_Stem006

Note: One can thaw multiple lines in the same 24/48 well plate, provided you clearly label the wells before starting and can handle multiple PBMCs without cross-contamination. While doing multiple PBMCs, place cells at least two wells apart.

Additional Note:

Version	Reason for Change	Author of change	Date Approved

1.0	N/A	N/A	

SOP: MS_Stem006- Culturing PBMC for Reprogramming

Culturing of PBMC for reprogramming			
SOP Number:	MS_Stem006	**Version Number:**	V1
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	See the list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of the SOP is to culture PBMC before iPSC reprogramming. This protocol can be used for PBMCs that have been freshly thawed from Liquid N_2 or isolated from fresh blood. This process is required before generating induced pluripotent stem cells (iPSCs).
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS

- All media used in the protocol should be at room temperature.
- ~~Do not keep any complete media in the 37°C water bath after taking out from the refrigerator~~ Never use 37°C water bath to warm complete medium, once taken out from the refrigerator.
- The growth kinetics of PBMC can vary between participants. Medical treatment (antibiotics or any life-long drugs), presence of infection, etc. may significantly influence the growth kinetics and timeline for PBMC growth in culture.

EQUIPMENT AND REAGENTS

Equipment

1. Class II Biosafety Cabinet (BSC) set up in the PC2 laboratory
2. Humified CO_2 Incubator set at 5% CO_2 and 37°C
3. Microscope
4. Refrigerated centrifuge with a swing-out bucket rotor, capable of low speeds (300 to 1000 g) with 15- or 50-mL tube holders.
5. Table-top microfuge
6. Micropipettes range from 0.5 to 1000 µL along with sterile-filtered tips
7. Pipet-Aid (cordless), with disposable serological pipettes (5- and 10-mL)
8. Refrigerator (2-8°C)
9. Bucket/beaker with disinfectant (Bleach/Trigene) for tip discard
10. Test-tube stands to hold 50-, 15-ml falcon tubes.

Reagents

1. StemSpan II (StemCell Technologies, Cat # 09655)
2. Erythroid Expansion Supplement 100X (StemCell Technologies, Cat # 02692)
3. RPMI-1640 (Thermofisher, Cat # 12633012)
4. Heat inactivated FBS or normal FBS
5. 70% Ethanol spray bottle for surface cleaning
6. 1:100 dilution of 5% sodium hypochlorite or 1:10 dilution of Trigene as a disinfectant.
7. Penicillin - Streptomycin (PS) Solution 10,000 IU (Thermofisher, Cat # 15140122)

Disposable

1. Autoclaved or sterile clear Eppendorf tubes (1.5-mL)
2. Falcon tubes (15- or 50-mL)
3. Tissue wipes
4. 48W or 24W Tissue Culture plate (Any brand)

Personal Protective Equipment

1. Laboratory coat/Lab-gowns in PC2 lab
2. Non-powdered, nitrile or equivalent gloves
3. Covered shoes

Procedure (Process time: 5-10 days)

1. StemSpan II complete medium can be prepared the day before culturing: Mix StemSpan II basal media with erythroid expansion supplement 100X (98 mL of StemSpan II and 1 mL of erythroid expansion supplement and 1 mL PS). Calculate the volume required. The complete medium has a 1-week shelf-life and must be discarded after a week. Store at 4°C

2. Complete RPMI-1640 medium: Mix RPMI-1640 with 5% HI-FBS or normal FBS to make complete medium (94-mL RPMI + 5-mL FBS + 1-mL PS, mix by inverting). Calculate the volume required.
3. For isolating PBMCs from fresh blood refer to SOP # MS_Stem003 or for thawing PBMCs from LN_2 refer to SOP # MS_Stem005
4. **Steps for freshly isolated PBMCs from blood:** Resuspend the PBMC pellet from fresh blood after the cell count in StemSpan II (as per SOP # MS_Stem003), to have a concentration of 1-4 million PBMCs per mL.
5. **Day 0**: In a fresh and sterile 24W/48W plate, seed 1-mL or 0.5-mL of PBMC suspension, respectively
6. Mix gently by moving the plate forward and backward to evenly distribute the cells in the tissue culture plate.
7. Replace the lid and observe the cells under a microscope before transferring the plate to the CO_2 incubator.
8. **Day 1**: Check the cells for any sign of contamination and check the quality of cells.
9. **Day 2**: Perform a media change by collecting the PBMCs in a sterile Eppendorf tube and centrifuging the contents at 300g x 5 min x RT.
10. Discard the supernatant and resuspend the pellet in fresh StemSpan II medium.
11. Gently mix the cells with the fresh medium and add the cells to a fresh well of the tissue culture plate and place the plate back in the CO_2 incubator.
12. **Day 4:** Perform media change as on day 2 and a cell count. There should be an increase in the PBMC number.
13. If no increase is observed in PBMC number, then continue to culture the PBMCs for a further 2 days.
14. If an increase is observed in PBMC number, then the cells are ready for reprogramming as per SOP # MS_Stem009.
15. **Steps for PBMCs culture for thawed PBMCs**: Thaw the PBMCs as per the SOP # MS_Stem005 and resuspend the cells as indicated.
16. Follow step 7-14 as above.
17. The only difference is thawed PBMCs may require anywhere from 9-11 days in culture to see good proliferation and viability before they can be used for reprogramming as per SOP # MS_Stem009.

    Notes: One may see a significant drop in cell viability and cell number (< 40% viability) but that will improve over time. Check the cells daily (especially for unwanted growth). If there is no appreciable increase in cell number or viability by day 10, discard the cells. Review your workflow before thawing a fresh PBMC vial.

Additional Note:

Version	Reason for Change	Author of change	Date Approved
1.0	N/A	N/A	

SOP: MS_Stem007- Reprogramming of PBMC to iPSC with Sendai Virus

Reprogramming of PBMC into induced pluripotent stem cells (iPSC) with Sendai virus			
SOP Number:	MS_Stem007	**Version Number:**	V1
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	See the list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of the SOP is to reprogram PBMC with Sendai Virus to generate induced pluripotent stem cells. This process is specifically for freshly isolated/ frozen PBMCs only.
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS

- Handle virus particles with the **UTMOST** care. Always keep the virus on ice while using in the BSC. Only trained researchers are permitted to work with this SOP. If you are not authorized to handle the Sendai virus, please contact the Laboratory Manager.
- Always check the Lot number of the Sendai virus kit as MOI (Multiplicity of Infection) can vary with Lot number.
- PBMC must be proliferating prior to reprogramming
- All media used in the protocol should be room temperature.
- ~~Do not keep any complete media in the 37°C water bath after taking out from the refrigerator~~ Never use 37°C water bath to warm complete medium, once taken out from the refrigerator.
- Always thaw medium supplements at 4°C overnight. Do not thaw them at RT before use. Avoid vigorous shaking of supplements. Mix only by gentle inverting multiple time till mixed uniformly.

EQUIPMENT AND REAGENTS

Equipment

1. Class II Biosafety Cabinet (BSC) set up in the PC2 laboratory
2. Humified CO_2 Incubator set at 5% CO_2 and 37°C
3. Microscope
4. Refrigerated centrifuge with a swing-out bucket rotor, refrigerated capable of low speeds (300 to 1000 g) with 15- or 50-mL tube holders.
5. Table-top microfuge
6. Micropipettes range from 0.5 to 1000 µL along with sterile-filtered tips
7. Pipet-Aid (cordless), with disposable serological pipettes (5- and 10-mL)
8. Refrigerator (2-8°C)
9. -80°C freezer, for virus storage
10. Bucket/beaker with disinfectant (Bleach/Trigene) for tip discard
11. Automated cell counter/Hemocytometer with a light-field microscope
12. Trypan blue
13. Test-tube stands to hold 50-, 15-ml falcon tubes.

Reagents

1. StemSpan II (StemCell Technologies, Cat # 09655)
2. Erythroid Expansion Supplement 100X (StemCell Technologies, Cat # 02692)
3. Sterile 1X PBS without Ca^{2+} and Mg^{2+} (Invitrogen Cat #) You can also buy 10X and dilute the same with sterile/autoclaved Mili Q water.
4. mTeSR1 Plus complete (StemCell Technologies, Cat # 05825)
5. ReproTeSR complete (StemCell Technologies, Cat # 05926)
6. Cytotunes iPSC 2.0 Sendai Reprogramming Kit (Thermofisher, Cat # A16517)
7. hESC-qualified Matrigel (Corning, Cat # 354277)
8. DMEM-F12 (Thermofisher, Cat # 11330032)
9. 70% Ethanol spray bottle for surface cleaning
10. 1:100 dilution of 5% sodium hypochlorite or 1:10 dilution of Trigene as a disinfectant.

Disposable

1. Autoclaved sterile Eppendorf (0.2-, 0.5- and 1.5-mL)
2. Falcon tubes (15- or 50-mL)
3. Serological Pipets (2-, 5- and 10- mL), sterile disposable. Large 25- and 50-mL pipets may be required.
4. Tissue wipes
5. 6W Sterile Tissue Culture Plates or 6 cm tissue culture dishes (Any brand)
6. 48W Tissue Culture Plate (Any brand)
7. Esky containing ice

Personal Protective Equipment

1. Laboratory coat/Lab-gowns in PC2 lab
2. Non-powdered, nitrile or equivalent gloves
3. Covered shoes
4. Eye protection, not mandatory while working in BSC

Procedure (Process time: 20-30 days, 10-50 min per day)

1. Steps 2-4 can be performed 1-2 days prior to reprogramming.
2. StemSpan II complete medium: Mix StemSpan II basal media with erythroid expansion supplement 100X (99 mL of StemSpan II and 1 mL of erythroid expansion supplement). Calculate the volume required. The complete medium has a 1-week shelf-life and must be discarded after a week. Store at 4°C
3. ReproTeSR medium: Mix ReproTeSR basal medium with its 25X supplement. Once thawed, aliquot the supplement and freeze for future use. Complete media has a shelf-life of 1 month and should be stored at 4°C.
4. mTeSR Plus medium: Mix mTeSR Plus basal with its supplement and store at 4°C for further use. Complete medium is stable for 1 month after reconstitution.
5. Before the start, PBMCs must be proliferating in culture as per SOP # MS_Stem006.
6. **Transduction day (day 0)**: Collect the PBMCs and perform a cell count. Plate 100,000 to 1 million PBMCs in a fresh well of a 48 W plate with 0.5-mL StemSpan II medium. Keep the plate in the CO_2 incubator while you prepare the virus.
7. Transfer the Cytotune Kit from the -80°C freezer to an Esky containing ice.
8. Spray the Cytotune kit with 70% ethanol, wipe with a tissue paper and place on ice in the BSC. ~~Spay the Cytotune kit with 70% and wipe with a tissue paper.~~ Open the Cytotune kit and collect the ~~3 colour-coded~~ three Eppendorf tubes containing the virus and thaw them in the 37°C water bath for 10-15 sec. Post-thawing, pulse spin the tubes in a bench-top microfuge and gently return them to the ice.
9. Calculate the exact volume of virus required to achieve a MOI of 5:5:3 (KOS: C-myc: KLf4) as per the formula below:

$$\text{Volume of Virus } (\mu L) = \frac{\text{MOI (CIU/cell)} \times \text{number of cells}}{\text{Titre of virus (CIU/mL)} \times 10^{-3}\ (\text{mL}/\mu L)}$$

10. Add the desired volume of the viruses to the PBMCs. This step must be finished in less than 3 minutes.
11. Mix the cells and virus in the plate evenly using a micropipette with a 0.2-mL filter tip. Return the plate to the incubator.
12. Seal the virus vials and return them to -80°C as soon as possible. Clean the work area and discard all the virus-infected disposables in disinfectant and keep them submerged for at least 20 minutes before disposing of the waste. Liquid waste down the sink and solid waste in autoclaving bags after disinfection.
13. **Post-transduction (day 1)**: Gently collect the transduced PBMC from the well and transfer to a sterile Eppendorf tube (1.5-mL), centrifuge at 300g x 5 min x RT, discard the supernatant. ~~Tap the pellet gently~~ Gently dislodge the pellet by tapping the tube (pellet may not be visible).
14. Add 0.5-mL of fresh StemSpan II complete medium and replate the transduced PBMC in a fresh well of the same (48W) plate. There may be significant cell death post-transduction. This is very normal.
15. **Day 3**: Prepare 1W of a 6 well plate or 6-cm Matrigel-coated plates, as per SOP# MS_Stem011.

16. Collect the transduced PBMCs in an Eppendorf and spin at 300g X 5 min x RT to collect the pellet. Resuspend the pellet in 1-mL fresh StemSpan II complete medium and plate the cells on Matrigel-coated plates and return to the incubator.
17. **Day 5**: Inspect the PBMC under the microscope. You may see flattened and attached PBMCs on the plate. Without removing existing medium, add 1-mL of complete ReproTeSR media to the plate and place it back in the CO_2 incubator for 2 days. If you do not see flattened cells, keep the plate for additional two days with StemSpan II complete medium. If still no attachment, is visible, you can still proceed with the exchange of media with ReproTeSR as stated above. In such conditions, care must be taken to find if there are any cells attached, more carefully.
18. **Day 7**: Observe the plate under the microscope. Carefully remove the media from the well and add 2-mL of ReproTeSR media to the well. The majority of floating PBMCs will be removed in this process. Take a photograph of the cells for your records. Return the cells to the incubator.
19. **Day 9-30**: Repeat the media change with ReproTeSR every alternate day for the next 15-20 days.
20. You should see iPSC-like colonies forming. Once a decent size is reached for multiple colonies on the plate, replace ReproTesR medium with complete mTeSR plus medium.
21. Culture for 4-5 days with a media change every alternate day.
22. When iPSC colonies are large enough, select multiple individual clones (10-20) to passage. Follow SOP # MS_Stem009 using dispase II (manual passage) for their first few passages before switching to ReLeSR (non-enzymatic passage) method.
23. Clones may die over the first few passages if they are not stable clones.
24. Maintain iPSC using SOP # MS_Stem008.

Variation (Process time: 20-30 days, 10-50 min per day)

There are multiple ways to make hiPSC (Lentiviral, Retroviral, mRNA-based, and other), episomal-based reprogramming is another good method to make footprint-free hiPSC lines. OriP/EBNA-1 (Epstein-Barr Nuclear Antigen-1) episome-based method is another good method (Yu et al., 2009, Science, 324, 797-801, Yu et al., 2011, PlosOne, 6 e17557). Episomal method utilizes 7 reprogramming genes (*OCT4, SOX2, NANOG, LIN28, c-MYC, KLF4* and *SV40LT*). One could also buy these episomes from Adgene website (www.adgene.com) and culture them in-house. However, this method is well established on fibroblasts and its efficiency in PBMCs reprogramming is under evaluation. Thermofisher and Stem Cell Technologies, both companies do sell similar products based on Episomal vectors.

The protocol stated here is based on fibroblast. Most of the steps are identical as above unless the user prefers to use commercially available kit.

1. Culture fibroblast in T-25 flask in Fibroblast Medium (DMEM +10% FBS + PS) for 2-3 days till they reach 70-90% confluency.
2. **Transduction Day 0:** Aspirate the spent medium from the fibroblasts in T75 flasks. Wash the cells in PBS without Calcium and Magnesium.
3. Add 2 mL of 0.05% Trypsin/EDTA to each flask and incubate the flasks at 37°C for approximately 4-5 minutes.

4. Add Fibroblast Medium to each flask. Tap the plate against your hand to ensure cells have been dislodged from the plate, and carefully transfer cells into a new 15-mL falcon tube.
5. Centrifuge at 300g X 5 min x RT, discard the supernatant and resuspend the cell in Fibroblast Medium and perform a cell count and viability assessment.
6. Transfer enough cells for 3 transfections (1-3 million) to a fresh 15-mL falcon tube, centrifuge the cell suspension (300g x 5 min x RT) and discard the supernatant. Dislodge the pellet gently. Cells are now ready for transfection.
7. Resuspend the pellet in Resuspension buffer R (comes with the Neon Transfection kit, Thermofisher) with a concentration of 1 x 10^6 cells/0.1-mL. If you use any other transfection method, the resuspension buffer.
8. Transfer the cell (100 µL per transfection) to a sterile 1.5-mL microfuge tube. As per the transfection system (Lonza or Thermofisher), follow the protocol as provided by the manufacturer along with the episomal vectors.
9. Once transfection is performed, transfer the transfected cells on Matrigel coated tissue culture plates with fresh Fibroblast Medium.
10. Transfer the plates in the CO_2 incubator overnight.
11. **Day 1 post-transfection**: Most of the fibroblast should have been attached. There would be some death. Replace the fibroblast medium with ReproTeSR medium and continue the culturing the cells as mentioned above.

Notes: There can be many steps in the above protocol that could significantly affect the reprogramming efficiency and generation of iPSCs.

1. The foremost being user handling and experience. The user must be trained and certified to do this process.
2. Sendai virus handling – Improper handling of Sendai virus could significantly reduce reprogramming efficiencies and hamper iPSC stability and number of clones generated.
3. Culturing medium – Improper thawing or storage of culture media could significantly hamper the generation or growth of iPSC colonies.
4. Matrigel coating of plates –Matrigel coating is another key component that can reduce iPSC growth.
5. Picking the right colony at the right time – this requires training and experience to identify newly formed iPSC colonies.
6. Contamination – Since this protocol is long (20-30 days) and requires multiple medium exchanges and the continuous movement of culture plates (BSC, microscope, CO_2 incubator), there can be a high chance of contamination. Spillage while moving plates is an important source of contamination. It is critical that operators are methodical in their cell culture technique and maintain a very clean workspace. If you are sick (cough/cold etc), it is highly recommended that you do not handle the cells.

Additional Note:

Version	Reason for Change	Author of change	Date Approved
1.0	N/A	N/A	

SOP: MS_Stem008 – Regular Maintenance of iPSCs

Regular maintenance of human induced pluripotent stem cells (iPSCs)			
SOP Number:	MS_Stem008	**Version Number:**	V1
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	See the list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of the SOP is to demonstrate maintenance of human induced pluripotent stem cells in culture
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS:

- All media used in the protocol should be at room temperature.
- ~~Do not keep any complete media in the 37°C water bath after taking out from the refrigerator~~ Never use 37°C water bath to warm complete medium, once taken out from the refrigerator.
- Always thaw medium supplements at 4°C overnight. Do not thaw them at RT before use. Avoid vigorous shaking of supplements to prevent frothing of the supplements. Mix only by gentle inverting multiple time till mixed uniformly.

EQUIPMENT AND REAGENTS

Equipment

1. Class II Biosafety Cabinet (BSC) set up in the PC2 laboratory
2. Humified CO_2 Incubator set at 5% CO_2 and 37°C
3. Microscope
4. Micropipettes range from 0.5 to 1000 µL along with sterile-filtered tips

5. Pipet-Aid (cordless), with disposable serological pipettes (5- and 10-mL)
6. Bucket/beaker with disinfectant (Bleach/Trigene) for tip discard

Reagents

1. mTeSR1 Plus complete (StemCell Technologies, Cat # 05825)
2. DMEM-F12 (Thermofisher, Cat # 11330032)
3. 70% Ethanol spray bottle for surface cleaning
4. 1:100 dilution of 5% sodium hypochlorite or 1:10 dilution of Trigene as a disinfectant.
5. Penicillin - Streptomycin Solution 10,000 IU (Thermofisher, Cat # 15140122)
6. hESC-Qualified Matrix, LDEV-free (Corning, Cat # 354277)

Disposable

1. Serological Pipets (2-, 5- and 10- mL), sterile disposable. Large 25- and 50-mL may be required
2. Tissue wipes
3. 6W Sterile Tissue Culture Plates or 6-cm tissue culture dishes (Any brand)

Personal Protective Equipment

1. Laboratory coat/Lab-gowns in PC2 lab
2. Non-powdered, nitrile or equivalent gloves
3. Covered shoes
4. Eye protection, not mandatory while working in BSC

Procedure (Process time: 10-20 min)

1. The following step can be performed a day or two in advance.
2. Complete mTeSR plus medium: Mix mTeSR plus supplement to the basal medium (500-mL) and add 5-mL of Pen-Strep (optional), mix well. If usage is low, aliquot the complete media in 50-mL falcons and store at -20°C. Frozen mTeSR plus is stable for 6 months. If usage is high, store the bottle at 4°C. Refrigerated mTeSR plus has a shelf-life of 1 month.
3. Take out the iPSC growing culture from the incubator and carefully observe under the microscope for spontaneous differentiation.
4. If there is excessing spontaneous differentiation, remove these areas using a 1-mL or 0.2-mL filtered micropipette tip under a dark field microscope.
5. Aspirate the spent medium along with the removed colony clumps, wash the wells with DMEM/F12 basal medium once or twice (depends on the amount of cleaning performed). A rough guide is not to see any floating clumps of cells in the culture plate.
6. Add fresh mTeSR plus medium and place the plate back in the incubator.
7. Medium changes are performed every 2 days, with an increasing volume to support iPSC growth and prevent spontaneous differentiation.
8. On weekend, double feed could be performed.
9. Always passage your cells on a 7-day cycle.

10. Passage cells as per SOP # MS_Stem009.

Notes: Many factors may impact the growth of iPSC cultures

1. Improper passage cycle. Always follow a 7-day cycle for maintaining cell lines.
2. Excessive spontaneous differentiation – over-crowded plates or less growth medium are main reasons for excessive spontaneous differentiation.
3. Improper removal of spontaneously differentiated areas from undifferentiated cells.
4. Continuous changes in components and change in culture practices may influence the growth kinetics of cell lines.
5. Karyotypic abnormalities within the cell lines.

Additional Note:

Version	Reason for Change	Author of change	Date Approved
1.0	N/A	N/A	

SOP: MS_Stem009 – Passaging of iPSC for Maintenance

Passaging of human induced pluripotent stem cells for maintenance			
SOP Number:	MS_Stem009	**Version Number:**	V1
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	See the list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of the SOP is outlining the process of passage for induced pluripotent stem cells in culture.
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS:

- All media used in the protocol should be room temperature.
- ~~Do not keep any complete media in the 37°C water bath after taking out from the refrigerator~~ Never use 37°C water bath to warm complete medium, once taken out from the refrigerator.
- Always thaw medium supplements at 4°C overnight. Do not thaw them at RT before use. Mix only by gentle inversion of tubes.
- Manual passaging using Dispase is the recommended method to passage cells for the first few passages on the parent plate to collect individual clones. Do not use ReLeSR for doing this step.

EQUIPMENT AND REAGENTS

Equipment

1. Class II Biosafety Cabinet (BSC) set up in the PC2 laboratory
2. Humified CO_2 Incubator set at 5% CO_2 and 37°C

3. Microscope
4. Micropipettes range from 0.5 to 1000 µL along with sterile-filtered tips
5. Pipet-Aid (cordless), with disposable serological pipettes (5- and 10-mL)
6. Bucket/beaker with disinfectant (Bleach/Trigene) for tip discard
7. Falcon (15-mL)
8. Sharp disposal bin

Reagents

1. mTeSR1 Plus complete (StemCell Technologies, Cat # 05825)
2. Dispase II (Sigma, Cat # D4693) or Dispase II solution (1mg/mL, Stem Cell Technologies, Cat # 07923)
3. ReLeSR (Stem Cell Technologies, Cat # 05872)
4. DMEM-F12 (Thermofisher, Cat # 11330032)
5. 70% Ethanol spray bottle for surface cleaning
6. 1:100 dilution of 5% sodium hypochlorite or 1:10 dilution of Trigene as a disinfectant.
7. Penicillin - Streptomycin (PS) Solution 10,000 IU (Thermofisher, Cat # 15140122)

Disposable

1. Serological Pipets (2-, 5- and 10- mL), sterile disposable. Large 25- and 50-mL may be required
2. Tissue wipes
3. 6W Sterile Tissue Culture Plates or 6 cm tissue culture dishes
4. Insulin Syringe or normal syringe with a 29 or 30G x 1.5-inch needle
5. Cell scraper (TPP, Cat # 99010)

Personal Protective Equipment

1. Laboratory coat/Lab-gowns in PC2 lab
2. Non-powdered, nitrile or equivalent gloves
3. Covered shoes
4. Eye protection, not mandatory while working in BSC

Procedure (Process time: 20-50 min, protocol dependent)

There are 2 methods of passaging hiPSC

a) Non-enzymatic method (ReLeSR)
b) Manual Method (Dispase II)

Manual Method using Dispase II (20-50 min)

1. Complete mTeSR plus medium can be prepared 1-2 days prior to passaging colonies: Mix mTeSR plus supplement with the basal medium (500-mL), add 5-mL of PS (optional), and mix well without agitation. If usage is low, aliquot the complete media in 50-mL falcons and store at -20°C. Frozen complete medium is stable for 6 months.

If usage is high, store the bottle at 4°C. Refrigerated mTeSR plus has a shelf life of 1 month.

2. Dispase II Solution (1mg/mL): You can purchase Dispase II solution from Stem Cell Technologies (ready to use). Alternatively, it can be made in-house from Dispase power (Sigma). Weigh 10 mg of Dispase and dissolve it in 10 mL of sterile DMEM/F12. Mix well to dissolve, filter sterilize the solution through a 0.22 µm syringe filter and store at 4°C. The enzyme solution has a shelf-life of 1 week. For long term storage, aliquot the enzyme solution and store at -20°C.
3. Remove the iPSC culture from the incubator and carefully observe it under the microscope (you are looking for spontaneous differentiation).
4. Aspirate the spent medium and wash the cells once with DMEM/F12 basal medium.
5. Add 1-mL of the Dispase solution to each iPSC well in a 6 well plate (or 1.5-mL to a 6-cm dish), gentle swirl and return to the incubator for 3-5 min (may vary slightly depending on the iPSC line).
6. Check the plate under the microscope – you should see "curling" of the colony edges.
7. Aspirate the enzyme and wash the cells thrice with 2-mL DMEM/F12.
8. Add 4-mL of DMEM/F12 and under the dark field microscope, using a 30G needle, manually cut the colonies to form a checkerboard. The size of the checkerboard should be uniform (approx. 200 µm by 200 µm). Cut only undifferentiated iPSC colonies. Do not disturb the differentiated colonies.
9. Using a cell scraper, scoop the colony clumps and transfer them to a freshly prepared Matrigel-coated tissue culture plate. Transfer ~50-60 clumps per plate.
10. Uniformly spread the iPSC colony clumps on the fresh tissue culture plate by back and forth motion followed by left and right motion.
11. Place the plate in the CO_2 incubator and do not disturb for 16-24 hours.
12. The next day, observe the plate under the microscope, looking for attachment of the small hiPSC clumps. Depending on the iPSC line, attachment ratios may be 30-75%.
13. Follow the SOP # MS_Stem008 for maintenance.

Non-enzymatic Method using ReLeSR (15-20 min)

1. Observe the growing iPSC culture under the microscope, looking for signs of spontaneous differentiation.
2. Aspirate the spent medium and wash the cells once with DMEM/F12.
3. Add 1-mL ReLeSR solution to each relevant well of the 6 well plate (or 1.5-mL to a 6-cm dish), gently swirl and leave in the BSC for ~1 min.
4. Aspirate the ReLeSR and transfer the plate to the incubator for 2-8 min (time may vary slightly depending on the hiPSC line but should be standardized for an iPSC line).
5. Inspect under the microscope – the colonies develop "cracks".
6. Add fresh mTeSR1 to the well and gently triturate the cells.
7. Transfer a small aliquot (containing ~100-200 clumps) to a fresh Matrigel-coated plate.
8. Ensure the colonies are evenly distributed across the plate by back and forth motion followed by left and right motion.
9. Return the plate to the CO_2 incubator and do not disturb for 16-24 hours.
10. The next day, inspect the small iPSC clumps under the microscope. 30-75% should have attached, depending on the cell line.
11. Follow the SOP # MS_Stem008 for maintenance

Notes: There are multiple reasons why iPSCs do not attach after passaging:

1. Clump size is too small. This is one of the most critical points as it reduces the seeding density in a freshly passaged plate.
2. Excessive exposure to Dispase or ReLeSR.
3. The plate is not properly coated with Matrigel.
4. Single-cell passaging is not a recommended method for maintaining iPSCs and should always be avoided.

Additional Note:

Version	Reason for Change	Author of change	Date Approved
1.0	N/A	N/A	

SOP: MS_Stem010 – Heat Inactivation of Fetal Bovine Serum

Heat Inactivation of Fetal Bovine Serum (FBS)			
SOP Number:	MS_Stem010	**Version Number:**	V1
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	As list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of this SOP is to heat inactivate fetal bovine serum (FBS) for use in various cell culture protocols.
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS

- Always thaw frozen fetal bovine serum (FBS) at 4°C.
- Gently mix the FBS bottle by inversion before use.
- Always aliquot FBS into smaller units, under sterile conditions.
- Always use a water-bath with clean water
- Avoid multiple freeze-thaw cycles of FBS

EQUIPMENT AND REAGENTS

Equipment

1. Class II Biosafety Cabinet (BSC) set up in the PC2 laboratory
2. Water-bath incubator, set at 56°C.
3. Pipet-Aid (cordless) with disposable serological pipettes (25- and 50- mL)
4. -20°C freezer
5. Stand for holding 50-mL falcons

Reagents

1. FBS (Hyclone, Catalogue may vary according to requirement and origin)
2. 70% Ethanol spray bottle for surface cleaning
3. 1:100 dilution of 5% sodium hypochlorite or 1:10 dilution of Trigene as a disinfectant.

Disposable

1. Serological Pipets (25- and 50-mL), sterile disposable
2. Falcon tubes (50-mL)
3. Tissue wipes

Personal Protective Equipment

1. Laboratory coat/Lab-gowns in PC2 lab
2. Non-powdered, nitrile or equivalent gloves
3. Covered shoes
4. Eye protection, not mandatory while working in BSC

Procedure (Process time: 30-45 min)

1. Set the water bath to 56°C.
2. Place a fully thawed, uniformly mixed, sealed bottle of FBS in the 56°C water-bath for 30 min.
3. Gently mix the contents of the bottle every 5 min for uniform heat inactivation.
4. After 30 min, wipe the FBS bottle to remove excess water and cool at RT.
5. Disinfect all items with 70% ethanol before placing them in the BSC (including FCS).
6. Create 40-mL aliquots of heat-inactivated FBS in 50-mL falcon tubes.
7. Tightly close and label the falcons with the Lot number and date and store them in a standing position in the -20°C.
8. Thaw aliquots of FBS at 4°C as and when required. Do not re-freeze thawed FBS.

Additional Note:

Version	Reason for Change	Author of change	Date Approved
1.0	N/A	N/A	

SOP: MS_Stem011 – Matrigel Coating for iPSC culture

Matrigel Coating for human induced pluripotent stem cell culture			
SOP Number:	MS_Stem011	**Version Number:**	V1
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	As list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of the SOP is to coat Matrigel on tissue culture plates for culturing human induced pluripotent stem cells.
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS:

- Always thaw Matrigel at 4°C over-night before first use.
- Do not thaw Matrigel at room temperature.
- Always keep Matrigel under cold conditions (or on ice in BSC).
- Always check and record the Matrigel Lot # before using a batch of Matrigel. Batch variations could affect iPSC growth kinetics.
- Do not freeze-thaw Matrigel.

EQUIPMENT AND REAGENTS

Equipment

1. Class II Biosafety Cabinet (BSC) set up in the PC2 laboratory
2. -20°C freezer
3. Refrigerator
4. Micropipette with sterile tips (1000 µL)
5. Esky with ice

6. Pipet-Aid (cordless) with disposable serological pipettes (5- and 10-mL)

Reagents

1. Matrigel, hESC-Qualified Matrix, LDEV-free (Corning, Cat # 354277)
2. 70% Ethanol spray bottle for surface cleaning
3. DMEM-F12

Disposable

1. Falcon tubes (5-, 25-, 50-mL)
2. Tissue culture plates (Size varies)
3. Tissue wipes

Personal Protective Equipment

1. Laboratory coat/Lab-gowns in PC2 lab
2. Non-powdered, nitrile or equivalent gloves
3. Covered shoes
4. Eye protection, not mandatory while working in BSC

Procedure (Process time: 15-30 min)

1. Disinfect the BSC content and all introduced items with 70% ethanol.
2. Remove the Matrigel aliquot from the freezer (-20°C) and let it thaw at 4°C.
3. In the BSC, transfer 25mL of ice cold DMEM-F12 into a 50 mL falcon.
4. Use a micropipette with a 1 mL filter tip to collect ~100-200 µL of DMEM-F12 and add it to the Matrigel aliquot.
5. Gently mix by pipetting and transfer the Matrigel content into the falcon of ice cold DMEM/F12.
6. Using a 10 mL serological pipette, mix the falcon tube content 3-4 times.
7. Transfer the required amount of diluted Matrigel per well or dish (the table below is a rough guide):

Tissue culture plate	Volume	Tissue culture plate	Volume
6 well plate	1 mL/ well	T-25 cm^2 flask	3 mL/flask
100 mm dish	6 mL/ dish	T-75 cm^2 flask	8 mL/flask

8. Replace the lids of the tissue culture plates and place them in the incubator.
9. The tissue culture plates will be ready to use after 2 hours but can be stored in the incubator for use within the week.

Notes: Matrigel-coated plates can also be stored in the refrigerator; however, the plates need to be sealed with parafilm. Care must be taken that the plates are stored on the flat surface that allows Matrigel to cover the entire bottom of the tissue culture plate. Plates can be used for about 2 weeks. Plates must first be thawed to room temperature before use. Never use a cold Matrigel plate for iPSC work.

Always check the Matrigel-coated plates for evaporation. Dried plates should not be used for iPSC maintenance and must be discarded.

The quality of the matrix coating significantly influences the iPSC growth kinetics and spontaneous differentiation.

Additional Note:

Version	Reason for Change	Author of change	Date Approved
1.0	N/A	N/A	

SOP: MS_Stem012- Cryopreservation of Human iPSC

Cryopreservation of Human iPSC (induced pluripotent stem cells)			
SOP Number:	MS_Stem012	**Version Number:**	V1
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	See the list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of the SOP is to cryopreserve PBMC
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS

- Keep clump size big before freezing.
- Cultures for freezing should be free from spontaneously differentiated areas.

EQUIPMENT AND REAGENTS

Equipment

1. Class II Biosafety Cabinet (BSC) set up in the PC2 laboratory
2. Refrigerated Centrifuge with swing-out bucket rotor, capable of low speeds (300 to 1000 g) with 15- or 50-mL tube holders
3. Micropipettes range from 20 to 1000 µL along with sterile-filtered tips

4. Pipet-Aid (cordless), with disposable serological pipettes (5- and 10-mL)
5. Refrigerator (2-8°C)
6. -80°C freezer for short-term storage of PBMCs
7. LN_2 for long-term storage of PBMCs
8. Bucket/beaker with disinfectant (Bleach/Trigene) for rinsing pipettes
9. Trypan blue
10. Nalgene Mr Frosty (1°C/min cryo-freezing container)
11. Stand for holding cryotubes

Reagents

1. mTeSR Plus (StemCell Technologies, Cat # 05825)
2. DMEM/F12 (Thermofisher, Cat # 11330032)
3. Dispase II (Sigma, Cat # D4693) or Dispase II solution (1mg/mL, Stem Cell Technologies, Cat # 07923)
4. 70% Ethanol spray bottle for surface cleaning
5. 1:100 dilution of 5% sodium hypochlorite or 1:10 dilution of Trigene as a disinfectant.
6. Cell culture or hybridoma grade DMSO (Sigma Cat # D2650-100mL) stored at RT and sealed with parafilm once opened.
7. CryoStor CS10 (StemCell Technologies, Cat # 07930)

Disposable

1. Serological pipets (5- and 10-mL, sterile and disposable). For larger blood volumes 25- and 50-mL may be required.
2. 2D bar-coded cryogenic vials (Thermofisher)
3. Tissue wipes
4. Cell scraper (TPP, Cat # 99010)

Personal Protective Equipment

1. Laboratory coat/Lab-gowns in PC2 lab
2. Non-powdered, nitrile or equivalent gloves
3. Covered shoes
4. Cryogloves and face shields when using LN_2
5. Eye protection, not mandatory while working in BSC

Procedure (Process time: 30-45 min)

1. Day before freezing, microscopically evaluate iPSC cultures. Clean/remove all spontaneously differentiated area from the tissue culture plate.
2. Wash the cells with DMEM/F12, once and add fresh mTeSR plus medium and place the plate back in the CO_2 incubator.
3. Next day, Aspirate the spent medium and wash the cells once with DMEM/F12 basal medium.

4. Add 1-mL of the Dispase solution to each well in a 6 well plate (or 1.5-mL to a 6-cm dish), gentle swirl and return to the incubator for 3-5 min (may vary slightly depending on the iPSC line).
5. Check the plate under the microscope – you should see “curling” of the colony edges.
6. Aspirate the enzyme and wash the cells thrice with 2-mL DMEM/F12.
7. Add 4-mL of DMEM/F12 and using a cell scraper, scoop the whole colonies (avoid breaking the colony to small clumps)
8. Transfer all the floating colonies in a 15-mL falcon tube with a 5-mL serological pipette.
9. Centrifuge the falcon tube at 100g x 3 min x RT
10. Carefully discard the supernatant and gently tap the falcon to dislodge the pellet.
11. Scan the bar-code of the cryotube and record all necessary information in the MS biobank storage database. The information recorded should contain, Type of cells with patient code, viability, number, date of freezing, and name/initials of the researcher.
12. Re-suspend the pellet in 1-mL cold CryoStor CS10. Mix the suspension with 5-mL serological pipette and transfer the contents in a 2D bar-coded cryovial and tightly screw the caps.
13. Transfer the cryovials into the pre-chilled Mr Frosty (each holds 18 tubes).
14. Place the Mr Frosty in the designated -20°C for temporary storage.
15. Remove all items and clean the BSC and working area with 70% ethanol and UV the BSC.
16. Transfer the Mr Frosty to the -80°C freezer for overnight storage.
17. The next day, transfer the cryovials to the vapour phase of LN_2 and record the storage details for each cryovial (number, position, and place in the LN_2 container).

Note: Avoid breaking iPSC colonies into smaller clumps. Keep them as big as possible for better thaw viability and attachment. Post-Dispase treatment, be gentle with the large iPSC clumps as they are prone to breaking.

Additional Note:

Version	Reason for Change	Author of change	Date Approved
1.0	N/A	N/A	

SOP: MS_Stem013- Thawing of Cryopreserved Human iPSC

Thawing of cryopreserved human iPSC			
SOP Number:	MS_Stem013	**Version Number:**	**V1**
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	See list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of the SOP is to thaw cryopreserved human iPSC for down-stream applications.
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS

- All media used in the protocol should be at room temperature.
- ~~Do not keep any complete media in the 37°C water bath after taking out from the refrigerator~~ Never use 37°C water bath to warm complete medium, once taken out from the refrigerator.

EQUIPMENT AND REAGENTS

Equipment

1. Class II Biosafety Cabinet (BSC) set up in the PC2 laboratory
2. Humified CO_2 Incubator set at 5% CO_2 and 37°C
3. Microscope

4. Refrigerated centrifuge with a swing-out bucket rotor, capable of low speeds (300 to 1000 g).
5. Table-top microfuge
6. Pipet-Aid (cordless), with disposable serological pipettes (5- and 10-mL)
7. Refrigerator (2-8°C)
8. Liquid N_2 container
9. Bucket/beaker with disinfectant (Bleach/Trigene) for tip discard
10. Test-tube stands to hold 15-, 50-ml falcon tubes.
11. Esky with ice

Reagents

1. mTeSR Plus (StemCell Technologies, Cat # 05825)
2. DMEM/F12 (Thermofisher, Cat # 11330032)
3. Rock Inhibitor, Y-27632 (StemCell technologies, Cat # 72304)
4. 70% Ethanol spray bottle for surface cleaning
5. 1:100 dilution of 5% sodium hypochlorite or 1:10 dilution of Trigene as a disinfectant.
6. Cell culture or hybridoma grade DMSO (Sigma Cat # D2650-100mL) stored at RT and sealed with parafilm once opened.

Disposable

1. Falcon tubes (15-, 50-mL)
2. Serological Pipets (2-, 5- and 10-mL), sterile disposable. Large 25- and 50-mL may be required
3. Tissue wipes
4. 6W or 6-cm Tissue Culture Plate/dish

Personal Protective Equipment

1. Laboratory coat/Lab-gowns in PC2 lab
2. Non-powdered, nitrile or equivalent gloves
3. Covered shoes
4. Cryoprotective shield and gloves mandatory while handing LN_2
5. Eye protection, not mandatory while working in BSC

Procedure (Process time: 15-30 minutes)

1. Make 10-mL of mTeSR plus medium containing 10 µM Rock Inhibitor (Ri).
2. In the BSC, add 9-mL of mTeSR plus media (containing Ri) to a 15-mL falcon and place this tube in the 37°C water bath before going to collect the iPSC from LN_2 storage.
3. Take a Matrigel-coated 6W or 6-cm tissue culture plate/dish and add 1.5-mL (6W) or 2-mL (6-cm) of mTeSR1 plus media with 10 µM Rock Inhibitor. Mix well and place the plate in the CO_2 incubator before going to collect the iPSC.
4. Fill an Esky with ice before going to the LN_2 storage room to collect the cryovial(s) of iPSC.
5. Find the location of the iPSCs to be thawed in the LN_2 storage database.

6. Wear a cryoprotective shield and gloves before taking out the desired iPSC vial from LN_2 storage and cross-check the vial with the box position. Scan the bar-code of the cryovial to reconfirm. Immerse the cryovial completely in ice and transport it to the lab.
7. In a beaker, collect 37°C water from the water-bath and immerse the cryovial. After 30 seconds gently stir the vial in the water bath.
8. While the cryovial is thawing, transfer the 15-mL falcon tube from the water bath to the BSC after cleaning with 70% ethanol.
9. When the cryovial content is ~80% thawed (a small frozen pellet remains), dry the vial, clean with 70% ethanol and transfer to the BSC.
10. Collect the content of the cryovial (should be 1-mL) with a 2-mL serological pipette and add the cells dropwise to 9-mL of mTeSR plus media.
11. Cap the falcon and mix the content by gentle turning and inversion.
12. Place the falcon tube in the swing-out rotor centrifuge and spin at 300 g x 3 min x RT.
13. Bring the 6W or 6-cm plate/dish from the CO_2 incubator to the BSC.
14. After centrifugation is complete, carefully remove and discard the supernatant from the 15-mL falcon into the bleach-containing discard container.
15. Gently finger-tap the base of the falcon to dislodge the iPSC pellet.
16. Add 1-mL of mTeSR1 plus media (containing Ri) to resuspend the pellet in the falcon tube. Do not triturate the iPSC clumps while mixing.
17. Transfer the cell suspension to the tissue culture plate/dish, ensuring uniformly distribution by back and forth motion followed by left and right motion.
18. Replace the lid and transfer the tissue culture plate to the CO_2 incubator.
19. For culturing iPSC refer to SOP # MS_Stem008

Note: One can thaw multiple lines in the same 6 well plate, provided you clearly label the wells before starting and can handle multiple cell lines without cross-contamination. Always thaw one iPSC vial in at least 2 wells with different densities. Divide the vial in 60:40 or 70:30 ratio within the 2 wells.
Post-thawing iPSC, one may see significant areas of spontaneous differentiation along with undifferentiated colonies. Remove the differentiated areas as frequently as required.

Additional Note:

Version	Reason for Change	Author of change	Date Approved
1.0	N/A	N/A	

SOP: MS_Stem014- Basic Characterization of Human iPSC

Basic characterization of human iPSC			
SOP Number:	MS_Stem014	**Version Number:**	**V1**
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	See list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of this SOP is to outline the basic characterization that is required to be perform as a quality check for human iPSCs.
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS

- All cell lines should be characterized before making freeze downs.
- Regular check of pluripotency status, chromosomal abnormalities and mycoplasma contamination should be done.

EQUIPMENT AND REAGENTS

Equipment

1. Class II Biosafety Cabinet (BSC) set up in the PC2 laboratory
2. Humified CO_2 Incubator set at 5% CO_2 and 37°C
3. Microscope

4. Refrigerated centrifuge with a swing-out bucket rotor, capable of low speeds (300 to 1000 g).
5. Table-top microfuge
6. Pipet-Aid (cordless), with disposable serological pipettes (5- and 10-mL)
7. Refrigerator (2-8°C)
8. Bucket/beaker with disinfectant (Bleach/Trigene) for tip discard
9. Test-tube stands to hold 15-, 50-ml falcon tubes
10. Esky with ice
11. All other instruments would be specific to each method

Reagents

1. PBS without Ca^{2+} and Mg^{2+}
2. 70% Ethanol spray bottle for surface cleaning
3. 1:100 dilution of 5% sodium hypochlorite or 1:10 dilution of Trigene as a disinfectant.
4. Antibodies (Oct4, Nanog, Tra-1-60, Tra-1-81, SSEA4, Sox2)
5. PCR reagents (Matermix, Water, Primers)
6. Mycoplasma Kit (Lonza, Cat # LT07-318)
7. Regents will be specific to each method used.

Disposable

1. Falcon tubes (15-, 50-mL)
2. Serological Pipets (2-, 5- and 10-mL), sterile disposable. Large 25- and 50-mL may be required
3. Tissue wipes
4. 6W or 6-cm Tissue Culture Plate/dish

Personal Protective Equipment

1. Laboratory coat/Lab-gowns in PC2 lab
2. Non-powdered, nitrile or equivalent gloves
3. Covered shoes
4. Eye protection, not mandatory while working in BSC

Procedure (Process time: Variable)

1. Basic characterization of iPSC cells should be performed using multiple methods and available protocols in the research laboratory.
2. **Evaluation of pluripotency**: Pluripotency evaluation must be done using a combination of PCR/ qPCR with immunofluorescence and flow cytometry.
    a. **For PCR/qPCR** – *POU5F1*, *SOX2*, *NANOG* must be done. A human ES line is vital for as a positive control. Negative control should be PBMCs.
    b. **Immunofluorescence** – Oct-4, SSEA4, Tra-1-80, Tra-1-61, Nanog, and Sox2. Any 4 markers should be used but **Tra-1-60 or Tra-1-81 is mandatory**.
    c. **Flow cytometry** – Oct-4, SSEA4, Tra-1-80, Tra-1-61, Nanog, and Sox2. Any 4 markers could be used **but Tra-1-60 or Tra-1-81 is mandatory**. A dual

staining of Tra-1-60 with a transcription factor is **mandatory**. A minimum of 10,000 gated events are required.

3. **Germ layer differentiation** – Embryoid bodies must be cultured for 7-10 day and germ layer could be evaluated either by immunofluorescence or PCR/qPCR. The panel of markers that must be done are:
    a. **Ectoderm**: *NES*, *PAX6*
    b. **Mesoderm**: *T*, *MESP1*, *GATA4*
    c. **Endoderm:** *AFP* and *HNF4A*
4. Alternatively, Commercial kit like STEMDiff Trilineage Differentiation kit (StemCell Technologies, Cat # 05230) can be used.
5. **Chromosomal instability**: G-banding pattern would be the standard method (Outsourced to RHH, TBA) but genome-based methods commercially available or in-house developed can also be used. However, these genome-based methods should be tested, validated, and discussed with Ashish Mehta.
6. **Mycoplasma testing**: Mycoplasma testing should be performed using Lonza MycoAlert Kit (Cat # LT07-318).
7. **Elimination of Sendai virus**: Verification of Sendai virus elimination should be performed by PCR. This test should be done around p10-p15 (cell line depended) post-generation of a new hiPSC line.
8. Above mentioned tests are **mandatory** for each cell line as a basic QC check. There are some other tests that can be performed, if required, depending on the study and PI.
9. **STR analysis:** This is not mandatory, but some journals may ask for this data during publications (Outsourced, TBA).
10. **Methylation Status**: To check methylation status of pluripotency genes only.
11. **PluriTest:** PluriTest is a commercial service from Thermofisher, which evaluates pluripotency of iPSC lines and provide comparison with well-established reference set of hESC/hiPSC line in their database. If this test is done, the basic characterization tests for pluripotency is not required (Points 2 and 3).

Additional Note:

Version	Reason for Change	Author of change	Date Approved
1.0	N/A	N/A	

SOP: MS_Stem015- Obtaining Participant Medical Information

Obtaining Participant Medical Information			
SOP Number:	MS_Stem015	**Version Number:**	**V1**
Effective Date:	1st Sept 2020	**Review Date:**	5th Oct-2023 to 30th Oct 2023

Author:	Ashish Mehta
Reviewed by:	See list above

Authorisation	
Name / Position:	Kaylene Young/ Theme Leader Brain Health and Disease Research, Menzies Institute for Medical Research, UTAS
Signature:	
Date:	

Purpose and Objective:
The purpose of the SOP is to outline the protocol for handling participant's medical information, enrolled in the research study and ensure the medical information is handled out in accordance with Good Clinical Practice Guidelines (2016), The Australian Code for the Responsible Conduct of Research (2018), UTAS Responsible Conduct of Research Framework. This SOP is based on the SWP124 (Accessing Electronic Heath Record, including DMR) and SWP118 (Disposal of confidential material) from the Clinical Research Facility (CRF), Menzies Institute for Medical Research.
Contacts:
Ashish Mehta (ashish.mehta@utas.edu.au)

CRITICAL STEPS

- All participant information is considered highly confidential and sensitive.
- Only authorized individuals (designated by the PI or clinician) will have access to them.
- All hard copies of the information should be stored in a locked cupboard.
- Online access to patient information needs to be done in a private area.

AUTHORIZATION

To access Electronic Medical Records (DMR), researcher/employees of UTAS must comply with:

1. **Ethics Approval:** Researcher/Employee accessing the records is named on the Ethics submission and/or amendments.
2. **PI Approval:** The researcher/employee has obtained the approval from the Principal Investigator of the study (in MSStem: Dr Kaylene Young).
3. **Clinician Approval:** The researcher/employee has obtained the approval from Primary Clinician of the study (in MSStem: Dr Bruce Taylor)
4. **Institutional Approval:** The researcher/employee has obtained the approval from the institution that owns the information (in MSStem: Tasmanian Health Services)
5. **Multiple Studies Approval:** In case the researcher wishes to access DME from multiple studies, ethics approval must be obtained for each.
6. Once approved, a unique personal log-in will be issued, solely for the study that has received Ethics approval. This access is subjected to audit and has an expiry date.

In order to obtain access to Electronic Health Records (e.g. DMR), complete "Accessing Electronic Health Records Request Form" (FN124), can be obtained as a soft copy from the Clinic SWP folder on Shared Drive or requested from the Clinical Research Facility Manager.

Once completed, the form needs to be sent to the Tasmanian Health Services (THS) either by post or email:

Postal address: Supervisor of Medical Records, 5th Floor, 25 Argyle Street, Hobart 7000 TAS.

Email: Dylan.arnold@this.tas.gov.au

NOTE: DO NOT DIRECTLY SEND THIS TO THE ABOVE ADDRESS. FORWARD THE COMPLETED FORM TO MANAGER, CLINICAL RESEARCH FACILITY (Ms. Jenifer Rayner)

RULES FOR USE

1. Access only information that is relevant to the study and a part of your professional duties
2. Access records on a computer that faces a private location (e.g. away from thoroughfares or open plan workspaces). If your work area is not private, Room 302 next to CRF Reception can be booked for the purpose of accessing sensitive information. To obtain access to the CRF booking, please contact CRF Manager.
3. Do not divulge, copy, release, sell, loan, alter or destroy any confidential information unless you are authorized to do so and is within the scope of your professional duties and have ethics approval.
4. Do not be careless while handling confidential information. For instance, while walking in public, always hold documents so that they cannot be read by unauthorized persons or carry them in a black/unlabelled folder.
5. Always log-off the DMR or other Electronic Health Records when you are not using them.
6. Never share your login credentials, regardless of their position.

7. Be prompt in reporting any activity or individual that you suspect may compromise the confidentiality of sensitive information.

NOTE: Every action undertaken by a user in the DMR system is logged, including the details of the date, time, user ID and the physical location of the user performing these actions. Beware that if the logs state you have searched information unrelated to your professional activity, it would be very difficult to refute it.

DISCLAIMER

1. Your obligations detailed within this document will continue after termination of your privileges as a researcher/employee at UTAS
2. Access privileges are subject to periodic review and revision
3. The University may at any time request to have your access coder or permission to confidential material revoked.

DISPOSAL OF CONFIDENTIAL MATERIAL

1. All printed documents including medical history, personal information that could identify the participant must be destroyed, if it not required.
2. Shed the confidential waste documents using the dedicated shredder in the Utilities Room (Rm 334A) as per the manufacturer's instructions.
3. Place these confidential waste documents into the locked receptacle labelled "Sucre Document Destruction" in the Supplies Room (Rm 335) for shredding by the designated contract waste disposal company.

Note: Remove all staples and adhesive labels before disposal.

Additional Note:

Version	Reason for Change	Author of change	Date Approved
1.0	N/A	N/A	