Recommended Guidelines for Handing Human iPSCs from the Cedars-Sinai iPSC Core

- Prior to culturing iPSCs received from the Cedars-Sinai RMI iPSC Core, review the information included in your Certificate of Analysis (COA) and SOPs provided in your shipment. The COA will contain culturing information specific to that cell line and the SOPs provide the best culturing practices that the cell lines have been adapted to. **Failure to follow these instructions and culture your iPSCs in the conditions outlined in the COA can result in poor recovery of the cell lines.** We cannot be held responsible for the loss of iPSCs as result of failure to follow our SOPs and COA.

- If you prefer to use different culture methods for the iPSCs that are different from our SOPs, you are most welcome to do so. However, please let the iPSC line acclimatize first to our recommended culture methods based on our SOPS and the COA specific to each cell line. We also recommend making an iPSC seed bank in your lab prior to experimentation (see below). This will save you further costs and from having to reorder vials.

- The iPSCs provided by us are cryopreserved in 1.5mL Nalgene cryovials and shipped on dry ice (domestic shipments) or in a vapor shipper (international shipments). If you do not plan on thawing your iPSCs immediately upon delivery, store your vials in vapor phase liquid nitrogen. Storage of the vials in liquid phase liquid nitrogen may result in damage to the cryovial and can compromise your sample.

- The investigator should create a stock (seed bank) of iPSCs as cryopreserved vials before beginning any experimentation.

iPSC Culturing FAQ

Thawing your iPSCs

- *What substrate should I thaw my iPSCs received from the Cedars-Sinai RMI iPSC Core onto?*

  iPSCs should be thawed directly onto Matrigel Growth Factor Reduced (GFR) Basement Membrane Matrix, *LDEV-Free (Corning Life Sciences, Catalog # 354230). Refer to your COA for the preferred coating concentration for your cell line. The iPSC Core will culture iPSCs on one of two Matrigel concentrations:

  - 0.5mg per 6-well plate or 0.083mg per well – referred to as “Regular Matrigel”
  - 1mg per 6-well palate or 0.17mg per well – referred to as “Double Matrigel”

  Specific instruction for preparing Matrigel coated plates are outlined in the standard operating procedures (SOPs) included in your shipment. Additional copies of these SOPs can be found on the Cedars-Sinai iPSC Core website under the Protocols tab.

- *What medium should iPSCs received from the Cedars-Sinai RMI iPSC Core be thawed into?*

  iPSCs should be thawed directly into mTeSR1 medium (STEMCELL Technologies, Catalog # 85850). Please do not use a medium other than mTeSR1 when thawing your iPSCs as this can affect attachment and cell recovery.

- *Should I add ROCK inhibitor to my recovery medium when thawing my cells?*

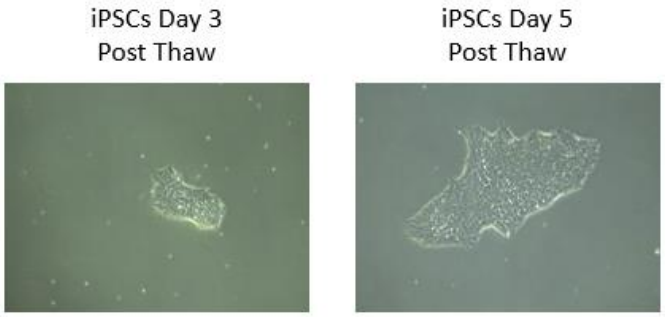
  No, ROCK inhibitor should not be added to your recovery medium.

- *How long does it take for my iPSCs to recover post thaw?*

  The exact recovery time can vary from line to line. Typically, an iPSC line will take 3-5 days to fully recover post thaw. The iPSC Core recommends looking at your cells in the microscope before changing your medium the day after thawing. If poor attachment is observed, wait two days after thawing to change the medium and continue to monitor the plate for a minimum of one week. iPSCs should be passaged 7 days after thawing regardless of colony size.

- *What should my iPSCs look like post thaw?*

  Most iPSC lines will exhibit normal PSC morphology (i.e. rounded shape, high nuclear to cytoplasmic ratio) immediately after thaw. Some cell lines will exhibit signs of stress (i.e. spikey morphology, spontaneous differentiation, small colony size). This is normal and the cell line will stabilize as you continue to culture it.



Maintaining your iPSCs

- *What conditions should be used for maintaining iPSCs received from the Cedars-Sinai RMI iPSC Core?*

  The iPSC Core maintains all iPSC lines in mTeSR1 medium on GFR Matrigel coated plates. Refer to your COA for the preferred coating concentration of Matrigel for your specific cell line. The iPSC Core does not use antibiotics or antifungals when culturing iPSCs. An investigator can add antibiotics for a short amount of time (~1-2 weeks) if contamination is suspected. It is not recommended that iPSCs be cultured in antibiotics long term.

- *My lab uses a different PSC medium. Can I culture my cells in a medium other than mTeSR1?*

  If an investigator wishes to culture their iPSCs in a different PSC medium (i.e. Essential 8 Medium), the iPSC Core recommends culturing your iPSCs in the recommended conditions (mTeSR1/Matrigel) for a **minimum of 2 weeks** post thaw to allow your cells to stabilize before transitioning your cells to a different medium. Transition your cells to the new medium slowly, beginning with a 75% mTeSR1/ 25% new medium mixture. Gradually increase the percentage of new medium while conversely decreasing the percentage of mTeSR1.

- *My lab uses a different coating substrate. Can I culture my cells on a substrate other than GFR Matrigel?*

  If an investigator wishes to culture their iPSCs on a different substrate, the iPSC Core recommends culturing your iPSCs in the recommended conditions (mTeSR1/Matrigel) for a **minimum of 2 weeks** post thaw to allow your cells to stabilize before transitioning your cells to a new substrate. On your next passaging day, prepare a Matrigel coated well of 6-well plate along with a coated well of your new substrate. Pass your cells onto both the Matrigel coated well and the new substrate coated well. Maintain the Matrigel coated well as backup until you are certain your cells have adapted to the new substrate. Once your cells have adapted to the new substrate, cryopreserve the backup cells.

- *How often should I change the medium for my iPSCs?*

  Medium should be changed every day. Investigators can choose to skip one day per week by doing a "double feed" (3-4mls per well of a 6-well plate) of mTeSR1 the day before. Skipping a medium change for more than one day per week is strongly discourage and may affect the quality of your cells.

- *How often should I check for/ remove spontaneous differentiation from my iPSCs?*

  The iPSC Core strongly recommends checking your cells daily for the presence of spontaneous differentiation. If observed, the spontaneous differentiation should be removed immediately.

- *How often should I passage my iPSCs?*

  The iPSC Core maintains a 7-day passaging schedule for all iPSCs.

- *What passaging method should I use for my iPSCs?*

  The iPSC Core utilizes 3 different passaging methods:

  - StemPro EZ Passaging Tool (Thermo Scientific, Catalog # 23181010)
  - Versene (Thermo Scientific, Catalog # 15040066) o
  - ReLeSR (STEMCELL Technologies, Catalog # 05872).

  Refer to your COA for the preferred passaging method for your specific cell line.