Frequently Asked Questions – NIGMS and NIA hiPSC

1. **What reagents should I have on hand to successfully culture iPSCs?**

Reagents and culture conditions are cell line specific. Refer to the appropriate Stem Cell Protocol and Certificate of Analysis for the specific cell line(s) you have requested for the specific culturing conditions the cell line(s) require by referring to the following links:
NIGMS- https://www.coriell.org/0/Sections/Collections/NIGMS/ipsc_list.aspx?PgId=696&coll=GM
NIA- https://www.coriell.org/0/Sections/Collections/NIA/ipsc_list.aspx?coll=AG

The table below provides a list of reagents necessary for culturing human iPSCs. There are no preferred vendors. Reagents can be obtained from a variety of vendors; the list below is given for convenience only.

Reagent	Potential Sources
6-well plates	Nunc, BD biosciences, Corning
0.1% gelatin	Millipore, Stem Cell Technologies
CF1 Mouse embryonic fibroblasts (MEFs)	ThermoFisher, Applied Stemcell, ATCC
DMEM/F12	Thermo Fisher Scientific , Stem Cell Technologies
Knock-out Serum Replacement	Thermo Fisher Scientific
mTeSR1	StemCell Technologies
Matrigel *see #2	Corning
E8	Thermo Fisher Scientific, StemCell Technologies
Vitronectin	Thermo Fisher Scientific, StemCell Technologies
L-glutamine	Thermo Fisher Scientific , Mediatech
Basic fibroblast growth factor (bFGF)	Thermo Fisher Scientific , Global Stem, Stemgent
Non-essential amino acids	Thermo Fisher Scientific , Mediatech
2-mercaptoethanol	Thermo Fisher Scientific
Rho Kinase (ROCK) Inhibitor	Stemgent, StemCell Technologies
Collagenase	Thermo Fisher ScientificSigma, Fisher
TrypLE Express	Thermo Fisher Scientific
Dispase	Thermo Fisher Scientific
Versene	Thermo Fisher Scientific

2. **If the iPSC ordered indicates to use Matrigel what type of Matrigel should I use?**

We recommend using only hESC qualified Matrigel. The other Matrigel matrices are not tested or suitable for any stem cell culture work. In house, we are using Corning catalog ID 354277. Please note that Matrigel concentration can vary from lot to lot. You must follow the manufacturer's instructions for the specific lot that you are using.

3. **Why do you suggest using a Rho Kinase (ROCK) Inhibitor in the recovery medium?**
   iPSCs have a very low viability following cryopreservation. Inclusion of the Rho Kinase (ROCK) Inhibitor in the recovery medium increases the viability of the cells increasing the number of colonies observed within the newly thawed colony and decreasing the number of days it takes for the colonies to develop.

4. **How do I know when it is time to passage my iPSCs?**
   iPSCs should be passaged for any of the following reasons:
   1. Individual colonies are large (over 700 microns)
   2. Colonies are close together and reaching confluence (~70-80%)
   3. Significant differentiation is observed

   Refer to specific stem cell protocol for the cell line you have ordered for more details.

5. **Why is the concentration of basic fibroblast growth factor (bFGF) variable?**
   Each iPSC line is grown in bFGF at the concentration specified by the submitter or due to the growth kinetics of the line. The concentration of bFGF for a specific line is indicated on the Certificate of Analysis.

6. **Do you add antibiotics to your media?**
   Coriell does not use antibiotics when culturing stem cells. Customers should be aware that inclusion of antibiotics in media may change growth characteristics of the cells. Also, continued culturing of cells in antibiotics can result in growth of antibiotic-resistant strains of bacteria

7. **Why do you recommend that I cryopreserve a small number of vials of stem cells for my laboratory?**
   Establishing a master stock is critical so that a vial can be recovered and expanded if a problem arises with the growing stem cells such as: contamination, spontaneous differentiation and changes in chromosomal integrity. If any of these occur, you can recover a vial of your master stock and expand it to make a working stock for the laboratory.

8. **Why do you recommend that I perform karyotype analysis on my stem cells?**
   Due to the possible genomic instability that can be introduced with the reprogramming process, iPSC lines need to have routine genomic analysis performed. Additionally, chromosomal abnormalities may arise if iPSC lines are not cultured appropriately. We recommend that you analyze the stem cells using G-band karyotype analysis when you create a master stock, a working stock and every 10 to 15 passages. We also recommend that karyotyping be performed if there is a noted change in morphology or growth kinetics.