Engineered iPS Cells Quick Start

Thank you for purchasing EditCo iPS Cells for your CRISPR experiment!

Your cells were created using high-quality chemically modified synthetic sgRNA and SpCas9 transfected as RNPs to ensure high editing efficiencies and without the use of any selection markers that can negatively affect cell biology. Knock-ins are generated using either ssDNA or plasmid, depending on the insert size. This Quick Start Guide can be used for all iPSC cell pools, clones, knockout and knock-in cells. In this guide, we include important information on how to store, resuscitate, and evaluate these cells. For additional details about your order, please reference the corresponding QC report.

Important Considerations

- **Please note that if the iPS cells are not recovered using the recommended conditions described in this Quick Start Guide, we cannot guarantee the viability and healthy recovery of the cells.** Please carefully follow the instructions included in Steps 2 and 3.

- **No antibiotic selection genes** were introduced when producing your cells. Broad-spectrum antibiotics/antimycotics can be added to the medium to prevent unwanted contamination. For more details on our iPS cells general editing process, please see our iPS Cell Tech Note.
    - **Note:** we use Pen/Strep (1x) for iPSC culture. We do not use antimycotics.

- **For any orders shipped on or before October 28, 2021 we have utilized StemFlex™ Medium and iMatrix-511** when culturing human iPS cells. **For any orders shipped after October 28, 2021 we have utilized mTeSR™ Plus Medium and iMatrix-511** when culturing human iPS cells. In either case, no pre-coating is necessary and iMatrix-511 is added to the cell suspension at passaging at 0.25ug/cm^2 according to Miyazaki et al.1 Please refer to either the StemFlex™ or mTeSR™ Plus protocols for details on how to culture and maintain your edited iPS cells.

- **iPS cells are incredibly sensitive and require daily maintenance.** It is vital to visualize your cells daily and clear any differentiated cells. Failure to maintain your cultures will reduce their pluripotency. Please see this guide from the New York Stem Cell Foundation for more information.

- **EditCo expands iPS cells using single cell passaging and not clump passaging.** iPS cells are adapted to single cell passaging and expansion methods during the gene editing process to fit EditCo's high throughput gene editing platform. Upon thawing of your cells you can use whichever method is suitable for your research needs.

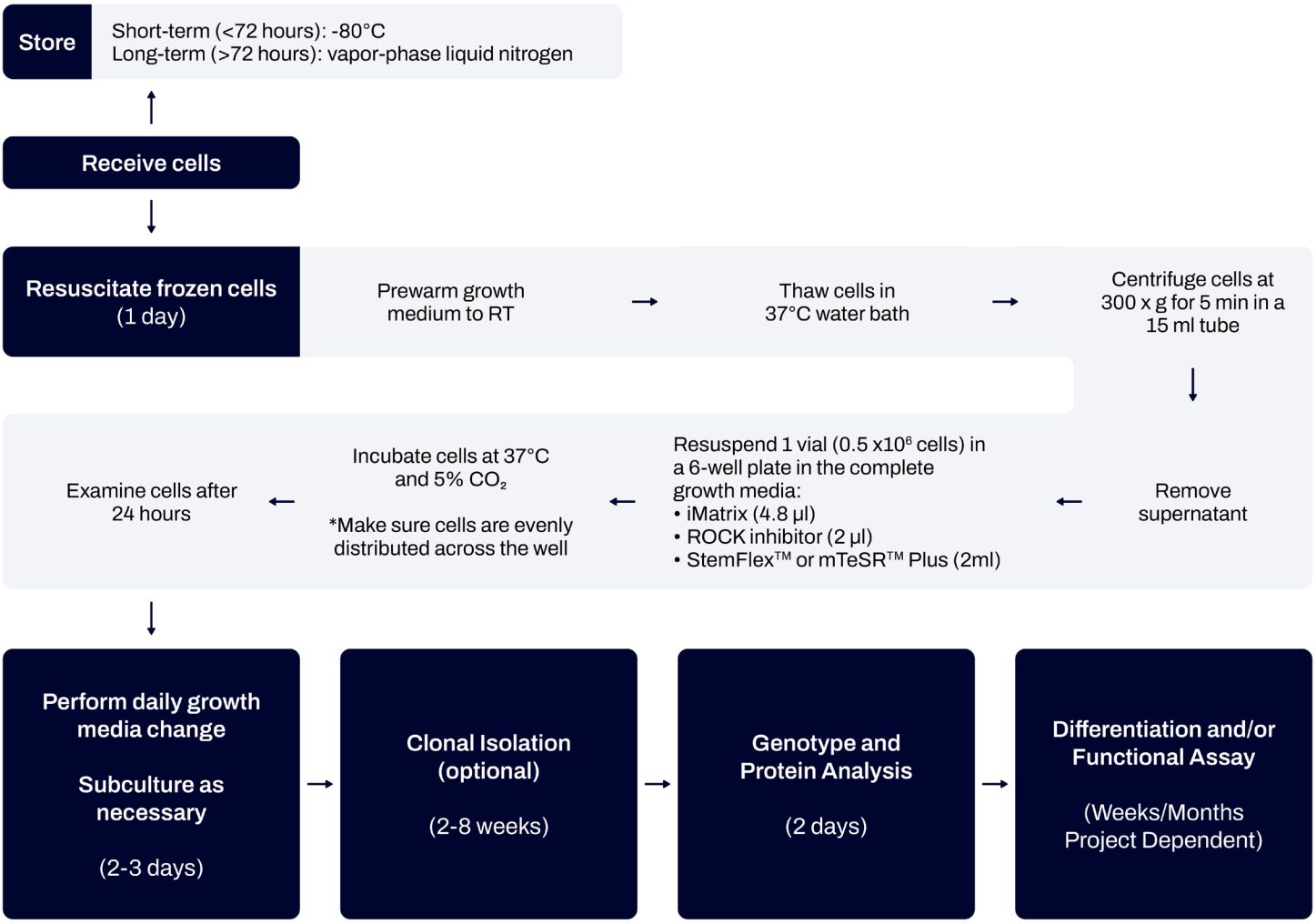
- **iPS cell line recovery and growth characteristics can vary from cell line to cell line.** Some iPS cell lines may take up to 5-10 days to fully recover from cryopreservation. It's essential to monitor the culture and maintain media changes for at least 10-14 days post-thaw to allow the cell line to recover completely.

- For pool populations, if the knockout or knock-in editing causes the cell growth dynamics to shift, the cell population may change over time. For clonal populations, the genotype of your edit is stable and will not change over time.

- Please refer to our Sample QC Report if you have any questions regarding the information included in your QC report.

1. Miyazaki, T., Isobe, T., Nakatsuji, N., & Suemori, H. (2017). Efficient Adhesion Culture of Human Pluripotent Stem Cells Using Laminin Fragments in an Uncoated Manner. Scientific reports, 7, 41165. https://doi.org/10.1038/srep4116

Workflow Schematic



Materials Required

Name
StemFlex™ Medium (Thermo Fisher Scientific, Catalog # A3349401) **Note: For any order shipped on or before October 28, 2021**
mTeSR™ Plus Medium (Stemcell Technologies, Catalog # 100-0276) **Note: For any order shipped after October 28, 2021**
iMatrix511 (Reprocell, Catalog # NP892-011)
Y-27632, ROCK Inhibitor (Stemgent, Catalog # 04-2012)
70% Ethanol (in a spray bottle)
NutriFreez® D10 Cryopreservation Medium (Biological Industries, Catalog# 05-713-1E)
StemPro™ Accutase™ Cell Dissociation Reagent (Thermo Fisher, Catalog# A1110501)
DMEM/F-12 (Thermo Fisher Scientific Catalog# 10565018)
Penicillin-Streptomycin 100X (5,000 U/mL) (Thermo Fisher; Catalog#15070063. - Optional)
Sterile 15 ml centrifuge tubes (Multiple vendors e.g, Thermo Fisher Scientific)
Sterile pipettes (Multiple vendors e.g, Thermo Fisher Scientific)
Tissue culture treated flasks, plates or dishes (Multiple vendors e.g, Thermo Fisher Scientific)
Water bath
Centrifuge
Biosafety Cabinet at appropriate containment level
Cell Incubator

Step 1. Receive Cells & Place in Storage

1. EditCo cells ship frozen. After receiving cells, immediately place them in storage. Cells should be frozen at -80°C for short-term storage (<72 hours) and placed in vapor-phase liquid nitrogen for long-term storage (>72 hours). With appropriate storage, cells can be kept for as long as needed.

   Important: Make a note of cells state upon delivery. They should still be frozen, tip the vial over to make sure the cryopreservation media is still solid. Please contact the Scientific Support Team at technicalsupport@editco.bio in case your cells arrive thawed or if there is any other visible damage.

2. If desired, cells can be thawed immediately after arrival. Please follow step 2 below. We highly recommend confirming the genotype of cells and their protein expression within 1-3 passages (see Step 3 below) and creating your own frozen cell stocks as soon as possible.

Step 2. Resuscitate the Frozen Cells

Note: Ensure all steps are completed using aseptic technique to prevent contamination.

1. Pre-warm complete StemFlex™ or mTeSR™ Plus medium to room temperature.
   (**Important:** make sure you use the same cell culture media that your iPS cells were grown in, **StemFlex™ medium for orders shipped on or before Oct 28, 2021, mTeSR™ Plus medium for orders shipped after October 28, 2021.**)

2. Remove a tube containing the frozen cells from storage and quickly place in a 37°C water bath. Please leave the top of the tube out of the water, **do not** submerge the entire tube in the water bath.

3. Rapidly thaw the cells over approx 1-2 minutes by gently swirling the tube in a 37°C water bath. Continuously monitor the tube until only small ice crystals remain.

4. Remove the tube from the water bath and spray it with 70% ethanol and wipe clean.

5. In a Biosafety hood, pipette all the contents of the tube into a 15 ml centrifuge tube and add 10 ml of pre-warmed complete mTeSR™ Plus or StemFlex™ medium to wash off the DMSO in the freezing medium.

6. Centrifuge the cell suspension at 300 X g for 5 minutes.

7. While the cells are spinning down, prepare the complete growth media as indicated below. We recommend a seeding density of 0.5 x106 viable cells per well of a 6-well plate (1x 10^5 cells per cm^2).

   Please thaw 1 vial containing 0.5 x10^6 cells in a 6-well plate:

Media Component	Volume
iMatrix	4.8 µl (0.25 µg/cm^2)
ROCK inhibitor	2 µl (final working concentration of 10 µM)
StemFlex™ or mTeSR™ Plus	2 ml

Please add iMatrix and ROCK inhibitor to complete StemFlex™ or mTeSR™ Plus media in a 15 ml conical flask.

8. Aseptically remove the supernatant from the centrifuged cell tube without disturbing the cell pellet.

9. Resuspend the cells gently in the complete cell growth medium containing iMatrix and ROCK inhibitor prepared previously.

10. Transfer the total volume of the cell suspension in the complete growth media to a 6-well plate.

11. Make sure the cells are evenly distributed across the well to allow optimal attachment and growth. Transfer the culture plate to an incubator set at 37°C and 5% CO_2. **Do not** disturb the plate for the next 24 hours.

12. Examine the cells after 24 hours to ensure they are healthy (e.g., clear borders, absence of contamination). Change fresh StemFlex™ or mTeSR™ Plus media the next day to remove the ROCK inhibitor.

    Important: iPS cell line recovery and growth characteristics can vary from cell line to cell line. For some iPS cell lines, it may take up to 5-10 days to fully recover them from cryopreservation. It is important to monitor the culture and maintain media changes for at least 10-14 days post thaw to allow the cell line to completely recover.

13. Perform daily medium change (StemFlex™ or mTeSR™ Plus only) and monitor the cell growth until the next passage.

14. Subculture as necessary. We recommend subculturing at 0.2-0.5 x 106 cells per well of a 6-well plate.

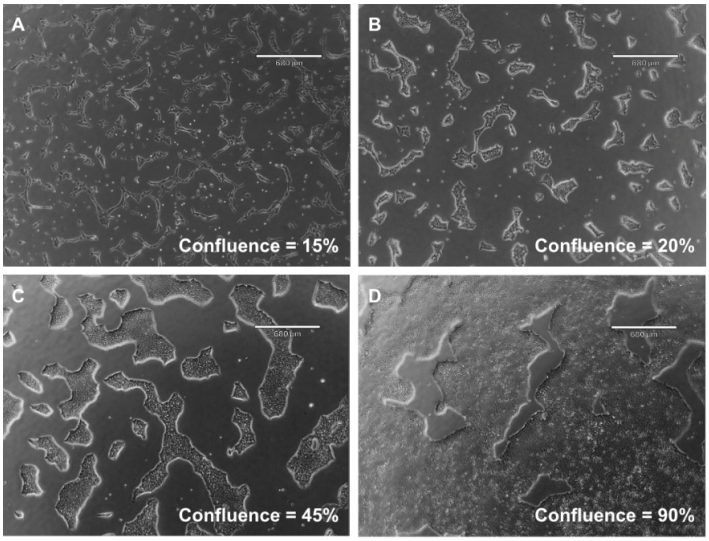


Figure 1: Example of brightfield images of healthy iPS cells at various time points post thaw.

A = 24 hours; B = 48 hours; C = 72 hours; D = 96 hours. Images captured are of an internally banked parental PGP-1 cell line at passage 27. Frozen iPS cells are thawed and plated in cell growth medium containing iMatrix511, and in the presence of Rock inhibitor (Figure 1A). After 24 hours of culturing, Rock inhibitor is removed and attached iPS cells grow (Figure 1 B, C, D). In time, healthy iPS cells become more confluent until they reach 80-90% confluency at around 96 hours post-thawing (Figure 1D) at which point they can be passaged. (scale bar 680 µm)

Important: iPS cell line recovery and growth characteristics can vary from cell line to cell line. In some cases, it can take up to 5-10 days to fully recover from cryopreservation. It's important to monitor and maintain culture and media changes for 10-14 days post thaw to allow the cell line to completely recover. Monitor the confluency during the 10 days. If after 10 days the cells are still experiencing issues, contact the Scientific Support Team at technicalsupport@editco.bio for further assistance.

Note: For more information on culturing iPS cells, please see the New York Stem Cell Foundation website or the mTeSR™ Plus or StemFlex™ protocols.

Step 3. Subculturing and Freezing

1. Prewarm complete StemFlex™ or mTeSR™ Plus medium, Accutase, and DMEM/F-12 to room temperature.

2. Aspirate media from the plate to be passaged and wash once with DMEM/F-12.

3. Add pre-warmed Accutase to the plate and place the plate in a 37°C incubator (see suggested volume below). Observe intermittently to determine when cells begin to detach (5-8 minutes). Try not to leave cells in Accutase for longer than 10 minutes.

Dish Size	Accutase
One well in 6-well dish	1 ml
T25 flask	2 ml
T75 flask	5 ml

4. When cells are completely detached from the dish by themselves, add the same volume of pre-warmed DMEM/F-12 to the plate to dilute Accutase.

5. Pipette the cell mixture in the plate up and down once to dissolve cell clumps (avoid making bubbles). Wash the bottom of the plate well to ensure detachment of cells. Transfer the cell suspension to a 15 ml conical tube. Count the cells using the cell counter.

6. Centrifuge tubes at 300g for 5 minutes.

For passage or expansion:

a. Aspirate the supernatant and re-suspend cells thoroughly in the complete mTeSR™ Plus or StemFlex™ medium containing iMatrix and ROCK inhibitor. Count the cells and seed them into new dishes in the density of 0.3-0.8 M cells in a well of a 6-well plate.

b. Incubate at 37°C and 5% CO_2, and change media after 24 hours to complete mTeSR™ Plus or StemFlex™ medium without ROCK inhibitor. Change media every 1-2 days thereafter until 80-90% confluency.

For freezing:

a. Aspirate the supernatant and re-suspend cells in NutriFreez® D10 Cryopreservation Medium (volume will be calculated based on the cell counting prior the spinning, make 1.2-1.5 M cells/ml this time).

b. Aliquot 1 ml of cell suspension in Cryopreservation Medium to each pre-labeled cryo-vial and place the vials in a freezing container and immediately place the container at -80 °C.

c. Transfer vials to LN_2 within 72 hr for long-term storage.

Step 4. Genotype & Analyze Protein

1. EditCo recommends genotyping your cells as soon as possible (within 1-3 passages). To evaluate the genotype of your edited cells you can either use Next Generation Sequencing (NGS) or Sanger sequencing.

   For single-guide knockouts and knock-in CRISPR edits, if you want to analyze genotypes using NGS, we recommend using CRISPResso. NGS primer sequences are provided in the QC report for your project.

   Alternatively, you can analyze single-guide, multi-guide, and knock-in CRISPR edits using EditCo's Inference of CRISPR Edits (ICE) tool, which relies on Sanger sequencing. Notably, EditCo's ICE tool is currently the only publicly available option for analyzing multi-guide derived CRISPR edits. For Sanger sequencing, PCR primers will be provided upon request. You can reach out to technicalsupport@editco.bio for Sanger primer suggestions for your order. Please note that our Sanger primer recommendations are computed using standard bioinformatic algorithms. They are not validated functionally by EditCo. Instructions on how to isolate genomic DNA, PCR-amplify the targeted region, and prepare for Sanger sequencing are available in our Genotyping protocol. Instructions on assessing knockout or knock-in editing efficiency using ICE are detailed in our ICE Knockout and Knock-in analysis protocols, respectively. For small knock-ins, we recommend identifying the editing genotype of the cells by Sanger sequencing and ICE analysis. For large knock-ins, the inserted sequence can be identified using junction PCR and Sanger sequencing of the PCR product.

2. For Knockout cells, protein abundance should be conducted to confirm the desired Knockout (e.g., western blot, flow cytometry, ELISA, immunostaining) within the first 7 days after thawing the cells. See our Tips and Tricks: Protein Analysis of Knockouts for an overview of different protein analysis techniques.

Step 5. Isolate Clones (Optional)

1. Clonal cell lines can be produced from a Cell Pool. Please follow the Limiting Dilution & Clonal Expansion protocol to isolate single cells via limiting dilution and generating monoclonal populations.

2. After generating clonal populations, we recommend identifying the editing genotype of the Cell Clone by Sanger sequencing and ICE analysis and validating protein depletion to confirm the desired knockout. For small knock-ins, we recommend identifying the editing genotype of the cell clone by Sanger sequencing and ICE analysis. For large knock-ins, the inserted sequence can be identified using junction PCR and Sanger sequencing of the PCR product. Appropriate downstream assays can be performed.

Appendix: Transitioning your cells from StemFlex™ to mTeSR™ Plus and vice versa

If you want to change the media you are using to grow your iPS cells we recommend you follow these guidelines:

- Ensure your iPS cells have completely recovered from thawing, look healthy and are proliferating at a normal rate (see Figure 1).
- Make sure you have made your own cell stock.
- Transitioning from media A to media B should be done by weaning out media A by replacing 50% of it with fresh media B every day.
- If cells are very healthy and have a high proliferation rate, you may need to do the media change more frequently.

Day	Media A to be replaced	New Media B to be Added	Final Media Composition
1	Retrieve 50% volume of media A	Add 50% volume media B	50% A + 50% B
2	Retrieve 50% volume of current media	Add 50% volume media B	25% A + 75% B
3	Retrieve 50% volume of current media	Add 50% volume media B	12.5 % A + 87.5% B
4	Retrieve 50% volume of current media	Add 50% volume media B	6.25% A + 93.75% B

Please note, this is just an example of a media transition. It is highly recommended to initially thaw and culture the cells as described in Step 2 and Step 3 of this quick start guide. Once the cells have recovered and look healthy, you can proceed with a slow media transition as detailedin the table above.

Important: EditCo cannot gaurantee the viability of the cells if culture conditions deviate from our recommendations in steps 2 and 3 of this quick start guide. We recommend following the workflow depicted in steps 1 through step 5 of this guide for optimal use of the Engineered Cells EditCo created for you.

Additional Information

For an up-to-date list of all protocols and other resources, please visit this link.

For technical assistance, contact our Scientific Support Team at technicalsupport@editco.bio.

For common FAQs, please visit this link.