Engineered iPS Cell Clones

Thank you for purchasing EditCo iPS Cell Clones for your experiment!

Your Cell Clones were created using high-quality synthetic chemically modified sgRNA and SpCas9 transfected as RNPs to ensure high on-target editing efficiencies. We are committed to providing you with high-quality clones containing your desired edit and without the use of any selection markers that can negatively affect cell biology. This Quick Start Guide can be used for both our Knockout and Knock-in Cell Clones. In this guide, we include important information on how to store, resuscitate, and evaluate your cells. For additional details about your order, please refer to the corresponding QC Report.

Materials Provided

Quantity	Name	Description	Storage
2 vials, 5 x 10^5 cells each	Edited Cell Clone 1	Independent clone isolated by single cell dilution from a pool generated by parental iPS cells electroporated with SpCas9 and target-specific sgRNA.	< 72 hours -80°C freezer > 72 hours vapor phase liquid nitrogen
2 vials, 5 x 10^5 cells each	Edited Cell Clone 2	Independent clone isolated by single cell dilution from a pool generated by parental iPS cells electroporated with SpCas9 and target-specific sgRNA.	< 72 hours -80°C freezer > 72 hours vapor phase liquid nitrogen
2 vials, 5 x 10^5 cells	Wild Type Cell Pool	Parental cells electroporated with SpCas9 only and confirmed to be unedited at target locus.	< 72 hours: -80°C freezer > 72 hours: vapor phase liquid nitrogen

Additional 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 Scientific, Catalog # A1110501)
DMEM/F-12 (Thermo Fisher Scientific, Catalog # 10565018)
Penicillin-Streptomycin 100X (5,000 U/mL) (Thermo Fisher Scientific, Catalog # 15070063). - Optional
Sterile 15 ml centrifuge tubes
Sterile pipettes
Tissue culture treated flasks, plates or dishes
Water bath
Centrifuge
Biosafety Cabinet at appropriate containment level
Cell Incubator

Workflow Schematic

1 DAY

Resuscitation of Frozen Cells

Warm & Examine after 24 hrs

Subculture as Necessary

1-2 WEEKS

Genotype & Protein Analysis (optimal)

PCR, Sanger, & ICE Analysis

Assess Protein Abundance (optimal)

WEEKS-MONTHS
(PROJECT-DEPENDENT)

Differentiation and/or Functional Assay

Important Considerations

- **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 general editing process, please see our iPS Cell Tech Note.
  - **Note:** we use Pen/Strep (1x) for iPSC culture. We do not use antimycotics.
- We highly recommend confirming the genotype of cells and their protein expression within 1-3 passages
(see Step 3).
- For clonal populations, the genotype of your edit is stable and will not change over time.
- **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 0.25 µg/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.

- We highly recommend creating your own frozen cell stocks as soon as possible.
- **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.

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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

Engineered iPS Cell Clones QC Reports

Note: You will receive one set of wild type controls (2 vials) per cell line in the order, regardless of the number of edits per cell type. If your order contains multiple edits and ships at different times, the wild type control information will be included in the QC report for the edited line that it has been shipped with.

Knockout iPS Cell Clones QC Report

A QC Report is provided with your order. Below is a **sample** analysis table and an explanation of each assay (by row number). Note that the assay results for your Knockout Cell Clones are in the QC report provided with your order and not depicted in the table below.

	Assay	Clone 1	Clone 2	Wild Type Cell Pool
1.	**Genotype/ICE analysis**	KO (-1)	KO (-2)	WT
2.	**Mycoplasma test**	Negative	Negative	Negative
3.	**Passage**	11	11	4

1. The edit that the clone contains. In the above example, clone 1 has a Knockout generated by a -1 deletion and clone 2 has a Knockout generated by a -2 deletion.*
2. The results of mycoplasma testing prior to shipment.
3. The total number of passages that the wild type and edited cell line have undergone at EditCo.

* A quantitative assessment of editing in expanded cells is conducted using EditCo's Inference of CRISPR Edits (ICE) analysis. Using Sanger sequenced data, the ICE software compares the sequence traces of amplicons generated from genomic DNA isolated from both the edited and unedited cells. For instructions on how to use and interpret ICE, please see our ICE Knockout and Knock-in analysis protocols. The results of this analysis can be seen using the ICE URL link included in the QC report.

Knock-in (small) iPS Cell Pools QC Report

A QC Report is provided with your order. Below is a **sample** analysis table for a Cell Clone containing an SNV edit (2 clones) and an explanation of several assays (by row number) included in the report. Note that the assay results for your Knock-In (small) Cell Clone are in the QC provided with your order and not depicted in the table below.

	Assay	Clone 1	Clone 2	Wild Type Cell Pool
1.	**Genotype/ICE analysis**	Lys123Ala	Lys123Ala	N/A
2.	**Mycoplasma test**	Negative	Negative	Negative
3.	**Passage**	5	5	5

1. The edit that the clone contains*. The position of the insert (C or N terminus) if it is a small tag. In the above SNV example, lysine was changed to alanine at position 123 (indicated as Lys123Ala).
2. The results of mycoplasma testing prior to shipment.
3. The total number of passages that the wild type and edited cell lines have undergone at EditCo.

*A quantitative assessment of editing is conducted using EditCo's Inference of CRISPR Edits (ICE) software. Using Sanger data, ICE compares the sequence traces of amplicons generated from genomic DNA isolated from both the edited and unedited cell populations. For a comprehensive guide on how to use and interpret ICE, please see our ICE Knockout and Knock-in analysis protocols. The results of this analysis are partially included in the QC report and can be seen using the ICE URL link included in the QC report.

Notes: The results of the optional tests for pluripotency (PluriTest) and genomic stability (KaryoStat) will also be included in the QC Report. If using the EditCo-supplied iPSC cell line, please see our website for further information.

Step 1. Receive Cells & Place in Storage

EditCo's Cell Clones 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.

If desired, cells can be thawed immediately after arrival. Please follow step 2.

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.
   (NOTE: make sure you use the same cell culture media that your iPS cells were grown in, **mTeSR™ Plus medium for orders shipped after October 28, 2021, and StemFlex™ medium for orders shipped on or before Oct 28, 2021.)**

2. Remove a tube containing the frozen cells from storage and quickly place in a 37°C water bath. Be careful not to submerge the cap of the 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™ Plus 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 x10^6 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)
mTeSR™ Plus or StemFlex™	2 ml

Please add iMatrix and ROCK inhibitor to complete mTeSR™ Plus or StemFlex™ 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 mTeSR™ Plus or StemFlex™ media the next day to remove the ROCK inhibitor.

    NOTE: 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 and monitor the cell growth until the next passage.
14. Subculture as necessary. We recommend subculturing at 0.2-0.5 x 10^6 cells per well of a 6-well plate.

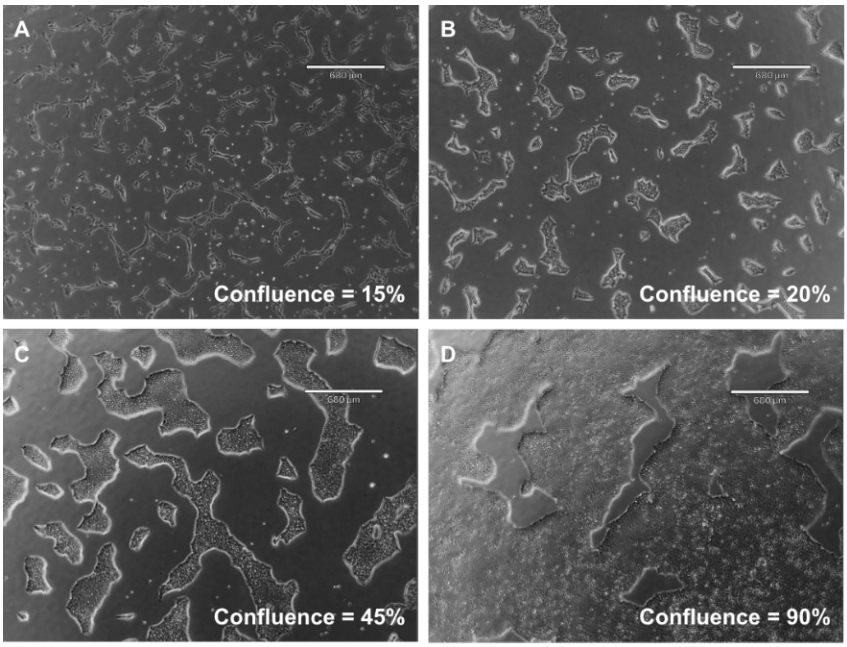

Figure 1: Example of brightfield images of healthy iPS cells at various time points post thaw.

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)

Note: 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 day and the cells are still experiencing issues, contact our technical support team at support@editco.com for further asssitance.

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 mTeSR™ Plus or StemFlex™ 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 within 1-3 passages. To genotypically evaluate your iPS Cell Clone, we recommend analyzing Sanger data using our free Inference of CRISPR Edits (ICE) tool. The PCR and Sequencing primer sequences are provided in the QC report.

   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 how to assess Knockout or Knock-in 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 Cell Clone by Sanger sequencing and ICE analysis.
2. For Knockout Clones it is recommended to measure protein abundance to confirm the desired knockout (e.g., Western blot, flow cytometry, ELISA, immunostaining) within 7 days. See EditCo's Tips and Tricks: Protein Analysis of Knockouts for an overview of different protein analysis techniques.

Step 5. Perform Functional Assay

1. Perform a functional assay on undifferentiated or differentiated cells, as appropriate.

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.

Note: 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 6 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.