

Oct 30, 2025

Protocol for Creating iPSCs from Erythroblasts

DOI

<https://dx.doi.org/10.17504/protocols.io.q26g7nndklwz/v1>

Petronio D. Zalamea¹, Jaime S. Messenger¹, Mia C. Zambrana¹, Ronald P. Hart¹

¹Department of Cell Biology & Neuroscience, Rutgers University, Piscataway, NJ 08854



Ronald P. Hart

Rutgers University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.q26g7nndklwz/v1>

Protocol Citation: Petronio D. Zalamea, Jaime S. Messenger, Mia C. Zambrana, Ronald P. Hart 2025. Protocol for Creating iPSCs from Erythroblasts. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.q26g7nndklwz/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: October 25, 2025



Last Modified: October 30, 2025

Protocol Integer ID: 230707

Keywords: induced pluripotent stem cells, iPSC, lymphocytes, erythroblasts, reprogramming, ipscs from erythroblasts genetics repository, erythroblasts genetics repository, expanded erythroblast, expanding erythroblast, pluripotent stem cell, induced pluripotent stem cell, cryopreserved lymphocyte, functional assays on specific cell type, production of ipsc, creating ipsc, specific cell type, initiating functional assay, genotype, ipsc colony

Funders Acknowledgements:

NIAAA

Grant ID: U10AA008401

NIMH

Grant ID: R01MH131296

BD² Breakthrough Discoveries for thriving with Bipolar Disorder

Grant ID: Team Lehner

Abstract

Genetics repositories usually store cryopreserved lymphocytes (CPL) from individual subjects. After GWAS or other types of genotyping, it is desirable to reprogram selected CPL with known diagnoses, genotypes, or other useful parameters into induced pluripotent stem cells (iPSC) for initiating functional assays on specific cell types. De-identified repository specimens are specifically excluded from human subjects regulations. We have optimized the production of iPSC by first expanding erythroblasts, which are then infected with commercial Sendai vectors expressing reprogramming factors. The expanded erythroblasts can be frozen as backup in case reprogramming fails to produce iPSC colonies. This protocol covers each step in the process.

Materials

Matrigel preparation:

- 5 mL stock bottle
- Ice-cold DMEM
- Matrigel

Preparation of CEPT:

- Chroman 1
- DMSO
- Emricasan
- trans-ISRIB
- Polyamine supplement

Expansion Medium:

- Stem Span XF
- Pen/Strep
- Ascorbic Acid
- SCF stock solution
- IL-3 stock solution
- EPO stock solution
- Dexamethasone
- IGF-1 stock solution

Stem Span Medium:

- Stem Span XF
- Pen/Strep

KSR Stock Medium:

- 80 mL DMEM
- 20 mL KnockOut Serum Replacement (KOSR)
- 1 mL GlutaMax (100x)
- 1 mL MEM Non-essential amino acids
- 180 μ L β -Mercaptoethanol (1000x)

Enriched Plating Medium:

- 10 mL KSR Stock Medium
- 50 μ L L-Ascorbic Acid
- 10 μ L SCF stock solution (50 μ g/mL)
- 10 μ L IL-3 stock solution (10 μ g/mL)
- 10 μ L EPO stock solution (2 U/ μ L)
- 10 μ L Dexamethasone
- 2 μ L IGF-1 stock solution (200 μ g/mL)

- 10 µL FGF stock solution (100 µg/mL)

Feeding Medium:

- 10 mL KSR Stock Medium
- 10 µL FGF stock solution (100 µg/mL)

MEF Medium:

- 435 mL DMEM
- 50 mL FBS
- 5 mL MEM Non-essential Amino Acids
- 900 µL β-Mercaptoethanol

Additional Materials:

- StemSpan™_XF (StemCell Technologies #100-0073)
- Pen/Strep (Gibco #15070-063)
- L-Ascorbic Acid (Sigma-Aldrich #50-81-7)
- SCF (Peprtech #300-07)
- IL-3 (Peprtech #200-03)
- EPO (R\0026D Systems #287-TC-500)
- Dexamethasone (Millipore Sigma #D8893-1MG)
- IGF-1 (Miltenyi Biotec # 130-138-933)
- DMEM, High glucose, with L-glutamine (Gibco #11965092)
- Fetal Bovine Serum (FBS) (Gibco #A52595-02)
- MEM Non-Essential Amino Acids (Gibco #11140-050)
- β-Mercaptoethanol (Gibco #21985-023)
- KnockOut Serum Replacement (KOSR) (Gibco #10826-010)
- GlutaMax(100x) (Gibco #35050061)
- bFGF (Peprtech #100-18B-250UG)
- polybrene-hexadimethrine bromide (Santa Cruz Biotechnology #sc-134220)
- Ultrapure Water with 0.1% Gelatin (Millipore #EX-006-B)
- Cyotune™.iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific #A16517)
- StemMACS™ iPS-Brew XF (iPS-Brew, Miltenyi Biotec #130-104-368)
- DMEM (Gibco #11965092)
- Corning® Matrigel® hESC-Qualified Matrix (Corning® #354277)
- DPBS without Ca and Mg (Gibco #14190144)
- Accutase® Cell Detachment Solution (Stemcell Technologies #07922)
- ReLeSR™ (Stemcell Technologies #100-0483)
- CEPT/polyamines (10,000x or 1000x; see protocol)
- MFreSR (Stemcell Technologies #05855)
- 0.4% Trypan Blue Solution (Invitrogen #T10282)
- Countess cell counting chamber slides (Invitrogen #C10283)
- Chroman 1, MedChem Express HY-15392
- Emricasan, SelleckChem S7775



- Polyamine supplement (1000X), Sigma-Aldrich P8483
- Trans-ISRIB, R\u0026D Systems 5284
- Dimethylsulfoxide (DMSO), Life Technologies A1516401
- Irradiated mEFs, R\u0026D systems PSC001
- EmbryoMax® Ultrapure Water With 0.1% Gelatin (Millipore #ES-006-B)
- Mr. Frosty™ Freezing Container (ThermoFisher 5100-0001)

Matrigel preparation

1. Thaw frozen 5 mL stock bottle on ice at 4°C overnight.
2. Dilute 1:1 with ice-cold DMEM. Aliquot 1 mL stock solutions and store at -20°C until ready to use.
3. Dilute 1 mL stock solution 1:35 with cold DMEM to prepare 35 mL working stock solution. This may be stored for up to 2 weeks at 4°C. Allow coated dish/plate to incubate for at least 1 hour at 37°C before starting. Use the following volumes of Matrigel for desired cell culture format:
10 cm dish – 3 mL Matrigel per dish
6 well plate – 1 mL Matrigel per well
12 well plate – 500 µL Matrigel per well
24 well plate – 250 µL Matrigel per well
96 well plate – 50 µL Matrigel per well

Preparation of CEPT

- 2 Make 0.5 mM (10,000X stock) Chroman 1.
 - Dissolve 5 mg of Chroman 1 in 22.91 mL of DMSO. Vortex until completely dissolved.
 - Store at 4°C for up to one month or -20°C for up to 1 year.Make 50 mM (10,000X stock) Emricasan
 - Dissolve 5 mg of Emricasan in 0.1756 mL of DMSO. Vortex until completely dissolved.
 - Store at 4°C for up to one month or -20°C for up to 1 year.Make 7 mM (10,000X stock) trans-ISRIB
 - Dissolve 10 mg of trans-ISRIB in 3.165 mL of DMSO. Gently warm at 45-60°C and vortex until completely dissolved.
 - Store at 4°C for up to one month or -20°C for up to 1 year.Dilute Chroman, Emricasan, and trans-ISRIB at 1:10,000 and polyamine supplement at 1:1,000 into desired medium.

Erythroblast Expansion

- 3
 1. Start with thawed, cryopreserved lymphocytes.
 2. To expand erythroblasts, place lymphocytes into a 12-well plate. (Note: Erythroblasts do not need Matrigel, they are free-floating cells.)
 3. Feed every other day with 2 mL Expansion Medium (double on Fridays for the weekend).
 4. Make and filter Stem Span Medium and Expansion Medium. Use a 50 mL Steriflip tube to filter media.
 5. Label one 15 mL conical tube per cell line.
 6. Gently mix the well using a P1000 to suspend the cells and transfer contents into the prelabeled tube.

7. Add 2 mL of Stem Span Medium to the tube and gently mix.
8. Add 1 mL of Expansion Medium to the well the cells were removed from to keep from drying out.
9. Centrifuge the 15 mL conical tube at 1000 rpm for 3 minutes.
10. Remove the supernatant and resuspend the cells using a P1000 in 1 mL fresh Expansion Medium and carefully transfer the contents into the same well on the 12-well plate.
11. Place the plate in an incubator at 37°C and 5% CO₂.
12. Let erythroblasts expand in culture for 1 week before starting the infection process.

Infection Protocol

- 4
 1. Label one 15 mL tube per cell line.
 2. Transfer cells from the well to one 15 mL tube using a P1000.
 3. Wash well 2x with 1 mL of Stem Span Medium and add to the 15 mL tube.
 4. Gently mix cell suspension.
 5. Use a 10 µL pipette tip to remove 10 µL of cell suspension.
 6. Combine with 10 µL of Trypan blue in an Eppendorf and mix.
 7. Dispense 10 µL of cells into a Countess slide.
 8. Place slide into Countess and press count button.
 9. Calculate the total amount of cells that are in the 15 mL tube.
10. Spin cells at 1000 rpm for 3 minutes.
11. Aspirate and discard supernatant. Resuspend cell pellet to 1×10^6 cells per mL using Stem Span Medium.
12. Mix gently and transfer 2 aliquots of 8×10^4 cells each into 2 pre-labeled Eppendorf tubes.
13. It is recommended to do 2 transfections per cell line.
14. Centrifuge in Mini-spin at 2000 rpm for 1 minute.
15. Remove and discard supernatant. Resuspend the cells in 250 µL of fresh EM Medium
16. Place into 1 well of the 24-well plate
17. Defrost the Sendai Virus Vectors according to the manufacturers protocol.
18. Mix and quickly spin down Sendai 2.0 vial to collect liquid
19. Make a master mix of Expansion Medium and polybrene-hexadimethrine bromide, per infection:
20. 2.5 mL EM medium
21. 1 µL polybrene-hexadimethrine bromide
22. Determine the multiplicity of infection (MOI) for each lot of virus and your cells, with a goal of hitting about MOI=5 for KOS, cMyc MOI=5 and Klf4 MOI=6.
23. Add the calculated volume of Sendai 2.0 vials to 250 µL per cell of Expansion medium with polybrene-hexadimethrine bromide line in Eppendorf tubes. Add 250 µL virus mix to each well that is being transduced
24. Place plate in an incubator at 37°C and 5% CO₂

25. Freeze remaining ERYB cells

Freezing ERYB cells

- 5
 1. Transfer contents of well (from 12 well plate; cells + Expansion Medium) into conical tube (would be about 2-3 mL of solution).
 2. Add 10 μ L Trypan Blue into a microcentrifuge tube and add 10 μ L of cells into same tube and dispense 10 μ L into a Countess slide.
 3. Determine how many vials are needed for freezing 1.5×10^6 cells per vial.
 4. Centrifuge tubes at 1000 rpm for 3 minutes.
 5. Aspirate supernatant.
 6. Add required volume of mFreSr to determine desired number of vials to have 1.5×10^6 cells in each.
 7. Place tube into a Mr. Frosty and freeze in a -80°C freezer until ready to be placed into LN_2 tank (at least overnight).

Plating mEFs

- 6
 1. Coat 100 μ L of 0.1% gelatin on 4 wells of 96-well plate per cell line, incubate for 1 hr.
 2. Thaw and seed irradiated mEFs onto 4 wells of 0.1% Gelatin coated 96-well plate
 3. Thaw frozen irradiated mEFs on ice and place inside hood until cells are thawed. Each vial contains 6 million cells in 1 mL.
 4. Clean outside of thawed mEF tube with 70% ethanol to prevent contamination.
 5. Once completely thawed, transfer half of the contents of the mEF vial (about 3 million cells) into a 15 mL conical tube and add 9 mL of mEF media to the tube.
 6. Pellet at 200 rcf (1336 rpm) for 3 minutes.
 7. Aspirate all gelatin solution from pre-warmed coated gelatin 96-well plates
 8. Resuspend pellets with 10 mL of mEF media
 9. Plate 100 μ L resuspended mEFs into each well of a warm gelatin coated 96-well plates
 10. Incubate the 96 well plates in a 37°C 5% CO_2 incubator overnight

Plating cells on 96-well plate

- 7
 1. Label one Eppendorf tube
 2. Make and filter KSR Stock Media for later use
 3. Make and filter Enriched Plating Medium containing growth factors for plating
 4. Add bFGF just before using media as it is unstable in culture media.
 5. Collect cells from one well and place them into the Eppendorf tube
 6. Centrifuge the tube at 2000 rpm for 1 minute.
 7. Resuspend cells in 100 μ L of Enriched Plating Medium

8. Prepare 96-well plate by aspirating the old medium and adding 100 μ L of Enriched Plate Medium to each well
9. Plate resuspended cells to 96-well plate at dilutions: 40%, 30%, 20%, 10%
10. Place the plate in an incubator at 37° C and 5% CO₂.

Feeding 96-well plate

- 8
 1. Exchange media every other day with 100 μ L of Feeding Medium
 2. Add bFGF just before using media as it is unstable in culture media
 3. After two weeks of feeding with Feeding Medium, transition to iPS Medium (iPS Brew) by aspirating the medium and replacing it with iPS-Brew.
 4. When colonies appear and reach a good size pick them onto an MG coated 24-well plate. When determining whether a colony is a good size and is a good colony to pick, it should not be too close to another colony, to ensure an individualized colony, and should have a darker color/complexion. About 2-3 mm diameter is best.

Colony Formation and Passaging

- 9
 1. Colony formation in 96-well plates should be transferred to a plate of larger area. Single colonies from cell lines should be transferred to a 24-well plate has previously been coated with Matrigel and since replaced with 1 mL of iPS Brew + Pen/Strep and CEPT.
 2. Wash the well containing colonies with DPBS before replacing with 100 μ L of iPS Brew.
 3. Prepare a glass 'picker' by exposing an autoclaved glass Pasteur pipet to a flame and bending the pipet into an L-shape. Bring the edge of the pipet to the flame and pull the edge of pipet off to produce a sharper and thinner edge for more precise picking.
 4. Disinfect the pipet by dipping it in 100% ethanol and drying off before putting it under a flame.
 5. Find desired colony and start to lift the colony off the plate by picking at the sides of the colony until the colony is completely detached from the plate.
 6. Using a 200 μ L micropipet, remove the medium containing the detached colony into a labelled well of a 24-well plate.
 7. Add 100 μ L of iPS Brew into the empty well.
 8. Place the 24-well plate into a 37° C incubator at 5% CO₂ and change medium every 24-48 hours with 0.5 mL iPS Brew with NO CEPT.
 9. Pick 2-3 colonies per cell line in case a colony dies during the expansion process.
 10. Feed 24-well plate every other day with 0.5 mL iPS Brew (double feed on Friday for the weekend) until the well becomes confluent.
 11. If in the 24-well plate there are a large number of differentiated cells, but colonies are still visible, transfer clean, undifferentiated colonies into a pre-coated Matrigel 12-well



plate using the same techniques as listed above with 1 mL of iPS Brew + Pen/Strep + CEPT

12. Replace medium every 24-48 hours with no CEPT/polyamines.
13. If there is little to no differentiation of cells/colonies in 12-well plate, passage the cells into a 6-well plate, coating 3 wells per cell line with Matrigel.
14. For standard passaging of colonies, coat a 6-well plate, assigned 3 wells for each cell lines, with Matrigel, and use ReLeSR as follows.
15. Aspirate old medium and add 1 mL DPBS for washing. Shake plate in a circular motion to wash off excess dead cells. Aspirate.
16. Add 1 mL ReLeSR for 1 minute (30 seconds if there is a lot of differentiation).
17. Aspirate ReLeSR, leaving only a thin layer on top of cells.
18. Incubate for 5 minutes in a 37°C, 5% CO₂ incubator. (4 minutes for differentiation)
19. Add 1 mL of IPSBrew and lightly tap the plate with the pipette aid to detach cells further from plate.
20. Distribute desired amount of cell solution for seeding into pre-coated MG plate with 2 mL iPSBrew + P/S + CEPT.
21. Once colonies have expanded to about 70-85% confluency without discernable differentiation in their designated 3 wells of a 6-well plate, you can begin the process of freezing down stock vials and producing RNA pellets.