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Mouse Embryonic Stem Cells Culture and Differentiation to Neural Stem Cells

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We use this protocol and it's working

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Abstract

The property of stem cell pluripotency observed in the inner cell mass of the developing mouse embryo at the blastocyst stage can be modeled in vitro via mouse embryonic stem cells (mESCs). mESCs can be cultured in gelatinized plates with media containing Leukemia inhibitory factor (LIF) and fetal bovine serum (LIF and serum), which maintains a dynamic and heterogeneous pluripotency state. These cells can also be cultured in media with two kinase inhibitors (2i) + LIF, which maintains a homogeneous naïve pluripotent state that is germline competent. These cells can be differentiated to the neural lineage by culturing them in neural stem cell (NSC) media, and NSCs can be isolated and cultured from this process. Here we describe protocols for culturing mESCs in LIF and serum or 2i + LIF conditions, and induction of neural differentiation to obtain and culture mESC-derived mouse NSCs.

Image Attribution

Images were obtained by Francisco Bustos, Sanford Research, Sioux Falls, South Dakota.

Protocol materials

- ☒ Mouse Laminin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L2020**
- ☒ DMEM, high glucose, no glutamine **Thermo Fisher Catalog #11960069**
- ☒ KnockOut[®]; Serum Replacement **Thermo Fisher Catalog #10828028**
- ☒ Fetal Bovine Serum, Regular, USDA Safety Tested **Corning Catalog #35-010-CV**
- ☒ MEM Non-Essential Amino Acids Solution (100X) **Thermo Fisher Catalog #11140050**
- ☒ L-Glutamine (200 mM) **Thermo Fisher Catalog #25030149**
- ☒ Penicillin-Streptomycin (10,000 U/mL) **Thermo Fisher Scientific Catalog #15140122**
- ☒ 2-Mercaptoethanol, 99%, pure **Thermo Fisher Scientific Catalog #AC125472500**
- ☒ ESGRO[®] Recombinant Mouse LIF Protein **Merck MilliporeSigma (Sigma-Aldrich) Catalog #ESG1107**
- ☒ Human LIF Recombinant Protein, PeproTech[®] **Thermo Fisher Scientific Catalog #300-05-25UG**
- ☒ GST-LIF recombinant protein **MRC-PPU Reagents and Services Catalog #DU1715**
- ☒ Gelatin from porcine skin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #G1890**
- ☒ PBS (10X), pH 7.4 **Thermo Fisher Catalog #70011069**
- ☒ 0.05% Trypsin-EDTA, phenol red **Invitrogen - Thermo Fisher Catalog #25300054**
- ☒ DMSO ≥ 99.9% **VWR International (Avantor) Catalog #MK494802**
- ☒ Externally and Internally Threaded Cryogenic Storage Vials **Thermo Fisher Catalog #12567501**
- ☒ DMEM/F-12, no glutamine **Thermo Fisher Catalog #21331020**
- ☒ Neurobasal[™] Medium **Gibco - Thermo Fisher Scientific Catalog #21103049**
- ☒ N2 supplement (100x supplement) **Gibco - Thermo Fisher Scientific Catalog #17502048**
- ☒ B-27[™] Supplement (50X), serum free **Gibco - Thermo Fisher Scientific Catalog #17504044**
- ☒ Glucose Solution **Thermo Fisher Catalog #A2494001**
- ☒ Gibco[™] Bovine Albumin Fraction V (7.5% solution) **Thermo Fisher Scientific Catalog #15260037**
- ☒ StemPro[™] Accutase[™] Cell Dissociation Reagent **Gibco - Thermo Fisher Scientific Catalog #A1110501**
- ☒ Phosphate-Buffered Saline, 1X without calcium and magnesium, PH 7.4 ± 0.1 **Corning Catalog #21-040-CV**
- ☒ CHIR99021 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #SML1046**
- ☒ PD 0325901 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #PZ0162**
- ☒ Gibco[™] DPBS (10X), calcium, magnesium **Fisher Scientific Catalog #14080055**
- ☒ Gibco[™] Mouse FGF-basic (FGF-2/bFGF) Recombinant Protein, PeproTech[®] **Fisher Scientific Catalog #4503310UG**
- ☒ Gibco[™] Mouse EGF Recombinant Protein, PeproTech[®] **Fisher Scientific Catalog #31509100UG**

 Gibco™ DMEM/F-12 HEPES Fisher Scientific Catalog #11-330-032

Troubleshooting



Mouse Embryonic Stem Cells Culture in LIF and Serum conditions

1w 5d

- 1 This protocol can be used to culture mouse embryonic stem cell lines including CCE, J1, E14TG2a, and others. 5d

Citation

Bustos F, Segarra-Fas A, Chaugule VK, Brandenburg L, Branigan E, Toth R, Macartney T, Knebel A, Hay RT, Walden H, Findlay GM (2018)

. RNF12 X-Linked Intellectual Disability Mutations Disrupt E3 Ligase Activity and Neural Differentiation.

<https://doi.org/10.1016/j.celrep.2018.04.022>

LINK

Citation

Villa F, Fujisawa R, Ainsworth J, Nishimura K, Lie-A-Ling M, Lacaud G, Labib KP (2021) . CUL2(LRR1) , TRAIIP and p97 control CMG helicase disassembly in the mammalian cell cycle.

<https://doi.org/10.15252/embr.202052164>

LINK

- 2 ESCs must be passed every  48:00:00 and media changed every  24:00:00 . 5d
However, occasionally cells can be grown for  48:00:00 without a media change if the plate is in low confluency.
- 3 ESCs can be grown in  10 mL of ES-DMEM + LIF in a 10 cm plate.
- 4  1000000 cells typically give a confluent plate after  48:00:00 , after which a 2d
1/10 or 1/20 splitting is normally sufficient for keeping stocks.

Preparation of ES-DMEM + LIF

5 For preparation of  600 mL **ES-DMEM +LIF**: DMEM containing  10 % (v/v) fetal bovine serum (FBS),  5 % (v/v) Knock-Out serum replacement,  50 ng/mL GST-tagged leukemia inhibitory factor (LIF),  100 U/mL penicillin/streptomycin,  2 millimolar (mM) glutamine,  0.1 millimolar (mM) minimum essential media (MEM) Non-essential amino acids,  1 millimolar (mM) sodium pyruvate and  0.1 millimolar (mM) β -mercaptoethanol (BME).

To a  500 mL

 DMEM, high glucose, no glutamine **Thermo Fisher Catalog #11960069** bottle add:

5.1  Fetal Bovine Serum, Regular, USDA Safety Tested **Corning Catalog #35-010-CV** :
 60 mL .

5.2  KnockOut™ Serum Replacement **Thermo Fisher Catalog #10828028** :
 30 mL .

5.3  MEM Non-Essential Amino Acids Solution (100X) **Thermo Fisher Catalog #11140050**
 :  6 mL .

5.4  L-Glutamine (200 mM) **Thermo Fisher Catalog #25030149** :  6 mL .

5.5  Penicillin-Streptomycin (10,000 U/mL) **Thermo Fisher Scientific Catalog #15140122**
 :  6 mL .

5.6  2-Mercaptoethanol, 99%, pure **Thermo Fisher Scientific Catalog #AC125472500** :
 4 μ L .

5.7  GST-LIF recombinant protein **MRC-PPU Reagents and Services Catalog #DU1715**

to [M] 50 ng/mL final concentration :  10 μ L of [M] 3.12 mg/mL stock.

Note

Alternative LIF sources:

-  ESGRO® Recombinant Mouse LIF Protein **Merck MilliporeSigma (Sigma-Aldrich) Catalog #ESG1107**

use [M] 500 U/mL final .

-  Human LIF Recombinant Protein, PeproTech® **Thermo Fisher Scientific Catalog #300-05-25UG**

use [M] 50 ng/mL final .

5.8 Filter in Stericup in biosafety cabinet to sterilize.

Equipment

Millipore® Stericup® Quick Release Vacuum Filtration System NAME

0.22 um polyethersulfone membrane filtration system TYPE

Millipore BRAND

All Photos(3) Key Documents COA/COQ View All Docum SKU

<https://www.sigmaaldrich.com/US/en/product/mm/s2gpu05re> LINK

0.22 um filter SPECIFICATIONS





Preparation of 0.1% Gelatin in PBS

6 Prepare 1% Gelatin.

Dissolve  10 g of

 Gelatin from porcine skin Merck MilliporeSigma (Sigma-Aldrich) Catalog #G1890

powder in  1000 mL ultrapure water.

6.1 Autoclave.

7 Prepare 0.1% Gelatin in PBS.

7.1 To a  500 mL bottle add:

7.2  45 mL  PBS (10X), pH 7.4 Thermo Fisher Catalog #70011069 .

7.3  425 mL ultrapure water.

7.4  50 mL of 1% Gelatin from step 1.

7.5 Filter in Stericup in biosafety cabinet to sterilize.

Equipment

Millipore® Stericup® Quick Release Vacuum Filtration System NAME

0.22 um polyethersulfone membrane filtration system TYPE

Millipore BRAND

All Photos(3) Key Documents COA/COQ View All Docum SKU

<https://www.sigmaaldrich.com/US/en/product/mm/s2gpu05re> LINK

0.22 um filter SPECIFICATIONS



Mouse Embryonic Stem Cells Passaging (10cm plate)

15m

8 Aspirate the media.

9 Wash cells in 5 mL

Phosphate-Buffered Saline, 1X without calcium and magnesium, PH 7.4 ± 0.1 **Corning Catalog #21-040-CV**

twice.

10 Aspirate and add 2 mL

0.05% Trypsin-EDTA, phenol red **Invitrogen - Thermo Fisher Catalog #25300054**

11 Incubate 00:05:00 at 37 °C .

5m

12 Add 4 mL of ES-DMEM +LIF to the trypsinized cells and gently pipette 10 times to break up any remaining cell clumps.

13 Transfer to a 15 mL conical tube.

14 Centrifuge 1000 rpm, 25°C, 00:05:00 , aspirate, and resuspend in 1 mL of media.

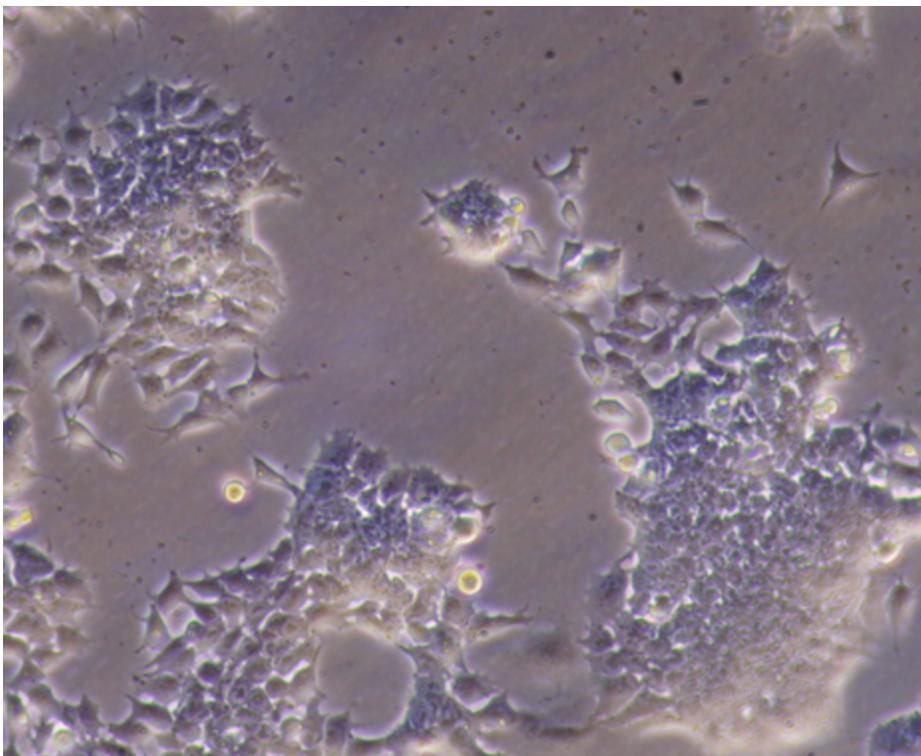
5m

15 Gelatinize plates: add 5 mL per 10 cm plate of 0.1 % (v/v) gelatin in PBS, spread and incubate for 00:05:00 , and aspirate.

5m

16 Count cells and plate desired seeding density in 7 mL ES-DMEM +LIF per 10 cm plate.

17



mESCs in ES-DMEM + LIF Media

Mouse Embryonic Stem Cells Freezing

18 Prepare **Freezing media** (80% ES-DMEM, 10% DMSO, 10% FBS).

19 For  10 mL of freezing media add:

19.1  8 mL ES-DMEM + LIF.

19.2  1 mL  DMSO ≥ 99.9% **VWR International (Avantor) Catalog #MK494802** .

19.3  1 mL  Fetal Bovine Serum, Regular, USDA Safety Tested **Corning Catalog #35-010-CV** .

20 Split cells normally (steps 7-12), centrifuge  1000 rpm, 00:05:00 , and gently resuspend in freezing media.

21 Transfer to

1d

 Externally and Internally Threaded Cryogenic Storage Vials **Thermo Fisher Catalog #12567501**

and incubate overnight in a:

Equipment	
Freezing container, Nalgene® Mr. Frosty	NAME
Cryopreservation	TYPE
Mr. Frosty	BRAND
C1562-1EA	SKU
https://www.sigmaaldrich.com/US/en/product/sigma/c1562?srsltid=AfmBOop_0lwqk5VONiPEPXRsjIThyq_U1vmTjaleiS_dhTHbNn_UcFG1	LINK

at  -80 °C for  24:00:00 .

22 Store at  -150 °C .

Mouse Embryonic Stem Cells Thawing

23 Thaw vial in a  37 °C water bath.

24 Prepare a tube with  9 mL ES-DMEM + LIF.

25 Once the cells have thawed, transfer them to the tube and centrifuge for  1000 rpm, 00:05:00 .

26 Aspirate supernatant.

27 Resuspend the pellet in ES-DMEM + LIF.

28 Transfer to a gelatinized plate.

Conversion to Naïve Pluripotency (2i + LIF)

1d 0h 10m

29 Protocol based on the following literature:



Citation

Nichols J, Jones K, Phillips JM, Newland SA, Roode M, Mansfield W, Smith A, Cooke A (2009)
. Validated germline-competent embryonic stem cell lines from nonobese diabetic mice.

<https://doi.org/10.1038/nm.1996>

LINK

Citation

Mulas C, Kalkan T, von Meyenn F, Leitch HG, Nichols J, Smith A (2019)
. Defined conditions for propagation and manipulation of mouse embryonic stem cells.

<https://doi.org/10.1242/dev.173146>

LINK

30 **Resuspension of 2i kinase inhibitors** (to make [M] 10 millimolar (mM) stocks):

 CHIR99021 Merck MilliporeSigma (Sigma-Aldrich) Catalog #SML1046

Molecular weight: [M] 465.34 g/mol

Resuspend  5 mg in  1074.5 μ L DMSO.

 PD 0325901 Merck MilliporeSigma (Sigma-Aldrich) Catalog #PZ0162

Molecular weight: [M] 482.19 g/mol

Resuspend  5 mg in  1036.9 μ L DMSO.

31 For preparation of  250 mL **N2B27 Media** ([M] 1 % (v/v) B27 supplement, [M] 0.5 % (v/v) N2 supplement , [M] 2 millimolar (mM) glutamine ; [M] 0.1 millimolar (mM) β -mercaptoethanol; [M] 100 U/mL penicillin/streptomycin in 1:1 DMEM/F12:Neurobasal medium)
In a  250 mL Stericup.

Equipment

Millipore® Stericup® Quick Release Vacuum Filtration System NAME

0.22 um polyethersulfone membrane filtration system TYPE

Millipore BRAND

All Photos(3) Key Documents COA/COQ View All Docum SKU

<https://www.sigmaaldrich.com/US/en/product/mm/s2gpu05re> LINK

0.22 um filter SPECIFICATIONS




add:

31.1  DMEM/F-12, no glutamine **Thermo Fisher Catalog #21331020** :  125 mL .

31.2  Neurobasal™ Medium **Gibco - Thermo Fisher Scientific Catalog #21103049** :
 125 mL .

31.3  N2 supplement (100x supplement) **Gibco - Thermo Fisher Scientific Catalog #17502048**
 :  1.25 mL .

31.4  B-27™ Supplement (50X), serum free **Gibco - Thermo Fisher Scientific Catalog #17504044**
 :  2.5 mL .

31.5  Penicillin-Streptomycin (10,000 U/mL) **Thermo Fisher Scientific Catalog #15140122**
 :  2.5 mL .

31.6  L-Glutamine (200 mM) **Thermo Fisher Catalog #25030149** :  2.5 mL .

31.7  2-Mercaptoethanol, 99%, pure **Thermo Fisher Scientific Catalog #AC125472500** :
 1.75 μ L .

32 For preparation of  30 mL **2i + LIF Media**. To  30 mL of N2B27 in  50 mL conical tube add :

32.1  9 μ L of  10 millimolar (mM) stock **CHIR99021** (from step 22) to  3 micromolar (μ M) final concentration.

32.2  3 μ L of  10 millimolar (mM) stock **PD0325901** (from step 22) to  1 micromolar (μ M) final concentration.

32.3  **GST-LIF recombinant protein MRC-PPU Reagents and Services Catalog #DU1715** to  50 ng/mL final concentration.

Note

From  3.12 mg/mL stock , dilute 1/10 in

 Phosphate-Buffered Saline, 1X without calcium and magnesium, PH 7.4 \pm 0.1 **Corning Catalog #21-040-CV**

and add  5 μ L of this dilution to the 2i + LIF media mix.

32.4 Filter through syringe filter into conical tube. Store at  4 $^{\circ}$ C .

33 **2i + LIF conversion (Do in 6-well or equivalent size plate).**

33.1 In gelatinized plates, seed  80000 cells per cm² in 6-well plates in ES-DMEM + LIF, and incubate  24:00:00 at  37 °C . 1d

33.2 Wash with  1 mL

 Phosphate-Buffered Saline, 1X without calcium and magnesium, PH 7.4 ± 0.1 **Corning Catalog #21-040-CV**

twice.

33.3 Add  200 μL 5m

 StemPro™ Accutase™ Cell Dissociation Reagent **Gibco - Thermo Fisher Scientific Catalog #A1110501**

and incubate at  37 °C for  00:05:00 .

33.4 Resuspend in  400 μL 2i+LIF media.

33.5 Centrifuge  0.7 x g, Room temperature, 00:05:00 . 5m

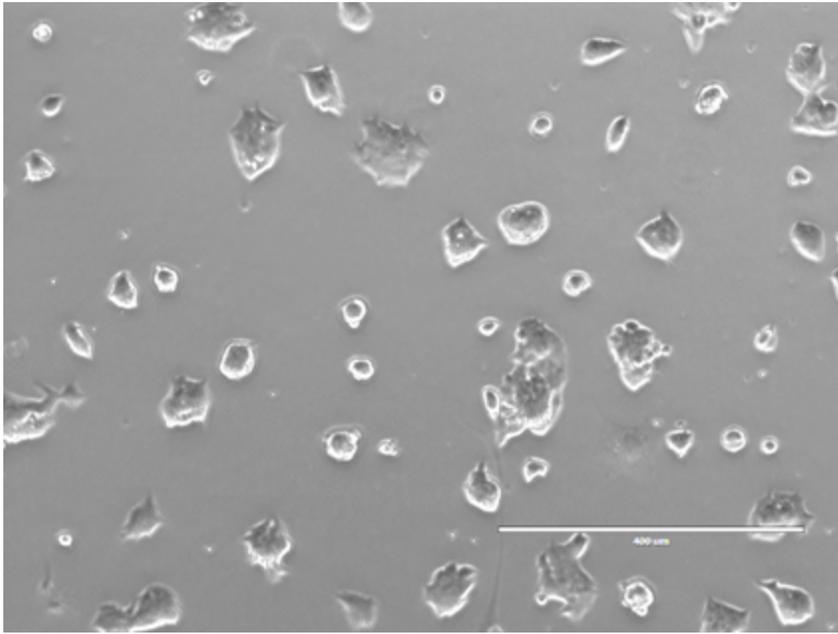
33.6 Aspirate supernatant and resuspend in  500 μL 2i+LIF media.

33.7 Add  100 μL of cell suspension (or  80000 cells per cm²) to new gelatinized plate.

33.8 Add  2 mL 2i+LIF media.

33.9 After 3 splits (steps 33.2 - 33.8 three times) cells are now converted to 2i + LIF (naïve pluripotency).

34



mESCs converted to naïve (2i + LIF) Pluripotency

Mouse Neural Stem Cell Differentiation from mESCs

1w 6d 3h 5m

35 Protocol based on the following literature:

Citation

Pollard SM, Benchoua A, Lowell S (2006). Neural stem cells, neurons, and glia.

<https://doi.org/>

LINK

Citation

Pollard SM (2013). In vitro expansion of fetal neural progenitors as adherent cell lines.

https://doi.org/10.1007/978-1-62703-574-3_2

LINK

36 For preparation of  530 mL (approximately) **NSC media** ( 1 % (v/v) B27 supplement,  0.5 % (v/v) N2 supplement ,  2.9 g/L glucose,  0.1 millimolar (mM) minimum essential media (MEM) Non-essential amino acids,  0.012 % w/v Bovine Albumin Fraction V,  0.1 millimolar (mM) β -mercaptoethanol;  100 U/mL penicillin/streptomycin in DMEM/F-12, HEPES).

To a  500 mL bottle of

 Gibco™ DMEM/F-12 HEPES Fisher Scientific Catalog #11-330-032

add:

36.1  7.25 mL  Glucose Solution Thermo Fisher Catalog #A2494001 .

36.2  5 mL
 MEM Non-Essential Amino Acids Solution (100X) Thermo Fisher Catalog #11140050

36.3  800 μ L
 Gibco™ Bovine Albumin Fraction V (7.5% solution) Thermo Fisher Scientific Catalog #15260037

36.4  5 mL
 Penicillin-Streptomycin (10,000 U/mL) Thermo Fisher Scientific Catalog #15140122

36.5  3.5 μ L
 2-Mercaptoethanol, 99%, pure Thermo Fisher Scientific Catalog #AC125472500 .

36.6  2.5 mL

 N2 supplement (100x supplement) **Gibco - Thermo Fisher**
Scientific Catalog #17502048

36.7  5 mL

 B-27™ Supplement (50X), serum free **Gibco - Thermo Fisher**
Scientific Catalog #17504044

36.8 Filter in Stericup in biosafety cabinet to sterilize.

Equipment

Millipore® Stericup® Quick Release Vacuum Filtration System NAME

0.22 um polyethersulfone membrane filtration system TYPE

Millipore BRAND

All Photos(3) Key Documents COA/COQ View All Docum SKU

<https://www.sigmaaldrich.com/US/en/product/mm/s2gpu05re> LINK

0.22 um filter SPECIFICATIONS



37 **Supplements:**

37.1 Epidermal growth factor (EGF) [M] 20 µg/mL stock

 Gibco™ Mouse EGF Recombinant Protein, PeproTech® **Fisher**
Scientific Catalog #31509100UG

: Resuspend $100\ \mu\text{g}$ in $200\ \mu\text{L}$ sterile ultrapure H_2O . Then add $4800\ \mu\text{L}$ of $0.1\ \%$ w/v BSA.

For a final concentration of $10\ \text{ng/mL}$ use $1\ \mu\text{L}$ of stock per $2\ \text{mL}$ media (1:2000).

37.2 Fibroblast growth factor 2 (FGF-2/bFGF) $20\ \mu\text{g/mL}$ stock

 Gibco™ Mouse FGF-basic (FGF-2/bFGF) Recombinant Protein, PeproTech® Fisher Scientific Catalog #4503310UG

Resuspend $10\ \mu\text{g}$ in $100\ \mu\text{L}$ sterile ultrapure H_2O . Then add $400\ \mu\text{L}$ of $0.1\ \%$ w/v BSA.

For a final concentration of $10\ \text{ng/mL}$ use $1\ \mu\text{L}$ of stock per $2\ \text{mL}$ media (1:2000).

37.3 Mouse Laminin Merck MilliporeSigma (Sigma-Aldrich) Catalog #L2020 stock is ~ $2\ \text{mg/mL}$

For a final concentration of $2\ \mu\text{g/mL}$ use $10\ \mu\text{L}$ of stock per $10\ \text{mL}$ of NSC media.

38 NSC differentiation.

38.1 From 2i+LIF cells.

38.2 In gelatinized plates, seed $1250\ \text{cells}$ per cm^2 in 2i + LIF media, and incubate for $24:00:00$ at $37\ \text{°C}$ in a 6-well plate.

1d

38.3 Wash gently with

 Phosphate-Buffered Saline, 1X without calcium and magnesium, PH 7.4 ± 0.1 Corning Catalog #21-040-CV

twice.

- 38.4 Change media for NSC media (without supplements), and incubate at  37 °C for  24:00:00 . 1d
- 38.5 Change media every  24:00:00 for  144:00:00 (6 days) . 1w
- 38.6 Replate  2000000 cells in a nongelatinized 10-cm plate (or less in a proportionally-sized plate) in NSC complete media: NSC media + EGF + FGF2 ( 1 µL of diluted stock of each per  2 mL media).
- 38.7 Grow neurospheres for  24:00:00 -  72:00:00 (until large spheres are formed). 4d
- 38.8 Harvest floating aggregates by transferring media with floating cells to a  15 mL conical tube.
- 38.9 Centrifuge  2000 rpm, 00:05:00 . 5m
- 38.10 Aspirate media (leave a little media on pellet).
- 38.11 Resuspend pellet in NSC complete media + Laminin.
- 38.12 Plate in 6-well plate coated with Laminin.

Note

Laminin coating (1/200):

- Make 1X PBS calcium, magnesium (PBS Ca₂₊,Mg₂₊):

Dilute

 Gibco™ DPBS (10X), calcium, magnesium Fisher Scientific Catalog #14080055

from 10X to 1X. Mix  50 mL

 Gibco™ DPBS (10X), calcium, magnesium Fisher Scientific Catalog #14080055

in  450 mL ultrapure H₂O. Filter in Stericup in biosafety cabinet to sterilize.

Equipment

Millipore® Stericup® Quick Release Vacuum Filtration System NAME

0.22 um polyethersulfone membrane filtration system TYPE

Millipore BRAND

All Photos(3) Key Documents COA/COQ View All Docum SKU

<https://www.sigmaaldrich.com/US/en/product/mm/s2gpu05re> LINK

0.22 um filter SPECIFICATIONS



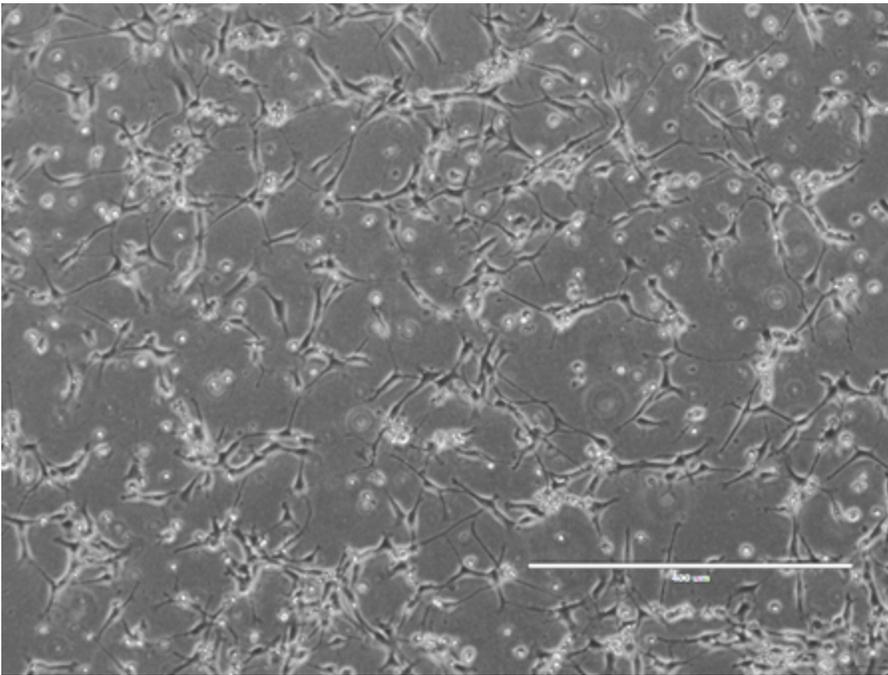
- For coating solution: add  5 μL of  Mouse Laminin Merck MilliporeSigma (Sigma-Aldrich) Catalog #L2020 to  995 μL 1X PBS Ca₂₊,Mg₂₊ in TC hood.
- Incubate  03:00:00 at  Room temperature or  37 °C before plating cells.

38.13 Grow until NSCs have migrated out of neurospheres, replacing media every day.

38.14 Split using Accutase to a new Laminin-coated plate/well.

38.15 For established NSCs, media will be NSC complete media + Laminin (optional if plate is laminin-coated).

38.16



Typical NSCs

Freezing Mouse Neural Stem Cells

39 Release with

 StemPro™ Accutase™ Cell Dissociation Reagent **Gibco - Thermo Fisher**
Scientific Catalog #A1110501

as above, but resuspend in freezing medium: [IM] 90 % (v/v) NSC media +

[IM] 10 % (v/v)  DMSO ≥ 99.9% **VWR International (Avantor) Catalog #MK494802**

40 Use  500 μL of suspension per freezing vial

 Externally and Internally Threaded Cryogenic Storage Vials **Thermo**
Fisher Catalog #12567501

41 Place vials in a

Equipment

Freezing container, Nalgene® Mr. Frosty

NAME

Cryopreservation

TYPE

Mr. Frosty

BRAND

C1562-1EA

SKU

[https://www.sigmaaldrich.com/US/en/product/sigma/c1562?](https://www.sigmaaldrich.com/US/en/product/sigma/c1562?srsltid=AfmBOop_0lwqk5VONiPEPXRsjIThyq_U1vmTjaleiS_dhTHbNn_UcFG1)

LINK

[srsltid=AfmBOop_0lwqk5VONiPEPXRsjIThyq_U1vmTjaleiS_dhTHbNn_UcFG1](https://www.sigmaaldrich.com/US/en/product/sigma/c1562?srsltid=AfmBOop_0lwqk5VONiPEPXRsjIThyq_U1vmTjaleiS_dhTHbNn_UcFG1)

at  -80 °C before moving to  -150 °C long-term.

Thawing Mouse Neural Stem Cells

5m

42 Thaw vial in a  37 °C water bath.

43 Prepare a tube with  5 mL NSC media.

44 Once the cells have thawed, transfer them to the tube and centrifuge for

5m

 1000 rpm, 00:05:00 .

45 Aspirate supernatant.

46 Resuspend the pellet in NSC complete media + Laminin.

47 Transfer to a

 Mouse Laminin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L2020** (LAM1)-coated well.

Protocol references

Pollard SM. In vitro expansion of fetal neural progenitors as adherent cell lines. *Methods Mol Biol.* 2013;1059:13-24. doi: 10.1007/978-1-62703-574-3_2. PMID: 23934830.

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