Protocol for Culturing Soriano Gene Trap ES cell lines

Cell Line Information

Soriano Gene Trap cell lines are feeder-dependent.

Parental ES cells: AK7.1, isolated from 129S4/SvJaeSor (light bellied agouti). Injection of ES cells into C57BL/6 blastocysts will produce agouti chimeras. This cell line is mycoplasma free.

SNL Feeder cells: SNL 76/7, feeder cells express leukemia inhibitory factor (LIF) and neomycin phosphotransferase (*Neo*). This cell line is mycoplasma free.

Reagents and Supplies:

Item	Vendor	Catalog Number
DMEM, high glucose, with glutamine	Gibco	11965-092
DMEM, with 25mM Hepes	Gibco	12430-054
Penicillin/Streptomycin, 100 u/ml	Gibco	15140-122
L-Glutamine	Gibco	25030081
Trypsin EDTA (0.05%) Trypsin EDTA (0.25%)	Gibco Gibco	25300-054 25200-072
Fetal Bovine Serum, Defined	Hyclone	SH30070.03
PBS (1X without Ca or Mg)	Gibco	14190-144
DMSO, 100 ml (2X Freezing medium: FBS with 20% DMSO)	Sigma	D2650
Mitocycin C 10x2mg (10µg/ml Inactivation media: add 2mg of Mitomycin C to 200 ml SNL feeder medium)	Sigma	M0503
2-Mercaptoethanol (1000x working soln: add 70µl 2-Mercaptoethanol to 9.93ml PBS. Store at 4°C)	Sigma	M-7522
Gelatin, 2% (0.1% working soln: add 25 ml of 2% solution to 475ml of PBS. Store at 4°C)	Sigma	G1393

Media

i) Soriano Gene Trap ES Cell Media (sterile filter through 0.2µM filter unit)

Reagent	Stock Conc.	Final Conc.	Volume
DMEM	1X	1X	407ml
FBS	100%	15%	75ml
100x Pen/Strep	100000 U/ml/ 100000ug/ml	50 U/ml 50ug/ml	2.5ml
2-Mercaptoethanol	1000x	1x, 0.1mM	0.5ml
100x L-Glutamine	200mM	2 mM	5 ml
Total Volume			**500ml**

ii) SNL Feeder Cell Media (sterile filter through 0.2μM filter unit)

Reagent	Stock Conc.	Final Conc.	Volume
DMEM	1X	1X	442.5ml
FBS	100%	10%	50ml
100x Pen/Strep	100000 U/ml 10000u/ml	50 U/ml 50u/ml	2.5ml
100x L-Glutamine	200 mM	2 mM	5ml
Total Volume			**500ml**

iii) SNL Inactivation Media (sterile filter through 0.2μM filter unit)

Reagent	Stock Conc.	Final Conc.	Volume
SNL feeder cell medium	1x	1x	200ml
Mitomycin C	2mg powder	10ug/ml	2mg
Total Volume			**200 ml**

*** You may use alternative Feeder cells such as MEFs. However, we strongly recommend that you substitute the ES cell media with 1000X LIF ***

Preparing SNL Feeder Cells

Thawing, Expanding and Treating Active SNL Cells

1. Thaw 1 vial of SNL cells (approximately 1.5-2 x 10^6 cells/vial) in a 37°C water bath and dilute into 10 ml of pre-warmed SNL feeder cell medium.
2. Pellet the cells by spinning for 4 minutes at 1000 rpm in a bench-top clinical centrifuge.
3. Aspirate off medium and gently resuspend cells in 5 ml of pre-warmed SNL feeder cell medium.
4. Transfer cell suspension to a 6 cm gelatinized dish, and grow at 37°C in a humidified 5% CO_2 incubator.
5. Change medium daily until confluent
6. When confluent, aspirate medium off and wash with 5 ml of pre-warmed PBS, pipetting it away from the cells. Rock dish gently and aspirate medium. Repeat.
7. Cover cells with 1 ml of 0.05% trypsin solution and incubate at 37°C for 4 minutes or until cells are uniformly dispersed into small clumps.
8. Add 5 ml of SNL Feeder medium to inactivate the trypsin, and pipette vigorously to make single cell suspension (we recommend 15 times).
9. Spin for 4 minutes at 1000 rpm.
10. Aspirate off medium and gently resuspend cells in 30 ml of pre-warmed SNL feeder cell medium.
11. Split the cell suspension onto three gelatinized 10 cm tissue culture dishes, and grow at 37°C in a humidified 5% CO_2 incubator.
12. Change medium daily until confluent (should take 2-3 days).
13. To mitotically inactivate, replace medium with 10 ml Inactivation medium (2mg of Mitomycin C to 200 ml SNL feeder medium), and incubate in a 37°C humidified 5% CO_2 incubator for 2.5 hours. Aspirate Inactivation medium, and rinse three times with pre-warmed PBS; aspirating completely between rinses. These dishes are now ready to use.
14. If you wish to freeze the cells for later usage, trypsinize and pellet the cells as before, but with 1.5 ml of 0.05% trypsin solution, and inactivate the trypsin with 8.5 ml medium.
15. For each 10 cm dish, count cells, and resuspend in an equal volume of SNL feeder cell medium and 2X Freezing medium; to a density of 1.5-2 x 10^6 cells/0.5 ml. Decant 0.5 ml aliquots into labeled cryovials.
16. Immediately place cryovials in a Styrofoam container or temperature controlled freezing vessel.

17. Freeze vials down to -80ºC freezer. After 24 hours, transfer cryovials to liquid or vapor-phase nitrogen for longer term storage.

Plating Mitotically Inactive SNL Feeder Cells

1. Coat a 6 cm tissue culture dish with 0.1% gelatin and aspirate off immediately before use.
2. Thaw 1 vial of mitotically inactive SNL feeder cells (approx. 1.5-2 x 10^6 cells) in a 37°C water bath and dilute into 10 ml of pre-warmed SNL feeder cell medium.
3. Pellet the cells by spinning for 4 minutes at 1000 rpm.
4. Aspirate off medium and gently resuspend cells in 5 ml of pre-warmed SNL feeder cell medium.
5. Transfer cell suspension to the 6 cm gelatinized dish, and grow at 37°C in a humidified 5% CO_2 incubator.

Thawing Soriano Gene Trap ES Cell Clones

1. Thaw 1 vial of ES cells (approximately 3 x 10^6 cells/vial) in a 37°C water bath and dilute (dropwise) into 10 ml of pre-warmed Soriano Gene Trap ES cell medium.
2. Pellet the cells by spinning for 4 minutes at 1000 rpm.
3. Aspirate off medium and gently resuspend cells in 5 ml of pre-warmed Soriano Gene Trap ES cell medium.
4. Aspirate the old medium from your 6 cm mitotically inactive SNL feeder cell dish.
5. Transfer the ES cell suspension to the feeder dish, and grow in a 37°C humidified 5% CO_2 incubator.
6. Change medium the following day to remove dead cells and residual DMSO.
7. Change medium daily until 80% confluent (approx. 1.5 x 10^7 cells); should take 2-4 days.
8. These cells are now ready for microinjection, and can be resuspended in 250-500µl microinjection medium (Hepes Buffered DMEM with 5% FBS; filtered through 0.2µM filter unit).

Passage and Expansion of Soriano Gene Trap ES Cell Clones

1. 1 day prior, prepare two 10 cm SNL Feeder dishes.
2. The next day, aspirate off old medium prior to plating ES cells.
3. On the day, wash the confluent 6 cm ES cell dish once with 5 ml PBS.
4. Cover cells with 1 ml of 0.25% trypsin solution and incubate at 37°C for 4-5 minutes or until cells are uniformly dispersed into small clumps.
5. Add 5 ml of Gene Trap ES cell medium; to inactivate the trypsin, and pipette vigorously to make single cell suspension (we recommend 10-15 times).
6. Spin for 4 minutes at 1000 rpm.
7. Aspirate supernatant and resuspend the pellet with 20 ml Soriano Gene Trap ES cell medium.
8. Transfer 10 ml of cell suspension onto each of the two 10 cm mitotically inactive SNL feeder cell dishes; prepared the day before.
9. Change medium daily until 80% confluent (should take 2-4 days).

Freezing Expanded Soriano Gene Trap ES Cell Clones

1. Wash the confluent 10 cm ES cell dishes once with 10 ml PBS each.
2. Cover cells with 1.5 ml of 0.25% trypsin solution and incubate at 37°C for 4-5 minutes or until cells are uniformly dispersed into small clumps.
3. Add 8.5 ml Gene Trap ES cell medium to inactivate the trypsin, and pipette vigorously to make single cell suspension (we recommend 10-15 times).
4. Spin for 4 minutes at 1000 rpm.
5. Aspirate supernatant and re-suspend pellet in an equal volume of Soriano Gene Trap ES cell medium and 2X Freezing medium (we would recommend 8-10 vials containing 0.5 ml aliquots; per 10 cm dish). Decant into labeled cryovials.
6. Immediately place cryovials in a Styrofoam container or temperature controlled freezing vessel.

Freeze vials down to ¯80ºC freezer. After 24 hours, transfer cryovials to liquid or vapor-phase nitrogen for longer term storage.