Culture mESCs:
F123 cells on feeders

KSR Media – filtered with a .22 um filter

*Warm KSR media in aliquots to avoid degradation of LIF protein

Stock Solution	Catalog	Final [ ]	Initial [ ]	1 bottle
DMEM	Corning 10013-CV or equivalent	~85%		500 mL
Knockout Serum replacement (KSR)	Invitrogen 10828028	~15%		90 mL
Non-essential amino acids	Gibco 11140-050 or equivalent	0.1 mM	10 mM	6 mL
100x Glutamax	Gibco 35050	1x	100X	6 mL
Beta-mercaptoethanol	Sigma M3148	50 uM	14.3M	4 uL
LIF	CellGuidance Systems GFM200**	1000U/mL		600,000U

**Use Quote CGS-30046, see F121-9 SOP for instruction

MEF Media – filtered with a .22 um filter

Stock Solution	Catalog	Final [ ]	Initial [ ]	1 bottle
DMEM	Corning 10013-CV or equivalent	90%		500 mL
FBS	***	10%		50 mL

*** 4DN reserved lot is available, Premium grade US origin, 500 ML, HI [Seradigm AB, **item: 97068-091**, **Quote:8030223666**, **reserve: 8003216852**, **Lot: 035B15**] Ordering instruction is here.

Other Reagents

Reagent	Catalog	Notes
Irradiated CF-1 MEFs	Global Stem GSC-6201G or equivalent	
Accutase	Life Tech A11105-01 or equivalent	Thaw O/N in fridge
Gelatin Type A	EMD Millipore SF008	Aliquot as needed

Culture F123 mESCs:

F123 mESCs should always be thawed onto a MEF feeder layer. F123 cells should be allowed to recover for at least 2 passages on MEFs. Once recovered, cells can be prepared for collection for RNA-seq.

MEFs can attach to the bottom of culture plate very quickly. So, putting the cells on plates for 30 minutes and then collecting the suspended medium can deplete most of the MEFs. Further passage F123 cells once on gelatin-only coated plates to dilute MEFs. F123 cells should not be passage on gelatin more than 2 passages as they will start to differentiate.

Plate MEFs at least 1 day before plating mESCs:

1. Add 0.1% gelatin type A to coated plates and incubate at 37°C for 20 minutes
2. Thaw a new vial of irradiated MEFs in the water bath for 2 minutes
3. Transfer MEFs to warm MEF media
4. Pellet the cells at 1000rpm for 5 minutes

5. Add enough MEF media to cell pellet to plate ~1 million cells/60 mm plate
6. Aspirate gelatin from coated plate
7. Add MEFs in media to plate
8. Shake plate back and forth for even distribution
9. Incubate at 37°C
   ** MEF cultures keep for ~10 days, no need to change media

Thaw F123 stocks and plate on MEF feeders:

****F123 stocks are in 10% DMSO and should be processed quickly to remove toxic DMSO

1. Thaw F123 cells in 37°C water bath
2. Immediately transfer F123 cells to warm KSR media
3. Immediately pellet the cells at 1000rpm for 5 min
4. Resuspend in KSR media
5. Aspirate MEF media from MEF wells
6. Transfer F123 cells to MEF feeder layer (~2 million cells/ 60mm dish)
7. Change media every 24 hours

Passaging F123 mESCs every ~2-3 days (when ~70% confluent to avoid differentiation):

1. Aspirate MEF media
2. Gently wash cells with DPBS
3. Add Accutase and incubate cells at 37°C for 1-10 minutes until lifted from plate
4. Add KSR media to dilute and inactivate Accutase

Plate	Vol Accutase	Vol Media to Dilute/Inactivate
10cm plate	1mL	~8mL
6 well plate	300 uL	~1.2 mL
12 well plate	150 uL	~600 uL

5. Pellet cells at 1000rpm for 5 minutes
6. Resuspend cells in KSR media
   - We typically split F123 cells 1:2 the first passage after thawing
   - Subsequent passages should have a 1:3 or 1:4 split depending on their recovery rate
7. Plate cells on MEF feeders or gelatin-only plates
8. Change the media every 24 hours

Make Frozen Cell Stocks:

When F123 cells are in logarithmic growth they are appropriate for frozen stocks. Passage F123 cells from MEF feeders as describe above. After pelleting cells, resuspend in freezing media (90% KSR Media + 10% DMSO). Freeze at -80°C for at least 24 hours before transferring to LN2 freezer for long-term storage.