Mouse iPS cell generation with STEMCCA

Plate 100,000 fibroblasts per well the day before infection in one well of a gelatin coated 6 well dish.
1. Prepare media: 1 cc complete MEF media* per well with 5ug/ml of polybrene.
2. Add 1cc media with polybrene to each well.
3. Leave first well without virus as a control.
4. Add 2-5 mcl STEMCCA lentivirus to each well.
5. After 24 hrs to stop the infection, aspirate off media and add 2 ml of fresh complete ES media** (without polybrene).
6. Change the media every 2 days and watch for colonies after days 16-30

Around day 20, or when colonies are largely visible, can begin picking colonies:
1. Place 4x microscope in tissue culture hood in UV for 1-2 hours to sterilize prior to picking.
2. Aspirate off media in original well. Wash once with PBS, add 2 ml of PBS.
3. When finished picking, aspirate off PBS in original plate and replace with 2ml of complete ES media if you want to keep the original plate in culture to watch for more colonies.
4. To prepare for picking: Fill several wells of a 96 well plate with 50mcl of 0.05% trypsin; and prepare a second 96 well plate (the day before picking) with mictomycin C-inactivated MEFs***.
5. Pick individual colonies using a 20mcl pipette set to 12mcl. Scrape the MEFs around the colony in a circular fashion and pipette up loose colony and place in the 96 well plate with trypsin.
6. Trypsinize colonies for 2-5 minutes.
7. Add 150 mcl of complete es media to trypsinized colonies (total volume in well should now be 200 mcl). Pipette up and down several times to form single cell suspension
8. In a 96 well plate previously plated with 15,000 MEFs per well, aspirate off old media and add the 200 mcl of picked colony with trypsin and complete ES media.
9. Re-feed the cells the following day with 200mcl of complete ES media.
10. Continue re-feeding the colonies every 2 days and pass into larger wells as they grow. Colonies should look exactly like ES colonies within 1 passage.

Media used:
* MEF Media (complete MEF media)
  500 mL DMEM (Gibco #11995 cat# 10569-044)
  50 mL FBS
  5 mL L-Glut (2mM final conc)
  5 mL P/S (final conc=1%)
** Embryonic Stem Cell Media (Complete ES media)
283 mL DMEM (Gibco #11995 cat# 10569- 044)
50 mL ESC qualified FBS (Hyclone SH30070.03E)
3.5 mL L-Glut (2mM final conc.)
3.5 mL P/S (final conc=1%)
350,000 Units of LIF (380 uL)
2.38 uL of stock BME (0.1 mM final conc.)

ES Media is modified from the ATCC suggested media for AINV15 mouse ESC; see: http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCC Num=SCRC-1002&Template=cellBiology

***For MEF preparation see separate MEF protocol for details.

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