XB2 Feeder Preparation

I. Growing "XB2" cells

Reagents

DMEM-10% FBS, Pen/Strep, L-glut
Trypsin

Protocol

- Thaw 1 vial of "XB2" (*not* "*XB2 feeder*") cells at 37°C. Add cells to 5 ml DMEM-10 in a T25.
- Split cells next day into a T75. When splitting, use 1 ml trypsin/T25 or 2ml trypsin/T75 and after dilution in DMEM-10, *always spin out cells* to get rid of trypsin. [the original protocol calls for 2 washes in PBS- 200 µg/ml EDTA, followed by PBS-EDTA + 250 µg/ml trypsin, but my way has been working]
- Split cells next day into 2-T162s. If want to continue carrying the line to freeze cells or do another feeder prep, also plate some back into a T75. When the T162's are confluent, proceed to making feeders. [Alternatively, split the T162s into 4 flasks and make feeders when they are confluent. I don't recommend making feeders from more than 4 flasks at a time. Once I have XB2 cells growing, I usually do enough feeder preps to accumulate 30 or more vials of feeders. Don't forget to freeze more XB2 cells when needed.]

II. Making "XB2 Feeder" cells

Reagents

DMEM-10% FBS, Pen/Strep, L-glut
Trypsin
500 µg/ml Mitomycin C (100X).
Sigma M-0503 (2 mg)
dissolve 2 mg powder in 4 ml ddH_20
filter sterilize
protect from light
store at 4°C
stable at 4°C for 3 months
[stable indefinitely at -80°C, but to store at -80°C, must rapid freeze to avoid precipitation]

Protocol to mitotically inhibit cells

- Aspirate media from confluent T162s.
- Replace with fresh DMEM-10 containing 5 µg/ml mitomycin C (25 ml/T162).

- Incubate cells for 3 hrs at 37°C.
- Wash cells 1X with DMEM-10 (10 ml/T162).
- Add DMEM-10 (10 ml/T162) and incubate 10 min at 37°C.
- Trypsinize cells using 2 ml trypsin/T162. Add 18 ml DMEM-10/T162.
- Count cells (let cells sit in DMEM-10 for at least 5 minutes after trypsinization to give them time to round up - they are much easier to count that way) and resuspend at 1 X 10^7 cells/ml.
- Aliquot cells 0.5 ml/vial = 5 x 10^6 cells/vial (call them "XB2 Feeders"), slow freeze at -80°C, and transfer to liquid N_2.
- 2 T162s may yield anywhere from 8-20 vials, depending upon their initial density, which is very hard to judge.