Culture of 3T3-J2 feeder cells

3T3-J2 are immortalized mouse fibroblasts used as feeders for human and mouse primary epithelial cells. In routine culture, they should be passaged every 3d day at 80-90 % confluency and split 1:6-1:10.

Growth medium: DMEM, 10 % FCS; Trypsin/EDTA: 0.05 % Trypsin, 0.02 % EDTA; 5 % CO^2, 37°C

Thawing and plating: Pre-warm 10 ml of medium, quickly thaw frozen cells and transfer into medium. Mix gently and spin 5 min at 1.000 rpm, RT. Aspirate off spnt and resuspend cells in 10 ml of medium, plate on 10 cm dish. Next day, change medium and add 12 ml of medium.

Routine passage: Upon 80-90 % confluency (should be ~3 mio. cells), aspirate off medium and wash 1 x with 2 ml of trypsin/EDTA. Remove completely. Add 2 ml of trypsin/EDTA and incubate 1-3 min in incubator. When cells come off, add 8 ml of medium, gently pipet up and down a few times to prepare a single cell suspension (check in microscope). Spin 5 min at 1.000 rpm, RT. Aspirate off spnt and resuspend cells in 10 ml of medium, plate ~300.000 cells/10 cm dish.

Freezing: Trypsinize cells as described above and count. Spin and resuspend cells at 4 mio cells/ml in normal medium. Slowly add 1 vol of 2x freeze medium (DMEM, 20 % FCS, 20 % DMSO) and mix gently. Freeze 2 mio cells/vial. Place in Nalgene container and freeze as indicated.

Mitomycin C (Sigma 50-07-7) treatment: **Wear gloves and avoid spills! Read safety sheet!** Treat cells at 80-90 % confluency. Replace growth medium with 10 ml/10 cm dish containing 4 µg/ml of mitomycin C. Incubate 4h at 37°C. Remove medium and wash cells 5 x with PBS. Trypsinize, count and collect all cells from one batch in 1 Falcon tube. Freeze as above at 1 mio cells in above freezing medium.

Quality control: before using MmC-treated cells, plate 1 vial and incubate for 5 days to make sure no proliferation occurs.

Seeding 3T3-J2 feeders for keratinocytes: Thaw cells as described and resuspend in 5 ml of keratinocyte medium (mouse or human, think about it!!!). Plate 1 mio cells ~ 2h before seeding keratinocytes. Add keratinocytes in 10 ml of medium. In low Ca2+ medium, feeders do not adhere very well. It may be necessary to re-add feeders in routine culture to maintain appropriate density.

Use of feeders for transfection experiments: feeders must be made resistant by transfecting in appropriate resistance gene before experiment. Test natural resistance of feeders before transfection, using appropriate concentration range of drug (consider Current protocols and/or other manuals). Transfect according to standard protocol. Next day, start selection at

drug conc. considerably higher than natural resistance. Isolate resistant colonies (appear after 8-12 days) and expand. Prepare stock and freeze as described before. It is possible to generate feeders with multiple resistances.