Preparation of Feeder plates for ES cell culture

Gelatinize Tissue Culture Plates

Gelatinize plates with 0.1% gelatin at room temperature for two hours.
(150 µl/well of 96 well plate; 12 ml/10 cm; 4 ml/6cm; 2 ml/well of 6 well; 0.5 ml/well of 4 well or 24 well).

Treat Confluent SNL76/7 Mouse Fibroblast Cells with Mitomycin C

1. Aspirate off media from the STO cell plate.
2. Mix 120 µl of mitomycin C (0.5 mg/ml in PBS) with 6 ml of STO media.
3. Add to the STO cells (6 ml/10 cm) and incubate at 37°C for two hours.

Make Feeder Plates

1. Wash cells with PBS.
2. Trypsinize cells with 0.8 ml Trypsin-EDTA at 37°C for 5 minutes.
3. Resuspend cells in 5 ml STO media, make single cell suspension and bring up to the volume to 10 ml/10 cm plate with STO media.
4. Count cells; (count all the 4 squares, divide the number by 4 and multiply 10^4, you have # of cells/ml).
5. Make cell suspension in 3.5×10^5 cells/ml.
6. Plate cells in gelatinized plates (150 µl/well of 96 well plate; 12 ml/10 cm; 4 ml/6cm; 2 ml/well of 6 well; 0.5 ml/well of 4 well or 24 well).