

SPLITTING GENE-TRAPPED ES CELLS:

1. Set-up 24-well replica plate: gelatinize and copy corresponding information. To gelatinize: Add enough 0.1% GELATIN in PBS to cover bottom of well. Let sit 10 min. and then suck off soln. with vacuum.

*SPLIT ONE PLATE AT A TIME TO AVOID THE PLATES DRYING OUT.

2. Suck off media in ES colony plate. Try not to touch bottom w/ pipette.
3. Wash w/ PBS. Stream PBS along side of well, not straight down so as to avoid harming the cells. Suck off PBS.
4. Add 100 λ of Trypsin using barrier tips. Be careful not to touch inside of Trypsin bottle. Incubate for a few minutes to activate Trypsin activity. tap plate gently against palm to break up clumps.
5. Add 1ml ES CELL MEDIA.
6. Using multichannel pipette, aspirate the mixture and transfer @ 1/2ml into replica plate.
7. Top off both plates by adding an additional 1/2ml of ES CELL MEDIA to bring the total vol. to 1ml.
8. Incubate. You are done.