

Oct 5. 01

9:45

- Take 1 cluster Fr. 10.5°C
- 1 " " 37°C.

- Aspirate med
- Vortex & left over med
- Transfer cells to 10 ml PBS
  - i.e. add 200 ul PBS → 10 ml.
  - 200 ul " → "
- spin at 1350 rpm 9'.

Decant

- Add .2 ml Tryp. 2.5' at 37°C.
- Add 2 ml D/S (D medium 5% serum)
- Syz vigorously 5X all singles. (Can syz maybe 2-3x only).

Count

10.5°C (50 ul in 20 ul)

1483  
1456  
1567  
1411

1479 × 2 × 400 = 1.18 × 10<sup>6</sup>/ml

Dilutions made in medium D/S

Make 1:1000 dil (ie. 1:10 → 1:10 → 1:10  
0.5 ml in 4.5 ml).

Plate. - 35 ml / T25 (414 cells)

- 37°C cluster. (Manipulate as 10.5°C)

1641  
1570  
1671  
1655

( 1:10 → 1:10 → 1:10 plate .3 ml T25