

Focus Formation Experiment

Background: "Mother cells" grow in flat monolayer, whereas transformed cells grow on top of one another, eventually forming foci. Mother cells take space that normally is taken by transformed cells. Thus forcing transformed cells to grow in focus formation.

Procedure

1. Collect trypsinized cells in Falcon tubes with 10ml media. Count both types of cells: mother and transforming using Coulter counter. Not necessary to count a blank in between each sample.
2. Plate 0.5×10^6 "mother cells" per P100. Make dilutions of transforming cells to be able to add 50-100 or 500-1000 transforming cells to mother cells. (Always resuspend cell suspension before aliquotting.) Add 10ml fresh media to each plate.
3. Feed every 3-4 days.
4. After 2 weeks, count foci, fix and stain.

Staining Focus Formation Experiment Plates

1. Wash carefully 2X cold DME (3-4ml each).
2. Wash 2X ice cold methanol.
3. Put on 2ml ice cold methanol 5-10min (to fix foci).
4. Aspirate. Rinse with ddH₂O.
5. Add 4ml 0.4% methylene blue/dd H₂O solution.
6. Wash several times 5ml ddH₂O.
7. Dry upside down, with bottom dish set at angle to cover.

Make photocopy and put with protocol.