

MAKING CELL SUBCLONES PROTOCOL

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GOALS

To reduce pre-existing genetic heterogeneity in cancer cells, cells will be seeded at a very low density: 1 cell/well in 96-well plates and then transferred to larger well plates for expanding into subclones.

MATERIALS

Repetitive pipette (Schiffman Lab) or multichannel pipette

Microcentrifuge tubes

Pipette basin

96-well plate (at least two plates for each cell line)

24-well plate

12-well plate

6-well plate

10 cm-culture dish

Cell lines of interest (Currently, we have HCC1806, MDA-MB-231, MDA-MB-231_mcherry, Hs578T_RFP/Luciferase cell clones)

Sterile phosphate-buffered saline (PBS), pH 7.4

Cell culture media containing 10% FBS

Trypsin solution

METHODS

Follow the “splitting cells” protocol to resuspend cells in culture media.

- 1.) Count cells.
- 2.) Based on cell counts (which should be approximately 5×10^5 – 10^6 cells/ml), serial dilute cells in microcentrifuge tubes and finally transfer to pipette basins to the cell density of 5 cells/ml.
 - For example, suppose your initial cell density is 5×10^5 cells/ml, 1:10 serial dilute cells to 5×10^4 , 5×10^3 , 5×10^2 cells/ml, final volume 1mL per dilution. Next, calculate the volume needed from the tube with 5×10^2 cells/ml and then add cell culture media containing 10% FBS to reach 5 cells/ml (repetitive pipette) or 10 cells/ml (multichannel pipette).
- 3.) Use a repetitive pipette or a multichannel pipette to transfer 200 ul of cells into at least two 96 well plates to reach 1 cells in 200 ul cell culture media per well.

Note: to reduce the edge effect caused by evaporation, fill the edge wells with 200 ul of PBS/well and only use the rest of 60 wells per plate.

Note: depends on cell line, each 60 wells might have ~10-20 wells that actually have 1 cell/well.
- 4.) Examine each well daily with the microscope at 100X magnification and mark the number of cells on the lids of 96-well plates to confirm the cell clones you pick are exactly expanding from 1 cell/well.

Note: Usually it will take up to ~1 week to see cells growing in well plate, depending on the growth rates of cells.

- 5.) Replenish cell culture media after seeing cells in the 96-well plates. When you see cells start to expand, change cell culture media every 2-3 days.
- 6.) After approximately 1 month, you should be able to see confluent cells in the wells. Add 100 ul trypsin/well to detach cells in 96 well plates and then incubate at 37°C for 5 min. Next, transfer the cell subclones into 24- or 12-well plates and add culture media.
- 7.) Label the cell subclones with the number of plates and their positions in each plate.
- 8.) When you see ~90% confluency of cell subclones, they can be transferred to 12-well plates or 6 well plates depending on their growth rates.
- 9.) After culturing in 6-well plates, cell clones can be finally transferred to 10 cm culture dishes or T-25 or T-75. And freeze the cell subclones when they reach confluency.

Reference

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3. http://home.sandiego.edu/~josephprovost/Single_cell_cloning_protocol.pdf