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 $5x10^5$–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 $5x10^5$ cells/ml, 1:10 serial dilute cells to $5x10^4$, $5x10^3$, $5x10^2$ cells/ml, final volume 1mL per dilution. Next, calculate the volume needed from the tube with $5x10^2$ 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^{o}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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2. https://www.addgene.org/protocols/limiting-dilution/
3. http://home.sandiego.edu/~josephprovost/Single_cell_cloning_protocol.pdf