Generation of stable cell lines

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1. Killing curve

Using your cell line of interest, determine the lowest concentration of antibiotics you will use for selection.

2. Transfection

Transfect cells in 35 mm dish with your plasmid and incubate for 2 days.

Trypsinize cells, transfer to 15-ml a conical tube, spin and remove S/N.

Resuspend cell pellet in 10 ml media.

Transfer individually 4 ml, 2 ml, 1 ml, 0.5 ml, 0.3 ml of suspended cells to 10 cm dish harboring selection antibiotics.

3. Selection

Wait until colonies form. The time required for colonies to form depends on which antibiotics you use.

N.B. For G418 (Geneticin), it usually takes more than a week; for puromycin, less than a week. Unless media turn orange, you don't have to change the media.

4. Picking up colonies

Remove media and wash in 10 ml PBS.

Add 1 ml of trypsin and incubate for 1 min (exact) at RT. Do not go overboard.

Remove trypsin and add 5 ml PBS.

Using 200p pipetman set at 100 μl, wiggle the tip around a colony a bit and suck up.

Release the sucked-up colony into a well of 24-well dish.