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Stable Cell Lines (Without Retroviral Infection) Protocol

Step 1, Standard Transfection:

1. Transfect the cells with the HuSH plasmid DNA using your standard protocol for transient transfection. After transfection, do not change the medium until the cells are ready to be passaged.

Step 2, Selection:

1. Passage the transfected cells (1:10 split) into a fresh vessel containing growth medium and 0.5-1.0 ug/ml puromycin (determined by killing curve using un-transfected cells).

2. Continue to grow and passage the cells as necessary, maintaining selection pressure by keeping 0.5-1.0 ug/mL puromycin in the growth medium.

3. After 4-7 days, a large number of the cells will be killed by the antibiotic, indicating that they did not take up or have lost the plasmid with the puromycin resistance cassette. The cells that remain growing in the puromycin-containing medium have retained the HuSH plasmid, which stably integrates into the genome of the targeted cells.

Step 3, Isolation:

1. Select clonal populations of cells by transferring a well-isolated single clump of cells (the clonal ancestor and cells divided from it) into a well of a 24-well plate.

2. Repeat to select 5-10 clonal populations. Continue growing these cells in selection medium for 1-2 additional passages

   At this time, each well contains a clonal population of stably transfected cells, which can be maintained in normal growth medium without the selection pressure of puromycin (although you may wish to grow the cells under “light pressure”, 0.2 ug/mL puromycin). These populations can be used for experiments or stored under liquid nitrogen in growth medium with 10% DMSO and 20% FBS for future use.