PROTOCOL FOR ESTABLISHING STABLE MAMMALIAN CELL LINES

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General Note: Plate cells at least 12hrs (but not over 24 hours) prior to transfection to achieve a 40-80% confluence at the time of transfection. Lower confluence will achieve higher percentage of transfection but may yield a lower absolute number of transfected cells. The following procedure can be scaled up or down proportional to the surface area of the plate or well with little or no change in the results. In general, lower amount of DNA is used for transfection when the establishment of stable lines is desired.

1. Plate cells in T-25 flasks at 12-16hrs prior to transfection (approx. 40-80% confluence).
2. Prepare two 1.5ml screw cap microfuge tubes or 2 wells of a 24 well plate with250 ul of OptiMEM or plain medium (i.e., DMEM without FBS) per transfection.
3. In one well or tube, add correct amount ug of plasmid (usually 1.0ug per flask) and mix well. (NOTE: some plasmid DNA may be linearized to increase the frequency of desired stable integration).
4. In the second well or tube, add correct amount of LipofectAMINE (usually 5 ul/ug DNA) and mix well.
5. Drip the DNA mix into the lipid mix, and let it sit in the hood for10-30 minutes while preparing the cells.
6. **Optional**: you can bypass **Steps 3-5** and prepare LipofectAIME/DNA complex by mixing: 250ul OptiMEM or DMEM, 1.0ug DNA, and 5.0ul LipofectAMINE per transfection. Mix well and let it sit in the hood for 10-30min. This direct mixing approach works equally effective in most cases.
7. Meanwhile, remove the medium from each well, and wash cells gently with 2.0-3.0ml of OptiMEM or DMEM (NOTE: serum free media).
8. [Optional: repeat the wash once. It is **NOT** desirable to repeat the wash on less adherent cells, such as HEK 293 cells].
9. Add 2.5ml OptiMEM or DMEM to each flask. Return flasks to incubator for 5-10min.
10. Add the Lipo/DNA mix to each flask. Rock gently and return flasks to the incubator.
11. After 3-6 hours, remove the medium and replace with 6.0-8.0ml of complete media (**NOTE**: If significant portion of the transfected cells is floating, do not remove Lipo/DNA-containg medium. Instead, just add 6.0ml of complete medium, and replace with fresh complete medium next morning).
12. On next day, transfected cells are trypsinized and resuspended in G418 (or appropriate selection drugs)-containing complete medium. Plate the cells into multiple 96-well plates by limit dilutions (i.e., plate cells at 100, 30, 10, 3, or 0.3 cells/well).
13. Stable clones should be formed at approximately two weeks after selection.