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H00010923-R01 Protocol

Protocol specific for SUB1 RNAi (H00010923-R01)

Optimization of the transfection condition

Generally, transfection optimization could be achieved by transfecting cells with siRNAs targeting endogenous genes such as Lamin A/C and GAPDH and then analyzing their expression by RT-PCR or Western blotting. Alternatively, assays using reporter genes including GFP and luciferease, which are faster and more quantitative than Western blot, facilitate time-consuming optimization experiment.

We isolated SiHa, HeLa and SK-OV-3 clones stably expressing Firefly Luciferase (FLuc) by transfecting those with a FLuc expression plasmid (hLuc/pcDNA3), and culturing in the presence of G418. With a siRNA/chimera targeting FLuc, these clones have been used to optimize siRNA/chimera transfection condition as well as selection of suitable transfection reagent for each cell lines. In order to avoid interfering endogenous miRNA network, it is important to keep the siRNA/chimera transfection concentrations as low as possible. We recommend using siRNA/chimera at a concentration lower than 5 nM, and where possible, at 1 nM.

Cell culture

It is important to select a cell line suitable for your RNAi experiment. Some cell lines are resistant to siRNA/chimera transfection. Further, a character of a cell line sometime varies among laboratories, which might be caused by repeated passages and cross contamination. We strongly recommend obtaining cell lines with good references from ATCC and other cell banks and to make a large cryostock. Thaw cells before each series of experiments and culture for 1 -2 weeks. After several experiments, cells should be discarded to avoid long-term passage.

 1) One day before transfection, plate cells at a density of 5x10 to the 4th -1x10 to the 5th cells per well in a 6-well plate containing 2 ml of DMEM medium containing 10% FCS without antibiotic (e.g. Streptomycin, penicillin, G418).

 2) Immediately after seeding cells, culture vessels should be shaken on bench back and forth and then from side to side several times slightly in a rough manner, resulting in even cell distribution on culture surface. In case of a 96-well plate, shaking does not improve cell distribution, or even cause centering cells in each well.

 3) On the day transfection, the cell culture should be 30% confluency. Please make sure that the CO2 incubator is installed horizontally because cell distribution in the culture vessel often influences RNAi efficiency.

Note: The cell density seeded is proportional to the culture surface area.

PBS washes immediate before transfection are unnecessary. siRNA/chimera transfections iRNA/chimera should be stored at 4 degrees C and at -20 degrees C in the case of dried and soluble forms respectively, but should not be refrozen for reuse. Small aliquots (10 uL) should be made.

Efficient RNAi can be achieved by using commercially available transfection reagent such as Lipofectamine 2000 and Lipofectamine RNAiMAX. From our experiences, we noticed that Lipofectamine RNAiMAX is superior to Lipofectamine 2000 to the extent of cytotoxicity and RNAi efficiency, and that cytotoxicity of Lipofectamine 2000 varies lot by lot. (Transfection protocol of Lipofectamine RNAiMAX can be obtained from the Invitrogen Website.) However, for cotransfection of siRNA/chimera and plasmid DNA, Lipofectamine 2000 should be used.

To achieve the maximal RNAi activity and lowest cytotoxicity, optimization of Lipofectamine 2000 volume is essential. Dilute siRNA/chimera with Opti-MEM I reduced serum medium (Invitrogen) up to 50 ul and vortex for a few seconds Dilute appropriate amount of Lipofectamine 2000 by Opti-MEM I reduced serum medium to 50 ul and vortex for a few seconds For SiHa and SK-OV-3 cells, dilute 1.0 -1.6 ul of Lipofectamine 2000 with Opti-MEM I reduced serum medium to 50 ul. For HeLa cells, diluted 0.2 - 0.4 ml of Lipofectamine 2000 with Opti-MEM I reduced serum medium to 50 ul. Combine diluted siRNA/chimera and Lipofectamine 2000, vortex for a few second, incubated at room temperature for 20 min, then added to a 2-ml culture. The amount of Lipofectamine 2000 should be changed in proportion to the culture medium volume.