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Transfection of mammalian cell lines with plasmids and siRNAs

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We use this protocol and it's working

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Abstract

This protocol details the transfection of mammalian cell lines with plasmids and siRNAs.

Lipofectamine 2000 (Invitrogen) or Fugene HD (Promega) or Fugene 6 (Promega) transfection reagents

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1 On the day before transfection, plate 100,000 HeLa cells per well in a 6 well dish. For other cell lines, the number of cells will need to be optimized to achieve 50-75% confluency on the day of transfection.

2 Warm Optimem (Gibco) and transfection reagent to Room temperature

3 Add 200 µL Optimem + 6 µL transfection reagent + 2 µg plasmid DNA to a round bottom polystyrene tube and mix gently.

4 Incubate 00:20:00 at Room temperature .

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5 Add dropwise to cells and mix gently. Return cells to incubator. Duration of transfection must be optimized for each plasmid and downstream application.

RNAiMax transfection reagent

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6 Warm Optimem (Gibco) and RNAiMax (Invitrogen) transfection reagent to Room temperature .

7 Combine 200 µL OPTiMEM, 5 µL RNAiMAX transfection reagent and 5 µL of 20 undetermined siRNA and mix gently.

8 Incubate 00:20:00 at Room temperature .

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9 Add transfection mix to 1.8 mL media containing 100,000 HeLa cells per well in 6-well dish and return to cell culture incubator for 24-72 hours (duration needs to be optimized for each target gene).