



Jun 05, 2024

Transfection of mammalian cell lines with plasmids and siRNAs

DOI

<https://dx.doi.org/10.17504/protocols.io.261ge55byg47/v1>

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Protocol Citation: Agnes Rocznia-Ferguson, Shawn M. Ferguson 2024. Transfection of mammalian cell lines with plasmids and siRNAs. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.261ge55byg47/v1>

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Protocol status: Working

We use this protocol and it's working

Created: May 24, 2024

Last Modified: June 05, 2024

Protocol Integer ID: 101278

Keywords: ASAPCRN, transfection of mammalian cell line, mammalian cell line, plasmid, sirnas this protocol detail, transfection, sirna, line

Funders Acknowledgements:

ASAP

Grant ID: 000580

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

20m

- 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 μ L Optimem + 6 μ L transfection reagent + 2 μ g plasmid DNA to a round bottom polystyrene tube and mix gently.
- 4 Incubate 00:20:00 at Room temperature . 20m
- 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

20m

- 6 Warm Optimem (Gibco) and RNAiMax (Invitrogen) transfection reagent to Room temperature .
- 7 Combine 200 μ L OPTiMEM, 5 μ L RNAiMAX transfection reagent and 5 μ L of 20 μ L siRNA and mix gently.
- 8 Incubate 00:20:00 at Room temperature . 20m
- 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).