Transfection of Cas9 RNP (ribonucleoprotein) into adherent cells using the Lipofectamine® RNAiMAX

New England BioLabs, Inc.

Abstract

Cas9 nuclease may be used *in vivo* to create targeted genome modifications. There are several ways in which to introduce Cas9-guide RNA complexes into cells. Here we present a method for the transfection of Cas9 RNP's into HEK293 FT cells using Thermo Fisher Lipofectamine® RNAiMAX. This is a 'reverse transfection' method that uses a final concentration of 10 nM RNP per transfection in a 96-well culture plate.

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Guidelines

Overview:

Cas9 nuclease may be used *in vivo* to create targeted genome modifications. There are several ways in which to introduce Cas9-guide RNA complexes into cells. Here we present a method for the transfection of Cas9 RNP's into HEK293 FT cells using Thermo Fisher Lipofectamine® RNAiMAX. This is a 'reverse transfection' method that uses a final concentration of 10 nM RNP per transfection in a 96-well culture plate.

Required Materials:

Cell Culture and Transfection

- HEK293 cells (or other cell line) at 70-90% confluency in a T-75 flask.
- EnGen™ Cas9 Nuclease NLS, *S. pyogenes* (M0646T or M0646M)
- sgRNA containing the targeting sequence in the region of interest
  - sgRNAs can be generated using the EnGen™ sgRNA Synthesis Kit, *S. pyogenes* (E3322S).
  - sgRNAs must contain the target sequences (20 nucleotides) adjacent to the Protospacer Adjacent Motif (PAM, NGG) in the target DNA. (1,2). See the EnGen sgRNA Synthesis Kit manual for further details.
- Lipofectamine RNAiMAX Transfection Reagent (ThermoFisher)

- Sterile 1X PBS without Ca2+ and Mg2+
- DMEM with Glutamax (or appropriate growth medium) with 10% FBS
- Optimem Reduced Serum Medium (ThermoFisher)
- 96-well culture plate

DNA Extraction and Genome Editing Analysis

- EnGen™ Mutation Detection Kit (E3321S)
- Epicentre QuickExtract™ DNA Extraction Solution (Epicentre #QE09050)

Before You Start:

- We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination. Further recommendations for avoiding ribonuclease contamination can be found here: https://www.neb.com/tools-and-resources/usage-guidelines/avoiding-ribonuclease-contamination.
- Transfection conditions may be highly variable. It is recommended to optimize your conditions for each cell type and Cas9 target you may have. This protocol follows conditions that have been optimized for a particular target and use of HEK293 cells.

Before start

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Materials

EnGen Cas9 NLS, S. pyogenes - 400 pmol M0646T by New England Biolabs

EnGen sgRNA Synthesis Kit, S. pyogenes - 20 rxns E3322S by New England Biolabs

EnGen Mutation Detection Kit - 25 rxns E3321S by New England Biolabs

Epicentre QuickExtract™ DNA Extraction Solution QE09050 by Epicentre

Protocol

RNP Complex Formation

Step 1.

Make a 3 µM working solution of sgRNA by diluting the stock with nuclease-free water.

RNP Complex Formation

Step 2.

Make a 3 µM working solution of Cas9-NLS by diluting with 1X Cas9 Reaction Buffer or Optimem.

RNP Complex Formation

Step 3.

Form the RNP complexes as follows below:

Component	Single Reaction	x3.3 (triplicates)
sgRNA (3 uM)	0.5 ul	1.65 ul
EnGen Cas9 NLS (3 uM)	0.5 ul	1.65 ul
Optimem	11.5	37.95 ul
Total	12.5 ul	41.25 ul

RNP Complex Formation

Step 4.

Gently mix the reaction and incubate at room temperature for 10 minutes.

DURATION

00:10:00

RNP Complex Formation

Step 5.

Form the liposome complexes as follows below.

Component	Single Reaction	x3.3 (triplicates)
RNP (120 nM)	12.5 µl	41.25 µl
RNAiMAX	1.2 µl	3.96 µl
	11.3 µl	37.29 µl
	12.5 µl	82.5 µl

NOTES

Breton Hornblower 03 Sep 2016

You can make a master mix of the RNAiMAX and Optimem and add this directly to the RNP tube from above.

RNP Complex Formation

Step 6.

Gently mix the reaction and incubate at room temperature for 20 minutes.

DURATION

00:20:00

Trypsinize and Prepare HEK293 Cells

Step 7.

Seed the cells so that they will be around 70-90% confluent on the day of transfection.

Trypsinize and Prepare HEK293 Cells

Step 8.

During the RNP/liposome incubation, trypsinize the cells, washing once to remove any traces of trypsin.

Trypsinize and Prepare HEK293 Cells

Step 9.

Resuspend the cells in 10 ml of media and count.

Trypsinize and Prepare HEK293 Cells

Step 10.

Calculate the dilution and volume needed to get the cells to 3.2 x 10^5 cells per ml. You will need 125 µl of cells per well.

Transfect Cells with Liposome Complexes

Step 11.

From each tube of RNP/liposome complex, aliquot 25 µl into 3 wells of a 96-well plate.

Transfect Cells with Liposome Complexes

Step 12.

Add 125 µl of cells (3.2 x 10^5 cells/ml) to each well containing RNP/liposome complex and pipette up and down gently a few times.

Transfect Cells with Liposome Complexes

Step 13.

Incubate the cells in a humidified 37 °C, 5% C02 incubator for 48-72 hours.

DURATION

48:00:00

Harvest DNA and Amplify Target Region

Step 14.

Gently aspirate the media from the cells and wash twice with 100 ul 1X PBS.

Harvest DNA and Amplify Target Region

Step 15.

Add 75 µl of Epicentre QuickExtract™ DNA Extraction Solution and shake/vortex for 5 minutes.

REAGENTS

Epicentre QuickExtract™ DNA Extraction Solution QE09050 by Epicentre

DURATION

00:05:00

Harvest DNA and Amplify Target Region

Step 16.

Transfer the solution to a PCR plate or tubes and place in a thermocycler, running the following program:

- 65°C for 15 min
- 95°C for 15 min
- Hold at 4°C

Harvest DNA and Amplify Target Region

Step 17.

Dilute the DNA 1:10 in nuclease-free water.

Harvest DNA and Amplify Target Region

Step 18.

Follow the protocol detailed in the EnGen Mutation Detection Kit (E3321S) manual.

REAGENTS

EnGen Mutation Detection Kit - 25 rxns E3321S by New England Biolabs