2

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Transfection of Cas9 RNP (ribonucleoprotein) into adherent cells using the Lipofectamine® RNAiMAX V.2

New England Biolabs[1]

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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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https://www.neb.com/protocols/2016/07/26/transfection-of-cas9-rnp-ribonucleoprotein-into-adherent-cells-using-the-lipofectamine-rnaimax

New England Biolabs 2022. Transfection of Cas9 RNP (ribonucleoprotein) into adherent cells using the Lipofectamine® RNAiMAX. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bhkuj4ww
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Transfection, riponucleoprotein, Cas9, Cas9 nuclease, lipofectamine

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MATERIALS

☒ EnGen Cas9 NLS, S. pyogenes - 400 pmol **New England Biolabs Catalog #M0646T**

☒ EnGen sgRNA Synthesis Kit, S. pyogenes - 20 rxns **New England Biolabs Catalog #E3322S**

☒ EnGen Mutation Detection Kit - 25 rxns **New England Biolabs Catalog #E3321S**

☒ Epicentre QuickExtract™ DNA Extraction Solution **Epicentre Catalog #QE09050**

☒ Opti-MEM™ Reduced Serum Medium **Thermo Fisher Scientific Catalog #31985062**

☒ HEK293 **ATCC Catalog #CRL-1573**

☒ DPBS no calcium no magnesium **Thermo Fisher Scientific Catalog #14190144**

☒ Lipofectamine™ RNAiMAX Transfection Reagent **Thermo Fisher Catalog #13778030**

☒ DMEM, low glucose, GlutaMAX™ Supplement, pyruvate **Thermo Fisher Catalog #21885108**

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)

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

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- 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.

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

RNP Complex Formation

1

Make a [M]**3 Micromolar (µM) working solution of sgRNA** by diluting the stock with nuclease-free water.

2

Make a [M]**3 Micromolar (µM) working solution of Cas9-NLS** by diluting with [M]**1 X Cas9 Reaction Buffer** or Optimem.

3

Form the RNP complexes as follows below:

A	B	C
Component	**Single Reaction**	**x3.3 (triplicates)**
sgRNA (3 µM)	0.5 µl	1.65 µl
EnGen Cas9 NLS (3 µM)	0.5 µl	1.65 µl
Optimem	11.5 µl	37.95 µl
Total	12.5 µl	41.25 µl

4

Gently mix the reaction and incubate at **Room temperature** for **00:10:00** .

5

Form the liposome complexes as follows below.

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A	B	C
Component	**Single Reaction**	**x3.3 (triplicates)**
RNP (120 nM)	12.5 µl	41.25 µl
RNAiMAX	1.2 µl	3.96 µl
Optimem	11.3 µl	37.29 µl
Total	12.5 µl	82.5 µl

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

6 

Gently mix the reaction and incubate at **Room temperature** for **00:20:00** .

Trypsinize and Prepare HEK293 Cells

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

8 

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

9 

Resuspend the cells in **10 mL** of media and count.

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

11 

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

12 

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

13

Incubate the cells in a humidified **37 °C** , 5% CO_2 incubator for 48-72 hours.

Harvest DNA and Amplify Target Region

14

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

15

Add **75 µL Epicentre QuickExtract™ DNA Extraction Solution** and shake/vortex for **00:05:00** .

16

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

- **65 °C** for **00:15:00**
- **95 °C** for **00:15:00**
- Hold at **4 °C**

17

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

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

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