Electroporation of Cas9 RNP (ribonucleoprotein) into adherent cells using the Neon® Electroporation

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 introduction of Cas9 RNP's into HEK293 FT cells using the Thermo Fisher Neon Electroporation System. This method uses a final concentration of 20 nM RNP per transfection in a 24-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 introduction of Cas9 RNP's into HEK293 FT cells using the Thermo Fisher Neon Electroporation System. This method uses a final concentration of 20 nM RNP per transfection in a 24-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.
- ThermoFisher Neon Transfection System 10 µl Kit (MPK1025)

- Sterile 1X PBS without Ca2+ and Mg2+
- DMEM with Glutamax (or appropriate growth medium) with 10% FBS
- 24-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.
- Please refer to the Neon Transfection System manual for proper usage of the equipment.

Before 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
- Please refer to the Neon Transfection System manual for proper usage of the equipment.

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

Electroporation

Step 1.

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

Electroporation

Step 2.

Set up the RNP formation reaction as follows below:

Component	7 µl reaction
Resuspension Buffer R	5.8 µl
EnGen Cas9-NLS (20 µM)	0.6 µl
sgRNA (20 µM)	0.6 µl

Electroporation

Step 3.

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

DURATION
00:20:00

Electroporation

Step 4.

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

Electroporation

Step 5.

Resuspend the cells in 10 ml of media and count.

Electroporation

Step 6.

Remove 1-2 x 10^6 cells to a sterile microfuge tube. (One tube of cells should be enough for 10 transfections).

Electroporation

Step 7.

Pellet for 5 min at 500 x g.

DURATION
00:05:00

Electroporation

Step 8.

Wash the cells once with 1X PBS.

Electroporation

Step 9.

Pellet for 5 min at 500 x g.

Electroporation

Step 10.

Resuspend the cells in 50 µl of Resuspension Buffer R.

Electroporation

Step 11.

Prepare a 24-well plate by adding 500 µl growth medium to the appropriate number of wells.

Electroporation

Step 12.

Add 5 µl of cells to each 7 µl RNP reaction.

Electroporation

Step 13.

Aspirate 10 µl of the RNP/cells mix into a 10 µl Neon tip.

Electroporation

Step 14.

Electroporate the cells under the following conditions: 1700V, 20 ms, 1 pulse.

Electroporation

Step 15.

Immediately transfer the cells to the prepared 24-well plate.

Electroporation

Step 16.

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

DURATION

48:00:00

Harvest DNA for on-target editing analysis

Step 17.

Gently aspirate the media from the cells.

Harvest DNA for on-target editing analysis

Step 18.

Wash with 100 µl 1X PBS. (1/2)

Harvest DNA for on-target editing analysis

Step 19.

Wash with 100 µl 1X PBS. (2/2)

Harvest DNA for on-target editing analysis

Step 20.

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

DURATION
00:05:00

Harvest DNA for on-target editing analysis

Step 21.

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 for on-target editing analysis

Step 22.

Dilute the DNA 1:10 in nuclease-free water

Harvest DNA for on-target editing analysis

Step 23.

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