Protocol for Electroporation of Cas12a Ribonucleoprotein (RNP) into adherent cells using the Neon® Electroporation

Overview

EnGen Lba Cas12a (Cpf1) (NEB #M0653) is a nuclease that may be used *in vivo* to create targeted genome modifications. There are several ways in which to introduce Cas12a-guide RNA complexes into cells. Here we present a method for the introduction of Cas12a RNP's into HEK293 FT cells using the Thermo Fisher® Neon Electroporation System. This method uses a guide RNA to protein ratio of 10:1. Actual pmols of RNA and protein may be optimized.

Cell Culture and Transfection

HEK293 cells (or other cell line) at 70-90% confluency in a T-75 flask
EnGen® Lba Cas12a (Cpf1) (NEB #M0653)
gRNA containing the targeting sequence in the region of interest
ThermoFisher Neon Transfection System 10 µl Kit (MPK1025)
Sterile 1X PBS without Ca^{2+} and Mg^{2+}

Trypsin to release the cells
DMEM with Glutamax (or appropriate growth medium) with 10% FBS
24-well culture plate, or desired plate

DNA Extraction and Genome Editing Analysis

EnGen Mutation Detection Kit (NEB #E3321)
Epicentre QuickExtract™ DNA Extraction Solution (Epicentre #QE09050)

- 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.
- Please refer to the Neon Transfection System manual for proper usage of the equipment.
- The Neon 10 µl Transfection System draws 10 µl of cells and transfection material into an electroporation pipette tip. This tip may be used twice for two sequential electroporations. Therefore, the volumes in this protocol are for duplicate reactions set up in the same tube, with an overage of 5 µl. However, volumes can be adjusted according to the user's needs.

Electroporation

1. Seed the cells so that they will be around 70-90% confluent on the day of transfection.
2. Set up the RNP formation reaction as follows below. (Resuspension Buffer R is included with the Neon transfection kit. It is not necessary to use the 10X buffer included with the EnGen LbaCas12a at this step.)

COMPONENT	AMOUNT
Resuspension Buffer R	7.0 µl
EnGen Lba Cas12a (Cpf1)(100 µM)	2.5 µl

COMPONENT	AMOUNT
gRNA (500 µM)	5.0 µl
Total Reaction Volume	**14.5 µl**

3. Gently mix the Resuspension Buffer R, EnGen Lba Cas12a, and gRNA and incubate at room temperature for 20 minutes.
4. During the incubation, trypsinize the cells, washing once to remove any traces of trypsin. Resuspend the cells in 5-10 ml of media. Dilute 20 µl of the cells with 20 µl of trypan blue. Determine the cell number and viability using a hemocytometer.
5. Calculate the number of cells you will need for the entire experiment (1-2 x 10^5 cells per duplicate transfection) and move those to a sterile microfuge tube. Pellet for 5 min at 500 x g. Wash the cells once with 1X PBS and repeat the centrifugation.
6. Calculate the volume of Resuspension Buffer R you will need to resuspend the cells (10.5 µl for duplicate transfections). Resuspend the cells in your calculated volume.
7. Prepare a 24-well plate by adding 500 µl growth medium to the appropriate number of wells.
8. Add 10.5 µl of cells to each 14.5 µl RNP reaction.
9. Aspirate 10 µl of the RNP/cells mix into a 10 µl Neon tip. Electroporate the cells under the following conditions: 1700V, 20 ms, 1 pulse.
10. Immediately transfer the cells to the prepared 24-well plate. Repeat with the next 10 µl and the same electroporation tip if desired.
11. Incubate the cells in a humidified 37°C, 5% CO_2 incubator for 48-72 hours.

Harvest DNA and Amplify Target Region

1. Gently aspirate the media from the cells and wash twice with 250 µl 1X PBS.
2. Add 50 µl of Epicentre QuickExtract™ DNA Extraction Solution and shake/vortex for 5 minutes. 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
3. Analysis of editing can be done following the protocol detailed in the EnGen Mutation Detection Kit (NEB #E3321) manual.