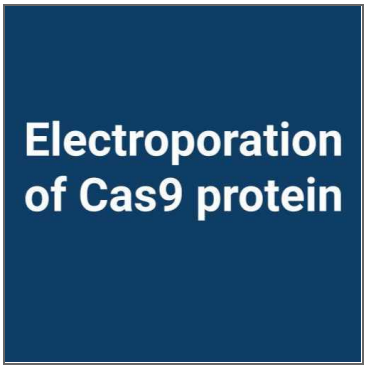

AUG 04, 2023

DOI:
dx.doi.org/10.17504/protocols.io.ewov1qykkgr2/v1

Protocol Citation: Jiuchun Zhang, Harper JW 2023. Electroporation of Cas9 protein into human pluripotent stem cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov1qykkgr2/v1

Protocol status: Working

Created: Jun 23, 2023

Last Modified: Aug 04, 2023

Electroporation of Cas9 protein into human pluripotent stem cells

Forked from Electroporation of Cas9 protein into human pluripotent stem cells

In 1 collection

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ABSTRACT

This protocol describes the electroporation of Cas9 protein into human pluripotent stem cells.

ATTACHMENTS

Electroporation_of_Cas9_protein_into_human_pluripotent_stem_cells.pdf

SAFETY WARNINGS

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

BEFORE START INSTRUCTIONS

Use ThermoFisher Kit to directly electroporate ESCs with Cas9 protein and sgRNA. Works better than plasmid transfection.

PROTOCOL integer ID:
83945

Keywords: Electroporation, Cas9, Cas9 protein, hPSCs, human pluripotent stem cells, ASAPCRN

1 10m

Add 10 µL buffer R to a sterile 1.5 ml tube. Add 6 µg purified Cas9 protein (2mg/ml). Then add 1.2 µg sgRNA. Pipet up and down to mix. Let it sit at Room temperature for 00:10:00. This is enough for 2 transfections (== one 6 well).

2

While waiting for the Cas9 to bind to sgRNA, individualize cells with Accutase. Neutralize Accutase with 5x volume E8 with Rock inhibitor.

3

Count cells. You will need $2x10^5$ for each transfection.

4

Spin down cells. Let it sit for a while so all the residue media can go down to the bottom of the tube. If the residue media is too much, take it out with a P200 pipet.

5

Resuspend cells to a concentration of $2x10^5$ per 5 µl (ie $4x10^7$ per ml) using buffer R.

> **Note**
>
> You don't have to take all the residue media off but you will need to take into account the volume of residue media so you are not too much off.

6

Prepare a 24 well matrigel coated plate. Add 0.5 mL 1 mL E8+ rock inhibitor (1:1000) to the wells you will use. Add HAS (1:2500) to each well. Each transfection goes into one well.

7

Wipe the Neon pipet station with EtOH and place it inside the hood.

8 Add 3 mL electrolytic buffer (buffer E) to the neon tube. Place the tube inside the station. You should feel a click before the tube is securely seated in the station.

9 Use program 13 from the optimization tab for electroporation parameter. Program 9 should also work.

10 When everything is ready, mix 10 µL - 11 µL of resuspended cells with the Cas9+RNA containing R buffer. The final volume should be in the range of 21 µL - 22 µL.

11 Take up a neon tip, pipet 10 µL cell protein mix and electroporate with program 13.

> **Note**
>
> It is important to pipet slowly to avoid air bubble formation. It is also important to insert the pipet slowly into the station, especially during the end of the insertion when you will feel a click. I normally help the pipet down slowly during the clicking so there is no sudden movement of the tip, which might create tiny air bubbles.

12 If you see air bubble in the tip, take it out, push everything out of the tip and repipet the mixture.

13 If you see sparking during the electroporation, your efficiency will reduce significantly.

14 Once electroporation is complete, push everything into one well of a 24 well plate. Do not pipet

up and down with Neon tip.

15 Repeat the same procedure with the same tip and the left over cell mixture. This is just a replicate.

16 Disperse cells evenly in the well and place cells in a low O2 incubator.

17 Put electroporated cells into low oxygen incubator for 2 days (Rm 329, key can be found at Melissa's desk).