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Cas9 RNP electroporation (suspension and adherent cells) V.4

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We use this protocol and it's working

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Abstract

This protocol, based on published work, demonstrates how to delivery Cas9 RNP-based gene editing reagents to cultured mamallian cells by electroporation with a Lonza 4d Nucleofector in the small scale 20 ul 16-well strip format. However, it can be upscaled accordingly for the use of the bigger 100 ul cuvettes with the same device.

Different cell lines need different nucleofector solutions, nucloefector programs and number of cells per reaction. Check **https://knowledge.lonza.com/** to find more information about your cell line of interest.

In addition, consider consulting some of the following papers:

1. RNP delivery paper upon which this work is based (Open Access):
https://elifesciences.org/content/3/e04766
2. Paper by an IGI post-doc that details the rationale behind HDR donor design:
https://www.ncbi.nlm.nih.gov/pubmed/26789497

Attachments

PDF

293T nucleofection p...

175KB

Materials

STEP MATERIALS

- Lonza Nucleofector 4d **Lonza Catalog #**AAF-1002X
- SF Cell Line 4D-Nucleofector® X Kit S (32 RCT) **Lonza Catalog #**V4XC-2032
- Lonza Nucleofector 4d **Lonza Catalog #**AAF-1002X
- SF Cell Line 4D-Nucleofector® X Kit S (32 RCT) **Lonza Catalog #**V4XC-2032

Protocol materials

SF Cell Line 4D-Nucleofector® X Kit S (32 RCT) **Lonza Catalog #**V4XC-2032

Lonza Nucleofector 4d **Lonza Catalog #**AAF-1002X

SF Cell Line 4D-Nucleofector® X Kit S (32 RCT) **Lonza Catalog #**V4XC-2032

Lonza Nucleofector 4d **Lonza Catalog #**AAF-1002X

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SF Cell Line 4D-Nucleofector® X Kit S (32 RCT) **Lonza Catalog #**V4XC-2032

Troubleshooting

Before start

You will need the following materials:

1. Purified Cas9-NLS protein, 40 µM in 1x Cas9 buffer
2. Purified sgRNA from in vitro transcription, >48 µM or synthetic
3. Single-stranded DNA HDR donor, 100 µM (as an IDT Ultramer) (optional)
4. Lonza 4D Nucleofector with X Unit
5. Lonza kit: electroporation solution and 16 reaction small-sized cuvettes (solution specific to your cell type)
6. 1x Cas9 buffer (20 mM HEPES-KOH pH 7.5, 150 mM KCl, 10% glycerol, 1 mM TCEP) and
   2x Cas9 buffer (40 mM HEPES-KOH pH 7.5, 300 mM KCl, 20% glycerol, 2 mM TCEP)

Prepare RNP Mix

1

For a standard reaction, we use 100 pmol Cas9 and 120 pmol sgRNA to form the RNP in a ≤ 5 ul volume. You will need a minimum sgRNA concentration of 48 uM. Mix the following in this order, add Cas9 to the sgRNA slowly while swirling the pipette tip:

	A	B	C	D
		Stock	**Final**	**Volume (ul)**
	Cas9 buffer	2x	1x	1.3
	sgRNA	100 uM	120 pmol	1.2
	Cas9	40 uM	100 pmol	2.5
				5

Protocol

NAME

In vitro transcription of guide RNAs and 5'-triphosphate removal

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Preview

Note

Optimal RNP formation requires the correct final buffer concentration. If your sgRNA is higher than 100 uM, dilute it with water before forming the RNP. If your sgRNA is as low as 48 uM you can omit the 2xCas9 buffer but this could impact the RNP formation and editing efficiency.

Note

Cas9-NLS is stored in -80°C, sgRNAs are prepped by runoff transcription, Cas9 buffer is kept at 4 °C. During the experiment, keep RNA and protein on ice while not in use.

2 Allow RNP to form for 10-20 minutes.

00:20:00

Prepare Cells

3 Count cells. (Trypsinize as needed)

4 Per reaction, pipette 200'000 cells into a collection tube

5 Spin 300 x g for 5 minutes to pellet cells softly. While the cells are spinning, prepare a culture plate and cuvette.

Note

Optimal spinning time and g force might vary between cell types

6 Prepare a 12-well-plate with 1mL media per well, and pre-warm in the incubator.

Note

For different cell types, use the appropriate culture vessel

Pre-Nucleofection

7 Prepare and label wells on 20uL nucleofection strips. Configure Lonza 4D using the recommended cell-type program. As an example, for K562 cells it is recommended to use the SF Cell Line kit and program FF-120

Lonza Nucleofector 4d **Lonza Catalog #**AAF-1002X

SF Cell Line 4D-Nucleofector® X Kit S (32 RCT) **Lonza Catalog #**V4XC-2032

Note

Commonly used cell types / standard programs:

	A	B	C
	Cell type	**Buffer**	**Pulse code**
	K562	SF	FF-120
	HEK-293T	SF	DG-130
	RPE1	P3	EA-104
	Jurkat	SE	CL-120
	HCT-116	SE	EN-113
	Stimulated T cell	P3	EH-115

8 Mix the Nucleofector solution and Supplement together for a total of 20 ul per reaction:

	A	B
		Volume (ul)
	Nucleofector Solution	16.4
	Supplement	3.6
		20

Note

Always prepare the mixture fresh because once the Supplement is added, it is stable for only 3 months.

9 Aspirate the media without disturbing the cell pellet. The pellet is soft so be careful.

10 Wash the pellet with PBS and repeat the centrifugation

11 Aspirate the PBS without disturbing the cell pellet and resuspend in the 20 ul complete Nucleofector solution

Nucleofection

12 Add the entire 5 µL RNP mix to the 20 µL cell suspension and mix gently.

13 If an HDR template is used, add it now. For single-stranded donor DNA, add 1uL of 100 uM stock (100 pmoles) to the cell suspension and mix well.

> Note
>
> Design the donor to match the guide, according to our NBT paper:
>
> https://www.ncbi.nlm.nih.gov/pubmed/26789497
>
> We order single-stranded donors from IDT, as "Ultramers" and resuspend them to 100 µM final concentration.

14 Transfer 25 µl nucleofection mixes to the multiwell nucleofector strip and cap. Pay attention to the orientation of the cap and cuvette in the nucleofector, which is noted in the manufacturer's instructions.

> Note
>
> Try to not introduce bubbles into the wells of the strip because this might interfere with the electroporation pulse. To remove bubbles, tap the strip on the bench top.

15 Insert the cuvette into the nucleofector and select desired well(s) and program(s). Electroporate.

16 Add 80uL of pre-warmed media to each well and then allow cells to sit in nucleofection strips for 10 minutes post-nucleofection. This is supposed to increase efficiency.

00:10:00

17 Gently pipette mixture out with a P200 into your pre-warmed 12-well plate. This should get the vast majority of cells, but if you wish, you may wash out the rest with media from the same well with more media.

Analysis of Editing

18 Allow cells at least 24 hours to settle and recover before attempted downstream analysis. Consider including non-electroporated controls to test viability. Generally, we check for editing 48-72h after nucleofection using amplicon next generation sequencing combined with CRISPResso2 analysis or using Sanger sequencing combined with an online analysis software (TIDE or ICE from Synthego).

24:00:00

For amplicon next generation sequencing of the target site(s), please see:

Protocol

NAME

PCR amplicon next generation sequencing

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