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In vitro assembling of RNP for nucleofection of hPSCs

In 1 collection

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ABSTRACT

This protocol describes the procedure for the in vitro assembly of ribonucleoprotein (RNP) which can be delivered into human pluripotent stem cells (hPSCs) using nucleofection.

General notes

1. Throughout this protocol, the term hPSC is used to collectively refer to both hiPSCs and hESCs. All described procedures have been tested and work equally well for hiPSCs and hESCs.

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COLLECTIONS

Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs

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MATERIALS TEXT

Item	Vendor	Catalog #
Cas9, purified protein, 40 µM	Macrolab, QB3 UC Berkeley	
Synthetic pegRNAs	IDT or Synthego	
Synthetic sgRNAs	Synthego	

1 Thaw Cas9 or PE2 protein **On ice** . We don't usually reuse leftover protein, since freezing and thawing compromises protein activity.

2 In each nucleofection reaction, prepare 10 µl RNP

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3 For regular **CRISPR/Cas9 editing,** use:
5 µl, autoclaved H2O
2 µl, 40 µM purified Cas9 protein
3 µl, 100 µM synthetic sgRNA

4 For prime editing **PE2 strategy**, use:
7-x µl, autoclaved H2O
x µl (90 pmol), purified PE2 protein
3 µl, 100 µM synthetic pegRNA

5 For prime editing **PE3 strategy**, use:
7-x µl, autoclaved H2O
x µl (90 pmol), purified PE2 protein
2 µl, 100 µM synthetic pegRNA
1 µl, 100 µM synthetic ngRNA

6 Pipet the proper amount of each component in the order showing above into a microcentrifuge tube. Mix using a P10/20 tip.

7 Incubate at **Room temperature** for **00:10:00** 10m to assemble RNPs. Once assembled, RNPs are stable at room temperature for 30 min, or at least 2h on ice.

8 If HDR template is desired, mix 100 pmol of template ssODN or 1 µg targeting vector with assembled RNPs right before mixing with cells.

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