Preparing mRNA for nucleofection of hPSCs

In 1 collection

Sep 06, 2022

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ABSTRACT

This protocol describes the standard procedure for preparing mRNA to 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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PROTOCOL CITATION

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COLLECTIONS

Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs

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Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs

MATERIALS TEXT

Item	Vendor	Catalog #
Synthetic pegRNAs	IDT or Synthego	
Synthetic sgRNAs	Synthego	

Note: This protocol makes reference to other protocols. Please check for any materials found in those protocols, which might not be listed here

1 Thaw mRNA and synthetic pegRNA/ngRNA **On ice**

2 In each nucleofection, use 4 µg total of mRNA

3 For prime editing **PE2 strategy**, use:
4 µg, IVT PE2 mRNA
1.5 µl, 100 µM Synthetic pegRNA

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4 For prime editing **PE3 strategy**, use:
4 µg, IVT PE2 mRNA
1 µl, 100 µM Synthetic pegRNA
0.5 µl, 100 µM, Synthetic ngRNA

5 Pipet the proper amount of each component into a microcentrifuge tube.

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