Preparing plasmids for nucleofection of hPSCs

In 1 collection

Sep 06, 2022

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ABSTRACT

This protocol describes the standard procedure for the preparation of plasmids 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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MATERIALS TEXT

Item	Vendor	Catalog #
Synthetic pegRNAs	IDT or Synthego	
Synthetic sgRNAs	Synthego	
pCMV-PE2	Addgene	132775

1 Thaw plasmids **On ice**

2 In each nucleofection, use 1 µg total of plasmid

3 For prime editing **PE2 strategy**, use:
500 ng pCMV-PE2
500 ng pU6-pegRNA

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4 For prime editing **PE3 strategy**, use:
500 ng pCMV-PE2
330 ng pU6-pegRNA
170 ng pBPK1520-ngRNA

5 Pipet the proper amount of each plasmid into a microcentrifuge tube. If the volume is too small, dilute in autoclaved water first.

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