Cas9 RNP nucleofection for CD34+ HSPCs using Lonza 4D Nucleofector

Forked from Cas9 RNP nucleofection for cell lines using Lonza 4D Nucleofector

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

STEPS MATERIALS

NAME	CATALOG #	VENDOR
SFEM II		Stemcell Technologies
CC110		Stemcell Technologies
Mobilized Peripheral Blood CD34 Stem/Progenitor Cells	mPB015F	

Thaw HSPCs, plate at 300,000 cells/mL in stem cell expansion medium

1

SFEM II
by Stemcell Technologies

CC110
by Stemcell Technologies

Mobilized Peripheral Blood CD34 Stem/Progenitor Cells
Catalog #: mPB015F

Count cells daily using hemacytometer

2 Log counts for day 0 (thaw day), day 1, and day 2 (electroporation day).

Generally, the count will decline slightly from day 0 to day 1, and recover from day 1 to day 2. The total number of cells on day 2 will be slightly higher than on day 0.

3

Prepare RNP mix

4

5 Add Cas9 to sgRNA slowly while swirling pipette tip, should take 30s to 1 minute.

6 00:20:00

7 Count cells using hemacytometer.

8 For each nucleofection, pipette 200k cells into a 15 mL conical.

> NOTE
> For replicates, you can multiply the amount of cells and RNP mix as needed and mix in a single tube before electroporating separately.

Prepare Cells

9 Spin 200 x g for 10 minutes to pellet cells softly. While the cells are spinning, prepare plate and cuvette.
00:10:00

10 Place a 96 well plate in the tissue culture incubator.

Nucleofection

11 Prepare and label wells on 20uL nucleofection strips. Configure Lonza 4d to zap with code ER100.

12 Pipette off media from cells, gently but completely, using a P200. The pellet is very soft so be careful.

13 Resuspend cells in 20 µL of P3 nucleofector solution using a P200.

14 Add the entire 10 µL RNP mix to the 20 µL resuspension and mix.

15 Add 1uL of 100uM donor DNA (100 pmoles) and mix well.

16 Add 27.5 µL of cells/RNP electroporation mix to the multiwell cuvette, and cap. Pay attention to the orientation of the cap and cuvette in the nucleofector, which is noted in the manufacturer's instructions.

17 Insert cuvette into nucleofector and zap.

18 Immediately layer electroporated cells with 72.5 µL of medium (SFEM II/Pen-strep/CC110) and rest for 5 minutes at room temperature.

19

Nucleofection

20 Pipette mixture out with a P200 into your pre-warmed 96 well plate. Wash each cuvette with an additional 100 µL of medium, and add that as well (final plating volume 200 µL).

21 Culture cells for several days in SFEM II/PenStrep/CC110 before genotying by desired method. Consider including un-zapped controls to test viability.

Alternatively, transfer cells to expansion medium after 1-2 days. We have used StemCell Erythroid Expansion supplement for this purpose. Otherwise, the cells will not expand substantially if left in CC110