



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

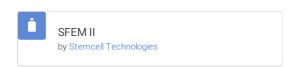
Working

We use this protocol in our group and it is working

STEPS MATERIALS

NAME V	CATALOG #	VENDOR V
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







Count cells daily using hemacytometer

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.

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Prepare RNP mix

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- 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

Q 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.

 $18 \qquad \text{Immediately layer electroporated cells with 72.5 } \mu\text{L of medium (SFEM II/Pen-strep/CC110)} \text{ and rest for 5 minutes at room temperature.}$

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Nucleofection

- 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

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