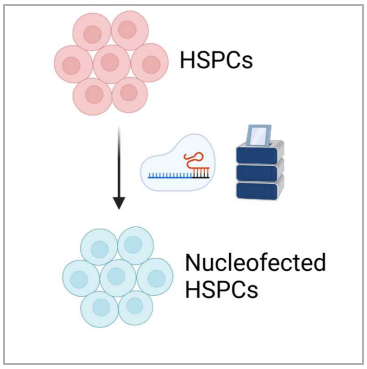

VERSION 2

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We use this protocol and it's working

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Cas9 RNP nucleofection (CD34+ HSPCs) V.2

Version 1 is forked from Cas9 RNP nucleofection for cell lines using Lonza 4D Nucleofector

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ABSTRACT

Protocol for nucleofection of human HSPCs with Cas9 RNP.

MATERIALS

- SFEM II **STEMCELL Technologies Inc.**
- CC110 **STEMCELL Technologies Inc.**
- Mobilized Peripheral Blood CD34 Stem/Progenitor Cells **Contributed by users Catalog #mPB015F**
- P3 Primary Cell 4D-Nucleofector™ X Kit S **Lonza Catalog #V4XP-3032**

Keywords: CRISPR, Cas, Cas9, electroporation, HSPC, CD34, nucleofection, genome editing

Media preparation

1 Ideally, StemSpan SFEM II **STEMCELL Technologies Inc. Catalog #09655** medium at 4 °C Overnight. Mix thoroughly before use.

** Do not refreeze. Store at 2 - 8°C for up to 1 month **

2 Thaw StemSpan™ CC110 1 mL **STEMCELL Technologies Inc. Catalog #2697** at Room temperature. Mix thoroughly before use.

3 Prepare the appropriate amount of supplemented media by adding CC110 (100X ; 10 uL for 1 mL) and P/S (100X ; 10 uL for 1 mL) to SFEM II media.

HSPCs thawing

4 Prepare a 37 °C water bath (in a beaker) in the bead bath.

5 Check that the vial is tightly capped and place in a 37 °C water bath.

6 After 2 min at 37°C, remove the vial from the water bath every 15-20 seconds and gently invert 3-4 times to check the level of thawing.

7 When the vial contents are 50-75% liquid, remove from the water bath and continue to gently invert the vial until the entire contents are liquid.

8 Gently invert 5 times to mix the cells and transfer the vial content to a 15mL tube at Room temperature.

9 Add 1 mL of media to rinse the vial and transfer any remaining cells to the 15mL tube. Also rinse the cap.

10 Slowly add 7 mL media (unsupplemented SFEMII) to the cells. Securely cap the tube and gently invert 3-5 times after adding all the media.

11 Centrifuge the tube at 200g x 8 minutes at Room temperature.

12 Resuspend the cells (pellet) in 2.5 mL of supplemented media.

** Resuspend in 1ml first, transfer to the plate, wash the tube further with 1ml and then 500 ul to make sure you are transferring as many cells as possible **

13 Count the cells using hemacytometer, adjust volume to plate 250,000 CD34+ per well of 6-well plate.

** 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 **

Preparation of RNP mix

14 For one reaction (200,000 HSPCs in small cuvettes) mix following components:

** first, dilute sgRNA in Cas9 Buffer 3X and then, slowly add Cas9 protein while mixing **

	A	B	C
	Cas9 3X buffer	1.5	

	A	B	C
	Ultrapure Water	0.375	
	Guide RNA (100 uM)	0.75	(75 pmol total)
	Cas9 (40uM)	1.875	(75 pmol total)
	total volume	4.5	

Note

Reactions can be prepared in 1.5 mL tubes. Cells resuspended in the nucleofection solution will be directly added to the RNP mix.

15 Incubate 15 min at RT

16 (Optionnal) Slowly add HDR donor (ssODN) protein while mixing.

** 1 uL ssODN at 100 uM = Final quantity: 100 pmol ssODN **

Preparation of nucleofection solution

17 For one reaction (200,000 HSPCs in small cuvettes) mix following components:

	A	B
	P3 Nucleofector™ Solution	16.4 uL
	Supplement solution	3.6 uL
	Total volume	20 uL

Prepare Cells

18 For one reaction, transfer 200,000 cells into a 15 mL Falcon tube.

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

19 Spin 300 x g for 6 minutes to pellet cells softly.

20 While the cells are spinning, prepare plate and cuvette. Place a 24-well plate with 1 mL per well of supplemented SFEMII in the incubator.

21 After centrifugation, discard supernatant and wash with PBS

** Cells can be transfered into a 1.5 mL Eppendorf tube to remove more supernatant in the next steps **

22 Spin 300 x g for 6 minutes to pellet cells softly.

Nucleofection

23 After centrifugation, discard supernatant completely and resuspend cells in the nucleofection solution

** The pellet is very soft so be careful **

24 Resuspend cells in the nucleofection solution.

25 Mix 20 uL of cells with the RNP mix

26 Add 24.5 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 **

27 Choose P3 cell solution and ER-100 code.

28 Insert cuvette into nucleofector and zap.

29 Wait 5 minutes at room temperature and then add 100 uL of supplemented SFEMII media to each cuvette.

30 Transfer the nucleofected cells into the pre-warmed 24-well plate. Wash each cuvette with an additional 100 µL of medium, and add that as well.

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