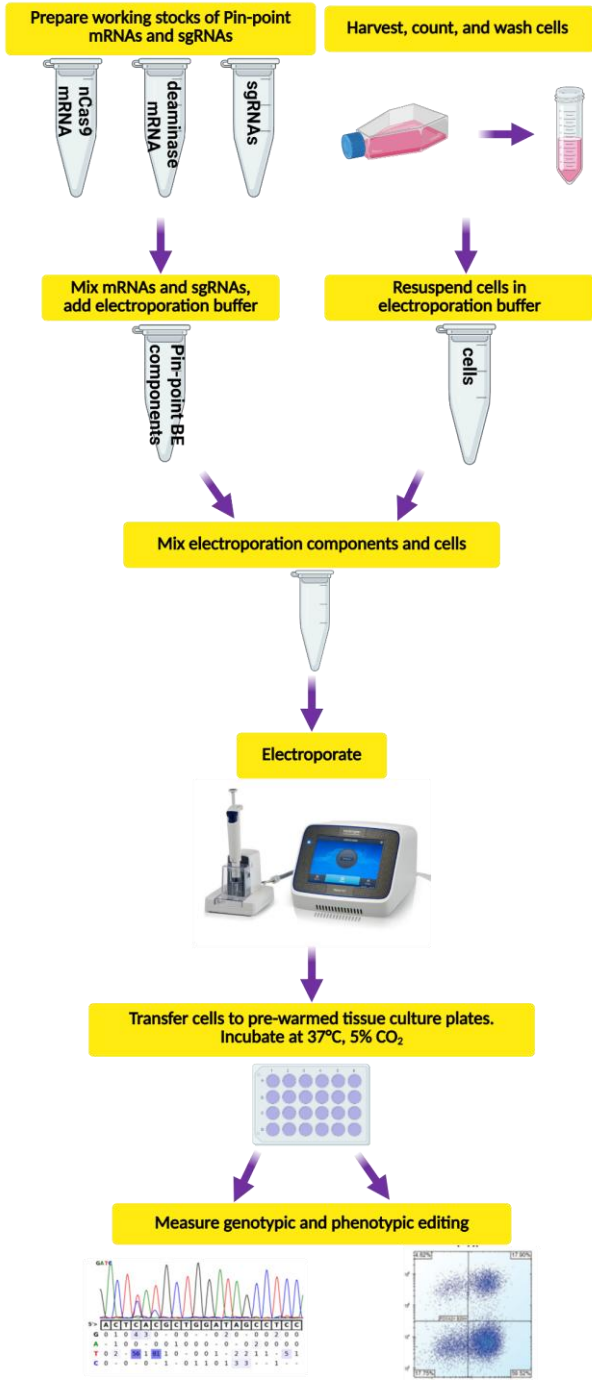


# Pin-point™ cytidine base editing (CBE) platform

## Short protocol for electroporation of human hematopoietic stem and progenitor cells (HSPCs) using the Neon™ Nxt Electroporation System

### Experimental workflow:



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The following is a protocol for delivering 5moU-modified [Pin-point CBE nCas9 mRNA](#) (Cat # PNP12744, PNP12746, PNP12748), [Pin-point CBE rAPOBEC deaminase mRNA](#) (Cat # PNP12745, PNP12747, PNP12749), and Pin-point CBE sgRNAs ([validated controls](#) or [custom](#)) to activated primary human HSPCs using the Neon Nxt Electroporation System. For more details, please refer to the [Pin-point platform CBE technical manual](#). (EP = electroporation)

Day -1	<b>Plate cells</b>	Thaw cryopreserved cells and seed at 200,000 cells/mL in appropriate culture medium. We recommend StemSpan™ SFEM II medium supplemented with StemSpan CD34+ expansion supplement, 1 µM SR1,1000 nM UM729 and Pen/Step.				
	<b>Prepare post-EP plates</b>	Add complete antibiotic-free cell culture medium to plates: 200 µL in 96-well plate or 500 µL in 24-well plate. Incubate at 37°C and 5% CO <sub>2</sub> .				
Day 0	<b>Prepare Pin-point base editing components</b>	Prepare working stock solutions of mRNAs and sgRNAs on ice according to the table below				
	<b>Prepare the cells</b>	Harvest and count the cells. Transfer the desired number of cells for electroporation into a 15 mL conical tube. Wash with PBS, centrifuge at 200 x g for 10 minutes. Resuspend cell pellet in buffer R at a final concentration of 1 x 10 <sup>7</sup> cells/mL				
	<b>Mix EP components</b>	Gently mix mRNAs + sgRNAs + cells:				
			<b>Pin-point component</b>	<b>Working stock*</b>	<b>Final amount per 10 µL EP</b>	<b>Volume per 10 µL EP</b>
<b>mRNA and sgRNA mix</b>		CBE nCas9 mRNA	2 µg/µL	2.8 µg	1.4 µL	
		CBE rAPOBEC deaminase mRNA	2 µg/µL	2 µg	1 µL	
	synthetic sgRNA	200 µM	6µM	0.3 µL		
Buffer R	-	-	to 5 µL			
<b>Cells</b>	Cells in buffer R	-	50,000 cells	5 µL		
* CBE nCas9 mRNA and CBE deaminase mRNA shipped at 2 µg/µL						
	<b>EP</b>	Electroporate on Neon Nxt Electroporation System using the following conditions: 1150V, 20ms, 2 pulses; Pipette cells into prepared plates. Incubate at 37°C, 5% CO <sub>2</sub> for 48-72 hours.				
Days 3 - 7	<b>Post-EP analysis</b>	Proceed with desired genotypic (Sanger sequencing) and/or phenotypic (flow cytometry or qPCR) analyses of base editing and protein expression levels.				

If you have questions or comments, please reach out to [Scientific Support](#).

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