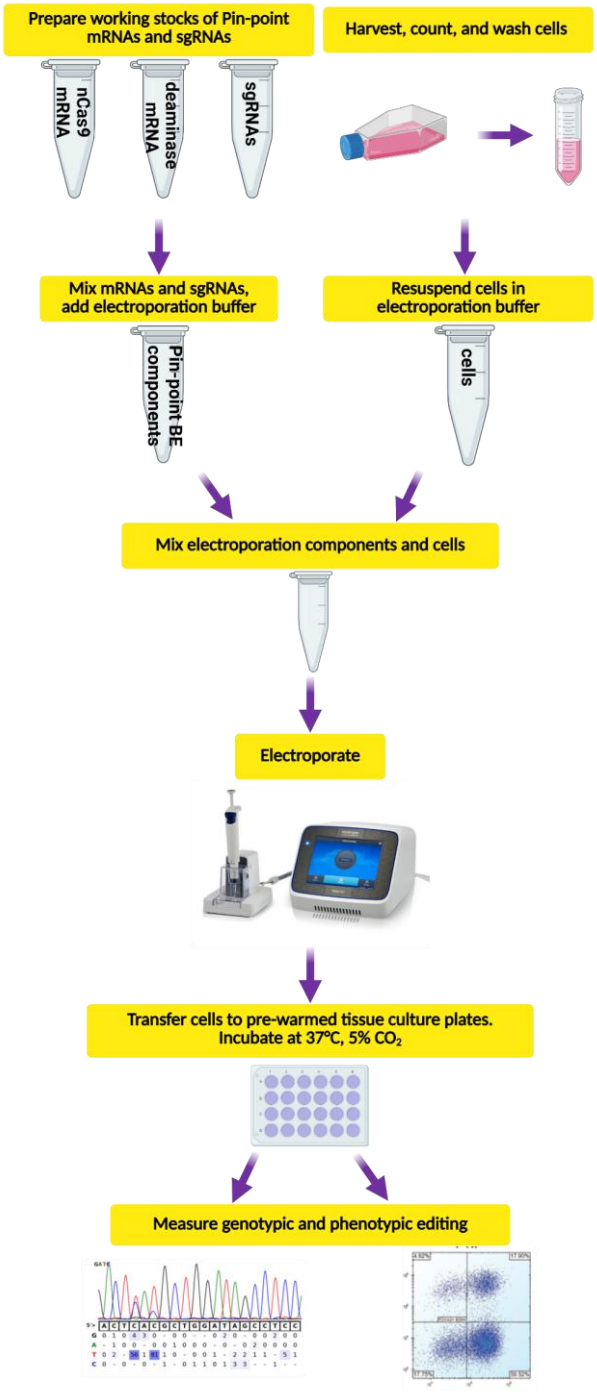
Pin-point™ adenine base editing (ABE) platform

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 ABE nCas9 mRNA (Cat # PNP12744, PNP12746, PNP12748), Pin-point ABE-exact deaminase mRNA (Cat # PNP13785, PNP13786, PNP13787), or Pin-point ABE-flex deaminase mRNA (Cat # PNP13777, PNP13778, PNP13779), and Pin-point sgRNAs (validated controls or custom) to stimulated human HSPCs using the Neon NxT Electroporation System. For more details, please refer to the Pin-point platform ABE technical manual.

(EP = electroporation)

<table>
<tr><td>Day -1</td><td>Plate cells</td><td>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.</td></tr>
<tr><td rowspan="4">Day 0</td><td>Prepare post-EP plates</td><td>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_2.</td></tr>
<tr><td>Prepare Pin-point base editing components</td><td>Prepare working stock solutions of mRNAs and sgRNAs on ice according to the table below.</td></tr>
<tr><td>Prepare the cells</td><td>Harvest and count the cells. Transfer the desired number of cells for electroporation into a centrifuge 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 10^7 cells/mL.</td></tr>
<tr><td>Mix EP components</td><td>Gently mix mRNAs + sgRNAs + cells:
<table>
<tr><th></th><th>Pin-point component</th><th>Working stock*</th><th>Final amount per 10 µL EP</th><th>Volume per 10 µL EP</th></tr>
<tr><td rowspan="4">mRNA and sgRNA mix</td><td>ABE nCas9 mRNA</td><td>2 µg/µL</td><td>1.5 µg</td><td>0.75 µL</td></tr>
<tr><td>ABE deaminase mRNA</td><td>2 µg/µL ABE-exact, 0.8 µg/µL ABE-flex*</td><td>1 µg ABE-exact, 0.4 µg ABE-flex</td><td>0.5 µL</td></tr>
<tr><td>synthetic sgRNA</td><td>200 µM</td><td>6.25µM</td><td>0.312 µL</td></tr>
<tr><td>Buffer R</td><td>-</td><td>-</td><td>to 5 µL</td></tr>
<tr><td>Cells</td><td>Cells in buffer R</td><td>-</td><td>50,000 cells</td><td>5 µL</td></tr>
</table>
* ABE nCas9 mRNA and ABE deaminase mRNA shipped at 2 µg/µL</td></tr>
<tr><td>EP</td><td>Electroporate 10 µL on Neon NxT Electroporation System using the following conditions: 1400V, 10ms, 3 pulses.
Pipette cells into prepared plates.
Incubate at 37°C, 5% CO_2 for 48-72 hours.</td></tr>
<tr><td>Days 3 - 7</td><td>Post-EP analysis</td><td>Proceed with desired genotypic (Sanger sequencing) and/or phenotypic (flow cytometry or qPCR) analyses of base editing and protein expression levels.</td></tr>
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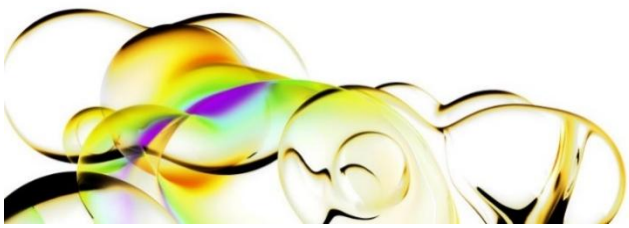

If you have questions or comments, please reach out to Scientific Support.