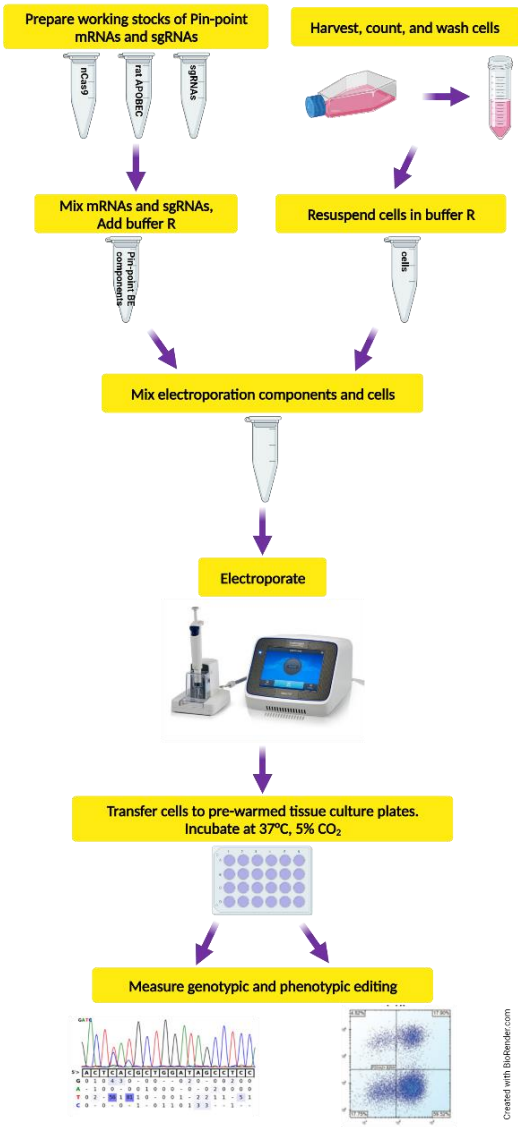
Pin-point™ base editing platform in HEK293T and human T cells
Quick protocol

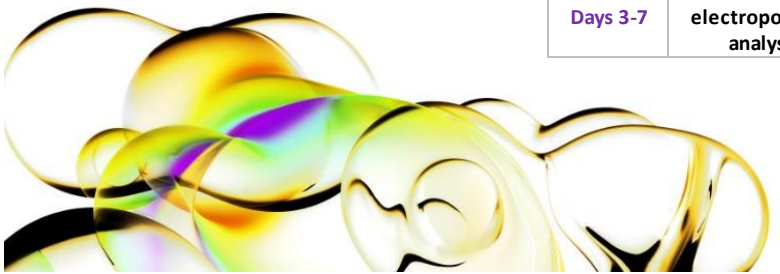
Version 3 September 2024



The following is a protocol for delivering unmodified Pin-point nCas9 mRNA (Cat # PNP12744, PNP12746, PNP12748), Pin-point rat APOBEC mRNA (Cat # PNP12745, PNP12747, PNP12749), and Pin-point sgRNAs (Cat # PNP-02000-01, PNP-02200-01, PNP-02300-01) to cultured mammalian cells using the Neon™ NxT Electroporation System. For more details, please see full protocol.

This protocol is written for electroporation of HEK293T cells and activated primary human T cells.

<table>
<tr><td>Day -2/-1</td><td>Plate cells</td><td>Seed cells at appropriate density.
HEK293T cells: 3 x 10^6 cells/10cm dish; ***Activated T cells***: 1 x 10^6 cells/mL</td></tr>
<tr><td rowspan="5">Day 0</td><td>Prepare post-electroporation plates</td><td>Add appropriate cell culture medium to plates
HEK293T cells: 96-well plates with 100µL medium per well; ***Activated T cells*** : 24-well plates with 500µL medium per well.
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 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 (avoid centrifuge speeds > 400 x g)
Resuspend cell pellet in R buffer: ***HEK293T cells***: 1 x 10^7 cells/mL; ***Activated T cells*** : 5 x 10^7 cells/mL.</td></tr>
<tr><td>Mix electroporation components</td><td>Gently mix mRNAs + sgRNAs + cells
HEK293T cells:
<table>
<tr><th>Pin-point component</th><th>Working stock*</th><th>Final amount per reaction</th><th>Volume per reaction</th></tr>
<tr><td>nCas9 mRNA</td><td>2 µg/µL</td><td>1 µg</td><td>0.5 µL</td></tr>
<tr><td>Rat APOBEC mRNA</td><td>200 ng/µL</td><td>100 ng</td><td>0.5 µL</td></tr>
<tr><td>synthetic sgRNA</td><td>100 µM</td><td>6 µM</td><td>0.6 µL</td></tr>
<tr><td>Buffer R</td><td>-</td><td>-</td><td>3.4 µL</td></tr>
<tr><td>Cells</td><td></td><td>50,000 cells</td><td>5 µL</td></tr>
</table>
* nCas9 mRNA and Rat APOBEC mRNA shipped at 2 µg/µl
Activated T cells:
<table>
<tr><th>Pin-point component</th><th>Working stock*</th><th>Final amount per reaction</th><th>Volume per reaction</th></tr>
<tr><td>nCas9 mRNA</td><td>2 µg/µL</td><td>1.56 µg</td><td>0.78 µL</td></tr>
<tr><td>Rat APOBEC mRNA</td><td>1 µg/µL</td><td>0.222 µg</td><td>0.222 µL</td></tr>
<tr><td>synthetic sgRNA</td><td>200 µM</td><td>2 - 6µM</td><td>0.1 µL of each sgRNA (0.3µL total when using 3 sgRNAs)</td></tr>
<tr><td>Buffer R</td><td>-</td><td>-</td><td>to 10 µL</td></tr>
<tr><td>Cells</td><td></td><td>250,000 cells</td><td>5 µL</td></tr>
</table></td></tr>
<tr><td>Electroporate</td><td>Electroporate on Neon NxT Electroporation System with the following conditions:
HEK293T cells: 1150V, 20ms, 2 pulses; ***Activated T cells***: 1600V, 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-electroporation analysis</td><td>Proceed with desired genotypic (Sanger sequencing) and phenotypic (flow cytometry) analyses of base editing levels.</td></tr>
</table>



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