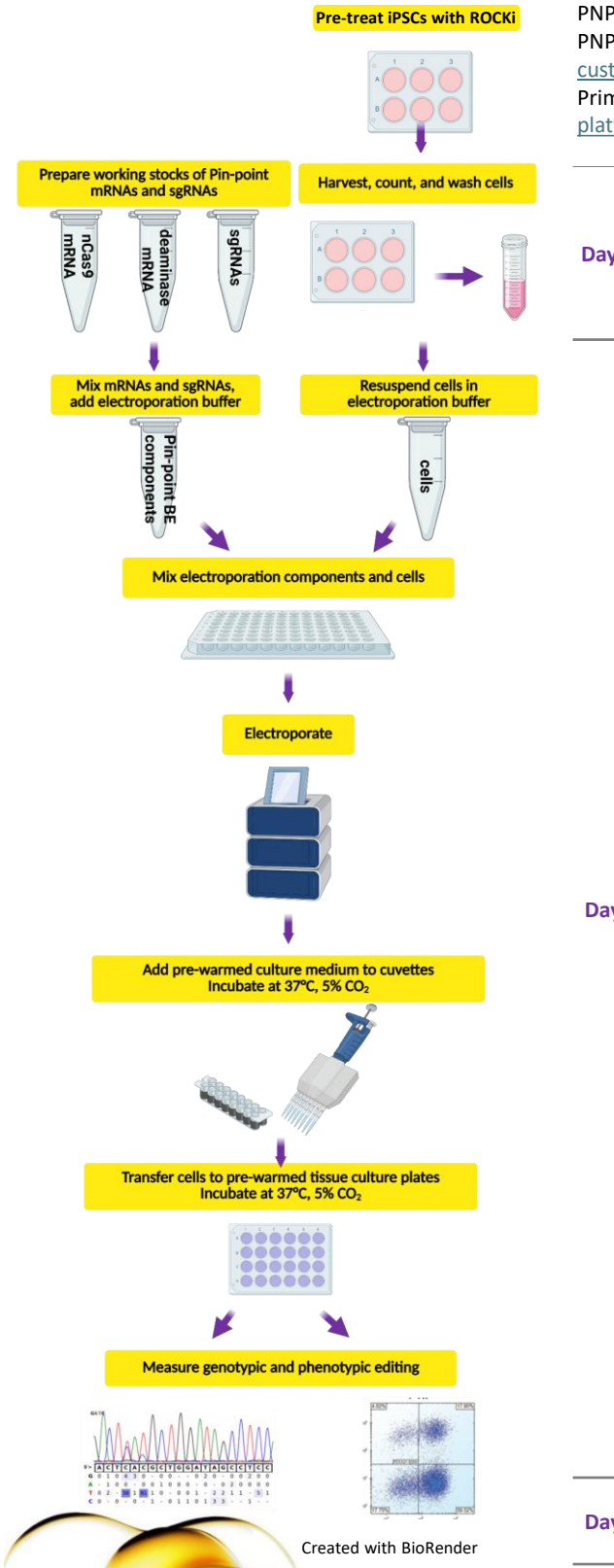
Pin-point™ cytidine base editing (CBE) platform

Short protocol for electroporation of human induced pluripotent stem cells (iPSCs) using the Lonza 4D-Nucleofector® System

Experimental workflow:



The following is a protocol for delivering unmodified Pin-point CBE nCas9 mRNA (Cat # PNP12744, PNP12746, and PNP12748), Pin-point CBE rAPOBEC deaminase mRNA (Cat # PNP12745, PNP12747, and PNP12749), and Pin-point CBE sgRNAs (validated controls or custom) to stimulated human iPSCs using the Lonza 4D-Nucleofector System with the P3 Primary Cell 96-well Nucleofector® Kit. For more details, please refer to the Pin-point platform CBE technical manual. (EP = electroporation)

<table>
<tr><td>Day -3</td><td>Plate cells</td><td>Coat plasticware with appropriate iPSC culture matrix (e.g Vitronectin XF).
Seed cells at appropriate density to achieve ~70% confluence at Day 0.
NOTE: We recommend mTeSR™ PLUS culture medium (STEMCELL Technologies)</td></tr>
<tr><td rowspan="5">Day 0</td><td>Prepare post-EP plates & treat iPSCs with ROCKi</td><td>2 hours prior to electroporation replace culture media with iPSC culture medium + Y-27632 (ROCKi) (10 µM).
Coat 12-well plates with iPSC culture matrix.
Add iPSC cell culture medium + ROCKi (10 µM) to matrix-coated plates: 1 mL per well of 12-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>Remove iPSC culture media and rinse cells twice with PBS.
Dissociate iPSC colonies by incubation at 37°C for 6-10 mins with Accutase (Gibco).
Add 1 mL iPSC culture medium to dissociated iPSC colonies and gently triturate to single cells by pipetting.
Count cells and transfer the desired number into a 15 mL conical tube: 2 x 10^5 cells/ 20 µL electroporation.
Centrifuge at 200 x g for 5 mins.
Resuspend cell pellet in P3 nucleofector solution 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 20 µL EP</th><th>Volume per 20 µL EP</th></tr>
<tr><td rowspan="4">mRNA and sgRNA mix</td><td>CBE nCas9 mRNA</td><td>2 µg/µL</td><td>2.56 µg</td><td>1.28 µL</td></tr>
<tr><td>CBE rAPOBEC deaminase mRNA</td><td>2 ug/µL</td><td>0.74 µg</td><td>0.37 µL</td></tr>
<tr><td>Synthetic sgRNA</td><td>200 µM</td><td>40 pmol</td><td>0.2 µL</td></tr>
<tr><td>P3 nucleofector solution</td><td>-</td><td>-</td><td>to 10 µL</td></tr>
<tr><td>Cells</td><td>Cells in P3 nucleofector solution</td><td>-</td><td>2 x 10^5 cells</td><td>10 µL</td></tr>
</table>
* CBE nCas9 mRNA and CBE deaminase mRNA shipped at 2 µg/µL</td></tr>
<tr><td>EP</td><td>Electroporate 20 µL on Lonza 4D-Nucleofector System using program CM-138.
Add 80 µL iPSC culture media + ROCKi (10 µM) to the cuvette.
Incubate at 37°C, 5% CO_2 for 5 mins.
Gently transfer cells into prepared plates and disperse evenly by tilting/rocking.
Incubate at 37°C, 5% CO_2.</td></tr>
<tr><td>Day 1</td><td>Change media</td><td>Remove iPSC culture media containing ROCKi and add fresh iPSC culture medium: 2 mL per well of 12-well plate.
Incubate at 37°C and 5% CO_2.</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) analyses of base editing levels.</td></tr>
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If you have questions or comments, please reach out to Scientific Support.