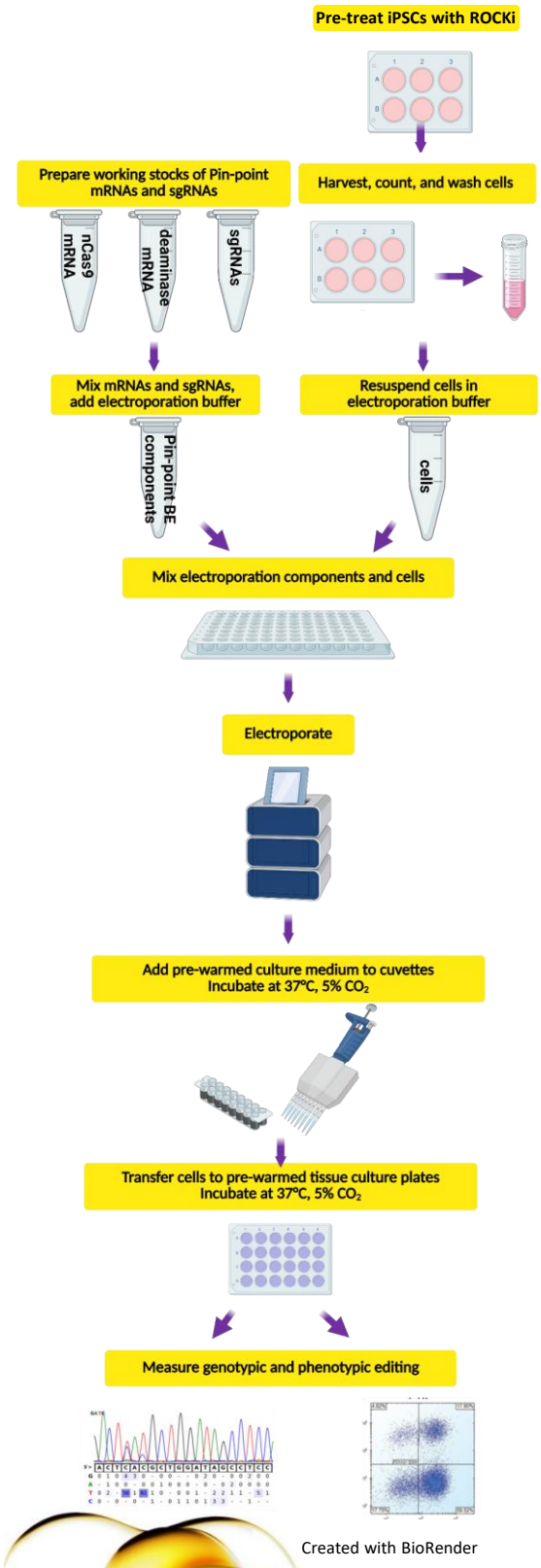
Pin-point™ adenine base editor (ABE) platform

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 ABE nCas9 mRNA (Cat # PNP13767, PNP13768, and PNP13769), Pin-point ABE-exact deaminase mRNA (Cat # PNP13781, PNP13782, and PNP13783) or Pin-point ABE-flex deaminase mRNA (Cat # PNP13773, PNP13774, and PNP13775), and Pin-point ABE 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 ABE technical manual.
(EP = electroporation)

Day -3	**Plate cells**	Coat plasticware with appropriate iPSC culture matrix (e.g Vitronectin XF). Seed cells at appropriate density to achieve ~70% confluence on Day 0. *NOTE: We recommend mTeSR™ PLUS culture medium (STEMCELL Technologies)*
Day 0	**Prepare post-EP plates & treat iPSCs with ROCKi**	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.
	Prepare Pin-point base editing components	Prepare working stock solutions of mRNAs and sgRNAs on ice according to the table below.
	Prepare the cells	Remove iPSC culture medium and rinse cells twice with PBS. Dissociate iPSC colonies by incubation with Accutase at 37°C for 6 - 10 mins. 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 centrifuge tube: 1 x 10^5 cells/ 20 µL electroporation. Centrifuge at 200 x g for 5 mins. Resuspend cell pellet in P3 nucleofector solution: 1 x 10^7 cells/mL.
	Mix EP components	Gently mix mRNAs + sgRNAs + cells:
	EP	Electroporate 20 µL on Lonza 4D-Nucleofector System using program CM-138 Add 80 µL iPSC culture medium + 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.
Day 1	**Change media**	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.
Days 3 - 7	**Post-EP analysis**	Proceed with desired genotypic (Sanger sequencing) and/or phenotypic (flow cytometry) analyses of base editing levels.

	Pin-point component	Working stock*	Final amount per 20 µL EP	Volume per 20 µL EP
mRNA and sgRNA mix	ABE nCas9 mRNA	2 µg/µL	1 µg	0.5 µL
	ABE deaminase mRNA	2 ug/µL ABE-exact, 0.2 ug/µL ABE-flex*	1 µg ABE-exact, 0.1 µg ABE-flex	0.5 µL
	Synthetic sgRNA	200 µM	40 pmol	0.2 µL
	P3 Nucleofector Solution	-	-	to 10 µL
Cells	Cells in P3 Nucleofector Solution	-	1 x 10^5 cells	10 µL

* ABE nCas9 mRNA and ABE deaminase mRNA shipped at 2 µg/µL

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