Pin-point™ base editing platform in human iPSCs
Quick protocol

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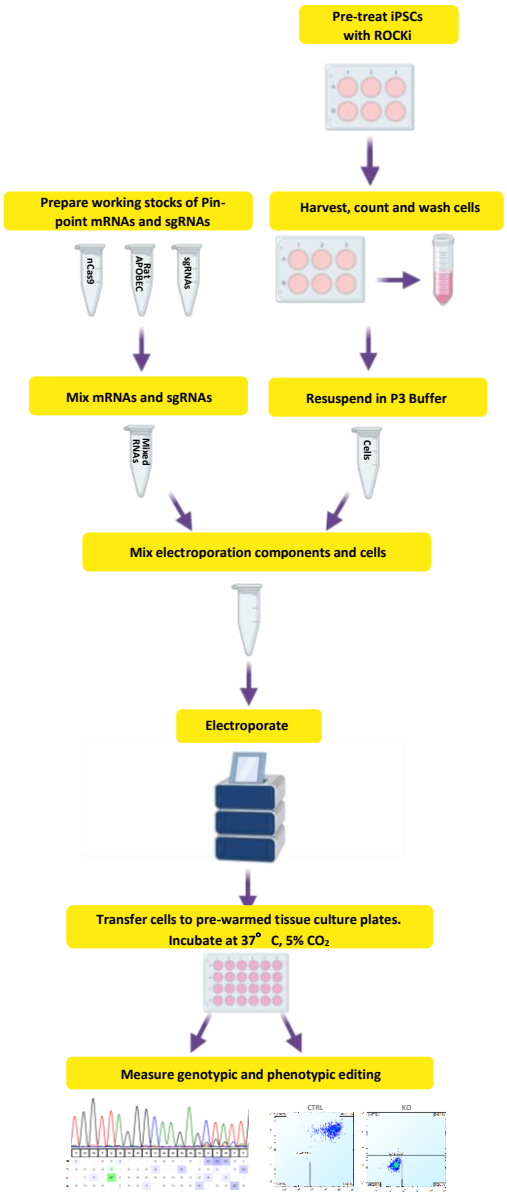

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The following is a protocol for delivering unmodified Pin-point nCas9 mRNA (Cat #PNP12744, PNP12746, and PNP12748), Pin-point rat APOBEC mRNA (Cat #PNP12745, PNP12747, and PNP12749), and Pin-point sgRNAs to cultured mammalian cells using the Lonza 4D-Nucleofector™ System with the P3 Primary Cell 96-well Nucleofector® Kit. For more details, please see our Pin-point platform technical manual.

This protocol is for electroporation of human iPSCs.

Day -3	**Plate cells**	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)*
Day 0	**Prepare post-electroporation 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 media and rinse cells twice with Phosphate Buffered Saline (Gibco). 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 live cells and transfer the desired number into a centrifuge tube: 2 x 10^5 cells/ 20 µL electroporation. Centrifuge at 200xg for 3 mins. Resuspend cell pellet in P3 complete buffer: 1 x 10^7 cells/mL.
	Mix electroporation components	Gently mix mRNAs + sgRNAs + cells:
	Electroporate	Electroporate 20 µL on Lonza 4D-Nucleofector™ System with appropriate program. *NOTE: We recommend program CM138 for iPSCs.* 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.
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-electroporation 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 electroporation	Volume per 20 µL electroporation
mRNA and sgRNA mix	nCas9 mRNA	2 µg/µL	2.56 µg	1.28 µL
	Rat APOBEC mRNA	2 ug/µL	0.74 µg	0.37 µL
	Synthetic sgRNA	200 µM	40 pmol	0.2 µL
P3 complete buffer + cells	P3 Buffer Supplement 1	- -	- -	16.4 µL 3.6 µL
	Cell suspension	-	2 x 10^5 cells	-

* nCas9 mRNA and Rat APOBEC mRNA shipped at 2 µg/µL

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