CRISPR-Cas9-mediated genome editing in primary murine bone marrow-derived macrophages.

By

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ABSTRACT

Mouse primary bone marrow-derived macrophages (BMDMs) are an important tool for studying macrophage physiology *ex vivo*. They recapitulate the complex biology of macrophages more closely than immortalized macrophage cell lines and can often be derived from mice carrying mutations of interest. However, the genetic manipulation of primary macrophages has remained challenging. Here, we present a protocol for efficient genome editing in primary mouse BMDMs using sgRNA-Cas9 ribonucleoprotein particles (RNP) assembled *in vitro*, including the methods for designing and synthesizing sgRNAs and RNP complex delivery. We also provide a rapid protocol to evaluate editing efficiency using routine Sanger sequencing and a readily available online analysis tool. The protocol requires no plasmid construction, can be performed in under a week, and results in up to 95% editing efficiency.

INTRODUCTION

Macrophages are innate immune cells that play critical roles in organogenesis, wound healing, and immunity[1,2]. Immortalized macrophage cell lines, such as RAW 264.7 cells, have advantages, including robust growth and effective methods for disrupting gene function including delivery of vectors for both RNA interference (RNAi) and CRISPR-Cas9[3,4]. However, their oncogenic transformation also alters their responses to many stimuli[5,6]. Primary macrophages more faithfully recapitulate *in vivo* macrophage function. However, disrupting gene function in primary bone-marrow derived macrophages (BMDMs) has been hampered by the relatively low potency of RNAi methods and the low efficiency of lentiviral transduction in these cells[7,8].

CRISPR-Cas9 is an effective method for gene disruption in a wide range of systems, including mammalian cells[9-12]. Streptococcus pyogenes Cas9 cleaves double-stranded DNA in mammalian cells with high-specificity when complexed with a target-specific single guide RNA (sgRNA). In initial studies, the Cas9 and sgRNA were delivered by plasmid or lentiviral vectors, an effective strategy in a number of cell types. Subsequently, it was determined that sgRNA-Cas9 complexes could be formed from purified components *in vitro* and delivered to the nucleus of many cell types efficiently through electroporation[13,14]. Here, we provide a detailed protocol for generating sgRNA-Cas9 ribonucleoprotein particles (RNPs) *in vitro* and delivering the RNPs to primary mouse BMDMs via electroporation. This method results in up to 95% editing of target genes with minimal cell death and yields > 1 x 10^6 cells per reaction. In addition, we provide a workflow for assessment of editing efficiency using routine Sanger sequencing and a well-established online chromatogram analysis tool known as Tracking of Indels by Decomposition (TIDE)[15].

PROTOCOL

1. Single guide RNA (sgRNA) design

1.1. Identify 20-nucleotide genomic target sequences which will be incorporated in the synthesized sgRNA. There are numerous free online design tools available including CRISPick from The Broad Institute or the CRISPR Design Tool from Synthego (Table 1).

1.2. Select 4-5 non-overlapping guides within each target gene, as Cas9 activity varies by guide and a priori predictions of highly active guides are imprecise. Ideally, choose guides that lie within the first large coding exon of a gene so that any translated protein is truncated early in the coding sequence. Although not mandatory, choosing guides that lie within the same exon will simplify the analysis of editing efficiency. To generate functional sgRNAs, it is critical to ensure proper orientation of the guide relative to the targeted genomic sequence. Most design tools provide the guide sequence in a 5' to 3' orientation regardless of which strand of the chromosome is being targeted. To confirm that the orientation is correct, verify that there is a Cas9 PAM sequence (nGG) immediately 3' of the targeted genomic sequence. Note that the PAM is not included in the sgRNA.

2. sgRNA synthesis (Figure 1). This step describes how to synthesize sgRNAs by using PCR to generate a template for *in vitro* transcription (IVT), carry out the IVT reaction, and purify the sgRNA using spin-columns. As an alternative to performing IVT, custom-designed synthetic sgRNAs are commercially available through several vendors.

2.1 Overlap-extension PCR to generate the IVT template. Each PCR reaction incorporates universal primers that encode the T7 RNA polymerase promoter and trans-activating CRISPR RNA (tracrRNA) along with the gene-specific primer encoding the guide (Figure 1A).

2.1.1 In this step, the overlapping guide-specific primer and tracrRNA sequences are annealed, and the overlap extended without amplification primers. Set up reactions: 1x PCR buffer with a

further 1 mM $MgCl_2$ added, 0.25 uL Herculase DNA polymerase, 0.5 mM each deoxynucleoside triphosphate (dNTP), 0.02 uM Guide-Specific Primer, and 0.02 uM T7 Reverse Long Universal Primer (Table 2) in a total volume of 46 uL. Perform 2 cycles of: 95 °C for 15 sec, 37 °C for 15 sec, 72 °C for 15 sec, and repeat the cycle once. Place reaction on ice.

2.1.2. To each reaction from step 2.1.1, add 2 uL of 25 uM universal forward amplification primer and 2 uL of 25 uM universal reverse amplification primer to bring final volume to 50 uL. Denature at 95 °C for 30 sec. Then cycle 35x: 95 °C for 15 sec, 65 °C for 20 sec, 72 °C for 15. Perform a final extension at 72 °C for 2 min.

2.1.3. Check the PCR product size on a 2% agarose gel. The product is 127 bp long (Figure 1B).

2.2. Purify the IVT template from step 2.1.3 using AMPure XP Reagent Beads, or similar, per manufacturer instructions, combining 50 uL of PCR product with 90 uL of beads. Elute DNA by adding 40 uL of elution buffer (5mM Tris, 0.1mM EDTA) pre-warmed to 65 °C.

2.3. Determine DNA concentration of the IVT template using UV absorbance at 260 nm. The concentration must be at least 50 ng/uL. If the yield is < 50 ng/uL, then repeat the PCR reaction and combine it with the product from step 2.2 to further concentrate the solution. PCR products can be stored at -20 °C.

2.4. IVT reaction

2.4.1. Several commercial kits are available; we recommend one designed to produce high yields, as the protocol requires high concentration sgRNA. Follow manufacturer instructions using 250 ng IVT template in a 20 uL reaction. Incubate the reaction at 37 °C for 4-16 h.

2.4.2. Quality control: Check the quality of the IVT product by running 1 uL of reaction on a 2% agarose 0.5x TB (Tris-Borate, no EDTA) gel. Though not mandatory, bands will resolve more sharply if using a urea-containing RNA sample buffer and if sample is denatured at 65 °C for 5

min prior to electrophoresis (Figure 1C).

2.5. If IVT reaction passes quality control, remove 5' triphosphate groups from sgRNA in IVT reaction to avoid activation of RIG-I and subsequent cell death: to each 20 uL IVT reaction, add 69 uL of RNase-free Milli-Q H_2O, and 3 uL of calf intestinal phosphatase in 1x reaction buffer. Incubate at 37 °C for 2 h.

2.6. Degrade the IVT template to avoid activation of cytosolic DNA sensors and cell death: to each reaction from step 2.5, add 1 uL DNase. Incubate at 37 °C for 15 min. The resulting IVT product can be stored at -20 °C for one day or -80 °C until column purification.

2.7. Purify IVT product using a spin column such as Qiagen RNeasy column. Bead purification kits produce inferior results.

2.7.1 If using Qiagen kit, bind RNA by adding 220 uL buffer RLT to the IVT mix, followed by 1 mL 100% ethanol and applying to the column. Then follow manufacturer instructions. Perform the final ethanol wash in a biosafety cabinet to ensure sterility.

2.7.2. Perform elution in a biosafety cabinet to ensure sterility. RNA is eluted in 30 uL of 10 mM Tris pH 8.0. Yield can be increased by 10-20% by re-applying the eluate to the column and performing a second elution.

2.8. Measure the concentration of sgRNA using UV absorbance att 260 nm. The sgRNA should be at a minimum concentration of 1100 ng/uL. Typical yields are >100 ug sgRNA.

2.9. Store the sgRNA at -80 °C. Make aliquots to avoid more than 3 freeze-thaw cycles.

3. Prepare for electroporation. All steps should be performed in a biosafety cabinet to maintain sterility.

3.1. Thaw cells 48 h before electroporation and plate on non-TC treated plates. Seed enough so that they are 80-90% confluent on the day of electroporation.

3.2. We use a Neon Transfection System with 10 uL tips. Set the electroporation parameters to 1900 v, 20 ms, 1 pulse. Wipe the surfaces of the Pipette Station with 70% ethanol and place it inside the biosafety cabinet.

3.3. Remove a transfection tube from the package, carefully keeping it sterile, and fill it with 3 mL of E Buffer. The E Buffer should be at room temperature at the time of electroporation. Place the tube in the pipette station and gently push down until it makes a clicking sound.

3.4. Sterilize 1 PCR tube per electroporation reaction by heating tubes in a thermocycler to 98 °C for 10 min. Place sterilized PCR tubes on ice.

3.5. For each electroporation reaction, prepare 2.5 uL of sgRNA at a concentration of 1100 ng/uL. Dilute the sgRNA in cold (4 °C) DPBS containing 0.9 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (DPBS + Ca/Mg). Leave diluted sgRNA on ice until ready to form RNP complexes.

3.6. Prepare 2 plates: a 12-well non-TC coated plate for electroporated cells and a 24 well plate for media. Aliquot 2 mL media per reaction into the 24-well plate.

3.7. Dilute Cas9 to 20 uM with cold (4 °C) DPBS + Ca/Mg. Per electroporation reaction, aliquot 1 uL of 20 uM Cas9 into sterile PCR tubes. Keep on ice until ready to form RNP complexes.

3.8. Prepare BMDMs for electroporation.

3.8.1 Remove growth media by aspirating. Wash the cells with DPBS without $CaCl_2$ and $MgCl_2$ (DPBS - Ca/Mg). Add DPBS + 1mM EDTA and incubate at room temperature until the cells begin lifting off the plate, about 5 min.

3.8.2. Pipette up and down to detach cells. Transfer cells to a low protein-binding 15 mL conical tube. Pellet cells at 500 x g for 5 min.

NOTE: Macrophages are adherent to non-TC coated plasticware. Use of low protein-binding centrifuge tubes dramatically improves cell recovery.

3.8.3. Work quickly to minimize the amount of time that the cells sit in DPBS + EDTA. Remove all but about 1 mL of supernatant. Resuspend cells in the remaining supernatant and transfer it to a low protein-binding microfuge tube.

3.8.4. Pipette up and down 10 times to create a single cell suspension. Remove a small aliquot for counting. Pellet the remaining cells at 500 x g for 5 min.

3.8.5. While cells are pelleting, count the cells and warm the Cas9 and sgRNA to room temperature.

3.8.6. Remove all the DPBS + EDTA supernatant from the pelleted cells. Resuspend the cells in DPBS + Ca/Mg and adjust concentration to 4 x 10^7 cells/mL (4 x 10^5 cells per reaction).

NOTE: Although each Neon kit is supplied with proprietary electroporation buffers (Buffer R, Buffer T), we have found no decrease in electroporation efficiency in head-to-head comparisons using DPBS + Ca/Mg.

3.8.7. Aliquot cells. If electroporating sgRNAs that target unique genes, aliquot cells into separate low protein-binding microfuge tubes for each targeted gene to avoid cross-contamination. Aliquoting cells for unique sgRNAs that target the same gene is not necessary. Set the cells aside at room temperature until electroporation.

4. Assemble the RNP.

4.1. Ensure the sgRNA and Cas9 are at room temperature to avoid precipitate formation. Add 2.5 uL of diluted sgRNA to the 1 uL Cas9 aliquots from step 3.7. Dispense the sgRNA slowly over 15 sec while stirring with the tip to mix and prevent precipitation.

4.2. Incubate 5-20 min at room temperature.

5. Electroporate the cells.

5.1. Prepare the electroporation tip. Press the plunger to extend the docking stem out of the pipettor.

Insert this stem into the electroporation tip. Important: Ensure the tip is secure and that the electrode plunger slides smoothly within the tip and extends slightly beyond the sheath. If not properly seated, air bubbles will be introduced into the tip resulting in tip failure.

5.2. Make sure the cells are fully suspended by pipetting up and down, then load the electroporation tip with cells, drawing the full 10 uL volume capacity of the tip and ensuring no bubbles are present.

5.3. Add the cells to the 3.5 uL of RNP from step 5 and pipette up and down 3 times to mix well. Draw 10 uL of the mixture into the tip, ensuring no bubbles are present.

5.4. Load the tip into the electroporation station, lowering it into the Buffer E. Avoid contact with all surfaces to keep the tip sterile. Push start on the touchscreen and wait for pulse to complete.

5.5. Remove the pipette from the electroporation station and eject the cells into a dry well of the labeled non-TC treated 12-well plate. Set aside the pipette with the electro-tip still attached, taking care that the tip does not contact anything and remains sterile.

5.6. Immediately add BMDM growth media (aliquoted in step 3.6) on top of the cells and gently shake the plate to disperse the cells.

5.7. Repeat steps 5.2-5.6 for every reaction.

5.7.1. If electroporating unique sgRNAs targeting the same gene, the electroporation tip can be re-used between sgRNAs; in most instances, 4-5 sgRNAs are tested per gene. If re-using tip, rinse tip 2 times in a 50 mL conical tube of room temperature DPBS + Ca/Mg.

5.7.2. If electroporating sgRNAs targeting different genes, we recommend using a fresh tip for each gene rather than re-using the tip.

5.8. Incubate electroporated cells at 37 °C with 5% CO_2.

5.9. Observe the viability of the cells 1-2 h post-electroporation. Optional: Add 5 ug/mL

gentamycin to the cells 1-2 h post-electroporation to help prevent contamination.

6. Assess editing efficiency

6.1. Most editing is complete by 48h. 48h post-electroporation, examine the cell monolayer. If the cells are ≥ 50% confluent, then proceed to step 6.2. If the cells are < 50% confluent wait an additional 1-2 days before proceeding to step 6.2. 1-2 x 10^5 cells are needed for genomic DNA analysis.

6.2. Extract genomic DNA (gDNA)

6.2.1. Wash cells once with DPBS - Ca/Mg. Add 1 mL DPBS + 1 mM EDTA. Incubate at room temperature until the cells begin lifting off the plate, about 5 min.

6.2.2. Pipette up and down to detach cells. Transfer cells to a low protein-binding microfuge tube.

6.2.3. Remove an aliquot of 1-2 x 10^5 cells. The remaining cells can be plated for use in experiments. We typically wait 5 days post-electroporation to allow decay of mRNA and protein before using cells in an experiment.

6.2.4. Pellet the aliquot of cells at maximum speed for 15 sec to pellet. Remove supernatant.

6.2.5. For genomic DNA analysis, resuspend the cell pellet in 50 uL of lysis buffer per 1 x 10^5 cells. Incubate at 98 °C for 10 min to lyse and inactivate endogenous DNase.

6.2.6. Place tubes on ice for 5 min to cool.

6.2.7. Add 2 uL of Proteinase K (20 mg/mL). Incubate 37 °C for at least 1 h. (Can be left overnight for convenience).

6.2.8. Incubate at 98 °C for 10 min to inactivate the Proteinase K.

6.2.9. Pellet debris for 2 min at maximum speed. Remove the supernatant which contains DNA.

6.3. Generate a PCR amplicon spanning the guide sites.

6.3.1. Design PCR primers that lie approximately 200 bp upstream of the most 5’ and 200 bp

downstream of the most 3' guide sites. Typical amplicon size is 400-600bp. Design a nested sequencing primer at least 125bp up or downstream from the nearest guide site and non-overlapping with PCR primer (Figure 2A).

6.3.2. Using the gDNA from step 6.2 as template, perform PCR to generate the amplicon spanning the guide sites.

6.4. Prepare PCR product for Sanger sequencing. We use the Exonuclease I/ Shrimp Alkaline Phosphatase (ExoI/SAP) method below and estimate DNA concentration relative to a known amount of DNA ladder. Commercial DNA purification kits also work well.

6.4.1. To 15 uL of PCR product, add 1 uL 10x ExoI reaction buffer, 7.5 uL MilliQ H_2O, 0.5 uL ExoI, and 1 uL SAP to degrade primers and dNTPs that interfere with sequencing.

6.4.2. Incubate at 37 °C for 30 min. Then incubate at 85 °C for 20 min.

6.5. Perform Sanger sequencing on the PCR product. TIDE requires high quality sequence chromatograms to accurately estimate editing efficiency. The sequence chromatogram for a non-edited locus should provide clear peaks with very little background (Figure 2C).

6.6. To estimate editing efficiency, analyze the Sanger sequencing chromatogram files for both the edited DNA and unedited control DNA using TIDE (Figure 2c, Table 1).

RESULTS

Generating sgRNAs takes 1-2 days and requires 2-3 h of hands-on time. The IVT template is a single PCR product that is 127 bp (Figure 1B). To reliably obtain high yields of IVT template, we usually purify two 50 uL PCR reactions, as described in step 2.2. The full length IVT product is a 98 nucleotide single-stranded RNA, which migrates similarly to 60-90 bp of double-stranded DNA (Figure 1C). Note that even with urea in the RNA gel loading buffer, it is normal to observe multiple bands and minor sgRNA-specific shifts on a non-denaturing agarose gel, likely due to

RNA secondary structure.

If successful editing has occurred, cells should be > 90% viable. The resulting pool of mutant cells should have a diverse set of random small insertions/deletions (indels) downstream of the guide site, created through non-homologous end joining of the double-stranded break. The edited loci of the pool of cells are simultaneously PCR amplified, resulting in a pool of amplicons with differing sequences. Upon sequencing the pool of amplicons, multiple nucleotides are observed at the position of, and each position downstream of, the Cas9 cleavage site (Figure 2C-E).

Editing efficiency varies both by gene and by guide, with editing efficiency estimated by TIDE ranging from 84% to 95% for 3 representative genes (Figure 2C-E). For some loci, we find that co-transfecting two active guides improves overall indel formation (data not shown). In addition, editing efficiency may also be modestly increased with a commercial electroporation enhancer. We observed that inclusion of the enhancer resulted in a modest increase in relative editing efficiency by about 20-30% with two different sgRNAs (Figure 3).

DISCUSSION

Cas9-sgRNA RNP delivery facilitates the efficient disruption of gene function in primary BMDMs. This enables rapid generation of mutants to screen for phenotypes of interest. It has the advantage of speed because it eliminates the need to construct plasmids or to prepare high-titer viral vectors typically needed to transduce BMDMs. The entire process of sgRNA preparation, RNP delivery, and recovery of mutants carrying indels can be completed in under a week. In addition, this protocol can be used with BMDMs derived from mice already carrying mutations, enabling the creation of double-mutant BMDMs. We have applied the method presented here in BMDMs from the widely available C57BL/6 mouse strain but expect it to function similarly across

different strains.

This method does have some limitations. The cost of the electroporation device and the consumables are significant. Costs can be somewhat reduced by re-using tips between unique sgRNAs targeting the same gene, as described in step 5.7.1. and by using standard PBS + Ca/Mg in lieu of proprietary buffers. This protocol also produces a limited number of mutant cells, with our standard conditions generating 4 x 10^5 cells per reaction. A modest scale-up may be possible, as we see no loss in efficiency with up to 2.4 x 10^6 cells per 10 uL reaction, while maintaining the same amount of RNP (data not shown), and larger 100 uL reaction electroporation formats are available.

Editing success varies by both guide and target gene. For a typical gene, we initially screen 4-5 sgRNAs to identify one that is active. Co-transfecting 2 active sgRNAs with distinct guide sequences results in better editing at some loci. However, note that when multiple guides are co-transfected TIDE may lose precision and alternative methods for assessing editing, such as immunoblot, may be needed. In addition, while some loci undergo robust editing regardless of the Cas9 source, we find improved editing at some, but not all, loci with commercial modified "second generation" Cas9 (data not shown).

In summary, Cas9-sgRNA RNP delivery is a fast and effective way to disrupt genes in primary BMDMs for small- to medium-scale experiments, allowing users to rapidly identify phenotypes in a cell type that closely recapitulates the complex biology of macrophages.

FIGURES AND TABLES

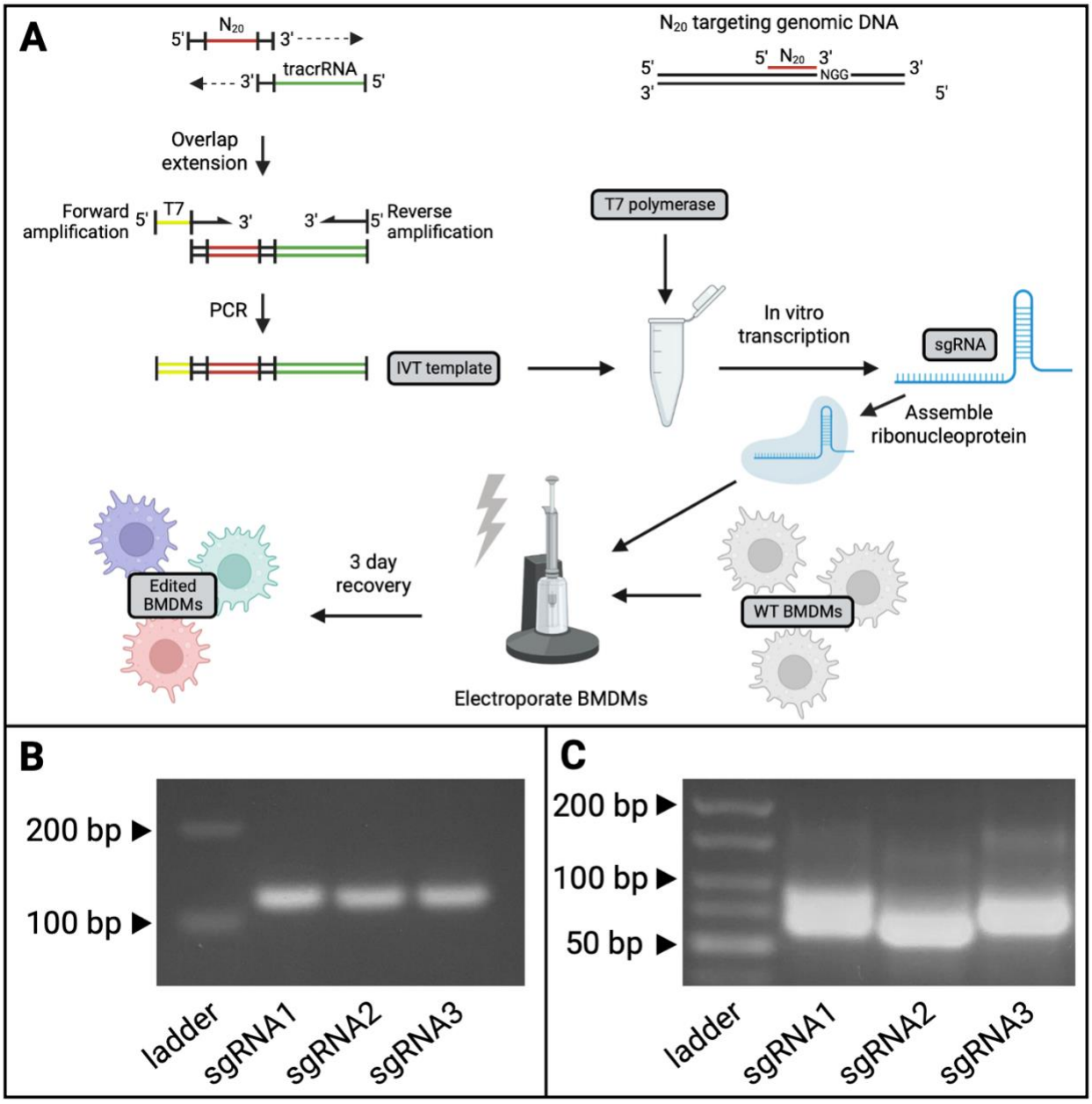

Figure 1. Overview of sgRNA-Cas9 editing process. **(A)** Schematic depicting the electroporation process. **(B)** Analysis of the PCR product IVT templates for Src sgRNAs 1, 2, and 3, resolved on a 2% agarose TAE gel. **(C)** IVT products for Src sgRNAs 1, 2, and 3, resolved on a 2% agarose 0.5x TB (Tris-Borate with exclusion of EDTA) gel. A dsDNA size ladder is shown. Created with BioRender.com.

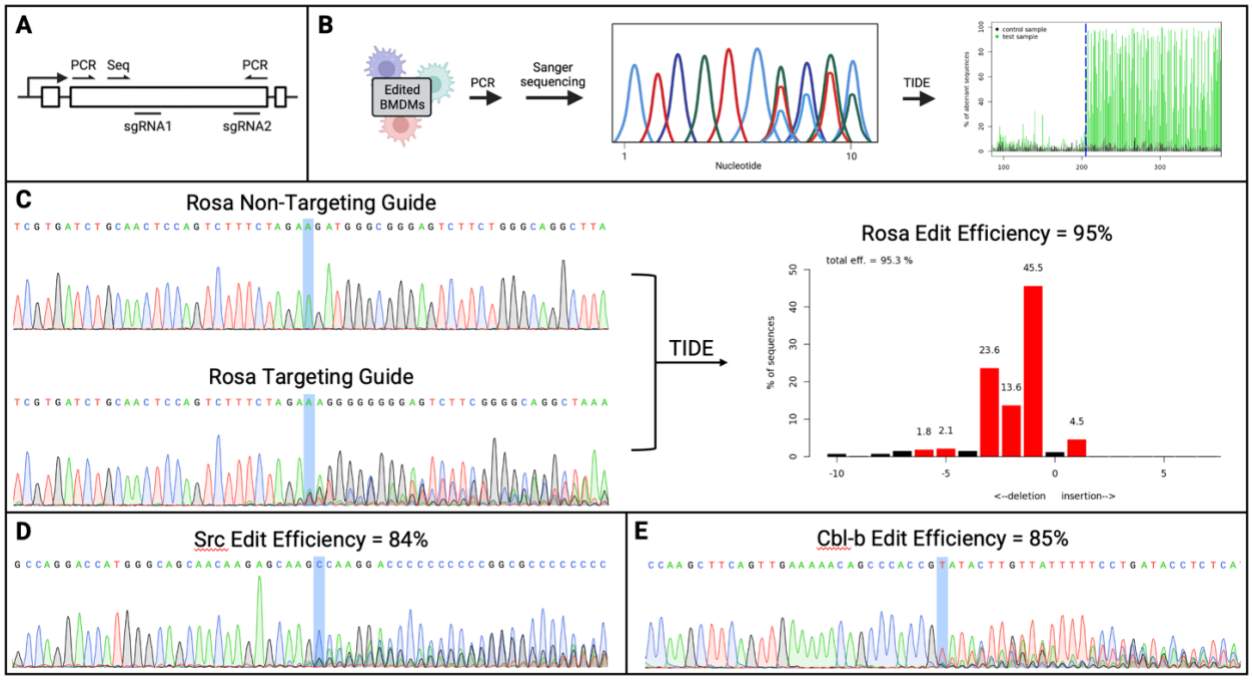

Figure 2. High editing efficiency achieved for multiple targeted genes. (A) Schematic of a targeted gene with locations of PCR and sequencing primers used to assess editing efficiency. Guide locations annotated. **(B)** Schematic of workflow for assessing editing efficiency. Using the genomic DNA of edited BMDMs as template, the targeted locus is amplified by PCR, the amplicon is sequenced, and TIDE is used to assess the editing efficiency. **(C)** Representative Sanger sequencing chromatogram of ROSA26 locus from BMDMs electroporated with a control non-targeting sgRNA-Cas9 RNP (top) and ROSA26-specific sgRNA-Cas9 RNP (bottom). The Cas9 cleavage site is highlighted with a vertical shaded bar on each chromatogram. Both chromatograms were used to generate the TIDE output (right) displaying the estimated edit efficiency and percentage of sequences harboring the indicated number of indels. **(D-E)** Sanger sequencing chromatograms of the **(D)** Src and **(E)** Cbl-b loci in edited BMDMs. The Cas9 cleavage site is highlighted with a vertical shaded bar on each chromatogram. Created with BioRender.com.

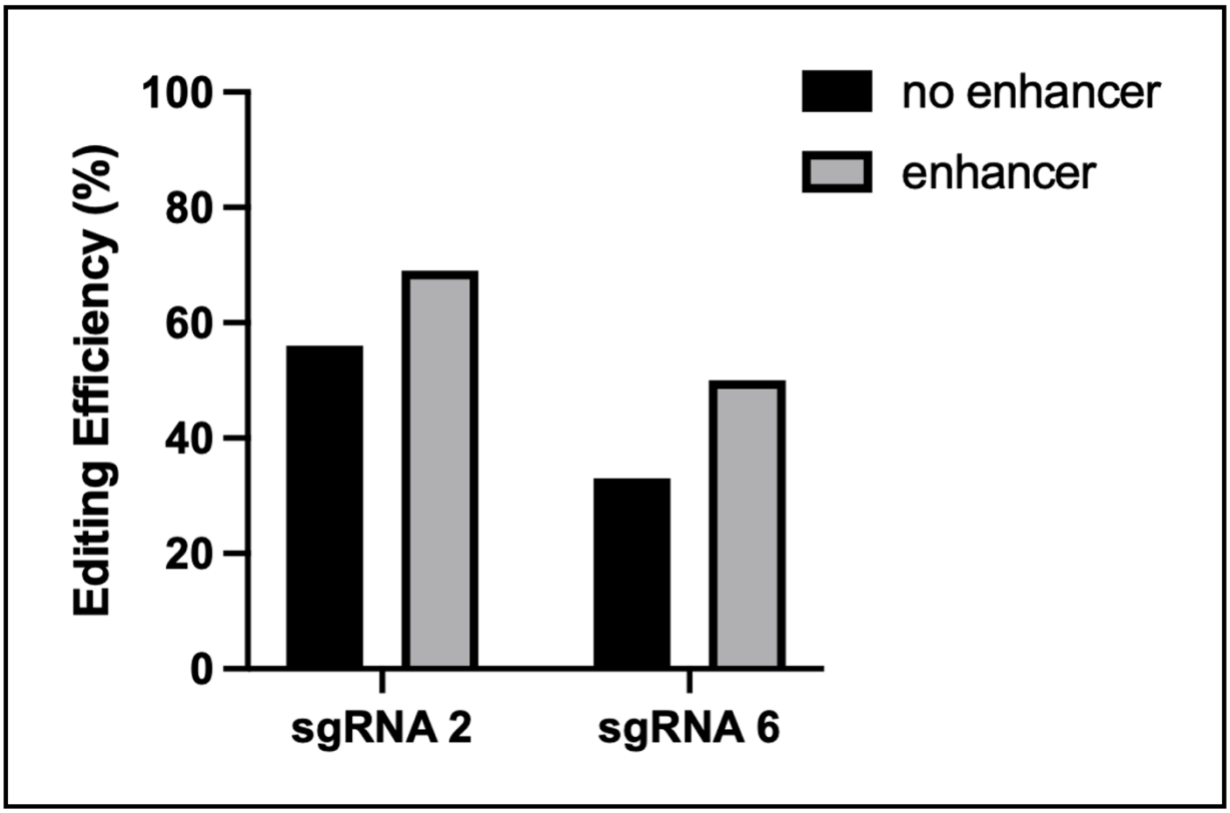

Figure 3. Commercial electroporation enhancer modestly increases editing efficiency. BMDMs were electroporated according to the protocol described here. For the BMDMs treated with the IDT enhancer, 1 uL of the enhancer was added to assembled RNPs to a final concentration of 4 uM prior to electroporation. Editing efficiency of the indicated sgRNA was evaluated as above.

Tool Name	URL
The Broad Institute - CRISPick	https://portals.broadinstitute.org/gppx/crispick/public
Synthego - CRISPR design tool	https://www.synthego.com/products/bioinformatics/crispr-design-tool
Tracking of Indels by Decomposition (TIDE)	http://shinyapps.datacurators.nl/tide/

Table 1. URLs of online tools.

Primer Name	Sequence
Universal Forward Amplification Primer	ggatcctaatacgactcactatag
Universal Reverse Amplification Primer	aaaaaagcaccgactcgg
T7 Reverse Long Universal Primer	aaaaaagcaccgactcggtgccacttttcaagttgataacggactagccttattttaacttgctatttctagctctaaaac
Gene-Specific Primer	ggatcctaatacgactcactatag[N_{20}]gttttagagctagaa
Gene-Specific Primer - Scramble	ggatcctaatacgactcactatagGCACTACCAGAGCTAACTCAgttttagagctagaa
Gene-Specific Primer - Rosa	ggatcctaatacgactcactatagCTCCAGTCTTTCTAGAAGATgttttagagctagaa
Gene-Specific Primer - Src Guide 5	ggatcctaatacgactcactatagCAGCAACAAGAGCAAGCCCAgttttagagctagaa
Gene-Specific Primer - Src Guide 6	ggatcctaatacgactcactatagAGCCCAAGGACGCCAGCCAGgttttagagctagaa
Gene-Specific Primer - Cbl-b Guide 3	ggatcctaatacgactcactatagAAAATATCAAGTATATACGGgttttagagctagaa
Gene-Specific Primer - Cbl-b Guide 4	ggatcctaatacgactcactatagGGTAAAATATCAAGTATATAgttttagagctagaa

Table 2. Primers and oligos used in PCR to generate template for IVT of the sgRNA. Guide sequences for Gene-Specific Primers are capitalized.

BMDM Growth media. Store at 4 C.	
DMEM	
Fetal bovine serum	10%
MCSF supernatant from 3T3-MCSF Cells	10%
L-glutamine	0.2 M
Sodium pyruvate	11 mg/mL
Lysis Buffer. Store at 4 C.	
Tris	20 mM
$MgCl_2$	5 mM
Triton-X 100	0.5%
2-mercaptoethanol (*add immediately prior to use*)	1%

Table 3. Compositions of BMDM growth media and lysis buffer.

Description	Vendor	Catalog #
Herculase DNA polymerase & buffer	Agilent	600677
Ampure XP Reagent Beads	Beckman Coulter	A63880
Ribolock	Thermo Fisher	EO0384
NEBuffer r2.1	NEB	B6002S
Calf intestinal alkaline phosphatase	NEB	M0525S
DNase	NEB	M0303S
RNeasy Mini Kit	Qiagen	74104
RNA loading dye	NEB	B0363S
S. pyogenes Cas9-NLS	IDT	1081059
S. pyogenes Cas9-NLS	University of California Macro Lab; Available to non-UC investigators.	
LoBind conical tubes 15 mL	Eppendorf	0030122216
LoBind Eppendorf tubes 2 mL	Eppendorf	022431102
HiScribe T7 High Yield RNA Synthesis Kit	NEB	E2040S
RNeasy Mini kit	Qiagen	74104
Alt-R Cas9 Electroporation Enhancer	IDT	1075915
Neon Transfection System	Thermo Fisher	MPK5000, MPP100, MPS100
Neon Transfection System 10 uL Kit	Thermo Fisher	MPK1025 or MPK1096
DPBS +Ca/Mg	Thermo Fisher	14040-133
DPBS -Ca/Mg	Thermo Fisher	14190-144
PBS + 1mM EDTA	Lonza	BE02017F
Proteinase K	Thermo Fisher	EO0491
ExoI	NEB	M0293S
SAP	NEB	M0371S
rCutSmart Buffer for ExoI	NEB	B6004S

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