ISSN 2583-9365

Original article

DOI: 10.48165/jlas.2026.9.2.2

Journal of Laboratory Animal Science

Year 2026, Volume-9, Issue-2 (Jul-Dec)

www.acspublisher.com

Development of cloning-free, PCR-based sgRNA synthesis using CRISPR/Cas9 for rapid Generation of Mice Models

Mahesh Sahare*, Vaishak Nair, Utkarsh Nikhade, Gamyashree K, Darshan L, Shilpa Kumari BA, Reena V, Mahima A V, Mohan GH

Mouse Genome Engineering Facility & Animal Care and Resource Center, the Institute for Stem Cell Science and Regenerative Medicine (in-Stem), and National Centre for Biological Sciences (NCBS), Bangalore-560065, INDIA.

ABSTRACT

The mouse models are vital for studying human biology, diseases, and therapeutic interventions. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology has revolutionized the creation of genetically modified animal models by enabling precise genome modifications across various species. The design and synthesis of guide RNAs (gRNAs) are essential steps in this system, but traditional methods require cloning crRNA and tracrRNA sequences into Cas expression vectors, a slow, labor-intensive process that often creates a bottleneck for large-scale applications. To address these challenges, we developed a rapid, PCR-based, cloning-free method for the synthesis of dsDNA transcription template. Our method creates in vitro transcription (IVT) templates with the T7 promoter, guide RNA, and tracrRNA scaffold using an overhang-extension PCR protocol. Single-stranded oligonucleotides are used to construct the tracrRNA sequence (80 bp), and overhang primers add the 5'- T7 promoter-crRNA-3' sequence to the double-stranded ~80 bp tracrRNA scaffold. This strategy eliminates the need for cloning, reducing gRNA synthesis time from weeks to about 6 hours. The activity of the synthesized gRNAs was confirmed through in vitro cleavage assays and the successful creation of mouse models. This optimized process offers a scalable, affordable way to accelerate CRISPR-based genome modification. Our PCR-based approach makes gRNA synthesis more accessible by drastically reducing time and labor requirements, offering considerable advantages across genetics, developmental biology, disease modeling, and transgenic core facilities.

Keywords CRISPR/Cas9, Single guide RNA (sgRNA), PCR-based synthesis, Genome editing, In-vitro Transcription (IVT)

Received 8-9-2025 Revised 30-9-2025 Accepted 2-10-25

INTRODUCTION

Mice models are crucial for understanding diseases and their molecular mechanisms, as well as for testing innovative therapeutic approaches under development. (Perlman et al., 2016, McGonigle et al., 2014). The CRISPR/Cas9 method is a revolutionary advancement in genome editing that provides exceptional precision and efficacy, par-

*Corresponding author.
Mahesh Sahare.
Mouse Genome Engineering Facility & Animal Care and Resource Center, the Institute for Stem Cell Science and Regenerative Medicine (inStem), and National Center for Biological Sciences (NCBS), Bangalore-560065, INDIA
Email- maheshsahare@instem.res.in

ticularly when generating mouse models (Gurumurthy et al., 2016). Compared to traditional embryonic stem cell-based mouse model generation, it has simplified the manufacturing of animal models, saving money and time. The CRISPR/Cas9 endonucleases are the most user-friendly gene-editing technology, which uses a simplified synthetic single-guide RNA (sgRNA) and the Cas9 protein to cleave the target DNA (Jinek M et al., 2012).

A single guide RNA (sgRNA), typically a 20-nucleotide sequence that matches the target DNA, directs the Cas9 endonuclease to the specific genomic location. There, ribonucleoprotein (RNP) complex recognizes a protospacer adjacent motif (PAM), typically the 5'-NGG-3' sequence, and causes a double-stranded DNA break (Cong et al., 2013). Generation of mouse models can be simplified by microinjecting a single guide RNA (sgRNA) and Cas9 protein into zygotes to create knockout mouse models. Additionally, knock-in mouse models (point mutations or reporter models) can be generated by providing a repair template or donor DNA, which is used to repair the double-stranded break induced by the sgRNA-Cas9 RNP complex via the homology-directed repair (HDR) mechanism (Quadros et al., 2017; Burgio et al., 2018).

Traditionally, sgRNA can be synthesized in laboratories through *in vitro* transcription utilizing a specific dsDNA template (derived from synthetic DNA oligonucleotides) or as a DNA expression vector, transcribed under the regulation of an RNA polymerase III promoter, which is either costly, time-consuming, laborious, and necessitates multiple steps to generate gRNAs (Mali et al., 2013). To address this limitation, recent studies in yeast (Hassan et al., 2021) and Xenopus tropicalis (Zheng et al., 2019) have shown that a PCR-based template synthesis approach can be used to prepare single-guide RNAs (sgRNAs).

Here, we demonstrate a PCR-based approach for assembling dsDNA templates for sgRNA synthesis, thereby avoiding the time-consuming and labor-intensive cloning process. In-house sgRNA synthesis protocols can accelerate the generation of mouse models in transgenic core facilities with minimal resources.

MATERIAL AND METHOD

Designing CRISPR RNA guides using CRISPOR web tool

Candidate guide RNA (gRNA) sequences were designed using CRISPOR, a guide RNA designing web tool (http://crispor.tefor.net/). This tool helps identify and evaluate potential target sequences within a user-defined sequence. We retrieved genomic sequences corresponding to the target gene from Ensembl (Harrison et al., 2004), and the sequences were annotated using "SnapGene software (www.snapgene.com)". Single guide RNA (sgRNA) sequence selection is carried out using CRISPOR online tool (https://crispor.gi.ucsc.edu/). The sgRNA targeting three candidate genes (*Serpina11, Wasp*, and *Tmem171*) is listed in Table 1.

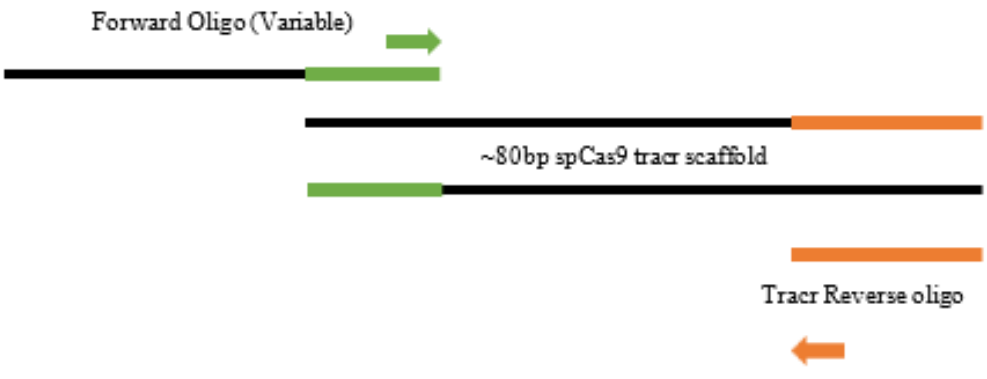
Oligonucleotide Design for In-Vitro Transcription Template

Single-guide RNA (sgRNA) synthesis requires a dsDNA template containing the guide RNA sequence and a Tracr sequence. We designed oligos for this template using SnapGene software version 6.2.

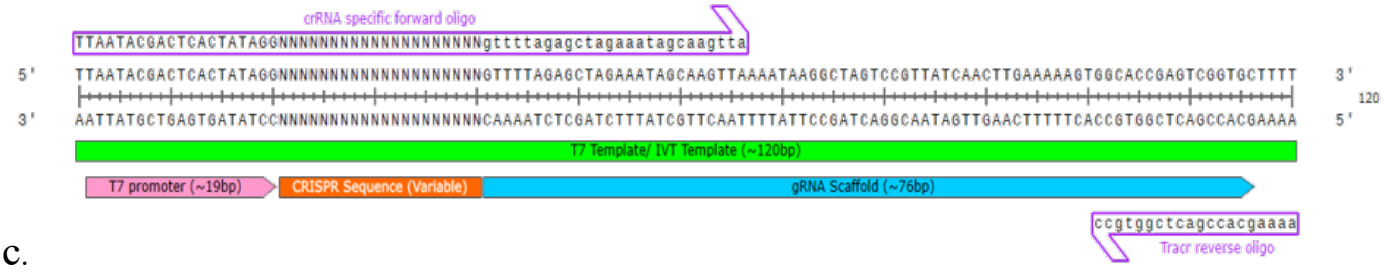
Two complementary oligonucleotides (forward and reverse) were synthesized to self-hybridize into the 80bp tracr scaffold. The forward oligo (spCas9 tracr_F, 80 nucleotides) was designed to include the spCas9 tracr sequence, while the reverse oligo (spCas9 tracr_R) was a reverse complementary sequence (**Table 2**). The tracr scaffold sequence was taken from plasmid pX330 spCas9-mSA (Plasmid #113096) (Gu et al., 2018).

We designed a forward primer that has 5'-T7 promoter-crRNA-tracr overlap-3' sequence. This forward primer has three parts: The T7 promoter sequence 5'-TTAATACGACTCACTATA-3', 20 nucleotides of the target-specific crRNA sequence, and an overlap region (~26 nucleotides) that matches the tracr scaffold. A truncated reverse primer (spCas9 tracr_reverse) was also synthesized to match part of the tracr scaffold (**Fig 1**).

a.



b.



c.

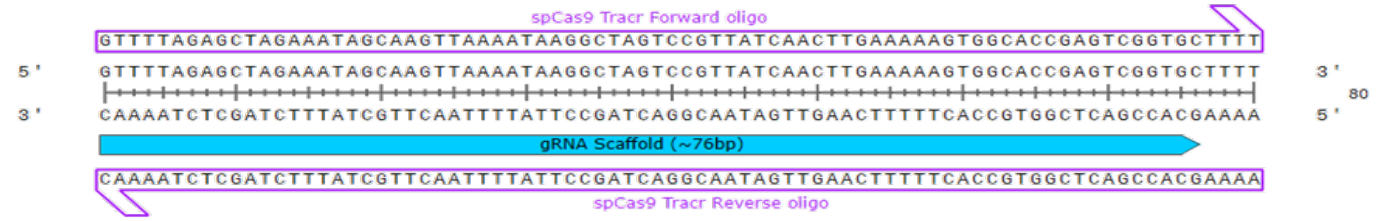


Fig 1: Primer design for cloning free T7 Template/ IVT template assembly. a) Primer schematics. The highlighted green region in the forward oligo is a tracr overlap (~26 bp) complementary to the ~80 bp Tracr scaffold. Forward oligo (~66nts) constitutes three parts i.e. 5'-T7 promoter (~19bp) - variable crRNA sequence (20bp) - tracr overlap (~26bp)-3'. **b)** Map of spCas9 tracr scaffold (~80bp). This scaffold can be ordered directly as DNA oligos, as shown in the map, and duplexed in-house for use as a T7 template. **c)** Map of a complete T7 template, 5'-T7 promoter (~19bp, annotated in pink) - variable crRNA sequence (20bp, annotated in orange) - tracr (~80bp, annotated in blue)-3'. The forward oligo needs to be changed according to the variable crRNA sequence. The remaining primers remain common throughout the reaction. The PCR is performed with crRNA-specific forward and tracr reverse oligos, with tracr scaffold (ordered as forward and reverse oligos, duplexed in-house as depicted in the b image) as a template. The end result of this PCR is the complete ~120bp double-stranded T7 template that can be used for In-Vitro transcription (IVT) for single guide RNA (sgRNA) synthesis.

Table 1. gRNA sequences used for gene targeting

Gene Name	Ensemble Gene ID	Sequence (5'-3')
Serpina11	ENSMUSG00000063232	5' Guide: GCTGAGGCCACCACGTGTCT 3' Guide: GTCCCCTGAGCCATGCTCGC
Wasp	ENSMUSG00000031165	5' Guide: GCACTTACTAGCCGAAGCCC 3' Guide: ACGATACAGATAGATTATGC
Tmem171	ENSMUSG00000052485	5' Guide: GAGGCGGAACTTCGTGTCAT 3' Guide: ATCATTTAGCAGACGAATTA

Table 2. *Oligonucleotide Design for double-stranded DNA template for sgRNA synthesis.*

Gene Name	Sequence (5'- 3')	Size (bp)
spCas9 tracr_F	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT	80
spCas9 tracr_R (Reverse complementary)	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC	80
Forward oligo (Variable)	TTAATACGACTCACTATAGGNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTA	64-66
spCas9 tracr_reverse	AAAAGCACCGACTCGGTGCC	20

The Tracr scaffold was assembled by mixed in equal amounts (100 µM) of forward and reverse tracr oligonucleotides. The total reaction volume was 10 µL, containing 1.0 µL SpCAS9 tracr_F, 1.0 µL SpCAS9 tracr_R, and 8.00 µL nuclease-free water (NFW). First, the mixture was denatured at 95 °C for 5 minutes. Then, it cooled to 25 °C at a ramp rate of about 0.3 °C per cycle. This created the ~80 bp double-stranded tracrRNA scaffold, which serves as a template for T7 template synthesis.

The complete T7/IVT template was synthesized by polymerase chain reaction (PCR). The reaction was catalysed by 2 units of high-fidelity Phusion polymerase (NEB Cat#

M0530L) in a 50 µL reaction, including dNTPs (0.2 µM), 1x HF reaction buffer. Double-stranded tracrRNA scaffold was added at 2 ng/µL as a template, along with the forward oligo at 0.25 µM. The PCR was performed with an enzyme activation step at 98°C for 1 min 2 s, followed by initial denaturation at 98°C for 5 min. This was followed by 35 cycles of 98°C for 30 s, 61°C for 30 s, and 72°C for 30 s. A final extension was carried out at 72°C for 5 min, and the reaction was held at 4°C indefinitely.

In-vitro transcription for sgRNA synthesis

We used the desired DNA template for in vitro transcription (IVT) with the MEGAshortscript T7 Transcription Kit (Invitrogen, Cat. no. AM1354) according to the manufacturer's guidelines. In a standard 20 µL reaction, we mixed 1–1.5 µg of purified 120bp dsDNA template with 2 µL of 10X MEGAshortscript T7 Reaction Buffer, 2 µL (75 mM) of each rNTP's, and 2 µL of T7 Enzyme Mix. Nuclease-free water was added to reach the final volume of 20 µL. The reaction was incubated at 37°C for 4 hours for RNA synthesis.

To remove any remaining DNA template, we added 3 µL of Turbo DNase I after transcription and incubated for 1 hour at 37 °C. We purified the sgRNAs using the MEGAclear™ Transcription Clean-Up Kit (Cat. no. AM1908). We confirmed purity and concentration with a Nanodrop reading, achieving an A260/A280 ratio of about ~2.0. We also visualized the RNA on a 1–2% agarose gel (Fig 2).

In-vitro cleavage assay

The targeting efficacy of the synthesized sgRNA was assessed using an *In-vitro* Cas9 cleavage assay. For this assay, a ribonucleoprotein (RNP) complex was assembled by mixing the sgRNA with the spCas9 endonuclease enzyme in a reaction cocktail containing 1X NEB Buffer r3.1 and nuclease-free water. The optimized working concentrations were 60 ng/µL for the sgRNA and 50 ng/µL for the Cas9 enzyme. This mixture was incubated at room temperature for ~20 minutes to facilitate the formation of the RNP complex. Once the RNP complex was formed, a target DNA substrate (100 ng) was added to the reaction, bringing the total volume to 10 µL. The reaction was then incubated at 37°C for 1 hour in a thermal cycler to allow cleavage of the target DNA by the RNA-guided Cas9 endonuclease. To terminate the cleavage reaction, RNase (1 mg/mL) and Proteinase K (0.5 mg/mL) were sequentially added to degrade the residual sgRNA and Cas9 protein, respectively. The reaction was analyzed on an agarose gel to observe the profile of post-cleavage fragments (Fig 3).

Detection of sgRNA activity in the developed knockout mice

Female Hybrid B6ND2 (C57BL/6N X DBA2) mice at 3–4 weeks of age were super-ovulated by intraperitoneal injection of 5 *IU* pregnant mare serum gonadotropin (PMSG) from Prospec Bio, followed 48 h later by injection of 5 *IU* human chorionic gonadotropin (HCG) from MSD Animal Health. Mouse zygotes were obtained by in vitro fertilization. One-cell-stage fertilized mouse embryos were injected with CRISPR components, mixed in microinjection buffer containing the final concentrations of Cas9 protein and sgRNA, 50 ng/µl and 25 ng/µl, respectively. The injected embryos were transferred into the oviduct of pseudo-pregnant females to allow further implantation and development. Microinjections and mouse transgenesis experiments were performed as described previously (Harms et. al. 2014). The resulting pups were genotyped to identify founders. After weaning, ear biopsies were collected, lysed, and subjected to DNA isolation, followed by PCR-based screening. The primer sequences used are provided in **Table 3**. The PCR confirmed founder were validated using sequence analysis by targeted base-pair regions amplification to detect the expected deletions, thereby confirming successful genome editing. The purified PCR product is sent for performing Sanger sequencing at the on-campus Next Generation Genomics Facility (NGGF).

Table 3. PCR primer sequences used for the amplification of specific genes and genotyping

Gene Name	Forward (5'-3')	Reverse (5'-3')	Product Size (bp) and Interpretation
Serpina11	CCCAAGAAAGCAGGGATGTA	TTCAATTCTGCGTCTCATGC	KO -ve: 1288 KO +ve: 802
Wasp	CAACCAGCCAGAGAATGGAT	TCCCCTCCTCCACTTTCTTT	KO -ve: 914 KO +ve: 505
Tmem171	ACCCACACCACCAGTCATTT	CCAGTGAGCTCATTCCCTTC	KO -ve: 1571 KO +ve: 483

RESULTS

Single guide RNA (sgRNA) synthesis

The simplified sequence of Tracr scaffold is necessary in creating the functional tracrRNA for the SpCas9 system (Jinek et al., 2012). The overlapping design of primers allowed for easy fusion of T7 promoter, CRISPR RNA and tracrRNA sequences into a full-length 120bp dsDNA template (T7 Template) via. overhang extension PCR. The T7 template was further used to synthesize a full-length sgRNA (100nts) via in vitro transcription. As shown in Fig 2, we ran the sgRNA after transcription against the 120 bp

dsDNA T7 template on an agarose gel for 20 minutes to confirm its successful synthesis.

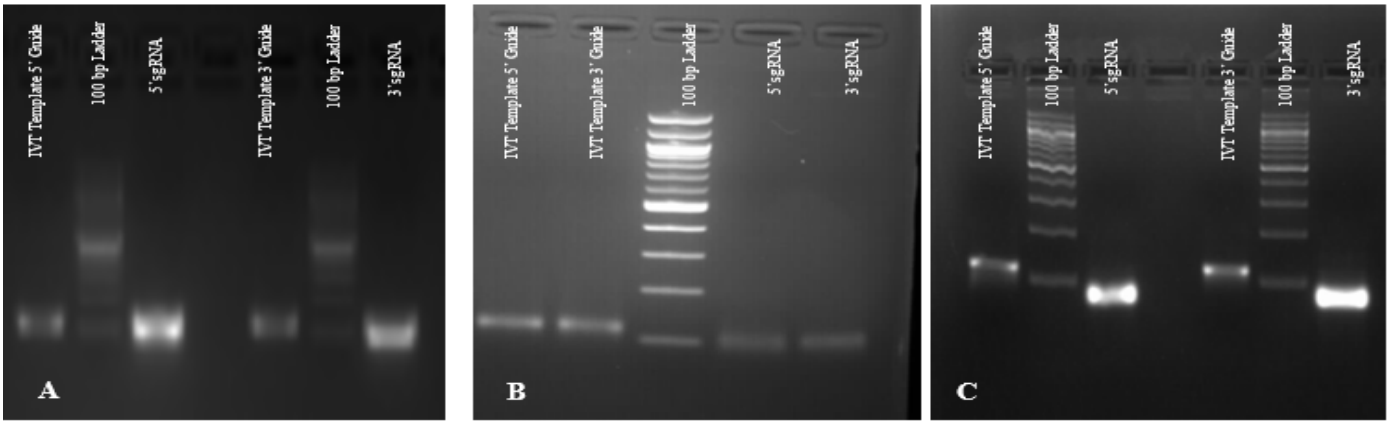


Fig 2. Pre-and post-transcription profiles of *Serpina11* (A), *Wasp* (B), and *Tmem171* (C) have been shown. The pre-transcription PCR product (120bp dsDNA transcription template) exhibited a single sharp band corresponding to the expected molecular weight (120 bp). Following in IVT, the sgRNA displays 100 nucleotides, maintaining its integrity, as evidenced by a well-defined band on agarose gels

In-vitro detection of sgRNA activity using the cleavage assay

To evaluate the quality of the synthesized sgRNAs, we performed In-vitro cleavage assay. In this assay, sgRNAs were synthesized via. IVT was incubated with Cas9 protein for ~20 minutes to facilitate the formation of a functional RNP complex. This was then further incubated with the PCR-amplified target DNA fragment. Further gel electrophoresis of the reaction products revealed clear evidence of target DNA cleavage (**Fig. 3**).

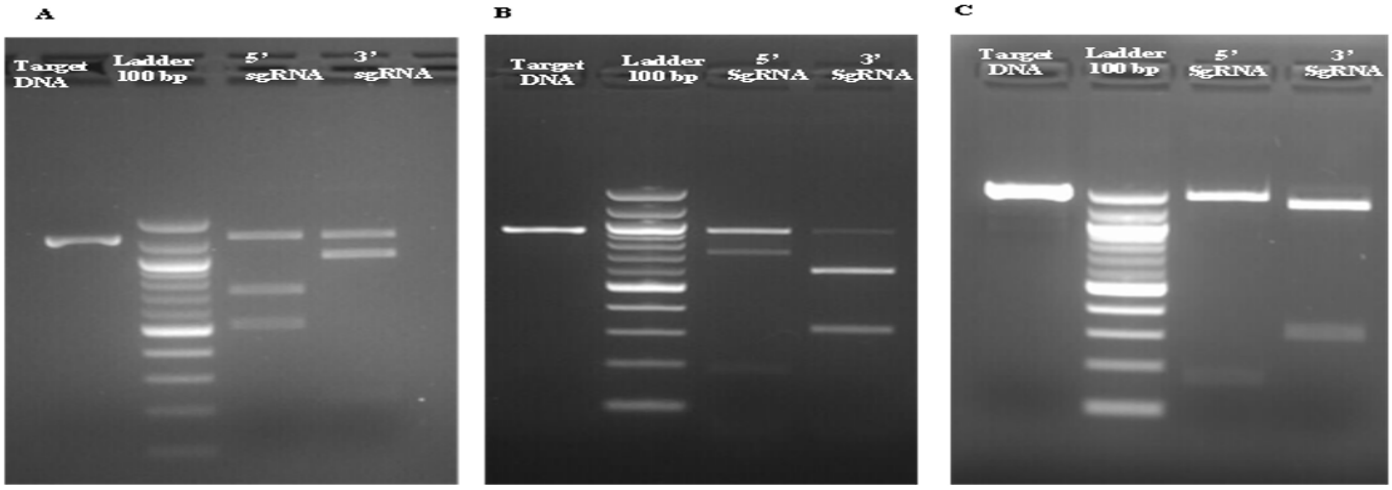


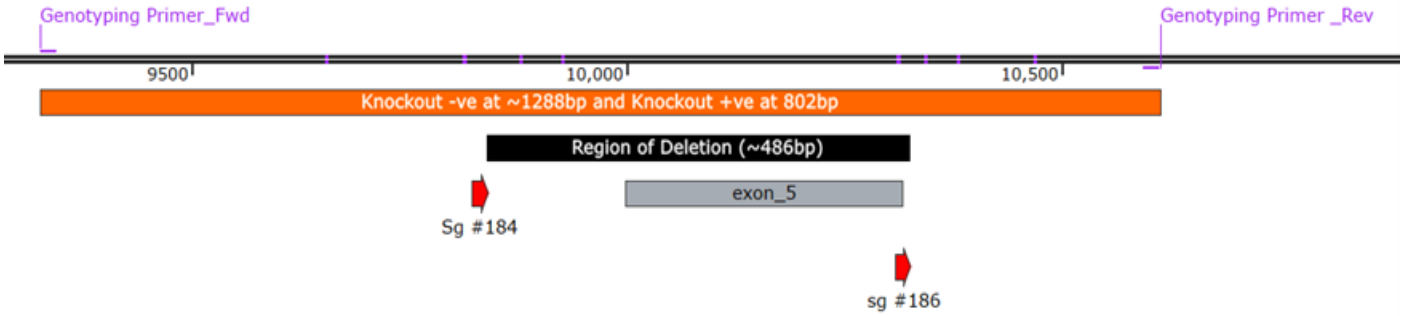
Fig 3. Analysis of In-Vitro cleavage assay. An sgRNA sequence was synthesized and tested against its target genes *Serpina11* (A), *Wasp* (B), and *Tmem171*. The target DNA template was amplified from genomic DNA of C5BL6NJ mice and incubated with the respective sgRNA and Cas9 protein. Primers sequences used for amplification of target DNA are mentioned (Table 3). Target DNA is loaded in lane 1, lane 2 is a 100bp DNA ladder, lane 3 is cleavage for 5' sgRNA, and lane 4 is cleavage for 3' sgRNA for the respective cleavage assay profile. A) Shows the cleavage profile of *Serpina11* target band (1299bp), displaying dropdown bands of 514 bp, 715 bp for the 5' guide and 1000 bp, 299 bp for the 3' guide. B) Shows the cleavage profile of the *Wasp* target band (914bp), displaying dropdown bands of 192 bp, 722 bp for the 5' guide and 600 bp, 314 bp for the 3' guide. c) shows the cleavage profile of *Tmem171* target band (1571bp), displaying dropdown bands of 174 bp, 1397 bp for 5' guide and 1262 bp, 309 bp for 3' guide.

In vivo detection of sgRNA activity through generating mouse models

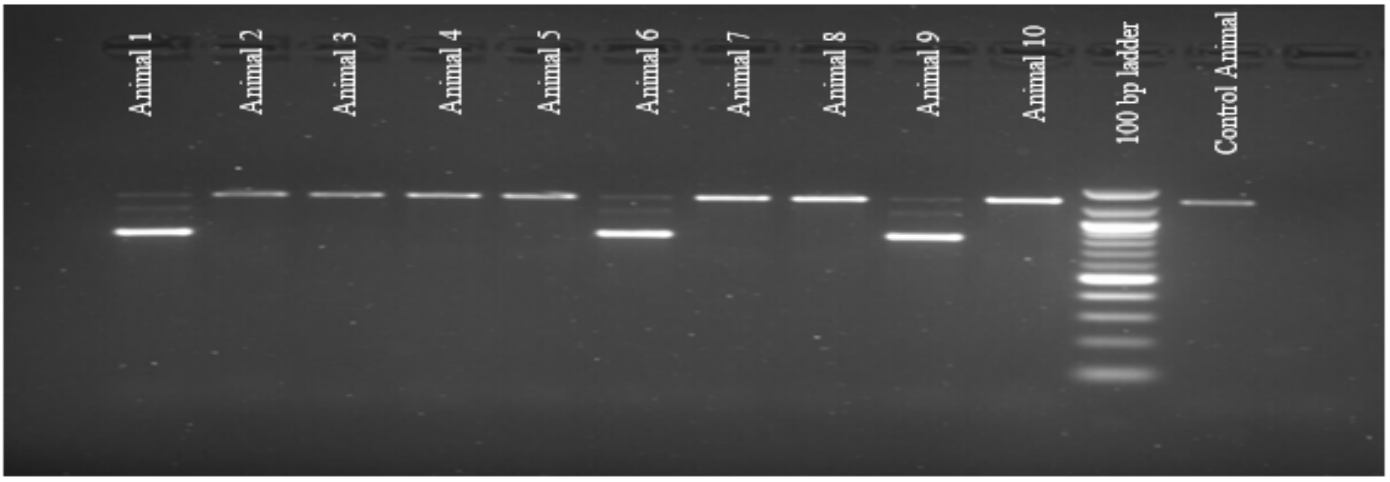
To evaluate the efficiency of PCR-based synthesized sgRNAs in mammalian systems, three candidate loci (*Serpina11, Wasp, Tmem171*) were selected. The targeting strategy is shown in **Fig 4a. Guide RNA and Cas9 protein RNP complexes targeting the** respective target loci were microinjected into fertilized mouse embryos. The founders were detected using PCR and positive animals were further confirmed via. sanger sequencing. The knockout strategy design and representative results of the (*Serpina11) gene are shown in Fig. 4. The results for Wasp* and *Tmem171 are shown in* **supplementary Fig. 1S and 2S**. Further, we have

observed successful editing efficiencies ranging from 3.7% to 15.6% for *Serpina11, Wasp, and Tmem171.* (**Table 4).**

a



b



c

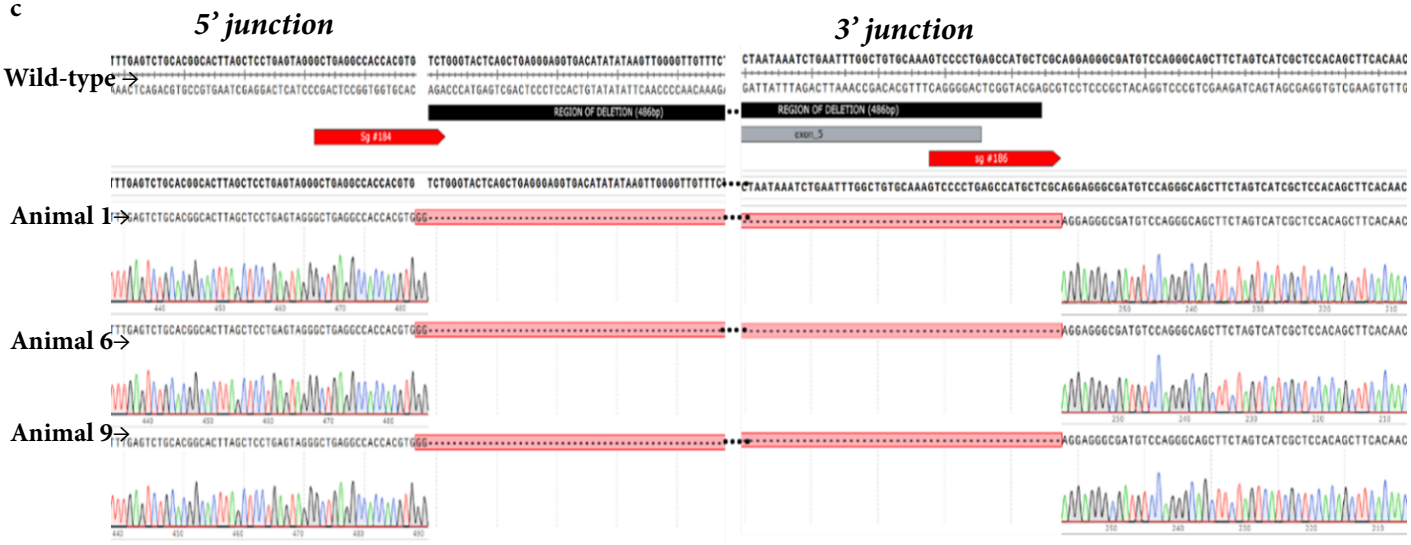


Fig 4. Schematic representation of strategy designing and genotyping of a CRISPR knockout mouse model using CRISPR/Cas9. a) Knock-out strategy and Genotyping design of *Serpina11* gene. Region of deletion is depicted in Black, Red arrows are the 5' and 3' guide RNAs in their respective orientation, and region of amplification is mentioned in orange (1288bp for wild-type/ Knockout negative allele and 802bp for knock-out positive allele. b) Gel image of animal screening. Positive animals in lanes 1, 6, and 9, Negative animals in lanes 2,3,4,5,7,8 and 10. Lane 11 is a 100 bp ladder, and Lane 12 is the negative control/Wild-type animal (C57BL/6NJ). c) Sanger sequencing of 5' junction and 3' Junction

Table 4. Mice model generation using in-house prepared sgRNA

Gene Name	Injected Embryos	Transferred (%)	Pups born (Overall Efficiency (%))	Founders rate (%)
Serpina11	220	166 (75.4)	54 (32.5 %)	2 (3.7 %)
Wasp	238	120 (50.4)	32 (26.6 %)	5 (15.6 %)
Tmem171	225	165 (73.3)	25 (15.5 %)	2 (8 %)

DISCUSSION

The CRISPR/Cas9 system has emerged as a powerful and straightforward method for genome manipulation, facilitating the creation of genetically modified animal models. Recently, cloning-free PCR strategies for synthesizing double-stranded DNA (dsDNA) templates for single-guide RNA (sgRNA) production have provided a rapid, efficient alternative to conventional plasmid-based methods. Herein, a modified overhang extension PCR method was developed to synthesize dsDNA transcription templates for sgRNA production, specifically aimed at generating mouse models. This technique enhances the existing overlap-extension PCR methodology by providing expedited template preparation for in vitro transcription (IVT) (Hu et al., 2019).

The sgRNAs generated using this strategy demonstrated high efficacy in *In-vitro* cleavage assays. To assess the in vivo editing efficiency, we generated mouse models targeting three candidate genes (*Serpina11, Wasp,* and *Tmem171*) using sgRNAs synthesized with this method. The high editing efficiency highlights its potential to replace commercial kits, which are costly and subject to long delivery times that limit experimental flexibility. In contrast, cloning-free PCR-based sgRNA generation enables same-day production of custom guides, reducing reagent use, eliminating bacterial culture steps, and lowering overall costs (Randazzo et al., 2021; Liu et al., 2022). By enabling rapid, in-house sgRNA synthesis and validation, this workflow streamlines genome engineering for applications in developmental biology, functional genomics, and translational research (Aida et al., 2015; Osha, 2023). Beyond its research applications, the simplicity and accessibility of this modified approach make it well-suited for undergraduate education, where integrating CRISPR-Cas9 technology has often been limited by resources and instructor expertise (Pieczynski et al., 2021).

In summary, cloning-free PCR-based workflows offer a rapid, accurate, and cost-efficient strategy for sgRNA synthesis, making them well-suited for scalable CRISPR/Cas9 genome editing applications.

ACKNOWLEDGMENTS

The authors would like to express their heartfelt gratitude to the Animal Care and Resource Center (ACRC) team at NSBS Bangalore. We also sincerely acknowledge Dr. Awadhesh Pandit and the Next Generation Genomics Facility (NGGF) at the Bangalore Life Science Cluster for their extensive assistance with sequencing."

ANIMAL ETHICS APPROVAL

The protocols for animal experiments were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), inStem Bangalore, which is registered with CPCSEA under registration number 109/GO/ReRcBiBt/S/99/CPCSEA. The approval for this study was granted under IAEC approval number INS-IAE 2023/06 (E2).

COMPETING INTEREST

The authors declare that they have no competing interests that could influence the work reported in this paper.

FUNDING

The work is funded by **SERB CRG grant (CRG/2022/007570-G),** the institute core fund, and National Centre for Biological Sciences (NCBS) and the Institute for Stem Cell Science and Regenerative Medicine (inStem), Bangalore-560065, INDIA.

REFERENCES

Aida T, Sato M, et al. (2015). Gene cassette knock-in in mammalian cells and animals using enhanced MMEJ. Nat. Commun. 6:8080.

Burgio G, Bayram Y, Arslan A (2018). Genome editing to create mouse models for biomedical research. NPJ Genom. Med. 3:16.

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. (2013). Multiplex genome engineering using CRISPR/Cas systems. Science 339(6121):819-823.

Gu B, Posfai E, Rossant J (2018). Efficient generation of targeted large insertions by microinjection into two-cell-stage mouse embryos. Nat. Biotechnol. 36(7):632-637.

Gurumurthy CB, Sato M, et al. (2016). Creation of CRISPR-based mouse models: A practical guide for biomedical researchers. Mamm. Genome 27(5-6):215-228.

Harms DW, Quadros RM, et al. (2014). Mouse genome editing using the CRISPR/Cas system. Nat. Protoc. 9(8):1956-1971.

Hassan W, Kazmi SK, Tahir MJ, et al. (2021). Global acceptance and hesitancy of COVID-19 vaccination: A narrative review. Narra J. 1(3):e57.

Hu X, Zhao Y, et al. (2019). Overlap extension PCR cloning: A simple, reliable, and high-throughput strategy for seamless cloning and protein expression. Methods Protoc. 2(2):19.

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337(6096):816-821.

Liu Y, Du Y, Xie W, Zhang F, Forrest D, Liu C (2019). Generation of conditional knockout mice by sequential insertion of two loxP sites in cis using CRISPR/Cas9 and single-stranded DNA oligonucleotides. Methods Mol. Biol. 1874:191-210.

Mali P, Yang L, Esvelt KM, et al. (2013). RNA-guided human genome engineering via Cas9. Science 339(6121):823-826.

McGonigle P, Ruggeri B (2014). Animal models of human disease: Challenges in enabling translation. Biochem. Pharmacol. 87(1):162-171.

Osha C (2023). Advances in mouse genome editing technology. Front. Genome Ed. 5:113.

Perlman RL, et al. (2016). Mouse models of human disease: An evolutionary perspective. Lab Anim. 45(6):237-239.

Pieczynski JN, Kee HL (2021). Designer babies?! A CRISPR-based learning module for undergraduates built around the CCR5 gene. Biochem. Mol. Biol. Educ. 49(1):80-93.

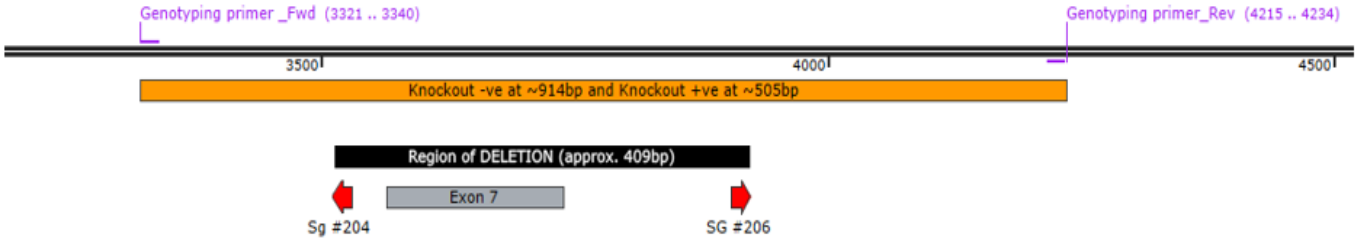
Quadros RM, Miura H, Harms DW, Akatsuka H, Sato T, Aida T, et al. (2017). Easi-CRISPR: A robust method for one-step generation of mice carrying conditional and insertion alleles using long ssDNA donors and CRISPR ribonucleoproteins. Genome Biol. 18:92.

Randazzo SG, Ticau S, Sorrells SF, et al. (2021). A convenient and cost-efficient method for the rapid generation of functional single guide RNAs for CRISPR/Cas9 gene editing. J. Biol. Methods 8(1):e147.

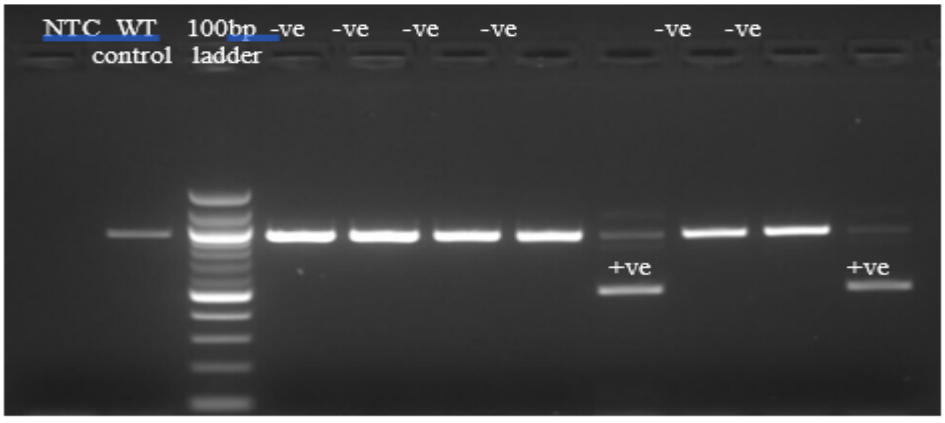
Zheng J, et al. (2019). Efficient genome editing in Xenopus tropicalis using PCR-based guide RNAs. Dev. Biol. 448(1):82-91.

Supplementary figures

A.



B.



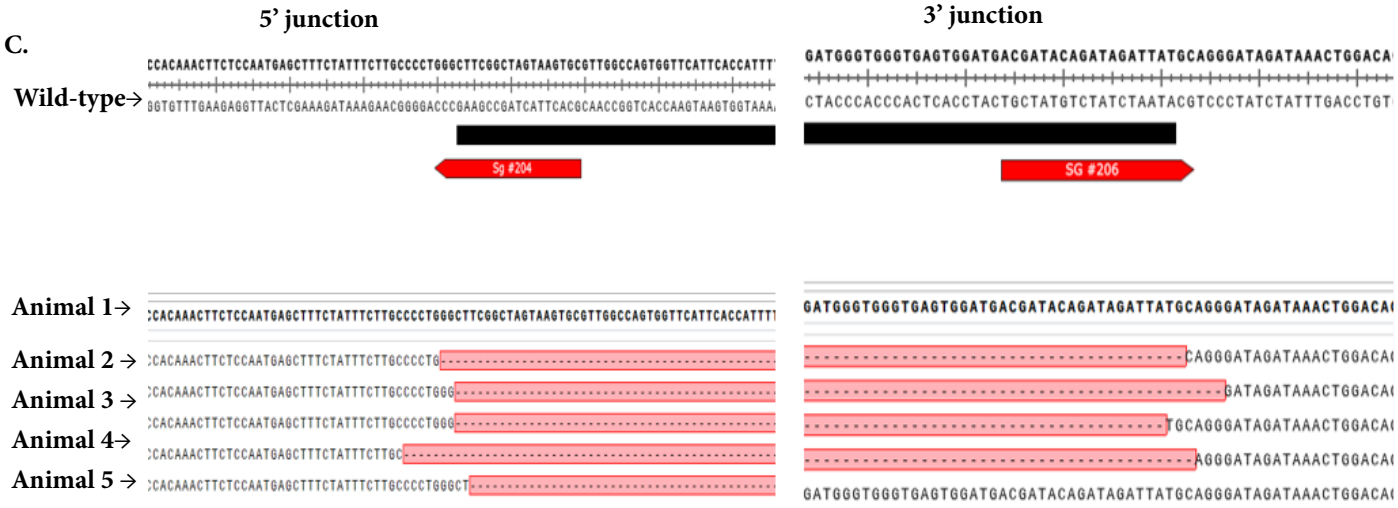
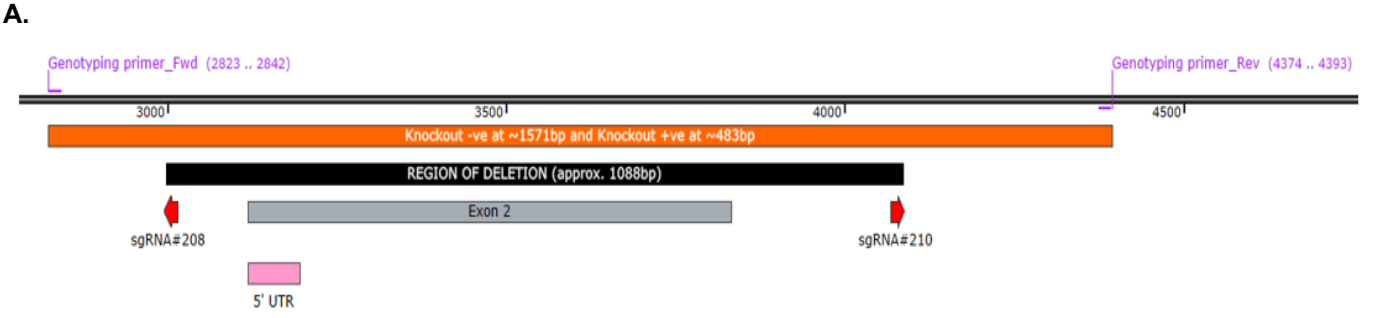
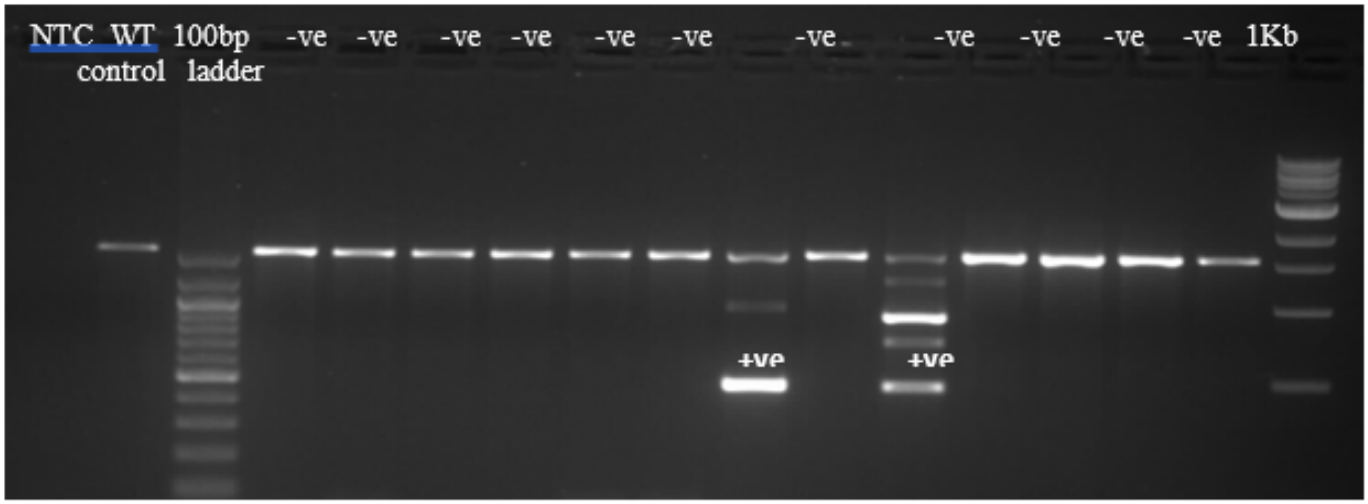


Fig 1S. Schematic representation of strategy designing and genotyping of a CRISPR knockout mouse model using CRISPR/Cas9. a) Knock-out strategy and Genotyping design of *Wasp* gene. Region of deletion is depicted in Black, Red arrows are the 5' and 3' guide RNAs in their respective orientation, and region of amplification is mentioned in orange (914 bp for wild-type/ Knockout negative allele and 409 bp for knock-out positive allele b). Gel image of animal screening. Positive animals in lanes 8 and 11, Negative animals in lanes 4, 5,6,7,9 and 10. Lane 3 is a 100 bp ladder, and Lane 2 is a negative control/Wild-type animal (C57BL/6NJ). c) Sanger sequencing of 5' junction and 3' Junction





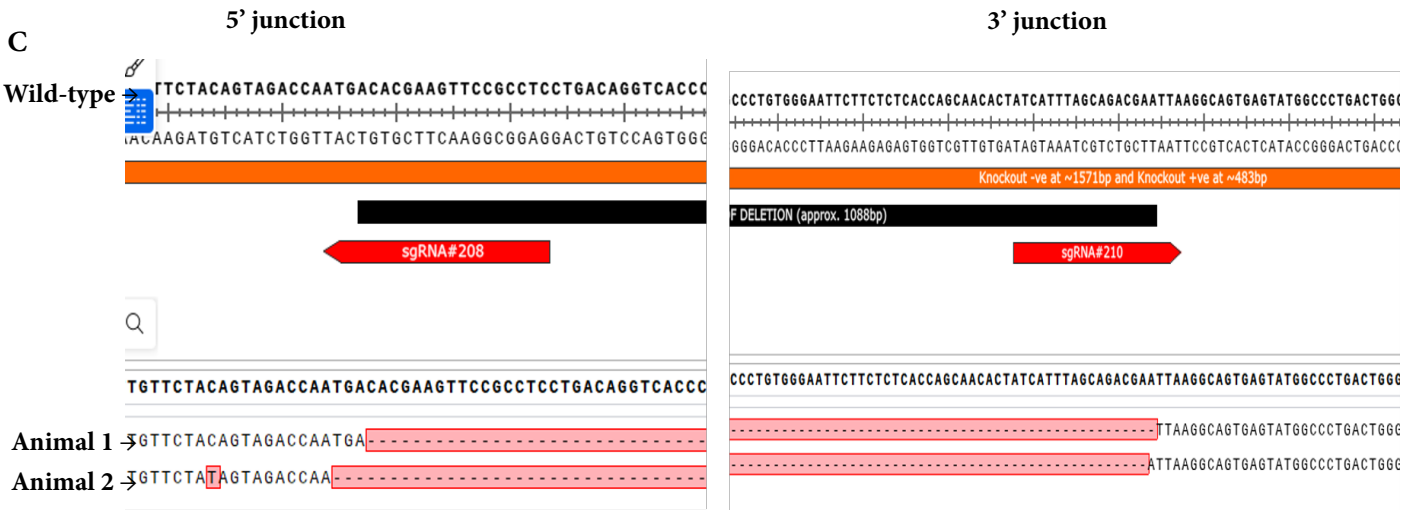


Fig 2S. Schematic representation of strategy designing and genotyping of a CRISPR knockout mouse model using CRISPR/ Cas9. a) Knock-out strategy and Genotyping design of *Tmem171* gene. Region of deletion is depicted in Black, Red arrows are the 5' and 3' guide RNAs in their respective orientation and region of amplification in mentioned in orange (1571 bp for wild-type/ Knockout negative allele and 483 bp for knock-out positive allele b) Gel image of animal screening. Positive animals in lanes 10 and 12, Negative animals in lanes 4, 5, 6, 7, 8, 9,11, 13, 14, 15 and 16. Lane 3 is 100 bp ladder and Lane 2 is Negative control/ Wild-type animal (C5BL6NJ). c) Sanger sequencing of 5' junction and 3' Junction.