Optimization of Embryo Electroporation to Enhance Genetic Modification in Rats Using the CRISPR/Cas9 Technique

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Background

CRISPR/Cas9 is a widely used technique for genetic modification that has enabled researchers to effectively alter the rat genome without embryonic stem cell targeting. Traditionally, reagents for the CRISPR/Cas9 system are delivered to rat embryos via a laborious and technically challenging microinjection technique.

Objective

The purpose of this study was to optimize a simple and high-throughput electroporation method to deliver CRISPR/Cas9 reagents into rat embryos. We compared different reagent media and electroporation at different embryonic developmental stages in order to optimize embryo survival and genome editing efficiency.

Materials and Methods

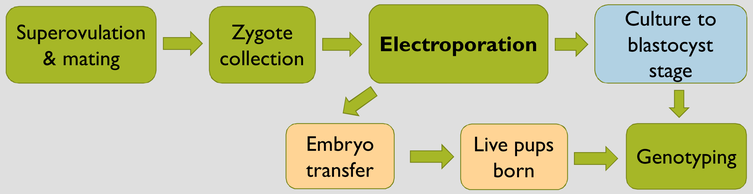

Figure 1. Mature female Sprague Dawley (SD) rats were superovulated and mated with SD stud males. Embryos were then collected at either the one or two-cell stage and subjected to electroporation to deliver genome editing reagents. The embryos were either cultured to the blastocyst stage and analyzed genetically (*in vitro* group) or transferred immediately after electroporation into pseudopregnant female SD rats. Live pups born to those surrogate dams were then analyzed genetically (*in vivo* group).

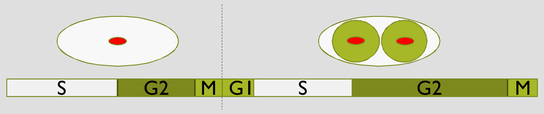

Figure 2. Schematic showing differences in the cell cycle of one-cell and two-cell stage embryo. It has been speculated that the longer G2 phase of two-cell embryos will increase the efficiency of knock-in strategies using CRISPR/Cas9.

Parameter	Poring Pulse	Transfer Pulse
Voltage	40/50 V	5 V
Pulse Length	3.5/0.5 ms	50 ms
Pulse Interval	50 ms	50 ms
Number of Pulses	4	5
Decay rate	10%	40%
Polarity	+	+/- (alternating)

Figure 3. Electroporation instrumentation and parameters.

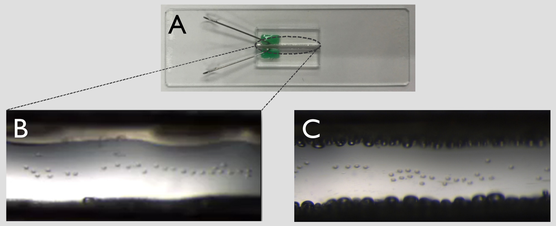

Figure 4. Electroporation slide and electroporation process.
(A) Electroporation slide. (B) Zygotes were placed between two electrodes. (C) Air bubbles were generated along electrodes when applying electrical pulses to zygotes.

Results

Knock-out electroporation

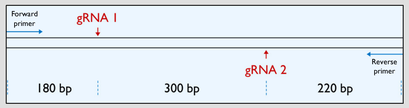

Figure 5. Schematic showing the two gRNA target sites.

Survival rate and genotyping efficiency among electroporation buffers.

Media	Embryos examined	Developed to blastocyst	Blastocysts genotyped	Full deletion
Water	45	19	17	4
TE buffer	45	24	21	5
Opti-MEM	45	27	24	5

Figure 6. Representative genotyping results. PCR product of wild type (non-edited) will be 700bp. PCR product with desired DNA deletion (del) will be 400bp.

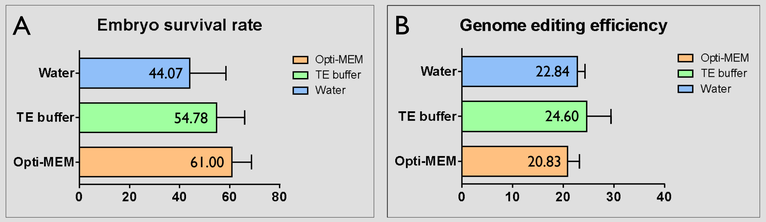

Figure 7. Electroporation survival rate and full deletion efficiency. (A) Embryo survival rate. (B) Genome editing efficiency.

In this project, we designed two CRISPR gRNAs to target the rat genome 300bp apart from each other. Because of the error-prone nature of the repair mechanism involved in CRISPR/Cas9 genome editing, a variety of genetic alterations are possible. However, for this experiment, we only screened for the 300bp deletion and therefore efficiency estimates are only for this particular genetic alteration and under-estimate the actual genome editing efficiency. Live pup genotyping analysis showed results similar to those seen in the *in vitro* group analysis.

Figure 8. Different developmental stages of rat embryos.

Figure 9. Live pups born from electroporated embryos.

Results

Knock-in electroporation

Survival rate and genotyping efficiency between one- and two-cell embryo electroporations.

Developmental stage	Embryos examined	Developed to blastocyst	Blastocysts genotyped	Knock-in
1-cell stage	71	20	20	2
2-cell stage	51	23	17	3

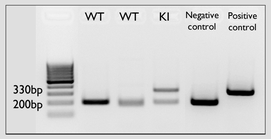

Figure 10. Representative genotyping results. The knock-in (KI) DNA sequence is 130bp long.

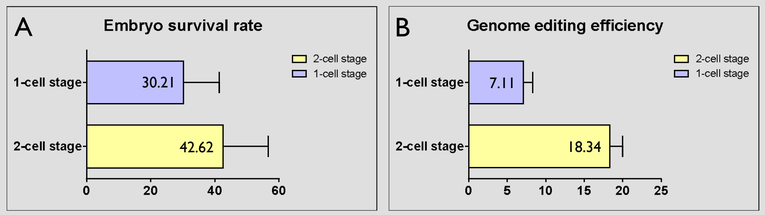

Figure 11. Electroporation survival rate and knock-in efficiency. (A) Embryo survival rate. (B) Genome editing efficiency.

There does not appear to be a difference in survival rate between embryos electroporated at a one-cell stage compared to those electroporated at a two-cell stage. While our hypothesis is that two-cell stage electroporation is more efficient than one-cell stage electroporation in terms of knock-in efficiency, our data represents only 2 experimental replicates and is not sufficient to determine statistical significance. We speculate that during the longer G2 phase seen in two-cell stage rat embryos, the chromatin is more open, therefore more accessible for the CRISPR/Cas9 reagents, leading to a higher knock-in efficiency.

Conclusions and Ongoing Studies

- None of the 3 electroporation mix buffers tested significantly impacted *in vitro* embryo survival or gene knock-out efficiency.
- Knock-in efficiency of two-cell stage embryos may be higher than one-cell stage embryos.
- On-going studies involve performing more embryo transfers in order to evaluate live pup rates and genetic knock-out/knock-in efficiencies with larger animal numbers.

Acknowledgements

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