# Generation of mutated mice combining CRISPR/Cas9 and electroporation

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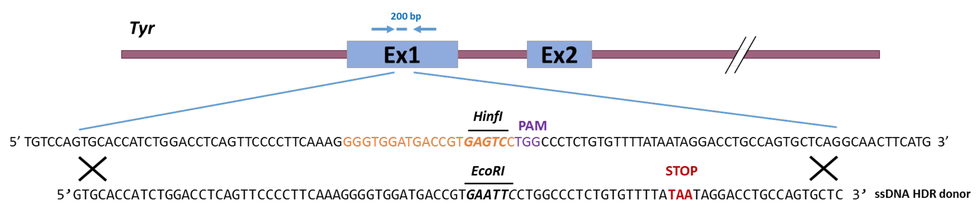
## INTRODUCTION

In order to improve and simplify our production of genetically engineering animals and to reduce the number of animals used (principles of the 3Rs), we assessed the benefice of fertilized eggs electroporation versus microinjection.

## ELECTROPORATION CONDITION VALIDATION

We used the Nepa21 electroporator and applied IDT electroporation conditions to edit the Tyrosinase gene with the approach already published by Chen and coll.(2016).

*Albinism is due to homozygous or compound heterozygous mutation in the tyrosinase gene and leads to an easy and simple coat color screen of the pups.*

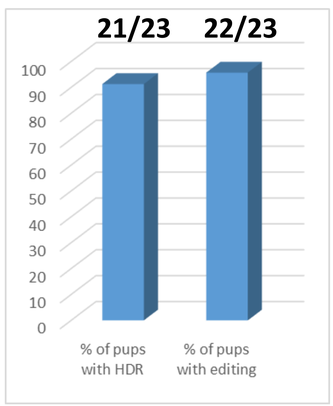



**PCR genotype performed on blastocysts:**

- 40 % of the blastocysts were edited (loss of HinfI site)
- 19 % of the blastocysts committed HDR (presence of EcoRI site)

**Phenotype and genotype of pups after reimplantation:**

Litter of edited pups

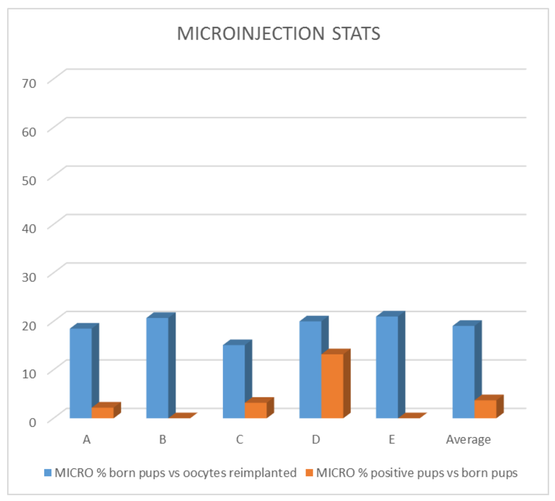


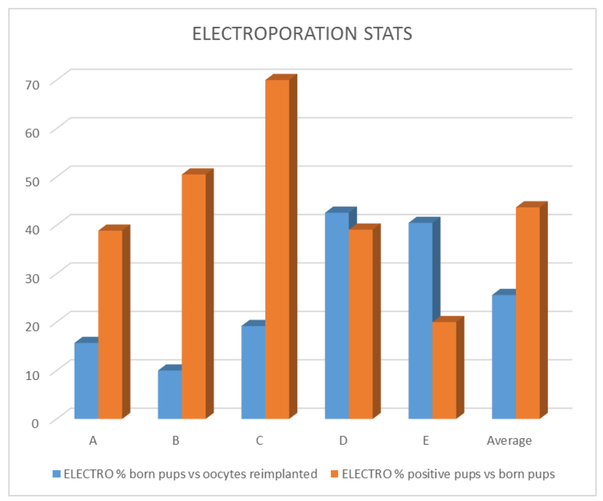

Percentage of edited pups

- Electroporation works really efficiently
- Better viability of oocytes after electroporation than after microinjection
- Our *home made* sgRNA were tested and are as efficient as dual RNA

## ELECTROPORATION VS MICROINJECTION

To compare benefices of electroporation vs microinjection we performed five projects in both conditions.




- **More than 10 X more positive pups** (X 11.8) obtained after electroporation.
- Sixteen times **less donor female** necessary to obtain one positive pup. **Reduction/minimize the number of animals** used per experiments (in accordance with **3Rs**).

## ADDITIONAL DATA

- We applied our condition to more than **20 projects** (including KO, point mutations and small knock-ins) in C57BL/6N eggs with the same success. On average more than **44 % positive pups** were obtained (between 6 and 18 pups born per project).
- The electroporation of **60 oocytes reimplanted in 3 foster females is enough** to obtain positive pups.
- Our electroporation conditions work also well in frozen fertilized oocytes (C57BL/6J) and can be applied in any genetic background.
- First positive results were obtained very recently on rat Sprague Dawley fertilized oocytes.

## CONCLUSION

- Electroporation allows the drastic reduction of the number of donor, foster females and fertilized oocytes.
- We consistently obtained high efficiency mouse genome editing for multiple genes, and we successfully generated mouse embryos with a variety of editing schemes, including indel mutations, point mutations, genomic deletions, and small precise insertions.
- Taken together, CRISPR-electroporation is a simple, economic, 3Rs friendly, high throughput, and highly efficient technique for genome editing *in vivo*, which replaces the traditional microinjection-dependent genome editing technique in mice and rat and could be applied in other mammalian species.

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EXCELLENCE IN MOUSE PHENOGENOMICS