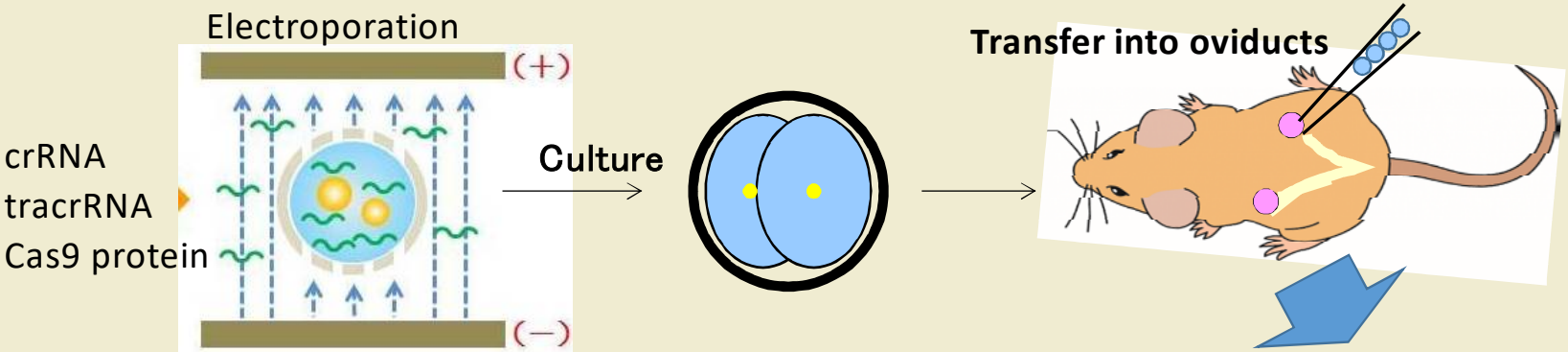
in vitro zygote electroporation

Producing genetically modified mice by electroporation



- No microinjection
- Easier and faster
- Better efficiency

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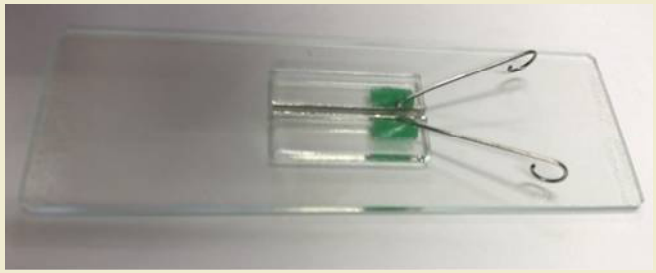
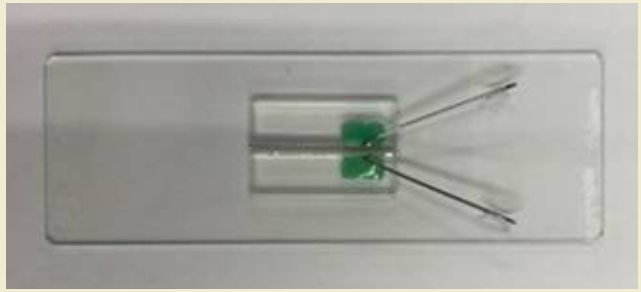
in vitro zygote electroporation

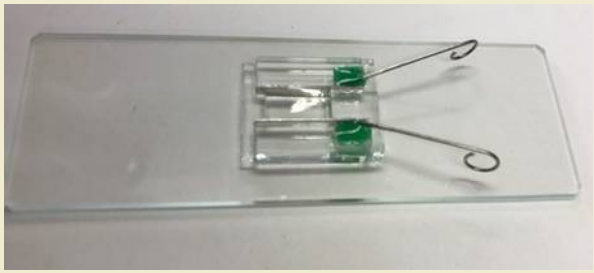
Electrode Types

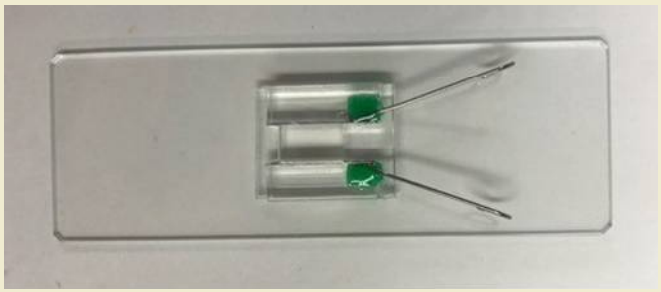




CUY501P1-1.5

- 1mm Gap
- 5 ul reagents
- 5 – 30 (or 50) zygotes
- Can reuse reagent





CUY505P5

- 1mm Gap
- 50 ul reagents
- 20 – 150 zygotes
- Better results

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Producing genetically modified mice by electroporation

Date	July 13, 2017
Name	Dr. Tomoharu Yasuda
Institution	Division of Immunology and Genomic Biology, Kyushu University
Electroporator	**NEPA21 TypeII**
Electrode	5 mm gap electrode (CUY505P5)
Electroporation buffer	1x OptiMEM
Volume in electrode	45 ul (+/-α)
Impedance	480-520 Ω
Zygotes	C57BL/6J, IVF, Stored in fridge for 1 day
Pre-electroporation wash	1x OptiMEM
Post-electroporation wash/culture	KSOMaa

Sample	Cas9 3NLS (IDT 1074181)	guide crRNA:tracrRNA (IDT)	Embryos electroporated	2-cell embryos (%)	Embryos transferred	Offspring (%)	Mutant/Offspring (%)
KO	100 ng/ul	36 ng/ul (1 uM)	88	54 (61%)	54	8 (15%)	8 (100%)
KO	100 ng/ul	36 ng/ul (1 uM)	88	52 (59%)	52	5 (10%)	5 (100%)
			176	**106 (60.2%)**	**106**	**13 (12.3%)**	**13 (100%)**

Data courtesy of Dr. Tomoharu Yasuda,
Division of Immunology and Genomic Biology,
Kyushu University

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in vitro zygote electroporation

Efficient generation of knock-in rats using CRISPR/Cas9
Long KIs over 1500 bases

Rat embryos were collected from the superovulated females and cultured in a modified Krebs-Ringer bicarbonate medium (ARK resource, Kumamoto, Japan).
For EP, 400ng/µl Cas9 mRNA, or 100ng/µl Cas9 protein, 200 ng/µl gRNA, and 40ng/µl lssDNA were electroporated into rat embryos.
Rat embryos that developed to the two-cell stage after the introduction of RNA and lssDNA were transferred into the oviducts of female surrogates anesthetized with isoflurane (DS Pharma Animal Health Co., Ltd., Osaka, Japan).

CONCLUSION : Efficient production of KI rats by electroporation and lss ODN is possible

Genes	KI lengths	Embryos electroporated	2cell embryos (%)	Embryos transferred	Pups delivered (%)	Mutant/offspring (%)	KI/offspring(%)
GeneV flox	674bp	160	77(48)	30	6(20)	6/6(100)	3/6(50)
GeneG flox	761bp	80	73(91)	60	27(45)	14/27(52)	2/14(7)
GeneP GFP	1421bp	180	131(72)	80	27(33)	27/27(100)	1/27(4)
GeneM 2A-iCre	1693bp	165	149(90)	60	11(18)	11/11(100)	1/11(9)

Data courtesy of Dr. Tomoji Mashimo,
Genome Editing Research and Development Center,
Graduate School of Medicine, Osaka University

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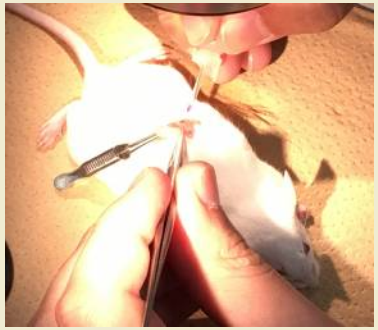
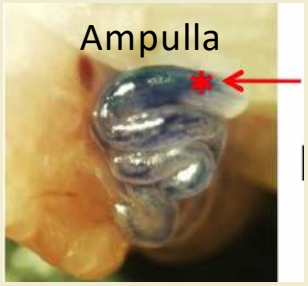
in vivo oviduct electroporation

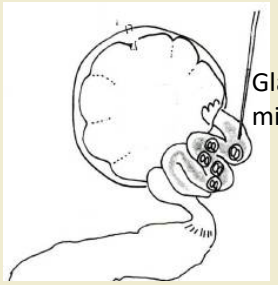
Genome-editing via Oviductal Nucleic Acids Delivery

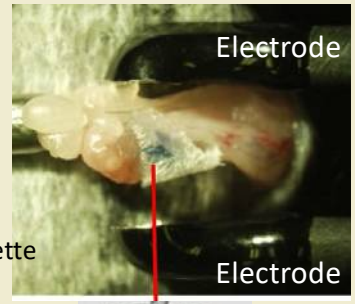
Expose the oviduct of a pregnant mouse

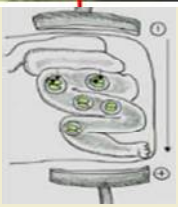


Inject DNA into the ampulla of oviduct









Electroporation
(transfer DNA into zygotes)

- **No embryo manipulation**
- **No pseudo-pregnant mice**
- **No vasectomized mice**
- **10-min treatment/mice**

Images courtesy of Dr. Shuji Takabayashi
Laboratory for Animal Resources Development
Hamamatsu University School Of Medicine

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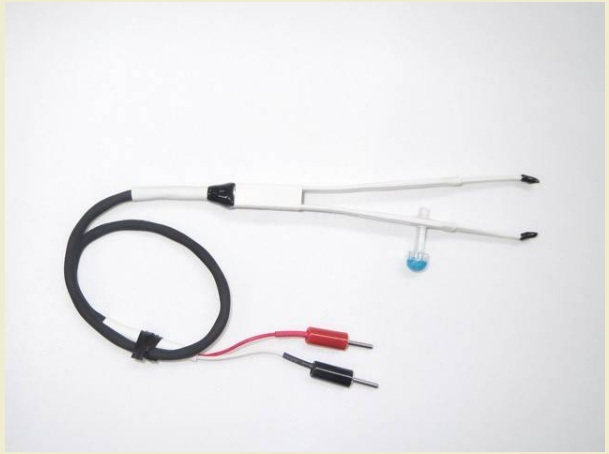
in vivo oviduct electroporation

Electrode Type



CUY652P2.5X4 (old model No. CUY562)

- **Concave platinum surface for maximum contact and minimum stress**

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in vivo oviduct electroporation

Concentrations of Cas9 protein and efficiencies of GM-mouse production

CAS9 conc. (ng/μl)	No. of ICR mice	No. of Off-spring	Ratio (%) Offsprings /mice	No. of KI Offspring (%)	No. of Indel mutant (%)
50	11	107	9.7	2 (1.9)	4(3.7)
100	7	47	6.7	20(42.6)	23(48.9)
500	6	45	7.5	28(62.2)	32(71.1)
1,000	7	47	6.7	28(59.6)	36(76.6)

Data courtesy of Dr. Shuji Takabayashi
Laboratory for Animal Resources Development
Hamamatsu University School Of Medicine

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