Generating genetically modified model marmoset

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CIEA
SINCE 1952

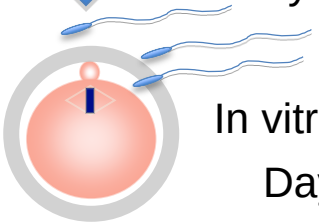
Overview of production of transgenic marmoset

Follicular stimulation
using FSH and hCG

Day -1-10

Oocytes collection & IVM

Day 11

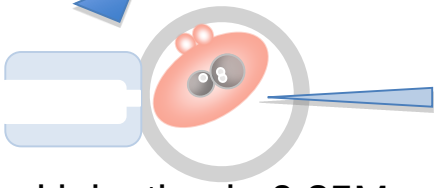


In vitro fertilization

Day 12

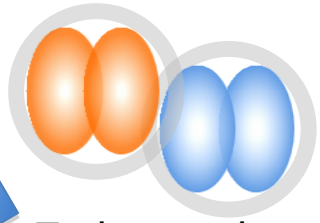
Confirm
pronucleus

Day 13

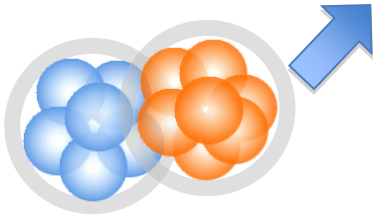


Viral injection in 0.25M sucrose
supplemented media

Day 14



Embryo culture
Day 14-20



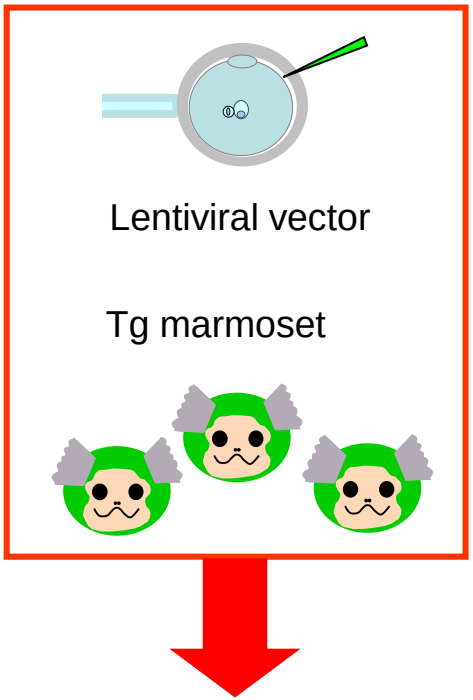
Select embryo that
express
Fluorescent protein

Embryo transfer
Day 18-21

Neonates
Additional 145days

Total around 167 days

Disadvantages of the viral vector technique for production transgenic animals



Difficult to

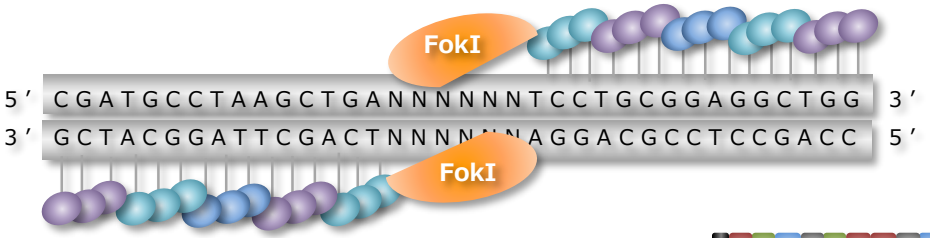
introduce DNA longer than 5kb

produce targeted gene knock out animals

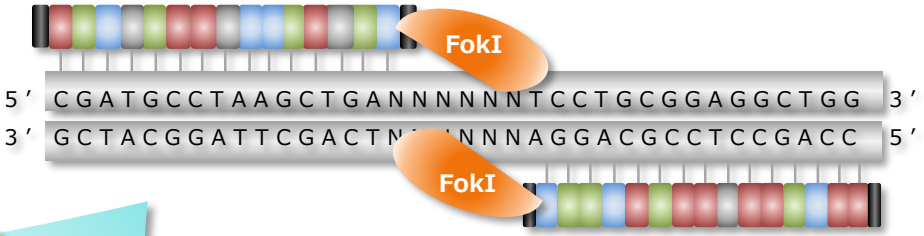
There are no pluripotent stem cells that have chimeric competent cells to produce targeted gene knockout animals in most of the mammal species including marmoset.

Production of target gene knock out marmoset using genome editing techniques

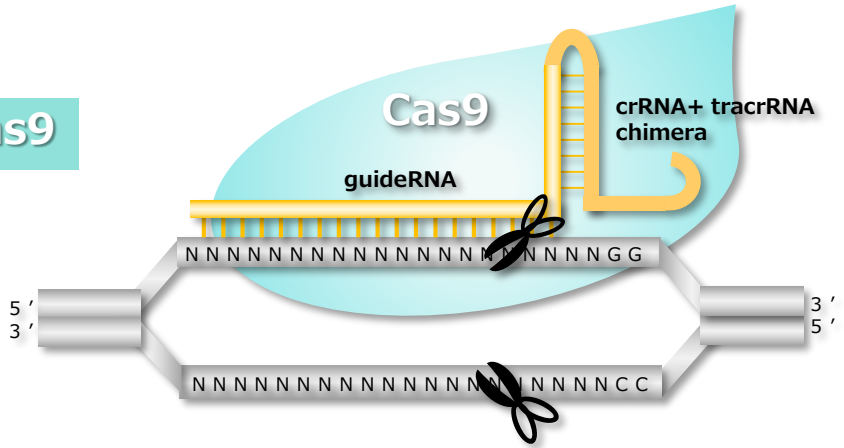
ZFN

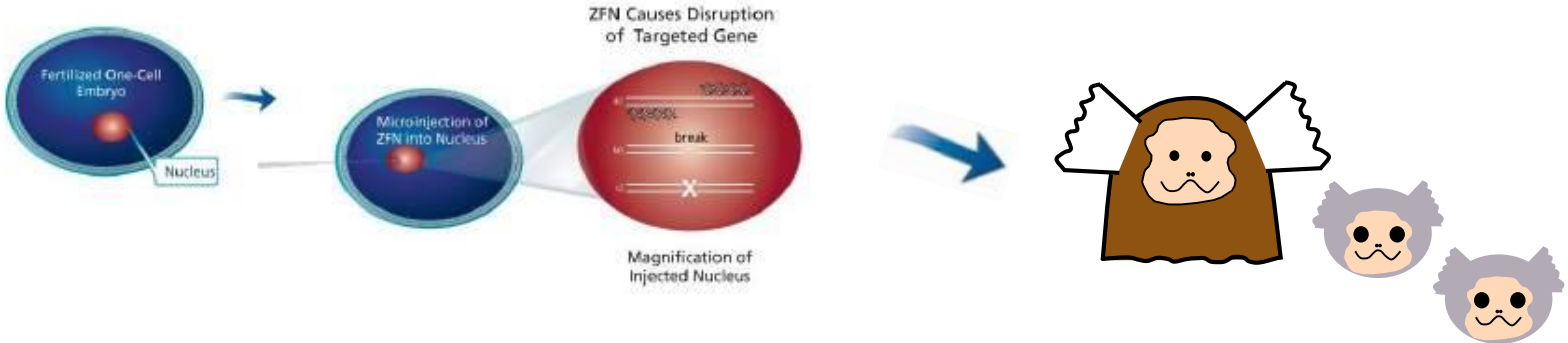


TALEN



CRISPR/Cas9





Production of Interleukin 2 receptor, common gamma chain gene (IL2Rg) knock out marmoset using ZFN and TALEN

Ken-ya Sato

Interleukin 2 receptor, common gamma (IL2Rγ)

- Common gamma chain for interleukin-2.4.7.9.15.21
- Responsible gene for X-linked severe combined immunodeficiency (X-SCID)

Application of the X-SCID marmoset

Transplantation immunology

Regenerative medicine

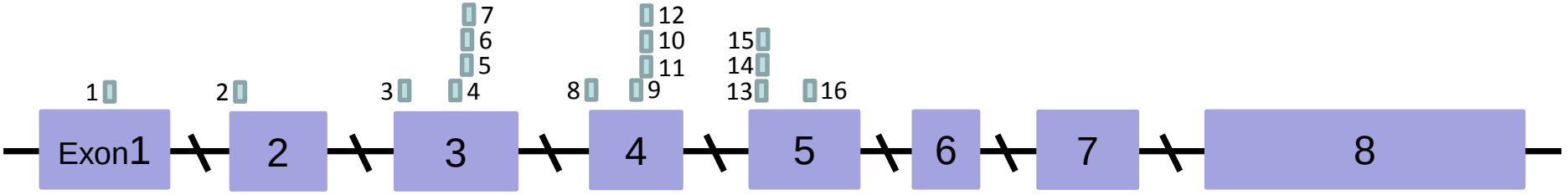
Oncology

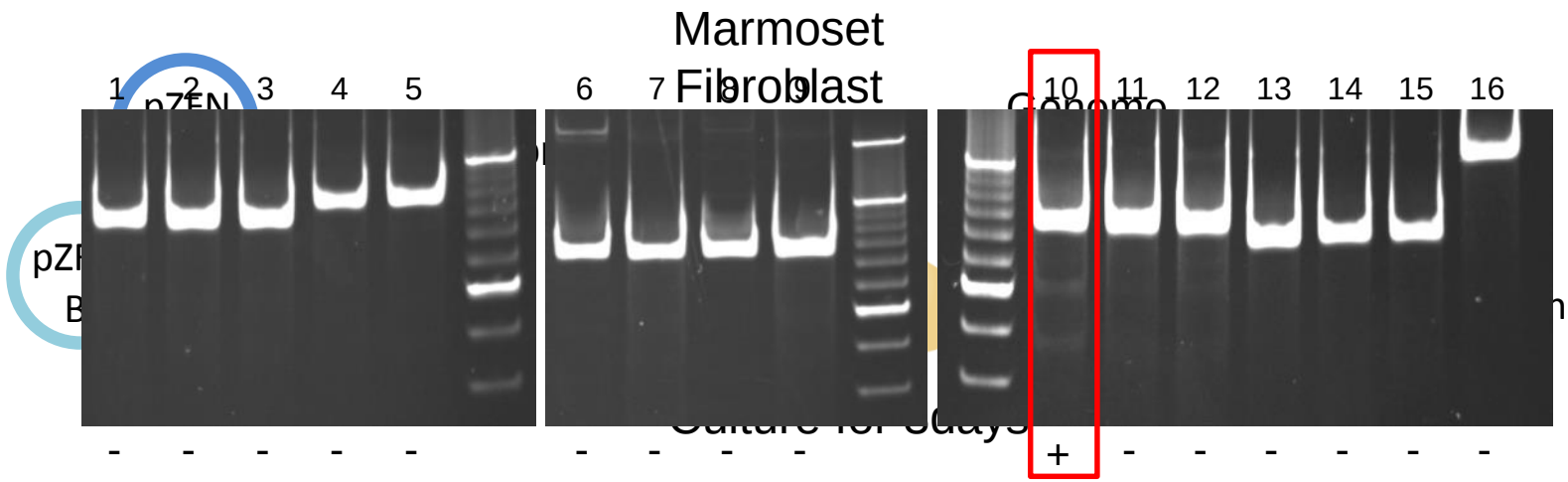
Hematology

The KO animal(s) would indicate phenotype from the neonate.

(Sato et al., Cell Stem Cells 2016)

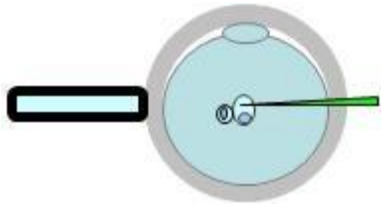
Screening of the most effective ZFNs or TALENs



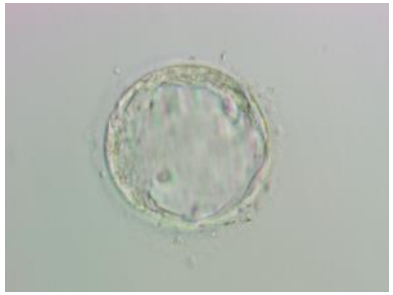


(Sato et al., Cell Stem Cells 2016)

Investigations of genome editing efficiency in the marmoset embryos

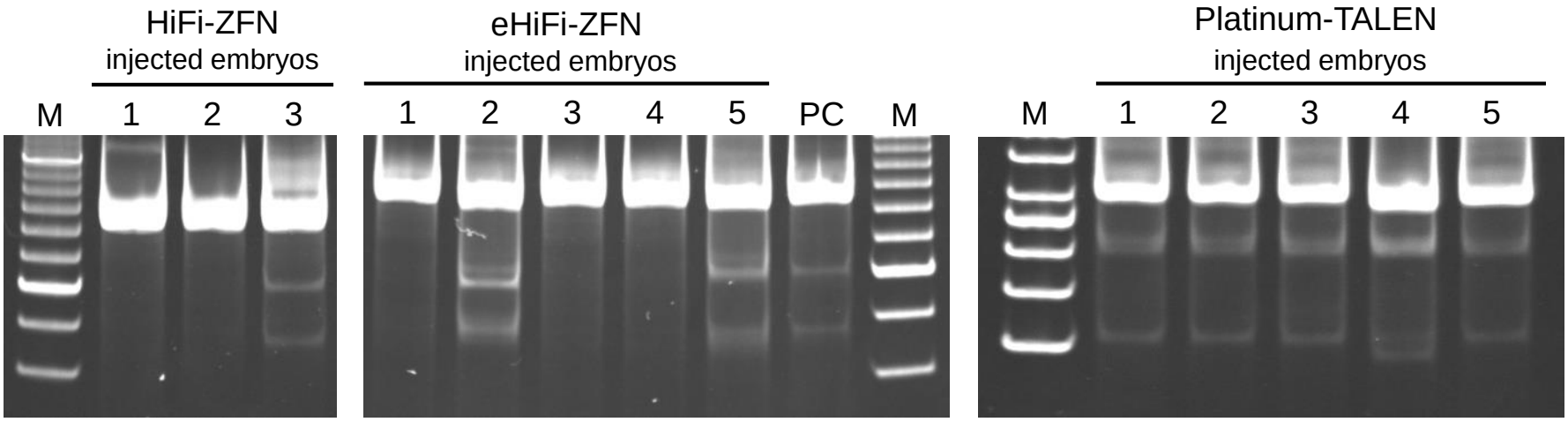


Microinjection



Culture the embryos until late stage

PCR
&
CEL-1 assay
(analysis for gene modification)



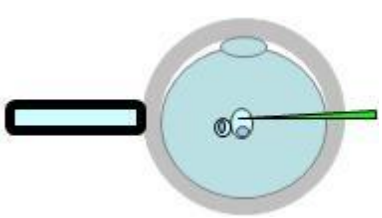
33.3% (1/3)

40.0% (2/5)

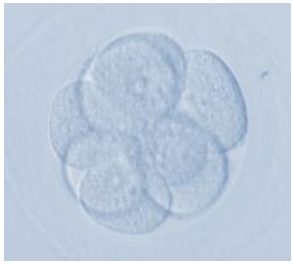
100% (5/5)

(Sato et al., Cell Stem Cells 2016)

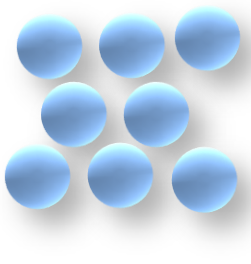
Estimation of mosaicism of the genome edited marmoset embryos



Microinjection



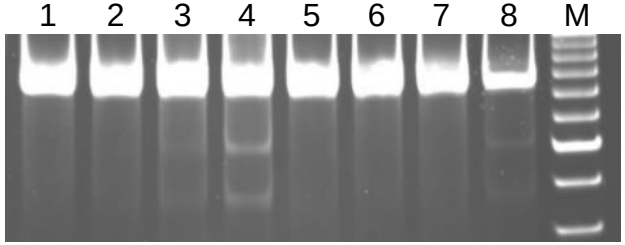
Culture embryos until 8 cell stage



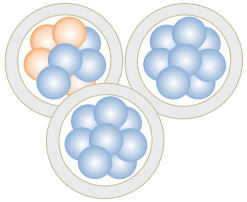
Split the blastomeres

PCR & CEL-1 assay

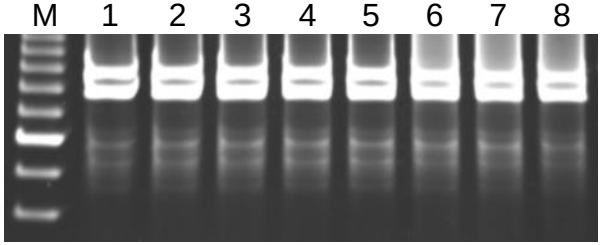
HiFi-ZFN



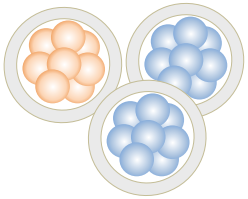
37.5% (3/8)



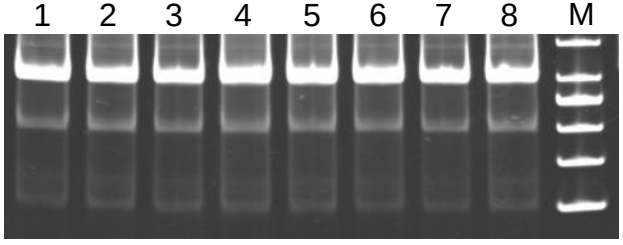
eHiFi-ZFN



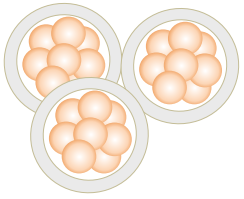
100% (8/8)



Platinum-TALEN



100% (8/8)

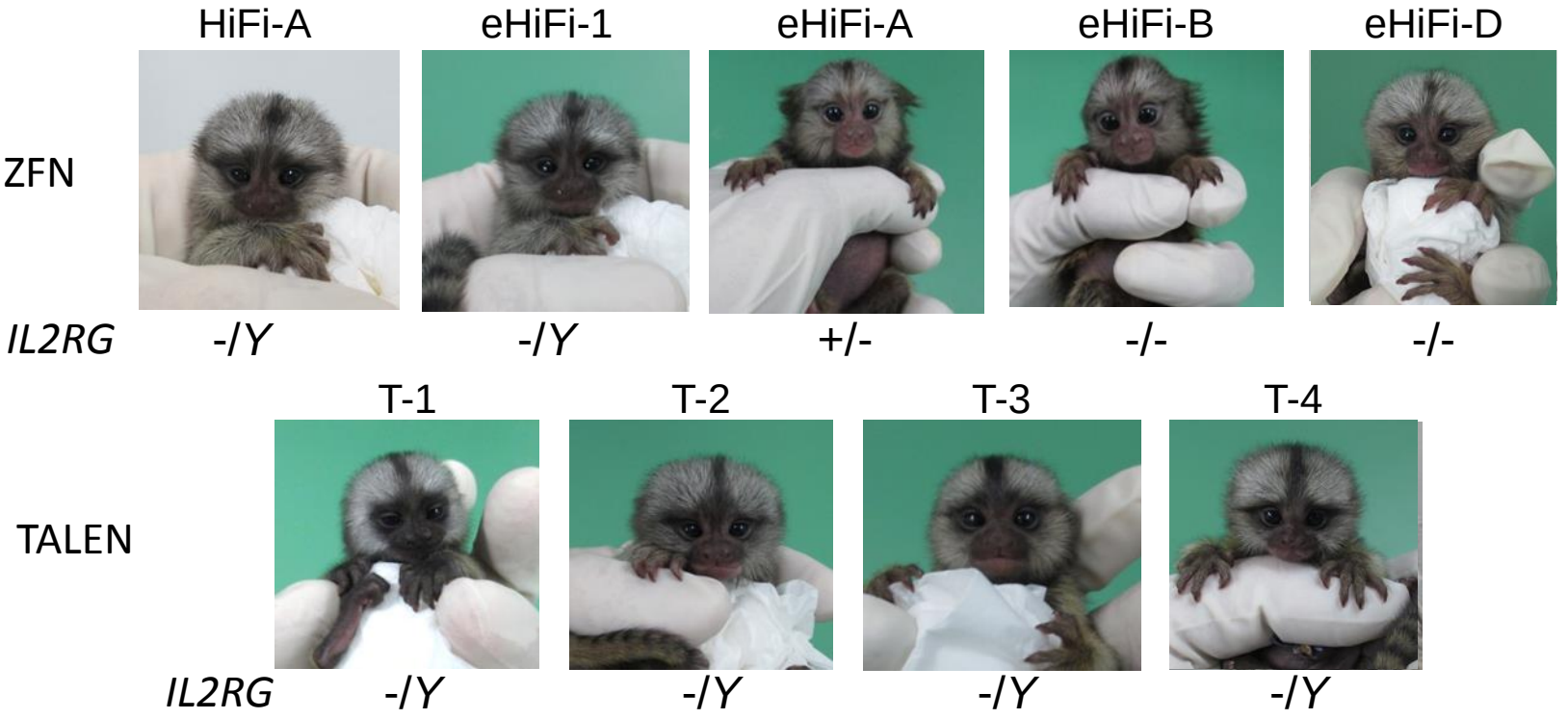


(Sato et al., Cell Stem Cells 2016)

Production of the Il2rg knock out marmosets

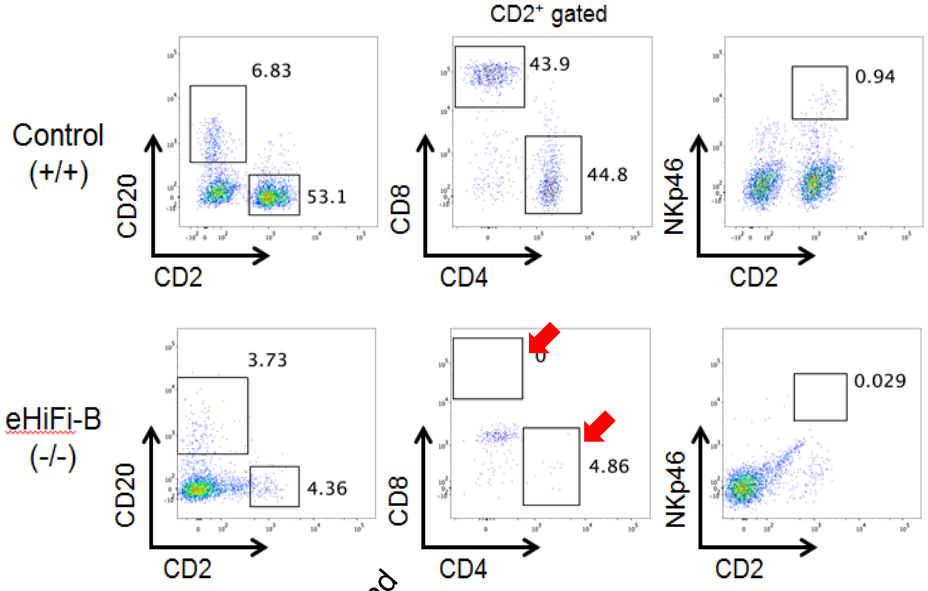
Construction	Injected Oocytes	Transferred embryos (%)	Recipients	Pregnancies (%)	Neonates	Mutant animals (%)
HiFi-ZFN	131	95 (72.5)	46	10 (21.7)	M:11, F:1	M:1 (8.3)
eHiFi-ZFN	58	42 (72.4)	38	5 (13.2)	M:2, F:3	M:1, F:3 (80)
TALEN-ex4	9	5 (55.6)	5	1 (20.0)	M:1	M:1 (100)
TALEN-ex2	52	37 (71.2)	24	4 (16.7)	M:3	M:3 (100)

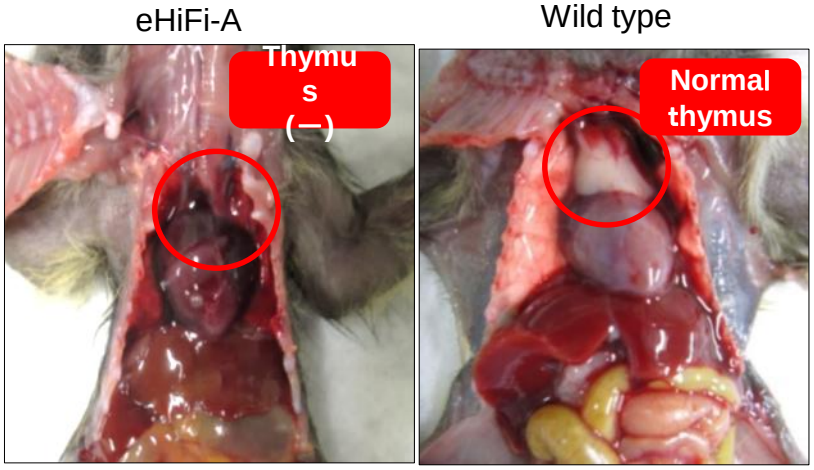
M: Male, F: Female

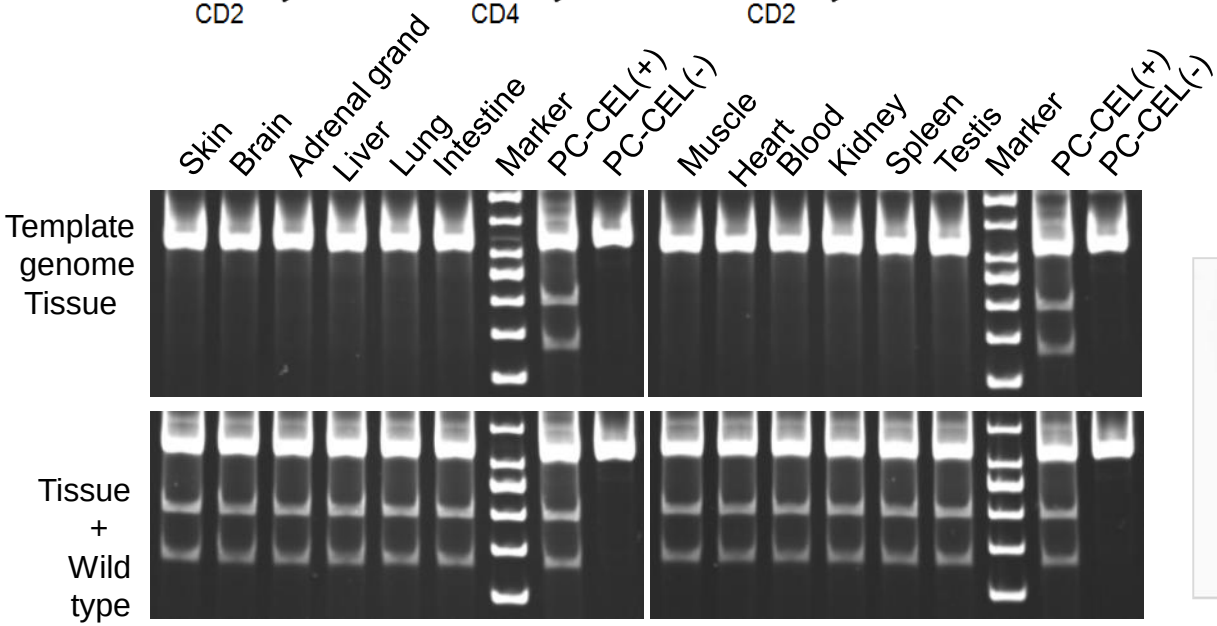


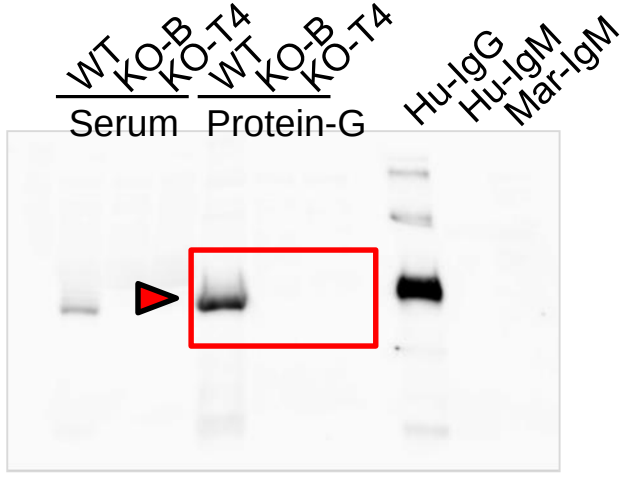
(Sato et al., Cell Stem Cells 2016)

Phenotypes of immunocompromised marmosets







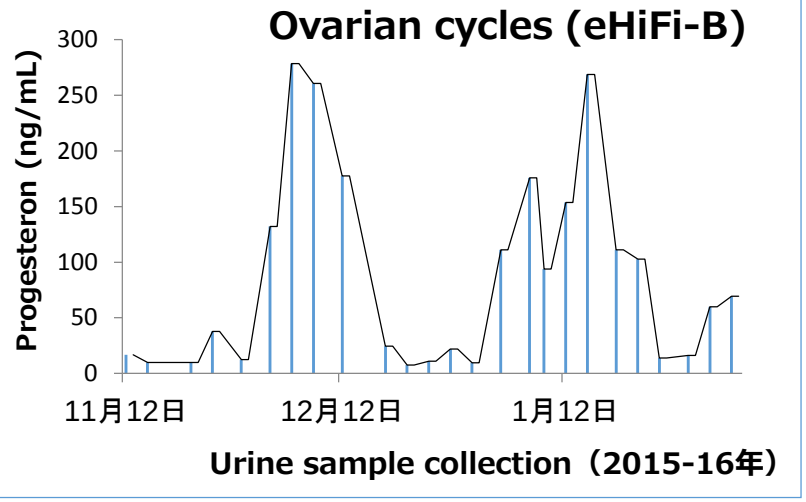


(Sato et al., Cell Stem Cells 2016)

Germline transmission of the KO gene

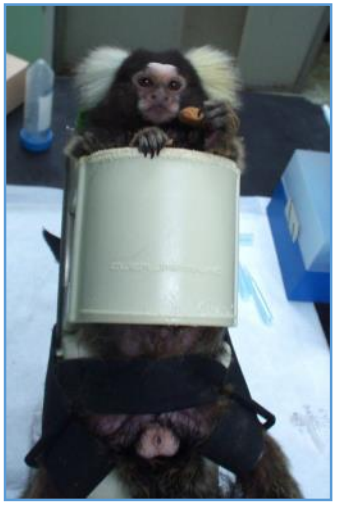
eHiFi-B (1.9yrs)

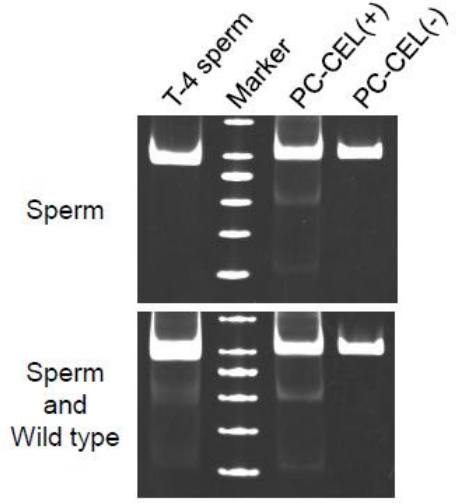
IL2RG(-/-)



T-4 (1.3yrs)

IL2RG(-/*Y*)

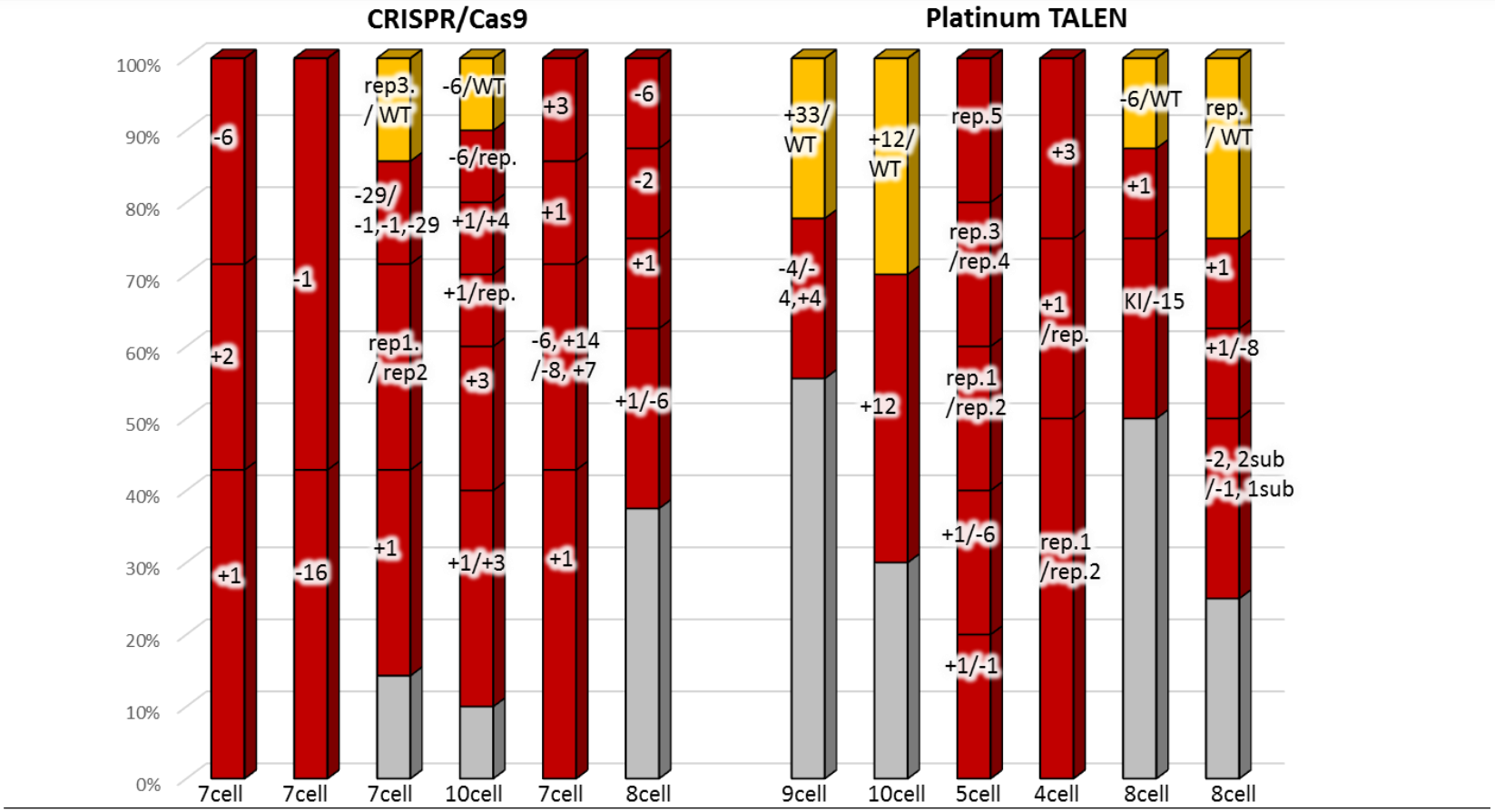





```
Wild type  CCTCCCAGAGGTTCAGTGTTTTGTGTTCAATGTCGAGTACATGAATTGCACTTGGAAC
T-4 sperm  CCTCCCAGAGGTTCAGTGTTTT----------------------GCACTTGGAAC (-25bp)
T-5 sperm  CCTCCCAGAGGTTCAGTGTTTTG-------TGTCGAGTACATGAATTGCACTTGGAAC (-7bp)
```

Modified genes are transferred to next generation

insulin gene mosaic mutation in marmoset embryo



When Cas9 mRNA was used, all embryos showed mosaicism

When Cas9 nuclease was used, the most of blastomeres showed bi-allelic modification.

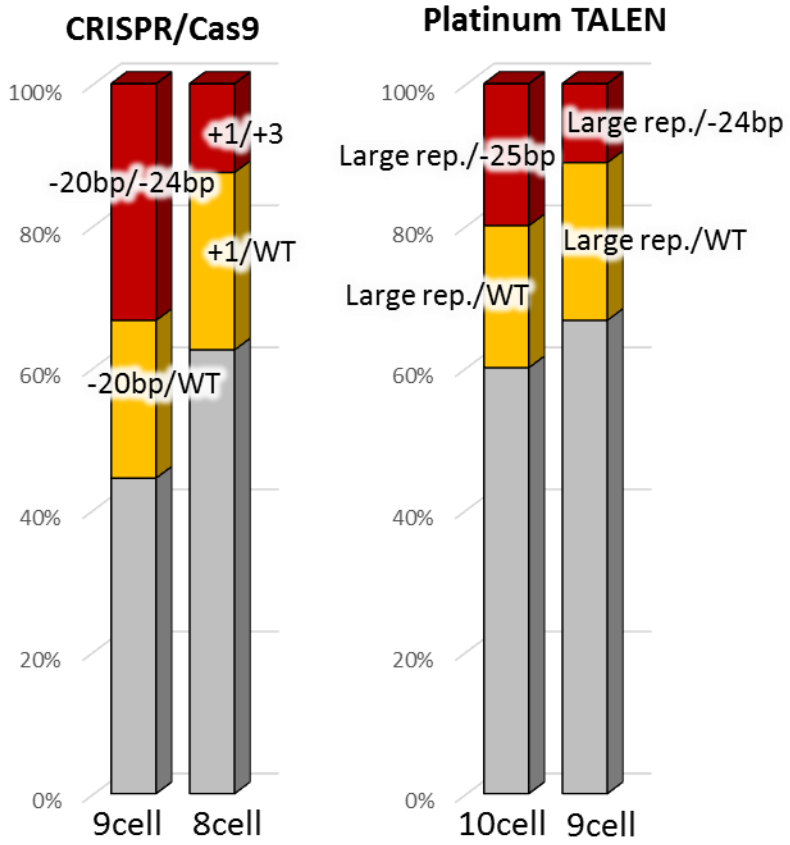
-difficult to obtain offspring from bi-allelic-modified embryo of c-*kit* gene?

-embryonic lethality?

	Injection	Transferred embryo	recipient	pregnancy	delivery	abortion	modified
Platinum TALENs	37	15	2	5	0	0	
CRISPR	14	9	2	0	2	0	

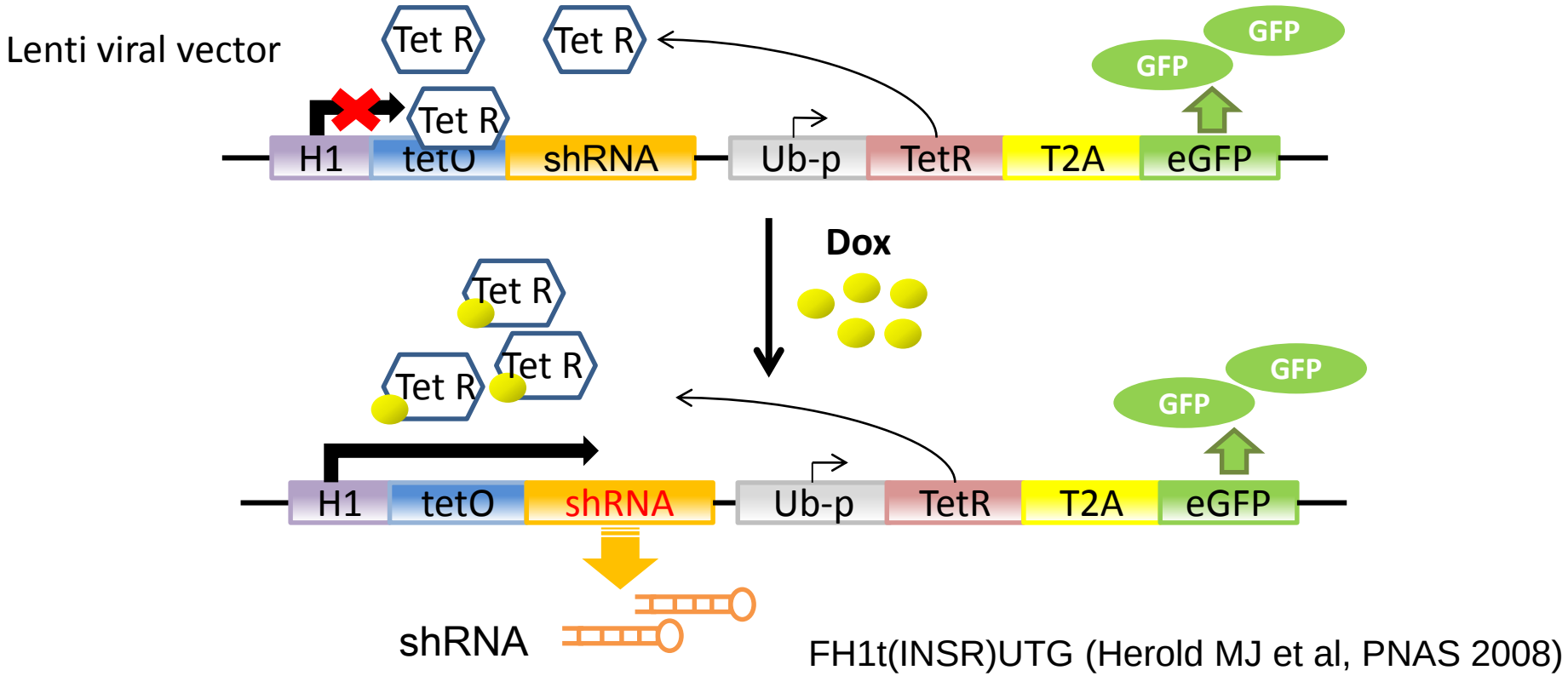
(Kumita et al., unpublished)

insulin gene mosaic mutation in marmoset embryo



Injection	Transferred embryo	recipient	pregnancy	delivery	abortion	modified
Platinum TALENs	16	6	2	5	0	0
CRISPR	9	6	2	0	2	0

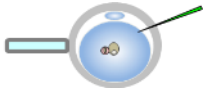
Target gene knock down marmoset model



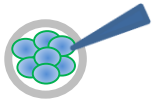
FH1t(INSR)UTG (Herold MJ et al, PNAS 2008)

Insulin receptor (INSR) KD diabetic model

Analysis of INSR-KD marmosets

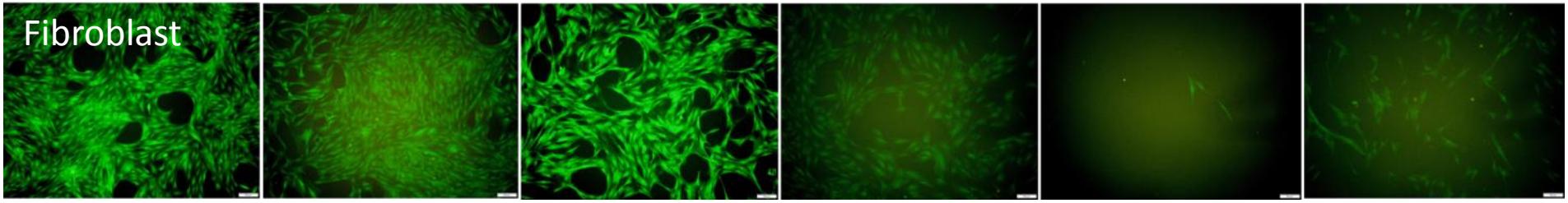


Pronuclear injection



Fertilized embryo injection

778 661 No ID 681 720 829



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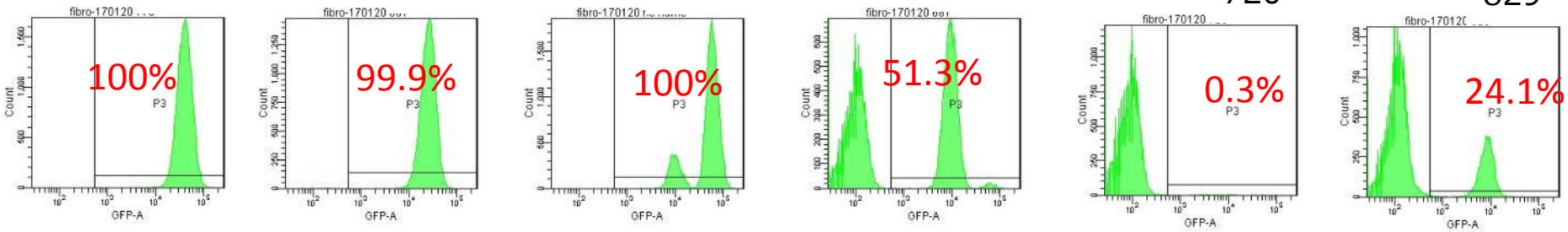
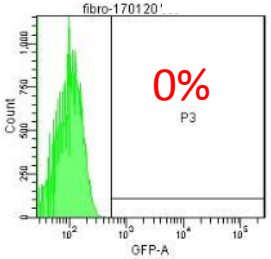
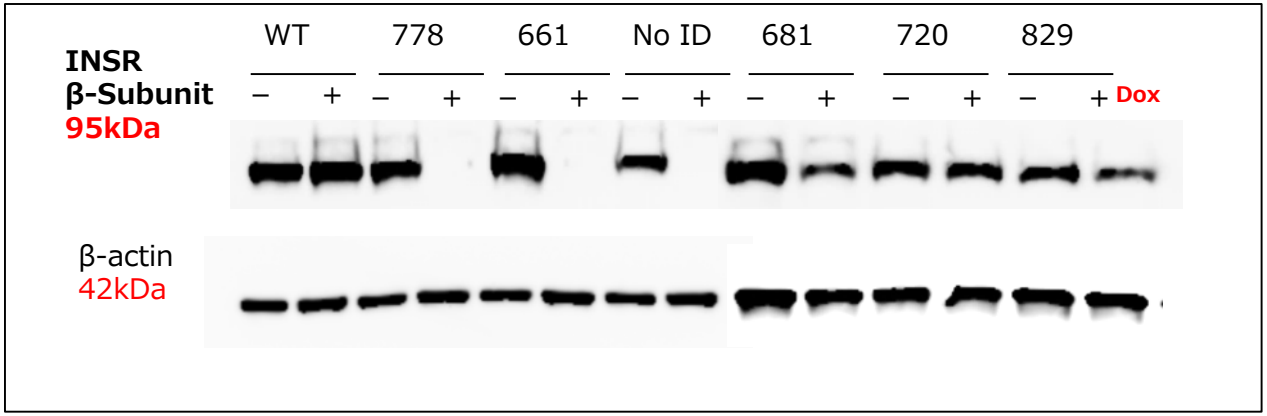


WT



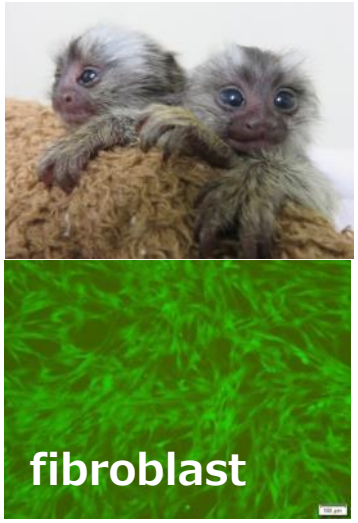
FACS analysis of GFP

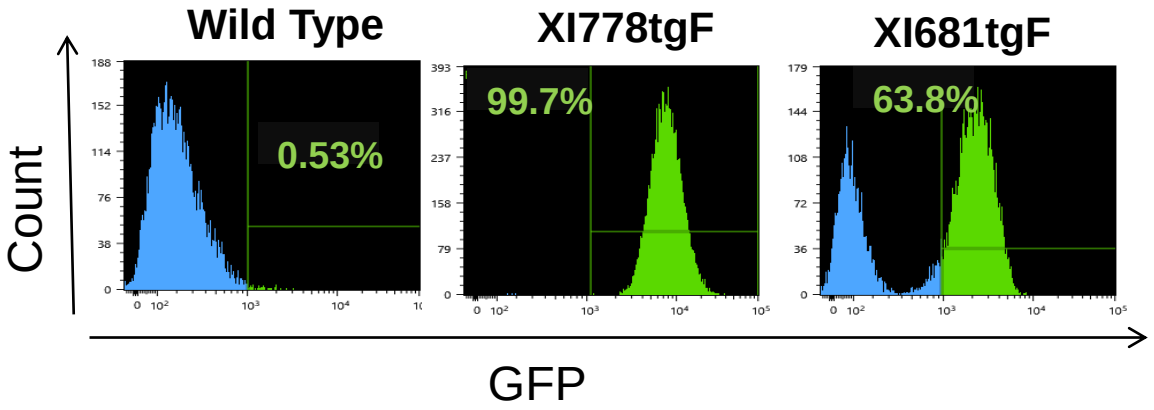
Verification of knockdown by western blotting.



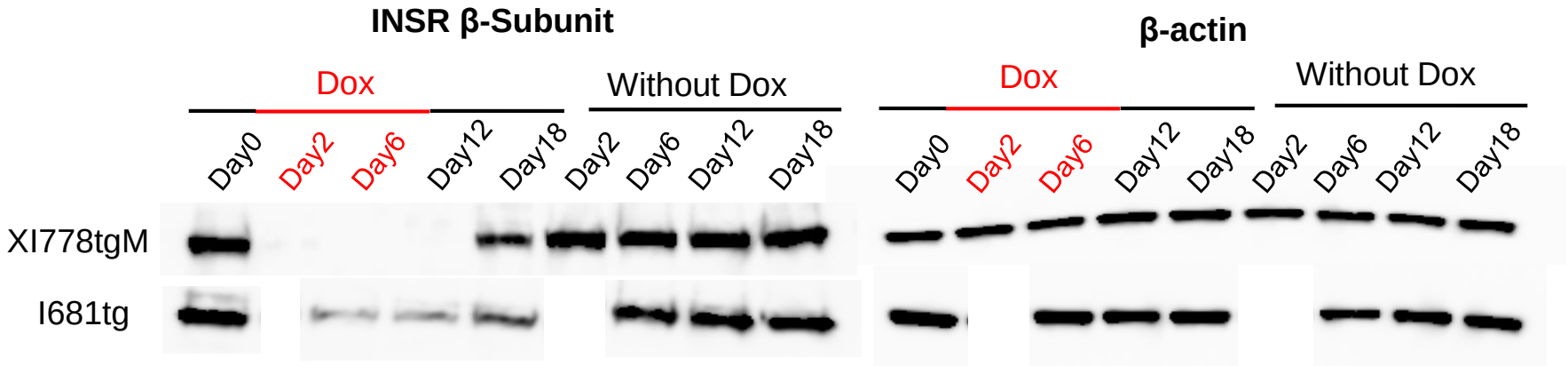
(Takahashi et al., Unpublished)

Time course of INSR knockdown in newborn fibroblast



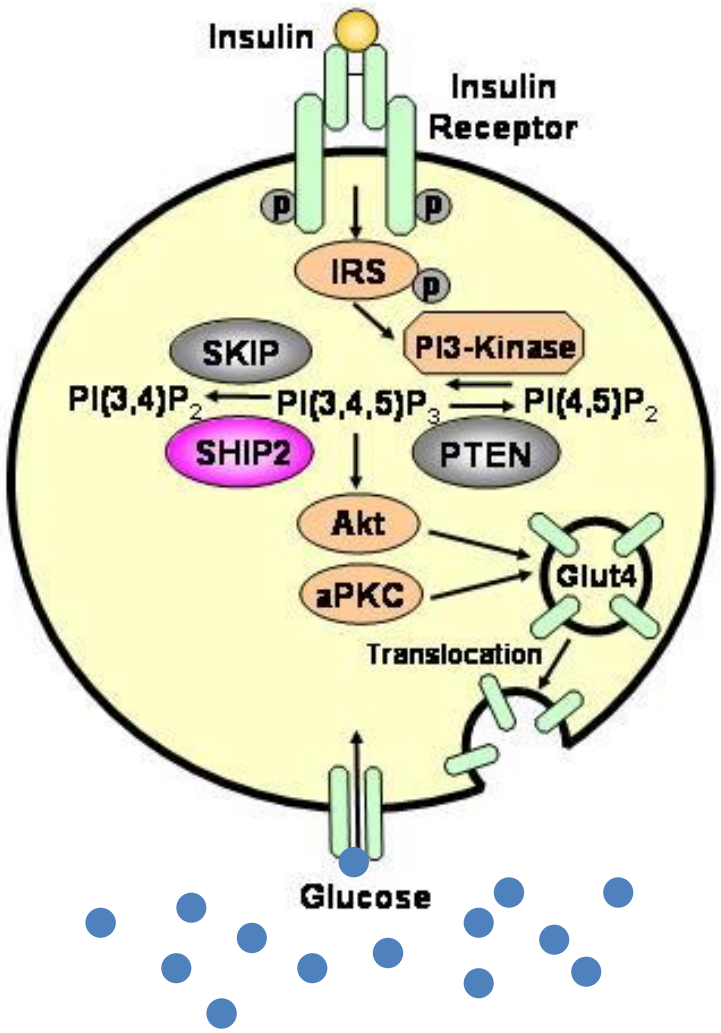


Dox administration



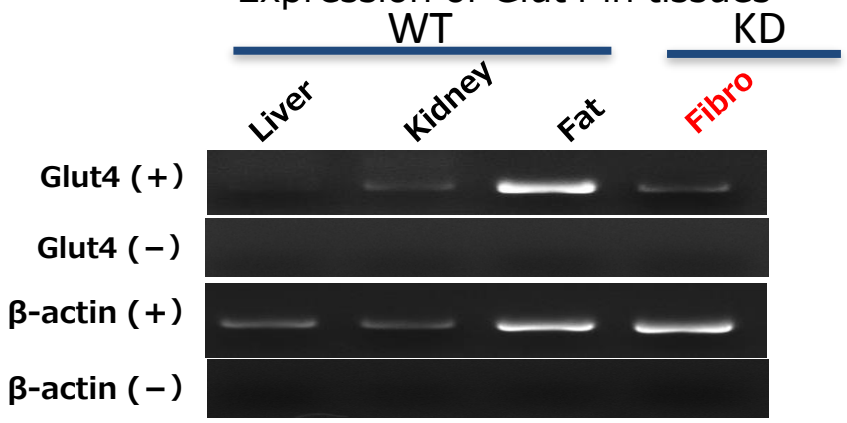
(Takahashi et al., Unpublished)

Glucose metabolism in Insulin receptor (INSR) KD

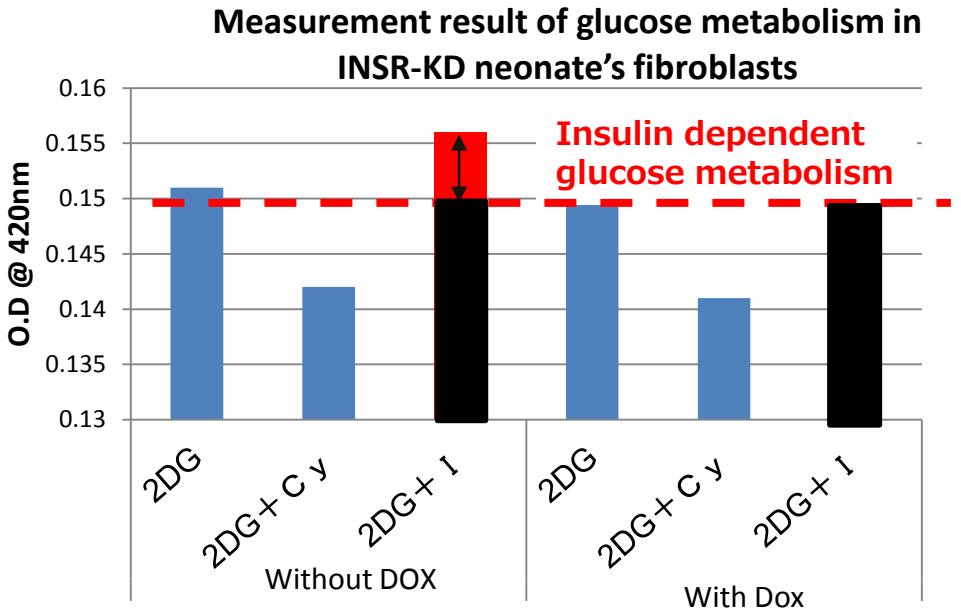


From http://www.pha.utoyama.ac.jp/clinphar/img/ResearchProjects/

Expression of Glut4 in tissues



Measurement of 2-deoxyglucose metabolic rate



Cy: Cytochalasin B
(Glucose uptake inhibitor)
I: Insulin

(Takahashi et al., Unpublished)

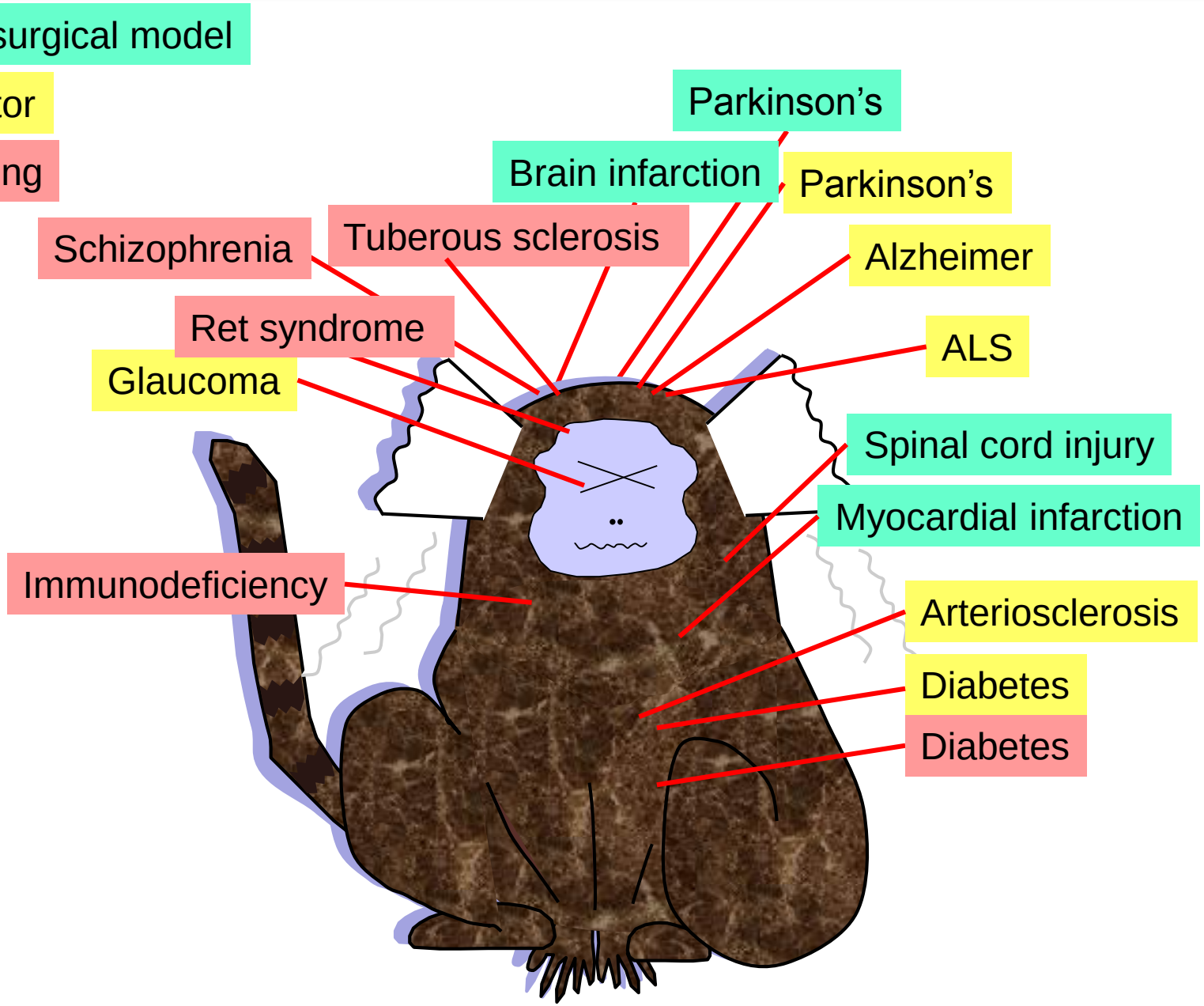
Summary

Both of ZFN and TALEN are effective strategies to produce target gene knock out marmosets.

Pre-evaluations of the genome editing tools are important to obtain target gene KO animals.

Target gene knock down would be another option to avoid embryonic lethality

Future perspective of marmoset disease models



Acknowledgements

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