New generation transgenic techniques in rabbits

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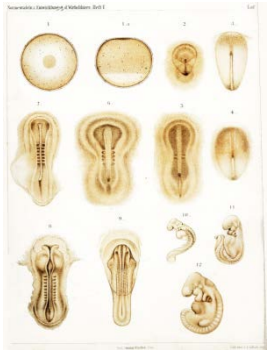
Why to use rabbits as model animals

• **Prenatal development (especially long lasting effects on health and complex disorders)**

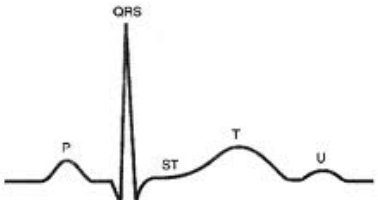


•**Diabetic pregnancy**

•**Atherosclerosis (Spontan mutants), transgenic rabbits**

•**Eye research (retinal degeneration)**

•**Arrhythmogenesis/ heart diseases**



•**Antibody production**

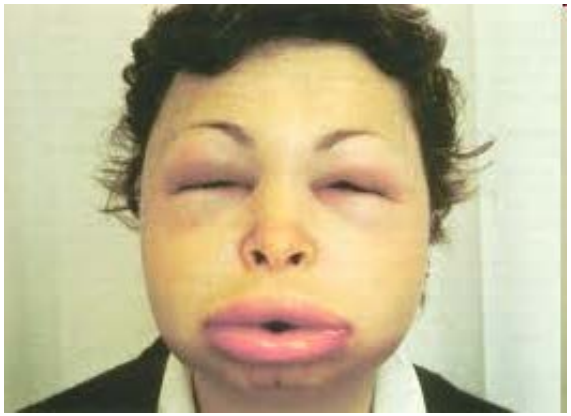
immun genes

Transgenic rabbit as a bioreactor

Ruconest- Human C1 inhibitor

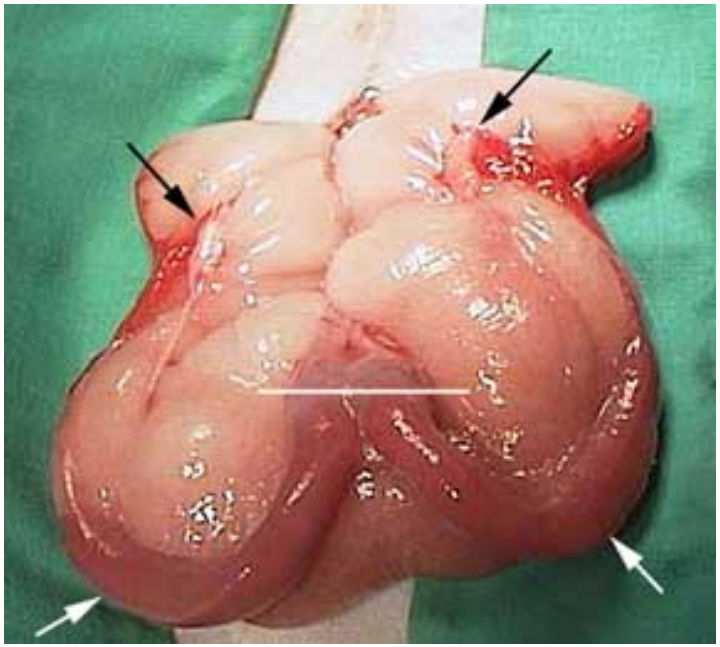
Produced in the milk of transgenic rabbits

For the treatment of hereditary angioedema



Alpha glucosidase produced in the milk of TR. Rabbits-
For Pompe disease
Clinical trials phase III

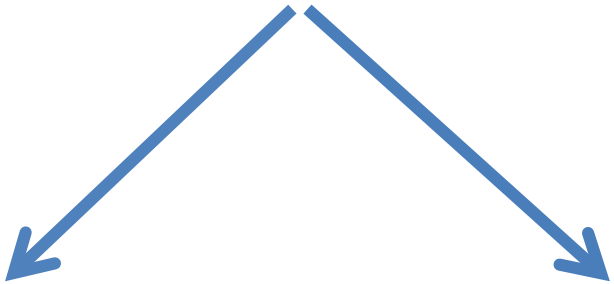
First generation transgenic method was microinjection in rabbits



Surgical transfer

Problems and solutions

Transgenic rabbits



Additive transgenesis

Problem: efficiency

Targeted transgenesis (knock-out, knock in, allele exchange)

Problem: ES cells

New technologies: Transposon transgenesis

nuclease technologies

Transposon mediated transgenesis

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RNA TRANSPOSON

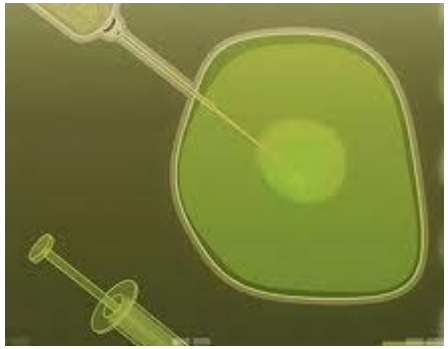
Cut and paste

DNA TRANSPOSON

Important in biotecnology

Transzposons are effective weapons in different fields of biotechnology

Transgenesis

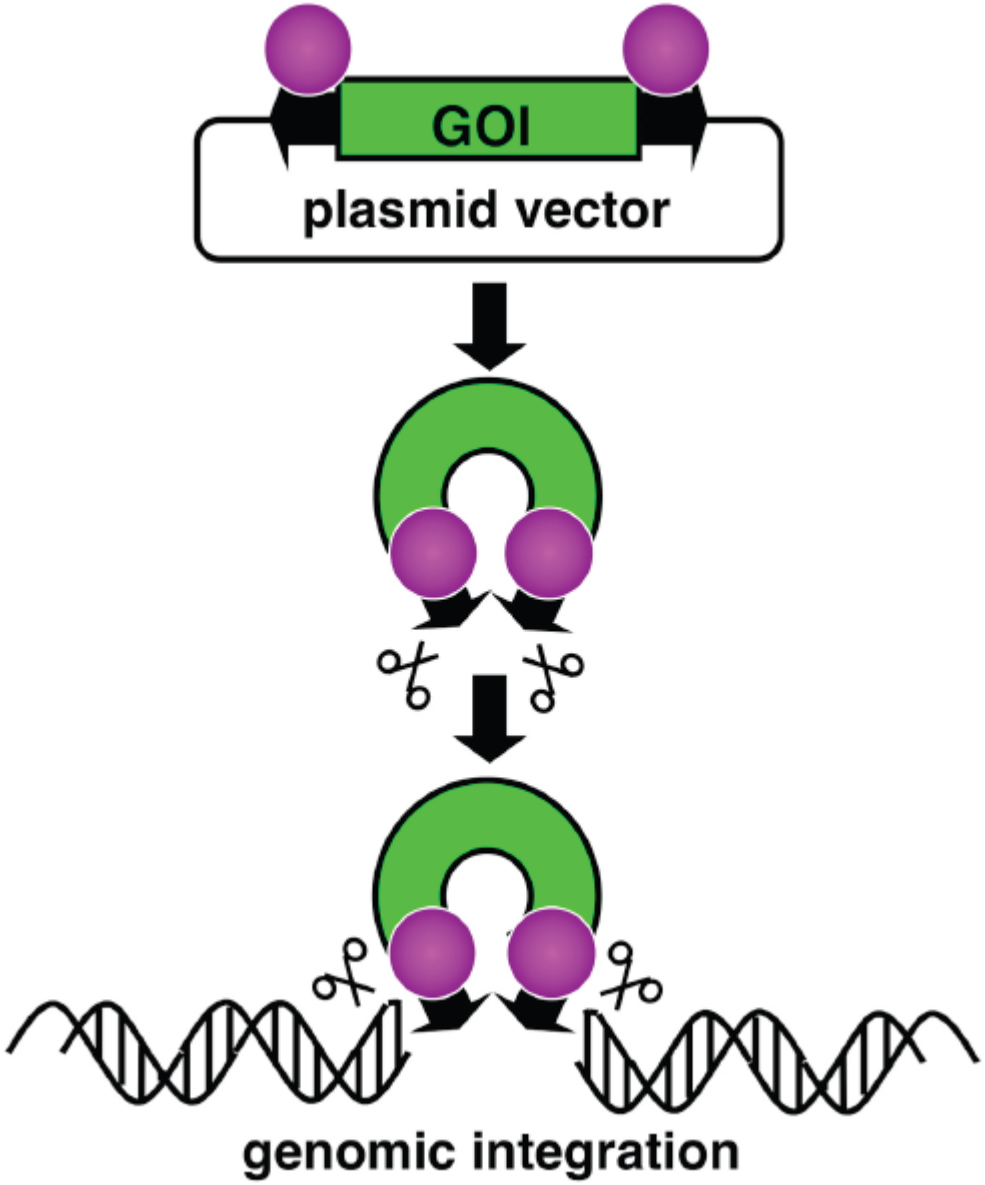


Transposon

Gene therapy

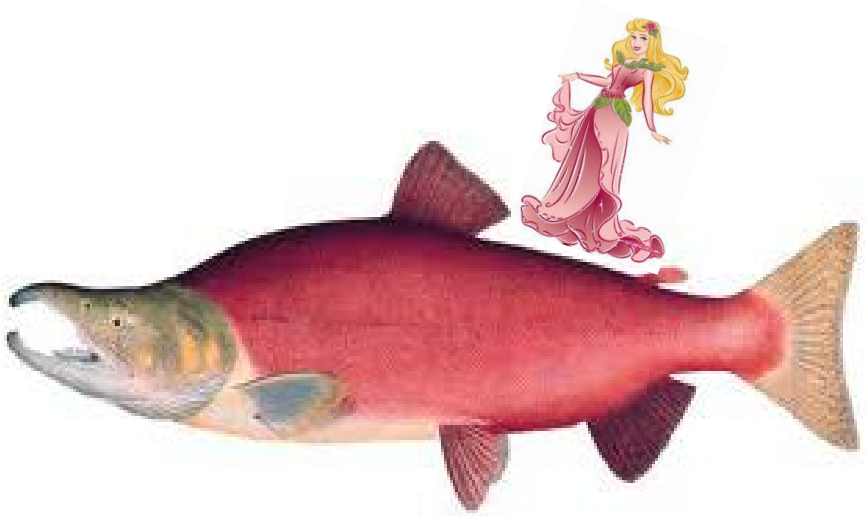
Insertional mutagenesis

How it works



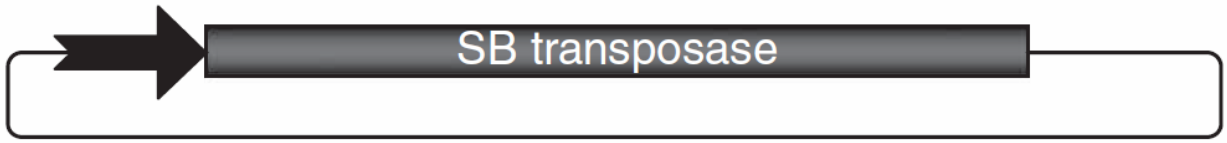
The Sleeping Beauty system

- **DNA transposon (Tc1/mariner)**
- **Reconstructed from salmon**
- **100X more efficient type by mutagenesis**
- **Integration to TA sequence**
- **Works in different species**



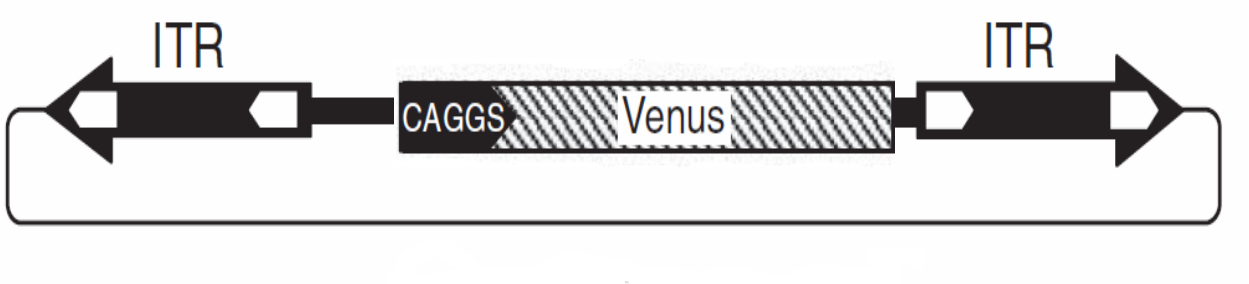
Elements of SB-100 System

1.



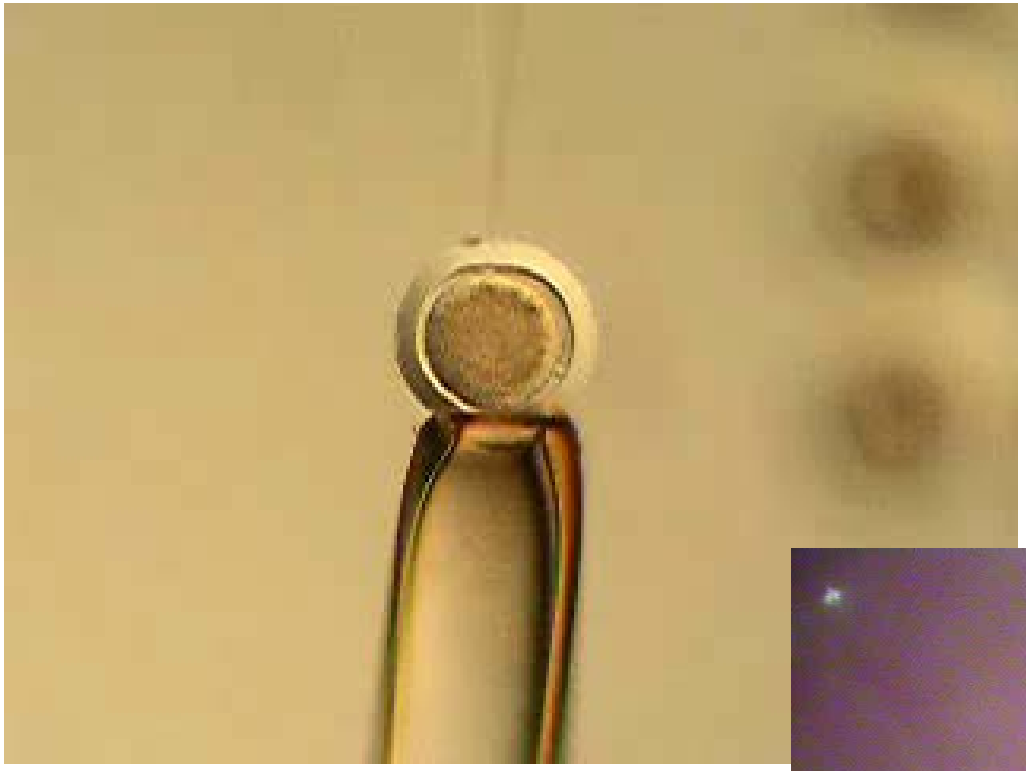
In vitro transcription

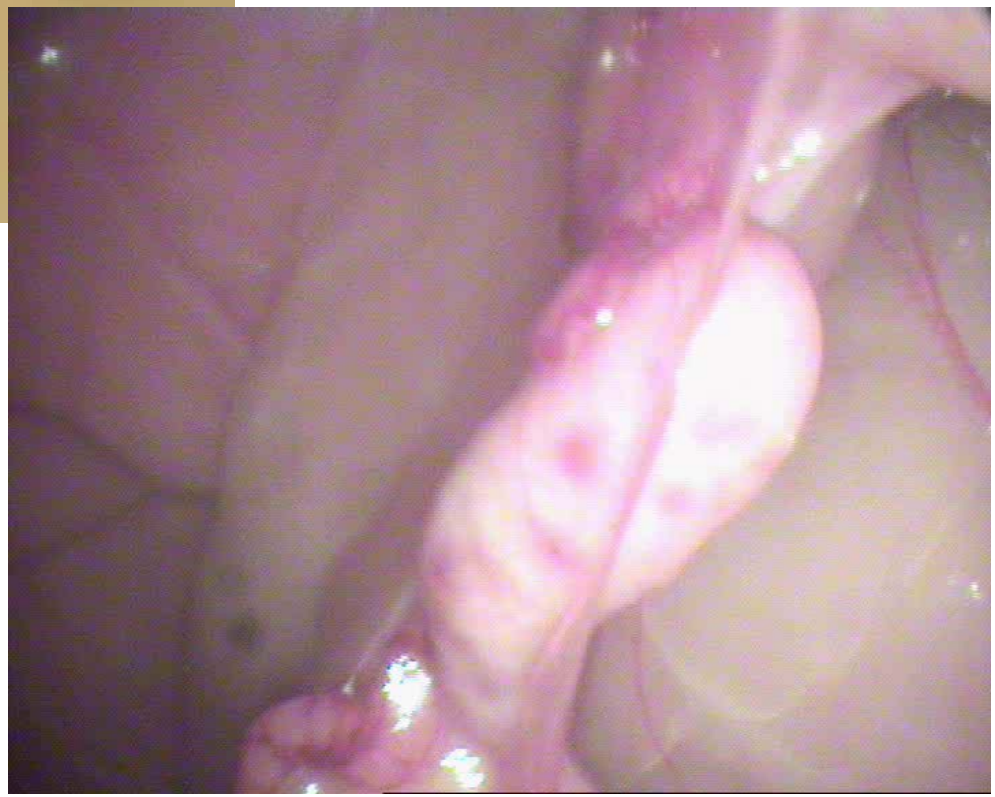
2.



Microinjection

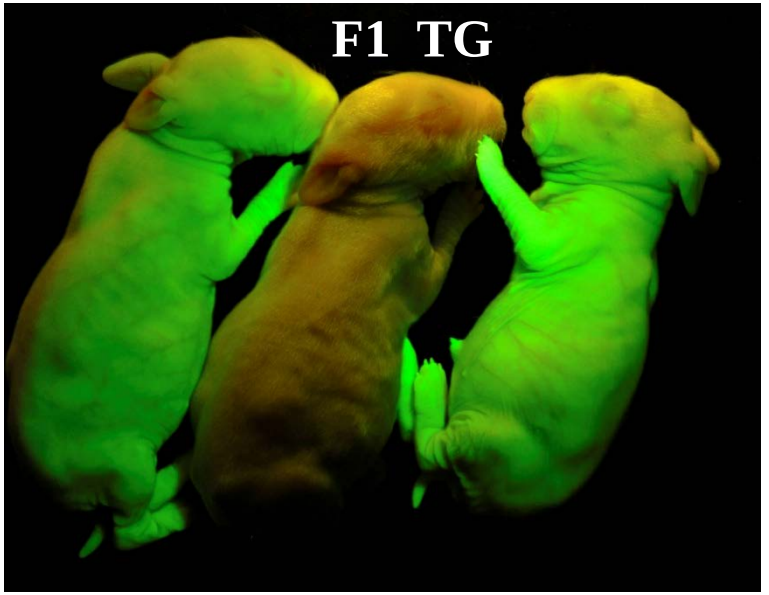
Embryo transfer





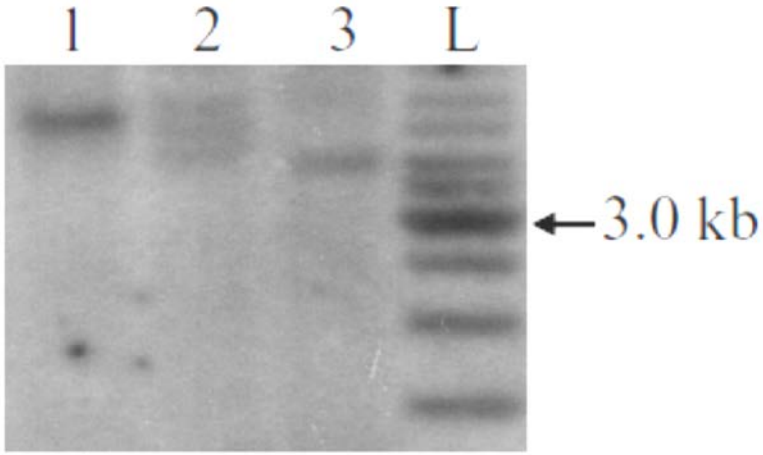
RESULTS

Embryos	Transferred	No. mothers	Efficiency		TG lines
644	472	25/10 40 %	7/46 15 %		4

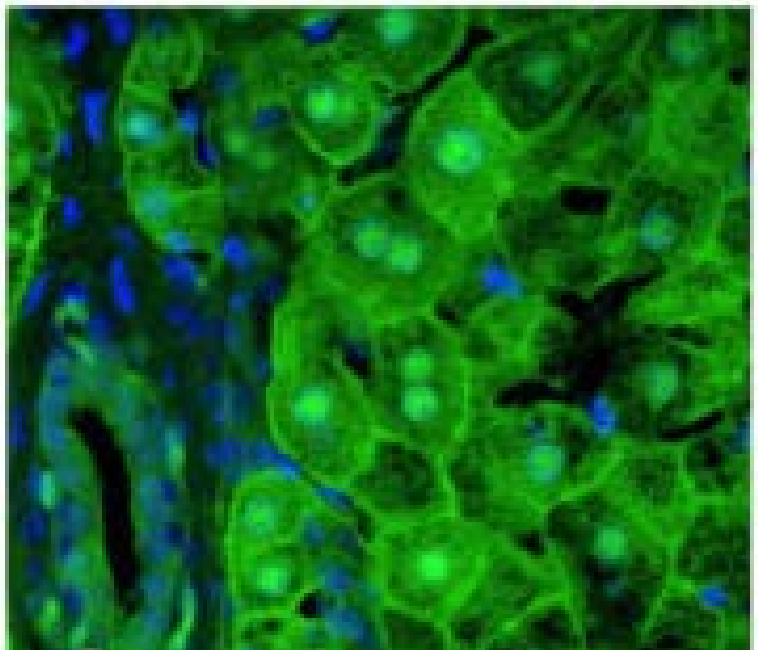


Determination of integration sites, copy number

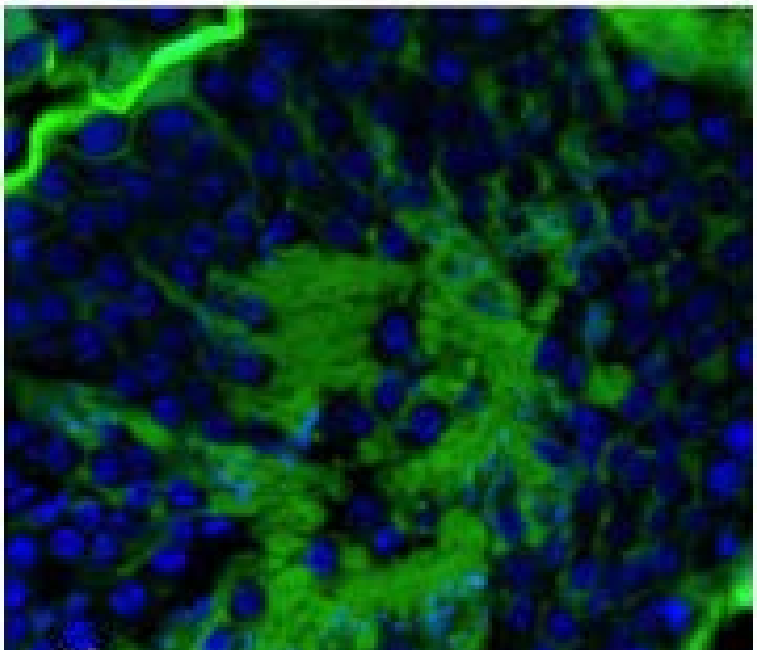
Founders	Integration site
SB3 JT	19 Chr
SB3 BT	8 Chr



Expression



Liver



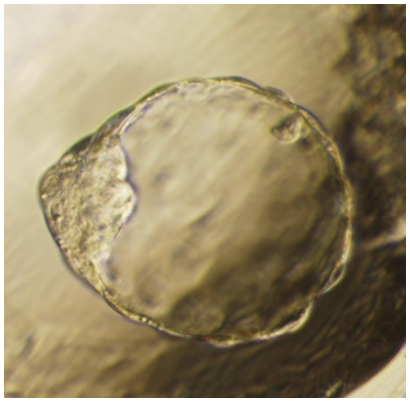
Testis

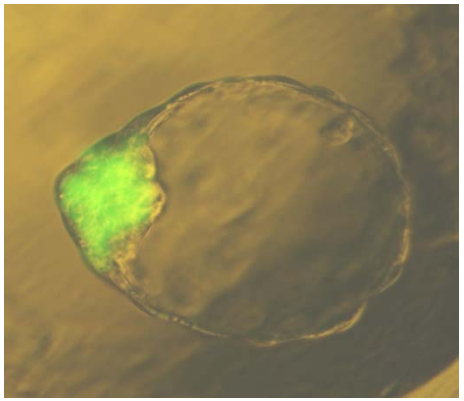
Summary

	Microinjection	Transposon
Efficiency	1-6 %	15%
Capacity	>2 Mb	<6 kb
Integration	randomly	Randomly (TA)
Form of integration	Concatamers	Single copy 1-3 integration sites
Expression	++++	+++++
Transgene transmission	+++++	++++

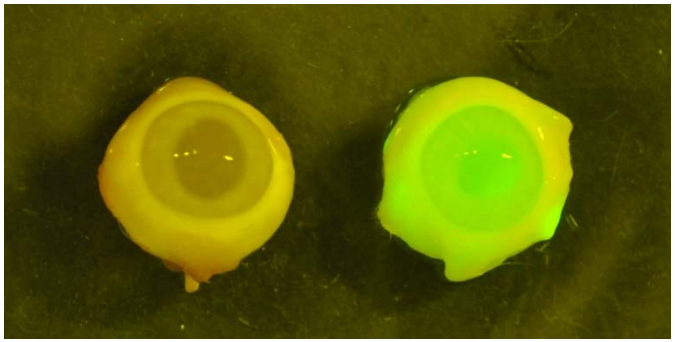
How to use our GFP rabbit

-Produce rabbit pluripotent stem cell lines

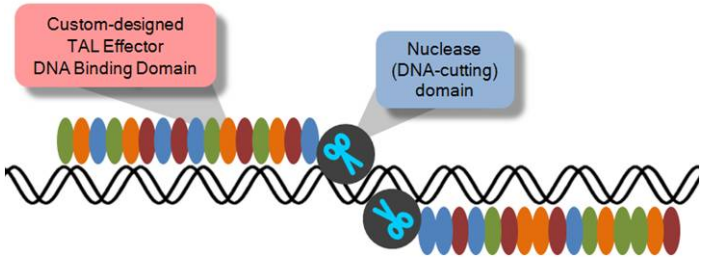


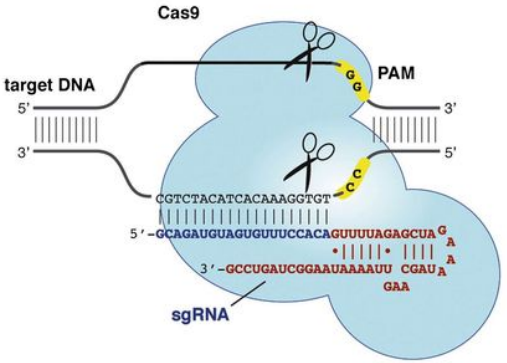


-experimental surgery methods (cornea, cartilage)



„Designer nuclease technology”



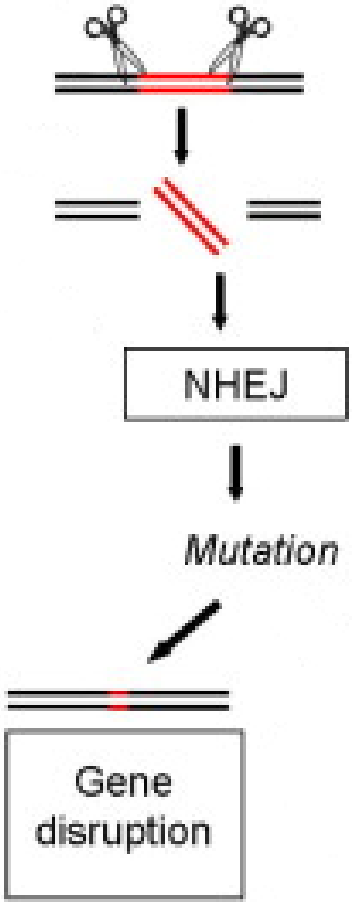


- Artificial systems based on natural protein systems
- Always harbour DNA binding part and a cleavage domain

Work as specific molecular scissors

Zinc finger nuclease
Talen nuclease
RNA mediated Crispr system

ZFN in rabbits- IGM targeted



IgM exon1

ZFN-L (18255) ZFN-R (18257) binding site (capital letters)

AGCCGGACCgtCAGGACcttcccGGTGGTGAAGAGAGGGGA

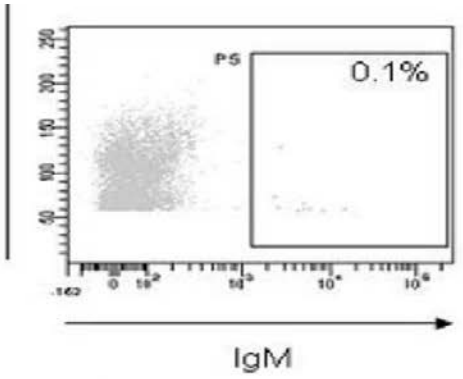
cagccggaccgtcaggacctcc-ggtggtgaagagaggggacaagtatatggccacct	Δ1
cagccggaccgtcagga----------caagtatatggccacct	Δ25
cagccggaccgtcagg----------tggtgaagagaggggacaagtatatggccacct	Δ10
cagccggaccgtcagtgaagacggtggtgaagaccggtggtgaagagaggggacaagta	+11 (Δ7+18)
cagccggaccgtcaggacctcccg-tggtgaagagaggggacaagtatatggccacct	Δ1
cagccggaccgtcaggaa-------gtggtgaagagaggggacaagtatatggccacct	Δ7 (Δ8+1)
cagccggaccgtcaggacc-----ggtggtgaagagaggggacaagtatatggccacct	Δ5
cagccggaccgtcaggacctcccoggtggtgaagagaggggacaagtatatggccacc	+1
cagccggaccgtcaggacccatgaattgaggtggtgaagaggggacaagtatatggcca	+5 (Δ5+10)
cagccggaccgtcag--------tggtggtgaagagaggggacaagtatatggccacct	Δ8 (Δ9+1)
cagccggaccgtcag----------gtgaagagaggggacaagtatatggccacct	Δ13

IgM exon2

ZFN-L (18276) ZFN-R (18277) binding site (capital letters)

AGCCGGTGGaGGCTGAaaccaaAGGGGCTGGGCCTGCGACC

aagccggtgg----------ggctgggcctgcgacctttagcatcagcagcat	Δ16
aagc----------agggctgggcctgcgacctttagcatcagcagcat	Δ19
aagccggtggaggctg----------ggcctgcgacctttagcatcagcagcat	Δ15
aagccggtggaggctgaaaccaaaagggctgggcctgcgacctttagcatcagcagca	+1



Frameshift mutation, early stop

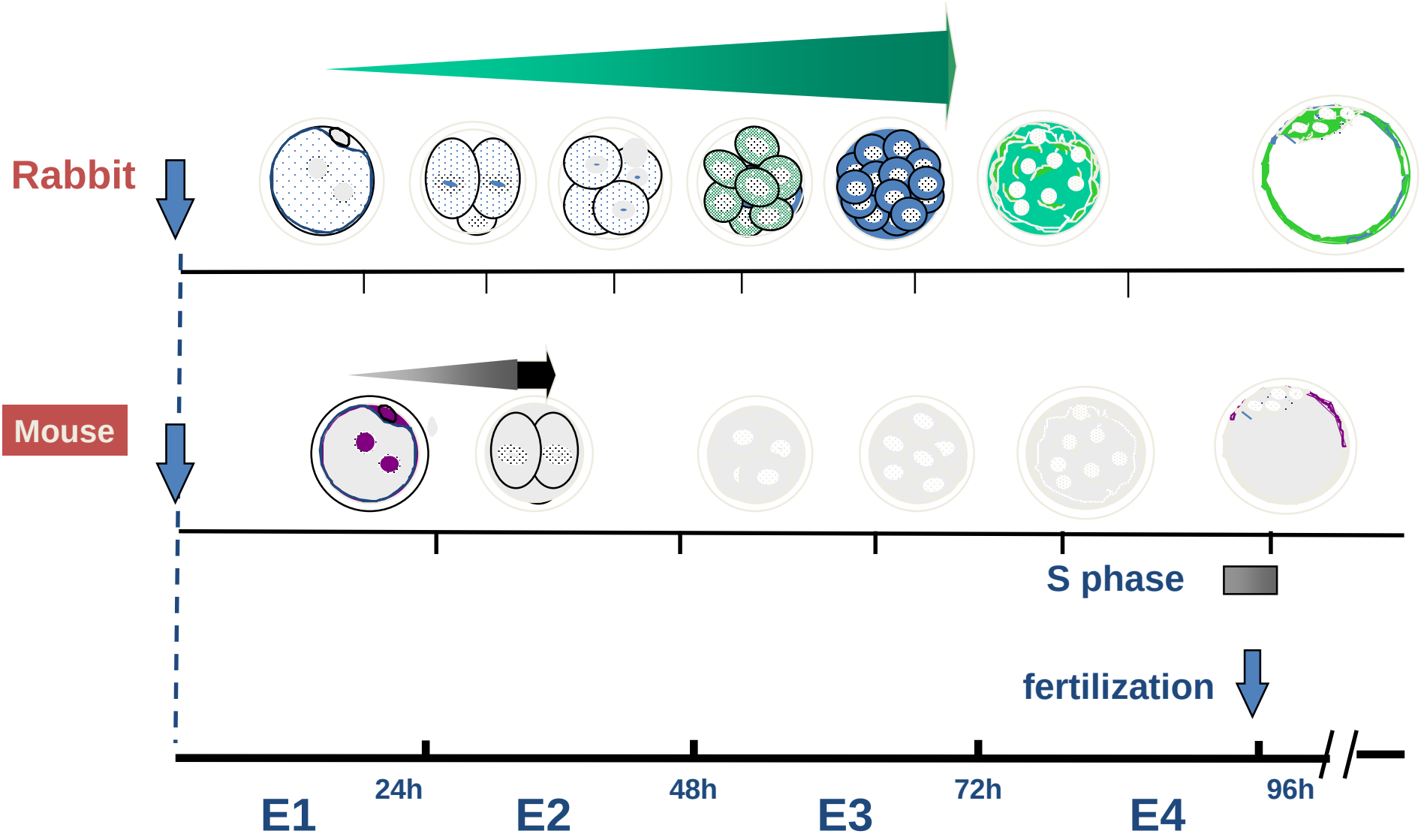
Targeted gene knock out

Our experiences with Zinc finger nuclease in rabbits

- **Very effective : 20-25% of founders are positive**
- **Founders are usually mosaic (less than 50% of F1 generation are mutant)**
- **Founders always transmit transgene to F1**
- **F1 generation can carry a different mutation than founder's ear. Special mosaicism.**

Δ2 mutation vs Δ4+1

Why we have mosaic founders?



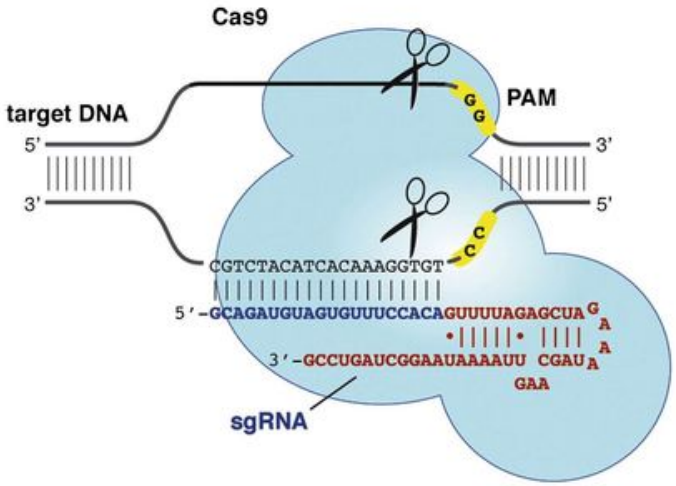
TALEN experiments in rabbits

One published paper with 2 different knock outs

TALEN rabbit experiments in our lab

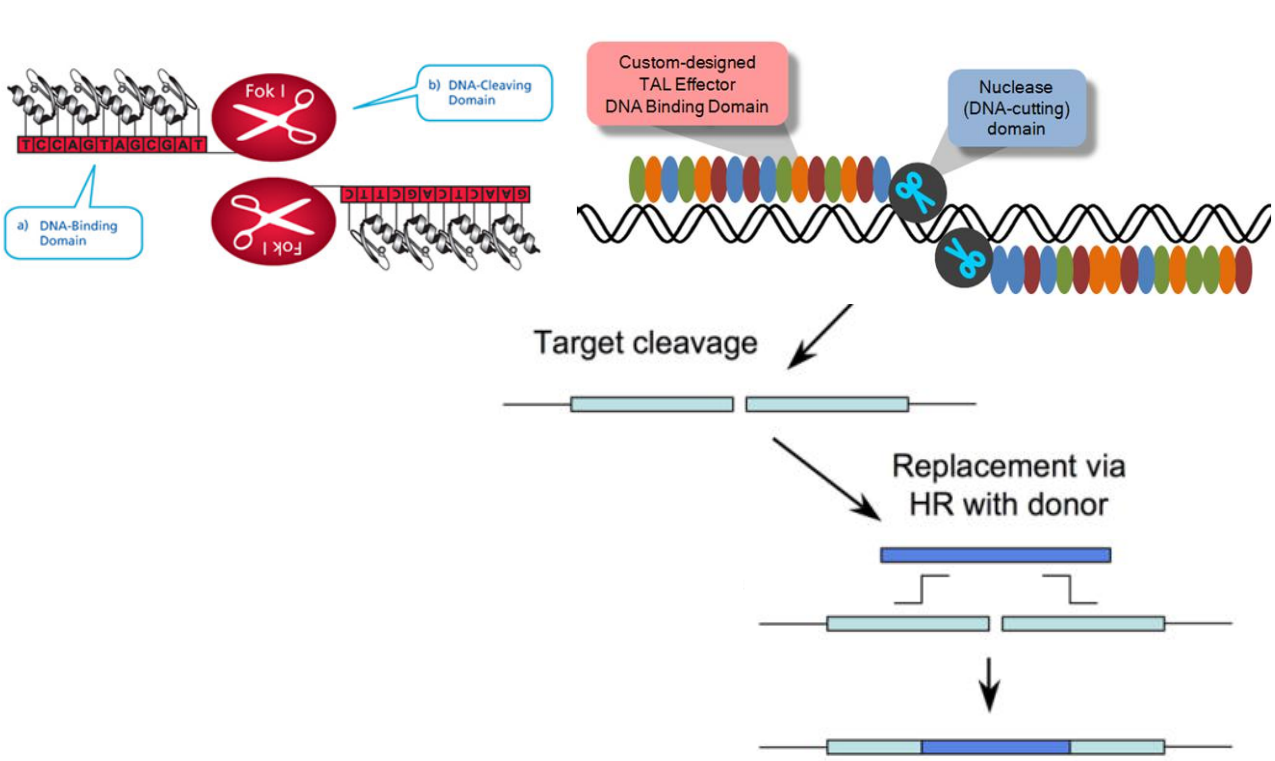
Flushed zygotes	Transferred zygotes	Recipients/ deliveries	Transgenics/new borns
213	178	10/5 50%	?/15

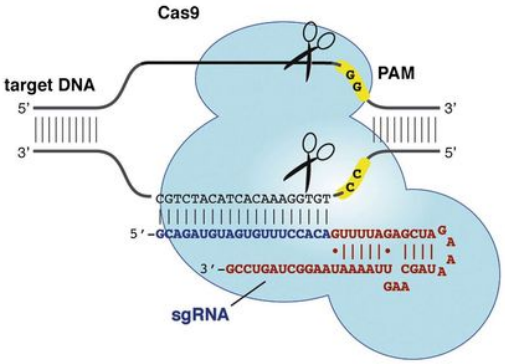
CRISP-R was extremly efficient in rabbits



Target gene	No. of embryos injected	No. of BL (%)	No. of BL sequenced	Mutants (%)	Bi-allelic mutations (%)
CD36	25	15 (60.0)	15	10 (66.7)	8 (80.0)
LDLR	18	11 (61.1)	10	5(50.0)	0 (0)
CFTR	60	33 (55.0)	19	11 (55.6)	4 (36.3)
APOE	67	33 (49.2)	23	16 (69.6)	16 (100)
APOCIII	38	13 (34.2)	9	4 (44.4)	0 (0)
LEP	18	12 (66.7)	12	2(16.7)	0 (0)
LEPR	19	13 (68.4)	10	1(10.0)	0 (0)
RyR2	20	13 (65)	10	10 (100.0)	8 (80.0)
SCARB1	25	16 (64.0)	8	2 (25.0)	0 (0)
Total	290	159 (54.8)	116	61 (52.6)	36 (59.0)

Targeted insertion is possible but not reported in rabbits





The result is homologous recombination and gene knock in or allele exchange

Nuclease technology is the ultimate technique to produce targeted gene modifications

- Relatively cheap
- Very specific
- Efficient in rabbits
- Any targeting event can be produced
- Even single bp exchange can be tested
- TRACELESS TECHNOLOGY
- Open a new era for large animal models

REGULATION???

Thank you for attention!