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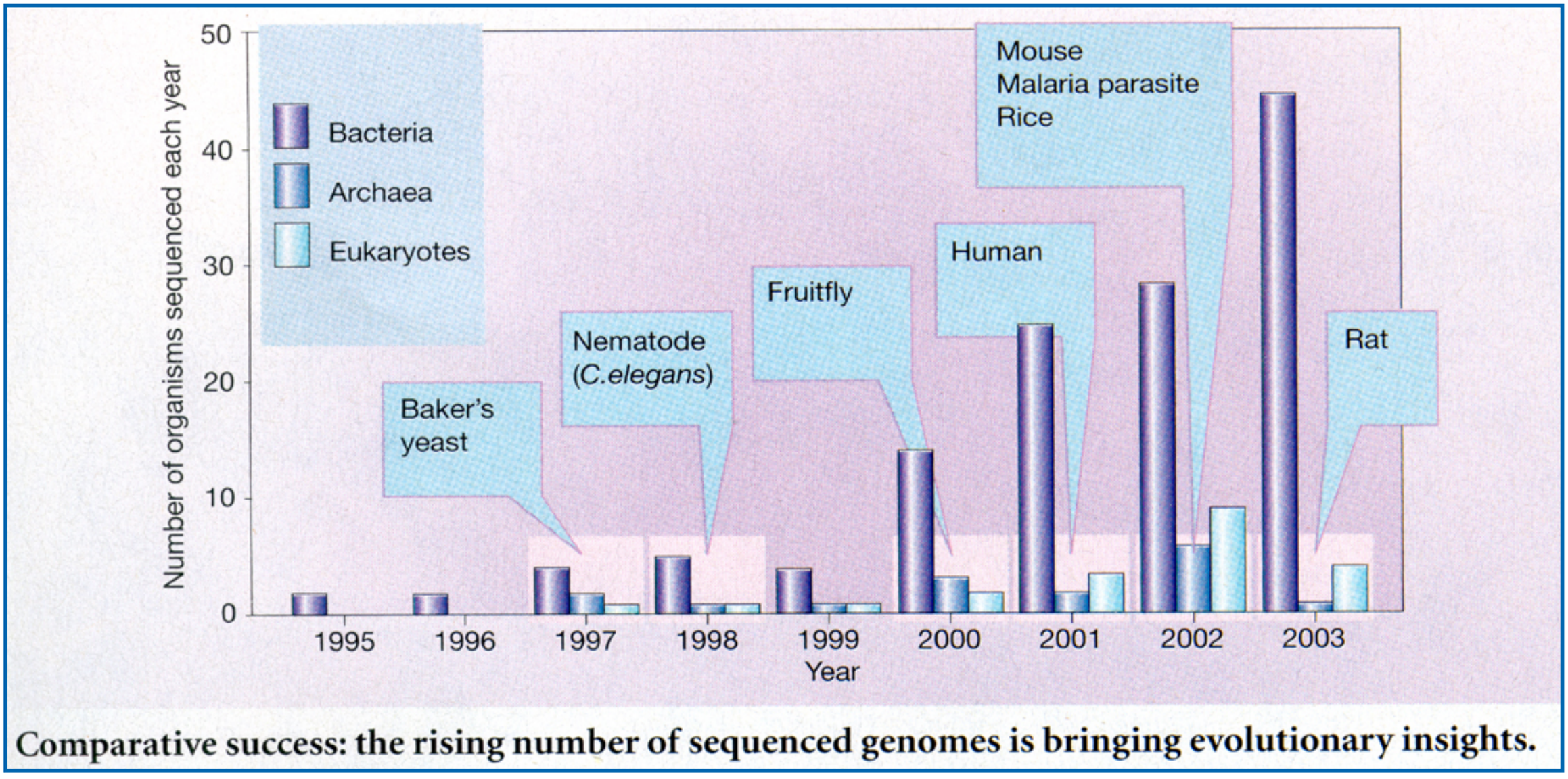
Stand und Perspektiven für das Gene Editing bei landwirtschaftlichen Nutztieren

Björn Petersen
Institute of Farm Animal Genetics
Friedrich-Loeffler-Institut (FLI)
Mariensee, Germany

SVT Frühjahrstagung, Zollikofen, 23.03.17

Current status genome sequencing



Comparative success: the rising number of sequenced genomes is bringing evolutionary insights.

Nature 426, 2003

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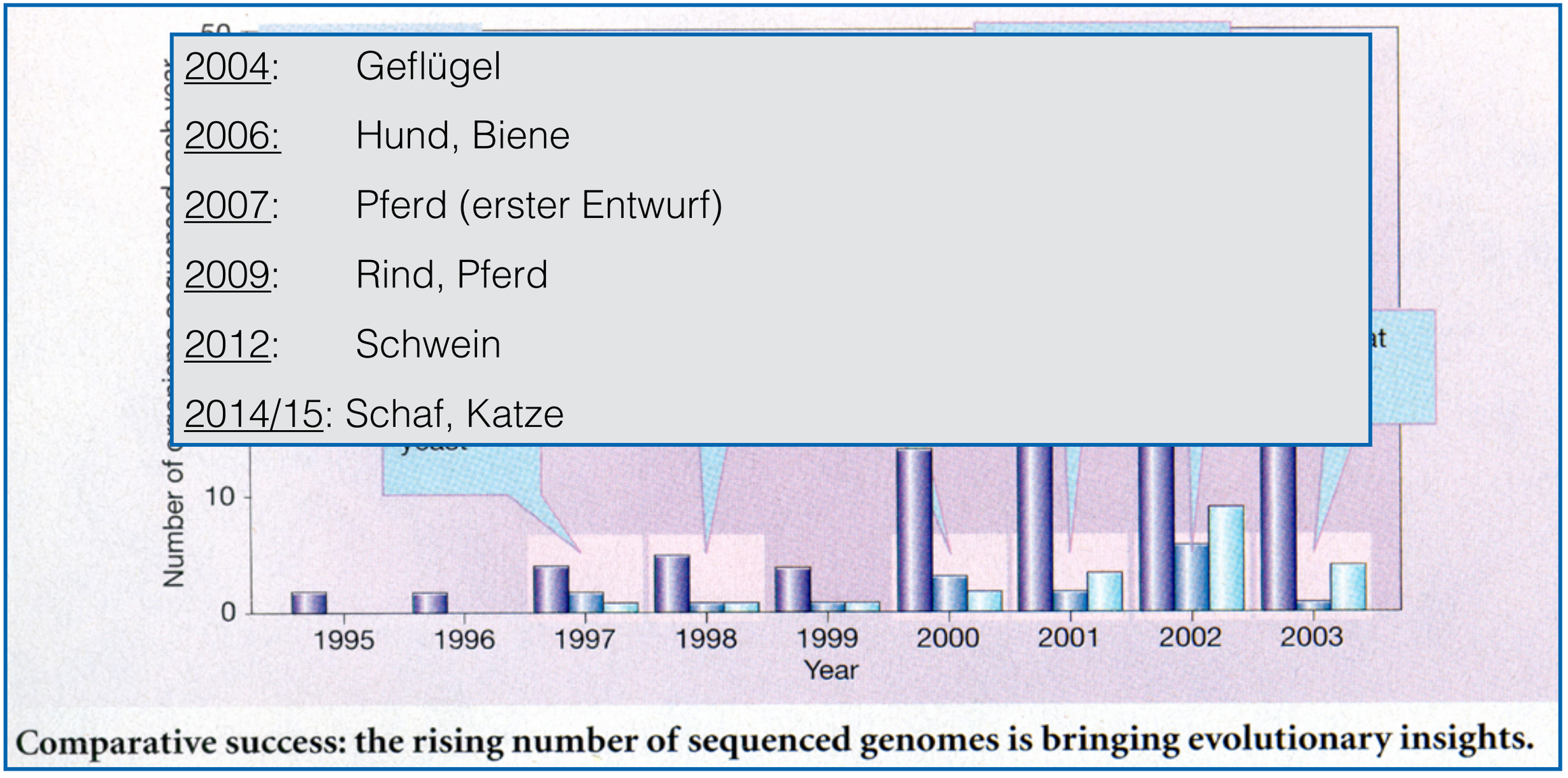
Current status genome sequencing



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Current status genome sequencing

2004: C

2006: P

2007: P

2009: P

2012: S

2014/15: S

T. J. Tabasco, female domestic Duroc pig, donor of porcine DNA blueprint

Nature 426, 2003

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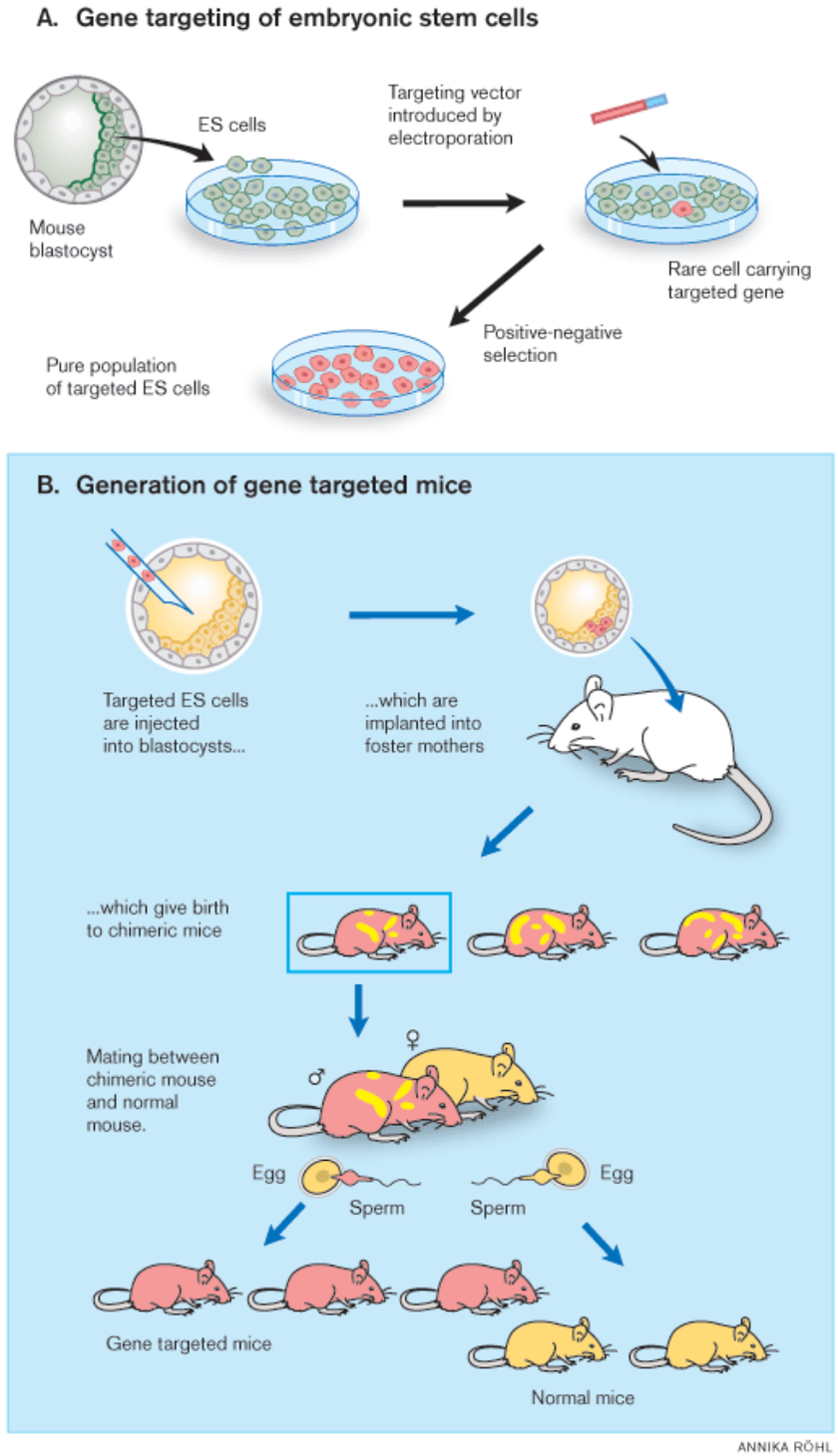
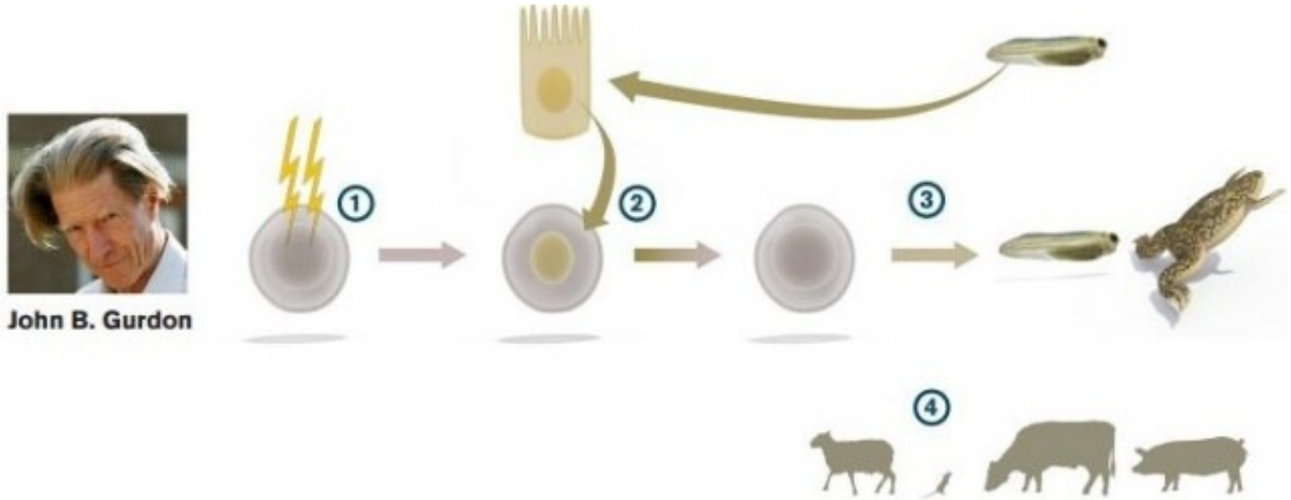
Genome Editing in mice (pre-GE era)



Quelle:http://www.nobelprize.org

Dolly breaks a dogma

Gurdon 1962



Sir John Gurdon, Nobel laureate 2012
(together with Shinya Yamanaka)

Dolly 1996-2003, with Sir Ian Wilmut

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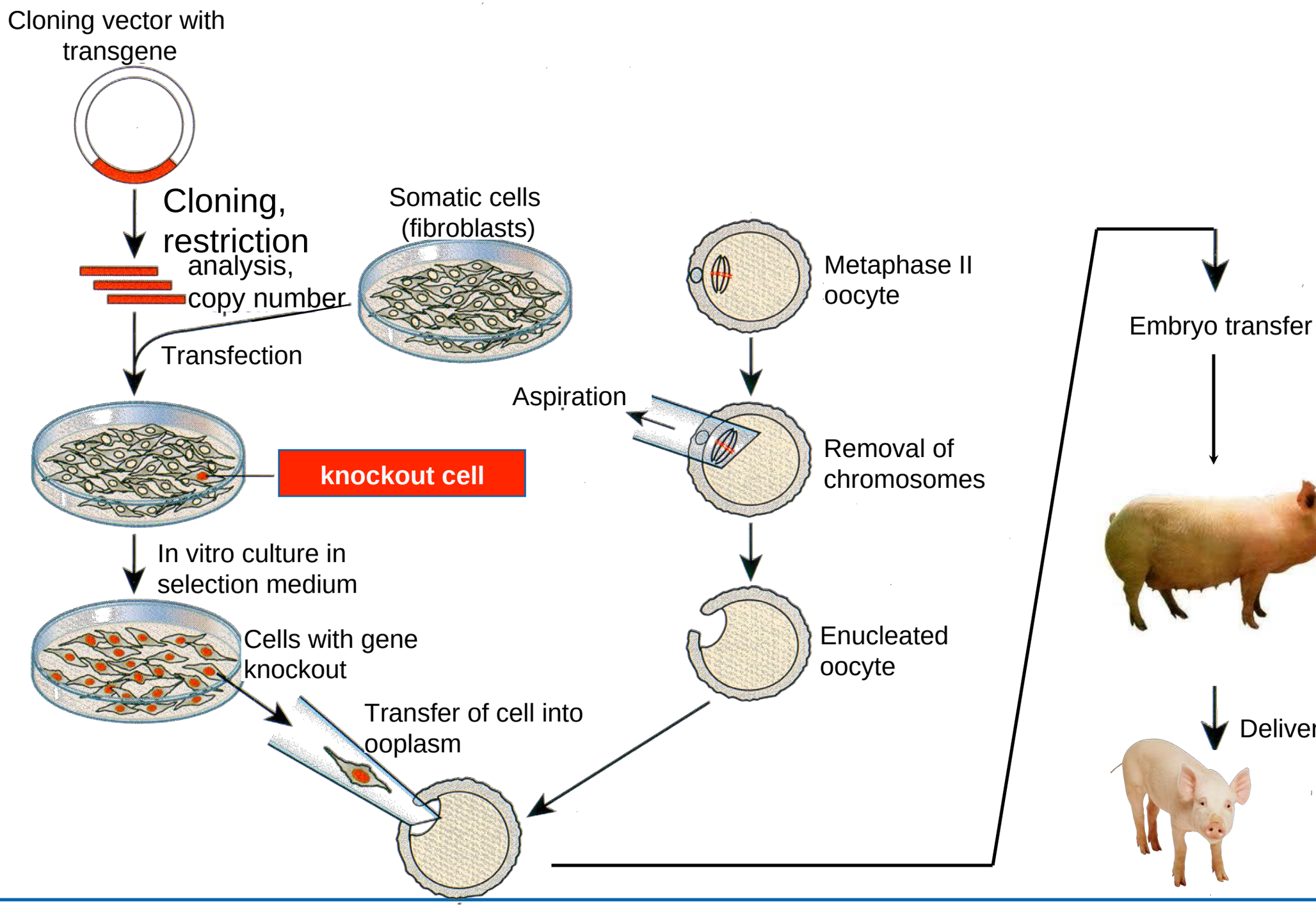
Production of knockout animals by somatic cell nuclear transfer



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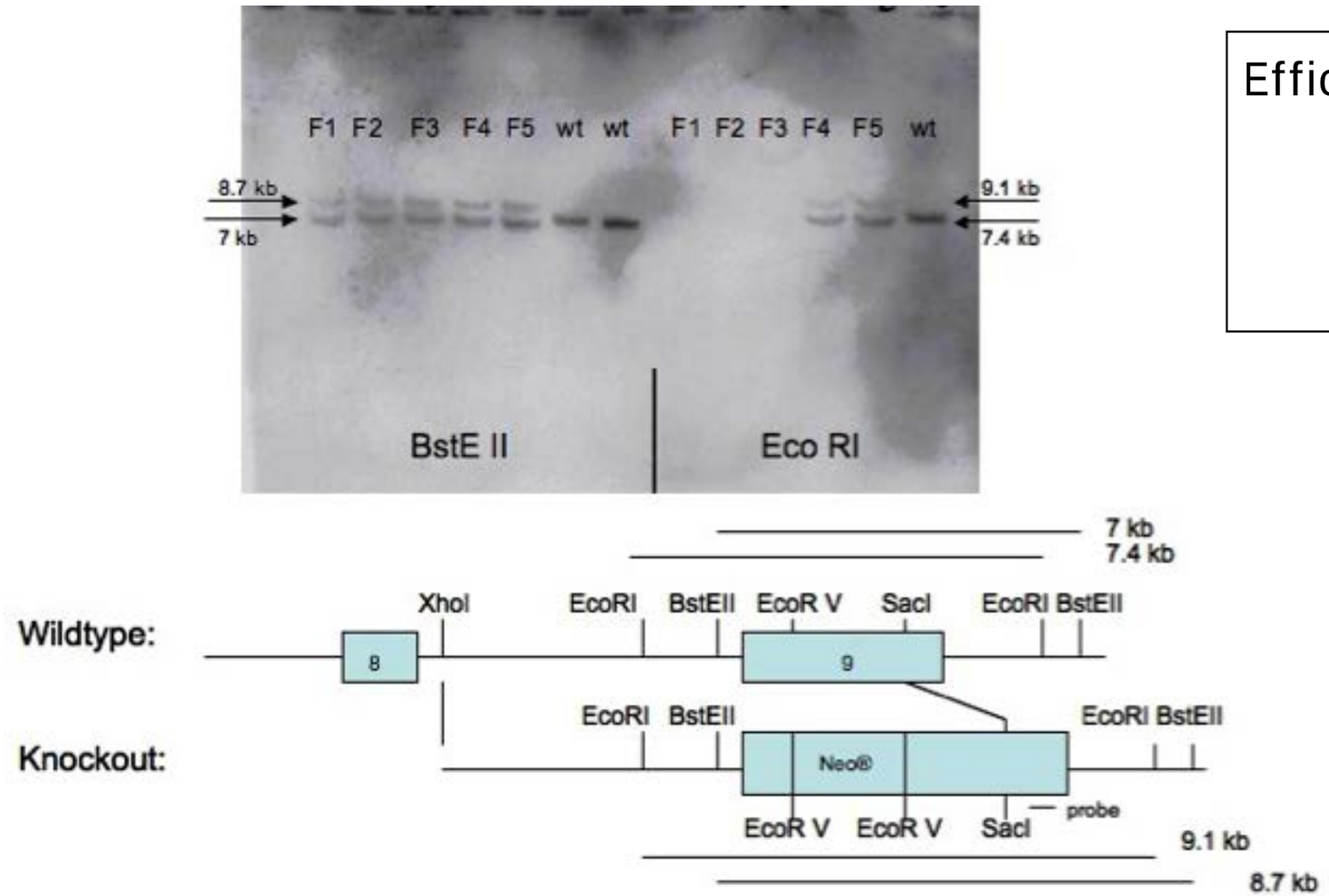
Gene knockout in farm animals until 2010



Efficiency of successful targeting:
$1x10^{-6}$

Efficiency of pig cloning:
$1\text{-}3x10^{-2}$

Feasible, but very inefficient!!
Only monoallelic KO achieveable.
Offspring are transgenic!

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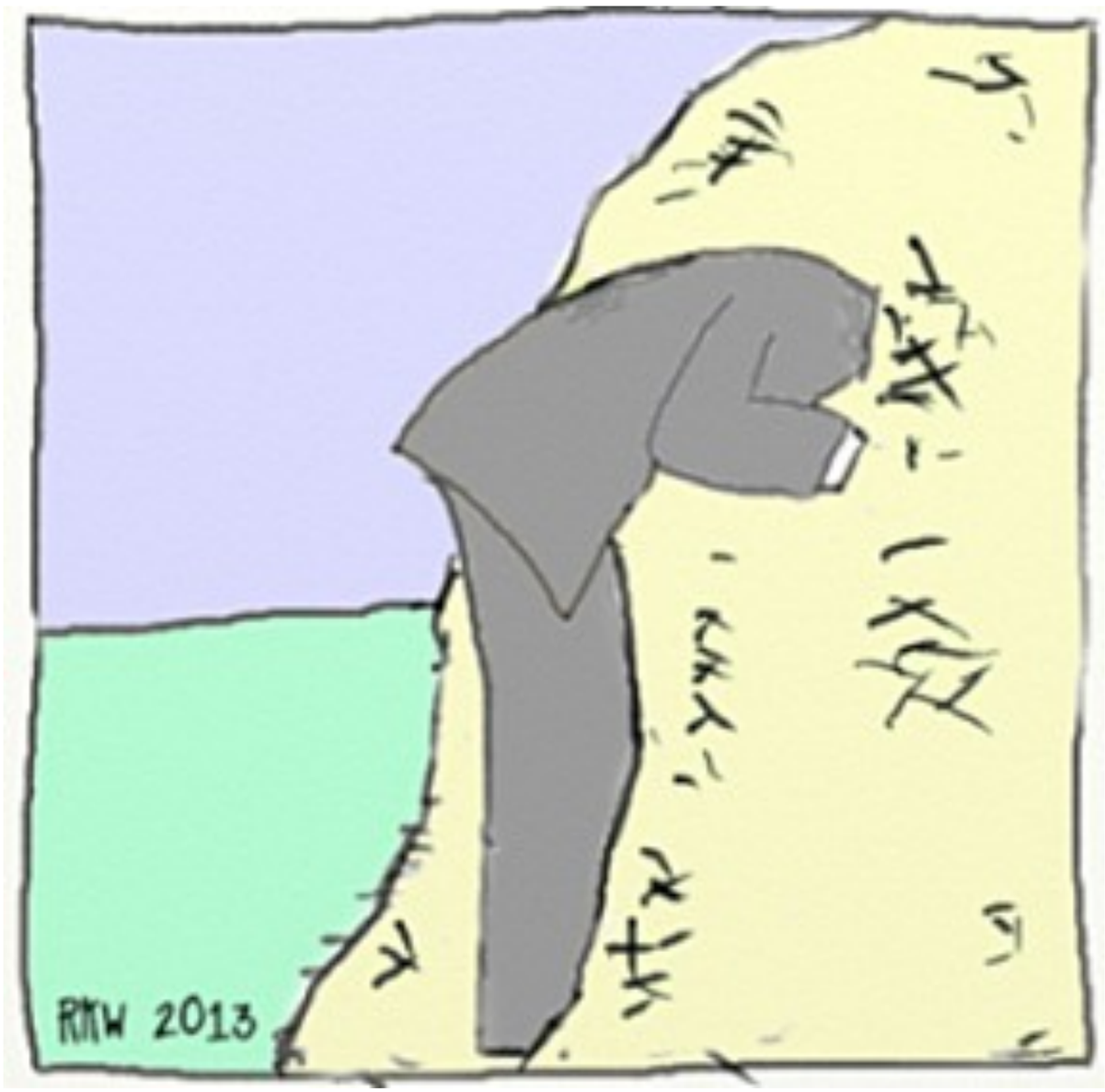
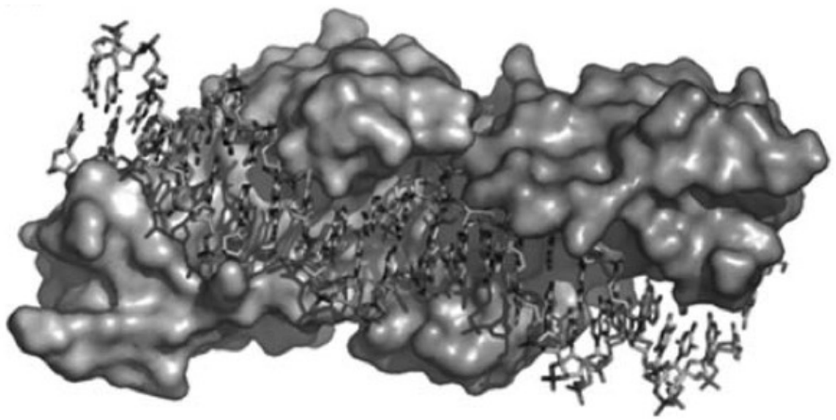


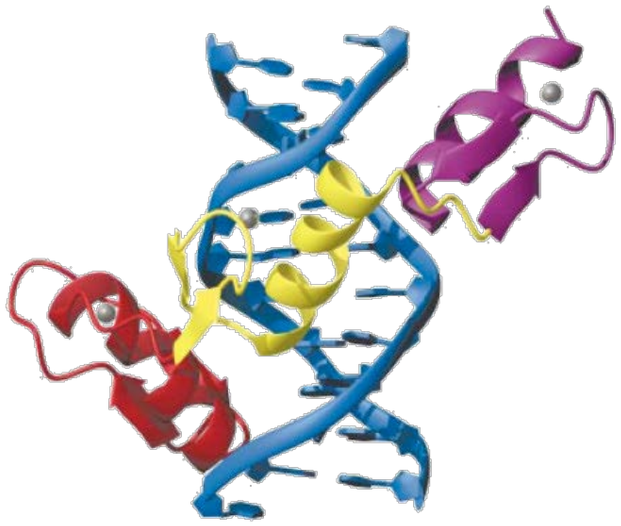
Finding the needle in the haystack!!

Gene editing tools

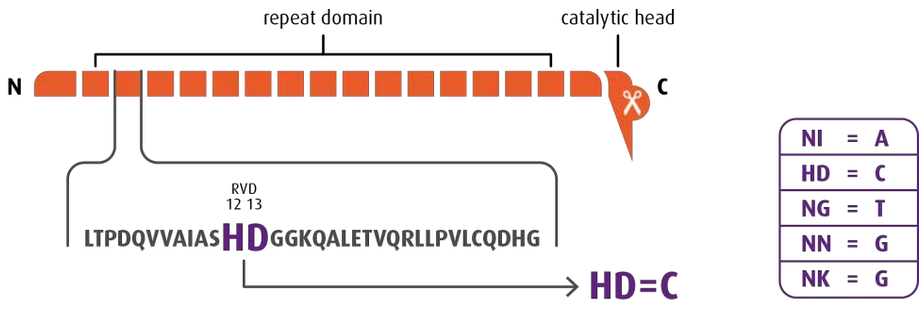
Classes of gene editing tools



Meganucleases



Zinc-Finger Nucleases



Transcription activator- like effektor nucleases (TALENs)

CRISPR/CAS

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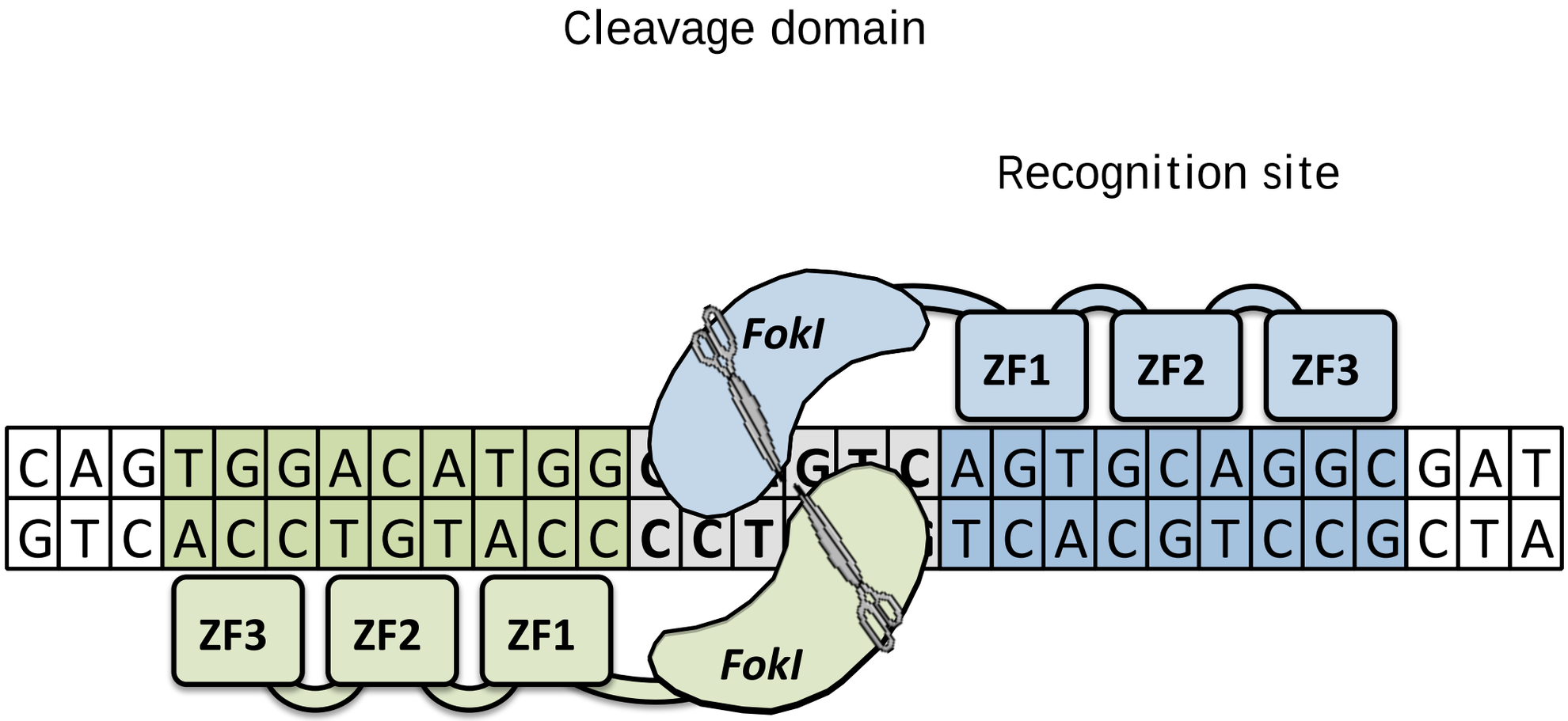
General mechanisms of gene editors



Gene editing starts with dsDNA cleavage

Petersen, B., and Niemann, H. (2015), Transgenic Res *24*, 381-396.

General mechanisms of gene editors

C	A	G	T	G	G	A	C	A	T	G	G	**G**	**G**	**A**
G	T	C	A	C	C	T	G	T	A	C	C	**C**	**C**	**T**

G	**T**	**C**	A	G	T	G	C	A	G	G	C	G	A	T
C	**A**	**G**	T	C	A	C	G	T	C	C	G	C	T	A

HDR

NHEJ

Petersen, B., and Niemann, H. (2015), Transgenic Res *24*, 381-396.

General mechanisms of gene editors

C	A	G	T	G	G	A	C	A	T	G	G	**G**	**G**	**A**
G	T	C	A	C	C	T	G	T	A	C	C	**C**	**C**	**T**

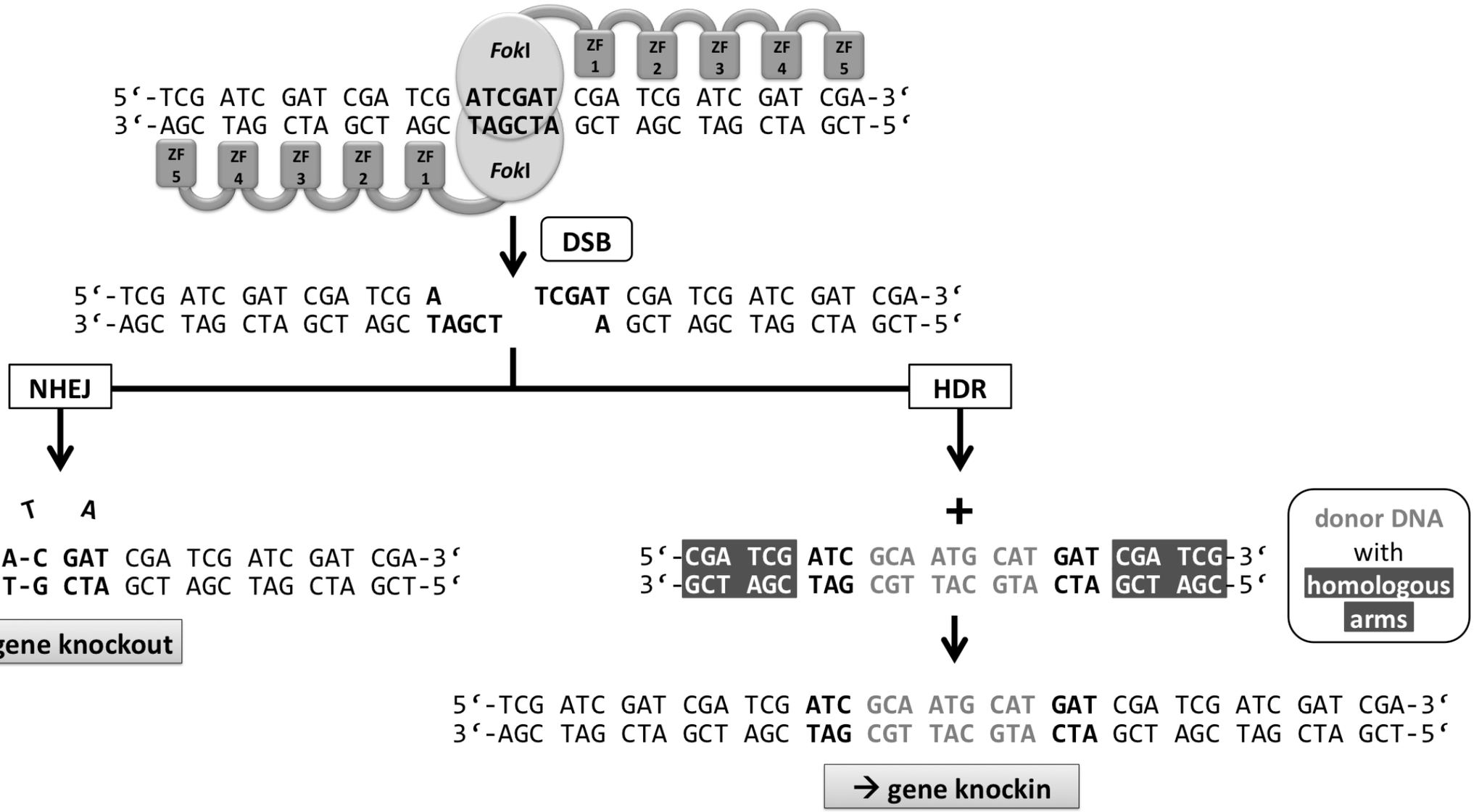
G	**T**	**C**	A	G	T	G	C	A	G	G	C	G	A	T
C	**A**	**G**	T	C	A	C	G	T	C	C	G	C	T	A

HDR

NHEJ

Petersen, B., and Niemann, H. (2015), Transgenic Res *24*, 381-396.

Gene knockout and knock-in by using GEs



Hauschild-Quintern, J., Petersen, B. et al. (2013),
Cellular and molecular life sciences : CMLS *70*, 2969-2983.

Genome Editing

- Use of Genome Editors to correct a genetic information

Es kann der Dümmste nicht in Frieden leben, wenn es dem bösen Nachbar nicht gefällt.

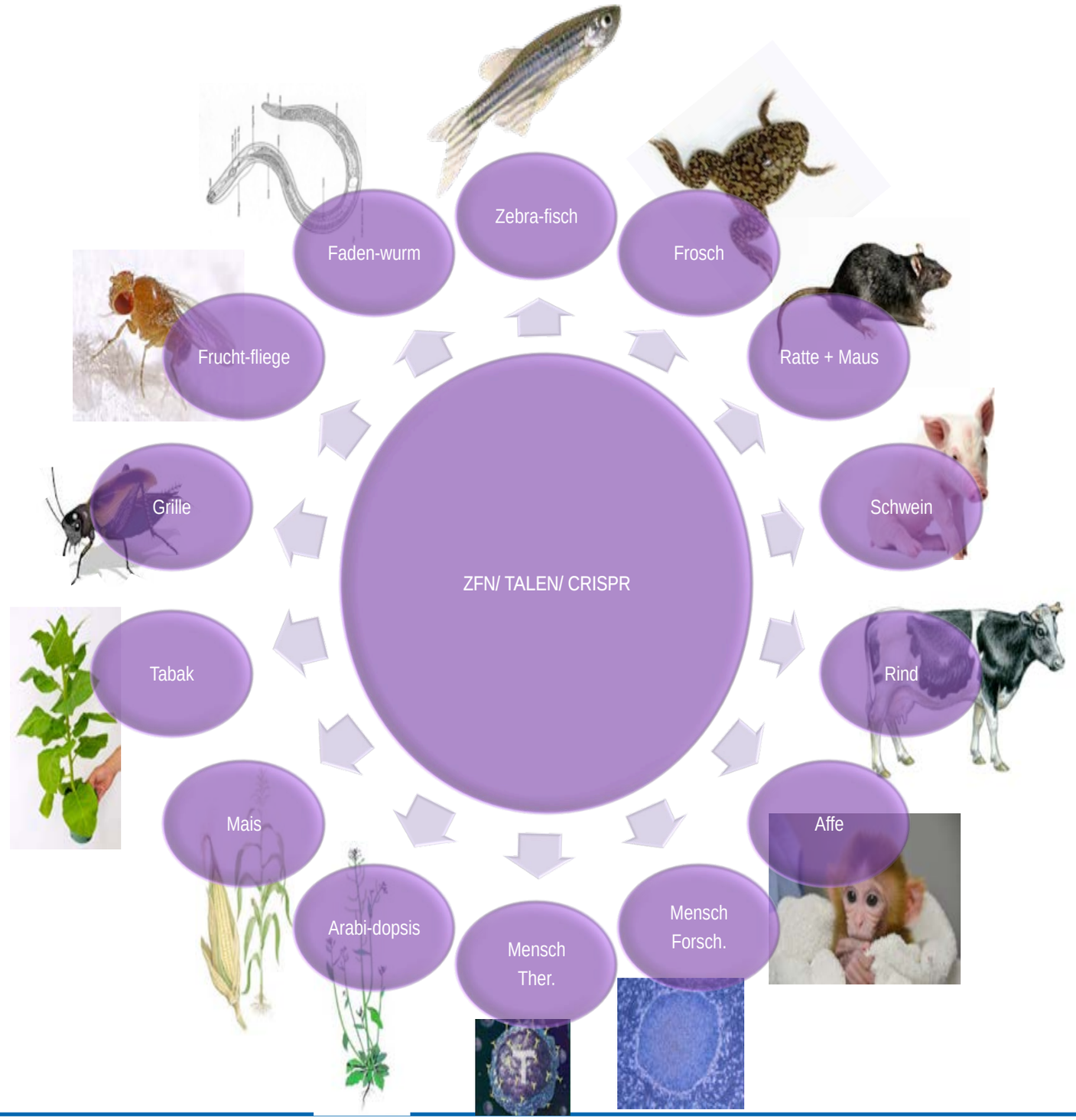
Es kann de mste nicht in Frieden leben, wenn es dem bösen Nachbar nicht gefällt.

kann der Frömmste nicht in Frieden

Es kann der Frömmste nicht in Frieden leben, wenn es dem bösen Nachbar nicht gefällt.

(Friedrich Schiller, Wilhelm Tell)

Universal application of Genome Editors



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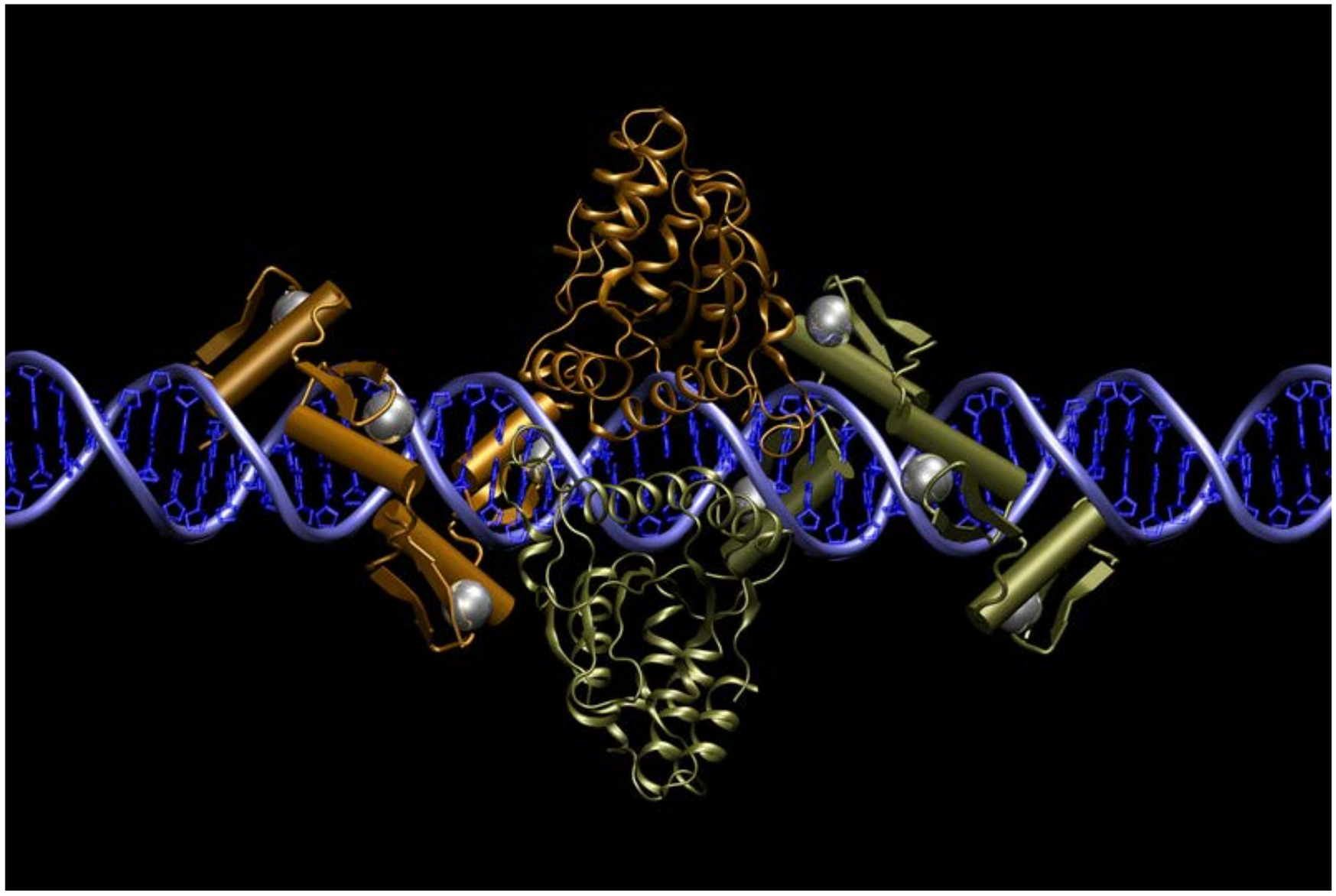
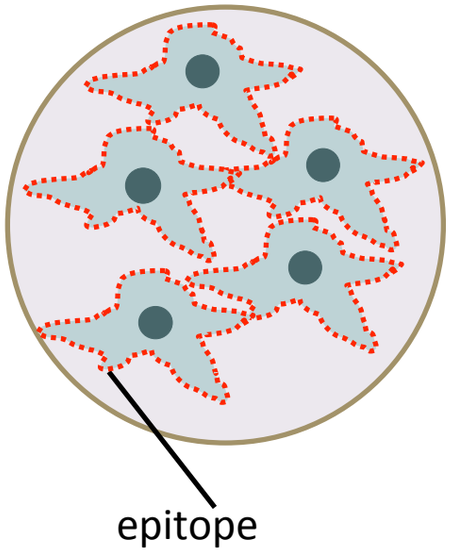
Gene Editing by ZFN



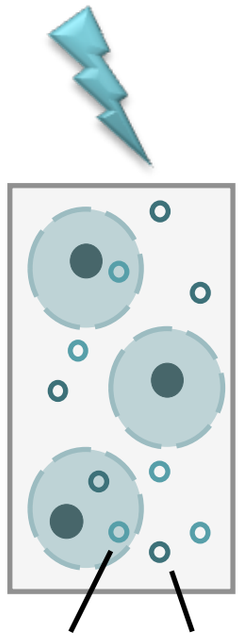
Generation of KO pigs by ZFN and SCNT

Cell culture of porcine fetal fibroblasts (WT)



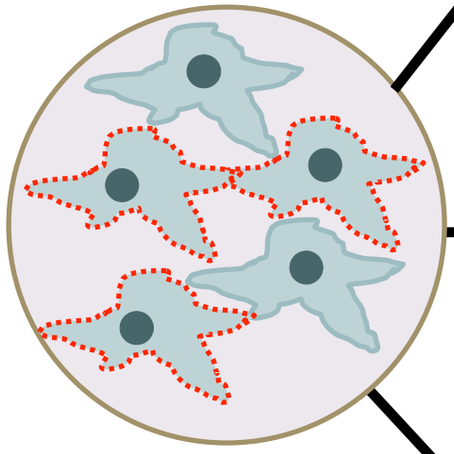
epitope

electro-poration

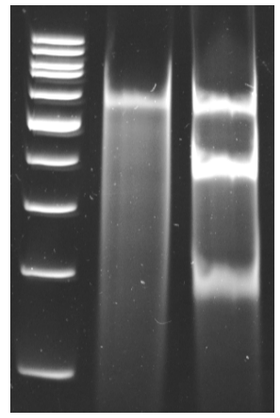


ZFN plasmids

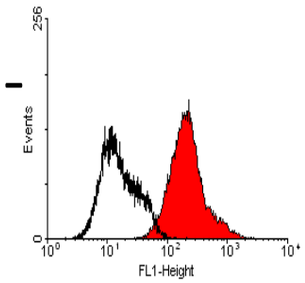
Cell culture: <u>mixture of edited and wt cells</u>



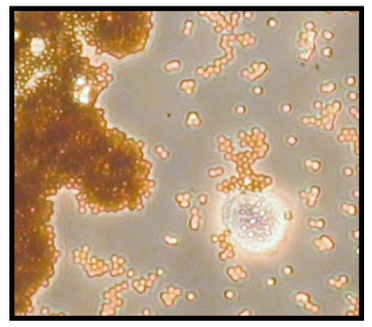
DNA-isolation
- Cel-I assay
- NHEJ



FACS analysis - biallelic KO



Selection of edited cells (FACS, Ab staining)



SCNT

Gene edited offspring

Hauschild, Petersen et al. (2011),
Proc Natl Acad Sci U S A 108 (29), 12013-17

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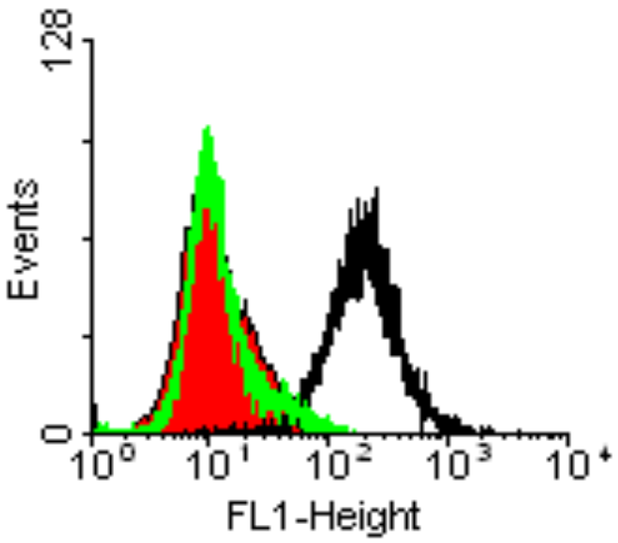
First pigs with a biallelic Ko of an endogenous gene (GGTA1) mediated by ZFNs

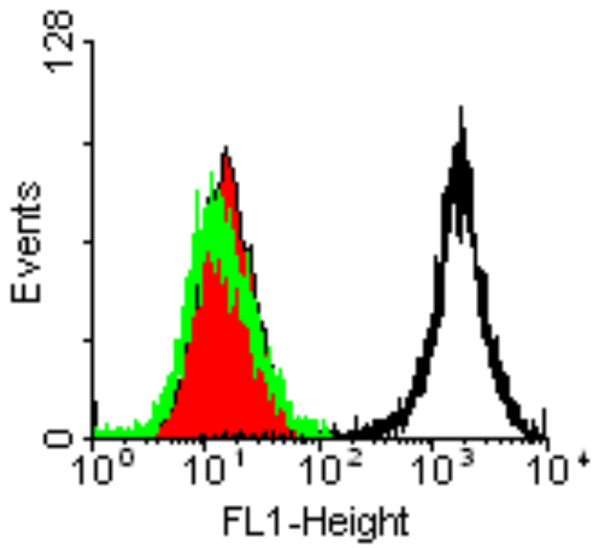
Lily
born December 16th 2010,
died 3 days later

- Fetal cells obtained from the first cloning served as donors in a recloning attempt (“Lily”)
- Cells from transfection E45 were also used as donor cells (“Lia”)

→5 pregnancies resulted in 9 living piglets

Lia
born January 6th 2011





ZFN-GalKO cells HR-GalKO cells Gal-positive cells

Hauschild, Petersen et al. (2011),
Proc Natl Acad Sci U S A 108 (29), 12013-17

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Sequencing of porcine GGTA1-locus

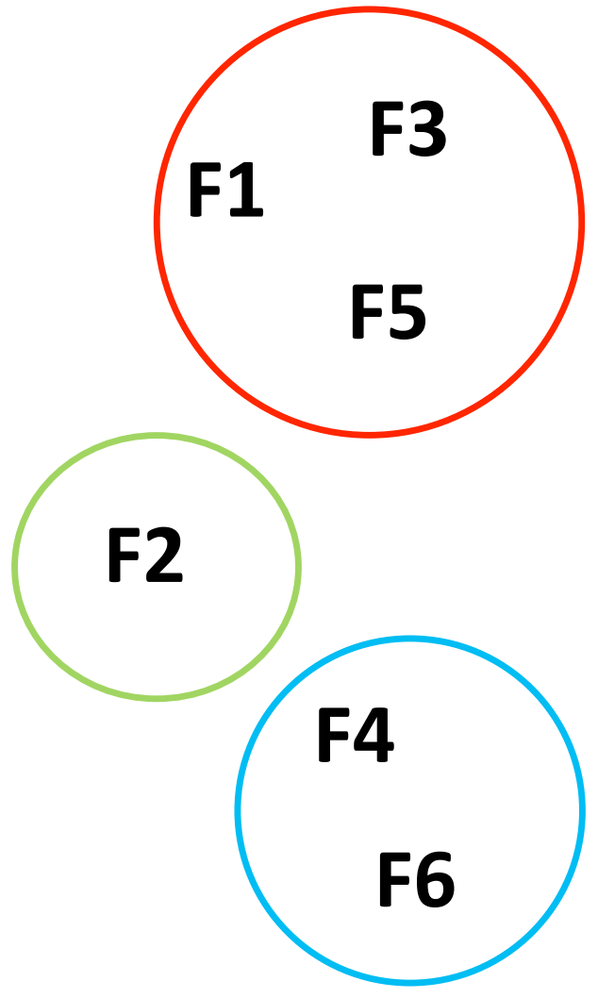
ZFN-GGTA1-23713 ZFN-GGTA1-23714

E41

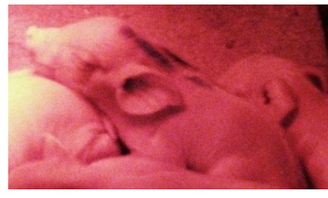
```
WT:   CGGTGGCTCAGCTACAGGCCTGGTGGTACAAGGCAC
C1F1: CGGTGGCTCAGCTACAG-CCTGGTGGTACAAGGCAC
      CGGTGGCTCAG-T------CTGGTGGTACAAGGCAC
C1F2: CGGTGGCTCAGCTACA----TGGTGGTACAAGGCAC
      CGGTGGCTCAGCTACAGGCCTGGTGGTACAAGGCAC
                       CCGG
C1F3: CGGTGGCTCAGCTACAG-CCTGGTGGTACAAGGCAC
      CGGTGGCTCAG-T------CTGGTGGTACAAGGCAC
C1F4: CGGTGGCTCAGCTACAG-CCTGGTGGTACAAGGCAC
C1F5: CGGTGGCTCAGCTACAG-CCTGGTGGTACAAGGCAC
      CGGTGGCTCAG-T------CTGGTGGTACAAGGCAC
C1F6: CGGTGGCTCAGCTACAG-CCTGGTGGTACAAGGCAC
```

E45

```
C2F1: CGGTGGCTCAGCTACAGGCCT------ACAAGGCAC
      CGGTGGCTCAGCTAC-----TGGTGGTACAAGGCAC
C2F2: ATGGACGTGGAT--(-96bp)--ATACGAGAGGCGG
```



➡ **Lia**

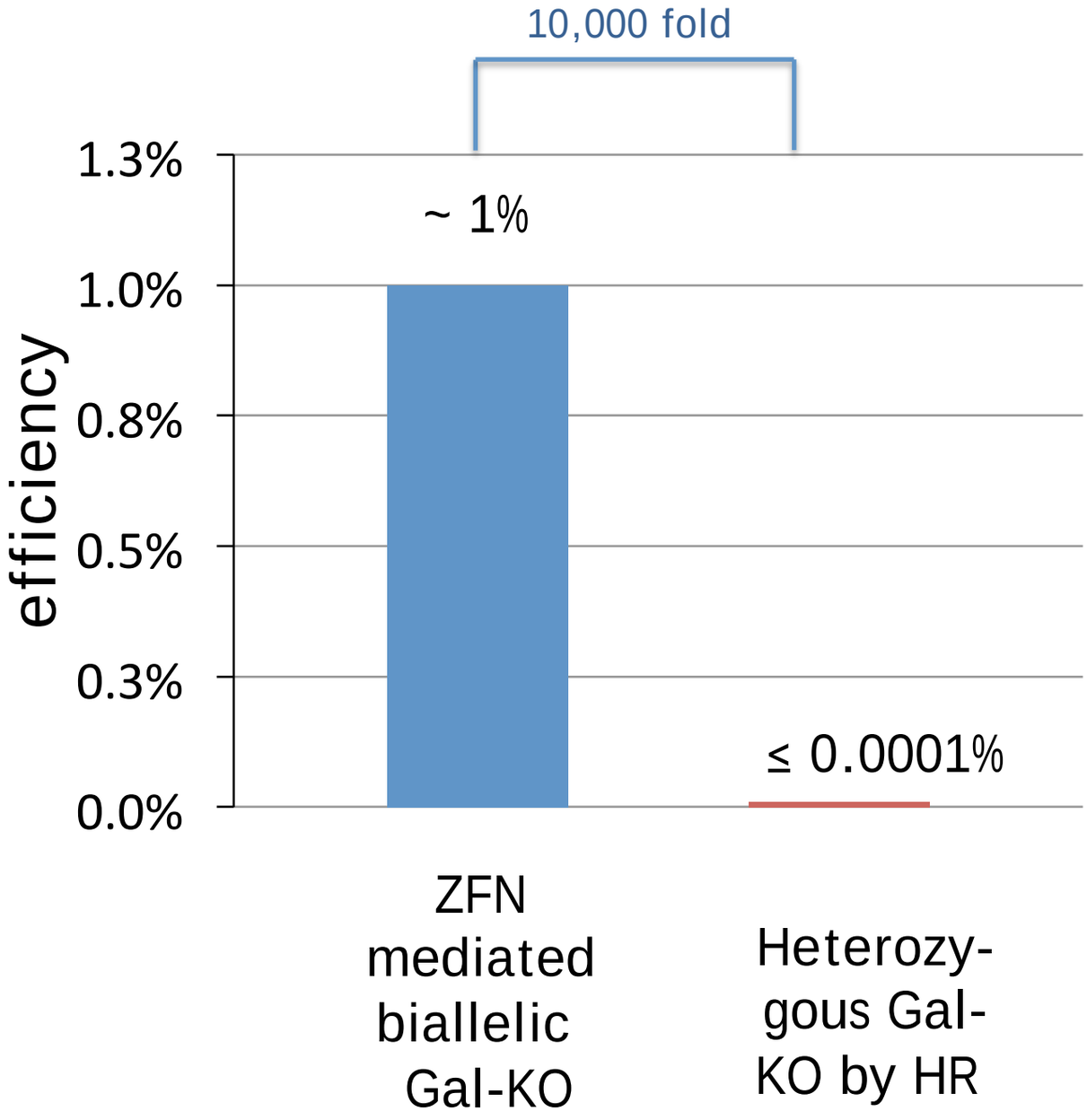


Hauschild, Petersen et al. (2011),
Proc Natl Acad Sci U S A 108 (29), 12013-17

Targeting of the porcine GGTA1-locus with ZFN vs. conventional targeting

- ~ 1% Gal-negative cells (biallelic KO)
- Compared to conventional heterozygous Gal-KO

Enhancement of more than 10,000 fold!



Hauschild, Petersen et al. (2011), Proc Natl Acad Sci U S A 108 (29), 12013-17

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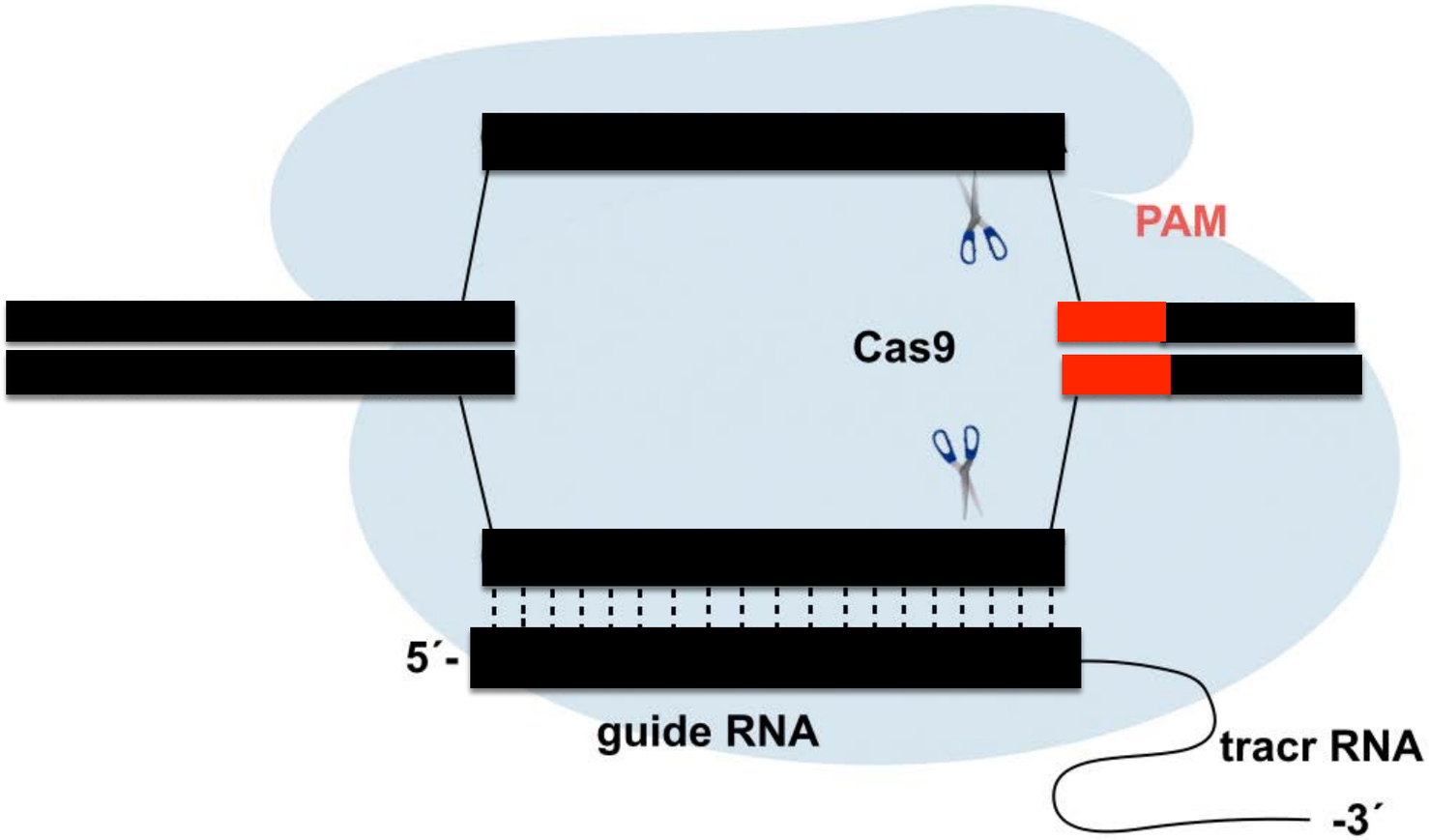
Genome Editing by CRISPR/Cas



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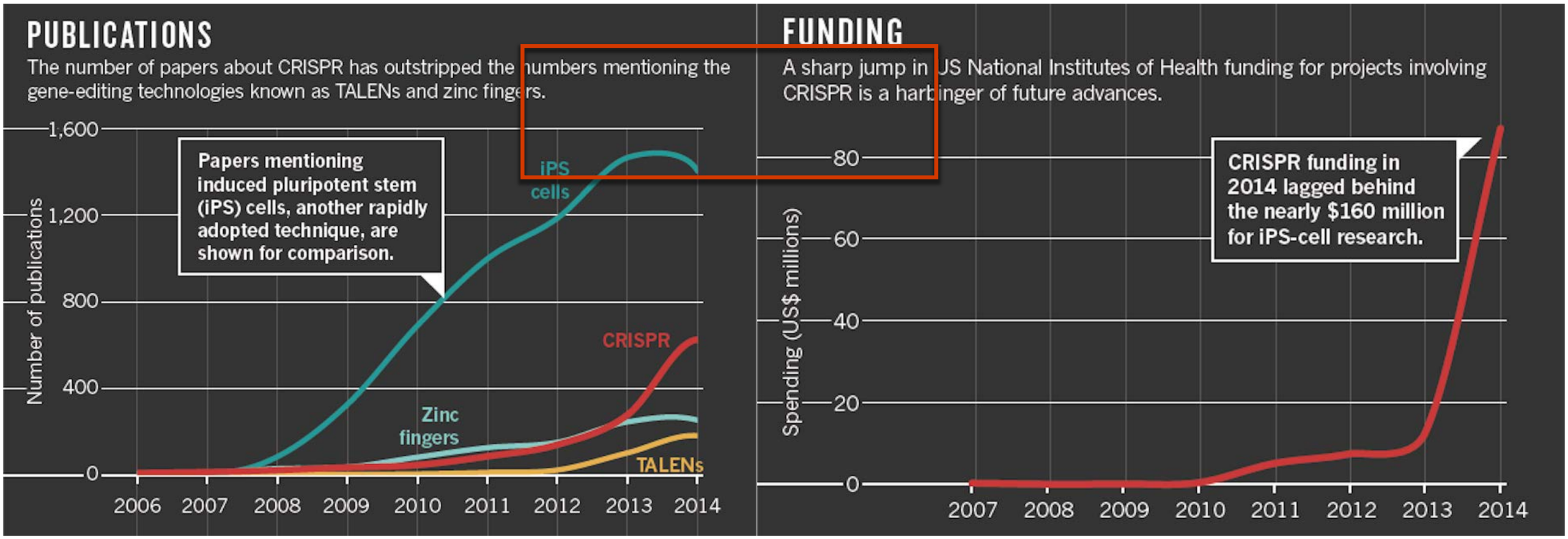
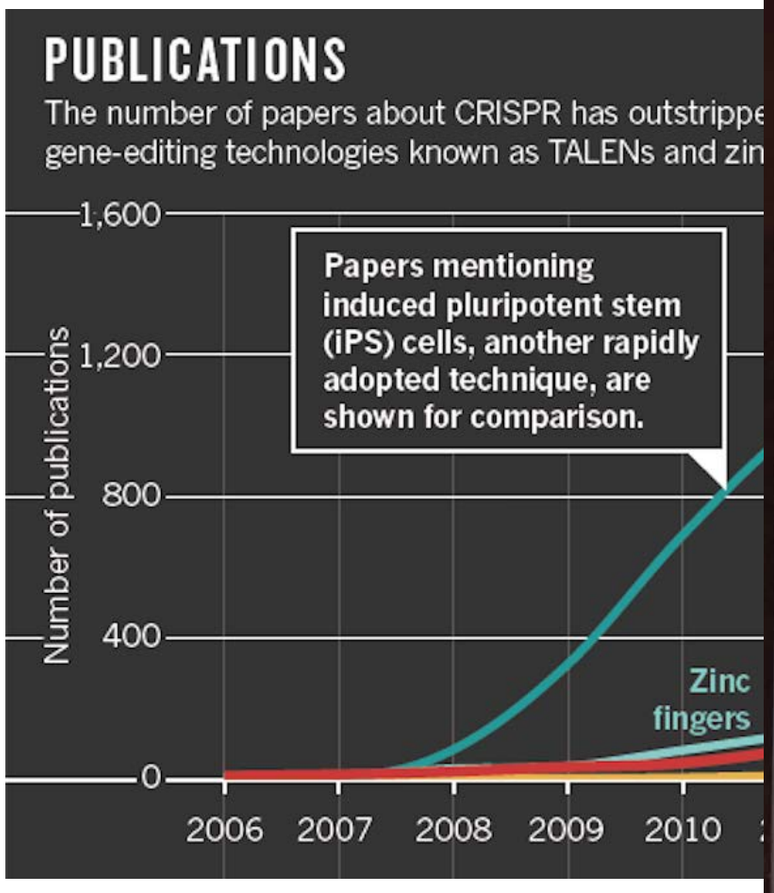
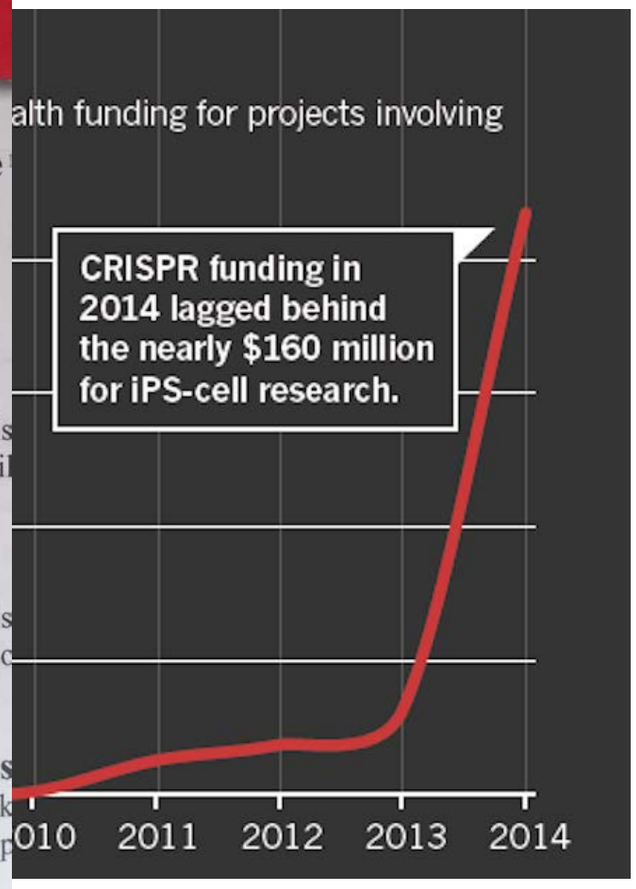
Importance of CRISPR/Cas



Importance of CRISPR/Cas





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Emmanuelle Charpentier

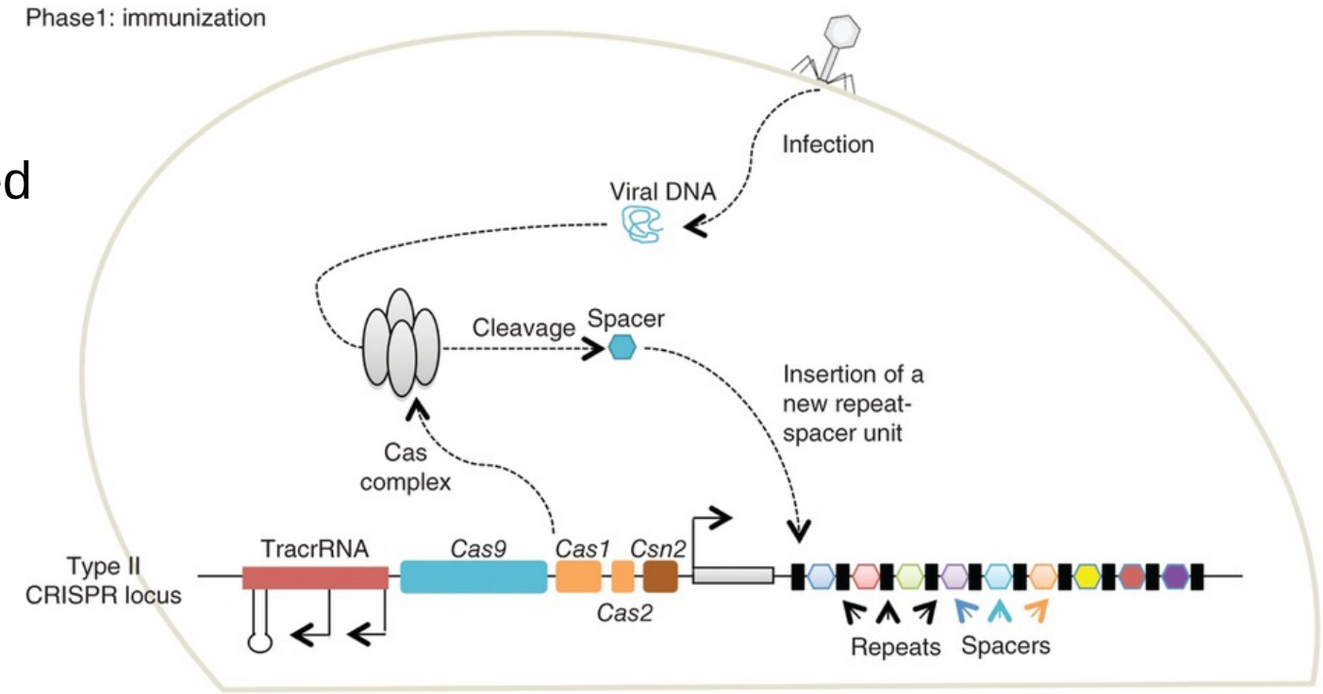
Max Planck Institute for Infection Biology, Berlin, Germany

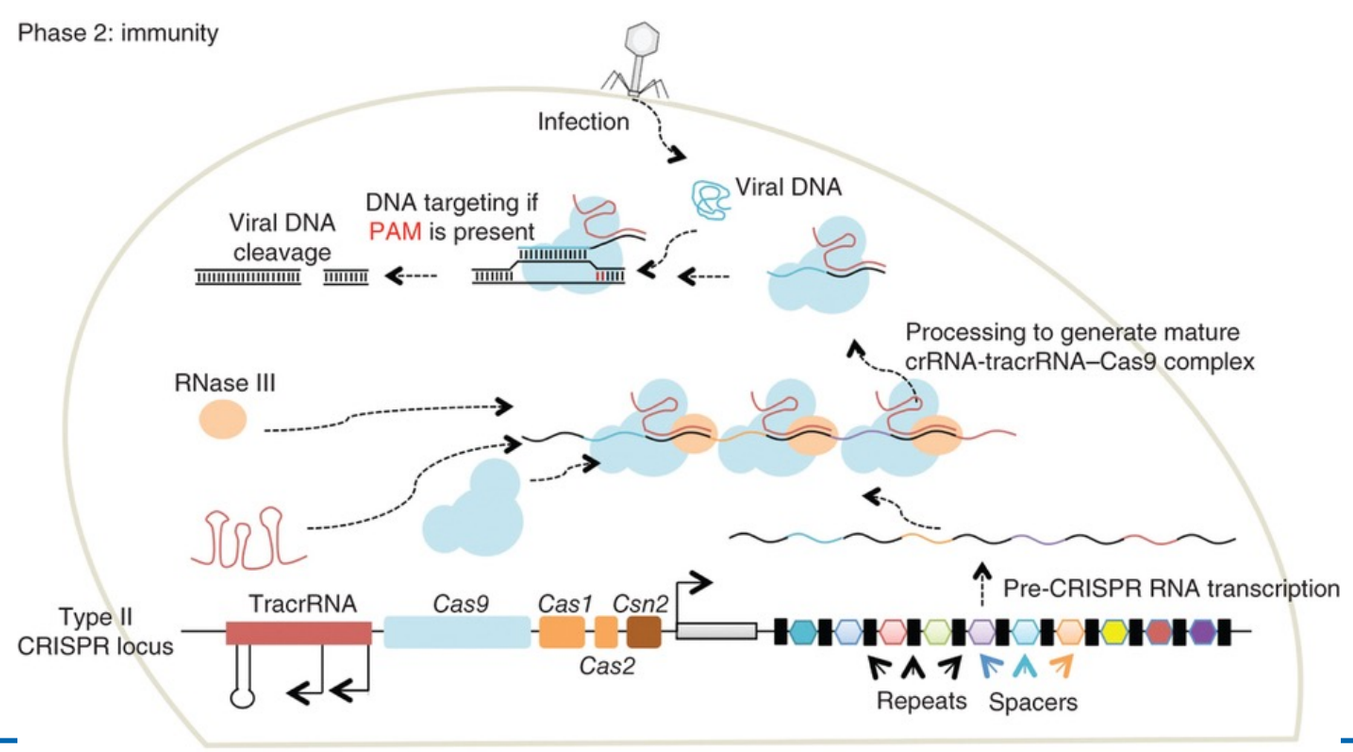
Jennifer Doudna

Doudna Lab
University of California
Berkeley, USA

CRISPRs: Hallmarks of acquired immunity in bacteria

CRISPR:
Clusters of regularly interspaced
short palindromic repeats





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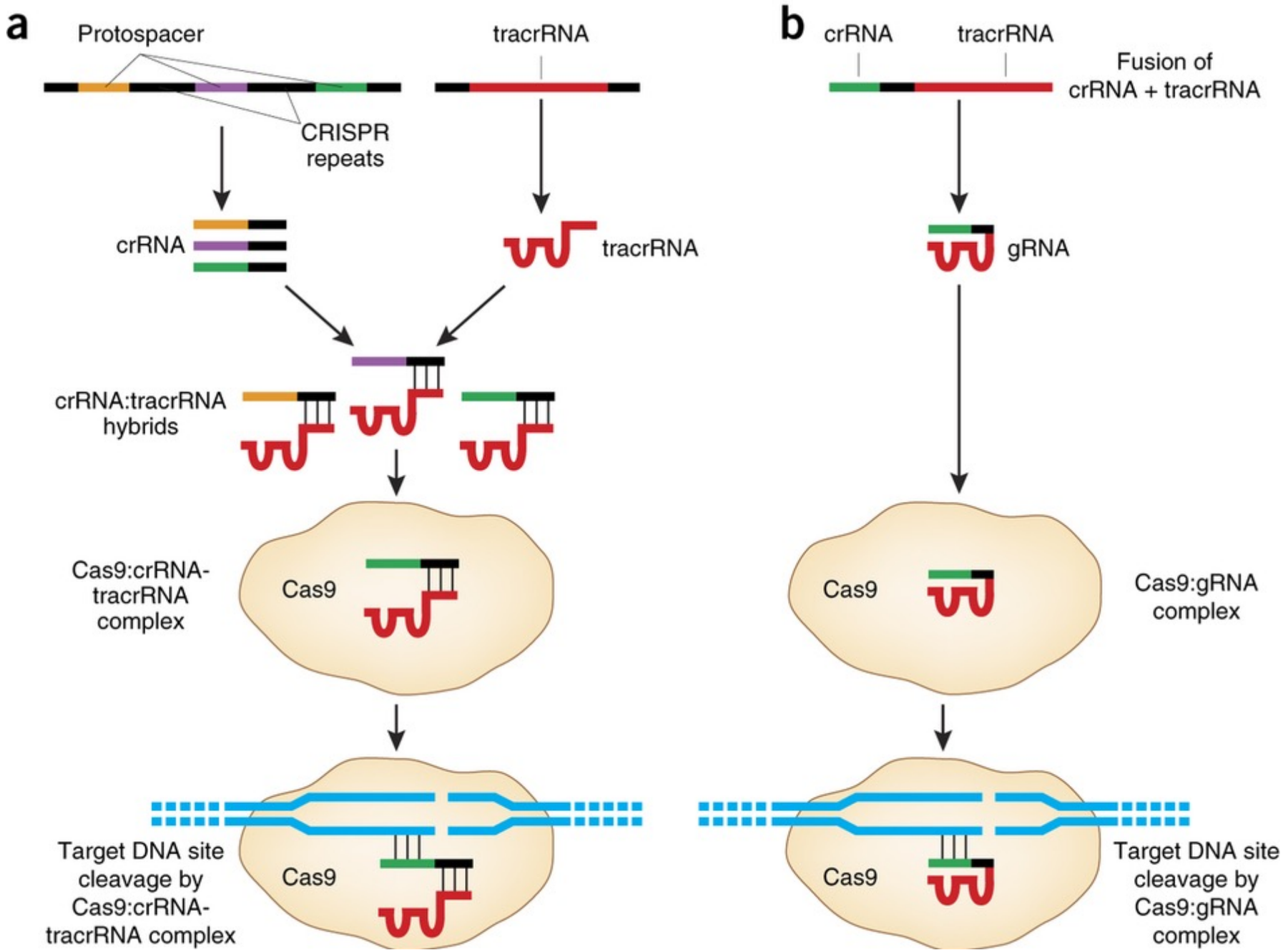
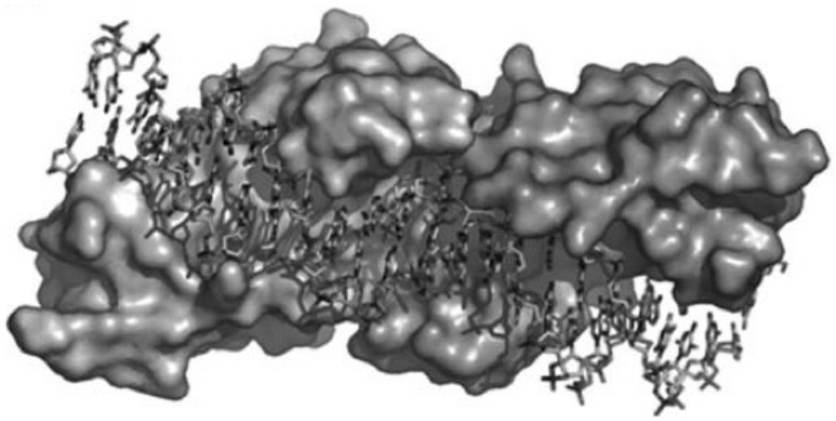
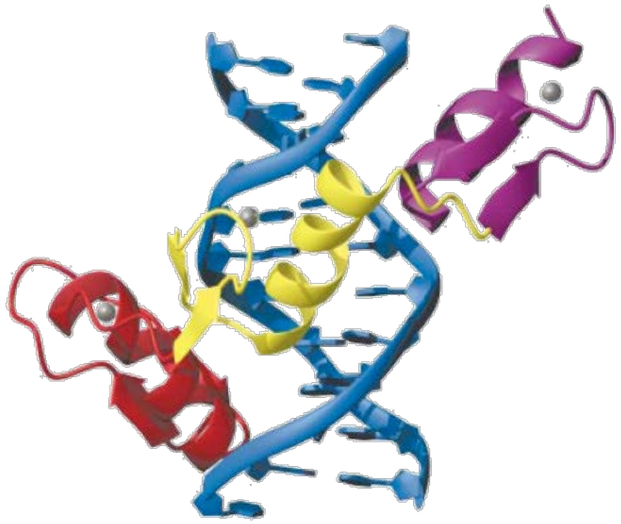

a
Protospacer
tracrRNA
CRISPR
repeats
crRNA
tracrRNA
crRNA:tracrRNA
hybrids
Cas9:crRNA-
tracrRNA
complex
Cas9
Target DNA site
cleavage by
Cas9:crRNA-
tracrRNA complex
b
crRNA
tracrRNA
Fusion of
crRNA + tracrRNA
gRNA
Cas9:gRNA
complex
Target DNA site
cleavage by
Cas9:gRNA
complex

Classes of gene editing tools

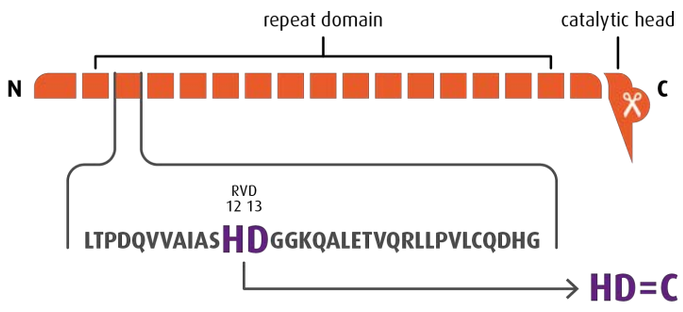


Meganucleases

Protein-based

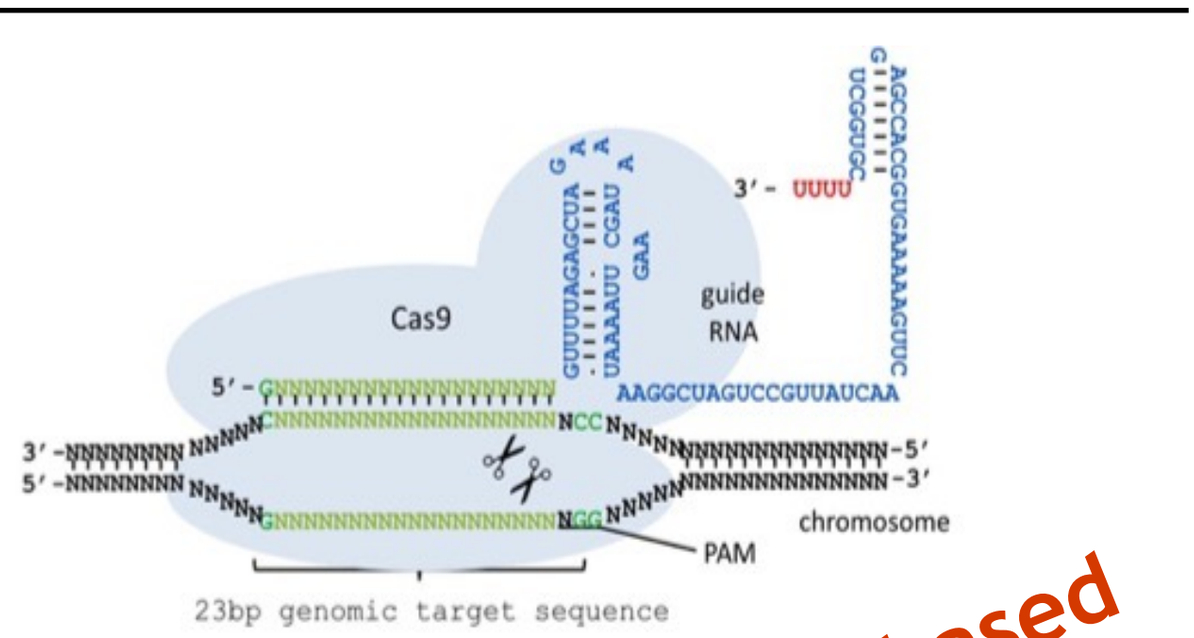


Zinc-Finger Nucleases



NI	=	A
HD	=	C
NG	=	T
NN	=	G
NK	=	G

Transcription activator- like effektor nucleases (TALENs)



CRISPR/CAS

RNA-based

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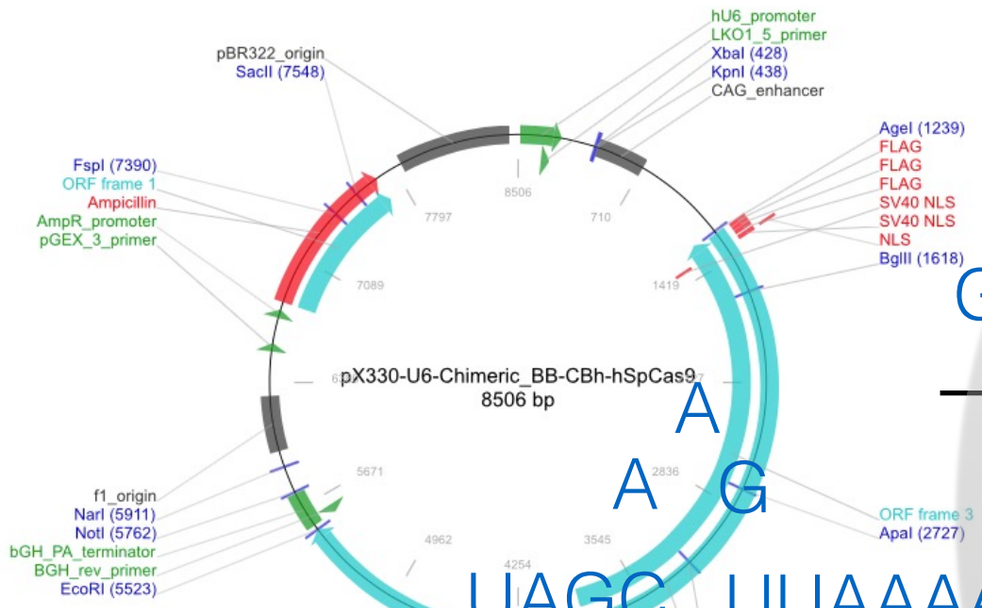
CRISPR/Cas in action



UCGGUGCUUU-3´

G

AGCCACGGUGAAAAAGUU C

A

A G

A

UAGC UUAAAAUAAGGCUAGUCCGUUAUC

AUCGAGAUUUUG GGACUUGAUGUUCCGAGCCG

5´-CACCGCCTGAACTACAAGGCTCGGC-3´

3´-CGGACTTGATGTTCCGAGCCGCAAA-5´

TARGETED LOCUS: 20nt PAM

ATTGCAGGGTTGCATTAGCCCTGAACTACAAGGCTCGGCAGGTCACCAA

TAACGTCCAACGTAATCGGACTTGATGTTCCGAGCCGTCCAGTGGTT

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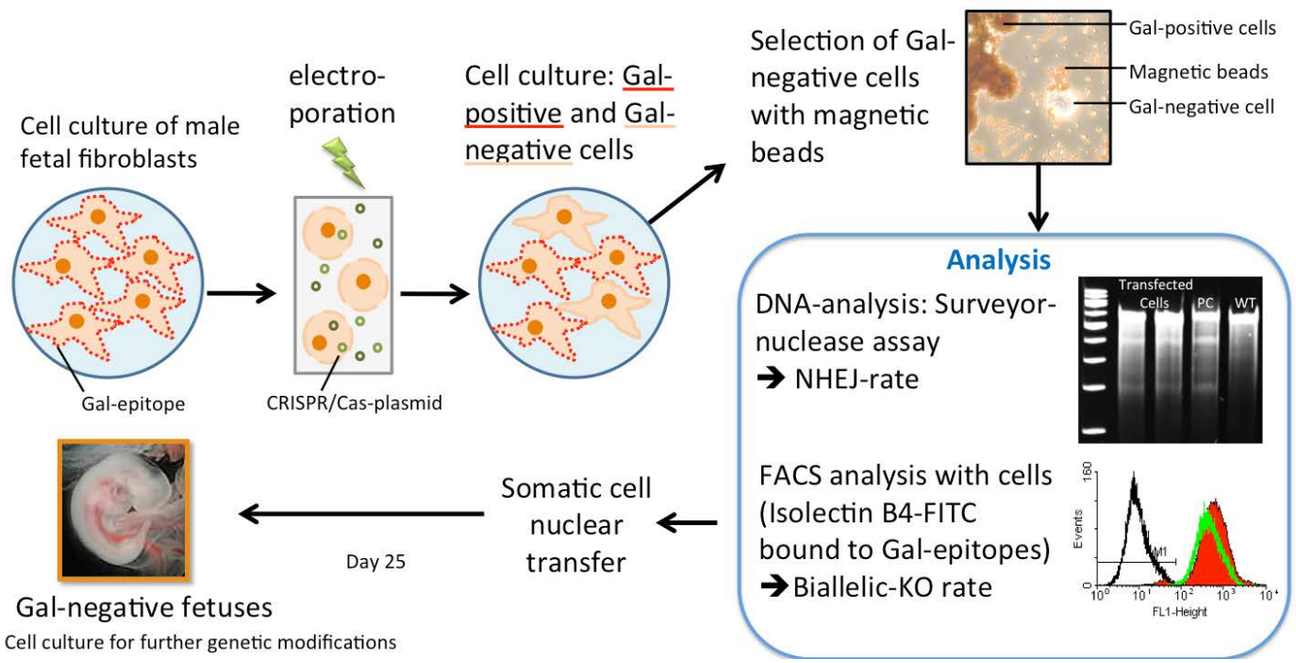
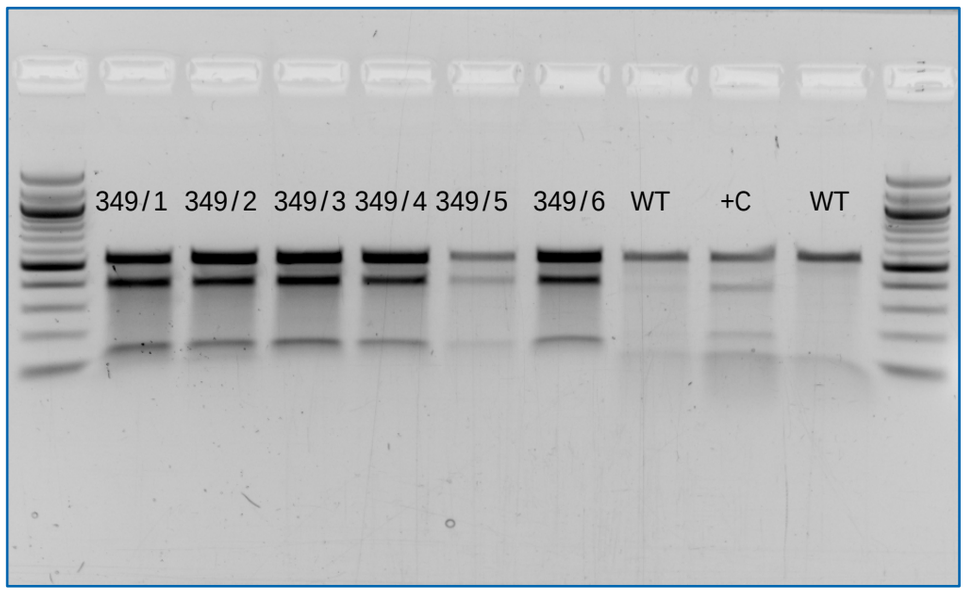
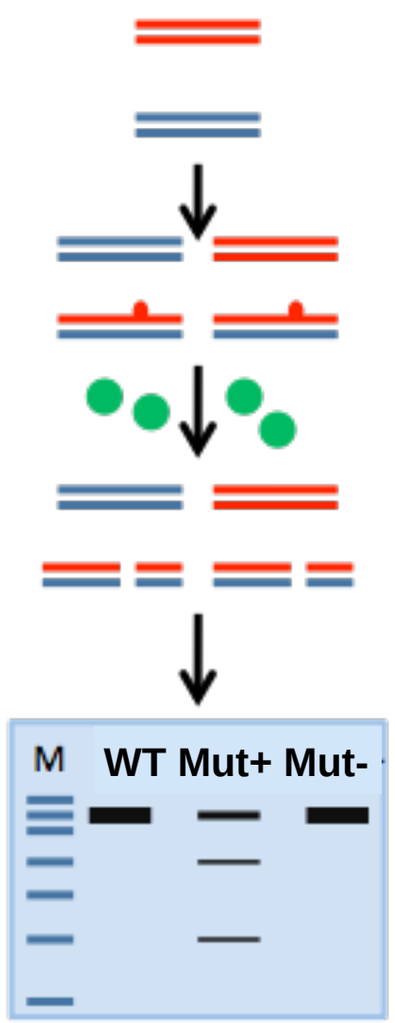
Targeting the porcine GGTA1-locus with CRISPR/Cas9





Surveyor nuclease assay of six cloned fetuses obtained from E18-cells as donor cells. (WT= wildtype control, +c= positive control biallelic GGTA1-KO cells, 349/1-6= fetal cells)

Surveyor Nuclease Assay (Cel-I):



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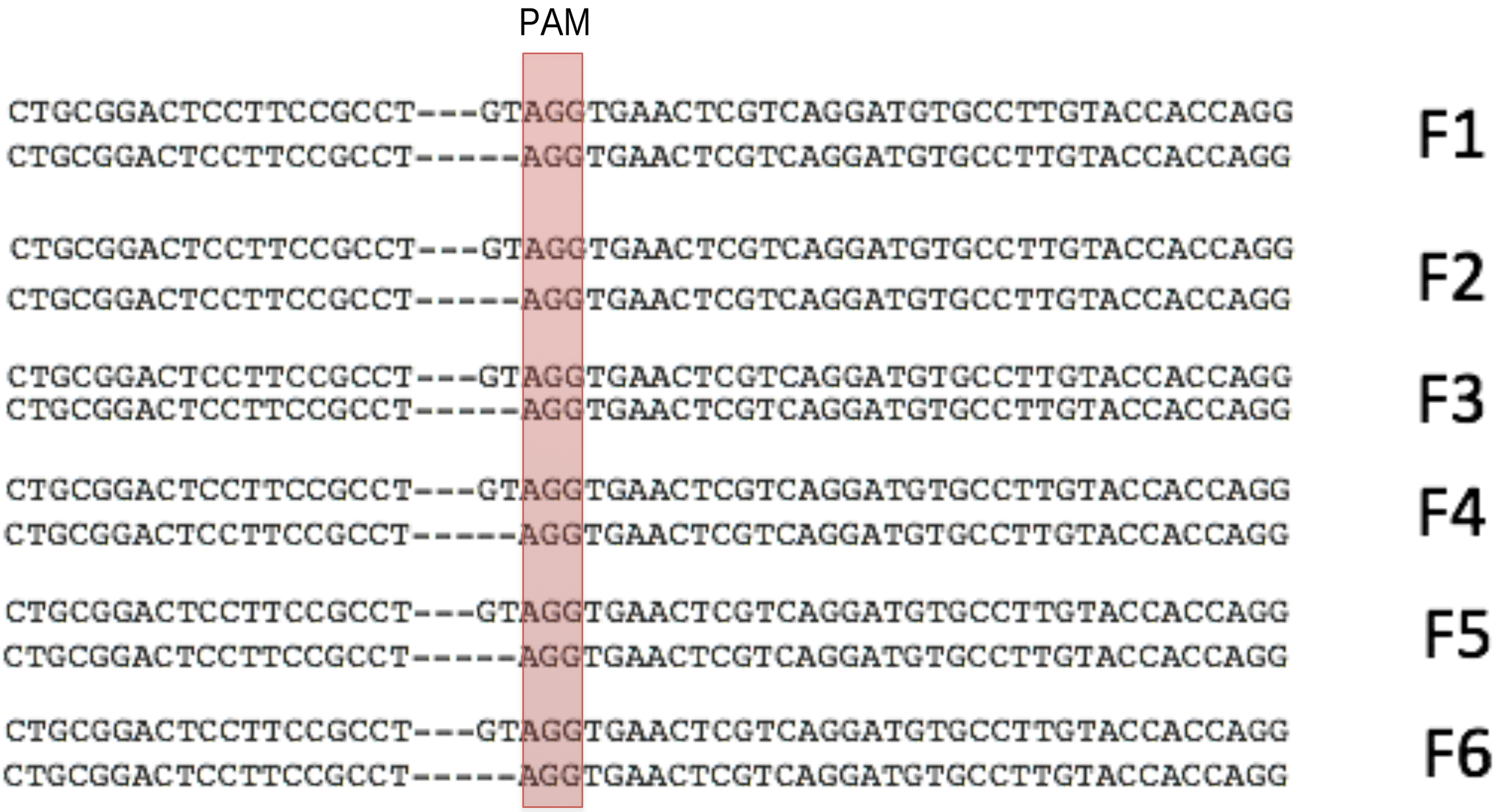
Sequence analysis of CRISPR/Cas9 modified alleles



Petersen et al., unpublished data

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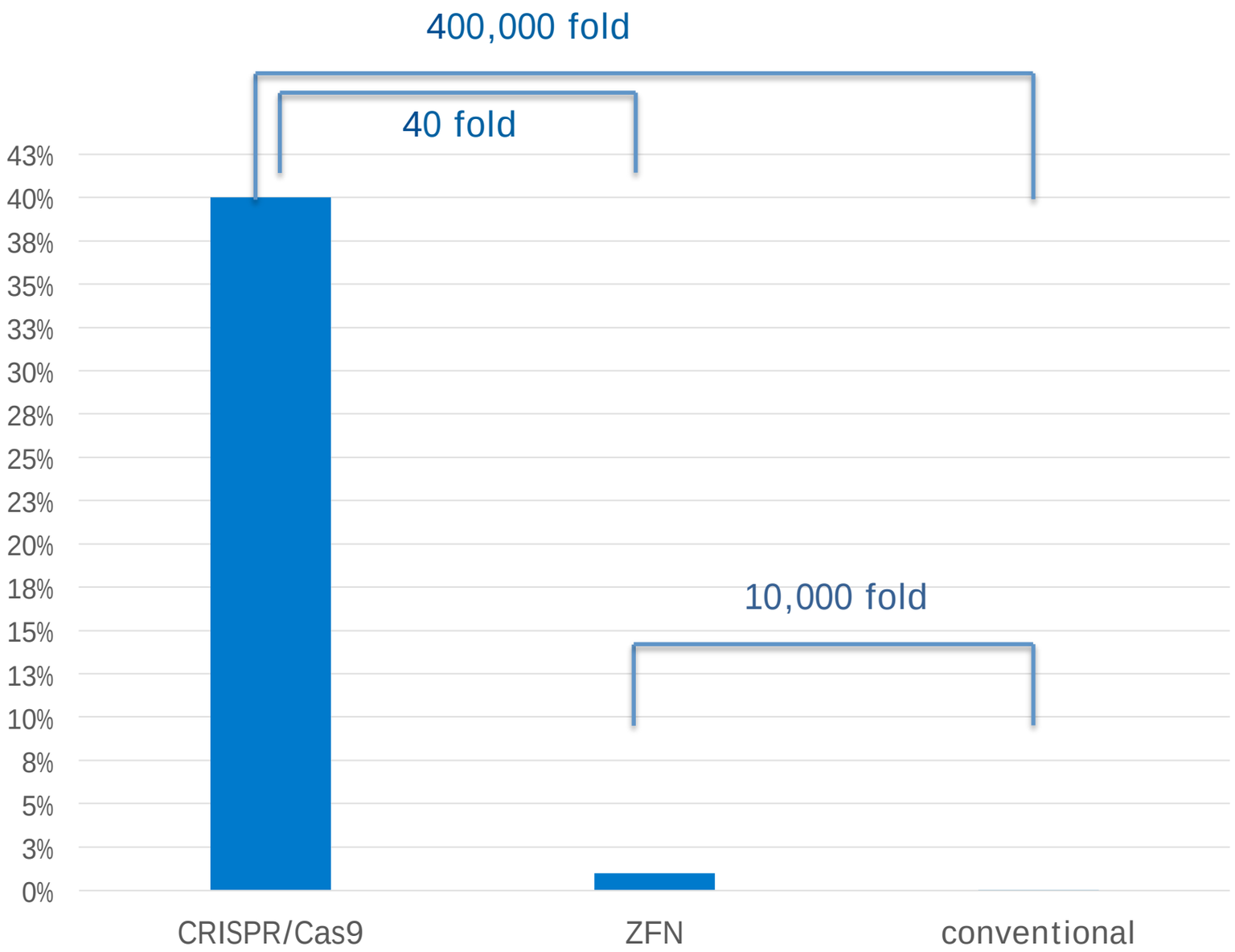
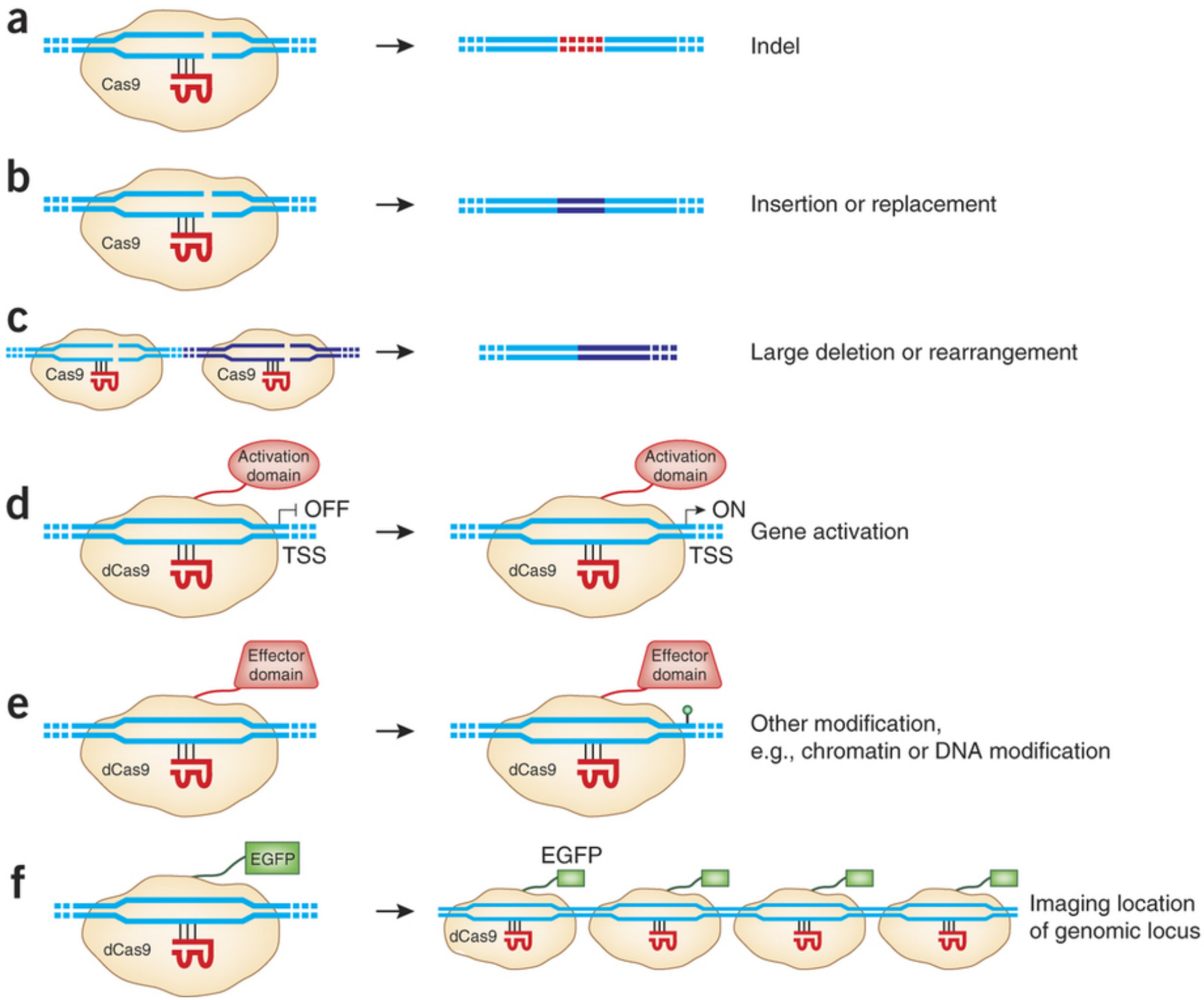
Targeting efficiency of the porcine GGTA1-locus



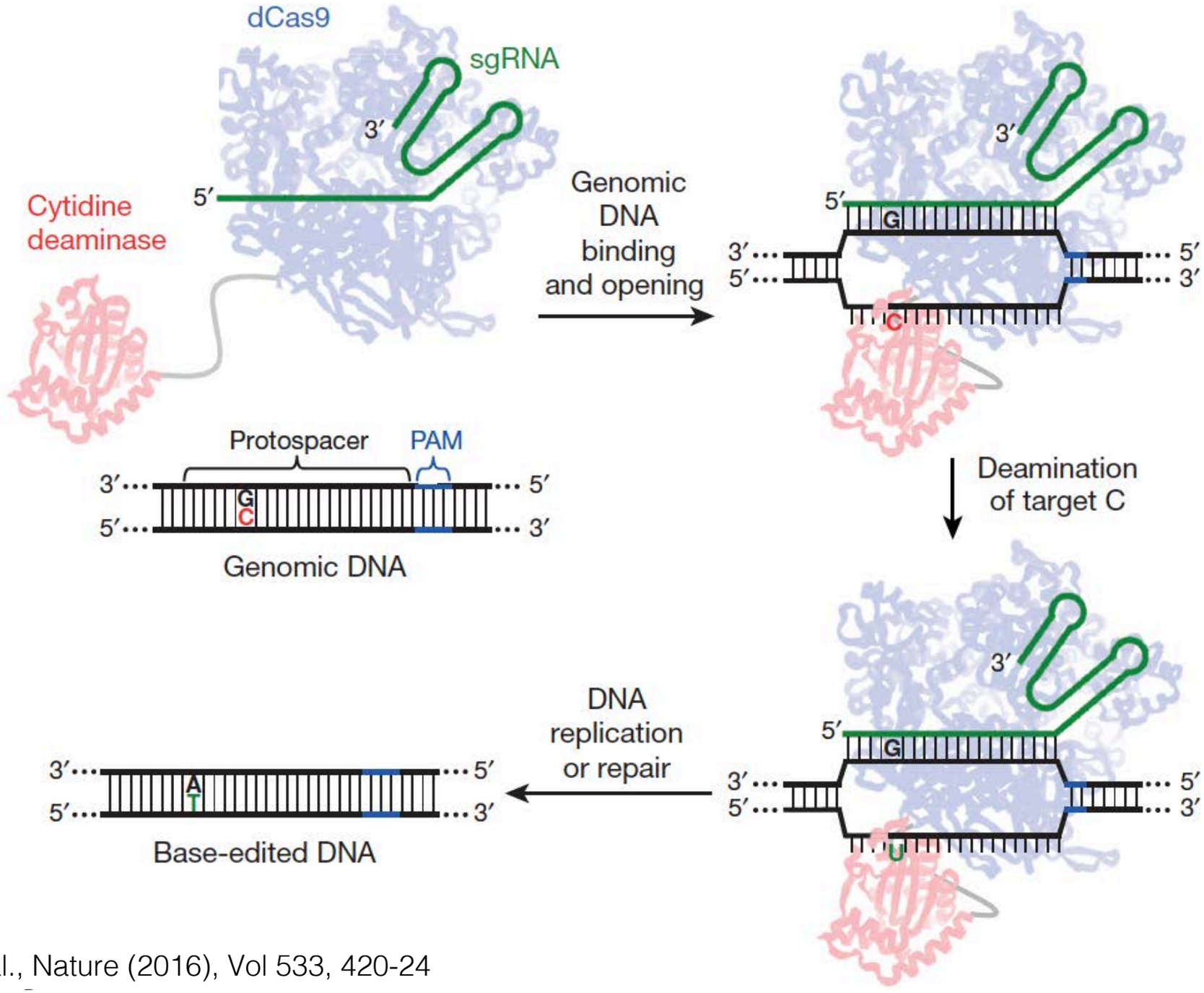
Petersen et al., unpublished data

Versatility of the CRISPR/Cas system



Sander & Joung, Nature Biotechnology 32, 347–355 (2014)

Programmable Editing without DNA cleavage



Komor et al., Nature (2016), Vol 533, 420-24

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Knockouts by cytoplasmic CRISPR/Cas microinjection

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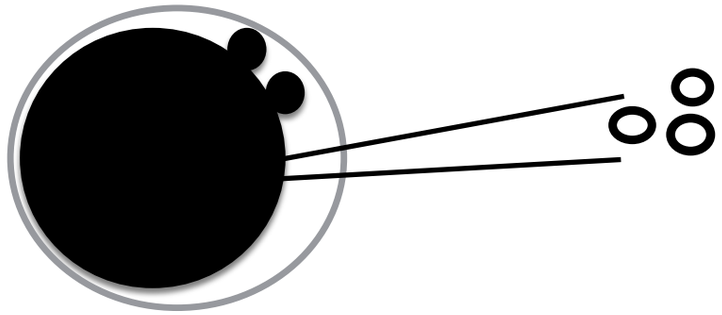
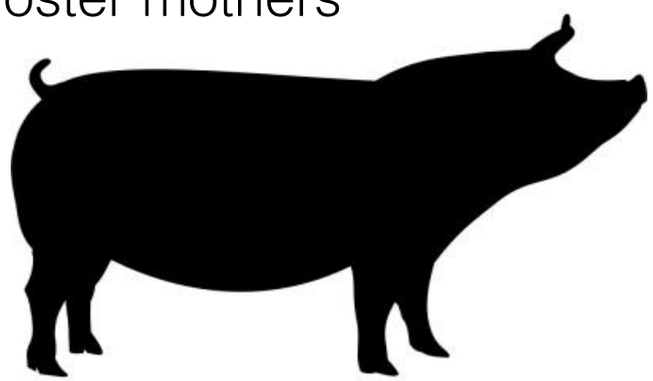
Knockouts by cytoplasmic CRISPR/Cas DNA microinjection



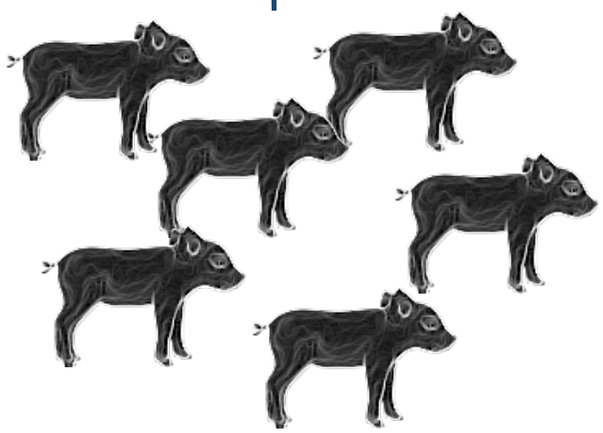
Intracytoplasmic microinjection of CRISPR/Cas DNA into *in vivo derived* zygotes

Transfer of microinjected zygotes into foster mothers



Termination of pregnancy around day 26 of gestation. Fetus analysis

Analysis of pigs, detection of bi- and mono-allelic KO-pigs and wild-type pigs



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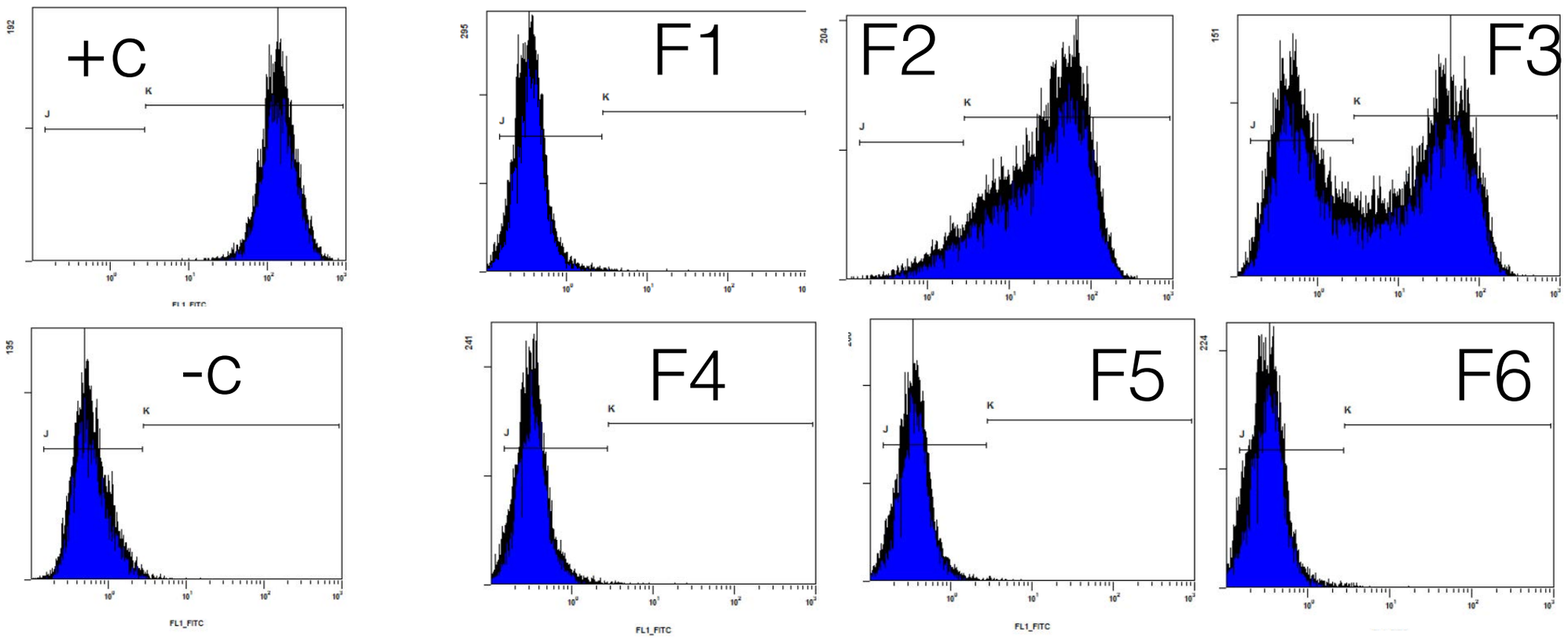
Generation of *GGTA1*$^{-/-}$ pigs by cytoplasmic microinjection

Recipient	transferred embryos (n)	pregnant
584	26	-
589	30	+
605	30	+
n=3	86	2/3 (66%)

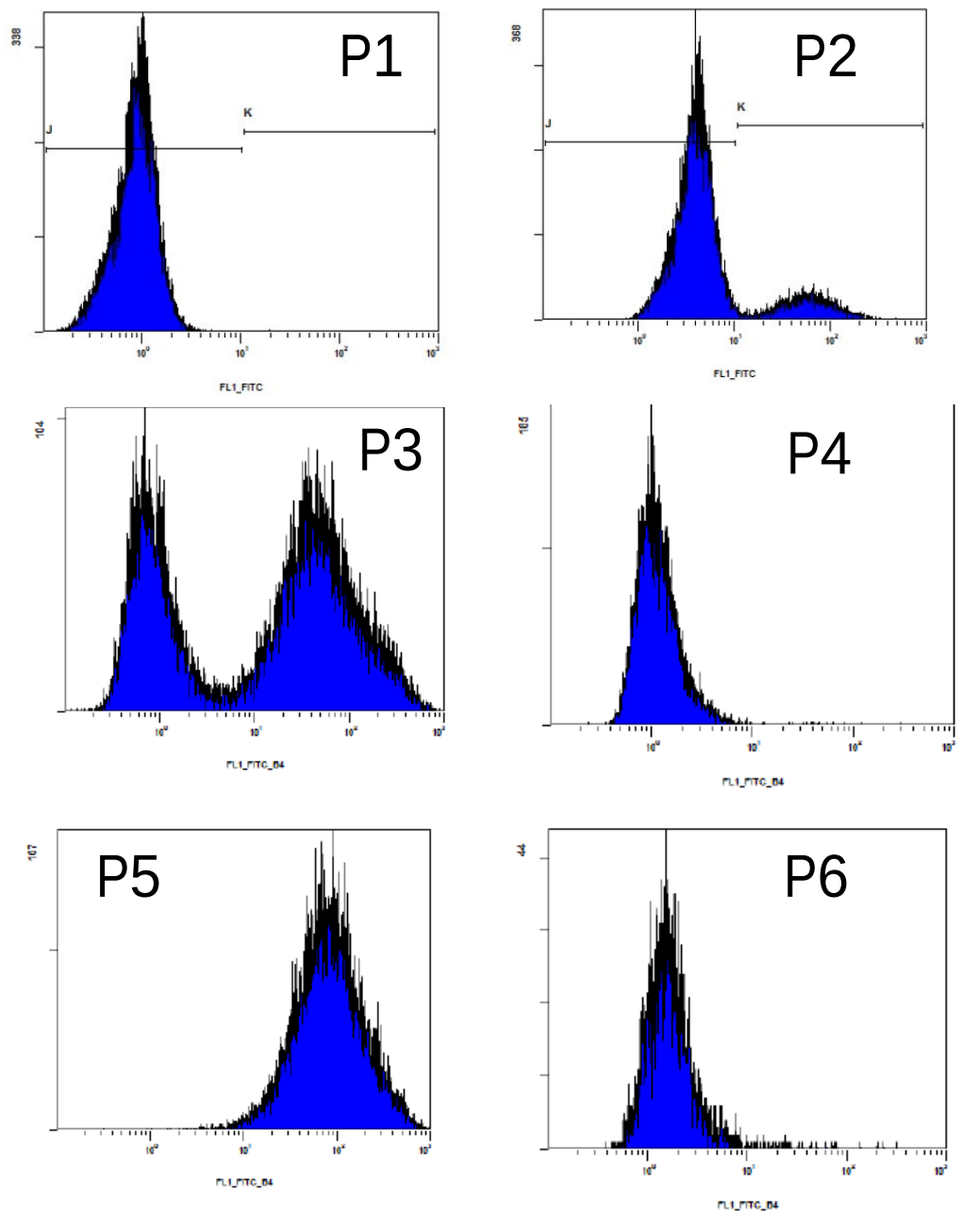
Recipient #605 was sacrificed on day 27 of gestation: 6 healthy fetuses were recovered.
#589 is allowed to go to term.
Delivery date 04.09.15, 6 healthy piglets



FACS after GS-IB4 staining. 4/6 (66%) of the fetuses have no Gal-epitopes indicating a biallelic KO of the GGTA1-locus

Generation of *GGTA1*$^{-/-}$ pigs by cytoplasmic microinjection

Recipient #589 farrowed on 04.09.15: 6 healthy piglets were born (5 males, 1 female).



Biallelic	Monoallelic or wt	Mosaic
7	1	4

58% carry a biallelic KO!

Petersen et al., Xenotransplantation (2016)

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Advantages and disadvantages GEs

Pros:

- Lack of vector backbone integration due to transient transfection
- No need for antibiotic selection cassette
- High efficiency of gene targeting
- Biallelic knockout possible in a single approach
- Targeting of several loci at once feasible
- Excision of several kb possible
- No need for SCNT to produce genetically modifed livestock

Cons:

- Quality of GE is highly dependent on the bioinformatic program (ZFN,TALENs).
- The use of specifically designed ZFNs can be very expensive.
- Off-target events can occur (though at low frequency)
- Cytotoxicity?
- Too easy to use?

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Applications of Gene Editing Tools in Farm Animals

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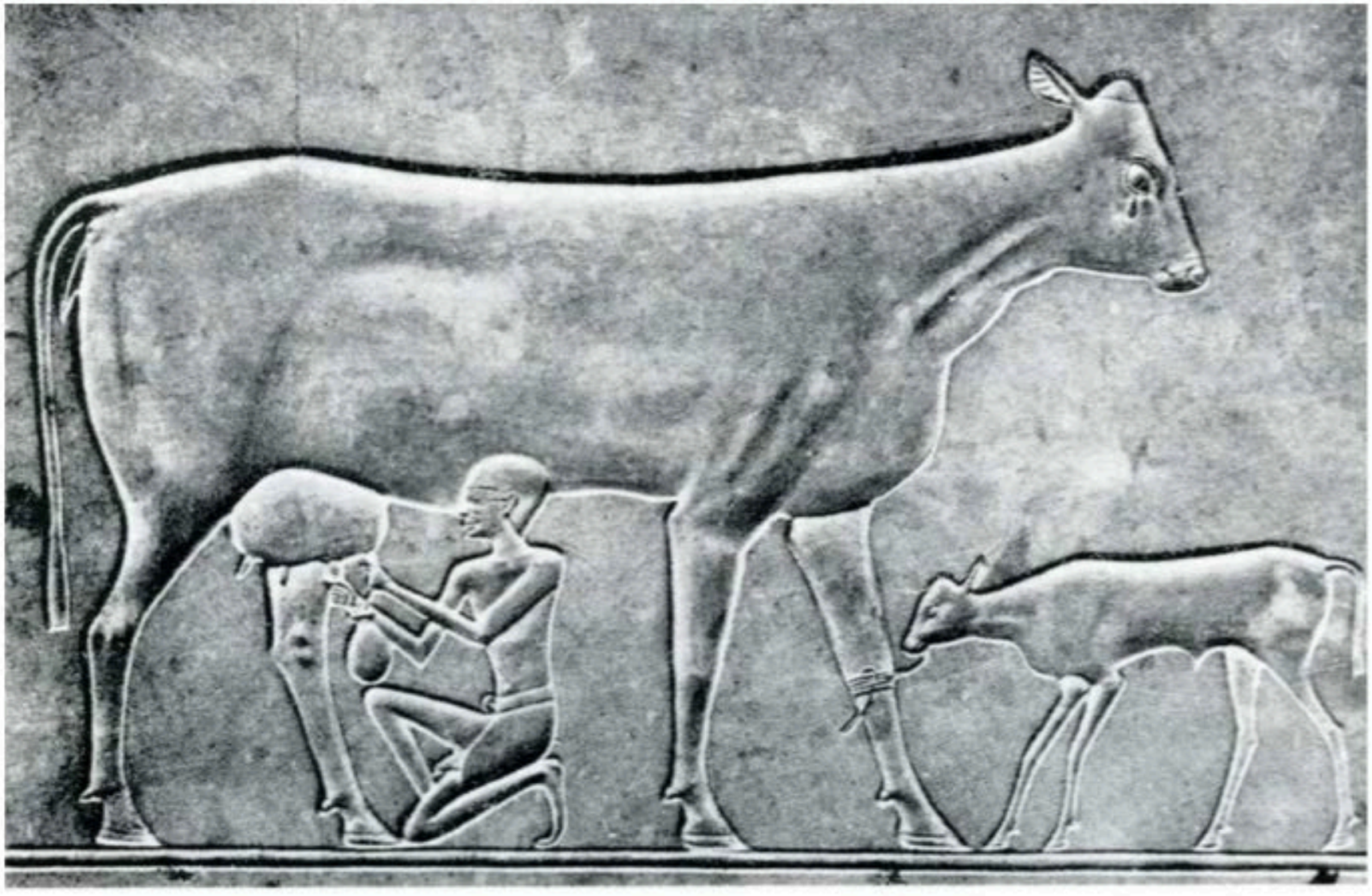
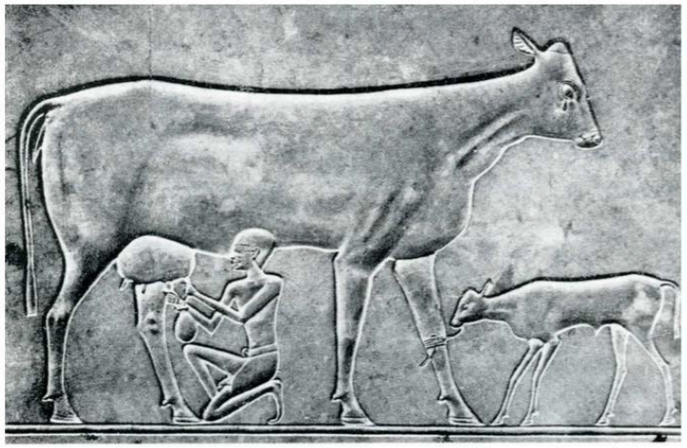


107. Melken einer hornlosen Kuh. Sarg der Kawit. 11. Dyn. [Foto: H. W. Müller, München]

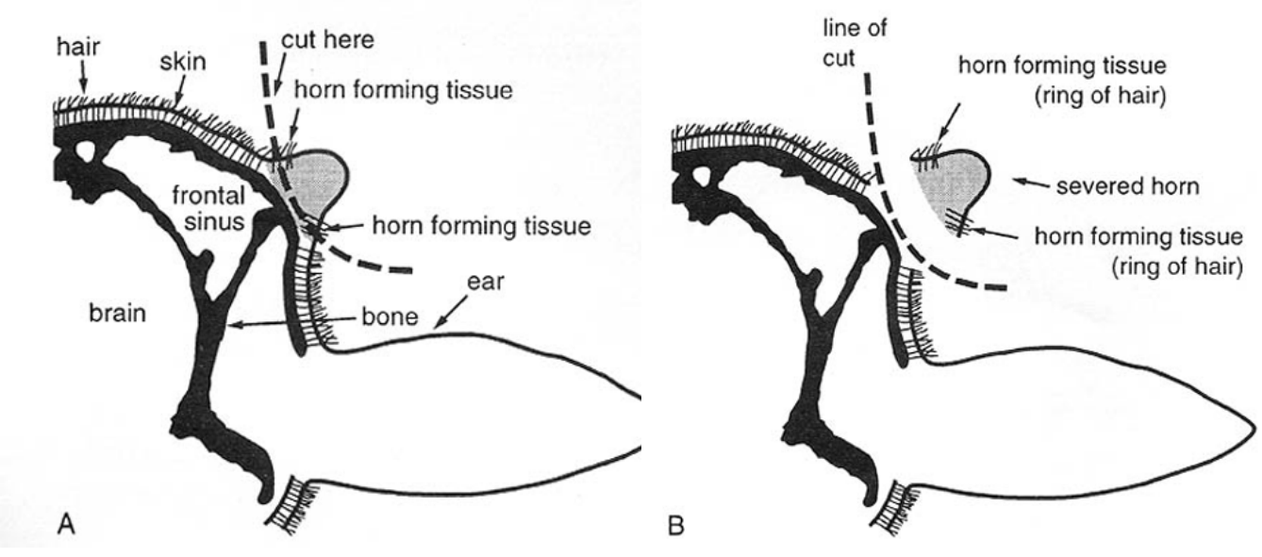
Polledness in cattle



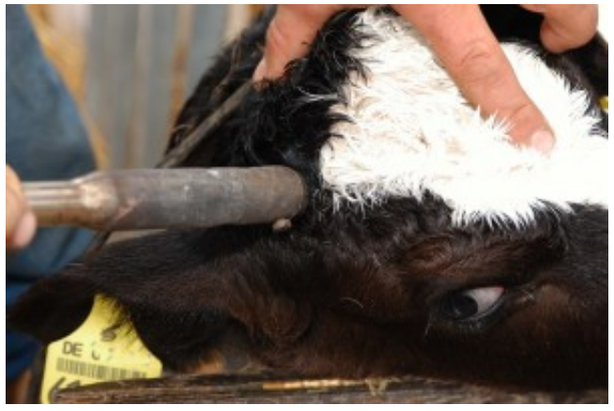
107. Melken einer hornlosen Kuh. Sarg der Kawit. 11. Dyn. [Foto: H. W. Müller, München]

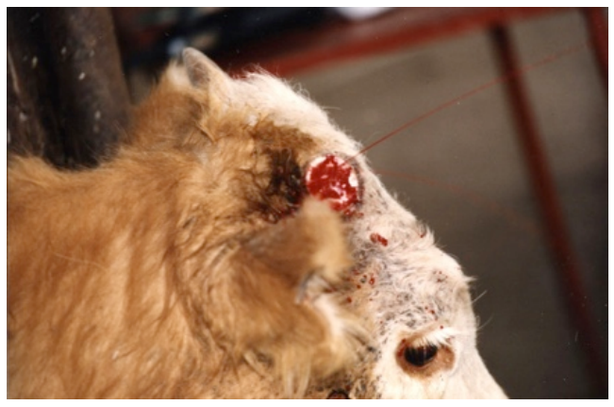
Kategorie	Rasse	Ursprungsland
komplett hornlos	Aberdeen Angus	Großbritannien
	Belted Galloway	Großbritannien
	Galloway	Großbritannien
	Swedish Red Polled (S)	Schweden
	Old Norwegian Red Vestland	Norwegen
> 20 % hornlos	Norwegian Red	Norwegen
	Welsh Black	Großbritannien
< 5 % hornlos	Holstein	USA/Deutschland/Niederlande/Frankreich
	Jersey	Großbritannien
	Simmental	Deutschland, CH
	Fleckvieh	Deutschland
	Ayreshire	Großbritannien
	Dexter	Großbritannien
	Charolais	Frankreich
	Limousin	Frankreich
	Salers	Frankreich
nicht hornlos	Highland Cattle	Großbritannien

Dehorning of cattle



- To protect the welfare of dairy farmers and cattle, horns are routinely manually removed from the majority of dairy cattle.
- Dehorning causes stress and pain to the animals,
  and adds expense to animal production
- Potential risk of infections
- Some breeds are naturally horn-free (e.g. Angus), a dominant trait referred to as *Polled*.
- Small fraction of Holsteins are hornless. Farmers are encouraged to increase their population by breeding (climbing to about 4% in 2015 from 3% in 2013).





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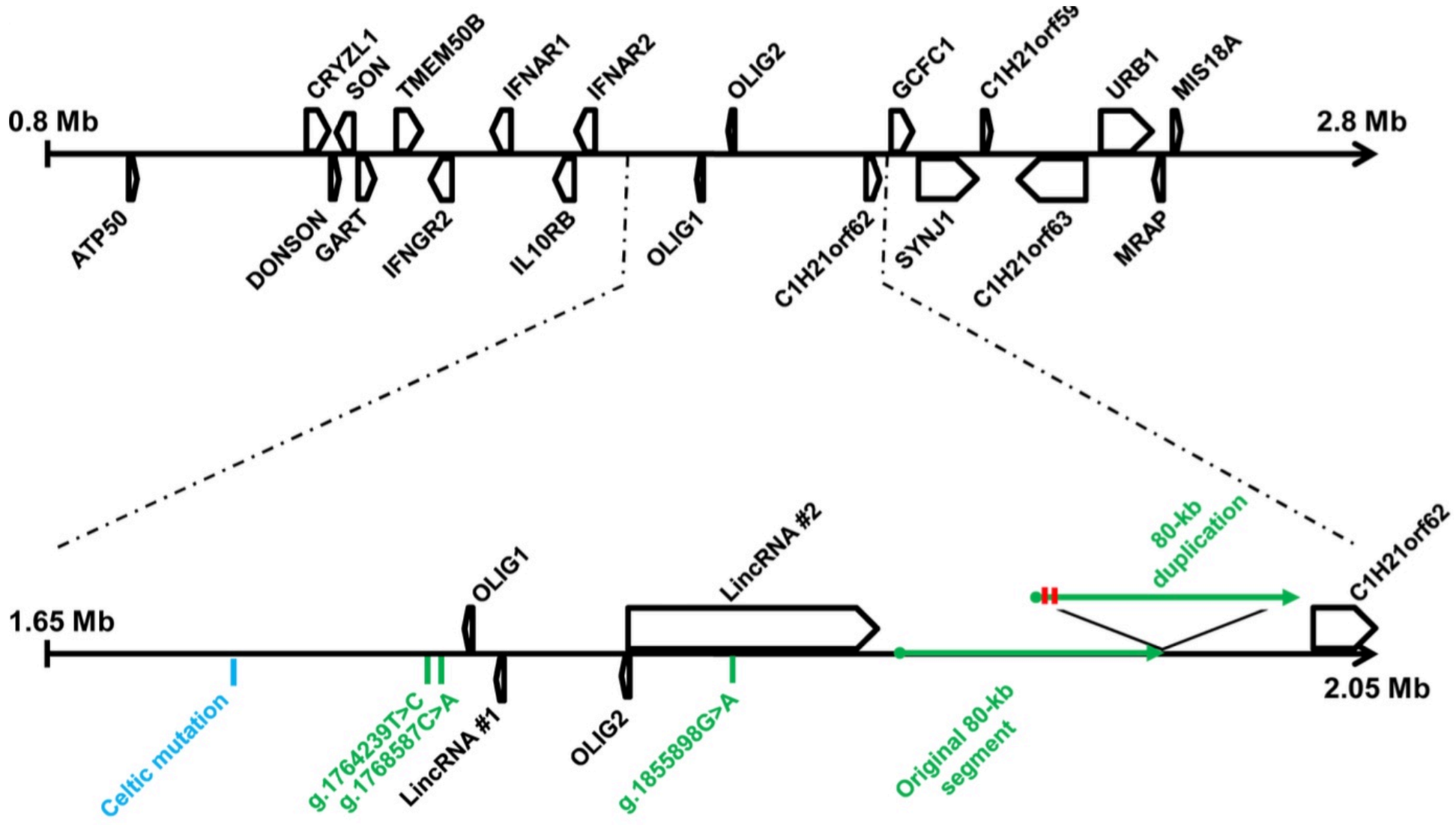
Candidate Mutations for a “hornless“ phenotype at the bovine polled locus



Keltische Variante (Celtic Mutation) besteht aus einer 212 Basen Insertion und 10 Basen Deletion, in Holstein Friesian ist eine 80 Kilobasen Duplikation vorherrschend. Im Anfangs-bereich der Duplikation finden sich zwei Sequenzvariationen zur Originalsequenz (rote Striche). Lokalisation Weitere 3 Kandidatenmutationen sind 1764239T>C, 1768587C>A, 1855898G>A.

(aus Allais-Bonnet, Grohs et al. 2013)

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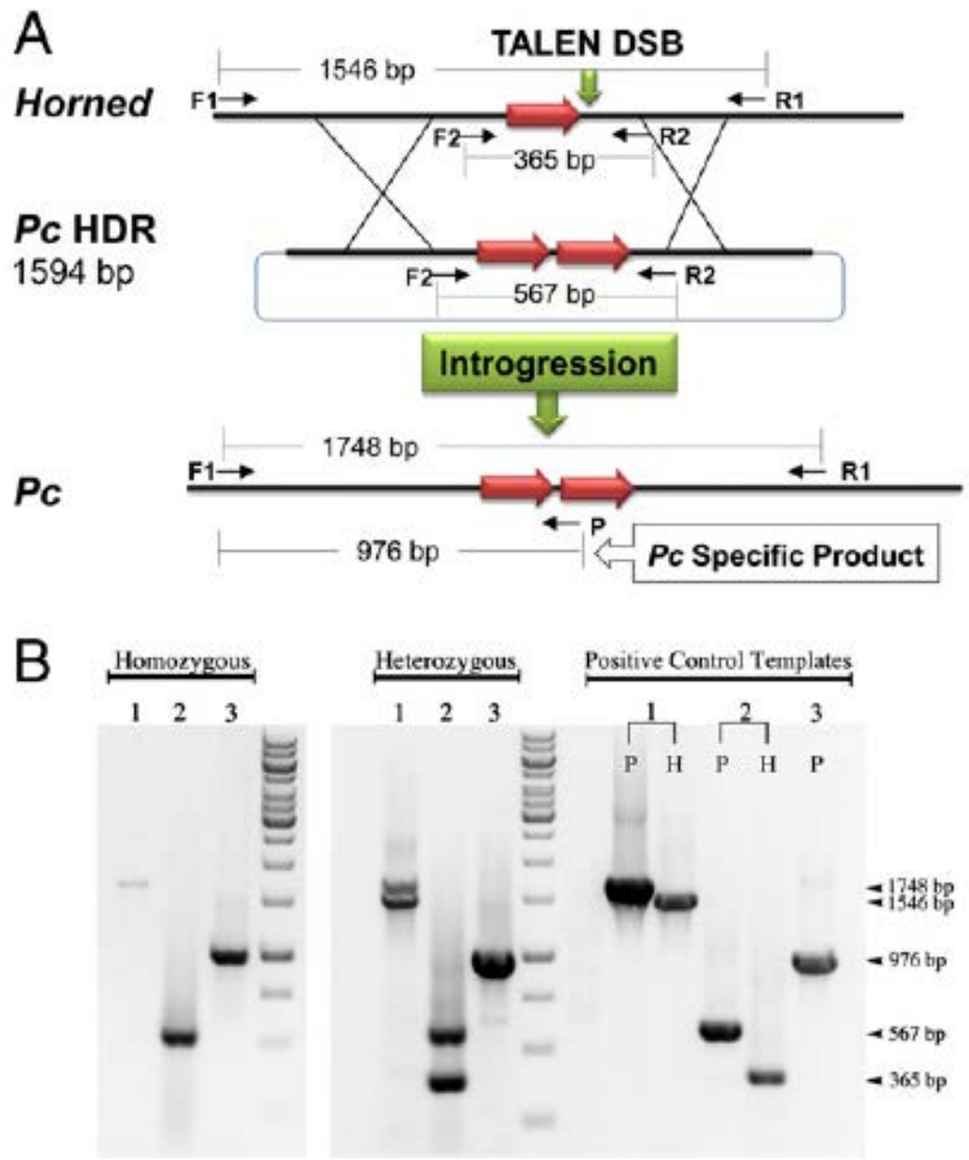
Nonmeiotic allele introgression by TALENs

- Meiotic introgression of the *Polled* trait is feasible by crossbreeding, but the genetic merit of the resulting animals would rank lower to the admixture of unselected alleles for net merit (i.e. milk production) into the population.

- Introgression of a polled allele (Celtic *Polled*) into horned bull fibroblasts by using TALEN mRNA

- TALENs cleaved the *Horned* locus and the *Polled* trait was integrated by HDR.

- 5/226 cell colonies were positive for the *Polled* trait by PCR. Three of them were homozygous for the *Polled* trait confirmed by sequencing.



Tan et al. 2013
Proc Natl Acad Sci USA 110:16526-31

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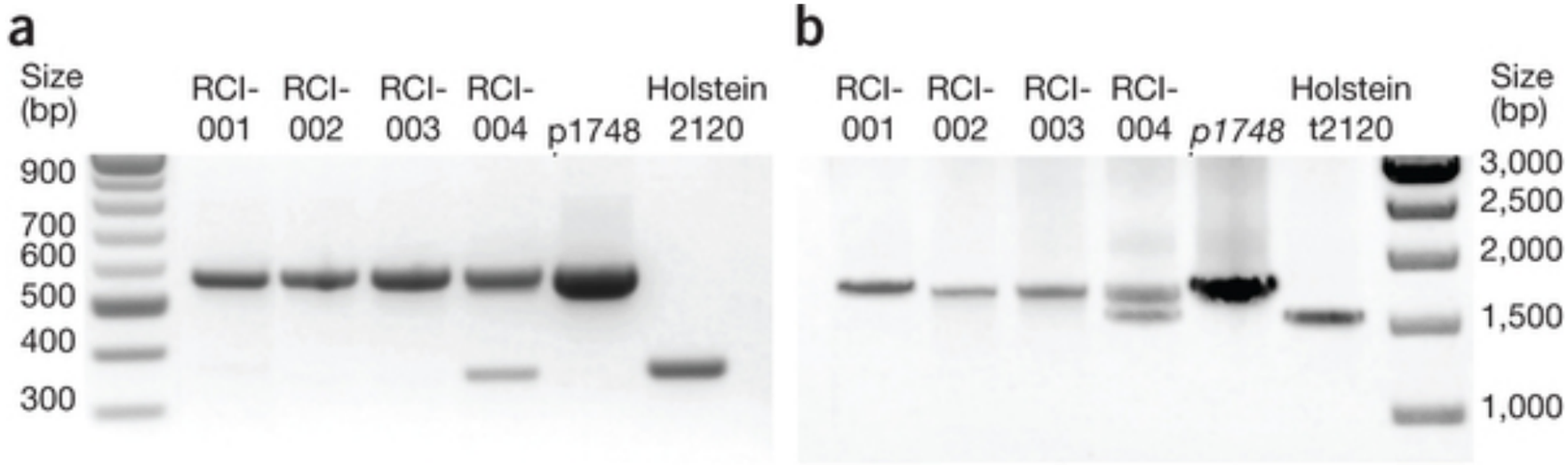
Gen Editing des Polled-Locus zur Produktion hornloser Rinder



Carlson et al., 2016, Nature Biotechnology 34, 479-481

Nonmeiotic allele introgression by TALENs

“Spotiguy“ and his clone. The first Holsteins that were modified to not grow horns.

Carlson et al., 2016, Nature Biotechnology 34, 479-481

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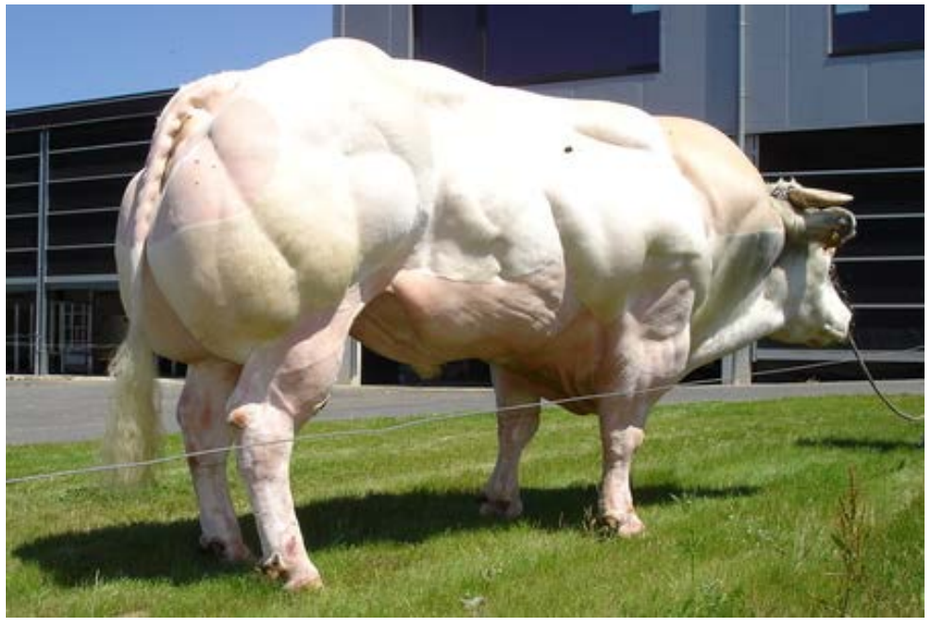
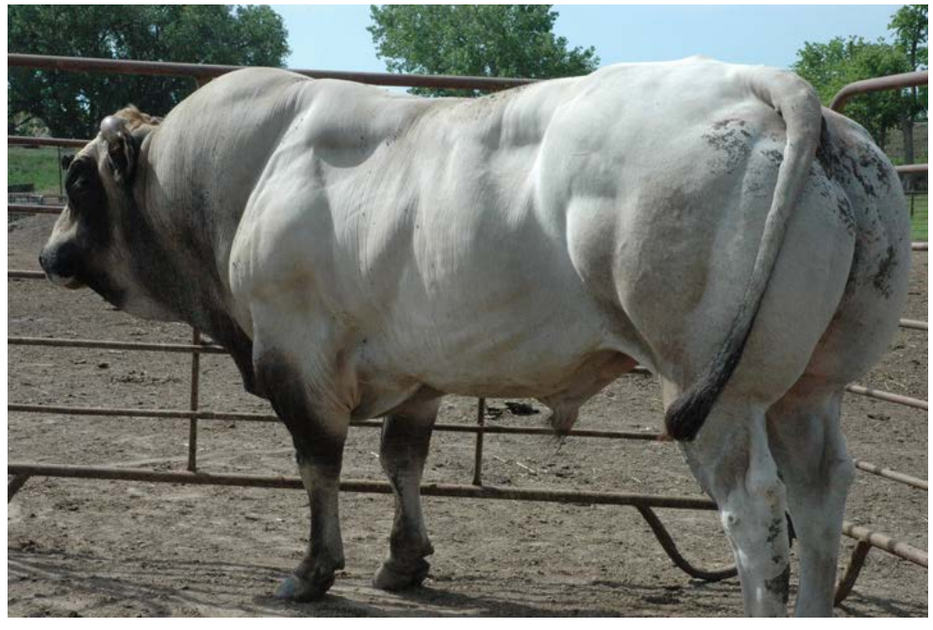
Disruption of bovine MSTN by GE

- Myostatin (*MSTN*) is known to be a negative regulator of muscle growth
- Inhibition by mutations in the *MSTN* gene causes double muscling in cattle, related with animal welfare issues
- Natural mutations are known in Blue Belgians and Piedmontese, leading to a 20% greater muscle mass compared to other breeds



Blue Belgian

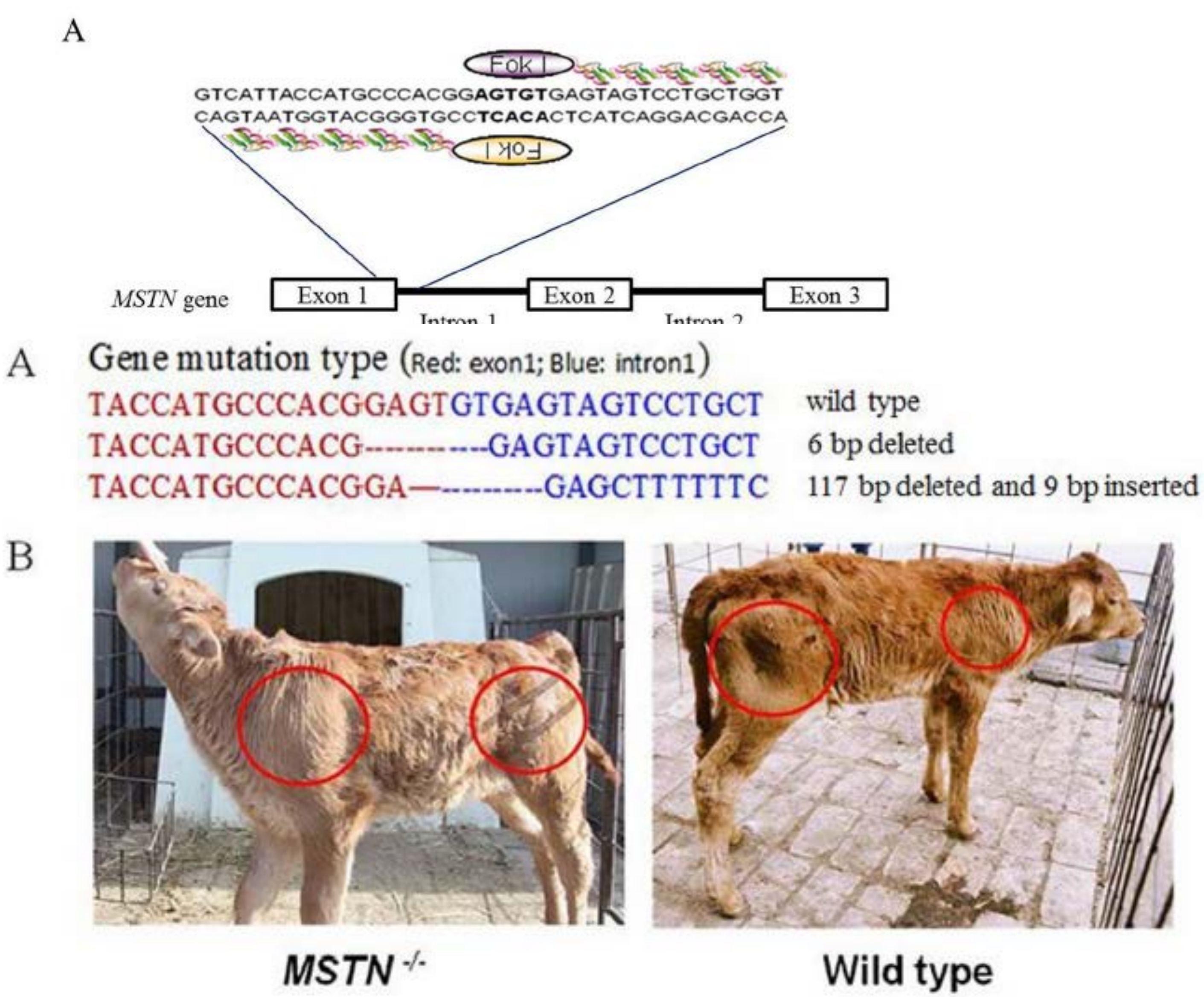


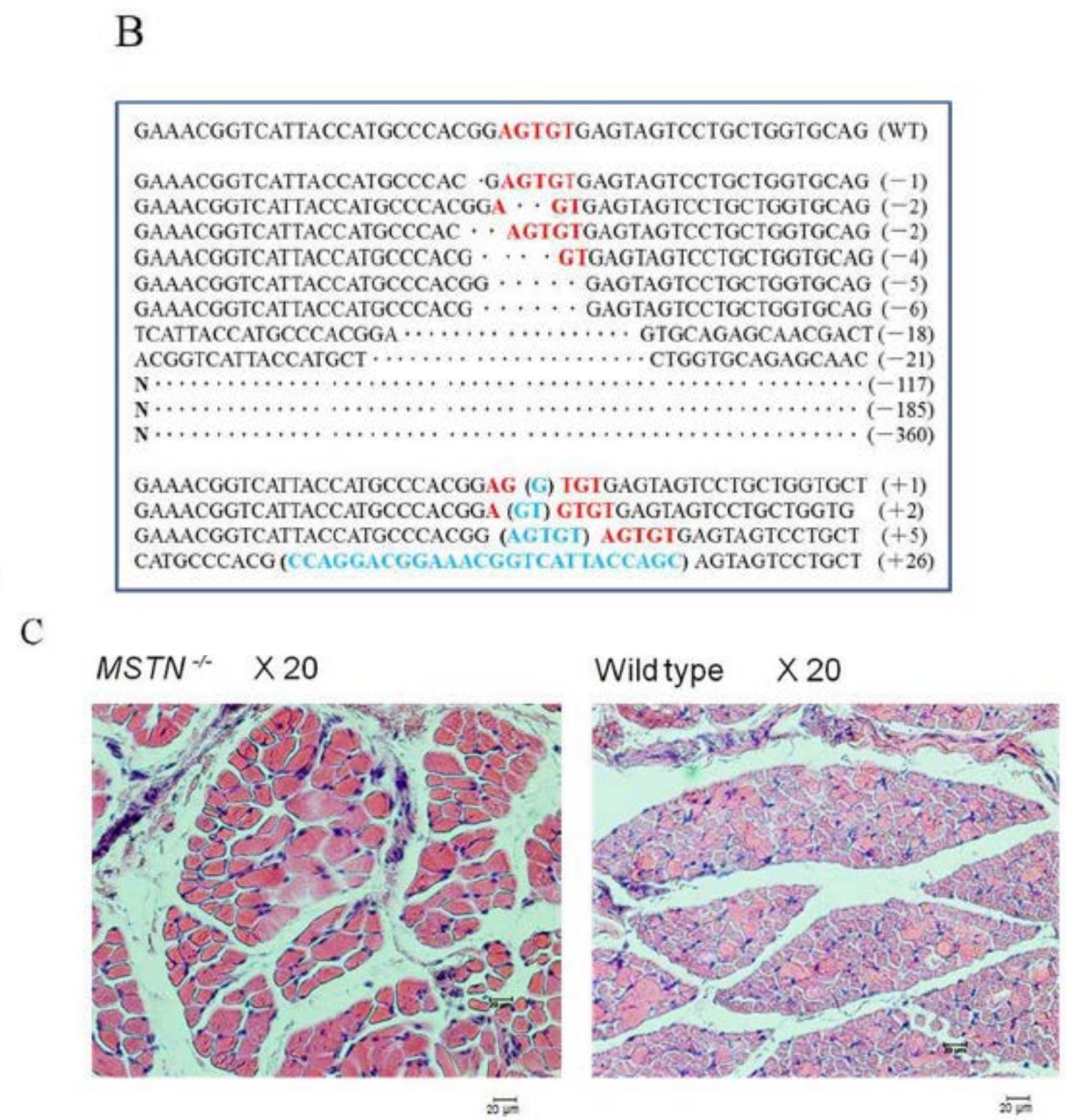
Piedmontese

Luo et al. (2014) PLoS ONE 9(4): e95225

Disruption of bovine MSTN by ZFN

- Biallelic disruption of *MSTN* by ZFN in Chinese Yellow Cattle
- Cells were used for SCNT, 35 pregnancies, 18 went to term





Luo et al. (2014) PLoS ONE 9(4): e95225

Disruption of bovine MSTN by TALEN

A:MSTN edited Nelore cattle after microinjection of TALEN mRNA into OPU-IVF zygotes. B) Live born bull and wildtype heifer calf.

Below: The MSTN editing events. Alignment of the bovine WT and the alleles present in the edited animals. The sequence of the bull shows that the animal is mosaic at the MSTN locus.

```
Nelore WT          GTGATGAACACTCCACAGAATCTCGATGCTGTCGTTACCCTCTAACTGTGGATTTTGA

Bull 1 Allele 1    GTGATGAACACTCCACAGAATCTCGATGCTGTCGTTACCCTCTAACTGTGGATTTTGA   WT
Bull 1 Allele 2    GTGATGAACACTCCACAGAATCTCGATGCTGT---TACCCTCTAACTGTGGATTTTGA   ΔR283
Bull 1 Allele 3    GTGATGAACACTCCACAGAATCTCGATGC-GTCGTTACCCTCTAACTGTGGATTTTGA   Δ1

Heifer Allele 1    GTGATGAACACTCCACAGAATCTCGATGCTGTCGTTACCCTCTAACTGTGGATTTTGA   WT
Heifer Allele 2    GTGATGAACACTCCACAGAATCTCGATGCTGTCGTTACCCTCTAACTGTGGATTTTGA   WT

Bull 2 Allele 1    GTGATGAACACTCCACAGAATCTCGATGCTGTCGTTACCCTCTAACTGTGGATTTTGA   WT
Bull 2 Allele 2    GTGATGAACACTCCACAGAATCTCGA---TGTCGTTACCCTCTAACTGTGGATTTTGA   ΔC281

Bull 3 Allele 1    GTGATGAACACTCCACAGAATCTCGATGCTGTCGTTACCCTCTAACTGTGGATTTTGA   WT
Bull 3 Allele 2    GTGATGAACACTCCACAGAATCTCGA----------------------AGGACAG---   Δ219 +7
```


Proudfoot et al. 2014 Transgenic Res.
Doi:10.1007/s11248-014-9832-x

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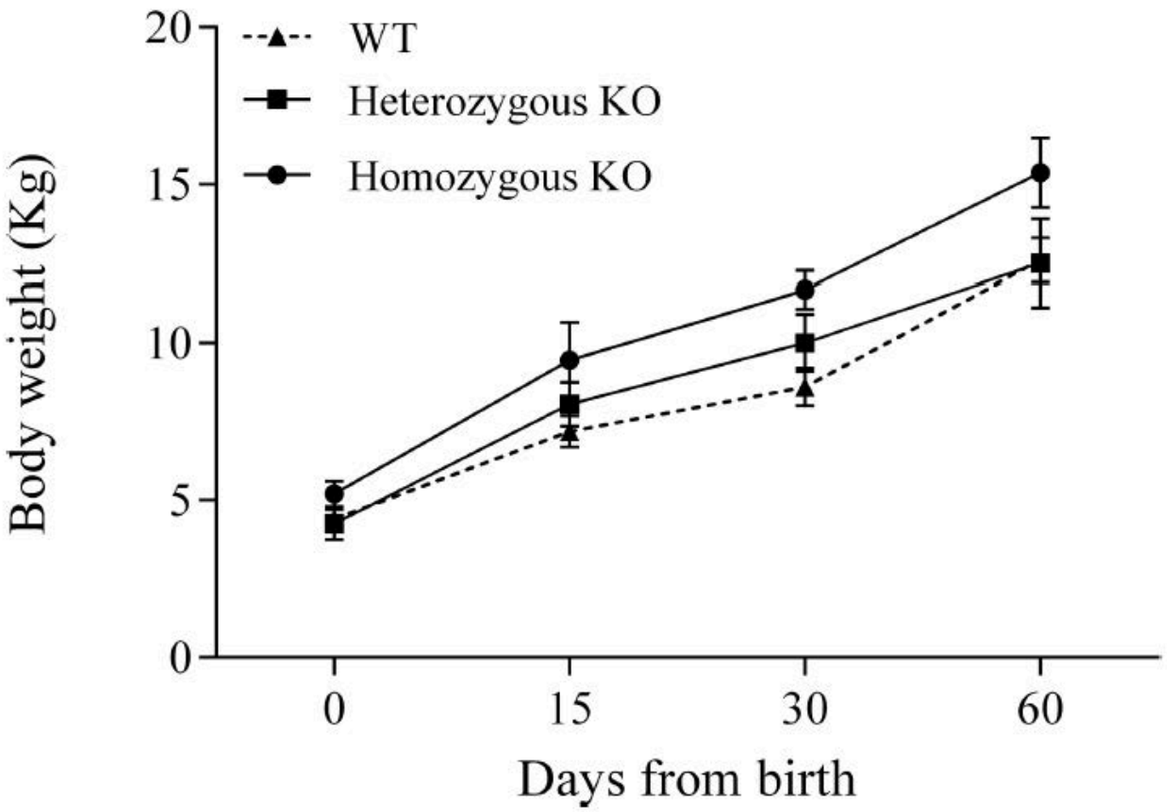
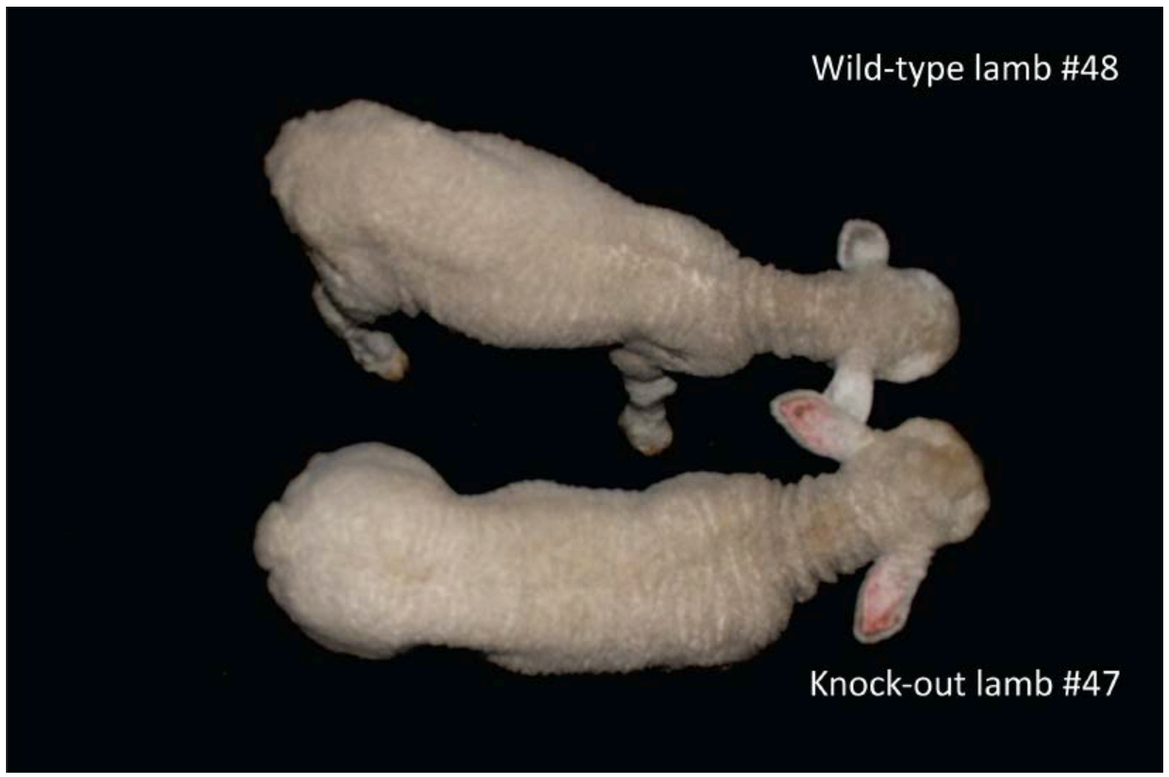
Disruption of ovine MSTN by CRISPR/Cas





- Targeting exon 1 of the ovine MSTN gene, intracytoplasmatic injection of Cas9mRNA and sgRNA
- 53 injected blastocysts transferred to 29 recipients, 19 became pregnant, 22 lambs born, 10 carried a mutation, 8 of them were biallelic, 5 homozygous.
- No significant difference in birth weight and size to wildtype controls.
- Significant higher weight gain in KO-lambs (20-30% heavier after 60 days).

Crispo et al. (2015)
PLoS ONE 10(8):
e0136690

MSTN knockout in pigs by TALENs

Produziert in Korea 2015

Nature, Juli 2015

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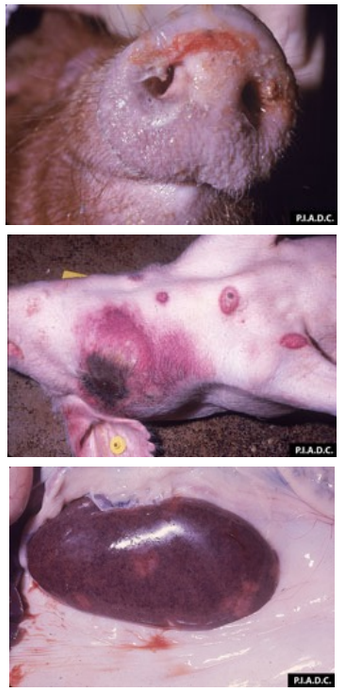
Pigs resilient to African Swine Fever

Warthog and Bushpig:

- ASF subclinical and persistent
- natural hosts
- transmission directly or via arthropod vector:Ornithodorus ticks

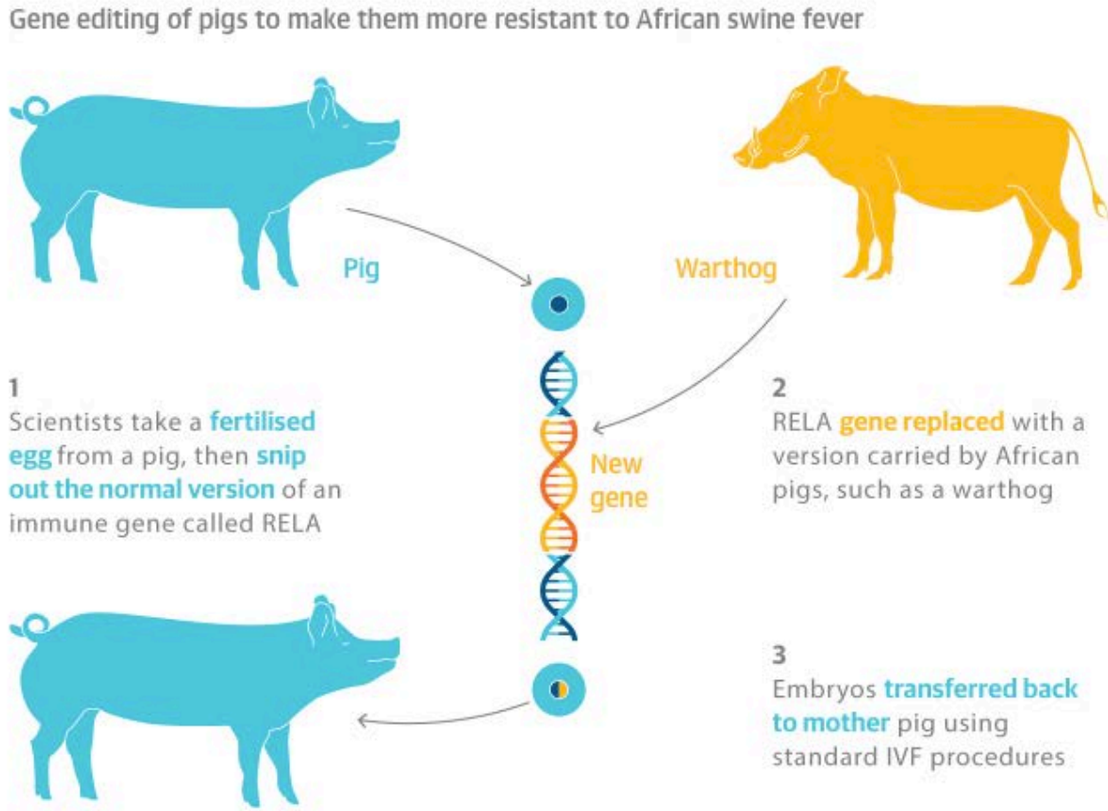
Domestic pig:



- acute, rapidly fatal hemorrhagic fever
- proinflammatory cytokine storm driven by infected macrophages
- widespread apoptosis of infected macrophages and uninfected lymphocytes
- severe hematological and vascular perturbations
- cardiovascular collapse

Palgrave et al. (2011),
JOURNAL OF VIROLOGY, June 2011, p. 6008–6014

Pigs resilient to African Swine Fever



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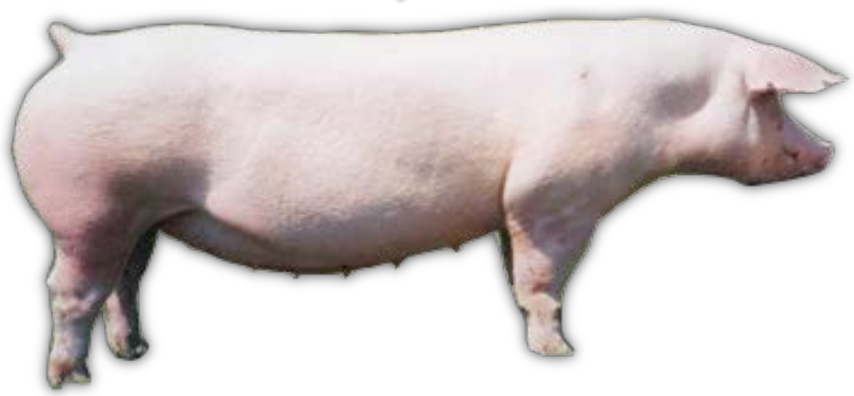
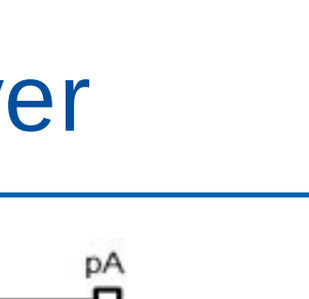
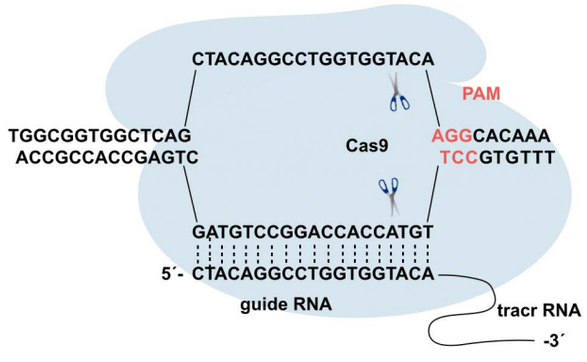
Pigs resilient to African Swine Fever

integrative, inducible CRISPR/Cas vector,
gRNAs targeting ASFV genomic region

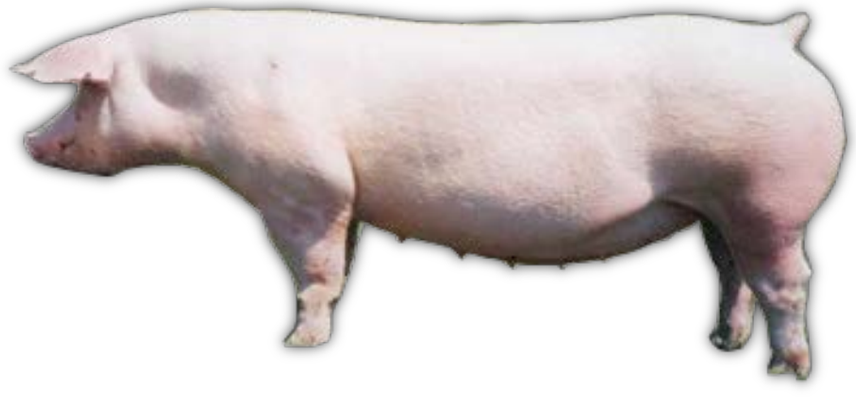




Transgenic leaky expression or upregulation by
Dox administration, cutting of viral DNA



ASF-resistant or resilient pig



Petersen et al., unpublished data

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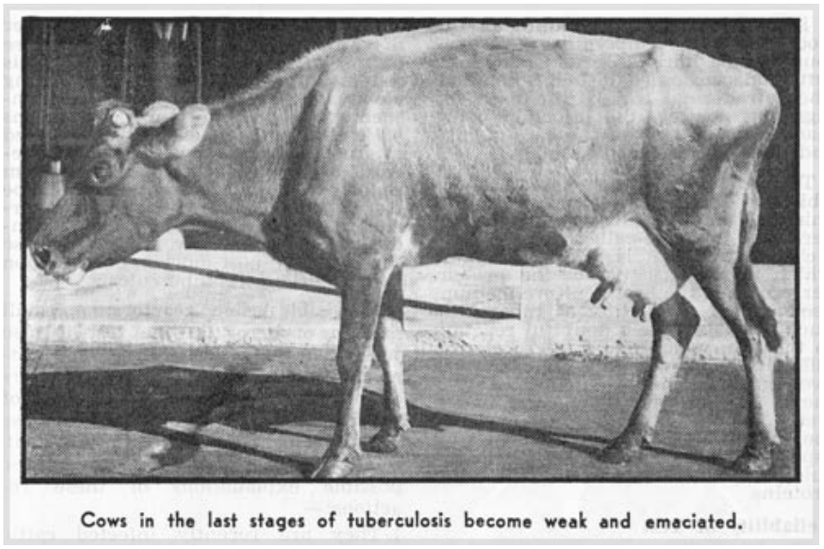
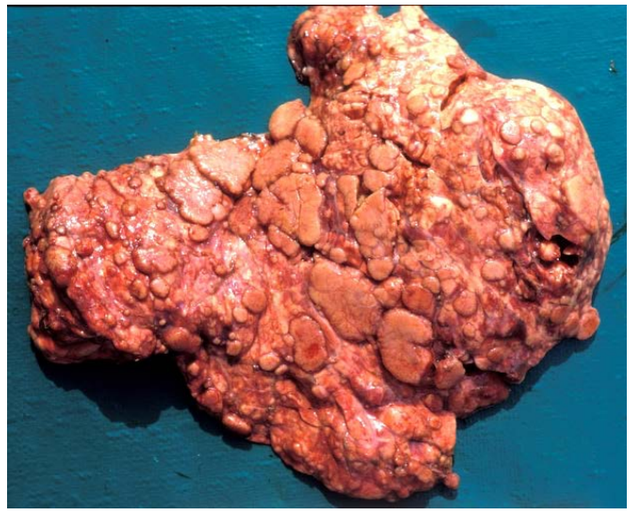
Increased Resistance against Tuberculosis

- Tuberculosis is a chronic infectious disease, caused by *Mycobacterium bovis* .
- *M.bovis* survives in cattle faeces for 1-8 weeks.
- *M.bovis* can infect humans.
- Spreads in breath, milk, saliva, urine, feaces, lesions, droppings.
- Mainly respiratory problems.
- Serious threat to agriculture in many areas, including New Zealand, England and Wales.
- In the UK over 26,000 cattle culled in 2013, which cost £100m



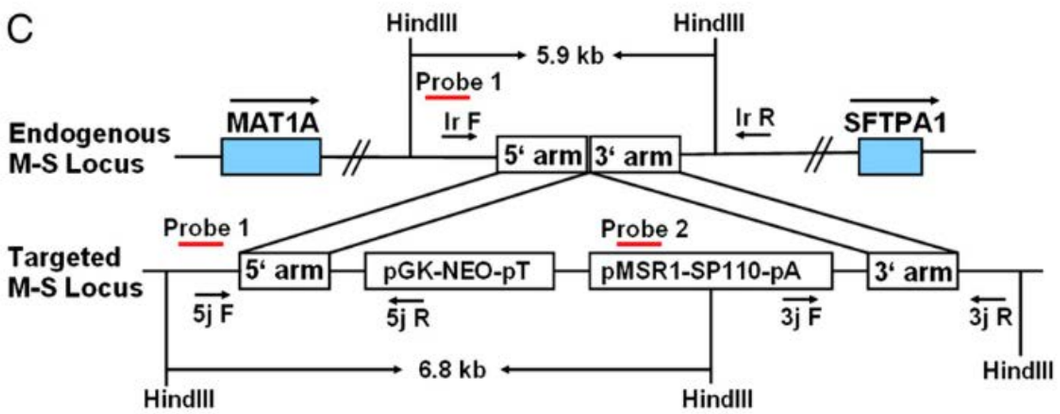
Cows in the last stages of tuberculosis become weak and emaciated.

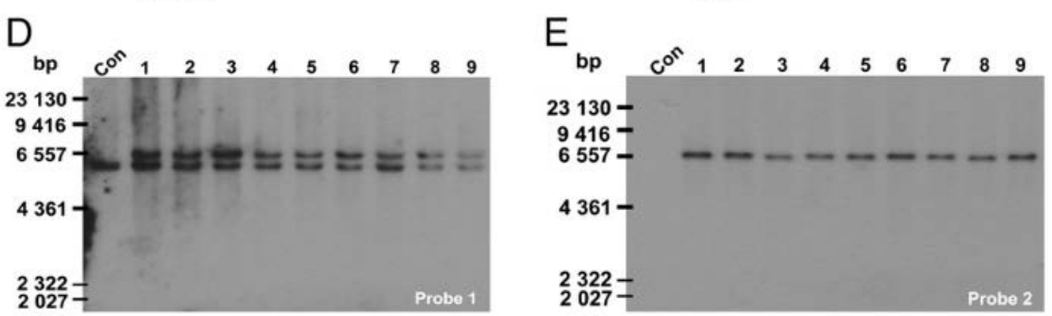


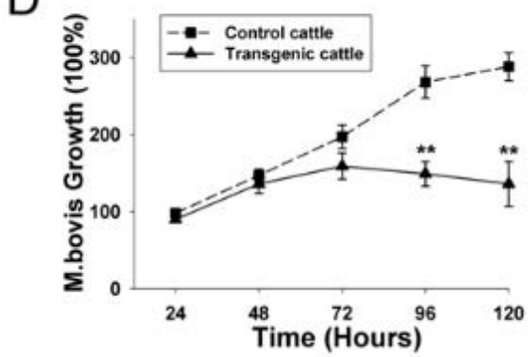
WU et al. Proc Natl Acad Sci USA 2015: 112: E1530-E1539.

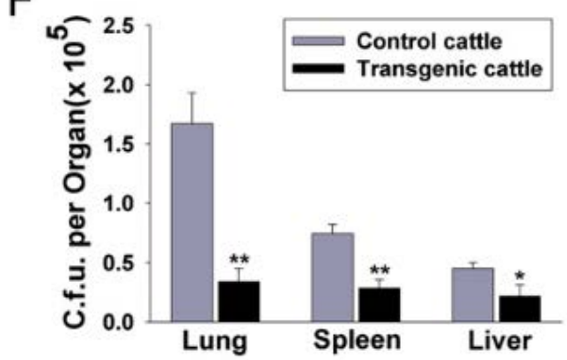
Increased Resistance against Tuberculosis

- The mouse *SP110* gene can control *M.bovis* growth in macrophages and induce apoptosis in infected cells.
- Transfer of the mouse *SP110* gene into the genome of Holstein-Friesian (Macrophage Scavenger Receptor (*MSR1)*-locus) by TALENs led to an increased resistance against *M.bovis* infection by macrophage-specific expression of *SP110*.









WU et al. Proc Natl Acad Sci USA 2015: 112: E1530-E1539.

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Increased Resistance against Tuberculosis

Table 2. Gross pathology of transgenic cattle challenged with *M. bovis* by endobronchial instillation

Animal	No. of lobes infected*	Lung score	No. of lymph nodes infected†	Lymph node score	Total pathology score	Mean‡
Transgenic 1	2	4	3	4	8	6.5
Transgenic 2	1	2	2	3	5	
Transgenic 3	0	0	0	0	0	
Control 1	5	21	6	14	35	32.0
Control 2	4	15	8	18	33	
Control 3	4	14	6	14	28	

*Lung lobes (left apical, left cardiac, left diaphragmatic, right apical, right cardiac, right diaphragmatic, and right accessory lobes) were examined for lesions using a gross pathology scoring system.
†Lymph nodes (mandibular, parotid, medial retropharyngeal, mediastinal, tracheobronchial, hepatic, mesenteric, and prescapular lymph nodes) were examined for lesions using a gross pathology scoring system.
‡Median values per group ($n = 3$). Only animals with lesions were taken into account.

WU et al. Proc Natl Acad Sci USA 2015: 112: E1530-E1539.

Increased Resistance against Tuberculosis

Table 3. Gross pathology of transgenic cattle challenged by transmission experiment

Animal	No. of lobes infected*	Lung score	No. of lymph nodes infected†	Lymph node score	Total score	Mean ± SD‡
Transgenic group 1	1	2	1	1	3	4.7 ± 2.1
	0	0	0	0	0	
	0	0	0	0	0	
Transgenic group 2	3	3	2	4	7	
	2	2	1	2	4	
	0	0	0	0	0	
Transgenic group 3	0	0	0	0	0	
	0	0	0	0	0	
	0	0	0	0	0	
Control group 1	4	12	6	13	25	17.8 ± 4.8
	4	13	5	10	23	
	3	10	3	8	18	
Control group 2	4	12	4	10	22	
	3	9	4	9	18	
	2	6	3	6	12	
Control group 3	3	8	4	9	17	
	3	7	2	5	12	
	2	7	3	6	13	

*Lung lobes (left apical, left cardiac, left diaphragmatic, right apical, right cardiac, right diaphragmatic, and right accessory lobes) were examined for lesions using a gross pathology scoring system.
†Lymph nodes (mandibular, parotid, medial retropharyngeal, mediastinal, tracheobronchial, hepatic, mesenteric, and prescapular lymph nodes) were examined for lesions using a gross pathology scoring system.
‡Median values per group ($n = 9$). Only animals with lesions were taken into account.

WU et al. Proc Natl Acad Sci USA
2015: 112: E1530-E1539.

PRRS resistant pigs after knockout of CD163 by CRISPR/Cas

Drei mit der CRISPR/Cas-Technik behandelte Ferkel wurden zusammen mit sieben normalen Tieren in einer Gruppe gehalten. Alle Tiere wurden mit dem PRRS-Virus infiziert. Nach fünf Tagen zeigten die normalen Ferkel die bekannten Symptome von PRRSV-Erkrankungen, die drei anderen jedoch nicht. Obwohl sie über 35 Tage zusammen mit den erkrankten Ferkeln gehalten wurden, blieben die editierten Schweine gesund und vital.

(PRRS: Porcine reproductive and respiratory syndrome virus, *CD163* is a macrophage differentiation antigen belonging to the scavenger receptor cysteine-rich (SRCR) family of membrane proteins)

Whitworth et al., Nature Biotechnology 34, 20–22 (2016)
Foto: University of Missouri

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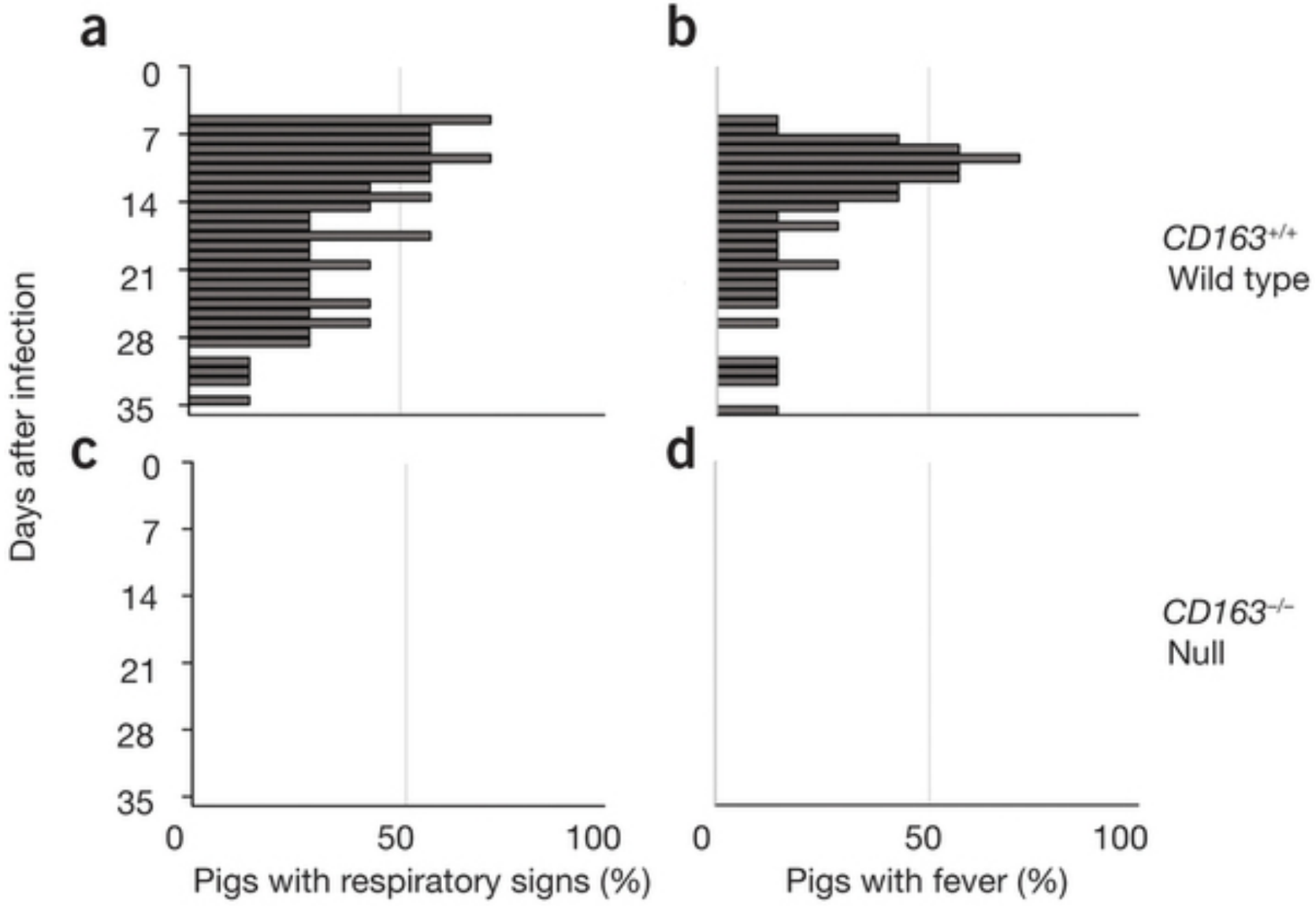
PRRS resistant pigs after knockout of CD163 by CRISPR/Cas

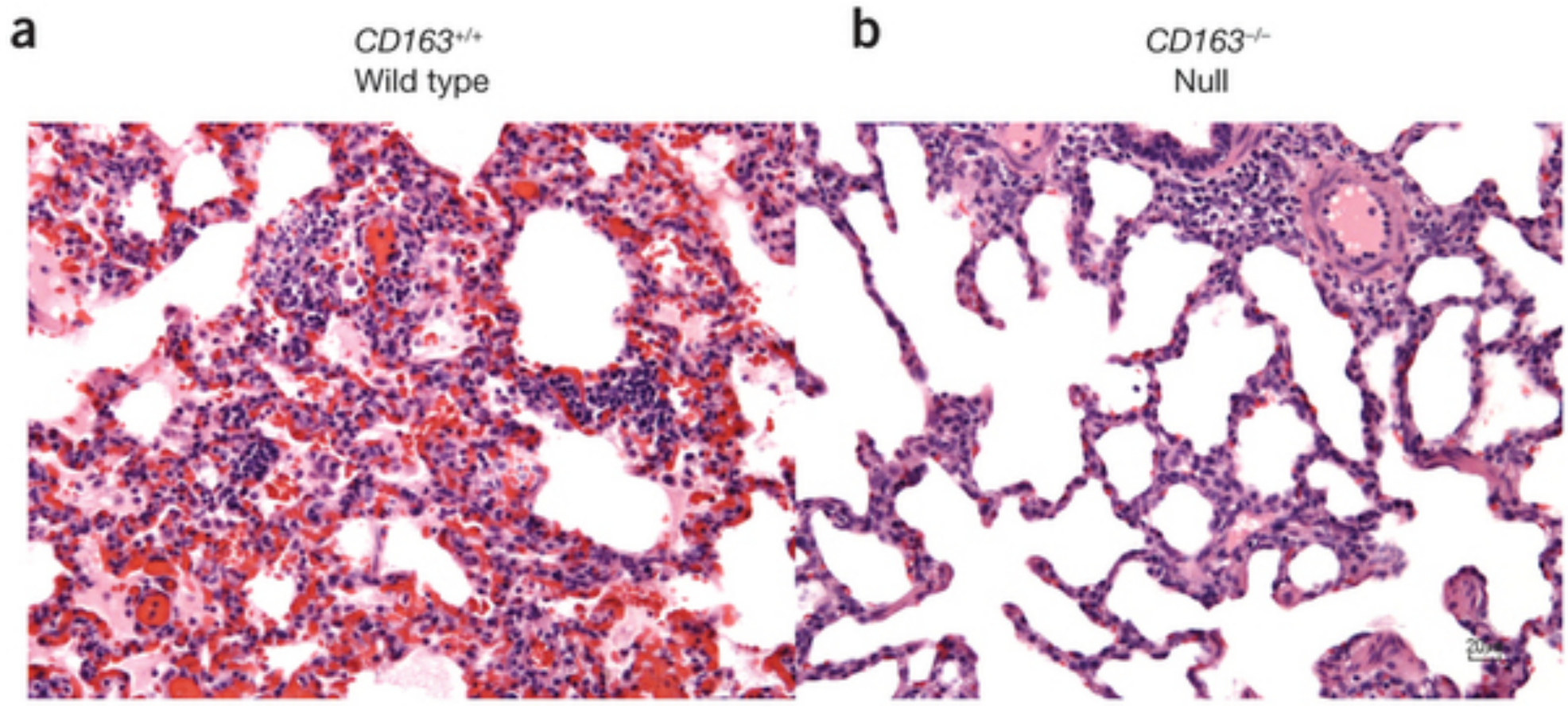


PRRS: Porcine reproductive and respiratory syndrome virus

Whitworth et al., Nature Biotechnology 34, 20–22 (2016)

PRRS resistant pigs after knockout of CD163 by CRISPR/Cas

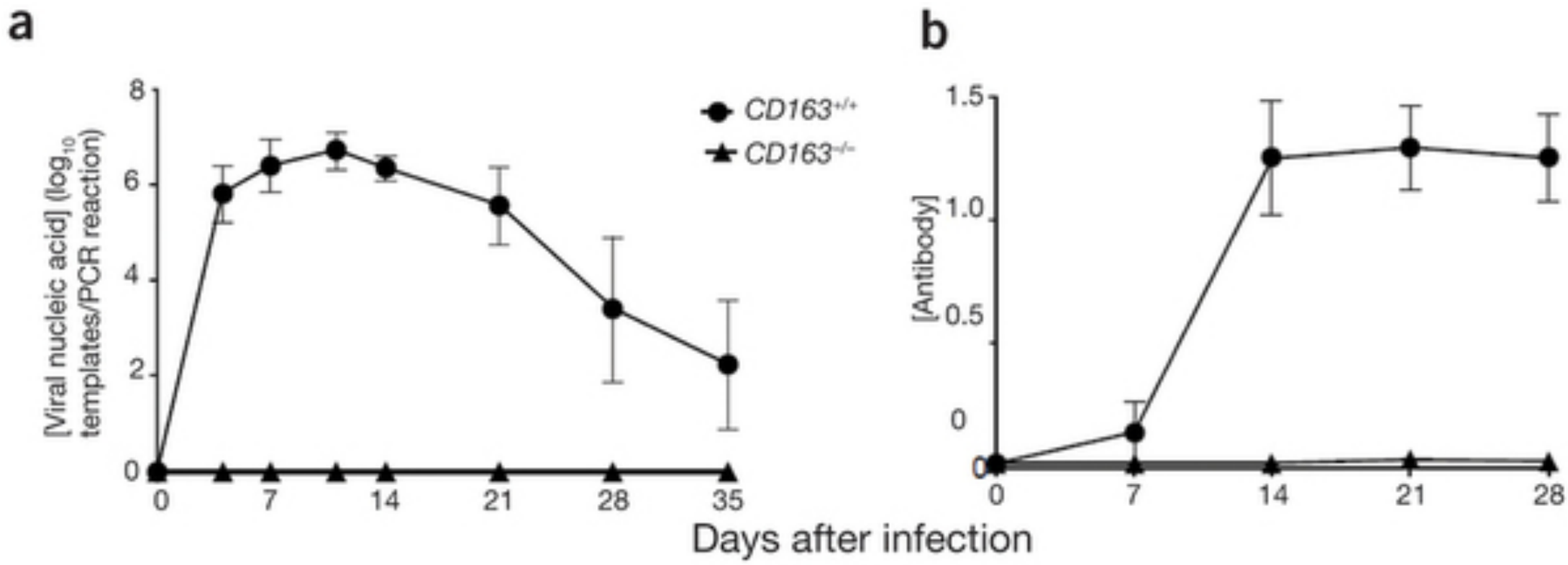
Mikroskopisches Bild in der Lunge von CD163+/+ und CD163-/-



Whitworth et al., Nature Biotechnology 34, 20–22 (2016)

PRRS resistant pigs after knockout of CD163 by CRISPR/Cas

PRRS specific DNA (a) und antibodies (b)



Whitworth et al., Nature Biotechnology 34, 20–22 (2016)

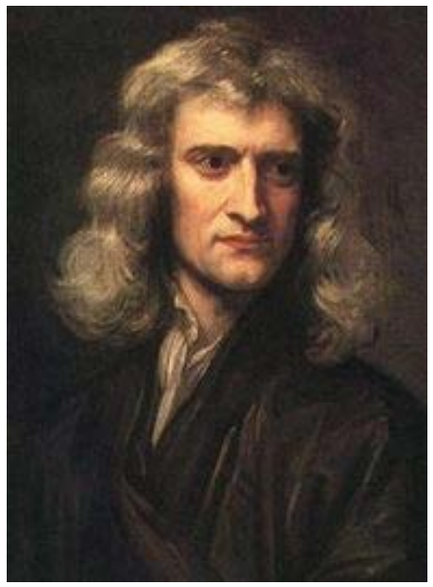
Gene-editing record smashed in pigs

Researchers modify more than 60 copies of the porcine endogenous retrovirus in effort to enable organ transplants into humans.

The gene-edited pigs will be raised in isolation from pathogens.

Also ~20 genes altered related to immunology and relevant for Xenotransplantation

Nature News 2015



What we know is a drop, what we don´t know is an ocean.

Isaac Newton (1643-1727)

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Gesetzliche Regulierung von genetisch veränderten Nutztieren

- FDA Final guidance on regulating genetically engineered animals (2009)

- EFSA Guidance on the risk assessment of food and feed from genetically modified animals including animal health and welfare aspects (2011)

- Codex Alimentarius, 2008. Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Animals. Codex Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organisation, Rome.

- DNA-Nukleasen: noch keine Regulierung, Tendenz geht z.Zt. dahin “Gene Editing” nicht gesetzlich zu regulieren (zumindest in USA).

To be a GMO or not to be a GMO

https://www.flickr.com/photos/securitydefenceagenda/8232274476

"There is no evidence that GM technologies are any riskier than conventional breeding technologies and this has been confirmed by thousands of research projects."

"Finally, we shouldn't forget that there are also other promising novel plant breeding technologies, post-GM, and we shouldn't make the mistake of regulating them to death as we have done with GM."

Anne Glover, former Chief Scientific Adviser to the President of the European Commission

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To be a GMO or not to be a GMO

Open letter to the Commission on new genetic engineering methods

27 January 2015

We call on the Commission to reject any attempt to exclude these new techniques from EU regulation.

In particular, we urge the Commission to ensure that:

- Organisms produced by these new techniques will be regulated as genetically modified organisms under existing EU regulations (Directive 2001/18). This means that they will require a full risk assessment before any approval or authorisation is given.
- Any food, feed and seeds as well as other breeding material produced using such new techniques will be labelled and fully traceable throughout the food and feed supply chain.
- Nothing in the TTIP and CETA negotiations will limit Europe's sovereignty and ability to regulate new genetic engineering methods and products as GMOs.
- Current GM health and environmental safety testing requirements are strengthened in light of the enhanced ability of these new techniques - individually or in combination - to alter the genetic code of plants, animals and other organisms.

To be a GMO or not to be a GMO

Die Europäische Kommission hat beschlossen, den Mechanismus für wissenschaftliche Beratung (SAM) um eine aktuelle wissenschaftliche Erläuterung zu den neuen Techniken der Pflanzen- und Tierzucht und bestimmten Anwendungen der Lebensmittelerzeugung zu bitten.
Der Mechanismus wurde unlängst eingerichtet, um die Kommission zeitnah mit hochwertiger, unabhängiger wissenschaftlicher Expertise zu versorgen.

Request to SAM HLG

SAM HLG is asked in the first instance and by March 2017 to provide an explanatory note on *new techniques in agricultural biotechnology including their potential agricultural application in synthetic biology and for gene drive, taking into consideration the most recent developments in the agricultural sector.*

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To be a GMO or not to be a GMO

Table 1 Comparison of SDN-1, -2, and -3 in relation to the legal interpretations (BVL, NGOs, BFN, NTWG, ZKBS, EFSA)

	BVL[1]	ZKBS[2]	NTWG[3]	EFSA[4,5]	NGOs[6]
SDN-1	Non GMO	Non GMO	Non GMO	Non GMO	GMO
SDN-2	Non GMO	Non GMO	Non GMO	Non GMO	GMO
SDN-3	GMO	GMO	GMO	GMO[b]	GMO
ODM	Non GMO[a]	Non GMO	Non GMO	Non GMO	GMO
RdDM	n.d	Non GMO	Non GMO	Non GMO	n.d
Interpretation	Process/product	n.d	n.d	n.d	Process

The classification refers to plants generated by using these techniques without stable integration of recombinant DNA

SDN site-directed nucleases, *ODM* oligonucleotide-directed mutagenesis, *RdDM* RNA-dependent DNA methylation, *n.d* no opinion given, *GMO* genetically modified organism, *BVL* German Federal Agency for Consumer Protection and Food Safety, *ZKBS* Zentrale Komission für biologische Sicherheit, *NTWG* New technology working group, *EFSA* European Food Safety Authority. *1* BVL 2015d, *2* ZKBS 2012, *3* Lusser et al. 2011, *4* EFSA 2012, *5* EFSA GMO unit 2015, *6* Krämer 2015, *7* Spranger 2015

[a] Serial steps should be considered separately

[b] Due to the known target site of the transgene lesser amounts of event-specific data might be necessary for the risk assessment

Summary and Conclusions

- Animal producers are challenged by climate change and burgeoning populations which requires development of biotechnologies that enhance productivity while reducing enviromental footprints and improving animal welfare.

- According to official data of FAO estimations, in order to feed a larger, more urban and richer human population, food production must be increased around 70% before the year 2050.

- Genome editing tools, such as ZFNs, TALENs and CRISPR/Cas offer sophisticated new opportunities to generate genetically modified animal models and have revolutionized the field of genetic modification.

- Genetically modified livestock can be generated by microinjection, without the need of true embryonic stem cells or induced pluripotent stem cells or SCNT technology, which has democratized genetic modification of livestock.

Summary and Conclusions

- Genome editing tools can now be used to elegantly knockout livestock genes or to precisely knock-in transgenes at specific genomic sites within the livestock genome.

- Specificity to avoid off-target events is still a challenge which has to be addressed and would be a critical prerequisite for employing these technologies in human patients.

- Gene editing dramatically shortens the introduction of genetic changes in livestock compared to breeding.

- Genome editing leaves no footprint in the genome, not distinguishable from natural mutations. Whether gene edited livestock is considered as GMO is currently a matter of debate. The approval of the AquAdvantage salmon could be a “game changer“.

Changed the societal question from:

If we could do it, would we want it?

To

Next year we will have it; will we allow it?

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F L I

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Klaus-Gerd Hadeler
SCNT Team
SVA Team

Thank you for your attention

„Success is the ability to go from one failure to another with no loss of enthusiasm“
(Winston Churchill)

Approved GM vertebrates

- GloFisch, genetically engineered zebrafish, no regulation necessary (FDA statement 2003)

- Atryn (antithrombin III), produced in the mammary gland of transgenic goats, approved by EMA (2006) and FDA (2009)

- Ruconest (C1 esterase inhibitor), produced in the mammary gland of transgenic rabbits, approved by EMA (2010)

- AquAdvantage salmon,added growth hormon from Pacific Chinook salmon, all year long expression, faster growth, approved by FDA (Nov.2015)

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Zusammenfassung und Schlussfolgerungen

- Die Genome der landwirtschaftlichen Nutztiere sind inzwischen sequenziert worden; damit liegen informative Genkarten vor, die züchterisch genutzt werden können (GBV).
- Neue molekulare Hilfsmittel wie z.B. DNA-Nukleasen erlauben zuverlässig präzise genetische Veränderungen (Gen Editing), die relativ einfach und effizient einzubringen sind. Die Risiken liegen vor allem bei der Induktion von „Off-target“ Mutationen.
- Die Nutzung der neuen genomischen Kenntnisse und Verfahren des Gen Editings erlauben die Entwicklung neuer Zuchtstrategien für die landwirtschaftliche Tierproduktion und die Biomedizin.
- Komplexe gesetzliche Regeln für die Anwendung transgener Tiere sind vorhanden. Der Einsatz von Gen Editing ist z.Zt. gesetzlich nicht geregelt, und könnte deshalb in der praktischen Anwendung der landwirtschaftlichen Tierzucht erfolgen.

Entwicklung der Nutztierzucht

- Domestikation
- Vermehrung „nützlicher“ Populationen
- Selektion nach dem Exterieur
- Selektion nach spezifischen Merkmalen
- Systematische Zucht auf der Basis von Populationsgenetik und Statistik
- Reproduktionsbiotechnologien (AI, ET, IVP, SCNT, etc.)
- Molekulargenetik und Genom basierte Zuchtkonzepte (SNPs, GBV, etc.)
- Zukünftig: ***Precision breeding***