Targeted agricultural production by genetically modified farm animals

- Status of livestock genomic maps
- Genetic modifications via DNA nucleases (Gene Editing)
- Perspectives: Precision breeding concepts

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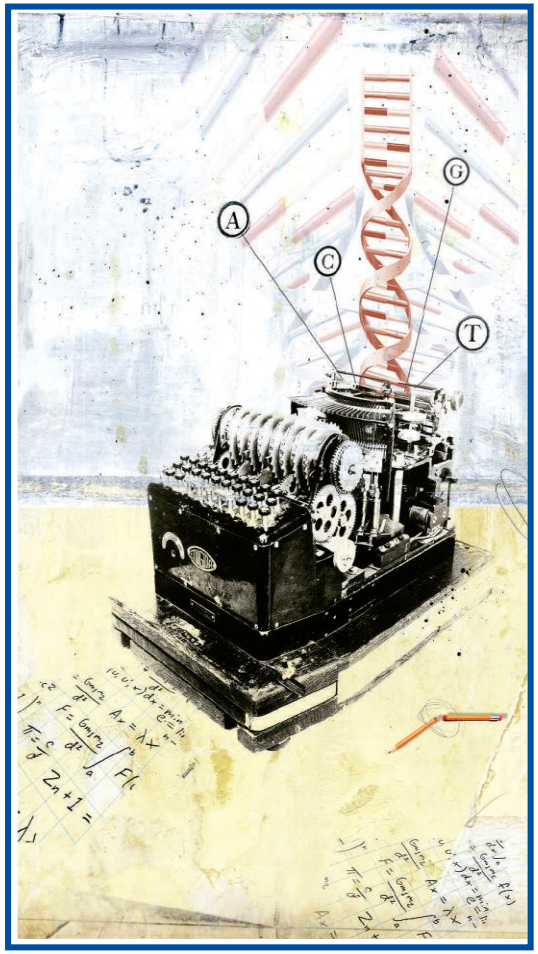
65th International Congress of Meat Science and Technology (ICoMST) | Potsdam, Germany, August 4 – 9th, 2019

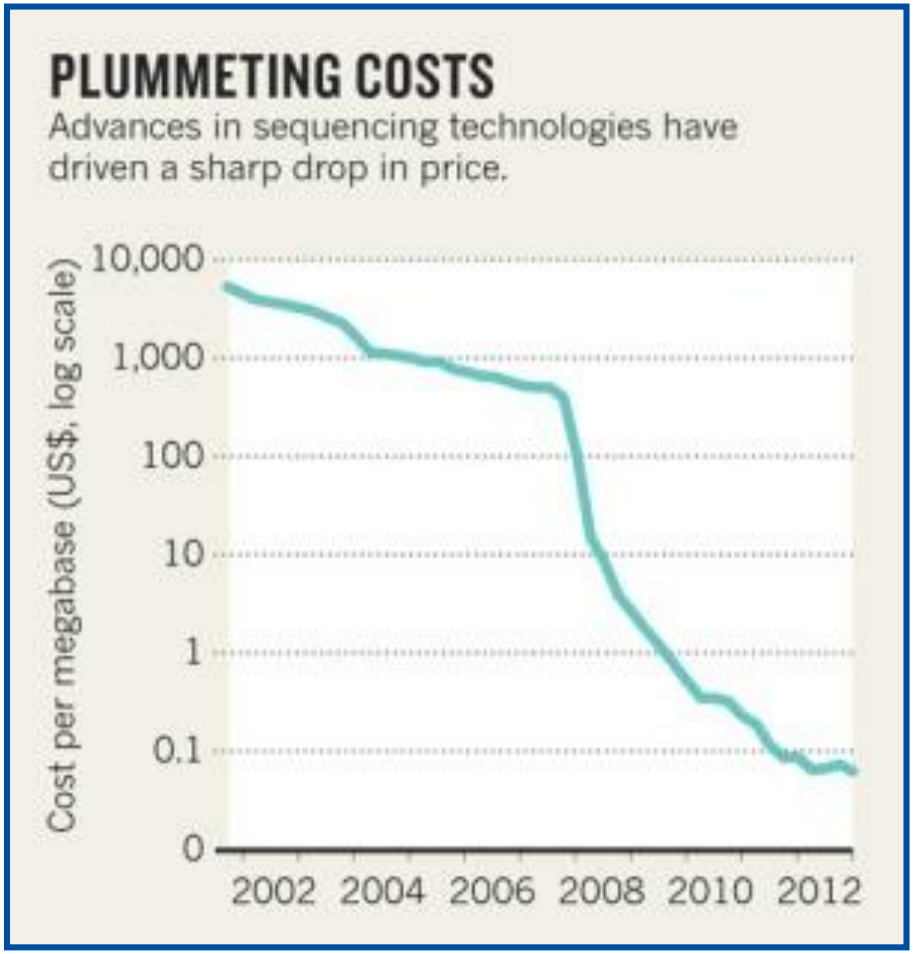
Current status of genome sequencing and annotation in farm animals

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

Nature 426, 2003

Rapid and significant reduction of sequencing costs





Nature 497, 2013

Features of the porcine genome

- Phylogenetic split between European and Asian wild boars ~1mill. year ago;
- Porcine genome contains ~21,640 protein coding genes, 380 pseudogenes, ~3000 nc RNAs, 197700 gene exons and ~26,500 gene transcripts;
- Only ~5% of the genome is actively transcribed into proteins;
- Extraordinary large repertoire of olfactory receptor genes;
- Porcine genome has 39 type I interferon genes (twice the number in humans and mice);
- 112 genomic positions where porcine protein has the same amino acid that is implicated in a human disease, incl. obesity, diabetes, Parkinson‘s or Alzheimer disease;
- Porcine genome has fewer PERV than many vertebrates, which in addition are also mostly defective.

Groenen et al., 2012 Nature 491, 393-398

Use of new genomic information

- **More precise breeding programs**
- ***Genomic breeding value; direct sequencing***

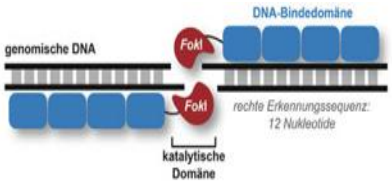
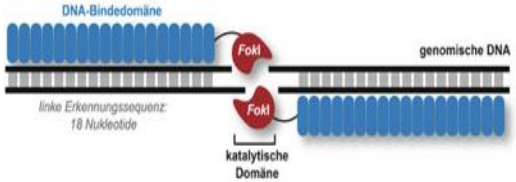
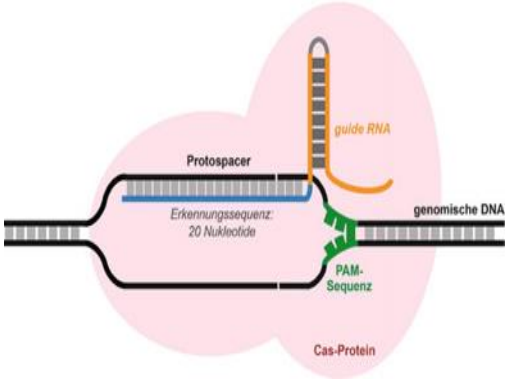
The genomic breeding value (GBV) avoids test bull maintenance, is much more precise and secure with regard to the prediction of the genetic gain and reduces costs.

- **Transcriptomics/Proteomics/Phenomics**
- **Production of genetically modified animals (Gene Editing)**
- **New knowledge about genetic diversity**
- **Descent studies**
- **Comparative genomics**

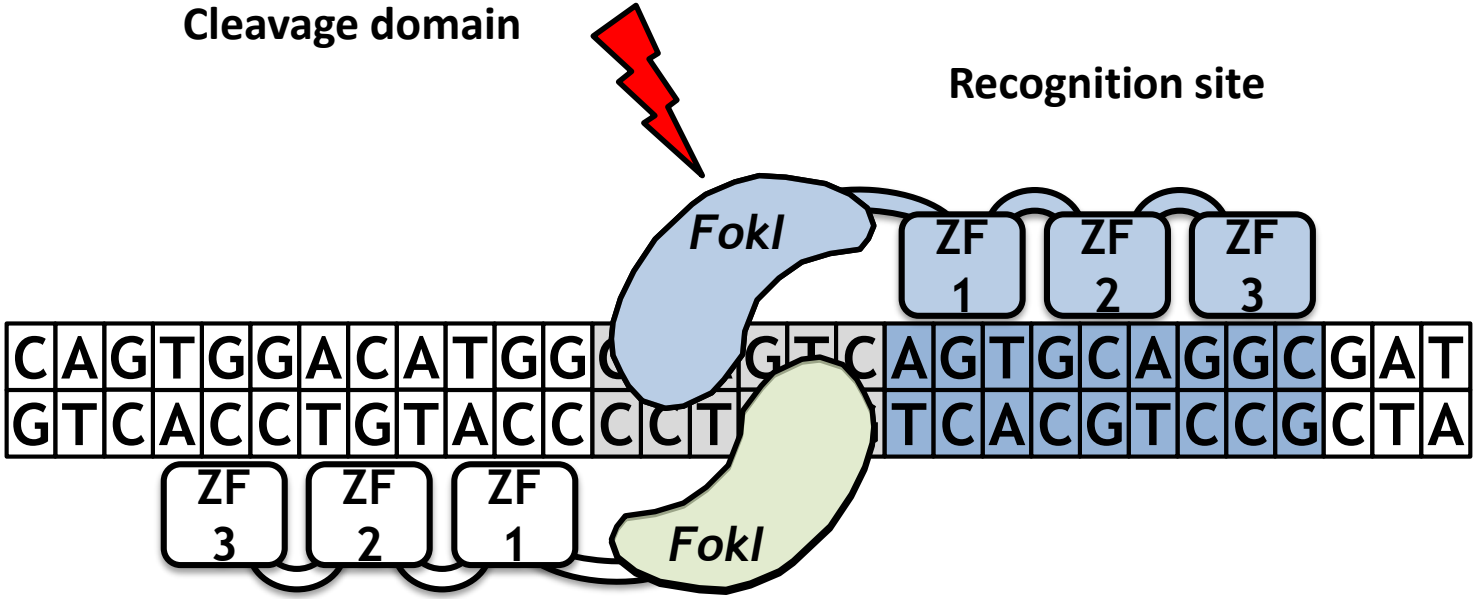
Novel tools for precise genetic engineering in domestic animals

- Designer nucleases (ZFN molecules, TALEN, CRISPR/Cas)
- Transposons (SB, PiggyBac, Tol 2)
- Pluripotent cells (iPS cells)

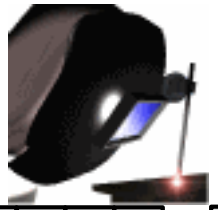
The different classes of gene scissors

	Zinc-finger-Nucleases (ZFN)	*TAL-Effektor-Nucleases (TALEN)*	*CRISPR/Cas-Nucleases (CRISPR)*
			
Recognition sequence:	**~24 bp**	**~36 bp**	**~22 bp**
Employed since:	**since 2003**	**since 2011**	**since 2013**
In practical use (clinic):	**since 2013**	**since 2016**	**since 2016**

Mechanism of Gene Editing Nucleases I



Mechanism of Gene Editing Nucleases II



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

Mechanism of Gene Editing Nucleases III

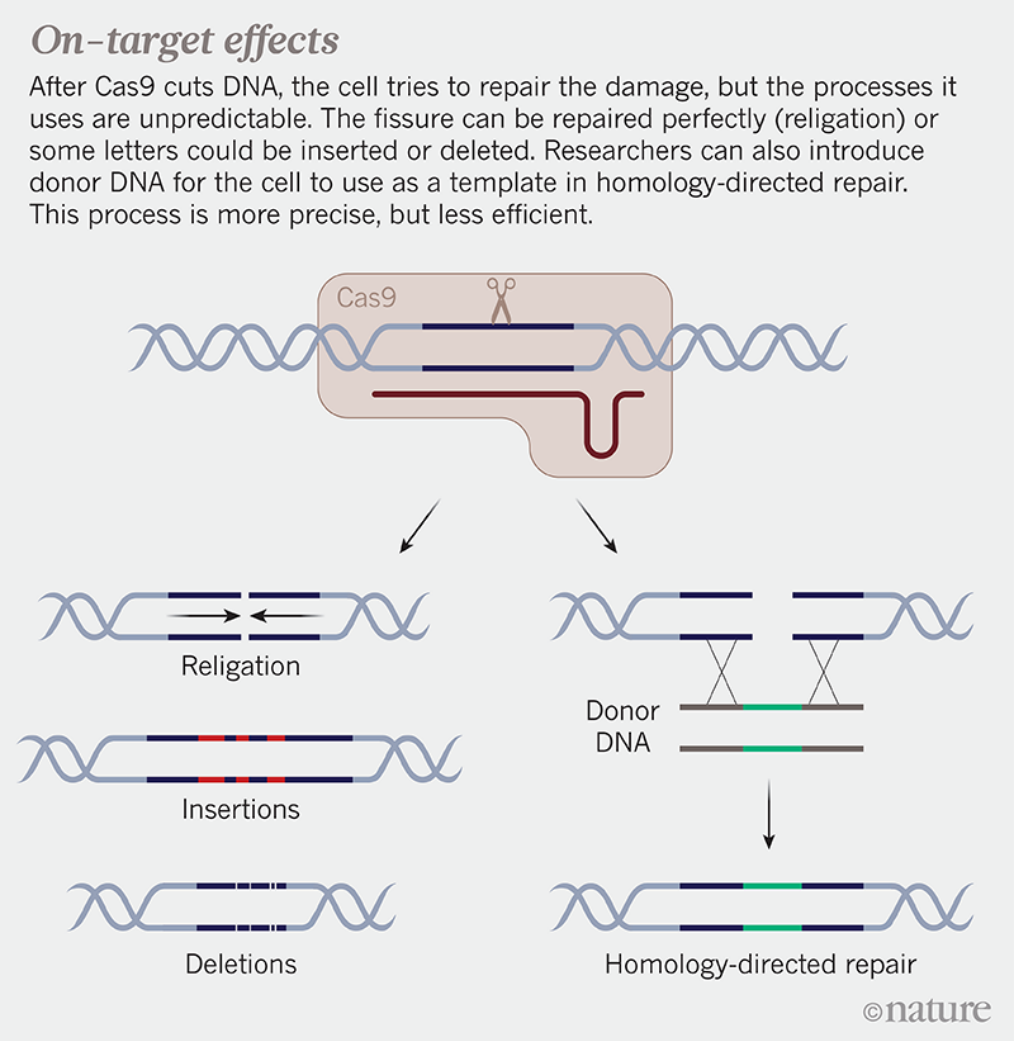
C	A	G	T	G	G	A	C	A	T	G	G	G	G	A
G	T	C	A	C	C	T	G	T	A	C	C	C	C	T

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

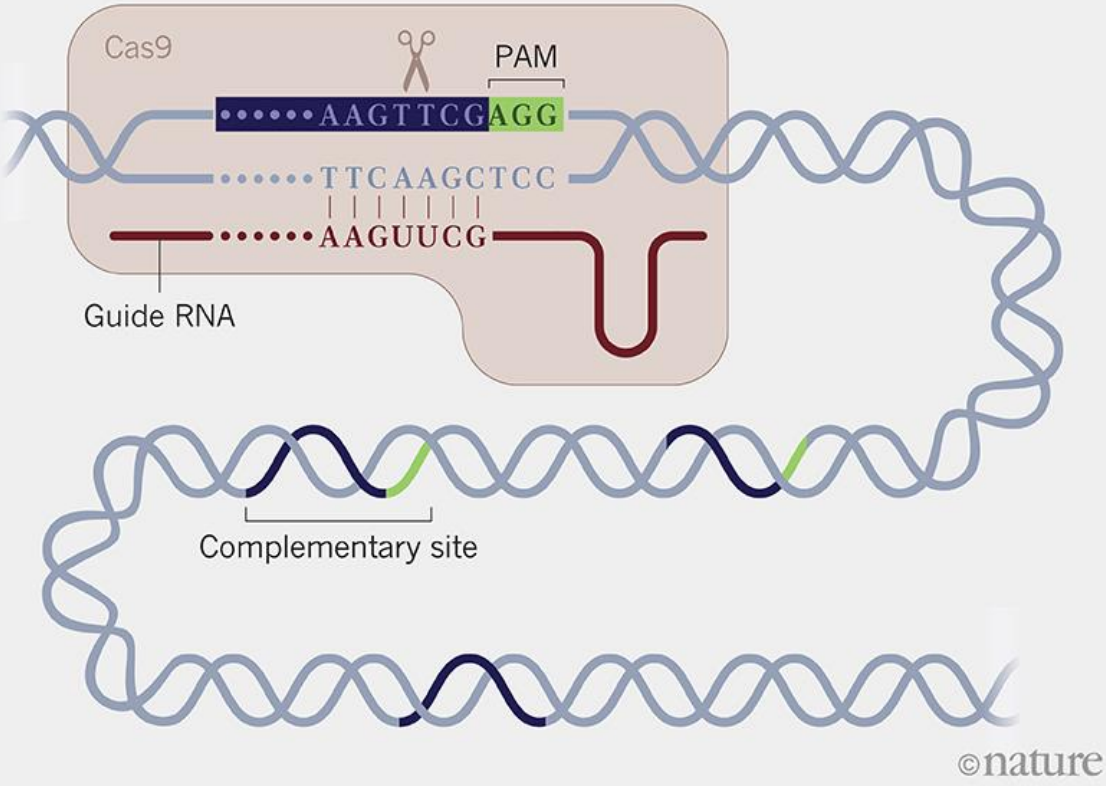
Mechanism involved in gene editing



Off targeting events in gene editing

Off-target effects

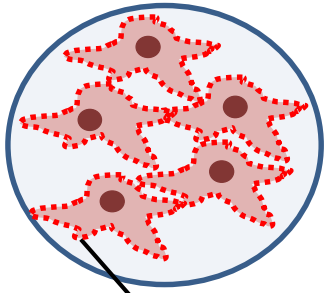
The Cas9 protein works like a pair of molecular scissors. A guide RNA sequence binds to a complementary DNA sequence that is adjacent to a string of letters known as the proto-spacer adjacent motif (PAM). But there can be many sites in the genome that contain the same or similar sequences, so Cas9 might cut in the wrong places.



©nature

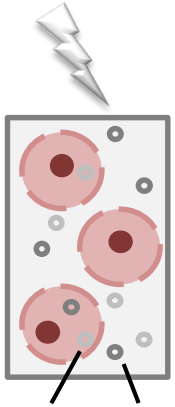
Strategy to arrive at pigs with homozygous knockout for α-gal

Cell culture of porcine fetal fibroblasts (WT)



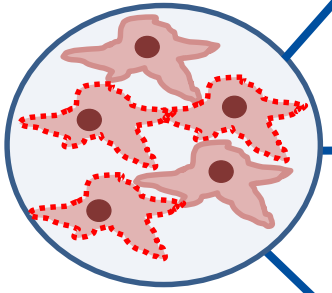
Gal-epitope

electro-poration

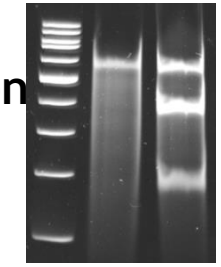


ZFN plasmids

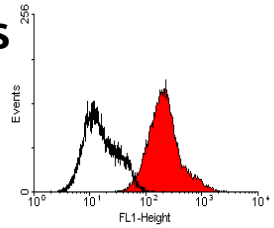
Cell culture: Gal-positive and Gal-negative cells



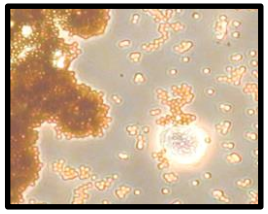
DNA-isolation
- Cel-I assay
- NHEJ



FACS analysis with cells (IB4-FITC)
- biallelic KO



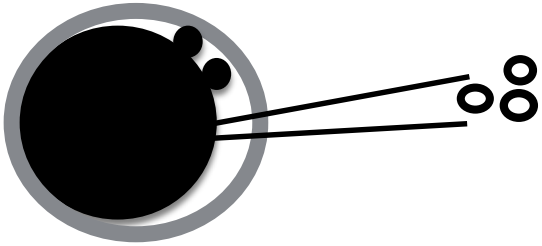
Selection for Gal-negative cells with magnetic beads



SCNT

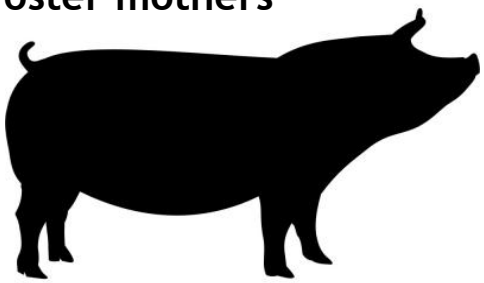
Gal-negative piglets

Production of GGTA1-KO pigs after injection of CRISPR/Cas plasmids

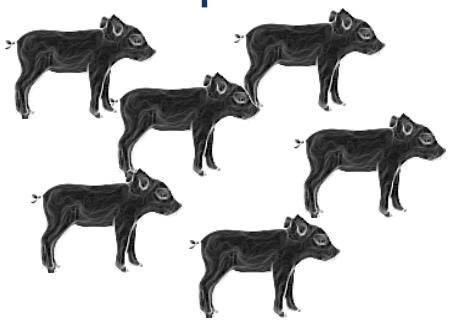


Intracytoplasmic microinjection of CRISPR/Cas DNA into *in vivo derived* zygotes

Transfer of microinjected zygotes into foster mothers



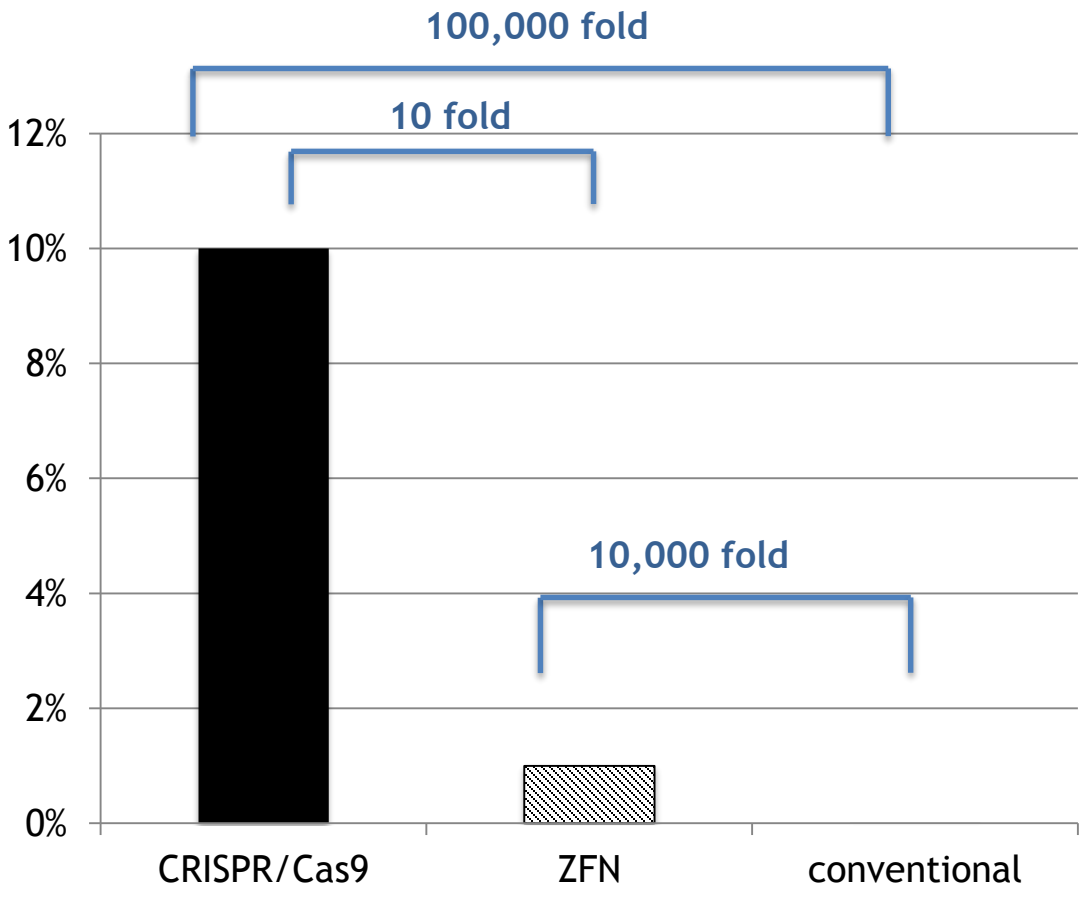
Termination of pregnancy around day 26 of gestation. Fetus analysis



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

Petersen et al., Xenotransplantation 23, 338-346, 2016

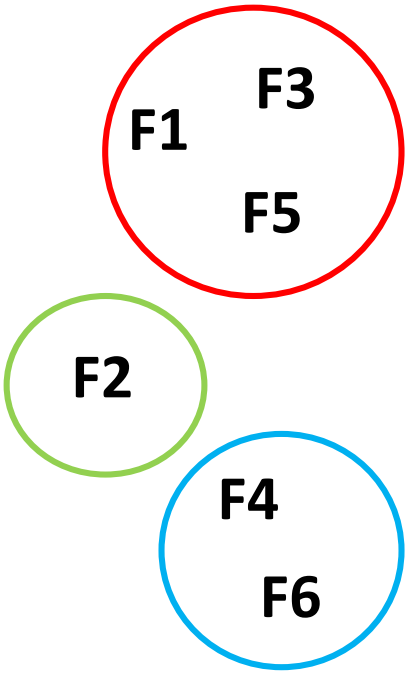
Bi-allelic targeting efficiency of the porcine GGTA1-locus by CRISPR/Cas or ZFN



Petersen et al., unpublished

Sequencing of porcine GGTA1-locus

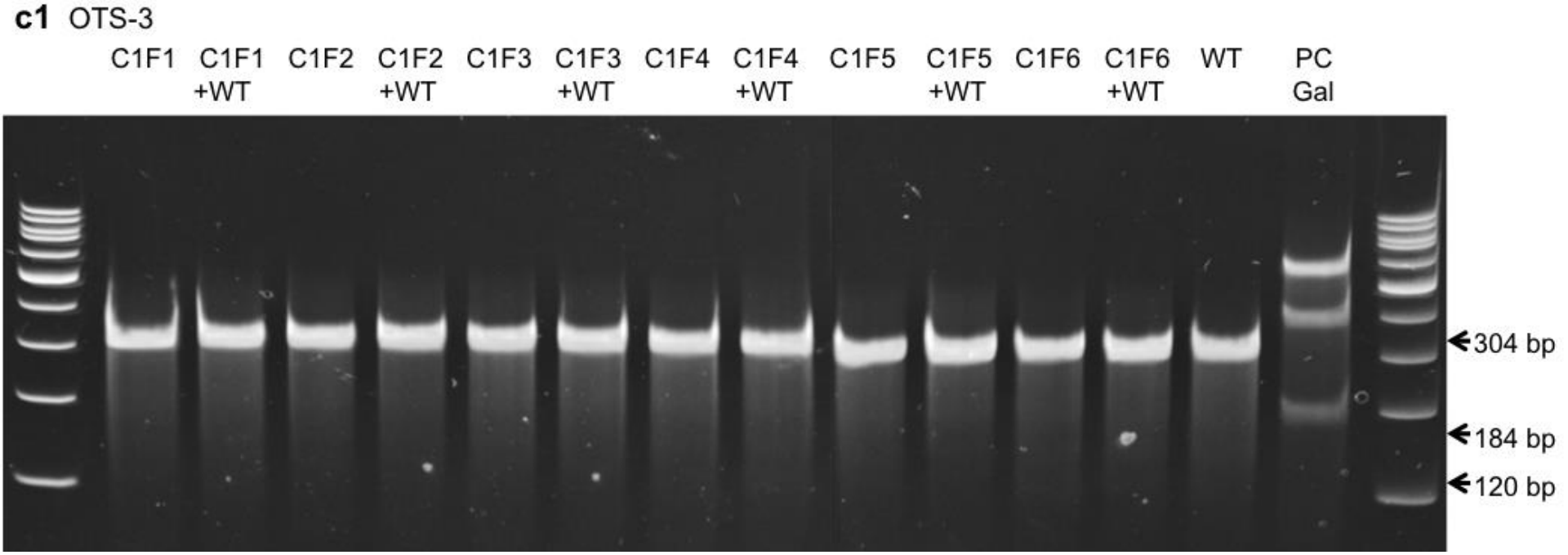
```
                ZFN-GGTA1-23713          ZFN-GGTA1-23714
      WT:CGGTGGCTCAGCTACAGGCCTGGTGGTACAAGGCAC
E41   C1F1:CGGTGGCTCAGCTACAG-CCTGGTGGTACAAGGCAC
           CGGTGGCTCAG-T------CTGGTGGTACAAGGCAC
      C1F2:CGGTGGCTCAGCTACA----TGGTGGTACAAGGCAC
           CGGTGGCTCAGCTACAGGCCTGGTGGTACAAGGCAC
                            CCGG
      C1F3:CGGTGGCTCAGCTACAG-CCTGGTGGTACAAGGCAC
           CGGTGGCTCAG-T------CTGGTGGTACAAGGCAC
      C1F4:CGGTGGCTCAGCTACAG-CCTGGTGGTACAAGGCAC
      C1F5:CGGTGGCTCAGCTACAG-CCTGGTGGTACAAGGCAC
           CGGTGGCTCAG-T------CTGGTGGTACAAGGCAC
      C1F6:CGGTGGCTCAGCTACAG-CCTGGTGGTACAAGGCAC
      C2F1:CGGTGGCTCAGCTACAGGCCT------ACAAGGCAC
E45        CGGTGGCTCAGCTAC-----TGGTGGTACAAGGCAC
      C2F2:ATGGACGTGGAT--(-96bp)--ATACGAGAGGCGG
```



Lia

Hauschild et al. PNAS 108, 12013 -12017 (2011)

Checking for off-target mutations



- The 10 most possible off-target sites were analyzed by PCR amplification and mutation detection assay (only one OTS is shown)

→ no off-target cleavage occurred

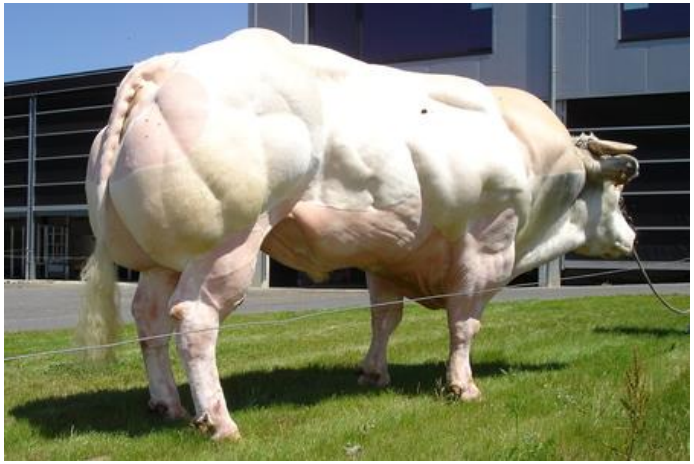
Hauschild et al. PNAS 108, 12013 –12017 (2011)

Application areas of DNA-nucleases (Gene Editing)

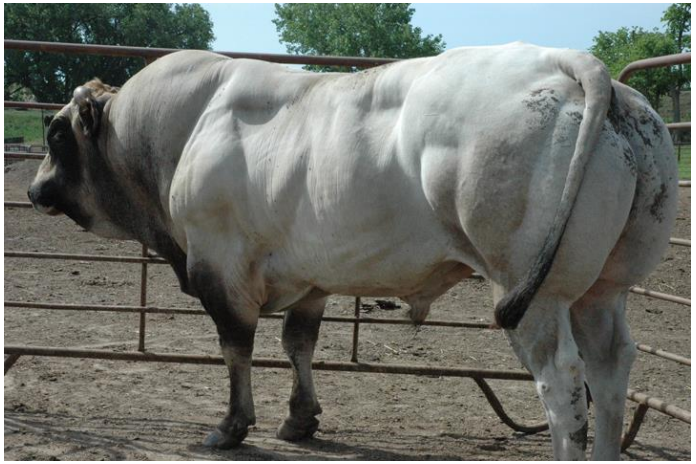
- Improved production of animal derived products
- Genetically determined disease resistance
- Production of hornless cattle
- Removal of allergenic molecules (egg white, milk)
- Sex determination
- Rescue of endangered breeds
- Pets (Minipigs, Koi, dogs)
- Disease models
- Biomedical applications (xenotransplantation, pharming)
- Vector control (insects, mosquitos)

Knockout of the MSTN gene via gene editing

- Myostatin (*MSTN,GDF-8*) is a negative regulatory element for growth of skeletal growth (Antagonist of IGF 1);
- The inhibition of skeletal growth by mutations of the *MSTN* gene causes the „double muscling" phenomon in cattle;
- Natural mutations are well-known for **Blue Belgian** and **Piedmontese** breeds (~20% more muscle).



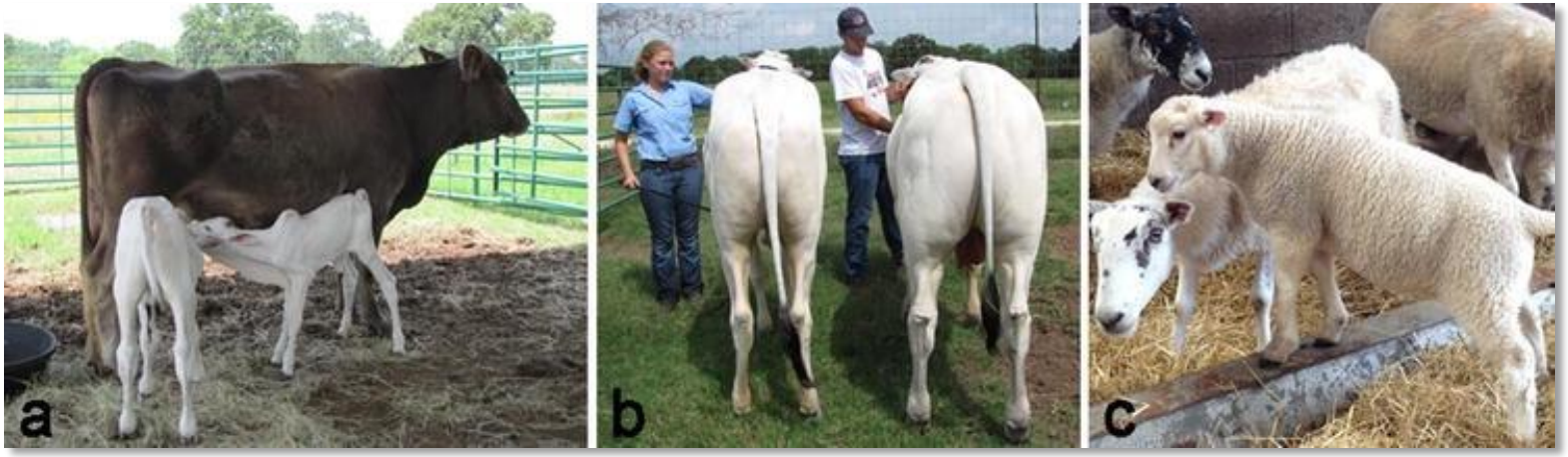
Blue Belgian



Piedmontese

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

Cattle and sheep with a TALEN mediated knockout for Myostatin

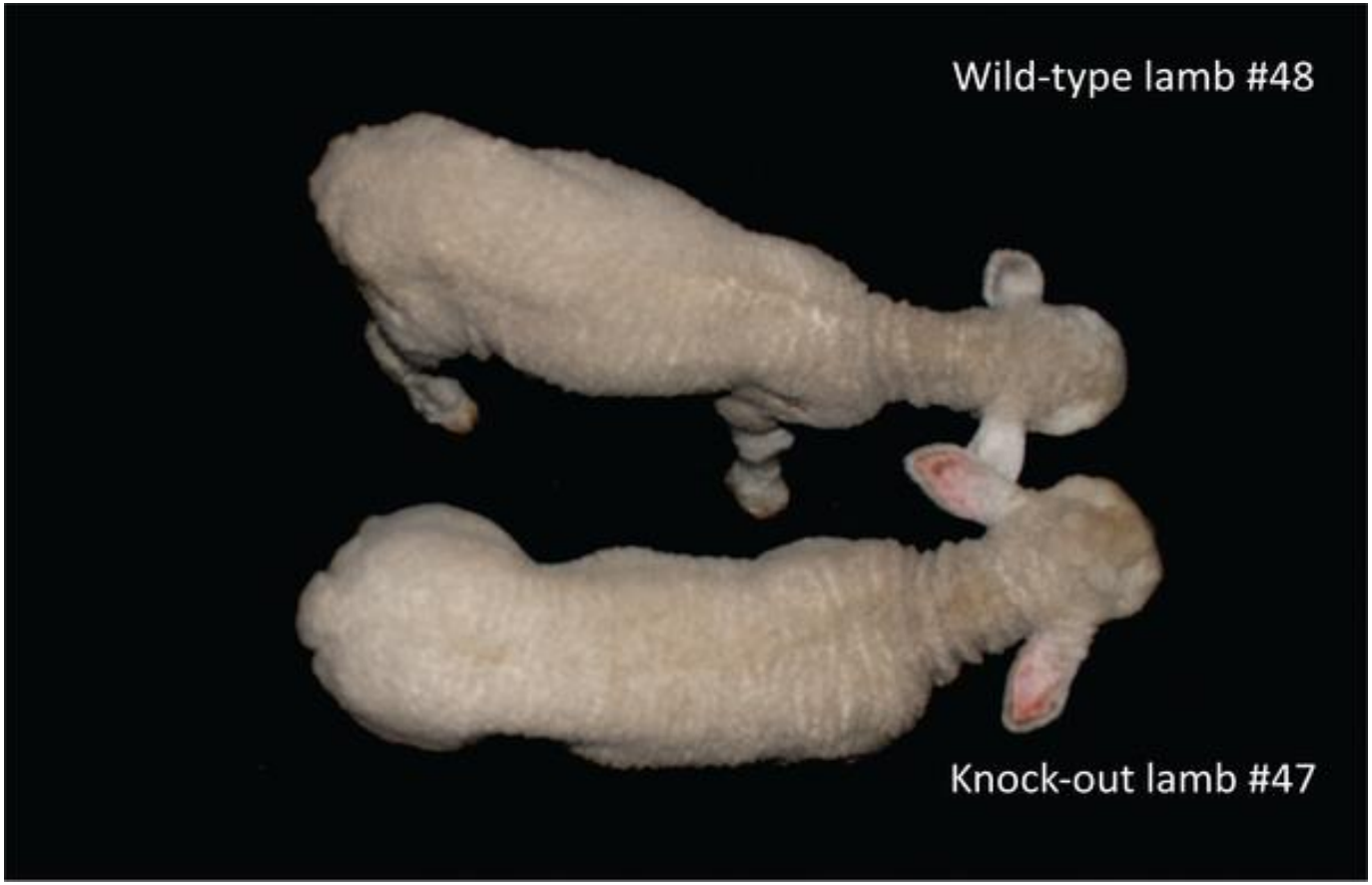


MSTN edited animals. A: The live born bull (bull #1: left) and heifer calf (right). B: The readily observed phenotypic difference between bull #1 (right) and the wild-type heifer (left). C: The edited lamb

```
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


Sheep WT           GTGATGAGCACTCCACAGAATCTCGATGCTGTCGTTACCCTCTAACTGTGGATTTTGA

Sheep Allele 1     GTGATGAGCACTCCACAGAATCTCGATGCTGTCGTTACCCTCTAACTGTGGATTTTGA   WT
Sheep Allele 2     GTGATGAGCACTCCACAGAATCTCGATGCTGT---TACCCTCTAACTGTGGATTTTGA   ΔR283
```

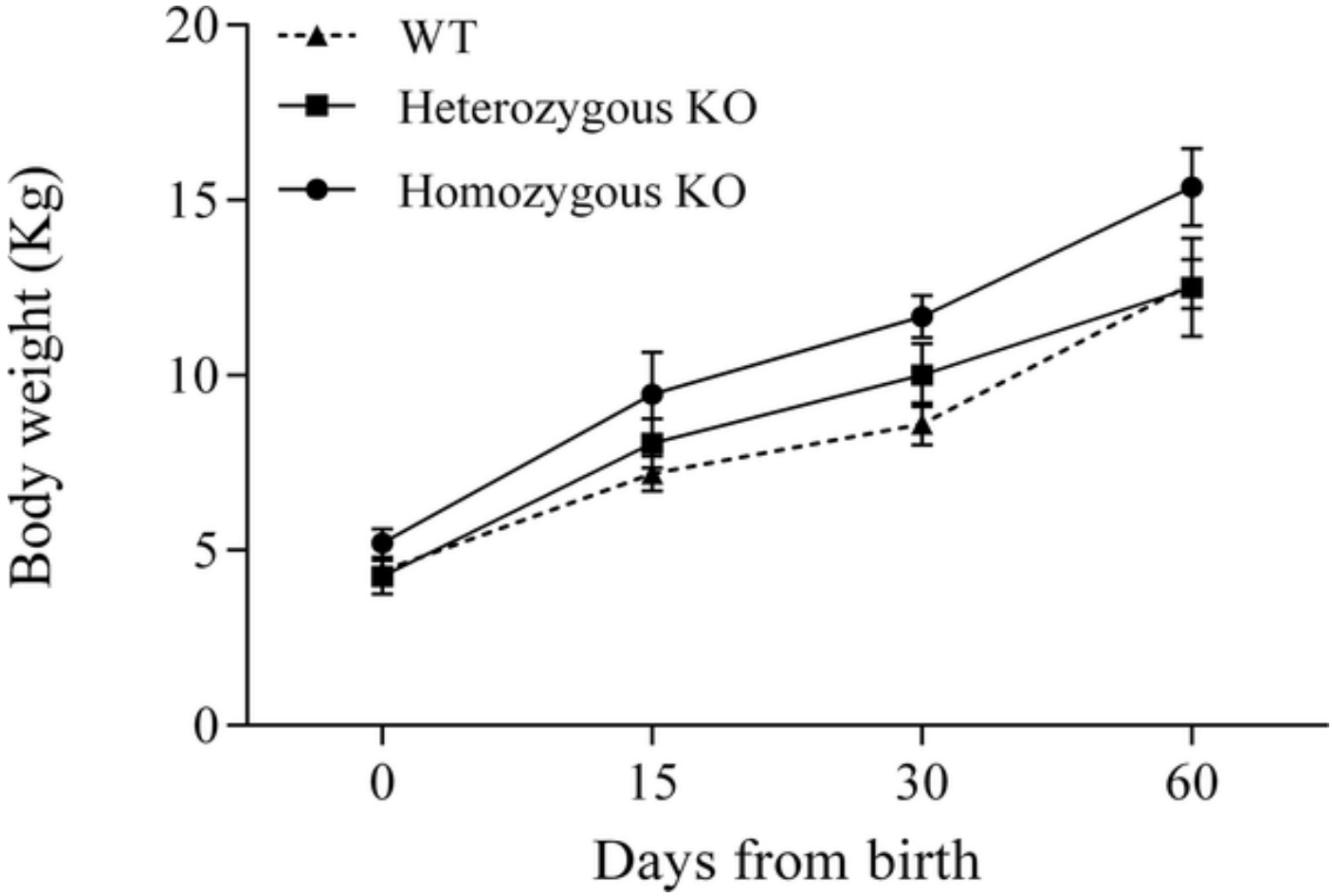
Proudfoot et al., 2015, Trans. Res.

Phenotype of WT- and MSTN-KO lambs at an age of 30 days



Crispo et al., 2015 Plos One, doi/10.1371/journal.pone.0136690

Body weight of lambs after knockout of the myostation locus via CRISPR/Cas



Crispo et al., 2015 Plos One, doi/10.1371/journal.pone.0136690

Myostatin knockout pigs, produced by genome edited via TALENs

Produced in Korea 2015

Nature, July 2015

Modification of porcine body composition

Reconstitution of *UCP1* using CRISPR/Cas9 in the white adipose tissue of pigs decreases fat deposition and improves thermogenic capacity

Qiantao Zheng[a,b,1], Jun Lin[c,d,1], Jiaojiao Huang[a,b,1], Hongyong Zhang[a,b], Rui Zhang[a,b], Xueying Zhang[e], Chunwei Cao[a,b], Catherine Hambly[f], Guosong Qin[a,b], Jing Yao[a,b], Ruigao Song[a,b], Qitao Jia[a,b], Xiao Wang[a,b], Yongshun Li[a], Nan Zhang[a], Zhengyu Piao[g], Rongcai Ye[c,d], John R. Speakman[e,f], Hongmei Wang[a,b], Qi Zhou[a,b], Yanfang Wang[h,2], Wanzhu Jin[b,c,2], and Jianguo Zhao[a,b,2]

UCP1 (Uncoupling protein 1):

- localized in inner mitochondrial membrane, generates heat by uncoupling ATP-synthesis from proton transit through membrane;

- Is non-functional in the domestic pig

Zheng et al. PNAS E9474-9482, 2017

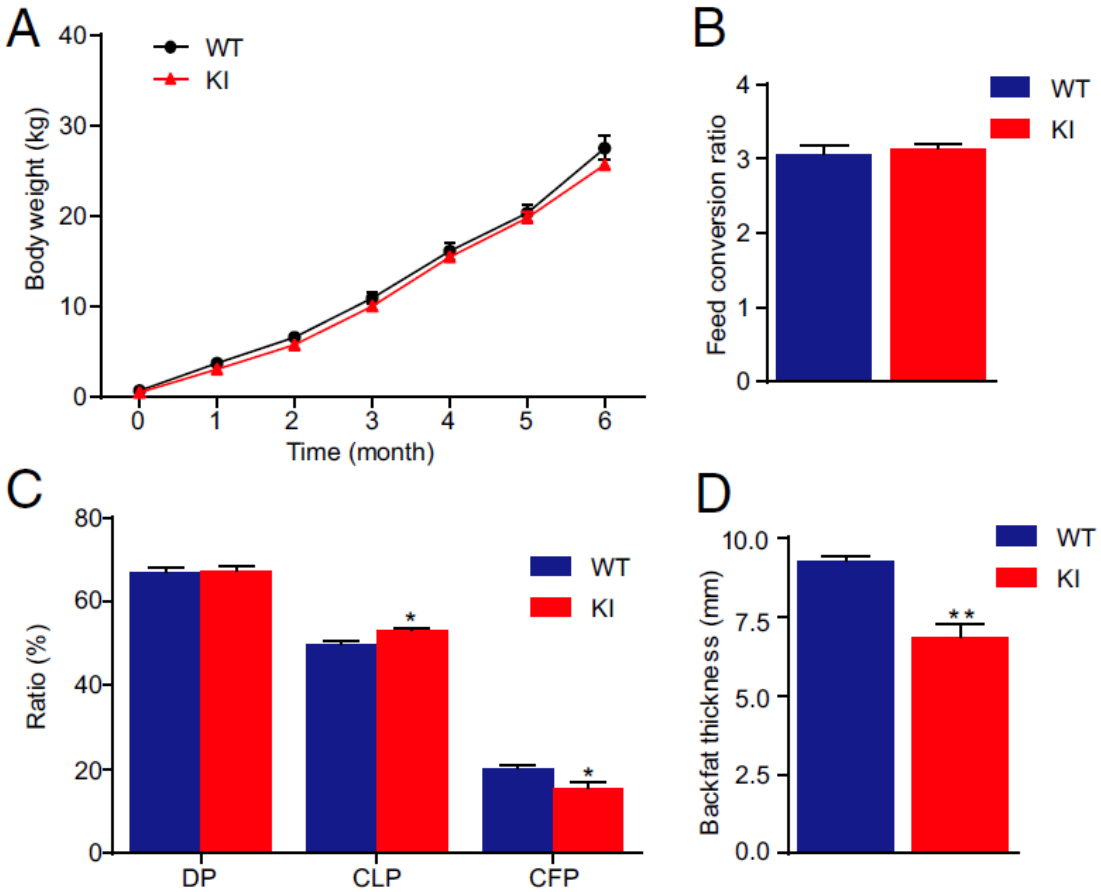
Modification of porcine body composition

- Insertion of mouse UCP1 into pUCP1 locus via CRISPR/Cas led to:

- Improved body temperature abilities upon cold temperature exposure
- Significant reduction of with body fat,
- Enhanced lean carcass

Zheng et al. PNAS E9474-9482, 2017

Modification of porcine body composition



Reduction of white fat upon UCP1 expression in pigs

Zheng et al. PNAS E9474-9482, 2017

Production of ß-lactoglobulin free (allergen-free) milk by cows genetically modified by CRISPR/Cas zygotic injection

SCIENTIFIC REPORTS

OPEN

Cattle with a precise, zygote-mediated deletion safely eliminate the major milk allergen beta-lactoglobulin

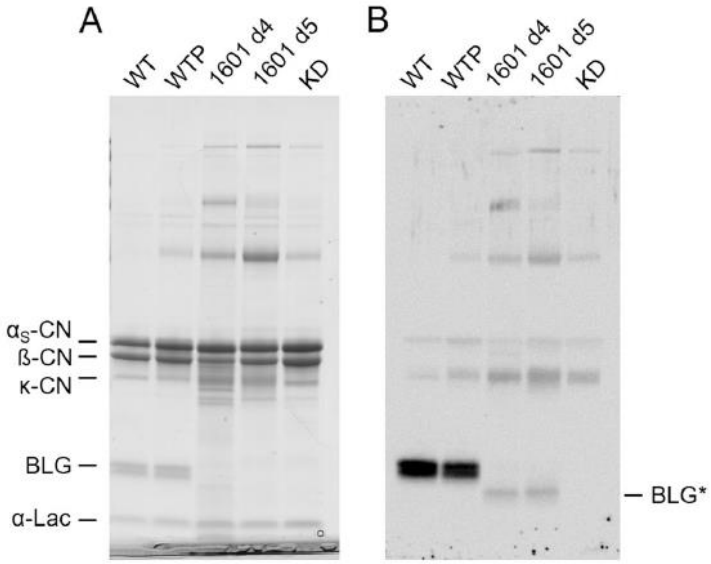
Received: 22 January 2018
Accepted: 19 April 2018
Published online: 16 May 2018

Jingwei Wei[1], Stefan Wagner[1,2], Paul Maclean[1], Brigid Brophy[1], Sally Cole[1], Grant Smolenski[1,3], Dan F. Carlson[4], Scott C. Fahrenkrug[4], David N. Wells[1] & Götz Laible[1]

Production of ß-lactoglobulin free (allergen-free) milk by cows genetically modified by CRISPR/Cas zygotic injection

1601 1602

Figure 2. Cattle, genome-edited for a precise disruption of the *LGB* gene. Shown are the 1601 female and 1602 male with biallelic edits of a repair template-directed nine bp deletion at the age of 19 months that were produced by zygote-mediated HDR editing.



A: Coomassie Blue staining
B: Western Blotting/SDS-PAGE separation of proteins

Wei et al., 2018, Scientific Reports

Targeted microRNA expression in dairy cows leads to the production of β-lactoglobulin-free, Casein enriched milk

Cow	Milk	Total Casein, mg/g	Whey, mg/g			
			α-Lac	BLG-A	BLG-b	Total
miRNA 6-4	Induced, day 1	98.2	3.9	0.0	0.0	3.9
miRNA 6-4	Induced, day 2	96.8	3.5	0.0	0.0	3.5
miRNA 6-4	Induced, day 3	106.6	4.3	0.0	0.0	4.3
miRNA 6-4	Induced, day 4	128.6	5.3	0.0	0.0	5.3
WT-1	Natural, day 69	39.6	1.5	5.7	0.6	7.8
WT-2	Induced, day 5	38.8	1.5	7.6	0.7	9.8
WT-3	Induced, day 5	32.5	1.5	7.3	0.9	9.4
WT-4	Colostrum, day 1	48.1	1.7	10.1	4.0	15.7
SEM-*	-	1.27	0.09	0.12	0.11	0.12

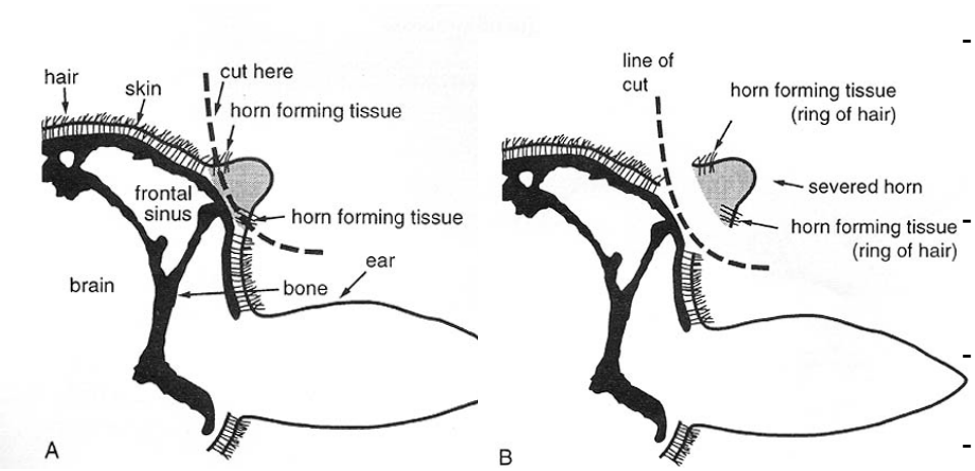
Jabed A et al. PNAS 2012;109:16811-16816

Horn and hornless cattle breeds (Polled Locus)

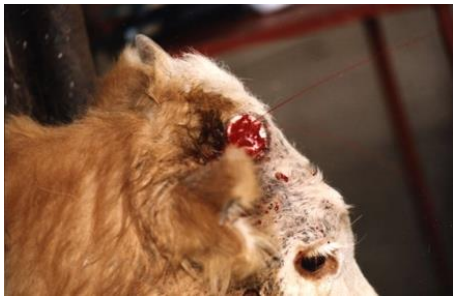
Belted Galloway

Category	Breed
Polled	Aberdeen Angus Belted Galloway Galloway Swedish Red Polled (S) Old Norwegian Red Vestland
>20% Polled	Norwegian Red Welsh Black
<5% Polled	Holstein Jersey Simmental Fleckvieh Ayreshire Dexter Charolais Limousin Salers
horned	Highland Cattle

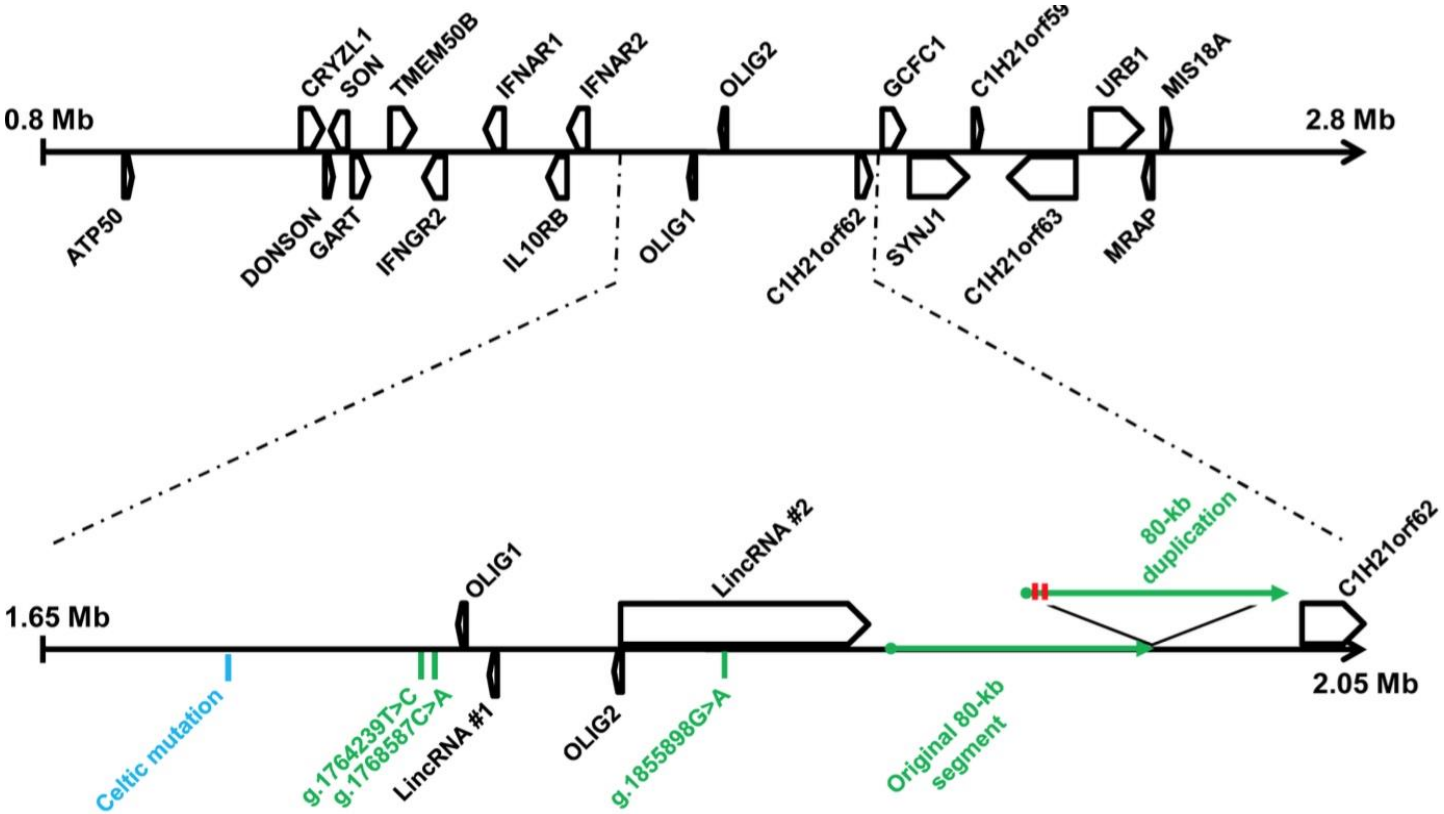
Dehorning in cattle



- 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).



Candidate mutations for phenotype „Hornless“ within Polled Locus



Celtic Mutation consists of 212 bases insertion and 10 bases deletion; in Holstein Friesian cattle an 80 kb duplication is prevalent. In the starting ares of the duplication two sequence variations compared to the original sequence are found (red lines). Localisation of 3 other candidate mutations are 1764239T>C, 1768587C>A, 1855898G>A.

Gene-editing of Polled locus: Spotiguy, born 2015, with two of his clones

Recombinetics

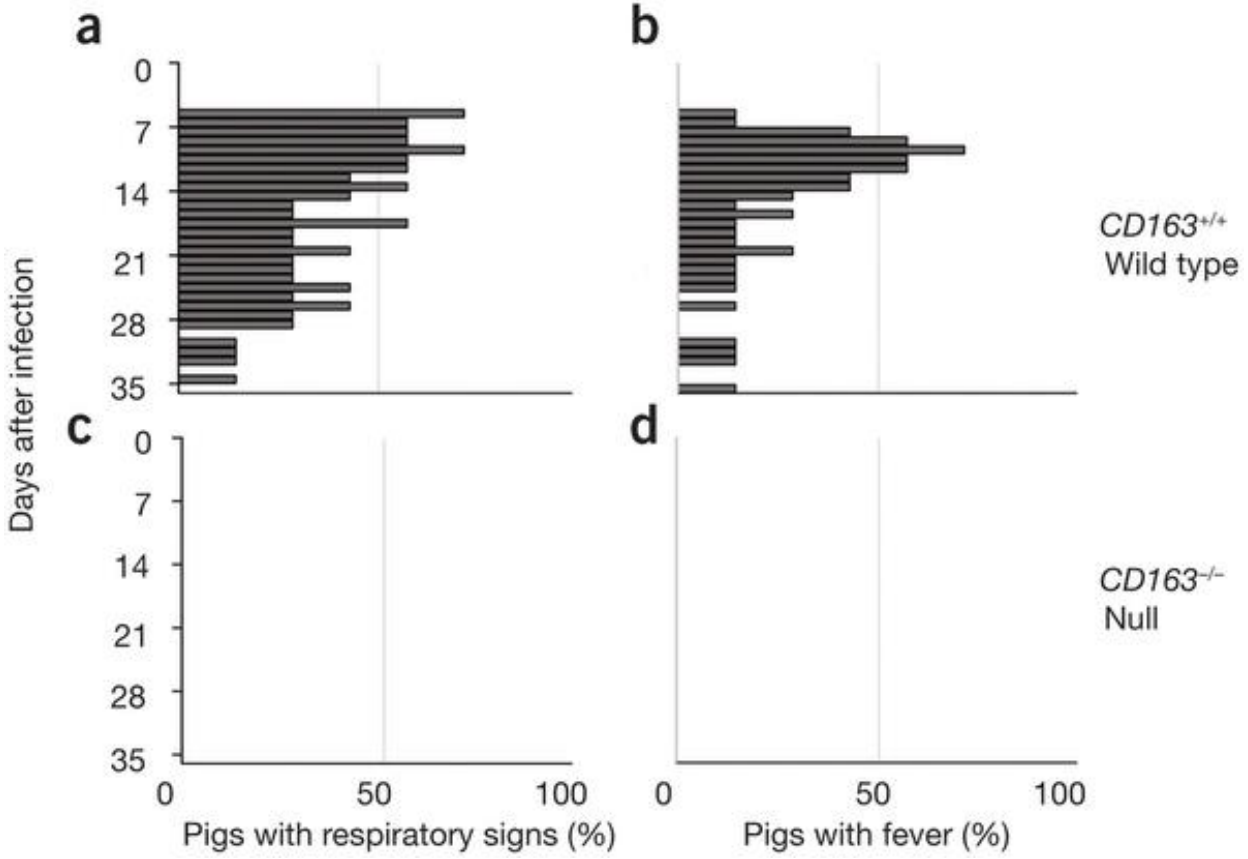
PRRS resistant pigs after CRISPR/Cas mediated Knockout of CD 163

Three CD163-KO piglets were kept together with seven WT-animals, all were infected with PRRS virus. WT-animals showed typical PRRS symptoms while the CD163-KO piglets remained healthy over the 35 days observation period.

(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

PRRS resistant pigs after CRISPR/Cas mediated Knockout of CD 163

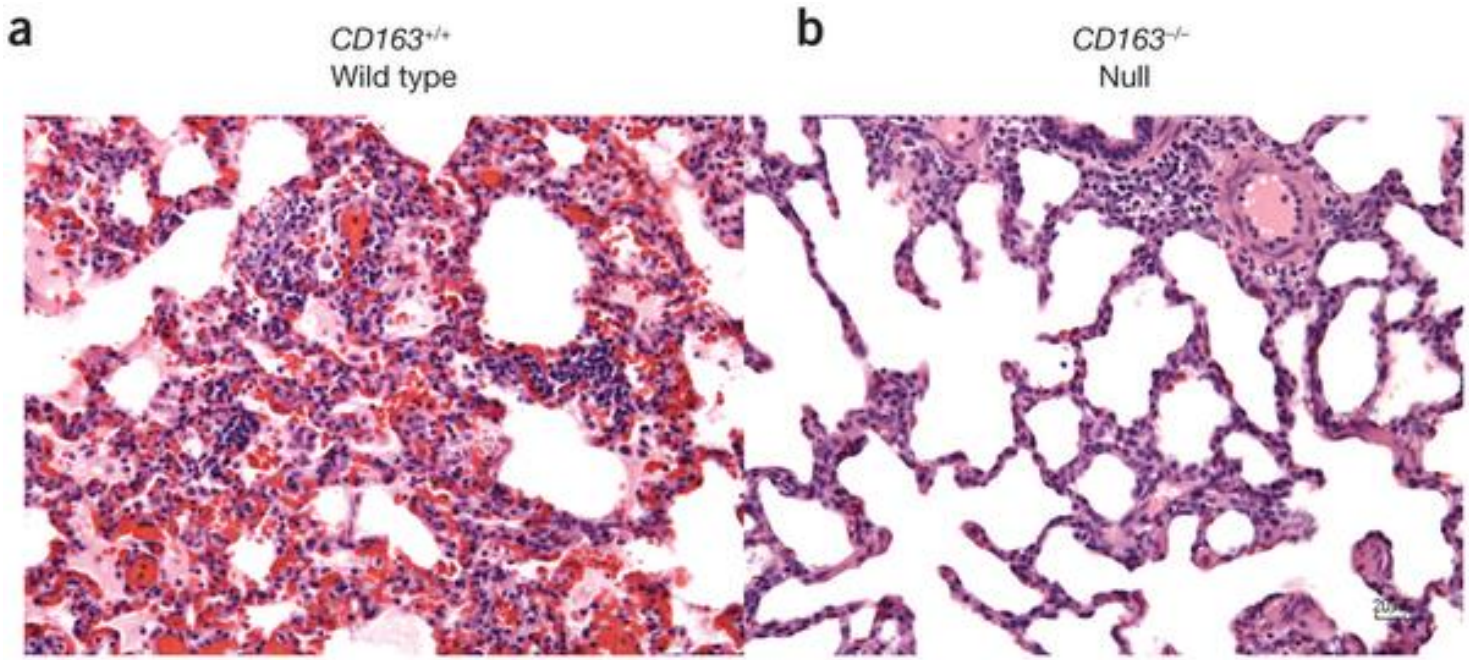


PRRS: Porcine reproductive and respiratory syndrome virus

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

PRRS resistant pigs after CRISPR/Cas mediated knockout of CD 163

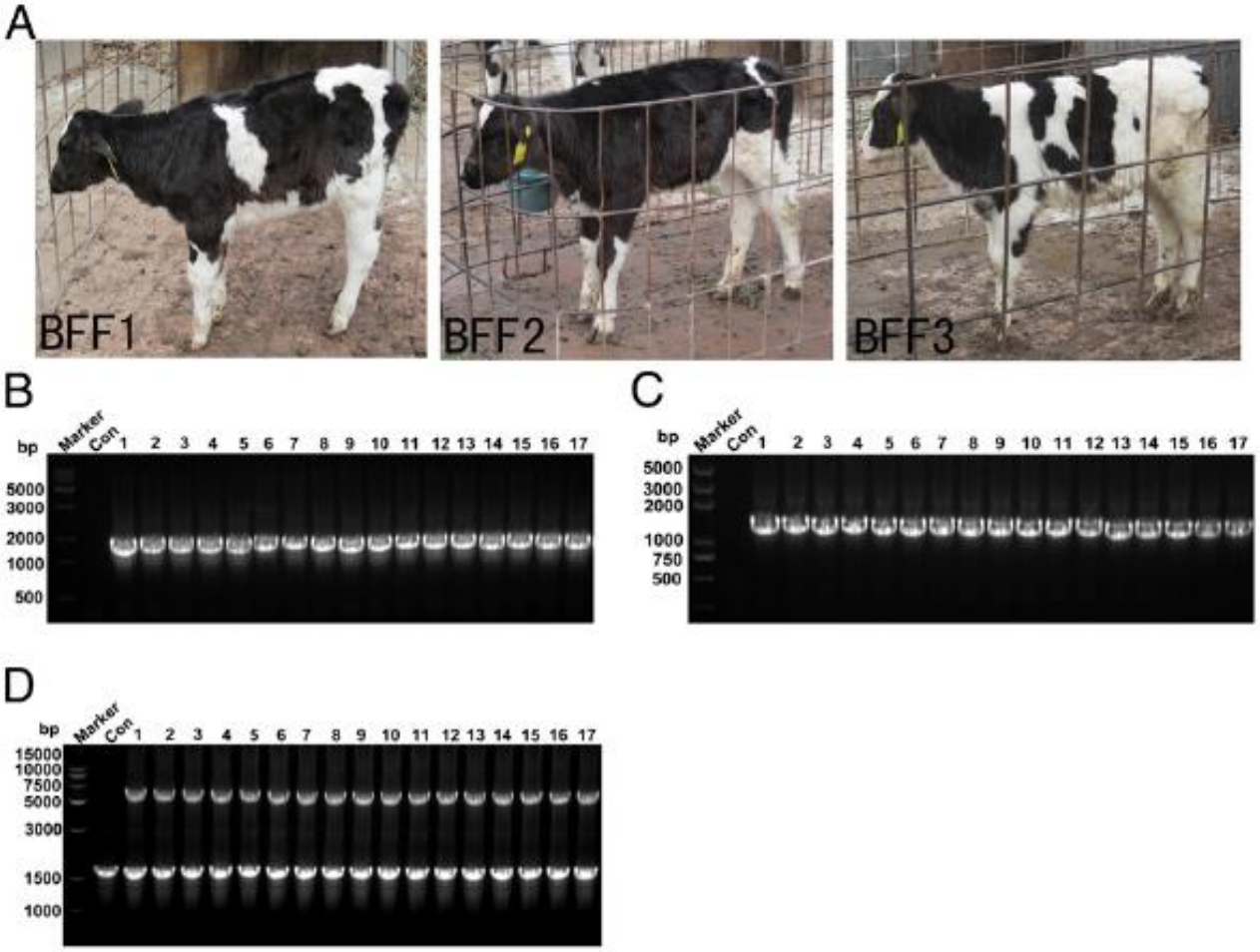
Microsopic image of the lung of CD163+/+ und CD163-/- pigs



PRRS: Porcine reproductive and respiratory syndrome virus

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

Cattle with genetic resistance against infections with Mycobacterium tuberculosis, mediated by Gene Editing



Wu et al., 2015, PNAS

Cattle with genetic resistance against infections with Mycobacterium tuberculosis, mediated by Gene Editing

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., 2015, PNAS

Gene-editing record smashed in pigs

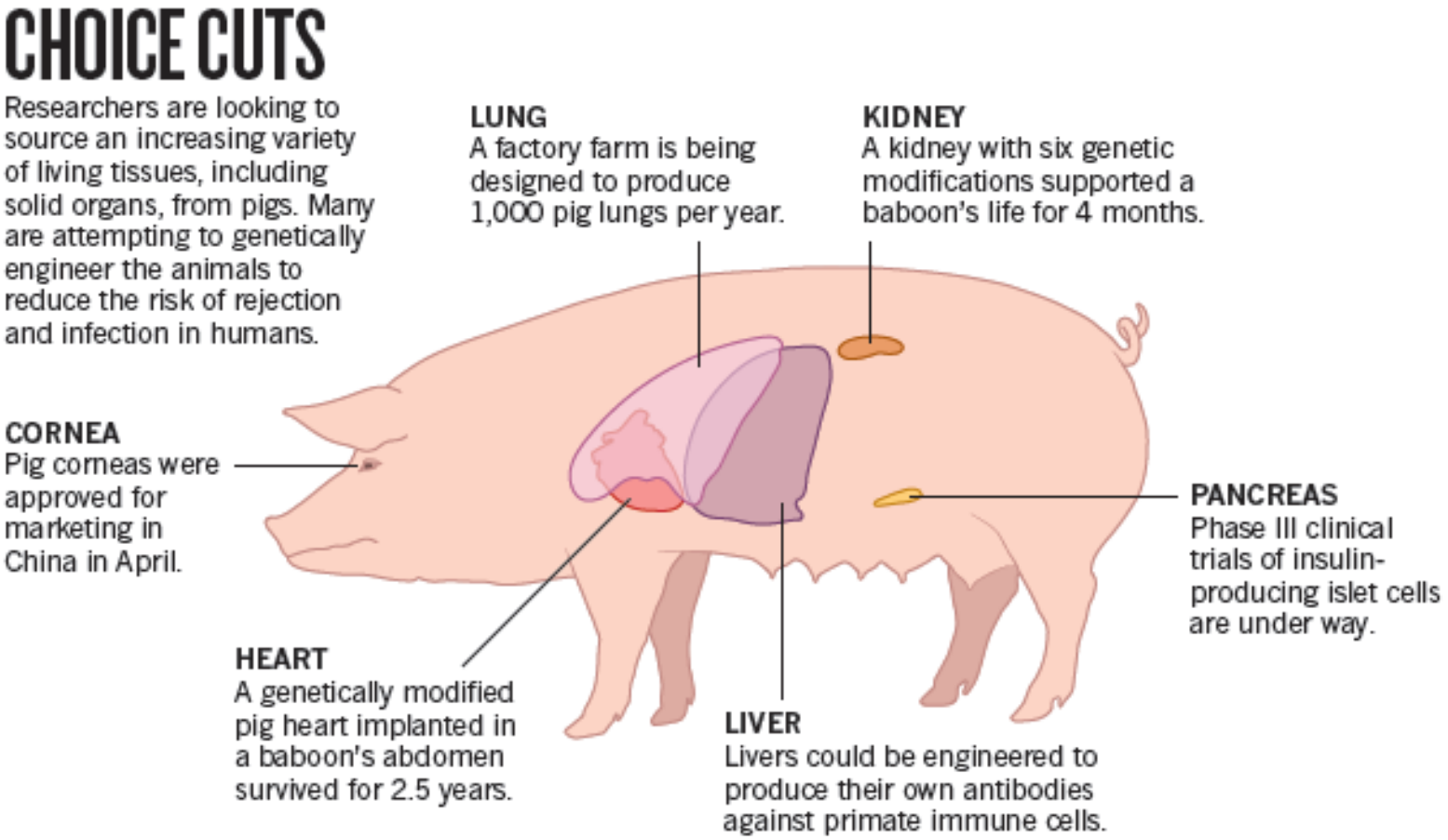
Researchers modify more than 60 genes (PERV) in effort to enable organ transplants into humans.

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

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

Nature News 2015.18525

Toward the tailored donor pig



Nature 527, Nov. 2015

Summary and conclusions

- The genomes of farm animals have been sequenced and annotated, thus informative gene maps are available that can be used for future oriented breeding concepts (GBV).
- Novel molecular tools such as DNA-nucleases are compatible with the efficient and reliable induction of precise genetic modifications (Gene Editing). The potential risks include „Off-target“ mutations, but recent research has shown that this is neglible.
- The application of the new genomic knowledge and gene editing tools allow for the development of novel breeding targets both for agriculture and biomedicine (**precision breeding**).
- Complex legal regulations are in place for the commercial use of transgenic farm animals. In Europe, the practical application of Gene Editing is currently dealt with as GMOs after the final legal ruling of the European High Court.

Evolution of farm animal breeding

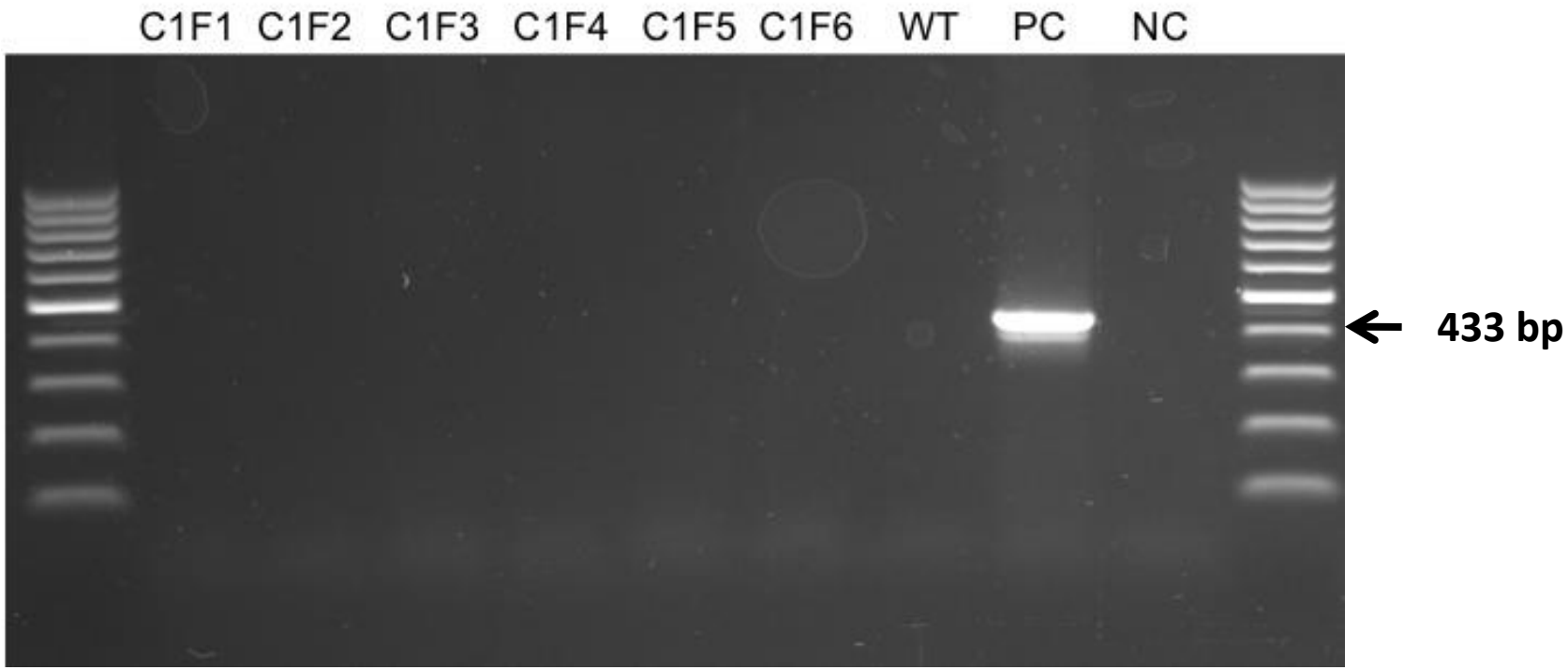
- Domestication
- Multiplication of „useful“ populations
- Selection acording to the phenotype
- Selection according to specific traits
- Systematic breeding on the basis of population genetics and statistics
- Reproductive biotechnologies (AI, ET, IVP, SCNT, etc.)
- Molecular genetics and genome based breeding concepts (SNPs, GBV, etc.)
- Future: ***Precision breeding***

Thank you for your attention

Keine Angst vor großen Tieren – Not afraid of large animals

Thanks very much for your interest and valued attention

Verification of lack of plasmid integration

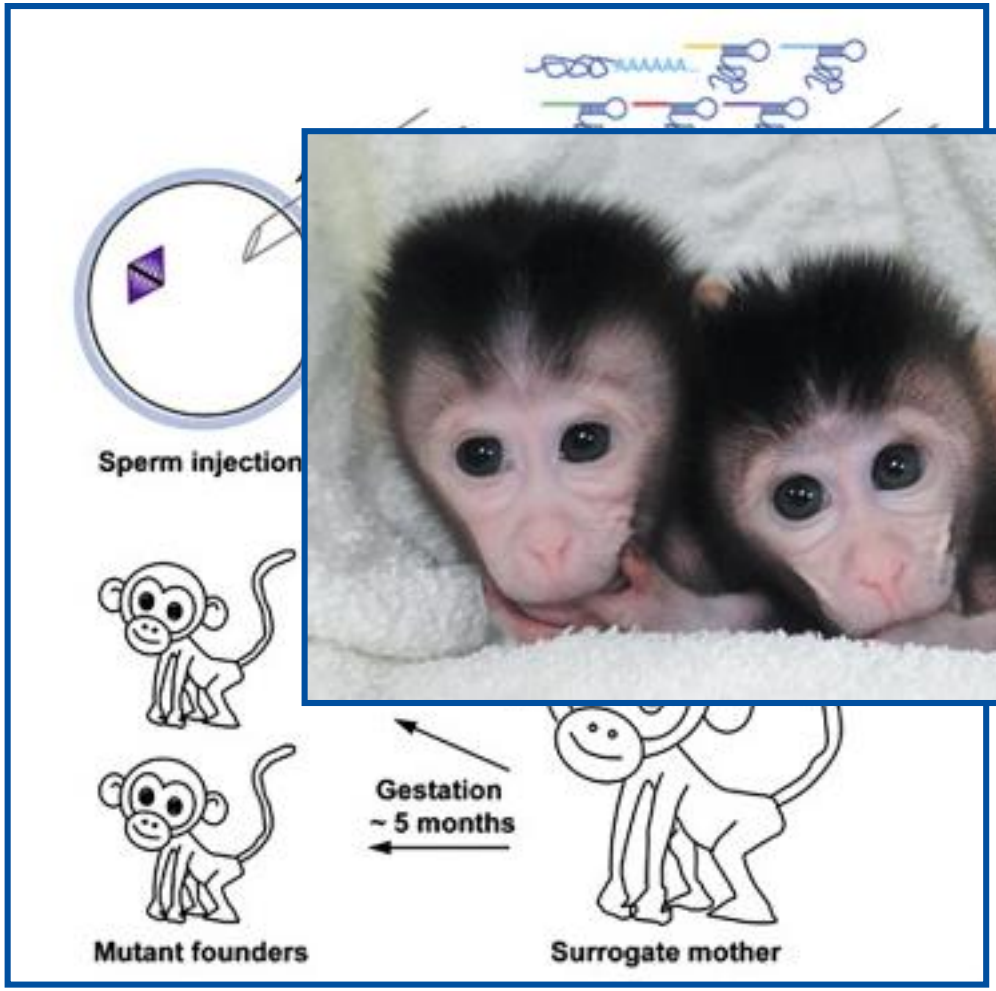


- PCR amplification of *FokI* only in PC containing plasmid DNA

→ no plasmid integration occurred

Hauschild et al. PNAS 108, 12013 –12017 (2011)

Successful application of CRISPR/Cas9 in primates (Cynomolgus monkeys (*Macaca fascicularis*))



ons in two
enes: *Ppar-γ*,
involved in
ulating
sm; and *Rag1*,
involved in
e function.

Niu et al., Cell 158, 836-843, 2014

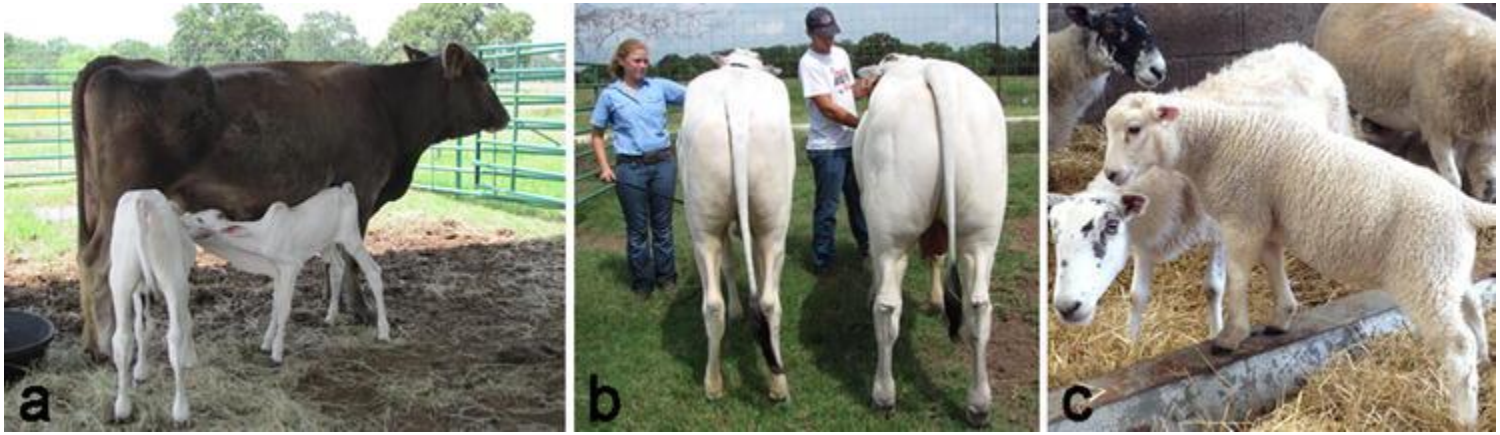
Advantages and disadvantages of DNA nucleases for HR in farm animals

- Lack of vector backbone integration due to transient transfection
- No need for antibiotic selection cassette
- High efficiency of gene targeting
- Biallelic knockout possible in one shot
- Somatic donor cells seem to be less stressed than after conventional targeting
- In conjunction with the magnetic bead selection a highly effective approach to produce pure cell populations with the desired KO.
- Control of off-target cleavage is required.
- Quality of ZFNs is highly dependent on the bioinformatic program.
- The use of specifically designed ZFNs can be very expensive.

Anwendungperspektiven für genetisch veränderte Nutztiere

- Landwirtschaftliche Perspektiven
- Wachstum und Entwicklung
- Krankheitsresistenz (Mx-Gen, IgA, BSE, PRRS, TB, etc)
- Laktation (Menge, Bestandteile)
- Wollproduktion
- Reproduktion
- Umweltverbesserungen
- Diätetische Verbesserungen
- Biomedizinische Perspektiven
- Gene Pharming
- Humaner Blutersatz
- Xenotransplantation
- Inhibitoren von Nervengasen (Biowaffen)
- Grundlagenforschung
- Modelle für humane Erkrankungen

Rinder und Schafe mit TALEN induziertem Knockout für Myostatin




```
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

Sheep WT           GTGATGAGCACTCCACAGAATCTCGATGCTGTCGTTACCCTCTAACTGTGGATTTTGA

Sheep Allele 1     GTGATGAGCACTCCACAGAATCTCGATGCTGTCGTTACCCTCTAACTGTGGATTTTGA   WT
Sheep Allele 2     GTGATGAGCACTCCACAGAATCTCGATGCTGT---TACCCTCTAACTGTGGATTTTGA   ΔR283
```

Proudfoot et al., Trans. Res. 24, 2015

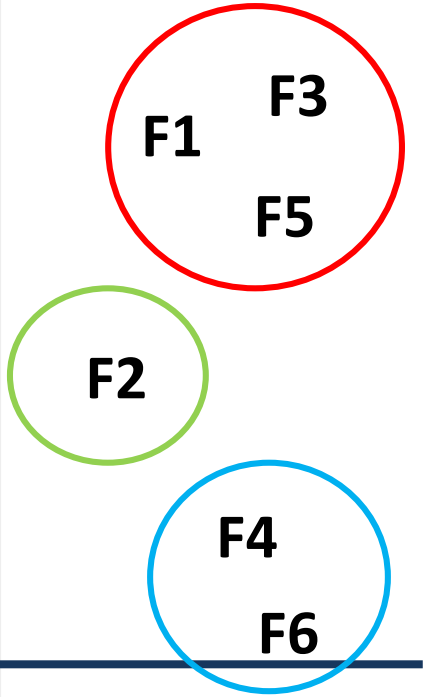
CRISPR/Cas für das Editing des Myostatin Gens

Beagles named Hercules, at left, and Tiangou are the world's first gene-edited dogs.

Sequenzierung des ZFN mutierten Gal-Locus

```
                ZFN-GGTA1-23713          ZFN-GGTA1-23714
      WT:CGGTGGCTCAGCTACAGGCCTGGTGGTACAAGGCAC
E41   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
```

WT

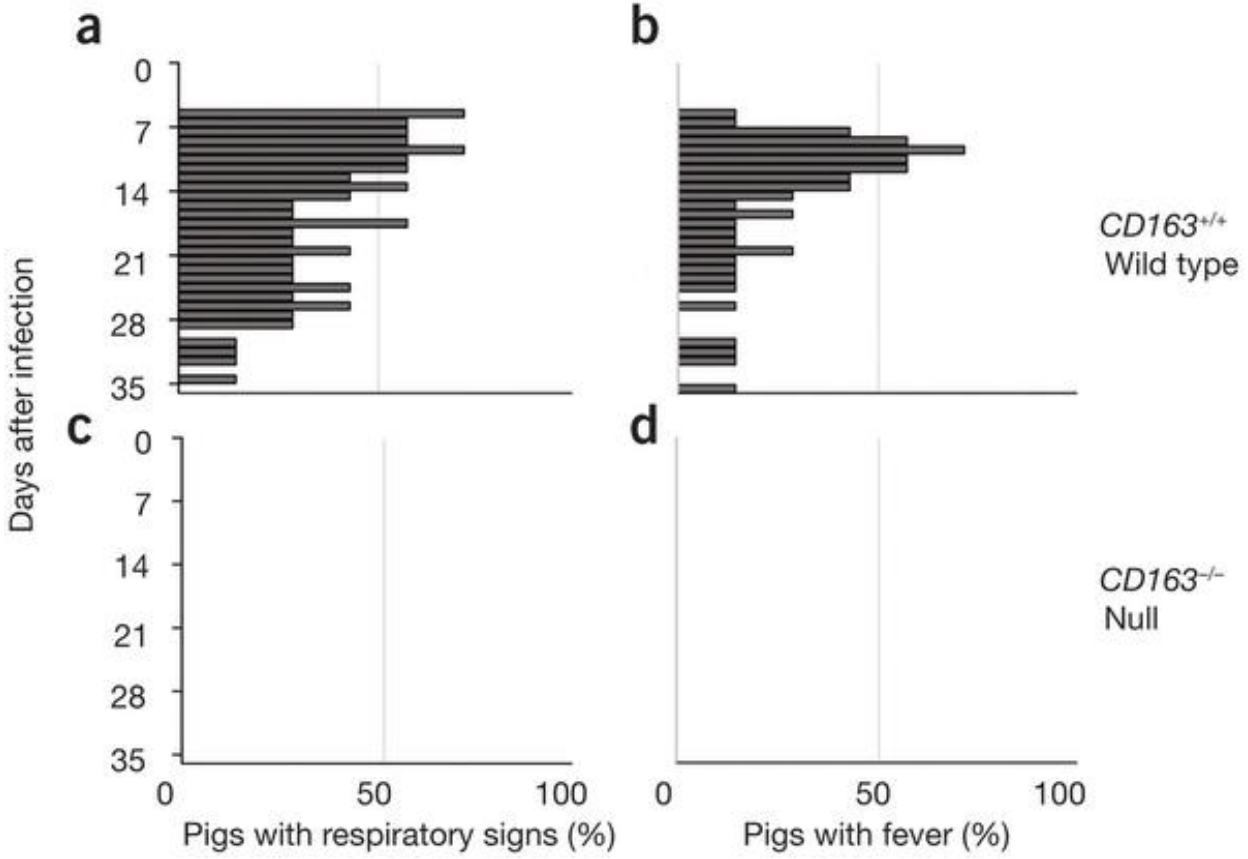


➡Lia

Hauschild et al. PNAS 108, 12013 –12017 (2011)

→ alle Mutationen führten zum Gal-Knock-out

PRRS resistente Schweine nach CRISPR/Cas vermitteltem Knockout von CD 163

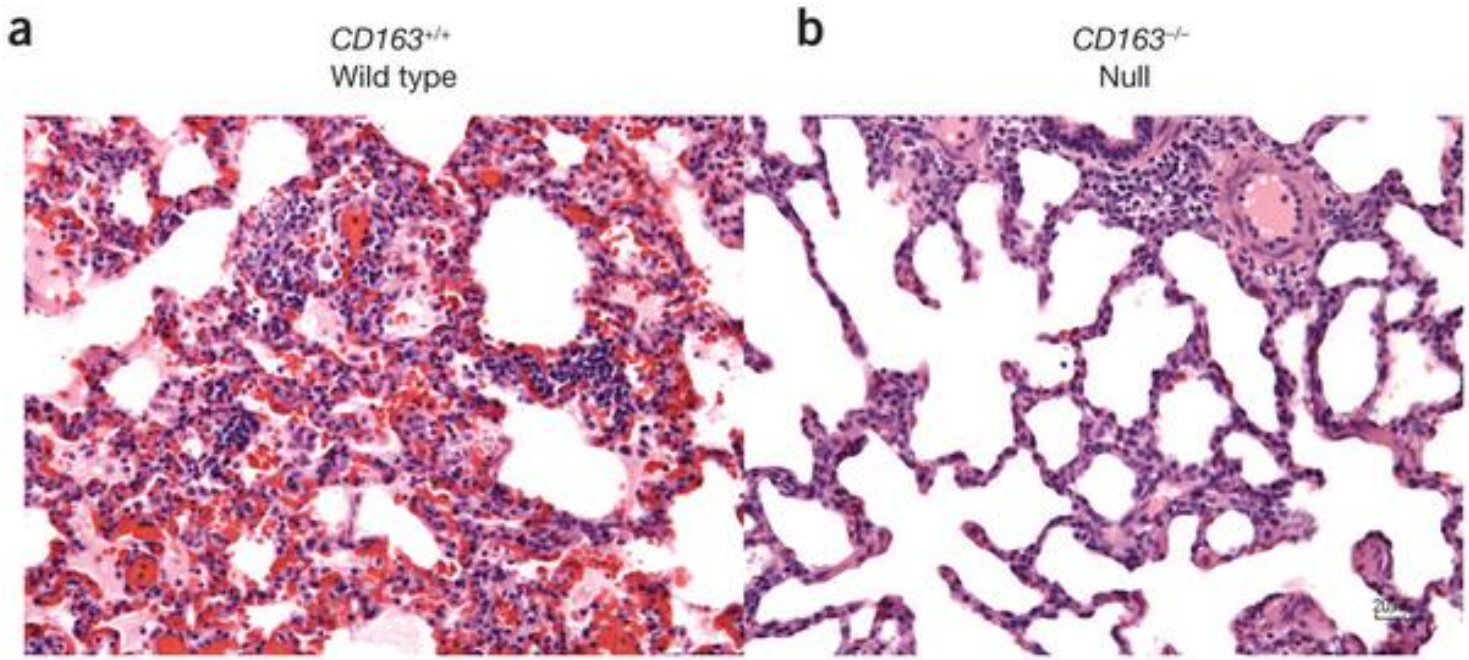


PRRS: Porcine reproductive and respiratory syndrome virus

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

PRRS resistente Schweine nach CRISPR/Cas vermitteltem Knockout von CD 163

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



PRRS: Porcine reproductive and respiratory syndrome virus

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

CRISPR/Cas 9 nickase mediated genome editing in bovine fibroblasts to induce resistance against Tuberculosis

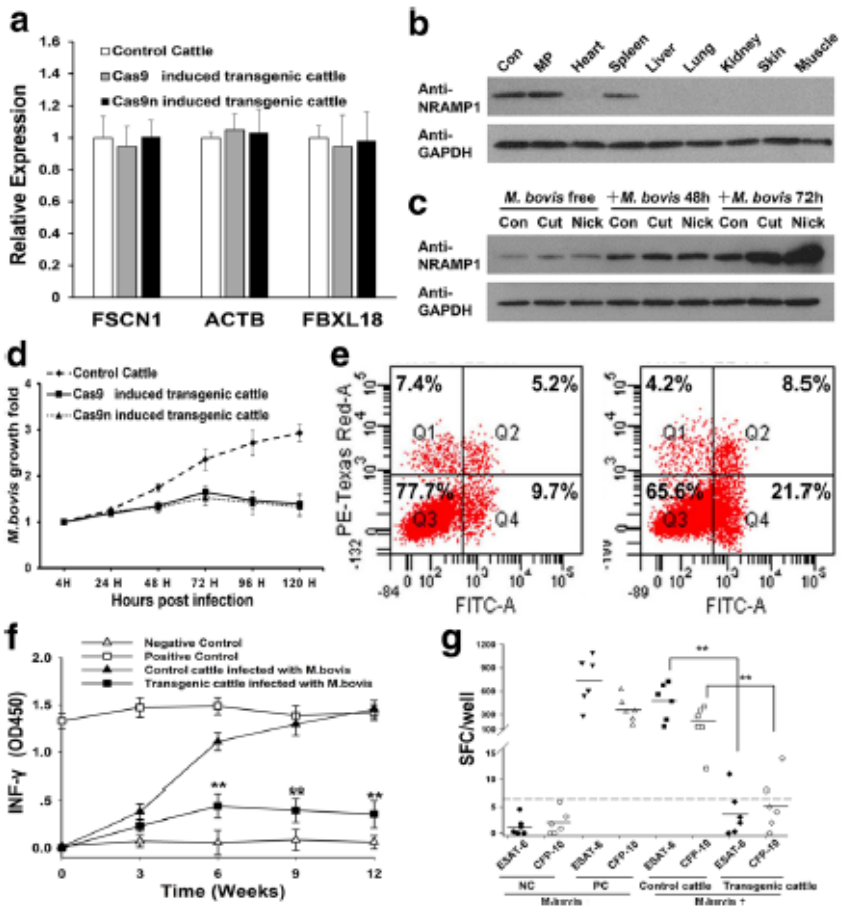
Table 1 Summary of nuclear transfer results from gene-targeted bovine fetal fibroblast cells

Nuclear donor	Cas9 nuclease					Cas9 nickase				
	Typical colonies suitable for SCNT				Total	Typical colonies suitable for SCNT				Total
Cell clone	45–57	45–86	45–136	45–159	-	45–113	45–187	45–243	45–351	-
Embryos obtained	632	578	644	531	2385	568	674	527	665	2434
Blastocysts (%)	137 (21.7)	143 (24.7)	126 (19.6)	119 (22.4)	525 (22.0)	163 (28.7)	210 (31.2)	157 (29.8)	223 (33.5)	753 (30.9)
Recipients	46	49	41	37	173	54	68	51	75	248
Pregnancies (%)	6 (13.0)	9 (18.4)	2 (4.9)	5 (13.5)	22 (12.7)	9 (16.7)	18 (26.5)	11 (21.6)	23 (30.7)	61 (24.6)
Calves at birth	1	2	0	1	4	2	5	3	6	16
Survived for 3 months	0	1	0	1	2	1	3	2	3	9

NRAMP1: natural resistance-associated macrophage protein-1, Inserted into targted locus on chromosome 25, at FSCN1/ACTB

Gao et al., Genome Biology 18:13, 2017

CRISPR/Cas 9 nickase mediated genome editing in bovine fibroblasts to produce Tuberculosis resistant cattle



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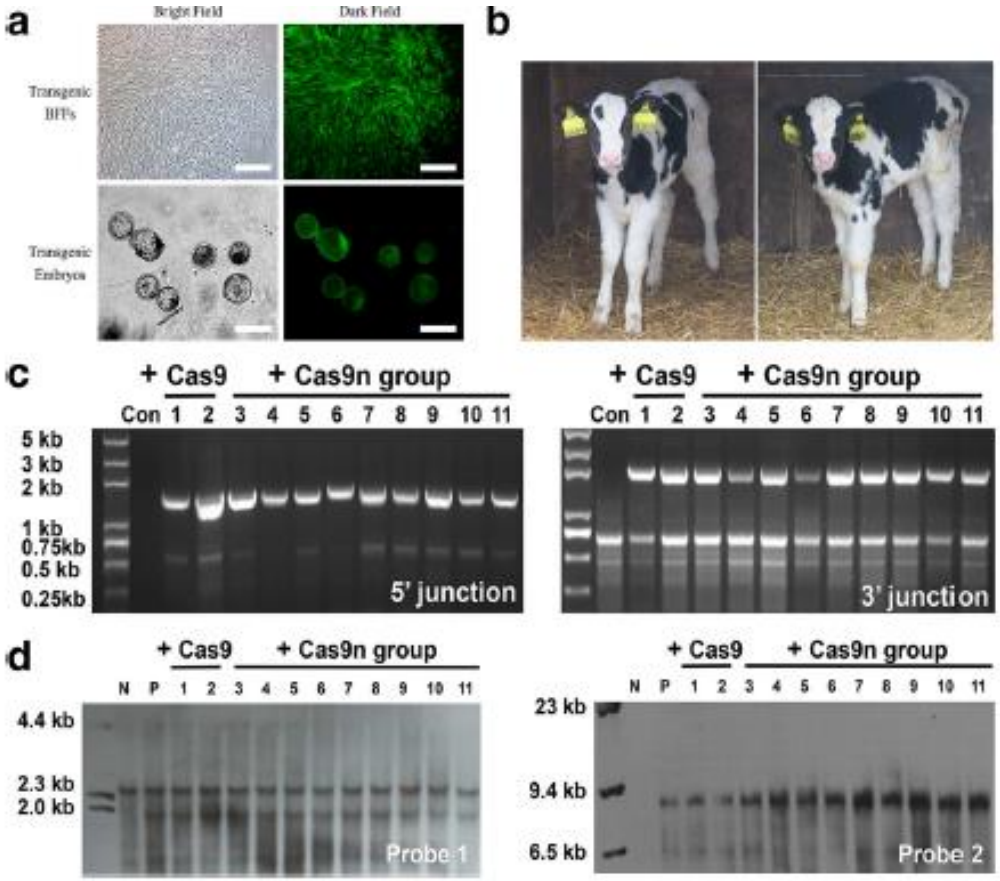

NRAMP1: natural resistance-associated macrophage protein-1, inserted into targted locus on chromosome 25, at FSCN1/ACTB

Gao et al., Genome Biology 18:13, 2017

Ausschalten multipler Genloci (PERV)

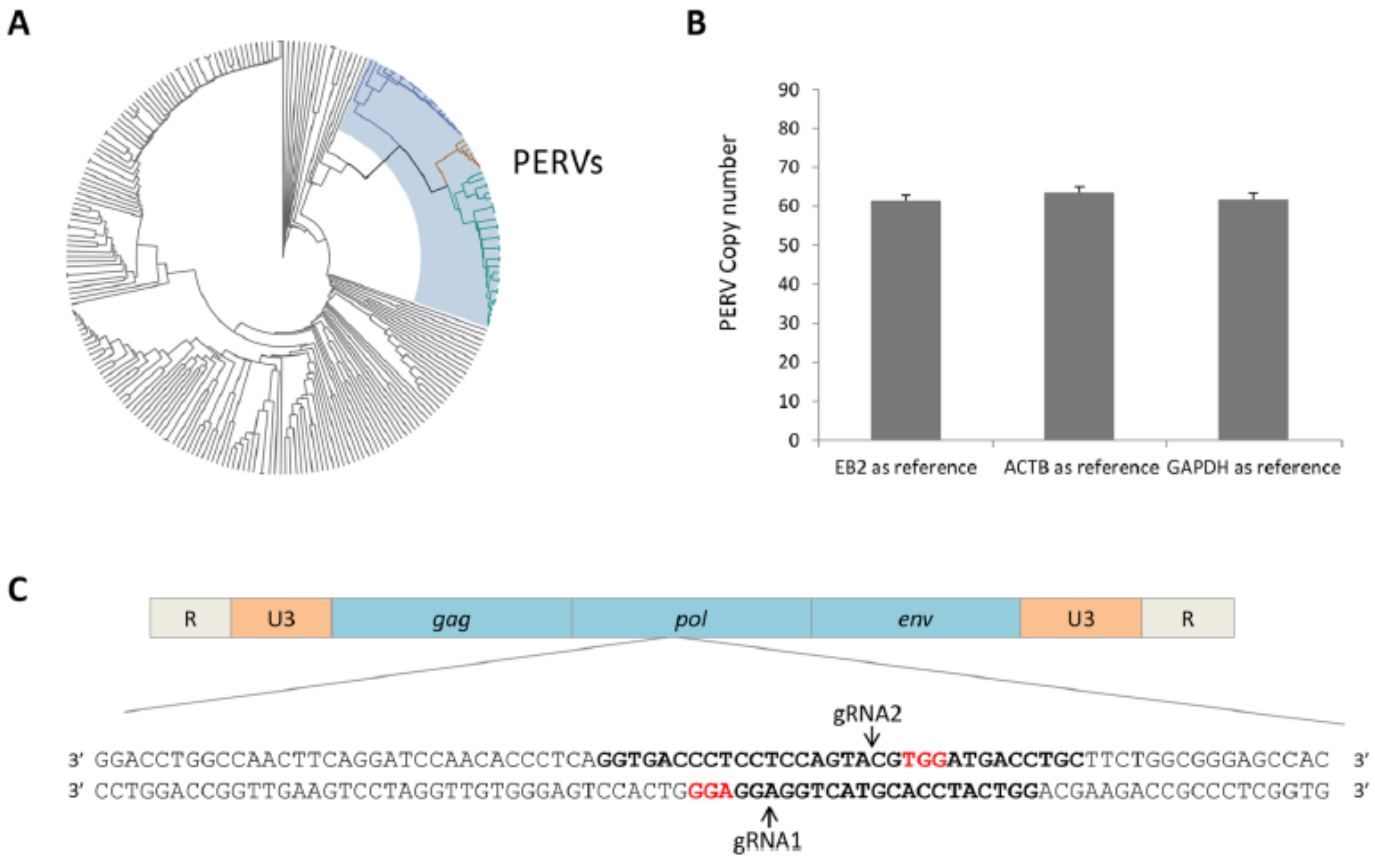

Fig. 1. CRISPR-Cas9 gRNAs were designed to specifically target the *pol* gene in 62 copies of PERVs in PK15 cells. (A) Phylogenetic tree representing endogenous retroviruses present in the pig genome. PERVs are highlighted in blue. (B) Copy number determination of PERVs in PK15 cells

Yang et al., Science Express, 11.10. 2015

Chancen und Risiken von DNA Nukleasen zur Induktion von genetischen Modifikationen bei Nutztieren

- Keine genomische Integration des Vektors (transiente Transfektion);
- Keine Antibiotika Selektionskassette;
- Hohe Effizienz beim Gen Targeting;
- Biallelischer Gen Knockout möglich in einem Ansatz
- DNA Nukleasen können in Wirtszellen (Oozyten, Embryonen) injiziert oder in somatische Zellen transfiziert werden;
- Somatische Zellen scheinen weniger „gestresst“ zu sein als nach konventionellem Gen Targeting;
- In Verbindung mit der Selektion über magnetische Kugeln sehr effektiv zur Produktion reiner Zellpopulationen mit dem angestrebten Knockout;
- **Risiken**
- Kontrolle von Off-target Mutationen
- Qualität der ZFNs ist abhängig vom bioinformatorischen Programm;
- Die Verwendung genspezifischer DNA Nukleasen kann mit patentrechtlichen Limitationen verbunden sein, kann ggf. teuer sein.

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 in EU: EN-NBTs, EU-GH Urteil abwarten. In USA und anderen Ländern geht Tendenz eindeutig dahin, "Gene Editing" (im engeren Sinne) **nicht** gesetzlich zu regulieren.
- **Deutschland:** Selbstverpflichtungserklärung der Zuchtorganisationen: GE nur bei Eigenschaften, die auch auf natürlichem Weg in der Zucht vorkommen könnten.

„Freiwillige Selbstverpflichtung zum Gene Editing in der Rinder- und Schweinezucht - Für einen verantwortungsvollen Umgang mit den neuen Züchtungstechniken“

Der Einsatz des Gene Editings an Tieren sollte auf Merkmale begrenzt sein, die Schäden, Schmerzen und Leiden beim Tier verhindern können oder helfen, die Anforderungen der Gesellschaft im Hinblick auf Umwelt, Klimaschutz und Produktqualität zu erfüllen (Ablehnung des Myostatin-KOs)

Nach aktuellem Wissensstand kommen als Zielmerkmale in der Rinder- und Schweinezucht grundsätzlich Folgende in Frage:

- *Merkmale mit hoher Tierwohlrelevanz (z.B. Erbfehler)*
- *Krankheitsresistenz/-toleranz (u.a. Zoonosen)*
- *Tiergesundheit/Robustheit*
- *Ressourceneffizienz, Umweltwirkung*
- *Produktqualität (z.B. Fleischbeschaffenheit, Inhaltsstoffe, sensorische Produkteigenschaften wie Vermeidung von Geruchsabweichungen).*

Dokumentation der Targets und Sicherung des Freiseins von Off-Target Effekten.

Gene-edited CRISPR mushroom escapes US regulation

A fungus engineered with the CRISPR–Cas9 technique can be cultivated and sold without further oversight.

Nature 532, 293, 2016

Gene-editing research in human embryos gains momentum

Fredrik Lanner, a stem-cell biologist at the Karolinska Institute in Stockholm, is preparing experiments that involve editing genes in human embryos.

Experiments are now approved in Sweden, China and the United Kingdom.

Nature 532, 289, 2016