The perspectives of genetically modified livestock in agriculture and biomedicine

- Agricultural perspectives
- Biomedical perspectives

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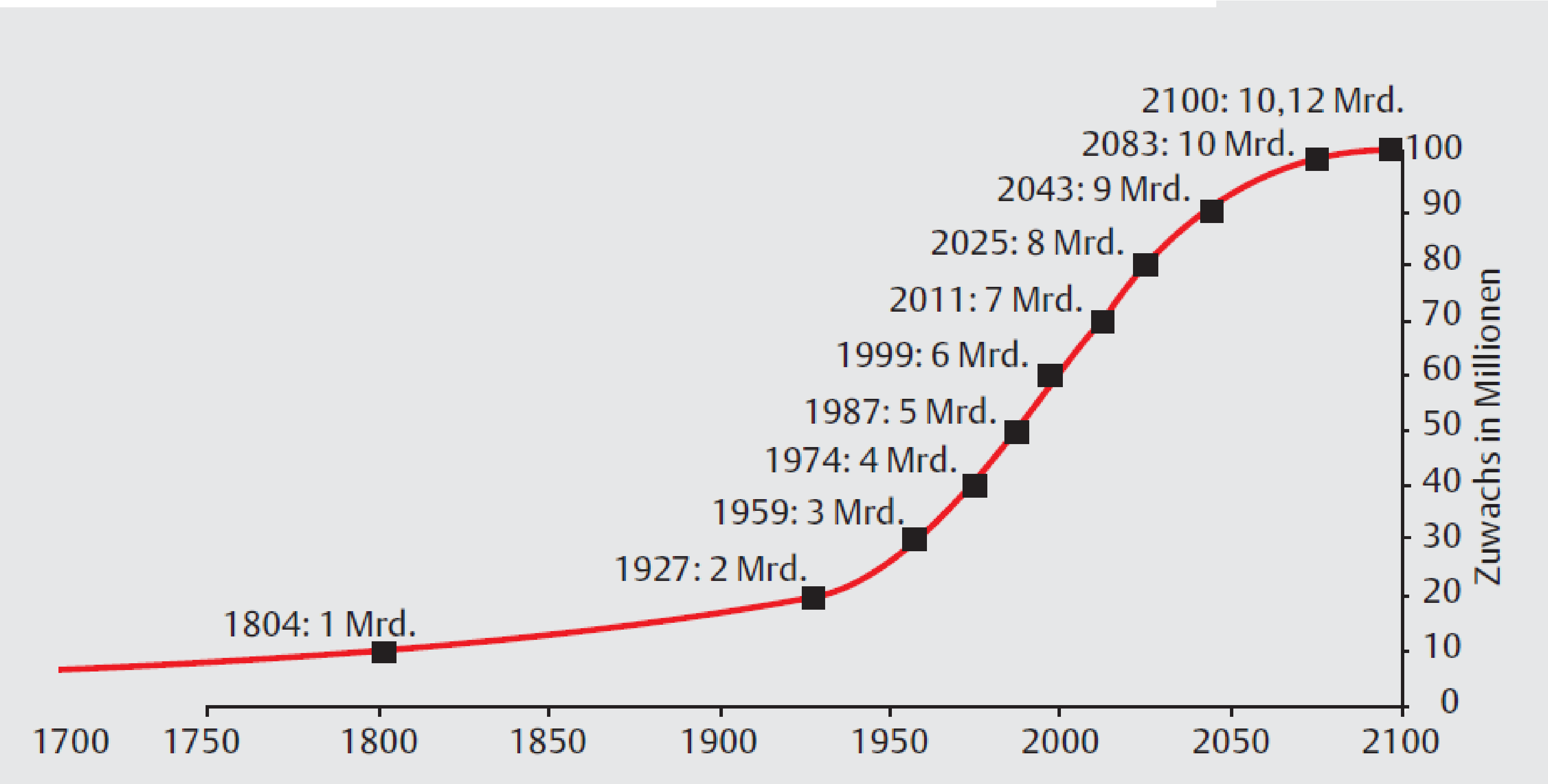
FABRE TP Session, 67th Annual EAAP-meeting, Belfast (UK)| August 30th, 2016

Projected increase in human global population



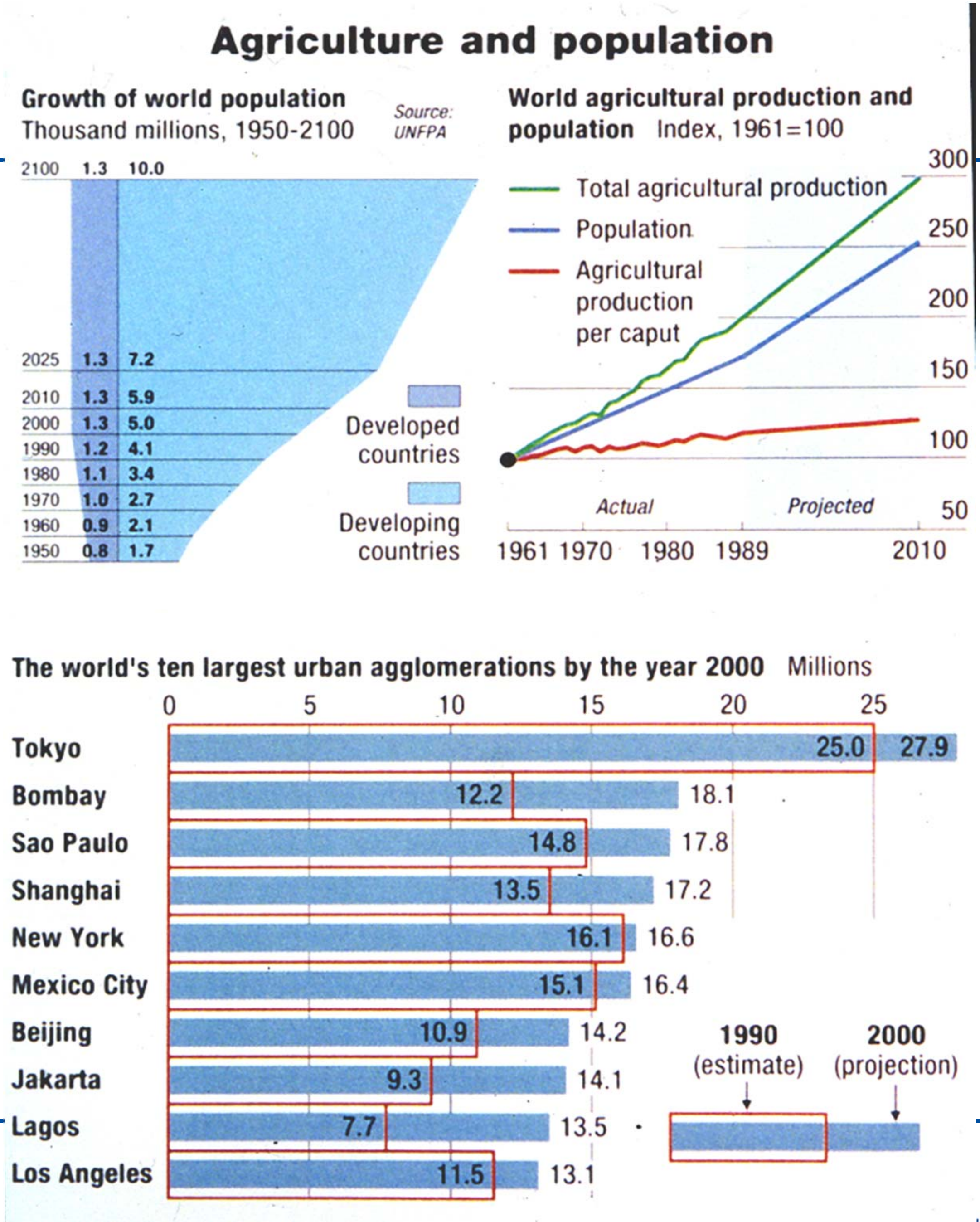
Quelle: UN, World Population Prospects: The 2010 Revision, 2011

Selected constraints of agricultural production

- ~5% of global land is usable for agriculture
- Increase in affluency if several parts of the world is associated with changes in diet towards more valuable animal proteins
- Environmental constraints of livestock production (~1/3 of climate relevant emission comes from agriculture)
- FAO: 1.3 kg CO_{2eq}/milk (Northern America, Europe);
  7.5 CO_{2eq}/milk (Africa)

- **Consequences**: Food production needs to be doubled or tripled

- **Need**: higher productivity without detrimental side effects (sustainable intensification).



Agriculture and population

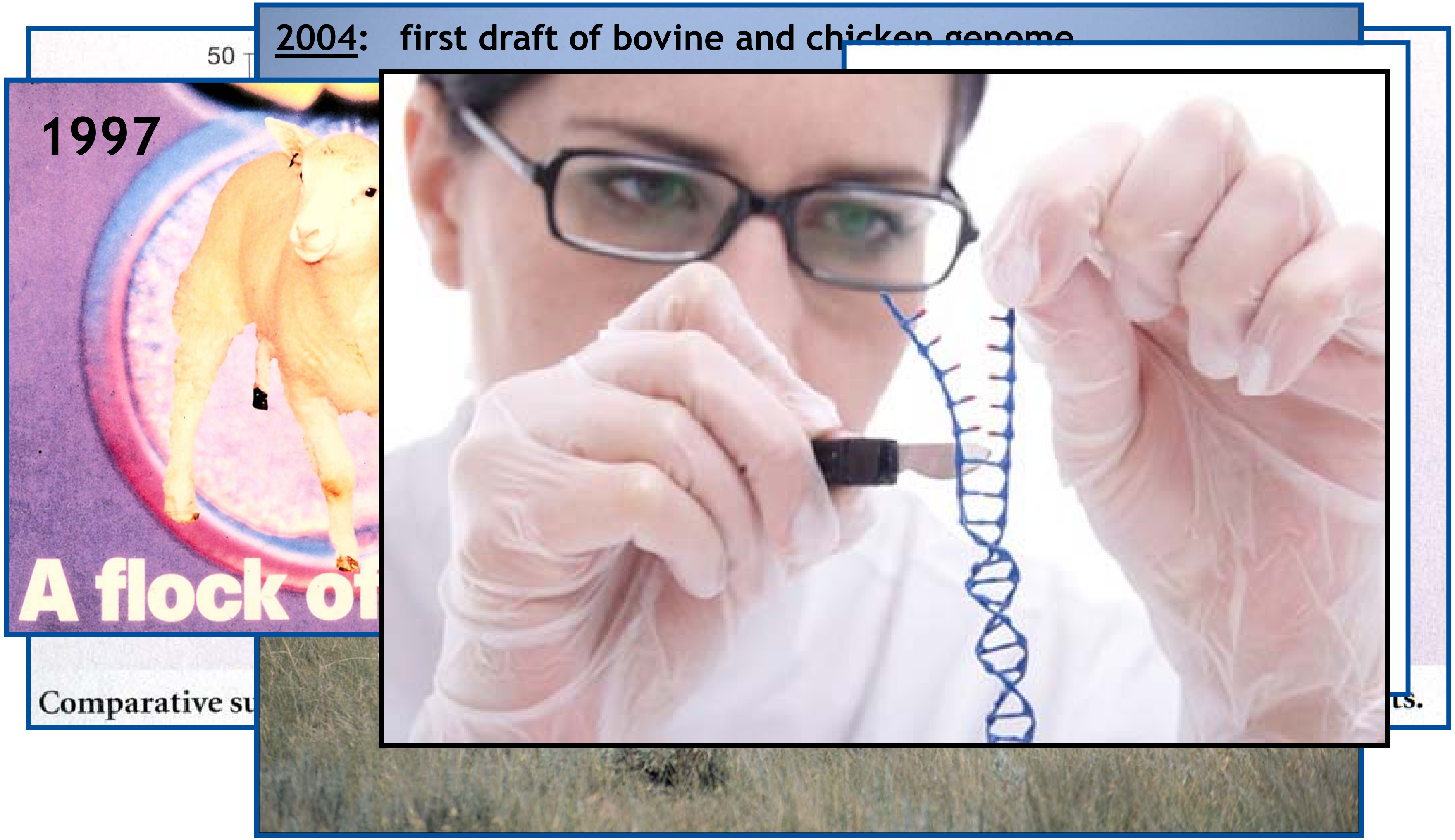
Growth of world population
Thousand millions, 1950-2100

Source: UNFPA

World agricultural production and population Index, 1961=100

The world's ten largest urban agglomerations by the year 2000 Millions

A new era in biology: Genome sequencing, somatic cloning and embryonic stem cells



2004: first draft of bovine and chicken genome

Nature 426, 2003

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Agricultural perspectives of genetically modified farm animals

- Growth and development (myostatin, GH, GHrec, IGF)
- Wool production
- Lactation (amount, composition)
- Hornless cattle (Polled locus)
- Disease resistance (Mx-gene, IgA, BSE, TB, PRRS, etc)
- Reproduction
- Environmental improvements (Phytase)
- Dietetic improvements
- Skewing the gender

Improvements in economically important parameters of growth hormone transgenic swine

Constructs	mMTI-bGH*	hMTI-pGH(cDNA)**
Weight gain	+ 23	10 - 20%
Feed efficiency	+ 18	10 - 15%
Backfat thickness	7.5 mm (from 21 mm)	significantly reduced
„Side effects “ GH)	+ + +	- (30 - 40ng/ml

*Pursel et al. 1990;   **Seamark and Nottle (BresaGen)

Cattle and sheep with TALEN induced knockout of the myostatin gene

```
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

Myostatin knockout pigs after employing TALEN

Produced in Korea 2015

Nature, July 2015

Gene-edited minipigs as pets

BGI announced its plan to sell the micropigs as pets at a summit in Shenzhen, China.

Cyranoski, Nature 526, 2015

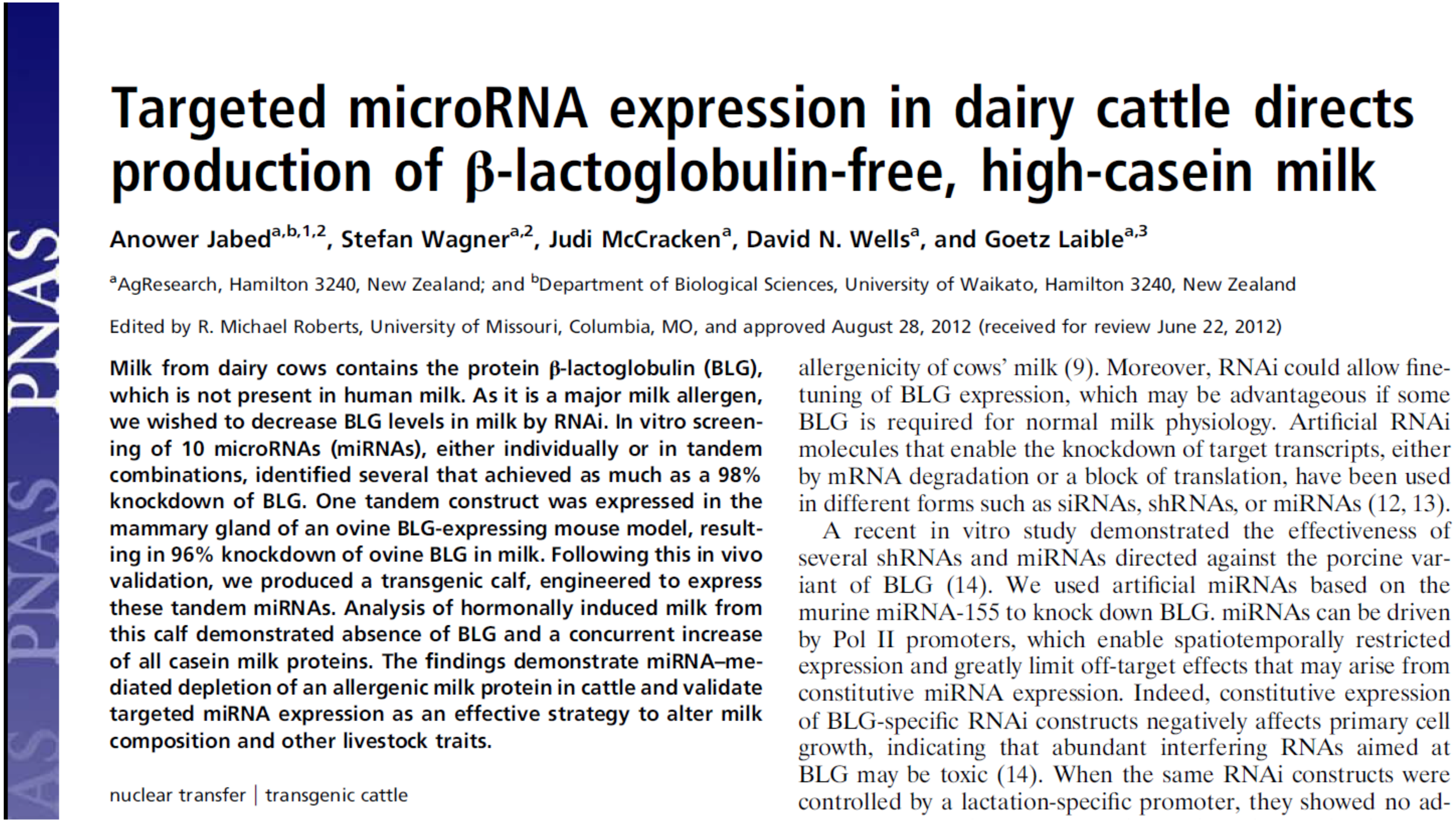
Transgenic animals with improved fibre production

Introduced modification	Application	Species	Reference
Ovine insulin-like growth factor 1	6.2% more fleece	Sheep	Damak et al. 1996
Ovine growth hormone	Improved wool production	Sheep	Adams et al. 2002
Ovine keratine intermediate filament	Improved wool processing and wearing properties	Sheep	Bawden et al. 1998
Bacterial serine transacetylase and O-acetylserine sulfhydrylase	Improved wool production	Sheep	Ward 2000

Genetically modified animals with improved milk production

Introduced modification	Application	Species	Reference
Bovine α-lactalbumin	Increase milk yield and piglet survival	Pig	Wheeler et al. 2001
Bovine b-and k-casein	Improved milk composition	Cattle	Brophy et al. 2003
siRNA-ß-lactoglobulin	reduced allergenicity	Cattle	Jabed et al. 2012
ß-lac-GFP-neo-Lycostaphin	improved udder health	Cattle	Wall et al. 2005
hlysozyme in ß-cas locus	improved udder health	Cattle	Liu et al. 2014

Production of ß-lactoglobulin free milk in siRNA-ß-lac transgenic cows



Targeted microRNA expression in dairy cattle directs production of β-lactoglobulin-free, high-casein milk

Anower Jabed[a,b,1,2], **Stefan Wagner**[a,2], **Judi McCracken**[a], **David N. Wells**[a], **and Goetz Laible**[a,3]

[a]AgResearch, Hamilton 3240, New Zealand; and [b]Department of Biological Sciences, University of Waikato, Hamilton 3240, New Zealand

Edited by R. Michael Roberts, University of Missouri, Columbia, MO, and approved August 28, 2012 (received for review June 22, 2012)

Milk from dairy cows contains the protein β-lactoglobulin (BLG), which is not present in human milk. As it is a major milk allergen, we wished to decrease BLG levels in milk by RNAi. In vitro screening of 10 microRNAs (miRNAs), either individually or in tandem combinations, identified several that achieved as much as a 98% knockdown of BLG. One tandem construct was expressed in the mammary gland of an ovine BLG-expressing mouse model, resulting in 96% knockdown of ovine BLG in milk. Following this in vivo validation, we produced a transgenic calf, engineered to express these tandem miRNAs. Analysis of hormonally induced milk from this calf demonstrated absence of BLG and a concurrent increase of all casein milk proteins. The findings demonstrate miRNA–mediated depletion of an allergenic milk protein in cattle and validate targeted miRNA expression as an effective strategy to alter milk composition and other livestock traits.

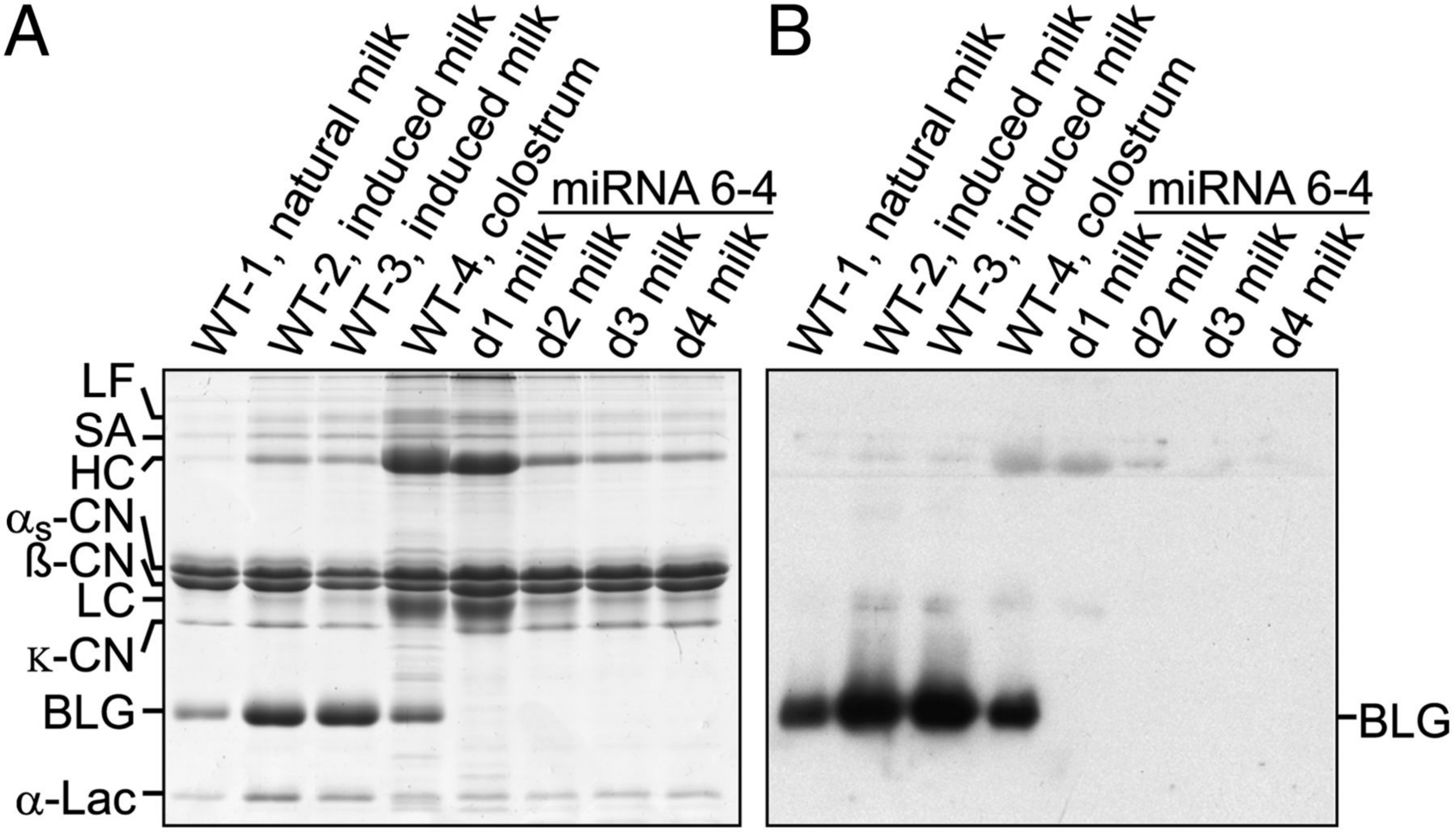
nuclear transfer | transgenic cattle

allergenicity of cows' milk (9). Moreover, RNAi could allow fine-tuning of BLG expression, which may be advantageous if some BLG is required for normal milk physiology. Artificial RNAi molecules that enable the knockdown of target transcripts, either by mRNA degradation or a block of translation, have been used in different forms such as siRNAs, shRNAs, or miRNAs (12, 13).

A recent in vitro study demonstrated the effectiveness of several shRNAs and miRNAs directed against the porcine variant of BLG (14). We used artificial miRNAs based on the murine miRNA-155 to knock down BLG. miRNAs can be driven by Pol II promoters, which enable spatiotemporally restricted expression and greatly limit off-target effects that may arise from constitutive miRNA expression. Indeed, constitutive expression of BLG-specific RNAi constructs negatively affects primary cell growth, indicating that abundant interfering RNAs aimed at BLG may be toxic (14). When the same RNAi constructs were controlled by a lactation-specific promoter, they showed no ad-

Jabed et al. 2012, PNAS 109, 16811-6

miRNA-mediated depletion of BLG in bovine milk



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

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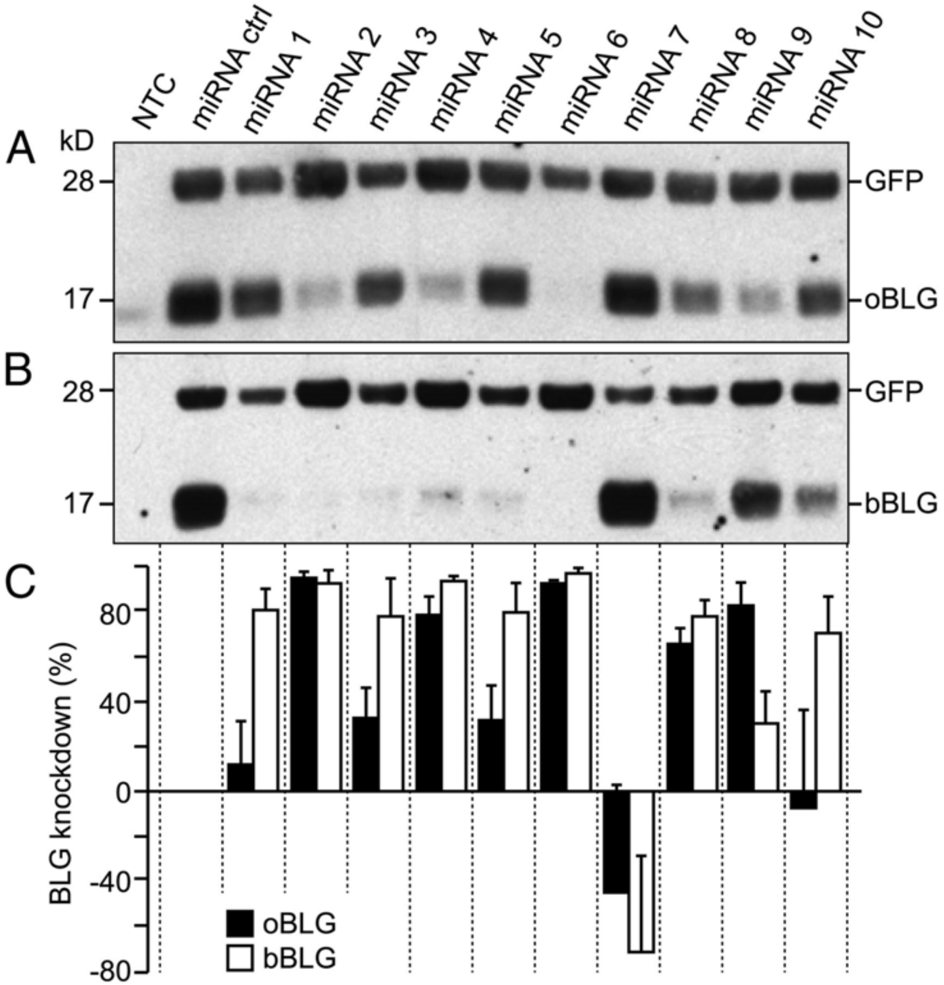
Targeted microRNA expression in dairy cattle directs production of ß-lactoglobulin-free, high-casein milk

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

In vitro knockdown of BLG in COS-7 cells.



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

Production of cattle with elevated concentration of ß- and k-Casein in milk

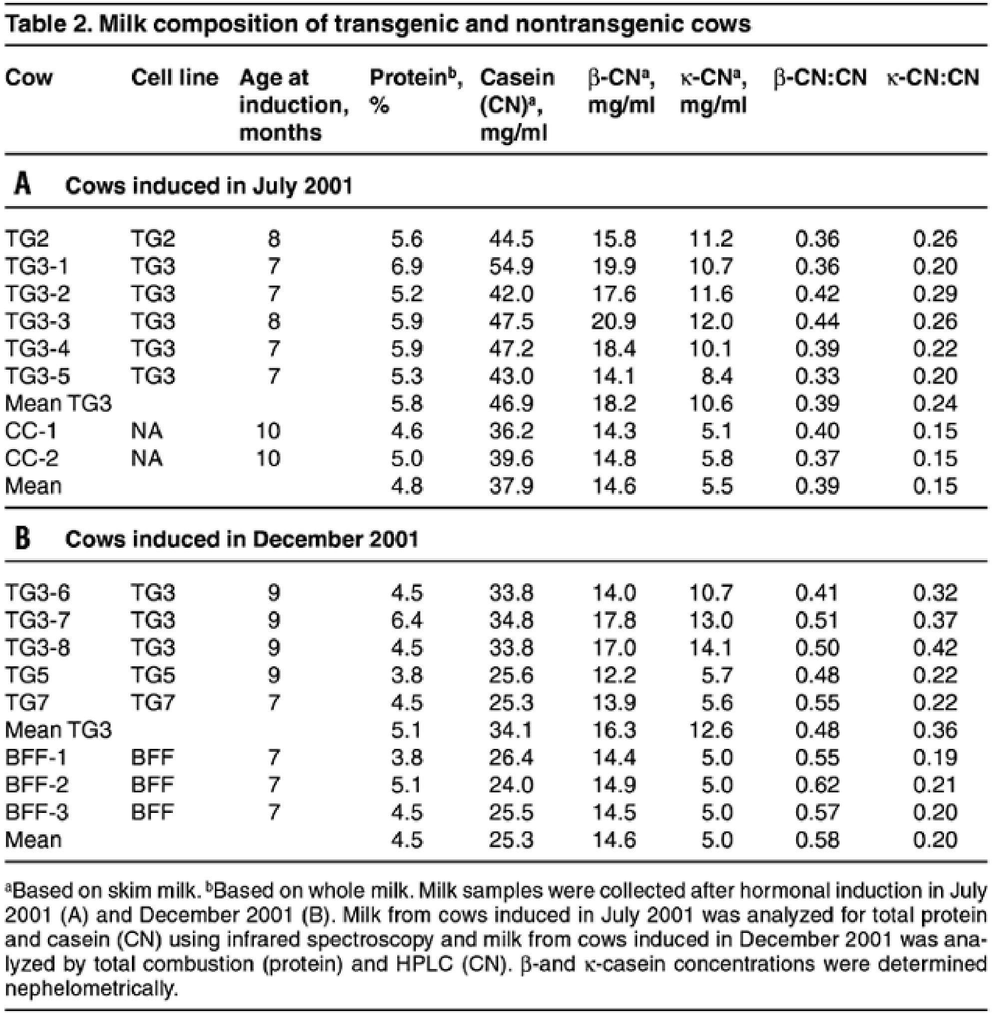


Table 2. Milk composition of transgenic and nontransgenic cows

Cow	Cell line	Age at induction, months	Protein[b], %	Casein (CN)[a], mg/ml	β-CN[a], mg/ml	κ-CN[a], mg/ml	β-CN:CN	κ-CN:CN
A Cows induced in July 2001								
TG2	TG2	8	5.6	44.5	15.8	11.2	0.36	0.26
TG3-1	TG3	7	6.9	54.9	19.9	10.7	0.36	0.20
TG3-2	TG3	7	5.2	42.0	17.6	11.6	0.42	0.29
TG3-3	TG3	8	5.9	47.5	20.9	12.0	0.44	0.26
TG3-4	TG3	7	5.9	47.2	18.4	10.1	0.39	0.22
TG3-5	TG3	7	5.3	43.0	14.1	8.4	0.33	0.20
Mean TG3			5.8	46.9	18.2	10.6	0.39	0.24
CC-1	NA	10	4.6	36.2	14.3	5.1	0.40	0.15
CC-2	NA	10	5.0	39.6	14.8	5.8	0.37	0.15
Mean			4.8	37.9	14.6	5.5	0.39	0.15
B Cows induced in December 2001								
TG3-6	TG3	9	4.5	33.8	14.0	10.7	0.41	0.32
TG3-7	TG3	9	6.4	34.8	17.8	13.0	0.51	0.37
TG3-8	TG3	9	4.5	33.8	17.0	14.1	0.50	0.42
TG5	TG5	9	3.8	25.6	12.2	5.7	0.48	0.22
TG7	TG7	7	4.5	25.3	13.9	5.6	0.55	0.22
Mean TG3			5.1	34.1	16.3	12.6	0.48	0.36
BFF-1	BFF	7	3.8	26.4	14.4	5.0	0.55	0.19
BFF-2	BFF	7	5.1	24.0	14.9	5.0	0.62	0.21
BFF-3	BFF	7	4.5	25.5	14.5	5.0	0.57	0.20
Mean			4.5	25.3	14.6	5.0	0.58	0.20

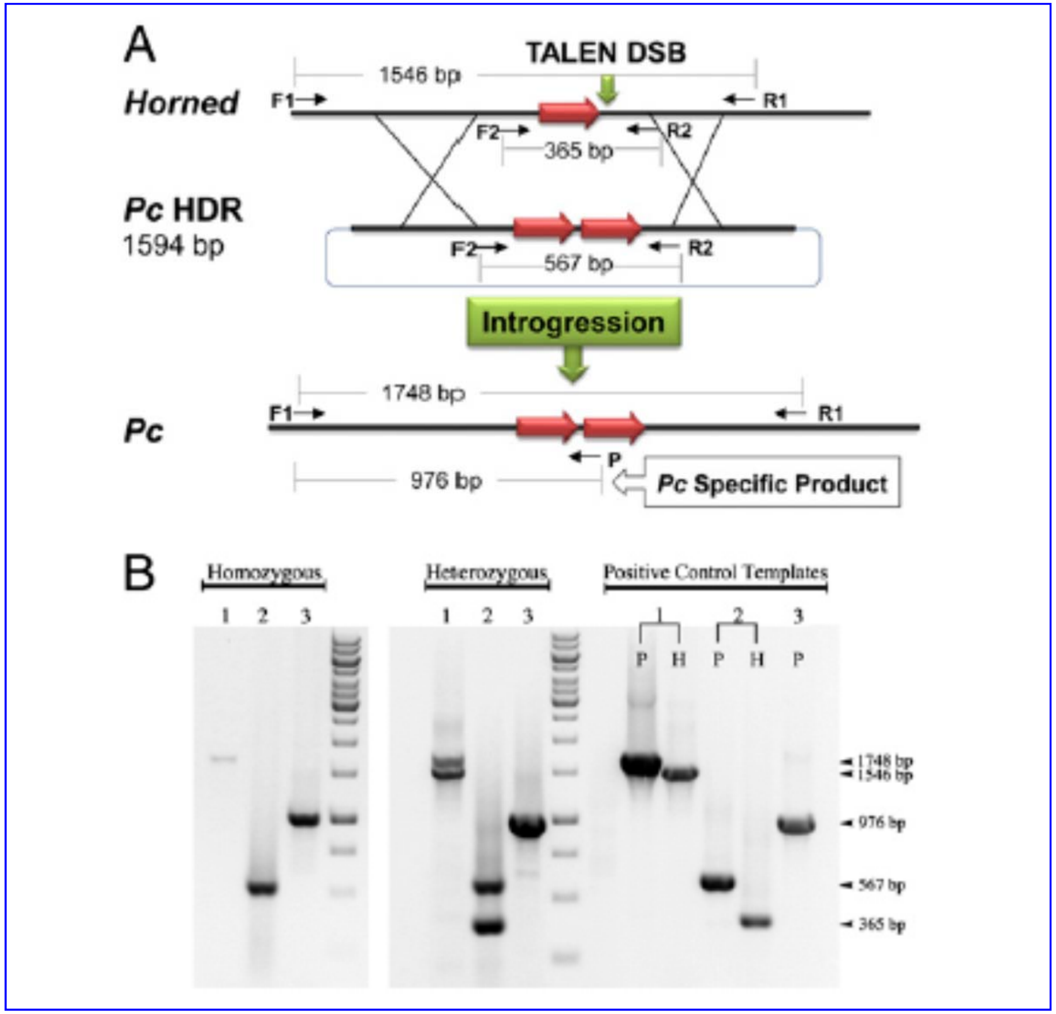
[a] Based on skim milk. [b] Based on whole milk. Milk samples were collected after hormonal induction in July 2001 (A) and December 2001 (B). Milk from cows induced in July 2001 was analyzed for total protein and casein (CN) using infrared spectroscopy and milk from cows induced in December 2001 was analyzed by total combustion (protein) and HPLC (CN). β-and κ-casein concentrations were determined nephelometrically.

Brophy et al. 2003, Nature Biotechnology 21, 157-162

Milk from hLZ transgenic goats helps children with diarrhea

Maga et al., 2014

TALEN induced mutation of the Polled locus to produce cattle without horns



Tan et al., PNAS 110, 16526-531; 2013

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

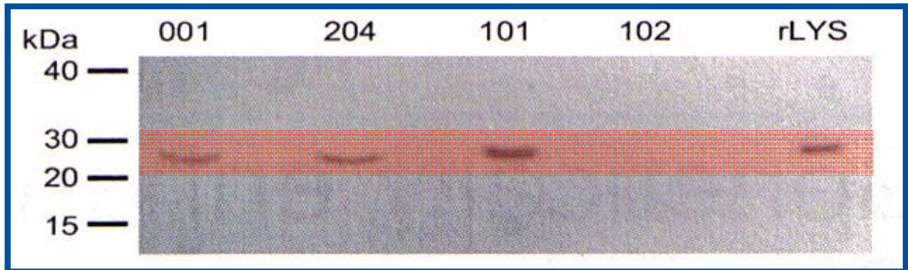
Carlson et al., Nature Biotechnology 2016

Approaches towards animals transgenic for enhanced disease resistance

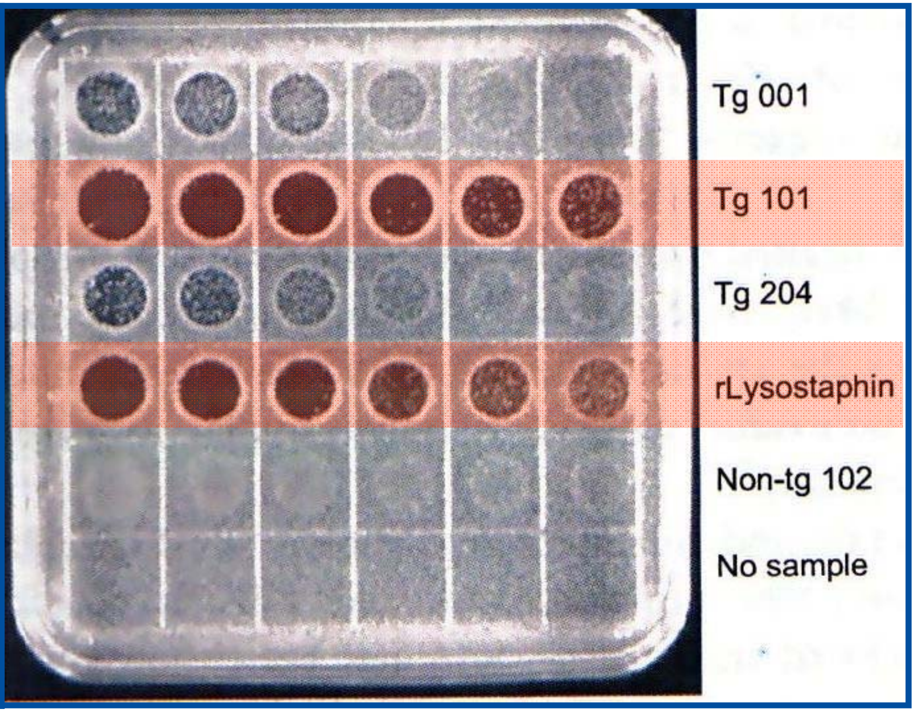
- General:
- Mx 1 protein (pigs)
- Immunoglobulin A (pigs)
- Visna virus envelope (sheep)
- Transmissible Gastroenteritis virus (TGEV; mammary gland specific mouse model)
- Knockout of specific genes (f.ex. Prion; PRRS, PERVs)
- siRNA mediated knockdown of pathogenic virus expression

- Mammary gland:
- α-Lactalbumin (pigs)
- Lysozyme (goats, cattle): antimicrobial effects
- Lactoferrin (cattle): bacteriostatic, bacteriocidic, iron provider
- Lycostaphin-transgenesis: St. aureus resistant cows

Transgenic cattle with mit Lysostaphin induced resistance of the mammary gland against infections with *St. aureus*



			Assay method	
			ELISA Mean ± s.e.m. (µg/ml)	Lysis assay Mean ± s.e.m. (µg/ml)
ID (Sex)	Generation	Founder ID		
001 (F)^a	Founder	001	0.87 ± 0.08	0.08 ± 0.01
101 (F)^a	Founder	101	11.03 ± 0.38	2.50 ± 0.20
204 (F)^a	Founder	204	0.92 ± 0.03	0.13 ± 0.01
214 (M)^b	G_1	004	5.2 ± 0.5	na
215 (F)^b	G_1	004	5.6 ± 0.9	na
312 (F)^b	G_1	104	13.8 ± 4.4	na



resistance against *St. aureus* infusions: Tg: 18/21(85.3%) (Infection only after very high doses) vs. WT 13/47 (31.7%)

Wall et al., 2005, Nature Biotechnology

Mastitis resistant cows by transgenic expression of human lysozyme expression from the ß-casein locus

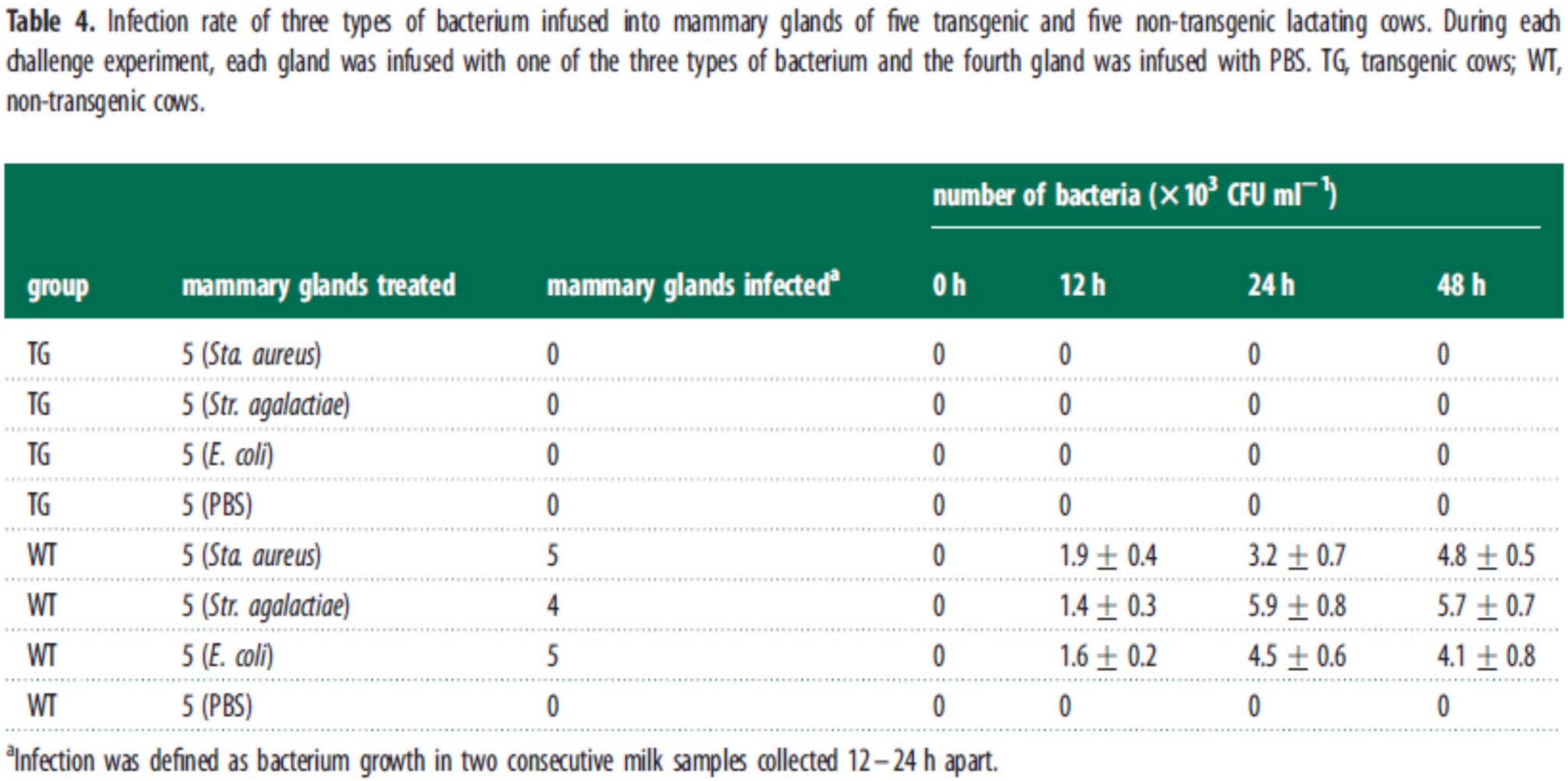


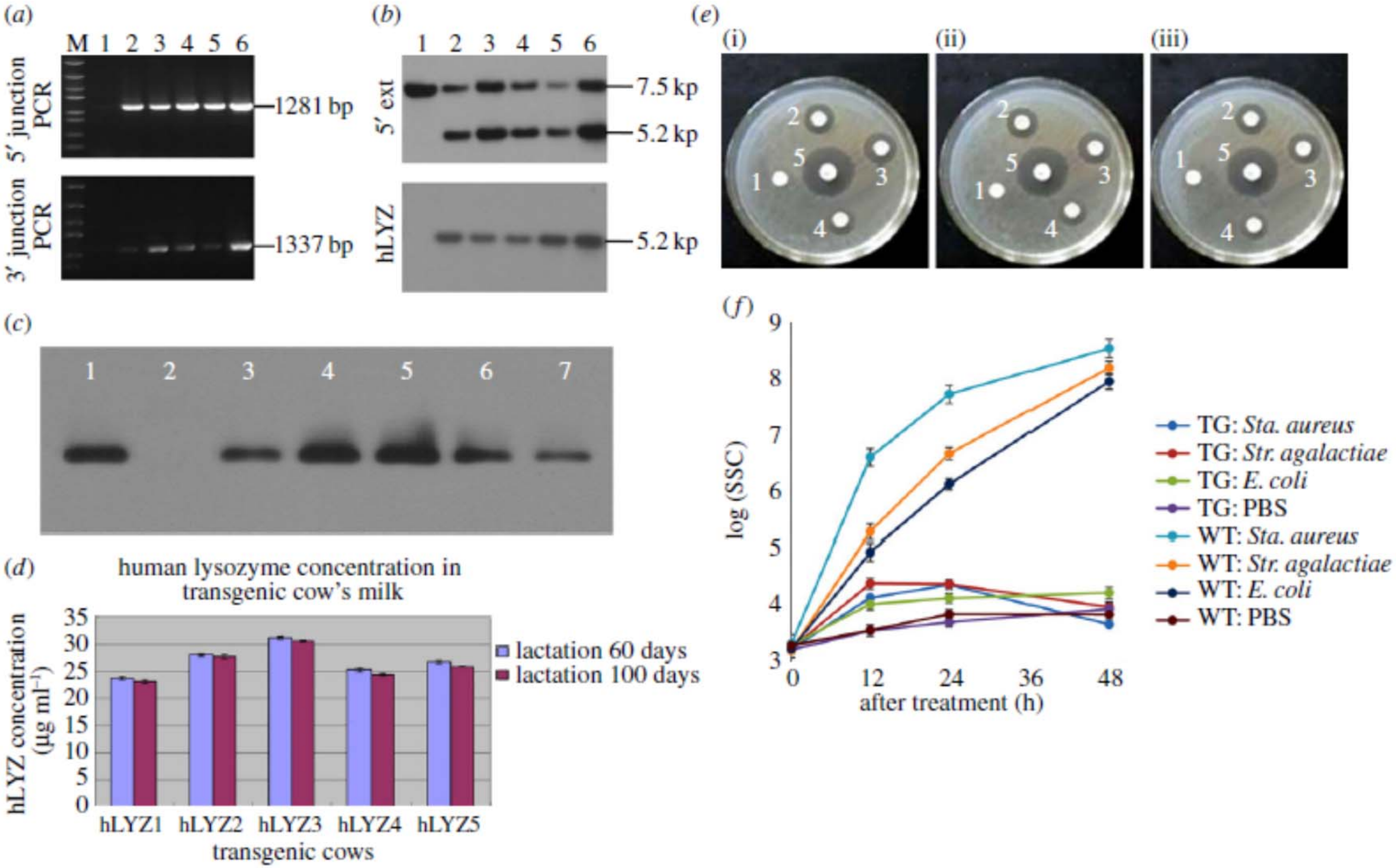
Table 4. Infection rate of three types of bacterium infused into mammary glands of five transgenic and five non-transgenic lactating cows. During each challenge experiment, each gland was infused with one of the three types of bacterium and the fourth gland was infused with PBS. TG, transgenic cows; WT, non-transgenic cows.

			number of bacteria ($\times 10^3$ CFU ml^{-1})			
group	mammary glands treated	mammary glands infected[a]	0 h	12 h	24 h	48 h
TG	5 (*Sta. aureus*)	0	0	0	0	0
TG	5 (*Str. agalactiae*)	0	0	0	0	0
TG	5 (*E. coli*)	0	0	0	0	0
TG	5 (PBS)	0	0	0	0	0
WT	5 (*Sta. aureus*)	5	0	1.9 ± 0.4	3.2 ± 0.7	4.8 ± 0.5
WT	5 (*Str. agalactiae*)	4	0	1.4 ± 0.3	5.9 ± 0.8	5.7 ± 0.7
WT	5 (*E. coli*)	5	0	1.6 ± 0.2	4.5 ± 0.6	4.1 ± 0.8
WT	5 (PBS)	0	0	0	0	0

[a] Infection was defined as bacterium growth in two consecutive milk samples collected 12–24 h apart.

Liu et al., Proc. R. Soc. B. 281, 2014

Mastitis resistant cows by transgenic expression of human lysozyme expression from the ß-casein locus



Liu et al., Proc. R. Soc. B. 281, 2014

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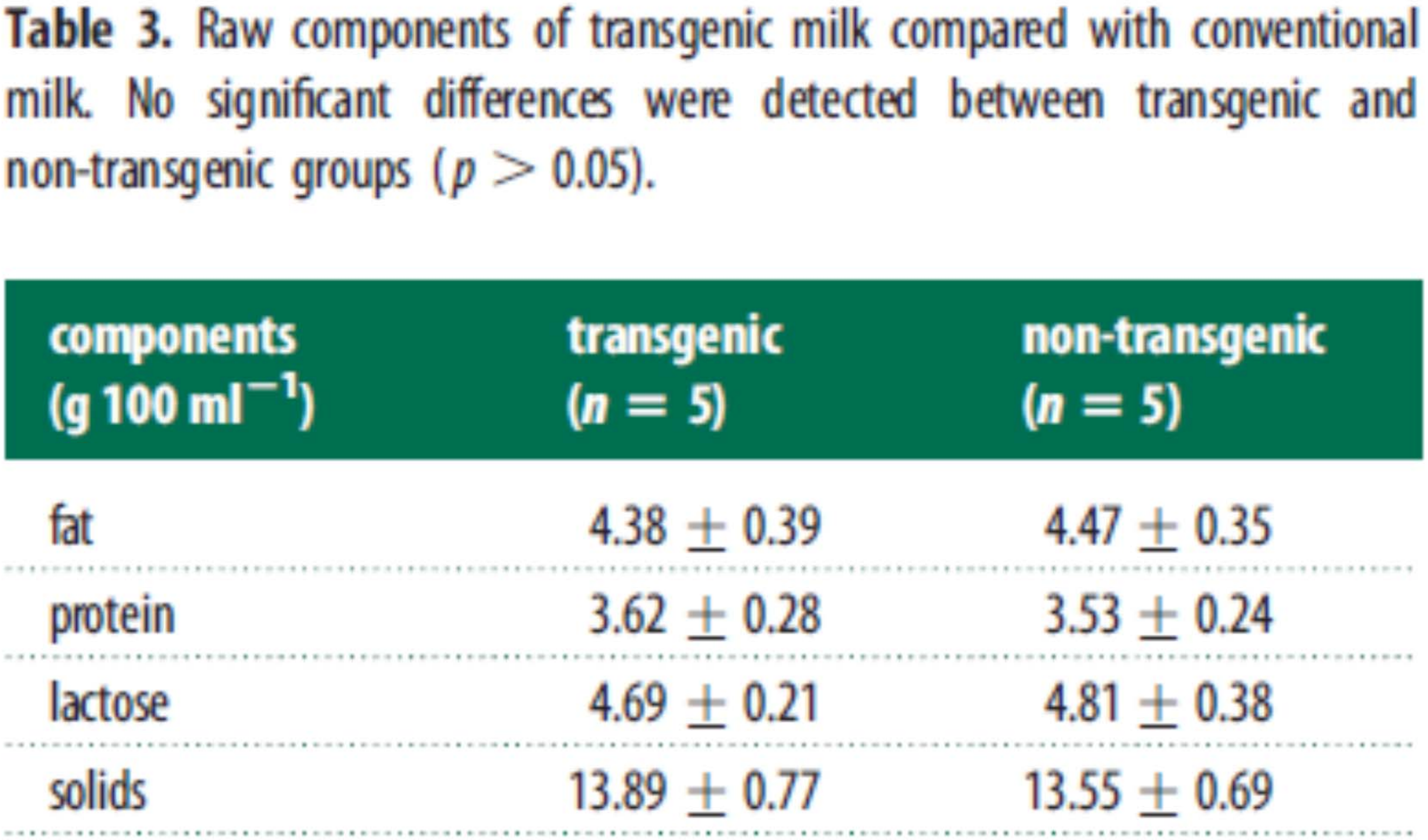


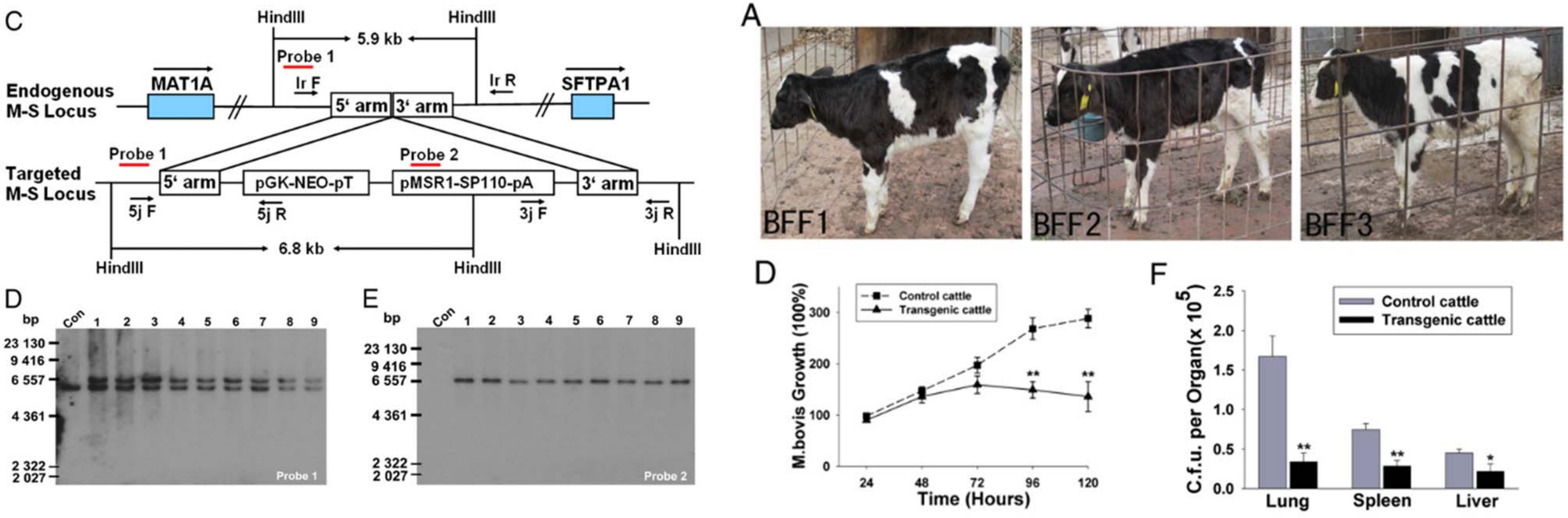
Table 3. Raw components of transgenic milk compared with conventional milk. No significant differences were detected between transgenic and non-transgenic groups ($p > 0.05$).

components ($g\ 100\ ml^{-1}$)	transgenic ($n = 5$)	non-transgenic ($n = 5$)
fat	4.38 ± 0.39	4.47 ± 0.35
protein	3.62 ± 0.28	3.53 ± 0.24
lactose	4.69 ± 0.21	4.81 ± 0.38
solids	13.89 ± 0.77	13.55 ± 0.69

Liu et al.,Proc. R. Soc. B. 281, 2014

Increased Resistance against Tuberculosis via gene editing (TALEN) and transgenic technology

- 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.

Cattle resistant against mycobacterium tuberculosis infection, produced via gene editing and transgenic technologies

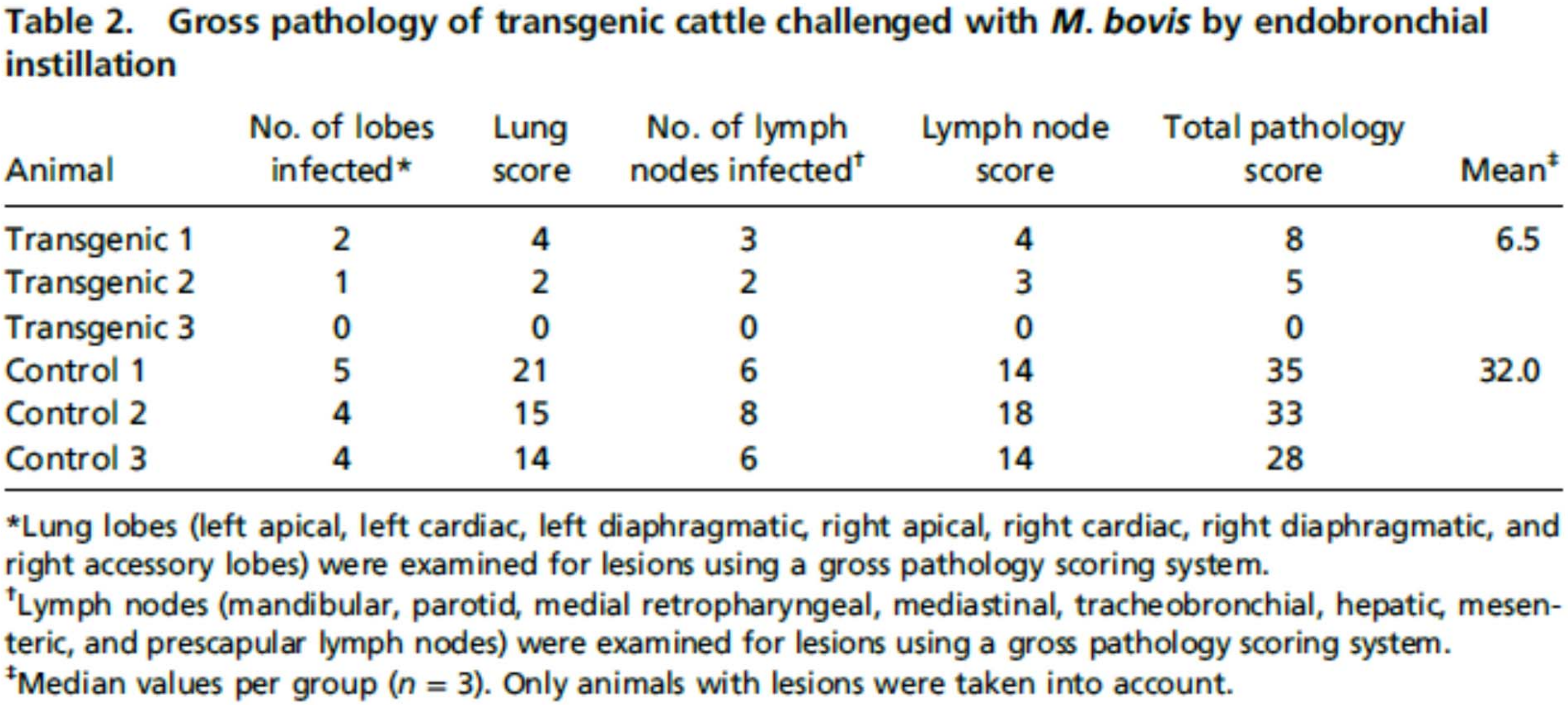


Table 2. Gross pathology of transgenic cattle challenged with *M. bovis* by endobronchial instillation

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.

Cattle with resistance to BSE after knockout of the prion locus

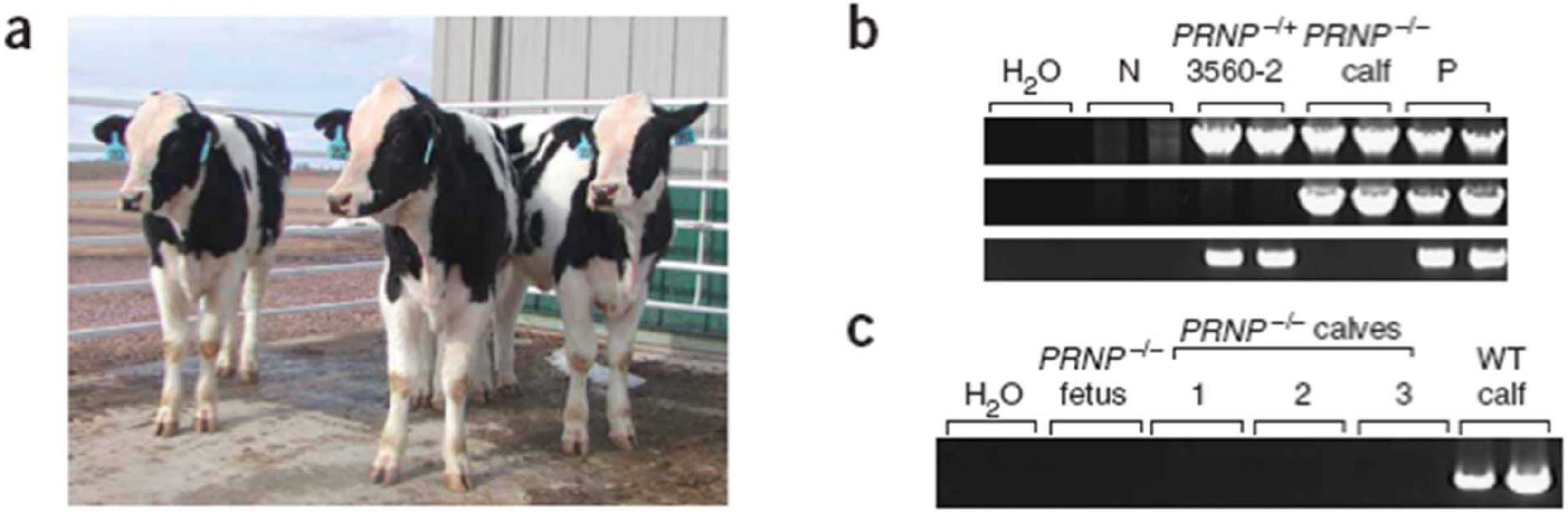


Figure 1 Generation of *PRNP*$^{-/-}$ cattle. **(a)** *PRNP*$^{-/-}$ cattle at 13 months of age. **(b)** Verification of *PRNP*$^{-/-}$ genotype in the ear biopsy fibroblasts by genomic PCR. P, positive control[6]; N, negative

Richt et al., 2007, Nature Biotechnology

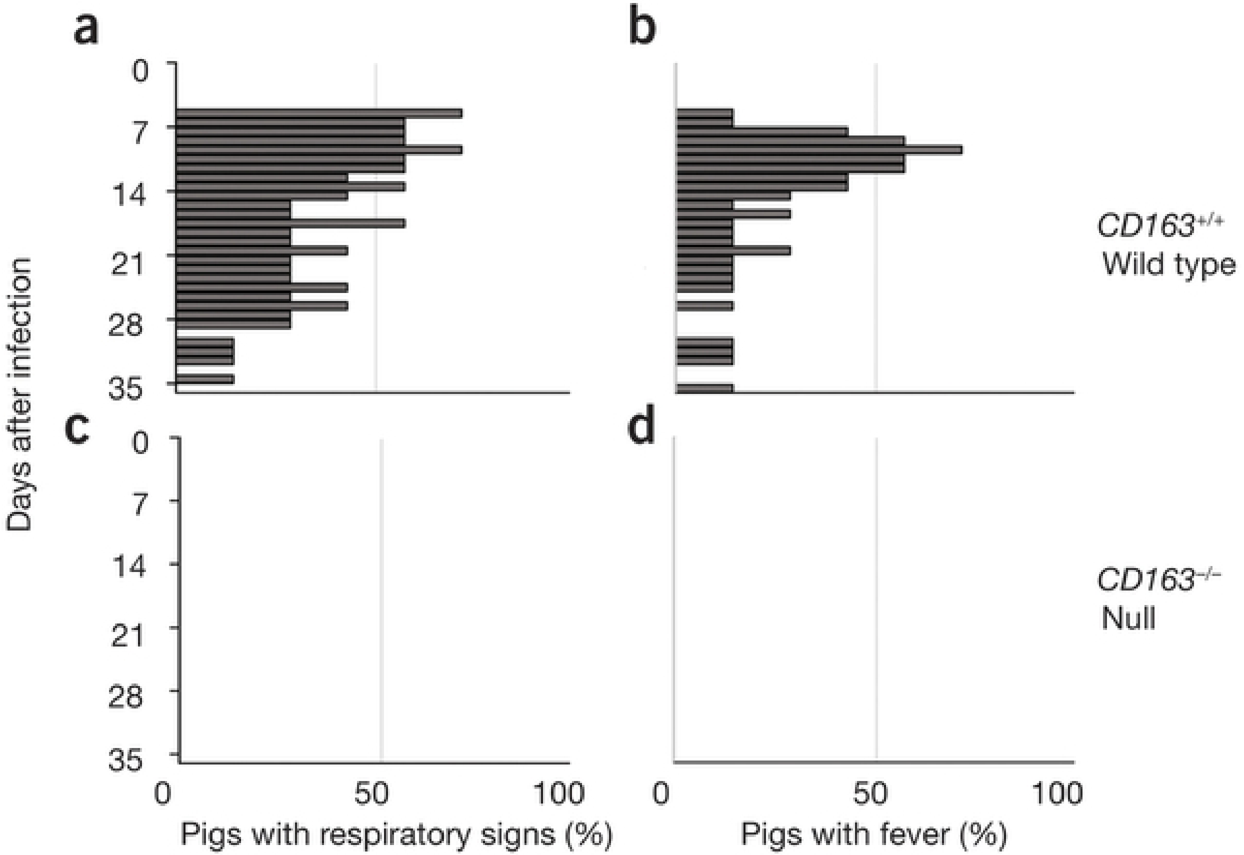
PRRS resistant pigs after CRISPR/Cas mediated knockout of CD 163

CRISPR/Cas-derived CD 163 knockout piglets were kept together with WT-piglets. All animals were infected with PRRS virus. Five days later, the WT-piglets showed typical PRRSV symptoms, while CD 163 KO piglets remained completely healthy.

(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)

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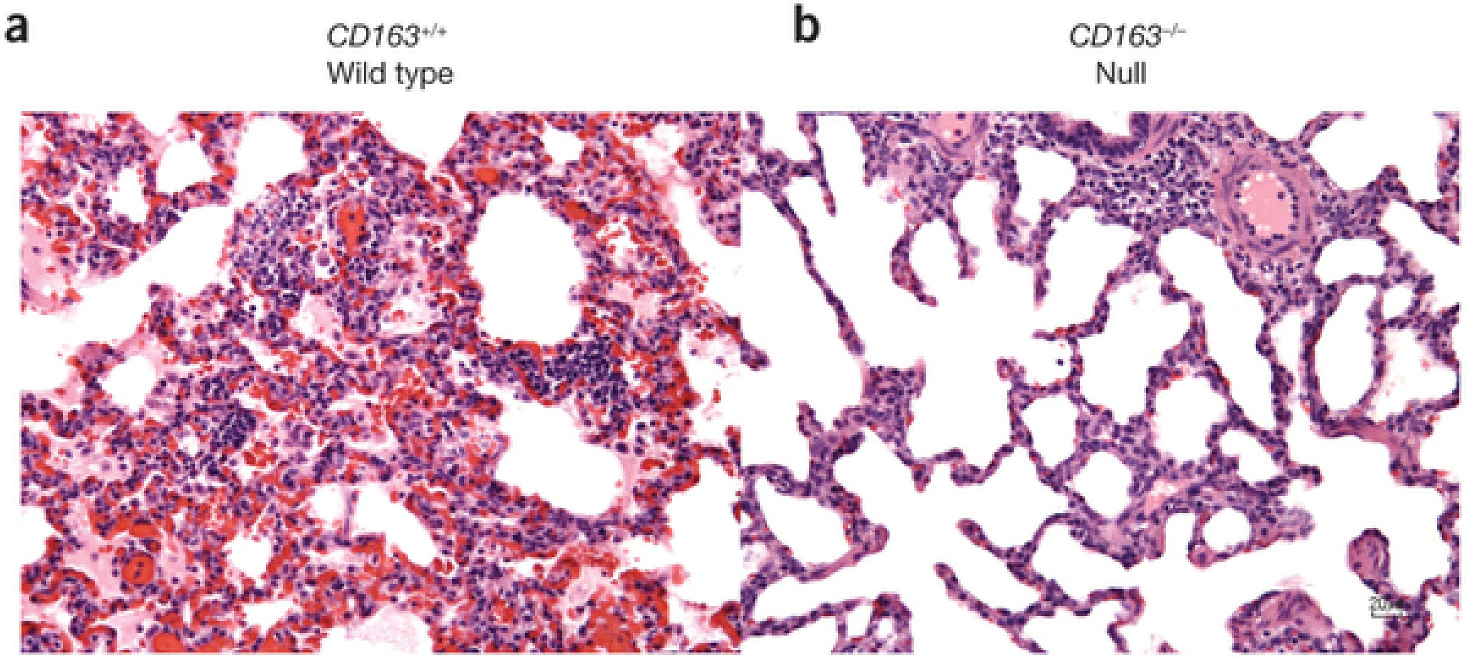
PRRS resistant pigs after CRISPR/Cas mediated knockout of CD 163

Microscopic image of the lung of CD163+/+ and CD163-/- pigs

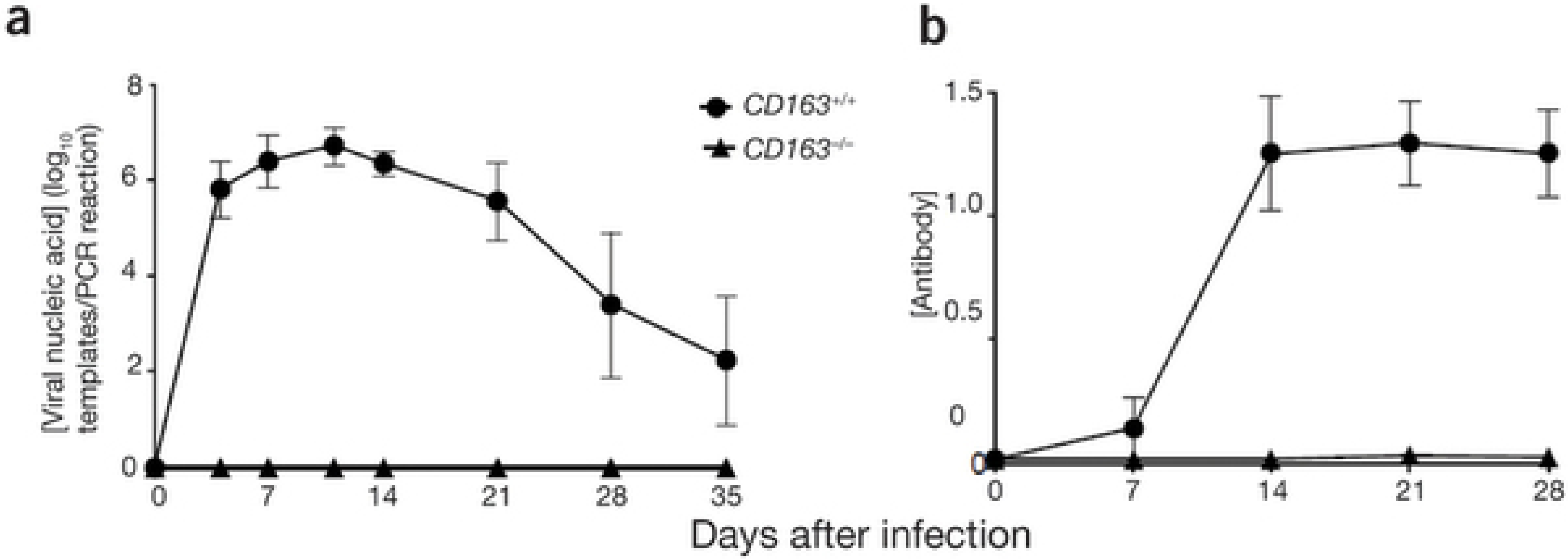


PRRS: Porcine reproductive and respiratory syndrome virus

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

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

PRRS specific DNA (a) und antibodies (b)



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Gene-editing record smashed in pigs

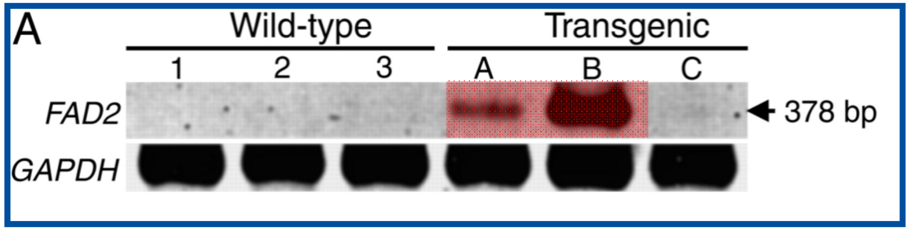
Researchers modify more than 60 genes (PERV) in effort to enable organ transplants into humans.

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

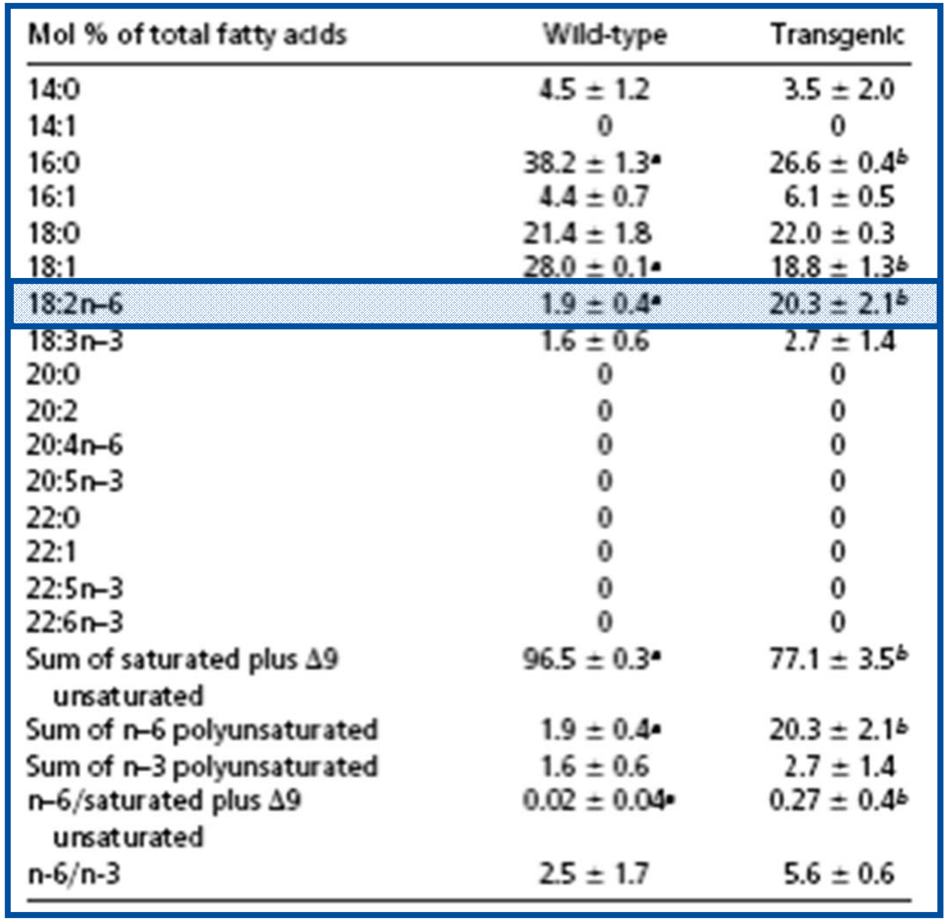
Also ~20 genes altered related to immunology and relevant for Xenotransplantation

Nature News 2015,18525

Spinach desaturase expression in transgenic pigs alters fatty acid profile in skeletal muscle



Desaturase expression in transgenic pigs leads to meat with more poly-unsaturated fatty acids.



Mol % of total fatty acids	Wild-type	Transgenic
14:0	4.5 ± 1.2	3.5 ± 2.0
14:1	0	0
16:0	38.2 ± 1.3^a	26.6 ± 0.4^b
16:1	4.4 ± 0.7	6.1 ± 0.5
18:0	21.4 ± 1.8	22.0 ± 0.3
18:1	28.0 ± 0.1^a	18.8 ± 1.3^b
18:2n–6	1.9 ± 0.4^a	20.3 ± 2.1^b
18:3n–3	1.6 ± 0.6	2.7 ± 1.4
20:0	0	0
20:2	0	0
20:4n–6	0	0
20:5n–3	0	0
22:0	0	0
22:1	0	0
22:5n–3	0	0
22:6n–3	0	0
Sum of saturated plus Δ9 unsaturated	96.5 ± 0.3^a	77.1 ± 3.5^b
Sum of n–6 polyunsaturated	1.9 ± 0.4^a	20.3 ± 2.1^b
Sum of n–3 polyunsaturated	1.6 ± 0.6	2.7 ± 1.4
n–6/saturated plus Δ9 unsaturated	0.02 ± 0.04^a	0.27 ± 0.4^b
n-6/n-3	2.5 ± 1.7	5.6 ± 0.6

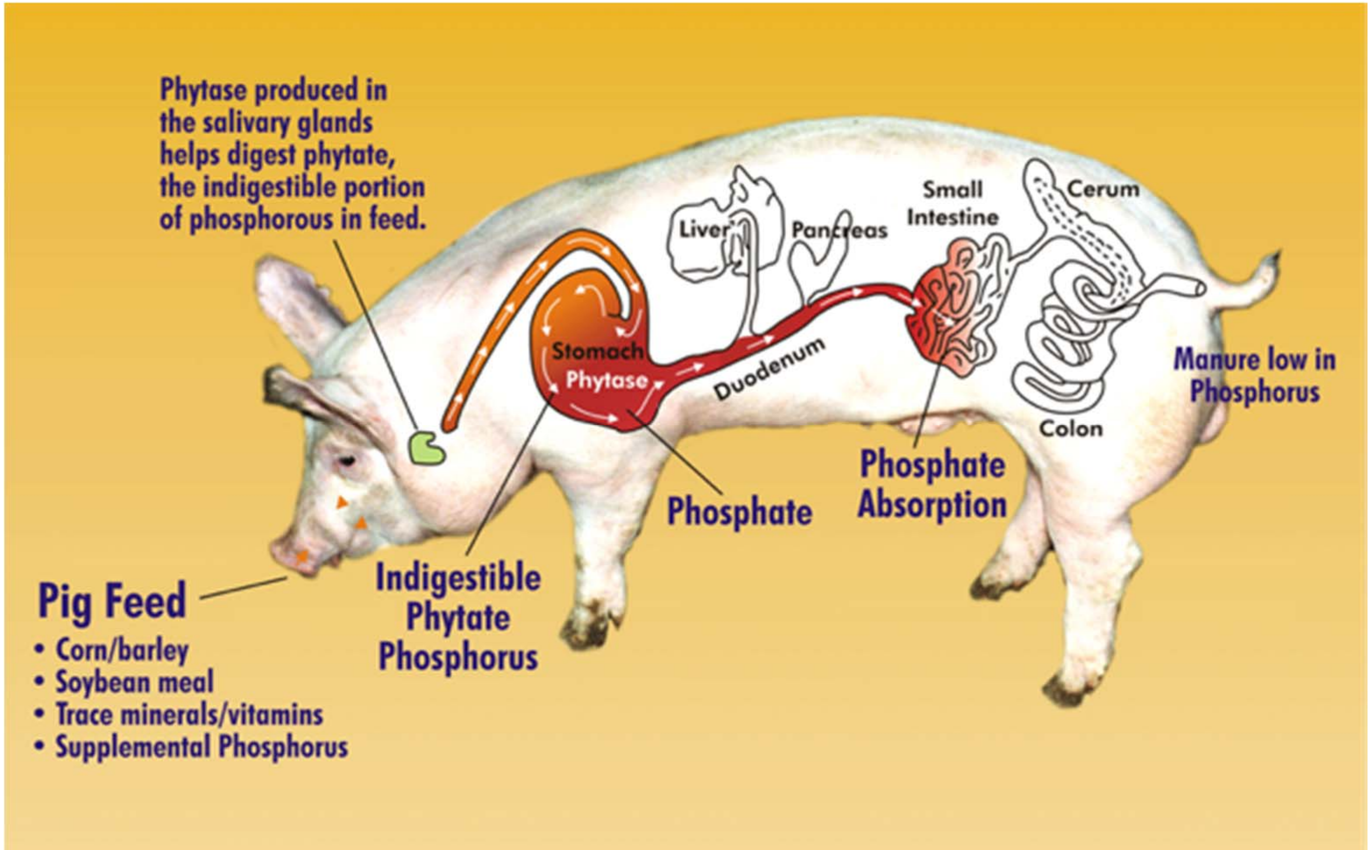
Saeki et al., 2004, PNAS

n-3 and n-6 fatty acids concentration and n-6/n-3 ratios in tail samples from *hfat-1* transgenic and wild-type piglets

Fatty acids in tails	**Transgenic piglets (*n* = 8)**	**Wild-type piglets (*n* = 8)**
ALA (18:3 *n*-3, %)	0.94 ± 0.10	0.63 ± 0.04
EPA (20:5 *n*-3, %)	4.21 ± 0.60	0.26 ± 0.07
DPA (22:5 *n*-3, %)	1.69 ± 0.19	0.35 ± 0.05
DHA (22:6 *n*-3, %)	1.75 ± 0.23	0.95 ± 0.21
Total *n*-3 FA (%)	8.59 ± 0.84	2.18 ± 0.25
Total *n*-6 FA (%)	14.28 ± 1.31	18.46 ± 1.41
n-6/*n*-3 ratio	1.69 ± 0.30	8.52 ± 0.62

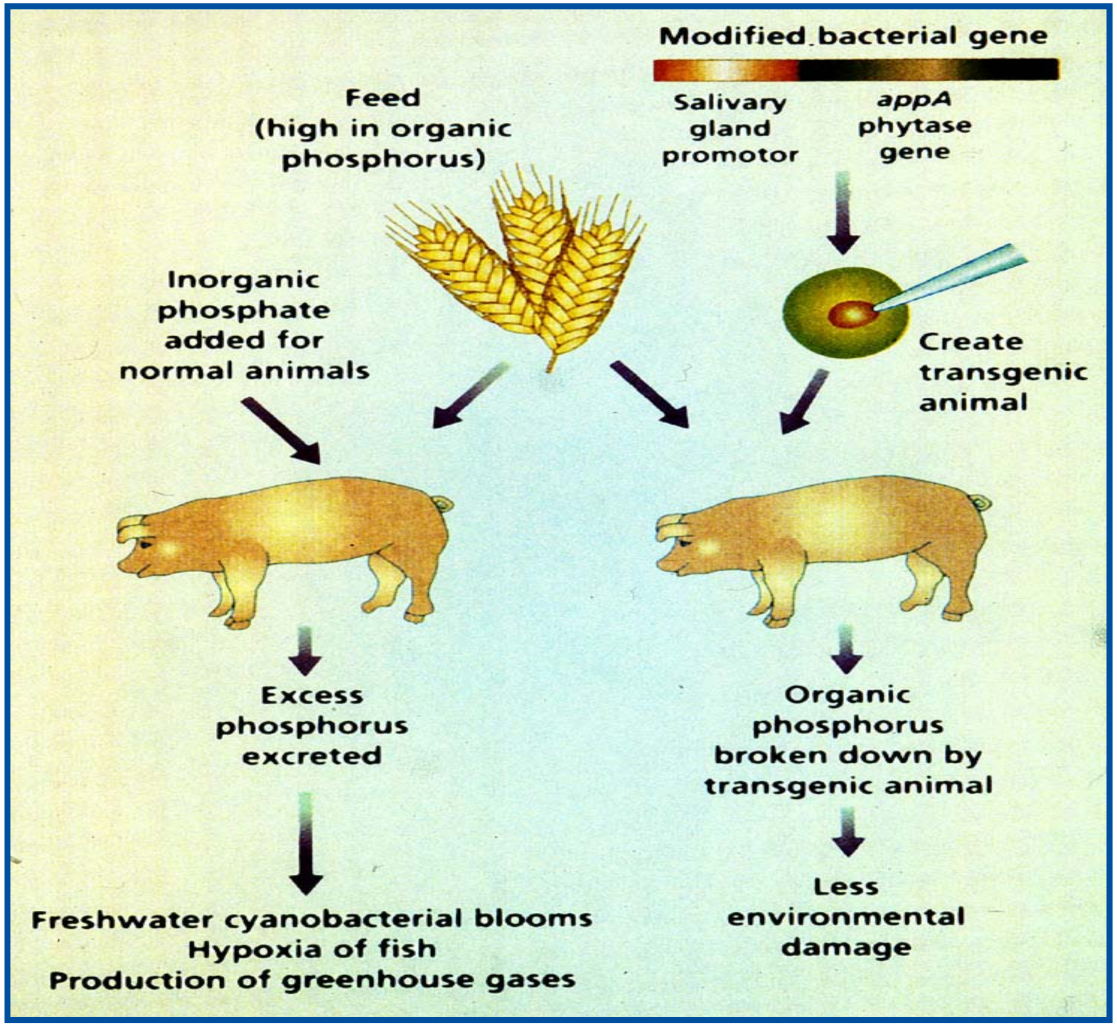
Lai et al. 2006, Nature Biotechnology

Transgenic pigs with expression of Phytase in salivary gland



Golovan et al., 2001, Nature Biotechnology

Transgenic swine expressing Phytase in the salivary gland



Golovan et al., Nat. Biotechol. 2001, 19, 741-745

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Expression of Phytase in the salivary gland of transgenic pigs

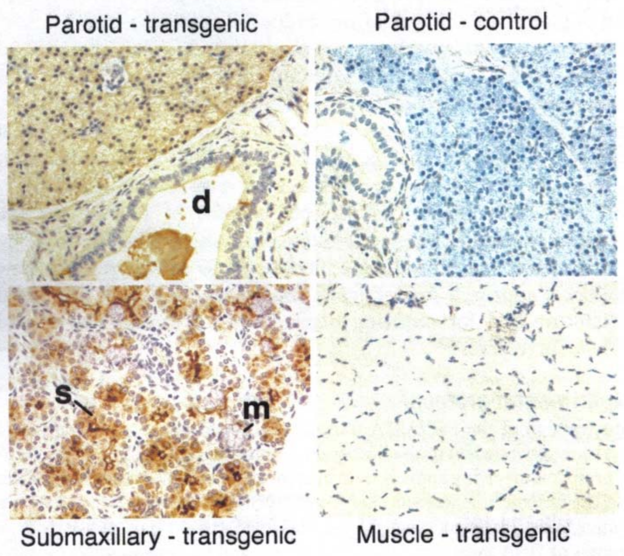


Table 1. True phosphorus digestibility (%) of transgenic phytase pig line WA using soybean meal as the sole source of phosphorus

Pigs	Non-transgenic	Transgenic
Weanling	48.5 ± 5.4^{a} ($n = 16$)	87.9 ± 3.4^{b} ($n = 14$)
Growing-finishing	51.9 ± 10.3^{a} ($n = 16$)	98.8 ± 3.4^{b} ($n = 14$)

[a,b] Means in the same row with different superscript letters differ ($P < 0.01$). True digestibility is the percentage of total phosphorus digested and absorbed from the diets corrected for endogenous phosphorus released from the gastrointestinal tract. Data represent mean ± s.e.m., as determined by a regression analysis technique[40,42].

Expression of phytase in the salivary gland of transgenic pigs

Reduction of anorganic phosphorus feeding via improved metabolism

Reduction of phosphorus excretion by up to 75%

Reduction of costs

Environment protection

Golovan et al. (2001), Nature Biotechnol. 19, 741-745

Transgenic animals with agriculturally important traits

- **Lactational performance**

Transgenic cattle: lactoferrin, lysozyme, caseins; but problems in some of the mouse models with milk production, Mariensee: Lactase transgenic mice with significant effects on lactose levels

Transgenic pigs: bovine α-lactalbumin: elevated lactose levels better piglet performance.

- **Dietetic improvements**

Transgenic pigs >unsaturated fatty acids by introduction of spinach desaturase gene

- **Wool shearing**
- **Reproductive performance**

(Estrogen receptor gene, inhibin reduction?)

Biomedical perspectives of genetically modified farm animals

- Biomedical perspectives

  Gene Pharming (rec. Proteins, mAbs)
  Human blood substitute
  Xenotransplantation
  Inhibitors of chemical weapons

- Basic research

  Epigenetic reprogramming
  Models for human diseases

Approved GM vertebrates

- GloFisch, genetically engineered zebra**fish, 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)

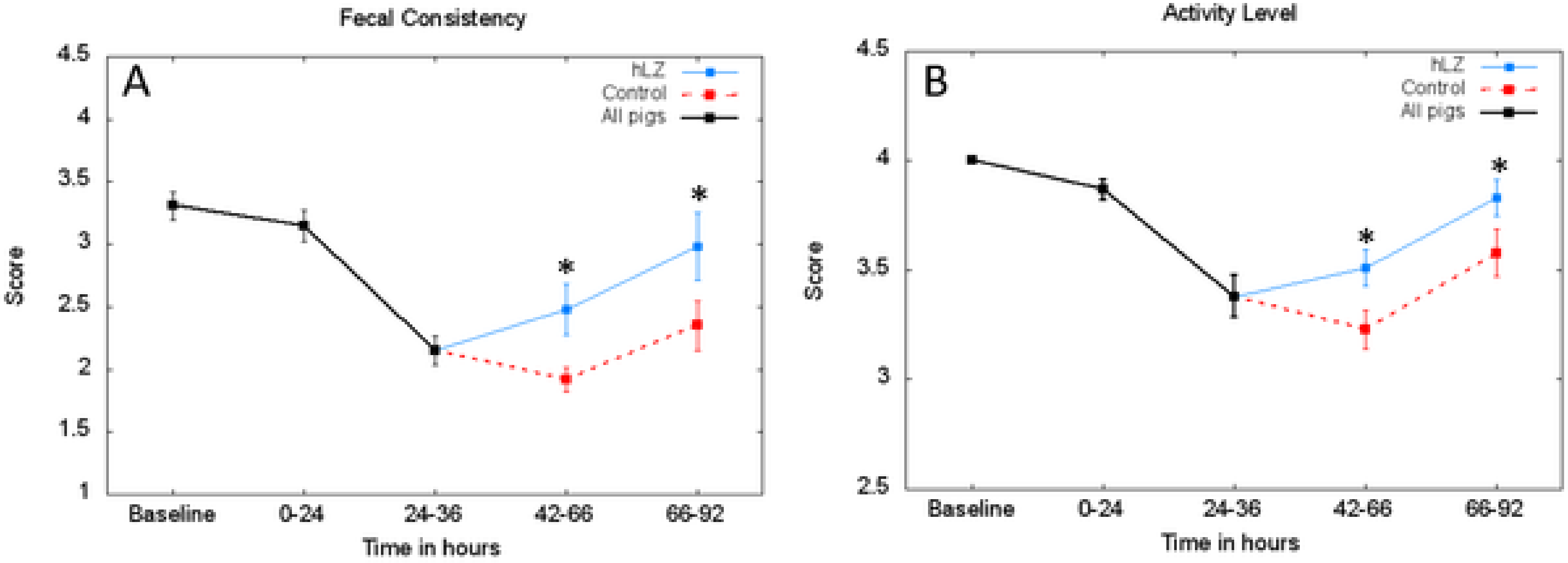
- AquAdvantage salmon,added growth hormon from Pacific Chinook salmon, all year long expression, faster growth, approved by FDA (Nov.2015)

Transgenic chickens are the latest animals engineered to produce ‘farmaceutical’ drugs.

US government approves transgenic chicken

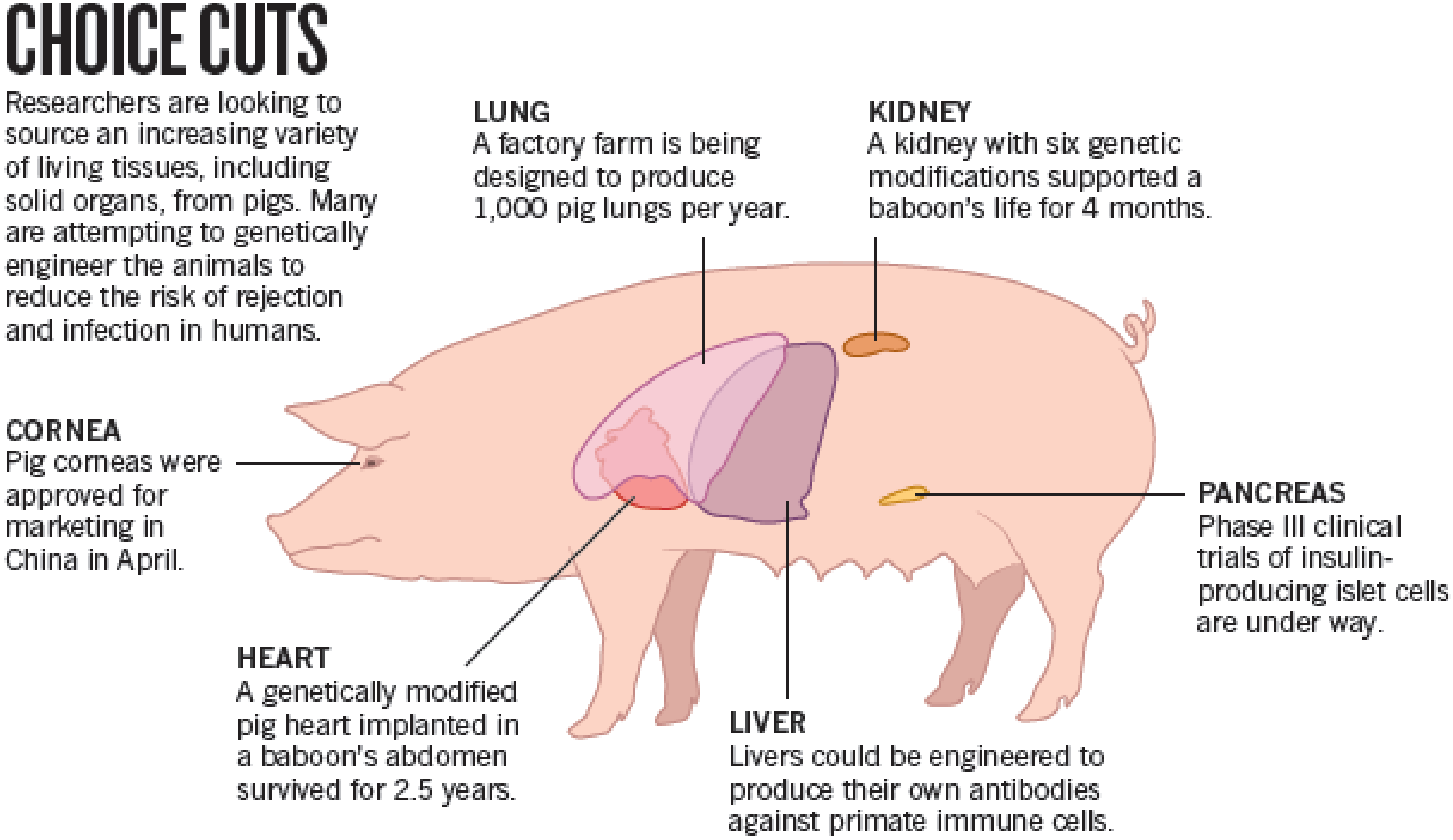
Nature 9.12.2015

Pigs fed hLZ- milk have improved fecal consistency and activity scores



Cooper CA, Garas Klobas LC, Maga EA, Murray JD (2013)
PLoS ONE 8(3): e58409. doi:10.1371/journal.pone.0058409
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0058409

Towards the ultimate donor pig



CHOICE CUTS

Researchers are looking to source an increasing variety of living tissues, including solid organs, from pigs. Many are attempting to genetically engineer the animals to reduce the risk of rejection and infection in humans.

LUNG
A factory farm is being designed to produce 1,000 pig lungs per year.

KIDNEY
A kidney with six genetic modifications supported a baboon's life for 4 months.

CORNEA
Pig corneas were approved for marketing in China in April.

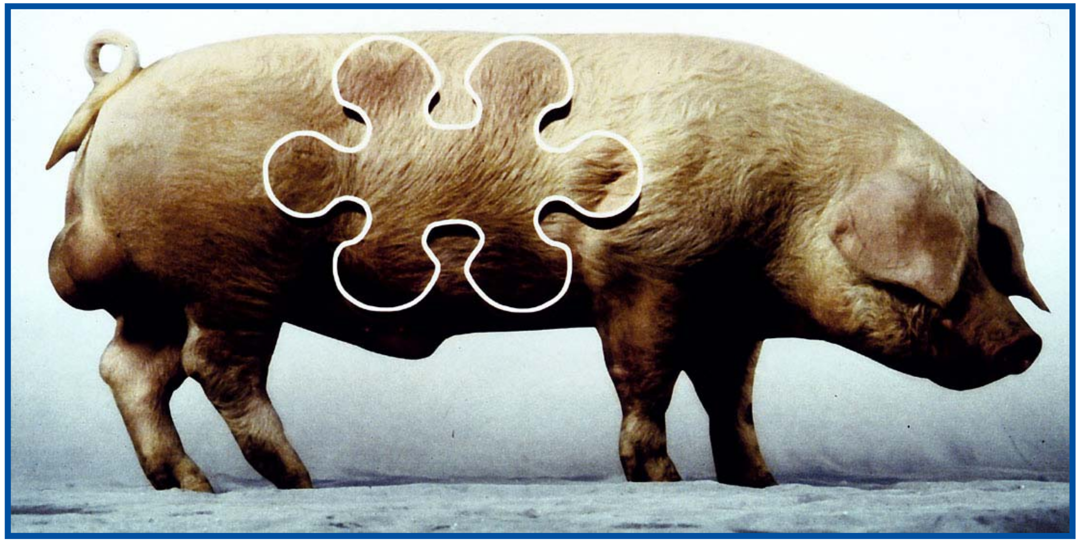
PANCREAS
Phase III clinical trials of insulin-producing islet cells are under way.

HEART
A genetically modified pig heart implanted in a baboon's abdomen survived for 2.5 years.

LIVER
Livers could be engineered to produce their own antibodies against primate immune cells.

Nature 527, Nov. 2015

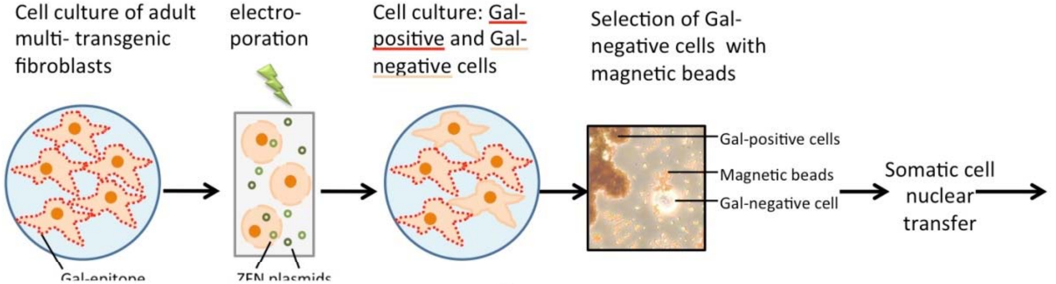
The domestic pig as a potential donor for human organs



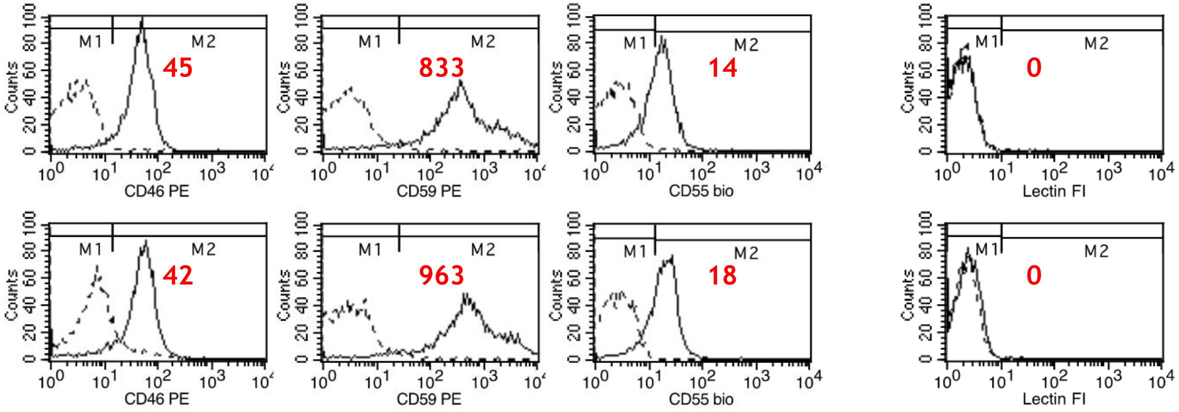
I like pigs;
dogs look up to us,
cats look down to us,
pigs treat us as equal.
(Winston Churchill)

- Domesticated species
- High fertility, great abundance, rapid growth
- Genetics, anatomy, physiology not too different from human
- Strict hygienic conditions possible
- Previous success with porcine insulin, heart valves, skin patches
- Genetic modifications possible

Multi-transgenic pigs (GGTA1-KO/hCD46/hCD55/hCD59/hA20/hHO-1) for improved xenotransplantation results



Cell culture of adult multi- transgenic fibroblasts → electro- poration → Cell culture: Gal-positive and Gal-negative cells → Selection of Gal-negative cells with magnetic beads → Somatic cell nuclear transfer →



SPBL
tg 229/1

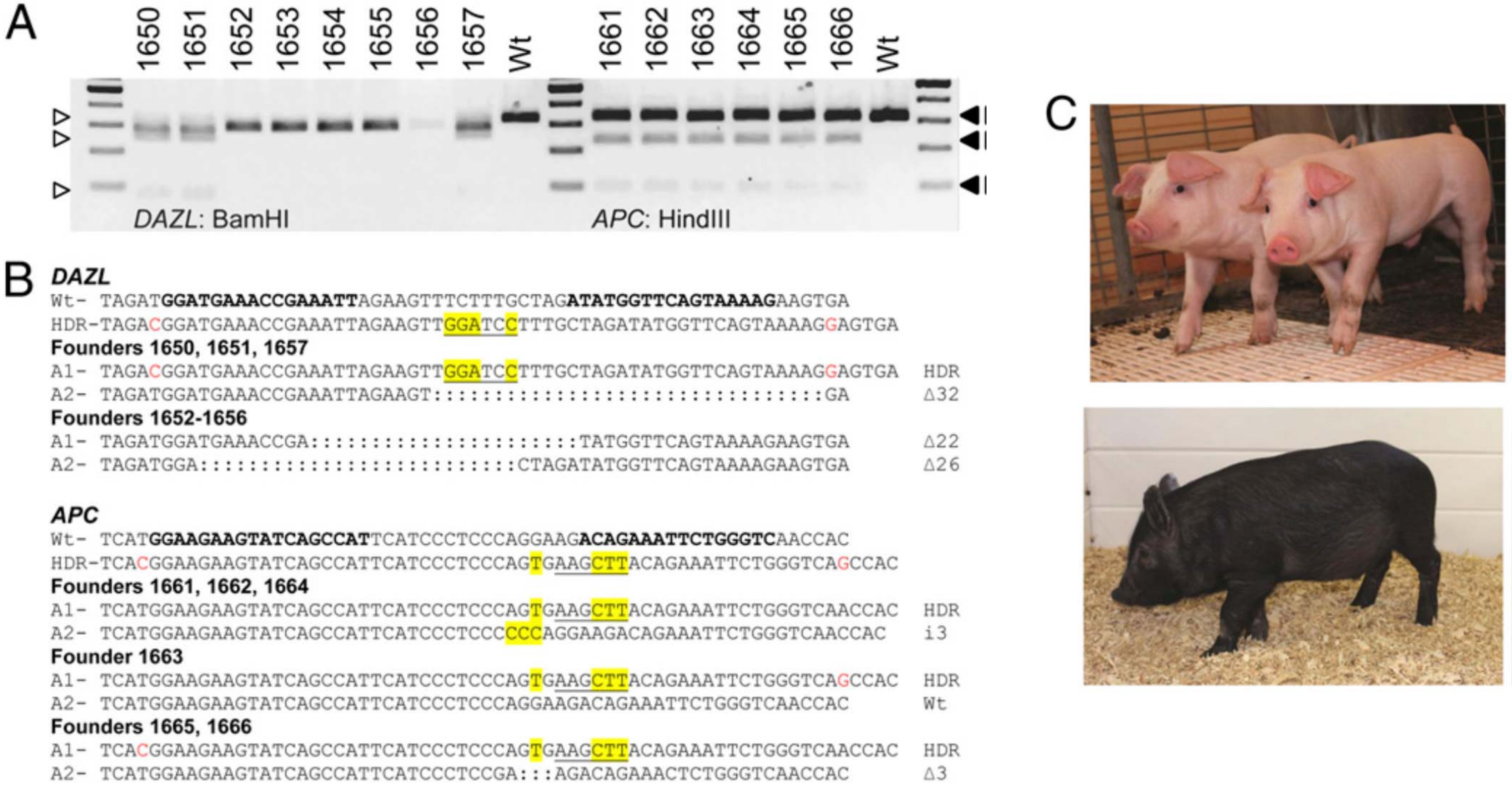
SPBL
tg 229/2

Liveborn multi-transgenic piglets "Thomas" and „Müller".

Donor cells	Recipients	Transferred embryos	Pregnant (PR)	Liveborn offspring	Ongoing pregnancies	Delivery dates
164/3 (recloning 1706GalKo)	10	983 (Ø = 98)	7 (70%)	4	3	15.11.14 17.01.15 18.01.15

K.Fischer et al. Scientific Reports 2016

Successful use of TALEN in livestock



DAZL

Wt- TAGAT**GGATGAAACCGAAATT**AGAAGTTTCTTTGCTAG**ATATGGTTCAGTAAAAG**AAGTGA

HDR-TAGACGGATGAAACCGAAATTAGAAGTTGGATCCTTTGCTAGATATGGTTCAGTAAAAGGAGTGA

Founders 1650, 1651, 1657

A1- TAGACGGATGAAACCGAAATTAGAAGTTGGATCCTTTGCTAGATATGGTTCAGTAAAAGGAGTGA HDR

A2- TAGATGGATGAAACCGAAATTAGAAGT::::::::::::::::::::::::::::::::GA Δ32

Founders 1652-1656

A1- TAGATGGATGAAACCGA::::::::::::::::::::::TATGGTTCAGTAAAAGAAGTGA Δ22

A2- TAGATGGA::::::::::::::::::::::::::CTAGATATGGTTCAGTAAAAGAAGTGA Δ26

APC

Wt- TCAT**GGAAGAAGTATCAGCCAT**TCATCCCTCCCAGGAAG**ACAGAAATTCTGGGTC**AACCAC

HDR-TCACGGAAGAAGTATCAGCCATTCATCCCTCCCAGTGAAGCTTACAGAAATTCTGGGTCAGCCAC

Founders 1661, 1662, 1664

A1- TCATGGAAGAAGTATCAGCCATTCATCCCTCCCAGTGAAGCTTACAGAAATTCTGGGTCAACCAC HDR

A2- TCATGGAAGAAGTATCAGCCATTCATCCCTCCCCCCAGGAAGACAGAAATTCTGGGTCAACCAC i3

Founder 1663

A1- TCATGGAAGAAGTATCAGCCATTCATCCCTCCCAGTGAAGCTTACAGAAATTCTGGGTCAGCCAC HDR

A2- TCATGGAAGAAGTATCAGCCATTCATCCCTCCCAGGAAGACAGAAATTCTGGGTCAACCAC Wt

Founders 1665, 1666

A1- TCACGGAAGAAGTATCAGCCATTCATCCCTCCCAGTGAAGCTTACAGAAATTCTGGGTCAACCAC HDR

A2- TCATGGAAGAAGTATCAGCCATTCATCCCTCCGA:::AGACAGAAACTCTGGGTCAACCAC Δ3

Tan et al., PNAS 110, 16526-531; 2013

Application perspectives for genetically modified farm animals

- **Agricultural perspectives**
  - Growth and development (myostatin, GH, GHrec, IGF)
  - Wool production
  - Lactation (amount, composition)
  - Hornless cattle (Polled locus)
  - Disease resistance (Mx-gene, IgA, BSE, TB, PRRS, etc)
  - Reproduction
  - Environmental improvements (
  - Dietetic improvements
- **Biomedical perspectives**
  - Gene Pharming (rec. Proteins, mAbs)
  - Human blood substitute
  - Xenotransplantation
  - Inhibitors of chemical weapons
- **Basic research**
  - Epigenetic reprogramming
  - Models for human diseases

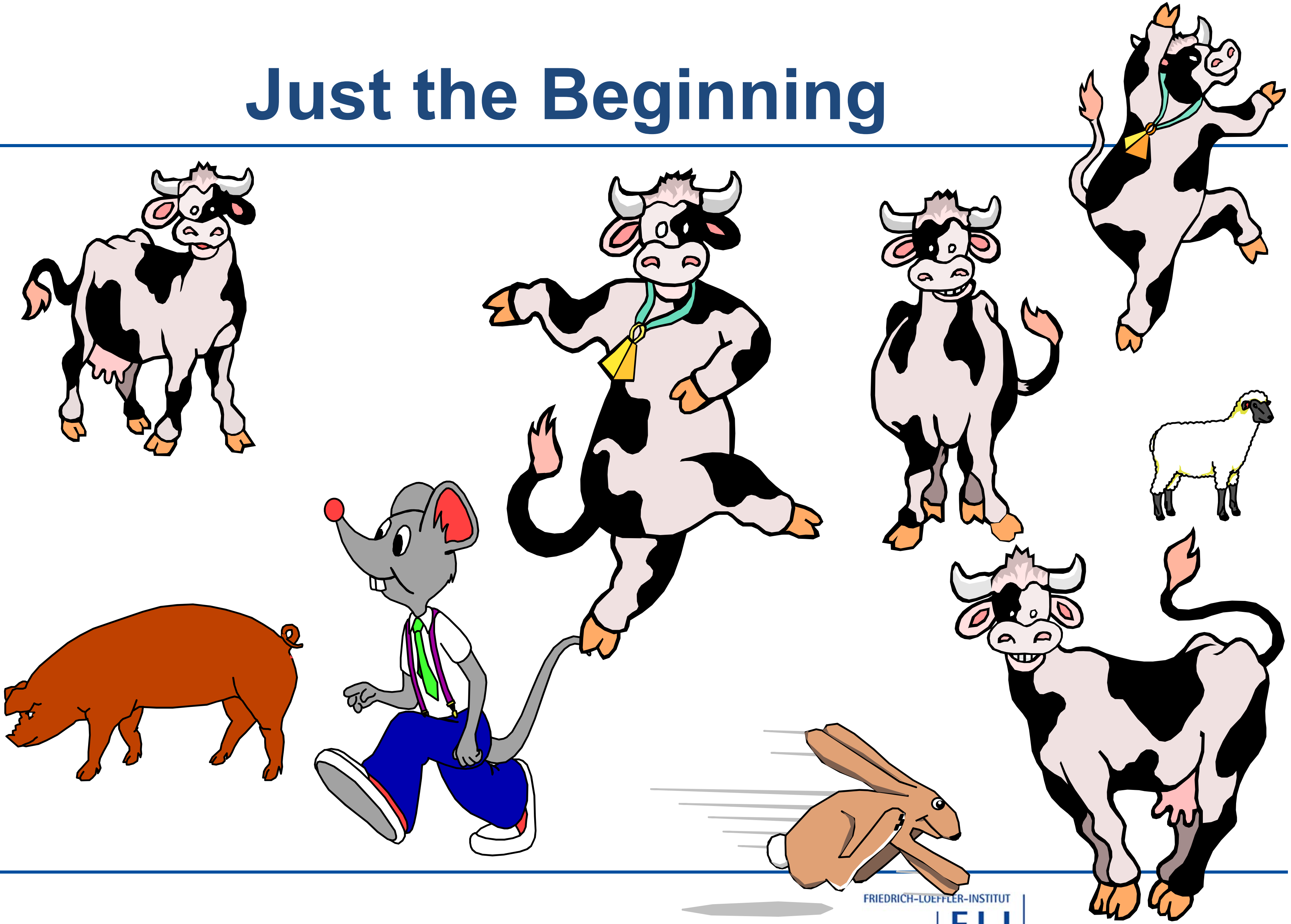
Evolution of farm animal breeding

- Domestication
- Proliferation of „useful“ populations
- Selection according to the Exterieur
- Selection according to specific traits
- Systematic breeding based on population genetics and statistics
- Reproductive technologies (AI, ET, IVP, SCNT, etc.)
- Molecular genetics and genome based breeding concepts (SNPs, GBV, etc.)
- Now entering the era of *Precision breeding*

The future : Targeted and diversified dairy production

- Full fat normal milk
- Defatted milk (knock-out of key lipid enzymes)
- Curd production (enhanced casein expression)
- Cheese production (enhanced casein expression)
- Coffee whitener and Creme liquor (ß-casein)
- Hypo-allergenic milk (reduced or omitted ß-lactoglobulin)
- Lactose free or –reduced milk (α- lactalbumin knock-out, additional lactase expression)
- Infant milk (enhanced lactoferrin expression)
- Improved udder health (lysozyme, etc.)
- Pharmaceutical proteins

Just the Beginning



Thank you for your attention.

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Aquabounty salmon: The first approved genetically engineered animal product

PAUL DARROW/NYT/REDUX/EYEVINE

AquAdvantage Atlantic salmon (at back) grow to twice the size of an normal Atlantic salmon (*Salmo salar*) over the same time.

The practical use of this new genomic information

- Better targeted breeding programmes
  Genomic breeding values; Direct sequencing
- Transcriptomics/Proteomics/Phenomics
- **Production of genetically modified (transgenic) animals**
- New knowledge on genetic diversity
- Descent studies
- Comparative genomics

Summary and Conclusions

- The genomes of farm animals have been sequenced and annotated; informative gene maps are available that can be used for breeding purposes (GBV).
- Novel molecular tools, incl. DNA-nucleases such as ZFNs, TALEN, CRISPR/Cas are compatible with precise genetic modifications (gene editing), that can be induced easily and with high efficiency.
- The use of the new genomic information and gene editing tools allow the development of novel breeding strategies, both for agricultural and biomedical purposes.
- Gene editors are also beneficial in human medicine.
- A complex and complicated legal framework is in place for commercial use of transgenic animals. The application of gene editing is not (yet) legally regulated and could thus be immediately employed in future oriented animal breeding systems.

Novel perspectives for animal breeding in agriculture and biomedicine

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