Regulation of Genetic Modified Animals in China

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Current Status of Genetic Modified Animals in China

The Key Special Project:

“Breeding and Cultivation of Novel GM varieties”

budget of 17.6 billion RMB

launched in The 11th Five-year Plan (since 2008),

the largest civil research project (space technology)

The Key Special Project:

“Breeding and Cultivation of Novel GM varieties”

Cattle

Swine

Goat/Sheep

The status of GM livestock studies in China

In 1999, the first GM cattle of China was born in Shanghai Medical Genetics Institute.

GM sheep successfully bred by China Agricultural University, Northwest A&F University, CAS, Xinjiang Acadamy of Animal Science

Studies of the methodology

Studies of genes with potential applications

Studies with breeding purpose

Studies of safety assessment issues

The Processes of Bio-safety Evaluation and Administration on Genetic Modified Organisms in Agriculture:

Experimental Study

Inter-medium Experiment

Environmental Release

Field Trial

Safety Certificate

Genetic Modified Animals

Cross-breeding with GMA.

An Outline of the Requirements on The Processes of Bio-safety Evaluation on Genetic Modified Animals in Agriculture:

- Information of Molecular Features
  - Bio-Information analysis on the bio-safety
- Genetic Stability Analysis
  - Information of gene integrity and expression during generation
- Animal Health Analysis
  - Behavior, growth, development, anatomy, reproduction.
- Environment Safety
- Food Safety

1. Information of Molecular Features

Study the integrity and expression of the foreign gene or fragments based on gene, transcription, and translation.

Information of expression vectors

Information of integrity of targeted gene in the animal genome

Information of expression of targeted fragments in the animal

2. Genetic Stability Analysis

Main Consideration: the integrity and expression of the genes in the animals during generations

Integrity

Expression

Traits

No less than 3 G

3. Animal Health Analysis

General Index:

Behavior, growth, development, anatomy, reproduction.

Physiological Index:

Parameters in clinical and anatomic traits:

Other parameters may be applied

Environment Safety

The abilities of the animals on survival competition and adaptation to environment

Evaluation of the animals on functional efficacy

Effect of the gene shifts on environment

Effect of the animals on bio-diversity

Food Safety Evaluation

Evaluation of Toxicity on the expressed products

Evaluation of Allergy

Analysis of Main Nutrient Integrants

Evaluation of Safety on Whole Food

90 day feeding test by rats

Nutrition Evaluation

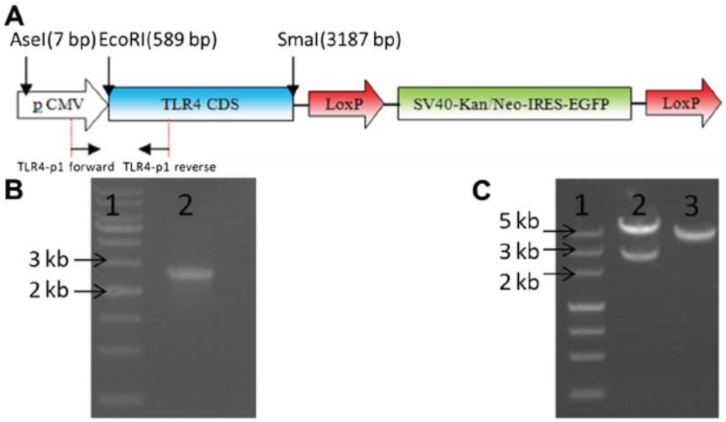
Evaluation on the effect of process on the animal products

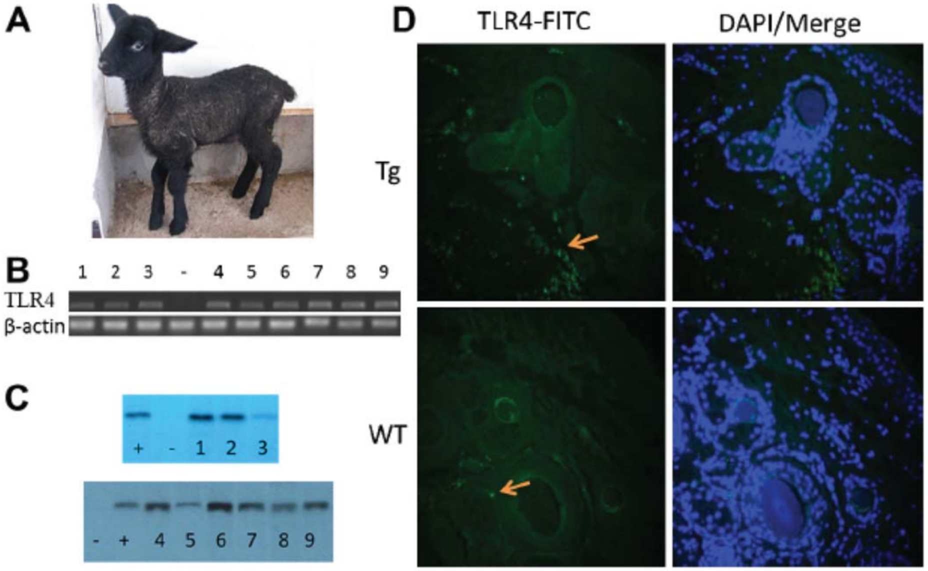
Progresses on the characterization of cloning events

Progress #1:

To get rid off the resistance/marker genes

Sheep:





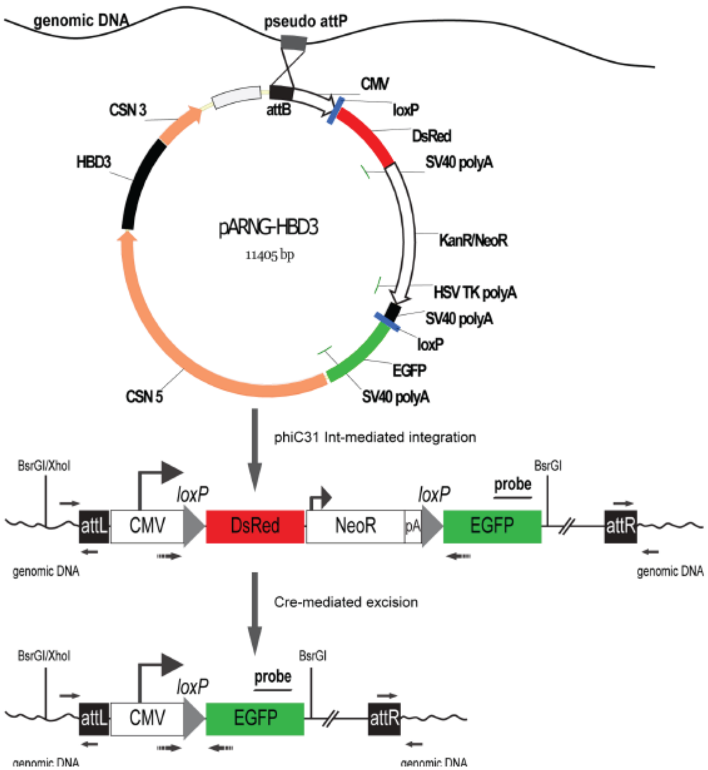
S Deng, et al., Theriogenology, 80:50-57, 2013

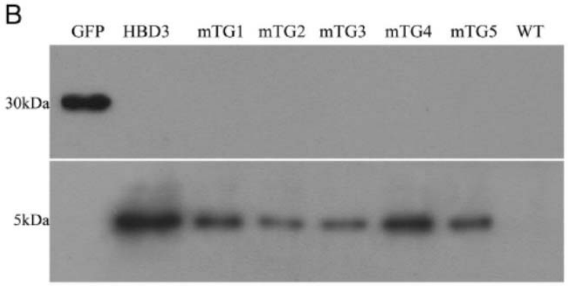
Progresses on the characterization of cloning events

Progress #1:

To get rid off the resistance/marker genes

Cattle:

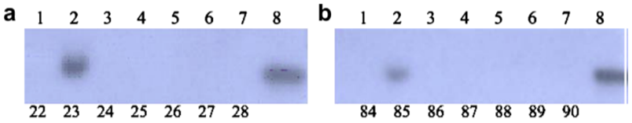




Y. Yu, et al., Plos One, 8:62457, 2013

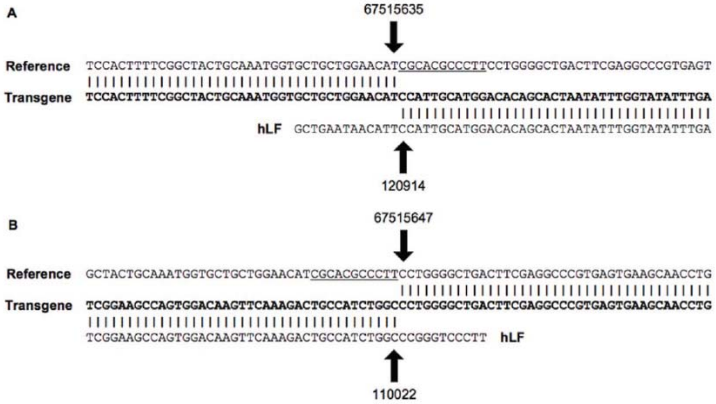
Progress #2:
To confirm the insertion of the gene on the host genome

Southern blot confirmation

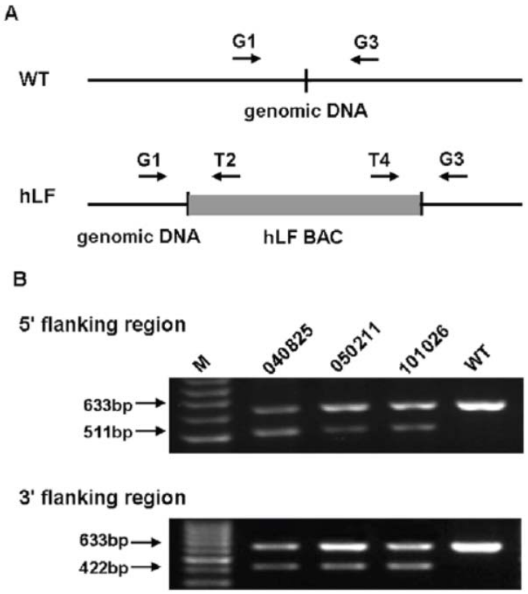


A Southern blot (transgenic goat to express human lactoferrin). J Zhang et al. PE&P, 2008

Whole genome sequence



Sequence results of the transgenic cattle.
J Zhang et al. Plos One, 2012

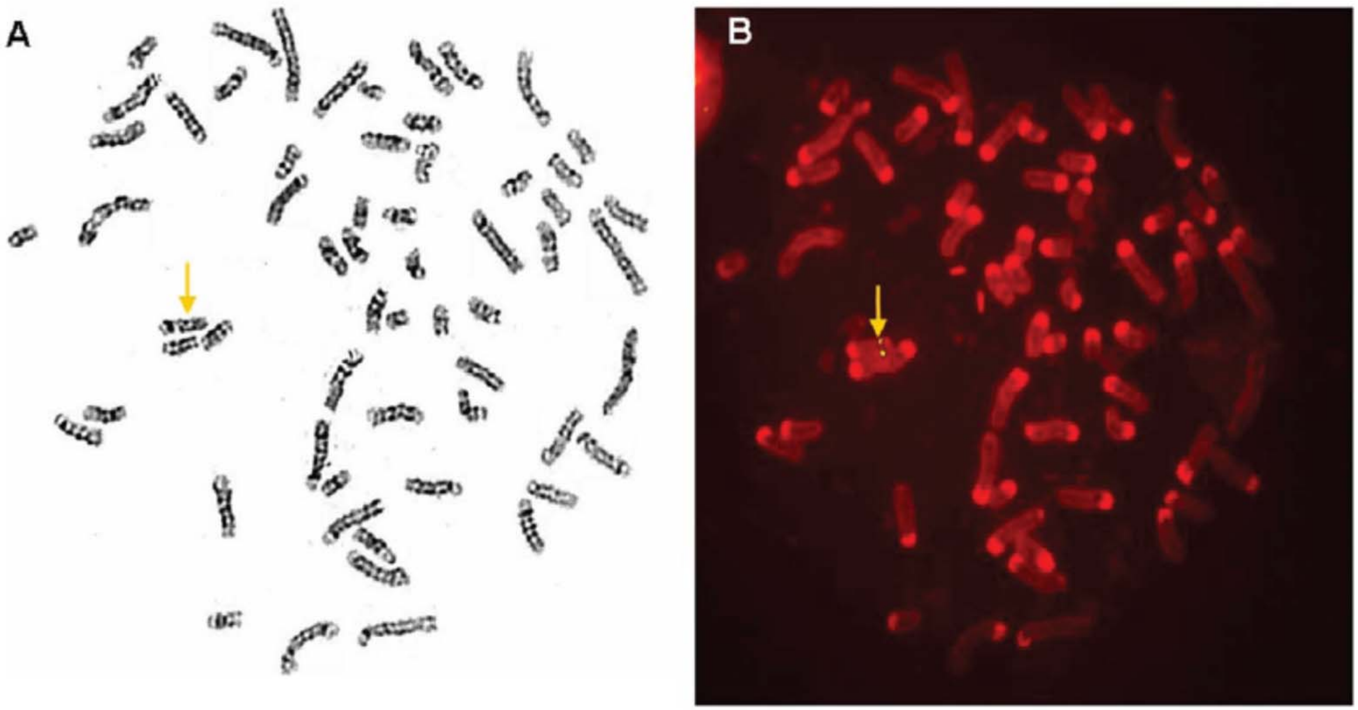


Confirmed by a PRC analysis. J Zhang et al. Plos One, 2012

Progress #2:

To confirm the insertion of the gene on the host genome

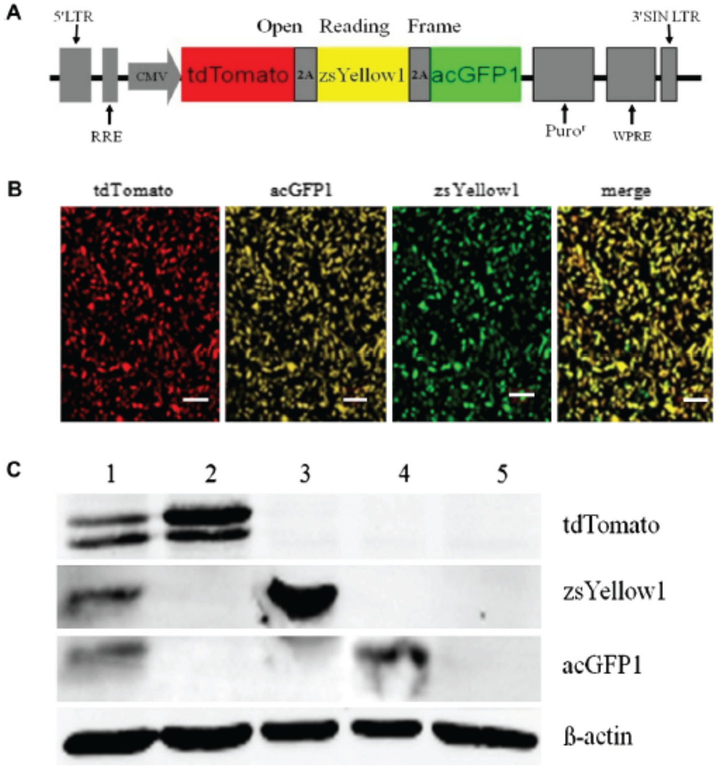
Verification of the transgene chromosomal location by FISH.
J Zhang et al. Plos One, 2012

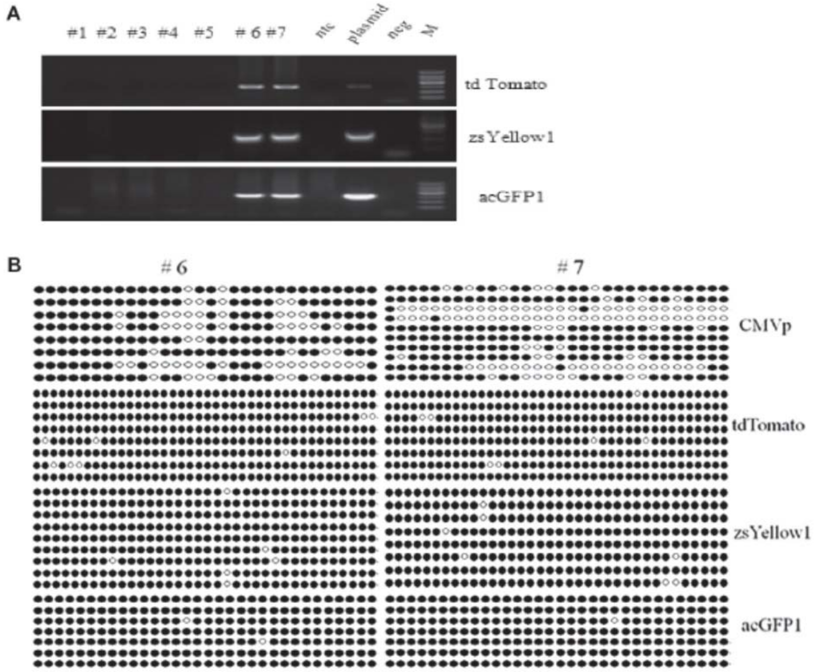


Progress #3:

To evaluate the modification of the promoters of inserted genes

Sheep:



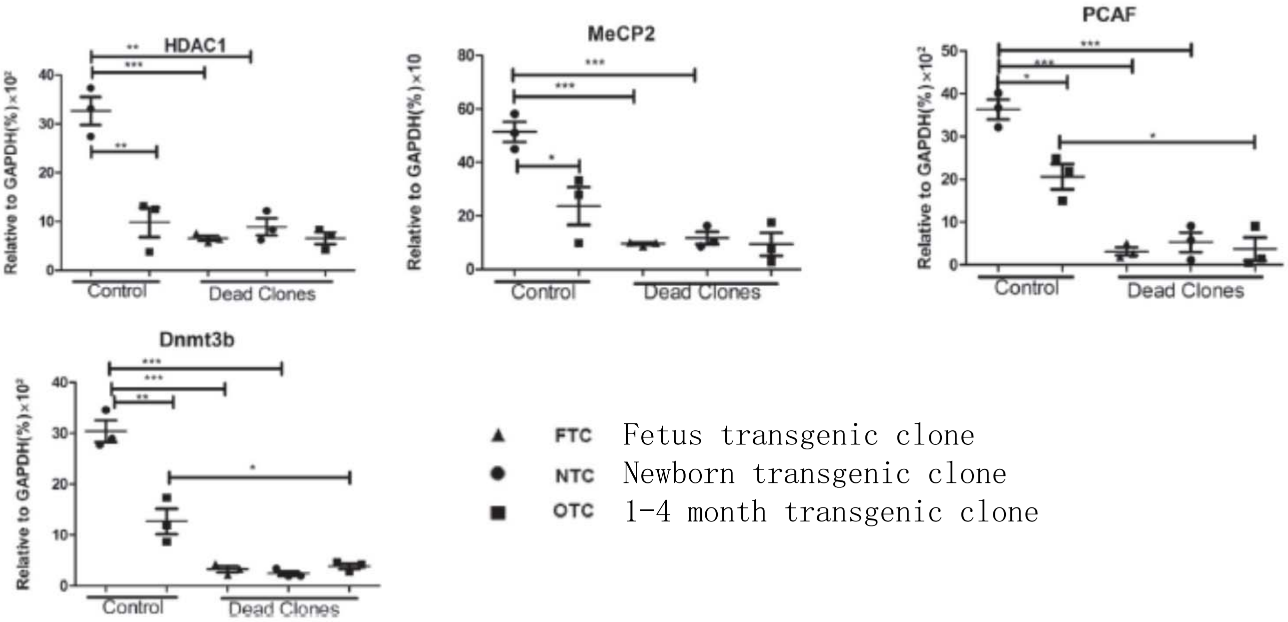


Y Tian et al. BBRC 434: 681-687, 2013

Progress #4:

To evaluate the epigenetic effect of cloning/transgene

Goat:



▲ FTC Fetus transgenic clone
● NTC Newborn transgenic clone
■ OTC 1-4 month transgenic clone

L Meng et al. Theriogenology 81: 459-466, 2014

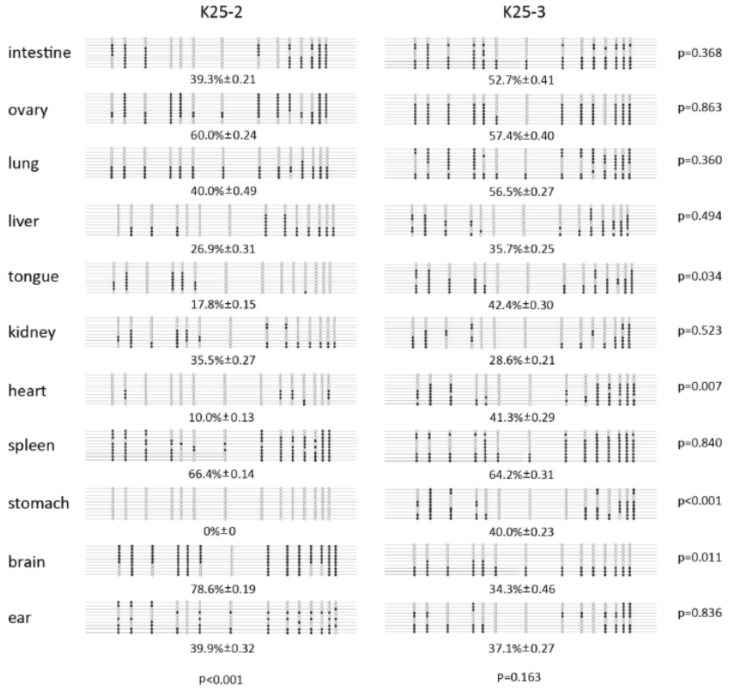
Progress #4:

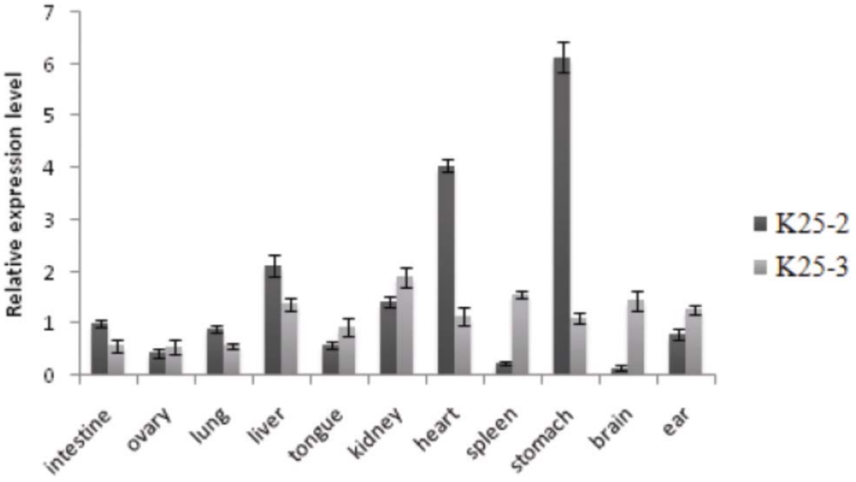
To evaluate the epigenetic effect of cloning/transgene

Pig: CMV-GFP **Position effect**

K25-2: L1M LINE element (retrotransferase, inactive)
K25-3: EST EV932945 (Aatf, active)

Variegation of CMV promoter methylation





Z Yin et al. GMR 11: 355-369, 2012

Progress #5:

To observe the gene expression changes at the whole genome

Cattle: Fat-1

$$\omega\text{-6} \xrightarrow{\text{Fat-1}} \omega\text{-3}$$

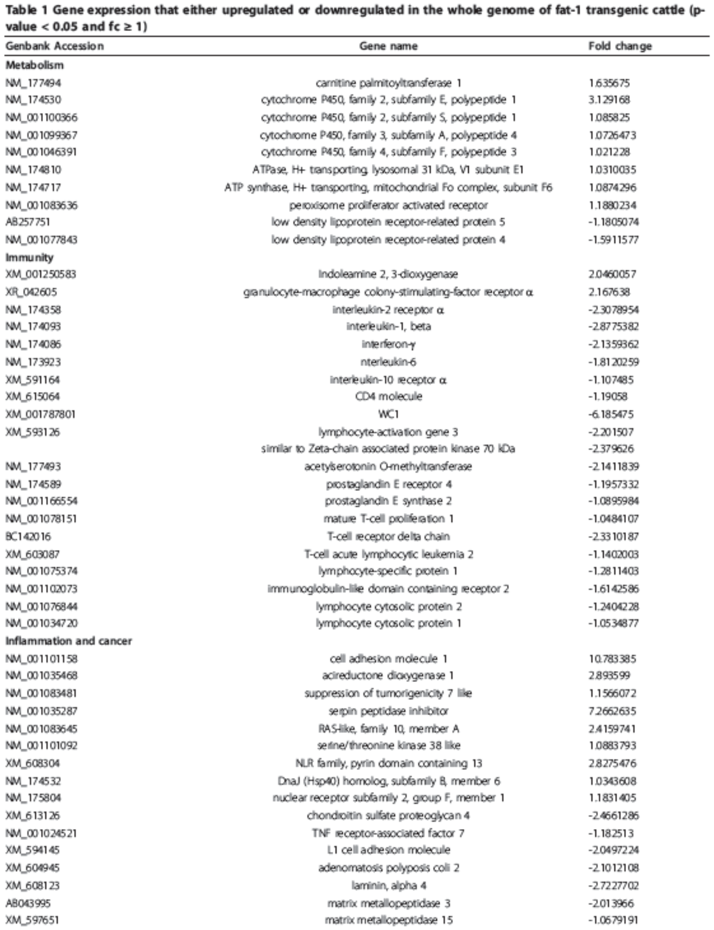


Table 1 Gene expression that either upregulated or downregulated in the whole genome of fat-1 transgenic cattle (p-value < 0.05 and fc ≥ 1)

Genbank Accession	Gene name	Fold change
Metabolism		
NM_177494	carnitine palmitoyltransferase 1	1.635675
NM_174530	cytochrome P450, family 2, subfamily E, polypeptide 1	3.129168
NM_001100366	cytochrome P450, family 2, subfamily S, polypeptide 1	1.085825
NM_001099367	cytochrome P450, family 3, subfamily A, polypeptide 4	1.0726473
NM_001046391	cytochrome P450, family 4, subfamily F, polypeptide 3	1.021228
NM_174810	ATPase, H+ transporting, lysosomal 31 kDa, V1 subunit E1	1.0310035
NM_174717	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit F6	1.0874296
NM_001083636	peroxisome proliferator activated receptor	1.1880234
AB257751	low density lipoprotein receptor-related protein 5	-1.1805074
NM_001077843	low density lipoprotein receptor-related protein 4	-1.5911577
Immunity		
XM_001250583	Indoleamine 2, 3-dioxygenase	2.0460057
XR_042605	granulocyte-macrophage colony-stimulating-factor receptor α	2.167638
NM_174358	interleukin-2 receptor α	-2.3078954
NM_174093	interleukin-1, beta	-2.8775382
NM_174086	interferon-γ	-2.1359362
NM_173923	nterleukin-6	-1.8120259
XM_591164	interleukin-10 receptor α	-1.107485
XM_615064	CD4 molecule	-1.19058
XM_001787801	WC1	-6.185475
XM_593126	lymphocyte-activation gene 3	-2.201507
	similar to Zeta-chain associated protein kinase 70 kDa	-2.379626
NM_177493	acetylserotonin O-methyltransferase	-2.1411839
NM_174589	prostaglandin E receptor 4	-1.1957332
NM_001166554	prostaglandin E synthase 2	-1.0895984
NM_001078151	mature T-cell proliferation 1	-1.0484107
BC142016	T-cell receptor delta chain	-2.3310187
XM_603087	T-cell acute lymphocytic leukemia 2	-1.1402003
NM_001075374	lymphocyte-specific protein 1	-1.2811403
NM_001102073	immunoglobulin-like domain containing receptor 2	-1.6142586
NM_001076844	lymphocyte cytosolic protein 2	-1.2404228
NM_001034720	lymphocyte cytosolic protein 1	-1.0534877
Inflammation and cancer		
NM_001101158	cell adhesion molecule 1	10.783385
NM_001035468	acireductone dioxygenase 1	2.893599
NM_001083481	suppression of tumorigenicity 7 like	1.1566072
NM_001035287	serpin peptidase inhibitor	7.2662635
NM_001083645	RAS-like, family 10, member A	2.4159741
NM_001101092	serine/threonine kinase 38 like	1.0883793
XM_608304	NLR family, pyrin domain containing 13	2.8275476
NM_174532	DnaJ (Hsp40) homolog, subfamily B, member 6	1.0343608
NM_175804	nuclear receptor subfamily 2, group F, member 1	1.1831405
XM_613126	chondroitin sulfate proteoglycan 4	-2.4661286
NM_001024521	TNF receptor-associated factor 7	-1.182513
XM_594145	L1 cell adhesion molecule	-2.0497224
XM_604945	adenomatosis polyposis coli 2	-2.1012108
XM_608123	laminin, alpha 4	-2.7227702
AB043995	matrix metallopeptidase 3	-2.013966
XM_597651	matrix metallopeptidase 15	-1.0679191

Cell adhesion molecule 1 +10.8 fold

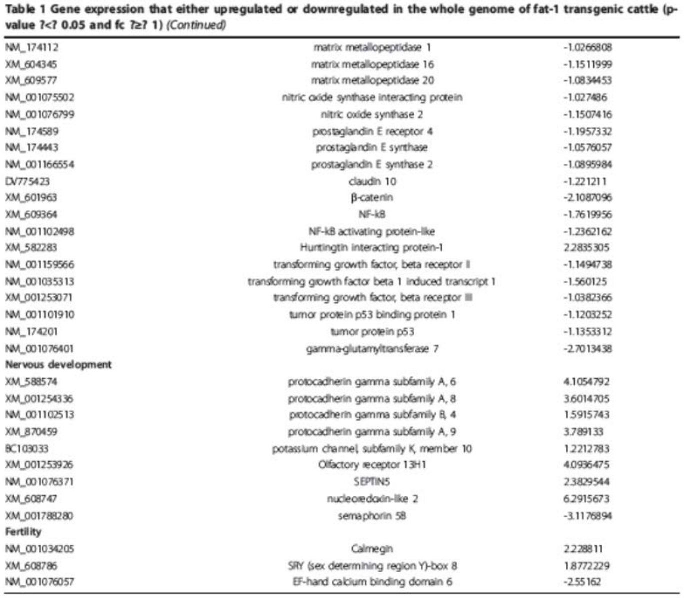


Table 1 Gene expression that either upregulated or downregulated in the whole genome of fat-1 transgenic cattle (p-value ?<? 0.05 and fc ?≥? 1) *(Continued)*

NM_174112	matrix metallopeptidase 1	-1.0266808
XM_604345	matrix metallopeptidase 16	-1.1511999
XM_609577	matrix metallopeptidase 20	-1.0834453
NM_001075502	nitric oxide synthase interacting protein	-1.027486
NM_001076799	nitric oxide synthase 2	-1.1507416
NM_174589	prostaglandin E receptor 4	-1.1957332
NM_174443	prostaglandin E synthase	-1.0576057
NM_001166554	prostaglandin E synthase 2	-1.0895984
DV775423	claudin 10	-1.221211
XM_601963	β-catenin	-2.1087096
XM_609364	NF-kB	-1.7619956
NM_001102498	NF-kB activating protein-like	-1.2362162
XM_582283	Huntingtin interacting protein-1	2.2835305
NM_001159566	transforming growth factor, beta receptor II	-1.1494738
NM_001035313	transforming growth factor beta 1 induced transcript 1	-1.560125
XM_001253071	transforming growth factor, beta receptor III	-1.0382366
NM_001101910	tumor protein p53 binding protein 1	-1.1203252
NM_174201	tumor protein p53	-1.1353312
NM_001076401	gamma-glutamyltransferase 7	-2.7013438
Nervous development		
XM_588574	protocadherin gamma subfamily A, 6	4.1054792
XM_001254336	protocadherin gamma subfamily A, 8	3.6014705
NM_001102513	protocadherin gamma subfamily B, 4	1.5915743
XM_870459	protocadherin gamma subfamily A, 9	3.789133
BC103033	potassium channel, subfamily K, member 10	1.2212783
XM_001253926	Olfactory receptor 13H1	4.0936475
NM_001076371	SEPTIN5	2.3829544
XM_608747	nucleoredoxin-like 2	6.2915673
XM_001788280	semaphorin 5B	-3.1176894
Fertility		
NM_001034205	Calmegin	2.228811
XM_608786	SRY (sex determining region Y)-box 8	1.8772229
NM_001076057	EF-hand calcium binding domain 6	-2.55162

T Guo et al. Lipids in Health and Diease 10:244, 2011

Progress #6:
To characterize the bioactivity of the transgene

Pig: Fat-1 ω-6 $\xrightarrow{\text{Fat-1}}$ ω-3

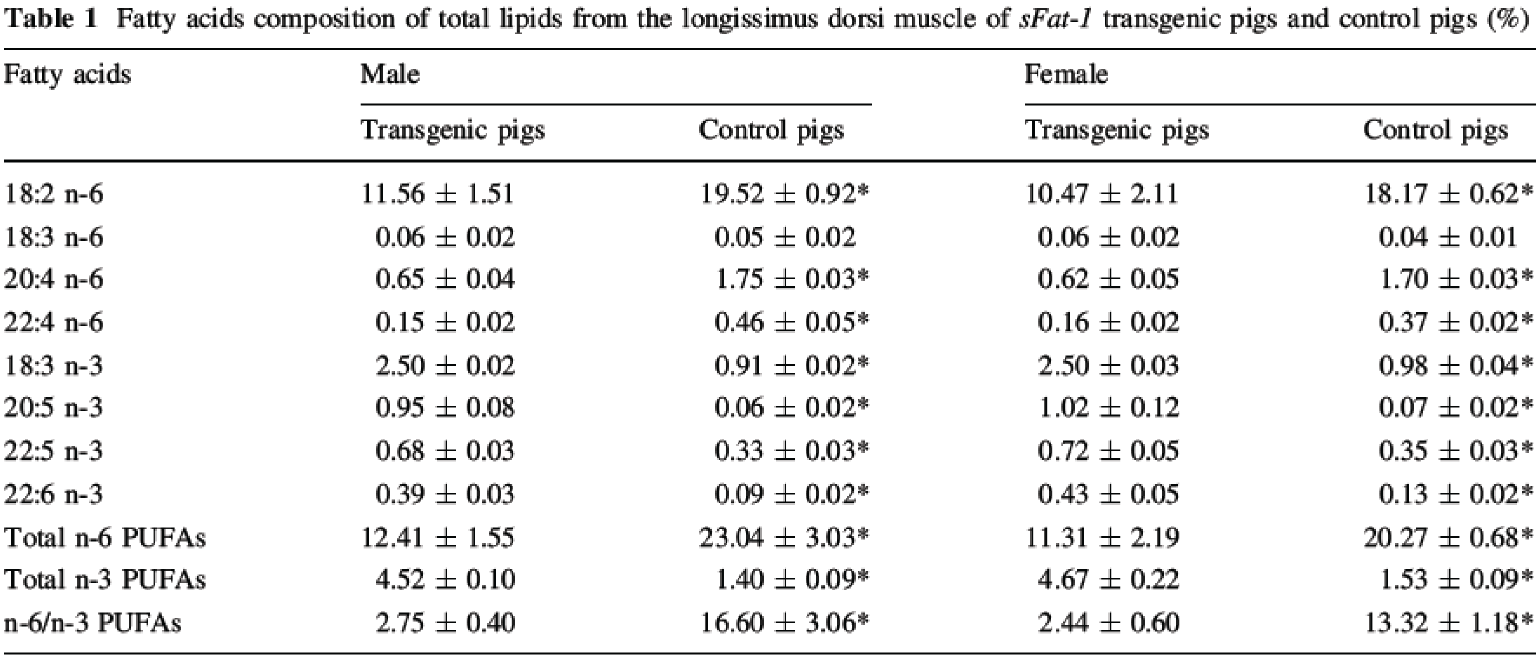


Table 1 Fatty acids composition of total lipids from the longissimus dorsi muscle of *sFat-1* transgenic pigs and control pigs (%)

Fatty acids	Male		Female	
	Transgenic pigs	Control pigs	Transgenic pigs	Control pigs
18:2 n-6	11.56 ± 1.51	19.52 ± 0.92*	10.47 ± 2.11	18.17 ± 0.62*
18:3 n-6	0.06 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.04 ± 0.01
20:4 n-6	0.65 ± 0.04	1.75 ± 0.03*	0.62 ± 0.05	1.70 ± 0.03*
22:4 n-6	0.15 ± 0.02	0.46 ± 0.05*	0.16 ± 0.02	0.37 ± 0.02*
18:3 n-3	2.50 ± 0.02	0.91 ± 0.02*	2.50 ± 0.03	0.98 ± 0.04*
20:5 n-3	0.95 ± 0.08	0.06 ± 0.02*	1.02 ± 0.12	0.07 ± 0.02*
22:5 n-3	0.68 ± 0.03	0.33 ± 0.03*	0.72 ± 0.05	0.35 ± 0.03*
22:6 n-3	0.39 ± 0.03	0.09 ± 0.02*	0.43 ± 0.05	0.13 ± 0.02*
Total n-6 PUFAs	12.41 ± 1.55	23.04 ± 3.03*	11.31 ± 2.19	20.27 ± 0.68*
Total n-3 PUFAs	4.52 ± 0.10	1.40 ± 0.09*	4.67 ± 0.22	1.53 ± 0.09*
n-6/n-3 PUFAs	2.75 ± 0.40	16.60 ± 3.06*	2.44 ± 0.60	13.32 ± 1.18*

M Tang et al. Transgenic Res 20: 749-758, 2011

Progress #6:

To characterize the bioactivity of the transgene

Cow: human lysozyme knock-in/Zinc-finger nucleases/b-casein

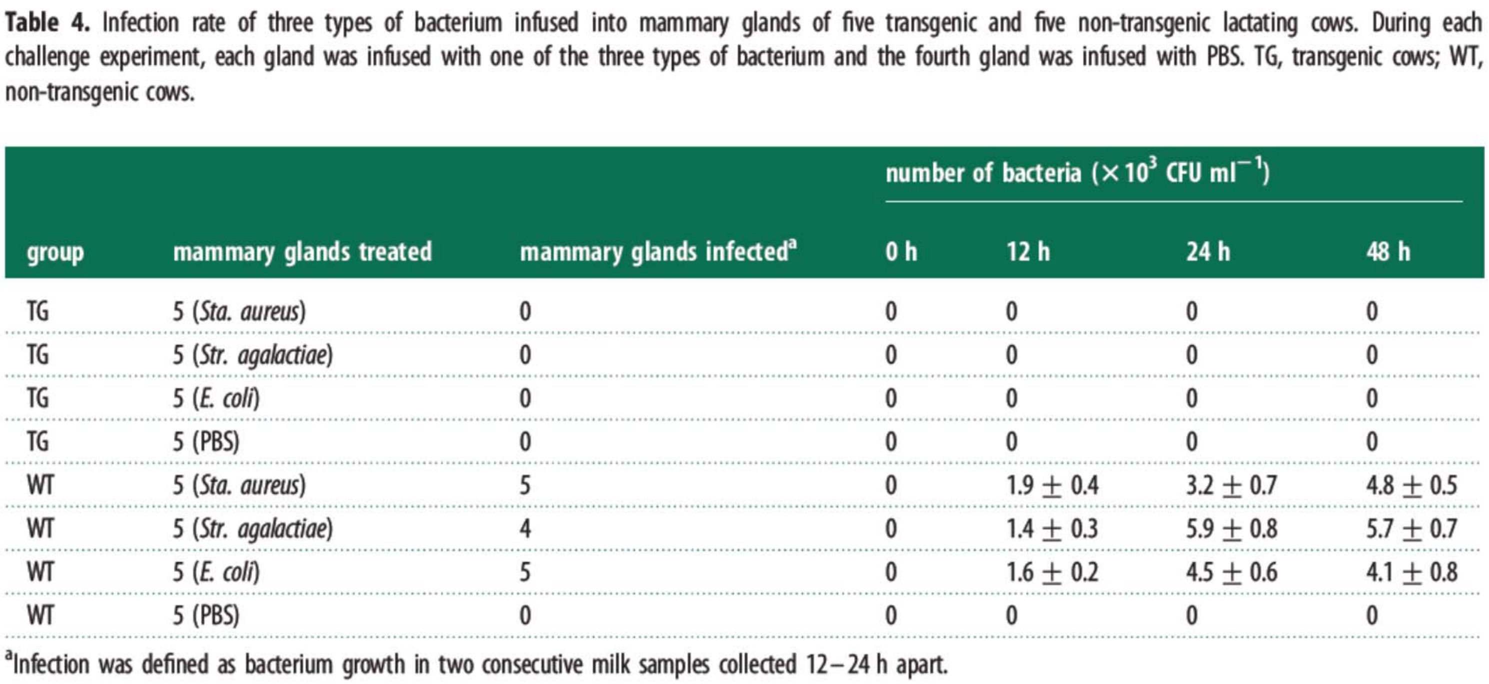
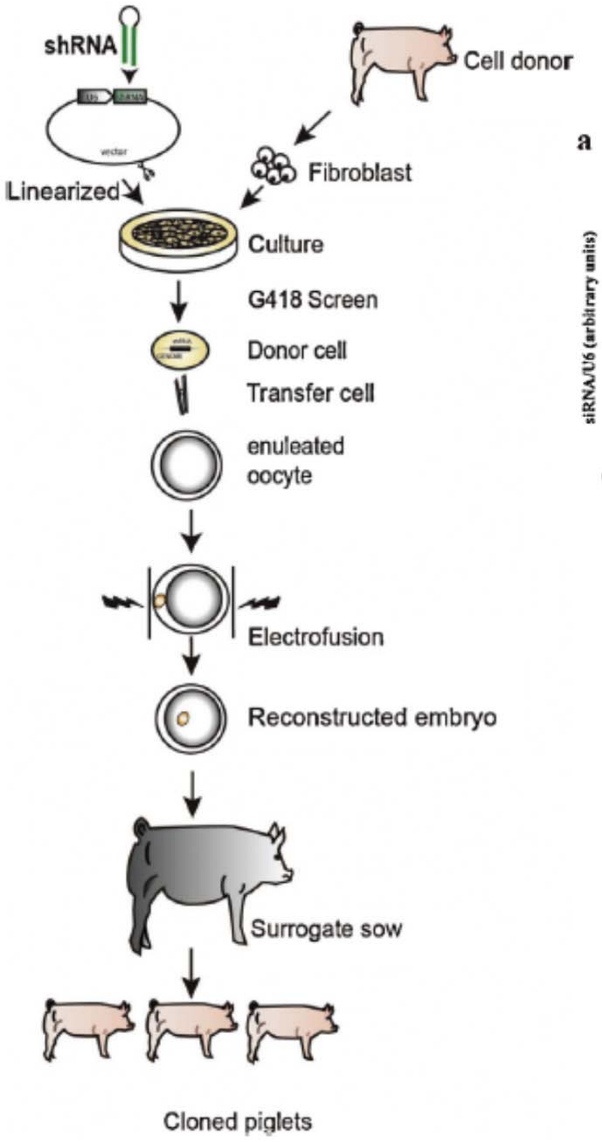


Table 4. Infection rate of three types of bacterium infused into mammary glands of five transgenic and five non-transgenic lactating cows. During each challenge experiment, each gland was infused with one of the three types of bacterium and the fourth gland was infused with PBS. TG, transgenic cows;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

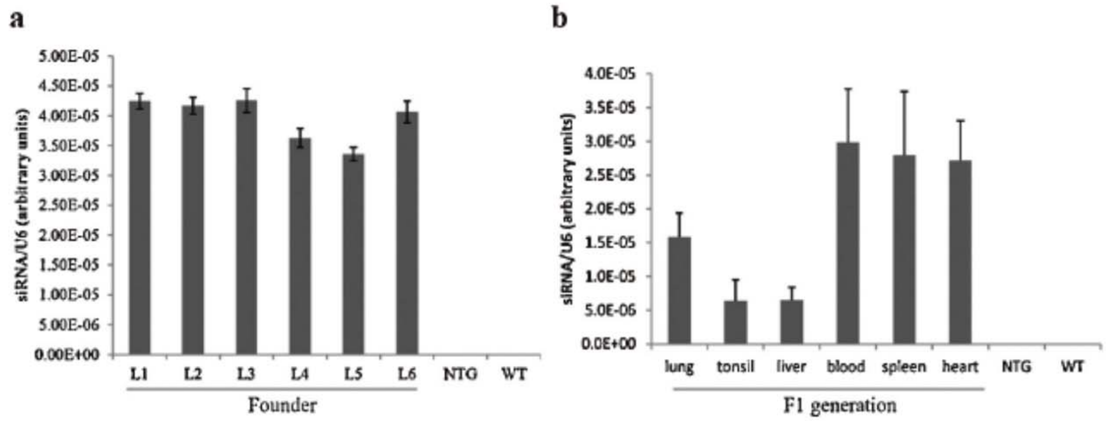
			number of bacteria ($\times 10^3$ CFU ml^{-1})			
group	mammary glands treated	mammary glands infected[a]	0 h	12 h	24 h	48 h
TG	5 (*Sta. aureus*)	0	0	0	0	0
TG	5 (*Str. agalactiae*)	0	0	0	0	0
TG	5 (*E. coli*)	0	0	0	0	0
TG	5 (PBS)	0	0	0	0	0
WT	5 (*Sta. aureus*)	5	0	1.9 ± 0.4	3.2 ± 0.7	4.8 ± 0.5
WT	5 (*Str.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

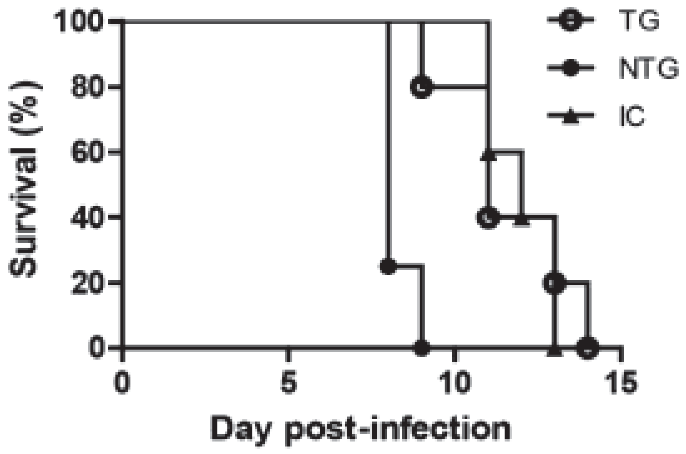
X Lu et al. Proc. R. Soc B 281: 2013368, 2014



The level of shRNA in the transgenic pigs



The transgenic pigs showed resistance to the virus challenge

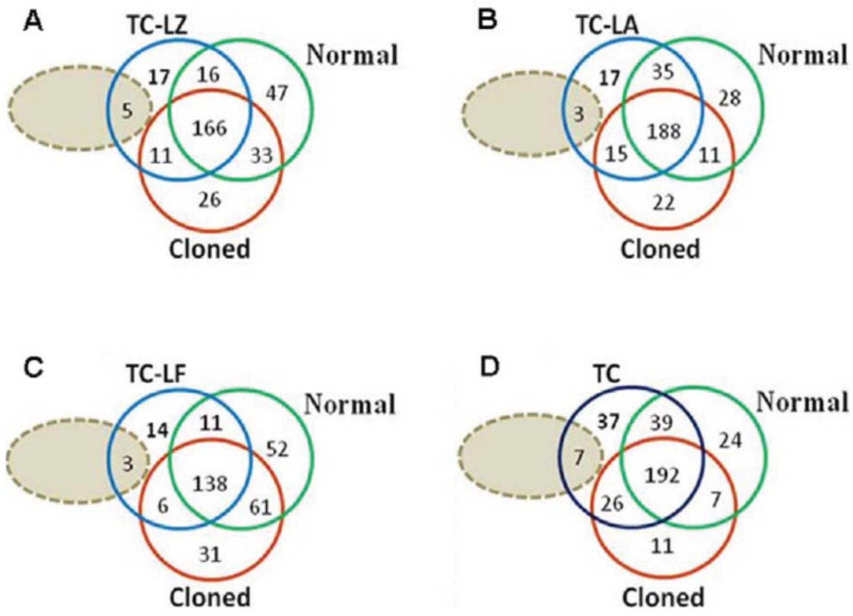


L Li et al. J of Biotechnology 171: 14-24, 2014

Progress #7:
To evaluate the productivity

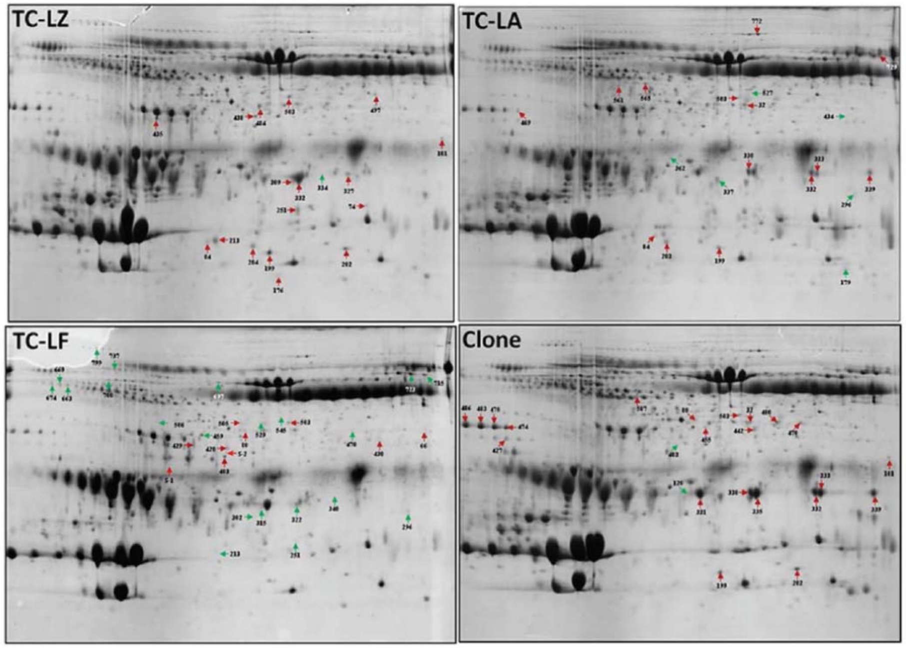
Cow: TC-LA: h-alpha-lactalbumn; TC-LF: h-lactoferrin; TC-LZ: h-lysozyme
TC: trangenic cloned, C: cloned, N: normal

Whey Protein Profiles



2D-Nano-LC-MS/MS

Whey Protein Profiles/MALDI-TOF/TOF



R Zhang et al. Plos One 7: 49697, 2012

Progress #8:

To assess the safety of meat from transgenic cattle

rhLF (lactoferrin)

	Meat powder from conventional cow		Meat powder from transgenic cow	
Macronutrients:	similar		similar	
Minerals:	similar		similar	
Amino acid:	similar		similar	
Energy (kJ/100g):	1660		1660	
Vitamins:	VA (4.79 ug/100g)		1.75	
Rat 90-day feeding:	5%	10%	5%	10%
Weight gain (male, g):	435.2±23.1	435.3±42.6	440.9±26.4	450.7±19.1
(female, g):	231.6±18.8	235.1±15.2	235.4±24.8	236.2±24.4
Hematological parameters:			almost similar	
Blood biochemical components				
Histopathological observations				

S Liu et al. Food and Chemical Toxicology 57: 314-321, 2013

Open questions

1. Transgenic animals for bioreactor

2. Transgenic animals for disease models

   High risk of gene drift

Thanks

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