The Advance of GMO Cattle in China

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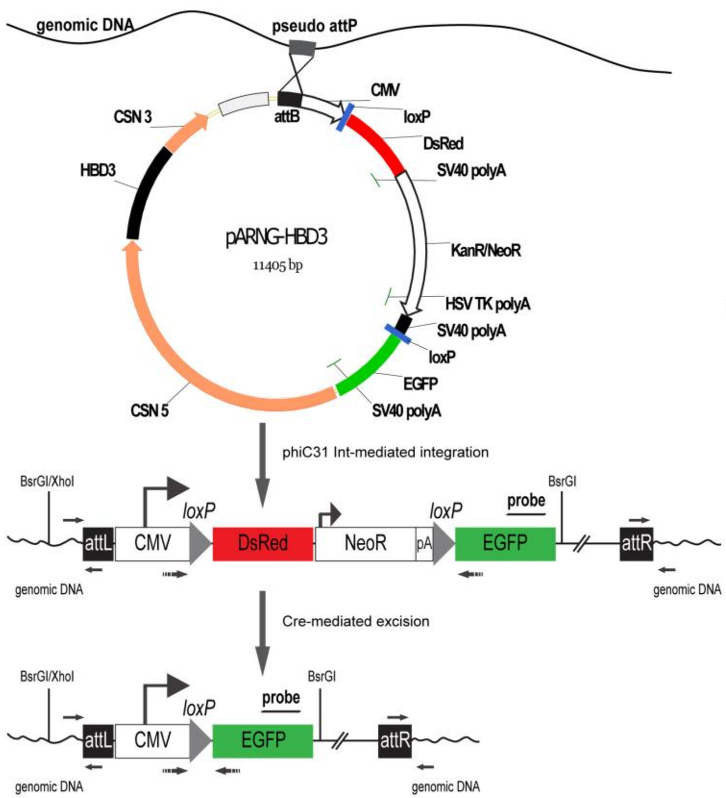
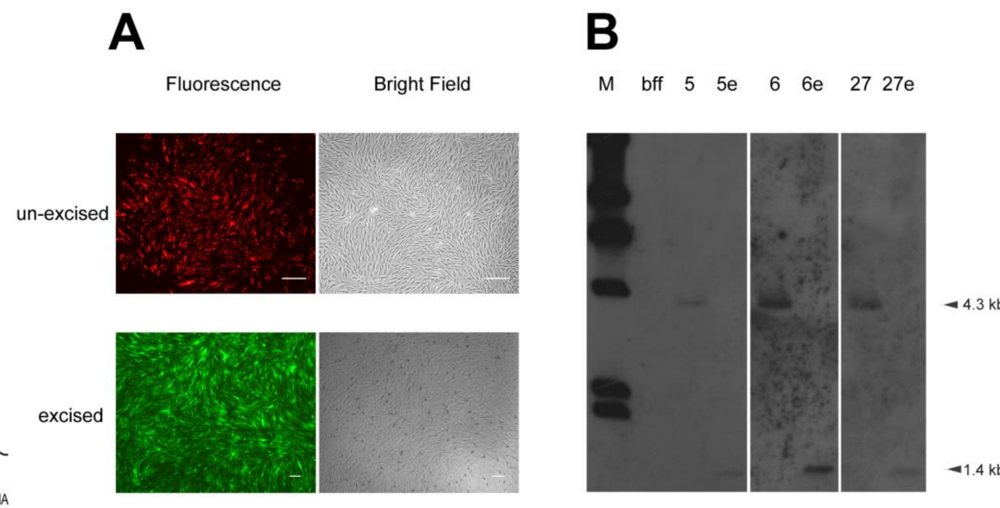
Jun 25, 2017

1. Methods in improving GMO cattle

A Site-Specific Recombinase-Based Method to Produce Antibiotic Selectable Marker Free Transgenic Cattle



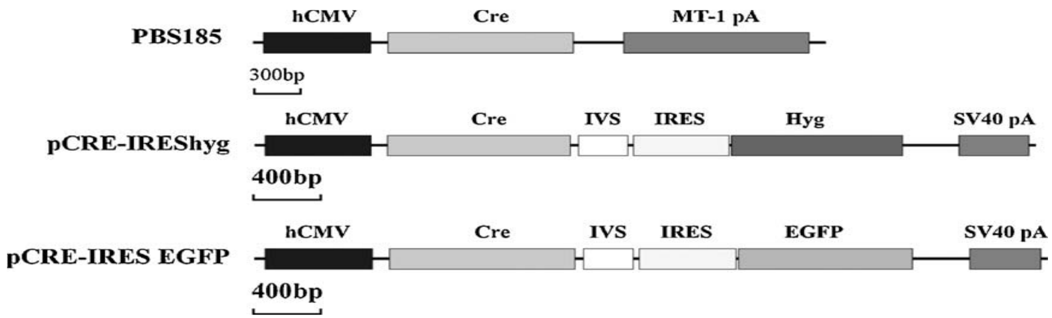
Schematic overview of the fluorescence double reporter construct pARNG-HBD3.



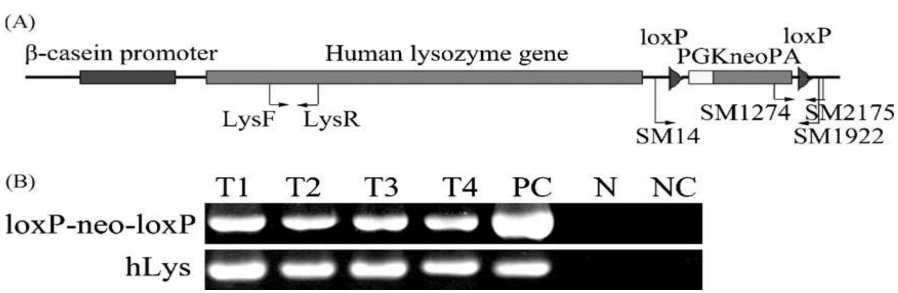
Identification of excision of the antibiotic selectable neoR marker in the transgenic cells

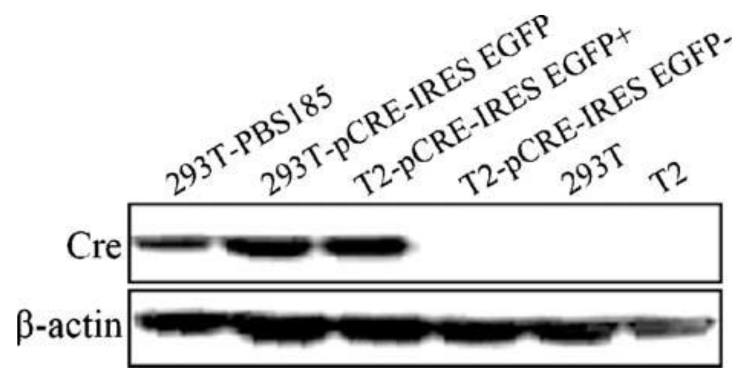
1. Methods in improving GMO cattle

Removal of selectable marker gene from fibroblast cells in transgenic cloned cattle by transient expression of Cre recombinase and subsequent effects on recloned embryo development



Construct of the transient Cre and GFP expression vector. IVS, synthetic intron; IRES, internal ribosome entry site; Hyg, hygromycin B phosphotransferase gene.





Expression of Cre recombinase in mammalian cells.

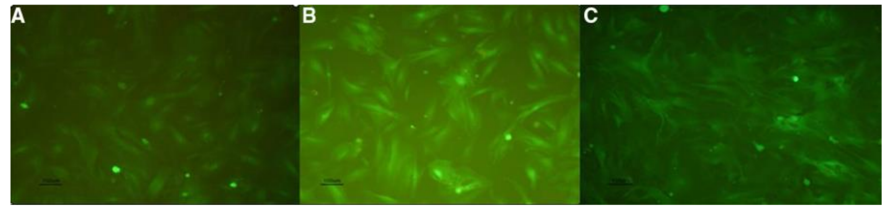
Theriogenology 72 (2009) 535–541

1. Methods in improving GMO cattle

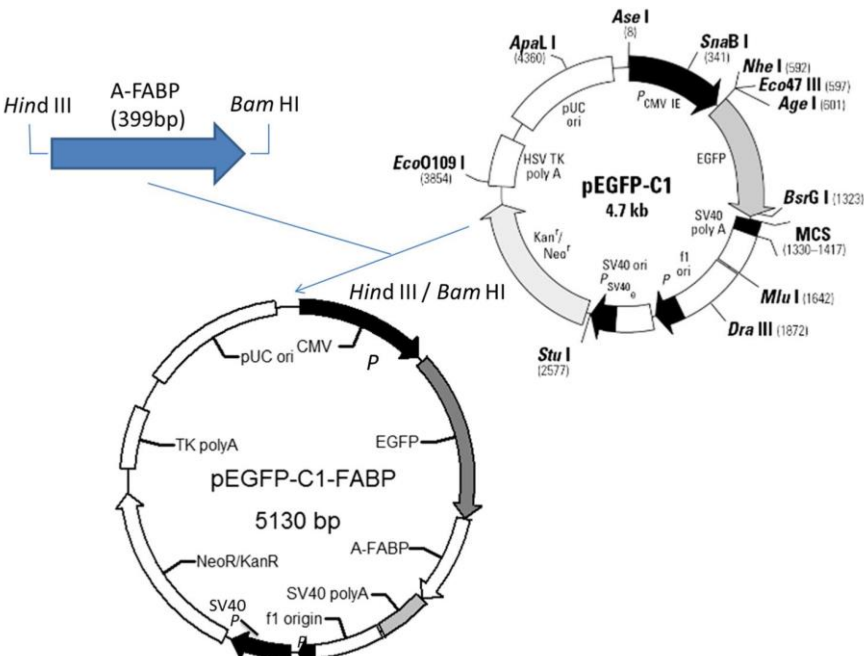
Screening somatic cell nuclear transfer parameters for generation of transgenic cloned cattle with intragenomic integration of additional gene copies that encode bovine adipocyte-type fatty acid-binding protein (A-FABP)

>Bos taurus fatty acid binding protein 4, adipocyte(FABP4), mutant cds mRNA
atgtgtgatgcatttgtaggtacctggaaacttgtctccagtgaaaactttgatgattacatgaaaga
agtgggcgtgggctttgctaccaggaaagtggctggcatggccaaacccactttgatcatcagtttg
aatgggggtgtggtGacGatAaaGtcTgaGagTacGtttaaaaatactgagatttccttcaaa
ttgggccaggaatttgatgaaatcactccagatgacaggaaagtcaagagcatcgtaaacttaga
tgaaggtgctctggtacaagtacaaaactgggatggaaaatcaaccaccataaagagaaaact
catggaCgaCaaAatggtCctCgaGtgtgtcatgaatggtgtcactgccaccagagtttatgag
agagcataa

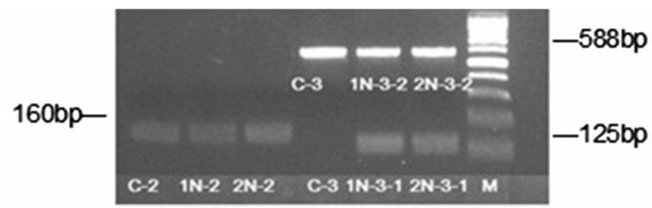
Mutant bovine *A-FABP* cDNA sequence synthesized.



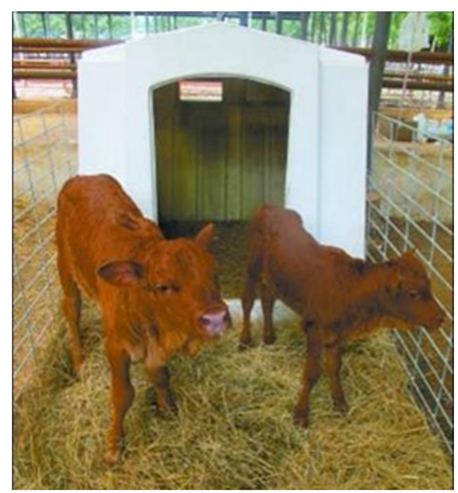
Generation of transgenic bovine fetal fibroblasts.



Construction of the recombinant plasmid (eGFPC1-FABP)



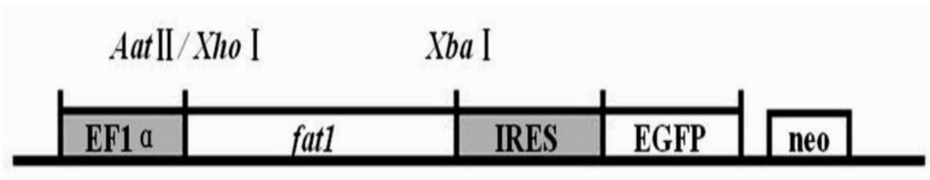
Identification of transgenic cattle by PCR analysis



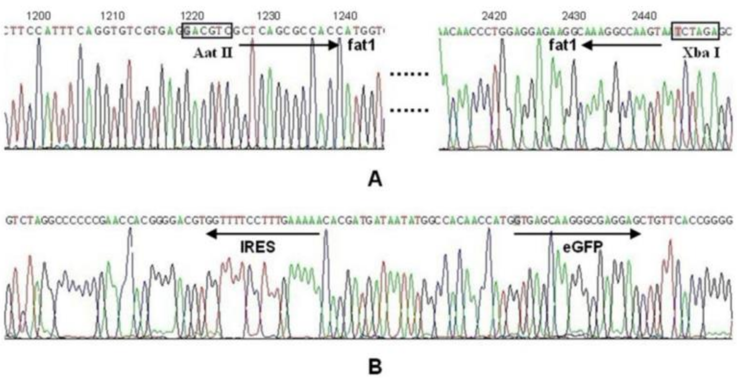
Mol Biol Rep (2017) 44:159–168

1. Methods in improving GMO cattle

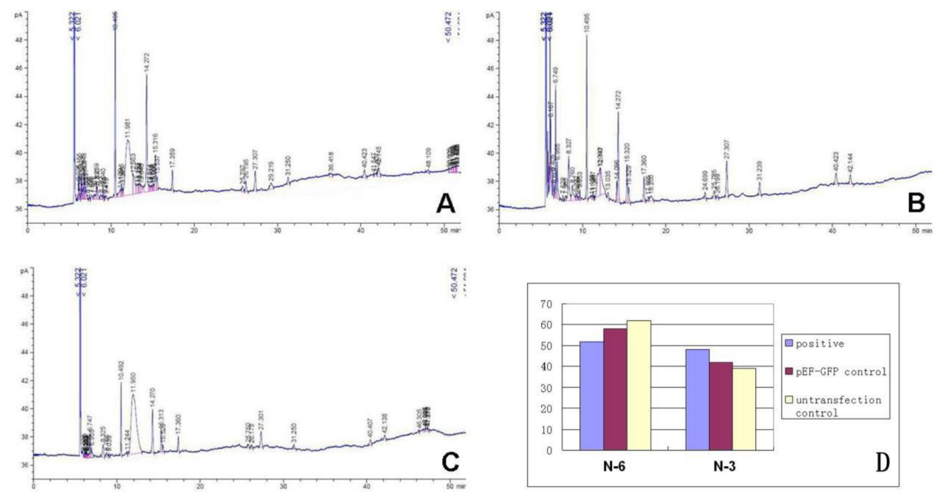
Construction of *fat1* Gene Expression Vector and Its Catalysis Efficiency in Bovine Fetal Fibroblast Cells



Structure of pEF-GFP-*fat1* vector. In this vector, *fat1* gene is driven by the EF1 promoter, followed by IRES and eGFP, containing the neo gene.



Results of sequencing of recombinant expression vector pEF-GFP-*fat1*

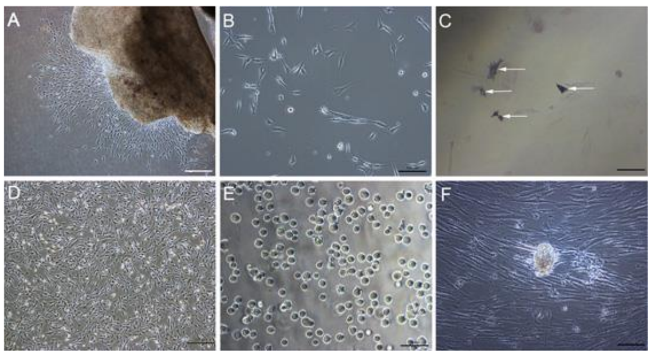


Results of gas chromatograph showing fatty acids profiles of total lipids extracted from cells. A : positive cells. B : pEF-GFP ransgenic. C : non-transgenic control. D : concentration of n-6/n-3 PUFAs in positive cells, pEF-GFP control and non-transgenic control (%).

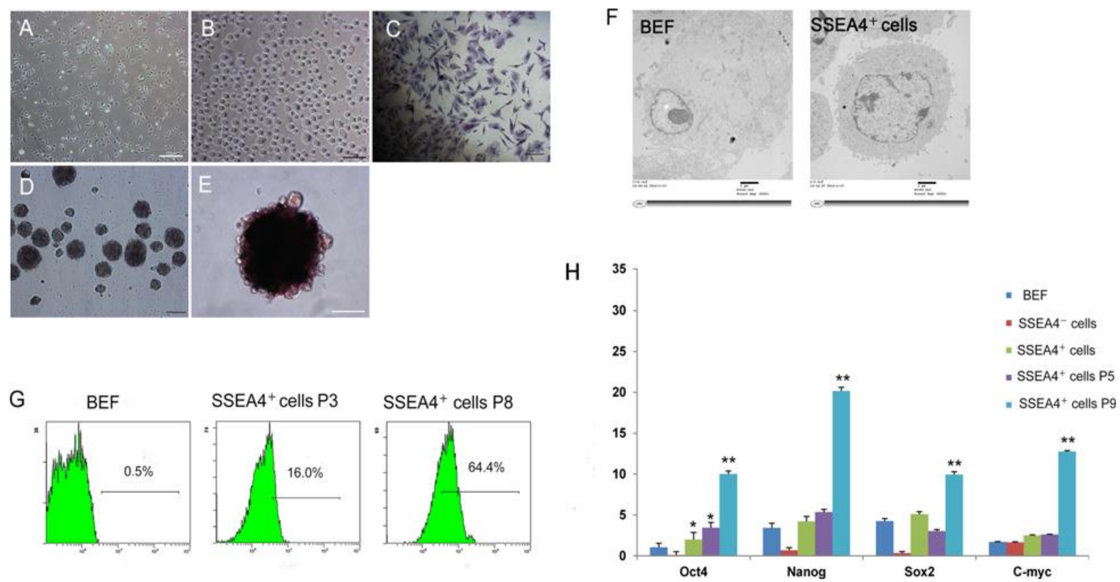
Asian-Aust. J. Anim. Sci. Vol. 25, No. 5 : 621 - 628 May 2012

1. Methods in improving GMO cattle

Application of a Novel Population of Multipotent Stem Cells Derived from Skin Fibroblasts as Donor Cells in Bovine SCNT



Culture of bovine embryonic fibroblasts

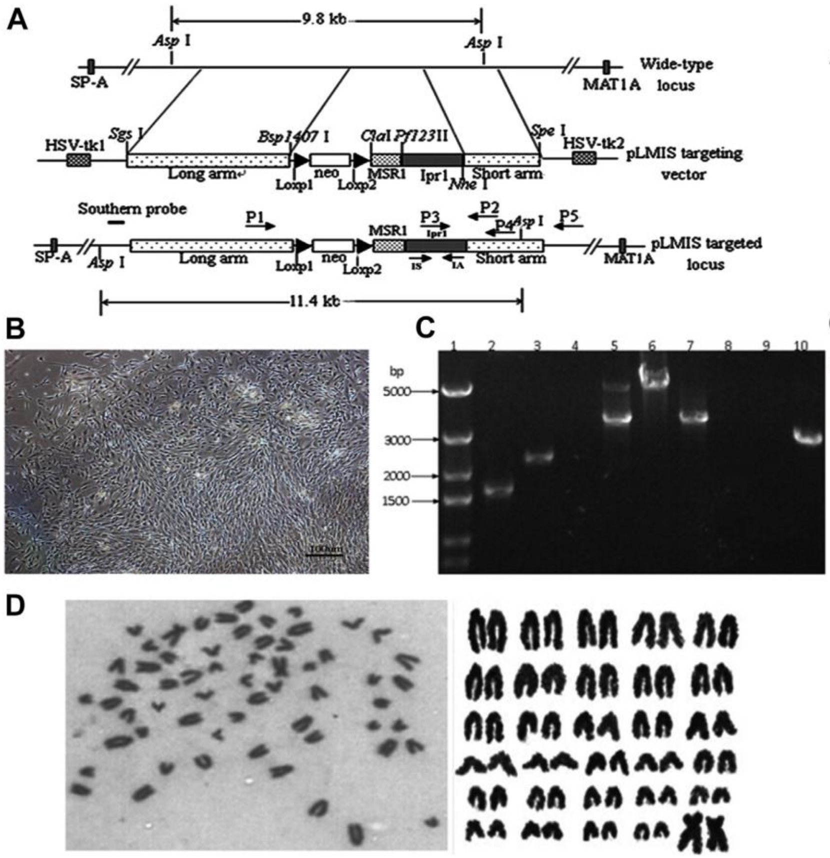


Isolation of SSEA-4$_+$ cells

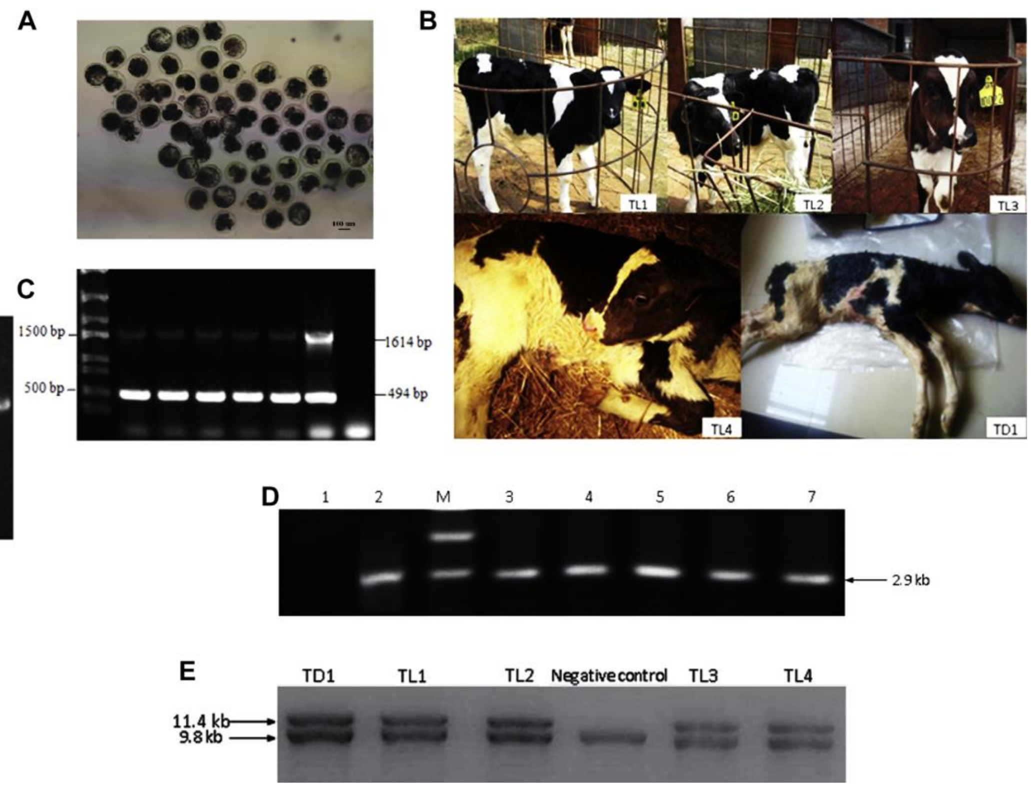
PLOS ONE |DOI:10.1371/journal.pone.0114423 January 20, 2015

1. Methods in improving GMO cattle

Transgenic cattle produced by nuclear transfer of fetal fibroblasts carrying Ipr1 gene at a specific locus



Preparation of Ipr1-transgenic nuclear donor cells.

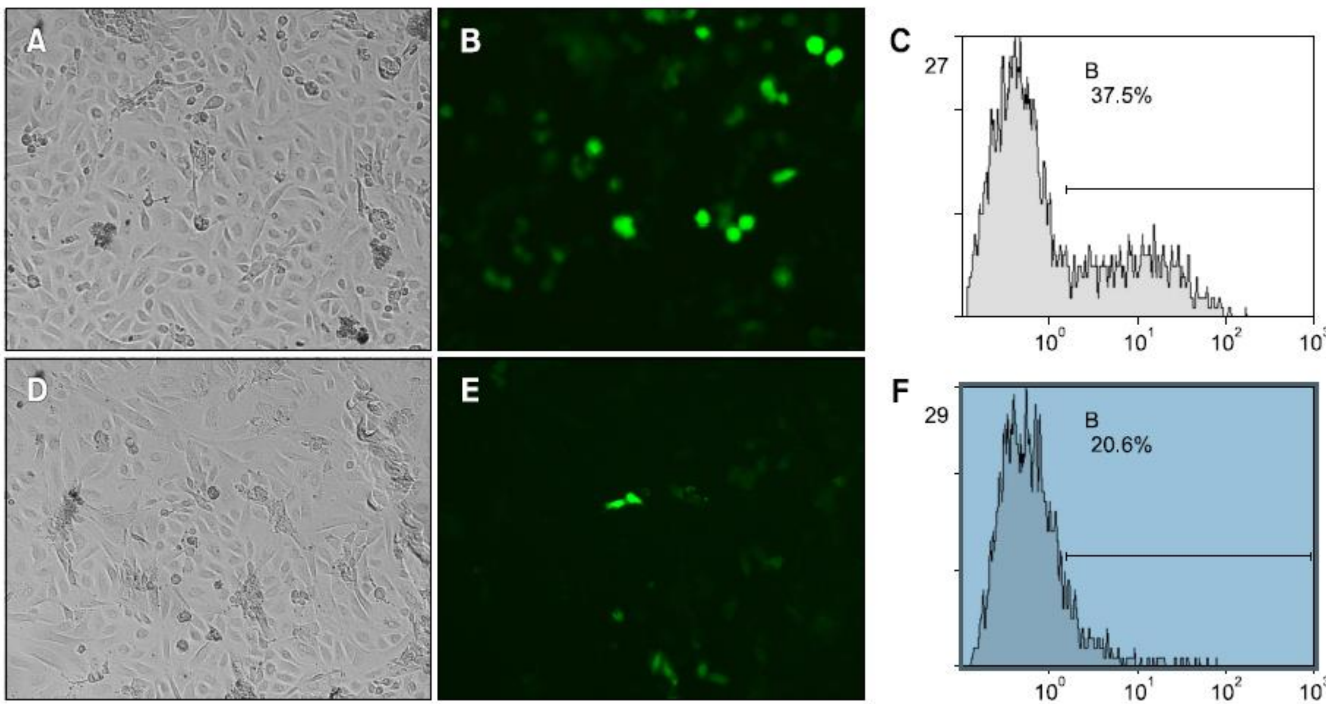


Production of Ipr1-transgenic cattle.

Theriogenology 84 (2015) 608－616

1. Methods in improving GMO cattle

Improved development of somatic cell cloned bovine embryos by a mammary gland epithelia cells in vitro model

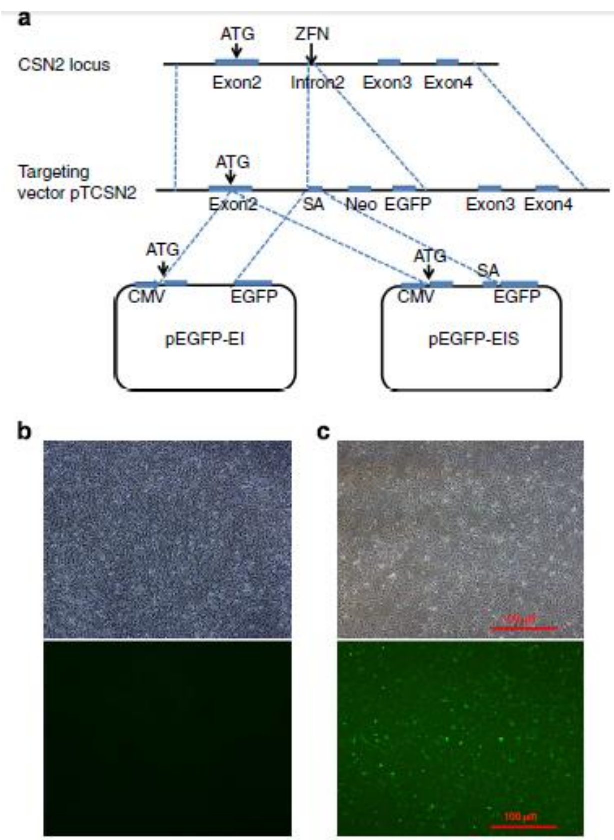


Detection of transgenic efficiency of hTERT-bMGEs and bMGEs by fluorescence images and FACS analysis. hTERT-bMGEs group was a mammary gland specific expression vector (pEBH) encoding human lysozyme (*hLYZ*) and GFP transfer into hTERT-bMGEs, which was obtained by transfecting the adenovirus-mediated hTERT gene into bovine mammary gland epithelia cells (bMGEs), with the bMGEs used as a control.

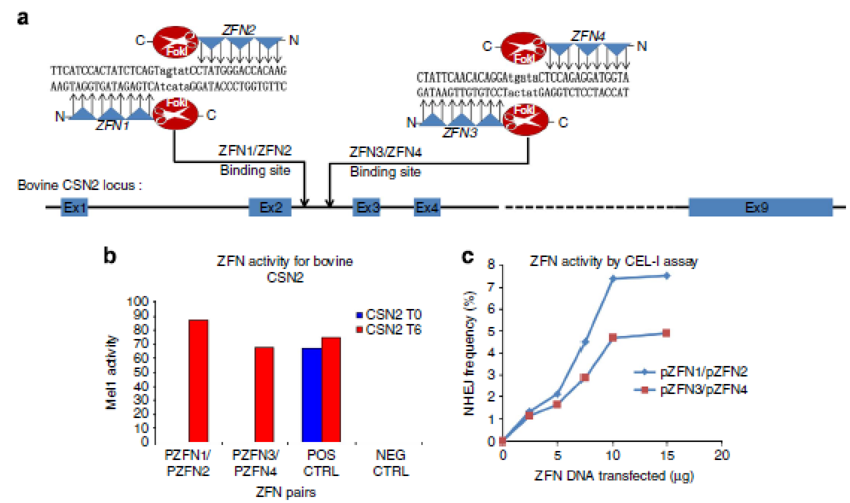
J Vet Sci 2016, 17(2), 145-152

1. Methods in improving GMO cattle

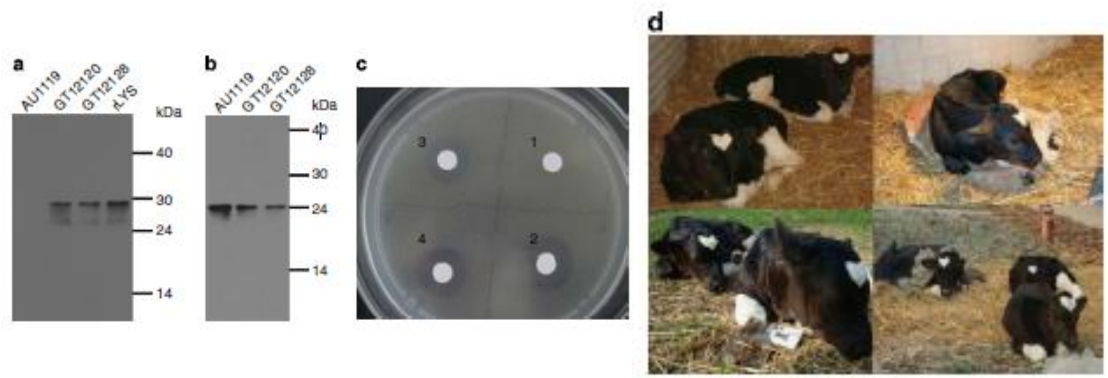
Zinc-finger nickase-mediated insertion of the lysostaphin gene into the beta-casein locus in cloned cows



Validation of splice acceptor activity in bovine fetal fibroblasts.

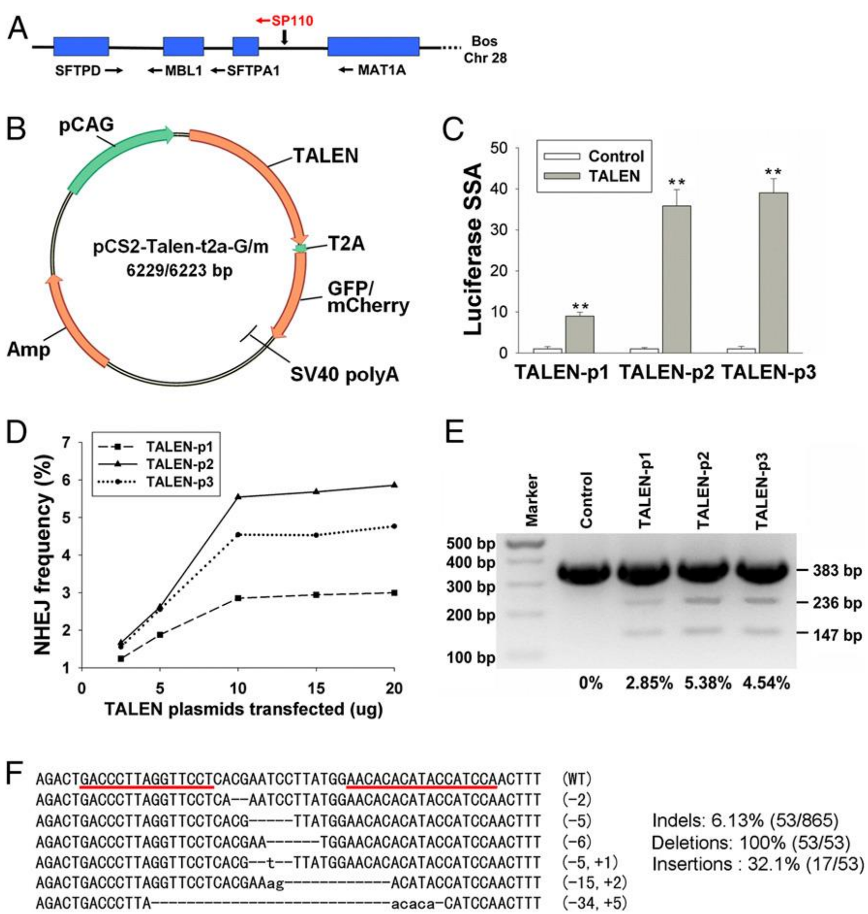


Targeting the bovine CSN2 locus with ZFNs and ZFNickases

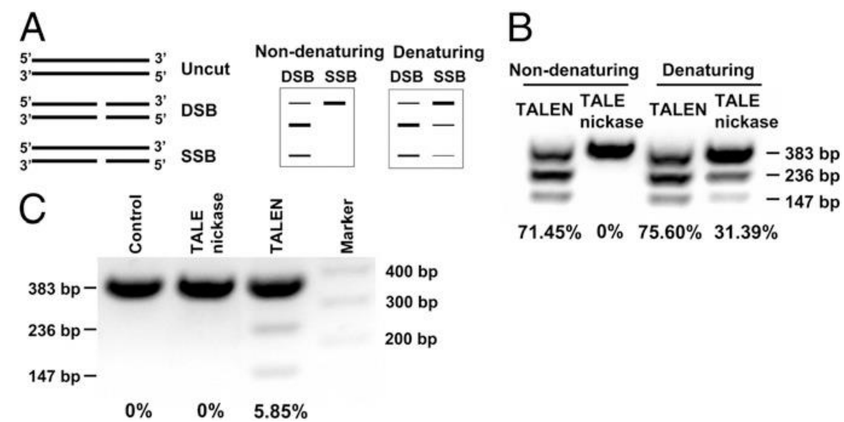


1. Methods in improving GMO cattle

TALE nickase-mediated SP110 knockin endows cattle with increased resistance to tuberculosis



Activity assessment of TALENs

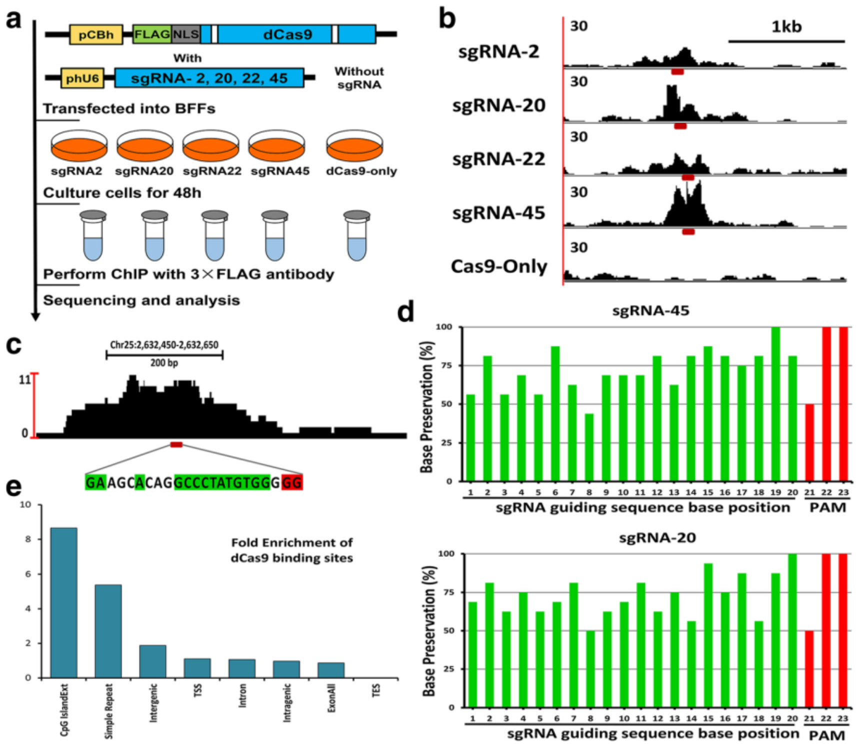


TALE nickase induced an SSB at the M-S locus and eliminated the NHEJ repair pathway

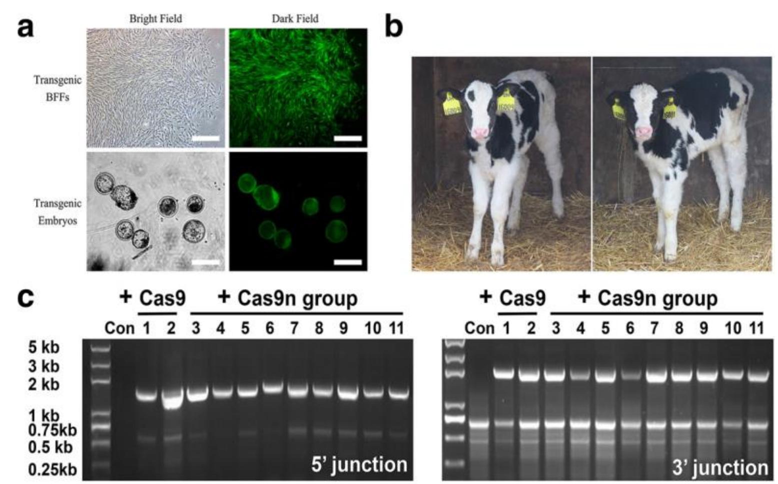
E1530–E1539 | PNAS | Published online March 2, 2015

1. Methods in improving GMO cattle

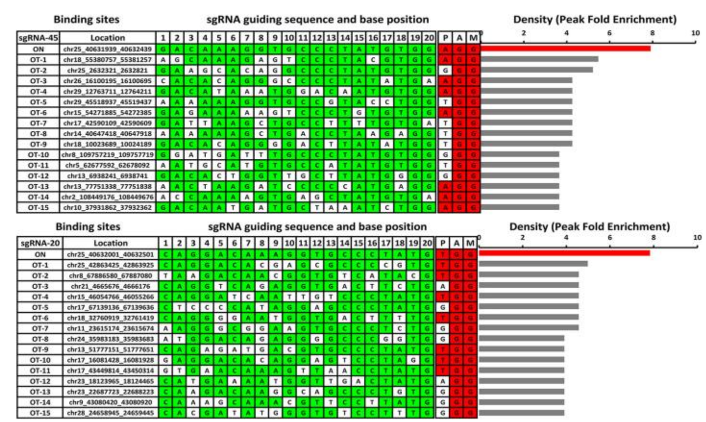
Single Cas9 nickase induced generation of NRAMP1 knockin cattle with reduced offtarget effects



Process and characteristics of dCas9 binding in BFFs



Assessment of the transgenic cattle

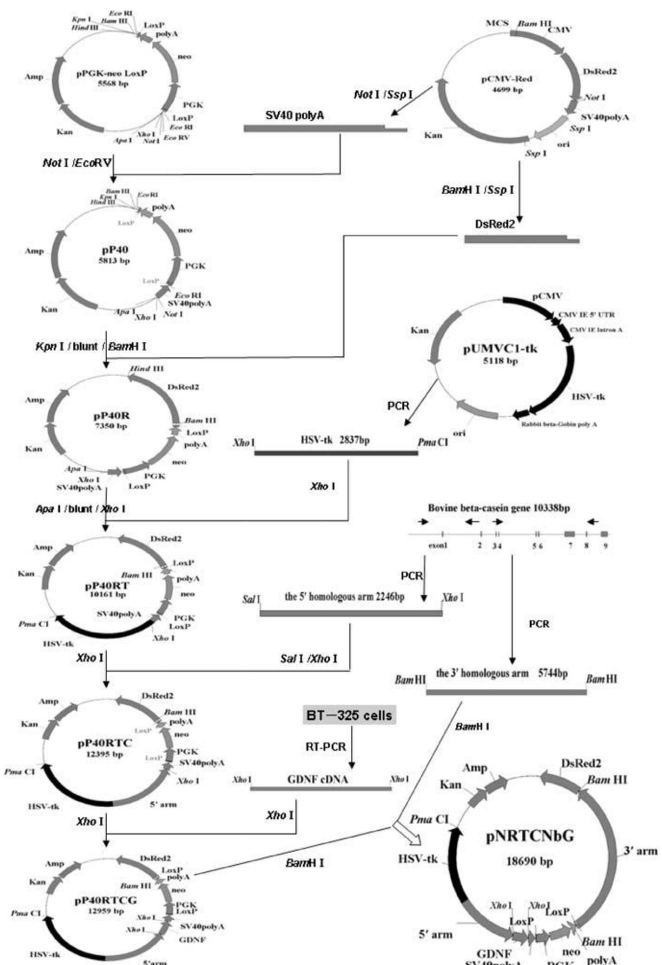


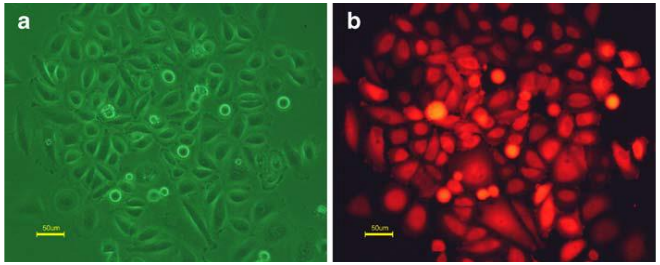
Off-target sites of sgRNA 45 and 20 with the top 15 ChIP-seq binding densities

Gao et al. Genome Biology (2017) 18:13

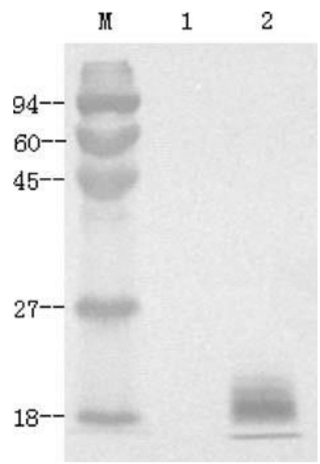
2. Mammary Glad Reactor

Construction of Targeting Vector for Expressing Human GDNF in Cattle Mammary Gland





G418 resistant cell clones transfected with pNRTCNbG.

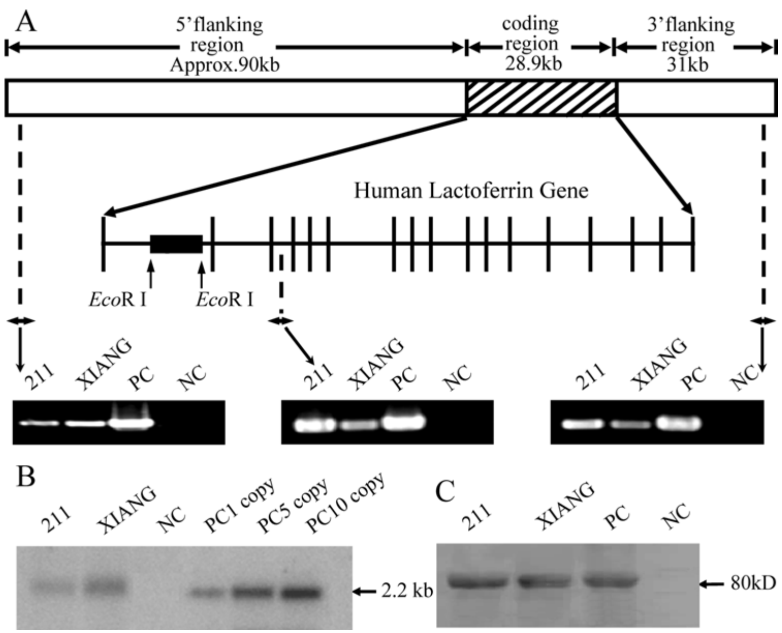


GDNF protein secretion detected by western blot. M: marker.
1: Supernatant of induction culture of the Bcap-37 cells at 48 h.
2: Supernatant of induction culture of the transgenic Bcap-37 cells at 48 h

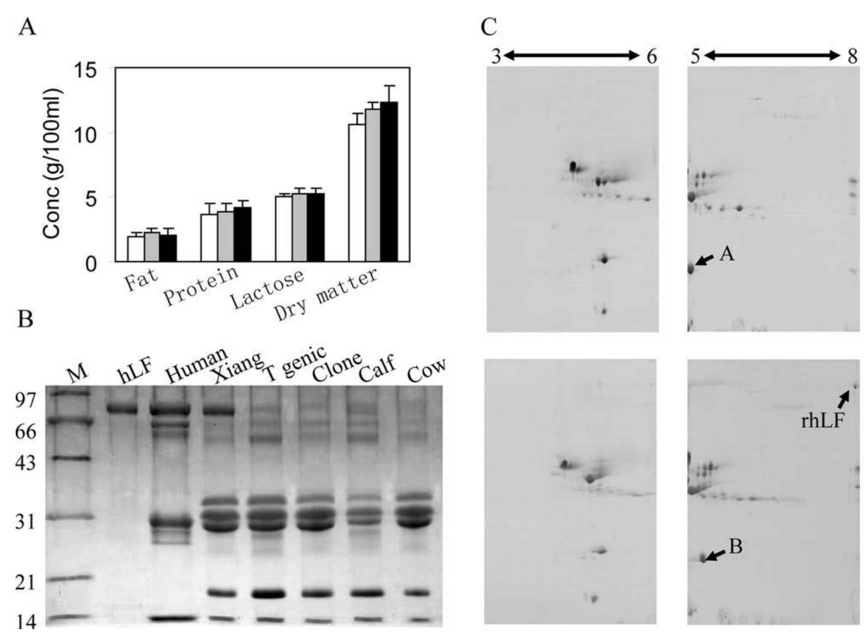
Appl Biochem Biotechnol (2009) 159:718–727

2. Mammary Glad Reactor

Cattle Mammary Bioreactor Generated by a Novel Procedure of Transgenic Cloning for Large-Scale Production of Functional Human Lactoferrin



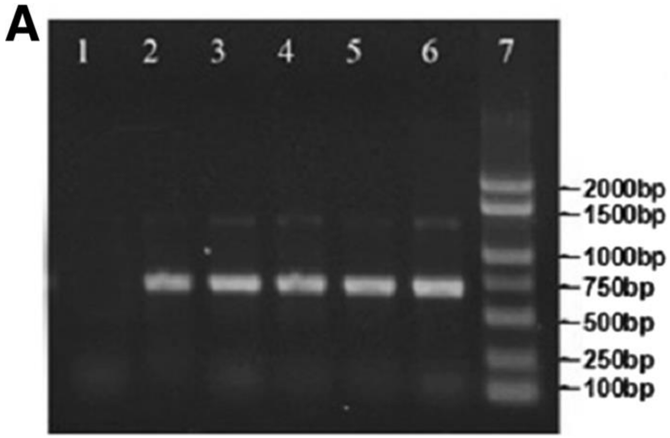
Results of determination of the hLF transgene

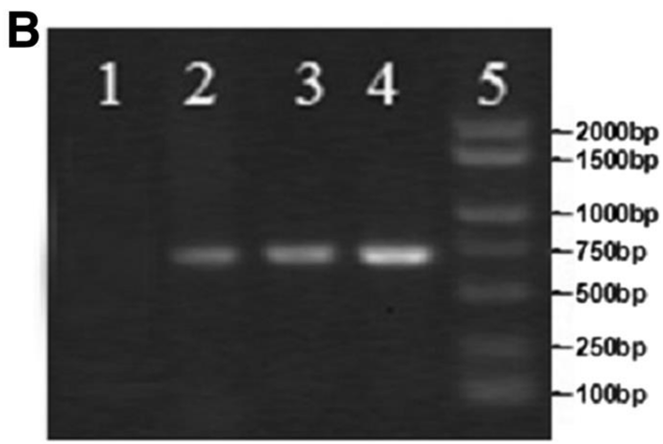


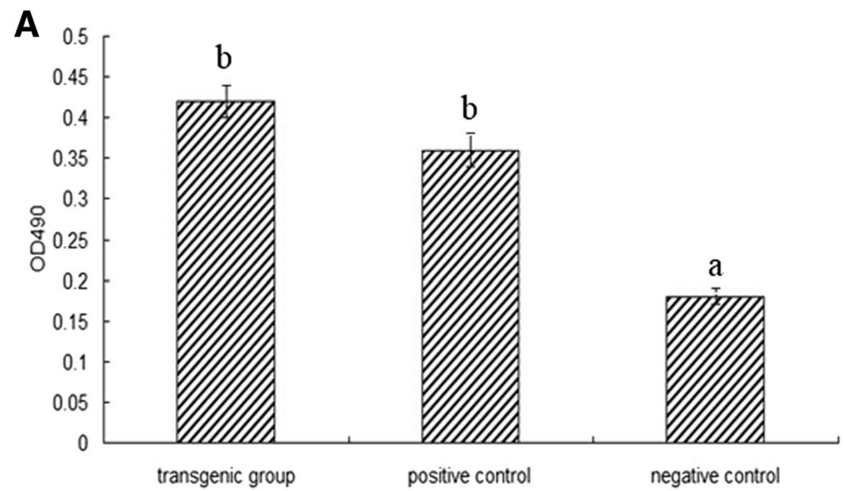
Composition analysis of transgenic milk.

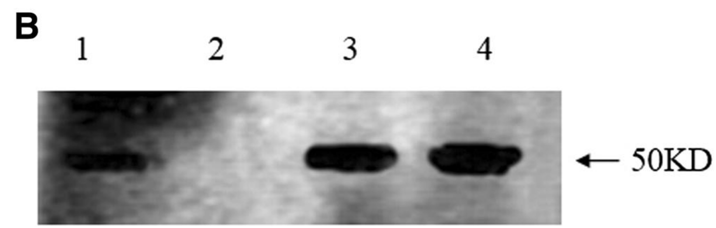
3. Trait Improvement

A transgenic ginseng vaccine for bovine viral diarrhea







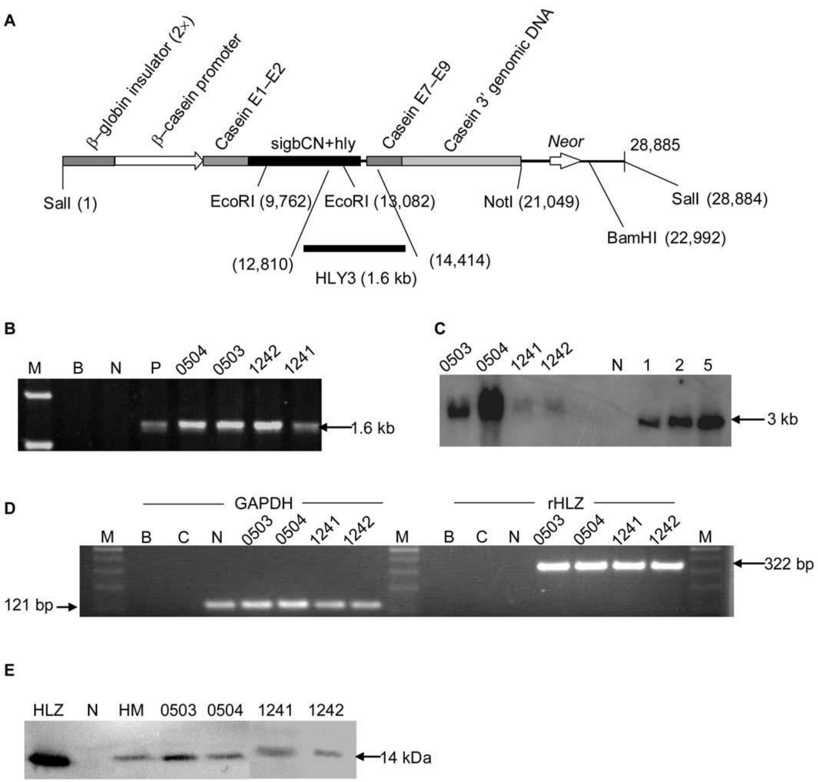


we report the use of Panax ginseng as an alternative production platform for the expression of glycoprotein E_{rns} via Agrobacteriummediated transformation.

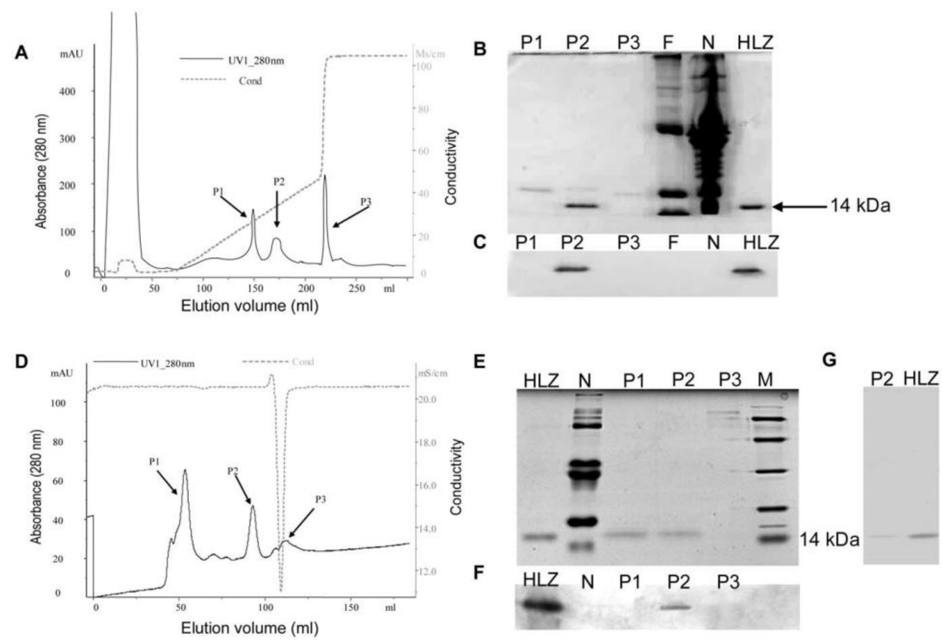
Gao et al. Virology Journal (2015)

3. Trait Improvement

Characterization of Bioactive Recombinant Human Lysozyme Expressed in Milk of Cloned Transgenic Cattle



Expression of rHLZ

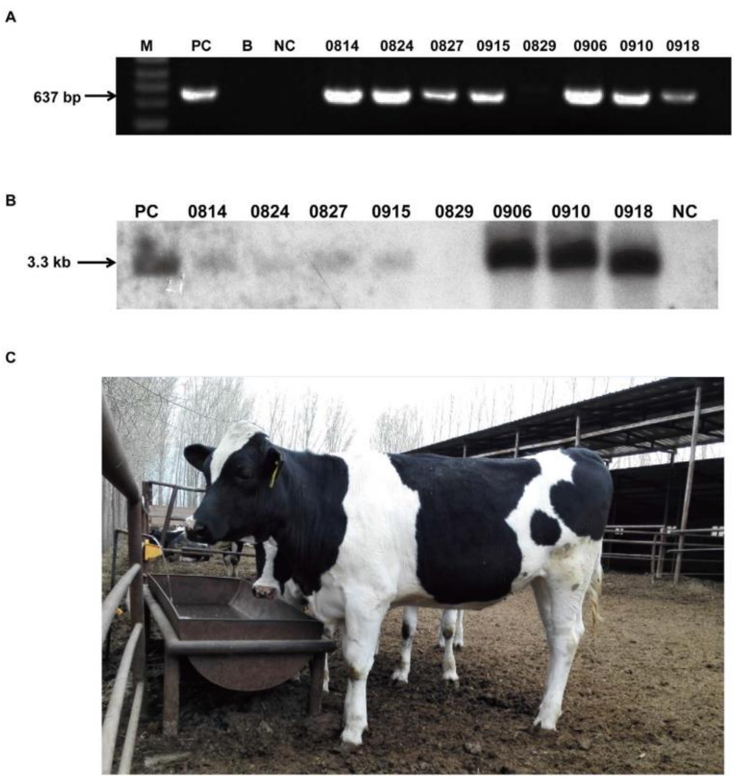


Purification of rHLZ by cation-exchange chromatography and gel filtration chromatography.

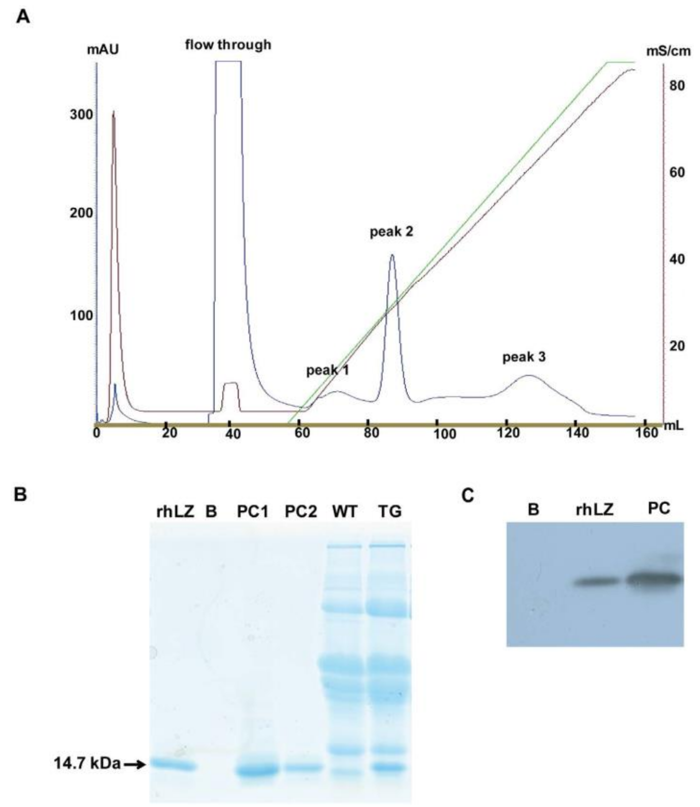
PLoS ONE March 2011 | Volume 6 | Issue 3 | e17593

3. Trait Improvement

Large-scale production of functional human lysozyme from marker-free transgenic cloned cows



Integration of transgene in genome of transgenic cloned cows.

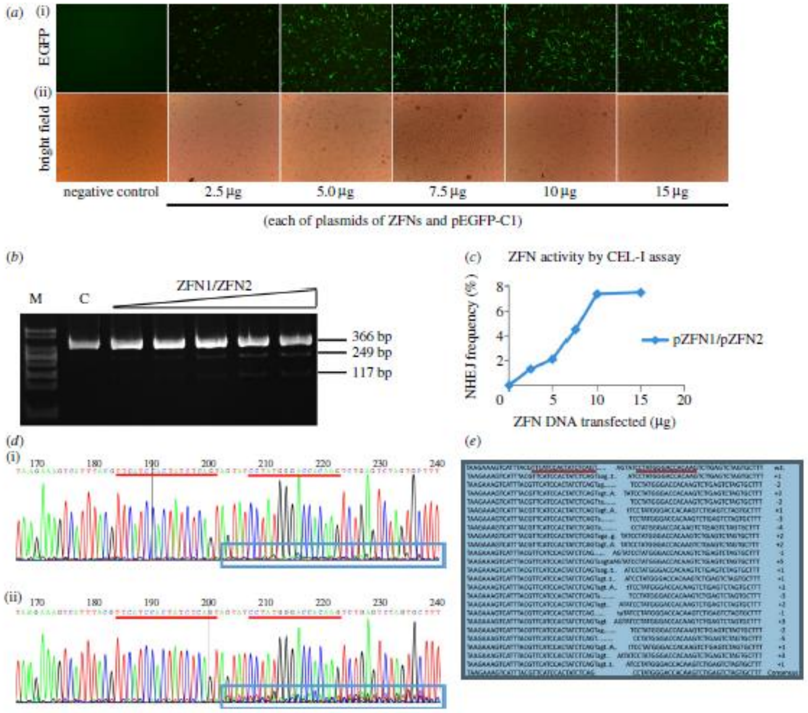


Identification of purified rhLZ from transgenic milk.

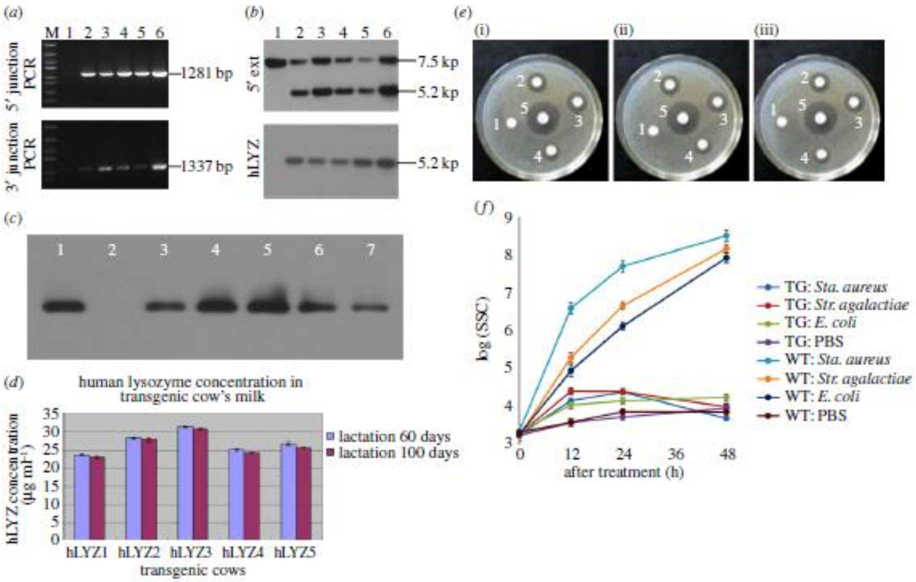
Scientific Reports | 6:22947 | DOI: 10.1038/srep22947/2016

3. Trait Improvement

Generation of mastitis resistance in cows by targeting human lysozyme gene to b-casein locus using zinc-finger nucleases



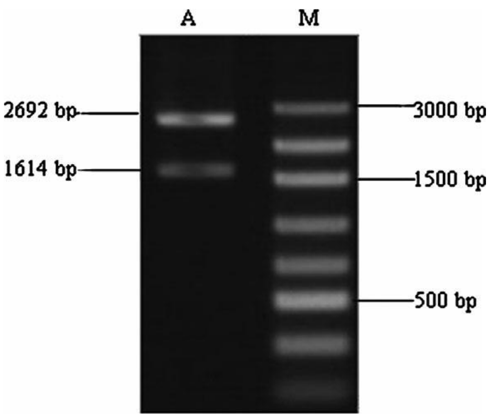
Validation of ZFNs for the CSN2 locus in bovine fibroblasts



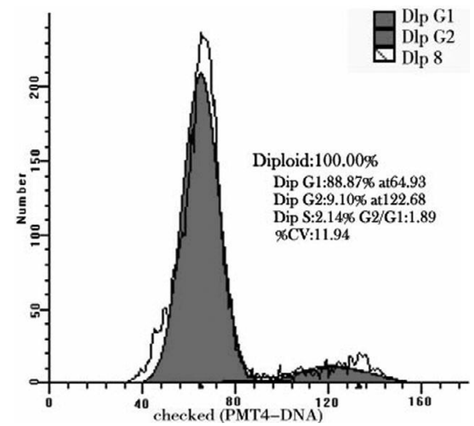
Liu X et al. 2014

3. Trait Improvement

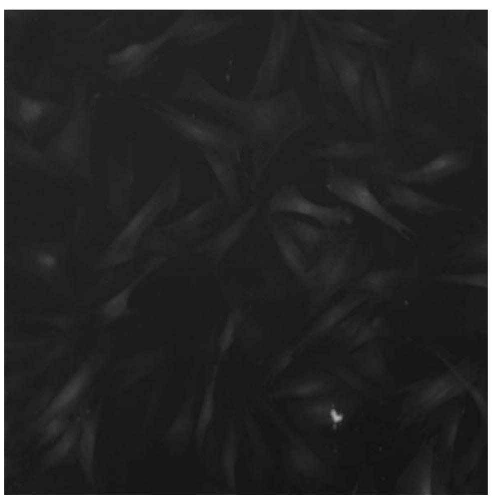
Construction of Ipr1 expression vector and development of cloned embryos *in vitro*



The restriction endonuclease double digestion of pIpr1. Lanes: A, pIpr1 was digested with *Eco*RI and *Pst*I; M, DNA marker VII (TIANGEN).



Karyotype analysis of donor cells by flow cytometry.



Expression of the *eGFP* gene in G418-resistant cells.

Table 1 *In vitro* development of bovine somatic cloned embryos

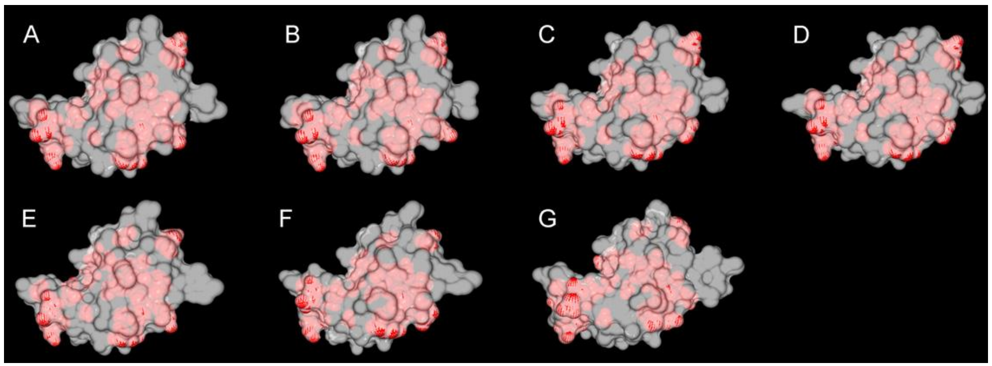
Donor cells	No. couples	No. embryo fused (%)	No. cleavages (%)	No. blastocysts (%)
Transgenic cells	60	43 (71.7)	35 (81.4)	8 (18.6)[a]
Non-transgenic cells	60	45 (75.0)	38 (84.5)	14 (31.1)[b]

[a,b]Values for individual different letters in the same column are significantly different ($P < 0.05$).

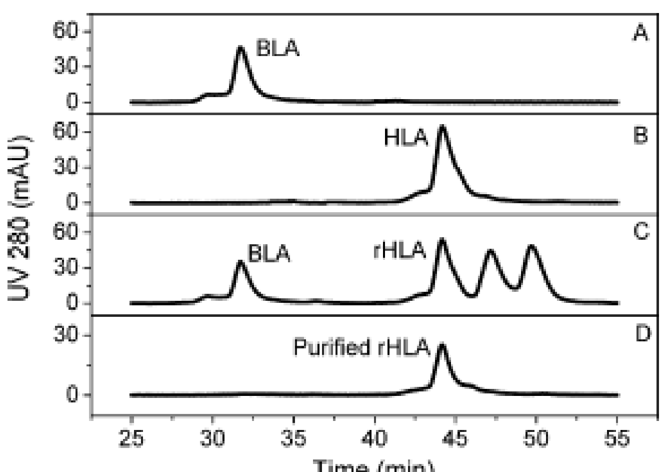
Zygote **21** (August), pp. 265–269. 18.06.2011

3. Trait Improvement

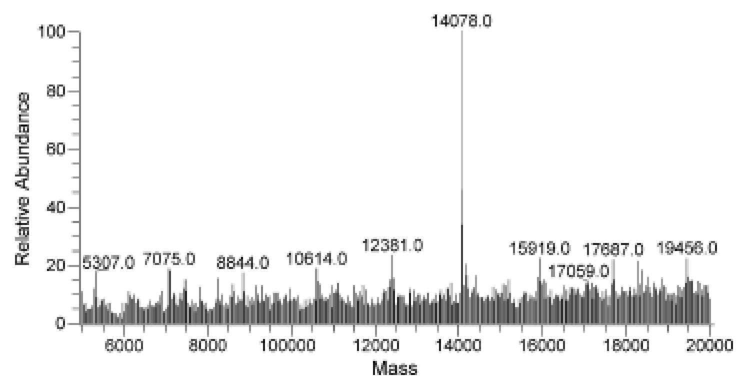
Efficient separation of homologous α-lactalbumin from transgenic bovine milk using optimized hydrophobic interaction chromatography



A schematic diagram of three-dimensional molecular structures of BLA (A–F for chain A–F) and HLA (G) to illustrate the influence of different amino acids on the protein surface properties. Solution structure of BLA (PDB code: 2G4N) and HLA (PDB code: 1A4V) [12].



HPRPC chromatograms of whey protein standards (A–B), acid whey (C) and purified rHLA product (D).

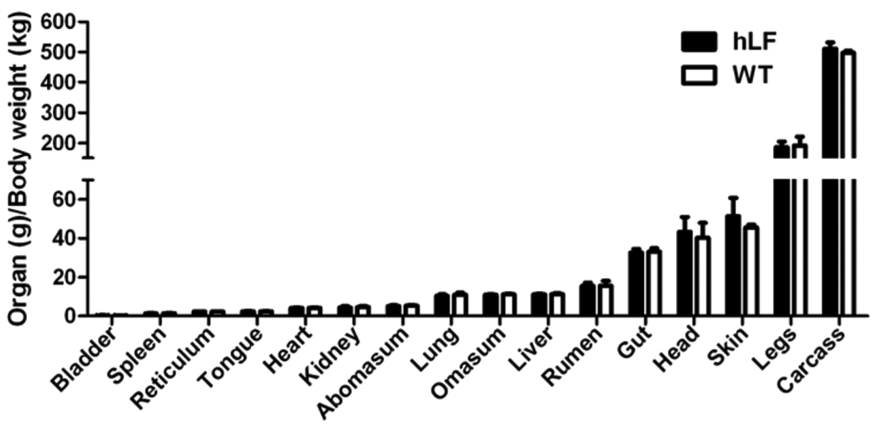


Molecular weight analysis of rHLA by mass spectrometry

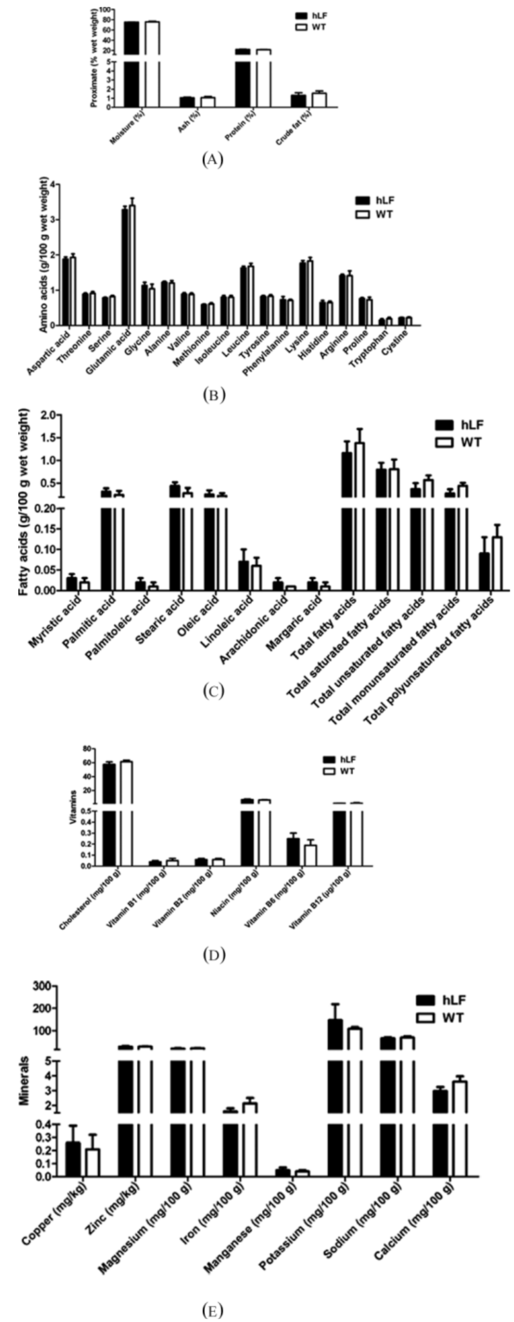
Journal of Chromatography A, 1217 (2010) 3668–3673

3. Trait Improvement

Nutritional Composition Analysis of Meat From Human Lactoferrin Transgenic Bulls



Proportions (mean SD) of organ or body part (g) over body weight (kg) for hLF bulls (n?6) and WT bulls (n?3).

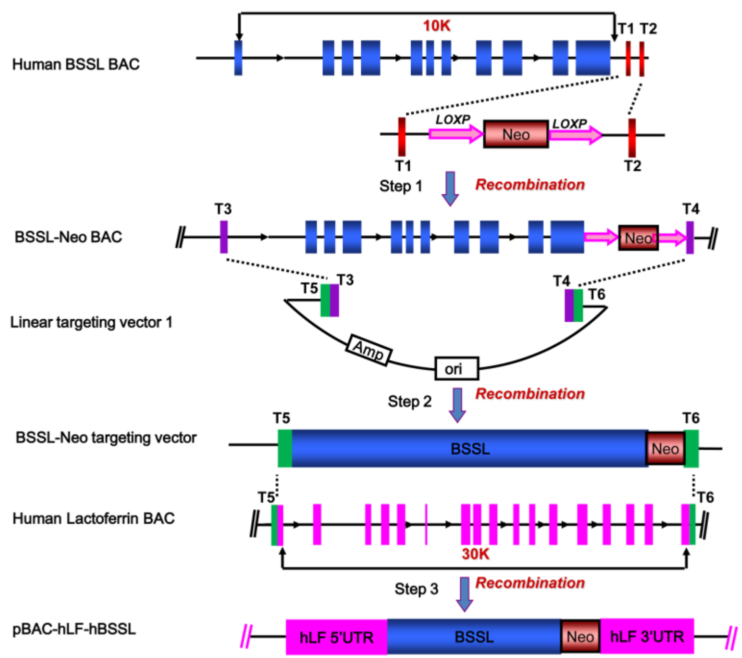


Meat nutritional composition analysis of hLF bulls versus WT bulls

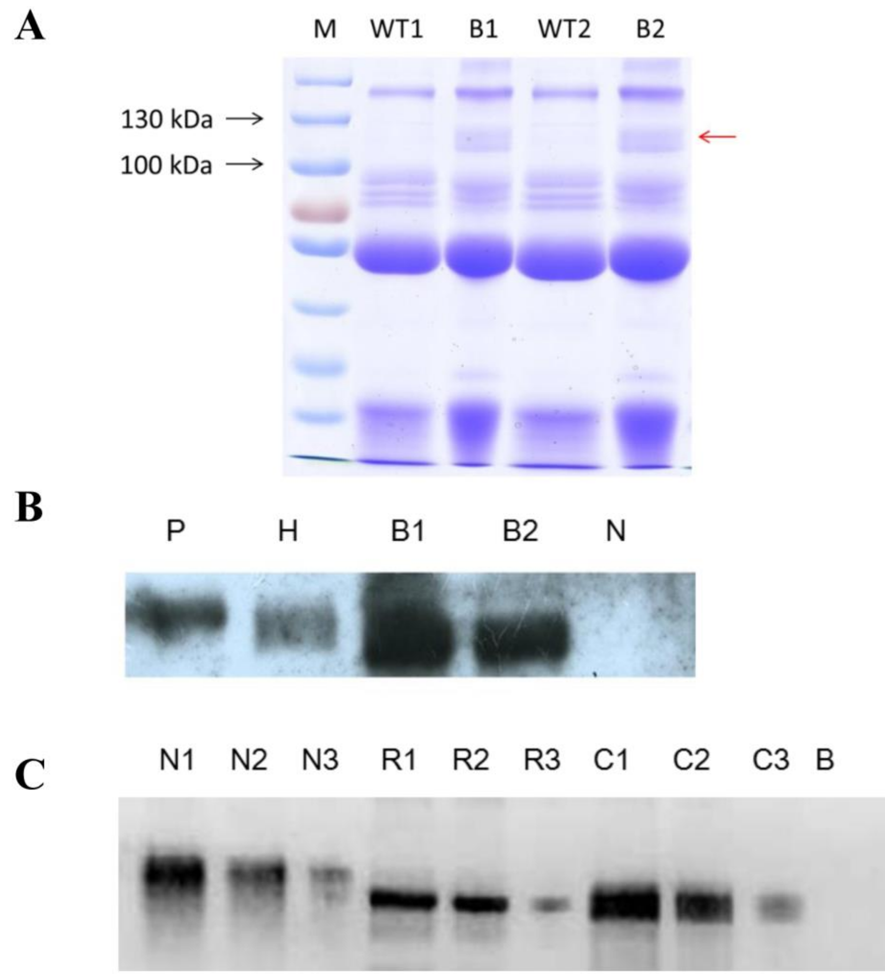
J Lipid Res. 2017 Jun 16

3. Trait Improvement

Purification and characterization of recombinant human bile salt stimulated lipase expressed in milk of transgenic cloned cows



Construction of the *hBSSL* expression vector pBAC-hLF-hBSSL and strategy for replacing 28.9 kb of hLF genomic sequence with 9,8 kb of hBSSL genomic DNA on the hLF BAC

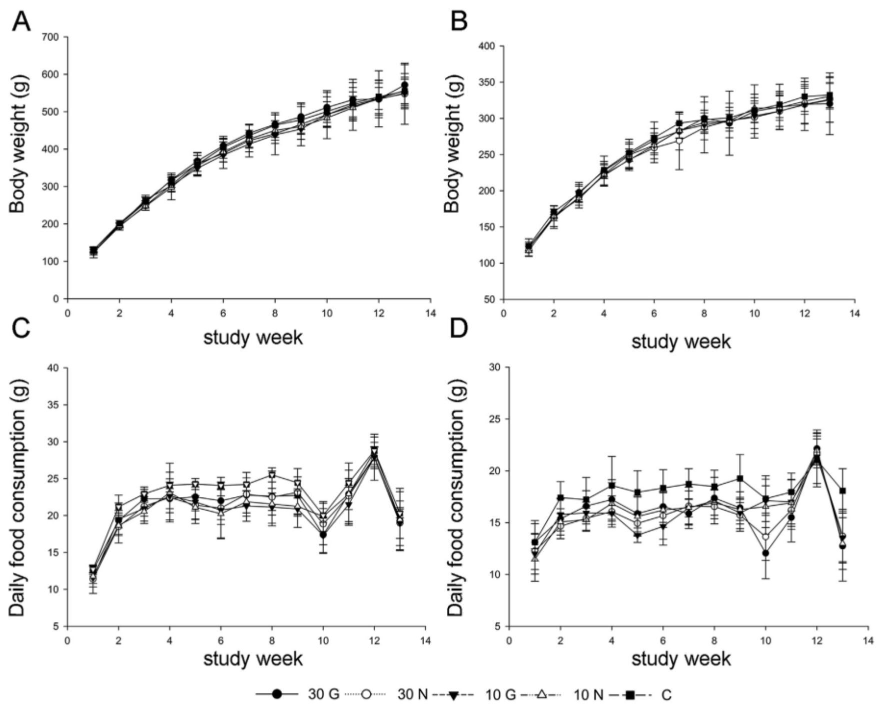


Expression of rhBSSL in transgenic milk.

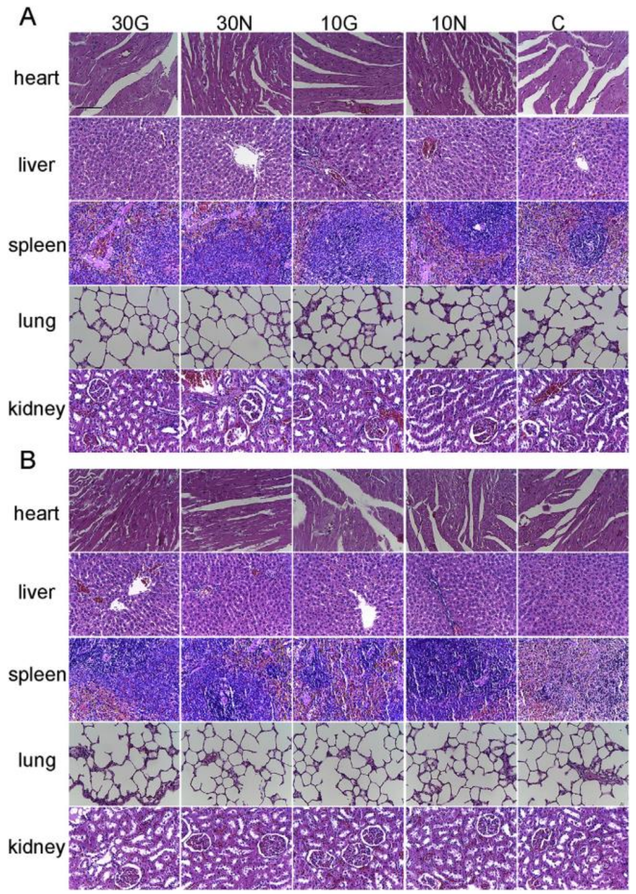
PLOS ONE | May 5, 2017

3. Trait Improvement

Safety assessment of genetically modified milk containing human beta-defensin-3 on rats by a 90-day feeding study



Body weight and daily food consumption

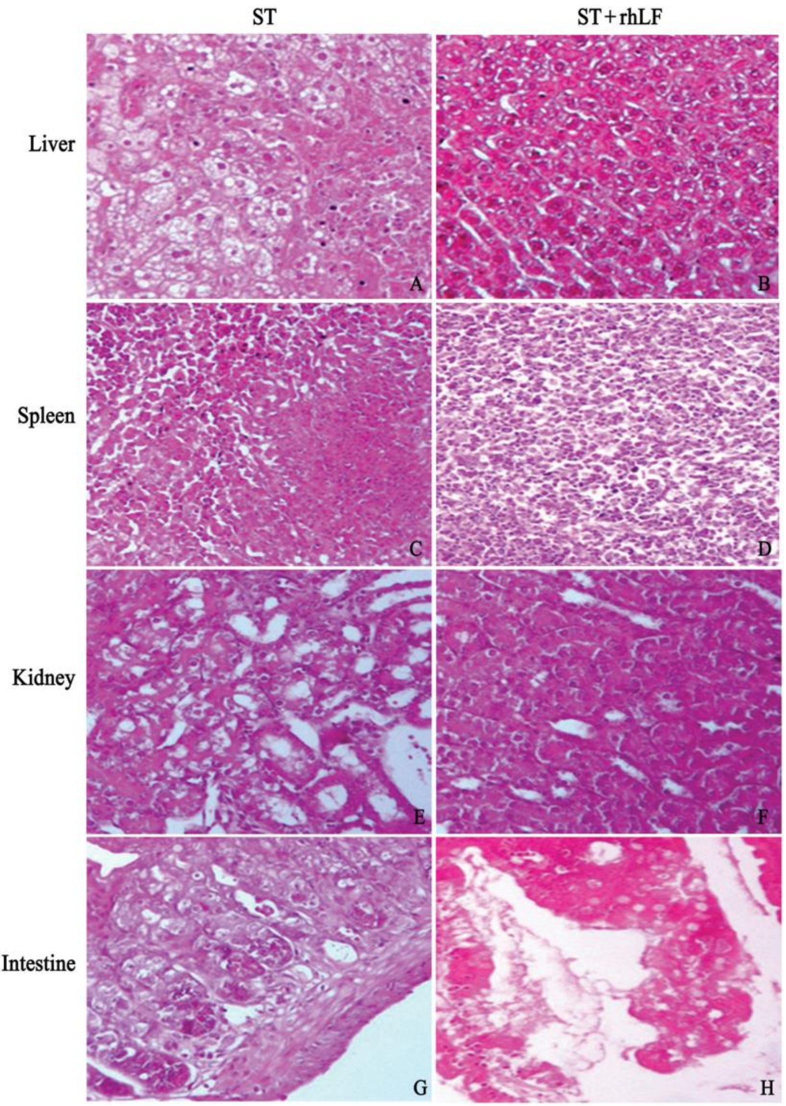


Histopathological results of main organs by HE staining

Food and Chemical Toxicology 100 (2017) 34e41

3. Trait Improvement

The effect of recombinant human lactoferrin from the milk of transgenic cows on Salmonella enterica serovar typhimurium infection in mice

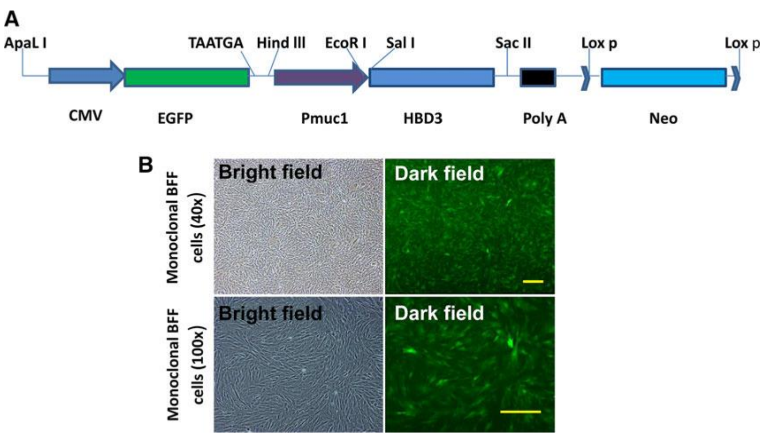


the present study demonstrated that recombinant human lactoferrin has considerable protective efficacy at combating Salmonella enterica serovar typhimurium infection in vivo. Therefore, rhLF might also have protective effects on inhibiting enteropathogenic bacteria and preventing foodborne disease. Further work is needed to determine the large potential applications of this valuable protein.

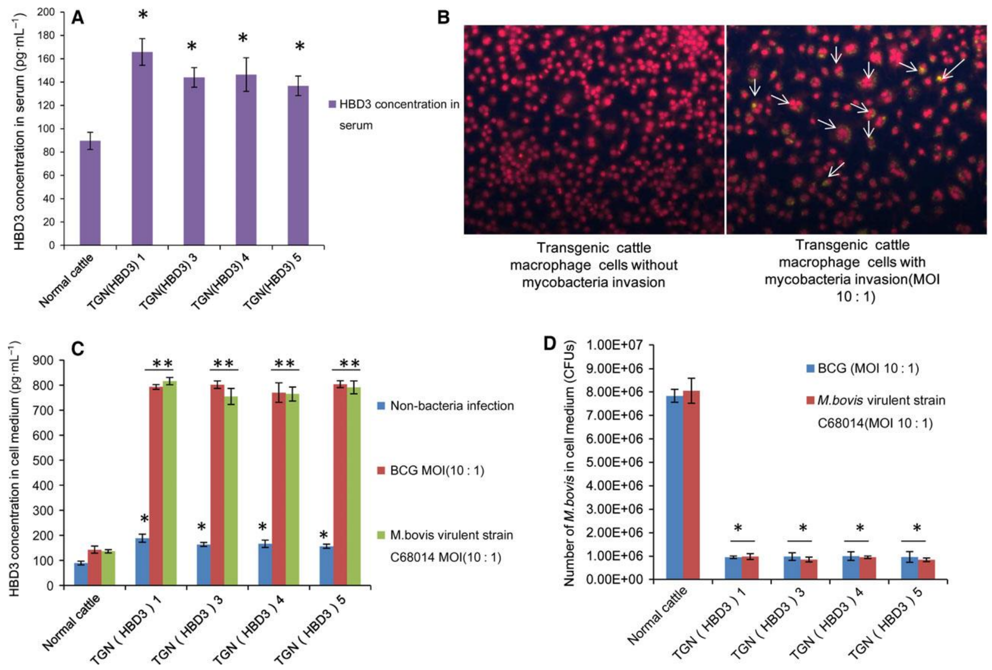
Food Funct., 2016, 7, 308–314

3. Trait Improvement

Generation of transgenic cattle expressing human b-defensin 3 as an approach to reducing susceptibility to Mycobacterium bovis infection



Construction of the expression vector and monoclonal BFF cell selection.

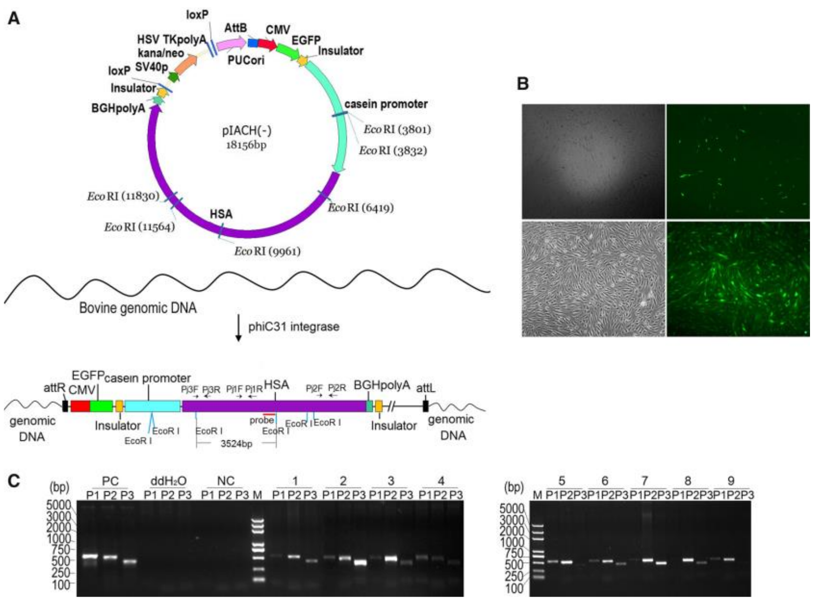


Anti-mycobacterial analysis of the four cattle that did not die

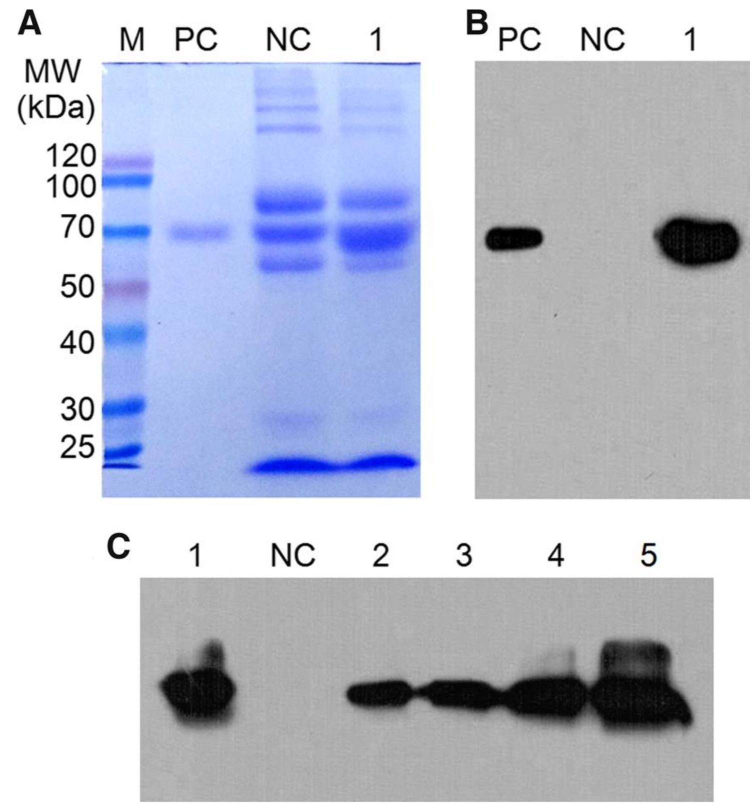
The FEBS Journal 283 (2016) 776–790

3. Trait Improvement

Production of transgenic cattle highly expressing human serum albumin in milk by phiC31 integrase-mediated gene delivery



Cell transfection and detection of HSA gene integration in the clonal cells

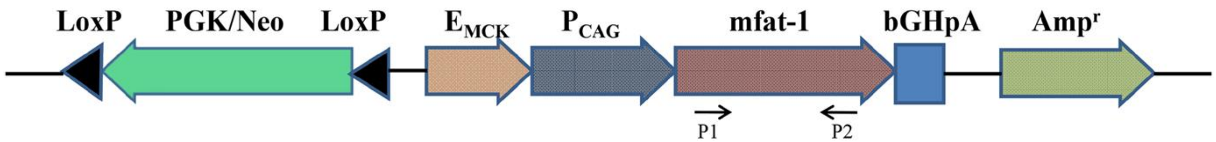


Analysis of recombinant HSA expression in the milk of transgenic cattle.

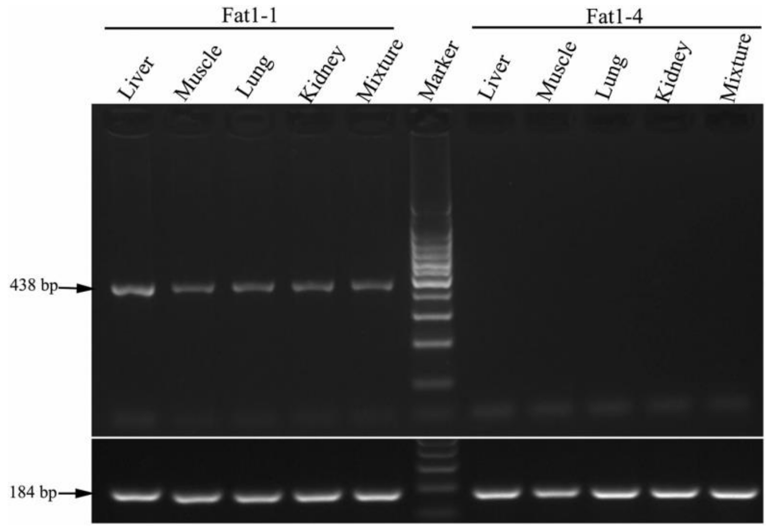
Transgenic Res (2015) 24:875–883

3. Trait Improvement

Production of transgenic beef cattle rich in n-3 PUFAs by somatic cell nuclear transfer



Schematic diagram of constructs for expression of mfat- 1 gene.



Detection of mfat1 expression by RT-PCR

Biotechnol Lett (2015) 37:1565–1571

Table 3 Fatty acids composition analysis

Fatty acids[a]	Fat1-1$^{(+)}$ (%)	Fat1-2$^{(+)}$ (%)	Fat1-10$^{(+)}$ (%)	Fat1-15$^{(+)}$ (%)	Fat1-4$^{(-)}$ (%)
MA (14:0)	3.02 ± 0.02	2.6 ± 0.32	5.17 ± 0.13	2.56 ± 0.08	4.07 ± 0.12
PA (16:0)	33.97 ± 0.75	28.5 ± 1.25	27.18 ± 1.02	30.82 ± 0.94	24.98 ± 1.2
PLOA (16:1)	6.3 ± 0.04	5.57 ± 0.12	3.1 ± 0.09	4.9 ± 0.65	3.64 ± 0.55
SA (18:0)	9.98 ± 0.32	12.86 ± 0.28	9.24 ± 0.17	13.09 ± 0.44	8.44 ± 0.37
OA (18:1 n-9)	44.86 ± 1.80	47.9 ± 1.87	31.18 ± 1.75	46.8 ± 1.50	30.28 ± 1.91
LA (18:2 n-6)	0.78 ± 0.05	1.05 ± 0.03	1.5 ± 0.03	0.9 ± 0.11	1.6 ± 0.09
ALA (18:3 n-3)	0.35 ± 0.02	0.5 ± 0.02	0.64 ± 0.05	0.36 ± 0.03	0.18 ± 0.03
ARA (20:0)	0.06 ± 0.01	0.08 ± 0.01	0.22 ± 0.02	0.06 ± 0.01	0.58 ± 0.03
EIA (20:1)	0.56 ± 0.02	0.64 ± 0.05	0.7 ± 0.04	0.4 ± 0.04	5.55 ± 0.06
EPA (20:5 n-3)	0.02 ± 0.01	0.02 ± 0.01	0.18 ± 0.03	0.03 ± 0.01	ND
DHA(22:6 n-3)	0.3 ± 0.04	0.33 ± 0.02	0.78 ± 0.05	0.18 ± 0.01	0.12 ± 0.01
Total n-3 fatty acids	0.69 ± 0.02	0.82 ± 0.07	1.58 ± 0.13	0.57 ± 0.03	0.3 ± 0.04
Total n-6 fatty acids	0.78 ± 0.05	1.05 ± 0.03	1.5 ± 0.03	0.9 ± 0.11	1.6 ± 0.09
n-6/n-3 ratio[b]	1.13 ± 0.07	1.28 ± 0.32	0.95 ± 0.06	1.58 ± 0.12	5.33 ± 0.08

14:0 Myristic acid (MA), *16:0* Palmitic acid (PA), *16:1* Palmitoleic acid (PLOA), *18:0* Stearic acid (SA), *18:1 n-9* Oleic acid (OA), *18:2 n-6* Linoleic acid (LA), *18:3 n-3* α-Linolenic acid (ALA), *20:0* Arachidic acid (ARA), *20:1* Eicosenoic acid (EIA), *20:5 n-3* Eicosapentaenoic acid (EPA), *22:6 n-3* Docosahexaenoic acid (DHA)

[a] The hindquarter muscle samples from dead transgenic positive (fat1-1, fat1-2, fat1-10 and fat1-15) and negative calves (fat1-4) were used for fatty acid analysis. Each value represents mean ± SD of three (n = 3) measurements

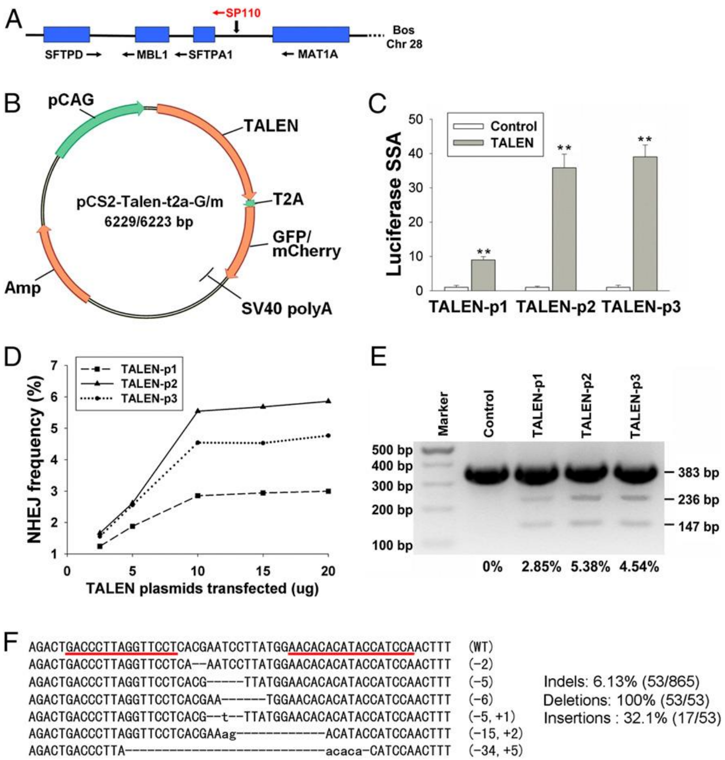
[b] n-6/n-3 ratio was calculated from n-6 fatty acid (LA) versus n-3 fatty acids (ALA, EPA and DHA)

(+) Represents transgenic positive cattle

(−) Represents transgenic negative cattle

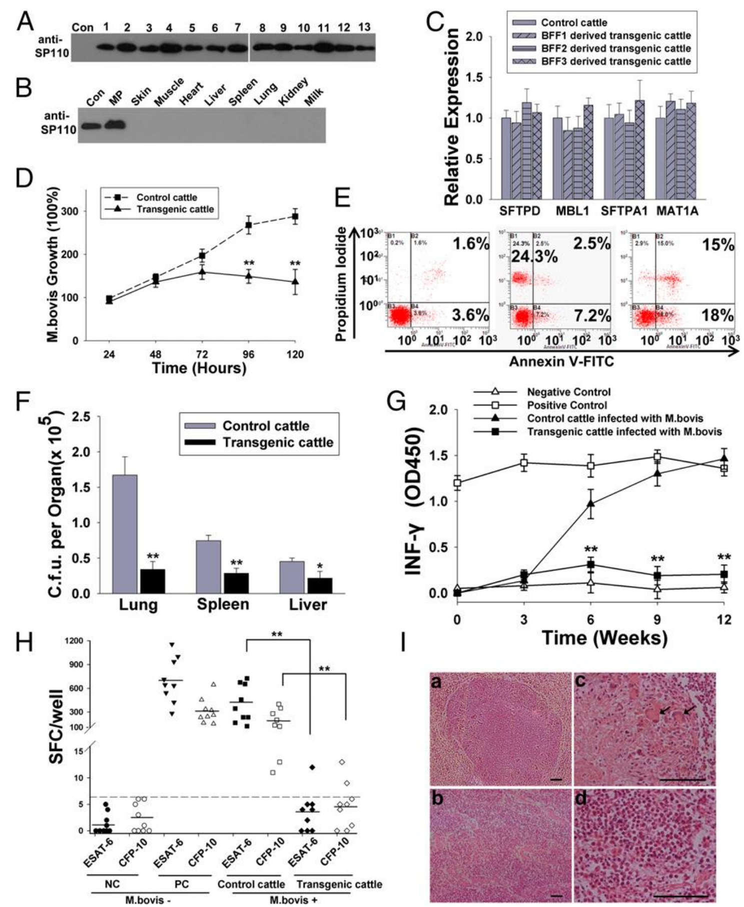
3. Trait Improvement

TALE nickase-mediated SP110 knockin endows cattle with increased resistance to tuberculosis



Activity assessment of TALENs

Assessment of the ability of transgenic cattle to resist tuberculosis



E1530–E1539 | PNAS | Published online March 2, 2015

3. Trait Improvement

Supplementation transgenic cow's milk containing recombinant human lactoferrin enhances systematic and intestinal immune responses in piglets

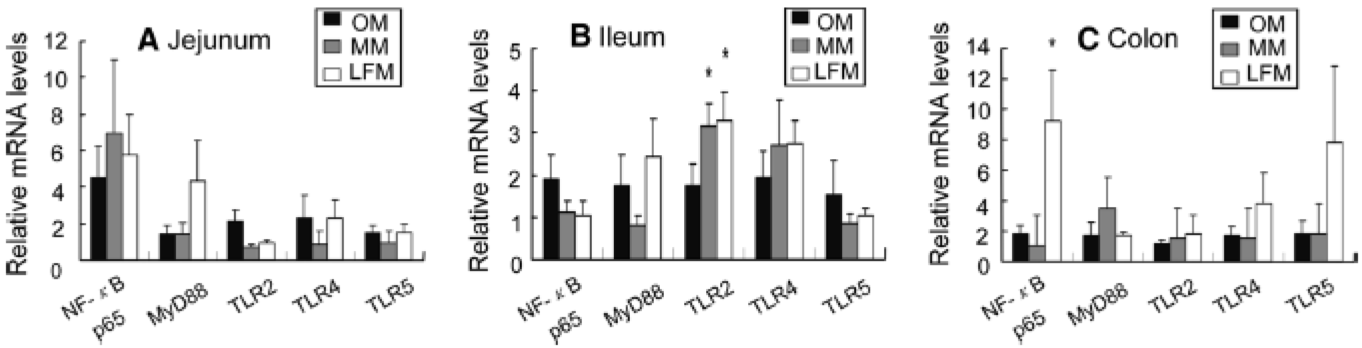
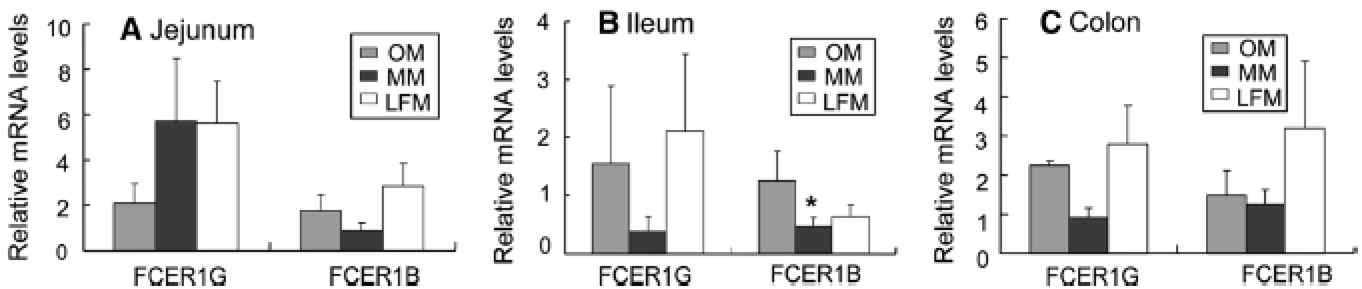


Fig. 1 Relative mRNA levels of *NF-κB p65*, *MyD88*, *TLR2*, *TLR4*, and *TLR5*. *A* Jejunum, *B* Ileum, *C* Colon. * The difference compare to O group at $P < 0.05$. *OM* ordinary milk, *MM* a 1:1 mix of ordinary milk and rhLF milk, *LFM* rhLF milk



Relative mRNA levels of FCER1G and FCER1B. A Jejunum, B Ileum, C Colon.

Mol Biol Rep (2014) 41:2119–2128

3. Trait Improvement

Fat-1 transgenic cattle as a model to study the function of ω-3 fatty acids

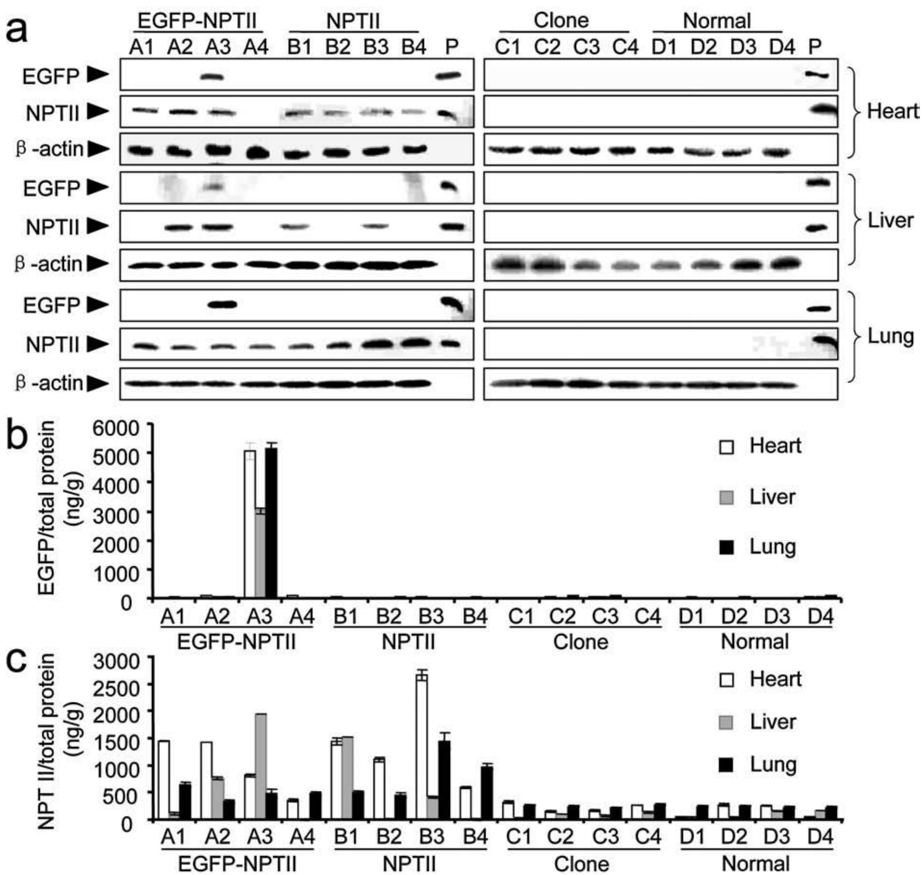
Table 1 Gene expression that either upregulated or downregulated in the whole genome of fat-1 transgenic cattle (p-value ?<? 0.05 and fc ?≥? 1) *(Continued)*

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

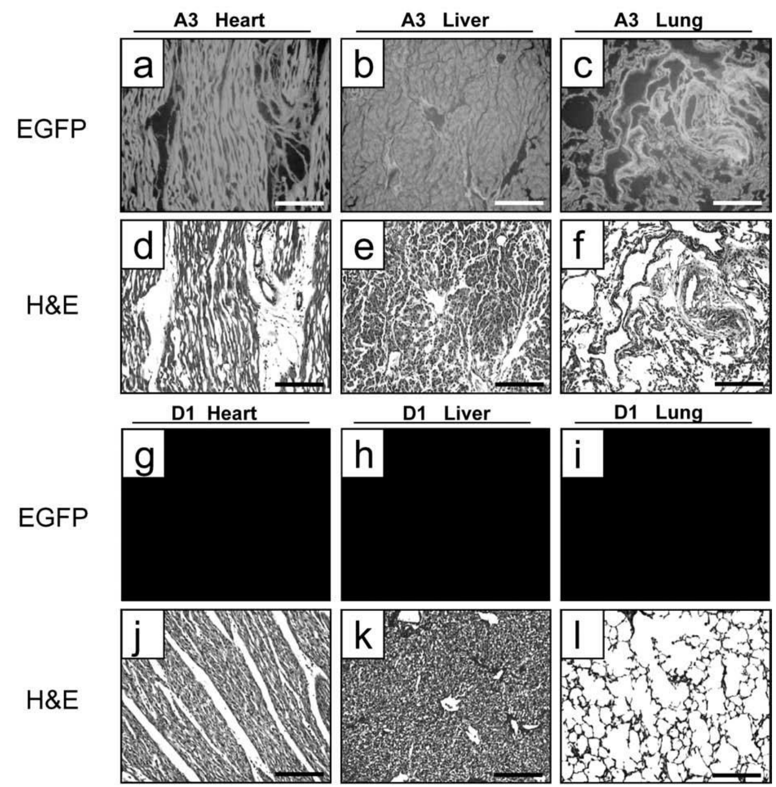
Guo et al. Lipids in Health and Disease 2011, 10:244

4. Genome Stability

Expression of EGFP and NPTII Protein Is Not Associated with Organ Abnormalities in Deceased Transgenic Cloned Cattle



Detection of EGFP and NPTII protein in heart, liver, and lung samples in the four groups.

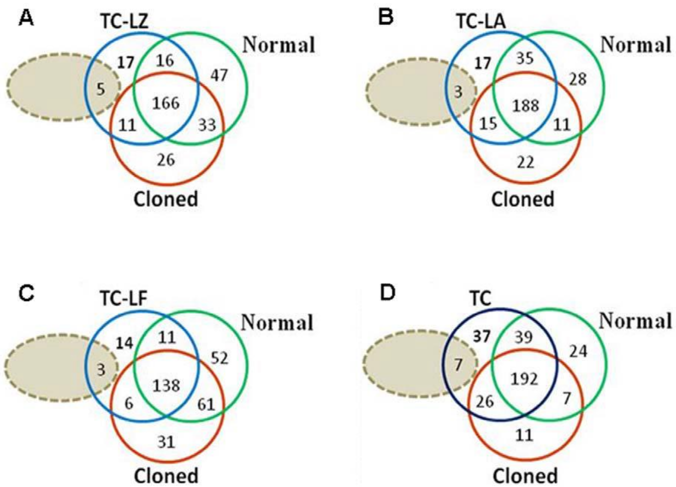


Histological analysis of EGFP by direct fluorescence under blue light. Paraffin sections were dyed by H&E (hematoxylin-eosin). D1 was a normal age-matched nontransgenic cow.

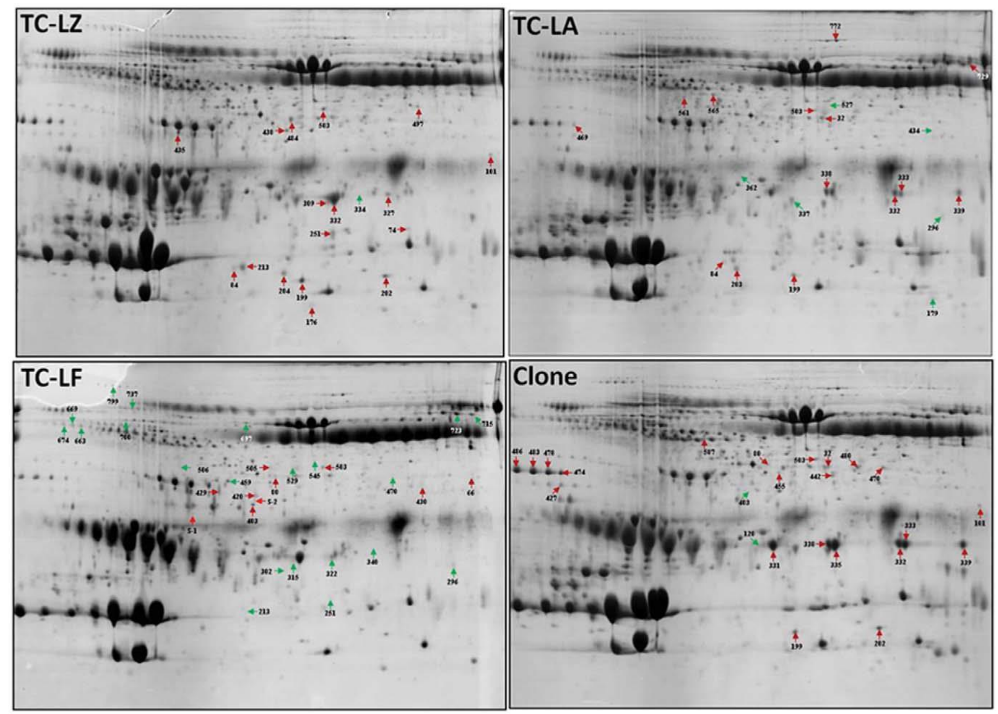
CLONING AND STEM CELLS Volume 10, Number 4, 2008

4. Genome Stability

Comprehensive Assessment of Milk Composition in Transgenic Cloned Cattle



Venn diagrams representation of whey protein profiles comparison of the TC-LZ (A), TC-LA (B), TC-LF (C) and all TC (D) groups were created with respect to the C and N groups, respectively.

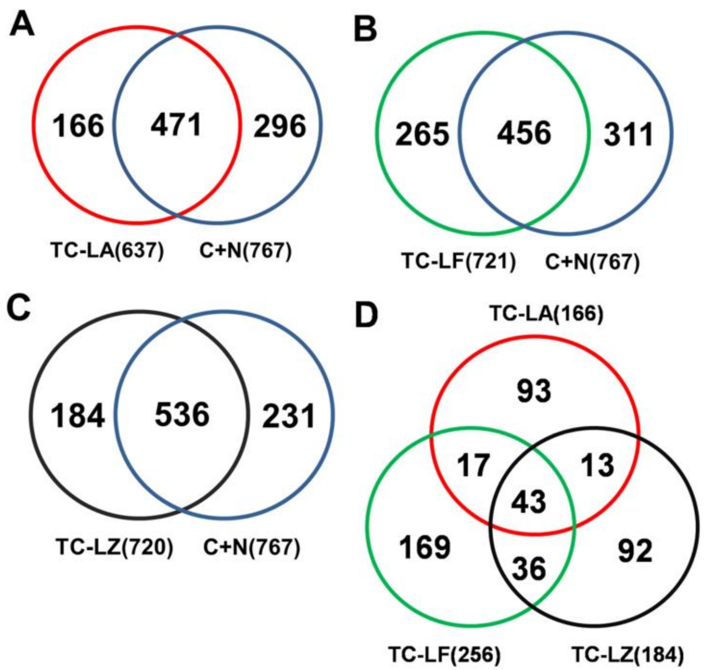


Differentially expressed protein spots with great than 2-fold changes (p#0.05) in the TC and the C group compared to N group

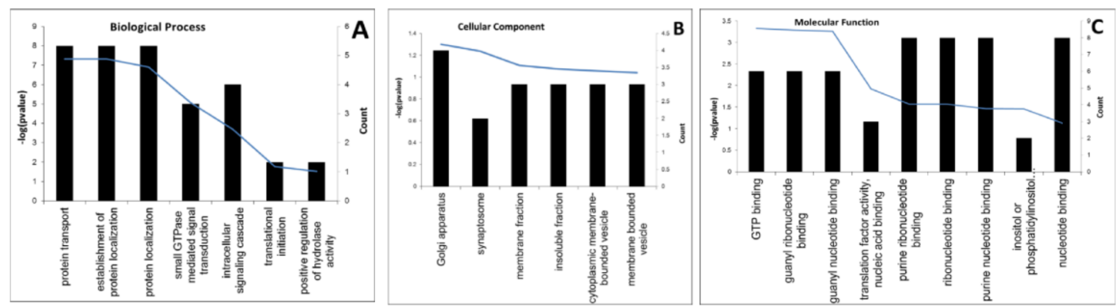
PLOS ONE November 2012 | Volume 7 | Issue 11 | e49697

4. Genome Stability

Comparative Proteomics of Milk Fat Globule Membrane Proteins from Transgenic Cloned Cattle



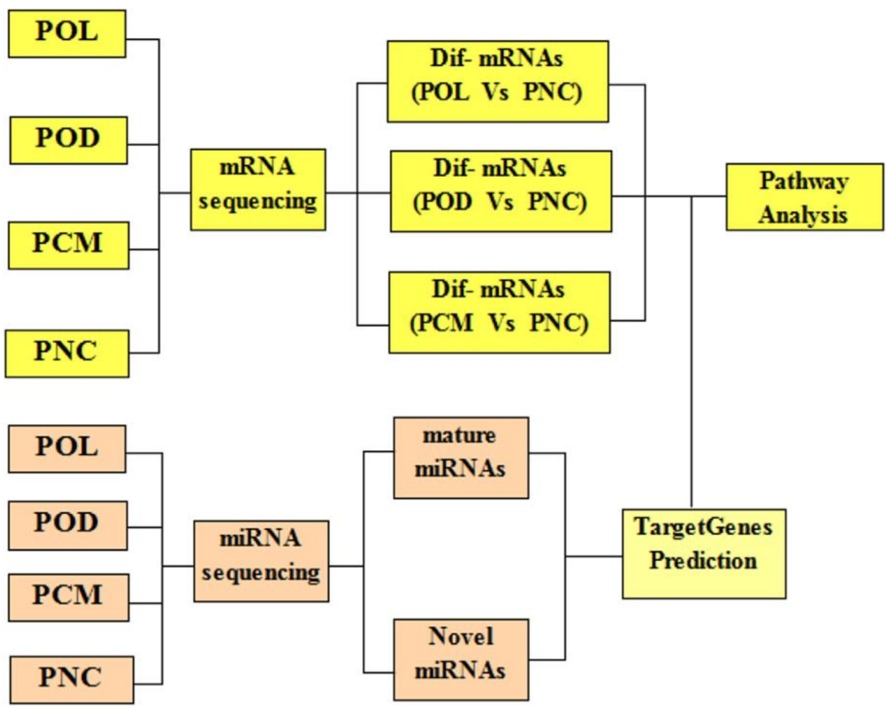
Comparison of MFGM proteins from the different TC and control lines



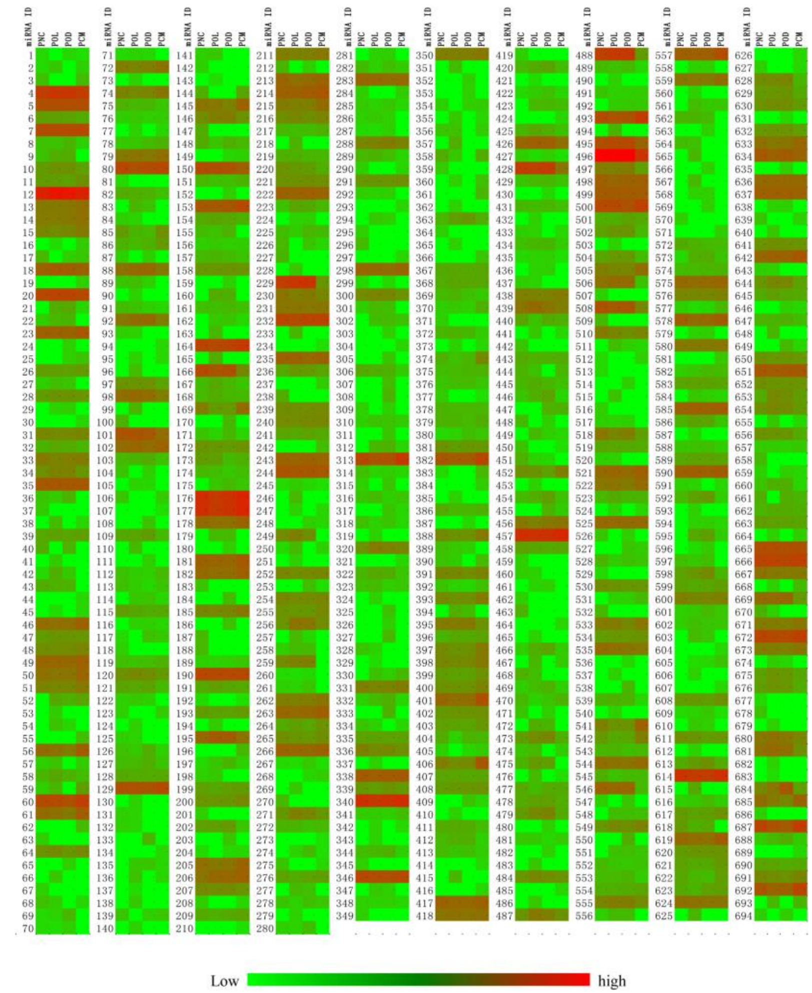
Functional analysis of MFGM proteins specifically expressed in the transgenic cloned animals

4. Genome Stability

Differentially expressed microRNAs and affected signaling pathways in placentae of transgenic cloned cattle

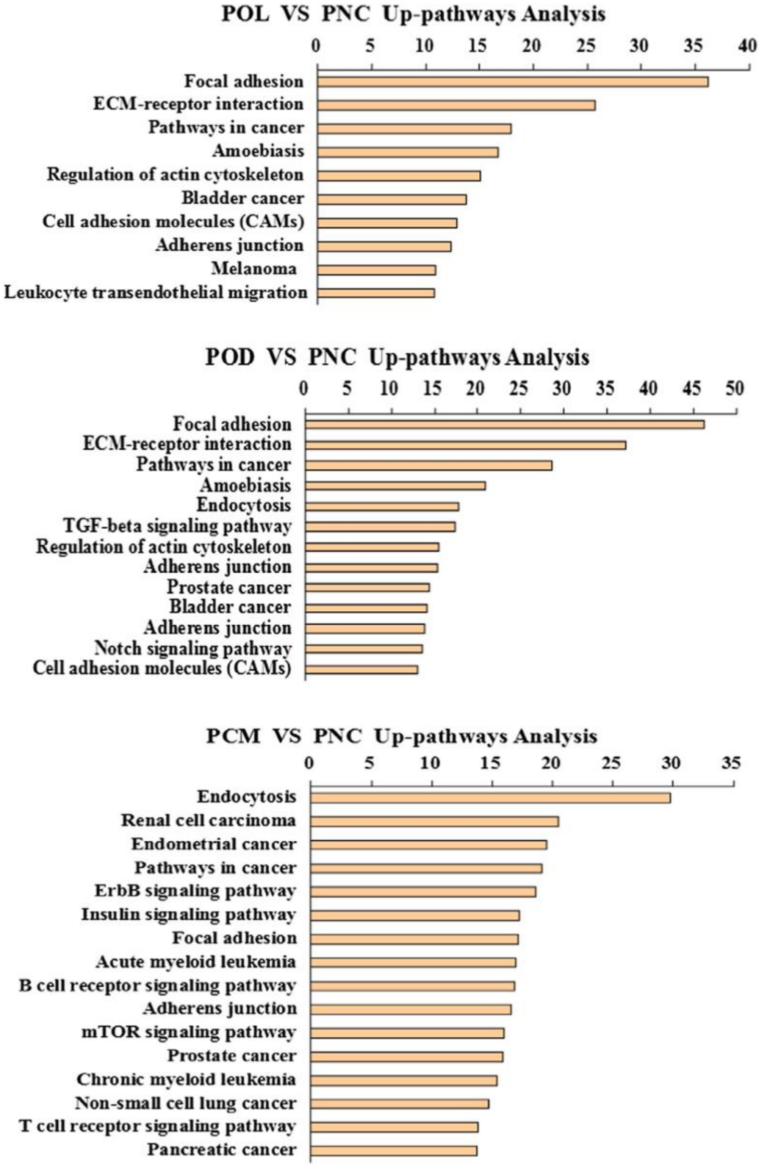


Flow diagram of bioinformatics analysis. miRNAs, microRNA

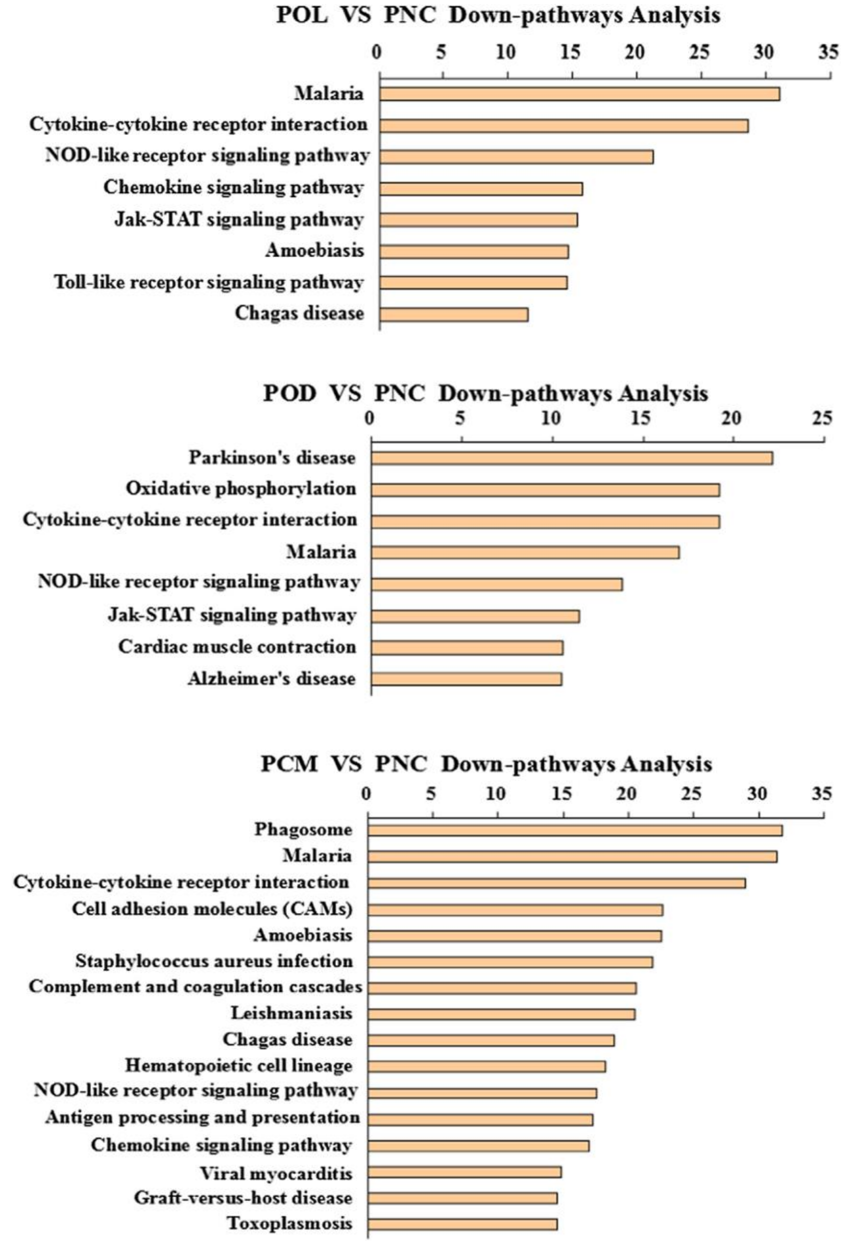


Differential expression levels of 694 miRNAs in four placental samples

F.-J. Liu et al. / Theriogenology 82 (2014) 338–346



Significantly upregulated signaling pathways analysis

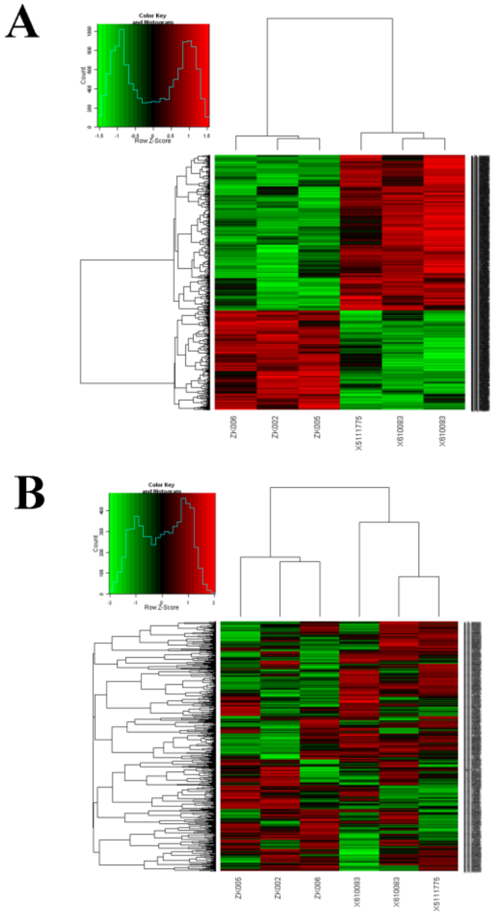


Significantly downregulated signaling pathways analysis

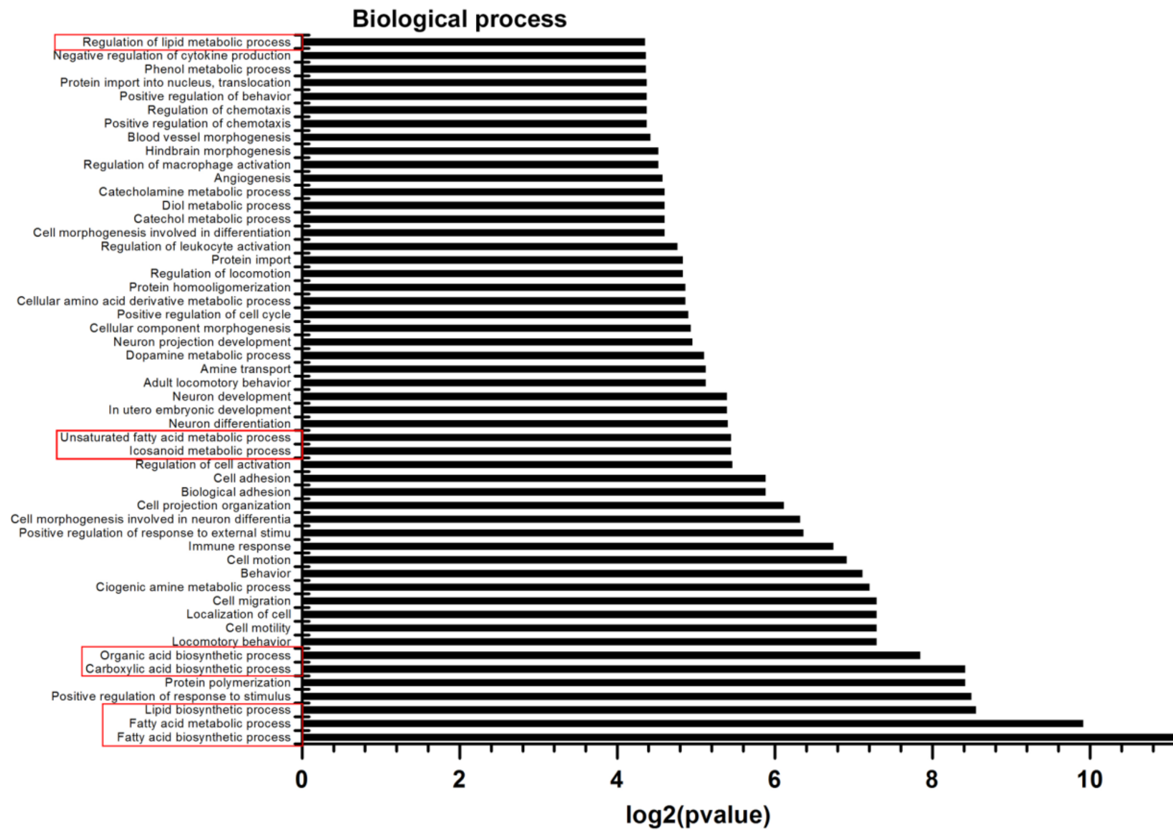
F.-J. Liu et al. / Theriogenology 82 (2014) 338–346

4. Genome Stability

Microarray Analysis of the Gene Expression Profile and Lipid Metabolism in Fat-1 Transgenic Cattle



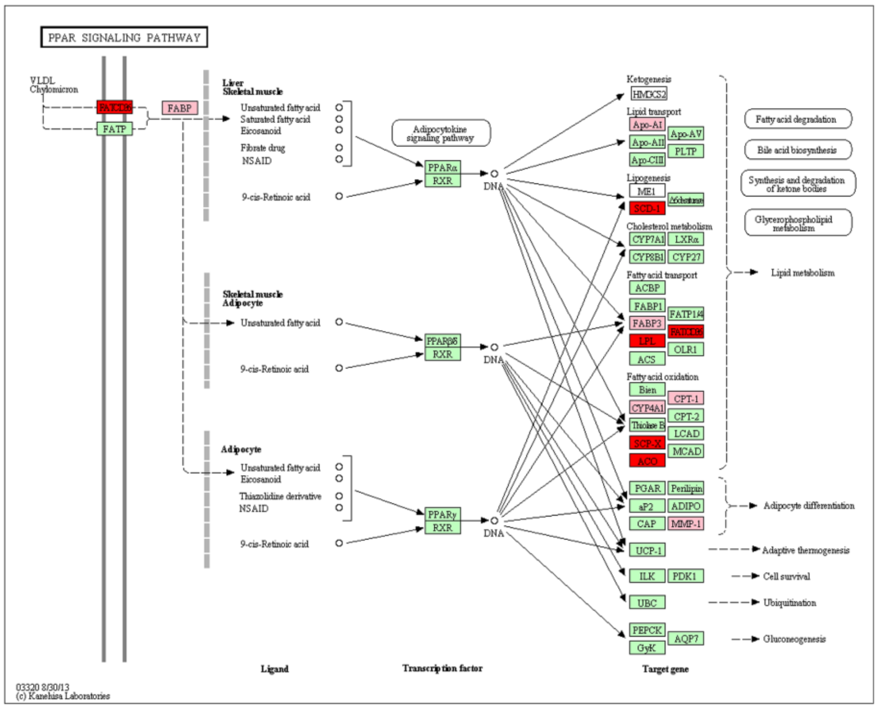
The hierarchical clustering analysis of fat-1 transgenic cattle and wild-type cattle

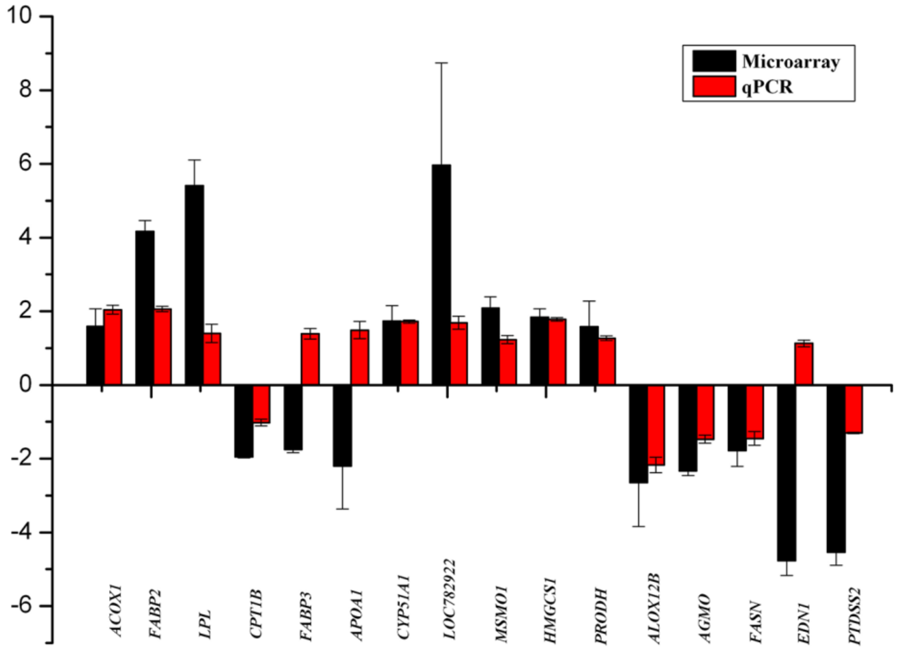


Microarray biological process (GO Ontology) classification

PLOS ONE October 1, 2015

Microarray Analysis of the Gene Expression Profile and Lipid Metabolism in Fat-1 Transgenic Cattle



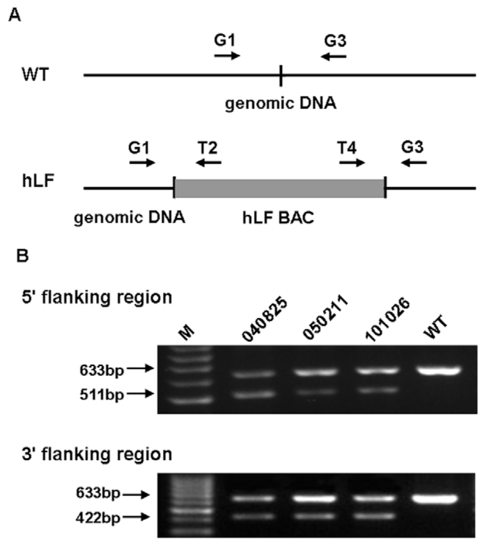


The significantly enriched genes in the 'PPAR signaling pathway'

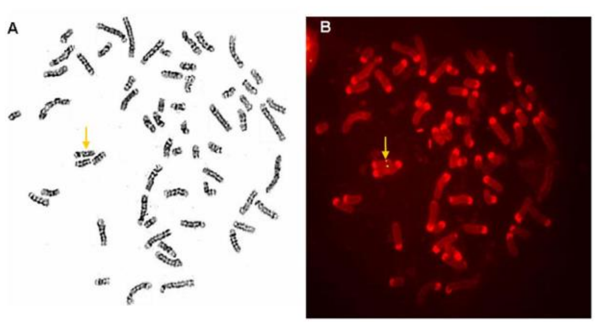
The significantly enriched genes in the 'PPAR signaling pathway'

4. Genome Stability

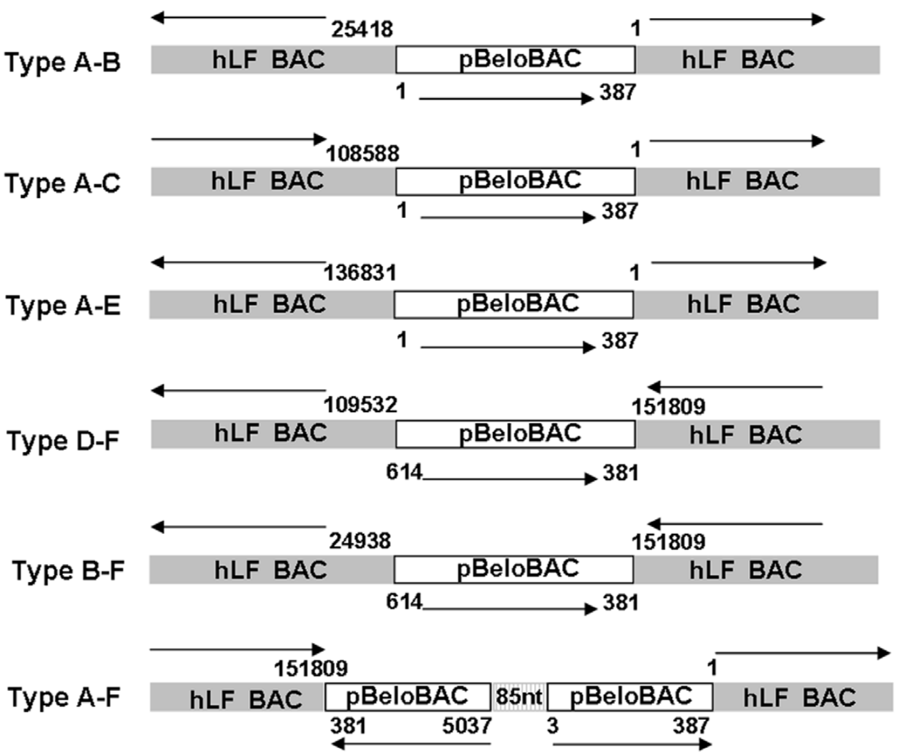
Molecular Characterization of Transgene Integration by Next-Generation Sequencing in Transgenic Cattle



Verification of the integration sites of the transgene by PCR.



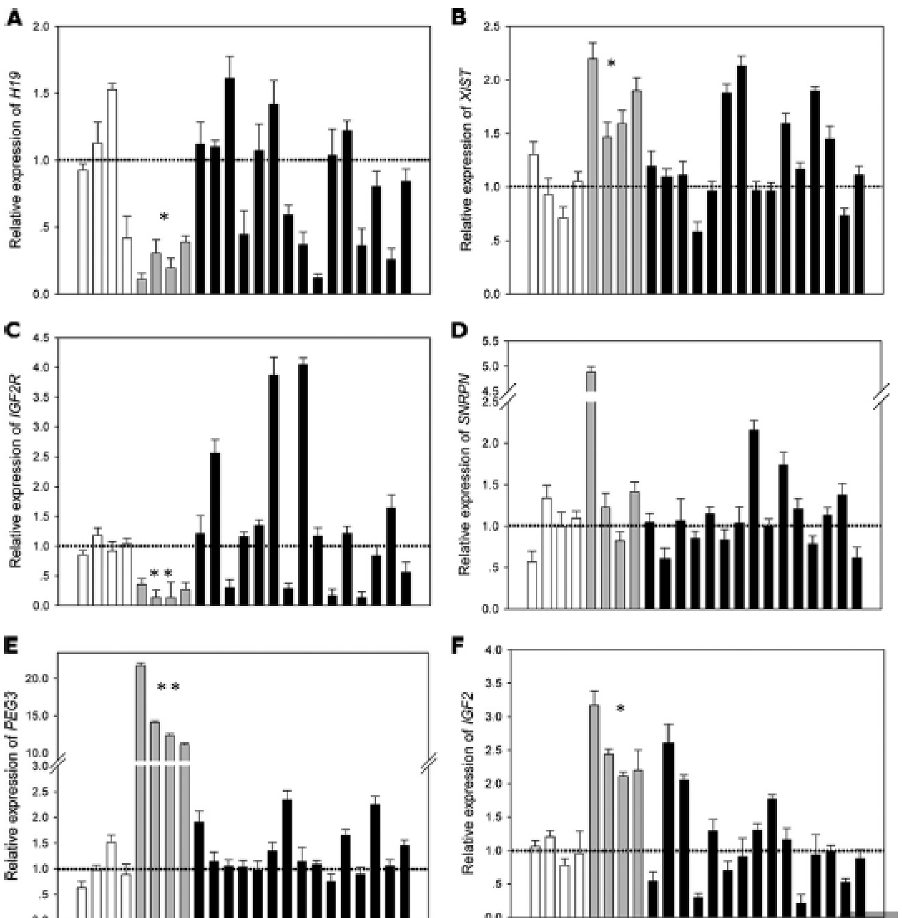
Verification of the transgene chromosomal location by FISH analysis



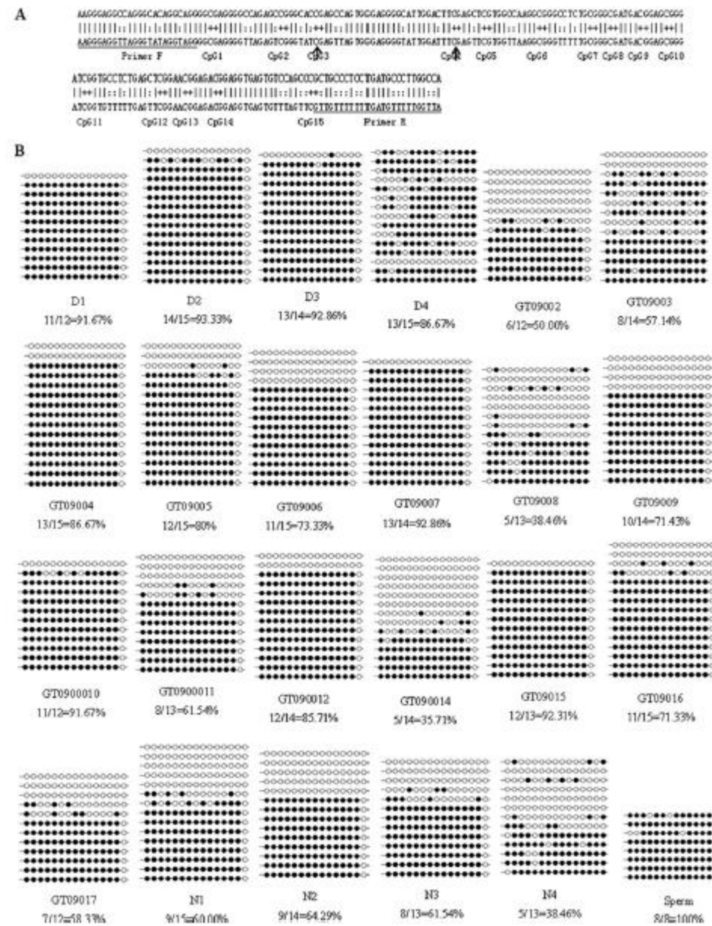
Schematic representation of the BAC rearrangements in transgenes

4. Genome Stability

Expression and methylation status of imprinted genes in placentas of deceased and live cloned transgenic calves



Relative mRNA levels of six imprinted genes in bovine placentas which were analyzed by quantitative real-time PCR



DNA methylation status of *H19* DMR was analyzed by BSP.

Theriogenology 75 (2011) 1346–1359

Conclusion:

1. Many new methods have been used to the GMO cattle breeding.
2. Mammary Reactor has been used to produce active reagents for industry.
3. Many genes have been transduced into cattle to improve its traits.
4. Genome stability is widely considered.

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