

Cloning, Sequence Analysis and Identification of a Nonsense Mutation-mediated mRNA Decay of Porcine *GSTM2* Gene

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Abstract The glutathione S-transferase mu 2 gene (*GSTM2*) encodes a GST functioning in the elimination of electrophilic compounds and the regulation of cell growth. In this study, the sequence of porcine *GSTM2* gene that contains the complete sequence encoding a protein of 218 amino acids was cloned. The deduced amino acid sequence shared 76%, 78% and 76% identity with that of human, mouse and rat, respectively. mRNA expression analysis showed that the porcine *GSTM2* gene was expressed at a high level in liver and testis, at a medium level in *longissimus dorsi* muscle, adipose tissue, spleen and lung, at a low level in kidney, and at a very low level in heart and embryo. A nonsense mutation (CGA→TGA) resulted from C27T substitution in the fifth exon to produce a premature translation termination codon was identified, and it was discovered that nonsense-mediated mRNA decay might have an effect on the regulation of porcine *GSTM2* gene expression. This polymorphism was analyzed in Large White, Landrace, Meishan and Qingping pig populations using the *Taq* I-polymerase chain reaction-restriction fragment length polymorphism method. The result showed that allele *C* had a higher frequency than allele *T* in each population.

Keywords *GSTM2*; pig; NMD; gene expression; PCR-RFLP

Glutathione S-transferase (GST) is biotransformation enzyme that exists widely in various mammalian tissues. It catalyzes the combination of glutathione and diverse electrophilic compounds, which leads to the disoxidation, inactivation and elimination of the latter [1]. Eight distinct classes of the soluble cytoplasmic mammalian GSTs have been identified: alpha, kappa, mu, omega, pi, sigma, theta and zeta [2]. The GST mu 2 gene (*GSTM2*) encodes a GST that belongs to the mu class. The mu class of enzymes functions in the elimination of free radicals, peroxides, electrophilic reagents and heavy metals, the participation of cell protection and the regulation of cell growth. In human, the genes encoding the enzymes of the mu class are organized in a gene cluster on chromosome 1p13.3 [3].

Nonsense-mediated mRNA decay (NMD) degrades selectively and rapidly the aberrant mRNA that contains the premature translation termination codon (PTC), avoiding

or reducing the production of truncated protein that might do harm to the cells. It is a conservative surveillance mechanism of the wide existence of eukaryotic cells and a key link in the qualitative control of mRNA after transcription [4,5].

Recent research proved that NMD played an important and extensive role in the regulation of gene expression [4, 5]. For example, among 6000 genes of *Saccharomyces cerevisiae*, at least 225 were directly or indirectly influenced by NMD. NMD is necessary for the maintenance of the length of telomeres and the assembly of the functional kinetochore of eukaryotic cells [6,7]. It also plays an important part in the etiological factors of inherited disease and cancer [8]. According to present research data, PTCs exist in all mRNA suffering NMD. Therefore, PTC is the sign of NMD [9].

There are several ways of producing PTC. One is DNA mutation, for instance, the base substitution of DNA makes the sense codon into one of the three termination codons, or the mutation of frame shift produces the terminator. Another one is in the processing of pre-mRNA, when an

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anomaly happens and the intron containing the termination codon remains. Moreover, some physiological transcripts might also contain PTC, such as non-coding transcripts and transcripts containing upstream open reading frames [10].

In the present study, we cloned a porcine *GSTM2* gene, identified a PTC in the coding region and analyzed the possible existence of NMD.

Materials and Methods

Animals

Large White, Landrace and Meishan pigs were selected from Jingpin pig station at Huazhong Agricultural University (Wuhan, China). Qingping pigs were managed by the Qingping research farm of Hubei province in China.

cDNA and DNA samples

Total RNAs were extracted with Trizol reagent (Gibco BRL, Carlsbad, USA) from various tissues (heart, liver, spleen, lung, kidney, adipose tissue, *longissimus dorsi* muscle, embryo and testis) of Large White, Landrace and Meishan pigs. Primary cDNA was synthesized in a final volume of 25 μ l: 5 μ l of 5 \times reaction buffer, 1 μ g of total

RNA, 0.5 mM each dNTP, 25 U of RNasin (40 U/ μ l), 2 μ l of 10 μ M oligo(dT)₁₅ and 200 U of Moloney murine leukemia virus reverse transcriptase (200 U/ μ l; Promega, Madison, USA).

SMART (switching mechanism at the 5' end of the RNA transcript) cDNA was synthesized for RACE (rapid amplification of cDNA ends)-PCR (polymerase chain reaction) using a SMART PCR cDNA Synthesis Kit (Clontech, San Jose, USA) following the manufacturer's instructions.

Genomic DNA was isolated from the blood samples of all pigs by phenol/chloroform extraction and ethanol precipitation [11].

PCR amplification and sequence analysis

To amplify the cDNA sequence of the porcine *GSTM2* gene, primer pair 1 (**Table 1**) was designed based on two pig expressed sequence tags (GenBank accession Nos. BP447344 and BP168412). To obtain the 5' full-length cDNA, primer pair 2 (**Table 1**) was used to amplify the 5' end of the gene with the SMART amplified cDNA from the pigs' *longissimus dorsi* muscle as template. Primer pairs 3, 4 and 5 (**Table 1**) were designed to amplify introns of the porcine *GSTM2* gene according to the above cDNA sequences we had obtained. PCR amplification was carried out in a 25 μ l reaction mixture containing 50 ng of cDNA or DNA as template, 0.5 μ l of each primer (5 μ M),

Table 1 Primer sequences and polymerase chain reaction annealing parameters

Name	Sequence (5'→3')	Annealing parameter
Primer pair 1	GTAGCCTCTCCCTTCGG TCACTCCATCTCCTGCTTC	56.0 °C
Primer pair 2	AACGCAGAGTACGCGGG GGCTTCTGTCATAGTCGGGAG	56.5 °C
Primer pair 3	GTAGCCTCTCCCTTCGG GGATTTGTCACTCAGCCAC	57.0 °C
Primer pair 4	TTCCAAATCCTCCTAAGTGC TTCTCCTCCTCTGTCTCCC	57.0 °C
Primer pair 5	GCTCGCAAGCACAACAT AGCCAGGAAATCCACATAG	57.0 °C
Primer pair 6	GTAGCCTCTCCCTTCGG GGATTTGTCACTCAGCCAC	56.5 °C
Primer pair 7	CTGCCCTACTTAATTGACGG GCTGACGCCTGAGATGC	59.0 °C
Primer pair 8	GCTCAGACAGCAGCAACA AGCCAGGAAATCCACATAG	56.0 °C
G3PDH	ACCACAGTCCATGCCATCAC TCCACCACCCTGTTGCTGTGA	56.5 °C

1.5 μl of each dNTP (2 mM), 2.5 μl of 10 \times PCR buffer with Mg^{2+} and 1U of *Taq* DNA polymerase (Biostar International, Toronto, Canada). The PCR conditions were as follows: 94 °C initial denaturation for 4 min, 35 cycles of 94 °C denaturation for 50 s, annealing for 50 s, and 72 °C extension for 1 min, followed by a 10 min extension at 72 °C. The products were cloned into the pMD 18-T cloning vector (TaKaRa, Dalian, China) and sequenced using M13-forward and M13-reverse primers.

The nucleotide sequence of the porcine *GSTM2* gene was compared with the GenBank at the National Center for Biotechnology Information using BLASTN and BLASTX searches of the “nr” database (<http://www.ncbi.nlm.nih.gov>). The prediction of the biophysics characteristics of the putative *GSTM2* protein was carried out by the ProtParam tool on the ExPASy proteomics server (<http://www.expasy.org/tools/protparam.html>). The analysis of protein structural domains and functional sites was carried out by the Prosite tool (<http://www.expasy.org/prosite/>). Multiple sequence alignments were carried out using the CLUSTALW 1.83 program (<http://www.ebi.ac.uk/clustalw/>) and the unrooted phylogenetic tree was constructed by DNASTar’s LaserGene software (DNASTar, Madison, USA).

mRNA expression analysis

The tissue distribution of porcine *GSTM2* mRNA was determined by semiquantitative reverse transcription-PCR using primer pair 6 with the cDNA from various tissues as templates (Table 1) [12]. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*) was used as an internal control over the template level. The primer pair for *G3PDH* is shown in Table 1. PCR amplification was carried out in a 25 μl reaction mixture as above. The expression level of *GSTM2* relative to *G3PDH* was analyzed with GeneTools from SynGene (Synoptics, Cambridge, UK).

Analysis of gene polymorphisms and identification of NMD

Polymorphisms were detected based on the sequence comparison. The distribution of polymorphisms was analyzed in genomic DNA from Large White, Landrace, Meishan and Qingping pig populations by means of the PCR-restriction fragment length polymorphism (RFLP) technique using primer pair 7 (Table 1).

We selected five pigs with genotype *CT*, analyzed the genotype of this polymorphism site in their cDNA from *longissimus dorsi* muscle by PCR-RFLP using primer pair 8 (Table 1) in order to detect whether allele *T* exists in

GSTM2 cDNA or not.

The reaction mixture and conditions for PCR amplification were as above. PCR products were digested with 5 U of *Taq* I at 65 °C for 5 h in a volume of 10 μl .

Results

PCR amplification and sequence analysis

The cDNA sequences amplified by primer pairs 1 and 2 were assembled into a sequence of 763 bp (GenBank accession No. DQ988117) which contained the complete coding sequence of porcine *GSTM2* gene. The coding sequence had 657 nucleotides, encoding a protein of 218 amino acids. We inferred the ATG codon at nucleotide residue 81–83 to be the true start site of translation, which was homological to that of other species. In addition, it contained the ANNATG start sequence which is typical in vertebrates [13].

Alignment analysis revealed that porcine *GSTM2* gene was composed of eight exons and seven introns. We obtained a DNA sequence of 2523 bp of porcine *GSTM2* (GenBank accession No. DQ988118) which lacked the eighth exon and the seventh intron with primer pairs 3, 4 and 5. The locations of splice donor/acceptor sites in all introns followed the consensus “GT/AG” rule [14] (Table 2).

Similarity comparison showed that the deduced amino acid sequence of porcine *GSTM2* shared 76%, 78% and 76% identity with that of human, mouse and rat, respectively. Primary structure analysis revealed that the molecular weight of the putative *GSTM2* protein was 25.7 kDa and its theoretical pI was 6.91. Prosite analysis predicted there were six potential casein kinase II phosphorylation sites, two protein kinase C phosphorylation sites, an N-myristoylation site, a cAMP- and cGMP-dependent protein kinase phosphorylation site, a tyrosine sulfation site and an amidation site.

Using the porcine *GSTM2* sequence and other sequences available in the GenBank database, we constructed a phylogenetic tree (Fig. 1) using DNASTar software. The results revealed that the porcine *GSTM2* had a closer genetic relationship with the *GSTM2* of mouse, rat, human and macaque than with that of frog.

mRNA expression analysis

The tissue expression profile analysis showed that the porcine *GSTM2* gene was expressed at a high level in liver and testis, at a medium level in *longissimus dorsi* muscle,

Table 2 Summary of the structure and splicing junctions of porcine *GSTM2* gene

Exon number	Splicing acceptor site	Exon length (bp)	Splicing donor site	Intron number	Intron length (bp)
1	None	116	CATCCGCGGGgtgagg	1	268
2	tcccagCTGGCCCACG	76	ATGGGGGACGgtaatg	2	419
3	ccacagCTCCCCACTA	65	CTCCCCAATgtaggt	3	285
4	ttgcagCTGCCCTACT	82	CACAACATGTgtgagt	4	97
5	ccgcagGTGGGGAGAC	101	CCCTGACTTTgtgagt	5	722
6	cctcagGAGAAACTGA	96	AGGGGACAAAGtaagg	6	85
7	ctgcagCTCACCTATG	111	?	7	?
8	?	?	None	None	None

?, information not known.

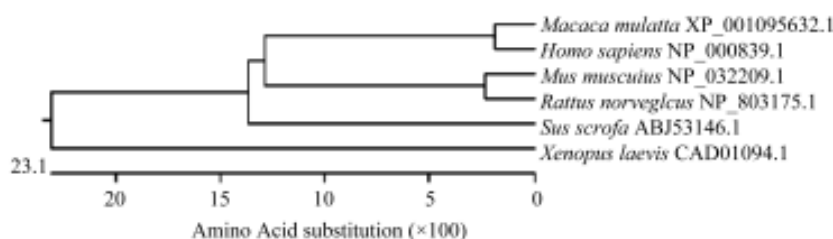


Fig. 1 Phylogenetic tree for six kinds of *GSTM2*

The sequences used for analysis are derived from GenBank, and their accession numbers are shown on the right-hand side. The bootstrap confidence values are shown at the nodes of the tree. The horizontal branch lengths are proportional to the estimated divergence of the sequence from the branch point.

adipose tissue, spleen and lung, at a low level in kidney, and at an even lower level in heart and embryo (**Fig. 2**).

Discovery of PTC and analysis of gene polymorphisms

To search for different *GSTM2* alleles in pig populations, the DNA sequence of *GSTM2* obtained was investigated in three different individual pigs representing three breeds (Large White, Landrace and Meishan). We found many mutations. One of them was special in that the nonsense mutation (CGA→TGA) resulted from C27T substitution in the fifth exon produced a PTC, and a truncated protein of 95 amino acids would be produced if translated; and this single nucleotide mutation disrupted a restriction site for endonuclease *Taq* I. We analyzed this polymorphism in genomic DNA from the four pig populations by means of the *Taq* I-PCR-RFLP technique (**Table 3**) using primer pair 7 (**Table 1**). The size of the PCR product was 504 bp. As shown in **Fig. 3**, genotype *CC* had two fragments (299 bp+205 bp), genotype *CT* had three fragments (504 bp+299 bp+205 bp), and genotype *TT* (504 bp) was not found. Allele *C* had a higher frequency than allele *T* in each population (**Table 3**).

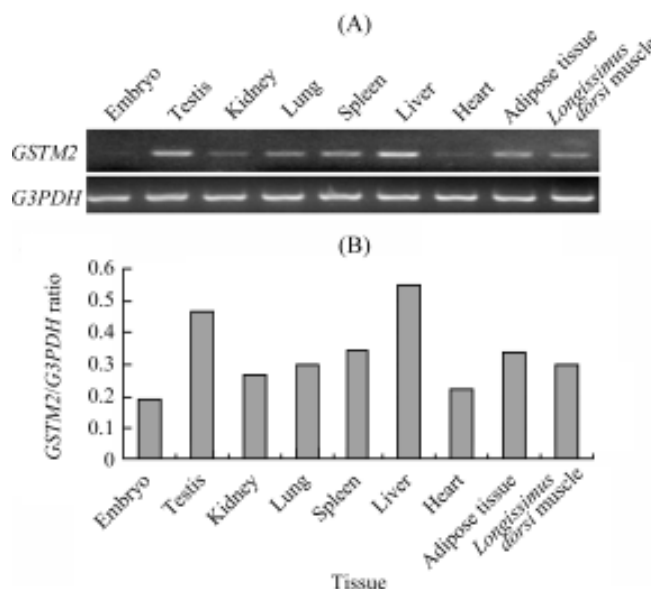
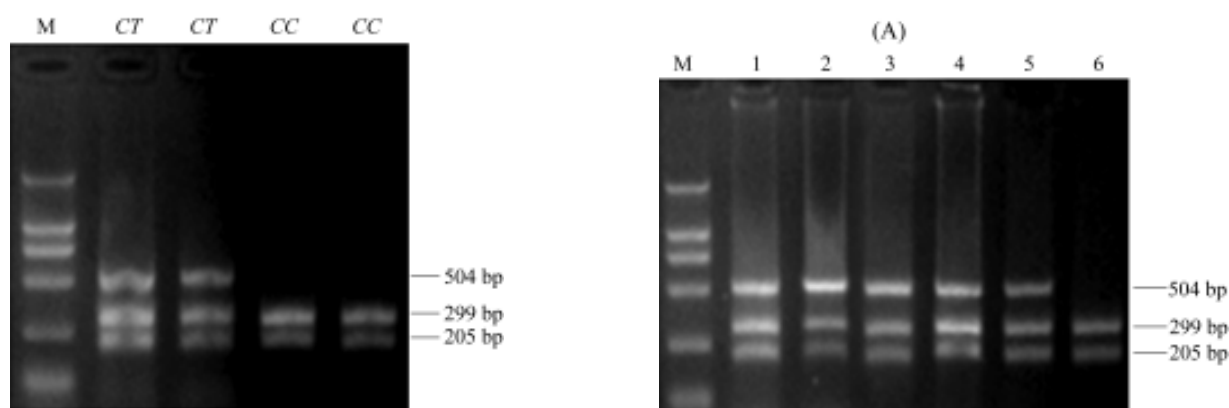


Fig. 2 Tissue expression profile analysis of the porcine *GSTM2* gene

(A) Gel electrophoresis of reverse transcription-polymerase chain reaction. (B) The expression level of *GSTM2* relative to *G3PDH* in various tissues analyzed with GeneTools from SynGene (Synoptics, Cambridge, UK).

Table 3 Polymorphism of *GSTM2* *Taq* I-polymerase chain reaction-restriction fragment length polymorphism with primer pair 7 in different pig populations

Population	No. of pigs	Genotype frequency			Allele frequency	
		<i>CC</i>	<i>CT</i>	<i>TT</i>	<i>C</i>	<i>T</i>
Large White	48	27	21	0	0.78	0.22
Landrace	41	29	12	0	0.85	0.15
Meishan	38	11	27	0	0.64	0.36
Qingping	37	20	17	0	0.77	0.23

**Fig. 3** Restriction pattern of the amplification with primer pair 7 digested with *Taq* I

M, DL2000.

Identification of NMD and mRNA expression analysis of porcine *GSTM2* with different genotypes

As we failed to find genotype *TT* in all populations, in order to detect whether allele *T* exists in cDNA or not when it exists in genomic DNA, we selected five pigs with genotype *CT*, analyzed the genotype of this polymorphism site in their cDNA by *Taq* I-PCR-RFLP using primer pair 8 with the cDNA from *longissimus dorsi* muscle as template. The size of the PCR product was 497 bp. If there is allele *T* in cDNA, there must be a fragment of 497 bp after the digestion of PCR product with *Taq* I. However, the PCR products of these five pigs were all digested into two fragments (302 bp+195 bp) and there was no fragment of 497 bp (Fig. 4), which suggested that there was only allele *C* and no allele *T* in *GSTM2* cDNA of all these five pigs. Therefore it was proved that allele *T* was absent in porcine *GSTM2* cDNA when it existed in genomic DNA. This result indicated that, because of the existence of PTC, the mRNA of allele *T* might be degraded by NMD path.

The expression of *GSTM2* in the cDNA from *longissi-*

Fig. 4 Genotyping of *GSTM2* in genomic DNA and cDNA of the five pigs by *Taq* I-polymerase chain reaction (PCR)-restriction fragment length polymorphism

(A) Genotyping of *GSTM2* in genomic DNA with primer pair 7. M, DL2000; 1–5, primer pair 7 PCR products digested with *Taq* I of the five pigs with genotype *CT*; 6, *CC* homozygote control. (B) Genotyping of *GSTM2* in cDNA with primer pair 8. M, DL2000; 1–5, primer pair 8 PCR products digested with *Taq* I of the five pigs in (A).

mus dorsi muscle of pigs with genotype *CC* and genotype *CT* was detected by semiquantitative reverse transcription-PCR using primer pair 6. The result showed that

GSTM2 gene was expressed at a higher level in pigs with genotype *CC* than with genotype *CT* (Fig. 5).

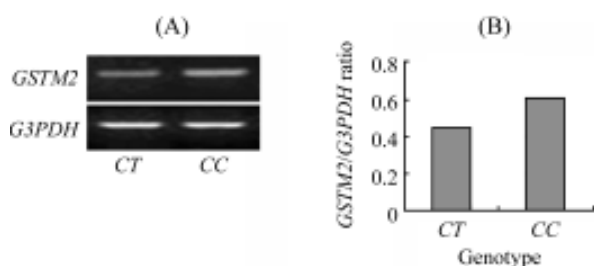


Fig. 5 mRNA expression analysis of porcine *GSTM2* with different genotypes

(A) Gel electrophoresis of reverse transcription-polymerase chain reaction. (B) The expression level of *GSTM2* relative to *G3PDH* analyzed with GeneTools from SynGene (Synoptics, Cambridge, UK).

Discussion

In previous research in our laboratory, we isolated several porcine genes by various methods, such as mRNA differential display and suppression subtractive hybridization [15,16]. In this research, we first cloned the porcine *GSTM2* gene using *in silico* cloning and PCR.

GSTs play an important role in cellular detoxification by catalyzing the conjugation of glutathione with hydrophobic xenobiotic and endobiotic compounds [17,18]. This detoxification process is an important cellular defense mechanism which can protect the cell from a wide range of toxic and carcinogenic substances [19,20]. Further studies indicated that every subclass of GST had substrate specificity. The class mu isoenzymes are most effective in detoxifying against styrene oxide, 1-nitropyrene oxide, epoxides such as benzo(a)pyrene 7,8-diol-9,10-epoxide and endogenous reactive species such as lipid and DNA hydroperoxides [17,21,22]. In addition, it was suggested that the lack of GST mu expression was linked to cancer susceptibility [22].

In our study, we discovered a PTC in porcine *GSTM2* gene and found that NMD might occur in the regulation of porcine *GSTM2* gene expression. At present, there is no report on this PTC and NMD effect in the *GSTM2* gene from pig or other species.

It is regarded that the NMD mechanism which monitors and eliminates PTC-mRNA is beneficial to both the effectual synthesis of functional protein and self-protection of cells, for PTC-mRNA translation generally produces truncated

protein which often has no activity or even does harm to the normal function of cells [23]. In our study, porcine *GSTM2* has a deduced protein of 218 amino acids, whereas its PTC-mRNA translation, if possible, will produce a truncated protein of 95 amino acids.

More researches show that in mammalian cells whether nonsense mRNA is degraded by the NMD path or not is decided by the relative distance between PTC and the downstream exon-exon junction which is adjacent to the PTC or nearest to the 3' end. When this distance is more than 50–55 bp, the nonsense mRNA will be degraded by the NMD path. When it is less than 50–55 bp, it will not be degraded [24,25]. In our research, in the porcine *GSTM2* gene the distance between the PTC we have found and the downstream exon-exon junction was more than 50 bp. This further suggested that the nonsense mRNA might be degraded by the NMD path, therefore we did not detect the corresponding allele *T* in cDNA. Of course, more work is needed to confirm this.

According to present results, many gene mutations which lead to human diseases are nonsense mutations [23]. NMD is closely related to many human diseases [26–28]. The function of *GSTM2* and the association between PTC and diseases implies that the existence of PTC in porcine *GSTM2* might relate to certain disease. In this study, we failed to find the *TT* homozygote genotype of the nonsense mutation site. Thus, it is worth increasing samples to find a *TT* homozygote, and investigating its biological effect and its relationship with diseases. Moreover, in our study it has been discovered that there was difference between the expression levels of *GSTM2* genotype *CC* and genotype *CT*. We will further study the influence of this difference on the porcine phenotype, particularly on the important economic traits.

References

- 1 Richard CS, Peter WJ, Anthony AF. Glutathione S-transferase genetics and role in toxicology. *Toxicol Let* 2000, 112: 357–363
- 2 Fanucchi MV, Buckpitt AR, Murphy ME, Storms DH, Hammock BD, Plopper CG. Development of phase II xenobiotic metabolizing enzymes in differentiating murine clara cells. *Toxicol Appl Pharmacol* 2000, 168: 253–267
- 3 Pearson WR, Vorachek WR, Xu SJ, Berger R, Hart I, Vannais D, Patterson D. Identification of class-mu glutathione transferase genes *GSTM1-GSTM5* on human chromosome 1p13. *Am J Hum Genet* 1993, 53: 220–233
- 4 Maquat LE, Carmichael GG. Quality control of mRNA function. *Cell* 2001, 104: 173–180
- 5 Couttet P, Grange T. Premature termination codons enhance mRNA decapping in human cells. *Nucleic Acids Res* 2004, 32: 488–494
- 6 González CI, Bhattacharya A, Wang W, Peltz SW. Nonsense mediated

- mRNA decay in *Saccharomyces cerevisiae*. *Gene* 2001, 274: 15–25
- 7 Dahlseid JN, Lew-Smith J, Lelivelt MJ, Enomoto S, Ford A, Desruisseaux M, McClellan M *et al.* mRNAs encoding telomerase components and regulators are controlled by UPF genes in *Saccharomyces cerevisiae*. *Eukaryot Cell* 2003, 2: 134–142
 - 8 Ionov Y, Nowak N, Perucho M, Markowitz S, Cowell JK. Manipulation of nonsense mediated decay identifies gene mutations in colon cancer cells with microsatellite instability. *Oncogene* 2004, 23: 639–645
 - 9 Liu HX, Cartegni L, Zhang MQ, Krainer AR. A mechanism for exon skipping caused by nonsense or missense mutations in BRCA1 and other genes. *Nature Genetics* 2001, 27: 55–58
 - 10 Ruiz-Echevarria MJ, Czaplinski K, Peltz SW. Making sense of nonsense in yeast. *Trends Biochem Sci* 1996, 21: 433–438
 - 11 Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning: A Laboratory Manual*. 2nd ed. New York: Cold Spring Harbor Laboratory Press 1989
 - 12 Kousteni S, Tura-Kockar F, Ramji DP. Sequence and expression analysis of a novel *Xenopus laevis* cDNA that encodes a protein similar to bacterial and chloroplast ribosomal protein L24. *Gene* 1999, 235: 13–18
 - 13 Kozak M. An analysis of 5′ noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res* 1987, 15: 8125–8128
 - 14 Breathnach R, Benoist C, O’Hare K, Gannon F, Chambon P. Ovalbumin gene: Evidence for a leader sequence in mRNA and DNA sequences at the exon–intron boundaries. *Proc Natl Acad Sci USA* 1978, 10: 4853–4857
 - 15 Liu YG, Xiong YZ, Deng CY. Isolation, sequence analysis and expression profile of a novel swine gene differentially expressed in the *longissimus dorsi* muscle tissues from Landrace×Large White cross-combination. *Acta Biochim Biophys Sin* 2005, 37: 186–191
 - 16 Huang T, Xiong YZ, Lei MG, Xu DQ, Deng CY. Identification of a differentially expressed gene *PPP1CB* between porcine *longissimus dorsi* of Meishan and Large White×Meishan hybrids. *Acta Biochim Biophys Sin* 2006, 38: 450–456
 - 17 Mannervik B, Danielson UH. Glutathione transferases-structure and catalytic activity. *CRC Crit Rev Biochem* 1988, 23: 283–337
 - 18 Armstrong RN. Structure, catalytic mechanism, and evolution of the glutathione transferases. *Chem Res Toxicol* 1997, 10: 2–18
 - 19 Hansson LO, Bolton-Grob R, Massoud T, Mannervik B. Evolution of differential substrate specificities in Mu class glutathione transferases probed by DNA shuffling. *J Mol Biol* 1999, 287: 265–276
 - 20 Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: Regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* 1995, 30: 445–600
 - 21 Ketterer B. Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis. *Mutat Res* 1988, 202: 343–361
 - 22 Seidegard J, Pero RW, Miller DG, Beattie EJ. A glutathione transferase in human leukocytes as a marker for the susceptibility to lung cancer. *Carcinogenesis* 1986, 7: 751–753
 - 23 Culbertson MR. RNA surveillance. Unforeseen consequences for gene expression, inherited genetic disorders and cancer. *Trends Genet* 1999, 15: 74–80
 - 24 Carter MS, Li S, Wilkinson MF. A splicing-dependent regulatory mechanism that detects translation signals. *EMBO J* 1996, 15: 5965–5975
 - 25 Nagy E, Maquat LE. A rule for termination-codon position within intron-containing gene: When nonsense affects RNA abundance. *Trends Biochem Sci* 1998, 23: 198–199
 - 26 Holbrook JA, Neu-Yilik G, Hentze MW, Kulozik AE. Nonsense-mediated decay approaches the clinic. *Nat Genet* 2004, 36: 801–808
 - 27 Noensie EN, Dietz HC. A strategy for disease gene identification through nonsense-mediated mRNA decay inhibition. *Nat Biotechnol* 2001, 19: 434–439
 - 28 Montagna M, Agata S, De Nicolo A, Menin C, Sordi G, Chieco-Bianchi L, D’Andrea E. Identification of BRCA1 and BRCA2 carriers by allele-specific gene expression (AGE) analysis. *Int J Cancer* 2002, 98: 732–736

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