DOI: 10.1007/s11427-006-2029-7

The single nucleotide polymorphisms of chicken melanocortin-4 receptor (*MC4R*) gene and their association analysis with carcass traits

QIU Xuemei[1, 2], LI Ning[1], DENG Xuemei[3], ZHAO Xingbo[3], MENG Qingyong[1] & WANG Xiuli[2]

1. State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing 100094, China;
2. College of Life Science and Technology, Dalian Fisheries University, Dalian, 116023, China;
3. College of Animal Science and Technology, China Agricultural University, Beijing 100094, China

Correspondence should be addressed to Li Ning (email: ninglbau@public3.bta.net.cn)

Received December 9, 2005; accepted February 6, 2006

Abstract Mutations of the melanocortin-4 receptor (*MC4R*) gene are associated with the appetite, obesity and growth in pig, mice and human. But little is known about the function of chicken *MC4R* gene. In this study, F_2 chicken resource population derived from broilers crossing to Silky was screened for the polymorphisms of the *MC4R* gene using PCR-single strand conformation polymorphism (PCR-SSCP) and DNA sequencing methods. Four single nucleotide polymorphisms (SNPs) sites were found. The mutation (C→T) in the 5′regulation region of chicken *MC4R* gene results in one more NF-E2 and cap transcription factor binding sites in the mutation allele than in the wild allele. One missense mutation (G→A) occurs in the coding region (61nt), which changes the glycine to arginine. Moreover, in the coding region there are 2 synonymous mutations, one G→T mutation at 315nt and one C→T mutation at 336nt. Least square analysis of the SNPs and carcass traits showed that *BB*, *DD* and *FF* genotypes are significantly associated with body weight, carcass weight (or half carcass weight), and leg muscle weight (P<0.05 or P<0.01). But no significant association between the genotypes and abdominal fat weight is found. The results present the evidence that the chicken *MC4R* gene can be selected as the major candidate gene for the carcass traits such as body weight and growth.

Keywords: melanocortin-4 receptor, SNPs, chicken, carcass traits.

The melanocortin-4 receptor (MC4R), one of the five members of the melanocortin family, is a peptide secreted in the ventromedial hypothalamus[1]. MC4R belongs to the G-protein coupled receptors (GPCRS) super family and is a transmembrane neuron receptor[2,3]. When coupled with alpha melanocyte-stimulating hormone (α-MSH), MC4R can inhibit the increase of the body weight[4]. In the mammals, MC4R can mediate the function of leptin and is a vital signal factor for the regulation of energy balance and energy dynamic balance[2]. Its main function is to control the appetite, body weight and energy metabolism[5,6]. Dubern *et al.* (2001) [7] searched for the mutations in *MC4R*, agouti-related protein (AGRP), and α-MSH

genes in 63 severely obese children by direct sequencing of the *MC4R* encoding sequence and SSCP analysis of AGRP and α-MSH genes. The results showed that expression of the obese phenotype was variable in mutation-positive family members. And they made a conclusion that the *MC4R* mutations may be a non-negligible cause of severe obesity in children with variable expression and penetrance. Rosmond *et al*.[8] (2001) studied the missense mutation of the *MC4R* gene in human. Their findings suggested that the missense mutation could contribute to the variability in body mass, abdominal fat distribution, leptin concentrations and diurnal cortisol levels. So they proposed the hypothesis that the inactivation of the *MC4R* by gene targeting could cause obesity. Hussar *et al*. (1997)[9] found that inactivation of the *MC4R* by gene targeting resulted in a maturity onset obesity syndrome of mice. Kwan *et al*.[10] (2000) studied one missense mutation in the pig *MC4R* gene. Their results showed that *MC4R* genotypes are significantly associated with backfat and growth rate in a number of lines as well as feed intake overall.

The chicken *MC4R* gene is a single copy gene with only one exon. Its coding sequence is 996 bp in length. The chicken *MC4R* gene encodes a 332 amino acid protein, sharing 86.4%—88.1% identity with human and mouse analogs. The chicken *MC4R* gene is expressed in adrenal, gonads, spleen, adipose tissues, as well as in the brain[11]. Compared with the abundant research in the mammals[12−18], little is reported about the detail of the function of the chicken *MC4R* gene. According to the characteristics of chicken growth and development and the expression of the *MC4R* gene, it is reasonable to select the *MC4R* gene as the candidate gene for the growth and carcass traits in chicken[19,20]. In this study, we searched the SNPs of the *MC4R* gene and analyzed their association with growth and carcass traits in the China Agricultural University (CAU) chicken resource population. The objective is to lay a foundation for the better understanding of the chicken *MC4R* gene and its further application for the chicken molecular breeding.

1 Materials and methods

1.1 Materials

(1) Experimental animals. The China Agricultural University (CAU) chicken resource population was established by reciprocal crossing of Broilers and Chinese Silky chicken. A total of 730 F_2 chickens from 4 lines were used in this study.

(2) Main reagents and apparatus. The main reagents were Taq E and protein K (MERCK Co.), 10×PCR buffer (500 mmol/L KCl, 100 mmol/L Tris-HCl, 15mmol/L $MgCl_2$, 1% glutin) and dNTPs (Amersham Pharmacia Co.), and gene clean III kit(Qbiogene Co.). Other reagents were purchased from the Supply Department of CAU. The main apparatus were Gene Amp PCR System 9700 (PERKIN ELMER), DYY—III2 electrophoresis system(Beijing Liuyi Instrument Factory) and ABI 377 DNA sequencer (PERKIN ELMER).

(3) Primers. According to the chicken *MC4R* gene sequence (GenBank accession No. AB012211), 11 pairs of primer were designed by Oligo primer software (version 6.0) and synthesized by Shanghai Sangon Bio. Co. The primer sequences, location and size of the amplified fragments are shown in Table 1.

1.2 Methods

(1) Collection of samples and carcass traits records.

The F_2 chickens were slaughtered at age of 12 weeks and blood was collected and stored at −20℃. The body weight, breast muscle weight, leg muscle weight, half carcass weight, carcass weight and abdominal fat weight were scored. The methods and procedures for the carcass trait were shown by Wang *et al*. (2000)[21].

(2) DNA extraction. The genomic DNA was extracted by the traditional phenol/chloroform method[22] and dissolved in sterile water with concentration of 100 ng/μL and stored at −20℃.

(3) PCR reaction. PCR amplification was performed in a reaction volume of 25 μL including 50 ng of genomic DNA, 25 pmol of each primer, 2.5 μL 10 × PCR buffer and 1.5 unit Taq DNA polymerase. The condition for PCR was as follows: 5 min at 94℃; 35 cycles of 30 s at 94℃, 30 s at 56℃, 30 s at 72℃; and a final 7 min extension at 72℃.

(4) SSCP analysis and DNA sequencing. 1 μL of the PCR product was diluted with 5 μL of loading

Table 1 Sequence and location of the primers of chicken *MC4R* gene

Primers	Sequence of primers	Location	Size of PCR products（bp）
P1	forward primer: 5′-AAGCTTGCGCACATCCAAGT-3′		
	reverse primer: 5′-GCTGCCGAGCAGAAACTAAT-3′	–608—–377	232
P2	forward primer: 5′-GCAGCAGTCACTTGAGCATT-3′		
	reverse primer: 5′-GCGCTCTCAAAGATGCAGAT-3′	–380—–180	201
P3	forward primer: 5′-CTTTGAGAGCGCAAACCAGT-3′		
	reverse primer: 5′-TGCTGGGTGAAATTCATCTT-3′	–191—18	210
P4	forward primer: 5′-CTCCAGCCTCTCCATTTCTG-3′		
	reverse primer: 5′-GCGAATGGAGGTTCTTGTTC-3′	28—229	202
P5	forward primer: 5′-GCCAAGAACAAGAACCTCCA-3′		
	reverse primer: 5′-AATTGATGCAAGCAGGGAAC-3′	205—408	204
P6	forward primer: 5′-AGTTCCCTGCTTGCATCAAT-3′		
	reverse primer: 5′-CAGATGATGACAACGCTGCT-3′	388—584	197
P7	forward primer: 5′-CATTCTCATGGCATCCCTTT-3′		
	reverse primer: 5′-GGATTGTAGGGGCAGGAGAT-3′	612—821	210
P8	forward primer: 5′-AGGGGCCATCACTCTCACTA-3′		
	reverse primer: 5′-GAGCTCCTGACTCCGAAATG-3′	723—924	202
P9	forward primer: 5′-GGAGTCAGGAGCTCAGGAAA-3′		
	reverse primer: 5′-GGAGGGAAGGGAAAGAGAAA-3′	911—1116	206
P10	forward primer: 5′-CCTTCCCTCCCATCTTTGTA-3′		
	reverse primer: 5′-TGCACTGTAACTCAGGCAAGTT-3′	1107—1337	231
P11	forward primer: 5′-TGCATATTGGTTGATAATGTTCC-3′		
	reverse primer: 5′-CAGGATTGCTGCTGTTTGAG-3′	1334—1546	213

buffer (98% formamide, 10 mmol/L EDTA pH8.0, 0.025% Xylene cyanol FF, 0.025% bromophenol blue, 2% Glycerol). After denaturation at 98℃ for 10 min, the mixture was immediately placed on ice for 10 min and then loaded on a 15% acrylamide/bisacrylamide (arc∶bis=29∶1) gel. After running at 10 V/cm for 14—16 h, the gel was stained by using the silver staining method. For each homozygote 3 PCR products were purified, recovered and sequenced by the ABI377 sequencer.

(5) Prediction of the transcription factor binding sites. Prediction of the transcription factor binding sites was carried out on line at the website of http://www.cbrc.jp/research/db/TFSEARCH.html.

(6) Statistical analyses of the association between the SNPs and carcass triats. The PROC-GLM procedure of SAS software package (version 8.2) was carried out for the association between the SNPs and carcass traits. The linear model was as follows: $y = \mu + j + s + r + e$, where y is carcass traits, μ is the least square mean of the carcass traits, j is the effect of genotype, s is the effect of sex., r is the effect of reciprocal crossing, and e is the residual effect.

2 Results

2.1 PCR-SSCP results and analysis

PCR amplification and SSCP analysis were carried out on the 2224 nt of chicken *MC4R* gene using 11 pairs of primer. The results showed that 3 pairs of primer (P1, P4 and P5) could result in the polymorphisms. The primer pair P1 is located in the 5′ regulation region of the chicken *MC4R* gene and can yield 232 bp fragments. Among the three genotypes identified by SSCP (Fig. 1(a)), the homozygote *AA* is defined as the wild genotype, *BB* as mutation genotype. The results of PCR-SSCP of primer pair P4 are shown in Fig. 1(b). The homozygote *CC* is consistent with the sequence of GenBank (AB012211). Fig. 1(c) shows the result of PCR-SSCP of primer pair P5.

2.2 The result of cloning and sequencing of polymorphic fragments

Each homozygote genotype found in each primer pair has been sequenced 3 times at least. Comparison of the sequences with the known chicken *MC4R* gene sequence (GenBank accession no. AB012211) showed 4 polymorphic sites. The location of the 4 polymor-

phic sites, substitution of nucleotide sequence and amino acid sequence, as well as the nucleotide sequence of different genotypes are listed in Table 2. In this study, the first nucleotide of the coding region of the chicken *MC4R* gene is defined as +1. A C→T mutation, located at –524, is found in the fragment amplified by primer pair P1. The G→A mutation at +61 is a missense mutation, which makes the 61st amino acid change from glycine (G) to arginine (R). Two mutations, G→T mutation at +315 and C→T mutation at +336, exist in the PCR fragments of primer pair P5. They are synonymous mutations. The sequencing results of different genotypes are shown in Fig. 2.

2.3 Prediction analysis of the potential transcription factor binding sites in the 5′ regulation region of chicken MC4R gene

The potential transcription factor binding sites in the 5′ regulation region of chicken *MC4R* gene were analyzed on line through the website of http://www.cbrc.jp/research/db/TFSEARCH.html. The results show that in the 5′ regulation region of chicken MC4R there are one more NF-E2 and cap transcript factor binding sites in the mutation *BB* genotype than in the wild *AA* genotype (Table 3).

2.4 Least square analysis of the association between different genotypes of the chicken MC4R gene and carcass traits

Table 4 shows the results of least square analysis of the association between the different genotypes in the 5′ regulation region of the chicken *MC4R* gene and carcass traits in the F2 resource population. The results showed that the body weight, half carcass weight and leg muscle weight of the *BB* genotype are significantly higher than those of *AA* and *AB* genotype ($P<0.05$).

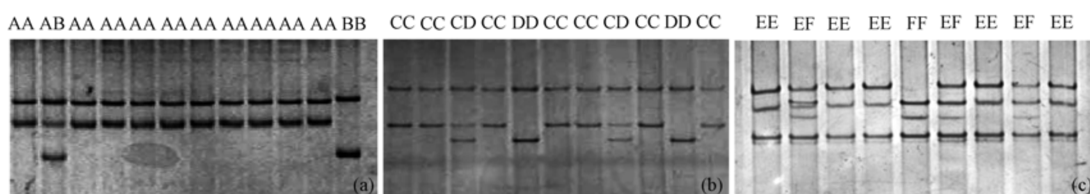

Fig. 1. SSCP analysis of different primer pairs. (a)SSCP analysis of primer pair P1, (b) SSCP analysis of primer pair P4, (c) SSCP analysis of primer pair P5.

Table 2 Comparison result of the sequences of different genotypes

Genotype	Nucleotide sequence	Location of mutation	Change of nucleotide	Change of amino acid
GenBank	**actttctgca gtgccgagtc ccattggctg**			
AA	actttctgca gtgccgagtc ccattggctg			
BB	actttctgca gtgctgagtc ccattggctg	–524	C→T	
GenBank	**ttctggaacc agagcaacgg actgcacagg**			
CC	ttctggaacc agagcaacgg actgcacagg			
DD	ttctggaacc agagcaacag actgcacagg	61	G→A	G21R
GenBank	**ctgctaaaca atacagatac agacgcacag**			
EE	**ctgctaaaca atacagatac agacgcacag**			
FF	**cttctaaaca atacagatac agatgcacag**	315; 336	G→T; C→T	L105L; D112D

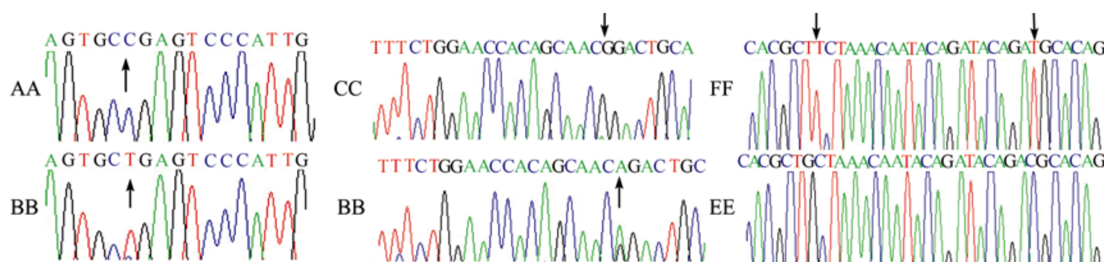

Fig. 2. Partial sequencing results of different genotypes.

Table 3 The prediction results of the transcription factors in the 5′regulation region of the chicken *MC4R* gene

The binding sites of *AA* genotype	The binding sites of *BB* genotype
	HSF
HSF	HSF
HSF	HSF
HSF	HSF
HSF	NF-E2
ADR1	ADR1
CCAAT	cap
cap	CCAAT
GATA-1	cap
NF-1	GATA-1
cap	NF-1
cap	cap
CdxA	cap
STRE	CdxA
NF-Y	STRE
cap	NF-Y
	cap

But for the breast muscle weight and abdominal fat weight traits, the difference among the 3 genotypes does not reach the significant level (P>0.05).

The results of least square analysis of the association between the different genotypes for primer pair P4 of the chicken *MC4R* gene and carcass traits in the F2 resource population are listed in Table 5. The body weight and half carcass weight of the *DD* genotype are significantly higher than those of *CC* genotype (P<0.05). But for the breast muscle weight, leg muscle weight and abdominal fat weight traits, the difference among the 3 genotype is not significant (P > 0.05).

The least square analysis was also carried out about the association between the different genotypes for primer pair P5 of the chicken *MC4R* gene and carcass traits. The results in Table 6 show that the chicken with *FF* genotype have significantly higher body weight, carcass weight, breast muscle weight and leg muscle weight than that with *EE* genotype (P<0.05). The abdominal fat weight has no significant difference among the 3 genotypes *EE*, *EF* and *FF* (P>0.05).

3 Discussion

In the mammals, the *MC4R* gene functions to control the appetite, body weight, energy metabolism and obesity[7−13]. According to this, we studied the chicken *MC4R* gene for its effect on the growth and carcass traits. Through the PCR-SSCP analysis of the whole sequence of chicken *MC4R* gene, 4 SNPs were found, 1 in the 5′ regulation region and 3 in the coding region.

The regulation sequence DNA may contain the information for the selective transcription of the gene. It can interact with regulation protein to modify the helix structure so as to be more easily distinguished by the regulation protein. The C→T mutation found in this study results in one more NF-E2 protein binding site and one transcription initializing factor cap signal. The increase of the transcription factor and the change of their sites may increase the quantity of the expression of the *MC4R* gene [23,24]. This may lead to the difference of MC4R protein so as to affect the growth and carcass performance. The result of least square analysis

Table 4 The least square analysis of the SSCP polymorphism in the 5′ regulation region and carcass traits (average value ± standard error)

Genotype	Number	Body weight	Half carcass weight	Breast muscle weight	Leg muscle weight	Abdominal fat eight
AA	195	1660.16±27.53a[a)]	1167.62±21.93a	93.28±2.30a	126.01±3.06a	45.14±2.86a
AB	50	1599.20±42.08a	1101.12±33.53a	91.91±3.52a	122.00±4.67a	41.84±4.37a
BB	18	1777.62±70.18b	1269.88±55.92b	100.68±5.86a	145.28±7.80b	49.89±7.29a

a) Least squares means within a row without a common superscript letter differ significantly. The same for the following tables.

Table 5 The least square analysis of the SSCP polymorphism of primer pair P4 of *MC4R* and carcass traits (average value ± standard error)

Genotype	Number	Body weight	Half carcass weight	Breast muscle weight	Leg muscle weight	Abdominal fat weight
CC	310	1623.49±21.10a	1135.29±16.67a	91.42±1.643a	126.70±2.07a	46.58±2.13a
CD	260	1685.45±22.39b	1177.89±17.69b	94.09±1.74a	129.64±2.19a	45.70±2.26a
DD	37	1717.62±49.07b	1213.88±38.78b	93.62±3.82a	133.16±4.82a	41.68±4.96a

Table 6 The least square analysis of the SSCP polymorphism of primer pair P5 of *MC4R* and carcass traits (average value ± standard error)

Genotype	Number	Body weight	Carcass weight	Breast muscle weight	Leg muscle weight	Abdominal fat weight
EE	101	1605.84±29.30a	1024.80±20.85a	89.68±2.29a	124.35±2.87a	42.70±3.03a
EF	247	1665.94±21.05b	1061.83±14.98b	93.10±1.64b	129.18±2.06b	47.78±2.17a
FF	168	1697.45±26.03b	1078.83±18.52b	96.65±2.033b	131.75±2.55b	44.78±2.69a

confirmed the significant association between the *BB* genotype and body weight, half carcass weight and leg muscle weight. But little is done about the structure and regulation elements of the *MC4R* gene. So it is unclear about the exact mechanism of the effect of the nucleotide change in the 5’ regulation region on the chicken carcass traits and further study should be conducted.

There are 3 polymorphic sites in the coding region of the chicken *MC4R* gene. The missense mutation (G→A) at +61 changes the amino acid glycine to arginine. This amino acid change may result in the change of the activity of the MC4R protein so as to affect its function. Results of the least square analysis of relationship between the different genotypes and carcass traits showed that the missense mutation can affect the chicken growth and carcass traits. The chickens with *DD* genotype have significantly higher body weight and half carcass weight (P<0.05). The other 2 mutations, G→T mutation at +315 and C→T mutation at +336, are synonymous mutations with no change of the amino acid sequence. But body weight, carcass weight, breast muscle weight, leg muscle weight of the mutation *FF* genotype are significantly higher than those of *EE* genotype (P<0.05). All these results show that the mutations can affect the yield of chicken meat and increase the chicken body weight. But the mechanism is still not known.

According to the above results, the mutations in the 5′ regulation and coding region of the chicken *MC4R* gene have significant effect on the body weight and carcass traits and little effect on the abdominal fat weight. This is not consistent with the majority of the references which show that the *MC4R* mainly affects the body weight and obesity [10−13]. But the results in this study confirm the reports by Takeuchi *et al.* (1998), that is, the chicken *MC4R* gene is expressed in brain, fat, adrenal and gonad tissue, while the mammal *MC4R* gene is only expressed in brain, there is significant difference between them[14].

The results in this study show that the mutations in the 5′ regulation region and coding region of the chicken *MC4R* gene are the main reasons for the variance of growth and carcass traits. The results of least square analysis also confirm the significant association between the polymorphisms and body weight, growth and carcass traits. So the *MC4R* gene is the major candidate gene for the chicken growth and carcass traits. The further study of the *MC4R* gene would lay a good foundation for chicken molecular breeding and production.

Acknowledgements This work was supported by the Funds for Creative Research Groups of National Natural Science Foundation of China, the Special Funds for Major State Basic Research of China (Grant No. G20000161).

References

1 Yeo G S, Farooqi I S, Challis B G, et al. The role of melanocortin signalling in the control of body weight: Evidence from human and murine genetic models. QJM, 2000, 93(1): 7－14[DOI]

2 Andersson L. Melanocortin receptor variants with phenotypic effects in horse, pig, and chicken. Ann N Y Acad Sci, 2003, 994: 313－318

3 Xu B, Goulding E H, Zang K, et al. Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci, 2003, 6(7): 736－742[DOI]

4 Sinha P S, Schioth H B, Tatro J B. Roles of the melanocortin-4 receptor in antipyretic and hyperthermic actions of centrally administered alpha-MSH. Brain Res, 2004, 1001(1－2): 150－158[DOI]

5 Hoggard N, Hunter L, Duncan J S, et al. Regulation of adipose tissue leptin secretion by alpha-melanocyte-stimulating hormone and agouti-related protein: Further evidence of an interaction between leptin and the melanocortin signalling system. J Mol Endocrinol, 2004, 32(1): 145－153[DOI]

6 Costa J L, Brennen M B, Hochgeschwender U. The human genetics of eating disorders lessons from the leptin/melanocortin system. Child Adolesc Psychiatr Clin N Am, 2002, 11(2): 387－397[DOI]

7 Dubern_B, Clement K, Pelloux V, et al. Mutational analysis of melanocortin-4 receptor, agouti-related protein, and alpha-melanocyte-stimulating hormone genes in severely obese children. J Pediatr, 2001, 139(2): 204－209[DOI]

8 Rosmond R, Chagnon M, Bouchard C, et al. A missense mutation in the human melanocortin-4 receptor gene in relation to abdominal obesity and salivary cortisol. Diabetologia, 2001, 44(10): 1335－1338[DOI]

9 Huszar D, Lynch C A, Fairchild-Huntress V. Targeted disruption of the melanocortin-4-reeptor results in obesity in mice. Cell, 1997, 88(1): 131－141[DOI]

10 Kim K S, Larsen N, Short T, et al. A missense variant of the porcine melanocortin-4 receptor (MC4R) gene is associated with fatness, growth, and feed intake traits. Mammalian Genome, 2000, 11(2): 131－135[DOI]

11 Takeuchi S, Takahashi S. Melanocortin receptor genes in the chicken——Tissue distributions. Gen Comp Endocrinol, 1998, 112(2): 220－231[DOI]
12 Srinivasan S, Lubrano-Berthelier C, Govaerts C, et al. Constitutive activity of the melanocortin-4 receptor is maintained by its N-terminal domain and plays a role in energy homeostasis in humans. J Clin Invest, 2004, 114(8): 1158－1164[DOI]
13 Ma L, Tataranni P A, Bogardus C, et al. Melanocortin 4 receptor gene variation is associated with severe obesity in Pima Indians. Diabetes, 2004, 53(10): 2696－2699
14 Lubrano-Berthelier C, Le Stunff C, Bougneres P, et al. A homozygous null mutation delineates the role of the melanocortin-4 receptor in humans. J Clin Endocrinol Metab, 2004, 89(5): 2028－2032[DOI]
15 Starowicz K, Bilecki W, Sieja A, et al. Melanocortin 4 receptor is expressed in the dorsal root ganglions and down-regulated in neuropathic rats. Neurosci Lett, 2004, 358(2): 79－82[DOI]
16 Valli-Jaakola K, Lipsanen-Nyman M, Oksanen L, et al. Identification and characterization of melanocortin-4 receptor gene mutations in morbidly obese finnish children and adults. J Clin Endocrinol Metab, 2004, 89(2): 940－945[DOI]
17 Valle E, Habermann F A, Moore S S, et al. Genomic localization and SNP discovery in the bovine melanocortin receptor 4 gene (MC4R). Anim Genet, 2004, 35(4): 351－352[DOI]
18 Houston R D, Cameron N D, Rance K A. A melanocortin-4 receptor (MC4R) polymorphism is associated with performance traits in divergently selected Large White pig populations. Anim Genet, 2004, 35(5): 386－390[DOI]
19 Qiu X M, Li N, Deng X M, et al. Progress in Candidate Genes Influencing Meat Quality Traits in Chickens. Hereditas (Beijing) (in Chinese), 2002, 24(5): 571－574
20 Qiu, X M, Li N, Wu C X, et al. Chromosomal Mapping of Chicken *MC4R* Using a Radiation Hybrid Panel and the Comparative Analysis of the Gene Homologous Regions Between Chicken and Human Chromosome. Acta Genet Sin (in Chinese), 2004, 31(12):1356－1360
21 Wang Q G, Li N, Deng X M, et al. Single nucleotide polymorphism analysis on chicken extraceullar fatty acid binding protein gene and its associations with fattiness trait. Sci China Ser C-Life Sci, 2001, 44(4): 429－434
22 Sambrook J, Fritsch E F, Maniatis T, Molecular Cloning: A Laboratory Manual, 2nd. New York: Cold Spring Harbor Laboratory Press, 1989
23 Doevendans P A, van Bilsen M. Transcription factors and the cardiac gene programme. Int J Biochem Cell Biol, 1996, 28(4): 387－403[DOI]
24 Cormier P, Pyronnet S, Salaun P, et al. Cap-dependent translation and control of the cell cycle. Prog Cell Cycle Res, 2003, 5: 469－475