Genetic polymorphism of GHR\(\text{Ssp}\) and Pit1\(\text{Stu}\) loci of twin and multiple births in local peranakan Ongole cattle

A Anggraeni\(^1\), C Talib\(^1\), S A Asmarasari\(^1\), F Saputra\(^1\) and R Misrianti\(^2\)

\(^1\)Indonesian Research Institute for Animal Production, Ciawi, Bogor 16720
\(^2\)Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau

Email: ria.anneke@yahoo.co.id

Abstract. Twin or multiple births may happen in beef cattle. Growth family genes directly control on growth traits, but also can affect on fertility of animal. This study aimed to identify genetic polymorphisms of GHR and Pit1 genes in local Peranakan Ongole (PO) cattle from twin and multiple births (M) compared to single birth (S). PO cattle as samples originated from four provinces of South Kalimantan, South Sumatra, Central Java and East Java. SNP variants of the GHR\(\text{Ssp}\) locus at exon 8 (A/T) and Pit1\(\text{Stu}\) locus at exon 3 (C/T) were analyzed by PCR RFLP technique. Allelic frequenciest of the GHR exon 8 g.914T>A locus of the two birth types were high for T allele (M = 78.1–100%, S = 71.4–100%) over A allele. Meanwhile the Pit1 exon 3 c.577C>T locus was monomorphic for the finding only C allele (100 %) without T allele. Some genetic indexes were also calculated for heterozygosity observation (Ho), heterozygosity expectation (He), effective number of alleles (Ne), fixation index (Fis) and polymorphism of information content (PIC). These values were not different between single and multiple births. The conclusion was that allelic frequenciest and genetic index values were almost similar between the two birth types of the local PO cattle.

1. Introduction

Cattle is well known as a uniparous animal that means it is very common for a cow to give birth only one calf \([1,2]\). However twin or multiple births in cattle can occur at a very low rate around 0.5–4%, depending on the influences of genetic and environmental factors \([3,4]\). Reproductive traits, especially twin and multiple births, in cattle are not affected by a single gene \([5,6]\), but they follow quantitative traits under the affect of many genes and their interaction to the environment \([7,8]\). Multiple births can reduce reproductive efficiency and productivity of the cows, such as increasing placental retention incidences, prolonging uterine involution, increasing calf mortality, and decreasing reproductive performances \([4]\). However multiple births in beef cattle could be beneficial under an intensive cow rearing management. Production efficiency increased due to lower production input costs for the maintenances during pregnant and lactating cows \([9]\).

By the existence of the current development of molecular marker technology makes possible to identify genes affecting quantitative traits, including also reproductive traits in livestock \([10]\). Phenotypic variations of these traits were the accumulation effects of a number of genes (multi genes) and their interactions that could be identified properly \([11]\). From the in vitro fertilization studies in cows proved the concurrent influences of the genetic polymorphisms of a number of reproductive and
growth genes to fertilization rates and embryo survivals [12]. Many single nucleotide polymorphisms (SNPs) in some candidate genes are possible in affecting growth and reproduction traits. A number of growth and reproductive genes were predicted to result in twin and multiple births in cattle. A number of the growth family genes, beside of controlling the growth traits, can also affect fertility traits in cattle, such as GH, GHR, GHRH, and Pit-1 genes [12–15]. Growth hormone (GH) either directly or indirectly affects various aspects of animal lactation, growth, and reproduction. Growth Hormone (GH) gene plays a key role in postnatal growths, muscles, bones, adipose tissues, and udder glands [14]. Growth Hormone Receptor (GHR) gene has a functional position as a candidate gene in influencing reproduction, milk production, and milk quality in dairy cows [13]. Meanwhile, Pit1 gene, also known as POU1F1 gene, was involved in various important signaling pathways for the mechanisms of growths, pituitary and mammary glands, and preproduction [12,15].

Peranakan Ongole (PO) cattle is one of the local beef cattle breeds spreads widely in various parts of Indonesia. Whereas PO cross cattle is quite common in Central Java as the result of the previous AI program. These cattle is one of potential local beef cattle breeds that should be more increased their population through a proper breeding program. The ability of a cow to come into calving at a yearly regular interval could be a determining factor for the success of a beef cattle farming. Another aspect to be interesting is to explore possible opportunity for improving twin and multiple births in cattle. Exploration of genetic polymorphism of the growth family genes may provide preliminary information to determine the chances of twin and multiple births in PO cattle. This study therefore aimed to study genetic polymorphism of GHR and Pit1 genes from twin and multiple (historical) births compared to single birth of the local PO cattle from a number of regions in Indonesia.

2. Materials and methods

2.1. Location and period
This research provided both field researches and laboratory researches conducted from 2010–2013. The field researches were done by visiting to a number of the origin area where PO cows previously informed getting the cases of calving twin or multiple births. The research locations were five districts from four provinces providing Hulu Sungai Tengah Regency (South Kalimantan), Palembang (South Sumatra), Kendal (Central Java), and Probolinggo and Pasuruan (East Java) as shown in table 1.

Laboratory researches were done for molecular analysis to detect genetic variants of the GHR and Pit1 genes by PCR-RFLP method. Molecular analysis were carried out at IRIAP Molecular Laboratory in Ciawi, Bogor and at Molecular Genetics Laboratory of the Faculty of Animal Husbandry of Bogor Agriculture University in Dramaga, Bogor, West Java, Indonesia.

2.2. Research materials
Animals as research samples were local Peranakan Ongole (PO) cattle and PO cross cattle from Central Java (table 1). Bloods as sources of DNA samples were collected from the cows of calving twin or multiple births and their offsprings, both male and female. Bloods of the cows calving single birth at the same location were also collected considered as animal control. A cow was defined for calving twin or multiple births (M) if that cow at least once time calved twin or multiple births during her life. While a historical twin and multiple cattle was those of both female and male offsprings of the twin or multiple calving cows. A single calving cow (S) was a cow giving a single birth in the same location.

The total number of the PO cattle as research samples were successively from Hulu Sungai Tengah in South Kalimantan by 31 hd. (M=17, S=14), Palembang in South Sumatra by 22 hd. (M=13, S=9), Kendal in Central Java by 23 hd. (M=16, S=7), Probolinggo by 13 hd. (M=8, S=5), Pasuruan by 26 hd. (M=16, S=10) in East Java. The bloods were collected for DNA extraction and identification of genetic polymorphism of the GHR and Pit1 genes.
Table 1. Local PO cattle as observation animal based on location and birth type.

| Province          | Regency / City      | Twin / Multiple Births (head) | Single birth (head) |
|-------------------|---------------------|------------------------------|--------------------|
| South Kalimantan  | Hulu Sungai Tengah | 17                           | 14                 |
| South Sumatera    | Palembang           | 13                           | 9                  |
| Central Java      | Kendal*             | 16                           | 7                  |
| East Java         | Probolinggo         | 8                            | 5                  |
|                   | Pasuruan            | 16                           | 10                 |
| Total             |                     | 70                           | 45                 |

Note: *) PO and PO cross cattle.

2.3. Blood collection
Blood sample was taken from a jugular vein or coccyx vein for 1–2 ml using a vacutainer measuring 21G X 1 ½ or a 10 ml syringe containing heparin anti-coagulant agent. The blood sample was added with 8 ml of absolute ethanol (EtOH) containing 1 mM EDTA at a total concentration of more than 50% so that the sample could last a long time.

2.4. DNA extraction
DNA extraction was done by referring to the method of Sambrook et al. [16] with a little modification using cell lysis buffer (350 μl 1xSTE and 40 μl 10% SDS) and 20 μl proteinase-K. DNA was purified by a phenol-chloroform method, namely by adding 40 μl 5 M NaCl and 400 μl phenol and chloroform iso amyl alcohol (CIAA). DNA was precipitated with 40 μl 5 M NaCl and 800 μl absolute ethanol. The precipitated DNAs were washed by adding 400 μl 70% ethanol and centrifuged at 12000 rpm for 5 minutes. Ethanol was evaporated using a vacuum pump. DNA was then dissolved with 80 μl 80% TE buffer.

2.5. Polymerase chain reaction (PCR)
Polymerase Chain Reaction (PCR) reaction was carried out on a thermocycler machine using the taq polymerase RBC enzyme and its buffer. The bovine genomic DNAs were used as a template in the DNA amplification reaction (PCR reaction) with the primary pairs (mix) of the nucleotide sequences (Forward and Reverse) for the GHR and Pit1 genes as shown in table 2.

Table 2. Primary sequences for amplifying the Pit1 and GHR genes.

| Gene | Length of amplicon | Region | Primer sequences |
|------|--------------------|--------|------------------|
| GHR  | 230                | Exon 8 | F: CTG TGG AAT ACT TGG GCT AGC AGT GAC AAT AT |
|      |                    |        | R: GTC TCT CTG TGG ACA CAA CA |
| Pit1 | 234                | Exon 3 | F: AAC TGA GAC TGG TGC CAC |
|      |                    |        | R: CTA ATA CTG AAA GCT CTA CA |

Source: *)[17]; **) [18].

The PCR reaction was carried out for a total volume of 25 μl of the solution mixture consisting of DNA Taq Polymerase and 10X Taq Polymerase buffer (100 mM tris-Cl, pH 8.3; 500 mM KCl; 15 mM MgCl2; 0.01 % gelatin); dNTP’s mix (dGTO, dATP, dTTP and dCTV) (Pharmacia); and sterile dH2O. The PCR reaction in the thermocycler machine was designed by temperatures for pre-denaturation of 93°C, denaturation of 94°C, annealing of 58–60°C, elongation of 72°C and post-PCR of 4°C. For propagation, the cycle was repeated 33 times.

2.6. DNA amplification
DNA extraction was carried out by electrophoresis on 1.5% agarose gel run at a voltage of 100 v for 40 minutes. Optimization of the targetted DNA segments of both GHR and Pit1 genes was carried out by...
polymerase chain reaction (PCR) method. The reagent used for amplification was by mixing 2 μl of the DNA sample with 25 pmol primer, 200 μM dNTP mixture, 1 mM MgCl₂, and 0.5 unit taq polymerase and by buffering them in a total solution of 25 μl. In vitro amplification using a thermal cycler machine was carried out under initial denaturation at a temperature of 94°C for 5 minutes. Amplification was carried out in 35 cycles, including denaturation at 94°C for 45 seconds, primer attachment at 60°C for 45 seconds, and elongation of new DNAs at 72°C for 1 minute. The final extension was carried out at 72°C for 5 minutes.

2.7. RFLP

Genotyping DNA fragments was done by restriction fragment length polymerase (RFLP) method. DNA fragments from PCR products were restricted by specific enzymes then different banding patterns from the electrophoresis results were detected. The band pattern detection technique was done manually based on the lengths of the observed DNA fragments using the silver staining method.

2.8. Data analysis

Data from genotyping results were calculated for genotype frequency, allele frequency, heterozygosity observation (Ho), heterozygosity expectation (He), effective number of allele (Ne), fixation index (Fis) and polymorphism of information content (PIC) by the POPGENE32 software version 1.32. Genotype frequency and allele frequency were calculated by formula of Nei and Kumar [19]. The values of heterozygosity observation (Ho) and heterozygosity expectations (He) referred to Nei and Kumar [19], and the degree of polymorphism was calculated by PIC value. The results of the frequencies of genotype and allele as well as the values of genetic indexes of the twin and multiple (historical) births were then compared to those of the single birth of PO and PO cross cattles in the same location.

3. Results and discussion

3.1. Genetic polymorphism

3.1.1. Growth Hormone Receptor Gene. Amplification of the DNA fragments of the GHR gene on exon 8 resulted in PCR products of the length of 230 bp. Nucleotide sequences of the GHR gene fragment on the exon 8 was completely known from the data sources on the gene bank access number DQ168861. PCR products were restricted by Ssp1 enzyme to identify a base mutation at the GHR|Ssp1 exon 8 (T/A) locus. The Ssp1 enzyme recognized at the cutting site bases of 5'.... AAT * ATT .... 3' between T and A bases. Genotyping the GHR gene on exon 8 fragment yielded three genotypes of TT, AT, and AA (figure 1). TT genotype presented two bands with the lengths of 200 bp and 30 bp. For the DNA fragment was not cut off by the Ssp1 enzyme resulted AA genotype as presented by only one band of 230 bp. Meanwhile, AT genotype resulted three bands, namely 230 bp, 200 pb, and 30 pb.

![Figure 1.](image-url)
Table 3. Genotype frequency and allele frequency of the GHR exon 8 g.914T>A locus of the PO cattle based on location and birth type.

| Birth type          | Location         | Genotype frequency | Alle frequency |
|---------------------|------------------|--------------------|----------------|
|                     |                   | AA     | AT    | TT    | A    | T    |
| Twin and multiple   | Hulu S Tengah (17)| 0.00 (0)| 0.1176 (2)| 0.8824 (15) | 0.0588 | 0.9412 |
| birth              | Palsembang (13)  | 0.00 (0) | 0.1538 (2) | 0.8462 (11) | 0.0769 | 0.9231 |
|                   | Kendal (16)*     | 0.00 (0) | 0.4375 (7) | 0.5625 (9) | 0.2188 | 0.7812 |
|                   | Probolinggo (8)  | 0.00 (0) | 0.1250 (1) | 0.8750 (7)  | 0.0625 | 0.9375 |
|                   | Pasuruan (16)    | 0.00 (0) | 0.0000 (0) | 1.0000 (16) | 0.0000 | 1.0000 |
| Single birth       | Hulu S Tengah (14)| 0.00 (0) | 0.0714 (1) | 0.9286 (13) | 0.0357 | 0.9643 |
|                   | Palsembang (9)   | 0.00 (0) | 0.1111 (1) | 0.8889 (8)  | 0.0556 | 0.9444 |
|                   | Kendal (7)*      | 0.00 (0) | 0.5714 (4) | 0.4286 (3)  | 0.2857 | 0.7143 |
|                   | Probolinggo (5)  | 0.00 (0) | 0.2000 (1) | 0.8000 (4)  | 0.1000 | 0.9000 |
|                   | Pasuruan (10)    | 0.00 (0) | 0.0000 (1) | 1.0000 (0)  | 0.0000 | 1.0000 |

Note: Number in a bracket for the number of PO cattle as samples. *) PO and PO cross cattle.

Identification of genotype variants of this GHR exon 8 g.914T>A locus showed that the PO cattles from twin and multiple (historical) births were dominated by the TT genotype (0.5625–1.00), lowed for AT genotype (0.00–0.4375), and blanked AA genotype (0.00) as shown in table 3. Similar condition was found from single birth as population control. The GHR exon 8 g.914T>A locus was also dominated by TT genotype (0.4286–1.00), lowed AT genotype (0.00–0.5714), and none of AA genotype (0.00). These lead into high frequencies of T allele against A allele for both birth types. Twin and multiple births had the frequencies of T allele by 0.7812–1.00 and A allele by 0.00–0.2188, while those for single birth for T allele by 0.7143–1.00 and A allele by 0.00–0.2857 respectively.

3.1.2. Pit-1 gene. Amplification of the DNA fragment of the Pit1 gene exon 3 resulted in the PCR products of the length of 234 bp. Genetic polymorphism of the Pit1|Stu1 exon 3 (C/T) locus followed the method of [20]. Genetic polymorphism of the Pit1 exon 3 fragments occurred for the existence for a point mutation between C to T bases. Genotyping DNA fragments of the Pit1|Stu1 exon 3 (C/T) locus from the previous study resulted in three genotypes i.e., CC, AC and AA. AA genotype presented only one band of 234 bp. AA cattle had a base mutation at position 37th base, so that the restriction site was not recognized by the Stu1 enzyme. Contrarily CC genotype had two bands with the fragments of 197 bp and 37 bp due to no base mutation at the restriction site. Further AC genotype had three bands of 234 bp, 197 bp, and 37 bp. Genotyping results of the DNA fragments of the Pit1 exon 3 c.577C>T locus for all of the observed PO and PO cross cattles across birth types and locations proved that resulted in only CC genotype, presented by two bands of 197 bp and 37 bp. Contrarily neither AC nor AA genotypes were identified. So all the observed cattles in this study produced only C allele (1.00) without A allele (0.00).

Nei [21] states that an allele is said polymorphic for possessing allele frequency equal to or less than 0.99 (99%), instead an allele is monomorphic for presenting allele frequency ≤0.01 (1%). Based on these statements, it could be stated that all the observed cattles were monomorphic for the Pit1 exon 3 c.577C>T locus. Previous study in FH and Jersey cattles reported genetic polymorphisms of the Pit1 exon 3 c.577C>T locus that resulted in CC, CA and AA genotypes [20]. The AA cows expressed a positive effect on fertility traits as indicated by higher in vitro embryonal fertilization (blastocyst) rates than those CC and AC cows [22].

3.1.3. Genetic indexes. The values of homozygosity observation (Ho), homozygosity expectation (He), effective allele number (Ne), Fixation index (Fis), and Polymorphic information content (PIC) for Pit1|Stu1 exon 3 C>T locus of the PO cattle based on location and birth type is presented in table 4. The Pit1 exon 3 c.577C>T locus was not calculated for population indexes due to it was monomorphic.
Heterozygosity is used to estimate the level of genetic polymorphism in a population. If the observed heterozygosity value (Ho) was close to 0 meaning a low level of the heterozygosity, whilst this value was closer to 1 meaning high level of the heterozygosity. In other condition, a population is stated to have a high heterozygosity if the values of Ho>He. The Pit1 exon 3 c.577C>T locus of twin and multiple births had the lowest heterozygosity observation (Ho) of the PO cattle from Hulu Sungai Tengah (Kalsel) (Ho = 0.1176), but the highest one was cross from Kendal (Central Java) (Ho = 0.4375). The same was true for single births. The exception was for the PO cattle from Pasuruan with Ho = 0.00 as this Pit1 exon 3 c.577C>T locus was monomorphic for both birth types.

| Birth type            | Location   | Ho      | He      | Ne      | PIC     | FIS    |
|-----------------------|------------|---------|---------|---------|---------|--------|
| Twin and multiple birth | Hulu S Tengah | 0.1176  | 0.1141  | 1.1245  | 0.2237  | -0.0625|
|                        | Palembang  | 0.1875  | 0.1754  | 1.2047  | 0.3111  | -0.1034|
|                        | Kendal*    | 0.4375  | 0.3528  | 1.5193  | 0.5253  | -0.2800|
|                        | Probolinggo | 0.1250  | 0.1250  | 1.1327  | 0.2338  | -0.0667|
|                        | Pasuruan   | 0.0000  | 0.0000  | 1.0000  | 0.0000  | -0.0447|
| Single birth           | Hulu S Tengah | 0.1325  | 0.1311  | 1.0000  | 0.1113  | -0.0515|
|                        | Palembang  | 0.1165  | 0.1112  | 1.0000  | 0.2923  | -0.1133|
|                        | Kendal*    | 0.5714  | 0.4396  | 1.6897  | 0.5983  | -0.4000|
|                        | Probolinggo | 0.2000  | 0.2000  | 1.2195  | 0.3251  | -0.1111|
|                        | Pasuruan   | 0.0000  | 0.0000  | 1.0000  | 0.0000  | -0.0541|

Table 4. Genetic indexes of the GHR exon 8 g.914T>A locus of the PO cattle based on location and birth type.

Note: Ho for heterozygosity observation, He for heterozygosity expectation, Ne for number of effective allele, PIC for polymorphic information content, and FIS for fixation index.
*) PO and PO cross cattle.

Fis value is a measure of the deviation of the genotype frequency from the panmictic frequency indicating whether heterozygous deficiency or excess. If Fis was positive indicating heterozygous excess instead of heterozygous deficiency for a negative value when this value were compared to the Hardy-Weinberg equilibrium expectation [15]. The Fis values of the PO cattle at all locations were negative for both twin and multiple births (Fis = -0.0447 - -0.2800) and single births (Fis = -0.0515 - -0.4000). PIC value can range from 0 to 1. Based on the PIC classification, it is stated that polymorphism is low if PIC value is <0.25, moderate polymorphism if 0.25<PIC value<0.50 and high if the PIC value>0.50. Based on this classification, PO cattle of both single and multiple births had high polymorphism from Palembang (South Sumatra) and Kendal (Central Java), as well as that of single birth from Probolinggo (PIC = 0.2923–0.5983). Further PO cattle of both birth types from other locations had low polymorphisms (PIC = 0.0000–0.2338). Ne value shows the effective population size that can describe the rate of inbreeding of a population. By excepting PO cattle from Pasuruan (East Java), Ne value of twin and multiple births were the lowest from Hulu Sungai Tengah (Ne = 1.1245) and the highest from Kendal (Ne = 1.5193). Ne values with the same pattern were found for single births.

Based on the population index values presented by homozygosity observation (Ho), homozygosity expectation (He), effective allele numbers (Ne), fixation index (Fis), and polymorphic information content (PIC) of the Pit1 exon 3 c.577C>T locus of twin and multiple births were almost the same to those of single birth of PO and PO cross cattle cows in each location.

4. Conclusion
The GHR|Ssp exon 8 (T/A) and Pit1|Stu1 exon 3 (C/T) loci of the PO cattle from twin and multiple births had almost similar frequencies of the alleles and genotypes to single birth. Allelic frequencies of the GHR exon 8 g.914T>A locus of both birth types were high for T allele (M = 78.1–100%, S = 71.4–100%) over A allele. Genetic index values were also almost similar between the two birth types. Meanwhile Pit1 exon 3 c.577C>T locus was monomorphic with the only C allele (100%) without the T allele. The similar conditions of both genetic polymorphisms and genetic indexes between the two birt
types indicated the two loci could not be considered as early indicators of genetic markers for exploring potency of twin and multiple births of the observed PO cattle.

References

[1] Çobanoğlu Ö 2010 Twinning in cattle: desirable or undesirable? J. Biol. Environ. Sci. 4 1–8
[2] Anggraeni A, Talib C, Asmarasari S A, Herawati T and Andreas E 2017 Genetic polymorphisms of IGF1, GH, and OPN genes in crosses Peranakan Ongole cattle based on birth type in Central Java J. Ilmu Ternak dan Vet. 22 165–72
[3] Sawa A, Bogucki M and Sylvia K C 2012 Reproduction performance of cows with single, twin and triplet calves Acta Vet. Brno 81 347–52
[4] Wakehaure R and Ganguly S 2016 Twinning in Cattle: A Review ARC J. Gynecol. Obstet. 1 1–3
[5] Morris C A, Day A M, Amyes N C and Hurford A P 1992 Ovulation and calving data from a herd selected for twin calving New Zel. J. Agric. Res. 35 379–91
[6] Gregory K E, Bennett G L, Van Vleck L D, Echternkamp S E and Cundiff L V. 1997 Genetic and environmental parameters for ovulation rate, twinning rate, and weight traits in a cattle population selected for twinning J. Anim. Sci. 75 1213–22
[7] Komisarek J and Dorynek Z 2002 Genetic aspects of twinning in cattle J. Appl. Genet. 43 55–68
[8] Meuwissen T H E, Karlsen A, Lien S, Olsaker I and Goddard M E 2002 Fine mapping of a quantitative trait locus for twinning rate using combined linkage and linkage disequilibrium mapping Genetics 161 373–9
[9] Karlsen A, Ruane J, Klemetsdal G and Heringstad B 2000 Twinning rate in Norwegian cattle: Frequency, (co)variance components, and genetic trends J. Anim. Sci. 78 15–20
[10] Dekkers J C 2004 Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. J. Anim. Sci. 82 313–28
[11] Horikawa Y, Gu J and Wu X 2008 Genetic susceptibility to bladder cancer with an emphasis on gene–gene and gene–environmental interactions Curr. Opin. Urol. 15 493–8
[12] Khatib H, Maltecca C, Monson R L, Schutzkus V, Wang X and Rutledge J J 2008 The fibroblast growth factor 2 gene is associated with embryonic mortality in cattle J. Anim. Sci. 86 2063–7
[13] Fontanesi L, Scotti E, Tazzoli M, Beretti F, Dall’Olio S, Davoli R and Russo V 2007 Investigation of allele frequencies of the growth hormone receptor (GHR) F279Y mutation in dairy and dual purpose cattle breeds Ital. J. Anim. Sci. 6 415–20
[14] Sami A J, Nazir M T, Jabeen Z and Shakoori A 2011 Gene study within the 5’ flanking regions of growth hormone gene of first exon in bos indicus African J. Biotechnol. 10 332–6
[15] Selvaggi M and Dario C 2011 Analysis of two Pit1 gene polymorphisms: Single nucleotide polymorphisms (SNPs) distribution patterns in Podolica cattle breed African J. Biotechnol. 10 11360–4
[16] Sambrook J, Fritsch E F and Maniatis T 1989 Molecular Cloning: A Laboratory Manual, 2nd ed (New York: Cold Spring Harbor)
[17] Blott S, Kim J J, Moisio S, Schmidt-Künstel A, Cornet A, Berzi P and Coppeters W 2003 Molecular dissection of a quantitative trait locus: a phenylalanine-to-tyrosine substitution in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition Genetics 163 253–66
[18] Huang M S , Sage A P, Lu J, Demer L L and Tingut Y 2008 Phosphate and pyrophosphate mediate PKA-induced vascular cell calcification Biochem. Biophysic. Res. Com. 374 553–58
[19] Nei M and Kumar S 2000 Molecular Evolution and Phylogenetics (England: Oxford University Press)
[20] Huang W X, Maltecca C and Khatib H 2008 A proline-to-histidine mutation in POU1F1 is associated with production traits in dairy cattle Anim. Genet. 39 554–7
[21] Nei M 1987 Molecular Evolutionary Genetics (New York: Columbia University Press)
[22] Khatib H, Huang W, Wang X, Tran A H, Bindrim A B, Schutzkus V, Monson R L and Yandell B S 2009 Single gene and gene interaction effects on fertilization and embryonic survival rates in cattle J. Dairy Sci. 92 2238–47