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A Study on Polymorphisms in Five Markers MR1-MR5 of Pit-1 Gene and Their Effects on Growth Rate and Carcass Traits in Egyptian Domestic Chickens

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Abstract

Chicken Pit-1 gene could be a candidate locus that affects important economic production traits. So, the present work was conducted to detect and analyze the different patterns (genotypes) in five reported polymorphisms (MR1-MR5) of such genes and investigate their effects on growth rate and carcass characteristics (Breast and thigh weights) in five different chicken breeds (Fayoumi, Dokki-4, White Leghorn, El-Salam and Inshas). Blood samples were collected from five breeds. DNA was isolated, and different patterns for MR1 and MR2-MR5 were analyzed by simple-PCR and PCR-RFLP, respectively. Sequence analysis and alignment of different detected patterns were performed. The results detected several patterns in five markers MRI-MR5 of the Pit-1 gene, two different types in MR1, MR2, MR4 and MR5, and four types in MR3. Sequence analysis identified various single nucleotide polymorphisms (SNPs) in MR patterns generating heterozygous and homozygous genotypes. Except for MR2, heterozygous genotypes were strongly associated with improving growth rate and carcass characteristics in some chicken breeds of MR1, MR3, and MR5 and all five breeds of MR4 as compared to homozygous genotypes. However, the results clarified a positive relationship between homozygous genotypes and improving the economic production traits in some breeds of MR1, MR3, and MR5 and all breeds of MR2 with respect to heterozygous genotypes. The present investigation proved that the detected SNPs in different patterns of the Pit-1 gene could be beneficial markers for selection of Egyptian chicken breeds for enhancing the growth rate and carcass characteristics. On the other hand, some homozygous genotypes are helpful in the successful breeding program. Therefore, the use of Pit-1 variations can be valuable for improving the important productivity traits in the chicken.

Introduction

The genetic improvement of native Egyptian chicken breeds is very important for improving their productivity performance. Generally, native chicken breeds in most countries were found to play a critical role in their economy. This is due to the taste and eggs of such native chickens, which are suitable and in demand for native consumers. Moreover, most indigenous poultry breeds were revealed to have good adaptability to climatic conditions and low nutritional regimes (Liverpool-Tasie *et al.*, 2019; Thu *et al.*, 2021). In Egypt, native chicken breeds are popularly raised and characterized by egg and meat quality, but these breeds show low growth rate due to low heterogeneity, despite using some solutions that include feeding, better health protection, and changing husbandry to improve chicken productivity. However, using genetic tools is a suitable method for such a purpose. Using candidate genes provides better tools for improving economically essential traits in

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poultry and animals (Rodbari *et al.,* 2011; Thu *et al.,* 2021). This is because the knowledge about the function of genes gave the good selection of individuals with the best breeding traits involving growth rates, body weight, and carcass traits (Manjula *et al.,* 2018; Thu *et al.,* 2021). These traits have considered a significant number of objectives, all related to reducing costs (Rodbari *et al.,* 2011). Pit-1 gene or pituitary-specific transcriptional factor could be a candidate locus that affects the above traits because it was clear that this factor has a major role as a transcriptional activator for pituitary gene transcription and in cell differentiation during organogenesis of the anterior pituitary (Zhang *et al.,* 2021).

In chicken, the Pit-1 gene was found on chromosome 1, comprising 7 exons and 6 introns (Bello *et al.,* 2020; McElroy *et al.,* 2006). Its mRNA was revealed to be present in any cell type of the pituitary. In contrast, the particular protein was found to be mainly expressed in lactotrophs, somatotrophs, and thyrotrophs that secrete prolactin (PRL), growth hormone (GH) and thyroid-stimulating hormone (TSH) (Iguchi *et al.,* 2017). Moreover, it is a transcription factor that binds and transactivates GH, PRL, and TSH hormone chain promoters encoding genes (Hassan *et al.,* 2022). Most genetic polymorphisms observed in this gene could control the hormone expression mentioned above and affect metabolic activity and skeletal muscle development of chickens (Bhattachyra and Chatterjee, 2013; Thu *et al.,* 2021).

Therefore, this study aimed to identify and analyze the association between the presence of single nucleotide polymorphisms (SNPs) in the Pit-1 gene and growth rate as well as carcass traits in five strains of chickens, including two Egyptian native strains, one imported strain, and two hybrid strains developed from Egyptian native and imported strains.

Materials and Methods

Chicken populations

Five chicken populations including two Egyptian native strains (Fayoumi and Dokki-4), one imported strain (White Leghorn) and two hybrid strains (El-Salam and Inshas) were selected. El-Salam strain is a result of mating between imported Nichols and Egyptian native Mamourah strains. Inshas Strain was produced from crossing between Egyptian native Sinai and imported White Plymouth Rock strains. About 125 broilers from the above strains (n = 25 per each) were used in this study. All chickens were raised under the same environment and management. The experimental diets were formulated according to the requirements of National Research Centre, Egypt. The broilers were fed on a diet which contained 23% crude protein, 5.76% crude fat, 4.2% crude fiber and 3000 Kcal/kg a representative energy. Live body weights were measured at the first day and day 30 to determine the growth rate. Chickens were slaughtered on day 30 and the carcass was eviscerated and dissected. The breast and thigh weights of each bird were measured.

Ethics approval and consent to participate

The Ethics of Medical Research Committee with number 19165, National Research Centre, Al Buhouth St. Dokki- Cairo, Egypt has approved this study.

Blood sample collection and DNA extraction

Blood samples were collected from brachial veins of 125 broilers by standard venipuncture and transferred into tubes containing heparin anticoagulant factor. Genomic DNA was extracted from blood samples using the salting-out method with some modifications (Javanrouh and Jelokhani-niaraki, 2020). The purity and the relative integrity of genomic DNA were detected by 1.5% agarose gel electrophoresis. Also, the concentration of DNA was quantified by NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The final DNA concentration ranged from 2-10 ng/µL.

Single nucleotide polymorphisms (SNPs) selection

In the Pit-1 gene, five SNPs that could be easily genotyped by either PCR-RFLP or simple PCR were selected. The characteristics of these SNPs are recorded in Table 1. The polymorphisms were 57 bp indel (MRI) according to Nie *et al*. (2005,) and 4 SNPs (MR2-MR5) were released by NCB1 with the accession number of Rs13905611, Rs13687125, Rs13687127, and Rs13687128, respectively. To amplify specific fragments that cover the markers from MRI-MR5, four primer pairs (Table 1) were used (where the primers for MR2 and MR3 have the same sequences). These primers were designed and recorded by Nie *et al*. (2008).

Genotyping by PCR-RFLP procedure

The polymerase chain reaction (PCR) was performed in a 25 µL mixture containing 50 ng of genomic DNA, 1x PCR buffer, 12.5 pmol of primers (PR1-PR4), 100 µM of each dNTP, 1.5 mM $MgCL_2$ and 1 U Taq DNA polymerase. The PCR cycles was performed as follows: 94°C for 3 min, followed by 35 cycles of 30 s at 94°C, 45 s at 58-62°C, 1 min at 72°C and a final elongation of 5 min at 72°C. The genotypes of MRI was detected on 2% agarose gel electrophoresis for PCR product, which amplified by PRI without cutting by restriction enzymes, while PCR products of PR2-PR5 were digested by *Taq1*, *MspI*, *EcoRI*, and *Tas1* cutting enzymes at 37°C overnight, respectively. The mixture of digestion consisted of 8 µL of PCR products, 1x digestion buffer and 3 U of each enzyme. Polymorphic

genotypes for MR2-MR5 resulted from digestion mixture were identified under UV light after detection on 2.0% agarose gel electrophoresis at 120 V, 60 mA for an hour.

Table 1. PCR-RFLP primers, restriction enzymes, and detailed information for MR1- MR5 of the chicken Pit-1 gene

Markers	Variation	Region	Primer sequence (5'-3')	Restriction enzyme	Amplicon (bp)
MR1 (PR1)	57 bp indel	Intron 2	F: gtcaaggcaaatattctgtacc R: tgcatgttaatttggctctg	//	387 or 330
MR2 (PR2)	C/T	Intron 5	F: ggaccctctctaacagctctc R: gggaagaatacagggaaagg	*Taq1*	599
MR3 (PR3)	A/G	Intron 5	The same sequences of MR2 primer	*Msp1*	599
MR4 (PR4)	C/T	Intron 5	F: ggggattttgccactttaggg R: tgggtaaggctctggcactgt	*EcoR1*	442
MR5 (PR5)	C/T	Exon 6	F: tgggaagaacagtttatggc R: tggctagcttgtaagggaatc	*Tas1*	483

*Marker 1 (MRI) was reported by Nie *et al*. (2008). Markers from MR2 to MR5 had been released by NCBI with accession numbers of Rs13905611, Rs13687125, Rs13687127, and Rs13687128, respectively, according to Nie *et al.* (2002).

Sequence analysis

Different genotypes of MR1, MR2, MR3, MR4, and MR5 were purified from PCR product using Qiaquick PCR Purification Kit (Qiagen, Germany). Sequence analysis was performed in Macrogen incorporation (Seoul, Korea), and aligned by cluster-wide analysis using Codon Code Aligner software (Codon Code Corporation, USA).

Statistical analysis

The present data were analyzed using the ANOVA option of the General Linear Model (GLM) procedure of SAS software to determine the total variance at $P < 0.05$ (SAS, 2004).

Results

Genetic polymorphisms:

Frequency distributions of different patterns

Amplification of MR1-MR5 fragments was performed using the PCR technique (Figure 1; A, B, C, and D, respectively). Different patterns indicating the genetic polymorphisms of the Pit-1 gene were detected by simple PCR for the MR1 locus and by PCR-RFLP for MR2-MR5 loci in Fayoumi, Dokki-4, Leghorn, El-Salam and Inshas chicken breeds (Table 2, Figure 1 and 2).

The result of MR1 showed two patterns, P1 and P2, according to the sizes of amplified PCR products (Figure 1; A). Pattern 1 at 387 bp was observed in all chickens of five tested breeds. Fayoumi breed showed the highest frequencies of such patterns (1.0), followed by Dokki-4 (0.76) Leghorn (0.44), El-Salam (0.5), and Inshas (0.68). Pattern 2 at 330 bp was demonstrated in all chicken breeds except the Fayoumi strain. Leghorn and El-Salam showed higher frequencies (0.48 and 0.44, respectively) compared to Dokki-4 and Inshas breeds (0.24 and 0.32, respectively).

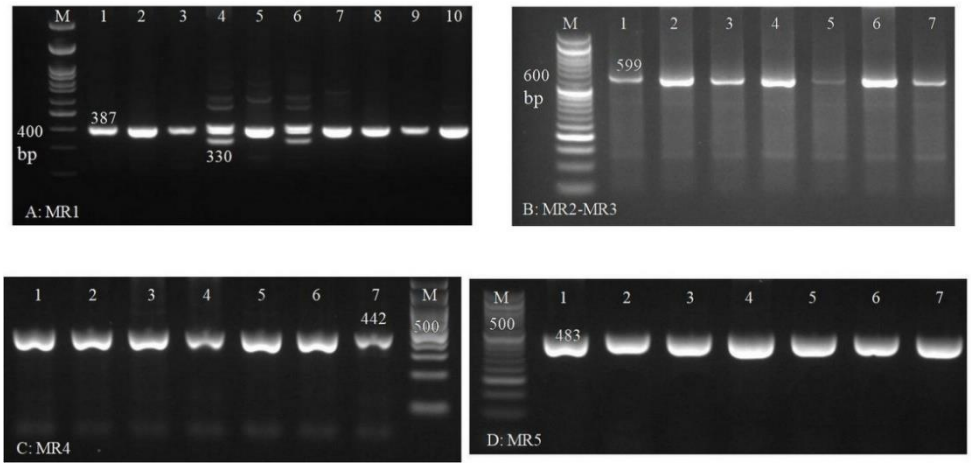

Figure 1. Shows PCR products of MR1-MR5 markers of the Pit-1 gene. A: indicates MR1 at sizes 387 and 330 bp; B: indicates MR2 and MR3 at size 599 bp; C: indicates MR4 at size 442 bp; and D: indicates MR5 at size 483bp. Lane M: indicates 100 or 50 bp ladder.

Table 2. Frequency distributions of different patterns for MR1-MR5 of Pit-1 gene observed in different chicken breeds

Breed	MR1			
	P1 (387 bp)		P3 (387, 330 bp)	
	No.	Freq.	No.	Freq.
Fayoumi	25	1.00	0	0.00
Dokki-4	19	0.76	6	0.24
Leghorn	11	0.44	12	0.48
El-Salam	12	0.50	11	0.44
Inshas	17	0.68	8	0.32

Breed	MR2			
	P1 (599 bp)		P2 (599, 459, 140 bp)	
	No.	Freq.	No.	Freq.
Fayoumi	17	0.68	8	0.32
Dokki-4	12	0.48	13	0.52
Leghorn	18	0.72	6	0.24
El-Salam	21	0.84	3	0.12
Inshas	20	0.80	4	0.16

Breed	MR3							
	P1 (599bp)		P2 (599, 483, 324, 275 bp)		P3 (483,116 bp)		P4 (324, 275 bp)	
	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.
Fayoumi	7	0.30	8	0.32	6	0.24	2	0.08
Dokki-4	10	0.40	4	0.16	4	0.16	6	0.24
Leghorn	5	0.20	3	0.12	17	0.68	0	0.00
El-Salam	13	0.52	1	0.04	10	0.40	0	0.00
Inshas	8	0.32	9	0.36	5	0.20	1	0.04

Breed	MR4			
	P1 (442 bp)		P2 (442, 252, 190 bp)	
	No.	Freq.	No.	Freq.
Fayoumi	5	0.20	13	0.52
Dokki-4	6	0.24	28	0.72
Leghorn	9	0.36	14	0.56
El-Salam	11	0.44	14	0.56
Inshas	8	0.32	16	0.64

Breed	MR5			
	P1 (483 bp)		P2 (483, 260, 223 bp)	
	No.	Freq.	No.	Freq.
Fayoumi	13	0.52	10	0.40
Dokki-4	6	0.24	19	0.76
Leghorn	15	0.60	8	0.32
El-Salam	21	0.84	4	0.16
Inshas	15	0.60	10	0.40

* P1=Pattern 1 P2=Pattern 2 P3=Pattern 3 P4=Pattern 4.

MR2 locus clarified two patterns, P1 and P2. Pattern 1 (599 bp) was found in all five chicken breeds (Figure 1; B). The El-Salam breed (0.84) followed by the Inshas breed (0.8) possessed higher frequencies of this pattern as compared to Fayoumi (0.68), Dokki-4 (0.48), and Leghorn (0.72) breeds. Pattern 2 (599, 459, and 140 bp), which resulted from digestion by *Taq1* enzyme, was detected in the five strains, where the Dokki-4 strain showed the highest frequency (0.52), while the El-Salam breed had the lowest frequency (0.12). The frequencies of Fayoumi, Leghorn, and Inshas breeds were 0.32, 0.24, and 0.16, respectively (Figure 2; A).

MR3 showed four patterns, P1 (599 bp), P2 (599,483, 324, and 275 bp), P3 (483 and 116), and P4 (342 and 275 bp), P2-P4 appeared from digestion by *Msp1* enzyme. Regarding P1, the El-Salam breed showed the highest frequency (0.52), while the Leghorn breed had the lowest frequency (0.2). The other three breeds (Fayoumi, Dokki-4, and Inshas) were observed to have 0.3, 0.4, and 0.32 frequencies, respectively. In P2, the Inshas breed followed by the Fayoumi breed was shown to contain the highest frequencies (0.36 and 0.32, respectively), whereas the El-Salam breed had the lowest frequency (0.04). The remaining two breeds, Leghorn and Dokki-4 had 0.12

and 0.16 frequencies, respectively. Pattern 3 showed the highest frequency in the Leghorn strain (0.68), while the Dokki-4 strain showed the lowest frequency (0.16). The remaining three breeds (Inshas, Fayoumi, and El-Salam) had 0.20, 0.24, and 0.40 frequencies, respectively. Pattern 4 was observed in three breeds only; Dokki-4 showed the highest frequency (0.24) followed by Fayoumi and Inshas breeds with frequencies of 0.08 and 0.04, respectively (Figure 2; B).

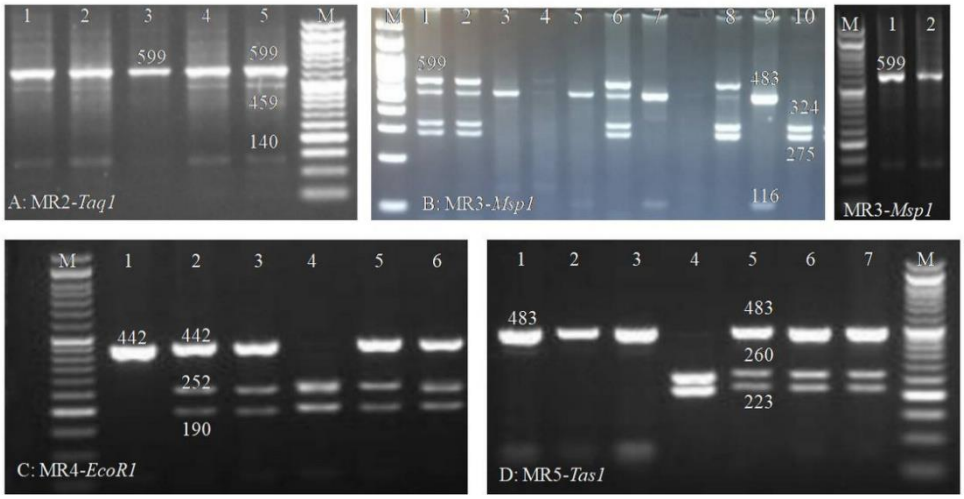

Figure 2. The electrophoresis patterns were produced after digestion of PCR-amplified fragments of MR2-MR5 of the Pit-1 gene with different restriction enzymes showing different bands. Lane M: 100 or 50 bp ladder.

MR4 possessed two patterns, P1 at 442bp and P2 at 442, 252, and 190bp. Pattern 1 was detected in the studied five breeds, where El-Salam breed followed by Leghorn breed had higher frequencies (0.44 and 0.36, respectively) as compared to the other three breeds, Inshas (0.32), Dokki-4 (0.24), and Fayoumi (0.2). In P2 that resulted from digestion by *EcoR1* enzyme, Dokki-4 strain showed the highest frequency (0.72), followed by Inshas strain (0.64) concerning the other three breeds, where the frequencies of P2 in Fayoumi, Leghorn, and El-Salam were 0.52, 0.56, and 0.56, respectively (Figure 2; C).

MR5 showed two patterns, P1 (483bp) and P2 (483, 260, and 223bp). For P1, El-Salam breed was demonstrated to contain the highest frequency (0.84), while the Dokki-4 strain had the lowest frequency (0.24). The frequencies of these patterns in the other three breeds, Fayoumi, Leghorn, and Inshas, were 0.52, 0.6, and 0.6, respectively. For P2, Dokki-4 strain had the highest frequency (0.76), while El-Salam strain had the lowest frequency (0.16). The frequencies of P2 that resulted from digestion by *Tas1* enzyme was detected in the other three strains, Fayoumi (0.4), Inshas (0.4), and Leghorn (0.32) (Figure 2; D).

b. Sequence analysis

The sequence analysis and alignment of MR1 identified three adjacent single nucleotide polymorphisms (A/G, G/T, and G/A) in pattern 2 (330 bp) of intron 2 of the Pit-1 gene as compared to pattern 1 (387bp) at positions 85, 102, and 109, respectively, of the amplified fragments (Figure 3; A and B). In addition, the sequence analysis clarified the nucleotide missing in P2 (57 nucleotides) within a coding region at position 182-238 bp (Figure 3; C, supplementary material).

The sequence analysis and alignment of MR2 revealed a deletion in P2 where nucleotide C at position 293 of P1 was deleted at P2. In addition to twelve nucleotide polymorphisms at different positions of P2 of intron 5 of the Pit-1 gene as compared to pattern 1 (the wild-type allele) at position 57(G/C), 124(G/T), 418(T/C), 422(G/A), 434(A/T), 453(A/G), 516(C/G) and five similar SNPs at positions 87, 142, 232, 357and 421(C/T) (Figure 4; A and B, supplementary material).

The sequence and alignment analysis of MR3 at intron 5 of the Pit-1 gene showed two adjacent nucleotide polymorphisms (G/A) in pattern 2 at positions 28 and 33 compared to pattern 1 (the wild-type allele). In addition, pattern 3 revealed one SNP (G/A) at position 441 compared to pattern 1. Moreover, in pattern 4 of MR3, the results clarified one SNP (C/T) in comparison to pattern 1 at position 220 of the amplified fragments (Figure 5; A, B, C and D, supplementary material).

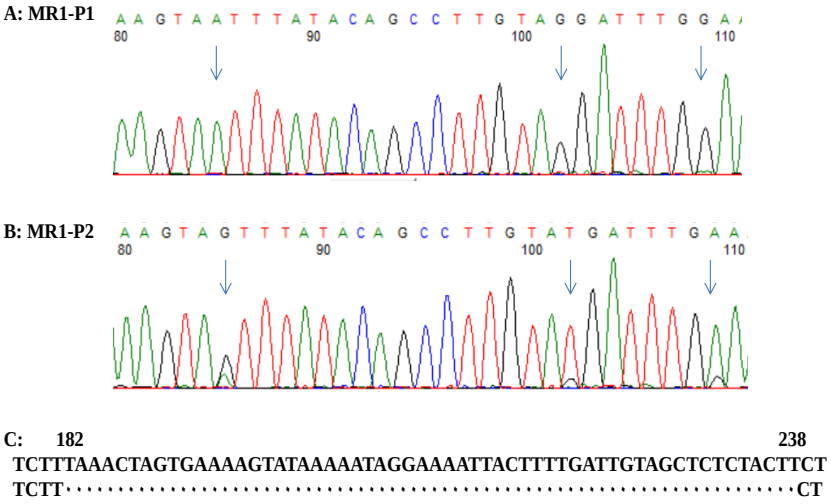

Figure 3. Sequence analysis of MR1 represented two different patterns (P1 and P2), where A: represented P1 (wild type allele) and B: represented P2 (mutant allele) with three single nucleotide polymorphisms (SNPs). C: Shows the alignment of P1 and P2 to show the nucleotide missing in the band 330 bp as one of PCR product of MR1 that differ from the other band 387 bp of PCR product of the same marker by missing of 57 nucleotides at positions 182-238 bp.

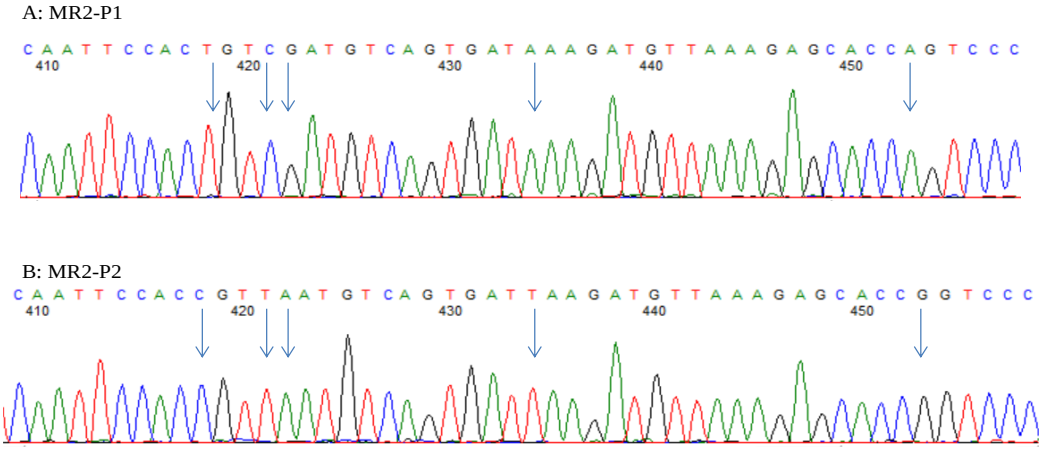

Figure 4. Sequence analysis of MR2 represented two different patterns (P1 and P2), where A: represented P1 (wild type allele) and B: represented P2 (mutant allele) with some single nucleotide polymorphisms (SNPs).

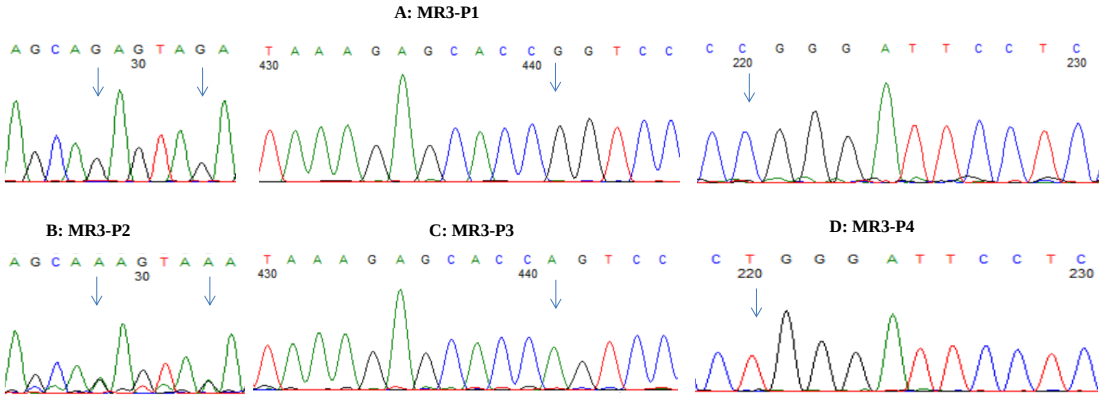

Figure 5. Sequence analysis of MR3 represented four different patterns (P1, P2, P3, and P4), where A represented P1, the wild type allele and B, C, and D represented the mutant alleles (P2, P3, and P4) with different types of single nucleotide polymorphisms (SNPs).

The sequence analysis and alignment of MR4 identified a SNP (G/A) at position 137 in pattern 2 of intron 5 of the Pit-1 gene compared to wild-type pattern P1 (Figure 6; A and B, supplementary material).

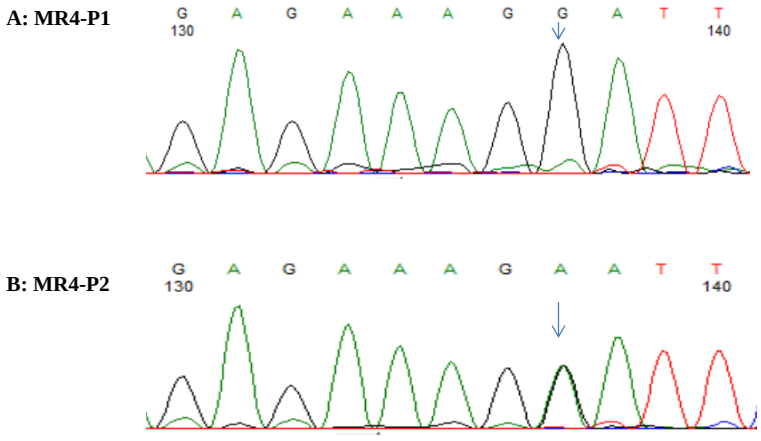

Figure 6. Sequence analysis of MR4 represented two different alleles (P1 and P2), where A: represented P1, the wild-type allele, and B: represented the P2 mutant allele with one single nucleotide polymorphism (SNP).

The sequence analysis of MR5 showed one single nucleotide polymorphism (SNP) (C/T) at position 177 in pattern 2 of exon 6 in the Pit-1 gene compared to wild-type pattern P1. (Figure 7; A and B, supplementary material).

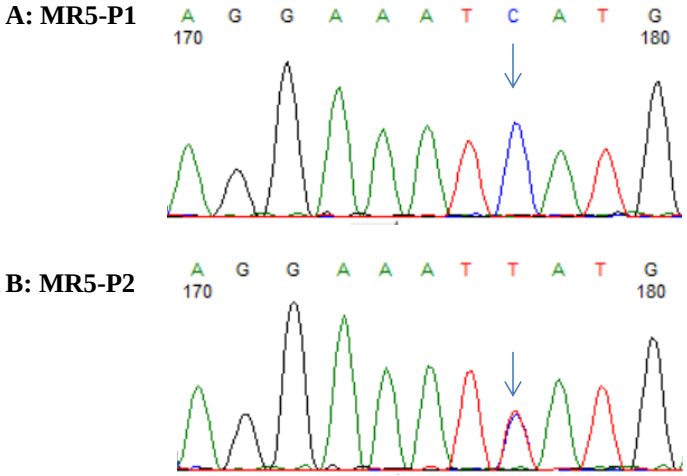

Figure 7. Sequence analysis of MR5 showed two different patterns (P1 and P2), where A: represented P1 wild-type allele and B: represented the P2 mutant allele with one single nucleotide polymorphism (SNP).

Association among different patterns of MR1-MR5 and economic traits

In the present study, the association of breeds and other patterns of MR1-MR5 of the Pit-1 gene with economical features that included growth rate and carcass characteristics, especially breast and thigh weight were investigated (Table 3a, b and Table 4).

In MR1, P1 of Dokki-4, El-Salam and Inshas breeds showed higher economic trait values than P2. In contrast, Leghorn birds that carry P2 were discriminated with improving growth rate and thigh weight with respect to the birds that have P1, although the breast weight in these birds was the best. The analysis of MR2 declared that the birds of five breeds that carry P1 had higher rates of economic trait values than those that have P2. In contrast, the Inshas breed that carries P1 showed the highest value of growth rate, as well as El-Salam and Leghorn breeds were discriminated with the highest values of breast and thigh weight, respectively.

Table 3a. Association of different patterns of MR1, MR2, MR4, and MR5 in Pit-1 gene with growth rate and carcass traits in five Egyptian domestic chicken breeds

	Breed	Growth rate	Carcass traits		Growth rate	Carcass traits	
			Breast wt./g	Thigh wt./g		Breast wt./g	Thigh wt./g
MR1		P1 (387 bp)			P2 (387, 330bp)		
	Fayoumi	117.5±0.31^{b}	26.3±1.11^{b}	8.2±0.39^{c}	N/A	N/A	N/A
	Dokki-4	73.1±3.22^{a}	17.3±0.40^{a}	4.0±0.37^{a}	23.1±1.44^{a}	1.7±0.17^{a}	1.2±0.34^{a}
	Leghorn	131.9±3.11^{c}	14.3±0.52^{a}	6.5±0.53^{b}	143±3.61^{d}	13±0.60^{c}	8.0±0.60^{d}
	El-Salam	139.3±3.50^{c}	15.3±1.92^{a}	6.2±0.12^{b}	128±3.73^{c}	11±0.62^{c}	5.6±0.31^{c}
	Inshas	196±5.72^{d}	17.7±0.74^{a}	7.7±0.39^{c}	92.5±4.92^{b}	8.3±1.0^{b}	3.6±0.19^{b}
MR2		P1 (599bp)			P2 (599, 459,140bp)		
	Fayoumi	79.9±1.81^{a}	9.0±0.64^{b}	5.5±0.26^{b}	35.3±0.01^{a}	4.2±0.02^{a}	2.6±0.03^{b}
	Dokki-4	46.1±2.90^{a}	3.3±0.35^{a}	2.6±0.24^{a}	38.5±0.02^{a}	3.2±0.02^{a}	2.6±0.01^{b}
	Leghorn	224±5.61^{b}	20.0±0.10^{c}	12±0.90^{d}	59.8±0.02^{b}	7.0±0.03^{c}	4.0±0.02^{c}
	El-Salam	204±12.0^{b}	21.9±1.20^{c}	10.7±0.54^{c}	150±0.01^{d}	3.2±0.04^{a}	1.6±0.01^{a}
	Inshas	241±18.0^{b}	21.2±1.0^{c}	9.3±0.41^{c}	101±0.03^{c}	4.3±0.01^{b}	1.9±0.02^{a}
MR4		P1 (442bp)			P2 (442,252,190bp)		
	Fayoumi	23.5±0.53^{a}	4.8±0.18^{a}	1.8±0.23^{a}	58.8±1.30^{a}	8.8±0.46^{b}	4.6±0.45^{a}
	Dokki-4	23.1±1.33^{a}	2.9±0.30^{a}	1.2±0.10^{a}	61.6±3.74^{a}	4.4±0.49^{a}	4.24±0.30^{a}
	Leghorn	116.7±2.91^{b}	11.7±0.52^{c}	5.8±0.34^{c}	170.5±2.81^{c}	15±0.79^{d}	8.6±0.90^{c}
	El-Salam	122.5±3.62^{b}	11±0.60^{c}	5.3±0.44^{c}	155.8±4.54^{b}	14±0.55^{c}	6.9±0.40^{b}
	Inshas	112.9±12.0^{b}	8.7±0.49^{b}	3.7±0.30^{b}	185.8±4.90^{e}	16.4±0.70^{d}	7.2±0.40^{bc}
MR5		P1 (483bp)			P2 (483, 260, 223bp)		
	Fayoumi	63.5±1.34^{b}	7.2±0.52^{b}	4.3±0.16^{b}	49.36±1.10^{a}	5.5±0.42^{b}	3.4±0.14^{b}
	Dokki-4	23.1±1.40^{a}	1.7±0.20^{a}	1.3±0.10^{a}	73.1±4.42^{b}	5.3±0.44^{b}	4±0.29^{bc}
	Leghorn	176±4.53^{c}	17.1±0.80^{c}	9.9±0.66^{b}	104.7±1.60^{c}	9.2±0.54^{c}	5.3±0.50^{d}
	El-Salam	233.7±3.80^{d}	21±1.0^{d}	10.2±0.41^{d}	44.5±1.33^{a}	4.0±0.23^{a}	2.0±0.10^{a}
	Inshas	174.3±6.74^{c}	15.3±0.50^{c}	6.8±0.30^{c}	113.1±1.80^{e}	9.9±0.38^{c}	4.5±0.53^{cd}

*wt.=weight/g P1=Pattern 1 P2=Pattern 2. The data had been expressed as means ± SE.
The values with different superscript letters (a,b,c,d,e) within a column represent significant statistical differences ($P < 0.05$).
**N/A means that the pattern is not apparent in this breed

Regarding MR3, P1 of Dokki-4, Leghorn and El-Salam breeds revealed higher values of economic traits than P2. In addition, the characteristics of economic traits in P1 of Dokki-4 and El-Salam breeds had higher values than those found in P3. While the characteristics of economic traits in P3 of the El-Salam breed were observed to be higher compared with those in P2. On the other hand, P4 of the Dokki-4 breed showed higher economic trait values than those recorded in P2 or P3. However, Fayoumi and Inshas birds with P4 were observed to have lower economic trait values than breeds with other patterns, P1, P2, and P3.

Concerning MR4, the birds of all five breeds that carry P2 had been discriminated with improving economic traits compared to those found in P1, where the growth rate, breast weight and thigh weight were higher in P2 than those recorded in P1.

In MR5, the economic trait values in P1 were higher in four breeds, Fayoumi, Leghorn, El-Salam and Inshas, compared with those in P2. In contrast, the birds of the Dokki-4 breed with P2 were discriminated with improving economic traits than those found in P1, where the growth rate, breast weight and thigh weight were higher in P2 than those observed in P1.

Table 3b. Association of different patterns of MR3 in Pit-1 gene with growth rate and carcass traits in five Egyptian domestic chicken breeds

	Breed	Growth rate	Carcass traits		Growth rate	Carcass traits		Growth rate	Carcass traits		Growth rate	Carcass traits	
			Breast wt./g	Thigh wt./g		Breast wt./g	Thigh wt./g		Breast wt./g	Thigh wt./g		Breast wt./g	Thigh wt./g
MR 3		P1 (599 bp)			P2 (599, 483, 324, 275 bp)			P3 (483,116 bp)			P4 (324, 275bp)		
	Fayoumi	37.5 ^{a} ±1.80	5.2±0.26^{ab}	2.4±0.10^{ab}	41.16±0.90^{d}	5.9±0.32^{c}	2.8±0.12^{c}	30.6±0.74^{b}	4.4±0.25^{ab}	2.1±0.10^{abc}	10.5±0.53^{b}	1.15±0.10^{a}	0.73±0.10^{a}
	Dokki-4	38.4 ^{a} ±2.42	2.9± 0.32^{a}	2.1±0.10^{a}	15.4±1.0^{b}	1.2±0.11^{a}	0.84±0.07^{a}	15.4±1.0^{a}	1.2±0.11^{a}	0.8±0.10^{ab}	23.1±1.23^{c}	1.8±0.19^{b}	1.3±0.11^{b}
	Leghorn	59.8±1.50^{b}	6±0.26^{b}	3.2±0.25^{b}	35.9±0.90^{c}	3.6±0.14^{b}	1.9±0.15^{b}	203.4±5.11^{e}	20.4±1.74^{d}	10.9±0.81^{d}	N/A	N/A	N/A
	El-Salam	139±4.12^{d}	13.5±0.60^{d}	6.5±0.41^{d}	11.14±0.31^{a}	1±0.02^{a}	0.5±0.02^{a}	111.3±3.23^{d}	10.4±0.50^{c}	5±0.29^{c}	N/A	N/A	N/A
	Inshas	101.5±2.51^{c}	5.2±0.34^{ab}	2.4±0.11^{ab}	41.16±0.90^{d}	5.9±0.32^{c}	2.8±0.12^{c}	30.6±0.71^{b}	5.7±0.26^{b}	2.4±0.24^{bc}	1.0±0.15^{a}	1.0±0.14^{a}	1.0±0.12^{ab}

*wt.=weight/g P1=Pattern 1 P2=Pattern 2 P3=Pattern 3 P4=Pattern 4. The data had been expressed as means ± SE.
The values with different superscript letters (a,b,c,d,e) within a column represent significant statistical differences ($P < 0.05$).
**N/A means that the pattern is not apparent in this breed

Table 4. Effect of homo- and heterozygous genotypes of five markers MR1-MR5 of Pit-1 gene on economic traits in five Egyptian domestic chicken breeds

Marker	Pattern	Genotypes (SNPs)	Economic traits including growth rate and carcass characteristics (breast weight and thigh weight).
MRI	P1 P2	GG, TT, AA AG, GT, GA	1- P1 genotypes were strongly associated with improving economic traits in Dokkki-4, El-Salam and Inshas breeds than P2 genotypes. 2- P2 genotypes were strongly linked with improving economic traits (except breast weight) in the Leghorn breed with respect to P1 genotypes.
MR2	P1 P2	TT, CC, GG, AA, AA CT, TC, AG, AT, GA, CG, GC, GT	1- P1 genotypes were strongly correlated with improving economic traits in all five breeds compared with P2 genotypes.
MR3	P1	GG, GG, AA, TT	1- P1 genotypes were valuable than P2 genotypes for economic traits in Dokki-4, Leghorn and El-Salam breeds. 2- Also, P1 genotypes in Dokki-4, breed was better than P3 and P4 genotypes of such breed for economic traits.
	P2	GA, GA	3- P2 genotypes were more favorable than P1 genotypes in Fayoumi and Inshas breeds for economic Traits. 4- Also, P2 genotypes were better in Fayoumi breed than P3 and P4 genotype for economic traits.
	P3	GA	5- P3 genotype of the Leghorn breed was strongly linked with improving economic traits than those found in Pl and P2 genotypes
	P4	CT	6- P4 genotype was not favorable for economic traits in the Inshas breed compared to P1, P2, and P3 genotypes.
MR4	P1 P2	GG GA	1- The P2 genotype was demonstrated to be strongly correlated with improving the economic traits in all five breeds compared with the P1 genotype.
MR5	P1	TT	1- The P1 genotype was strongly related to improving economic traits in studied breeds (except the Dokki-4 breed) with respect to the P2 genotype.
	P2	CT	2- The P2 genotype was clarified to be strongly associated with improving the economic traits in the Dokki-4 breed as compared to the P1 genotype.

Discussion

The presence of genetic variants in five markers (MR1-MR5) of the Pit-1 gene could be based on simple PCR products as in MRI or PCR-RFLP as in the other four markers (MR2-MR5). The polymorphisms in the Pit-1 gene can be utilized in selection and association with important economic traits, such as growth rate and carcass traits.

The present results detected different patterns (genotypes) in the previously mentioned five markers, two different patterns in MR1, MR2, MR4, and MR5 and four in MR3. This study is considered the first to discover different patterns, especially in four markers (MR2-MR5) of the chicken Pit-1 gene. Shahnaz *et al.* (2008) and Kulibaba *et al.* (2015) detected different chicken growth hormone gene patterns using the PCR-RFLP procedure and explained that the presence of different patterns in chicken genes might refer to insertions or duplication of some sequences suggesting two or three restriction sites and consequently consisting different fragment patterns on original amplicons.

In addition, inducing gene duplication must be accompanied by altering the restriction site in the duplicated fragment leading to different patterns (Gootwine *et al.*, 1993; Ofir and Gootwine, 1997). Moreover, gene duplication leads to the generation of several SNPs or alleles in one specimen (Wallis *et al.*, 1998; Maniou *et al.*, 2004; Abdelmoneim *et al.*, 2017). These observations were compatible with our results, where we detected three SNPs in P2 of MR1 (A/G, G/T, and G/A), a deletion at position 293, and twelve SNPs in P2 of MR2 (G/C, C/T (at different five positions), G/T, T/C, G/A, A/T, A/G, and C/G), and two SNPs in P2 of MR3 (G/A and G/A) and one SNP at both P3 and P4 of MR3 (G/A and C/T, respectively).

The gene duplication was also revealed in different animal species, where growth hormone gene duplication in sheep and goats was previously

mentioned (Gootwine *et al.,* 1993; Ofir and Gootwine, 1997). Furthermore, a series of growth hormone gene duplications were detected in the marmoset, rhesus, spider monkey, and human (Golos *et al.,* 1993; Wallis and Wallis, 2001; Wallis and Wallis, 2002).

Our findings were similar to Nie *et al.* (2008) that reported two patterns (P1, 387 bp and P2, 330 bp) in MR1 of the Pit-1 gene in Chinese chicken breeds and considered the only one that identified these two patterns in MR1 of chicken Pit-1 gene. Therefore, the present study adds a new pattern in MR1 that consists of 387 bp and 330 bp together in the same bird. Shahnaz *et al.* (2008) and Nie *et al.* (2002) observed the generating of new fragments in the chicken GH gene and detected novel sites at 560bp of such gene. These genetic events might be attributed to inducing duplication (gene duplication), insertion of some sequences and other substitutions spread among the introns (Shahnaz *et al.,* 2008; Kulibaba *et al.,* 2015).

In the present findings, the length variation considering nucleotide missing (57 nucleotides) in the fragment 330bp compared with the fragment 387bp of the MR1 marker was similar to that demonstrated by Farag *et al.* (2018) on the KAP1.1 sheep gene. They confirmed that three fragments (alleles) varied in length (A, 341bp; B, 311bp; and C, 281bp), where the nucleotide missing in B and C alleles was 30 and 60 nucleotides, respectively, at different positions of the sequence alignment. These length variations in alleles of the same gene were explained by Rogers *et al.* (2007), who speculated that the inducing of such genetic events might be due to unequal crossover and gene conversion causing nucleotide missing.

According to the present study, the heterozygous genotypes of MR1 (AG, GT, and GA), MR3 (GA, and CT), MR4 (GA), and MR5 (CT) in the Pit-1 gene were associated strongly with improving growth traits and carcass characteristics in some Egyptian domestic chicken breeds, in addition to homozygous genotypes of MR4 in all studied breeds. These findings were documented by Nie *et al.* (2008), who revealed that in some Chinese chicken breeds, different SNPs in MR2, MR3, MR4, and MR5 of the Pit-1 gene. These heterozygous genotypes were significantly correlated with body weight, average daily gain, shank diameter and length, and hatch weight than homozygous genotypes. Moreover, in the Iranian commercial Broiler line, Rodbari *et al.* (2011) found that Pit-1/*MsP1* heterozygous genotype (AB) was strongly associated with body weight, carcass weight and breast muscles compared to the observed in homozygous genotypes AA and BB.

The present results were also supported by Xu *et al.* (2012), who detected seventeen SNPs in the Pit-1 gene from two strains (SD02 and SD03) of 126 Erlang mountainous chickens. Only two SNPs (T/C and C/T) were clarified to be strongly associated with carcass quality traits in two strains compared with those of homozygous genotypes. Moreover, in the strain SD03, the TC genotype was significantly linked with body weight, carcass weight and breast muscle weight, whereas the CT genotype was significantly related to body weight. However, in SD02 chicken, the two genotypes TC and CT were not significantly correlated with any carcass characteristics.

Furthermore, Manjula *et al.* (2018) identified that the heterozygous genotype (TC) of intron 5 in the Pit-1 gene in Korean native chicken was strongly correlated with body weight at 2 weeks, weight gain from hatch to 2 weeks and from16 to 18 weeks concerning other homozygous genotypes. The study on Podolica Young bulls by Selvaggi *et al.* (2011) reported that the polymorphism of Pit-1/*Hinf1* was significantly linked to growth traits, and Pit-1/*Taq1* was strongly correlated to early development from birth to weaning. The polymorphisms of the Pit-1 gene were also documented by their significant association with body weight in pigs (Song *et al.,* 2005) and dwarfism in humans (Sobrier *et al.,* 2016).

Concerning the homozygous genotypes in pattern P1 of each of MR1 (GG, TT and AA), MR2 (TT, CC, GG, AA, and AA), MR3 (GG, GG, AA, and TT), and MR5 (TT), the results clarified a positive relationship between these genotypes and improving the economic production traits in some breeds of MR1, MR3 and MR5 and all breeds of MR2 with respect to heterozygous genotypes.

Our results were similar to the data obtained by Thu *et al.* (2021) on Noi native chickens, where they found in Pit-1/*BspH1* loci (TT and CT genotypes) that carcass weight, the average breast weight, and average thigh weight were higher in birds with TT homozygous genotype than birds with CT heterozygous genotype.

In a previous study on the Iranian commercial broiler line, Rodbari *et al.* (2011) revealed in Pit-1/*Msp1* loci (CC and AB genotypes) that there were higher values of carcass weight, dramatic weight, wing weight and back weight of birds that bear CC homozygous genotype than birds that bear AB heterozygous genotype.

The current findings were also supported by the results obtained by Jin *et al.* (2018) on some Chinese chicken strains, where the birds with TT homozygous genotype of Pit-1 gene manifested higher significant values of body weight and body weight gain compared to the birds bearing CT heterozygous genotype. The birds with GG homozygous genotype of Pit-1 gene were discriminated with more significant body weight and feed intake ($P < 0.05$) than the birds with AG heterozygous genotype.

Conclusion

The present investigation proved that the detected SNPs in different patterns of the Pit-1 gene could be

useful markers for selecting Egyptian chicken breeds to enhance the growth rates and carcass characteristics. On the other hand, some homozygous genotypes, such as P1 of each locus in five selected loci (MR1-MR5) of the Pit-1 gene are helpful in the successful breeding program. Therefore, the use of Pit-1 variations can be valuable for improving the essential productivity traits in the chicken.

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Conflict of interest
The authors declare that they have no conflict of interest.

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Supplementry Material

Sequenc analysis of MR1

MR1-P1

AGAGTAACATACTTAGCCTAGGATGTGAGTGGGATGCTAGAATACCTTTAAGTGTAAAAATACATGATCATCT
GTATGAAAGTAATTTATACAGCCTTGTAGGATTTGGAAAAATCATACACTGACTGAAAGTGTTTCTGGACTTTA
AAGCTATCCCACTGTCTTTAAACTAGTGAAAAGTATAAAAATAGGAAAATTACTTTTGATTGTAGCTCTCTACTT
CTAATCTTGATCCTAAATGT

MR1-P2

AGAGTAACATACTTAGCCTAGGATGTGAGTGGGATGCTAGAATACCTTTAAGTGTAAAAATACATGATCATCT
GTATGAAAGTAGTTTATACAGCCTTGTATGATTTGAAAAAATCATACACTGACTGAAAGTGTTTCTGGACTTTA
AAGCTATCCCACTGTCTT……CTAATCTTGATCCTAAATGT

Sequenc analysis of MR2

MR2-P1

ACCTGGACACAGTACTCTAGATGGGGCCTCACAAGAGCAGAGTAGAGAGGGACAAT**G**ACCTCCCTGTCCCTG
CTGGCCATCCCTCT**C**CTGGTGGAGCCCAAGATACCATTTGCTTTCTGAGCT**G**CAAGAGCACACTGCTGG**C**TCAA
GTTCAGTTTTTCATCGTCAGGACCCCCAGCTCCTTCTCTGCAGGGCTACTCTCAAGGACTGCTCCTTCCAGTCTG
TATAAATGCC**C**GGGATTCCTCCAGCCTAAGTGCAAAACCCTGCACTTTGCTGTGTTGAACCTCATTATGTT**C**ACC
CAGGCCCACTTTTCAAGTCTGTTGAGGATCCTCTGAATGGCATCCCTTCCTTCCACTGTG**C**CAACCACACCACTC
AGCTTGGCATCATCAGCAAACTTGCTGAGAGTGCACTCAATTCCAC**T**GT**CG**ATGTCAGTGAT**A**AAGATGTTAAA
GAGCACC**A**GTCCCAAGAAAGACCTCTGGGGGATGCCACTCATTACCACCTGGACATAGAACCATTAGTAA**C**CA
CCCTTTGTCTGCGGCCTTT

MR2-P2

ACCTGGACACAGTACTCTAGATGGGGCCTCACAAGAGCAGAGTAGAGAGGGACAAT**C**ACCTCCCTGTCCCTGC
TGGCCATCCCTCT**T**CTGGTGGAGCCCAAGATACCATTTGCTTTCTGAGCT**T**CAAGAGCACACTGCTGG**T**TCAAG
TTCAGTTTTTCATCGTCAGGACCCCCAGCTCCTTCTCTGCAGGGCTACTCTCAAGGACTGCTCCTTCCAGTCTGT
ATAAATGCC**T**GGGATTCCTCCAGCCTAAGTGCAAAACCCTGCACTTTGCTGTGTTGAACCTCATTATGTT**.**ACCC
AGGCCCACTTTTCAAGTCTGTTGAGGATCCTCTGAATGGCATCCCTTCCTTCCACTGTG**T**CAACCACACCACTCA
GCTTGGCATCATCAGCAAACTTGCTGAGAGTGCACTCAATTCCAC**C**GT**TA**ATGTCAGTGAT**T**AAGATGTTAAAG
AGCACC**G**GTCCCAAGAAAGACCTCTGGGGGATGCCACTCATTACCACCTGGACATAGAACCATTAGTAA**G**CAC
CCTTTGTCTGCGGCCTTT

Sequenc analysis of MR3

MR3-P1

TACTCTAGATGGGGCCTCACAAGAGCA**G**AGTA**G**AGAGGGACAATGACCTCCCTGTCCCTGCTGGCCATCCCTC
TCCTGGTGGAGCCCAAGATACCATTTGCTTTCTGAGCTGCAAGAGCACACTGCTGGCTCAAGTTCAGTTTTTCA
TCGTCAGGACCCCCAGCTCCTTCTCTGCAGGGCTACTCTCAAGGACTGCTCCTTCCAGTCTGTATAAATGCC**C**G
GGATTCCTCCAGCCTAAGTGCAAAACCCTGCACTTTGCTGTGTTGAACCTCATTATGTTCACCCAGGCCCACTTT
TCAAGTCTGTTGAGGATCCTCTGAATGGCATCCCTTCCTTCCACTGTGCCAACCACACCACTCAGCTTGGCATCA
TCAGCAAACTTGCTGAGAGTGCACTCAATTCCACTGTCGATGTCAGTGATAAAGATGTTAAAGAGCACC**G**GTC
CCAAGAAAGACCTCTGGGGGATGCCACTCATTACCACCTGGACATAGAACCATTAGTAACCACCCTTTGTCTGC
GGCCTTTCCCTGAAATTCTTCCCCA

MR3-P2

TACTCTAGATGGGGCCTCACAAGAGCA**A**AGTA**A**AGAGGGACAATGACCTCCCTGTCCCTGCTGGCCATCCCTC
TCCTGGTGGAGCCCAAGATACCATTTGCTTTCTGAGCTGCAAGAGCACACTGCTGGCTCAAGTTCAGTTTTTCA
TCGTCAGGACCCCCAGCTCCTTCTCTGCAGGGCTACTCTCAAGGACTGCTCCTTCCAGTCTGTATAAATGCCTGG
GATTCCTCCAGCCTAAGTGCAAAACCCTGCACTTTGCTGTGTTGAACCTCATTATGTTCACCCAGGCCCACTTTT
CAAGTCTGTTGAGGATCCTCTGAATGGCATCCCTTCCTTCCACTGTGCCAACCACACCACTCAGCTTGGCATCAT
CAGCAAACTTGCTGAGAGTGCACTCAATTCCACTGTCGATGTCAGTGATAAAGATGTTAAAGAGCACCAGTCC
CAAGAAAGACCTCTGGGGGATGCCACTCATTACCACCTGGACATAGAACCATTAGTAACCACCCTTTGTCTGCG
GCCTTTCCCTGAAATTCTTCCCCA

MR3-P3

TACTCTAGATGGGGCCTCACAAGAGCAGAGTAGAGAGGGACAATGACCTCCCTGTCCCTGCTGGCCATCCCTC
TCCTGGTGGAGCCCAAGATACCATTTGCTTTCTGAGCTGCAAGAGCACACTGCTGGCTCAAGTTCAGTTTTTCA
TCGTCAGGACCCCCAGCTCCTTCTCTGCAGGGCTACTCTCAAGGACTGCTCCTTCCAGTCTGTATAAATGCCCG
GGATTCCTCCAGCCTAAGTGCAAAACCCTGCACTTTGCTGTGTTGAACCTCATTATGTTCACCCAGGCCCACTTT
TCAAGTCTGTTGAGGATCCTCTGAATGGCATCCCTTCCTTCCACTGTGCCAACCACACCACTCAGCTTGGCATCA
TCAGCAAACTTGCTGAGAGTGCACTCAATTCCACTGTCGATGTCAGTGATAAAGATGTTAAAGAGCACC**A**GTC
CCAAGAAAGACCTCTGGGGGATGCCACTCATTACCACCTGGACATAGAACCATTAGTAACCACCCTTTGTCTGC
GGCCTTTCCCTGAAATTCTTCCCCA

MR3-P4

TACTCTAGATGGGGCCTCACAAGAGCAGAGTAGAGAGGGACAATGACCTCCCTGTCCCTGCTGGCCATCCCTC
TCCTGGTGGAGCCCAAGATACCATTTGCTTTCTGAGCTGCAAGAGCACACTGCTGGCTCAAGTTCAGTTTTTCA
TCGTCAGGACCCCCAGCTCCTTCTCTGCAGGGCTACTCTCAAGGACTGCTCCTTCCAGTCTGTATAAATGCC**T**G
GGATTCCTCCAGCCTAAGTGCAAAACCCTGCACTTTGCTGTGTTGAACCTCATTATGTTCACCCAGGCCCACTTT
TCAAGTCTGTTGAGGATCCTCTGAATGGCATCCCTTCCTTCCACTGTGCCAACCACACCACTCAGCTTGGCATCA
TCAGCAAACTTGCTGAGAGTGCACTCAATTCCACTGTCGATGTCAGTGATAAAGATGTTAAAGAGCACCGGTC
CCAAGAAAGACCTCTGGGGGATGCCACTCATTACCACCTGGACATAGAACCATTAGTAACCACCCTTTGTCTGC
GGCCTTTCCCTGAAATTCTTCCCCA

Sequenc analysis of MR4

MR4-P1

TGACCTTCCATGAGTAAAGTTTTATCCCTAGTGAGGTGCTGTGATTAGCAAATGAGCAATGAAGCATTTTACAA
CCCCTGTTCACTCCTACCTGAGAAGCATCCTCTTAGAGGAATATATACTTTGAAAGAGAAAGGATTCATATCTTC
ATTTTATTTCTTATATCAAAGGCAATAGCATTCAAAAAAGCAAAGCTCAAGTTGAATGCGAGAGGAGAAGGTA
TGAGTTGTCATTGTGGGGCTCAGATTGTGGGGCCAGAGAACAGCCTACATCTCTGGGAGATGGCATTGACAG
AGGATGTGATTGCTATTCAAAGAACAGGCACTTTGCTGTGGCAAGTAGGGCCTGACAGTGCCAAGTTTGTGCC
CTAAAGTGGCAAAATCCCCCAGCACG

MR4-P2

TGACCTTCCATGAGTAAAGTTTTATCCCTAGTGAGGTGCTGTGATTAGCAAATGAGCAATGAAGCATTTTACAA
CCCCTGTTCACTCCTACCTGAGAAGCATCCTCTTAGAGGAATATATACTTTGAAAGAGAAAGAATTCATATCTTC
ATTTTATTTCTTATATCAAAGGCAATAGCATTCAAAAAAGCAAAGCTCAAGTTGAATGCGAGAGGAGAAGGTA
TGAGTTGTCATTGTGGGGCTCAGATTGTGGGGCCATAGAACAGCCTACATCTCTGGGAGATGGCATTGACAGA
GGATGTGATTGCTATTCAAAGAACAGGCACTTTGCTGTGGCAAGTAGGGCCTGACAGTGCCAAGTTTGTGCCC
TAAAGTGGCAAAATCCCCCAGCACG

Sequenc analysis of MR5

MR5-P1

TGATAACCTCTAAAACACTGCTGTTGTCGTTTCTGATTTCCAGAAATACTATACAAAAAGCCTGAATGTGTCAG
AAAAGTTTCTAATAGTGATATTTTTCCCTTTGTACAGTATTTCTGCCAAAGAAGCCCTGGAGAGGCACTTTGGA
AACAAAGCAAGCCTTCTTCTCAGGAAAT**C**ATGAGGATGGCTGAGGGGCTTAATCTTGAGAAAGAAGTCGTAA
GAGTTTGGTTTTGCAACAGAAGACAAAGGGAAAAAAGAGTGAAGACAAGTCTGCATCAGAACGCCTTTAGCT
CTATTATCAAGGAGCACCATGAGTGCCGGTAAAGCTTTTTCATGCGTAGCTGTGGATTTCTGTTTTGCTTTATTT
TAAGTACTTTGTATATACTTATTTTCATGAAGAAAAAATGGTAAATCACTTGATTCCCTAAAGCCAAAGCCAAA

MR5-P2

TGATAACCTCTAAAACACTGCTGTTGTCGTTTCTGATTTCCAGAAATACTATACAAAAAGCCTGAATGTGTCAG
AAAAGTTTCTAATAGTGATATTTTTCCCTTTGTACAGTATTTCTGCCAAAGAAGCCCTGGAGAGGCACTTTGGA
AACAAAGCAAGCCTTCTTCTCAGGAAAT**T**ATGAGGATGGCTGAGGGGCTTAATCTTGAGAAAGAAGTCGTAA
GAGTTTGGTTTTGCAACAGAAGACAAAGGGAAAAAAGAGTGAAGACAAGTCTGCATCAGAACGCCTTTAGCT
CTATTATCAAGGAGCACCATGAGTGCCGGTAAAGCTTTTTCATGCGTAGCTGTGGATTTCTGTTTTGCTTTATTT
TAAGTACTTTGTATATACTTATTTTCATGAAGAAAAAATGGTAAATCACTTGATTCCCTAAAGCCAAAGCCAAA