

## Development of molecular markers among Barred Plymouth rock, Korean Ogol Chicken and White Leghorn

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### Abstract

To identify germline chimeric chicken using germ cell transplantation method, the testcross, spends much time, labor and cost to perform, is the only way for distinguishing germline chimeric chicken from normal one. And to enhance the method, development of breed-specific molecular markers have been needed. We have just identified breed-specific sequence polymorphisms among Barred Plymouth rock, Korean Ogol Chicken and White Leghorn in *PMEL17* and *MC1R* gene the loci of which are identical to *dominant white* and *extended black* loci. These sequence polymorphism will be very useful for screening germline chimera.

▶ **Key words** : molecular marker, germline chimeric chicken, polymorphisms, *PMEL17* gene, *MC1R* gene

### Introduction

Efficient identification of germline chimera from many candidate recipients is the old homework in germ cell transplantation technologies in chicken. But the testcross method spends much time, labor and cost to perform. For enhancing this, some DNA

markers have been developed using SNPs (Harumi et al., 2004). However, these markers are not breed-specific, selection of chickens with specific allele was required before producing germline chimeric chicken.

Plumage color of chicken is a major factor characterizing the breeds. *Dominant white(I)* and *extended black(E)* are major loci affecting plumage color of chicken. Recent study revealed that the *dominant white* and *extended black* locus are equivalent to *PMEL17* and *MC1R* gene, respectively. Moreover, the sequence polymorphisms in these genes reported to associated with pigmentation patterns of chickens(Kerje et al., 2003, 2004).

In this study, we identified sequence polymorphisms among Barred Plymouth rock, Korean Ogol Chicken and White Leghorn. This result can be effectively applicable in screening germline chimera in chicken.

### Materials and Methods

#### *Animal*

Ten Korean Ogol Chicken (KOC), ten White Leghorn (WL) and ten Barred Plymouth Rock (BPR) chickens were used in this study. White Leghorn and Korean Ogol chickens were maintained at the University Animal Farm, Seoul National University, Korea.

#### *Sequencing of genomic DNA and cDNA*

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Blood samples were collected from 30 chickens (ten WL, ten KOC, ten BPR). Genomic DNA was isolated using PUREGENE<sup>®</sup> DNA Purification Kit(Gentra Systems) and mixed among each breed samples.

The PMEL17 gene sequence was amplified by PCR. The first cycle in the PCR was 10 min at 94°C followed by 35 cycles each consisting of 30s at 94°C, 30s at 65°C and 1min at 72°C and ending with an incubation for 10 min at 72°C. Then the gene sequence was sequenced by method of Kerje et al.(2004). MC1R gene sequence was amplified by PCR. The first cycle in the PCR was 5 min at 94°C followed by 35 cycles each consisting of 45s at 94°C, 45s at 61°C and 1.5min at 72°C and ending with an incubation for 5 min at 72°C. Then the gene sequence was sequenced by method of Kerje et al.(2003).

## Result

We identified 12 breed-specific polymorphic region among Barred Plymouth rock, Korean Ogol Chicken and White Leghorn. In PMEL17 gene, 8 sequence polymorphisms were identified at nucleotide position of 680, 798, 1811, 2184, 3236, 3246, 3270 and 3671 in gallus gallus PMEL17 gene sequence (accession number: AY636124). And in MC1R gene, 4 sequence polymorphisms were identified at nucleotide position of 69, 213, 635 and 636 in gallus gallus MC1R gene sequence (accession number: NM\_001031462). White Leghorn has different sequence at these nucleotide position from the others, but Barred Plymouth Rock and Korean Ogol Chicken have identical sequences.

## 적 요

Dominant white와 Extended black 은 닭의 깃털 색깔에 관여하는 유전자로서 각각 PMEL17과 MC1R 유전자를 암호화하며 이들의 염기서열 다형은 닭의 깃털 색깔 양상과 매우 밀접하게 연관되어있다. 본 실험은 이러한 점에 착안하여 PMEL17과 MC1R 유전자의 염기서열에서 Barred Plymouth rock, Korean Ogol Chicken and White Leghorn의 품종 특이적인 염기서열 다형을 확인하였다. PMEL17 유전자에서 8개, MC1R 유전자에서 4개씩 모두 12개의 다형이 확인되었다. White

Leghorn은 모든 염기서열 다형 위치에서 다른 두 종의 닭들과 다른 염기를 가지고 있었다. 그러나 Barred Plymouth Rock과 Korean Ogol Chicken은 모든 염기서열 다형 위치에서 같은 염기를 가지고 있었다.

## Reference

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