## QTL fine mapping of entire chromosome: F2 broiler x layer cross identifying a single QTL on GGA4 affecting body weight

A previous microsatellite QTL analysis in an F2 broiler x layer chicken cross identified a single QTL on GGA4 affecting body weight[1]. The male-line broiler was from the same line as used in the SNP project. Starting with 256 randomly selected SNPs on GGA4 that are polymorphic between the layer and broiler lines used for the SNP project, 47 assays were designed using the SNPlex™ Genotyping System v2.0 (Applied Biosystems). Our F2 experimental cross (n = 466) was typed for these SNPs, and any informative SNPs were merged with the genotype data from 26 polymorphic microsatellite markers to give a higher density linkage map of the QTL region on GGA4. Genetic linkage maps were estimated for both sexes using *CriMap*[2]. QTL analysis was done in *QTL Express*[3] using the sex-averaged linkage map of 54 markers.

Of the 47 SNPlex assays, 7 failed, 11 were monomorphic, 1 was heterozygous in all F2, and the remaining 28 were informative. None of these 28 informative SNPs were line specific (i.e. both lines fixed for alternative alleles), and only four SNPs had line specific genotypes (e.g. one line homozygous and the other line partly heterozygous and partly homozygous for the other allele). The joint linkage map for GGA4 contained 54 markers spanning a total of 276 cM (sex-averaged map, Figure S1), with the female map longer than the male map by about 19%, contrary to expectations from the whole genome map[4].

These analyses provided evidence of two QTLs affecting body weight (Table S6). Their combined additive genetic effect of 230 g was similar to the previous estimate[1] from a single QTL of 249 g, at an average body weight of 2.0 kg. Together, these QTLs account for about one-third of the difference between broiler and layer lines at 6 weeks of age. The benefits of this new data are reflected in the improved genetic information content in areas of GGA4 with gaps in the microsatellite map (Figure S2). In the past, PIC values exceeding 0.5 to 0.6 were rare, but using the additional SNP data, they no longer are. The average marker interval is 5.2 cM for GGA4 in its entirety, but 4.3 cM for the q-arm that

has both microsatellite and SNP markers (6.9 and 3.7 cM for microsatellite and SNPs individually). Further benefits are expected when characterizing an Advanced Intercross Line[5], since identification of all recombinations in the 8-10th generation from the F2 should contribute to fine mapping of the QTL.

## References


1. Sewalem, A. et al. Mapping of Quantitative Trait Loci (QTL) for body weight at 3, 6 and 9 weeks of age in a broiler layer cross. *Poult. Sci.* **81**, 1775-1781 (2002).
2. Green, P., Falls, K. & Crooks, S. *CriMap version 2.4* (Washington University School of Medicine, Saint Louis, 1990).
3. Seaton G., Haley C.S., Knott S.A., Kearsey M. & Visscher P.M. QTL Express: mapping quantitative trait loci in simple and complex pedigrees. *Bioinformatics* **18:** 339-340 (2002).
4. Groenen, M.A. & Crooijmans, R.P. Structural genomics: integrating linkage, physical and sequence maps. In *Poultry Genetics, Breeding and Biotechnology* (eds. Muir, W.M. & Aggrey, S.E.) 497-536 (CABI Publishing, Wallingford, 2003).
5. Song, J.Z., Soller, M. & Genezi, A. The full-sib intercross line (FSIL): a QTL mapping design for outcrossing species. *Genet. Res.* **73**, 61-73 (1999).

**Table S6.** QTL analysis for 6-week body weight of chicken chromosome 4, based on 26 microsatellites, with and without 28 novel SNP markers.

| | **26 microsatellites** | **26 microsatellites + 28 SNPs** |
|---|---|---|
| Test statistic 2 vs. 0 QTL | 21.5*** | 22.6*** |
| Test statistic 2 vs. 1 QTL | 9.6*** | 10.2*** |
| Position of QTL 1 | 98 | 100 |
| Position of QTL 2 | 240 | 237 |
| Additive effect of QTL1, g | 79±18 | 81±15 |
| Dominance effect of QTL 1, g | 17±26 | 14±26 |
| Additive effect of QTL2, g | 161±20 | 152±19 |
| Dominance effect of QTL2, g | -42±35 | -20±31 |

*** $P<0.001$

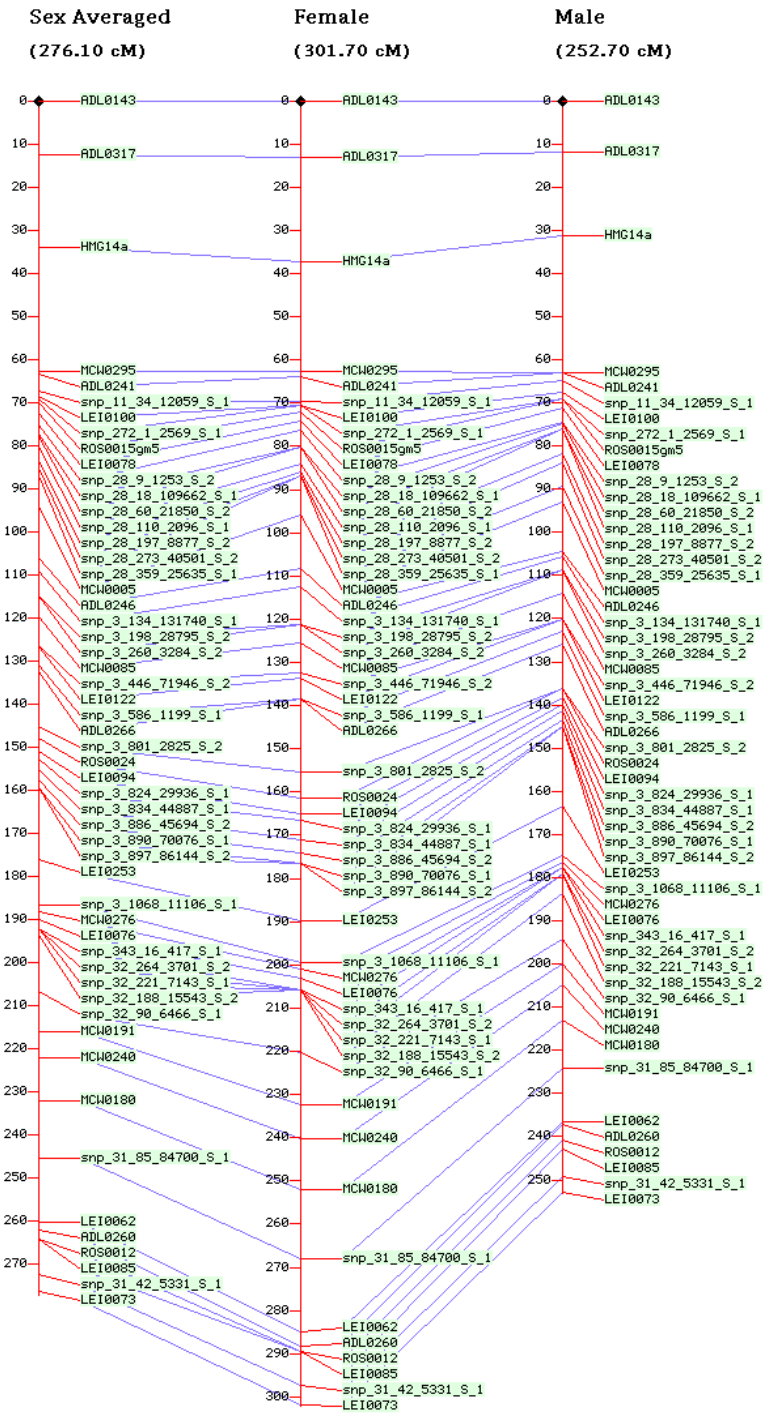


**Figure S1.** Sex-averaged and sex-specific linkage maps for chicken chromosome 4 using 26 microsatellites and 28 novel SNP markers.

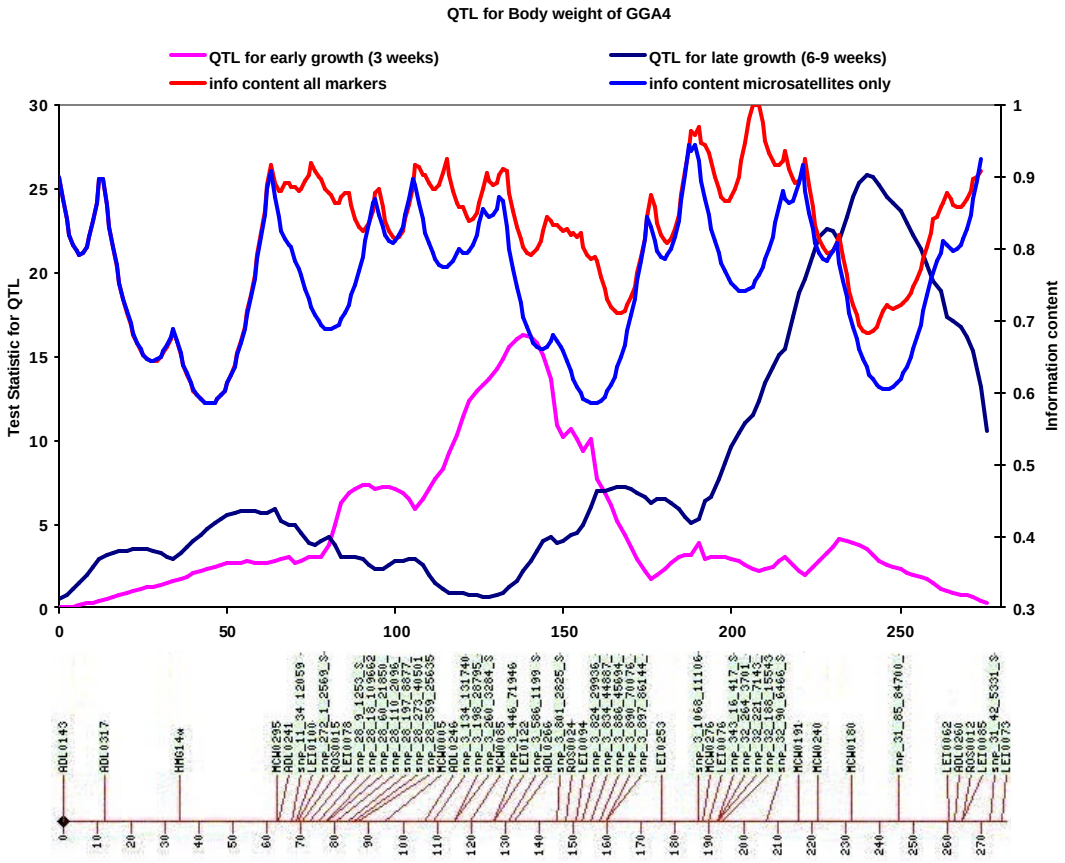


**Figure S2.** Information content from scan along GGA4 for QTL affecting body weight. The blue line shows information content using 26 microsatellite markers, and the red line shows information content using an additional 28 novel SNP markers. Test statistic for QTL affecting early growth (purple) and late growth (dark blue) are also shown. Marker positions are indicated under the x-axis.

## QTL mapping to a specific region: SNP analysis of QTL in the TGFB2 region on GGA3 in broiler-Leghorn F2 cross

Some TGF-ß gene SNPs are associated with QTLs for important agronomic traits like antibody kinetics and body composition[1,2]. To refine the QTL regions within or near the TGFB2 gene on GGA3, we tested some additional SNPs from the SNP project on F2 resource populations[3] that were generated by crossing sires from a broiler breeder male line with dams from two genetically distinct highly inbred (> 99%) chicken lines[4], the Leghorn G-B2 and the Fayoumi M15.2. Fayoumi was imported to the U.S. from Egypt in 1954 because of reported resistance to avian leucosis. The F1 birds were intercrossed, within dam line, to produce two F2 populations.

We measured body weight at two-week intervals up to 8 weeks of age, as well as length, bone mineral content and bone mineral density of the tibia at 8 weeks of age[1]. To refine the QTL, we selected four regions (two on each side of TGFB2), spaced 10 cM apart and with 4 SNPs per region (16 SNPs in total). Ten of the 16 SNPs were informative in the F2 resource population, five in the broiler-Leghorn cross, and five in the broiler-Fayoumi cross (Table S7). In addition, five novel SNPs were identified. One SNP in each region was selected for PCR-RFLP typing of 386 F2 individuals from the broiler-Leghorn cross. An analysis of these SNP-trait associations (Table S8) indicate that the skeletal QTL is most likely to be between SNP2 and TGFB2, a region containing the following candidate genes: usherin isoform A, estrogen-related receptor gamma, lysophospholipase-like 1, zinc transporter 8, bifunctional amino acyl-tRNA synthetase. A separate, growth-related QTL may be present between SNP1 and SNP2.

## References


1. Li, H., et al. Chicken quantitative trait loci for growth and body composition associated with transforming growth factor-ß genes. *Poult. Sci.* **82,** 347-356 (2003).

2. Zhou, H. & Lamont, S.J. Association of transforming growth factor ß genes with quantitative trait loci for antibody response kinetics in hens. *Anim. Genet.* **34,** 275-282 (2003).
3. Deeb, N. & Lamont, S. J. Genetic architecture of growth and body composition in unique chicken populations. *J. Heredity* **93,** 107-118 (2002).
4. Zhou, H. & Lamont, S. J. Genetic characterization of biodiversity in highly inbred chicken lines by microsatellite markers. *Anim. Genet.* **30**, 256-264 (1999).

**Table S7**: Primer and SNP information from the SNP project and the Iowa State University (ISU) F2 resource population.

| Primer Set | Primer Sequences | Position on GGA3 | Predicted in SNP dataset | ISU Resource Population (F/B/L) |
|---|---|---|---|---|
| Ch3IL | F: 5'-ATCTTCCTGAGTGGAGTAGTTCT-3' | 10708457 | G>A (JF/L) | G>G>G |
| | R: 5'-CGTAACCTAACCAAAAAGTAAAA-3' | 10708476 | C>T | T>T>T |
| | | 10708495 | 14 bp del in JF | no deletion |
| | | 10708551 | A>C (JF/L) | A>C>C |
| | | 10708573 | | G>G/A>A |
| | | 10708769 | | **G>G/A>A** |
| Ch3IIB | F: 5'-GCAAGGTAGCAAGGTTTATAGTA-3' | 13210964 | | G>A>A |
| | R: 5'-TTGCATTGTATTTCATATGATTC-3' | 13211017 | | **7 bp deletion in Broiler** |
| | | 13211080 | T>C (JF/B) | C>T>T |
| | | 13211096 | C>T (JF/B) | T>C>C |
| | | 13211116 | | A>G>G |
| | | 13211180 | 7 bp del in B | 7 bp del in Fayoumi |
| | | 13211190 | 1 bp del in B | 1 bp del in Fayoumi |
| Ch3IIIL | F: 5'-ACAGTCTGCATATCCAACACTAC-3' | 18263896 | T>G (JF/L) | T>T>G |
| | R: 5'-GTGAAAGCCATGTTAGAGATAAG-3' | 18263990 | A>G (JF/L) | **A>A>G** |
| | | 18264025 | A>G (JF/L) | A>A>G |
| | | 18264067 | C>T (JF/L) | C>C>T |
| Ch3IVL2 | F: 5'-TTGTAGGTAACAAATGACAGGAT-3' | 20576320 | 1 bp del in L C>T>T | no deletion |
| | R: 5'-AAGCAATGCTGTATCAGAGAGTA-3' | 20576375 | (JF/L/S) | C>C>C |
| | | 20576509 | G>A (JF/L) | **G>G>A** |
| | | 20576531 | G>A (JF/L) | G>G>G |

**Notes:** JF/L = Jungle Fowl to Leghorn nucleotide change; JF/B = Jungle Fowl to broiler nucleotide change; S = Silkie; F/B/L = nucleotide change from Fayoumi to broiler sire to Leghorn in the Iowa State University resource population; the **three SNPs and an indel in bold** (one per amplicon; referred to as SNP1, 2, 3, and 4 in the text and Table S8) were used for F2 genotyping of the ISU broiler-Leghorn resource population.

**Table S8**: Associations (*P* value) of SNPs with chicken skeletal and growth traits in a broiler-Leghorn F2 cross.

| Trait | *P* value | | | | |
|---|---|---|---|---|---|
| | **SNP1** | **SNP2** | **TGFB2** | **SNP3** | **SNP4** |
| **Skeletal Traits** | | | | | |
| BMC (g) | NS | 0.03 | 0.02 | NS | NS |
| BMD (g/cm2) | NS | 0.1 | 0.05 | NS | NS |
| TBL (mm) | NS | NS | 0.05 | 0.17 | NS |
| **Growth** | | | | | |
| BW (g) 2 wk | 0.19 | NS | NS | NS | NS |
| BW (g) 4 wk | 0.13 | NS | NS | NS | NS |
| BW (g) 6 wk | 0.14 | 0.07 | NS | NS | NS |
| BW (g) 8 wk | 0.14 | 0.04 | NS | NS | NS |

Location of SNPs noted in Table S7; NS = $P > 0.20$; BMC = bone mineral content; BMD = bone mineral density; TBL = tibia length; BW = body weight; TGFB2 data comes from Li et al. 2003.

## Application of SNPs for candidate gene association: Cytokines

Cytokines control the immune response, and in mammals, polymorphisms in cytokine genes are associated with disease resistance or susceptibility[1]. We identified 326 SNPs in some 12 pro-inflammatory, Th1, Th2 and Treg cytokine genes previously characterized in our laboratory. Forty such SNPs that mapped to coding sequences or known regulatory regions were amplified by PCR of genomic DNA from each of 8 inbred White Leghorn (Layer) lines. SNPs were identified by direct sequencing of the PCR products, and 32 of them were informative (Table S9). Six segregated between eight inbred layer lines (Table S10), and they mapped correctly in the genome when their segregation was analyzed in backcross mapping populations (Compton reference populations line $6_1$ x line $7_2$ and line 15I x line N - PMID 1353476)[2]. Four of the SNPs, in the Th2 cytokine genes IL-4 and IL-13 that drive antibody responses, segregated between the inbred Layer lines N and 15I that show differential antibody responses to vaccination[3]. They are therefore candidate SNPs for the differential responses between these two lines.

## References


1. Gallagher, G., Eskdale, J. & Bidwell, J.L. Cytokine genetics - polymorphisms, functional variations and disease associations. In *The Cytokine Handbook, 4th Ed.* (eds. Thomson, A.W. & Lotze, M.T.) 19-55 (Academic Press, London, 2003).
2. Bumstead, N. Genomic mapping of resistance to Marek's disease. *Avian Pathol.* **27**, S78-S81 (1998).
3. Bumstead, N. et al. *EU Project FAIR3 PL96-1502 New molecular approaches for improved poultry vaccines* (Institute for Animal Health, Compton, 2000).

**Table S9**. Details of SNPs identified within cytokine genes. The cytokines are grouped according to function. B-L-S = broiler-layer-Silkie, i.e. the number of SNPs identified in a particular line for each cytokine gene. Forty of these SNPs were in coding or regulatory regions. Of these, 32 were informative. Of these, 6 segregated between our inbred lines, and their id numbers are given.

| Cytokine gene | No. of SNPs (B-L-S) | No. of informative SNPs | No. of segregating SNPs | SNP # |
|---|---|---|---|---|
| **Pro-inflammatory** | | | | |
| IL-6 | 0-0-6 | 0 | 0 | - |
| **Th1** | | | | |
| IL-2 | 0-0-2 | 1 | 0 | - |
| IL-12α | 18-3-12 | 19 | 1 | snp.43.100.1355.S.1 |
| IL-12β | 17-10-33 | | 0 | - |
| IL-18 | 25-0-19 | 0 | 0 | - |
| **Th2** | | | | |
| IL-4 | 3-13-12 | 5 | 4 | snp.103.50.22506.S.3<br>snp.103.50.22726.S.3<br>snp.103.50.22795.S.3<br>snp.103.50.22884.S.3 |
| IL-5 | 14-2-9 | 0 | 0 | - |
| IL-13 | 0-2-15 | 4 | 1 | snp.103.50.16122.S.3 |
| **Treg** | | | | |
| IL-10 | 2-0-3 | 0 | 0 | - |
| **Others** | | | | |
| IL-3 | 9-6-9 | 0 | 0 | - |
| IL-15 | 15-14-24 | 0 | 0 | - |
| GM-CSF | 10-8-10 | 3 | 0 | - |

**Table S10**: SNPs in cytokine genes that are polymorphic between layers with different MHC haplotypes. SNPs are shown as nucleotide changes, with positions in the chicken genome indicated by chromosome and base number. BLS refers to the sequence in broiler, layer, and/or Silkie (BLS = change in broiler, layer or Silkie respectively, - = no change, x = not sequenced). The gene in which each SNP is located is indicated. Under MHC haplotype, - = not determined. The four SNPs in bold were used for Backcross genotyping of the Compton Mapping (Nx15I) and MDV Mapping (6x7) populations.

| | | | | | Line (MHC B Haplotype) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SNP Number** | **Chr. - Base No.** | **SNP** | **BLS** | **Gene** | **6 (2)** | **7 (2)** | **15I (15)** | **N (21)** | **0 (21)** | **W (14)** | **B4** | **B12** | **Notes** |
| snp.43.100.1355.S.1 | 9-21724516 | T>C | BXX | IL-12A | C | - | - | C | - | T | C | C | Promoter |
| snp.103.50.16122.S.3 | 13-15971216 | **G>C** | XXS | IL-13 | C | G | C | G | G | G | C | C | Promoter |
| snp.103.50.22506.S.3 | 13-15977600 | T>C | X-S | IL-4 | C | C | C | T | C | C | C | C | Promoter |
| snp.103.50.22726.S.3 | 13-15977820 | **C>T** | X-S | IL-4 | T | T | T | C | - | T | T | T | Promoter |
| snp.103.50.22795.S.3 | 13-15977889 | **T>C** | XXS | IL-4 | C | T | C | C | C | T | C | C | Met>Thr |
| snp.103.50.22884.S.3 | 13-15977978 | **G>A** | XXS | IL-4 | G | G | A | G | G | G | G | G | Intronic |

## Application of SNPs for candidate gene association: The MHC

DNA from eight 15-B congenic lines[1,2] was analyzed. The DNA was purified from whole blood cells using the QIAamp DNA Blood Minikit (QIAGEN, Valencia, CA), and then used as a template in a standard PCR reaction with the primers given in Table S11. When the SNP generated a restriction site, the PCR product was further analyzed by restriction fragment length polymorphism (RFLP). When the SNP produced no restriction site, the PCR product was directly sequenced with an ABI 3100 (both strands). We had previously sequenced numerous MHC-encoded genes from different haplotypes of White Leghorn (layer) chickens. We could therefore easily determine that some of the nucleotides in the MHC-encoded genes with SNPs from broiler, layer, and Silkie were also polymorphic between our haplotypes. Moreover, these SNPs can be used to distinguish between lines of White leghorn chickens that are resistant or susceptible to commercially important pathogens like Marek's Disease Virus. The combined results from both studies are shown in Table S12.

## References


1. Shen, P.F., Smith, E.J. & Bacon, L.D. The ontogeny of blood cells, complement and immunoglobulins in 3- to 12-week-old 15I5-B congenic white Leghorn chickens. *Poult. Sci.* **63,** 1083-1093 (1984).
2. Bacon, L.D., Ismail, N. & Motta, J.V. Allograft and antibody responses of 15I5 B congenic chickens. *Prog. Clin. Biol. Res.* **238,** 219-233 (1987).

**Table S11**. Details of primers and methods used to analyze SNPs in the MHC.

| SNP# | Chr. - Base No. | Forward Primer | Reverse primer | SNP Detection Method |
|---|---|---|---|---|
| snp.26856.S.1 | MHC-26856 | GCCTGAACCTTGATGTCCTTA | TTAGGGGACCGATGCTATG | RFLP (MnlI) |
| snp.36295.S.2 | MHC-36295 | ACAACGACAGCCCTAAGCACA | GGCAGCCGATGGAACCTAC | RFLP (MaeII) |
| snp.67126.S.2 | MHC-67126 | CACGTGGAGGGACAGCGGTCA | GGGACACTGAGCCGCACGCA | Sequencing |
| snp.67152.S.2 | MHC-67152 | CACGTGGAGGGACAGCGGTCA | GGGACACTGAGCCGCACGCA | Sequencing |
| snp.67164.S.2 | MHC-67164 | CACGTGGAGGGACAGCGGTCA | GGGACACTGAGCCGCACGCA | Sequencing |
| snp.67221.S.2 | MHC-67221 | CACGTGGAGGGACAGCGGTCA | GGGACACTGAGCCGCACGCA | Sequencing |
| snp.67272.S.2 | MHC-67272 | CACGTGGAGGGACAGCGGTCA | GGGACACTGAGCCGCACGCA | Sequencing |
| snp.64376.S.2 | MHC-64376 | CCCTTTGGCTGCGAGGATCTC | CGCTCACTCCACGCCAAC | RFLP (BstNI) |
| snp.69245.S.1 | MHC-69245 | TGGGGGCCGTTCTAAA | GCTCCAGGCAGACCTACATAG | RFLP (DsaI) |

**Table S12**: SNPs in the chicken MHC that are polymorphic between layers with different MHC haplotypes. SNPs are shown as nucleotide changes, with position in the genome indicated by chromosome and base number, except for those labeled MHC, which are numbered according to EMBL Acc. No. AL023516. BLS refers to the sequence in broiler, layer, and/or Silkie (BLS = change in broiler, layer or Silkie respectively, - = no change, x = not sequenced). The gene in which each SNP is located is indicated and the amino acid residue encoded is shown in bold (where applicable). Under MHC haplotype, - = not determined.

| | | | | | MHC B Haplotype | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SNP Number** | **Chromosome - Base No.** | **SNP** | **BLS** | **Gene** | **2** | **4** | **5** | **12** | **13** | **14** | **15** | **19** | **21** | **Notes** |
| snp.7544.2.239.S.3 | Un-151325532 | C>T | XXS | TAP1 exon 10 (RD**P**RI) | C | C | - | C | C | C | C | C | C | non-coding |
| snp.7544.2.566.S.3 | Un-151325859 | A>G | XXS | TAP1 exon 11 (AE**R**VV) | G | A | - | G | A | G | G | G | A | non-coding |
| snp.7544.2.576.S.3 | Un-151325869 | T>C | XXS | TAP1 exon 11 (VV**L**EG) | T | T | - | C | T | T | C | C | T | non-coding |
| snp.368.11.10208.S.2 | 16-168065 | A>G | -LX | BNK exon 6 (RL**H**P) | G | G | - | G | - | G | G | G | G | His>Tyr |
| snp.368.11.11881.S.2 | 16-169738 | C>T | XLX | BNK intron 1 | T | T | - | T | - | T | T | T | C | non-coding |
| snp.368.12.1112.S.2 | 16-171995 | T>C | XLS | Blec 5'UTR | C | C | - | C | - | C | C | C | C | non-coding, possible NF-AT site |
| snp.368.12.1115.S.2 | 16-171998 | G>C | XLS | Blec 5'UTR | C | C | - | C | - | C | C | C | G | non-coding |
| snp.368.14.2060.S.2 | 16-178072 | T>C | XLX | Tapasin exon 5 (RV**S**VR) | C | C | - | C | - | C | T | C | C | non-coding |
| snp.368.14.2069.S.2 | 16-178081 | G>A | XLX | Tapasin exon 5 (VR**L**LL) | G | G | - | G | - | G | G | G | A | non-coding |
| snp.26856.S.1 | MHC-26856 | G>A | BXX | B-NK exon 4 (AE**E**DH) | A | - | A | G | A | - | A | G | A | Glu>Lys |
| snp.36295.S.2 | MHC-36295 | A>G | XLX | Tapasin exon 5 (GD**I**YS) | G | - | G | A | G | - | G | A | G | Ile>Val |
| snp.64376.S.2 | MHC-64376 | A>G | XLX | TAP1 exon 9 (AR**Q**VG) | G | - | G | A | G | - | G | A | G | Gln>Arg |
| snp.69245.S.1 | MHC-69245 | G>A | BXX | TAP2 exon 1 (GP**R**GA) | G | - | G | G | G | - | G | G | G | Arg>His |
| snp.67126.S.2 | MHC-67126 | G>A | XLX | TAP1 exon 2 (QRF) | G | - | G | G | G | - | A | G | G | non-coding |
| snp.67152.S.2 | MHC-67152 | A>C | XLX | TAP1 exon 2 | C | - | A | A | C | - | C | C | C | non-coding |
| snp.67164.S.2 | MHC-67164 | A>G | XLX | TAP1 exon 2 | A | - | A | A | A | - | G | A | G | non-coding |
| snp.67221.S.2 | MHC-67221 | C>T | XLX | TAP1 exon 2 | C | - | C | C | T | - | T | C | T | non-coding |
| snp.67272.S.2 | MHC-67272 | T>C | XLX | TAP1 exon 2 | T | - | T | T | C | - | C | T | T | non-coding |