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GPh1.6

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Candidate SNPs for chicken immune response genes

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Chicken MHC and *Rfp-Y* region

Chicken microchromosome 16 (GGA16):

MHC complex – NOR – *Rfp-Y* region

- **MHC (B complex):** 19 genes, 92 kb (Kaufman *et al.* 1999, Immunol Rev, 167:101-117)
- ***Rfp-Y* region:** MHC-like, contains at least 2 non-classical class I genes and 3 non-classical class II genes (Miller *et al.*, 1994, Proc Natl Acad Sci, 91:4397-4401; Miller *et al.*, 1996, Proc Natl Acad Sci, 93:3958-3962)

Analised genes

- **B complex:**
 - *Tapasin*
 - *TAP1*
 - *TAP2*
- ***Rfp-Y* region:**
 - *YFV*
 - *YFVI*

Abbreviations

■	B	Broiler
■	OVA	Layer
■	R	Breeder
■	PPP	Pepoi
■	PER	Ermellinato di Rovigo
■	PRL	Robusto Lionato
■	PRM	Robusto Maculato

Methods

- Design of specific primer pairs
- Genomic DNA PCR
- Sequencing
- In silico analysis  **PolyBayes**

Tapasin

- **Product** of 448 bp: Exon 5 – Intron – Exon 6
- 66 animals analysed
- 98% homology with **BLASTn** vs AJ004999 and AL023516
- From alignment with **ClustalW**: nucleotide differences due to heterozygosity

ClustalW alignment (example)

```
OVA15      CCRTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
OVA18      CCRTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
R1         CCRTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
PER315     CCGTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
PRL106     CCGTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
OVA5       CCGTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
PER314     CCGTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
OVA12      CCGTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
OVA16      CCGTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
B1         CCGTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
R8         CCGTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
B4         CCRTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
B6         CCRTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
B5         CCRTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
R3         CCRTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
B8         CCGTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
B13        CCGTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
R7         CCGTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
R5         CCGTGGTTAGGAGGGTCTGAGGGACATCAGGACCATGGCCTGGGACAATGGGAGATCATG 300
**  ****
```

PolyBayes rules

- **Bayesian-statistical formulation** that calculates the SNP probability score $P(\text{SNP})$
- **Is based on:**
 - Alignment depth
 - Base calls
 - Base quality values
 - Base composition
 - A priori polymorphism rate
- **Output:** list of candidate polymorphic sites, each with an associated SNP probability score

PolyBayes results

SNP position	Transition/Transversion	P(SNP)
93	C/T	0.999999992848812
94	G/A	1
113	C/T	1
125	C/T	0.999999998917252
161	C/T	1
167	C/T	1
176	G/A	1
196	G/A	1
217	C/T	0.99997349531803
243	G/A	1
370	C/T	1
404	C/T	0.999932085883546



12 SNP sites identified

TAP1

- **Product** of 431 bp: Exon 5 – Intron – Exon 6
- 67 animals analysed
- 98% homology with **BLASTn** vs AL023516
- **ClustalW**

PolyBayes results

SNP Position	Transition / Transversion	P(SNP)
68	A/G	1
89	C/T	1
96	C/T	1
110	C/T	1
111	A/G	1
116	G/T	1
136	G/A	0.999552795039223
146	T/C	1
197	C/T	0.9999999978104289
210	G/A	0.99
250	C/T	1
257	C/T	0,992578909243932
274	G/A	1
331	G/A	1
340	G/A	0,998486641554802
367	C/T	1
368	C/T	1
372	A/G	0.999999982112263



18 SNP sites identified

TAP2

- **Product** of 399 bp: Exon 4 – Intron – Exon 5 – Intron – Exon 6
- 66 analysed animals
- 97% homology with **BLASTn** vs AL023516
- **ClustalW**

PolyBayes results

SNP Position	Transition / Transversion	P(SNP)
22	A/C	1
23	G/A	1
37	G/A	1
41	G/A	1
60	A/C/T	1
61	G/A	1
89	C/T	1
97	A/C	1
98	G/A/-	1
116	C/T	0.996341190717853
131	G/A	1
134	G/A	1
143	G/A	1
157	G/A	1
180	G/A	1
188	C/T	1
212	A/T	1
248	G/A	1
261	C/G	0.999639450470216
300	C/T	1
336	C/G	1



21 SNP sites identified

YFVI

- **Product** of 198 bp: Exon 2 - Intron
- 56 analysed animals
- 98% homology with **BLASTn** vs AF218784
- **ClustalW**

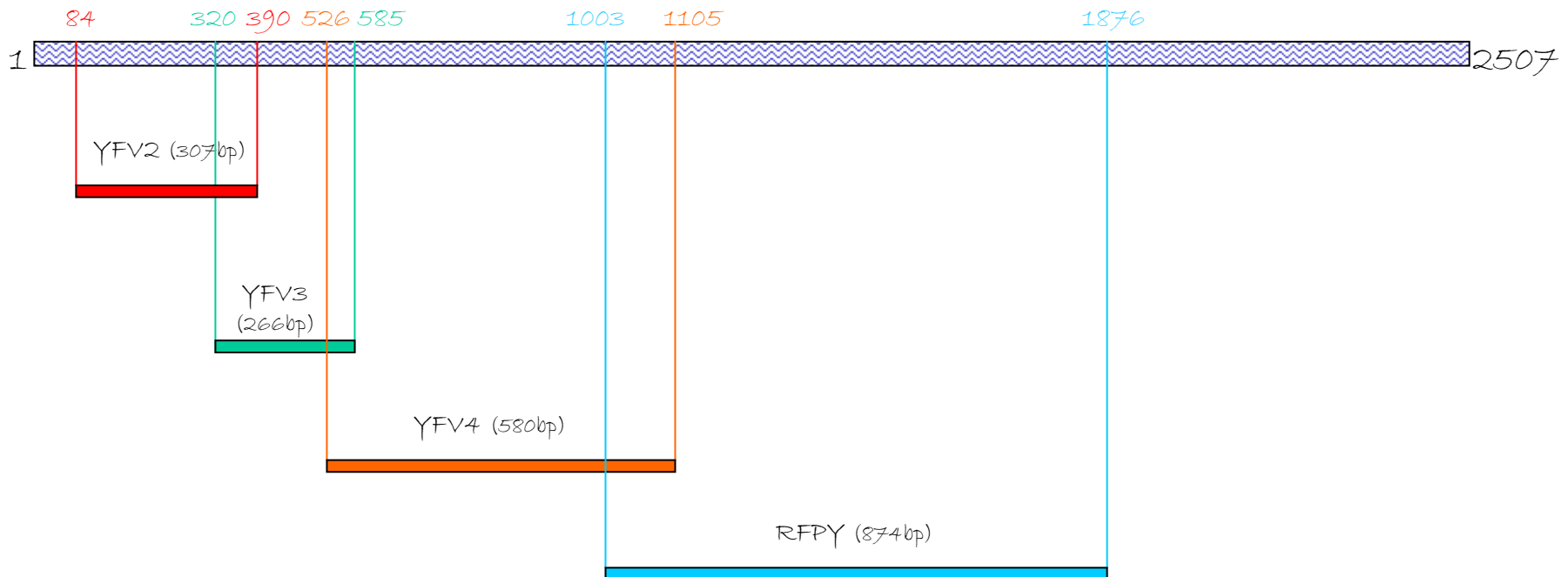
PolyBayes results

SNP Position	Transition / Transversion	P(SNP)
65	C/T	0.999999999761322
122	G/A	1



2 SNP sites identified

YFV



Total product: 1791 bp (from exon 1 to exon 6)

YFV

- Alignment depth:
 - ❖ YFV2 ➡ 24
 - ❖ YFV3 ➡ 28
 - ❖ YFV4 ➡ 22
 - ❖ RFPY ➡ 5
- 95% homology with **BLASTn** vs AF218783
- **ClustalW**

ClustalW alignment (example)

```
B7      CAGAAGGCCCRGGGCGGTGAGCGGGATTTTGACTGGTTCCTGAGCAGGCTGCCGGAACGC 240
B8      CAGAAGGCCCRGGGCGGTGAGCGGGATTTTGACTGGTTCCTGAGCAGGCTGCCGGAACGC 240
OVA11   CAGAAGGCCCAGGCGCGGTGAGCGGGATTTTGACTGGKKCCTGRGCAGGCTGCCGGAACGC 240
PRM328  CAGAAGGCCCAGGCGCGGTGAGCGGGATTTTGACTGGKKCCTGRGCAGGCTGCCGGAACGC 240
OVA12   CAGAAGGCCCAGGCGCGGTGAGCGGGATTTTGACWGGKKCCTGRGCAGGCTGCCGGAACGC 240
OVA13   CAGAAGGCCCAGGCGCGGTGAGCGGGATTTTGACWGGKKCCTGRGCAGGCTGCCGGAACGC 240
B9      CAGAAGGCCCRGGGCGGTGAGCGGGATTTTGACTGGKKCCTGAGCAGGCTGCCGGAACGC 240
OVA9     CAGAAGGCCCAGGCGCGGTGAGCGGGATTTTGACAGGGGCCTGGGCAGGCTGCCGGAACGC 240
OVA16   CAGAAGGCCCAGGCGCGGTGAGCGGGATTTTGACAGGGGCCTGGGCAGGCTGCCGGAACGC 240
OVA18   CAGAAGGCCCAGGCGCGGTGAGCGGGATTTTGACAGGGGCCTGGGCAGGCTGCCGGAACGC 240
PER314  CAGAAGGCCCAGGCGCGGTGAGCGGGATTTTGACAGGGGCCTGGGCAGGCTGCCGGAACGC 240
PER316  CAGAAGGCCCAGGCGCGGTGAGCGGGATTTTGACAGGGGCCTGGGCAGGCTGCCGGAACGC 240
PPP350  CAGAAGGCCCGGGGCGGTGAGCGGGATTTTGACAGGGGCCTGGGCAGGCTGCCGGAACGC 240
OVA14   CAGAAGGCCCAGGGTGGTGAAGCGGGATTTTGACTGGTTCCTGAGCAGGCTGCCGGAACGC 240
B15     CAGAAGGCCCGGGATGGTGAAGCAGGATTTCTCGGGTTCCTGAGCAGGCTGCCGGAACAC 240
*****  **  *****  *****  *  **  *****  *****  *
```

PolyBayes results

- Identified **90** SNP sites:

Transition/Transversion	No. of sequences
G/A	30
C/T	23
G/C	8
A/T	5
C/A	15
G/T	7
A/C/T	1
A/G/T	1

- P(SNP) is **>0.99** for every SNP site

Conclusions

- International Chicken Polymorphism Map Consortium (2004, Nature, 432:717-722): 5 SNPs kb⁻¹
- Exception: GGA16 → higher number of SNPs (>12 SNPs kb⁻¹)
- MHC genes in this work:



the high number of SNPs
within GGA16 is confirmed

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