doi:10.1111/j.1365-2052.2003.01082.x

A radiation hybrid map of chicken chromosome 15

D. G. J. Jennen*, R. P. M. A. Crooijmans*, M. Morisson†, A. E. Grootemaat*, J. J. van der der Poel*, A. Vignal† and M. A. M. Groenen*

*Animal Breeding and Genetics Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen University, Wageningen, The Netherlands. †Laboratoire de génétique cellulaire, Institut national de la recherche agronomique, Castanet-Tolosan, France

Summary

We have constructed a radiation hybrid (RH) map of chicken chromosome (GGA) 15. This map can be used as a resource to efficiently map genes to this chromosome. The map has been developed using a 6000 rad chicken–hamster whole-genome radiation hybrid panel (ChickRH6). In total, six microsatellite loci, 18 sequence tagged sites (STSs) from BAC end sequences and 11 genes were typed on the panel. The initial framework map comprised eight markers, and an additional 23 markers were then added to generate the final map. The total map length was 334 centiRay$_{6000}$ (cR$_{6000}$). The estimated retention frequency for the data set was 18%. Using an estimated physical length of 21 Mb, the ratio between cR$_{6000}$ and physical distance over GGA15 was estimated to be 0.063 Mb/cR$_{6000}$. The present map increases the marker density and the marker resolution on GGA15 and enables fast mapping of new chicken genes homologous to genes from human chromosomes 12 and 22.

Keywords chicken, chicken chromosome 15, framework map, radiation hybrid panel.

Radiation hybrid (RH) mapping has proven to be an efficient way for the construction of physical maps with a resolution intermediate between genetic maps and bacterial artificial chromosome (BAC) contigs. The mapping is performed by simple polymerase chain reaction (PCR), therefore RH markers need not to be polymorphic. Whole-genome RH panels are available for several domestic animals, e.g. cow (Womack *et al.* 1997), pig (Yerle *et al.* 1998) and horse (Kiguwa *et al.* 2000). A chicken whole-genome RH panel was created by Morisson *et al.* (2002), by using 6000 rad of gamma rays. This panel, called ChickRH6 consists of 90 hybrid clones. We report here the first application of this panel for the construction of a RH map of chicken chromosome (GGA) 15. This chromosome, containing quantitative trait loci for fatness traits (Ikeobi *et al.* 2002; Jennen *et al.* in press) has been used previously in a comparative mapping study (Jennen *et al.* 2003a).

A detailed description of the microsatellite markers used for the construction of the RH map of GGA15 have been published by Groenen *et al.* (2000). Genes previously mapped on GGA15 (*CRYBB1, HIRA, PITPNB*, and *TBX3*) and STSs derived from BAC end sequences of GGA15 BAC contigs were described by Jennen *et al.* (2003a). From the comparative mapping information described by Jennen *et al.* (2003a) seven chicken orthologues of human genes located in human on chromosomes 12 and 22 were used in this study. Primers were designed from sequences of these chicken orthologues, available in public databases (Table 1). Primer pairs that gave a clear amplification product in chicken and not in the hamster DNA control were used for RH typing. Ten to 25 ng of each panel DNA in a 384-well plate was amplified in a 6 μl reaction mixture containing 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH = 8.3), 1 mM tetra-methylammoniumchloride (TMAC), 0.1% Triton X-100, 0.01% gelatine, 0.2 mM of each dNTP, 0.125 U Silverstar polymerase (Eurogentec, Liege, Belgium) and 1.2 pmol of each primer. Amplification was carried out under the conditions as follows: denaturation at 95 °C for 2 min, then 35 cycles at 95 °C for 30 s, optimal annealing temperature (45–60 °C) for 45 s and 72 °C for 60 s. Scoring of the panel was performed as a plus–minus screening on an ethidiumbromide stained 1.5% agarose gel in 0.5X TBE. Each marker was typed in duplicate independently.

A framework map was constructed, using the Carthagene program (Schiex & Gaspin 1997). From the previously constructed BAC contigs (Jennen *et al.* 2003a), eight markers (one per BAC contig) were used to compute a 1000 : 1 framework map (LOD score greater than 3.0). The

Address for correspondence

D. G. J. Jennen, Animal Breeding and Genetics Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands.
E-mail: danyel.jennen@wur.nl

Accepted for publication 29 November 2003

Table 1 Characteristics of sequence tagged site markers developed in chicken genes.

Gene	Accession number		Human cytogenetic map position	PCR size (bp)	Forward primer (5′–3′)	Reverse primer (5′–3′)
	Chicken	Human[1]				
ACADS	BU305889	NM_000017	12q22-qter	>800[2]	CATTAGTGCATTCCTTGTTC	ATGAGCGATGTTTGTTGCAG
TBX5	AF069396	NM_000192	12q24.1	343	AAACTTCACCAGCGGAAGAG	TGGAACATGCTATGGGTGTC
TCF1	X67690	NM_000545	12q24.2	422	TGCTGCCATCCACTCATAAC	TGTCTTGGCATTTTCTGCTG
LIMK2	D26310	NM_005569	22q12.2	112	AAACTGGGTCCAGTGGATTC	CCACACATTACCTAGGACTC
NF2	AJ393948	NM_000268	22q12.2	167	GATGAGGTCTGAAGAGACAG	CCTTCTGTTCCATCAGTCGC
SERPIND1	AF061728	NM_000185	22q11.21	176	TAAAGCAGAGAACACCACCG	TGCTATTGAGTCCATTCACG
SNRPD3	AJ397202	NM_004175	22q11.23	135	TTTGGCCTTGACCAGTATGC	TGTGTAAGAGGAGTTCTGTC

[1]Accession number of human genes used in BLAST search to identify chicken orthologous genes.
[2]Fragment contains introns of unknown size.

framework map consisted of six microsatellite loci (*MCW0031, MCW0226, LEI0120, ADL0039, MCW0231*, and *MCW0080*), one STS from BAC end sequence (*ST15BE142*), and one gene (*TBX3*) (Fig. 1). Additional markers that have a LOD score greater than 5.0 with at least one framework marker, were placed on the map. Four previously mapped markers, including the gene *CRYBB1* did not meet this criterion and were excluded from the map. In order to avoid inflation of the map size, we chose to project additional markers at their most likely location without altering the multipoint distance between framework markers (Fig. 1). Twenty-three markers were consistently integrated into the framework map. Finally, the radiation hybrid map of GGA15 contained 31 markers with an average retention frequency of 18%. This is in good agreement with the retention frequencies found for microchromosomes by Morisson *et al.* (2002). The total map length was 334 centiRay$_{6000}$ (cR$_{6000}$). With an estimated physical length of GGA15 of 21 Mb (Jennen *et al.* 2003a), the ratio between cR$_{6000}$ and physical distance over GGA15 was estimated to be 0.063 Mb/cR$_{6000}$.

Our data clearly show that the previous assignment of *LIMK2* to GGA2 (Groenen & Crooijmans 2003) was not correct and that the gene is located on GGA15. As a result of sequencing errors, *LIMK2* was incorrectly linked to microsatellite marker *MCW0189*, which is located on GGA2. Furthermore, six previously unmapped genes, i.e. *ACADS, NF2, SERPIND1, SNRPD3, TBX5*, and *TCF1* were also mapped to GGA15. By mapping seven new genes, we also improved the comparative map of GGA15 with human and mouse. The number of conserved segments increased to at least 19 segments in the chicken–human–mouse comparison (Fig. 2). Using the same approach previously described (Jennen *et al.* 2003a), we estimated, for the whole chicken genome, the total number of conserved segments to be at least 1000 and the rate of chromosomal change in the chicken lineage to range from 2–3 rearrangements per million years because the divergence 300 Ma. This is slightly higher than the previous estimates (Jennen *et al.* 2003a).

Acknowledgements

This work was financially supported by the Netherlands Technology Foundation (STW; grant WBI.4706).

References

Groenen M.A.M. & Crooijmans R.P.M.A. (2003) Chapter 26. Structural genomics: integrating linkage, physical and sequence maps. In: *Poultry Genetics, Breeding and Technology* (Ed. by W.M. Muir & S.E. Aggrey), pp. 497–536. CAB International, Wallingford.

Groenen M.A.M., Cheng H.H., Bumstead N., *et al.* (2000) A consensus linkage map of the chicken genome. *Genome Research* **10,** 137–47.

Ikeobi C.O.N., Woolliams J.A., Morrice D.R., *et al.* (2002) Quantitative trait loci affecting fatness in the chicken. *Animal Genetics* **33,** 428–35.

Jennen D.G.J., Crooijmans R.P.M.A., Kamps B., *et al.* (2003a) Comparative map between chicken chromosome 15 and human chromosomal region 12q24 and 22q11-q12. *Mammalian Genome* **14,** 629–39.

Jennen D.G.J., Vereijken A.L.J., Bovenhuis H., *et al.* Detection and localization of quantitative trait loci affecting fatness in broilers. *Poultry Science* in press.

Kiguwa S.L., Hextall P., Smith A.L., *et al.* (2000) A horse whole-genome-radiation hybrid panel: chromosome 1 and 10 preliminary maps. *Mammalian Genome* **11,** 803–5.

Morisson M., Lemière S., Bosc S., *et al.* (2002) ChickRH6: a chicken whole genome radiation hybrid panel. *Genetics, selection, evolution* **34,** 521–33.

Schiex T. & Gaspin C. (1997) CARTHAGENE: constructing and joining maximum likelihood genetic maps. *Proceedings of International Conference on Intelligent Systems for Molecular Biology* **5,** 258–67.

Womack J.E., Johnson J.S., Owens E.K., Rexroad C.E., III, Schläpfer J. & Yang Y.-P. (1997) A whole-genome radiation hybrid panel for bovine gene mapping. *Mammalian Genome* **8,** 854–6.

Yerle M., Pinton P., Robic A., *et al.* (1998) Construction of a whole-genome radiation hybrid panel for high-resolution gene mapping in pigs. *Cytogenetics and Cell Genetics* **82,** 182–8.

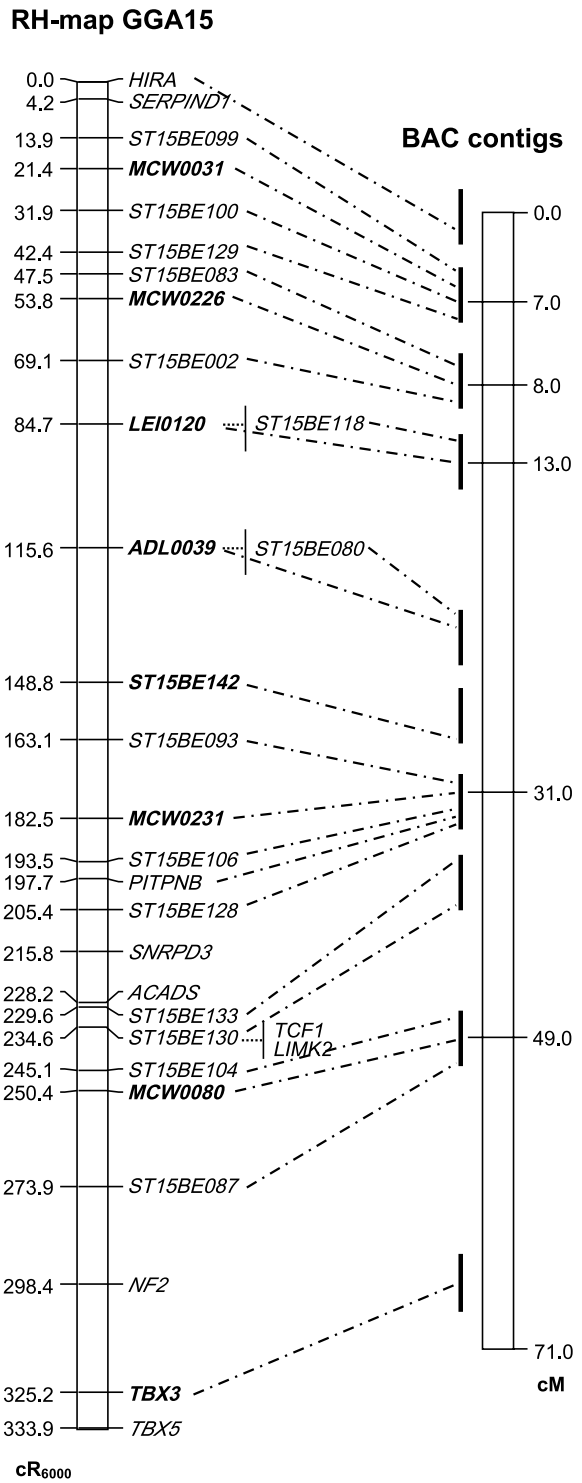

Figure 1 Radiation hybrid (RH) map of chicken chromosome 15 (GGA15) comprising 31 loci. The markers of the 1000 : 1 framework map (LOD score greater than 3.0) are highlighted in bold. Previously unmapped genes are shown in italics. The RH map is aligned with the GGA15 BAC contigs (not to scale). The relative positions of the anchor loci on the GGA15 linkage map is given in cM according to Groenen and Crooijmans (2003).

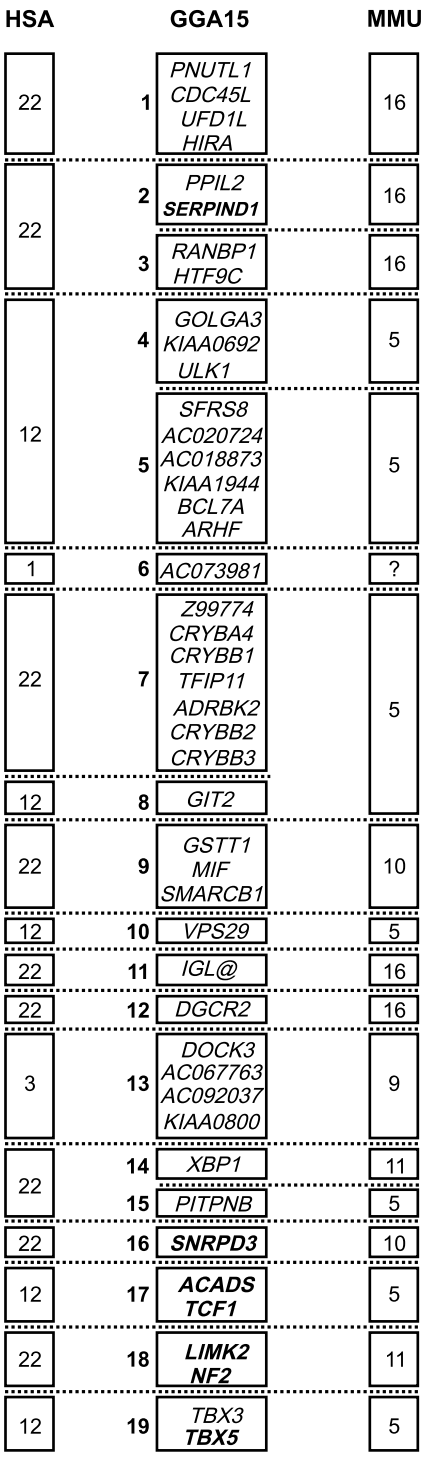

Figure 2 Comparative map of chicken chromosome 15 (GGA15) to human (HSA) and mouse (MMU). Previously unmapped genes are shown in bold. Chromosome segments in which the gene order in all three species is the same, are indicated by block 1–19. Positions of chromosomal rearrangements are indicated by dotted lines, with the chicken gene order as a start. The numbers of the human and mouse chromosomes are shown inside the vertical bars of HSA and MMU, respectively.