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Chromosomal assignment of chicken clone contigs by extending the consensus linkage map

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Summary

The bacterial artificial clone-based physical map for chicken plays an important role in the integration of the consensus linkage map and the whole-genome shotgun sequence. It also provides a valuable resource for clone selection within applications such as fluorescent *in situ* hybridization and positional cloning. However, a substantial number of clone contigs have not yet been assigned to a chromosomal location or have an ambiguous chromosome assignment. In this study, 86 single nucleotide polymorphism markers derived from 86 clones were mapped on the genetic map. These markers added anchoring information for 56 clone contigs and 13 individual clones, covering a total of 57 145 clones.

Keywords chicken, FPC, genetic map, physical map.

Introduction

Genetic and genomic research in chicken has been driven by its importance as a major worldwide food source (Speedy 2003) and its prominent role in research areas such as embryology and immunology (Brown *et al.* 2003). Several genomic resources have been built to facilitate this, including the consensus linkage map (Groenen *et al.* 2000), a large collection of expressed sequence tags (ESTs) (Boardman *et al.* 2002), a single nucleotide polymorphism (SNP) resource containing 2.8 million SNPs (International Chicken Polymorphism Map Consortium 2004) and the recently published whole-genome DNA sequence (International Chicken Genome Sequencing Consortium 2004).

As a result of the low number of interspersed repetitive elements in the chicken genome, this draft sequence is of high quality compared with the first draft sequences of the human genome (International Human Genome Sequencing Consortium 2001). However, the relatively low number of genetic markers and the presence of two distinct chromosomal subtypes in the chicken genome make it more difficult to assign a chromosomal location for the chicken sequence contigs.

The chicken karyotype consists of 38 pairs of autosomes and two sex chromosomes. The eight largest chromosomes and the Z-sex chromosome can be cytogenetically distinguished. However, the W chromosome and the other 30 pairs of chromosomes are small, lack a clear banding pattern and until recently, could not be unequivocally identified (Fillon *et al.* 1998; Masabanda *et al.* 2004). Sequence maps are currently only available for 30 chromosomes and efforts are ongoing to construct linkage and sequence maps for the remaining nine as well.

The chicken bacterial artificial chromosome (BAC) contig map covering 95% of the chicken genome (Wallis *et al.* 2004) provides an important intermediate resource to aid in this effort. This map of over 180 000 clones represents a 20-fold coverage of the chicken genome, and consists of 260 BAC contigs, of which 226 were assigned to a particular chromosome. However, 10% of the latter still have links to multiple chromosomes. To increase the integrity of this map, additional links between clone contigs and genomic location are necessary.

In this paper, we describe the anchoring of 86 additional clones to the genetic map, resulting in the extension of the genetic linkage map and chromosomal assignment for 56 contigs and 13 individual BAC clones.

Materials and methods

SNP discovery

Ninety clones were selected from 69 finger printed contigs (FPC) that were not unambiguously anchored to the genetic map (see Table 1 and ChickFPC website http://www.animalsciences.nl/ChickFPC). For CH261 and TAM32 clones, end sequences were downloaded from the NCBI website

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Table 1 Marker development data and results.

Clone contig	SNP[1]	STS[2]	BAC	Reference population	Number of animals	Method	Restriction enzyme	Chromosome	Position (cM)
ctg1301	*SCW0039*	BV210197	WAG-38K9	Wageningen	191	SNaPshot		GGA14	47 ± 9
ctg1801	*SCW0032*	BV210196	WAG-65N20	Wageningen	56	SNaPshot		GGA1	72 ± 5
ctg1901	*SCW0042*	BV209676	CH261-102C18	Wageningen	90	SNaPshot		GGA3	65 ± 22
ctg39101	*SCW0004*	BV210185	WAG-119K7	East Lansing	48	RFLP	*Msp*I	GGA2	250 ± 5
ctg39802	*SCW0010*	BV210187	WAG-37C7	Wageningen	44	SNaPshot		GGA4	115 ± 10
ctg41701	*SCW0011*	BV210165	WAG-41N15	Wageningen	44	SNaPshot		GGA4	80 ± 15
ctg41701	*SCW0037*	BV210189	WAG-29I8_2	Wageningen	157	SNaPshot		GGA4	45 ± 1
ctg41701	*SCW0038*	BV210188	WAG-29I8_1	Wageningen	56	SNaPshot		GGA4	57 ± 15
ctg41701	*SCW0041*	BV210178	WAG-73D19	Wageningen	191	SNaPshot		GGA4	35 ± 3
ctg42901	*SCW0048*	BV209672	CH261-163P5	Wageningen	191	SNaPshot		GGA1	44 ± 6
ctg42901	*SCW0050*	BV209669	CH261-189M5	Wageningen	191	SNaPshot		GGA1	54 ± 4
ctg45001	*SCW0051*	BV209675	CH261-31M21	Wageningen	147	SNaPshot		GGA2	403 ± 10
ctg47701	*SCW0001*	BV210180	WAG-41F8	Wageningen	56	SNaPshot		GGA1	260 ± 15
ctg52701	*SCW0018*	BV210174	WAG-110C15	Wageningen	56	RFLP	*Tsp*509I	GGA15	64 ± 1
ctg63807	*SCW0053*	BV209677	CH261-94C11	Wageningen	113	SNaPshot		GGA7	54 ± 8
ctg63814	*SCW0335*	BV209710	CH261-178I12	Wageningen	113	SNaPshot		W35	9
ctg63820	*SCW0245*	BV210266	CH261-69E18	Wageningen	135	SNaPshot		Unlinked[3]	
ctg63820	*SCW0329*	BV210267	CH261-31K3	Wageningen	113	SNaPshot		Unlinked[3]	
ctg63826	*SCW0246*	BV209683	CH261-162M20	Wageningen	56	SNaPshot		GGAZ	62 ± 24
ctg63827	*SCW0247*	BV209688	CH261-141O12	Wageningen	135	SNaPshot		W35	0
ctg63832	*SCW0345*	BV209705	CH261-62F14	Wageningen	57	SNaPshot		GGAZ	161 ± 30
ctg63838	*SCW0024*	BV210186	WAG-55M22	Wageningen	56	SNaPshot		GGA19	24 ± 12
ctg63839	*SCW0035*	BV210195	WAG-46K16	Wageningen	57	SNaPshot		GGA2	107 ± 17
ctg63840	*SCW0332*	BV209695	CH261-131P12	Wageningen	113	SNaPshot		GGA11	11 ± 11
ctg63840	*SCW0351*	BV209706	CH261-153F12	Wageningen	113	SNaPshot		GGA11	11 ± 11
ctg63845	*SCW0008*	BV210181	WAG-107C4	Wageningen	100	RFLP	*Bse*NI	GGA3	195 ± 15
ctg63868	*SCW0009*	BV210172	WAG-24L19	East Lansing	48	RFLP	*Sst*I	GGA4	243 ± 7
ctg63878	*SCW0043*	BV209679	CH261-104D13	Wageningen	134	SNaPshot		GGA1	376 ± 6
ctg63883	*SCW0049*	BV209668	CH261-176B5	Wageningen	147	SNaPshot		GGA13	11 ± 15
ctg63886	*SCW0352*	BV209702	CH261-160P22	Wageningen	57	SNaPshot		GGAZ	97 ± 24
ctg63895	*SCW0336*	BV209708	CH261-191C15	Wageningen	113	SNaPshot		GGAZ	131 ± 17
ctg63896	*SCW0250*	BV209681	CH261-40L10	Wageningen	57	SNaPshot		GGA18	45 ± 10
ctg63903	*SCW0251*	BV209682	CH261-8E24	Wageningen	191	SNaPshot		GGA9	114 ± 7
ctg63910	*SCW0359*	BV210403	CH261-66M16	Wageningen	91	SNaPshot		GGA27	63 ± 6
ctg63912	*SCW0046*	BV209674	CH261-142A15	Wageningen	191	SNaPshot		GGA2	92 ± 7
ctg63912	*SCW0344*	BV209689	CH261-42J19	Wageningen	57	SNaPshot		GGA4	159 ± 16
ctg63914	*SCW0252*	BV209686	CH261-131I1	Wageningen	90	SNaPshot		GGA3	204 ± 14
ctg63920	*SCW0030*	BV210192	WAG-90M16	Wageningen	191	SNaPshot		GGA8	94 ± 2
ctg63920	*SCW0031*	BV210191	WAG-48E16	Wageningen	134	SNaPshot		GGA8	91 ± 10
ctg63929	*SCW0343*	BV209711	CH261-37P4	Wageningen	56	SNaPshot		GGA4	85 ± 16
ctg63930	*SCW0357*	BV210397	CH261-48G12	Wageningen	91	SNaPshot		GGA27	63 ± 6
ctg63930	*SCW0361*	BV210398	CH261-72H13	Wageningen	57	SNaPshot		GGA27	63 ± 6
ctg63932	*SCW0349*	BV209707	CH261-115H6	Wageningen	56	SNaPshot		GGA9	10 ± 10
ctg63935	*SCW0330*	BV209697	CH261-49E12	Wageningen	57	SNaPshot		GGA19	17 ± 17
ctg63953	*SCW0353*	BV210399	CH261-17L7	Wageningen	57	SNaPshot		GGA14	64 ± 14
ctg63979	*SCW0342*	BV209712	CH261-32I19	Wageningen	56	SNaPshot		GGA7	71 ± 28
ctg63980	*SCW0033*	BV210179	WAG-93J15	Wageningen	191	SNaPshot		GGA19	0 ± 1
ctg63984	*SCW0338*	BV209709	CH261-7G4	Wageningen	56	SNaPshot		E22C19W28	16 ± 16
ctg63986	*SCW0327*	BV209699	CH261-27M9	Wageningen	56	SNaPshot		Unlinked[3]	
ctg63986	*SCW0350*	BV209698	CH261-138O8	Wageningen	113	SNaPshot		GGA8	Unknown
ctg63994	*SCW0254*	BV209687	CH261-58G24	Wageningen	191	SNaPshot		GGA1	30 ± 15
ctg63994	*SCW0346*	BV209694	CH261-63K17	Wageningen	113	SNaPshot		GGA1	39 ± 15
ctg63999	*SCW0002*	BV210166	WAG-71L23	East Lansing	48	RFLP	*Sau*3AI	GGA1	497 ± 8
ctg63999	*SCW0003*	BV210171	WAG-20B19	Wageningen	44	RFLP	*Eco*91I	GGA1	527 ± 6
ctg64003	*SCW0354*	BV210401	CH261-26C20	Wageningen	91	SNaPshot		W36	0 ± 0

Table 1 Continued

Clone contig	SNP[1]	STS[2]	BAC	Reference population	Number of animals	Method	Restriction enzyme	Chromosome	Position (cM)
ctg64003	*SCW0363*	BV210402	CH261-97O10	Wageningen	57	SNaPshot		W36	0 ± 0
ctg64012	*SCW0047*	BV209670	CH261-158P14	Wageningen	147	SNaPshot		GGA6	32 ± 8
ctg64015	*SCW0255*	BV209685	TAM32-22L22	Wageningen	100	SNaPshot		GGA14	19 ± 11
ctg64017	*SCW0331*	BV209693	CH261-50J10	Wageningen	57	SNaPshot		GGA3	4 ± 4
ctg64017	*SCW0348*	BV209692	CH261-85B24	Wageningen	56	SNaPshot		GGA3	15 ± 15
ctg64019	*SCW0334*	BV210268	CH261-145G22	Wageningen	113	SNaPshot		Unlinked[3]	
ctg64030	*SCW0034*	BV210194	WAG-92E21	Wageningen	191	SNaPshot		GGA2	92 ± 7
ctg64031	*SCW0256*	BV209684	CH261-142J16	Wageningen	191	SNaPshot		GGA22	10 ± 10
ctg64031	*SCW0340*	BV209704	CH261-17L23	Wageningen	113	SNaPshot		GGA22	17 ± 4
ctg64032	*SCW0355*	BV210404	CH261-31G15	Wageningen	91	SNaPshot		GGA26	24 ± 9
ctg64032	*SCW0362*	BV210405	CH261-82G18	Wageningen	34	SNaPshot		GGA26	24 ± 9
ctg64041	*SCW0036*	BV210190	WAG-77D19	Wageningen	34	SNaPshot		GGA2	19 ± 20
ctg64041	*SCW0337*	BV209700	CH261-6K16	Wageningen	113	SNaPshot		GGA2	24 ± 17
ctg64041	*SCW0341*	BV209701	CH261-18K20	Wageningen	113	SNaPshot		GGA2	28 ± 7
ctg64046	*SCW0044*	BV209673	CH261-121J20	Wageningen	91	SNaPshot		GGA2	0 ± 6
ctg64049	*SCW0339*	BV209703	CH261-13I10	Wageningen	56	SNaPshot		GGAZ	150 ± 19
ctg80002	*SCW0045*	BV209678	CH261-128J23	Wageningen	135	SNaPshot		GGA1	323 ± 11
ctg80002	*SCW0052*	BV209671	CH261-62J9	Wageningen	191	SNaPshot		GGA1	330 ± 8
ctg80002	*SCW0328*	BV209696	CH261-28H4	Wageningen	113	SNaPshot		GGA1	310 ± 19
ctg80023	*SCW0333*	BV209690	CH261-140F12	Wageningen	57	SNaPshot		GGA3	86 ± 55
ctg80023	*SCW0347*	BV209691	CH261-83M13	Wageningen	57	SNaPshot		GGA3	57 ± 13
ctg80082	*SCW0248*	BV209680	CH261-164M21	Wageningen	191	SNaPshot		GGAZ	36 ± 36
Singleton	*SCW0006*	BV210173	WAG-9D11	East Lansing	48	SNaPshot		GGA2	54 ± 8
Singleton	*SCW0007*	BV210167	WAG-34N9	East Lansing	48	SNaPshot		GGA2	160 ± 10
Singleton	*SCW0012*	BV210183	WAG-12E10	East Lansing	48	RFLP	*Bsp*LI	GGAZ	150 ± 7
Singleton	*SCW0014*	BV210176	WAG-50C3	East Lansing	48	RFLP	*Aci*I	GGAZ	0 ± 10
Singleton	*SCW0015*	BV210184	WAG-41O5	Wageningen	100	RFLP	*Bse*NI	GGA9	60 ± 10
Singleton	*SCW0016*	BV210168	WAG-21I3	Wageningen	44	RFLP	*Tsp*RI	GGAZ	80 ± 12
Singleton	*SCW0019*	BV210170	WAG-4I21	East Lansing	48	RFLP	*Sst*I	GGA12	26 ± 10
Singleton	*SCW0022*	BV210169	WAG-34F1	East Lansing	48	SNaPshot		GGAZ	200 ± 20
Singleton	*SCW0023*	BV210182	WAG-38G23	Wageningen	56	SNaPshot		GGA5	95 ± 15
Singleton	*SCW0025*	BV210177	WAG-28L9	Wageningen	56	SNaPshot		GGA17	27 ± 12
Singleton	*SCW0040*	BV210193	WAG-98G13	Wageningen	191	SNaPshot		GGA3	267 ± 7
Singleton	*SCW0367*	BV210400	CH261-175E23	Wageningen	57	SNaPshot		GGA1	149 ± 20
Singleton	*SCW0005*	BV210175	WAG-53H21	Wageningen	44	RFLP	*Sna*BI	GGA2	52 ± 8

[1]More information on the SNP can be found by concatenating the SNP ID to the following URL: 'https://acedb.asg.wur.nl/chickdb/generic/tree?class=Marker&name='.

[2]The STS ID is the NCBI STS accession number.

[3]Markers *SCW0245*, *SCW0327*, *SCW0329* and *SCW0334* did not show linkage to any other marker.

(http://www.ncbi.nlm.nih.gov), PCR primers were designed using the primer3 programme (Rozen & Skaletsky 2000) and PCRs were run on eight animals of the Wageningen reference population (Groenen *et al.* 1998). For WAG clones, BAC ends were sequenced (see *Materials and methods* Aerts *et al.* 2003) and PCR primers were designed using the primer3 program. PCRs were run on four animals of the Wageningen reference population (Groenen *et al.* 1998) and four animals of the East Lansing population (Crittenden *et al.* 1993). Accession numbers for all PCR products are presented in Table 1.

Standard PCR techniques were used to generate templates for resequencing. PCR volumes were 12 μl and contained 30 ng genomic DNA, 0.195 μM of each primer, 0.14 U/μl Taq (Silverstar, Eurogentec, Belgium), 1.071 mM tetramethylammoniumchloride, 0.186 mM dNTPs, 2.15% DMSO and 1X PCR buffer (1X PCR buffer contained 10 mM Tris–HCl pH 9.0, 1.5 mM $MgCl_2 \cdot 6H_2O$, 50 mM KCl, 0.01% (w/v) gelatin and 0.1% Triton X-100). PCR conditions were 95 °C for 5 min, 35 cycles of 95 °C for 30 s, annealing temperature for 30 s and 72 °C for 30 s, followed by 72 °C for 4 min. Annealing temperatures were 50 °C, 55 °C, 60 °C or a combination of five cycles at 58 °C and 30 cycles at 55 °C.

The PCR fragments were purified using Millipore PCR Cleanup Filter Plates (Millipore, Billerica, MA, USA).

Sequencing reactions were performed using the ABI BigDye Terminator Cycle Sequencing protocol (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Each reaction contained 1 μl PCR product (20 ng), 2 μl of one of the PCR primers as sequencing primer (1.6 pmol), 1 μl ABI BigDye v2.0, 3 μl BigDye dilution buffer and 3 μl MilliQ. The Millipore Sequencing Reaction Cleaning Kit (Millipore) was used for cleanup of the sequencing reaction. Sequences were generated using the ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

The SNP discovery on these sequences was performed using the Staden package (Staden *et al.* 2000) and/or the POSA perl objects (Aerts *et al.* 2004).

SNP genotyping

For SNPs genotyped with the restriction fragment length polymorphism (RFLP) method, the genomic region was amplified using the PCR protocol described above. PCR products were cut with the restriction enzyme mentioned in Table 1. Reactions were set up according to the protocol provided by the enzyme supplier. Restriction fragments were separated using standard agarose gel electrophoresis (1.5% multipurpose agarose, 0.5X TBE buffer, 45 min, 120 V) for visual bandcalling.

The AccuPrime (Invitrogen, Breda, The Netherlands) kit was used for PCR amplification for SNPs genotyped with the SnaPshot technology. Multiplex reactions of up to six sequence tagged sites (STS) with the same annealing temperature were performed in 20 μl and contained 60 ng template DNA, 10 μl AccuPrime SuperMix II and 0.2 μM of each primer. PCR conditions were 94 °C for 10 min, 41 cycles of 94 °C for 30 s, annealing temperature for 30 s and 68 °C for 3 min, followed by 68 °C for 2 min. PCR products were then pooled based on single-base extension (SBE) primer length into six super-pools of 17 assays. Genotyping was performed using the standard SNaPshot Multiplex Kit (Applied Biosystems) with the following modifications. For the *Exo*I treatment, 0.4 μl *Exo*I was used instead of 0.2 μl. For the SBE reaction, 4 μl Half Big Dye Buffer (GenPak, New Milton, UK) and 1 μl SNaPshot Ready Reaction Mix were used. The SBE reaction involved 40 cycles.

Genotype detection was achieved using the ABI Prism 3100 Genetic Analyzer (Applied Biosystems). The sample preparation scheme was modified using 2 μl SNaPshot product, 8 μl Hi-diformamide and 0.25 μl GeneScan-120 LIZ size standard. Genotypes were scored using Genemapper v3.0 (Applied Biosystems). The reference populations used for genotyping and the number of animals sampled are shown in Table 1.

Placement on genetic map

Genotyping was performed in either the East Lansing reference population (Crittenden *et al.* 1993) or the Wageningen reference population (Groenen *et al.* 1998; see Table 1). Markers that were genotyped on the East Lansing population were assigned to specific-linkage groups using MapManager (v2.6; Manly 1993). The CRIMAP program (http://compgen.rutgers.edu/multimap/crimap/) was used for markers genotyped on the Wageningen reference population. Map locations were calculated by comparing the new markers with the framework markers of the consensus linkage map (Groenen *et al.* 2000) using the CRIMAP 'build' option.

Results

Anchoring the clone contigs to a chromosomal location involved the following steps: (i) SNP discovery within a clone end-sequence in a small panel of animals, (ii) genotyping the SNP in one of the families that were used to build the consensus linkage map (Groenen *et al.* 2000) and (iii) multipoint linkage analysis to establish the genomic location of the SNP.

In total, 86 of 90 SNP markers were added to the linkage map (see Table 1), anchoring 56 different clone contigs and 13 singleton clones (Wallis *et al.* 2004) to 24 different chromosomes. As the 56 contigs contained 57 132 clones, mapping information was directly or indirectly added for 57 145 clones. Eighteen of the 56 contigs had no previous genetic mapping information.

Eight of the 90 SNP markers did not show linkage to any marker of the consensus linkage map and so could not be genetically mapped. However, both *SCW0335* (contig ctg63814) and *SCW0247* (ctg63827) and markers *SCW0354* and *SCW0363* (both ctg64003) showed linkage to each other. Two new linkage groups, W35 and W36, were created to reflect this. Interestingly, although clones CH261-69E18 and CH261-31K3 (both ctg63820) overlap according to the physical map published by Wallis *et al.* (2004), markers *SCW0245* and *SCW0329* did not show linkage to each other. Closer inspection of the restriction digestion fingerprints of these two clones suggested that these probably have been misassembled and do not overlap.

The number of links per chromosome for each contig as presented in Wallis *et al.* (2004) as well as the links identified in this paper are given in Table 2. For 16 of the 56 contigs, map assignments were generated in this study for more than one clone. For one of these contigs, clones were mapped to different chromosomes resulting in ambiguity of the chromosomal assignment for this contig (i.e. CH261-142A15 and CH261-42J19 in contig ctg63912). Furthermore, we found that 25% of the contigs that already contained mapping information were mapped to another chromosome using the 'majority rule' approach (Table 2) in which the chromosomal location was designated as the largest group of mapped clones.

Table 2 Number of links to chromosomes for the clone contigs tested.

Clone contig	Number of clones	Links to chromosomes Wallis *et al.* (2004)[1]	Links to chromosomes (this paper)[1]
ctg1301	1001	1(1);2(1);4(2);13(4);14(87);15(14);24(1)	14(1)
ctg1801	4104	1(166);3(3);4(1);6(3);7(3);10(8);11(2);13(2);15(1);23(2);W(1);Z (1)	1(1)
ctg1901	2160	1(5);2(3);3(16);5(2);10(3);14(1);16(2);24(19);26(1)	3(1)
ctg39101	4216	1(5);2(139);3(5);4(4);5(1);6(1);10(1);13(5);16(2);28(14)	2(1)
ctg39802	3458	1(4);2(4);3(1);4(91);5(5);7(2);8(1);10(10);11(1);15(1);16(2);17(1);24(2);26(1);28(1);32(1)	4(1)
ctg41701	2468	1(2);2(3);4(91);5(1);10(1);13(4);16(2);W(1);Z(1)	4(4)
ctg42901	1288	1(8);2(1);4(1);15(4);18(1);24(11)	1(2)
ctg45001	724	2(17);4(1);10(3);12(1);17(1)	2(1)
ctg47701	718	1(58)	1(1)
ctg52701	674	1(1);2(7);3(1);4(2);6(1);10(2);15(104);C15(3)	15(1)
ctg63807	2332	1(3);2(1);23(1);3(1);7(19);10(10);13(1);Z(1)	7(1)
ctg63814	53	Not mapped	W35(1)
ctg63820	84	Not mapped	Not mapped (2)
ctg63826	97	Not mapped	Z(1)
ctg63827	717	5(12);10(5)	W35(1)
ctg63832	63	Not mapped	Z(1)
ctg63838	495	1(1);8(10);19(23)	19(1)
ctg63839	3601	1(9);2(156);4(6);7(4);10(7);11(1);15(3);16(3);27(1);28(4);Z(1)	2(1)
ctg63840	349	1(1);2(1);4(1);11(2);16(2);17(1)	11(2)
ctg63845	33	Not mapped	3(1)
ctg63868	2903	1(3);2(14);3(2);4(148);10(1);16(2);28(6);Z(1)	4(1)
ctg63878	808	1(22);3(1);5(2);13(3);28(1)	1(1)
ctg63883	1031	1(1);2(1);13(14);16(4);28(2)	13(1)
ctg63886	129	10(9)	Z(1)
ctg63895	68	Z(1)	Z(1)
ctg63896	128	3(7);18(1)	18(1)
ctg63903	92	Not mapped	9(1)
ctg63910	10	Not mapped	27(1)
ctg63912	1012	2(1);8(1);10(8);16(2)	2(1);4(1)
ctg63914	2787	1(8);2(1);3(90);5(2);13(4);16(2);Z(3)	3(1)
ctg63920	2779	1(2);2(2);3(3);4(2);5(1);7(1);8(168);10(10);13(2);15(2);26(1);28(2);E50C23(1)	8(2)
ctg63929	46	Not mapped	4(1)
ctg63930	22	Not mapped	27(2)
ctg63932	352	9(4)	9(1)
ctg63935	162	Not mapped	19(1)
ctg63953	19	Not mapped	14(1)
ctg63979	16	Not mapped	7(1)
ctg63980	535	19(14);21(1)	19(1)
ctg63984	55	Not mapped	E22C19W28 (1)
ctg63986	448	1(1);8(5);23(1)	8(1)
ctg63994	370	10(3)	1(2)
ctg63999	2999	1(75);2(4);4(3);5(8);10(6);13(2);17(1);21(1);23(2);28(1);32(1)	1(2)
ctg64003	15	Not mapped	W36(2)
ctg64012	1331	6(6);8(4);10(2);16(2);17(2)	6(1)
ctg64015	723	2(4);5(1);10(3);13(9);14(43);C37(12)	14(1)
ctg64017	401	2(1);3(1);5(1);10(5);13(2);14(1);16(2)	3(2)
ctg64019	479	4(2);8(1);17(4);18(1)	Not mapped (1)
ctg64030	2160	1(1);2(84);10(25);14(4);17(1);20(1);26(1);28(1)	2(1)
ctg64031	117	Not mapped	22(2)
ctg64032	11	Not mapped	26(2)
ctg64041	144	2(1);5(1)	2(3)
ctg64046	320	2(20)	2(1)
ctg64049	259	Not mapped	Z(1)
ctg80002	4903	1(188);2(1);4(2);8(5);10(7);13(1);15(2);16(3);22(3);26(1);Z(1)	1(3)

Table 2 Continued

Clone contig	Number of clones	Links to chromosomes Wallis *et al.* (2004)[1]	Links to chromosomes (this paper)[1]
ctg80023	746	1(1);2(1);3(1);10(18);14(4);24(1)	3(2)
ctg80082	104	Not mapped	Z(1)

[1]The chromosome number is given, followed by the number of clone-chromosome links for that contig (in parentheses).

Discussion

In this project, map assignments have been added to a significant number of clones within contigs that were either not anchored or mapped ambiguously to multiple chromosomes. To this aim, clones were selected from these contigs to design genetic markers.

In Aerts *et al.* (2003), a similar but inverse approach was used; the Wageningen BAC library was screened using markers with known genetic locations. With the current approach, clones were mapped directly on the chicken consensus linkage map, resulting in new mapping information for >57 000 clones. In addition, the linkage map was extended with 86 new SNP markers and two new linkage groups (W35 and W36). Three markers (*SCW0245*, *SCW0329* and *SCW0334*) derived from two contigs (ctg63820 and ctg64019) were not linked to any existing linkage group of the consensus linkage map. These contigs, as well as the three assigned to the new linkage groups W35 and W36 (ctg63814, ctg63827 and ctg64003), most likely represent some of the as yet unidentified microchromosomes (GGA25, GGA29-31 and GGA33-38).

Clone selection and marker development took place in an early stage of building the physical map. This map changed significantly when contigs were merged and many singleton clones were inserted into existing contigs. As a result, several clones that were mapped in this paper were part of recently mapped contigs (Table 2: ctg47701 and ctg64046).

Although the clone-contig map published by Wallis *et al.* (2004) is a valuable resource for chicken genomic and genetic research, many ambiguities and inconsistencies still need to be resolved. For example, a considerable number of contigs contain clones that are mapped to different chromosomes. In these cases, the genomic position of the contig is typically assessed using a 'majority rule' approach: the chromosomal location is dictated by the largest group of clones that are mapped to the same chromosome. For large contigs containing a large number of links to a genomic position, this generally poses no problems. However, for FPC contigs with a relatively small number of links, the genomic location can often not be easily inferred. This is clearly demonstrated by the results shown in Table 2, where all contigs with more than 20 links to a particular chromosome were confirmed by the new mapping data.

Four reasons can account for these mapping inconsistencies within clone contigs: (i) a marker is wrongly assigned to a certain chromosomal location, (ii) a clone is erroneously found to be positive for a marker, (iii) a clone contig is chimeric and must be split into two or more subcontigs or (iv) a single clone is wrongly placed within a certain contig. Ctg64030 represents an example of the first type of error (Table 2); 71% ($n = 84$) of the marker data link this contig to chromosome 2, while 21% ($n = 25$) link it to chromosome 10. However, all links to chromosome 10 on this chromosome arise from chromosome walking data. These markers were not independently mapped to chromosome 10, but were assigned to that chromosome because they reside within a contig that was mapped to chromosome 10. As a result, a single wrong map assignment can cause all these markers to be mapped erroneously. Other contigs (ctg1901, ctg42901, ctg63827, ctg63912 and ctg63994) most likely also show this type of error (Table 2).

Clone JH089D18 in ctg1301 presents an example of the second type of inconsistency. The assignment of marker *ADL0255* to this clone may bee erroneous because other mapping data on the contig strongly suggest that the contig contains part of chromosome 14. Closer inspection of the clone fingerprints further supports assignment to chromosome 14.

The contigs that have links to multiple chromosomes (Table 2) probably represent examples of the third and fourth causes for mapping inconsistency. Although particular attention was paid towards avoiding these inconsistencies during assembly of the clone contigs (Wallis *et al.* 2004), the nature of building clone contigs based on clone fingerprints can lead to these errors.

Inspection of the mapping results for two clones in ctg63912 (CH261-42J19 and CH261-142A15) revealed no clear cause for the fact that they are mapped to two different chromosomes. In addition, the fingerprints of these clones fit those of the neighbouring clones. This suggests that a larger group of clones has to be removed from ctg63912.

As up to 25% of the markers were mapped to another chromosome than the previous chromosomal assignment of that contig using the 'majority rule' approach, it is clear that this 'majority rule' method cannot be applied without taking the actual clone-marker combinations into consideration. More specifically, a distinction should be made between markers that were linked to clones using PCR screening, overgo hybridization screening or dummy markers that were added when clones were part of the same chromosome walking contig. As described above, especially

the latter can cause clone contigs to be mapped to the wrong chromosome. Therefore, these links should not be used as sole evidence for map assignment, but rather be used as confirmation only. In addition, the integration of the physical map with other maps such as the sequence map can be used to confirm map assignments.

In conclusion, this paper presents new genetic mapping information that anchors clone contigs from the physical map (Wallis *et al.* 2004) to a genomic location. These results will be integrated in a subsequent release of the chicken genome map. To resolve all ambiguities and unmapped contigs, further mapping of clones to the consensus linkage map and the integration of the physical map with other maps are necessary.

References

Aerts J., Crooijmans R., Cornelissen S., Hemmatian K., Veenendaal T., Jaadar A., van der Poel J., Fillon V., Vignal A. & Groenen M. (2003) Integration of chicken genomic resources to enable whole-genome sequencing. *Cytogenetics and Genome Research* **102,** 297–303.

Aerts J., Jungerius B. & Groenen M. (2004) POSA: perl objects for DNA sequencing data analysis. *BMC Genomics* **5,** 60.

Boardman P., Sanz-Ezquerro J., Overton I., Burt D., Bosch E., Fong W., Tickle C., Brown W., Wilson S. & Hubbard S. (2002) A comprehensive collection of chicken cDNAs. *Current Biology* **12,** 1965–9.

Brown W., Hubbard S., Tickle C. & Wilson S. (2003) The chicken as a model for large-scale analysis of vertebrate gene function. *Nature Reviews Genetics* **4,** 87–97.

Crittenden L.B., Provencher L., Levin I., Abplanalp H., Briles R.W., Briles W.E. & Dodgson J.B. (1993) Characterization of a Red Jungle Fowl by White Leghorn backcross reference population for molecular mapping of the chicken genome. *Poultry Science* **72,** 334–48.

Fillon V., Morisson M., Zoorob R., Auffray M.D., Gellin J. & Vignal A. (1998) Identification of 16 chicken microchromosomes by molecular markers using two-colour fluorescence *in situ* hybridization (FISH). *Chromosome Research* **6,** 307–13.

Groenen M., Crooijmans R., Veenendaal A., Cheng H., Siwek M. & der Poel, J. (1998) A comprehensive microsatellite linkage map of the chicken genome. *Genomics* **49,** 265–74.

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

International Chicken Genome Sequencing Consortium (2004) Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* **432,** 695–716.

International Chicken Polymorphism Map Consortium (2004) A genetic variation map for chicken with 2.8 million single-nucleotide polymorphisms. *Nature* **432,** 717–22.

International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome. *Nature* **409,** 860–921.

Manly K.F. (1993) A Macintosh program for storage and analysis of experimental genetic mapping data. *Mammalian Genome* **4,** 303–13.

Masabanda J., Burt D., O'Brien P. *et al.* (2004) Molecular cytogenetic definition of the chicken genome: the first complete avian karyotype. *Genetics* **166,** 1367–73.

Rozen S. & Skaletsky H. (2000) Primer 3 on the WWW for general users and for biologist programmers. *Methods in Molecular Biology* **132,** 365–86.

Speedy A. (2003) Global production and consumption of animal source foods. *Journal of Nutrition* **13,** 4048S–53S.

Staden R., Beal K.F. & Bonfield J.K. (2000) The Staden Package, 1998. *Methods in Molecular Biology* **132,** 115–30.

Wallis J.W., Aerts J., Groenen M. *et al.* (2004) A physical map of the chicken genome. *Nature* **432,** 761–4.