

A first-generation map of the turkey genome

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Abstract: A primary linkage map of the domestic turkey (*Meleagris gallopavo*) was developed by segregation analysis of genetic markers within a backcross family. This reference family includes 84 offspring from one F₁ sire mated to two dams. Genomic DNA was digested using one of five restriction enzymes, and restriction fragment length polymorphisms were detected on Southern blots using probes prepared from 135 random clones isolated from a whole-embryo cDNA library. DNA sequence was subsequently determined for 114 of these cDNA clones. Sequence comparisons were done using BLAST searches of the GenBank database, and redundant sequences were eliminated. High similarity was found between 23% of the turkey sequences and mRNA sequences reported for the chicken. The current map, based on expressed genes, includes 138 loci, encompassing 113 loci arranged into 22 linkage groups and an additional 25 loci that remain unlinked. The average distance between linked markers is 6 cM and the longest linkage group (17 loci) measures 131 cM. The total map distance contained within linkage groups is 651 cM. The present map provides an important framework for future genome mapping in the turkey.

Key words: genetic map, *Meleagris gallopavo*, expressed sequence tag, RFLP.

Résumé : Une carte génétique primaire pour le dindon (*Meleagris gallopavo*) a été produite en analysant la ségrégation de marqueurs génétiques au sein d'une population issue d'un rétrocroisement. Cette famille de référence compte 84 individus issus d'un mâle croisé à deux femelles. L'ADN génomique a été digéré à l'aide de l'une de cinq enzymes de restriction et les polymorphismes de longueur des fragments de restriction (RFLP) ont été détectés par hybridation Southern à l'aide de 135 clones aléatoires provenant d'une banque d'ADNc préparée à partir d'embryons entiers. La séquence de 114 de ces clones a été déterminée par la suite. Des comparaisons de séquences ont été réalisées à même la banque de données Genbank avec le logiciel BLAST et les séquences redondantes ont été éliminées. Une forte similitude a été notée entre 23 % des clones du dindon et des séquences d'ARNm décrites chez le poulet. La présente carte, fondée sur des gènes exprimés, comprend 138 locus dont 113 forment 22 groupes de liaison et 25 demeurent non-associés. La distance moyenne entre les marqueurs est de 6 cM et le groupe de liaison le plus long (17 locus) totalise 131 cM. La distance génétique totale est de 651 cM. Cette carte fournit une ébauche importante pour de futurs travaux de cartographie génétique chez le dindon.

Mots clés : carte génétique, *Meleagris gallopavo*, étiquette de gène exprimé, RFLP.

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Introduction

A long-term goal for gene mapping of domestic animals is to identify sequences affecting traits of economic importance, such as efficient production, increased reproduction,

and disease resistance. In recent years, the genomes of many agriculturally important species have been mapped, primarily using anonymous genetic markers like microsatellites. However, there is increasing emphasis on the mapping of expressed genes. In mammals, comprehensive genetic maps have been generated for cattle (Barendse et al. 1997; Kappes et al. 1997) and pig (Rohrer et al. 1994, 1996). For chicken, three medium-resolution genetic maps (Compton, Bumstead and Palyga 1992; East Lansing, Levin et al. 1994; Crittenden et al. 1993; and Wageningen, Groenen et al. 1998) have recently been joined into a single second-generation map (Groenen et al. 2000). Because of the turkey's close evolutionary relationship to the chicken, comparative and other "post-genomics" studies involving the turkey have significant potential.

Genomics-based research on production traits and disease susceptibility has been indicated as a high priority for the poultry industry, and the chicken has been identified as a high-priority species for genome sequencing. In contrast, the turkey is one of only a few major agricultural animal species without a comprehensive genetic map. A number of microsatellite markers are being developed for the turkey, and many of these are publicly available (Huang et al. 1999;

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Reed et al. 2000a, 2002; K.M. Reed, unpublished data; Dranchak et al. 2003). Here we present a primary linkage map of the domestic turkey (*Meleagris gallopavo*), developed by segregation analysis within a backcross family, using genetic markers based on expressed genes.

Materials and methods

Reference family

The family used for linkage mapping was derived from a single F₁ sire (an intercross between two commercial lines, A and B) backcrossed to two dams from line B. Two to five millilitres of whole blood was collected from the grandparents and parents, and 1 mL of whole blood was collected from euthanized poults on the day of hatching. A total of 84 offspring were included in the analysis, 44 poults from one dam and 40 from the other.

cDNA preparation and cloning

RNA was isolated from a 24-day-old turkey embryo using a commercially available kit (Stratagene, La Jolla, Calif.) and a cDNA library was constructed by directional cloning into the Lambda ZAP vector (pBluescript SK (-), Stratagene). The amplified library was plated at low density (~200 pfu per 100-mm plate). Individual clones were picked from well-isolated plaques and stored in SM (sodium-magnesium) buffer at 4°C.

Restriction fragment length polymorphism (RFLP) analysis

Total genomic DNA was prepared from whole blood (Medrano et al. 1990) that had been stored frozen at -70°C. DNA samples representing grandparents, parents, and progeny were digested with one of five restriction enzymes (*EcoRI*, *HinfI*, *MspI*, *RsaI*, or *TaqI*), electrophoresed on 1% w/v agarose gels (1 µg per lane), and blotted onto charged nylon membranes (GeneScreen Plus, NEN/PerkinElmer Life Sciences, Boston, Mass.). Inserts from random cDNA clones were amplified using universal primers and were subsequently radiolabelled (³²P]dCTP) using a Prime-It II kit (Stratagene), following the manufacturer's recommendations. Blotted membranes were prehybridized for 1 h at 65°C in 1% SDS, 0.5% BSA, 0.2% Carnation™ nonfat dry milk in 1× SSPE (1×: 0.18 M NaCl, 10 mM NaPO₄, and 1 mM EDTA (pH 7.7)). Hybridizations were carried out overnight at 65°C in 8% sodium dextran sulfate, 0.1% SDS, 0.1% Carnation non-fat dry milk, 0.1% BSA, 50 µg yeast RNA/mL, 50 µg sodium heparin/mL, 1 mM Na₄P₂O₇, and 50 µg herring sperm DNA/mL in 2× SSPE. Membranes were subsequently subjected to four 1-h washes (65°C) in 0.2× SSPE containing 0.2% SDS. Washed membranes were covered with plastic wrap, sandwiched between two intensifying screens, and exposed to X-ray film for 1–4 days at -70°C. Following autoradiography, residual probe DNA were removed by rinsing used membranes in 0.2× SSPE with 0.2% SDS for 3–5 min at 95°C. Stripped membranes were covered with plastic wrap while still damp and stored for re-use at -20°C.

Linkage analysis

Marker genotypes were scored manually from autoradiographs. Genotypic data were initially analyzed using JoinMap version 1.4 (Stam 1993) and subsequently analyzed using JoinMap version 2.0 (Stam and Van Ooijen 1995), following input parameters suggested by Jermstad et al. (1998). Pairwise recombination estimates were made for all markers separately for the offspring of each dam (module JMREC), using LOD and recombination (rec) thresholds of 0.01 and 0.499, respectively. Preliminary linkage assessments were made on pooled data using module JMPWG for a range of LOD scores from 2.5 to 5.0 in increments of 0.5. Map distances were assigned for loci within each group, using module JMMAP with the following parameters: LOD threshold 0.1; rec threshold 0.49; jump threshold 3; triplet threshold 7, ripple value 3, and Kosambi's mapping function.

DNA sequencing

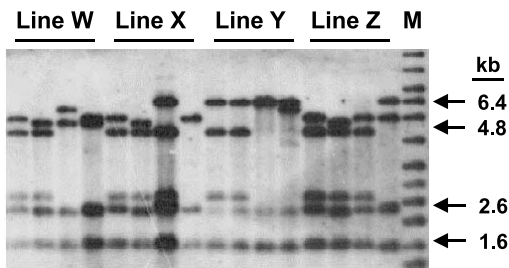
The cDNA clones were subjected to PCR to amplify the inserts for DNA sequencing. Each 25-µL reaction contained as template 1 µL of eluted phage, 1.5 mM MgCl₂, 2.5 pmol each primer (T3 and T7 flanking primers), 100 µM dNTP, and 0.35 U *Taq* DNA polymerase (Qiagen, Valencia, Calif.). Amplifications were performed in a Techne thermal cycler under the following reaction conditions: 15 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 56°C, 30 s at 72°C; and a final extension of 5 min at 72°C. PCR products were resolved on 1% w/v agarose gel. Sequencing templates were prepared from the amplified DNA using a QIAquick PCR purification kit (Qiagen) and analyzed on an automated DNA sequencer (ABI 373, Applied Biosystems, Foster City, Calif.) with vector-specific primers. DNA sequences were manually edited and checked for redundancy with Sequencher (Gene Codes Corp., Ann Arbor, Mich.). Nonredundant sequences were compared with GenBank entries by BLAST searches (BLASTn and tBLASTx, National Center for Biotechnology Information, Bethesda, Md.), using sequences deposited as of August 31, 2002.

Results

Initial screening for RFLP polymorphisms was done using DNA samples from several prospective pure-line grandparents and contemporary F₁ parents, including the individuals ultimately used to produce the backcross progeny of this report. Promising combinations of restriction enzymes and probes were subsequently used to produce autoradiographs from Southern blots containing DNA from offspring. Many probes revealed three or more alleles, suggesting reasonably high levels of DNA polymorphisms within "pure-lines" of turkeys. Several of the more highly polymorphic probes were used to examine the level of RFLP variation in samples from other lines. For example, one probe (Nte0728) revealed six alleles among 16 individuals drawn from four commercial lines (Fig. 1).

While examining autoradiographs, we noticed that some probes revealed sex-specific RFLP patterns. For example, Nte0786 revealed two distinct restriction fragments among females, whereas males have only one (Fig. 2). Linkage of the segregating fragment to the W chromosome (Fig. 3) was inferred, since in birds, females are the heterogametic sex

Fig. 1. Restriction fragment length variation within and between four commercial lines of turkey, as detected with the cDNA clone Nte0728. DNA samples were digested with *RsaI*. Shown are four males from each of four pure lines, W–Z. Approximate size (kb) is indicated for several restriction fragments.

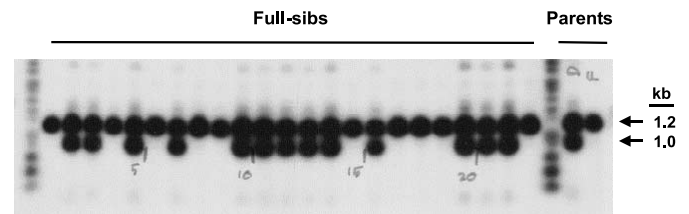


(ZW). The restriction patterns for Z-linked loci (Fig. 3) are more difficult to discern: males can be either heterozygous or homozygous for any polymorphic locus (as for autosomal loci), but females are hemizygous for either allele, and thus heterozygous females are absent. Based on this rationale, markers were tentatively assigned as autosomal, Z linked, or W linked. Subsequent probability assessments (Yang Da, University of Minnesota, personal communication), confirmed these assignments.

Linkage analyses were conducted using JoinMap versions 1.4 (Stam 1993) and 2.0. Our first map (not shown) was constructed using JoinMap version 1.4 (Stam 1993), but this version could accommodate only two alleles among offspring segregating either as backcross or F_2 mating configurations. JoinMap version 2.0 (Stam and Van Ooijen 1995) accommodates multiple alleles and therefore takes advantage of additional information. The analyses presented here are based on JoinMap version 2.0. Overall, the results of these two analyses were generally similar except that JoinMap version 2.0 added several new loci to existing linkage groups, and other linkage groups coalesced.

The current expressed sequence tag (EST)–RFLP linkage map (Fig. 3) contains 113 loci arranged in 22 linkage groups, with another 25 loci remaining unlinked. Anticipating that linkage groups will eventually be assigned to specific chromosomes, we have provided interim names to the linkage groups described herein, designating them alphabetically as A, B, etc., in order of descending size. An exception was made for linkage groups corresponding to sex chromosomes, designating these as Z and W. The longest linkage group (A, Fig. 3) encompasses 131 cM containing 17 loci. Two linkage groups (C and D, Fig. 3) are depicted as separate subgroups. In these cases, the loci were assigned to their respective linkage groups with a LOD score of 3.5 or greater, but JoinMap could not unambiguously order the loci within each group. Upon closer inspection, we observed that troublesome loci were represented by fewer informative meioses; they were either segregating as F_2 s (in which heterozygous offspring are excluded) or they were homozygous in the F_1 sire and were segregating in only one of the two dams. Hence, the subgroups depict our best overall estimate of map distances and order among the various loci in these linkage groups. In total, the 113 linked loci span a combined distance of 651 cM, separated by an average distance of 6 cM.

Fig. 2. Sex-specific marker segregation within members of a mapping family detected with the cDNA clone Nte0786. DNA samples were digested with *TaqI*. Shown are 23 offspring flanked on either side by a molecular weight (MW) ladder. The parents are shown to the right of the second MW ladder, with the dam to the left and the sire to the right. Because the restriction pattern for all females includes two fragments (1.2 and 1.0 kb), whereas males have only one fragment (1.2 kb), the segregating fragment (1.0 kb) is inferred to be W linked.



Long after the Southern blots had been produced and the genotypic data scored, PCR was used to amplify inserts from stored cDNA clones used to create the map. PCR products were purified on Qiagen PCR clean-up columns and the resulting DNA templates sequenced on automated DNA sequencers at the Advanced Genetic Analysis Center, University of Minnesota, using the T3 vector-specific primer.

Of the 135 clones attempted, 114 yielded useable sequence data, averaging 505 bases per clone (Table 1). Although all clones were sequenced from the 5' end of the directionally cloned cDNA inserts, sequence reads were typically long enough to include the entire insert. Searches of GenBank (blastn and tblastx) identified 26 (23%) cDNAs as highly similar to sequences from chicken, 58 (51%) as similar to sequences from other species, and 30 (26%) with no significant similarity to GenBank entries. Sequences from nine clones (8%) were found to be redundant. Autoradiographs used to detect RFLPs of redundant probes were reexamined and the redundancies confirmed. Additional redundancies were inferred by examining autoradiographs of closely linked markers. Genotypic data from redundant markers were removed and the linkage relationships recalculated.

Relationships between turkey and chicken genes and chromosome assignments were inferred by one of two methods. First, for each locus with DNA sequence data, the best match to a position in the human genome sequence was determined by BLAST. From the resulting human chromosome position (HSA in Table 1), the best corresponding position in the chicken genome was determined from the comparative map of Groenen et al. (2000). Alternatively the assignments to chicken chromosomes were made by comparing the list of genes identified in our cDNA clones to genes included in the ArkDB chicken database (www.thearkdb.org). Hypothesized chromosomal assignments are included for the sequenced loci in Table 1.

Discussion

This map of expressed genes illustrates an expanding effort at the University of Minnesota to create a comprehen-

Fig. 3. Linkage map of expressed genes in the turkey. Linkage groups are named alphabetically from largest to smallest, except for the sex chromosomes Z and W. Loci shown in blue represent sequenced cDNAs, whereas those shown in red have no sequence data available. This map encompasses 113 genes on 22 linkage groups, spanning 651 cM. Another 25 genes were analyzed but remain unlinked.

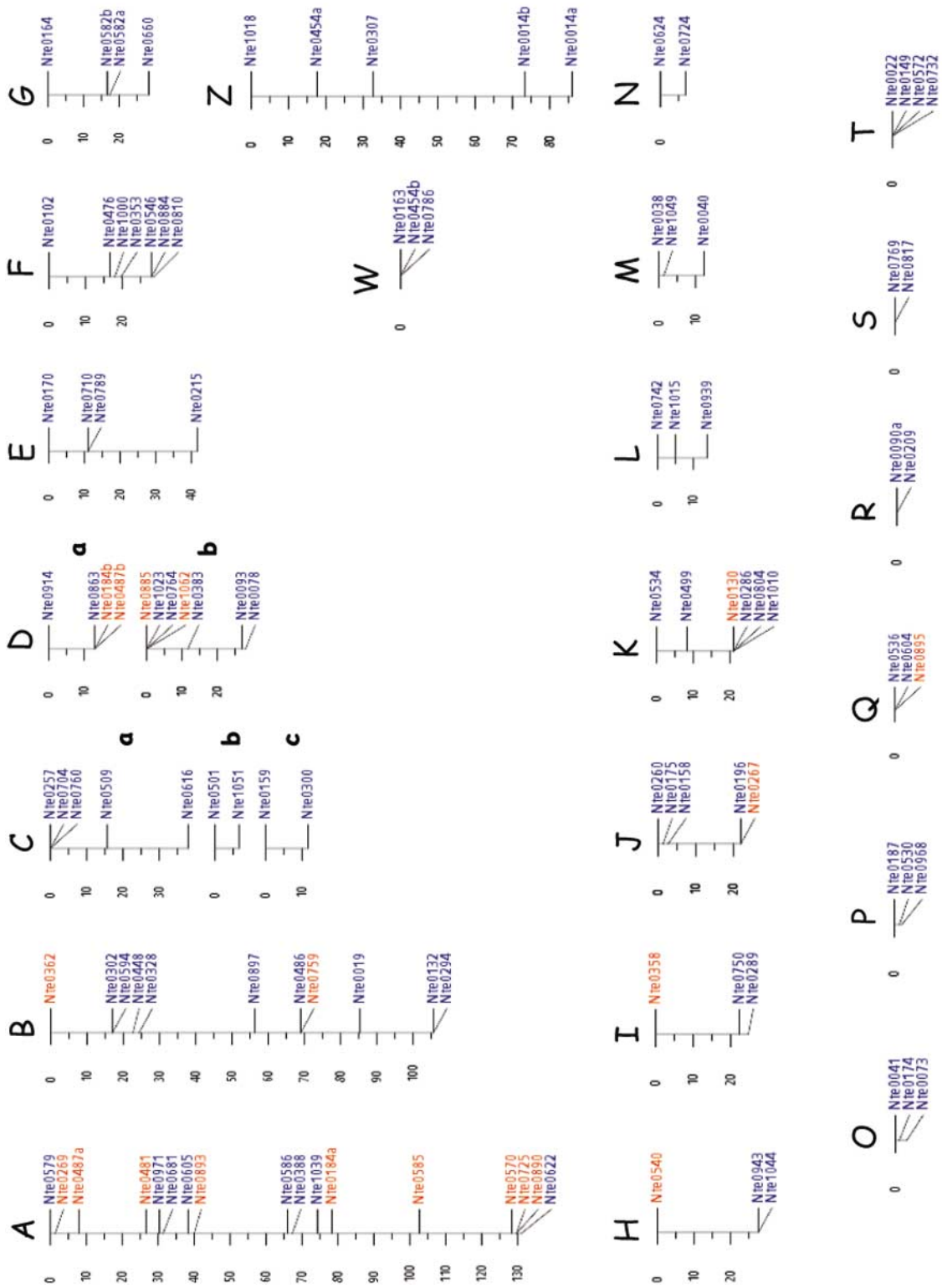


Table 1. Summary of locus information by linkage group.

Locus	Linkage group	cM	Enz	bp	GenBank accession No.	Sequence similarity	<i>E</i> value*	HSA	<i>E</i> value [†]	Chicken chromosome
<i>Nie0579</i>	A	0.0	<i>MspI</i>	436	BI335659	Phosphatase 1, catalytic subunit, γ isoform	6.0×10^{-9}	12q24.1	4.0×10^{-9}	1
<i>Nie0269</i>		1.2	<i>RsaI</i>	none		Eukaryotic translation elongation factor 1 δ	2.0×10^{-15}	7q11	8.0×10^{-11}	—
<i>Nie0487a</i>		7.9	<i>MspI</i>	none		Eukaryotic translation initiation factor 3, subunit 6	1.0×10^{-78}	6q13	3.0×10^{-63}	3
<i>Nie0481</i>		26.7	<i>HinfI</i>	none		Heparan sulfate proteoglycan 1	5.0×10^{-07}	8q21	2.0×10^{-06}	2
<i>Nie0971</i>		30.2	<i>TaqI</i>	492	BI335704		nss		nss	
<i>Nie0681</i>		31.3	<i>TaqI</i>	611	BI335672	Novel sequence	2.0×10^{-40}	7q34	2.0×10^{-15}	1/2
<i>Nie0605</i>		38.4	<i>EcoRI</i>	629	BI335666	Single-stranded DNA binding protein	0.12		nss	
<i>Nie0893</i>		40.2	<i>MspI</i>	none		<i>Dromaius novaehollandiae</i> (CRI) partial gene sequence			nss	
<i>Nie0586</i>		66.3	<i>TaqI</i>	179	BI335661					
<i>Nie0388</i>		67.6	<i>MspI</i>	595	BI335643					
<i>Nie1039</i>		74.6	<i>RsaI</i>	627	BI335711					
<i>Nie0184a</i>		78.6	<i>MspI</i>	none						
<i>Nie0585</i>		102.8	<i>MspI</i>	none						
<i>Nie0570</i>		128.3	<i>HinfI</i>	none						
<i>Nie0725</i>		129.5	<i>MspI</i>	none						
<i>Nie0890</i>		129.5	<i>MspI</i>	none		Identical RFLP pattern as <i>Nie0725</i>				
<i>Nie0622</i>		130.7	<i>MspI</i>	587	BI335669	<i>Meleagris gallopavo</i> 18S ribosomal RNA gene	0.0	13	0.0	16/16
<i>Nie0362</i>	B	0.0	<i>TaqI</i>	none						
<i>Nie0302</i>		17.1	<i>RsaI</i>	455	BI335638	Chicken brain tubulin, α chain	1.0×10^{-131}	2q35	8.0×10^{-63}	7/15
<i>Nie0594</i>		17.1	<i>MspI</i>	226	BI335663	Chicken brain tubulin mRNA (RFLP distinct from <i>Nie0302</i>)	1.0×10^{-42}		nss	
<i>Nie0448</i>		22.7	<i>EcoRI</i>	345	BI335644	Chicken nonhistone protein HMG-17 gene	1.0×10^{-166}	Multiple	1.0×10^{-23}	—/UN
<i>Nie0328</i>		24.3	<i>EcoRI</i>	670	BI335640	ATP synthase β -subunit	1.0×10^{-97}	12q13	5.0×10^{-19}	E22C19W28
<i>Nie0897</i>		56.4	<i>MspI</i>	515	BI335697	Novel sequence	nss		nss	
<i>Nie0486</i>		69.2	<i>TaqI</i>	464	BI335647	Chicken mRNA for ferritin H chain protein	0.0	11q13	2.0×10^{-36}	5
<i>Nie0759</i>		69.2	<i>TaqI</i>	ns						
<i>Nie0019</i>		85.5	<i>HinfI</i>	622	BI335604	RNA polymerase II transcriptional regulation mediator (MED6)	2.0×10^{-21}	14q23	2.0×10^{-05}	5
<i>Nie0132</i>		105.7	<i>MspI</i>	494	BI335617	Eukaryotic translation initiation factor 5 (eIF-5)	1.0×10^{-34}	14q32	2.0×10^{-14}	5/5
<i>Nie0294</i>		105.7	<i>MspI</i>	717	BI335636	EIF-5 (RFLP similar to <i>Nie0132</i>)	4.0×10^{-57}	14q32	7.0×10^{-37}	5/5
<i>Nie0257</i>	Ca	0.0	<i>TaqI</i>	375	BI335631	Novel sequence	nss		nss	
<i>Nie0704</i>		0.0	<i>HinfI</i>	442	BI335675	Novel sequence	nss		nss	
<i>Nie0760</i>		0.0	<i>HinfI</i>	541	BI335682	Chicken hemoglobin α -A mRNA	0.0	16p13.3	8.0×10^{-05}	3/14

Table 1 (continued).

Locus	Linkage group	cM	Enz	bp	GenBank accession No.	Sequence similarity	E value*	HSA	E value†	Chicken chromosome
<i>Nie0509</i>		15.7	<i>RsaI</i>	611	BI335651	Human <i>faul</i> gene	1.0×10^{-19}	11q12	2.0×10^{-36}	1/E49C20W
<i>Nie0616</i>		38.0	<i>HinfI</i>	649	BI335668	Eukaryotic translation elongation factor 1 γ	1.0×10^{-38}	7q33	9.0×10^{-39}	21
<i>Nie0501</i>	Cb	0.0	<i>MspI</i>	628	BI335650	<i>Homo sapiens</i> hypothetical protein BC004923	0.12		nss	1
<i>Nie1051</i>		6.6	<i>TaqI</i>	476	BI335715	Human NADH dehydrogenase (ubiquinone) 1 α subcomplex	3.0×10^{-17}	1p21	3.0×10^{-13}	1
<i>Nie0159</i>	Cc	0.0	<i>TaqI</i>	547	BI335621	<i>Homo sapiens</i> BAC clone RPI1-168P10 from 7	2.0×10^{-6}	7p14	1.0×10^{-06}	1
<i>Nie0300</i>		11.6	<i>TaqI</i>	510	BI335637	<i>Mus musculus</i> mRNA for type I interferon IFN α 2	4.0×10^{-04}		nss	
<i>Nie0914</i>	Da	0.0	<i>TaqI</i>	657	BI335699	Novel sequence	nss		nss	
<i>Nie0863</i>		13.1	<i>MspI</i>	466	BI335695	Goose β -actin	6.0×10^{-86}	1p35	1.0×10^{-15}	5/E54/2
<i>Nie0184b</i>		13.1	<i>MspI</i>	none		Distinct RFLP pattern from <i>Nie184a</i>				
<i>Nie0487b</i>		13.1	<i>MspI</i>	none		Distinct RFLP pattern from <i>Nie487a</i>				
<i>Nie0854</i>		13.1	<i>MspI</i>	258	BI335694	Goose β -actin (identical RFLP pattern as <i>Nie0863</i>)	8.0×10^{-10}		nss	2
<i>Nie0885</i>	Db	0.0	<i>MspI</i>	none		Identical RFLP pattern as <i>Nie1023</i>				
<i>Nie1023</i>		0.0	<i>MspI</i>	373	BI335710	<i>Mus musculus</i> ribosomal protein L44	7.0×10^{-76}	14q22	5.0×10^{-70}	5
<i>Nie0764</i>		0.0	<i>MspI</i>	478	BI335683	Rat nucleolar protein	6.0×10^{-04}		nss	
<i>Nie1062</i>		0.0	<i>MspI</i>	none						
<i>Nie0383</i>		11.5	<i>HinfI</i>	340	BI335642	Novel sequence	nss		nss	
<i>Nie0093</i>		26.7	<i>RsaI</i>	640	BI335613	Chicken fibroblast growth factor (FHF2)	0.0	Xq24-27	1.0×10^{-62}	4
<i>Nie0078</i>		27.6	<i>MspI</i>	475	BI335610	<i>Gallus gallus</i> mRNA for diaphanous homologue	1.0×10^{-147}		nss	
<i>Nie0170</i>	E	0.0	<i>TaqI</i>	656	BI335624	Novel sequence	nss		nss	
<i>Nie0710</i>		10.9	<i>MspI</i>	424	BI335676	Human ribosomal protein L34	2.0×10^{-68}	8q22	7.0×10^{-54}	2
<i>Nie0789</i>		10.9	<i>MspI</i>	419	BI335687	Human ribosomal protein L34	2.0×10^{-68}	8q22	7.0×10^{-57}	2
<i>Nie0215</i>		41.7	<i>HinfI</i>	596	BI335630	Chicken anchorin CII (ANXA5) gene	1.0×10^{-135}		nss	4
<i>Nie0102</i>	F	0.0	<i>RsaI</i>	494	BI335614	Novel sequence	nss		nss	
<i>Nie0476</i>		16.6	<i>RsaI</i>	632	BI335646	Chicken ribosomal protein L5 (RPL5)	0.0	22q13	6.0×10^{-43}	1/8
<i>Nie1000</i>		17.8	<i>RsaI</i>	310	BI335705	Chicken ribosomal protein L5 (RPL5)	1.0×10^{-149}	22q13	1.0×10^{-48}	1/8
<i>Nie0353</i>		19.4	<i>MspI</i>	575	BI335641	Frog fizzy1, cell cycle protein	2.0×10^{-15}		nss	

Table 1 (continued).

Locus	Linkage group	cM	Enz	bp	GenBank accession No.	Sequence similarity	<i>E</i> value*	HSA	<i>E</i> value†	Chicken chromosome
<i>Nie0546</i>		27.8	<i>TaqI</i>	640	BI335656	Glutathione phospholipid hydroperoxide peroxidase (PHGPx)	6.0×10^{-13}	1q23	4.0×10^{-13}	8
<i>Nie0884</i>		27.8	<i>TaqI</i>	603	BI335696	Human cysteine-rich intestinal protein CRIP1	3.0×10^{-45}	22q11.2	1.0×10^{-34}	1
<i>Nie0810</i>		28.6	<i>TaqI</i>	415	BI335689	Novel sequence	nss		nss	
<i>Nie0164</i>	G	0.0	<i>MspI</i>	974	BI335623/BI335716	Human <i>KIAA0136</i> gene	3.0×10^{-12}	21q21	3.0×10^{-12}	4
<i>Nie0582b</i>		16.7	<i>MspI</i>			Distinct RFLP pattern from <i>Nie582a</i>				
<i>Nie0582a</i>		17.2	<i>MspI</i>	588	BI335660	Roundabout (axon guidance receptor) homolog 1	1.0×10^{-50}	3p13	4.0×10^{-13}	4/E16C17W
<i>Nie0660</i>		28.1	<i>MspI</i>	660	BI335671	Novel sequence	nss		nss	22
<i>Nie0540</i>	H	0.0	<i>MspI</i>	ns						
<i>Nie0943</i>		27.5	<i>MspI</i>	363	BI335702	Human <i>fau 1</i> gene	6.0×10^{-20}	11q12	2.0×10^{-36}	1/E6 × 1004W23
<i>Nie1044</i>		27.5	<i>MspI</i>	471	BI335712	<i>Mus musculus</i> zinc finger protein (C2H2 type) 276 (<i>Zfp276</i>)	0.001		nss	
<i>Nie0358</i>	I	0.0	<i>MspI</i>	none						
<i>Nie0750</i>		22.4	<i>MspI</i>	622	BI335681	<i>Homo sapiens</i> involucrin (IVL) cDNA	0.12		nss	
<i>Nie0289</i>		24.7	<i>HinfI</i>	426	BI335634	Chicken ubiquitin I (<i>Ubf</i>) gene	2.0×10^{-95}	12q24.3	3.0×10^{-28}	1/UN
<i>Nie0260</i>	J	0.0	<i>TaqI</i>	275	BI335717	Chicken ubiquitin I (<i>Ubf</i>) gene	2.0×10^{-84}	12q24.3	2.0×10^{-62}	1/UN
<i>Nie0175</i>		1.2	<i>TaqI</i>	480	BI335626	Novel sequence	nss		nss	
<i>Nie0158</i>		2.5	<i>TaqI</i>	376	BI335620	Human RNA-binding protein S1	2.0×10^{-26}	4p15.3	2.0×10^{-26}	4
<i>Nie0196</i>		21.8	<i>MspI</i>	289	BI335628	Chicken glutathione S- transferase (CL 3)	6.0×10^{-82}		nss	3
<i>Nie0267</i>		21.8	<i>MspI</i>	none		Identical RFLP as <i>Nie0196</i>				
<i>Nie0534</i>	K	0.0	<i>MspI</i>	650	BI335653	Human GTP-binding protein RAB6C	1.0×10^{-109}	2q14	1.0×10^{-90}	1
<i>Nie0499</i>		8.2	<i>MspI</i>	266	BI335648	Chicken mRNA for Hsp47, heat shock protein	4.0×10^{-59}		nss	
<i>Nie0130</i>		20.9	<i>TaqI</i>	none						
<i>Nie0286</i>		20.9	<i>EcoRI</i>	593	BI335633	Chicken β -globulin	1.0×10^{-164}	11p15	4.0×10^{-10}	1,5/1
<i>Nie0804</i>		20.9	<i>RsaI</i>	451	BI335688	Chicken hemoglobin β chain	1.0×10^{-127}	11p15	3.0×10^{-16}	5/1
<i>Nie1010</i>		20.9	<i>RsaI</i>	588	BI335707	Chicken hemoglobin β chain	0.0	11p15	2.0×10^{-11}	5/1
<i>Nie0742</i>	L	0.0	<i>HinfI</i>	334	BI335680	Novel sequence	nss		nss	
<i>Nie1015</i>		5.0	<i>MspI</i>	413	BI335708	Ribosomal protein L35a	1.0×10^{-63}	16p12	3.0×10^{-25}	E35C18W14
<i>Nie0939</i>		14.1	<i>RsaI</i>	465	BI335701	Chicken ubiquitin-ribosomal fusion protein	1.0×10^{-179}	14q22	2.0×10^{-73}	5
<i>Nie0038</i>	M	0.0	<i>EcoRI</i>	641	BI335606	Chicken ribosomal Protein S4	0.0	20p12	4.0×10^{-50}	3
<i>Nie1049</i>		1.2	<i>EcoRI</i>	523	BI335714	Chicken ribosomal Protein S4	0.0	12	5.0×10^{-34}	1

Table 1 (continued).

Locus	Linkage group	cM	Enz	bp	GenBank accession No.	Sequence similarity	E value*	HSA	E value†	Chicken chromosome
<i>Nie0040</i>		12.1	<i>EcoRI</i>	200	BI335607	Chicken hemoglobin β chain	1.0×10^{-41}		nss	
<i>Nie0624</i>	N	0.0	<i>TaqI</i>	358	BI335670	Human α-tubulin	7.0×10^{-39}	12q12	5.0×10^{-33}	E22C19W28 /15
<i>Nie0724</i>		6.9	<i>MspI</i>	597	BI335677	Novel sequence	nss		nss	
<i>Nie0041</i>	O	0.0	<i>TaqI</i>	744	BI335608	Chicken cystatin gene	0.0		nss	
<i>Nie0174</i>		0.9	<i>RsaI</i>	418	BI335625	Novel sequence	nss		nss	
<i>Nie0073</i>		3.0	<i>TaqI</i>	608	BI335609	Mammalian histone H3, family 3A	1.0×10^{-13}	2q31	1.0×10^{-13}	7
<i>Nie0187</i>	P	0.0	<i>HinfI</i>	120	BI335627	Novel sequence	nss		nss	
<i>Nie0530</i>		1.3	<i>RsaI</i>	231	BI335652	Novel sequence	nss		nss	
<i>Nie0968</i>		1.9	<i>TaqI</i>	523	BI335703	Ribosomal protein S18	3.0×10^{-71}	17p12	2.0×10^{-35}	E21E31C25 W12
<i>Nie0536</i>	Q	0.0	<i>MspI</i>	544	BI335654	Human ubiquinol-cytochrome c reductase core protein I	4.0×10^{-55}		nss	
<i>Nie0604</i>		0.0	<i>MspI</i>	571	BI335665	Novel sequence	nss		nss	
<i>Nie0895</i>		0.0	<i>RsaI</i>	ns						
<i>Nie0090</i>	R	0.0	<i>EcoRI</i>	137	BI335612	Novel sequence	nss		nss	
<i>Nie0209</i>		0.0	<i>HinfI</i>	447	BI335629	Novel sequence	nss		nss	
<i>Nie0769</i>	S	0.0	<i>HinfI</i>	341	BI335684	<i>Mus musculus</i> ES cells cDNA	7.0×10^{-11}		nss	
<i>Nie0817</i>		0.0	<i>HinfI</i>	653	BI335691	Novel sequence	nss		nss	
<i>Nie0022</i>	T	0.0	<i>MspI</i>	473	BI335605	Ribosomal protein S14	1.0×10^{-18}	1p36.1	7.0×10^{-79}	E54
<i>Nie0149</i>		0.0	<i>MspI</i>	436	BI335618	Ribosomal protein S14	1.0×10^{-106}	1p36	4.0×10^{-68}	E54
<i>Nie0572</i>		0.0	<i>MspI</i>	279	BI335658	Ribosomal protein S14	2.0×10^{-38}	5q22.1p36.1	2.0×10^{-25}	E48C28W13 W27/5
<i>Nie0732</i>		0.0	<i>MspI</i>	532	BI335679	Ribosomal protein S14	1.0×10^{-111}	1p36.1	3.0×10^{-72}	E54
<i>Nie0163</i>	W	0.0	<i>EcoRI</i>	666	BI335622	Ribosomal protein L17	3.0×10^{-82}	15q22.1p22	3.0×10^{-57}	—
<i>Nie0454b</i>		0.0	<i>MspI</i>							
<i>Nie0786</i>		0.0	<i>TaqI</i>	480	BI335686	Novel sequence	nss		nss	
<i>Nie1018</i>	Z	0.0	<i>TaqI</i>	629	BI335709	Novel sequence	nss		nss	
<i>Nie0454a</i>		17.5	<i>MspI</i>	589	BI335645	AD-012 Human adrenal gland protein	2.0×10^{-47}	9p12	6.0×10^{-06}	Z
<i>Nie0307</i>		32.6	<i>TaqI</i>	454	BI335639	Chicken purpurin	0.0		nss	
<i>Nie0014b</i>		73.2	<i>HinfI</i>			RFLP detected with different RE than <i>Nie0014a</i>				
<i>Nie0014a</i>		85.9	<i>HinfI</i>	377	BI335603	Myocyte-specific enhancer (MEF2C)	1.0×10^{-41}	7q11.2	6.0×10^{-54}	—
<i>Nie0083</i>	Unlink ^d		<i>MspI</i>	795	BI335611	Human FEZ1	2.0×10^{-13}	8p21	1.0×10^{-13}	1
<i>Nie0113</i>			<i>RsaI</i>	683	BI335615	Mammalian ligitin (LGTN)	7.0×10^{-28}	1q31	4.0×10^{-07}	—
<i>Nie0114</i>			<i>RsaI</i>	668	BI335616	Novel sequence	nss		nss	
<i>Nie0152</i>			<i>RsaI</i>	568	BI335619	Ribosomal protein L11	3.0×10^{-82}	5q23.1p35	1.0×10^{-25}	E48C28W13 W27/5

Table 1 (concluded).

Locus	Linkage group	cM	Enz	bp	GenBank accession No.	Sequence similarity	HSA	<i>E</i> value*	<i>E</i> value†	Chicken chromosome
<i>Nie0276</i>			<i>MspI</i>	379	BI335632	Murine mRNA L27 ribosomal protein	11p15	6.0×10^{-24}	2.0×10^{-13}	1/5
<i>Nie0292</i>			<i>MspI</i>	657	BI335635	Novel sequence		nss	nss	
<i>Nie0500</i>			<i>MspI</i>	796	BI335649	Mouse SHYC	15p11.1	4.0×10^{-11}	1.0×10^{-10}	—
<i>Nie0537</i>			<i>HinfI</i>	498	BI335655	Novel sequence		nss	nss	
<i>Nie0571</i>			<i>MspI</i>	435	BI335657	Novel sequence		nss	nss	
<i>Nie0593</i>			<i>MspI</i>	362	BI335662	Ribosomal protein L32 (RPL32)	7p12	3.0×10^{-58}	2.0×10^{-44}	2
<i>Nie0600</i>			<i>TaqI</i>	306	BI335664	Novel sequence		nss	nss	
<i>Nie0612</i>			<i>RsaI</i>	586	BI335667	Novel sequence		nss	nss	
<i>Nie0698</i>			<i>TaqI</i>	433	BI335673	Ribosomal protein L31(RpL31)	2q36	3.0×10^{-86}	2.0×10^{-60}	7
<i>Nie0700</i>			<i>MspI</i>	492	BI335674	Chicken myosin alkali 1 chain (MELC)	2q34	1.0×10^{-157}	5.0×10^{-09}	7
<i>Nie0710</i>			<i>MspI</i>	424	BI335676	Ribosomal protein L34	8q22	5.0×10^{-77}	7.0×10^{-54}	2
<i>Nie0728</i>			<i>RsaI</i>	633	BI335678	Chicken Jun-binding protein mRNA	19p13.2	0.0	4.0×10^{-41}	E25C31
<i>Nie0753</i>			<i>EcoRI</i>	563	BI335718	ATP synthase, H ⁺ transporting, subunit c	18q21	2.0×10^{-66}	2.0×10^{-51}	2
<i>Nie0773</i>			<i>RsaI</i>	548	BI335685	Ribosomal protein L28(RPL28)	19q13.4	1.0×10^{-22}	5.0×10^{-09}	3/E25C31
<i>Nie0815</i>			<i>TaqI</i>	545	BI335690	Novel sequence		nss	nss	
<i>Nie0836</i>			<i>TaqI</i>	488	BI335692	Novel sequence		nss	nss	
<i>Nie0843</i>			<i>EcoRI</i>	652	BI335693	Human CGI-121 protein mRNA	10p11.2	6.0×10^{-13}	3.0×10^{-08}	2
<i>Nie0902</i>			<i>MspI</i>	420	BI335698	Eukaryotic translation initiation factor 3		2.0×10^{-17}	nss	
<i>Nie0920</i>			<i>TaqI</i>	663	BI335700	<i>Homo sapiens</i> NRAS-related gene	1p11	1.0×10^{-106}	7.0×10^{-49}	—
<i>Nie1004</i>			<i>HinfI</i>	676	BI335706	Quail α -tropomyosin, intron IV		1.0×10^{-04}	nss	
<i>Nie1047</i>			<i>TaqI</i>	443	BI335713	Rat adenylate kinase 2(AK2)	1p31	9.0×10^{-48}	3.0×10^{-22}	8

Note: Included are the position within the linkage group (cM), the restriction enzyme used to detect the locus (Enz), the length of cDNA sequence obtained (bp), GenBank accession number, and summary results of the sequence BLAST searches. Chicken chromosome assignments were inferred from the comparative map of Groenen et al. (2000) or were based on mapped gene positions obtained from the ArkDB chicken database (indicated in bold). nss, sequences that produced no significant similarity.

*The sequence with the highest similarity (*E* value) is given for each locus with sequence data.

†The best human chromosome (HSA) position match (*E* value) was determined by BLAST comparisons with the human genome.

sive linkage map of the turkey genome. This research will use marker loci previously developed for turkey and other avian species (chicken and quail, Reed et al. 2000b) and also includes development of new microsatellite loci. The long-term objective of this project is to develop a linkage map to support QTL mapping and comparative genomics, and much work remains to be done.

The current map consists of 22 linkage groups with an additional 25 unlinked loci. Linkage groups encompass 651 cM, and if we ascribe 20 cM to each unlinked marker, total coverage is approximately 1150 cM. In comparison to the length of the comprehensive chicken map (3800 cM, Groenen et al. 2000), the current turkey map represents coverage of approximately 14–30%.

The 22 linkage groups of the current map should evolve substantially as new markers are added. Genomes of the turkey ($2n = 80$) and the chicken ($2n = 78$), include a small number of macrochromosomes, many microchromosomes, and the Z–W sex chromosomes. In the chicken, chromosomes 1, 2 and Z are metacentric, whereas the turkey has only two large metacentric chromosomes believed to correspond to chromosomes 1 and Z (Schmid et al. 2000). These authors also point out that the Z chromosome is the fifth-largest chromosome in chicken, whereas it is the fourth-largest chromosome in turkey. A major karyotypic difference between these two species is that turkey chromosomes 3 and 6 are believed to represent a centric fission of chicken chromosome 2 (Schmid et al. 2000). The degree to which syntenic groups are conserved between these related species should become more apparent as marker density on the turkey map is increased.

For the most part, comparisons between turkey and chicken chromosomes are indirect, being inferred from the chicken–human comparative map (Groenen et al. 2000) following comparisons with human sequences using BLAST. Despite the shortcomings of this approach, certain turkey linkage groups (or portions thereof) can be tentatively assigned as corresponding to specific chicken chromosomes. For example, the terminal region of linkage group B (from Nte0486 at 69.2 cM) likely corresponds to Gga 5.

As described above, the turkey linkage group Z almost certainly corresponds to the Z chromosome. This assignment is further supported by DNA sequence and genetic linkage. Based on sequence similarity, Nte0454a (at 17 cM on group Z, Fig. 3) corresponds to human AD-012 on chromosome 9 (9p12). This portion of HSA 9 appears orthologous with a portion of the Z chromosome of chickens (Groenen et al. 2000; Schmid et al. 2000). In addition, AD-012 appears linked to MSU0067 near one end of the chicken Z chromosome (Lawson and Ellegren, personal communication).

With only about 30 genes located on the chicken Z chromosome (Schmid et al. 2000), it is worthwhile pointing out one other Z-linked gene identified in turkey. The sequence of Nte0307 is nearly identical to a cDNA sequence encoding a purpurin precursor in chicken (Berman et al. 1987). The first 360 bases of Nte0307 corresponds to the last 120 codons of the chicken sequence, a region containing only six synonymous nucleotide substitutions. To our knowledge, the purpurin gene has not been mapped in chicken, but these data suggest that it is also likely to be located on Gga Z.

Among species, comparisons of chromosomal organization based on gene sequences alone can be problematic, especially when dealing with gene families. For example, the sequences of two turkey clones (Nte0289 and Nte0260) correspond to the ubiquitin I (*UBI*) gene of the chicken. The sequences are partially overlapping, containing the extreme 3' end of the coding region, and differ at 10 positions in the 132-bp region of overlap. Separate RFLP loci were scored for these two clones, and linkage analysis placed these in separate linkage groups (I and J). Ubiquitin is a multigene family in humans and our data suggest a similar situation in the turkey.

Another problematic example is illustrated by Nte0622. Mapping to linkage group A (Table 1), the sequence of Nte0622 is similar to an 18S ribosomal RNA gene. In chicken, the 18S ribosomal gene maps to a microchromosome, Gga 16 (Bloom and Bacon 1985). Given our random selection of markers, it is unlikely that linkage group A, our largest turkey linkage group, corresponds to a microchromosome. The resolution of ambiguities such as these will be greatly facilitated by the future sequencing of the chicken genome and by expanded mapping of the turkey.

Because the map presented in this study was developed using RFLPs, a method requiring significant quantities of genomic DNA, only limited amounts of DNA remain from the original mapping families. Future mapping efforts at the University of Minnesota, emphasizing microsatellite markers, will focus on new F₂ reference families developed from commercial lines at Nicholas Turkey Breeding Farms. Nevertheless, efforts are being made to incorporate polymorphic markers common to both mapping populations. As the next generation map is developed, it should be possible to build a consensus map that encompasses genes mapped in the present study. By including expressed genes from the turkey, sequence comparisons to the growing database of chicken ESTs will greatly facilitate comparisons between these avian relatives.

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