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Short Communication

A well-tested set of primers to amplify regions spread across the avian genome

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1. Introduction

DNA markers have been used to examine a broad range of biological problems, including those in phylogenetics, population genetics and the identification of individuals. Nuclear loci provide a rich source of information regarding evolutionary history and they allow researchers to examine many unlinked markers that have the potential to provide insight the coalescent process as well as introgression or hybridization events (e.g. Maddison, 1997; Nichols, 2001). Information about the nuclear genome can be collected by sequencing or by examining single nucleotide polymorphisms (SNPs) (Sunnucks, 2000; Avise, 2004). Unlike mitochondrial regions where virtually universal primers are available for vertebrates (e.g., Sorenson et al., 1999), extensively tested primer sets for nuclear regions that are likely to work on the majority of species within a group are not available.

In phylogenetic studies, nuclear sequence data have provided additional insights into avian evolution (e.g., Avise, 2004; Hackett et al., 2008). The nuclear genome is heterogeneous, consisting of coding regions, introns, untranslated regions (UTRs), and intergenic regions. The markers exhibit varying patterns of molecular evolution (e.g., mutation rates, base composition, etc.), and thus can be used at a variety of different taxonomic levels for different types of phylogenetic questions. In addition to substitutional variation, non-coding regions often exhibit length variation, providing indel (insertion–deletion) characters that can be added to the region's information content (e.g., Fain and Houde, 2004).

Population-level questions can be examined using non-coding regions such as introns. Substitutional (and indel) variation of multiple individuals of a single species or a set of closely related species provide insights into population divergence times and ancestral population sizes (e.g., Jennings and Edwards, 2005; Aitken et al., 2004). SNPs present in these rapidly evolving regions are increasingly being used as markers because they provide an opportunity to assess a large number of unlinked loci for a range of population questions (Brumfield et al., 2003; Morin et al., 2004). In addition to population genetics, SNPs also have the potential to be used in parentage studies and for quantitative genetic studies in wild populations.

In birds, there are several studies which have published sets of primers to amplify nuclear regions, particularly intron regions (e.g., Friesen et al., 1999, 1997; Primmer et al., 2002; Waltari and Edwards, 2002; Slade et al., 1993; Backström et al., 2008). However, many of these primers have been tested on a limited set of

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Table 1
Characteristics of the loci included in this study.

Locus	Chr[a]	Main regions[b]	bp in Gallus[c]	% Coding[d]	Exon rate[e]	Intron rate[e]	UTR rate[e]	Mix rate[e]	Effort[f]
ACO1	Z	Intron 9	1060	3	–	3.02	–	–	H
ALDOB	Z	Introns 3–7	2440	24	0.91	3.14	–	2.49	H
ARNTL	5	Intron 12	550	19	–	2.87	–	–	H
BDNF	5	Exon 1	690	100	0.72	–	–	–	H
CLOCK[g]	4	Intron 10	670	10	–	2.97	–	–	M
CLOCK[g]	4	3′ UTR	530	<1	–	–	1.14	–	H
CLTC	19	Introns 6–7	1930	22	0.91	3.07	–	2.59	H
CLTCL1	15	Intron 7	730	42	0.79	2.89	–	2.44	H
CRYAA	1	Intron 1	1190	21	0.76	3.71	–	3.05	H
CSDE1	26	Intron 5	470	27	0.30	3.14	–	2.38	M
CYP19A1[h]	10	5′ UTR	530	22	1.47	–	2.34	1.79	H
EEF2	28	Introns 5–9	1700	33	0.85	3.79	–	2.72	H
EGR1	13	Exon 2, 3′ UTR	1740	71	1.04		1.17	1.13	H
FGB	4	Introns 4–7	2580	22	0.92	2.86	–	2.53	H
GARS	2	Intron 11	303	19	–	2.69	–	–	M
GH1	27	Introns 2–3	1030	24	1.22	3.34	–	2.74	H
HMGN2	23	Introns 2–5	1600	13	0.69	3.88	–	3.47	H
HOXA3	2	Intron 1	1460	6	–	2.51	–	–	H
IRF1	13	Intron 2	920	5	–	3.49	–	–	H
IRF2	4	Intron 2	700	12	–	2.06	–	–	H
NAT15	14	Intron 4	1010	12	–	3.16	–	–	M
MB	1	Intron 2	950	3	–	1.96	–	–	H
MUSK	Z	Intron 3	700	28	0.94	3.16	–	2.72	H
MYC	2	Intron 2, Exon 3, 3′ UTR	1240	45	0.86	2.69	0.89	1.30	H
NGF	26	Exon 4	750	95	1.67	–	–	–	H
NTF3	1	Exon 2	730	100	1.01	–	–	–	H
PARK7	21	Intron 2	700	14	–	3.29	–	–	M
PAXIP1	2	Intron 20	480	<1	–	2.53	–	–	M
PCBD	6	Introns 2–3	1190	11	0.73	3.06	–	2.68	H
PER2	9	Intron 9	510	9	–	2.96	–	–	M
RAPGEF1	17	Intron 18	1270	<1	–	2.83	–	–	M
RHO	12	Introns 1–3	1890	45	1.09	3.42	–	2.66	H
SPIN1	Z	Intron 2	940	14	–	3.34	–	–	H
TGFB2	3	Intron 5	560	3	–	2.67	–	–	H
TPM1	10	Intron 6	490	<1	–	1.34	–	–	H
TXNDC12	8	Intron 6	479	5	–	2.81	–	–	M
VIM	2	Intron 8	500	2	–	3.08	–	–	M

[a] Chromosome in the chicken genome.
[b] Since exons in the 5′ UTR of the chicken genome are not always annotated, our numbering system considered the first coding exon to be exon 1 (based on Build 2.1, assembly of the chicken genome released on May 2006 by the Genome Sequencing Center at Washington University in Saint Louis), and that the intron was numbered by the preceding exon. For previously published regions, published exon and intron designations were used [MYC (Harshman et al., 2003); NGF (Bertaux et al., 2004), and TPM1 (Primmer et al., 2002)]. For PAXIP1, the chicken genome lacked the 3′ end, so numbering was based on the human genome.
[c] Length in the chicken of the longest amplifiable region (excluding primer sequence). For MYC and GH1, this only includes regions amplified in the nested or semi-nested PCR.
[d] The percent of amplified product that is coding exon in the chicken.
[e] The rate is the slope of the best fit line (constrained to go through the origin) of the *p*-distance regressed against the *p*-distances for all exons included in Hackett et al. (2008). For rates targeting a single region (e.g, a single intron) that have less than 20% exon, rates are only given for the intron. Mix is the rate of the combined region that contained introns, exons and/or UTRs.
[f] High (H) effort are primers tested in ~200 species; Moderate (M) effort are tested in 42 species.
[g] The two CLOCK segments are discontiguous and so are not included in Fig. 2.
[h] The amplified region includes the entire 5′ UTR as well as upstream 5′ non-coding sequence.

taxa, and thus may not work in many groups. Here we present broadly tested primers for 36 nuclear loci (Table 1; including introns, exons, and UTRs) located on 21 chromosomes in the chicken genome (Fig. 1). We have already collected a substantial dataset from these regions (e.g., Chojnowski et al., 2008; Yuri et al., 2008; Hackett et al., 2008), providing outgroups and a backbone for future studies that utilize the same regions.

2. Loci and primers

The primers presented here were tested either on 199 species (Table S1) representing all avian orders and most non-passerine families (High Effort in Table 1) or else on 42 selected species (Table S1) representing all major avian groups (Moderate Effort in Table 1). The primers presented here expand those published previously (Harshman et al., 2003; Barker, 2004; Driskell and Christidis, 2004; Zink et al., 2006; Cox et al., 2007; Barker et al., 2008; Chojnowski et al., 2008; Yuri et al., 2008; Hackett et al., 2008) in several major ways. First, we provide additional primers to expand the utility of published loci, including alternative primers and internal primers that are anchored in highly conserved regions and can also be used both for nested PCR and to obtain shorter segments. Second, we provide well-tested primers that can be used to amplify segments of 16 additional loci. Finally, we provide detailed maps of all regions with multiple primers, indicating the locations of these primers. Based upon our tests, we include the primers (Table 2) that are most likely to successfully amplify their target regions in the widest range of avian taxa; problems we have identified in the use of these primers are described below.

Several strategies were used for the development of novel loci. Work on some loci was initiated before the publication of the chicken (*Gallus gallus*) genome, and these loci were selected from among the available chicken sequences that included intron and

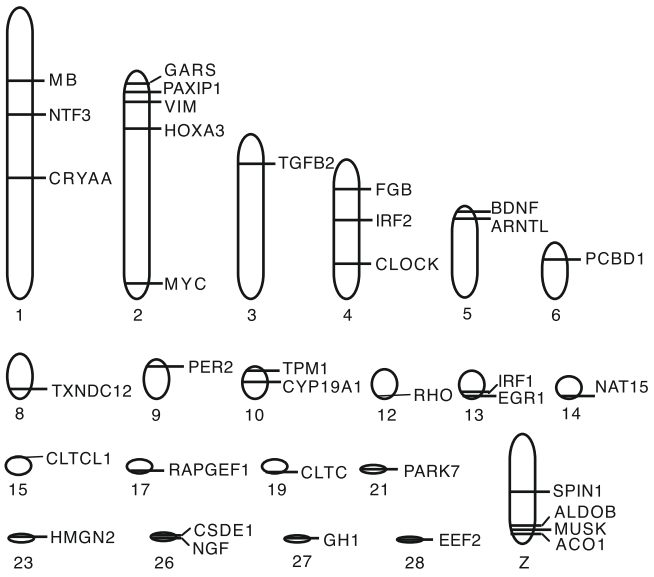

Fig. 1. Karyotype of the chicken (*Gallus gallus*) with loci included in this study designated using the HUGO (Human Genome Organisation) name (Table 1). Locations of specific loci were determined using MapViewer at the National Center for Biotechnology Information.

exon structure (and introns of a suitable length). Primers were then developed by comparing sequences from chicken, human, and when available, reptiles or other avian species. Later loci were selected as they had regions (particularly introns) that were of a suitable length in the chicken genome, and additional sequence data was available from at least one other avian species (typically *Taenopygia guttata*, *Melopsittacus undulatus* and *Columba livia*). Four introns were selected for development because they were identified as non-canonical (U12-dependent) introns in human genes (Levine and Durbin, 2001); primers for these were developed by comparing human sequences with the chicken orthologs. All non-canonical introns used here (those in GARS, NAT15, PAXIP1, and RAPGEF) have AT-AC terminal dinucleotides rather than the GT-AG terminal dinucleotides found in canonical introns.

Basic chromosome structure appears largely conserved within birds (e.g. Griffin et al., 2007), suggesting that most loci that are unlinked in the chicken genome (Fig. 1) will remain unlinked in other avian lineages. The 36 loci represent a wide variety of genes (Table S2), and the amplified regions include differing amounts of coding and non-coding sequence (Table 1). Evolutionary rates vary (Table 1) though in almost all cases introns evolve at higher rates than either UTRs or coding regions.

For many loci, the primers we present amplify a single region, while for other loci we present a suite of primers that allow amplification of larger regions (or the targeting of only a subset of the region we amplified) that can include combinations of introns, coding exons and UTRs (Fig. 2). Amplification of longer regions is advantageous when a large amount of data from a single locus is desired, for example to reduce variance in gene tree estimation (e.g., Chojnowski et al., 2008) or to obtain a large amount of data likely to have evolved under similar models of evolution to examine patterns of sequence evolution (e.g., Lake, 1997). Furthermore, larger regions also allow comparison of patterns of change across different types of data (e.g., coding vs. non-coding segments). We emphasize, however, that the regions selected are biased towards introns because we have found that avian introns are useful for phylogenetics at both deep and shallow level.

Amplification of a single region (one intron) is relatively straightforward in most cases, generally requiring a single primer set. For regions with more than one primer pair (most of these span multiple introns and/or exons) the position of the different primers relative each other and to intron/exon structure are presented (Fig. 2). Most primers were used for PCR and sequencing but a few were used primarily for sequencing to allow double-stranded contigs to be obtained. The sequencing primers presented in Table 2 include only those that are in highly conserved regions (primarily exons) that should also be useful for PCR across a broad range of taxa if amplification of smaller regions was desired. A few primers were used in nested PCR strategies (see below). To obtain complete double-stranded sequences for longer introns, it was often necessary to use primers that were not conserved across taxa (not included in Table 2). However, these primers can be readily developed for specific taxa once sequencing with the PCR primers has been completed.

In general we used standard PCR conditions (annealing temperatures and Mg^{++} concentrations are noted in Table 2). However in a few cases, alternative PCR strategies (e.g., adjusting magnesium levels, use of specialized polymerases, nested PCR) were required to amplify regions for a small proportion of taxa. There were certain primer sets that generally required use of particular PCR strategies or that were not robust in certain taxonomic groups. There are two paralogs of ALDOB that occasionally amplify with exon-anchored ALDOB primers. To obtain the majority of the region, a semi-nested PCR strategy using Ald.3F and Ald.7intR followed by amplification using Ald.3F and Ald.7R increased specificity. The region amplified using primers Ald.6F and Ald.8R can be used without nesting, though amplification of a smaller product may occur under some conditions. Nested PCR was used to amplify the entire region of GH1 (GH-F874 and GH-R3108, followed by GH-F897 and GH-R1925) while a semi-nested strategy was used for MYC (MYC-FOR-01 and MYC-REV-47 followed by MYC-FOR-02 and MYC-REV-47).

For some regions, one primer set worked much of the time, but an alternative set was often necessary to achieve amplifications across all taxa. This included amplification of FGB intron 5 (Fib5 and Fib6 worked much of the time, but Fib.5F2 and Fib.6R2 often worked when those primers failed), MUSK (MUSK-I3F2 and MUSK-I3R2 were most robust), ACO1 (ACO1-I9F and ACO1-I9R were used most commonly), and IRF1 (about half of the taxa amplified with IRF1.2F and IRF1.3R, while the remainder were amplified with IRF1.2F2 and IRF1.3R2). For MB, the use of MYO2 and MYOintR avoided amplification of a homopolymer run in many taxa, decreasing the need to clone products due to length polymorphisms. In addition, for some taxa, amplification of two smaller regions (MYO2 and MYOintR plus MYOintF and MYO3F) was necessary. For these regions, the specific primer combination that was most successful depended strongly upon phylogeny so it may be necessary to try multiple combinations. Alternatively, some primer sets amplified less exon relative to intron, such as in CLTCL1, where both CLTCL1.e8Rnew and CLTCL1.e8Ralt amplified robustly when paired with CLTCL1.e7F, but use of CLTCL1.e8Rnew gave almost 150 bp more of exon sequence.

Some primer sets failed to work, or amplified poorly, in specific groups. For example, in CLTC, CLTC.e7Falt and CLTC.e8Ralt amplifies intron 7 from the paralog CLTCL1 in passerines; for passerines, primers CLTC.e7Fpass2 and CLTC.e8Rpass amplified the correct paralog, but were not very robust. The CLK UTR primers (CLK.21F and CLK.3′UTR) amplified poorly in Coraciiformes and Psittaciformes, while Ald.6F and Ald.8R amplified weakly in Psittaciiformes. The 3′ end of RHO (covering all primers amplifying exons 2–4) did not amplify from ratites (but did amplify from Tinamiformes). CYP19A1 and TPM1 amplified robustly in only 75% of the taxa (150 species), though there was little phylogenetic pattern to which taxa amplified and which did not. The published primers for the SPINZ (Z-linked copy of spindling) occasionally amplify SPINW instead.

Table 2
Primers used to amplify regions from Table 1. For regions with more than two primers (except CLOCK), see Fig. 2 for primer locations.

Locus	Primer name	Sequence (5′–3′)	Use[a]	Annealing T° (Mg^{++} conc.)	Authors[b] or references
ACO1	ACO1-I9F	CTGTGGGAATGCTGAGAGATTT	1	55° (2.0 mM)	FKB
	ACO1-I9R2	CAACTTTGTCCTGGGGTCTTT	1		FKB
	ACO1-I9F2	CTCCTCTCAGGATCCAGACTT	1	55° (2.0 mM)	FKB
	ACO1-I9R	CTGCAGCAAGGCACAACAGT	1		FKB
ALDOB	AldB.3F	GCCATTTCCAGCTCTCATCAAAG	1	55° (1.5 mM)	RTK & ELB
	AldB.7intR	CAYAGGAATGAATCRRGAATCAC	3		RTK & ELB
	AldB.7R	AGCAGTGTCCCTTCCAGGTASAC	1	58° (1.5 mM)	RTK & ELB
	AldB.6F	GAGCCAGAAGTCTTACCTGAYGG	1	50° (1.5 mM)[c]	RTK & ELB
	AldB.8R	GCTCKCCCGTATGAGAAGGTCAGYTT	1		RTK & ELB
	AldB.4F	GCAGGAACAAATGGAGAAACSAC	2		RTK & ELB
	AldB.5F	GAGCGCTGTGCCCAGTACAAGAA	2		RTK & ELB
	AldB.7F	GTTCTGGCTGCTGTCTACAAGGC	2		RTK & ELB
	AldB.4R	GTSGTTTCTCCATTTGTTCCTGC	2		RTK & ELB
	AldB.5R	ACTTGTTGGCAGATGCTGGCGTA	2		RTK & ELB
	AldB.6R	AGCGCTGGAGRTCATGGTCT	2		RTK & ELB
ARNTL	ARNTL.12F	TGGTTCAGTTTCATGAACCCTTG	1	55° (2.0 mM)	RTK & ELB
	ARNTL.13R	CCTGAAGCACRCTGTCCATGCT	1		RTK & ELB
BDNF	ChickBDNF-5′	ATGACCATCCTTTTCCTTACTATG	1	55° (2.0 mM)	Sehgal and Lovette (2003)
	ChickBDNF-3′	TCTTCCCCTTTTAATGGTTAATGTAC	1		Sehgal and Lovette (2003)
CLOCK	CLK.10F	CATGTGGATGATCTAGATAATCTGGC	1	55° (2.0 mM)	RTK & ELB
	CLK.10R	GYAATGTGTTTGCAGCCAAATCCA	1		RTK & ELB
	CLK.21F	CCTTCCAAAGCTCAGCCACAGTA	1	57° (2.0 mM)	RTK & ELB
	CLK.3′UTR	GCCTACAGATAACAGATTACGTTTCATGC	1		RTK & ELB
CLTC	CLTC.e6Fnew	CTACATGAACAGAATCAGTGGAGAGAC	1	64° (1.5 mM)	RTK, JLC, & ELB
	CLTC.e7Rnew	GCTGCCACTTTTGCTGCCTCTGAATA	1		RTK, JLC, & ELB
	CLTC.e7Falt	CAGAATCCTGATCTAGCTTTACGAATGGC	1	53° (1.5 mM)	RTK, JLC, & ELB
	CLTC.e8Ralt	CATTTCTCCAGAAGTTGTTTGCGTCC	1		RTK, JLC, & ELB
	CLTC.e7Fpass2	CAGGTGCTCTCAGTGTGTGTGGAAGA	1	52° (2.5 mM)	RTK, JLC, & ELB
	CLTC.e8Rpass	TGWGCTGGAACACTCTGGAACCG	1		RTK, JLC, & ELB
CLTCL1	CLTCL1.e7F	CACCAATGTTCTGCAGAATCCTGA	1	55° (2.5 mM)	RTK, JLC, & ELB
	CLTCL1.e8Rnew	CCAGCTTATCTTCCTTNAGCCATTTCTC	1		RTK, JLC, & ELB
	CLTCL1.e8Ralt	GGCTGAGCTGGTACACTCTGGAACTTCC	1	58° (2.0 mM)	RTK & ELB
CRYAA	CRY.1F	TTACTATYCAGCACCCCTGGTTCAA	1	63° (1.5 mM)	RTK & ELB
	CRY.2R	CTGTCTTTCACTGTGCTTGCCRTGRAT	1		RTK & ELB
CSDE1	CSDE.5F	CTGGTGCTGTAAGTGCTCGTAAC	1	59° (2.5 mM)	RTK, JVS, & ELB
	CSDE.6R	CCAGGCTGTAAGGTTTCTAGGTCAC	1		RTK, JVS, & ELB
CYP19A1	CYP.5UTRF	GAACTCATTCAATGCTGCCATRTGTG	1	52° (2.0 mM)	RTK & ELB
	CYP.1R	GGCCCTGGTATTGATGATGTTTCTTCAT	1		RTK & ELB
EEF2	EEF2.5F	GAAACAGTTTGCTGAGATGTATGTTGC	1	60° (1.5 mM)	RTK & ELB
	EEF2.7R	GGTTTGCCCTCCTTGTCCTTATC	1		RTK & ELB
	EEF2.6F	CCTTGAYCCCATCTTYAAGGT	1	58° (1.5 mM)	RTK & ELB
	EEF2.9R	CCATGATYCTGACTTTCARGCCAGT	1		RTK & ELB
	EEF2.7F	GACGCGATCATGACCTTCAAGAAAGA	2		RTK & ELB
	EEF2.8F	ACCTGCCTTCTCCTGTCACAG	2		RTK & ELB
	EEF2.6R	CACCTTRAAGATGGGRTCAAG	2		RTK & ELB
	EEF2.8R	TATGGCRGCCTCATCATCAGG	2		RTK & ELB
EGR1[d]	Z1F	AGAAACCAGCTATCCCAAYCAA	1	60° (2.0 mM)	Chubb (2004)
	Z9R	CTCAATTGTCCTTGGAGAAAAGG	1		Chubb (2004)
	Z5F	CCTTTTCTCCAAGGACAATTGA	1	45° (2.0 mM)	Chubb (2004)
	Z10R	AAAACAAAACTTCTGCCAC	1		Chubb (2004)
	Z3F	CCCTATGCCTGCCCAGTGGAGTCC	2		Chubb (2004)
	Z7R	CGTGAAAACCTCCGGTCACAG	2		Chubb (2004)
FGB	Fib3	CTGTAATATCCCGGTGGTTTCAGG	1	55° (2.0 mM)	FKB
	Fib4	ATTTCAGATGTTTCACCTCCCTTTC	1		FKB
	Fib5	CGCCATACAGAGTATACTGTGACAT	1	54° (2.0 mM)	FKB
	Fib6	GCCATCCTGGCGATTCTGAA	1		FKB
	Fib.5F2	GTACCTCATCCAGCCAGATCCT	1	54° (2.0 mM)	SH, SR, RCKB, & JH
	Fib.6R2	TTCTGAATCAAAGTCCAGCC	1		SH, SR, RCKB, & JH
	Fib.6F	TTGCAAAGAGTGGAGGGAAG	1	53.8° (2.0 mM)	KJM
	Fib.8R	CCATCCACCACCATCTTCTT	1		KJM
	Fib.7R	TTGGCTGATTTTGTCATTTCC	2		KJM
	Fib.7F	TGATGGAAGGAGCTTCACAG	2		KJM
	Fib.7intF	CCTACTCAGAAGACAGGAGCTCA	2		KJM
	Fib.7intR	TGAGCTCCTGTCTTCTGAGTAGG	2		KJM
GARS	GARS.11F	GTTCCTCTYATAGCTGAGAAGC	1	62° (2.5 mM)	RTK, VHT, & ELB
	GARS.12R	CGTCCTTCTTRTAAGCTTTGCC	1		RTK, VHT, & ELB
GH1	GH-F874	CCTTCCCWGCCATGCCCCTTTCCAACC	3	70–60° (2.0 mM)[e]	TY & MJB
	GH-R3108	CCGTAGTTCTTCAGCAGGGCSTCCTCG	3		TY & MJB
	GH-F897	TGTTTGCCAACGCTGTGCTGAGG	1	60° (1.5 mM)	TY & MJB
	GH-R1925	TCCCTTCTTCCAGGTCCTTTART	1		TY & MJB
	GH-F1391	GATGTCTCCACAGGAACGYA	2		TY & MJB
	GH-R1476	GATTTCTGCTGGGCATCATCCTTCC	2		TY & MJB

(*continued on next page*)

Table 2 (*continued*)

Locus	Primer name	Sequence (5′–3′)	Use[a]	Annealing T° (Mg^{++} conc.)	Authors[b] or references
HMGN2	HMG17.2F	GCTGAAGGAGATACCAARGGCGA	1	62° (1.5 mM)	RTK & ELB
	HMG17.4R	CTTTGGAGCTGCCTTTTTAGG	1		RTK & ELB
	HMG17.3F	AACGGAGATCGGCGAGGTTATC	1	58° (1.5 mM)	RTK & ELB
	HMG17.6R2	AGCACCTTCGGCTTTCTG	1		TY
	HMG17.6R3	TAGAGTCACCAGAAGTACACAGTTATC	1		TY
	HMG17.4F	AAACCTGCYCCTCCRAAGCCAGAGCC	2		RTK & ELB
	HMG17.5F	AAAACGGAGATGCCAAAACAGAC	2		TY
	HMG17.3R	GATAACCTCGCCGATCTCCGTT	2		RTK & ELB
	HMG17.5R	CTGGTCTGTTTTGGCATCTCC	2		RTK & ELB
HOXA3	HOXA3.1F	GTCTCGGCAAAACACAAAGC	1	55° (2.0 mM)	KJM
	HOXA3.2R	AGCTGGGCACTTGTGTAAGC	1		KJM
IRF1	IRF1.2F	GCATGAGACCCTGGTTGG	1	52.5° (2.0 mM)	KJM
	IRF1.3R	ATGCTTAGCTGCATGTTTCC	1		KJM
	IRF1.2F2	TGCAGATCAATTCCAATCAAATAC	1	51.5° (2.0 mM)	KJM
	IRF1.3R2	CAGGCGTCTTTCTCCATGTC	1		KJM
IRF2	IRF2.2F	ATGTCTTTGGGTCGGGTTTA	1	55.5° (2.0 mM)	KJM
	IRF2.3R	GAAACTGGGCAATTCACACA	1		KJM
NAT15	NAT.4F	ATCAGAGGGGTTCTCAAAGATGG	1	62° (2.0 mM)	RTK, VHT, & ELB
	NAT.5R	AGAGAAGGCTCTGGGCTTGTCGGTA	1		RTK, VHT, & ELB
MB	MYO2	GCCACCAAGCACAAGATCCC	1	52° (2.0 mM)	Slade et al. (1993)
	MYO3F	TTCAGCAAGGACCTTGATAATGACTT	1		Heslewood et al. (1998)
	MYOintF	ATAAACCAGCCCATGCAGCCT	1	52° (2.0 mM)	SH, SR, RCKB, & JH
	MYOintR	CCAGACTAAGAAATAGGTTGC	1		SH, SR, RCKB, & JH
MUSK	MUSK-I3F	CTTCCATGCACTACAATGGGAAA	1	50° (2.0 mM)	FKB
	MUSK-I3R	CTCTGAACATTGTGGATCCTCAA	1		FKB
	MUSK-I3F2	AAATAACCCGACCACCTGTAAA	1	50° (2.0 mM)	FKB
	MUSK-I3R2	TAGGCACTGCCCAGACTGTT	1		FKB
MYC	MYC-FOR-01	TAATTAAGGGCAGCTTGAGTC	3	53° (1.75 mM)	MJB, CH, & WH
	MYC-REV-47	CTATAAAGACTTTATTAAAGGTATTTACAT	1		MJB, CH, & WH
	MYC-FOR-02	TGAGTCTGGGAGCTTTATTG	1	55° (1.7 mM)	MJB, CH, & WH
	MYC-FOR-03	AGAAGAAGAACAAGAGGAAG	2		MJB, CH, & WH
	MYC-FOR-05	CACAAACTYGAGCAGCTAAG	2		MJB, CH, & WH
	MYC-REV-04	GGCTTACTGTGCTCTTCT	2		MJB, CH, & WH
	MYC-REV-06	TTAGCTGCTCAAGTTTGTG	2		MJB, CH, & WH
NGF	AllNGF5′	GGTGCATAGCGTAATGTCCATG	1	52° (2.0 mM)	Sehgal and Lovette (2003)
	AllNGF3′	ATAATTTACAGGCTGAGGTAG	1		Sehgal and Lovette (2003)
NTF3	ChickNT3-5′	ATGTCCATCTTGTTTTATGTG	1	50° (2.0 mM)	Sehgal and Lovette (2003)
	ChickNT3-3′	GTTCTTCCTATTTTTCTTGAC	1		Sehgal and Lovette (2003)
PARK7	PARK.2F	GCAGGCCTRRCTGGAAAAGARCC	1	56° (1.5 mM)	RTK & ELB
	PARK.3R	TTCTGAGCTCCWAGRTTACC	1		RTK & ELB
PAXIP1	PAX.20F	CCCTCAGACACTGGATTAYGAATCAT	1	60° (2.5 mM)	RTK, VHT, & ELB
	PAX.21R	CCAAGGATTCCGAAGCAGTAAG	1		RTK, VHT, & ELB
PCBD1	PCBD.2F	AGAGCTGTGGGGTGGAACGAGGTGGA	1	64° (1.5 mM)	RTK & ELB
	PCBD.4R	TCRTGGGTGCTCAAGGTGATGTGAAC	1		RTK & ELB
	PCBD.3F	CYAGAGTGGCTCTACARGCAGAA	2		RTK & ELB
	PCBD.3R	CCTTRTTGTACACRTTGAACC	2		RTK & ELB
PER2	PER.9F	CATCTTCAYCCAAATGACAGACC	1	55° (2.0 mM)	RTK & ELB
	PER.10R	CCTGATTGGTGAATAGTCAAAAGG	1		RTK & ELB
RAPGEF1	RAP.18F	GCCCATCAAGAAGCTRCARTACAGAT	1	62° (2.5 mM)	RTK, VHT, & ELB
	RAP.19R	CTGGGRAGTGGCAAAACTTCTCAT	1		RTK, VHT, & ELB
RHO	Rhod.5′F	CACCTCRCAARCCGCAGCCAT	1	57° (1.5 mM)	RTK & ELB
	Rhod.ex1R	GTAGCAAAGAAGCCTTCRAYGTAGC	1		RTK & ELB
	Rhod.1F	GAACGGGTACTTTGTCTTTGGAGTAAC	1	64° (1.5 mM)	RTK & ELB
	Rhod.1R	CCCATGATGGCGTGGTTCTCCCC	1		RTK & ELB
	Rhod.2F	GAAATTGCTCTCTGGTCRCTGGTYGT	1	60° (1.5 mM)	RTK & ELB
	Rhod.4R	AAAGAANGCYGGGATGGTCATGAAGA	1		RTK & ELB
	Rhod.3F	CTGAAGCCAGAGRTCAACAACGAAT	2		RTK & ELB
	Rhod.3R	ATCCCRCACGAGCACTGCAT	2		RTK & ELB
SPIN1	Spin319F	TATGGACTAGAACTGCACAAAG	1	60° (2.0 mM)	Handley et al. (2004)
	Spin472R	AGACCATCCCCCTCCATTCATC	1		Handley et al. (2004)
TGFB2	TGFB2.5F	GAAGCGTGCTCTAGATGCTG	1	58° (2.0 mM)	Primmer et al. (2002)
	TGFB2.6R	AGGCAGCAATTATCCTGCAC	1		Primmer et al. (2002)
TPM1	Trop.6aF	AATGGCTGCAGAGGATAA	1	60° (4.0 mM)	Primmer et al. (2002)
	Trop.6bR	TCCTCTTCAAGCTCAGCACA	1		Primmer et al. (2002)
TXNDC12	TXN.6F	GGAAACCCCAGCTACAAGTATTTC	1	57° (1.5 mM)	RTK, JVS, & ELB
	TXN.7R	GGCCTCCTTCATCCCTTGG	1		RTK, JVS, & ELB
VIM	VIM.8F	GACCGTGGAAACTAGAGATGGAC	1	57° (1.5 mM)	RTK, JVS, & ELB
	VIM.9R	GTCATCGTGATGCTGGGAAGTTTC	1		RTK, JVS, & ELB

[a] Use refers to the way in which we used primer: 1 is a primer we used for both PCR and sequencing, 2 indicates primers used primarily for sequencing but likely to work for PCR as well; type 3 primers were used for PCR to produce an initial product used as template in a nested or semi-nested PCR strategy.

[b] For primers published by our group, primer authors are provided if there are questions about the primers or locus.

[c] For this set of ALDOB primers we used a 10 s annealing time to limit amplification of a paralog.

[d] A gene map has been published in Chubb, 2004 and so is not shown in Fig. 1.

[e] A touchdown PCR was used for this locus, stepping down from 70° to 60°, see also Yuri et al. (2008).

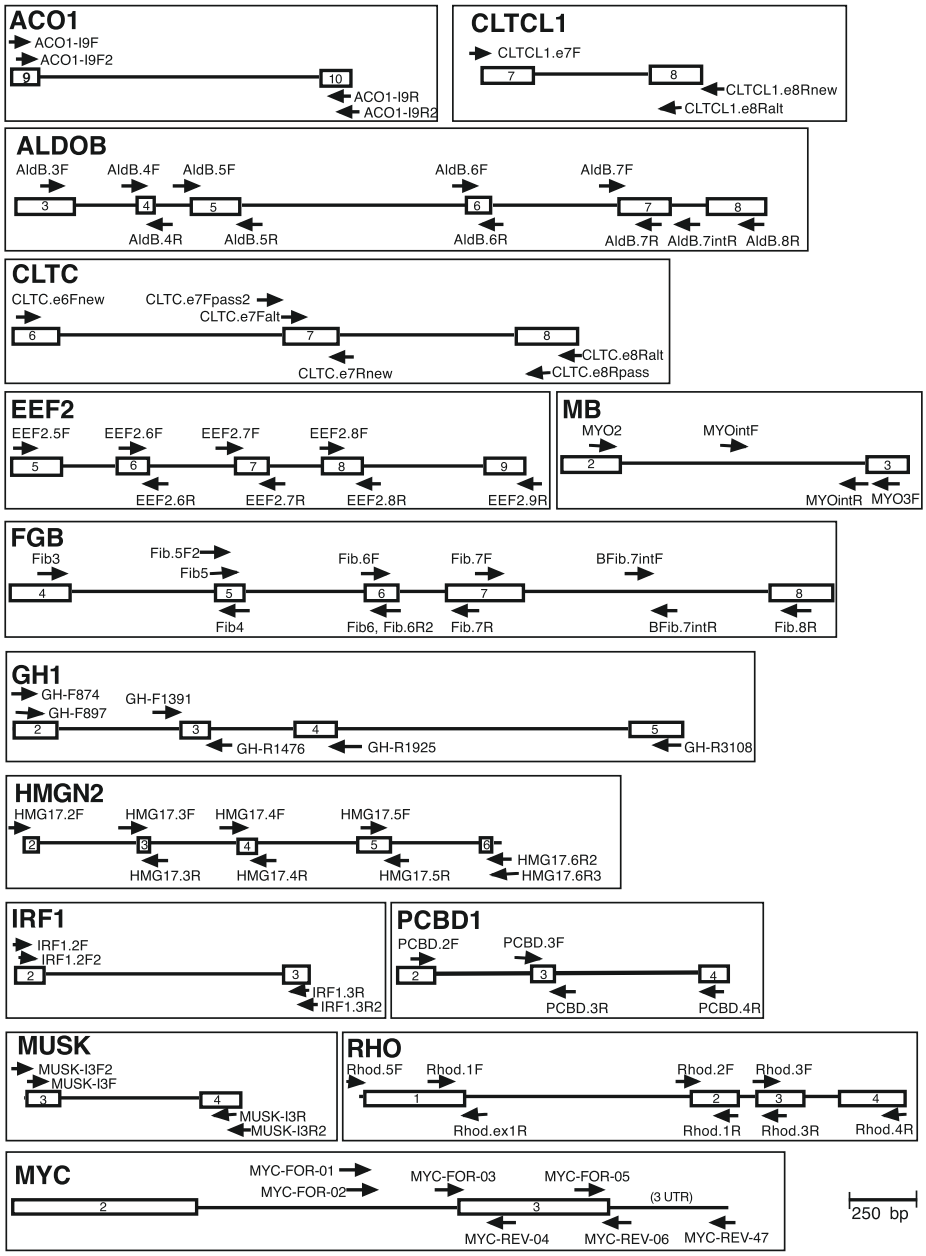

Fig. 2. Primer locations for regions where we include more than two primers, scaled to the same length. Exons are boxes, with the exon number inside. Primer arrows are not proportional to primer length though the arrow tip indicates the 3′ end of the primer. A single arrow was used when the 3′ end of two primers differed by less than 10 bp. For some longer introns, additional non-conserved primers (not shown) were used for sequencing.

3. Conclusions

Given the importance of comparing unlinked loci, resources such as the one we have developed here should have broad usefulness and a high probability of being used successfully. We hope future studies can build upon the sequences we have already collected in many avian taxa, and can contribute to a better understanding of avian evolution, ecology and conservation. In addition, we hope to stimulate the development of additional well-tested loci that can be added to the resource that we have begun. Most importantly, we hope that this resource stimulates other groups to compile similar easy to use resources in a single location for other taxonomic groups.

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Marjorie Barrick Museum (University of Nevada, Las Vegas), Museum of Southwestern Biology (University of New Mexico), Museum of Vertebrate Zoology (University of California, Berkeley), Museum Victoria, National Museum of Natural History, San Francisco Zoological Garden, and Zoological Museum University of Copenhagen.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2008.11.018.

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