*Defining Turtle Diversity: Proceedings of a Workshop on Genetics, Ethics, and Taxonomy of Freshwater Turtles and Tortoises*
*H. Bradley Shaffer, Nancy N. FitzSimmons, Arthur Georges, and Anders G.J. Rhodin, Eds.*
*Chelonian Research Monographs 4:124-141 • © 2007 by Chelonian Research Foundation*

# A Compendium of PCR Primers for mtDNA, Microsatellite, and Other Nuclear Loci for Freshwater Turtles and Tortoises

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**ABSTRACT. – Molecular markers have proven to be a powerful tool for research on turtles. In particular, the application of the polymerase chain reaction (PCR) has increased the availability of molecular technologies while decreasing the cost. However, the cost, time, and expertise associated with developing and testing primers for a particular species can still present a significant barrier, especially to researchers less experienced with molecular methods. In this paper we provide the primer sequence, genomic location, and taxa for 202 PCR primers spanning the entire mitochondrial genome. We also report primers for 11 nuclear coding genes and introns. Finally, we provide primer sequence, amplicon size, and number of observed alleles for 181 microsatellite loci from all major clades of living turtles. We hope that this nearly comprehensive compilation of freshwater turtle and tortoise PCR primers can reduce some of the initial difficulties for beginning turtle geneticists and further facilitate research in existing labs.**

**KEY WORDS. – Reptilia; Testudines; turtle; PCR; primer; mtDNA; nuclear DNA; microsatellite; STR**

The power and utility of genetic tools for the study of turtle biology and conservation is evidenced by the extensive and rapidly growing literature on the past, present, and future use of such molecular tools reviewed in this volume. The increasing availability and decreasing cost of molecular technologies, specifically the polymerase chain reaction (PCR), is making genetic analysis more accessible to researchers. However, the cost, time, and expertise associated with developing and testing primers for a particular species can still present a significant barrier, especially to researchers inexperienced with molecular methods. In this paper we hope to reduce some of the initial difficulties or frustration for turtle biologists by providing a thorough compilation of published (and some unpublished) information on PCR primers developed specifically for turtle studies.

We have organized our discussion of molecular markers into three categories: (1) mitochondrial DNA, (2) nuclear loci (including both protein-coding genes and introns), and (3) microsatellite loci (Simple Tandem Repeats). For each marker type we provide a brief description of its strengths and limitations, and the kind of study for which it may be most appropriate. In Tables 1 and 2 we list each primer's region (gene or locus), name (or names), primer sequence, original reference, and a selective (non-exhaustive) list of citations for studies that have used that primer. Because some primers have been used on multiple taxa, we have also included a list of species (when possible) or a summary of the major clades in which the primers have been successfully applied. For the mitochondrial primers (Table 1), we include the orientation and 5' primer position relative to the published *Chrysemys picta* mitochondrial genome (Mindell et al., 1999) along with a genomic map (Fig. 1A-E) to compare primer coverage and provide estimates of predicted product size of different primer combinations. Due to space limitations not all primers are depicted in the figure. We encourage readers to consult the figure to find primers in the region of interest and then reference the tables for a more complete listing of available primers in that region and taxa in which those have been used. Our summary of primers focuses on freshwater turtles and tortoises, and largely excludes the extensive literature on marine turtles. However, because of the demonstrated inter-species cross-amplification of many microsatellite loci, we have also included a non-exhaustive list of marine turtle primers. Also for the microsatellite markers, we have included an indication of the expected size and level of variation of the amplified product in the target species and a list of non-target species in which the locus has been tested and failed to amplify.

A paper of this nature (reporting a set of available primers) will already be out of date on the day it is published. This is unavoidable in a hard copy publication, but can be avoided by establishment of an open access database for turtle researchers to report their primers as they are devel-

oped, provided of course that researchers are willing to post their new primers or the application of existing primers to new species on the database. We have established such a database in a companion website for this publication, which can be accessed at http://www.csuchico.edu/biol/personnel/engstrom/turtleprimers.htm. We hope that compiling this information in a single reference will aid in the rapid diffusion of information on new, useful primers and new applications of existing primers. We hope that this will facilitate research and accelerate progress toward understanding the phylogeny and population genetics of turtles, by guiding researchers to molecular markers that will (1) be applicable their particular study animal, (2) harbor levels of variation appropriate to their question, and (3) be comparable to previous studies. However, to ensure that appropriate credit accrues to the researchers who have performed the hard work of developing markers, we remind anyone using primers listed in this publication or the companion website to cite the primary references for those primers or to contact those who developed previously unpublished primers for updated citation information. We reiterate that publications by the original developers of the molecular markers should be considered the primary references, NOT this summary report or its companion website.

## Mitochondrial DNA

Mitochondrial DNA (mtDNA) sequence data have been and continue to be particularly informative in both in phylogeography and in systematics (Hillis et al., 1996). The mitochondrial genome has a highly conserved gene content and gene order (Boore, 1999, but see Parham et al., 2006a,b), lacks introns, lacks significant recombination (Avise, 1994, 2004; Moore, 1995; Sunnucks, 2000), and is present in multiple copies per cell, thus rendering the acquisition and analysis of mtDNA sequence data relatively easy and straightforward compared with the more complex nuclear genome (see below). The overall rate of nucleotide substitution in the mitochondrial genome is relatively rapid (Brown et al., 1979), providing a rich source of variable characters. However, this organelle also offers a mix of fast evolving genes, useful for studies of recently diverged lineages (e.g., within species, among closely related species), and slowly-evolving genes suitable for studies of more ancient divergences (e.g., among genera or families [Graybeal, 1994]). Mitochondrial DNA has a small effective population size relative to the nuclear genome, resulting in a shorter average coalescent time (Moore, 1995), albeit with a high variance (Hudson and Turelli, 2003). This combination of attributes renders mtDNA useful for a wide variety of genetically based studies. However, as a maternally-inherited, single locus, mtDNA provides a somewhat limited perspective on the evolutionary and ecological history of a species. The demonstration of hybridization (Parham et al., 2001; Stuart and Parham, 2007; Spinks et al., unpubl. data) and potential differences in male and female behavior (FitzSimmons et al., 1997), for example, may often require nuclear data to test mtDNA-based hypotheses. Thus, while mtDNA has provided and will continue to provide an invaluable tool, it is also important to identify independent markers that complement those in the mitochondrial genome.

The 202 turtle mtDNA primers listed in Table 1 have been used to amplify and sequence all regions of the turtle mtDNA genome, including all 13 protein-coding genes, 22 tRNAs, 2 rRNAs, and the control region/d-loop. The most frequently used genes in deep phylogenetic studies are the slowly evolving 12s rRNA (e.g., Shaffer et al., 1997), and moderately evolving Cytochrome *b* (Cytb) (e.g., Shaffer et al., 1997; Spinks et al., 2004). Cytb, NADH 4 (also commonly abbreviated ND4) and other protein coding genes have been most useful for studies among closely related species (e.g., Caccone et al., 1999a,b; Engstrom et al., 2002; Feldman and Parham, 2002) or for phylogeographic studies within species (e.g., Starkey et al., 2003; Spinks and Shaffer, 2005). The control region is widely used in population and intraspecific level studies because of its high rate of mutation (Stewart and Baker, 1994; Starkey et al., 2003; Pearse et al., 2006); however, some studies have noted equal or greater levels of variation in protein coding genes (Spinks and Shaffer, 2005).

## Nuclear Loci

Because of the recognized limitations of mtDNA, increased attention is being paid to the nuclear genome as an additional, independent source of data for phylogenetic, phylogeographic, and population genetic analyses (e.g., Bruford and Wayne, 1993; Groth and Barrowclough, 1999; Hare, 2001). The three sources of nuclear data most commonly used include size polymorphisms at microsatellite loci (discussed below), and sequence data from nuclear protein-coding genes and introns. In contrast to mtDNA, nuclear protein-coding genes and introns tend to evolve more slowly (Prychitko and Moore, 1997, 2000; Groth and Barrowclough, 1999; Birks and Edwards, 2002; Caccone et al., 2004; Engstrom et al., 2004; Fujita et al., 2004), making them less prone to excessive homoplasy—a common problem among mitochondrial genes over deeper divergences. Nuclear introns have the further advantage of being free from many of the evolutionary constraints imposed on protein-coding sequences, resulting in little base compositional bias, relatively low transition/transversion ratio, and little among-site rate heterogeneity (Armstrong et al., 2001; Prychitko and Moore, 2003; Fujita et al., 2004). One disadvantage of nuclear DNA is that the slow rate of evolution, which minimizes homoplasy on long timescales, can also reduce variation on shorter timescales (Birks and Edwards, 2002). This characteristic can limit its utility in phylogeographic and population genetic studies of turtles (Spinks and Shaffer, 2005).

Because they can be more difficult to develop compared with mtDNA loci, relatively fewer primers have been de-

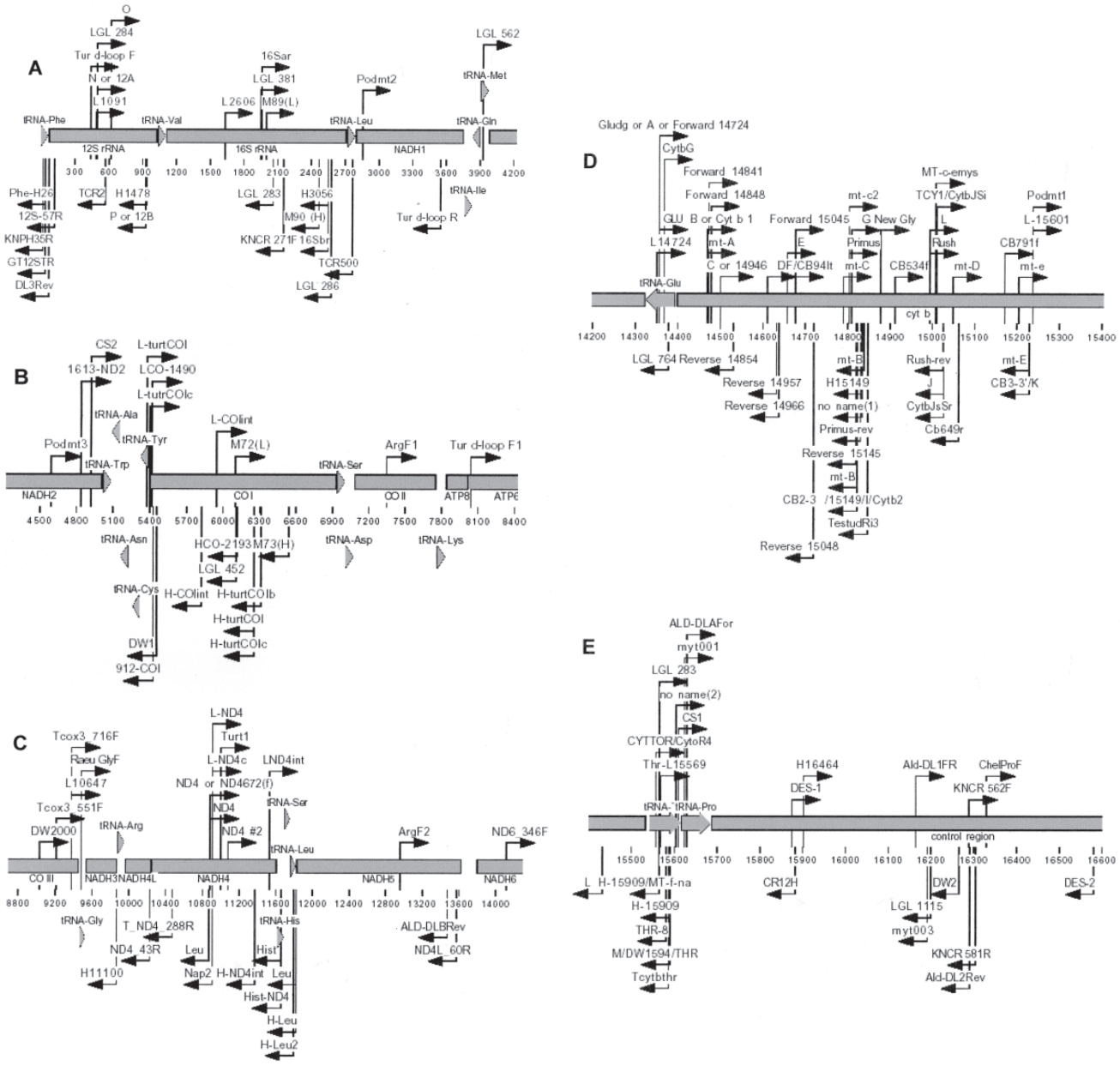


**Figure 1**. The five panels depict map of 5' position and orientation of turtle primers listed within Table 1 relative to the sense strand (L) and loci of the *Chrysemys picta* mitochondrial genome (GenBank accession AF069423).

scribed for nuclear protein coding genes and introns. In Table 2 we list primers for 6 introns and 3 protein-coding genes. Intron sequence has shown great utility in interspecific phylogenetics (Engstrom et al., 2004; Fujita et al., 2004), but due to their lack of functional constraint they can be difficult to align across deep phylogenies (Fujita et al., 2004; but see Loytynoja and Goldman, 2005) Protein-coding genes have proven useful in interspecific phylogenies at many levels (Georges et al., 1998), and will be crucial in testing the location of the root of the turtle tree (Krenz et al., 2005; Near et al., 2005) and in understanding the placement of turtles relative to other amniotes (Hedges and Poling, 1999). Because nuclear introns and protein coding genes are bi-parentally inherited, detection of heterozygotes is a useful tool in the identification of interspecific hybrids (Stuart and Parham, 2007; Spinks, unpubl. data). Another less-explored source of nuclear gene data is the rapidly growing field of developmental genetics. Many genes have been cloned from complementary DNA (cDNA) libraries constructed for studies of sex determination (e.g., Valenzuela et al., 2006), morphological development and gene expression (Chien et al., 2005, 2006) and chromosome evolution (Kuraku et al., 2005, 2006; Matsuda et al., 2005). Complimentary DNA is synthesized using the enzyme *reverse transcriptase* to make DNA copies of all of the mature mRNA transcribed in a tissue sample. Although primers for detection of genes identified in these cDNA libraries have been published, we have decided not to include this exten-

**Table 1.** Primers currently available for amplification of mitochondrial loci of tortoise and freshwater turtles. Each primer is listed by locus, strand orientation (O*) (H = heavy, L = light), and 5' position relative to the *Chrysemys picta* mitochondrial genome (GenBank accession AF069423) except in cases where the primer does not align with *Chrysemys*, in which case the primer is aligned either with mitochondrial genomes of *Dogania subplana* (NC002780) and indicated with a "D" or *Pelomedusa subrufa* (AF039066) and indicated with a "P". No location is given for several primers designed for amplification of the control region in kinosternid turtles, which did not align well with other turtle genomes. Groups of taxa successfully amplified and associated references are listed in the final columns. Contact information for unpublished primer sequences: TNE (tengstrom@csuchico.edu), NNF (Nancy.FitzSimmons@canberra.edu.au), MRJF (mf@txstate.edu). Key to taxa: CR = Suborder Cryptodira, Chely = Family Chelydridae, TE = Superfamily Testudinoidea, Test = Family Testudinidae, Geo = Family Geoemydidae, Emy = Family Emydidae, TR = Superfamily Trionychoidea, Car = Family Carettochelyidae, Trio = Family Trionychidae, K = Superfamily Kinosternoidea, Derma = Family Dermatemydidae, Kino = Family Kinosternidae, Platy = Family Platysternidae, C = Superfamily Chelonioidea, Chelo = Family Cheloniidae, Dermo = Family Dermochelyidae, PL = Suborder Pleurodira, Cheli = Family Chelidae, P = Superfamily Pelomedusoidea, Pelo = Family Pelomedusidae, Podo = Family Podocnemididae.

| Primer Location | O* | Pos.+ | Primer Name | Primer Sequence (5'-3') | Orig. Ref. | Taxa | References Citing Primer |
|---|---|---|---|---|---|---|---|
| tRNA-Phe | L | 19 | L1 | AAAGCACGGCACTGAAGATGC | 135 | Geo | |
| tRNA-Phe | H | 28 | KNPH 35R | GCCGTGCTTTGATATAAGCT | 148 | Kino | |
| tRNA-Phe | H | 50 | GT12STR | ATCTTGGCAACTTCAGTGCC | 28 | Test | 23, 27 |
| tRNA-Phe | H | 50 | Phe-H26 | TACCCATCTTGGCAACTTCAGTGCC | 119 | Test | |
| 12S rRNA | H | 78 | DL3Rev | AATATTTGAGTTGTCGTGGG | 15 | Test | |
| 12S rRNA | H | 128 | 12S-57R | GATACTTGCATGTGTAAGTTT | 148 | Kino | |
| 12S rRNA | H | 143 | H10 | TTCACTGGTTATGCAGATACTT | 135 | Geo | |
| 12S rRNA | L | 497 | N/12SA | AAACTGGGATTAGATACCCCACTAT | 120 | TE | 150, 154 |
| 12S rRNA | L | 491 | L1091 | AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT | 82 | Geo, Test, Cheli | 4, 25, 27, 74, 75, 90, 98, 118, 147, 163, 164, 172 |
| 12S rRNA | L | 501 | LGL 284 | TGGGATTAGATACCCCACTAT | 33 | Test | 114 |
| 12S rRNA | L | 508 | 12SXLF | GATTAGATACCCCACTATGCTTAG | 153 | Geo | |
| 12S rRNA | H | 582 | TCR2 | GCTCGTAGTTCTCTGGCGG | 113 | Podo | 151 |
| 12S rRNA | L | 626 | O | CCTAGAGGAGCCTGTTC | 150 | TNE | |
| 12S rRNA | H | 939 | P/12SB | GAGGGTGACGGGCGGTGTGT | 120 | TE | 150, 154 |
| 12S rRNA | H | 947 | H1478 | TGACTGCAGAGGGTGACGGGCGGTGTGT | 82 | Geo, Test, Cheli | 4, 25, 27, 74, 75, 118, 147, 163, 164, 172 |
| 12S rRNA | L | 1058 | L2 | AAAGCATTCAGCTTACACCTGA | 135 | Geo | |
| 16S rRNA | H | 1255 | H1 | TTTCATCTTTCCTTGCGGTAC | 135 | Geo | |
| 16S rRNA | L | 1639 | L2606 | GGCCTAAAAGCAGCCACCTGTAAAGACAGCGT | 70 | Geo | 74, 75 |
| 16S rRNA | L | 1954 | LGL 381 | ACCCCGCCTGTTTACCAAAAACAT | 16 | Emy | |
| 16S rRNA | L | 1958 | 16Sar/AR | CGCCTGTTTATCAAAAACAT | 120 | Test | 27, 28, 118 |
| 16S rRNA | L | 2002 | M89 (L) | AGGAGTGATGCCTGCCCAGTGAC | 63 | PL | |
| 16S rRNA | H | 2073 | LGL 283 | TGATTATGCTACCTTTGCACRGT | 33 | Test | 114 |
| 16S rRNA | L | 2124 | L3 | GTCTCTTACAAATAATCAGTGA | 135 | Geo | |
| 16S rRNA | H | 2207 | H2 | AAGTTCCACAGGGTCTTCTCG | 135 | Geo | |
| 16S rRNA | H | 2485 | M90 (H) | CCTTAATAGCGGCTGCACCATTAGGA | 63 | PL | |
| 16S rRNA | H | 2560 | 16Sbr/BR | CCGGTCTGAACTCAGATCACGT | 120 | Test | 27, 28, 90, 118 |
| 16S rRNA | H | 2562 | H3056 | CTCCGGTCTGAACTCAGATCACGTAGG | 70 | Geo | 74, 75 |
| 16S rRNA | H | 2589 | LGL 286 | AGATAGAAACCGACCTGGAT | 16 | Emy | |
| ND1 | L | P2457 | Podmt2 | TTGCTGTAGAATCTGACATCC | 151 | Podo | |
| ND1 | H | P3549 | Tur d-loop R | GGAAGTGTATATGAAACCTGGGT | 174 | Pelo | |
| ND1 | L | 2899 | ND1F | GGMTAYATACAACTTCGAAAAGG | 153 | Geo | |
| ND1 | L | 3169 | L11 | TCCGGTTGAGCTTCAAACTC | 135 | Geo | |
| ND1 | H | 3340 | H3 | ACTATTCCTGCTCAGGCTCCG | 135 | Geo | |
| ND1 | H | 3829 | ND1R | GGTTTTAGCCTCTATTATTCACCC | 153 | Geo | |
| tRNA-Met | L | 3928 | LGL 562 | TAAGCTATCGGGCCCATACC | 114 | Test | |
| ND2 | L | 4374 | L4 | ACCTGACAAAAACTAGCCCCA | 135 | Geo | |
| ND2 | H | 4506 | H11 | GTAGTTGGGTTTGGTTTAGTCC | 135 | Geo | |
| ND2 | L | 4842 | 1613-ND2 | CTAAGCCTATTCTTCTA | 149 | Emy | |
| ND2 | L | 4923 | CS2 | GGACGCCATAACACAAT | 167 | Chely | |
| ND2 | H | 5084 | ND2R | GAGGTTCTATCTCTTGTTTGGGGC | 153 | Geo | |
| tRNA-Tyr | L | 5379 | L-turtCOI | ACTCAGCCATCTTACCTGTGATT | 157 | Geo | 24, 126, 153 |
| tRNA-Tyr | L | 5396 | L-turtCOIc | TACCTGTGATTTTAACCCGTTGAT | 157 | Geo | 24, 126, 153 |
| CO I | L | 5420 | L5 | THTTCTCYACTAACCATAAAG | 135 | Geo | |
| CO I | L | 5424 | LCO-1490 | GGTCAACAATCATAAAGATATTGG | 51 | Geo | 45, 125 |
| CO I | H | 5436 | 912-COI | GTGGTTGGTTGAGAATAATCA | 149 | Emy | |
| CO I | H | 5486 | H4 | ACTATTCCTGCTCAGGCTCCG | 135 | Geo | |
| CO I | H | 5839 | H-COIint | TAGTTAGGTCTACAGAGGCGC | 157 | Geo | 24, 126 |
| CO I | L | 5956 | L-COIint | TGATCAGTACTTATCACAGCCG | 157 | Geo | 24, 126 |
| CO I | L | 6106 | M72 (L) | TGATTCTTCGGTCACCCAGAAGTGTA | 63 | PL | |
| CO I | H | 6128 | LGL 452 | ACTTCAGGGTGCCCAAAGAATCA | 114 | Test | |
| CO I | H | 6131 | HCO-2193 | TAAACTTCAGGGTGACCAAAAAATCA | 51 | Geo | 45, 125 |
| CO I | H | 6265 | H-turtCOI | CCCATACGATGAAGCCTAAGAA | 157 | Geo | 24, 126 |
| CO I | H | 6272 | H-turtCOIc | TGGTGGGCTCATACAATAAAGC | 157 | Geo | 24, 126 |
| CO I | H | 6326 | H-turtCOIb | GTTGCAGATGTAAAATAGGCTCG | 157 | Geo | 24, 126 |
| CO I | L | 6337 | L12 | CTCATCCCCAACAGGAGTAAAA | 135 | Geo | |
| CO I | H | 6551 | M73 (H) | CCTATTGATAGGACGTAGTGGAAGTG | 63 | PL | |
| CO I | H | 6579 | H5 | AAATCYTGCTATGATGGCGAA | 135 | Geo | |
| CO II | L | 7594 | L6 | AAACAGACGCARTCCCAGGCAC | 135 | Geo | |
| CO II | H | 7795 | H12 | GTCATCCTGTTTAGCTTCTCTAG | 135 | Geo | |
| ATPase 8 | L | 8659 | L13 | GCCTCTACCTACAAGAAAC | 135 | Geo | |
| ATPase 6 | H | 8766 | H6 | GTTATTAGTAGTTGCTGCTGTGC | 135 | Geo | |
| CO III | L | 9038 | DW 2000 | ACAGGCGTAATCCTACTAA | 168 | Trio | |
| CO III | L | 9209 | TCox3_551F | CTACAAGCCATAGAGTATTACGAAGC | TNE | Trio, Geo | |
| CO III | L | 9379 | TCox3_716F | CTTTGGGTTTGAAGCAGCTGC | TNE | Trio, Geo | |
| CO III | L | 9386 | L10647 | TTYGAAGCMGCMGCMTGATACTG | 106 | Emy | 107 |
| tRNAGly | L | 9481 | New Gly | ATAAGTACAATGMYTTCCA | 5 | Test | 20 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| tRNA-Gly | L | 9482 | Raeu GlyF | CCAATACAAATGACTTCCAATC | TNE | Trio | |
| tRNA-Gly | L | 9483 | TGlyF1 | TAGTAYAARTGACTTCCAATCA | TNE | Trio, Geo | |
| tRNA-Gly | L | 9485 | L7 | AGTACAAATGACTTCCAATCA | 135 | Geo | |
| tRNA-Gly | L | 9492 | TGlyF2 | TGACTTCCAATCAYTMAGTTT | TNE | Trio, Geo | |
| NADH3 | H | 9717 | H13 | GAAGAATCGAATTGAGAATGG | 135 | Geo | |
| NADH3 | H | 9884 | H11100 | TCTGCYCAYTCTARKCCTCCYTG | 106 | Emy | 107 |
| tRNA-Arg | L | D9924 | ArgF1 | GATTGATAAAACATGGTTACCC | TNE | Trio | |
| tRNA-Arg | L | 9929 | ArgF2 | TAAAACATGGTTACCCTATGACACC | TNE | Trio | |
| NADH4 | H | D10286 | Raeu ND4-42R | GTATCATATGTGTGTTGGTTTGG | TNE | Trio | |
| NADH4 | H | 10239 | ND4_43R | GGTTTAGGTTTTGTAGGTGGCTTG | TNE | Geo | |
| NADH4 | H | 10483 | T_ND4_288R | TAGGATTATTAGTGGAGTAAGTCAGC | TNE | Trio, Geo | |
| NADH4 | L | 10508 | L15 | GAACCCCTATCACGAAAACG | 135 | Geo | |
| NADH4 | H | 10677 | H7 | TTTGATTWCCTCATCGTGTGTG | 135 | Geo | |
| NADH4 | L | 10886 | ND4 | CACCTATGACTACCAAAAGCTCATGTAGAAGC | 5 | Emy, Geo, Test | 20, 41, 45, 46 |
| NADH4 | L | 10892 | ND4/ ND4_672(f) | TGACTACCAAAAGCTCATGTACAAGC | 43 | Emy, Trio | 42, 44, 152 |
| NADH4 | L | 10910 | L-ND4 | GTAGAAGCCCCAATCGCAG | 157 | Geo | 24, 126, 153 |
| NADH4 | L | 10918 | L-ND4c | CCAATCGCAGGATCAATAATC | 157 | Geo | 24, 126 |
| NADH4 | H | 10921 | Nap2 | TGGAGCTTCTACGTGRGCTTT | 5 | Test | 20 |
| NADH4 | L | 11000 | Turt1 | GATCCTCTATCAAAAACACT | MRJF | | |
| NADH4 | L | 11079 | ND4 #2 | TACGACAAACAGACCTAAAATC | 5 | Test | 96 |
| NADH4 | H | 11389 | H-ND4int | GGTTAGCTCTCCTATTAGGTTGAT | 157 | Geo | 24, 126 |
| NADH4 | L | 11534 | L-ND4int | ACCCATACACGAGAACATCTACT | 157 | Geo | 24, 126 |
| tRNA-His | H | 11674 | Hist-ND4 | CCTATTTTAGAGCCACAGTCTAATG | 43 | Trio | 42 |
| tRNA-His | H | 11675 | Hist | CCTATTTTTAGAGCCACAGTCTAATG | 44 | Trio | |
| tRNA-Leu | L | 11772 | L8 | AGGATAGAAGTAATCCAATGG | 135 | Geo | |
| tRNA-Leu | L | 11775 | LGL 763 | AATAGTTTATCCRTTGGTCTTAGG | 34 | Test | 114 |
| tRNA-Leu | H | 11821 | H-Leu2 | ATTTGCACCAAGGGTTAATGG | 157 | Geo | 24, 126 |
| tRNA-Leu | H | 11836 | H-Leu | ATTACTTTTACTTGGATTTGCACCA | 157 | Geo | 24, 126, 153 |
| tRNA-Leu | H | 11837 | Leu | CATTACTTTTACTTGGATTTGCACCA | 5 | Geo, Test | 20, 45, 46, 96, 125 |
| NADH5 | L | P11901 | Podmt3 | TCACAGACATAACCATAAGCAC | 151 | Podo | |
| NADH5 | H | 11956 | H15 | GCTGTTTTTACGGCTGTTTTTTG | 135 | Geo | |
| NADH5 | L | 12454 | ND5_619F* | ACCACGTTTAGGTTCATTTTCATTAC | 45 | Emy | *“Leu” in 45 |
| NADH5 | L | 12812 | L16 | CATACACGCCTTCTTTAAAGC | 135 | Geo | |
| NADH5 | H | 12899 | H8 | TATCTTTCGAATTGCTTGTTC | 135 | Geo | |
| NADH5 | H | 13488 | ALD-DLBRev | ACGATGTGCAGTGGGAGTGGTTG | 119 | Test | |
| NADH5 | H | 13590 | ND5_1755R | AGATTAAGGAGATTCGGTGGAG | TNE | Trio | |
| NADH6 | L | 14118 | ND6 346F | GAATAAGCAAAAACCACTAACATACCCCC | 44 | Trio | |
| tRNA-Glu | L | 14349 | L14724 | CGAAGCTTGATATGAAAAACCATCGTTG | 105 | Emy, Test, Geo | 4, 76, 87, 88, 104, 114 |
| tRNA-Glu | L | 14358 | GLU | TGACATGAAAAAYCAYCGTTG | 116 | Test | 25, 118 |
| tRNA-Glu | L | 14358 | Gludg/GLUDGE/A/ Forward 14724 | TGACTTGAARAACCAYCGTTG | 120 | CR,PL | 7-9, 27, 28, 44, 45, 89, 118, 150, 154 |
| tRNA-Glu | L | 14368 | CytbG | AACCATCGTTGTWATCAACTAC | 154 | Emy, Geo, Test | 36, 77, 90, 103, 153 |
| tRNA-Glu | L | 14369 | L9 | AACCACCGTTCTATTCAACTA | 135 | Geo | |
| tRNA-Glu | L | 14370 | L14735t | CCATCGTTGTAATCAACTAC | 76 | Geo | |
| tRNA-Glu | H | 14381 | LGL 764 | TTACAACGATGGTTTTTCATRTCA | 34 | Test | 114 |
| Cyt b | L | 14462 | MT-a | CTCCCAGCCCCATCCAACATCTCAGCATGATGAAAC | 60 | Test | |
| Cyt b | | 14462 | mt-a-neu | CTCCCAGCCCCATCCAACATCTCAGCATGATGAAACTTCG | 56 | Geo | |
| Cyt b | L | 14462 | L14841 | AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA | 82 | Emy, Chely | 1, 78, 138 |
| Cyt b | L | 14471 | B/Cyt b 1 | CCATCCAACATCTCAGCATGATGAAA | 120 | TE | 1, 94, 150 |
| Cyt b | L | 14473 | Forward 14841 | ATCCAACATCTCAGCATGATGAAA | 8 | Test | 7, 9 |
| Cyt b | L | 14476 | mt-A | CAACATCTCAGCATGATGAAACTTCG | 93 | Geo, Emy | 1, 13, 14, 91, 92, 144 |
| Cyt b | L | 14478 | Forward 14848 | CATCTCAGCATGATGAAACTTCGGA | 8 | Test | 7, 9 |
| Cyt b | H | 14532 | Reverse 14854 | TGTAGGATTAAGCAGATGCCTAGT | 8 | Test | 7, 9 |
| Cyt b | H | 14513 | H16 | CTAATAGTGATCCGAAGTTTCAT | 135 | Geo | |
| Cyt b | L | 14581 | C / 14946 | ACTAGCATTCTCATCAGTAG | 150 | Test | 7-9 |
| Cyt b | L | 14612 | D | CGAGATGTTAATAACGGCTG | 150 | TE | |
| Cyt b | H | 14635 | Reverse 14957 | AAGTCATCCGTATTGTACGTCTCG | 8 | Test | 7, 9 |
| Cyt b | H | 14641 | Reverse 14966 | TCGGATAAGTCACCCGTACTG | 8 | Test | 7, 9 |
| Cyt b | L | 14658 | E | GCGCCTCATTCTTCTTTATCT | 150 | TE | |
| Cyt b | L | 14678 | Forward 15045 | TGCATTTACCTCCAYATYGGCCG | 8 | Test | 7, 9 |
| Cyt b | L | 14678 | “F”/CB94lt | TGCATCTACCTTCACATYGGMCG | 150 | TE | 44 |
| Cyt b | H | 14723 | Reverse 15048 | GGTAAGAGCCGTARTAAAGTC | 8 | Test | 7, 9 |
| Cyt b | L | 14792 | mt-C | TAYGTCCTACCATGAGGACAAATATCATTCTGAGG | 170 | Emy, Geo | 11, 66, 91, 171 |
| Cyt b | L | 14804 | Primus | TGAGGCCAAATATCCTTCTGAGGTGCAACCG | 45 | Emy | |
| Cyt b | L | 14805 | mt-c2 | GAGGACAAATATCATTCTGAGG | 13 | Geo | |
| Cyt b | L | 14804 | G | TGAGGACAAATATCATTCTGAGGGGCTGCAG | 150 | TE | |
| Cyt b | H | 14824 | Reverse 15145 | TCAGAATGATATTTGTCCCCATGGT | 8 | Test | 7, 9 |
| Cyt b | H | 14827 | mt-B | ACCTCAAAAGGATATTTGTCCTCA | 14 | Geo | |
| Cyt b | H | 14827 | CB2-3'/15149/”I”/ Cytb2 | CCCTCAGAATGATATTTGTCCTCA | 121 | Emy, Test, Trio | 9, 14, 27, 28, 44, 89, 94, 150 |
| Cyt b | H | 14834 | Primus-rev | CGGTTGCACCTCAGAAGGATATTTGGCCTCA | 45 | Emy | |
| Cyt b | H | 14836 | no name(1) | AACTGCAGCCCCTCAGAATGATATTTGTCCTCA | 138 | Chey | |
| Cyt b | H | 14837 | H15149 | AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA | 82 | Emy, Test | 4, 14, 78, 87, 88 |
| Cyt b | H | 14837 | H15149 | TAACTGTAGCCCCTCAGAATGATATTTGTCCTCA | 76 | Geo | |
| Cyt b | H | 14843 | mt-B | TTGTGATTACTGTAGCACCTCAAAATGATATTTGTCCTCA | 170 | Emy, Geo | 14, 66, 91, 171 |
| Cyt b | H | 14850 | TestudRi3 | AGTAGGTTGGTGATGACAGTGGC | 13 | Geo | |
| Cyt b | H | 14852 | H15197 | CCGATATAAGGGATTGCTGA | 76 | Geo | |
| Cyt b | L | 14912 | CB534f | GACAATGCAACCCTAACACG | 44 | Trio | |
| Cyt b | L | 14995 | Rush | TTCCTACATGAAACCGGATCAAACAACCCAAA | 45 | Emy | |
| Cyt b | L | 14996 | H | TTCCTWCACGAAACAGGNTCAAACAA | 150 | Test | |
| Cyt b | L | 15009 | MT-c-emys | CCGGATCAAACAAYCCAACAGG | 60 | Test | |
| Cyt b | L | 15011 | TCR1/CytbJSi | GGATCAAACAACCCAACAGG | 113 | Emy, Geo, Test, Podo | 14, 36, 77, 103, 151, 154 |
| Cyt b | H | 15027 | Rush-rev | GTTGGGTTGTTTGATCCGGTTTCATGTAGAAA | 45 | Emy | |
| Cyt b | H | 15030 | J | CCTGTTGGGTTYTTTGAKCC | 150 | TE | |
| Cyt b | H | 15030 | CytbJsSr | CCTGTTGGGTTGTTTGATCC | 154 | Emy, Geo, Test | 36, 77, 103 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Cyt b | L | 15050 | mt-D | AAAATCCCATTCCACCCCTACTACTCCACAAAAGA | 170 | Emy, Geo | 66, 91, 154, 171 |
| Cyt b | H | 15066 | CB649r | GGGTGGAATGGGATTTTGTC | 44 | Trio | |
| Cyt b | L | 15089 | Mau-F | CTAGGCCTCATCTTAATACT | 56 | Geo | |
| CytB | H | 15149 | Ri-neu | GTGAAGTTGTCTGGGTCTCCTAG | 56 | Geo | |
| Cyt b | L | 15171 | CB791f | CACCMGCYAACCCACTATC | 44 | Trio | |
| Cyt b | L | 15206 | mt-e | AAACCAGAATGATACTTCCTATTTGC | 13 | Geo | |
| Cyt b | H | 15231 | mt-E | GCAAATAGGAAGTATCATTCTGG | 13 | Geo, Test | |
| Cyt b | H | 15232 | CB3-3'/"K" | GGCAAATAGGAARTATCATTC | 121 | Trio | 150 |
| Cyt b | L | 15237 | L-15601 | CCATTCTACGCTCAATCCC | 91 | Emy | 57-59, 91 |
| Cyt b | L | 15237 | Podmt1 | CAATGCTGCGATCCATCC | 151 | Podo | |
| Cyt b | H | 15435 | L | TCTTCTACTGGTTGTCCTCCGATTCA | 150 | TE | |
| Cyt b | L | 15457 | L14 | AGCAGCCTCCATCCTTTTACTT | 135 | Geo | |
| tRNA-Thr | L | 15557 | CYTTOR/ CytoR4 | GCTTAACTAAAGCACCGGTCTTG | 28 | Test | 15, 23, 91 |
| tRNA-Thr | L | 15567 | LGL 283 | TACACTGGTCTTGTAAACC | 87, 91 | Emy | 89, 91, 114 |
| tRNA-Thr | L | 15569 | Thr-L15569 | CATTGGTCTTGTAAACCAAAGACTG | 119 | Test | |
| tRNA-Thr | H | 15569 | H-15909/MT-f-na | AGGGTGGAGTCTTCAGTTTTTGGTTTACAAGACCAATG | 91 | Emy, Geo, Test | 56-60, 91, 144 |
| tRNA-Thr | H | 15585 | THR-8 | GGTTTACAAGACCAATGCTT | 154 | Emy, Geo, Test | 36, 77, 91, 103, 153 |
| tRNA-Thr | H | 15591 | Tcytbthr | TTCTTTGGTTTACAAGACC | 44 | Trio | |
| tRNA-Thr | H | 15593 | H-15909 | CAGTTTTTGGTTTACAAGACCAATG | 14 | Geo | |
| tRNA-Thr | H | 15593 | "M"/DW1594/THR | TCATCTTCGGTTTACAAGAC | 150 | Emy, Geo, Test, Trio | 36, 45, 77, 91, 103, 154, 168 |
| tRNA-Thr | L | 15565 | TCRThr | AAAGCAYTGGTCTTGTAAACC | TNE | Chelo, Podo, Trio | |
| tRNA-Thr | L | 15573 | PounCRThr | GGTCTTGTAAACCAAAAACTG | TNE | Podo | |
| tRNA-Thr | H | 15593 | H9 | CAATCTTTGGTTTACAAGACC | 135 | Geo | |
| tRNA-Thr | L | 15605 | no name(2) | TCTTCCTAGAATAATCAAAAG | 139 | Chely | 138 |
| tRNA-Thr | L | 15609 | CS1 | CTAGAATAATCAAAAGAGAAGG | 167 | Chely | |
| tRNA-Pro | L | 15624 | myt001 | GAGAAAGACTTAAACCTTC | 164 | Test | |
| tRNA-Pro | L | 15629 | ALD-DLAFor | AGACTCAAACCCTCATCTCCGG | 119 | Test | |
| tRNA-Pro | H | | DW1 | CCCTTTGATAAAAGATACGGATCTTACGGC | 165 | Kino | 166 |
| Control | L | 15863 | Tur d-loop F | GGCTATGTACGTCGTGCATTCAT | 174 | Pelo | |
| Control | L | 15876 | DES-1 | GCATTCATCTATTTTCCGTTAGCA | 155 | Emy | 152 |
| Control | L | 15780 | MS1F | CAAGGGTGGATCGGGCATAAC | 54 | Emy | |
| Control | H | 15884 | CR12H | ATGAATGTACAATTATACATA | 93 | Emy, Geo | 66, 91, 92, 164, 171 |
| Control | H | 15884 | CR12H | ATGAATGTACAATTATACAT | 92 | Emy | |
| Control | L | 15902 | H16464 | CTTACTAACAAGGTTGCTAATT | 105 | Test | 114 |
| Control | L | P15949 | Tur d-loop F1 | TCTTCAGGATACCTCTGGCTGTT | 174 | Pelo | |
| Control | L | 16048 | KNCR 271F | ATCGTTATACATGGTTATCTATT | 148 | Kino | |
| Control | L | 16088 | EbF1 | CGAGARATAAGCAACCCTTGT | 2 | Emydoidea | |
| Control | L | 16163 | L10 | AACTGATTTATTCTGGCCTCT | 135 | Geo | |
| Control | L | 16166 | Ald-DL1FR | GATCTATTCTGGCCTCTGG | 119 | Test | |
| Control | H | 16176 | TCR500 | CCCTGAAGAAAGAACCGAGGCC | 44 | Podo, Trio, Chelo | |
| Control | H | 16188 | MS1R | GTGCCTGAAAAAACAACCACAGG | 54 | Emy | |
| Control | H | 16194 | myt003 | GACAAAACAACCAAAGGCCAG | 164 | Test | |
| Control | H | 16202 | LGL 1115 | ATGACCCTGAAGAAAGAACCAG | 87 | Emy, Chely | 89, 104, 114, 138, 139, 164 |
| Control | H | 16237 | PounCR500 | GAACCAGAGGCCTCTTAAAAAG | TNE | Podo | |
| Control | H | 16269 | DW2 | GATTAATAGTCTAGAACTTACTGACCAAAGGC | 165 | Kino | 166 |
| Control | L | 16288 | KNCR 562F | GGTCTTACTTGCATATCGTAG | 148 | Kino | |
| Control | H | 16294 | Ald-DL2Rev | TAAAAGCGCAATATGCCAGG | 119 | Test | |
| Control | H | 16308 | KNCR 581R | CTACGATATGCAAGTAAGACC | 148 | Kino | |
| Control | L | 16332 | ChelProF | CCGGTCCCCAAAACCGGAAC | 3 | Kino | 148 |
| Control | H | 16374 | H14 | CAGTCTTCATTGAGTTGGCAG | 135 | Geo | |
| Control | H | 16583 | EbR1 | ATTTAGGGGTTGYCGAGA | 2 | Emydoidea | |
| Control | H | 16585 | DES-2 | GGATTTAGGGGTTTGACGAGAAT | 155 | Emy | 152, 153 |

sive list of potentially very useful loci in this review because these primers have not been tried on genomic DNA, and cDNA cloning techniques are not as accessible to many molecular biologists. However, we strongly encourage readers to consult the original references and explore the utility of this rich source of phylogenetically informative genetic loci.

## Microsatellite Simple Tandem Repeat (STR) Loci

Microsatellites have become popular genetic markers for determining population structure and revealing differentiation among populations and individuals (Bruford and Wayne, 1993). Microsatellites, or simple tandem repeats (STRs), are non-coding repetitive DNA sequences composed of a variable number of tandemly repeating motifs. On average, STRs have mutation rates between $10^{-2}$ and $10^{-5}$ per gamete per generation (Page and Holmes, 1998) and thus can provide the resolution to differentiate individuals and populations, even within small geographic areas. Microsatellites are bi-parentally inherited (unless associated with a sex chromosome) and co-dominant, thereby allowing both alleles at a locus to be identified in heterozygotes. Microsatellites are generally considered selectively neutral (but see McGaugh et al., this volume) and their simple Mendelian transmission makes them useful for assessing genetic diversity. In freshwater turtles and tortoises, microsatellites have been used in studies of population genetics (e.g., Ciofi et al., 2002; Kuo and Janzen, 2004), conservation genetics (e.g., Sites et al., 1999; Cunningham et al., 2002; Pearse et al., 2006), as well as paternity and mating systems (e.g., Valenzuela, 2000; Roques et al., 2006; Pearse et al., in press). In addition, STRs are well-suited to address future concerns in turtle biology such as interspecies hybridization (Roy et al., 1994, 1996; Williams et al., 2005) and forensic detection of wildlife poaching (e.g., Manel et al., 2002).

The process of finding microsatellite markers can unfortunately be very time-consuming and expensive. The methods for locating STR loci have improved (Zane et al.,

**Table 2.** Primers currently available for amplification of nuclear loci of tortoise and freshwater turtles. Groups of taxa successfully amplified and associated references are listed in the final columns (cited references listed below). Key to taxa: CR = Suborder Cryptodira, Chely = Family Chelydridae, TE = Superfamily Testudinoidea, Test = Family Testudinidae, Geo = Family Geoemydidae, Emy = Family Emydidae, TR = Superfamily Trionychoidea, Car = Family Carettochelyidae, Trio = Family Trionychidae, K = Superfamily Kinosternoidea, Derma = Family Dermatemydidae, Kino = Family Kinosternidae, Platy = Family Platysternidae, C = Superfamily Chelonioidea, Chelo = Family Cheloniidae, Dermo = Family Dermochelyidae, PL = Suborder Pleurodira, Cheli = Family Chelidae, P = Superfamily Pelomedusoidea, Pelo = Family Pelomedusidae, Podo = Family Podocnemididae.

| Target Locus | Primer Name | Length (bp) | Primer Sequence(5'-3') | Ref. | Taxa | References citing primer |
|---|---|---|---|---|---|---|
| Actin intron | ACT I-5' | 20 | GCTGTTTTCCCGTCCATTGT | 121 | Test | 26 |
| Actin intron | ACT II-3' | 24 | GTCCTTCTGCCCCATACCSACCAG | 121 | Test | 26 |
| aldolase intron | Ald1-5' | 23 | TGTGCCCAGTATAAGAAGGATGG | 121 | Test | 26 |
| aldolase intron | Ald2-3' | 29 | CCCATCAGGGAGAATTTCAGGCTCCACAA | 121 | Test | 26 |
| Calmodulin intron | cal1 | 23 | GCCGAGCTGCARGAYATGATCAA | 38 | Test | 26 |
| Calmodulin intron | cal2 | 26 | GTGTCCTTCATTTTNCKTGCCATCAT | 37 | Test | 26 |
| c-mos oncogene | G136 (F) | 20 | AAGCAGGTGAAGAAATGCAG | 63 | PL | |
| c-mos oncogene | G137 (R) | 19 | TCCAATCTTGCACACACCC | 63 | PL | |
| c-mos oncogene | CM1 | 23 | GCCTGGTGCTCCATCGACTGGGA | 12 | Test | 90 |
| c-mos oncogene | CM2 | 25 | GGGTGATGGCAAAGGAGTAGATGTC | 12 | Test | 90 |
| c-mos oncogene | Cmos1 | 26 | GCCTGGTGCTCCATCGACTGGGATCA | 90 | Test | |
| c-mos oncogene | Cmos3 | 23 | GTAGATGTCTGCTTTGGGGGTGA | 90 | Test | |
| Creatine kinase intron 6 | CK6-5' | 24 | GACCACCTCCGAGTCATCTCBATG | 121 | Test | 26 |
| Creatine kinase intron 6 | CK7-3' | 21 | CAGGTGCTCGTTCCACATGAA | 121 | Test | 26 |
| GAPDH | GapdH950 | 27 | CATCAAGTCCACAACACGGTTGCTGTA | 55 | Emy | 152 |
| GAPDH | GapdL890 | 26 | ACCTTTAATGCGGGTGCTGGCATTGC | 55 | Emy | 152 |
| HNF-1a intron 2 | HNFAL-F | 20 | GCAGCCCTCTACACCTGGTA | 131 | Geo | 153 |
| HNF-1a intron 2 | HNFAL-R | 20 | CAATATCCCCTGACCAGCAT | 131 | Geo | 153 |
| ITS-1 | RNA-1 | 29 | TCCGTAGGTGAACCTGCGGAAGGATCATT | 95 | Test | 26 |
| ITS-1 | RNA-2 | 29 | CACGAGCCGAGTGATCCACCGCTAAGAGT | 95 | Test | 26 |
| ITS-1 | RNA-3 | 19 | GCGTTCCGGCGCGGAGGTT | 95 | Test | 26 |
| ITS-1 | RNA-4 | 19 | AAACCTCCGCGCCGCAACG | 95 | Test | 26 |
| R35 Intron 1 | R35 Ex1 | 21 | ACGATTCTCGCTGATTCTTGC | 61 | Emy, Geo, Trio | 36, 44, 152-154 |
| R35 Intron 1 | R35 Ex2 | 24 | GCAGAAAACTGAATGTCTCAAAGG | 61 | Emy, Geo, Trio | 36, 44, 152-154 |
| R35 Intron 1 | L-R35int | 25 | AGCATTACTACATTTTGATGCAATG | 158 | Geo | |
| R35 Intron 1 | H-R35int | 21 | CCAGCAAAGGACTCACTTGTA | 158 | Geo | |
| R35 Intron 1 | R35In1CF | 20 | TTKVTGBAATKTATGGRRAG | 153 | Geo | |
| R35 Intron 1 | R35In1CR | 20 | CTYYCCATAMATTVCABMAA | 153 | Geo | |
| RAG1 | RAGF1 | 20 | CCWGAWGARATTCAGCAYCC | 83 | TE | |
| RAG1 | RAGF2 | 21 | GAGATCATTYGAAAAGGCACC | 83 | TE | |
| RAG1 | RAGF3 | 21 | AGAACCTGCATCCTRAAGTGC | 83 | TE | |
| RAG1 | RAGF5 | 21 | GAGATGTCAGYGAGAAGCATG | 83 | TE | |
| RAG1 | RAGR1 | 22 | GCAAGATCTCTTCATCRCATTC | 83 | TE | |
| RAG1 | RAGR2 | 22 | GATGTTCAGGAAGGATTTCACT | 83 | TE | |
| RAG1 | RAGR3 | 21 | CTCAGGATGGCTGTCAGAGTC | 83 | TE | |
| RAG1 | RAGR4 | 21 | TGCAACACAGCTCTGAATTGG | 83 | TE | |
| RAG1 | RAGR5 | 20 | GACATCCTCCATTTCATAGC | 83 | TE | |
| RAG2 | F2 (Rag2) | 23 | CAGGATGGACTTTCTTTCCATGT | 90 | Test | |
| RAG2 | F2-1(Rag2) | 19 | TTCCAGAGCTTCAGGATGG | 90 | Test | |
| RAG2 | R2-1(Rag2) | 25 | CAGTTGAATAGAAAGGAACCCAAGT | 90 | Test | |
| Reelin intron 61 | RELN61F | 30 | TGAAAGAGTCACTGAAATAAACTGGGAAAC | 153 | Geo | |
| Reelin intron 61 | RELN61R | 26 | GCCATGTAATTCCATTATTTACACTG | 153 | Geo | |

2002), yet, even for the experienced worker, laboratory procedures may require substantial time and money. Commercially, it can cost from $10,000 to $15,000 per species to develop an STR library. In addition, even after loci have been identified, there is no guarantee the loci will be polymorphic (i.e., exhibit multiple alleles) and therefore be informative to the research question. Although costs are high in the development phase, this expense is offset by relatively low costs associated with later phases (i.e., genotyping) and by the potential utility of the markers for future studies of the target species or other closely related taxa. Because the cost of commercially synthesized primers is low (ca. $0.30/bp), assessing the utility (i.e., polymorphism) of primers already developed for taxa closely related to the target species is far more cost effective. In Table 3 we have compiled the primer sequences for 160 STR loci from all major clades of turtles. Many of these loci have already exhibited successful amplification in other species.

Turtles are suggested to have conservative genomes and therefore may be particularly well suited to inter-species primer amplification (Avise et al., 1992; FitzSimmons et al., 1995; King and Julian, 2004). However, conservation of the sequence flanking the STR (i.e., where the primer attaches) does not necessarily imply that the STR motif has also been conserved. Therefore, we offer a few cautionary tales to stress the importance of sequencing polymorphic loci developed in a non-target species before making assumptions regarding utility of a marker, even if it is to be used in a closely related species. Sequencing also allows for uniformity of datasets by different researchers. For example, despite hundreds of millions of years of evolutionary change, primers developed for a microsatellite locus in *Chelonia mydas* amplify the same locus (verified by comparing flanking sequences) in *Gopherus agassizii* and exhibit moderate variability, although the repeat motif is dramatically different (Edwards et al., 2004) (Tables 3 and 4). Motif changes can also be observed within a genus (e.g., locus GP81 identified in *Gopherus polyphemus* and successfully amplified in *Gopherus agassizii* [Tables 3 and 4; Schwartz et al., 2003]), or even within a species (e.g., locus GP61 originally

**Table 3.** Primer pairs developed for microsatellite loci in turtles. Taxa Described = original species in which the loci were identified. Additional Taxa = Taxa in which the locus has been successfully amplified. Additonal References = Studies in addition to the original reference which have used the locus. Key to Taxa: Apsp = *Apalone spinifera;* Caca = *Caretta caretta*; Cain = *Carettochelys insculpta*; CHEL = Famly Cheloniidae; Chmy = *Chelonia mydas*; Chpi = *Chrysemys picta*; Chru = *Chelodina rugosa*; Chse = *Chelydra serpentina*; Deco = *Dermochelys coriacea*; DERM = Family Dermochelyidae; DIPS = *Dipsochelys* spp.; ELSE = *Elseya* sp.; Embl = *Emydoidea blandingii*; Emma = *Emydura macquarii*; EMYD = Famly Emydidae; Erim = *Eretmochelys imbricata*; Erma = *Erymnochelys madagascariensis*; GEOC = *Geochelone* spp.; Gera = *Geochelone radiata*; Glin = *Glyptemys insculpta*; Glmu = *Glyptemys muhlenbergii*; Goag = *Gopherus agassizii*; GOPH = *Gopherus* spp.; Gopo = *Gopherus polyphemus*; Grko = *Graptemys kohnii*; Leke = *Lepidochelys kempi*; Leol = *Lepidochelys olivacea*; Mate = *Malaclemys terrapin*; Poex = *Podocnemis expansa*; Tegr = *Testudo graeca*; Tehe = *Testudo hermanni;* Teho = *Testudo horsfieldii*; Tema = *Testudo marginata*; Tewe = *Testudo weissingeri*; Teor = *Terrapene ornata*. Information for unpublished primer sequences: Unpub01 = Arthur Georges(georges@aerg.canberra.edu.au) primers for *Chelodina rugosa* purchased under contract from Jane Hughes (Griffith U) optimized by Erika Alacs (U. Canberra); Unpub02 = FitzSimmons et al. (Nancy.Fitzsimmons@canberra.edu.au); Unpub03 = FitzSimmons and Georges; Unpub04 = Peter H. Dutton (Peter.Dutton@noaa.gov).

| Locus | GenBank Acc. No. | Repeat Motif | Forward and Reverse Primer sequence (5'_3') | Amplicon Size (bp) | Obs. Alleles | Taxa Descr. | Add. Taxa | Orig. Ref. | Add. Refs. |
|---|---|---|---|---|---|---|---|---|---|
| Ah01 | | GA | F: TGCAGTTTGCTGAGCTTAGAG<br>R: TGTTGGCTGGTCTCATGTTC | 120-160 | 6 | Teho | | 79 | |
| Ah02 | | GA | F: AGGGGTGGGGATAGATTG<br>R: GCAGAGAGCAGAGGTTTGACC | 123-137 | 7 | Teho | | 79 | |
| BTCA2 | AY335787 | $(CA)_8N_{14}(CA)_7$ | F: CTTAAAAAGACATTAAAATATCTT<br>R: AACTCTCCCTAAAACCACAG | 184–192 | 3 | Embl | Chpi, Chse | 97 | |
| BTCA5 | AY335788 | $(GA)_{11}$ | F: GCTGCTTAGCACAACTCATAA<br>R: CTTTTGTATTTAATCCATGATGAA | 146–154 | 3 | Embl | Chpi, Chse | 97 | |
| BTCA7 | AY335789 | $(CA)_{12}$ | F: TGGAATTAGATGTTTTGCAGTT<br>R: TCATTTCTGTTTTCCACACTG | 154–158 | 2 | Embl | Chpi, Chse | 97 | |
| BTCA9 | AY335790 | $(CA)_9$ | F: TACTCAAGATTTGAAGCAGATACA<br>R: GGCTTGATTCTACTGTCACTTAC | 148–184 | 9 | Embl | Chpi, Chse | 97 | |
| BTGA2 | AY335791 | $(GGA)_5N_3(GA)_3$ | F: ATGATCTAATGGTCCCTTCTG<br>R: CTGTTAGCTTATTCTTCTGCAA | 144–148 | 3 | Embl | Chpi, Chse | 97 | |
| BTGA3 | AY335792 | $(GA)_{11}$ | F: CCTAGATTTTGTCTGGCTATTA<br>R: TATCTCAGTAATAATCCCCTTAG | 108 | 1 | Embl | Chpi, Chse | 97 | |
| BTGA4 | AY335793 | $(GA)_{11}$ | F: CTCATAAAGTAAGGACGGGAA<br>R: CCTAGAGATGGAATCTTTGTATT | 146–154 | 3 | Embl | Chpi, Chse | 97 | |
| Cc117 | | $(CA)_{17}$ | F: TCTTTAACGTATCTCCTGTAGCTC<br>R: CAGTAGTGTCAGTTCATTGTTTCA | | | Caca | CHEL, DERM | 49, 50 | 32, 48, 50 |
| Cc-136 | | $(GT)_{21}$ | F: ACCAATCATTCAATGCCTTAGG<br>R: CTTTGCTAGGTATTTATACACACAG | 124-228 | 44 | Caca | | Unpub02 | |
| Cc141 | | | F: CAGCAGGCTGTCAGTTCTCCAC<br>R: TAGTACGTCTGGCCTGACTTT | | | Caca | | Unpub02 | 19, 32, 110 |
| Cc7 | | $(CA)_{14}$ | F: TGCATTGCTTGACCAATTAGTGAG<br>R: ACATGTATAGTTGAGGAGCAAGTG | 165-217 | 20 | Caca | EMYD, GOPH | 47 | 19, 32, 39, 40, 110 |
| Ccar176 | AF333763 | | F: GGCTGGGTGTCCATAAAAGA<br>R: TTGATGCAGGAGTCACCAAG | 186–220 | 16 | Caca | | 110 | |
| CCM2 | | | F: TGGCACTGGTGGAAT<br>R: TGACTCCCAAATACTGCT | | | Caca | | 53 | 19, 110 |
| Ci-107 | | $(CT)_6T(CT)_3(CA)_{10}TA(CA)_8$ | F: CCAGGAATTTCTTCATGCCAC<br>R: GTTTAACATGCCTTGGCTCCTTC | 288 | 1 | Cain | | Unpub03 | |
| Ci-123 | | $(CA)_3CG(CA)_{13}$ | F: GTTTGCAGGCAACCATCATATAGTC<br>R: GGAACATTTCAACCCATCAGG | 172 | 1 | Cain | | Unpub03 | |
| Ci-124 | | $(CA)_4CN(CA)_{32}$ | F: AAACAAATCTGCTATCATGCC<br>R: GTGGAGATACAACCTTTATGATGAC | 150-210 | 16 | Cain | | Unpub03 | |
| Ci-125 | | $(CA)_{17}$ | F: ACACAGCATATTATGATTTGG<br>R: TTGTGTCTTTGCTATTTTAGTC | 194-196 | 2 | Cain | | Unpub03 | |
| Ci-126 | | $(CA)_{16}$ | F: GGGATCAAACCATGCAAGTATG<br>R: GTTTTCCAGATTTGTCCCTCCA | 192-194 | 2 | Cain | | Unpub03 | |
| Ci-128 | | $(CA)_{17}$ | F: GTTTCCATCCCTATTAAGTTATCAC<br>R: TTATGGGAGTTGCTCTTTGCC | 283 | 1 | Cain | | Unpub03 | |
| Ci-130 | | $(CA)_{12}GA(T)_7$ | F: GTTTACAATACCTGCACTTTCTC<br>R: TTAGGCAATTAACACTTCTC | 103 | 1 | Cain | | Unpub03 | |
| Ci-145 | | $(CA)_{13}$ | F: GTTTGGGCACCTGTCTCTTATAG<br>R: GGGCTTTCAGGCATCTTCAC | 147 | 1 | Cain | | Unpub03 | |
| Cm3 | | $(CA)_{13}$ | F: AATACTACCATGAGATGGGATGTG<br>R: ATTCTTTTCTCCATAAACAAGGCC | | | Chmy | CHEL, DERM | 49, 50 | 32, 48, 137 |
| Cm58 | | $(CA)_{13}$ | F: GCCTGCAGTACACTCGGTATTTAT<br>R: TCAATGAAAGTGACAGGATGTACC | | | Chmy | CHEL, DERM, Goag | 49 | 41, 48, 137 |
| Cm72 | | $(CA)_{33}$ | F: CTATAAGGAGAAAGCGTTAAGACA<br>R: CCAAATTAGGATTACACAGCCAAC | | | Chmy | CHEL, DERM | 49 | 48, 137 |
| Cm84 | | $(CA)_{15}$ | F: TGTTTTGACATTAGTCCAGGATTG<br>R: ATTGTTATAGCCTATTGTTCAGGA | | | Chmy | CHEL, DERM | 49 | 32, 73, 80, 137 |
| Cp10 | | | F: GGTGCAGCAAGTTCAGGAGAC<br>R: GGTGTTAATGCACTGGAGAATCA | | ~24 | Chpi | | 129 | |
| Cp2 | | | F: CTCTAAGGGTTGCACTTCTCAAA<br>R: GAGGTGGCATCAAAACATCAT | | ~24 | Chpi | | 129 | |
| Cp3 | | | F: ATCTTTAAGTCTGTGAACTTCAGGG<br>R: CTGTCTCATGCAAAGCTGGTAG | | ~24 | Chpi | | 129 | |
| Dc107 | | | F: GTCACGGAAAGAGTGCCTGC<br>R: CAATTTGAGGTTATAGACC | 158–186 | 11 | Deco | Caca | Unpub04 | 19 |
| Dc99 | | | F: CACCCATTTTTTCCCATTG | 130–140 | | Deco | | 19 | 32 |
| Eb05 | AF416293 | AAT | F: GCCAGGAACAATGTTTTA<br>R: TTGGCATTCTACACATAATAA<br>R: ATTTGAGCATAACTTTTCGTGG | 45–57 | 5 | Embl | Chse, Gopo | 115 | |
| Eb09 | AF411049 | CA | F: TTGAATTAGCTCATAAGCAC<br>R: TCATAATGTGAATTGGTCTC | 128–160 | 15 | Embl | Gopo | 115 | 108 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Eb11 | AF411050 | CA | F: GAGGATCAGAATGTTCAGAC<br>R: TCTGACTTGAATTAAACCTC | 172–204 | 13 | Embl | | 115 | 108 |
| Eb12 | AF416294 | CA | F: GTCCCTAGATTTAACTGATAAACTTG<br>R: AGGGTGGAGGAAGAGGAATAG | 119–141 | 7 | Embl | Chse, Chpi | 115 | |
| Eb15 | AF411051 | CA | F: AATTGATCCCTTGATCCG<br>R: TCAGGACTATGAGGAAGC | 147–186 | 34 | Embl | Chse, Chpi, Gopo | 115 | |
| Eb17 | AF416295 | AAT | F: CCCACAAAAGTAGACACCTAT<br>R: GGCACTGAAATAAGAGAAAGTA | 94–109 | 5 | Embl | Chse, Chpi, Gopo, Trsp | 115 | 108 |
| Eb19 | AF416296 | AAT | F: AGGGCTCTGAAGCACTAAAGTAA<br>R: CTCCGGCTTTTCATTTGTGT | 100–109 | 3 | Embl | Chse, Chpi, Apsp | 115 | 108 |
| Ei8 | | $(CA)_{19}$ | F: ATATGATTAGGCAAGGCTCTCAAC<br>R: AATCTTGAGATTGGCTTAGAAATC | | | Erim | CHEL, DERM | 49 | 32, 73, 80, 108, 137 |
| GAL100 | | $(CA)_{26}$ | F: TCTTAATAAATTCCATGAGTTGAGCT<br>R: AGGGTGATTTCATAAACAAACAGAA | 100-156 | 19 | GEOC | DIPS | 31 | 15, 119 |
| GAL127 | | $(CA)_{21}$ | F: TAACTATAAACATCAACTGGCAGAA<br>R: GTTTAGTGTCATCTGCATATGC | 97-175 | 31 | GEOC | | 31 | 15, 62 |
| GAL136 | | $(CA)_{20}$ | F: ATGAGATGTATGTACAGAAAATATA<br>R: CTGGAGGGAAGTAAGAATC | 73-101 | 12 | GEOC | DIPS | 31 | 15, 119 |
| GAL159 | | $(CA)_{24}$ | F: AATATTTGAAGATACTCATCCTCGA<br>R: TTATGTGCTTGTGTCATCTTTTT | 83-123 | 19 | GEOC | | 31 | 15 |
| GAL247 | | $(CA)_{39}$ | F: ATTAACTGATTTGAGCAGTCATCCA<br>R: TGCTGTGAATAGTAACTGAGC | 69–93 | 3 | GEOC | DIPS | 119 | |
| GAL263 | | $(CA)_{17}$ | F: GGGAAAGTACTATTTCCAGAGCTGG<br>R: GCTGAGGCTAGCTAATTTTTATGT | 80-164 | 25 | GEOC | DIPS | 31 | 15, 119 |
| GAL45 | | $(CA)_{17}$ | F: TATCTCCTTCCACACGGAGATGGG<br>R: CCCCAAAGTAAAGTTAGCTCTCTCA | 87-123 | 13 | GEOC | | 31 | 15 |
| GAL50 | | $(CA)_{24}$ | F: TGGGACAGGCAAACTAACAAAACTT<br>R: TGCAGAAGTTAATCCCTTTCTCCTT | 96-182 | 37 | GEOC | DIPS | 31 | 15, 119 |
| GAL73 | | $(CA)_{24}$ | F: ATTATGTGCTTGTGTCATCTTTTTC<br>R: TTGAAGATACTCATCCTCGATACA | 78-126 | 20 | GEOC | DIPS | 31 | 15, 119 |
| GAL75 | | $(CA)_{24}$ | F: GAAGCCATTTACCACAAACTTATT<br>R: GTTACCATAGCATTCCTGATTATAG | 73-149 | 22 | GEOC | | 31 | 15 |
| GAL85 | | $(CA)_{22}$ | F: TGTGGGGCATGGAAGGGCC<br>R: CACCAAGAGAGGAAAATAATGCTGGG | 81–91 | 3 | GEOC | DIPS | 119 | |
| GAL94 | | $(CA)_{18}$ | F: CTTCTATTTCCCAACCATCT<br>R: AACTTTATATTTGTGTGCATATT | 85-111 | 13 | GEOC | DIPS | 31 | 15, 119 |
| GmuA18 | AF337648 | $(GT)_{14}$ | F: TATCAGGGAAAGCAATGTAAGG<br>R: AGTGAAACAAGCAGTTATGGTG | | | Glmu | EMYD | 81 | |
| GmuA19 | AF517227 | $(GA)_7(GT)_{14}$ | F: TAAGAGACAGATGCTCAGCAAG<br>R: GTACATAACACGCACCCAATG | | | Glmu | Teor | 81 | 84 |
| GmuA32 | AF517228 | $(GT)_{33}$ | F: TTATATTGCCTGCTGCTATCAC<br>R: ATGAAAGTGTGCCTTTCACTG | | | Glmu | EMYD | 81 | |
| GmuB08 | AF517229 | $(TAC)_{10}$ | F: CTCTGAGACCCTTATTCACGTC<br>R: AGCCTTTGTCTGTAAGCTGTTC | | | Glmu | Teor | 81 | 79, 84, 140 |
| GmuB12 | AF517230 | $(TAC)_7$ | F: TCAATCTTCCAGCCTAACTGTG<br>R: AGGGATGTGTTTTGCAACTGG | | | Glmu | Teor, Tegr | 81 | 84 |
| GmuB21 | AF517231 | $(TAC)_{10}$ | F: CTAGTTCGAAACAGGACCGTTG<br>R: CCACACGACAGTTTGATGTCAG | | | Glmu | Teor, Glin | 81 | 84, 160 |
| GmuB67 | AF517232 | $(TAC)_{13}$ | F: ACTCAAGCACTGACACACAATC<br>R: CCAGTATTTGTGAGAATTTCCTTC | 151–168 | 3 | Glmu | Teor | 81 | 84 |
| GmuB80 | AF517233 | $(ATCT)_{16}$ | F: TTATTGTGCATTGTATCATGGG<br>R: CGCTACCATCATGTAACTAAGAG | | | Glmu | EMYD | 81 | |
| GmuB91 | AF517234 | $(TAC)_6$ | F: TCAGGGAAGCAATAGAACACTC<br>R: TCTCATCCCTAAGTAAACCCAC | 139–142 | 2 | Glmu | Teor | 81 | 84 |
| GmuD107 | AF517250 | $(ATCT)_{15}$ | F: GACAAACATGAACAGGAGAAGAG<br>R:ATTAGAGAGACAGATAGATAGGACTTG | 189–209 | 5 | Glmu | EMYD | 81 | |
| GmuD114 | AF517251 | $(ATCT)_{13}$ | F: ATAGACATAGTGCATATAGACATAGCC<br>R: ACGTTCTTGCAGGGTCAGAG | 92–128 | 6 | Glmu | EMYD | 81 | 141 |
| GmuD121 | AF517252 | $(ATCT)_8$ | F: GGCAAATATCCAATAGAAATCC<br>R: CAACTTCCTCGTGGGTTCAG | 138–154 | 5 | Glmu | Teor | 81 | 84 |
| GmuD16 | AF517235 | $(ATCT)_{19}$ | F: ATCCCTGAAATTTTGTGTGTTC<br>R: TTTACTCTAGAAGGGGCAATCC | 188–228 | 9 | Glmu | EMYD, Glin, Tegr | 81 | 84, 160 |
| GmuD21 | AF517236 | $(ATCT)_{15}$ | F: GCAGTTAGGCATTACTCAACATC<br>R: AGGGTATGAATACAGGGGTGTC | 163–199 | 5 | Glmu | Teor | 81 | 84 |
| GmuD28 | AF517237 | $(ATCT)_{15}$ | F: AGCTGTTTGTCATCATACACTCTC<br>R: TGGCCCTCATGTTTTATAAGTG | 208–236 | 6 | Glmu | EMYD | 81 | |
| GmuD40 | AF517238 | $(ATCT)_{22}$ | F: TTTGTCATATCATCCACTCACC<br>R: TTTGTCACAGATGGGAATTAGC | 157–201 | 9 | Glmu | EMYD, Glin | 81 | 160 |
| GmuD51 | AF517239 | $(ATCT)_{52}$ | F: GTTGGGCACTAGATAGATTCG<br>R: CATTCAAGTCAACGGAAAGAC | 307–359 | 10 | Glmu | EMYD, Tegr | 81 | 79, 140, 141 |
| GmuD55 | AF517240 | $(ATCT)_{10}$ | F: GTGATACTCTGCAACCCATCC<br>R: TTGCATTCAGAATATCCATCAG | 212–224 | 4 | Glmu | Teor | 81 | 84 |
| GmuD62 | AF517241 | $(ATCT)_{11}$ | F: GGTGGTATAGAAAATCCTAAAATGG<br>R: GTGCAAACTGTCTGGAAATAGG | | | Glmu | Teor | 81 | 84 |
| GmuD70 | AF517242 | $(ATCT)_8$ | F: AGTGTAGTCATGGCATAGAGAGG<br>R: ATCAAATTCTTCCAACCCTACC | 185-205 | 5 | Glmu | EMYD | 81 | |
| GmuD79 | AF517243 | $(ATCT)_{10}$ | F: GCCCTGTTCCATTCTTATTCTG<br>R: ATCCCCTTAGTCGTCTCTTTTC | 164–192 | 3 | Glmu | Teor | 81 | 84 |
| GmuD87 | AF517244 | $(ATCT)_{22}$ | F: AAACCCTAAGACATCAGACAGG<br>R: CAAATCCAGTACCCAGAAAGTC | 260–292 | 8 | Glmu | Teor, Glin | 81 | 79, 84, 140 |
| GmuD88 | AF517245 | $(ATCT)_{18}$ | F: AACAATGCCTGAAAATGCAC<br>R: TAGGCTACCTCTGAAAATGCTG | 154–178 | 17 | Glmu | Teor | 81 | 84 |
| GmuD89 | AF517246 | $(ATCT)_7$ | F: GCTCGCTGTAACTAGCTCTAACTC<br>R: CCAGGCAGCTTTGTTTAATG | 112–124 | 3 | Glmu | EMYD | 81 | |
| GmuD90 | AF517247 | $(ATCT)_9$ | F: ATAGCAGGACAATTACCACCAG<br>R: CCTAGTTGCTGCTGACTCCAC | 122–134 | 3 | Glmu | Teor | 81 | 84 |

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|---|---|---|---|---|---|---|---|---|---|
| GmuD93 | AF517248 | $(ATCT)_{18}$ | F: AGACTCTCTTGACCAGATTTTCTC<br>R: TCTGCCTTCTATCACTCTCCTG | 185–389 | 10 | Glmu | Teor, Glin | 81 | 84, 140, 141 |
| GmuD95 | AF517249 | $(ATCT)_{17}$ | F: AGGTACGAGACAGGACAAAGTG<br>R: TGAATGCAGTGTAACATTTGAG | 153–177 | 4 | Glmu | Teor | 81 | 84 |
| *Goag*3 | AY317141 | $(CAA)_6$ | F: CTGATTGGTCTGACTCCCT<br>R: CCTGATTGCTTCCTGACAC | 375-381 | 3 | Goag | | 40 | |
| *Goag*32 | AY317147 | $(AC)_6$ | F: GTGCTGCCTTGATAAGTAA<br>R: ATAGTTTTCTTTCCTACACAT | 177-179 | 2 | Goag | | 40 | |
| *Goag*4 | AY317142 | $(CAA)_{24}$ | F: CTCAACAAAAGGTAAGTGATG<br>R: GCATAAAAGTAAACAGTAAAGTA | 110-188 | 17 | Goag | | 40 | |
| *Goag*5 | AY317143 | $(GAT)_{17}$ | F: AGGCAAGTGGGTGGTAATG<br>R: GCGATTTTGAGGCTTCTTTC | 257-365 | 27 | Goag | | 40 | |
| *Goag*6 | AY317144 | $(TC)_8(AC)_{11}$ | F: TAAGGGCTATGAGGAAGAAT<br>R: GTAATGGTGTGGGTGGGA | 360-442 | 15 | Goag | | 40 | |
| *Goag*7 | AY317145 | $(AC)_3(GC)_5(AC)_{11}$ | F: TCAATCCATTAGTCTTCACCC<br>R: TTTCTGTTTATGCTCCGTATTA | 261-281 | 8 | Goag | | 40 | |
| *Goag*8 | AY317146 | $(CA)_{14}TA(CA)_3$ | F: ATGCTGACAATAGAACAAGA<br>R: ACATCTGGGGCTAAAGTG | 192 | 1 | Goag | | 40 | |
| GP102 | AF546890 | $(GT)_5(CT)_{13}(CA)_5$ | F: AGCTGCCTGACTGCTATGCT<br>R: GCATAATCAGCATCAACAACAAA | 299–339 | 15 | Gopo | GOPH, Grko | 146 | 54, 109, 145 |
| GP15 | AF546895 | $(GA)_{15}(GT)_8$ | F: CCTATTTTTCCCCCTCACAGT<br>R: GAAAATAAAAACAGTCCCAACCA | 207–269 | 19 | Gopo | GOPH, Grko | 146 | 54, 109, 145 |
| GP19 | AF546891 | $(GT)_9/(GT)_3(GA)_6$ | F: GCAGGACAGTGCCACACTA<br>R: CAGCCATATTAATGACAATCTG | 252–256 | 3 | Gopo | GOPH, Grko | 146 | 54, 109, 145 |
| GP26 | AF546892 | $(GT)_{12}$ | F: GACAACCATCTTTACCCACA<br>R: TCCCAAGACATAAGTCAGTAGC | 358–370 | 6 | Gopo | GOPH, Grko | 146 | 54, 109, 145 |
| GP30 | AF546889 | $(GT)_{13}$ | F: GAATGCAGCACTGCTTGGTA<br>R: CGAAGAGGGAGCACGTTTAG | 194–232 | 10 | Gopo | GOPH, Grko | 146 | 54, 109, 145 |
| GP55 | AF546893 | $(GT)_9$ | F: TTAGGGATTTTCTGTCTACTTCAG<br>R: CGCAATGTGACACGCTATT | 265–271 | 2 | Gopo | GOPH, Grko | 146 | 54, 109, 145 |
| GP61 | AF546896 | $(GT)_{12}$ | F: GCATTAAACCATTGTGCCTCA<br>R: AGTGGTGGTCGAAGTGGAAC | 197–245 | 7 | Gopo | GOPH, Grko | 146 | 54, 109, 145 |
| GP81 | AF546894 | $(GT)_{11}(GA)_{10}$ | F: TCACACAAACCCCATCCATA<br>R: TCCATTGAATTGCCATCTGA | 397–415 | 7 | Gopo | GOPH, Grko | 146 | 54, 109, 145 |
| GP96 | AF546888 | $(GA)_{11}$ | F: TCAGTTACCGGATAATGTTCAGTG<br>R: TGCTGTTACCTCGTGCATGT | 141–157 | 8 | Gopo | GOPH, Grko | 146 | 54, 109, 145 |
| Klk314 | | $(CA)_5$ | F: GGTGCCAAGGAGGACGCTG<br>R: CATGCTCGCCCCTGGAAAG | 109 | 1 | Leke | | 80 | |
| Klk315 | | $(CA)_8$ | F: AGACAAACTCCCCCTTGCTAGG<br>R: CCCAGAAGGTGAAGAAATACCAAA | 135 | 10 | Leke | Leol | 80 | |
| Klk316 | | $(CA)_{22}$ | F: TACATCCATACATGCAGCCCCCTGA<br>R: GGTGCTAGGGTGAGTATTGAGCACT | 132 | 3 | Leke | Leol | 80 | 32 |
| Klk325 | | $(CA)_8$ | F: CCCAGTTCCTTTCAACCAAGTA<br>R: CTTGAGCTTTAACAGATGACAAAA | 155 | 1 | Leke | | 80 | |
| MR-1 | AY934859 | (AC)11 | F: TTTCTGCACCTGCTTAACTT<br>R: CTCATGGAGGTGGTGTTACT | 222–234 | 5 | Mari | | 101 | |
| MR-2 | AY934860 | (AC)9 | F: ACGGAATCCTGATTAATTCC<br>R: CTTCCCTCAATACAATGGTT | 199–229 | 5 | Mari | | 101 | |
| MR-3 | AY934861 | GT)8 | F: CATTTTCTTTATCGCCTCAC<br>R: CTTTCACAGCACAAGTCTCA | 181–189 | 5 | Mari | | 101 | |
| MR-5 | AY934862 | (GA)18 | F: TCTAGGGTCGCCCCTGTAGG<br>R: CTGGGAATGTTCTGCGGTTG | 149–189 | 10 | Mari | | 101 | |
| MR-8 | AY934863 | (GT)32(GA)12 | F: TGCCCTCTGATGCTCTGGTG<br>R: GCCCAAATGTCTACAACTGTGG | 154–194 | 18 | | | 101 | |
| MR-9 | AY934864 | (CT)16 | F: CCAATGCTCCAGGCGTG<br>R: GCCAGTCTTACTGCTGAACC | 97–105 | 5 | | | 101 | |
| OR-1 | AY325422 | $(CAAA)_{16}$ | F: CCCCTTGTGTTCTGAAATCCTATGA<br>R: CAGGCATAGGGAAAAATCAGAGGTA | 150–202 | 24 | Leol | Chmy, Erim | 1 | |
| OR-2 | AY325423 | $(GT)_8GCC(GT)_5$ | F: GCTCCTGCATCACTATTTCCTGTT<br>R: TGCTGCCCCCACACCCTCTG | 153–185 | 12 | Leol | Chmy, Erim, Deco | 1 | |
| OR-3 | AY325424 | $(TC)_9(AC)_6GC(AC)_2$ | F: TTGTTTTATTTTTATTGGTCATTTCAG<br>R: GCACCTTTTCACGTTGTCCACATGT | 146 | 1 | Leol | Chmy, Erim, Deco | 1 | |
| OR-4 | AY325425 | $(TG)_9/(TG)_{23}$ | F: AGGCACACTAACAGAGAACTTGG<br>R: GGGACCCTAAAATACCACAAGACA | 122–172 | 18 | Leol | Chmy, Erim | 1 | |
| OR-7 | AY325427 | $(GT)_6(GA)_7$ | F:GGGTTAGATATAGGAGGTGCTTGATGT<br>R: TCAGGATTAGCCAACAAGAGCAAAA | 185–219 | 16 | Leol | Chmy, Erim, Deco | 1 | |
| OR-8 | AY325428 | $(TC)_{23}$ | F: GCACTGGTGGGAAAATATTGTTGT<br>R: GCTGGGCTAATAAAATGTTGTGCA | 148–166 | 8 | Leol | Chmy, Erim, Deco | 1 | |
| PE1075 | AF141138 | $(AC)_{11}$ | F: ATGAGCCTGAAGAGTTGGAA<br>R: AACTTAGGCTGCATGAGTTG | 247–283 | 6 | Poex | | 161 | 127, 128 |
| PE344 | AF141136 | $(AG)_{13}$ | F: ATCCTGAGTTTAAAGGTGA<br>R: AACTCTTCAAACTCCTCTAG | 144–208 | 10 | Poex | | 161 | 127, 128 |
| PE519 | AF141137 | $(CT)_7(CA)_8(CG)_2(CA)_8$ | F: GCTGAGCTAGACTAACATGC<br>R: GTAAATTGCCATACTTGGAG | 239–327 | 8 | Poex | | 161 | 127, 128 |
| Pod1 | | $(CA)_{32}$ | F: GATCTTTCTTTACAGGTGCAGTTC<br>R: CACAACTAAATTACAGCACTCCG | 154-204 | 21 | Poex | | 151 | 127, 128, 161 |
| Pod128 | | $(GT)_{27}(GC)_7$ | F: GTGTCAGGGCTACCATCAAGATTG<br>R: CCAGTAAAATTCACTACCAGCATG | 140-209 | 23 | Poex | | 151 | 127, 128, 161 |
| Pod147 | | $(GT)_{16}(A)_{20}$ | F: GTGACAGCAGCATCTCATTTTCTC<br>R: ATGACACATTACCATCCCATAGG | 181-249 | 19 | Poex | | 151 | 127, 128, 161 |
| Pod62 | | $(GT)_{11}(TA)_5$ | F: ATGAGTGTGGAATGAGAGGAAC<br>R: CCCATCCACAGAAGCAAATTCC | 182-214 | 9 | Poex | | 151 | 127, 128, 161 |
| Pod79 | | $(CT)_{13}(CA)_{16}$ | F: GGGAGAGCATTGCTGGTTGGTG<br>R: CAATGTCATCACCGCAGAACCC | 220-260 | 16 | Poex | | 151 | 127, 128, 161 |
| Pod91 | | $G_9(GT)_{17}(GA)_8$ | F: TCATTTTGGTTAGAAGTGAAGGC<br>R: GGTTGTTCATCTTTTAGATTCACC | 111-255 | 40 | Poex | | 151 | 127, 128, 161 |

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|---|---|---|---|---|---|---|---|---|---|
| RAD14 | AY900651 | $(CT)_{12}(AC)_{14}$ | F: GATCCCCAACTGTCACCAC<br>R: AAAATGTTGCTCTCCTAAATGC | 218–262 | | Gera | | 122 | |
| RAD27 | AY900652 | $(TG)_7TA(TG)_{16}$ | F: AAAATCTACCAAGGTCTGCAAAG<br>R: TTACAGAGCATCAGCAAGGC | 230–270 | | Gera | | 122 | |
| RAD284 | AY900653 | $(GT)_{22}$ | F: GTGCTGAACAGAGGCTGATG<br>R: CACACACACAGACAGAAGATTATT | 209–243 | | Gera | | 122 | |
| RAD313 | AY900654 | $(GT)_{12}GAG(GT)_3(GA)_8(GT)_5(GA)_{11}$ | F: AGTTGTTTTCCCACCCCC<br>R: TCCCCAAGACACCTGCTG | 220–292 | | Gera | | 122 | |
| RAD542 | AY900655 | $(CA)_{13}$ | F: TCCTGTGATTGTTTCATAGAACG<br>R: TCTGCTCCTTCCTGTGTGC | 148–196 | | Gera | | 122 | |
| RAD573 | AY900656 | $(CA)_8(TGCA)_2(CA)_2CG(CA)_4$ | F: TGAACAGAACGATCCTCCCC<br>R: GGGAAAGCCAGGGCACTAG | 199–225 | | Gera | | 122 | |
| RAD891 | AY900657 | $(CT)_{12}(CA)_6CG(CA)_9$ | F: TATTCACCCACGAAAGCTCA<br>R: GGTTGTTGGAGAAAGGAGGA | 194–242 | | Gera | | 122 | |
| RAD932 | AY900658 | $(GT)_{15}$ | F: GGTAGATAGTTCCTTCAGCCTTG<br>R: TCCCCTCTTTTTCTGTCTCATAG | 152–204 | | Gera | | 122 | |
| T12 | | $(CAG)_9/GAG/(CAG)_3$ | F: GGGATCACTCGGCCACTCTGG<br>R: ACCCAAGAATACCCGTCACCG | 157-163 | 3 | Chru | | Unpub01 | |
| T14 | | $(TGC)_8$ | F: TAGGCTCAGGGATATGATAGC<br>R: CTCCAGCGACAGTTGCAACAG | 120-129 | 4 | Chru | | Unpub01 | |
| T17 | | $(TGC)_7$ | F: AACAGTATTATGGATGCAGAC<br>R: GACACAAAAGGTACCATTCCC | 121-130 | 4 | Chru | | Unpub01 | |
| T26 | | $(GCA)_7$ | F: CAGTGATTTTTGCTACCAAGG<br>R: GCAAAACAGTATTATGGATGC | 158-167 | 4 | Chru | | Unpub01 | |
| T42 | | $(ACC)_8$ | F: CCAAACTTGAACACTGCTGTG<br>R: GGACTCCCAGATTATGGTCTC | 158-164 | 2 | Chru | | Unpub01 | |
| T44 | | $(AGC)_7$ | F: AAGGCAGTTGAGAACCAGGTG<br>R: GTAGATGCCACCCATGTTGTC | 131-142 | 5 | Chru | | Unpub01 | |
| T50 | | $(GCA)_8$ | F: TGCTGCCTGCCATTAGCTTAC<br>R: CTGCATTTGAGCAATTCGCTG | 134 | 1 | Chru | | Unpub01 | |
| T58 | | $(CAC)_7$ | F: TCCTGAAAGGGTGGGCAAAGG<br>R: CTAGATGATTCTCAGTCTTTC | 157-166 | 3 | Chru | | Unpub01 | |
| T79 | | $(TGC)_7C(TGC)_1$ | F: TTCCCCCCACAAGTCACTTTC<br>R: TGTATTACTCTCCGTGTCTCG | | | | | Unpub01 | |
| T80 | | $(TGC)_7$ | F: CTCACCTGCAGCCTCTTTCTC<br>R: AGGACCTTTCAGGACCCTCAC | 138-159 | 6 | Chru | | Unpub01 | |
| T87 | | $(TGC)_9$ | F: CAGCACTGATCTGCAAGTACC<br>R: GCTACACCAGTTTCACTCTGC | 136-148 | 3 | Chru | | Unpub01 | |
| TerpSH1 | AY156709 | $(AGAT)_{15}$ | F: CCACTGGGATCTAATCACTT<br>R: GGCAACTTAGCAT | 254–302 | 12 | Mate | | 68 | 69 |
| TerpSH2 | AY156710 | $(AGAT)_{12}$ | F: GCCAGCAGGAGTAATG<br>R: CTATTAGGGCAGAGACGAG | 171–227 | 12 | Mate | | 68 | 69 |
| TerpSH3 | AY156711 | $(CAAA)_{14}$ | F: TCCCCCAATGCACAC<br>R: CTGC*CCAATCCATTTAGA | 283–311 | 8 | Mate | | 68 | 69 |
| TerpSH5 | AY156713 | $(CTAT)_{12}$ | F: TTGCTGCTATATGCTTAAT<br>R: CCTCCCTGCCTATTGA | 157–189 | 8 | Mate | | 68 | 69 |
| TerpSH7 | AY156715 | $(AGAT)_{13}$ | F: CACACACACTGTATTTTGATA<br>R: CTATGCCCTTTCTAGTTTG | 97–137 | 10 | Mate | | 68 | 69 |
| TerpSH8 | AY156716 | $(GA)_{19}$ | F: CCAAATTAAATATCTACC<br>R: AGCCTTTCCAGTATTCAGTA | 193–221 | 14 | Mate | | 68 | 69 |
| Test10 | AY822052 | $(AC)_{15}(TA)_2(GA)_2$ | F: AGACTCTCTGTGATGGTAATAGCA<br>R: GATTTTCATTGGCATATAAGACACA | 194–228 | 10 | Tehe | | 52 | |
| Test21 | AY822048 | $(CA)_{10}(CT)_5$ | F: AAACTGGCTGAAACCCAGC<br>R: TTGGGAGTTTGACTGATCTAGGA | 203–235 | 9 | Tehe | | 68 | |
| Test56 | *AY822049* | $(CT)_6GCT(CA)_{12}$ | F: GATATGCAGGCAAACAGGCT<br>R: CAGGAATCTGTGCATGATTGA | 199–205 | 3 | Tehe | | 68 | |
| Test71 | AY822050 | $(AC)_9$ | F: GATTGTGGTCACATATAGAGGAGG<br>R: TGTTGTACTTAGCTGTTCTGATCTATT | 126–130 | 3 | Tehe | | 68 | |
| Test76 | AY822051 | $(CA)_8$ | F: GAATTCTAACTTTTCTCTGTGGAGC<br>R: TCTTATTGCATATCTGAGTACAGAAGA | 116–118 | 2 | Tehe | | 68 | |
| Test88 | AY822053 | $(TC)_{10}(AC)_8$ | F: TTTCCACAGAAAGGAGGAGC<br>R: CAAATTGAATAAACAGAGTTTTCCC | 181–209 | 5 | Tehe | | 68 | |
| tle10f | | AC | F: TTCTGCTTCTGTGGTTCCACC<br>R: CTGTATTTCAAGGACTCTGCC | 139-155 | 6 | Emma | ELSE | Unpub01 | |
| tle13.1 | | TG | F: TGGGTCTAATTCAGTGAAGAG<br>R: TGAGTTTCAGGCATCTCCTCG | 197-221 | 20 | Emma | ELSE | Unpub01 | |
| tle13.3 | | TG | F: GTGTCAGCCCTCCAGAATGTC<br>R: TCAACGAGAAGCAAATTGAAG | 110-168 | | Emma | | Unpub01 | |
| tle6.2 | | GT | F: GTTTACAGTTCACCTCTTCAG<br>R: TCAATCTAACGTAATTGTGCC | 97-129 | 22 | Emma | | Unpub01 | |
| tle7.2 | | CA | F: ACAGCCATCACGTTTAGCCAC<br>R: GCCAATTTGTTTACATATCCC | 121-141 | 12 | Emma | | Unpub01 | |
| tle16.31 | | AC | F: GACCCTAATCCCCTCCTAATCC<br>R: CCAACCCTTCTGACTCTCACTC | 231-309 | 36 | Emma | ELSE | Unpub01 | |
| tle19.1 | | CTT | F: CTACCACCTGCTTTACCAACC<br>R: GTGAAACCCGATGCTCTTGAACC | 181-202 | 8 | Emma | ELSE | Unpub01 | |
| tle19.3 | | AC | F: CAGCGTTTTGCCCATGGTAAG<br>R: GTGCTAAACCAGTCTCATTGTG | 253-299 | 24 | Emma | | Unpub01 | |
| tle23.41 | | $(GCT)_4CCT(GCT)_4$ | F: CACCCAAGAATACCCGTCACC<br>R: GTACACCCAATGATCACTCG | 176 | 1 | Emma | | Unpub01 | |
| tle28.21 | | AC | F: GCTTTGCCTATCATCCTCTTGC<br>R: CCTGGTCTCATTCAGAAAGG | 133-173 | 17 | Emma | ELSE | Unpub01 | |
| tle31.1 | | $(TC)_{14}(AC)_{10}$ | F: TAACGGAAGGTCTTCAAAGGTC<br>R: GTAGTGTGTCCCAGGCGATTCGAC | 270-384 | 26 | Emma | | Unpub01 | |
| TWS190 | DQ398951 | (TC)9 | F: TTGTTCTGCCATCAGTCAGC<br>R: ATCCCCTTACCACCAACTCC | 091–097 | 3 | Tewe | Tema | 130 | |

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|---|---|---|---|---|---|---|---|---|
| TWL61 | DQ398949 | (CA)13 | F: CCAACCCTGTAGGACTGAAGC<br>R: GTTCCGAGCACTGCAACC | 137–171 | 9 | Tewe | Tema | 130 |
| TWR106 | DQ398952 | (CT)11(CA)19 | F: ACAATCCCACACTCCTTTGC<br>R: CTCACCTTTGGCCCTTCC | 171–227 | 10 | Tewe | Tema | 130 |
| TWL221 | DQ398955 | (TG)12 (TCTG)6TC | F: TGCTGGCTGAAGTTTACAGAG<br>R: CCAGAAGCTGAAGCAACTCC | 217–267 | 6 | Tewe | Tema | 130 |
| TWMD51 | DQ398956 | (AC)7 | F: CACTGGGCAGAAACCAGAAG<br>R: GCTGCATGTGGCTCTTTTAC | 249–251 | 2 | Tewe | Tema | 130 |
| TWI61 | DQ398953 | (GT)11(GA)10 | F: TATTTCAGGCGTGGAGCAAC<br>R: CAATGGGCTACTTGCCTACC | 242–344 | 19 | Tewe | Tema | 130 |
| TWT113 | DQ398954 | (TC)10 | F: CTTTTAGGCTGGGCTGATTG<br>R: ATGCAACCCCAGTACCTCTG | 276–286 | 5 | Tewe | Tema | 130 |
| TWQ113 | DQ398950 | (CT)12 | F: CAGAGGACGTGAGCGAGAG<br>R: TTGAGGATGTTGTAGAGGATGC | 281–293 | 6 | Tewe | Tema | 130 |
| 59HDZ131 | DQ464448 | (CA)12 | F:AAGTTCAGACTGGGCAGGG<br>R:CCACCTTCAGACACACACTCAC | 204–220 | 4 | Erma | | 136 |
| 59HDZ188 | DQ464447 | (CA)9 | F:CTCAAACCAGGGGCTAAAG<br>R:CTATTTCAGGCTGTGGGAGG | 208–214 | 3 | Erma | | 136 |
| 59HDZ196 | DQ464449 | (GT)21 | F:AGGATTCAAACAGTGGAGTGC<br>R:CCCAGACAATGACTAACAAACC | 196–220 | 5 | Erma | | 136 |
| 59HDZ234 | DQ464450 | (CTTT)5 | F:CTCCCACGAAATCTCATGC<br>R:TGTAAGATGCTGGCAAAAGTG | 231–235 | 3 | Erma | | 136 |
| 59HDZ242 | DQ464451 | (GT)17 | F:AGCGGAGAGAGGGGGAAC<br>R:TGAAACAAAGGGGCAATCC | 078–094 | 5 | Erma | | 136 |
| 59HDZ327 | DQ464452 | (TC)8(AC)7AAAA(TC)8(AC)8AATT(TC)9TT(TC)8(AC)11 | F:ACACAGGGTCCATCCACTTC<br>R:TCAGCAAAACAAGCAACGAG | 308–316 | 4 | Erma | | 136 |
| 59HDZ397 | DQ464453 | (GT)7 | F:GAACGCACCAGAACGCAG<br>R:CCCAGAACGCTCCTACATTG | 140–160 | 4 | Erma | | 136 |
| 59HDZ499 | DQ464454 | (CA)9GA(CA)3 GC(CA)14 | F:GTGAGCCCCCAAATSCCC<br>R:TGCTGGACAACTAATCTTTTCTATC | 187–205 | 8 | Erma | | 136 |
| 59HDZ669 | DQ464455 | (GT)9 | F:CCAGGACATCTTAGACTACTGTTCC<br>R:CACTATTTAGGCTTTTCATTCTGC | 225–229 | 4 | Erma | | 136 |
| 59HDZ777 | DQ464456 | (CA)20 | F:GAAAAAAAAAGGGGTGGGG<br>R:AGGGAGTTAGGGGTTGTAGGAG | 134–148 | 7 | Erma | | 136 |
| 59HDZ897 | DQ464457 | (GT)13 | F:TGTGTGGAGAGGGATGGTTC<br>R:GTATGCTTAACCCCCACCTC | 147–159 | 6 | Erma | | 136 |

described in *G. polyphemus* [Schwartz et al., 2003]). Locus GP61 exhibits two different motif states in *G. agassizii*; alleles having greater than 16 repeats have a simple dinucleotde motif, $(GT)_{16+}$, but alleles that score in the range of 10–12 repeats possess a compound motif, $(GT)_4AT(GT)_6$ (Edwards, unpubl. data; Tables 3 and 4). For this locus a single *G. agassizii* individual can be homozygous for either motif or heterozygous for both motifs. Knowledge of the different allelic states can help researchers choose the best model for their analysis, such that an infinite allele model might be a better choice for analyses of these data than a stepwise model of evolution.

While motif differences among species may not affect the utility of a marker within a species, changes that occur across populations within a species might reveal more significant evolutionary changes that would be masked during fragment analysis without subsequent sequencing. For example, locus Goag05 was originally described in *Gopherus agassizii* from samples collected in the Sonoran Desert (Tables 3 and 4; Edwards et al., 2003). Fragment analysis of this locus in *G. agassizii* samples collected from the Mojave Desert reveal amplicon lengths in the range of those observed in the Sonoran samples. However, comparison of locus sequences from both populations revealed fixed differences in the motif indicating that there has been significant evolutionary change between the populations and that gene flow does not occur (Edwards, unpubl. data; Tables 3 and 4). It might also be implied that the motif observed in the Mojave Desert samples is derived from the Sonoran Desert motif. The nucleotide sequence of the flanking regions surrounding the motif also revealed single nucleotide polymorphisms (SNPs) between the two populations. Although microsatellites are generally best applied to genetic studies within a species, these examples suggest that sequencing STR loci and their flanking regions can reveal potentially neutral, autosomal SNPs that imply deeper evolutionary changes and are applicable for inter-species phylogenetic studies.

The development of molecular tools for freshwater turtles and tortoises is not complete. Obviously there is great potential in exploring and applying entirely new molecular techniques, such as sequencing entire mitochondrial genomes (Parham et al., 2006a,b), development of additional informative nuclear markers (Fujita et al., 2004), or microarrays and beyond. Indeed, there are many questions and many species that will require development of new markers or new approaches. However, there is still much to be learned about the biology and conservation of freshwater turtles and tortoises by simply applying the wide array of molecular markers that are already available today. For the majority of common

**Table 4.** Observed motif differences from cross-species amplification of microsatellite loci.

| Locus | Species | Motif |
|---|---|---|
| Cm58 | *Chelonia mydas* | $(CA)_{13}$ |
| | *Gopherus agassizii* | $(CA)_2CG(CT)_4$ |
| GP81 | *Gopherus polyphemus* | $(GT)_{11}(GA)_{10}$ |
| | *Gopherus agassizii* | $(GT)_9GACA(GA)_8$ |
| GP61 | *Gopherus polyphemus* | $(GT)_{12}$ |
| | *Gopherus agassizii* (allelic state 1) | $(GT)_{16+}$ |
| | *Gopherus agassizii* (allelic state 2) | $(GT)_4AT\ (GT)_6$ |
| Goag05 | *Gopherus agassizii* (Sonoran) | $(GAT)_{6\text{-}38}$ |
| | *Gopherus agassizii* (Mojave) | $GACGAA(GAT)_2GACGAA$ |

applications in most species, all the tools needed already exist and are consolidated here. It should be noted, however, that the tables we provide here are incomplete, as many researchers have not included in their publications information such as GenBank accession numbers, STR motifs, expected amplicon size, or other species that a primer might have utility in. We urge those in the research community contributing such data to the scientific literature to include as much information as possible. We are entering a new era in which the cost and time associated with the development of molecular markers should not hinder researchers hoping to apply molecular approaches to important challenges in turtle biology and conservation.

*Acknowledgments*. — This material is based upon work supported by the NSF under grant # DEB-0507916 for the Turtle Genetics workshop held from 7–12 August 2005 at Harvard University. Additional financial support for the workshop came from the Museum of Comparative Zoology (Harvard University), Chelonian Research Foundation, and Conservation International. We thank the many individuals contributed unpublished information for this compilation and we especially thank Brad Shaffer for providing the impetus for this work and Anders Rhodin for his careful and dedicated editing of this volume.

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