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# Universal Primers for Amplification of the Complete Mitochondrial 12S rRNA Gene in Vertebrates

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**Hurng-Yi Wang, Mung-Pei Tsai, Ming-Chung Tu and Sin-Che Lee (2000)** Universal primers for amplification of the complete mitochondrial 12S rRNA gene in vertebrates. *Zoological Studies* **39**(1): 61-66. The conserved regions of *tRNA*$^{PHE}$ and *16S rRNA* in the vertebrate mitochondrial genome were compared in order to design the primers, 12SR and 12SL. These universal primers can be broadly used to amplify a 1.3-kb DNA fragment by polymerase chain reaction (PCR) over a wide range of major vertebrate lineages represented by the species listed in the text. There is little length variation of the PCR product among different taxa. Further sequence analysis revealed that the fragment contains complete lengths of *12S rRNA* and *tRNA*$^{VAL}$, and that the length of *16S rRNA* is 200 bp. In tests through all representative taxa investigated, the above 2 primers could amplify the complete 12S rRNA gene from all representative taxa investigated. As the 12S rRNA gene is widely used for phylogenetic analyses among different hierarchies, the use of these primer sets for study of higher-category phylogenies in vertebrates now becomes possible.

**Key words:** 12SR and 12SL, Primers, PCR amplification, Vertebrates.

Molecular markers are used as tools for estimating the phylogenetic relationships of different kinds of organisms (Avise 1994). Although various techniques, such as allozyme analysis, RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), and mini- and microsatellites, have been employed for molecular systematics, most investigators are examining DNA sequence polymorphism, which is the most fundamental unit of molecular variation. So it is important to choose an appropriate genetic marker for phylogenetic analysis. Ribosomal RNA (rRNA) genes, including 4 nuclear rRNA genes and 2 mitochondrial rRNA genes, are some of the most widely used genetic markers for phylogenetic analyses. The mitochondrial rRNA genes, including *12S* and *16S*, evolve much more rapidly than the nuclear rRNA genes. As *rRNAs* (both among and within genes) evolve at different rates, *rRNA* sequences have been used to infer phylogenies across a very broad spectrum, from studies among the lineages of life to relationships among closely related species and populations (Hillis and Dixon 1991).

Of the rRNA genes, *12S mitochondrial rRNA* has been widely used to study the phylogenetic relationships among different levels of taxa such as families (Alves-Gomes et al. 1995, Douzery and Catzeflis 1995, Ledje and Arnason 1996), genera (Gatesy et al. 1997, Murphy and Collier 1997), and species (Murphy and Collier 1996, Halanych and Robinson 1997). Because of the broad spectrum of phylogenetic analyses of *12S rRNA*, especially in vertebrates, using PCR to amplify the complete region of this gene for further analyses will be very useful. As reported in this paper, we designed primer pairs based on sequences of conserved regions of *tRNA*$^{PHE}$ and *16S rRNA* of the mitochondrial genome from GenBank, and used them to amplify the complete 12S rRNA and partial 16S rRNA genes from major lineages of vertebrates.

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