Supplement to Hugall, A.F., R. Foster and M.S.Y. Lee 2007. Calibration Choice, Rate Smoothing, and the Pattern of Tetrapod Diversification According to the Long Nuclear Gene RAG-1. Systematic Biology (accepted).

Primers

Because of the deep divergences for many sampled taxa, numerous primers had to be tried for most taxa and new primers developed for several. Page 2 of this supplement shows a schematic map of RAG-1 with primer sites and sequences.

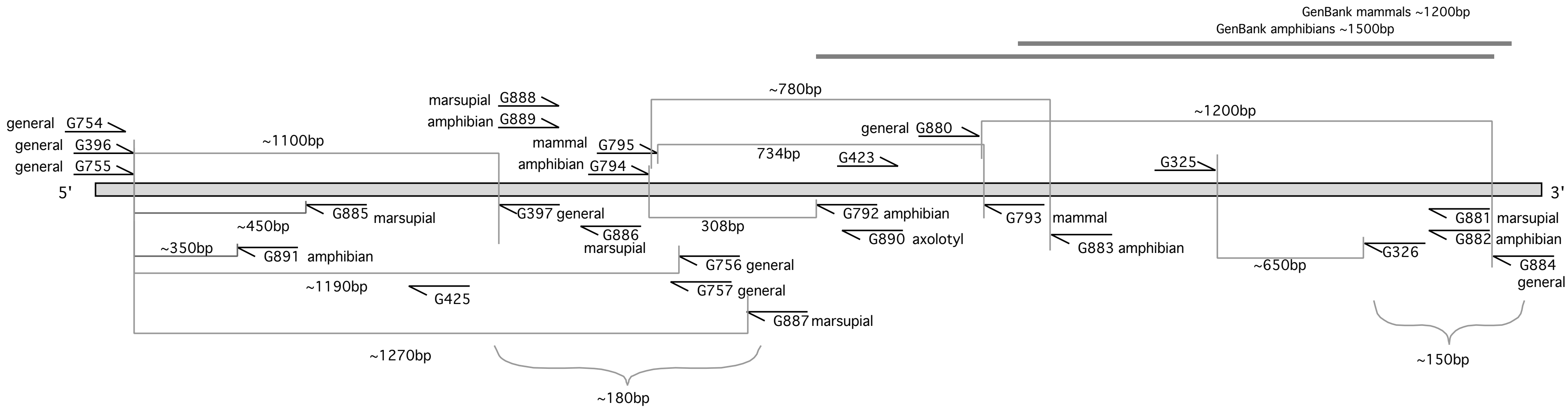
Molecular methods

Total cellular DNA was extracted from frozen or ethanol preserved liver tissue using the salting out method of Miller et al. (1988). PCR amplifications were performed using AmpliTaq Gold with GeneAmp (Roche) reagents, in accordance with the manufacturer's instructions, in 50µl reactions (50 -100ng of DNA, 2mM $MgCl_2$). Amplification was carried out on an Eppendorf Mastercycler and comprised an initial denaturation and polymerase activation step of 95 °C for 9 min. followed by 35 cycles of 94 °C for 45 s, 55 °C for 45 s, 72 °C for 60 s and a final extension step of 72 °C for 6 min. Primers used are listed in Table 2. PCR products were purified for sequencing with an UltraClean PCR clean-up kit (Mo Bio Laboratories) and cycle sequenced in both directions using Big Dye 3.1 (Perkin Elmer) chemistry, following the manufacturer's protocols. Cycle sequencing products were precipitated with isopropanol and analysed with a Prism 3700 Genetic Analyser (ABI). Sequences were edited using SeqEd v1.0.3 (ABI).

Miller S.A., D.D. Dykes and H.F. Polesky.1988. A simple salting out procedure for extracting DNA from nucleated cells. Nucleic Acids Research 16: 1215.

RAG-1 ~3300bp

If the arrows overlap then so do the primers
Length somewhat schematic



Written 5' to 3'

G754	CATCCACATACTAAATTYTCNGARTGGAA	Tm ~ 72°C
G755	AAGTTTTCAGAATGGAAGTTYAAGCTNTT	Tm ~68°C
G756	TCTCCACCTTCTTCYTTNTCAGCAAA	Tm ~ 73°C
G757	TCTTCTTTCTCAGCAAAAGCYTTNACYTG	Tm ~75°C
G792	GCATGCAATGGTTGAAARATYTG	Tm ~ 73°C
G793	GAGTTGGGCTTGGCTTCYTCRAA	Tm ~ 68°C
G794	ACTAGGAGAGCACAAAARCAYCG	Tm ~ 65°C
G795	CAGAAACACCGTCTAAGRGARCT	Tm ~ 62°C

G880	TCYTGTGATGGRATGGGAGAYGT
G881	GAGGTGTACAACCAATGRTGYTT
G882	GATGTATAGAGCCAGTGRTGYTT
G883	TCATGGTCAGATTCATCAGCNARCAT
G884	GCATTATGAGCGTTCATGAAYTTYTG
G885	CAAAACTGGGTGGGGTGGAT
G886	AGTTCTCTTAGACGATGTTTYTG
G887	TCATCTGCTTGTCGATGCTCRTT
G888	AAAGGTGGCAGGCCCCGGCARCA
G889	AAAGGTGGACGCCCTAGGCARCA
G890	GCAAGCAAAGGCTGGAAGATCTG
G891	CCACATATACGGCAAAGNTG

Tm low 60s to mid 70s

Some example combinations

G754,755,396 with G886, 756,757,887 marsupial first 3rd
G888 with 793 masupial middle 3rd
G889 with G883 amphibian middle 3rd
G889 with G890 axolotyl middle 3rd
G880 with 881, 884 last 3rd marsupials
G880 with 882, 884 last 3rd amphibians