

The chloroplast PCR primer database: tools for comprehensive phylogeographic analysis of a whole genome



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The data collected:

A data base is presented which collects published primer information for chloroplast DNA. Additional primers were designed in order to fill gaps where no or little primer information could be found. Chloroplast genes evolve slowly, and many primers have been designed to work across species ('universal primers', e.g. Taberlet et al. 1991, Grivet et al. 2001). Amplicons are either the genes themselves, typically in studies of sequence variation in higher-order phylogeny, or spacers, introns, and intergenic regions (e.g. Graham and Olmstead 2000, Small et al. 1998, Shaw et al. 2005) in studies of phylogeographic patterns within and among species. The current list of 'generic' primers consists of more than 500 sequences.

The methods:

Alignments of fully sequenced chloroplast genomes (retrieved from GenBank 1998-2005), and primer design, were done using standard methods (software: PC/Gene and OMIGA, Accelrys, UK). BLASTALL (NCBI, USA) was used to search for homologies of the primers in 8 chloroplasts (from GenBank, July 2005, except *Populus*): *Nicotiana tabacum*, *Atropa belladonna*, *Spinacia oleracea*, *Arabidopsis thaliana*, *Populus trichocarpa* (Heinze et al. unpublished), *Oryza sativa*, *Pinus thunbergii*, and *Marchantia polymorpha*, with a cut-off E value of 0.5.

Using the database:

Our experience shows that many of the primers can be combined into pairs for PCR quite freely when 'generic' PCR conditions ("stepdown" or "two-step PCR") are applied. With this set of primers it becomes possible to study the whole chloroplast genome for variation in a comprehensive way for many taxa (table: successful amplification with 75 primer pairs from *Fraxinus excelsior*; see also list below right).

primer F/P	primer R/M	amplification	approx. length (bp)
atpB-CP	atpB-M	++	1000
atpB	atpB-M	++	5515-10116
atpB	atpB-M	++	555
atpB	atpB-Fluorescence	++	555
atpB	atpB	++	2100000-21000
atpB1	atpB1	++	1000000-1000
atpB2	atpB2	++	1000000-1000
atpB3	atpB3	++	1000000-1000
atpB4	atpB4	++	1000000-1000
atpB5	atpB5	++	1000000-1000
atpB6	atpB6	++	1000000-1000
atpB7	atpB7	++	1000000-1000
atpB8	atpB8	++	1000000-1000
atpB9	atpB9	++	1000000-1000
atpB10	atpB10	++	1000000-1000
atpB11	atpB11	++	1000000-1000
atpB12	atpB12	++	1000000-1000
atpB13	atpB13	++	1000000-1000
atpB14	atpB14	++	1000000-1000
atpB15	atpB15	++	1000000-1000
atpB16	atpB16	++	1000000-1000
atpB17	atpB17	++	1000000-1000
atpB18	atpB18	++	1000000-1000
atpB19	atpB19	++	1000000-1000
atpB20	atpB20	++	1000000-1000
atpB21	atpB21	++	1000000-1000
atpB22	atpB22	++	1000000-1000
atpB23	atpB23	++	1000000-1000
atpB24	atpB24	++	1000000-1000
atpB25	atpB25	++	1000000-1000
atpB26	atpB26	++	1000000-1000
atpB27	atpB27	++	1000000-1000
atpB28	atpB28	++	1000000-1000
atpB29	atpB29	++	1000000-1000
atpB30	atpB30	++	1000000-1000
atpB31	atpB31	++	1000000-1000
atpB32	atpB32	++	1000000-1000
atpB33	atpB33	++	1000000-1000
atpB34	atpB34	++	1000000-1000
atpB35	atpB35	++	1000000-1000
atpB36	atpB36	++	1000000-1000
atpB37	atpB37	++	1000000-1000
atpB38	atpB38	++	1000000-1000
atpB39	atpB39	++	1000000-1000
atpB40	atpB40	++	1000000-1000
atpB41	atpB41	++	1000000-1000
atpB42	atpB42	++	1000000-1000
atpB43	atpB43	++	1000000-1000
atpB44	atpB44	++	1000000-1000
atpB45	atpB45	++	1000000-1000
atpB46	atpB46	++	1000000-1000
atpB47	atpB47	++	1000000-1000
atpB48	atpB48	++	1000000-1000
atpB49	atpB49	++	1000000-1000
atpB50	atpB50	++	1000000-1000
atpB51	atpB51	++	1000000-1000
atpB52	atpB52	++	1000000-1000
atpB53	atpB53	++	1000000-1000
atpB54	atpB54	++	1000000-1000
atpB55	atpB55	++	1000000-1000
atpB56	atpB56	++	1000000-1000
atpB57	atpB57	++	1000000-1000
atpB58	atpB58	++	1000000-1000
atpB59	atpB59	++	1000000-1000
atpB60	atpB60	++	1000000-1000
atpB61	atpB61	++	1000000-1000
atpB62	atpB62	++	1000000-1000
atpB63	atpB63	++	1000000-1000
atpB64	atpB64	++	1000000-1000
atpB65	atpB65	++	1000000-1000
atpB66	atpB66	++	1000000-1000
atpB67	atpB67	++	1000000-1000
atpB68	atpB68	++	1000000-1000
atpB69	atpB69	++	1000000-1000
atpB70	atpB70	++	1000000-1000
atpB71	atpB71	++	1000000-1000
atpB72	atpB72	++	1000000-1000
atpB73	atpB73	++	1000000-1000
atpB74	atpB74	++	1000000-1000
atpB75	atpB75	++	1000000-1000

primer F/P	primer R/M	amplification	approx. length (bp)
atpB76	atpB76	++	1000000-1000
atpB77	atpB77	++	1000000-1000
atpB78	atpB78	++	1000000-1000
atpB79	atpB79	++	1000000-1000
atpB80	atpB80	++	1000000-1000
atpB81	atpB81	++	1000000-1000
atpB82	atpB82	++	1000000-1000
atpB83	atpB83	++	1000000-1000
atpB84	atpB84	++	1000000-1000
atpB85	atpB85	++	1000000-1000
atpB86	atpB86	++	1000000-1000
atpB87	atpB87	++	1000000-1000
atpB88	atpB88	++	1000000-1000
atpB89	atpB89	++	1000000-1000
atpB90	atpB90	++	1000000-1000
atpB91	atpB91	++	1000000-1000
atpB92	atpB92	++	1000000-1000
atpB93	atpB93	++	1000000-1000
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atpB100	atpB100	++	1000000-1000
atpB101	atpB101	++	1000000-1000
atpB102	atpB102	++	1000000-1000
atpB103	atpB103	++	1000000-1000
atpB104	atpB104	++	1000000-1000
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