

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 unpublished), *Oryza sativa*, *Pinus thunbergii*, and *Marchantia polymorpha*, with a cut-off E value of 0.5.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)
atpA	atpA	++	1000
atpB	atpB	++	5515-10161
atpE	atpE	++	555
atpH	atpH	++	2100-2400
atpI	atpI	++	1000-1500
atpJ	atpJ	++	1000-1500
atpK	atpK	++	1000-1500
atpL	atpL	++	1000-1500
atpM	atpM	++	1000-1500
atpN	atpN	++	1000-1500
atpO	atpO	++	1000-1500
atpP	atpP	++	1000-1500
atpQ	atpQ	++	1000-1500
atpR	atpR	++	1000-1500
atpS	atpS	++	1000-1500
atpT	atpT	++	1000-1500
atpU	atpU	++	1000-1500
atpV	atpV	++	1000-1500
atpW	atpW	++	1000-1500
atpX	atpX	++	1000-1500
atpY	atpY	++	1000-1500
atpZ	atpZ	++	1000-1500
atp1	atp1	++	1000-1500
atp2	atp2	++	1000-1500
atp3	atp3	++	1000-1500
atp4	atp4	++	1000-1500
atp5	atp5	++	1000-1500
atp6	atp6	++	1000-1500
atp7	atp7	++	1000-1500
atp8	atp8	++	1000-1500
atp9	atp9	++	1000-1500
atp10	atp10	++	1000-1500
atp11	atp11	++	1000-1500
atp12	atp12	++	1000-1500
atp13	atp13	++	1000-1500
atp14	atp14	++	1000-1500
atp15	atp15	++	1000-1500
atp16	atp16	++	1000-1500
atp17	atp17	++	1000-1500
atp18	atp18	++	1000-1500
atp19	atp19	++	1000-1500
atp20	atp20	++	1000-1500
atp21	atp21	++	1000-1500
atp22	atp22	++	1000-1500
atp23	atp23	++	1000-1500
atp24	atp24	++	1000-1500
atp25	atp25	++	1000-1500
atp26	atp26	++	1000-1500
atp27	atp27	++	1000-1500
atp28	atp28	++	1000-1500
atp29	atp29	++	1000-1500
atp30	atp30	++	1000-1500
atp31	atp31	++	1000-1500
atp32	atp32	++	1000-1500
atp33	atp33	++	1000-1500
atp34	atp34	++	1000-1500
atp35	atp35	++	1000-1500
atp36	atp36	++	1000-1500
atp37	atp37	++	1000-1500
atp38	atp38	++	1000-1500
atp39	atp39	++	1000-1500
atp40	atp40	++	1000-1500
atp41	atp41	++	1000-1500
atp42	atp42	++	1000-1500
atp43	atp43	++	1000-1500
atp44	atp44	++	1000-1500
atp45	atp45	++	1000-1500
atp46	atp46	++	1000-1500
atp47	atp47	++	1000-1500
atp48	atp48	++	1000-1500
atp49	atp49	++	1000-1500
atp50	atp50	++	1000-1500
atp51	atp51	++	1000-1500
atp52	atp52	++	1000-1500
atp53	atp53	++	1000-1500
atp54	atp54	++	1000-1500
atp55	atp55	++	1000-1500
atp56	atp56	++	1000-1500
atp57	atp57	++	1000-1500
atp58	atp58	++	1000-1500
atp59	atp59	++	1000-1500
atp60	atp60	++	1000-1500
atp61	atp61	++	1000-1500
atp62	atp62	++	1000-1500
atp63	atp63	++	1000-1500
atp64	atp64	++	1000-1500
atp65	atp65	++	1000-1500
atp66	atp66	++	1000-1500
atp67	atp67	++	1000-1500
atp68	atp68	++	1000-1500
atp69	atp69	++	1000-1500
atp70	atp70	++	1000-1500
atp71	atp71	++	1000-1500
atp72	atp72	++	1000-1500
atp73	atp73	++	1000-1500
atp74	atp74	++	1000-1500
atp75	atp75	++	1000-1500

<http://bfw.ac.at/200/1859.html>

total number of entries

587

results of BLASTALL homology searches:

anchored in <i>Nicotiana tabacum</i>	575
anchored in <i>Atropa belladonna</i>	530
anchored in <i>Spinacia oleracea</i>	427
anchored in <i>Arabidopsis thaliana</i>	421
anchored in <i>Populus trichocarpa</i>	427
anchored in <i>Oryza sativa</i>	395
anchored in <i>Pinus thunbergii</i>	301
anchored in <i>Marchantia polymorpha</i>	226
present in all 8 chloroplast genomes	152
present in <i>Nicotiana</i> and <i>Atropa</i>	530
present in <i>Nicotiana</i> , <i>Atropa</i> , <i>Spinacia</i>	421
present in <i>Nicotiana</i> <i>Atropa</i> <i>Arabidopsis</i> <i>Populus</i>	369
present in <i>Arabidopsis</i> and <i>Populus</i>	372
present in all but <i>Oryza</i> , <i>Pinus</i> , <i>Marchantia</i>	333
present in all but <i>Pinus</i> and <i>Marchantia</i>	289
present in all but <i>Marchantia</i>	152
within trn genes	100
within photosystem genes (psa, psb)	83
within ribosomal proteins	
and RNA polymerase (rpl, rps, rpo)	92
within ATPase genes (atp)	28
not anchored in identified genes	165
within ycf genes	122
within rbcL	35
within NADH-specific dehydrogenase (ndh) genes	18

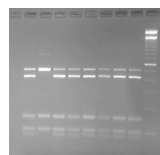
Variation in chloroplast DNA fragments

Efficient methods for analysing polymorphisms are necessary - traditional sequencing may not be an option in large-scale studies. Alternatives are simple PCR-RFLP in gels, or denaturing high-performance liquid chromatography for simultaneous detection and analysis of polymorphisms.

Agarose PCR-RFLP

After PCR, samples are scanned on agarose gels for successful amplification. An aliquot of the PCR is treated with restriction enzymes. Restriction polymorphisms and major insertions-deletions can be detected in high-percentage agarose gels.

Example: Polymorphism in *Fraxinus excelsior* - second lane shows different banding pattern



References

- Graham, S.W. and Olmstead, R.G. (2000) Utility of 17 chloroplast genes for inferring the phylogeny of the basal angiosperms. *American Journal of Botany*, **87**, 1712-1730.
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- Taberlet, P., Gielly, L., Pautou, G. and Bouvet, J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.*, **17**, 1105-1109.

Denaturing HPLC

A mixture of the PCR products of sample and a standard plant is heated and cooled to encourage heteroduplex DNA formation, then applied to a silica or polymer column (Varian HELIX system). At a certain temperature specific for each fragment, heteroduplex molecules start to melt and are eluted with different patterns (additional peaks).

Example: dHPLC analysis of *Fraxinus excelsior* chloroplast PCR fragment

