

Chloroplast genome research - A PCR primer database for the comprehensive analysis of a whole genome

B. HEINZE, A. CASTILLO, C. CALDERÓN, B. FUSSI and W. ARTHOFER

An internet data base is presented which collects published primer information for chloroplast DNA (<http://bfw.ac.at/200/1859.html>). 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 phylogenetic studies (sequencing of chloroplast genes), or spacers, introns, and intergenic regions in studies of phylogeographic patterns within and among species (e.g. GRAHAM and OLMSTEAD 2000, SMALL et al. 1998, SHAW et al. 2005). The current list of such primers consists of more than 500 sequences.

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 taba-*

cum, *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 unpublished), *Oryza sativa*, *Pinus thunbergii* and *Marchantia polymorpha*, with a cut-off E value of 0.5. The chloroplast of black cottonwood (*Populus trichocarpa*) clone 'Nisqually-1' has been shotgun sequenced to a depth >400X and may become the new 'gold standard' for chloroplast DNA research (TUSKAN et al. 2006, HEINZE et al. unpublished).

Efficient methods for analysing polymorphisms are necessary - traditional sequencing may not be an option in large-scale studies, or when searching for very polymorphic regions in the chloroplast. Alternatives are simple PCR-RFLP in gels, or denaturing high-performance liquid chromatography. The most common polymorphisms between related lines are SNPs or small insertions-deletions (HEINZE and CALDERÓN, unpublished), and the two methods presented differ in their sensitivity to detect those polymorphisms. PCR-RFLP is amenable to comparisons across laboratories, if certain standards in the procedure are met.

References

- GRAHAM, S.W. and R.G. OLMSTEAD, 2000: Utility of 17 chloroplast genes for inferring the phylogeny of the basal angiosperms. *American Journal of Botany*, 87, 1712-1730.
- GRIVET, D., B. HEINZE, G.G. VENDRAMIN and R.J. PETIT, 2001: Genome walking with consensus primers: application to the large single copy region of chloroplast DNA. *Molecular Ecology Notes*, 1, 345-349.
- SHAW, J., E.B. LICKEY, J.T. BECK, S.B. FARMER, W. LIU, J. MILLER, K.C. SIRIPUN, C.T. WINDER, E.E. SCHILLING and R.L. SMALL, 2005: The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Am. J. Bot.*, 92, 142-166.
- SMALL, R.L., J.A. RYBURN, R.C. CRONN, T. SEELANAN and J.F. WENDEL, 1998: The tortoise and the hare: Choosing between non-coding plastome and nuclear ADH sequences for phylogeny reconstruction in a recently diverged plant group. *American Journal of Botany*, 85, 1301-1315.
- TABERLET, P., L. GIELLY, G. PAUTOU and J. BOUVET, 1991: Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.*, 17, 1105-1109.
- TUSKAN, G.A., S. DIFAZIO, S. JANSSON et al., 2006: The Genome of black cottonwood, *Populus trichocarpa* (Torr. and Gray). *Science*, 313, 1596-1604.

Autoren: Dr. Berthold HEINZE and Mag. Barbara FUSSI, Federal Research Centre for Forests, Department of Genetics, Hauptstraße 7, A-1140 VIENNA; Almudena CASTILLO and Carmen CALDERÓN BSc, present address: Institute for Sustainable Agriculture (IAS-CSIC), Alameda del Obispo s/n, E-14080 CÓRDOBA; Dr. Wolfgang ARTHOFER, present address: Universität für Bodenkultur, Institut für Forstentomologie, Forstpathologie und Forstschutz, Hasenauerstraße 38, A-1190 WIEN. berthold.heinze@bfw.gv.at

