## Protocols

1. DNA extraction
2. PCR
3. Sequence analysis

## 1. DNA extraction

Material is shock-frozen in liquid nitrogen, and stored at -80 °C. The frozen material is ground using a high-throughput disruptor (TissueLyser, Qiagen, Germany), and the DNA is extracted using the InnuPrep Plant DNA Kit (Macherey-Nagel) following the instructions of the producer. Around 50-100 mg of plant material is sufficient. The kit is based on affinity to a column, whereby the DNA binds to the matrix, such that contaminants can be eluted. Subsequently, the DNA can be eluted by elution buffer or nuclease-free water. Typical volumes are 100 µl of eluate. Quality is controlled by spectrophotometry via the Nano-Drop. The device is first initialised by pipetting 1.5 µl of nuclease-free water. Then the device is calibrated by pipetting 1.5 µl of the blank (the solvent for the DNA), then the DNA sample is measured (1.5 µl). The values give the concentration of DNA (in ng/µl) and its purity (determined as $A_{260}/A_{280}$ measuring contamination by proteins and $A_{260}/A_{230}$ measuring contaminatoin by polysaccharides). The $A_{260}/A_{280}$ should be ideally 1.8, higher values indicate RNA contamination, lower values indicate protein contamination. The $A_{260}/A_{230}$ value should be between 1.8 and 2.2. The concentration should then be adjusted to 50 ng / µl for optimal use in genomic PCR.

## 2. PCR

Sample volume is 10 µl. Each sample with 1 µl buffer (10x), 1 µl BSA (10 mg / ml), 0.2 µl of each primer (10 pmol), 0.2 µl dNTPs (10 mmol each), and 0.1 µl Taq (New England Biolabs, Frankfurt) and ~0.5 µl template (50 ng/µl).

**Table 1:** PCR protocol for amplification of tnrH-psbA

| step | | temperature | Time |
|---|---|---|---|
| initial denaturation | | 95°C | 2:00 min |
| denaturation | 35x | 95°C | 0:30 min |
| annealing | | 60°C | 0:30 min |
| elongation | | 68°C | 1:00 min |
| final elongation | | 68°C | 5:00 min |
| storage | | 12°C | - |

**Table 2:** oligonucleotide primers for typical plant barcodes.

| target | Sequence | Amplicon (bp) |
|---|---|---|
| | | |
| rbcLa* | F: 5-ATGTCACCACAAACAGAGACTAAAGC-3<br>R: 5-CGTGGTGGACTTGATTTTAC-3 | 599 |
| matK | F: 5-ACCCAGTCCATCTGGAAATCTTGGTTC-3<br>R: 5-CGTACAGTACTTTTGTGTTTACGAG-3 | 878 |
| trnL-F igs | F: 5-CGAAATCGGTAGACGCTACG-3<br>R: 5-ATTTGAACTGGTGACAGAG-3 | 803-1209 |
| trnH-psbA igs** | F: 5-GTTATGCATGAACGTAATGCTC-3<br>R: 5-CGCGCATGGTGGATTCACAATCC-3 | 420-446 |

*source for the oligonucleotide primers for rbcLa

Levin RA, Wagner WL, Hoch PC, et al. (2003) Family Level Relationships of Onagraceae Based on Chloroplast rbcL and ndhF Data. Am J Bot 90, 107-115

**source for the oligonucleotide primers for tnrH-psbA

Sang T et al. (1997) Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). Am J Bot 84, 1120.

Tate JA, Simpson BB (2003): Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. Systematic Botany 28, 723–737.

**Trouble shooting for recalcitrant samples (e.g. dried material from commercial samples).** Here, the DNA can be extracted using the Invisorb® MSpin Plant Mini Kit (Stratec Molecular) following the instructions of the producer. Further, for the PCR 10 mg/ml of bovine serum albumin were added.

## 3. Sequence analysis

The sequences are processed like explained in the sequence tutorial. Basically, the quality of the sequences is assessed by visual inspection of the chromatogramms, by BLAST search of the sequence and validation of the sequence identity, and by alignment of the two reads (forward and reverse complement) using the Alignment Explorer of the freeware MEGA (http://www.megasoftware.net/). In addition, the NCBI database can be searched by BLAST for homologues, which can be selected by help of the Taxonomy View tool to get a relevant collection of homologues from related taxa. The alignment can then be trimmed and used to construct and bootstrap a Neighbor-Joining (NJ) tree.