A Simple and Rapid Protocol for Preparation of Crude Drug DNA Suitable for PCR

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A protocol for rapid purification of DNA from a variety of herbal crude drugs, starting from samples as small as 1 mg, has been developed. Neither phenol extraction nor alcohol precipitation is involved in the procedure. Total nucleic acids are first extracted with the lysis buffer containing SDS. After CHCl_3 extraction, DNA is bound to glass fiber fleece in the presence of guanidine thiocyanate, while unbound impurities are washed away. The entire procedure can be carried out in 1.5-ml microtubes. The purified DNA obtained serves as a template for PCR.

Key words DNA purification protocol; molecular authentication; herbal crude drug

Correct identification of crude drugs is a prerequisite for chemical and pharmacological investigations of natural medicines, and also for their clinical applications. To this end, DNA profiling (DNA-based polymorphic assay) has several advantages over morphological and chemical assays, including: 1) not the phenotype but the genotype is directly assayed and therefore the result is not influenced by environmental factors; 2) one or more gene sequences with the appropriate evolutionary rate for solving the particular problem can be selected for the assay; and 3) the required sample amount is very small, especially if the polymerase chain reaction (PCR) is involved in the assay. Because of these potential merits, the authentication of crude drugs using DNA markers is attracting increased attention.¹⁾

Extraction of DNA from crude drugs is difficult due to the presence of large amounts of phenolic compounds and/or polysaccharides, and various laborious steps must be incorporated to obtain DNA suitable for PCR amplification. Thus the establishment of a simple but reliable procedure that allows extraction of DNA of purity sufficient for PCR amplification from crude drugs is desirable. In this communication, we describe a simple, rapid, small-scale protocol for the extraction of crude drug DNA suitable for PCR. The present method is adapted from that of Kikuchi *et al.*,²⁾ with significant modifications. Although the method was originally developed for DNA extraction from the crude drugs "Atractylodes rhizome" (*Byaku-jutsu*) and "Atractylodes lancea rhizome" (*So-jutsu*), it is also applicable to various other crude drugs of plant origin.

Powdered crude drugs (10–1 mg) were homogenized with 100 μl lysis buffer comprising 400 mM tris-HCl, pH 8.0, 150 mM NaCl, 60 mM EDTA, 10 mM 2-mercaptoethanol, 1% polyvinylpyrrolidone and 1% SDS using a microhomogenizer in a 1.5-ml microtube and incubated at 60 °C for 20 min with occasional vortexing. Then 50 μl of 3 M sodium acetate was added to the homogenate which was further incubated in ice for additional 20 min. The slurry was extracted with 200 μl CHCl_3 and centrifuged at 2000 *g* for 15 min at room tem-

perature. The aqueous layer was transferred to a new microtube and further purified using either a High Pure PCR Product Purification Kit (Roche) or Sephaglas BandPrep Kit (Pharmacia) according to the protocol supplied by the manufacturer. The DNA content in the preparation was estimated fluorometrically using a DyNA Quant 200 Fluorometer (Pharmacia). Calf thymus DNA was used as the standard.

Using the described protocol, DNA 3 μg and 0.4 μg was obtained from powdered Atractylodes rhizome 10 mg and 1 mg, respectively. To evaluate whether the DNA preparation was suitable for use as a PCR template, the 5'-region (260 bp) of the chloroplast *trnK* gene was amplified using a primer set designed based on the *trnK* sequence of *Atractylodes* species.³⁾ PCR was carried out for 30 cycles in 25 μl PCR buffer containing 5 nmol of each dNTP, 10 pmol of each primer, 10 ng of template DNA, and 1 unit of *Taq* DNA polymerase (Roche). At1F=5'-GGGTTGCTAACTCCAA-CGGTA-3' and At1R=5'-CGACCCAACTTGAAATTGAC-3' were used as a forward and reverse primer, respectively, to amplify the region. Each PCR cycle consists of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 1 min.

As shown in Fig. 1, no amplification product was detected when DNA preparation from the crude drug Atractylodes rhizome was applied to PCR prior to the final purification step (lane C). This was presumably due to the presence of impurities inhibiting DNA polymerase activity in the DNA preparation. A detectable amount of the PCR product was accumulated after the second round of PCR, in which 5 μl of the first PCR mixture was used as template of the reamplification (data not shown). However, it is not desirable to repeat PCR not only because it is time-consuming but also because

Fig. 1. Agarose Gel Electrophoresis of PCR Products Obtained from Fresh Leaves of *Atractylodes japonica* and the Crude Drug Atractylodes Rhizome (*Byaku-jutsu*)

Amplification was carried out with 10 ng of template DNA and the primer set designed to amplify the 260-bp 5'-region of the chloroplast *trnK* gene. The product was separated on 2% agarose gel and visualized by ethidium bromide staining. Lane A, 100-bp ladder size marker; lane B, template DNA was prepared from fresh leaves of *A. japonica*; lane C, template DNA was extracted from the crude drug Atractylodes rhizome; lanes D and E, template DNA was extracted from the crude drug Atractylodes rhizome and further purified using (lane D) the Sephaglas BandPrep Kit (Pharmacia) or (lane E) the High Pure PCR Product Purification Kit (Roche).

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the chance of incorporation of mismatched nucleotides into the PCR product increases proportionally to the number of PCR cycles. Therefore we incorporated a final purification step based on the specific binding of DNA to the surface of glass fibers or silica materials in the presence of chaotropic ions.⁴⁾ We examined two commercially available kits, High Pure PCR Product Purification Kit (Roche) and SephaGel BandPrep Kit (Pharmacia), for this purpose. The former kit was originally designed to separate PCR products from other PCR components including oligonucleotide primers, while the latter was designed to elute DNA from agarose gel, although both kits are based on essentially the same principle. When crude drug DNA purified using these kits was used as the PCR template, the amplified fragment of the expected size (260 bp) was clearly detected after one round of PCR (Fig. 1, lanes D and E). Either kit works well, but the High Pure PCR Product Purification Kit protocol is simpler and faster. We prepared DNA from two specimens of *Atractylodes lancea* rhizome and four specimens of *Atractylodes rhizome* obtained at a local market using the present protocol and obtained the 260-bp fragment of the chloroplast *trnK* gene from all DNA preparations (data not shown).

To clarify whether the above procedure is applicable to various crude drugs other than those from *Atractylodes* species, DNA was prepared from six different crude drugs and used as templates for random amplified polymorphic DNA (RAPD) analysis. Amplification was carried out in a 25- μ l reaction volume containing 5 nmol of each dNTP, 20 pmol of a 12-mer arbitrary primer, 0.5 unit of *Taq* DNA polymerase (GIBCO BRL), and 20 ng of template DNA for 40 cycles of 94 °C for 30 s, 38 °C for 30 s, and 72 °C for 90 s. As shown in Fig. 2, RAPD patterns specific to each crude drug species were obtained from these crude drugs. This indicates that the described protocol is applicable not only to crude drugs from *Atractylodes* species but to a variety of herbal crude drugs.

In conclusion, the present protocol allows rapid and easy purification of DNA suitable for PCR analysis from a variety of herbal crude drugs without phenol extraction or alcohol precipitation. It is also suitable for automation, and can facilitate studies of the integration of DNA genetic markers in crude drug authentication.

Fig. 2. RAPD Profiles of DNA Prepared from Various Herbal Crude Drugs

Amplification was carried out using 20 ng of template DNA and a 12-mer arbitrary primer (5'-ACTGGCCGAGGG-3'). The PCR products were separated by 1.0% agarose gel electrophoresis and detected with a FluorImager 595 (Molecular Dynamics) after SYBR gold staining. Size markers, λ DNA digested with *Hin* dIII. Control represents the amplification product of PCR without adding template DNA.

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