Rapid DNA Extraction from Plants

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1 Introduction

The first step of RAPD fingerprinting is the preparation of the target DNA template. Intuitively, minimal DNA template preparation should be necessary for RAPDs since, theoretically, PCR may amplify a single DNA molecule. It seems one would simply homogenize tissue and allow the PCR to "find" and amplify the target DNA. Indeed, many rapid DNA isolation methods designed for use with PCR actually involve little isolation of DNA. Rather, they employ a "grind and use" process (e.g.,Wang et al. 1993) or minimal purification (e.g., Edwards et al. 1991). These methods are very fast, require little tissue and amplify well with plants that are amenable to DNA extraction and that contain few interfering secondary metabolites. However, the resulting DNA templates are not very pure and may not be stable for long periods of time. Recently, DNA purity has been implicated as one of the most important factors in RAPD reproducibility (McClelland and Welsh 1994). McClelland and Welsh (1994) suggest that researchers use only high quality templates to assure reproducible RAPDs. The assay they suggest consists of replicate RAPD PCRs in which the DNA concentration is titrated over two orders of magnitude. If the DNA is of adequate quality the replicates should yield identical RAPD fingerprints. In addition, Heinze (1994) found that the addition of RNAse A improved RAPD reproducibility with gymnosperm embryos. However, the basal method was a crude prep similar to that of Edwards et al. (1991). Thus, it seems likely that there could be several interactive factors involving DNA purity or lack thereof in RAPD reproducibility. It seems prudent to researchers performing RAPD PCR to assure themselves that the isolated DNA is of sufficient quality to ensure reproducible RAPD fingerprints.

The objective of this chapter is to describe a rapid CTAB miniprep that yields relatively pure DNA from plants and that can also be used

with fungi and fish. The procedure features the cationic detergent CTAB (hexadecyltrimethylammonium bromide) in the homogenization buffer, one chloroform extraction, and an alcohol precipitation. This procedure was published by Stewart and Via (1993) and has its origins in previously published procedures (Murray and Thompson 1980; Doyle and Doyle 1987, 1990). The procedure described in this chapter further streamlines the Stewart and Via (1993) miniprep procedure.

2 Materials

Equipment

cordless electric drill capable of speeds between 500 and 1000 rpm (e.g., Black and Decker CD 2000 "Ranger")

Supplies

disposable pellet pestles (e.g., Bio-Ventures catalog# P-50 Murfreesboro, Tennessee, USA). Alternatively, one may use a 1000 µl plastic pipette tip that has been pushed onto a deburring tool as a homogenizing pestle (Stewart and Via 1993). The end o the pipette tip is crimped upward when pressed on the tool (th tip is pressed against the bottom of the pipette tip box), thereb creating a "blade" for homogenization.

Reagents and solutions

homogenization buffer (amounts stated are for 1 l; all reagent may be purchased through Sigma Chemicals):100 mM Tris-HC (15.76 g in 800 ml of water, pH adjusted to 8.0 with NaOH), 20 ml EDTA (40 ml of 0.5 M EDTA, pH 8.0), 2 % w/v CTAB (20 g), 1.42 l NaCl (81.8 g), 2 % w/v PVP-40 (polyvinylpyrrolidone, averag molecular weight 40000) (20 g), 5 mM ascorbic acid (0.88 g 4.0 mM DIECA (diethyldithiocarbamic acid) (0.69 g). Bring vo ume to 1 l. This solution may be kept indefinitely at room tempe ature. Just prior to use, add 6 µl 2-mercaptoethanol per ml homogenization buffer.
extraction solution: chloroform:isoamyl alcohol (24:1). This m be stored indefinitely at room temperature in a sealed containe ethanol or isopropanol

3 Experimental Procedure

1. Harvest between 0.02 and 0.2 g fresh leaf, callus, embryo, sti (or appropriate animal tissue) into a microfuge tube. Place on i These may be sufficiently stable for hours to days, depend upon species.

Note: For plants, best quality and yield is obtained when newly flushing leaves are used. However, the procedure has been used with a plethora of tissues, including herbarium specimens.

Caution: All subsequent steps should be performed in a fume hood.

2. Aliquot 500 µl of homogenization buffer (including the 2-mercaptoethanol) into each microfuge tube. For tough leaves from woody plants (exemplified by oaks or cranberry) heating the buffer to 65°C may increase yield.
3. Chuck pestle directly onto drill and homogenize tissue. The disposable pestles may be reused after decontamination with 10 % commercial bleach followed by subsequent washes with water.
4. Incubate tubes at 65°C for 5–60 min. The incubation time is not crucial for most species and can be determined by investigator convenience.
5. Add 500 µl extraction solution to tubes and mix. For most plant species this may consist of ten inversions. For recalcitrant species (such as woody plants) placing tubes horizontally on a shaker (500 rpm) for 5–10 min may increase yield.
6. Centrifuge at 3000 *g* for 5 min. Slightly higher *g* or longer centrifugation periods may be necessary to keep the interface compact and the top layer clear if many (>12) samples are centrifuged at a time.
7. Pipette top (aqueous) layer (about 400 µl) into a fresh tube.
8. Add 800 µl ethanol or 270 µl isopropanol and invert several times to precipitate DNA. The sample may be allowed to set at room temperature or 4°C for 5–60 min to increase DNA yield. Longer periods will not affect DNA quality, but may slightly increase yield.
9. Centrifuge at 13000–16000 *g* for 10–20 min at room temperature.
10. Decant supernatant, air-dry pellet, and resuspend in 50–500 µl water.

4 Results and Comments

This method has been used with success in several plants (*Arabidopsis*, banana, rapid cycling *Brassicas*, canola, chestnut, cranberry, and tobacco, to name a few), fungi (such as *Russula* spp.) and fish (such

as *Rivulus marmoratus*). DNA yields, as quantified using Hoechst 33258 dye fluorometry, are typically between 1and 50 µg depending on species, starting material and extraction conditions mentioned earlier. This represents enough DNA for between 40 and 2000 RAPD PCRs. RAPD fingerprints using this method compare favorably to those using very pure DNA isolated by the method of Doyle and Doyle (1990) and subsequent purification through a CsCl ethidium bromide gradient (Stewart and Via 1993). This method has recently been used to isolate DNA for restriction digests and subsequent Southern blotting (e.g., our lab; May et al. 1995). Restrictability is another indication of high quality DNA. The prepared DNA is stable in time. For example, cranberry DNA templates that were extracted over 2 years ago and stored at -20°C yield identical RAPD fingerprints now as when the DNA was first extracted. In addition, they continue to pass the McClelland and Welsh (1994) test and produce reproducible RAPD fingerprints when template amounts are varied over two orders of magnitude (5–500 ng). In summary, the CTAB miniprep method is rapid (one person can process 100–200 samples in a typical workday) and yields adequately pure DNA for RAPD fingerprinting.

References

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