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*Commentary*

# Microprep Protocol for Extraction of DNA from Tomato and other Herbaceous Plants

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The extraction of DNA from plant tissue is a critical and often very time-consuming step in many plant molecular biology procedures. This is especially true for studies of molecular genetics, QTLs, or molecular-marker-based breeding where hundreds or even thousands of plant samples need to be analyzed in a short period of time. Many protocols are laborious and are limited by the need for large amounts of plant tissue.

Based on the methods originally described by Murray et al. (1980), we have developed a procedure in our laboratory that maximizes the number of plant samples one person can extract and that yields sufficient DNA for 50 to 100 PCR reactions or two to four Southern blots. The use of very small, new leaves makes it possible to extract DNA from seedlings only one to three weeks old, reducing the need for large amounts of greenhouse space. The entire procedure can be done in 1.5-mL microcentrifuge tubes, eliminating the need for large centrifuges. Using this procedure, one person can isolate DNA from several hundred plants per day.

## Materials and Solutions

*Drill*: Heavy duty household drill with keyless chuck (so pestles can be easily replaced) and plastic drill bit / pestles (VWR Scientific, catalog no. KT95050-99).
*Microcentrifuge*: with a fixed-angle rotor, capable of 10,000 rpm and holding as many samples as possible.

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**Abbreviation:** PCR, polymerase chain reaction.

*DNA extraction buffer*: 0.35 M sorbitol, 0.1 M tris-base, 5 mM EDTA, pH 7.5.
*Nuclei lysis buffer*: 0.2 M tris, 0.05 M EDTA, 2 M NaCl, 2 % CTAB.
*Sarkosyl*, 5 % w/v.
*Microprep buffer*: 2.5 parts DNA extraction buffer, 2.5 parts nuclei lysis buffer, 1.0 part 5% Sarkosyl. Add 0.3 to 0.5 g sodium bisulfite/100 mL buffer immediately before use (can be increased to avoid color in final product).

For 75 extractions: 25 mL DNA extraction buffer; 25 mL nuclei lysis buffer; 10 mL Sarkosyl; 60 mL microprep buffer; add 0.2 g sodium bisulfite.

## Protocol

- Collect 50-100 mg of leaf tissue (approximately 4–8 new leaflets, up to 1.5 cm long) from a 1- to 3-week-old tomato seedling[1] and nestle loosely in the bottom of a 1.5 mL Eppendorf tube.[2]
- Prepare fresh microprep buffer (see recipe below); keep at room temperature.
- Add 200 µL of buffer and grind tissue with power drill and plastic bit, rinsing pestle with water between samples; add another 550 µL of buffer and either vortex lightly or shake entire rack by hand.
- Incubate in 65 °C waterbath for 30–120 minutes.
- Fill the tube with chloroform:isoamyl (24:1). Mix well. (This can be done by vortexing each tube or sandwiching tubes between two racks and vigorously inverting or shaking up and down 50–100 times).
- Centrifuge tubes at 10,000 rpm for 5 minutes.
- Pipet off aqueous phase (usually approximately 0.5 mL) into new microfuge tubes. Add 2/3 to 1 times the volume of cold isopropanol to each tube. Invert tubes repeatedly until DNA precipitates.
- Immediately spin at 10,000 rpm for 5 minutes (no more), pour off isopropanol and wash pellet with 70% ethanol.[3]
- Dry pellet by leaving tubes upside down on paper towels for approximately 1 h or placing on sides in seed dryer for 15 minutes (longer if necessary).
- Resuspend DNA in 50 µL of TE at 65 °C for 15 min.
- Spin 10 min at 10,000 rpm, store at 4 °C for up to 1 week or -20 °C for longer storage.
- For RFLP use, digest 15 to 25 µL for one Southern blot (can expect 5–10 µg DNA, use 15–20 units of enzyme). For PCR, use 1 µL.

**Notes**

1. If only PCR is needed, one can use as little as 1 cotyledon resuspended in 50 µL of TE in the resuspension step, but use 5 µL to PCR.
2. Tissue can be harvested and kept at room temperature for up to 3 hours or stored at 4 °C for up to 3 days.

**Troubleshooting**

| Problem | Possible solution |
|---|---|
| Low concentration of DNA | |
| | Grind longer. |
| | Use younger tissue. |
| DNA does not form firm pellet | |
| | After spin, replace 500 µL isopropanol with 70 % v/v EtOH; gently mix; re-spin. |
| DNA does not digest | |
| | Dry pellet longer before resuspension (remove alcohol residue). |
| | Take only the aqueous layer of the chloroform gradient; do not take any interface, and avoid chloroform residue. |
| | Spin for shorter time after isopropanol precipitation; avoid spinning down starch, etc. |
| DNA does not PCR | |
| | See solutions for not digesting. |
| | Use different amount of DNA (probably less). |

3. Can stop here, storing pellet in 70% v/v EtOH at -20 °C indefinitely.

## Comments

Yield should be approximately 10 to 20 µg of DNA per plant, enough for two to four Southerns or 50 to 100 PCR reactions. The number of samples can be maximized by using two drills with drill stands concurrently and an on/off switch operated by a foot pedal. Because the procedure can be used on very young seedlings, minimal time is wasted waiting for plants to grow. Since one person can extract DNA from several hundred seedlings in one day, whole populations can be processed quickly, and results from PCRs or Southern blots can be available in a few days. This protocol is known to work on tomato, pepper, potato, apple, tobacco, strawberry, *Arabidopsis*, and artichoke.

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### Reference

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