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A rapid and low-cost genomic DNA extraction protocol for plant tissue

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Abstract

Genomic DNA extraction is the first step in almost every plant molecular biology technique. This includes routine PCR genotyping and marker-assisted breeding. Long-established methods rely on CTAB or phenol-chloroform extraction. Both give high DNA purity, but both are slow. Both also depend on hazardous organic solvents. It uses a sodium dodecyl sulfate (SDS) based extraction buffer, called the Rapid Buffer. A sodium borate (SB) buffer is used alongside it for agarose gel electrophoresis. We give full recipes for both buffers. We also give a complete fifteen-step extraction procedure, with the reasoning behind each step. The article covers agarose gel preparation, supporting laboratory calculations, a comparison with conventional methods and practical guidance on troubleshooting and safety. The protocol avoids phenol and chloroform entirely. It uses small reaction volumes, so many samples can be processed in parallel. The full procedure takes well under an hour of active bench time. This makes it well suited to routine genotyping, high-throughput screening and teaching laboratories.

Keywords: DNA, extraction, PCR, breeding, genotyping

Introduction

Every study in plant molecular biology starts with the same question. How do you get clean, intact DNA out of a leaf? Different methods answer this question in different ways. Each one trades off speed, cost, safety and DNA purity. The cetyltrimethylammonium bromide (CTAB) method is one reference standard, popularized by Doyle and Doyle (1987). Organic phenol-chloroform extraction is another. Both give high-purity, high-molecular-weight DNA, but both are slow. They often need an hour or more of incubation and both use hazardous organic solvents. This complicates laboratory safety and waste disposal. It becomes a real bottleneck for laboratories processing many samples, whether for marker-assisted breeding, genetic diversity surveys or teaching.

Rapid, detergent-based methods ease this bottleneck. Anionic detergents such as sodium dodecyl sulfate (SDS) lyse plant cells and denature proteins just as well as CTAB, while avoiding organic solvents altogether. A single leaf sample can yield PCR-ready DNA in well under an hour (Edwards *et al.*, 1991). Interest in rapid, low-cost extraction methods continues today, especially for high-throughput genotyping (Yang *et al.*, 2025). A brief potassium acetate precipitation step can remove SDS-bound protein and lipid with no organic extraction step at all, using the same chemistry as alkaline plasmid preparation (Birnboim and Doly, 1979).

This article documents one such protocol in full. It is a Tris-EDTA-NaCl-SDS extraction buffer, called the Rapid Buffer. A companion sodium borate (SB) buffer is used for agarose gel electrophoresis of the extracted DNA (Brody and Kern, 2004). We give the complete recipes, the step-by-step procedure, the laboratory calculations and troubleshooting notes. These were compiled and cross-checked from laboratory notebook records. The aim is a single, reproducible bench reference for routine use, teaching and future comparison.

1. Overview of the Extraction Workflow

The protocol has three stages (Figures 1 and 2). First, plant tissue is lysed directly in the Rapid Buffer, a Tris-EDTA-NaCl-SDS solution. It ruptures cell membranes, denatures protein and protects the released DNA from enzymatic degradation (Section 3.1). Second, SDS-bound protein and cellular debris are removed by a brief potassium acetate precipitation and centrifugation step. The DNA in the resulting supernatant is then recovered by isopropanol

precipitation, washed and resuspended in water (Section 4). Third, DNA quality is checked by agarose gel electrophoresis, using a sodium borate (SB) running buffer (Sections 3.2 and 5). The entire procedure needs no phenol or chloroform. It can be completed in well under an hour of hands-on bench time.

The protocol is described here for plant leaf tissue in general. The Rapid Buffer formulation is consistent with SDS-based extraction methods validated for rice (*Oryza sativa*) leaf tissue; the underlying chemistry is broadly applicable to other species, although tissue with a higher phenolic or polysaccharide content may benefit from minor optimisation, such as a longer incubation or the addition of polyvinylpyrrolidone (Section 6).

2. Materials, Reagents and Equipment

2.1 Equipment

- Mortar and pestle
- Labelled 1.5–2 mL microcentrifuge (Eppendorf) tubes
- Water bath or dry bath capable of maintaining 65 °C
- Dry bath, for drying the DNA pellet
- Centrifuge capable of 10,000 rpm
- Vortex mixer
- Micropipettes and tips covering 10–1000 µL
- Agarose gel electrophoresis unit and power supply

2.2 Reagents and solutions

- Rapid Buffer, freshly supplemented with β-mercaptoethanol (Section 3.1)
- Potassium acetate solution
- Isopropanol
- 70% ethanol
- MilliQ (ultrapure) or nuclease-free water
- SB buffer, 1X working solution, for agarose gel electrophoresis (Section 3.2)
- Molecular-grade agarose
- A nucleic acid stain suitable for the electrophoresis unit available

3. Preparation of Buffers and Stock Solutions

3.1 The Rapid Extraction Buffer

The Rapid Buffer is a Tris-EDTA-NaCl-SDS extraction buffer that lyses plant cell walls and membranes, denatures cellular protein and protects genomic DNA from enzymatic degradation during extraction. Each component has a specific role. Tris-hydrochloride (Tris-HCl) buffers the solution at a stable, slightly alkaline pH of about 8.0, the conditions under which double-stranded DNA is most stable. Ethylenediaminetetraacetic acid (EDTA) chelates divalent metal ions, particularly Mg^{2+}, that DNA-degrading nucleases require as cofactors, so it inhibits their activity. Sodium chloride (NaCl) maintains ionic strength, helps neutralise the negatively charged DNA backbone and assists in separating DNA from protein and polysaccharides during later precipitation steps. Sodium dodecyl sulfate (SDS), a strong anionic detergent, dissolves lipid membranes and denatures protein, including histones and nucleases, releasing DNA into solution. Together, these four components lyse plant tissue efficiently while limiting DNA degradation.

The complete Rapid Buffer is made by combining four stock solutions in equal volumes and making up to a final volume with double-distilled water (ddH2O). Table 1 gives the composition of the buffer, the preparation of each stock solution and the final concentration of each component.

Table 1. Composition and preparation of the Rapid Buffer (200 mL)

Stock solution	Mol. wt. (g/mol)	Amount per 100 mL stock	Vol. used	Final conc.	Preparation notes
1 M Tris-HCl, pH 8.0	121.14	12.114 g	20 mL	100 mM	Dissolve in ~60 mL ddH2O; adjust to pH 8.0 with concentrated HCl; make up to 100 mL.
5 M NaCl	58.5	29.25 g	20 mL	500 mM	Dissolve in 80–90 mL ddH2O; make up to 100 mL.
0.5 M EDTA, pH 8.0	372.24*	18.612 g	20 mL	50 mM	Add to ~80 mL ddH2O; raise pH gradually with NaOH pellets while stirring, as the salt dissolves fully only near pH 8.0; make up to 100 mL.
10% (w/v) SDS	N/A	10 g	20 mL	1% (w/v)	Dissolve in ~80 mL deionized water, warming gently (40–

Stock solution	Mol. wt. (g/mol)	Amount per 100 mL stock	Vol. used	Final conc.	Preparation notes
					45 °C) to aid dissolution; make up to 100 mL. Do not autoclave.

**Molecular weight of the disodium salt, dihydrate.*

Combine 20 mL of each of the four stock solutions (80 mL total) and make up to a final volume of 200 mL with ddH2O. Store the combined stock at room temperature; SDS can precipitate from solution under refrigeration, so cold storage of the Rapid Buffer is not recommended unless the buffer is warmed to redissolve any precipitate before use.

Immediately before use, not in advance, supplement an aliquot of Rapid Buffer with β-mercaptoethanol (β-ME) at 2µL per 1mL of buffer (20 µL per 10 mL). β-ME is a reducing agent that prevents oxidation of phenolic compounds released from damaged plant tissue, which can otherwise discolour and contaminate the DNA preparation. Because β-ME loses reducing activity once mixed into the buffer, add it fresh and use the supplemented buffer the same day. β-ME is toxic and has a strong odour; handle it in a fume hood with gloves and eye protection (Section 7.3).

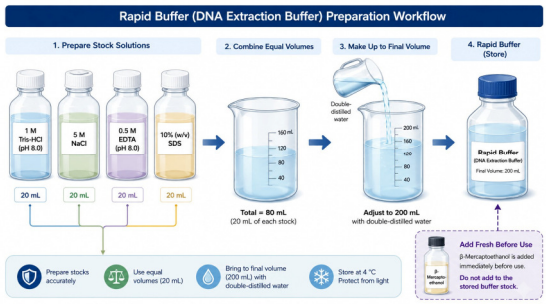

Fig. 1. Preparation of the Rapid Buffer. Four stock solutions (1 M Tris-HCl pH 8.0, 5 M NaCl, 0.5 M EDTA pH 8.0 and 10% w/v SDS) are prepared individually, combined in equal volumes and made up to 200 mL with double-distilled water. β-Mercaptoethanol is added fresh immediately before use and is not part of the stored stock.

3.2 The SB (Sodium Borate) Running Buffer

Sodium borate (SB) buffer serves as the electrophoresis running buffer for the agarose gels described in Section 5, in place of the more traditional Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE) buffers. SB is a well-established, low-ionic-strength alternative to Tris-based running buffers: it generates substantially less heat at a given voltage, which permits faster runs at higher voltage without overheating the gel and it needs neither Tris nor EDTA (Brody and Kern, 2004).

The SB buffer is prepared as a concentrated 20X stock and diluted to 1X immediately before use. To prepare 500 mL of 20X stock, dissolve 19 g of sodium borate in about 300 mL of distilled or deionized water, then make up to a final volume of 500 mL. Store the 20X stock at room temperature.

Working (1X) SB buffer is prepared from the 20X stock by simple dilution, using the relationship $C_1V_1 = C_2V_2$. Table 2 gives the volumes of 20X stock and water required for common working volumes.

Table 2. Dilution of 20X SB stock to 1X working buffer

Final volume (1X)	20X stock required	Water required
60 mL	3 mL	57 mL
100 mL	5 mL	95 mL
200 mL	10 mL	190 mL
500 mL	25 mL	475 mL
1000 mL	50 mL	950 mL

4. Genomic DNA Extraction Procedure

The complete extraction procedure comprises fifteen steps, from tissue sampling to final storage of the extracted DNA (Figure 2). All centrifugation steps are performed at 10,000 rpm. Because relative centrifugal force (RCF, in ×g) depends on the radius of the specific rotor used, Table 4 gives the formula for converting rpm to RCF if this is needed for reporting or reproducibility.

Step 1. Place the plant tissue sample in a mortar.

Step 2. Add 200 µL of Rapid Buffer (freshly supplemented with β-mercaptoethanol) and crush the tissue thoroughly with a pestle. Add a further 200 µL of the same buffer to bring the total volume to 400 µL. Beginning lysis during mechanical disruption limits the time nucleases have to act on the released DNA.

Step 3. Transfer the crushed extract to a labelled 1.5–2 mL microcentrifuge tube.

Step 4. Incubate at 65 °C for 15 min with the tube capped, to complete cell lysis, denature protein and inactivate nucleases.

Step 5. Add potassium acetate (5 M) solution at 32 µL per 100 µL of sample volume (128 µL for a 400 µL sample) and vortex 2–3 times. Potassium acetate precipitates SDS-protein and SDS-lipid complexes, the same chemistry used in alkaline plasmid preparation (Birnboim and Doly, 1979), while leaving

nucleic acids in solution.

Step 6. Centrifuge at 10,000 rpm for 10 min to pellet the precipitated complexes and cellular debris.

Step 7. Transfer the supernatant to a new labelled tube and discard the pellet.

Step 8. Add 400 µL of isopropanol, a volume equal to the Rapid Buffer used and mix by gentle inversion 2–3 times to precipitate the DNA. Avoid vigorous vortexing at this step, as it can shear high-molecular-weight DNA.

Step 9. Centrifuge at 10,000 rpm for 5 min to pellet the precipitated DNA.

Step 10. Carefully remove and discard the isopropanol supernatant without disturbing the DNA pellet.

Step 11. Wash the pellet with 200 µL of 70% (v/v) ethanol to remove residual salts and isopropanol.

Step 12. Centrifuge at 10,000 rpm for 3 min.

Step 13. Remove and discard the ethanol wash.

Step 14. Air-dry the pellet with the tube open in a dry bath for approximately 10 min, until the ethanol odour is no longer detectable. Avoid over-drying, as an excessively dry pellet becomes difficult to resuspend.

Step 15. Resuspend the pellet in 150 µL of MilliQ (or nuclease-free) water, tapping the tube gently until fully dissolved, then store the extracted genomic DNA at –20 °C.

The DNA obtained by this procedure is generally suitable for downstream applications such as PCR-based genotyping and restriction analysis without further purification. Applications requiring very high purity or very high molecular weight, such as long-read sequencing, may benefit from an additional purification step, as discussed in Section 6.

Fig. 2. The DNA extraction procedure (Steps 1–15), from tissue sampling to final storage. Plant tissue is crushed in Rapid Buffer and incubated at 65 °C (Steps 1–4); SDS-protein complexes are removed by potassium acetate precipitation and centrifugation (Steps 5–7); and DNA is recovered from the supernatant by isopropanol precipitation, washed with 70% ethanol, dried, resuspended in water and stored at –20 °C (Steps 8–15).

5. Assessment of DNA Quality by Agarose Gel Electrophoresis

5.1 Casting the Gel

Agarose gel electrophoresis confirms the presence, approximate size distribution and integrity of the extracted DNA (Voytas, 2000). To cast a gel: weigh the required mass of agarose (Section 5.2) into a clean flask; add the corresponding volume of 1X SB buffer (Section 3.2); heat the suspension in a microwave or on a hotplate until the agarose is fully dissolved and the solution runs clear, swirling periodically and taking care to avoid boil-over; allow the molten agarose to cool to approximately 55–60 °C before pouring, to avoid warping the casting tray; pour into a casting tray fitted with an appropriate well comb; and allow the gel to set at room temperature for 20–30 min before use. Lower-percentage gels (0.8–1.0%) resolve large, high-molecular-weight fragments such as genomic DNA better, while higher-percentage gels (2–3%) better resolve smaller fragments such as PCR products.

5.2 Calculating the Mass of Agarose Required

The mass of agarose required for a gel of a given percentage and volume is calculated as:

Mass of agarose (g) = (Gel percentage ÷ 100) × Gel volume (mL)

For example, a 2.0% gel with a volume of 60 mL requires (2.0 ÷ 100) × 60 = 1.2 g of agarose. Table 3 gives pre-calculated agarose masses for common gel percentages and casting volumes.

Table 3. Mass of agarose (g) required for common gel percentages and casting volumes

Gel percentage	30 mL	50 mL	60 mL	100 mL
0.8%	0.24 g	0.40 g	0.48 g	0.80 g
1.0%	0.30 g	0.50 g	0.60 g	1.00 g
1.2%	0.36 g	0.60 g	0.72 g	1.20 g
1.5%	0.45 g	0.75 g	0.90 g	1.50 g
2.0%	0.60 g	1.00 g	1.20 g	2.00 g
3.0%	0.90 g	1.50 g	1.80 g	3.00 g

5.3 Supporting Laboratory Calculations

Table 4 summarises additional formulas used throughout buffer preparation (Section 3) and gel casting (Section 5.2).

Table 4. Quick-reference formulas used in buffer and gel preparation (Sections 3 and 5)

Quantity	Formula
General dilution	$C_1V_1 = C_2V_2$, where C = concentration and V = volume
Mass required for a	Weight (g) = Molarity (mol/L) ×

Quantity	Formula
molar solution	Molecular weight (g/mol) × Volume (L)
Percentage weight/volume, % (w/v)	X% (w/v) = X g of solute per 100 mL of final solution
RCF from rpm	RCF (×g) = $1.118 \times 10^{-5} \times r \times N^2$, where r = rotor radius (cm), N = speed (rpm)

Extracted DNA can also be assessed spectrophotometrically alongside gel electrophoresis. Absorbance ratios (A260/A280) of approximately 1.8–2.0 generally indicate DNA reasonably free of protein contamination; ratios below this range may indicate residual protein, while ratios above it may indicate residual RNA (Green and Sambrook, 2012).

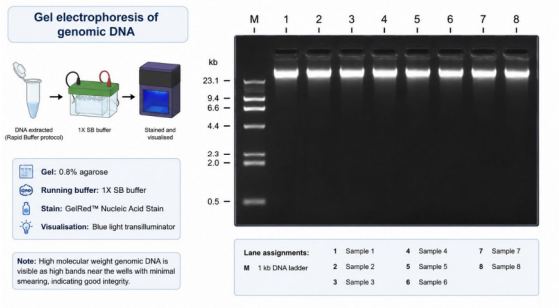

Fig. 3. Representative agarose gel electrophoresis pattern for DNA extracted with the Rapid Buffer protocol. Samples were run on a 0.8% agarose gel in 1X SB buffer alongside a DNA size ladder, stained with GelRed nucleic acid stain and visualised under blue-light transillumination. High-molecular-weight genomic DNA appears as a band near the wells in each sample lane, with minimal smearing, indicating good DNA integrity.

6. Comparison with Conventional Extraction Methods

Table 5 compares key features of the Rapid Buffer protocol described here with conventional CTAB (Doyle and Doyle, 1987) and organic phenol-chloroform extraction, the two methods most widely used for plant genomic DNA. Considerable variability exists even within a single named method. Published CTAB protocols, for example, differ considerably in reagent concentrations, incubation times and optional additives (Schenk *et al.*, 2023), so the comparison below reflects typical, representative implementations of each approach rather than a single definitive standard.

Table 5. Comparison of the Rapid Buffer protocol with conventional CTAB and organic phenol-chloroform DNA extraction methods

Feature	This protocol (Rapid Buffer)	Standard/classical approach	Comments
Lysis detergent	SDS (anionic)	CTAB (cationic); e.g. Doyle and Doyle (1987)	SDS lysis is rapid at 65 °C; CTAB protocols often use a similar or higher temperature but longer incubation.
Protein/lipid removal	Potassium acetate precipitation	Phenol:chloroform:isoamyl alcohol extraction	Avoids hazardous organic solvents and simplifies waste disposal; may retain marginally more residual protein.
Enzymatic digestion	Not included	Proteinase K digestion often included	Shortens protocol time and cost; Proteinase K can be added if very high purity is required.
RNA removal	Not included	RNase A treatment often included	Acceptable for PCR-based genotyping; RNA contamination can affect spectrophotometric DNA quantification.
DNA precipitation	Isopropanol (primary); ethanol (wash only)	Ethanol sometimes used for both precipitation and washing	Isopropanol requires a smaller volume relative to sample, useful for high-throughput processing.
Typical reaction scale	150–400 µL, microcentrifuge tubes	Often 1–10 mL or more	Smaller scale suits parallel processing of many samples.
Handling of phenolic compounds	β-Mercaptoethanol in buffer; no PVP	PVP commonly included for phenolic-rich tissue	Adequate for low-to-moderate phenolic tissue (e.g. rice leaves); PVP may be needed for phenolic-rich species.
Typical total protocol time	~15 min incubation plus brief centrifugation steps (well under 1 h)	Often 1–2 h or more	Faster turnaround supports high-throughput sample processing.

7. Practical Notes, Safety and Troubleshooting

7.1 Practical Tips and Common Pitfalls

- Always add β-mercaptoethanol to the Rapid Buffer fresh, immediately before use; do not prepare and store the supplemented buffer in advance.
- Label every tube clearly at each transfer step. The procedure involves several

sequential transfers between tubes and mislabelling is a common source of sample mix-up.

- Confirm that the SDS stock solution is fully dissolved, with no visible particulate, before combining it into the Rapid Buffer; incompletely dissolved SDS reduces lysis efficiency.
- Remove supernatants and washes completely and carefully at each step; residual liquid left behind, particularly after the ethanol wash (Step 13), can inhibit downstream enzymatic reactions such as PCR.
- Mix by gentle inversion rather than vortexing after isopropanol precipitation (Step 8); vigorous vortexing at this stage can shear high-molecular-weight DNA.
- Assess extracted DNA by both agarose gel electrophoresis and, where available, spectrophotometry (A260/A280) before proceeding to downstream applications.

7.2 Troubleshooting Guide

Table 6. Troubleshooting guide for common issues encountered during the extraction procedure (Section 4)

Issue observed	Likely cause(s)	Recommended action
Low DNA yield	Incomplete tissue crushing; insufficient lysis time or temperature; supernatant lost or incompletely transferred (Step 7)	Ensure thorough crushing; confirm the 65 °C bath has reached temperature before starting the incubation timer; transfer supernatant carefully and check tube labels at each step.
Degraded or smeared DNA on gel	Excessive vortexing after DNA precipitation; prolonged handling at room temperature; nuclease contamination	Mix by gentle inversion rather than vortexing after Step 8; minimise time samples spend at room temperature; use nuclease-free tubes, tips and water.
Pellet difficult to resuspend	Pellet over-dried at Step 14; insufficient mixing at Step 15	Dry only until the ethanol odour is no longer detectable, not longer; allow the tube to stand briefly at room temperature and tap gently to resuspend.
Discoloured or impure DNA	High phenolic or polysaccharide content in the starting tissue	Consider adding polyvinylpyrrolidone (PVP) to the Rapid Buffer or increasing β-mercaptoethanol content, for phenolic-rich tissue (Section 6).

7.3 Safety Precautions

- β-Mercaptoethanol is toxic and has a strong, persistent odour; handle only in a fume hood, wearing gloves and eye protection.
- Concentrated HCl, used to adjust the pH of the Tris-HCl stock, is corrosive; handle in a fume hood with appropriate personal protective equipment.
- SDS and EDTA powders can irritate the respiratory tract; weigh them in a ventilated area, avoid inhaling airborne particulate and use a mask if necessary.
- Isopropanol and ethanol are flammable; keep away from open flame and heat sources and store in appropriate flammable-liquid cabinets.
- Wear a laboratory coat, gloves and safety glasses throughout the procedure and dispose of all chemical waste according to institutional guidelines.

Conclusion

The Rapid Buffer protocol offers a fast, low-cost and comparatively safe route to PCR-ready genomic DNA from plant leaf tissue. It replaces organic-solvent extraction with a brief potassium acetate precipitation step. This removes phenol and chloroform from the workflow entirely. It also simplifies hands-on time and chemical waste disposal. No special equipment is needed beyond a centrifuge and a heat block. The small reaction volumes and short incubation times suit high-throughput work, including routine PCR-based genotyping, marker-assisted breeding and genetic diversity surveys. The protocol also suits teaching laboratories, where students must finish an extraction within a single class period.

These advantages come with trade-offs. The protocol omits enzymatic protein digestion and RNase treatment. The DNA obtained is generally suitable for PCR and restriction-based applications, but it may not match the purity of DNA from more elaborate CTAB or phenol-chloroform protocols. Tissue rich in phenolic compounds or polysaccharides may need extra optimisation, such as adding polyvinylpyrrolidone. Laboratories planning to use this protocol for sequencing, cloning or other purity-sensitive work should first validate DNA quality against their own requirements. With that caveat in mind, this protocol is a reliable and reproducible addition to the standard molecular biology toolkit.

Conflict of Interest

The author(s) declare no conflict of interest.

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