Buffer Preparation Recipes

1 M Tris-HCl, pH 8.0 (200 ml)

Reagent	Amount	Notes
Tris base	24.22 g	Dissolve in ~150 ml dH_2O, stir until dissolved
conc. HCl	—	Adjust pH to 7.8
dH_2O	up to 200 ml	Autoclave, store at 4 °C

0.5 M EDTA, pH 8.0 (100 ml)

Reagent	Amount	Notes
EDTA disodium salt	18.61 g	Dissolve in ~75 ml dH_2O with gentle heating
NaOH	~2 g (slightly less)	Required for dissolution; adjust to pH 8.0 with 1 M NaOH
dH_2O	up to 100 ml	Autoclave, store at 4 °C

CTAB Extraction Buffer (200 ml)

Reagent	Amount	Notes
CTAB (cetyltrimethylammonium bromide)	4 g	Dissolve with gentle heating
1 M Tris-HCl (pH 8.0)	20 ml	
0.5 M EDTA (pH 8.0)	8 ml	
NaCl (molecular grade)	17.24 g	
dH_2O	up to 200 ml	Autoclave, store at room temperature

Washing buffer:

76% ethanol, 10 mM ammonium acetate.

0.1× TE buffer:

10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)

DNA Extraction

Genomic DNA was extracted using the **CTAB method** (Doyle and Doyle, 1990). For each genotype, ten seeds were germinated on moistened filter paper, and bulked seedlings were used as the source of tissue. Several milligrams of fresh tissue were collected and ground in liquid nitrogen using 1.5 ml microtubes.

Each sample was mixed with 500 µl of CTAB extraction buffer and incubated at 65 °C for 30 min with occasional inversion. Following incubation, 500 µl of chloroform:isoamyl alcohol (24:1) was added under a fume hood, and the samples were gently inverted to form an emulsion. The mixture was centrifuged for 10 min at 11,000 rpm at room temperature, and the supernatant was transferred to fresh 1.5 ml tubes. DNA was precipitated by adding 350 µl of ice-cold isopropanol and gently inverting the tubes until DNA threads were visible. Samples were centrifuged for 5 min at 10,000 rpm, and the supernatant was discarded.

The DNA pellet was washed with 200 µl of washing buffer, centrifuged for 3 min at 10,000 rpm, and air-dried for ~40 min at 35 °C. The dried pellet was resuspended in 50 µl of 0.1× TE buffer and stored at 4 °C for at least 24 h to allow complete dissolution, followed by long-term storage at –20 °C.