**Edwards DNA Extraction**

**Background**

[Nucleic Acids Res.](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC333874/) 1991 Mar 25; 19(6): 1349. doi: [10.1093/nar/19.6.1349](https://dx.doi.org/10.1093%2Fnar%2F19.6.1349)

A simple and rapid method for the preparation of plant genomic DNA for PCR analysis.

[K Edwards](https://www.ncbi.nlm.nih.gov/pubmed/?term=Edwards%20K%5BAuthor%5D&cauthor=true&cauthor_uid=2030957), [C Johnstone](https://www.ncbi.nlm.nih.gov/pubmed/?term=Johnstone%20C%5BAuthor%5D&cauthor=true&cauthor_uid=2030957), and [C Thompson](https://www.ncbi.nlm.nih.gov/pubmed/?term=Thompson%20C%5BAuthor%5D&cauthor=true&cauthor_uid=2030957)

The extracted DNA will be used for Polymerase Chain Reaction (PCR) amplification of specific DNA in the sample. Because PCR is so sensitive, preventing sample contamination is critical. Gloves must be worn and care taken to prevent cross contamination during pipetting or other sample manipulation.

**Materials**

* Tweezers/scissors – for removing leaf tissue from a plant as necessary
* Gloves
* Microcentrifuge tube rack
* Microcentrifuge tubes
* Number of samples x 2 – label two tubes for each sample
* Plant sample: Leaf or leaf piece ~ 1.0 cm square
* Plastic pestle
* Edwards extraction buffer: 200mM Tris-HCl pH 7.5, 250mM NaCl, 25 mM EDTA, 0.5% SDS
* Isopropanol
* TE (Tris-EDTA) – 10mM Tris-HCl, pH7.5, 1mM EDTA

**Procedure**

# Place plant tissue sample into labeled 1.5 ml microcentrifuge tube

# Grind tissue:

Grind leaf at bottom of tube with sterile plastic pestle, briefly, but thoroughly.

1. Add 500 μL of Edwards extraction buffer at room temperature and grind further till no large leaf pieces are visible.

Note: Can leave sample at room temperature while other samples are prepared if doing more than one sample.

# Centrifuge for 5 minutes at maximum speed in microcentrifuge (13000 rpm)

# Prepare new 1.5 ml microcentrifuge tubes while samples are spinning. (Could also be done at start of the experiment).

Labeled for each sample – fill with 300 μL isopropanol

1. Remove samples from centrifuge.

Carefully, without transferring any of the plant material, remove 300 μL of supernatant and add to the appropriately labeled tube with isopropanol. Discard first tube.

Invert tube a few times and allow to sit for a minimum of 2 minutes

# Centrifuge samples for 10 minutes at maximum speed (13000 rpm)

# Discard supernatant

Can pour out into sink

Pellet most likely not visible

1. Dry pellet

Keep tube open, air dry inverted on clean paper towel for 10-15 minutes, making sure no liquid is present anymore.

1. Add 100 μL TE –

Store overnight at 4 C before first use to allow best resuspension of DNA.

Resuspend DNA by pipetting up and down or vortexing.

1. Store samples at -20 C for longer term storage.
2. Use 0.5 μL as template in a 20 μl PCR reaction.