# Skeletonema DNA extraction by Plant DNAzol

**Adrian Clarke**

## Abstract

**Citation:** Adrian Clarke Skeletonema DNA extraction by Plant DNAzol. **protocols.io**
dx.doi.org/10.17504/protocols.io.g6bbzan
**Published:** 09 Feb 2017

## Protocol

Preparations

**Step 1.**

- Prepare **Plant DNAzol** by adding RNAse (100ug/mL, make 0,6mL/sample)
- Prepare **Wash solution,** 1 part **Plant DNAzol** to 0,75 parts 99% ethanol (2x0,6mL/sample)
- Prepare **75% ethanol** solution (5*0,6mL/sample)

Pellet Skeletonema culture (1500 x g, 5 min), remove growth me and dissolve in f/2 or growth media ~250uL.

**Step 2.**

Use Swing-out rotor

Put liquid N2 in mortar, drop Skeletonema suspension into N2 and grind once its evaporated.

**Step 3.**

Transfer ground cells to 2mL eppendorf tube and add 600uL prepared DNAzol (with RNAse).

**Step 4.**

Mix by inversion; incubate at 25°C (RT) for 5 min with a little shake.

**Step 5.**

Add 600uL Chloroform, mix by vortexing in short bursts, and incubate at 25°C (RT) for 5 min with a little shake.

**Step 6.**

Centrifuge 10 min at 12000g

**Step 7.**

Transfer the supernatant (aqueous phase) to a fresh eppendorf tube. (Around 800 uL)

**Step 8.**

Add 1200uL 99% ethanol to aqueous phase, mix by inversion and incubate for 5 min at RT.

**Step 9.**

Adjust volume to match 75% of supernatant if using other than 800uL

Pellet DNA by centrifugation, 4 min, 7000g. Remove the supernatant.

**Step 10.**

Add 600uL Wash solution to percipitate, mix by pipetting,

**Step 11.**

Incubate samples 5 min at RT.

**Step 12.**

Pellet DNA by centrifugation, 4 min, 7000g. Remove the supernatant by pipett

**Step 13.**

Repeat the Washing Procedure one more time (Step 11-

**Step 14.**

. Add 600uL 75% ethanol solution

**Step 15.**

Pellet DNA by centrifugation, 4 min, 7000g. Remove the supernatant as much as possible.

**Step 16.**

Repeat step 15+16 three-four additional times.

**Step 17.**

Evaporate the remaining ethanol in speed

**Step 18.**

. Dissolve the DNA in 80uL Water

**Step 19.**

Centrifuge 12000g for 5 min

**Step 20.**

Take the supernatant (75uL); add 0,75 ul 10xTE.

**Step 21.**

Makes for 0.1xTE for storage in 4C or -20