Extraction of genomic DNA using "Edwards" buffer

Fabian Fink, 26.08.2014 modified after Edwars et al., 1991 Nucleic Acids Research

Abstract

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Before start

Prepare Edwards buffer

Reagent	Final conc.	[g] for 100 ml	molar mass	comment
Tris	200 mM	3,15	157,6 g/mol	
EDTA	25 mM	0,93	372,24 g/mol	EDTA-Disodium-Dihydrate
NaCl	250 mM	1,46	58,44 g/mol	
SDS	0,5% (w/v)	0,5		

Adjust to pH 8 (with HCl) before adding SDS, then fill up to 100 ml with ddH_2O.

Protocol

Step 1.

Grind tissue (fresh or frozen entire seedlings, 0.5-1 rosette of cauline leaves, inflorescence) in microfuge tube with autoclaved plastic pestle for 10-15 seconds.

Step 2.

Add 400 µl Edwards buffer.

Step 3.

Grind briefly to remove any remaining tissue from pestle.

Step 4.

Vortex 5 seconds.

The preps can be kept at room temperature (RT) until all preps are ready (up to 20).

Step 5.

Centrifuge 5 minutes in tabletop centrifuge at full speed (13.000 rpm).

Step 6.

Transfer 300 µl supernatant into a fresh microfuge tube.

Add 300 µl isopropanol and mix.

Leave for 2 minutes at RT.

Step 7.

Centrifuge 10 minutes at RT.

Step 8.

Decant off and discard all supernatant into S1-waste.

Step 9.

Wash pellet with 700 µl 70 % EtOH, then centrifuge for 5 minutes at full speed and discard EtOH.

Step 10.

Air dry DNA pellet at 37°C.

Step 11.

Resuspend pellet in 40 µl of sterile ddH2O (incubate at 37°C if necessary).