gDNA Extraction of Eucalypts pauciflora for full genome sequencing

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Abstract

This extraction protocol follows the DArT DNA Extraction protocol but contains modifications (including increasing the concentration of PVP to 4 %

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Protocol

Making buffers

Step 1.

Extraction Buffer

0.35 M sorbitol

0.1 M TrisHCl pH 8

5 mM EDTA pH 8

To make 500 mL:

31.9 g sorbitol

50 mL 1 M TrisHCl

5 mL 0.5 M EDTA

Fill up to 500 mL with deionised water

Lysis Buffer

0.2 M TrisHCl pH 8

0.05 M EDTA pH 8

2 M NaCl

2 % CTAB

To make 500 mL:

100 mL 1 M TrisHCl pH 8

50 mL 0.5 M EDTA pH 8

200 mL NaCl

10 g CTAB

Fill up to 500 mL with deionised water

Sarcosyl stock (5 %)

Fresh Working Buffer: Make up fresh working buffer (this lasts 3-4 days). Contains 0.5 % sodiumdisulfite and 4 % PVP-40 (K29-32) Sigma

To make 120 mL:

0.6 g sodiumdisulfite

4.8 g PVP-40

50 mL extraction buffer

50 mL lysis buffer

20 mL sarcosyl

To make 30 mL:

0.15 g sodiumdisulfite

1.2 g PVP-40

12.5 mL extraction buffer

12.5 mL lysis buffer

5 mL sarcosyl

Proteinase K (0.1 mg/mL of fresh working buffer). This is 30 uL of Qiagen proteinase K (20 mg/mL).

Extraction Day 1

Step 2.

Autoclave mortar and pestles before use. Prepare 2 mL tubes (sterilised) and 1.5 mL tubes (sterilised).

Extraction Day 1

Step 3.

Make up chloroform:isoamyl mixture (24:1) and 77 % ethanol. Place 100 % isopropanol in the freezer. Heat fresh working buffer to 65°C.

Extraction Day 1

Step 4.

Grind 1 leaf in mortar and pestle under liquid nitrogen to fine powder. Make sure material does not thaw. Place powder in 2 mL tubes and store in the freezer (-20°C) until needed.

Extraction Day 1

Step 5.

Aliquot 800 uL of fresh working buffer into each tube. Swirl fresh working buffer before adding to tubes.

Extraction Day 1

Step 6.

Add 8 uL of RNase to each tube. Incubate at 37 C for 20 mins. Mix well.

Extraction Day 1

Step 7.

Add 60 uL of proteinase K to each tube, mix well. Incubate for 10 min at 50 C. Inactivate at 65 C in the water bath.

Extraction Day 1

Step 8.

Incubate tubes at 65°C for 1 to 1.5 hours, invert tubes every 20 minutes.

Extraction Day 1

Step 9.

Cool down tubes for at least 5 minutes.

Extraction Day 1

Step 10.

Add 800 uL of chloroform:isoamyl alcohol (24:1). Invert tubes 30 times.

Extraction Day 1

Step 11.

Spin at 3000 x g RT for 20 minutes.

Extraction Day 1

Step 12.

Transfer water phase to fresh tube, careful not to transfer the chloroform or intermediate phase. Record the volumes added to each tube and add the same amount of ice cold isopropanol. Invert tubes 10 times.

Extraction Day 1

Step 13.

Leave tubes in fridge overnight.

DNA Ethanol Precipitation Day 2

Step 14.

The following day, spin tubes at 3,000 x g for 30 minutes. A pellet should appear at the bottom of tubes.

DNA Ethanol Precipitation Day 2

Step 15.

Discard the supernatant. Add 1.5 mL of 77 % ethanol. Vortex briefly and leave for 20 minutes.

DNA Ethanol Precipitation Day 2

Step 16.

Spin at 14,000 x g for 20 minutes.

DNA Ethanol Precipitation Day 2

Step 17.

Discard supernatant. Add 1.5 mL of 77 % ethanol, vortex briefly and leave in fridge overnight (can leave for two nights).

DNA Ethanol Precipitation Day 3

Step 18.
The following day, spin tubes at 14,000 x g for 20 minutes.

DNA Ethanol Precipitation Day 3

Step 19.
Discard supernatant. Add 1.5 mL of 77 % ethanol. Vortex briefly and leave for 20 minutes.

DNA Ethanol Precipitation Day 3

Step 20.
Spin at 14,000 x g for 20 minutes.

DNA Ethanol Precipitation Day 3

Step 21.
Discard supernatant. Dry pellet for 30 minutes on the bench (or until dried).

DNA Ethanol Precipitation Day 3

Step 22.
Re-suspend pellet in 50 µL of sterilised water.

DNA Ethanol Precipitation Day 3

Step 23.
When pellet is completely dissolved, run 1 µL on a 0.8 % agarose gel.

DNA Ethanol Precipitation Day 3

Step 24.
Compare the result of gel with Qubit for concentrations (ng/µL).

DNA Ethanol Precipitation Day 3

Step 25.
Store samples in freezer (-20°C) until putting through the zymo purification plate.

Zymo DNA Purification Day 4

Step 26.

NOTES
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This follows the protocol of Zymo Research Corp for Z-96 DNA Clean and Concentrator, however contains modifications.

Zymo DNA Purification Day 4

Step 27.
Take 25 uL of each sample and place in another labelled 1.5 mL (so that each sample is divided between two tubes). Add 125 µL of sterilised water to each tube. Mix well.

Zymo DNA Purification Day 4

Step 28.
Add 600 µL of the DNA binding buffer, vortex. Leave samples for 20 minutes.

Zymo DNA Purification Day 4

Step 29.
Place zymo plate on top of waste plate. Transfer samples to wells of zymo plate. Record which well contains which sample. Balance plate with a spare plate to 0.01 g.

Zymo DNA Purification Day 4

Step 30.
Spin at 3,000 x g for 5 minutes. Check that all the solution has gone through. If it has not, increase speed (3,500 to 5,000 x g) and spin again (multiple spins may be required for all the solution to go

through). Once all the solution has gone through, spin again at 3,000 x g for 2 minutes.

Zymo DNA Purification Day 4

Step 31.

Discard waste. Place another clean waste plate below the zymo plate. Make sure filters of zymo plate do not touch the bench when changing plates. Add 200 µL of wash buffer. Balance plate with a spare plate to 0.01 g.

Zymo DNA Purification Day 4

Step 32.

Spin at 3,000 x g for 2 minutes.

Zymo DNA Purification Day 4

Step 33.

Add 200 uL of wash buffer and spin at 3, 000 x g for 2 minutes.

Zymo DNA Purification Day 4

Step 34.

Check that all the solution has gone through. Spin (extra spin) at 3,000 x g for 2 minutes.

Zymo DNA Purification Day 4

Step 35.

Replace waste plate with a PCR plate. Make sure filters of zymo plate do not touch the bench when transferring.

Zymo DNA Purification Day 4

Step 36.

Add 20 µL of TE to each well. Allow to elute for 30 minutes.

Zymo DNA Purification Day 4

Step 37.

Spin at 3,000 x g for 2 minutes. All liquid should have passed through the wells into the PCR plate. Liquid should be clear. This is the first elution. Remove the first PCR plate and seal and label.

Zymo DNA Purification Day 4

Step 38.

Place zymo on a second PCR plate (for the second elution). Repeat steps 10 to 11, but only add 15 uL of TE to maximise concentration.

Zymo DNA Purification Day 4

Step 39.

For the third elution repeat steps 10-11, but add 10 µL of TE.

Zymo DNA Purification Day 4

Step 40.

Transfer samples from PCR plates into small sterilised tubes (600 µL). Mark the wells that have been used on the zymo and PCR plates and store until needed again.

Zymo DNA Purification Day 4

Step 41.

Run 1 µL of each sample on a 1 % agarose gel.

Zymo DNA Purification Day 4

Step 42.

Compare the result of gel with Qubit for concentrations (ng/µL).

Zymo DNA Purification Day 4

Step 43.

Store samples in freezer (-20°C).