Analysis of Leaf and Root Transcriptomes of Soil-Grown *Avena barbata* Plants.

Roots

CASE STUDY

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Overview

- **Keywords:** *Avena barbata*, climate change, ESTs, root
- **Aim of the study:** Generation of a large amount of cDNA sequence data for transcriptomic studies in *A. barbata*
- **Application:** Transcriptome analysis by Sanger sequencing and pyrosequencing
- **Sample:** *Avena barbata*
- **Sample type:** Root
- **Material:** FastPrep-24™ instrument
- **Buffer:** Modified CTAB (CetylTrimethylAmmonium Bromide) buffer: 50 mL of 0.1 M of aluminum ammonium sulfate and 0.5 mL of phenol: chloroform: isoamyl alcohol (25: 24: 1)

Protocol and Parameters

Total RNA was extracted from 200 mg of roots using a modified CTAB method.

1. 0.5 mL of modified CTAB buffer was added to the samples.
2. Samples were bead beaten for 30 seconds at 5.5 m/s in a FastPrep-24™ instrument.
3. Samples were centrifuged at 16,000 x g for 5 minutes at 4°C.
4. A second extraction with the modified CTAB buffer was conducted.
5. A 1 mL aliquot of chloroform was then added to the aqueous supernatant followed by a centrifugation at 12,000 x g for 5 minutes at 4°C.
6. 2 vols. of 30% (w/v) polyethylene glycol mw 6,000 in 1.6 M NaCl solution and 1 mL of linear acrylamide were added to the aqueous supernatant to precipitate the nucleic acids.
7. The RNA/DNA pellet was subsequently washed with 60% ice-cold ethanol and resuspended in diethylpyrocarbonate (DEPC)-treated water.

Conclusion

The results show that the FastPrep-24™ extraction method generates high-quality RNA for sequencing.

The combined use of pyrosequencing and Sanger sequencing was successful in generating a high number of expressed sequence tags (ESTs).

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