MP Biomedicals

Case study - *Roots*

Plant

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

Stéphanie M. Swarbreck, Erika A. Lindquist, David D. Ackerly, and Gary L. Andersen.
Plant Cell Physiol. 2011. Vol 52.

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 & pyrosequencing
- **Sample name:** *Avena barbata*
- **Sample type:** Root
- **Material:** FastPrep-24™ Homogenizer
- **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 (cetyltrimethylammonium bromide) method.

1. 0.5 ml of modified CTAB buffer was added to the samples,
2. Samples were bead beaten for 30 s at 5.5 m/s in a FastPrep-24™ instrument
3. Samples were centrifuged at 16,000 x g for 5 min 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 min at 4 °C.
6. 2 vols. of 30% (w/v) polyethylene glycol 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 a **good-quality RNA** for sequencing.
- The combined use of pyrosequencing and Sanger sequencing was successful in generating a **high number** of expressed sequence tags (ESTs).

Successful sample preparation using the MP Biomedicals FastPrep® product line has been highlighted in thousands of scientific articles. To access articles and other materials, visit www.mpbio.com/FastPrepLibrary.