



RNA extraction from Cyanobacteria with bead beating

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CONTEXT

Our research aims at developing Cyanobacteria as the future producer of renewable biofuels. The validity and reproducibility of gene expression studies depend on the quality of extracted RNA and the degree of genomic DNA contamination. Cyanobacteria are gram-negative prokaryotes that synthesize chlorophyll *a* and carry out photosynthetic water oxidation. These organisms possess an extended array of secondary metabolites that impair cell lysis, presenting particular challenges when it comes to nucleic acid isolation. With this work we identify and explore strategies for improved and lower cost high quality RNA isolation from Cyanobacteria¹.

MATERIAL

- Precellys®24 (mechanical cell disruption): "Trizol beads" sample and "PGTX beads" sample
- Precellys® kit: 03961-1-004 (glass beads 0.5 mm)
- Standard method: High temperature cell disruption 95°C ("PGTX95" sample)
- Sample: *Nostoc punctiforme* ATCC 29133 cells
- Buffer: Trizol or PGTX

PROTOCOL

- Precellys®24: 6500rpm, 2x20sec, 10s break.
- Centrifugation steps.
- Analysis: NanoDrop ND-1000 UV/Vis, automated electrophoresis system, PCR / RT-PCR.

1) Fernando Lopes Pinto et al. BMC Molecular Biology 2009, 10:79 doi:10.1186/1471-2199-10-79



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CONCLUSION

Cyanobacteria can be particularly resistance to both chemical and physical stress, making nucleic acid extraction particularly difficult.

The use of Precellys®24 greatly facilitated homogenization allowing increased RNA extraction - in both amount and purity.

RESULTS

Yield and absorption ratios for the 3 different extraction methods were determined and shown in the figure 1.

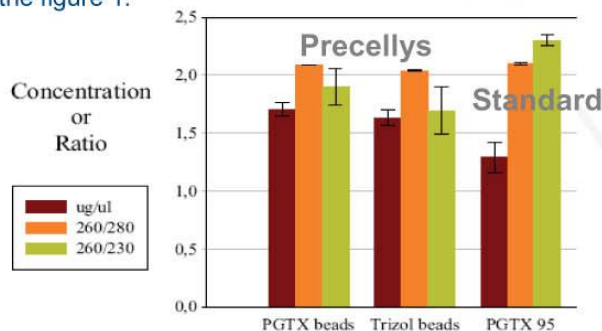


Figure 1: Extracted RNA yield and purity.

RNA integrity is checked and shown in the figure 2.

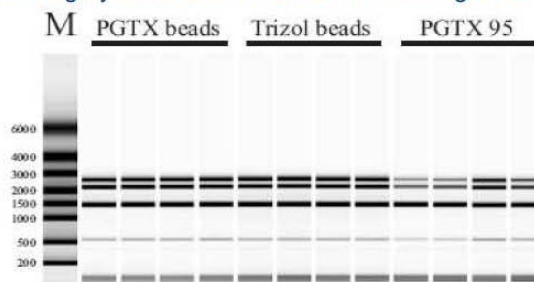


Figure 2: Gel image generated by the automated electrophoresis system.

It was possible to improve purity of isolated RNA by modifying protocol procedures. Further improvements, both in RNA purity and experimental cost, were achieved by using a new extraction solution, PGTX.



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