

Total RNA extraction from *Rhizobium etli* cell or bacteroid pellets

Centre of Microbial and Plant Genetics, Katholieke Universiteit Leuven, Belgium

CONTEXT

The laboratory is focused on the study of the genome-wide transcriptome of *R. etli* under diverse conditions.

Non-coding RNAs (ncRNAs) play a crucial role in the intricate regulation of bacterial gene expression, allowing bacteria to quickly adapt to changing environments. In this study, we have compared an extensive compilation of these non-coding RNA predictions to intergenic expression data of a whole-genome high-resolution tiling array in the soil-dwelling α -proteobacterium *Rhizobium etli* [1].

MATERIAL

- Precellys[®]24 homogenizer.
- Precellys[®] kit: 03961-1-005 (glass beads 0.1mm).
- Sample: *Rhizobium etli* cell pellets (from 20-40ml of bacteria culture) or bacteroid pellets (prepared from *Phaseolus vulgaris* root nodules), RNA stabilized, immediately frozen in liquid nitrogen and stored at -80°C.
- Extraction buffer: 1mL TRIzol Plus RNA Purification kit (Invitrogen).

PROTOCOL

- Precellys[®]24: 6500 rpm, 2x45 sec – 30 sec pause.
- Analyses: RNA isolation, RNA integrity, quantity and purity, cDNA synthesis & RNA detection by tiling microarray.

[1] Vercruyse et al. BMC Genomics 2010, 11:53
<http://www.biomedcentral.com/1471-2164/11/53>

Symbiotic and Pathogenic Interactions Group
Part of the Centre of Microbial and Plant Genetics

CMPG-SP

RESULTS

All samples had an RNA Quality Indicator value of 10, using Experion RNA StdSens Chips. The ncRNA peak could be detected in each sample (Figure 1). RNA quantity and purity was assessed using the NanoDrop ND-1000. The A260/A280 ratio and A260/A230 ratio of all samples were ≥ 2 .

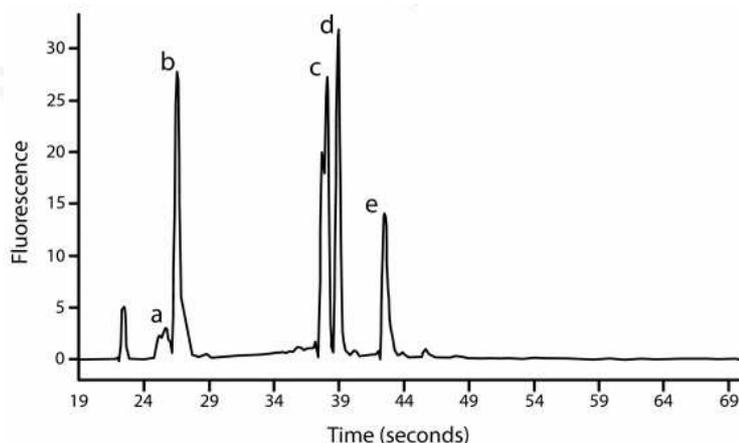


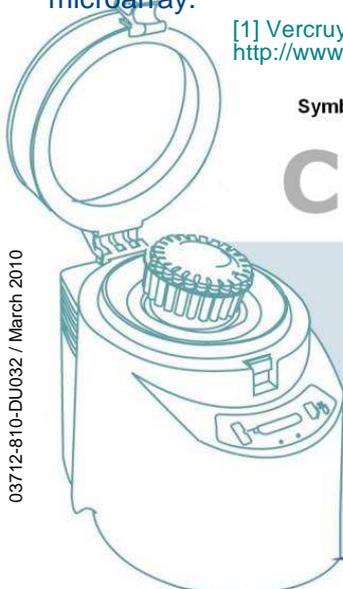
Figure 1: An example of high quality total RNA, illustrating the fragmentation 23S rRNA: (a) small RNA peak including 5S rRNA and the ncRNAs (b) 23S fragment of ~135 bp (c) two 23S fragments of ~1300 bp (d) 16S rRNA (e) intact 23S rRNA.

Samples were hybridized on a whole-genome tiling array covering the entire *R. etli* genome sequence (6.5 Mbp in total) and scanned by NimbleGen Systems.

The Precellys[®]24 homogenizer is an easy to use device that can be utilized to accomplish a thorough lysis of even stationary phase cells in combination with the lysing kit.

CONCLUSION

Transcriptomic studies require high amounts of pure intact RNA. High quality expression data were obtained using the Precellys[®]24 system allowing for an efficient and reproducible homogenization of different kinds of samples.



Problem



Solution

