MICROBIOLOGY

ROBUST AND UNBIASED MICROBIAL RNA EXTRACTION WORKFLOW WITH THE PRECELLYS® EVOLUTION HOMOGENIZER AND THE ZYMOBIOMICS DNA/RNA MINIPREP KIT

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/ CONTEXT

There is an increasing concern that the scientific world is undergoing a reproducibility crisis. Microbiomics is one of the fields that is facing the biggest challenges, because substantial bias can be introduced at each step of the workflow [1]. This has led researchers to question and validate their methodology using mock-microbial community standards, such as the ZymoBIOMICS® Microbial Community Standard (Zymo Research Corp., Irvine, CA). The ZymoBIOMICS® standard contains ten well-characterized organisms (i.e., 5 gram-positive, 3 gram-negative, and 2 yeast) that are mixed at defined proportions. This standard is comprised of microbes of various sizes and cell wall toughness, which makes it ideal for evaluating each step of the microbiomics workflow.

It has been reported that the choice of cell lysis technique during the nucleic acid extraction step can significantly influence results of Next Generation Sequencing (NGS) based microbiome analysis [2]. Mechanical lysis using bead beating-based homogenization has become the Gold Standard. Thanks to its powerful 3D-movement, the Precellys® Evolution offers great homogenization capabilities within a few seconds. Here, we use the ZymoBIOMICS® Microbial Community standard to show that the Precellys® Evolution bead-beating homogenizer combined with the ZymoBIOMICS® DNA/RNA Miniprep Kit can achieve complete lysis without compromising RNA integrity enabling accurate metagenome and microbiome analysis. Additionally, we evaluate the quality of the nucleic acid extracted with this workflow from this standard and 3 additional samples, including Gram-positive and Gram-negative bacteria and human fecal matter samples.

/ MATERIALS

- Homogenizer: Precellys® Evolution homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France)
- Sample Storage Buffer: Zymo Research DNA/RNA Shield (ensures the stabilization of DNA and RNA in any biological sample)
- Samples:
  - ZymoBIOMICS® Microbial Community Standard
  - *Listeria monocytogenes* cells (Gram-positive), 2. 10^8 cells/prep
  - *Escherichia coli* cells (Gram-negative), 3. 10^8 cells/prep
  - human fecal matter samples, 50 mg per prep
- **Extraction kit:** ZymoBIOMICS® DNA/RNA Miniprep Kit
- **Analysis instruments:** Agilent 2200 TapeStation, Thermo Scientific NanoDrop™ 2000, Illumina MiniSeq®

/ PROTOCOL

- All sample volumes were adjusted to 1 mL with DNA/RNA Shield™
- Samples were loaded into ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)
- Samples were homogenized using the Precellys® Evolution, with the following protocol:
  - 1 minute cycle at 9000 rpm
  - 120 second pause (at room temperature)
  - Repeat cycle 4 times
- DNA and RNA extraction was performed with ZymoBIOMICS® DNA/RNA Miniprep Kit, with a 100µL elution.
- Data analysis was performed with the Agilent Tapestation 2200 (High Sensitivty RNA ScreenTape) and Illumina MiniSeq®.

/ RESULTS

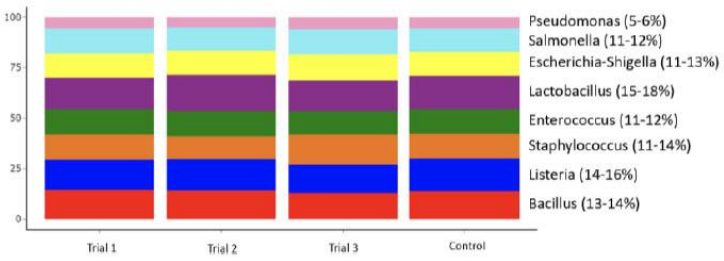

***Figure 1**: Microbial composition analysis (Genus) of the DNA extracted from 3 ZymoBIOMICS standard samples (Trial 1 – 3) that were mechanically lysed using the Precellys Evolution homogenizer .*

16S sequencing results show complete, unbiased lysis using the Precellys® Evolution Homogenizer. The microbial composition analysis (Genus) of the DNA extracted from 3 ZymoBIOMICS standard samples (Trial 1 – 3) that were mechanically lysed using the Precellys® Evolution homogenizer is closely aligned with the ZymoBIOMICS® Microbial Community Standard's theoretical composition, as can be seen in Figure 1. All 8 species in each sample were within 2-3% deviation of the theortical values.

/ RESULTS

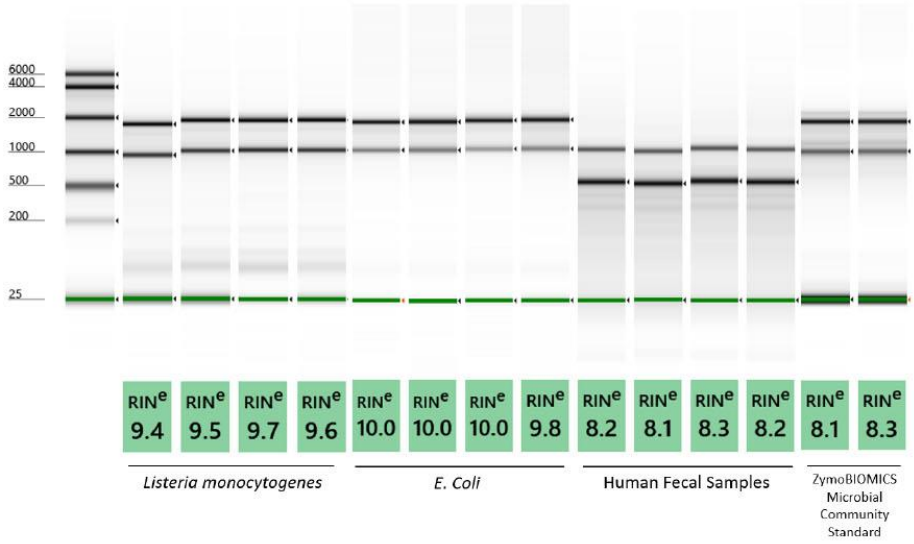

Figure 2 *: Agilent TapeStation 2200 results for ZymoBIOMICS® Microbial Community Standard, Escherichia Coli, Listeria monocytogenes, and human fecal matter samples.*

Lysis using the optimized protocol on the Precellys® Evolution homogenizer does not negatively impact the integrity of the nucleic acids. The purified RNA from all samples show strong ribosomal bands and high RIN, whether it is Gram-negative (easy-to-lyse) bacteria or Gram-positive (hard-to-lyse) bacteria. Complex samples, such as human fecal matter and the ZymoBIOMICS Microbial Community Standard which contain multiple microorganisms, inherently produce lower RIN scores due to the different sized ribosomal bands or the natural fragmentation characteristics of the sample type. Here, even these complex samples show RIN scores above 8.

Sample type	ng/uL	260/280	260/230
ZymoBIOMICS Standard	290.7	2.08	2.11
ZymoBIOMICS Standard	292.7	2.07	2.02
L. monocytogenes	196.7	2.06	1.99
L. monocytogenes	197.9	2.07	1.96
L. monocytogenes	186.7	2.08	1.99
L. monocytogenes	198	2.05	1.98
E. coli	280.8	2.12	2.35
E. coli	284	2.13	2.34
E. coli	284.2	2.13	2.32
E. coli	268.9	2.12	2.35
Fecal matter	257.5	2.11	2.24
Fecal matter	240.8	2.12	2.17
Fecal matter	329.9	2.12	2.17
Fecal matter	329.2	2.11	2.16

Figure 3: *Thermo Scientific NanoDrop™ Spectrometer results for the RNA extracted from ZymoBIOMICS® Microbial Community Standard, Escherichia coli cells, L. monocytogenes cells, and human fecal matter samples.*

Furthermore, as can be seen in **Figure 3**, Thermo Scientific NanoDrop™ Spectrometer results for the RNA isolated after lysing with the Precellys® Evolution homogenizer shows high purity and yield.

/ REFERENCES

1. Wesolowska-Andersen A, Bahl MI, Carvalho V *et al.* Choice of bacterial DNA extraction method from fecal material influences community structure as evaluated by metagenomic analysis. *Microbiome* 2(1), 19 (2014)

2. Costea PI. Zeller G, Sunagawa S *et al.* Towards standards for human fecal sample processing in metagenomic studies. *Nat. Biotechnol.* 35(11), 1069-1076 (2017)

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Zymo Research is a privately-held American manufacturer of molecular biology research tools used for DNA and RNA research and analysis. Their offices are headquartered at 17062 Murphy Ave., Irvine, CA92614, Tel:949-679-1190
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/ CONCLUSION

Here, we were able to show with the ZymoBIOMICS® Microbial Community Standard, that the use of Precellys® Evolution with the optimized protocol followed by nucleic acid extraction with the ZymoBIOMICS DNA/RNA Miniprep Kit constitutes a robust and unbiased workflow. Preventing bias is essential for accurate microbial analysis and profiling.

The complete workflow provided by Precellys® Evolution combined with ZymoBIOMICS DNA/RNA Miniprep Kit yields DNA and RNA of high purity and integrity, compatible with NGS analysis.

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