Precellys
The Science of Lysing

BEST PRACTICES FOR NGS SAMPLE PREPARATION

Over the past decade, the advent of next-generation sequencing techniques (NGS) has revolutionized the landscape of biology. Whole genomes and transcriptomes can now be sequenced much more efficiently in terms of time & cost than with earlier technologies, allowing scientists to deepen their understanding of many diseases such as cancer. NGS workflows generally begin with a sample preparation step, whose success is crucial to ensure reliable sequencing results. An efficient and uniform homogenization is needed to obtain an adequate quantity and quality of nucleic acids.

Mechanical lysis, and more specifically bead-beating technology, is considered the gold standard for sample homogenization. For this reason, Bertin Technologies has chosen 3-dimensional bead-beating technology to empower its range of homogenizers, the Precellys.

In this White Paper, we present best practices for NGS sample preparation steps with the Precellys homogenizers. Six optimized protocols, suitable for food, tissue, and fecal samples are presented to help researchers get high-quality, high-yield nucleic acids for their NGS workflows.

DISCOVER OPTIMIZED HOMOGENIZATION PROTOCOLS FOR NGS WORKFLOWS

SUMMARY

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

Zymo Research Corp (Irvine, CA) and Bertin Corp (Rockville, MD)

/ 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

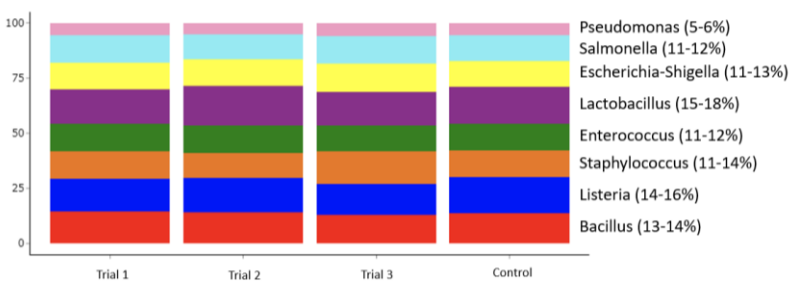

***Figure 1**: Microbial composition analysis (Genus) of the DNA extracted from 3 ZymoBIOMICS standard samples (Trial 1 – 3) that were mechanically lysed using the Precellys Evolution homogenizer .*

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.

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

Zymo Research Corp (Irvine, CA) and Bertin Corp (Rockville, MD)

/ RESULTS

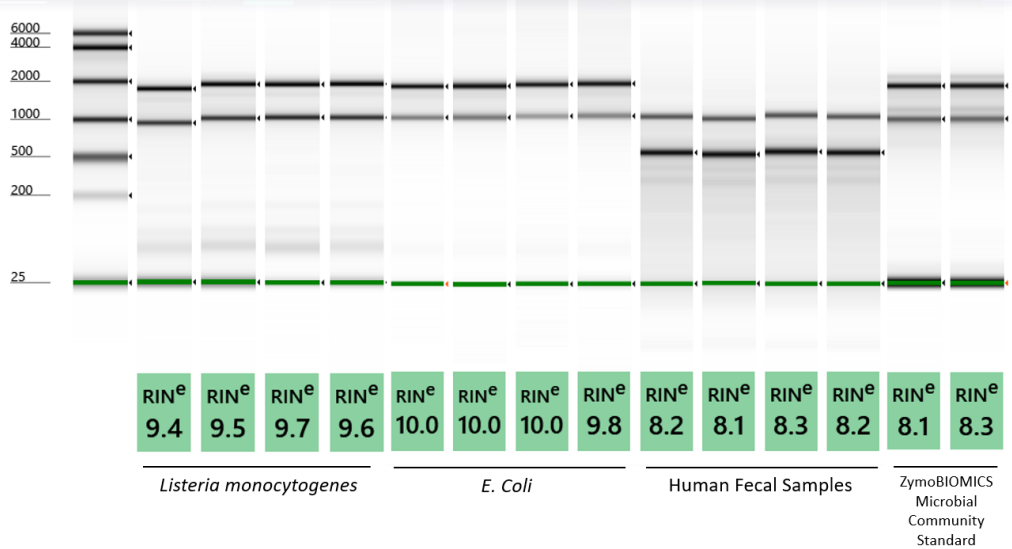

Figure 2 : *Agilent TapeStation 2200 results for ZymoBIOMICS® Microbial Community Standard, Escherichia Coli, Listeria monocytogenes, and human fecal matter samples.*

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)

ZYMO RESEARCH
The Beauty of Science is to Make Things Simple

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
www.zymoresearch.com

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.

PREC-026-DU145-A

MICROBIOTA ANALYSIS OF FERMENTED MILK SAMPLES WITH NEXT GENERATION SEQUENCING

Centre for Healthy Eating & Food Innovation, Maastricht University
Campus Venlo, Venlo, The Netherlands

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/ CONTEXT

Dadih is a traditional Indonesian fermented buffalo milk drink with numerous health benefits associated with several probiotic strains. The current process of dadih production is still mostly artisanal and does not rely on good hygiene practices. The Dadih Initiative was started in Indonesia in 2017 to standardize and optimize production and quality control of dadih. However, before expanding the production of dadih, additional insights into the nature of microorganisms present in the final product are needed. With the advent of next generation sequencing technologies (NGS), it is now possible to explore the microbiota composition of dadih samples with an unprecedented level of complexity. In this work, the microbiota composition of artisanal dadih samples was determined using 16S-rRNA-gene amplicon-sequencing of the V3-V4 region. Several sites of collection and production methods were evaluated, including samples made from pasteurized or raw milk, and using back-slopping (a practice which involves using previous fermentation as a starter culture) or not.

/ MATERIALS AND PROTOCOL

- Dadih samples were collected from two different regions in Bukittinggi city (Gadut - 2 separate producers - and Palupuh), and in Padang Panjang town. There were differences in back-slopping practice, where previous fermentations were used as a starter culture (five samples) or not (three samples). Table 1 gives an overview of the different samples obtained. Duplicate samples were stored frozen.
- **DNA extraction:** the samples were centrifuged at 14 500 g and subsequent DNA extraction from the pellet was performed using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research, Irvine, CA) according to manufacturer's instructions, and the **Precellys 24 tissue homogenizer** (Bertin Technologies, Montigny-le-Bretonneux, France), with 3 cycles of 30s at 6500 rpm, with 5 min cooling on ice in between.
- **PCR-amplifying the V3–V4 region of the 16S rRNA gene and next generation sequencing:** Illumina 16S rRNA gene amplicon libraries were generated and sequenced at BaseClear (Leiden, the Netherlands). In short, barcoded amplicons from the V3–V4 region of 16S rRNA genes were generated using a 2-step PCR.
- **Sequence processing and analyses:** The sequencing run was analysed with the Illumina CASAVA pipeline (v1.8.3) with demultiplexing based on sample-specific barcodes. Results can be found in Figure 1.

/ CUSTOMER

Maastricht University

(1) VENEMA, Koen et SURONO, Ingrid. S. Microbiota composition of dadih–a traditional fermented buffalo milk of West Sumatra. *Letters in applied microbiology*, 2019, vol. 68, no 3, p. 234-240.

/ RESULTS

	Site of collection	Pasteurized	Back slopping	Note
A	Gadut	Yes	Yes	Producer 1
B	Gadut	No	Yes	Producer 1
C	Palupuh	No	Yes	
D	Palupuh	Yes	No	
E	Padang Panjang	No	No	
F	Padang Panjang	Yes	No	
G	Gadut	Yes	Yes	Producer 2
H	Gadut	No	Yes	Producer 2

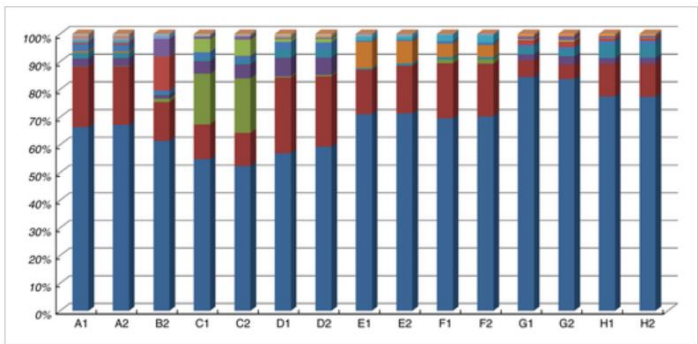

Figure 1: Relative abundance of major OTUs (at least 0·1% in any of the samples) in the duplicate dadih samples (coded according to Table 1).
Legend for microbial OTUs (at genus level, or higher if not available at genus level, indicated by f__: family, o__: order. When the same names are given, these are still different OTUs): Lactococcus ; Klebsiella ; f_Bifidobacteriaceae ; Leuconostoc ; f__Enterobacteriaceae ; Streptococcus ; f__Enterobacteriaceae ; f__Lactobacillaceae ; Lactobacillus ; f__Lactobacillaceae Acinetobacter ; f__ Oxalobacteraceae ; Vagococcus ; Acetobacter ; Proteus ; f__Enterococcaceae ; o__Lactobacillales, and Corynebacterium . From (1)

NGS results show that Lactococcus (52–83%) predominated in all samples, followed by Klebsiella (5–26%), and Lactobacillaceae, Bifidobacterium, Streptococcus, and Leuconostoc . Back-slopping practice was significantly correlated with higher levels of Lactobacillaceae, Pediococcus, species of the order Burkholderiales, and Serratia, but with lower levels of other Enterobacteriaceae (including Klebsiella), Streptococcaceae, Staphylococcus, and Brachybacterium. Taken together, fermentation results vary significantly from producer to producer and back-slopping practice should be recommended. These findings are instrumental in implementing future good manufacturing practices to produce a safe and healthy yoghurt-like dadih. The Precellys homogenizer is the ideal instrument for food quality control workflows, giving access to high-yield, high-quality nucleic acids suitable for Next Generation Sequencing (NGS) analysis.

PREC-026-DU151-B

TRANSCRIPTOMIC ANALYSIS OF CHEESE-RIPENING MICROBIAL COMMUNITIES WITH DUAL RNA-SEQ

Université Paris-Saclay, INRAE, AgroParisTech, UMR SayFood, Thiverval-Grignon, France

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/ CONTEXT

Ripening cultures containing fungi and bacteria have a crucial role in the production of smear-ripened cheeses. Unfortunately, little is known about the biotic interactions within these microbial communities at the cheese surface, except for the positive impact of the pH increase initiated by fungi on the growth of several acid-sensitive bacteria such as *Brevibacterium aurantiacum*, and *Hafnia alvei*. In this work, the biotic interactions of a cheese-ripening community composed of *Debaryomyces hansenii*, *Brevibacterium aurantiacum*, and *Hafnia alvei*, are explored thanks to a lab-scale mini-cheese model which aims to reproduce cheese-ripening conditions. The development of next generation sequencing techniques (NGS) over the past decade now allows researchers to analyze the transcriptome of multiple species present in the same sample simultaneously. Here, a relatively novel technique, dual RNA-seq, was used to capture the transcriptome of *D. hansenii*, B. *aurantiacum*, and *H. alvei* and analyze the metabolic interactions between these species at the cheese surface.

/ MATERIALS AND PROTOCOL

- Mini-cheese production: Different mini-cheeses were produced, with the complete community, with *D. hansenii* alone, with *D. hansenii* and *H. alvei*, and with *D. hansenii* and *B. aurantiacum*, ripened at 15°C for 21 or 28 days.
- Cheese samples homogenization: 500mg cheese samples were homogenized using the Precellys Evolution homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) in 7mL Precellys lysing tubes containing 0.1 mm-diameter and 0.5 mm-diameter beads zirconium beads (Biospec Products, Bartlesville, US) with 5 ml of UptiZol reagent (Interchim, Montluçon, France), and the following program: 2 cycles of 20s at 10 000 rpm , with a 5min break on ice after each cycle. The tubes were then stored at −80°C until the RNA extraction.
- RNA extraction: Phenol-chloroform RNA extraction, followed by DNAse treatment steps and an rRNA depletion step were performed according to the protocol described in [1].
- Dual RNA-seq: Directional RNA-seq libraries were constructed using the ScriptSeq V2 RNA-seq library preparation kit (Illumina), according to the manufacturer's recommendations (11 PCR cycles were performed). Libraries were pooled in equimolar proportions and sequenced (Single Read 75 pb) on an Illumina NextSeq500 instrument, using a NextSeq 500 High Output 75 cycles kit. Results related to iron-acquisition genes expression can be found in Figure 1.

/ RESULTS

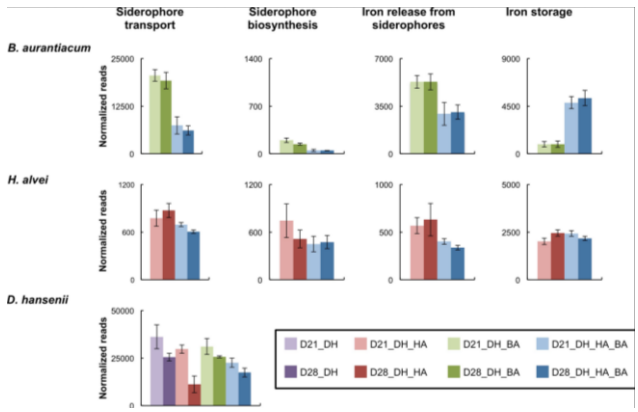

Figure 1:. Expression of genes involved in iron acquisition in the mini-cheeses. The expression level for each type of gene is represented as the sum of the sequencing reads (normalized against each species) that mapped to the corresponding genes. Bars are colored according to the biological conditions; D21 and D28 correspond to the sampling time (day 21 and day 28, respectively); DH, HA, and BA correspond to the presence of D. hansenii, H. alvei, and B. aurantiacum, respectively. The error bars represent the standard deviations (four cheese replicates). From [1].

Dual RNA-seq results indicate that the production of siderophores – small iron-chelating compounds that are commonly secreted by microorganisms to serve as iron carriers across cell membranes – by *H.alvei* increases iron availability for *B. aurantiacum*.

/ CUSTOMER

INRAE AgroParisTech

[1] PHAM, Nguyen-Phuong, LANDAUD, Sophie, LIEBEN, Pascale, *et al.* Transcription profiling reveals cooperative metabolic interactions in a microbial cheese-ripening community composed of Debaryomyces hansenii, Brevibacterium aurantiacum and Hafnia alvei. *Frontiers in microbiology*, 2019, vol. 10, p. 1901.

Dual RNA-seq analysis of mini cheese samples shows that iron acquisition plays an important role in the biotic interactions between cheese surface microorganisms. Using techniques such as dual RNA-seq can help researchers decipher the metabolic interactions within ripening cheese cultures, which in turn could help improve strain selection for cheese production. The Precellys Evolution homogenizer allows for efficient and uniform lysis of microbial cells, and gives access to high-quality RNA suitable for RNA-seq analysis.

PREC-026-DU152

MICRORNA EXPRESSION PROFILING OF INDIVIDUAL RAT HYPOTHALAMIC NUCLEI

Neuroendocrinologie Moléculaire de la Prise Alimentaire, University of Paris-Sud 11, UMR 8195, Orsay, France and Neuroendocrinologie Moléculaire de la Prise Alimentaire, CNRS, Centre de Neurosciences, Paris-Sud UMR8195, Orsay, France.

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/ CONTEXT

MicroRNAs (miRNAs) finely tune messenger RNA (mRNA) expression. As the brain is a highly heterogeneous tissue, physiologically relevant miRNA expression profiling greatly benefits from sampling brain regions or nuclei. MiRNA expression profiling from individual samples is also important for investigating potential differences between animals according to their physiological and pathophysiological status[1].

/ MATERIALS

Precellys 24 & Cryolys cooling option.

Precellys lysing kit: 03961-1-003 (CK14).

Sample: ~1-3 mm3 of frozen hypothalamic tissue.

Extraction solvent: 700µl QIAzol lysis reagent (Qiagen).

/ PROTOCOL

Precellys 24: 5500 rpm, 1x20 sec.

Small RNAs were purified using the miRNeasy Mini Kit (Qiagen) with the two-column system.

Small RNAs were recovered in a volume of 30µL of RNase-free H2O and size-fractionated on a denaturing urea (8M) polyacrylamide (17%) gel.

RNAs of 16-30 bases were eluted in 0.4M NaCl by overnight incubation under gentle shaking at +4°C, then precipitated with the addition of 3 volumes of ethanol in the presence of 0.04µg/µL glycogen. cDNA libraries were constructed using an Illumina-like protocol.

/ RESULTS

cDNA library sequencing using a GAIIX machine provided individual miRNA expression profiles from single hypothalamic nuclei with a read depth >10-5. Individual miRNA expression profiles are shown for five paraventricular nuclei (PVN). All harbored products from more than 200 miRNA genes, the twenty most abundant of which accounted for 78-85% of the whole profiles (figure 1).

Overall, our results showed that cDNA libraries can be constructed with RNAs of 16-30 bases that have been purified from individual rat hypothalamic nuclei.

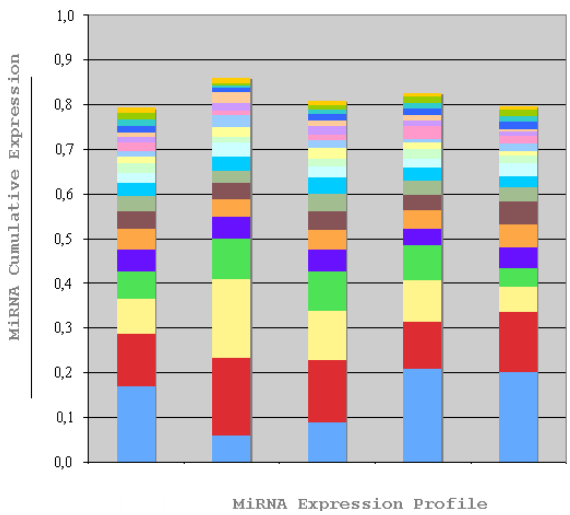

Figure 1: MiRNA expression in PVN profiles.

Expression of the 20 most highly expressed miRNAs are shown for the five PVN profiles. Different miRNAs are shown in different colors.

The quality of the cDNA libraries proved the quality and efficiency of the Precellys 24 when working with hypothalamic tissues.

Precellys 24 homogenizer alone can be used at least for hypothalamic tissues. For other tough tissues requiring a longer time of homogenization, Cryolys cooling option is strongly recommended.

03712-810-DU074

DNA EXTRACTION FROM FROZEN TUMOR SAMPLES USING THE MINILYS TISSUE HOMOGENIZER COMPARED TO THE MANUAL HOMOGENIZATION METHOD

Molecular Pathology Unit, Liverpool Clinical Laboratories , UK

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/ CONTEXT

Breast, ovarian, endometrial and lung tumor samples are routinely homogenized and processed for DNA in cancer research. In addition to local diagnostic requirements, DNA obtained from tumour samples is submitted to the 100,000 Genome Project that aims to use Whole Genome Sequencing (WGS) technique on patients, plus their families, with a rare disease or cancer. This project imposes high standards of DNA quantity and fragment length quality.

In this study, the Minilys tissue homogenizer was evaluated for tumor tissue sample homogenization and results were compared to those obtained following a manual sample homogenization method. The DNA yield and quality as well as hands-on time required were compared between the two methods.

/ MATERIALS

Samples: 4mm punch biopsies of frozen specimens

Buffer: Proteinase K buffer

For Minilys Method: Minilys homogenizer and 2mL CK28-R Precellys lysing kit.

For manual method: Mini plastic disposable pestle and mortar (optional, a razor blade or scalpel)

/ PROTOCOL

Manual method: The frozen biopsies were manually treated using the mini plastic disposable pestle/mortar. Samples not homogenized satisfactorily, were chopped up using razor blades/scalpels (treatment time: 5 to 10 minutes per sample). Each sample was then split in 2 tubes: one for storage and one for analysis. Tube for analysis was lysed overnight with 20 µl of Proteinase K (at 37°C) followed by a fluid extraction performed on the next day with a standard kit extraction.

Minilys method: The biopsies were placed into Precellys 2ml CK28-R tubes containing 180µl of ATL buffer. The samples were homogenized with Minilys for 2x20 seconds at 5,000 rpm and at the end of the run, 20µl of Proteinase K were then added directly into the tube for lysis (1h at 37°C). After lysis, each sample was split into 2 tubes: one for storage and one for analysis. Fluid extraction was then performed on the tube for analysis with a standard kit extraction.

/ RESULTS

- The processing time was significantly reduced when using Minilys for homogenization, as well as the post treatment time with Proteinase K (reduced to one hour vs overnight for the manual method).
- The yield of DNA recovery with Minilys was higher in 81% of the samples compared to the manual method. Nine out of eleven samples homogenized by the manual method didn't exceed the concentration of 15ng/µl while the lowest concentration found in samples homogenized by the Minilys was 3 times higher (49ng/µl). The average DNA yield recovery with the Minilys was 185.7ng/µl compared to 26.8ng/µl for the manual method. Therefore, only 1 sample needed to be treated.
- All DNA samples obtained with the Minilys showed good quality, including excellent fragment length (Figure 1) meeting the 100.000 genome requirements of >60% of fragments with a minimal length of 23kbp.

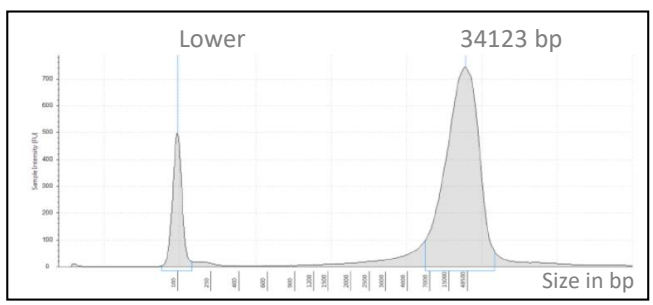

Figure 1. *Fragment length characterization using the TapeStation instrument (Agilent)*

/ CUSTOMER

Liverpool Clinical Laboratories

Dr. Alistair Reid
Consultant Clinical Scientist

The use of the Minilys tissue homogenizer to homogenize tumor samples proved to be an efficient method compared to manual sample preparation, and is now the reference method at Liverpool Clinical Laboratories:

- As DNA recovery yield is higher, DNA extraction no longer needs to be duplicated, reducing costs by half as only one DNA extraction kit per sample is needed.
- Hands-on time and total processing time were considerably reduced, thus saving both technical and human resources.
- The quality of the DNA samples obtained had optimal fragment length, leading to a high likelihood of successful WGS.

PREC-026-DU137

OPTIMIZED PROTOCOL FOR VIRAL METAGENOMICS STUDIES ON FAECAL SAMPLES WITH MINILYS HOMOGENIZER

University of Leuven, KU Leuven, Department of Microbiology, Immunology and Transplantation, Rega Institute, Laboratory of Viral Metagenomics, Leuven, Belgium

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/ CONTEXT

Next Generation Sequencing (NGS) has revolutionized the study of the human gut microbiome - the community of microorganisms (including bacteria, fungi, and viruses) present within the human gut. While in recent years, our understanding of the human microbiome has greatly improved, most studies have focused on bacteria, ignoring viral genomes. For this reason, little is known regarding the human virome –the viral component of the human microbiome. One of the main challenges in studying the role of the human gut virome in health is the absence of validated methods for high throughput and reproducible virome analysis.

In this study, the quantitative effects of different steps of sample preparation for virome analysis were evaluated with qPCR and next generation sequencing (NGS). Several procedures were tested for the homogenization, centrifugation, filtration, and random amplification steps, using a mock-virome (including 9 highly diverse viruses among which coronaviruses) and a bacterial mock-community. As a result, an optimized protocol for fecal sample preparation was created, the NetoVIR (Novel enrichment technique of VIRomes). The NetoVIR protocol makes use of the Minilys (Bertin Technologies, France) to efficiently and uniformly homogenize samples. Thanks to Minilys-powered homogenization and other optimized steps.

(Figure 1), the NetoVIR protocol can recover all viruses present in the mock-virome samples.

/ PROTOCOL

Homogenization: Mock-virome and bacterial mock-community were homogenized using a tissue homogenizer (Minilys, Bertin technologies, France). A 200 µL stock of mock-virome was subjected to different homogenization speeds (3000 rpm or 5000 rpm) with or without the presence of ceramic beads (Ø0.1 mm (CK01–2 ml, P000919-LYSK0-A) or Ø2.8 mm (CK28–2 ml, P000911-LYSK0-A)) and compared to a non-homogenized control (Figure 2). All samples were homogenized for 1 min.

Centrifugation and filtration: Samples were centrifuged using a bench top centrifuge (Heraeus pico 17, Thermoscientific). Two-hundred µl of mock-virome or bacterial mock-community was centrifuged at 100 g or 17000 g for 3 min or 30 min. For filtration, a 0.8-µm centrifugal (PES) filter (VK01P042, Sartorius), a 0.8-µm polycarbonate (PC) filter (ATTP14250, Millipore), as well as a 0.45-µm centrifugal filter (UFC40HV00, Millipore) and a 0.22-µm centrifugal filter (UFC40GV00, Millipore) were tested.

Nuclease treatment and DNA/RNA extraction: Samples were treated for 2 hours at 37 °C with a cocktail of 1 µl microccocal nuclease (NEB) and 2 µl of benzonase (Millipore) and 7 µl of homemade buffer (1M Tris, 100 mM CaCl2 and 30 mM MgCl2, pH 8) and extracted with the QIAamp Viral RNA Mini Kit (Qiagen) without carrier RNA.

Random amplification: Random amplification of nucleic acids was performed using the Whole Transcriptome Amplification Kit 2 (WTA2, Sigma Aldrich) according to manufacturer's instructions with the exception of the initial denaturation step which was performed at 95 °C instead of 70 °C in order to also denature double-stranded DNA or RNA to make it available for the amplification. In addition, the number of amplification cycles was varied between 7, 12, 17 and 22. WTA2 products were purified with the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions.

NGS sequencing: NGS library preparation was performed using the Nextera XT DNA Library Preparation kit (Illumina) as described in [1]. Sequencing was performed on a HiSeq™ 2500 platform (Illumina) for 2 × 150 cycles. Sequencing reads can be seen in Figure 3.

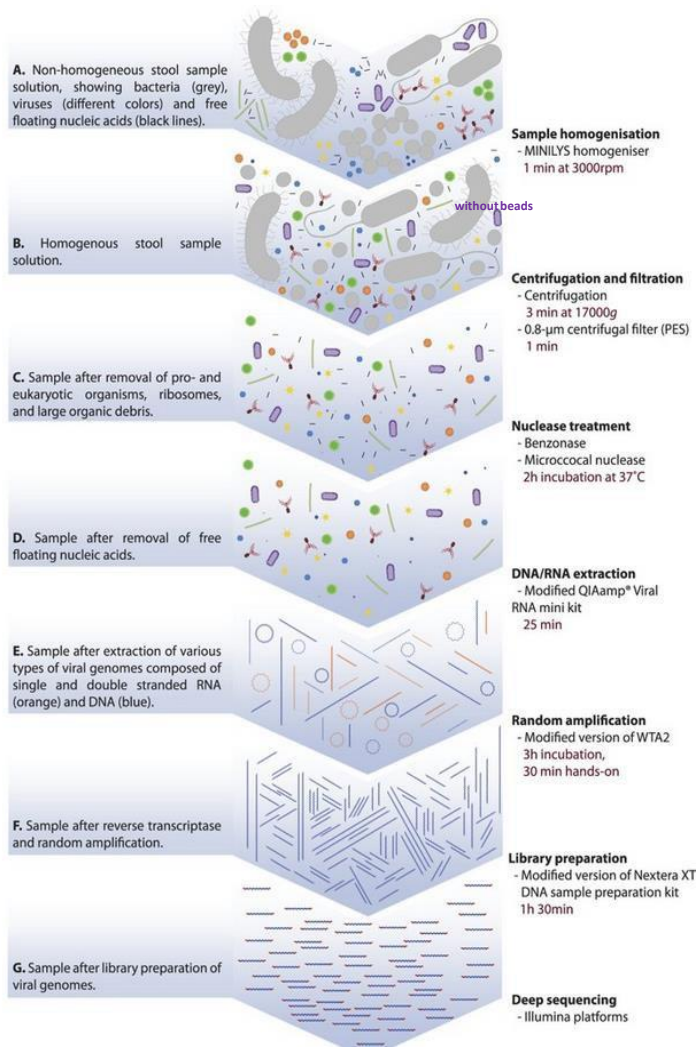

Figure 1: Description of the optimized NetoVIR protocol, On average, the protocol takes 8 h to complete. From [1]

OPTIMIZED PROTOCOL FOR VIRAL METAGENOMICS STUDIES ON FAECAL SAMPLES WITH MINILYS HOMOGENIZER

University of Leuven, KU Leuven, Department of Microbiology, Immunology and Transplantation, Rega Institute, Laboratory of Viral Metagenomics, Leuven, Belgium

/ RESULTS

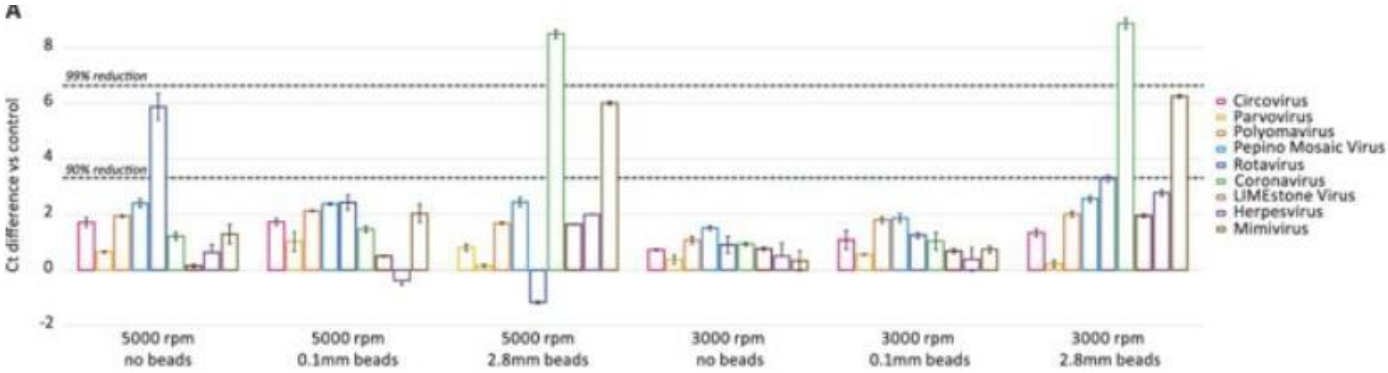

Figure 2: Ct differences vs control for different homogenization experiments performed on the mock-virome community. Standard deviations are based on three qPCR replicates. From [1].
Homogenization with Ø2.8 mm beads led to a destruction of viral particles irrespective of homogenization speed. The reduction was largest for coronavirus (99.5% and 99.6% and Ct differences of 8.5 and 8.9 for 5000 and 3000 rpm, respectively) and mimivirus (96.0% and 97.7% and Ct differences of 6.0 and 6.3 for 5000 and 3000 rpm, respectively). homogenization at 5000 rpm (without beads or with Ø0.1 mm beads) showed a larger reduction in viral particles than homogenization at 3000 rpm. Reduction of viral particles was lowest using 3000 rpm homogenization without beads.

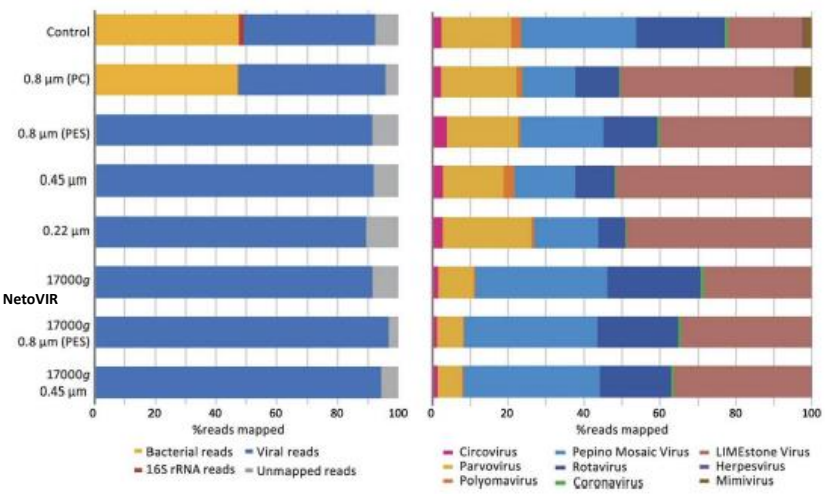

Figure 3: Left: NGS sequencing reads for sample consisting of pooled bacterial and viral communities: percentage of NGS sequencing reads for bacterial, 16S rRNA, viral and unmapped reads for the conditions tested, Right: Distribution of NGS sequencing reads for the mock-virome. From [1].
7 different workflows were tested including homogenization at 3000 rpm without beads and 17 amplification cycles, in combination with different conditions of filtration (0.8 PC/PES, 0.45 and 0.22-µm) and/or centrifugation (3 min at 17000 g).
The 0.8-µm PES filter plus centrifugation condition yielded the highest percentage of viral reads, of which most were attributed to pepino mosaic virus (33.9%), LIMEstone virus (32.9%) and rotavirus (20.6%.
The four protocols without centrifugation showed an expansion of the LIMEstone virus reads, mainly at the expense of rotavirus and pepino mosaic virus reads.
Based on these results, a favoured protocol named NetoVIR (Novel enrichment technique of VIRomes) was selected. NetoVIR consisted of homogenization at 3000 rpm for 1 min without beads, centrifugation for 3 min at 17000 g plus 0.8-µm PES filter filtration and 17 amplification cycles.

/ CUSTOMER

KU LEUVEN

[1] CONCEIÇÃO-NETO, Nádia, ZELLER, Mark, LEFRÈRE, Hanne, *et al.* Modular approach to customise sample preparation procedures for viral metagenomics: a reproducible protocol for virome analysis. *Scientific reports*, 2015, vol. 5, no 1, p. 1-14.

The NetoVIR protocol with Minilys homogenization allows for fast, reproducible and high throughput sample preparation for viral metagenomic studies. NGS results show that all viruses present in the mock virome sample can be recovered. It also seems like the ratio of viral versus bacterial and 16S rRNA genetic material is strongly altered in favor of viruses.

For most biological and environmental samples, obtaining an homogenous solution is essential for optimal and reproducible viral particles purification. This study shows how Minilys can homogenize fecal samples in a fast, reproductible way while introducing minimal bias.

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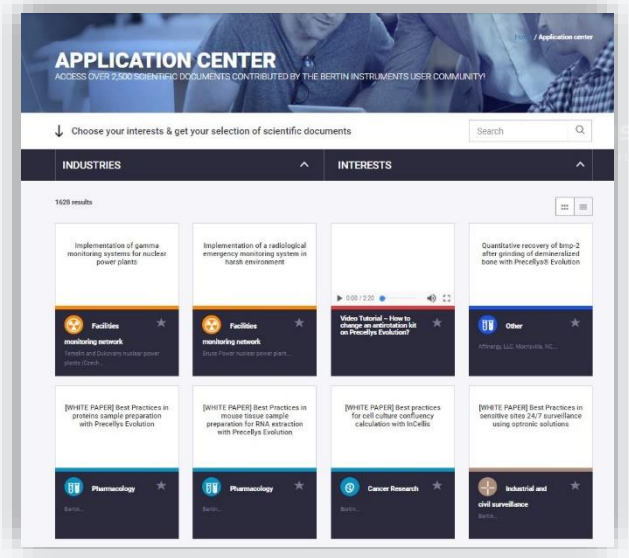

Precellys® Evolution is the most advanced homogenizer gathering high efficiency and versatility for all sample preparation needs:

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