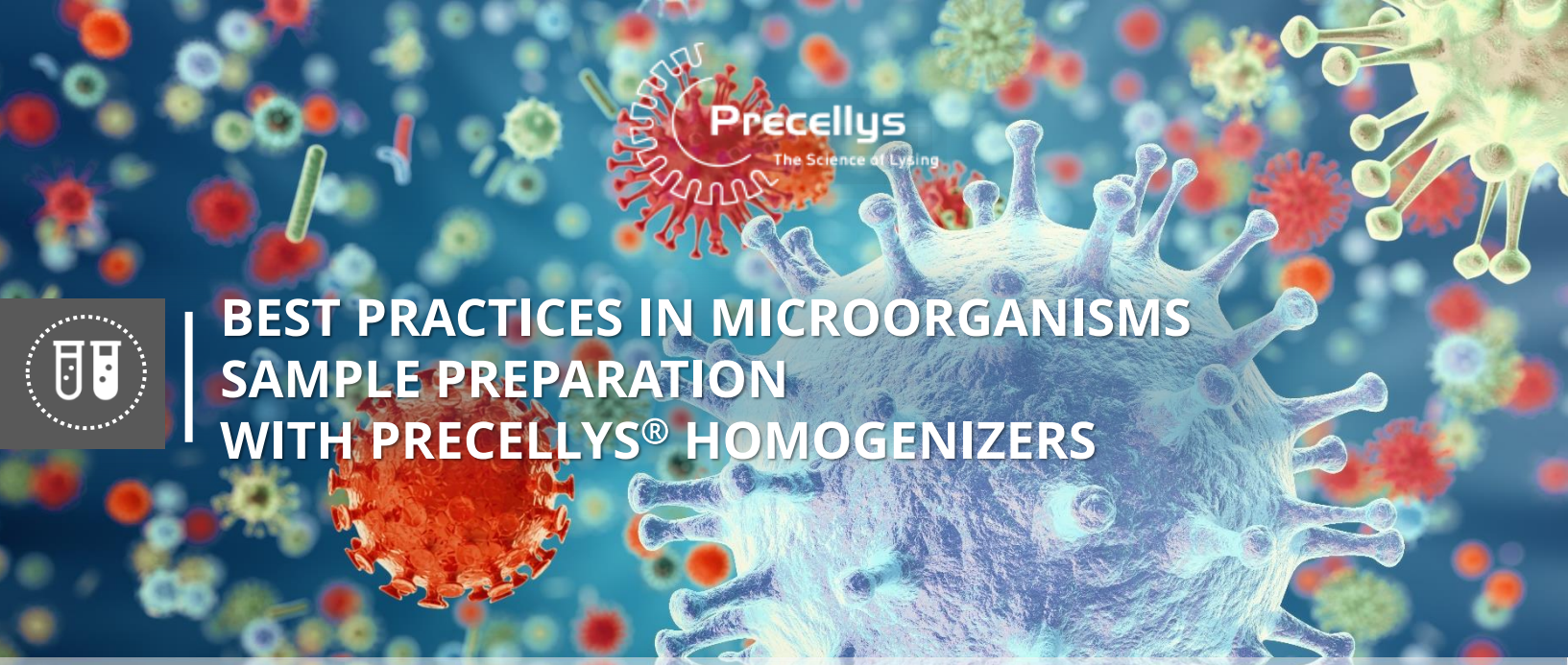

BEST PRACTICES IN MICROORGANISMS SAMPLE PREPARATION WITH PRECELLYS® HOMOGENIZERS

Microorganisms, like bacteria, fungi, archaea, algae, protozoa, and viruses, known as microbes play a critical role in many different phenomena. They are involved in climate change, nutrient cycling, biodegradation and diseases. In biotechnology, microbes can be used in a wide range of fields, such as the pharmaceutical industry to produce drugs, the environmental sector to help manage pollution and the food industry for food processing. The major goals of microbiology research are to decipher the mechanisms by which microorganisms affect health and diseases, as well as finding new applications to them.

Recently, the field of microbiology has been greatly advanced by new techniques enabling culture-independent analyses. In most studies, the composition of a microbial population is evaluated via NGS (Next Generation Sequencing), after which the results are compared to known bacterial sequence databases.

Reproducible microorganisms analysis depends on a robust and unbiased microbiome workflow. Cell lysing is an important step before proceeding to the extraction of DNA, RNA, proteins or live bacteria from samples. Mechanical lysis using beads (bead beating) is considered the gold standard method to lyse various types of cells and microbes. The Precellys tissue homogenizer is capable of performing cell and microbes lysis within minutes thanks to its 3D-bead beating technology.

THE PRECELLYS® IN COMBINATION WITH THE RIGHT LYSING KIT HOMOGENIZES ANY TYPE OF SAMPLE RAPIDLY

SUMMARY

bertin INSTRUMENTS

OZYME
Des femmes et des hommes au service de vos recherches

CNIM group

UNBIASED DNA EXTRACTION FROM THE ZYMOBIOMICS™ MICROBIAL COMMUNITY STANDARD

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

/ CONTEXT

Microbiome profiling via Next Generation Sequencing (NGS) techniques is rapidly changing the landscape of microbiology. However, to ensure accurate microbiome measurements, it is essential to ensure the microbiome workflow is unbiased and robust.[1] For instance, it has been reported that bias in microbiomics analyses can be introduced at every step[2-4] including the DNA extraction step if microbial cells are not uniformly and completely lysed. Mechanical cell lysis using beads of various composition (bead beating) is a generally accepted method used to lyse various microbes[5] and, although a variety of mechanical lysis devices and instruments are available, their performance greatly varies.[6-8] Until recently, there has not been a convenient way to assess the efficiency of lysis afforded by any particular instrument, however, with the release of the ZymoBIOMICS™ Microbial Community Standard (Zymo Research Corp., Irvine, CA) this is now possible. The ZymoBIOMICS™ Microbial Community is a mock microbial community standard comprised of ten well-characterized organisms (i.e., 5 gram-positive, 3 gram-negative, and 2 yeast) that are mixed at defined proportions. The standard contains microbes of different cell wall recalcitrance and of different cell size making it ideal for evaluating cell lysis efficiency. In this study, the efficiency of microbial lysis with the ZymoBIOMICS™ Microbial Community Standard was assessed using the Precellys® Evolution tissue homogenizer combined with Cryolys® Evolution cooling unit (Bertin Instruments, Rockville, MD).

/ MATERIALS

Instrument: Precellys® Evolution and Cryolys® Evolution (Bertin Instruments)

Sample: DNA of the twelve aliquots (75 µl each) of the ZymoBIOMICS™ Microbial Community Standard extracted using the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research Corp.) following the manufacturer's protocol.

Buffer: 725 µl of Lysis Solution per sample included in the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research Corp.)

/ PROTOCOL

Cell lysis

- Cell lysis was performed using a Precellys® Evolution set at maximum speed (10,000 rpm) fitted with a Cryolys® Evolution cooling unit set at 4 °C.
- Lysis of the standard was assessed in triplicate and several run where tested in order to validate the optimal run:
  - 1*60 sec, no break (total time 1 min)
  - 3*60 sec, 60 sec break (total time 5 min)
  - 5*60 sec, 60 sec break (total time 9 min)
- Extracted DNA was quantified using a Qubit™ dsDNA BR Assay Kit (Thermo Fisher Scientific) and the microbial composition of the extracted DNA determined via 16S rRNA gene sequencing using an Illumina® MiSeq™ (2x300bp).
- Sequencing and data analysis were performed by the ZymoBIOMICS™ Sequencing Service (Zymo Research).

/ PROTOCOL

Initial Control cell lysis

- The control cell lysis was performed as described in the cell lysis section using a Supplier X tissue homogenizer set at maximum speed (6.5m/s).
- Lysis of the control was optimized by using the following run: -5*60 sec, 300 sec break (total time 25 min)
- DNA extraction and sequencing were performed as explained in the Cell lysis protocol section.

/ RESULTS

DNA quantification indicated the yields of extracted DNA reached a maximum (compared to control) when lysed for 1 minute using the Precellys® Evolution tissue homogenizer (Figure 1). This indicates that efficient cell lysation can be achieved by homogenizing cells for 1 minute instead of 5 minutes which was previously reported. These data were supported by 16S sequencing data that showed the microbial composition reflected the theoretical closely indicating that complete lysis was achieved in as little as 1 minute of bead beating (Figure 2).

In addition, standard composition is consistent and stays stable with the theoretical value even after 5 minutes of homogenization using Precellys® Evolution tissue homogenizer.

UNBIASED DNA EXTRACTION FROM THE ZYMOBIOMICS™ MICROBIAL COMMUNITY STANDARD

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

/ RESULTS

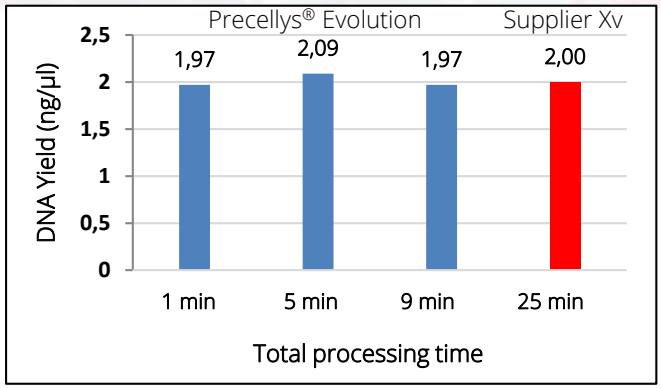

Figure 1. DNA concentrations achieved after homogenizing cells for 1, 3 and 5 minutes using the Precellys® Evolution tissue homogenizer representing a total processing time of 1, 5 and 9 minutes respectively. High yield DNA was obtained in as little as 1 minute cell lysis. The "Control" sample is the DNA yield obtained using an alternative homogenizer (Supplier X) and was achieved in approximately 5 minutes bead beating time and 25 minutes processing time in total.

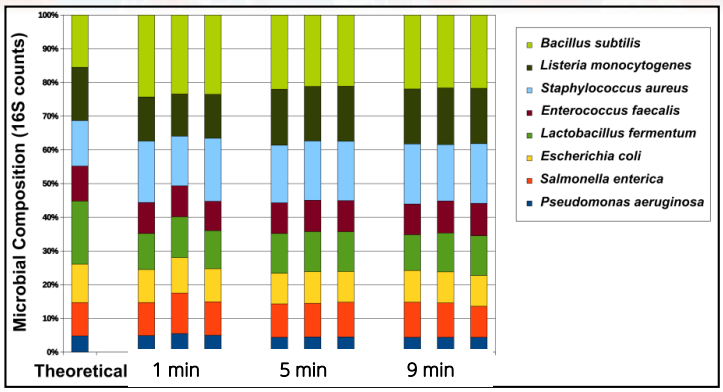

Figure 2. Microbial composition analysis (16S) closely matches the ZymoBIOMICS™ Microbial Community Standard's theoretical composition in as little as 1 minute using the Precellys® Evolution tissue homogenizer. DNA was extracted from ZymoBIOMICS™ Microbial Community Standard using the ZymoBIOMICS™ DNA Miniprep Kit and Precellys® Evolution tissue homogenizer and yields determined at the processing times indicated.

/ CONCLUSIONS

The Precellys® Evolution tissue homogenizer is capable of achieving complete, unbiased cell lysis in only 1 minute as assessed using the ZymoBIOMICS™ Microbial Community Standard in conjunction with the ZymoBIOMICS™ DNA Miniprep Kit. The optimized microbial lysis workflow reported here, serves as a validated procedure for accurate downstream microbiome measurements including amplicon sequencing, shotgun sequencing, RNA-based sequencing procedures. Not only does the workflow provide for efficient uniform microbial lysis and higher yields of inhibitor-free DNA, but it also facilitates reduced processing times with the entire extraction procedure requiring less than 20 minutes.

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VIABLE CULTIVABLE BACTERIA WITHIN TICKS AFTER HOMOGENIZATION WITH PRECELLYS 24

Tropical Infectious Diseases Research & Education Centre, University of Malaya, 50603 Kuala Lumpur, Malaysia.

/ CONTEXT

Disease transmission from rat to humans can occur through direct contact with rat excretion, contaminated environment or through ectoparasite vectors such as ticks. Ixodes sp. in particular, are three-host ticks commonly encountered in wild rats and have been documented to harbour Anaplasma sp. and Babesia sp. pathogens. Among those pathogens that are commonly found in the environment, bacterial pathogens of the genus Bacillus and Paenibacillus for example, are ubiquitous and several rare species have recently surfaced in the clinical setting as causative agents of human infections.

Wild rodents are often attracted to human dwellings by waste produced by human activity, this may aid the spread and transmission of tick-borne pathogens.

In light of this, an effort was initiated to study the cultivable bacteria within ticks collected from wild rodents.

/ MATERIALS AND PROTOCOL

Rodents were captured (Sungai Congkak Recreational Forest, Selangor, Malaysia) and identified (Table 1).

Ticks were collected from trapped rodents. Engorged adult ticks were collected from the rats and identified using morphological keys.

Collected ticks were individually surface sterilized with 70 % ethanol and rinsed thoroughly with nuclease-free water to remove environmental contaminants before homogenization.

Clean ticks were homogenized using Precellys® 24 as follows:

- Precellys® Lysing kit CK28, ref. P000911-LYSK0-A.0 (2.8 mm ceramic beads),
- 5500 rpm, 40 seconds in 500µl of sterile PBS.

Tick homogenates were inoculated onto Columbia agar with 5 % sheep blood and incubated at 37°C for 48 h under aerobic condition.

Bacterial cultures were purified until single colonies were obtained, followed by 16S rDNA sequencing for identification.

/ CUSTOMER

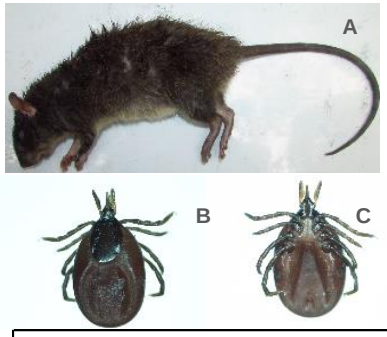
Shih-Keng Loong, Siti-Nabilah Ishak, Fang-Shiang Lim, Jing-Jing Khoo, Farah-Shafawati Mohd-Taib, Sazaly AbuBakar.

UNIVERSITY OF MALAYA

TIDREC@UM
TROPICAL INFECTIOUS DISEASES RESEARCH & EDUCATION CENTRE

/ RESULTS

One unique bacterial isolate originating from an engorged adult female *Ixodes granulatus* Supino (Fig.1, B et C) collected off a *Sundamys muelleri* rat (Fig. 1, A) was identified as *Paenibacillus lautus* via 16S rDNA, *ctpA* sequencing and biochemical tests. *P. lautus* isolate was susceptible to amikacin, ciprofloxacin, imipenem and meropenem. The isolate displayed resistance toward ampicillin, penicillin, clindamycin, chloramphenicol, rifampicin and trimethoprim-sulfamethoxazole, and intermediate-resistance toward erythromycin and gentamycin.



/ Fig. 1. *Sundamys muelleri*, Müller's giant Sunda Rat (A) and the collected engorged female adult *Ixodes granulatus*, dorsal (B) and ventral view (C).

Host		
Common name	**Scientific name**	**Number of ticks (n)**
Müller's giant Sunda rat	*Sundamys muelleri*	25
Whitehead's spiny rat	*Maxomys whiteheadi*	1
Rajah spiny rat	*Maxomys rajah*	1
Black rat	*Rattus rattus*	3

/ Table 1. Host of collected engorged female adult *Ixodes granulatus*.

/ CONCLUSION

The Precellys ® 24 coupled to the right set of lysing kits and parameters allow the extraction of living bacteria from ticks or infected tissue samples, ensuring their culture downstream to homogenization.

Paenibacillus lautus carried by ticks could potentially cause disease to humans thorough tick bites. In order to reduce the probability of interactions between wild rodents and human dwellings, an organized and monitored waste disposal could take place in public recreational areas.

PROTEIN EXTRACTION FROM E.COLI USING PRECELLYS® EVOLUTION

Institut Pasteur, PFPR, Paris, France (bellalou@pasteur.fr)

/ CONTEXT

The lab is responsible for screening a large number of E.coli culture conditions for the optimization of soluble protein production. Due to the flexibility and efficiency of the Precellys® Evolution, 3 volumes (1mL, 5mL, 10mL) of high concentrated E.coli cells were effectively lysed to extract soluble proteins for analytical or purification purposes. Different quantities of glass beads in Precellys® lysing tubes were evaluated.

/ MATERIALS

Precellys® Evolution.

Precellys® lysing kit: VK01_2mL (KT03961-1-005.2); Empty tube_7mL (KT03961-1-404.7) + 3.5g glass beads 0.1mm (KT03961-1-104.BK); Empty tube_7mL (KT03961-1-404.7)+2.4g glass beads 0.1mm; Empty tube_15mL (KT03961-1-406.15)+7g glass beads 0.1mm; Empty tube_15mL (KT03961-1-406.15)+4.8g glass beads 0.1mm (KT03961-1-104.BK)

Sample: E.coli cells (DMSO1230) were normalized to an OD600 of 50, and subsequently loaded into a Precellys ® lysis kit (1mL into 2mL tubes; 5mL into 7mL tubes; 10mL into 15mL tubes).

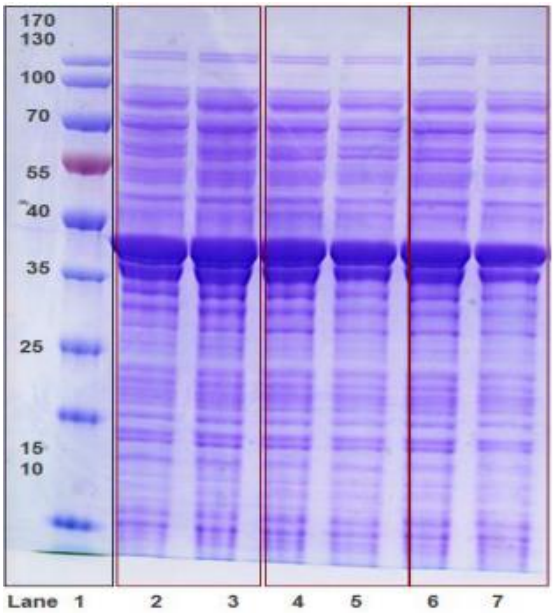
/ PROTOCOL

Precellys® Evolution (stored in a cold room): 9000rpm; 6x30sec (60s break) for 2mL and 7mL tubes; 9900rpm; 6x30sec (60s break) for 15mL tubes.

Analysis: After homogenization, the tubes were centrifuged for 15min at 5000 rpm. The supernatant contains the soluble protein fraction. The protein extracts were separated by SDS-PAGE, followed by Coomassie blue staining.

/ RESULTS

The gel picture obtained after homogenization on the Precellys® Evolution (Figure 1) shows an efficient extraction of protein into multivolume Precellys® lysing tubes (2mL, 7mL and 15mL). A higher quantity of glass beads improves the extraction of soluble protein.



The gel was stained with Coomassie Blue. Lane 1: molecular weight standard; Lanes 2 and 3: 1mL E.coli prep/0.7g glass beads_2mL; Lane 4: 5mL E.coli prep/3.5g glass beads_7mL tube); Lane 5: 5mL E.coli prep/2.4g glass beads_7mL tube; Lane 6: 10mL E.coli prep/7.0g glass beads_15mL tube; Lane 7: 10mL E.coli prep/4.8g glass beads_15mL tube.

/ CONCLUSION

The homogenizer Precellys® Evolution is suitable and convenient for high concentration (OD600=50) of E.coli cells lysis. Due to the flexibility of the Precellys® Evolution (2.0, 7.0 and 15mL Precellys® tubes), a scale-up volume of soluble proteins extracts can be carried out efficiently.
The Cryolys® cooling unit can be used to prevent degradation of thermo-sensitive samples.

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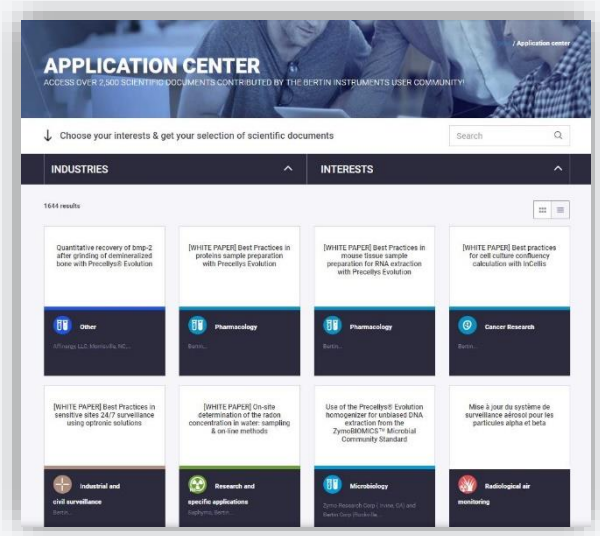

Precellys® Evolution is the most advanced homogenizer gathering high efficiency and versatility for all sample preparation needs:

- **Flexibility:** 24 x 2mL (or 0.5mL), 12 x 7mL, 6 x 15mL and 96 well-plate format
- **Efficiency:** up to 10 000 rpm speed to grind any type of sample
- **Integrity:** protect your molecules with the Cryolys® Evolution cooling unit

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Instrumentation : 01 30 85 92 88 - instrum@ozyme.fr

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