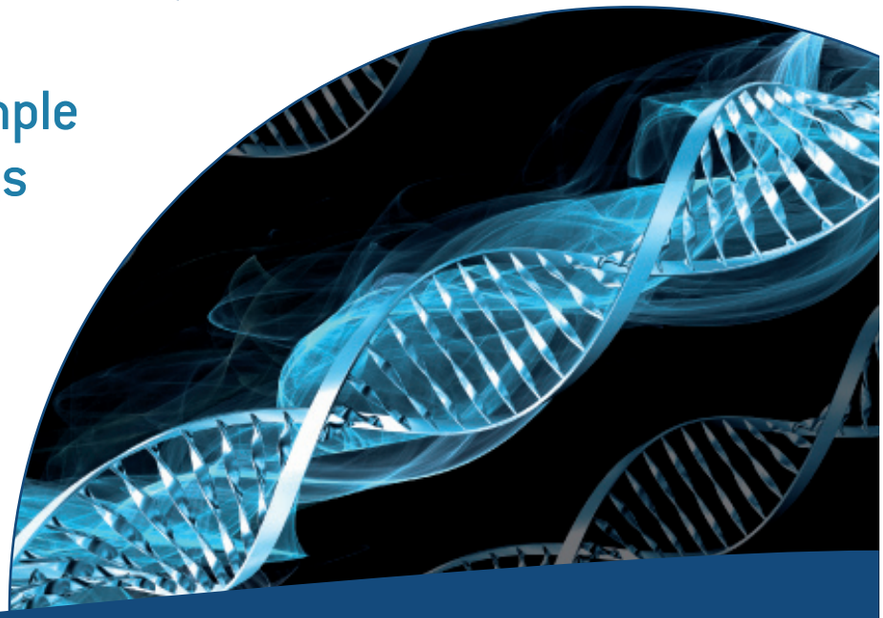
Precellys

The Science of Lysing

APPLICATION BOOKLET

Optimize your sample prep' with Precellys Homogenizers & Lysing Kits



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Precellys
The Science of Lysing

Find the Precellys product adapted to your needs!

Precellys 24
for high throughput

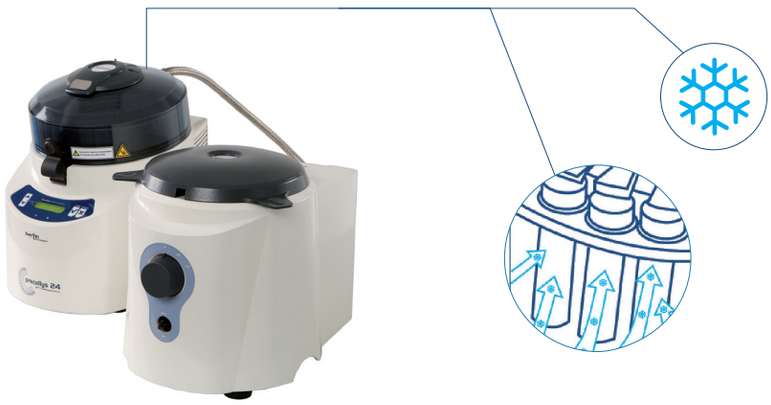
Precellys 24 Dual
for flexibility

Minilys
for personal use

Discover 3 different tissue homogenizers:

- different throughput
- different size of tubes / samples
- different budget

EQ03119-200-RD000.0 : Precellys 24
EQ03119-200-RD010.0 : Precellys 24 DUAL
EQ06404-200-RD000.0 : Minilys



Cryolys
for sensitive samples

The only efficient option for sensitive samples:

- compatible with Precellys 24 / Precellys 24-Dual
- cooling system before / during / after sample preparation process
- keep the extracted molecules their native properties

EQ05068-200-RD000.0 : Cryolys

Lysing Kits
Consumables

More than 25 references
of Lysing kits to fit with your samples type / samples size / method of analysis:

- different size / robsutness of tubes
- different type / size of beads

CRYOLYS
Cooling system for sensitive sample preparation

Native-state proteins

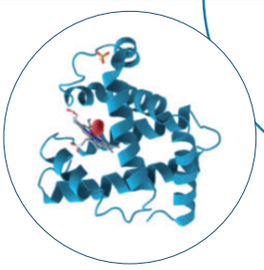
Stable RNA

Thermo-sensitive molecules

Enzymes

siRNA

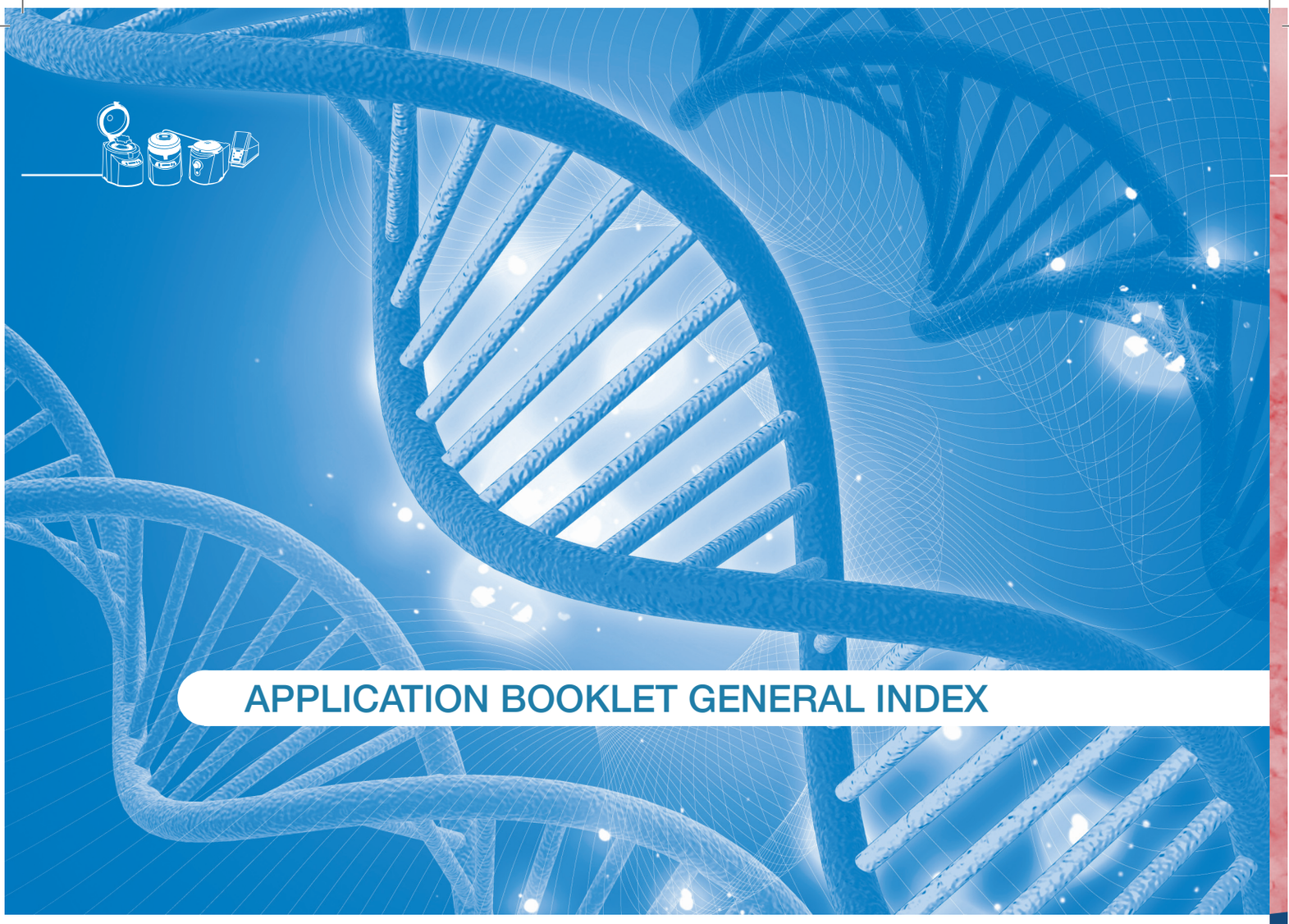
Metabolites



READ APPLICATIONS WITH CRYOLYS COOLING OPTION

READ SCIENTIFIC PUBLICATIONS WITH CRYOLYS COOLING OPTION

- Procedure for tissue sample preparation and metabolite extraction for high-throughput targeted metabolomics
  Werner Römisch-Margl., et al. (2010), Biomedical and Life Sciences; Metabolomics, DOI: 10.1007/s11306-011-0293-4Online First™
- Biochemical and behavioral characterization of the double transgenic mouse model
  Huaqi Xiong et al. (2011) (APPswe/PS1dE9) of Alzheimer's disease, Neurosciences bulletin August 1, 2011, 27(4):221-232. DOI:10.1007/s12264-011-1015-7
- Expression Profile of Genes Associated with the Dopamine Pathway in Vitiligo Skin Biopsies and Blood Sera
  Ene Reimann et al. (2012), Dermatology (DOI: 10.1159/000338023)
- Atorvastatin up-regulate toxicologically relevant genes in rainbow trout gills
  Kathrin Sabine Ellesat et al. (2012), Ecotoxicology DOI 10.1007/s10646-012-0918-z
- Abcg2 deficiency augments oxidative stress and cognitive deficits in Tg-SwDI transgenic mice
  Yu Zeng et al. (2012), Journal of Neurochemistry DOI: 10.1111/j.1471-4159.2012.07783.x
- Discovery and Optimization of New Benzimidazole- and Benzoxazole- Pyrimidone Selective PI3Kß Inhibitors for the Treatment of Phosphatase and TENsin homologue (PTEN)- Deficient Cancers
  Victor Certal et al. (2012), Journal of Medicinal Chemistry dx.doi.org/10.1021/jm300241b | J. Med. Chem. 2012, 55, 4788–4805
- Juan C. García-Cañaveras et al (2012) Targeted profiling of circulating and hepatic bile acids in human, mouse and rat using an UPLC-MRM-MS validated method
  ASBMB, October 2012, 53 (10), Print ISSN 0022-2275, Online ISSN 1539-7262
- Gastrointestinal Microbiota and Local Inflammation during Oxazolone-induced Dermatitis in BALB/cA Mice
  Lundberg, Randi et al (2012) - Comparative Medicine, Volume 62, Number 5, October 2012 , pp. 371-380(10)
- Human skin transcriptome during superficial cutaneous wound healing- Wound Repair and Regeneration
  Kristo Nuutila et al (2012). doi: 10.1111/j.1524-475X.2012.00831.x
- Intratesticular 13-cis retinoic acid is lower in men with abnormal semen analyses: a pilot study
  J. J. Nya-Ngatchou (2012)– Andrology DOI: 10.1111/j.2047-2927.2012.00033.x
- Crp induces switching of the CsrB and CsrC RNAs in Yersinia pseudotuberculosis and links nutritional status to virulence
  Ann Kathrin Heroven et al (2012) - Frontiers in Cellular and Infection Microbiology, doi: 10.3389/fcimb.2012.00158
- Early leptin blockade predisposes fat-fed rats to overweight and modifies hypothalamic microRNA
  Charlotte Benoit et al. (2013) - J Endocrinol April 10, 2013 JOE-12-0561 doi: 10.1530/JOE-12-0561



APPLICATION BOOKLET GENERAL INDEX

COMPOUND EXTRACTION FROM WHOLE LUNG OF RATS WITH PRECELLYS®24 DUAL – 7 ML VIAL.

Chiesi Farmaceutici, DMPK department, Parma, Italy

CONTEXT

The Company is focused on respiratory diseases. Our goal is the detection of chemicals entities as potential news drugs in biological fluids and tissues, as lung, bronchus and trachea after administration to animal.
The sample preparation involves homogenization of tissues, most of the times, **whole organ** hence the trials with **Precellys®24 Dual – 7 ml vial.**

MATERIAL

- Precellys®24 Dual vs Ultraturrax (usual device)
- Precellys® kit 7ML ceramic bead CK28 (réf.0904- 01)
- Samples: 8 whole lungs weights ranged from 1.2 to 1.4 g (4 samples by device)
- Buffer: Acetonitril / 0.9%NaCl mixture (50/50 v/v)

PROTOCOL

- Sampling : Whole lung (1 vol.) + Buffer (3 vol.)
- **Precellys®24 Dual** parameters: 6500rpm, 2x20 sec. vs Ultraturrax : around 40s
- Analyze : Injection of 10 µl of supernatant (after protein precipitation) in LC/MS/MS system (API3000 Triple Quadrupole – Applied Biosystems).

RESULTS

The analyte levels in the sample are almost the same, so no difference involving the two homogenizing techniques are detectable (See Figure 1).
Reproducibility is good in both cases, taking into account the individual variability.

Few residual cartilaginous fragments (probably bronchus/trachea) were observed in both methods (fewer amounts with Ultraturrax). **The results show that there is no impact on the analyte recovery from matrix.**

With both methods, temperature within the sample after processing could reach 70°C depending on the sample and protocols. Considering the stability of the analyte and the short time of exposure to this temperature (sample were preserved in ice before and after processing), sample degradation was not significant, but a refrigerating device could be useful in different situation.

Ultraturrax is fastidious and time consuming with cleaning steps and potential contamination between samples. **The spent time is at least 5 times less using Precellys®24 Dual.**

CUSTOMER

Chiesi

ILLUSTRATIONS

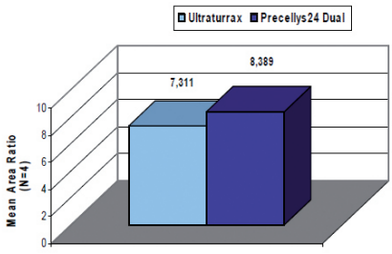


Figure 1

CONCLUSION

Precellys®24-Dual was successfully evaluated. As the results, **Precellys®24-Dual** is a suitable and reliable system for grinding whole organs with a fast process, without cross contamination, high reproducibility and comparable analyte levels extracted.
The use of Cryolys cooling option suits to reduce the increase temperature observed during processing.

03712-810-DU016

RNA EXTRACTION FROM MOUSE ARTERY

National Yang-Ming University - Taiwan

CONTEXT

The Center is focused on high-throughput genome analysis.
The goal of this work is to compare three equipments dedicated to the homogenization of mouse artery tissues to extract total RNA.

MATERIAL

- Precellys®24.
- Precellys lysing kits: 03961-1-003 (1.4mm ceramic beads)
- Competitor M and competitor Q.
- Sample: 30 mg of mouse artery tissues (triplicate).
- Buffer: Trizol.

PROTOCOL

- Precellys®24: 1.4mm ceramic beads, 250 µl Trizol, 6500 rpm, 2x10s (10s break) and 6500 rpm, 1x15s (8s break).
- Competitor M: 1.4 mm ceramic beads, 200 µl Trizol, 6500 rpm, 50 sec.
- Competitor Q: 5 mm metal bead, 350 µl Trizol, 30 1/s, 2 min.
- Total RNA extraction: Trizol extraction method.

RESULTS

The figure 1 illustrates the total RNA yield obtained from mouse artery tissues with the three homogenizers tested. The table 1 shows the RNA quality parameters for each RNA extracted.

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National Yang-Ming University VYM Genome Research Center
VYMGC 榮陽基因體研究中心

ILLUSTRATIONS

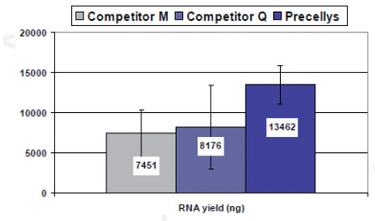

Figure 1

RNA yield (ng)

	A260/280	A260/230	Total RNA Conc. (ng/µl)	RNA yield	RIN
Competitor M	1,79	2,27	372,56	7451,20	6,53
Competitor Q	1,80	2,28	408,80	8176,00	7,43
Precellys	1,86	2,34	673,08	13461,60	7,30

Table 1

CONCLUSION

The best RNA yield is obtained with **Precellys®24** homogenizer compared both competitors. The RIN and the A260/280 show a good quality of RNA extracted.

Precellys®24 gives us a new approach in our sample preparation, combining efficiency and time for this sensitive sample.

03712-810-DU018

ANIMAL TISSUES

CHIKUNGUNYA VIRAL RNA AND VIRAL TITRATION IN TISSUE SAMPLES IN CYNOMOLGUS MACAQUES AS MODEL

CEA, Division of Immuno-Virology/Institute of Emerging Diseases and Innovative Therapies (iMETI), Fontenay-aux-Roses, France

CONTEXT

The *Chikungunya virus* (CHIKV) is a mosquito-borne alphavirus that induces in humans a disease characterized by fever, rash, and pain in muscles and joints. The aim of this study is to propose a new model for CHIKV infection in adult, immunocompetent cynomolgus macaques. CHIKV infection in these animals recapitulated the viral, clinical, and pathological features observed in human disease [1].

MATERIAL

- Precellys®24 homogenizer.
- Precellys® kit: 03961-1-002 (ceramic beads 2.8mm) for smooth tissues and 03961-1-001 (metals beads 2.8mm) for joint and muscle.
- Samples: Splenic, hepatic, muscular, and joint tissues from cynomolgus macaques inoculated with CHIKV (30 mg /~100mg).
- Buffer: 1 ml lysis buffer (Macherey Nagel) for Viral RNA extraction or 1 ml DMEM supplemented with 10% FCS for Viral titration

PROTOCOL

- Precellys®24: 5000 rpm, 2x20 sec or 2x10 sec
- Tissue viral RNA extraction: Nucleospin 96 RNA kit (Macherey Nagel), Relative quantitative RT-PCR simultaneously with CHIKV and GAPDH primers and probes.
- Determination of viral titers (TCID50/g): on mammalian BHK-21 cell lines based on their TCID50 using 4–5 replicates.

RESULTS

The figure 1 shows the infectious virus titers in spleen, liver, muscle, and joint collected from CHIKV-infected macaques, 6 and 44 dpi.

Our results provide insights into the pathogenesis of CHIKV. We have developed a relevant macaque model of CHIKV infection, in which we demonstrated long-term CHIKV persistence in various tissues and identified macrophages as cellular reservoirs during the late stages of CHIKV infection in vivo.

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cea

energie atomique • energies alternatives

ILLUSTRATIONS

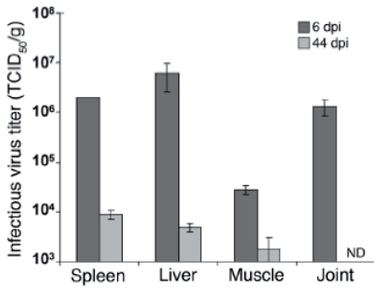

Figure 1: Tissues were collected at 6 dpi from macaques inoculated i.v. with 10^7 PFU CHIKV, or at 44 dpi from macaques inoculated i.v. with 10^6 PFU CHIKV, and the amount of infectious virus present in tissues was quantified by TCID50. Data are mean ± SEM of 2 independent virus titrations. The detection threshold was 700 TCID50/g.

[1] K.labadie et al, J Clin Invest. 2010;120(3):894– 906. doi:10.1172/JCI40104. http://www.jci.org

CONCLUSION

The **Precellys®24** is an ideal tool to evaluate both viral RNA and viral titers from various tissues of cynomolgus macaques inoculated with *Chikungunya* virus.
Precellys®24 is very easy to use, it is as simple as using a centrifuge. Comparing to the former method (mortar), we appreciate the cleanness and the cross-contamination free provided by consumables of **Precellys®24** lysing kit. The reproducibility given for various tissues of this study is also a strong advantage of this smart homogenizing equipment.

03712-810-DU033

TOTAL PROTEIN EXTRACTION FROM MICE HIPPOCAMPI AND PC12 CELLS

INSERM U862, Neurocentre Magendie, University of Bordeaux, Bordeaux, France

CONTEXT

Glucocorticoid hormones are released during the active phase of the circadian cycle and in response to stress. Stress-induced high levels of glucocorticoids have been shown to increase the memory of stress-associated events and to contribute to the development of stressrelated pathologies (depression, anxiety, drug abuse…) This study provides a complete molecular pathway (GR/Egr-1/MAPK/Synapsin-Ia-Ib) through which stress and glucocorticoids enhance the memory of stressrelated events.
The study involved total protein extractions both from mice hippocampi and PC12 cells using Precellys®24 homogenizer [1].

MATERIAL

- Precellys®24 homogenizer.
- Precellys® kit : 03961-1-003 (1.4mm ceramic beads).
- Samples: mice hippocampi and PC12 cells.
- Buffer: RIPA buffer containing protease and phosphatase inhibitors (Sigma).

PROTOCOL

- Precellys®24: 5000 rpm, 2x30 sec, 10 sec break.
- Centrifugation: 10000 rpm, 10 min, 4°C.
- SDS-PAGE, immunoblotting with relevant antibodies.

RESULTS

The figure below shows an example of total protein extraction from PC12 cells using Precellys®24.
Total extracts were analyzed by western blot. The corresponding X-ray films were quantified by densitometry.
Those results allowed to better understand the action of corticosterone in the molecular cascade mediating the enhancement of stress-related memories.

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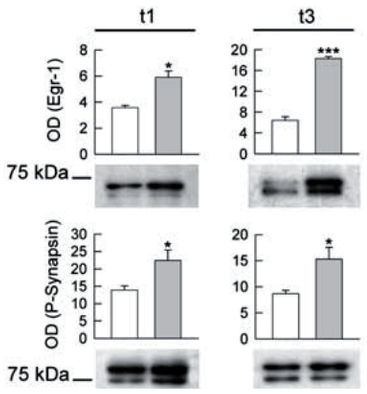
Instituts thématiques Inserm
Institut national de la santé et de la recherche médicale

ILLUSTRATIONS



*Corticosterone-induced phosphorylation of synapsin-I depends on Egr-1 activation. Total extracts from PC12 cells were analyzed by western blot 1 and 3 h after treatment with 10nM corticosterone (gray bars). The corresponding X-ray films were quantified by densitometry (optical density, OD, mean± s.e.m., n = 3). *P < 0.05; ***P < 0.001, in comparison with basal levels.*

[1] Revest et al. 2010. The enhancement of stress-related memory by glucocorticoids depends on synapsin-Ia/Ib. Molecular Psychiatry, n° 4 (April) doi:10.1038/mp.2010.40

CONCLUSION

Precellys® is well adapted for extraction of total proteins from brain samples and cell lines.

Sample homogenization is quick and efficient in term of quantity and quality of extracted proteins (e.g. phosphoproteins). With our protocol, we are able to treat 24 samples in less than 2 minutes by avoiding samples cross contamination.

03712-810-DU035

RNA EXTRACTION FROM MOUSE EMBRYONIC TOOTH GERMS IN 0.5ML VIAL

Functional Genomics Institute of Lyon, Lyon, France

CONTEXT

Our team aims at better understanding how a morphology arises during development in connection with gene expression, and how it changes during evolution. For that purpose, we are using the rodent molar as an organ model.
In order to study gene expression during tooth development, developing tooth germs (soft tissue) were dissected from mouse embryos (15-16 days post coïtum) and stored in RNA later.

MATERIAL

- Precellys®24.
- Precellys® kit: 03961-1-203 (ceramic beads 1.4mm – 0.5ml tubes).
- Sample: mouse embryonic tooth germs previously in RNA later, rough estimate: 0.75mg for 6 tooth germs.
- Buffer: 200 µl lysis buffer (RLT+ ß-mercaptoethanol).

PROTOCOL

- Precellys®24 parameters: 5500 rpm, 2x10 sec - 10s break.
- Purification method: Qiagen RNeasy Micro kit.
- Analysis: Agilent Bioanalyser 2100.

RESULTS

RNA extracted was of very good quality (RIN = 10). The yield was closed to theoretical yield for embryonic tissues (theoretical: 1.5 µg; obtained: 1 to 1.4 µg).
Note: Increasing lysis time (5500 rpm 2x 20s (10s) did not lead to any yield improvement.

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Institut de Génomique Fonctionnelle de Lyon

ILLUSTRATIONS

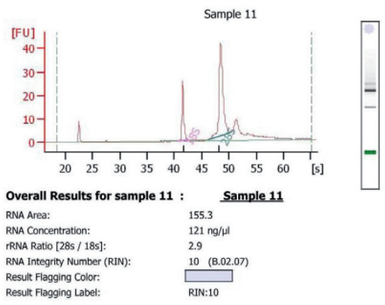

Figure 1: typical profile obtained on Agilent Bioalnalyser 2100 following lysis with Precellys®24 and purification with Qiagen RNeasy micro kit.

[1] K.labadie et al, J Clin Invest. 2010;120(3):894– 906. doi:10.1172/JCI40104. http://www.jci.org

CONCLUSION

The homogenization with **Precellys®24** is efficient and lead to a total RNA of good quality.

The **Precellys®Lysing kit 0.5mL** 1.4mm ceramics beads is appropriate to have a quick and effective homogenization of a low amount of sample (<1 mg).

03712-810-DU037

DROSOPHILA HOMOGENIZATION FOR LIPID ANALYSIS

Department of Developmental Biology, University of Regensburg, Germany

CONTEXT

Our research aim is to establish *Drosophila* as a model for Friedreich´s ataxia. This human neurological disorder is produced by the lack of the mitochondrial protein frataxin. Frataxin depletion results in a mitochondrial dysfunction and metabolic problems.
We wanted to study weather reduction of frataxin in *Drosophila* also induced some metabolic responses such as loss of lipid homeostasis.
In this work we have found that ubiquitous and glialtargetted reduction of frataxin expression leads to an increase in fatty acids [1].

MATERIAL

- Precellys®24 homogenizer.
- Precellys®lysing kit: 03961-1-002 (ceramic beads 2.8mm)
- Sample: *Drosophila* L3 larvae (15) or *Drosophila* adult heads (80).
- Buffer: Water.

PROTOCOL

- Precellys®24: 5500 rpm, 2x25 sec, 10s break.
- Centrifugation steps: 5000 rpm - 60s.
- Analysis of lipid content: Samples were delipidated according to Bligh and Dyer, 1959 for thin layer chromatography studies and gas chromatography coupled with mass spectrometry (GC/MS) was carried out after FA methyl ester derivatization according to Ecker et al., 2010

RESULTS

Quality and quantity of extracted lipids from *Drosophila* samples using Precellys®24 technology was sufficient on the one hand to have reliable and reproducible results from different biological replica (not illustrated) and on the other hand to observe clear differences between control flies and frataxindeficient individuals (Figure1).

Frataxin deficiency increases the amount of each fatty acid.
In conclusion, loss of frataxin affects lipid metabolism and catabolism provoking an accumulation of fatty acids. Moreover, triacylglicerides and other neutral or phospholipids are not so affected.

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ILLUSTRATIONS

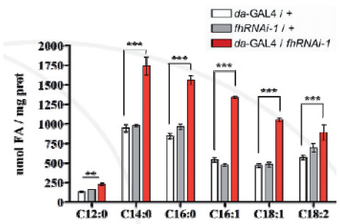

Figure 1: GC/MS analysis of fatty acids from Drosophila L3 larvae (Myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1) and linoleic acid (C18:2)).

[1] J.A. Navarro et al., Altered lipid metabolism in a Drosophila model of Friedreich's ataxia, Human Molecular Genetics, 2010 1–13 doi:10.1093/hmg/ddq183.

CONCLUSION

Precellys®24 provided us a complete fly homogenate containing lipid in the right range of both amount and purity, in order to carry out our experiments.
Sample preparation is not only easy but cross contamination free.

03712-810-DU038

ANIMAL TISSUES

QUANTIFICATION OF METHADONE AND ITS METABOLITE (EDDP) IN INSECT'S TISSUES

National Institute of Criminalistics and Criminology, University of Mons, Belgium

CONTEXT

Forensic entomotoxicology, a relatively new branch of forensic entomology studies the usefulness of insects as alternative toxicological samples.
Larvae are of most interest as they are present in a high number and are more visible on-site. To measure variability in a pool of larvae, single larva must be analyzed separately [1].
Sometimes, forensic case samples are insufficient and analysis of single specimen is also recommended .
In addition, it's important to use a simple and rapid method.

MATERIAL

- Precellys®48 homogenizer.
- Precellys® lysing kit: 03961-1-008 (metal beads 2.8mm).
- Sample: single larva (50 mg) from experiments (n=12) and authentic cases (n=7).
- Buffer: 500 µL of water deionized.

PROTOCOL

- Precellys®48: 4000 rpm, 1x30 sec.
- Liquid / liquid extraction: 500 µL of saturated ammonium chloride buffer (pH 9.2) + 4mL of 1- chlorobutane.
- Analysis: Liquid chromatography and mass spectrometry.

RESULTS

Figure 1 represents the results following the analysis of authentic cases. In a single third instar larva, we have quantified a high concentration of methadone (38.7 pg/mg larva) and EDDP (82.8 pg/mg larva) (Fig.1).
In all cases, both the parent drug and metabolite could be detected following the analysis of a single specimen which confirms variability of drug accumulation in entomological samples.
This method allows rapidly detecting methadone and EDDP in single larva on human remains of a young man suspected to consume heroin.

[1] M. Gosselin et al, Quantification of Methadone and its Metabolite 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine in Third Instar Larvae of Lucilia sericata (Diptera: Calliphoridae) Using Liquid Chromatography–Tandem Mass Spectrometry, Journal of Analytical Toxicology 2010;34:1-7.

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ILLUSTRATIONS

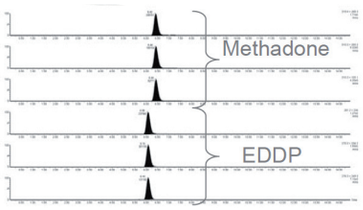

Figure 1. MRM chromatograms for (top to bottom) methadone, methadone, methadone-d9, EDDP, EDDP, and EDDP-d3 in a larva sampled on human remains.

CONCLUSION

Precellys®48 is an ideal tool for sample preparation in forensic entomotoxicology.
Precellys®48 is very easy to use, it is as simple as using a centrifuge. Comparing to the former method (mortar) or mixer, we appreciate the cleanness and the cross-contamination free provided by consumables of **Precellys®** lysing kit.

03712-810-DU039

EXTRACTION OF RNA FROM VARIOUS *CAENORHABDITIS* SPECIES (NEMATODES)

College of Life and Environmental Sciences, School of Biosciences, Univ. of Birmingham, Birmingham, UK.

CONTEXT

Ageing, immunity and stress tolerance are inherent characteristics of all organisms. These traits in *Caenorhabditis elegans* have been shown to be regulated at least in part by the daf-16 gene. Through our research we aimed to establish a correlation between the expression of daf-16 and observed phenotypes (lifespan, immunity and stress tolerance) among four closely related *Caenorhabditis* species (*C. elegans*, *C. briggsae*, *C. remanei* and *C. brenerri*).
For such studies we required optimal concentration of high quality extracted RNA with minimal genomic DNA contamination. The added complication of using entire organisms for RNA extraction also meant that a very effective method be used to produce homogenized samples for RNA Isolation.

MATERIAL

- Precellys®24 Homogenizer
- Precellys® lysing kit: 03961-1-004 (0.5mm glass beads)
- Sample & buffer: Suspension of M9 buffer with *C.elegans* from a full 9cm petri dish.

PROTOCOL

- Animals (either mixed stage, or staged, depending on requirements) were washed off petri plates using M9 buffer, gently pelleted by centrifugation (1500rpm for 1min) and washed three times with M9 buffer before resuspending in 500µl of M9 buffer.
- Precellys®24: 6400rpm, 2x10secs twice for a total time of 40secs.
- Analysis: RNA was isolated directly from the extract using Qiagen RNeasy kit before being checked for quality and quantity using NanoDrop analysis.

RESULTS

Employing this methodology we were able to successfully isolate required amounts and quality of RNA from four species of the *Ceanorhabditis* genus at various stages of development. On average a yield of 1.5 – 2 µg/µl of RNA was obtained per 9cm petri plate. This RNA was then used for RT-PCR to quantify gene expression studies as shown in Figure 1.

[1] AMRIT, F. R., BOEHNISCH, C. M. & MAY, R. C. (2010) Phenotypic covariance of longevity, immunity and stress resistance in the Caenorhabditis nematodes. PLoS One, 5, e9978.

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UNIVERSITY OF BIRMINGHAM

ILLUSTRATIONS

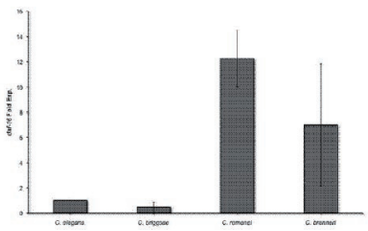

Figure 1: Expression levels of daf-16 (normalized to the reference gene gpd-3) among mixed populations - nematodes at various stages of development adapted from Amrit et al, 2010 [1].

CONCLUSION

The use of the **Precellys®24** to produce homogenized extracts for RNA isolation significantly improved the concentration and quality of extracted RNA as well as improving the speed of the isolation protocol.

03712-810-DU041

MAINTAINING BACTERIAL VIABILITY DURING TISSUE HOMOGENIZATION

Illawarra Health and Medical Research Institute, University of Wollongong, Australia

CONTEXT

In an animal model of invasive infections caused by *Streptococcus pyogenes*, bacteria will colonize various tissues and organs. Bacterial loads present in these tissues is determined by homogenization, serial dilution and plating onto nutrient agar.
This study investigates the use of the Precellys® 24 for tissue homogenization.

MATERIAL

- Precellys® 24 homogenizer.
- Precellys® kits: 03961-1-003 (1.4mm ceramic beads) and 03961-1-002 (2.8mm ceramic beads).
- Samples: skin (122.3 mg) and spleen tissues (38.6 mg).
- Bacteria culture: *S. pyogenes* was grown in Todd Hewitt Broth supplemented with 1% yeast extract at 37°C un til an A600 = 0.5.
- Buffer: 0.7% sterile saline (1000µl) inoculated with *S.pyogenes* (T0 Inoculum).

PROTOCOL

Note that pathogenic** S. pyogenes **must be processed under appropriate biosafety containment.

- Precellys® 24 parameters:
  - Spleen: 6000 rpm, 1x30 sec,
  - Skin: 6000 rpm, 2x30 sec or 6000 rpm, 4x30 sec.
- Analysis: assessment of *S.pyogenes* concentration (CFU/ml) by cultural method.

RESULTS

Following tissue homogenization, the number of viable bacterial cells (CFU/ml) present was determined by serial dilution in sterile 0.7% saline and plating onto nutrient agar. Results (see Figure1) indicate that for all treatment groups the type of bead and the processing time had no significant effect on the viability of *S. pyogenes* (t test p> 0.1).

CUSTOMER

University of Wollongong

ILLUSTRATIONS

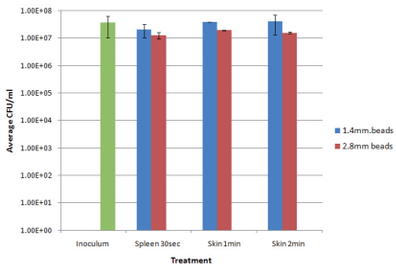

Figure 1: The effect of tissue homogenization on the viability of S. pyogenes. Data represents the mean CFU/ml from triplicate samples with error bars indicating the standard derivation. The inoculum represents the CFU/ml added to each sample.

CONCLUSION

Tissue homogenization (spleen & skin) using the **Precellys®24** with ceramics beads kits (1.4mm or 2.8mm) does not affect the viability of the Gram positive pathogen S.pyogenes present in these samples. The bacterial viability is maintained during tissue homogenization.
The potential to process up to 24 individual samples in a short time period with no risk of cross-contamination has considerable benefits over traditional homogenization methods.

03712-810-DU053

DRUG EXTRACTION FROM WHOLE BRAIN RAT WITH MINILYS

PharmOptima, Portage MI, USA

CONTEXT

PharmOptima focuses **in vivo and in vitro DM&PK and Discovery Services**, providing IND & NDA-enabling research protocols. DM&PK with an ocular focus is a part of the laboratory's routine research.
PharmOptima lab currently uses Precellys to homogenize a variety of different animal tissues. In this study, Minilys and probe sonicator have been compared to current method (Precellys). Advantages & drawbacks are discussed.

MATERIAL

- Minilys vs Precellys Dual homogenizer vs Probe sonicator.
- Precellys kit ceramic beads 7mL vial (Ref.03961-1-302) +2 beads (Ref.03961-1-106).
- Sample: 1 thawed whole brain rat (~1.7 g) into 7mL vial (Minilys & Precellys trials) or into 15mL vial (Probe sonicator trial).
- Buffer: 2 volumes of sodium chloride solution.

PROTOCOL

- Minilys & Precellys: 5000 rpm, 2x45sec (15sec break).
- Probe sonicator: several seconds.
- Analysis: liquid-liquid extraction; LC-MS/MS system.

RESULTS

The analyte levels by samples are equivalent. No difference involving the three homogenizing techniques are detectable (See Figure 1).
Despite one sample prep in 7mL vial, Minilys is preferred rather the probe sonicator method which is fastidious and time consuming with cleaning steps and potential contamination between samples.

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PharmOptima
DRUG DISCOVERY & OPTIMIZATION

ILLUSTRATIONS

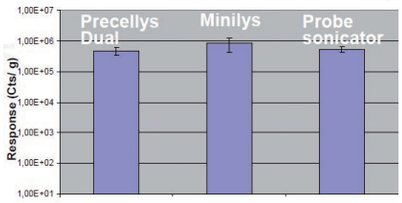
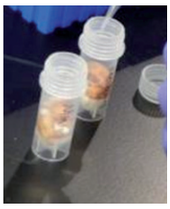
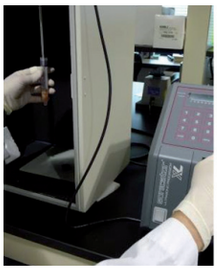


Figure 1: LC-MS/MS analysis from Precellys Dual, Minilys, and Sonicator sample.



Whole brain rat 7mL vial

Minilys



Probe sonicator

CONCLUSION

Precellys Dual and Minilys are suitable and reliable systems to extract Drug from animal tissues for DM&PK testing thanks grinding whole organs as brain rat with a fast process, cross-contamination free, high reproducibility and comparable drug levels extracted.
Minilys homogenizer is a real alternative for standardization of the sample preparation for laboratories with a low or medium throughput of sample.

03712-810-DU055

DRUG EXTRACTION FROM WHOLE IRIS-CILIARY BODY PORK

PharmOptima, Portage MI, USA

CONTEXT

PharmOptima focuses on **in vivo and in vitro DM&PK and Discovery Services,** providing IND & NDAenabling research protocols. High quality DM&PK and Ocular PK research are a part of the laboratory's daily activities.
PharmOptima, experienced in ophthalmology studies, currently uses Precellys to homogenize a variety of ocular tissues as cornea, conjunctiva, lens, irisciliary body, trabecular meshwork, sclera, retina, choroid, optic nerve. In this study, Minilys has been compared to current method (Precellys) to homogenize whole iris-ciliary body from pork.

MATERIAL

- Minilys vs Precellys Dual homogenizer
- Precellys kit ceramic beads 7mL vial (Ref.03961-1-302) +2 beads (Ref.03961-1-106).
- Sample: Thawed whole iris-ciliary body pork (~0.7 g) into 7mL vial.
- Buffer: 2 volumes of sodium chloride solution.

PROTOCOL

- Minilys & Precellys: 5000 rpm, 4x30sec (15sec break).
- Analysis: liquid-liquid extraction; LC-MS/MS system.

RESULTS

The analyte levels are equivalent. No difference involving the both homogenizer is detectable (See Figure 1).

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ILLUSTRATIONS

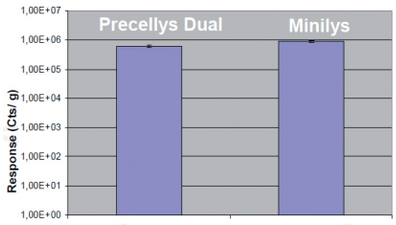
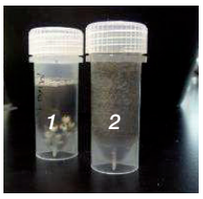
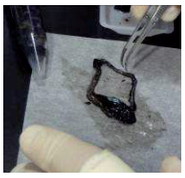


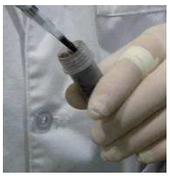
Figure 1: LC-MS/MS analysis from Precellys Dual and Minilys sample.



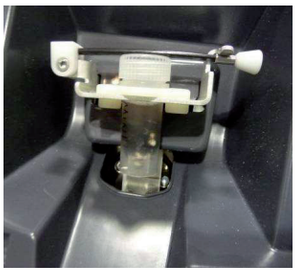
7mL vial



Whole iris-ciliary body / Before (1)



Whole iris-ciliary body / After (2)



Homogenization of whole iris-ciliary body into 7mL vial with Minilys

CONCLUSION

Precellys Dual and Minilys are suitable and reliable systems to extract Drug from ocular tissues for DM&PK testing thanks grinding whole organs as iris-ciliary body pork with a fast process, cross-contamination free, high reproducibility and comparable drug levels extracted.
Minilys homogenizer is a real alternative for standardization of the sample preparation for laboratories with a low or medium throughput of sample.

03712-810-DU056

DRUG EXTRACTION FROM BRAIN, MUSCLE RAT TISSUES

PharmOptima, Portage MI, USA

CONTEXT

PharmOptima focuses on in vivo and in vitro DM&PK and Discovery Services, providing IND & NDAenabling research protocols. High quality DM&PK and Ocular PK research are a part of the laboratory's daily activities.
PharmOptima lab currently uses Precellys to homogenize a variety of animal tissues. In this study, Minilys has been compared with Precellys to homogenize brain and rat muscle.

MATERIAL

- Minilys vs Precellys Dual homogenizer
- Precellys kit: 03961-1-009 (mix ceramic beads).
- Sample: Thawed brain (~450mg) and muscle (~200mg).
- Buffer: 2 volumes of sodium chloride solution.

PROTOCOL

- Brain tissue (triplicate):
  - Minilys/Precellys: 5000 rpm, 2x45sec (15sec break).
- Muscle tissue (triplicate):
  - Minilys: 5000 rpm, 3x40sec (15sec break).
  - Precellys: 5500 rpm, 3x30sec (15sec break).
- Analysis: liquid-liquid extraction; LC-MS/MS system.

RESULTS

The analyte levels by samples are equivalent. No difference involving the both homogenizers is detectable (See Figure 1).

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DRUG DISCOVERY & OPTIMIZATION

ILLUSTRATIONS

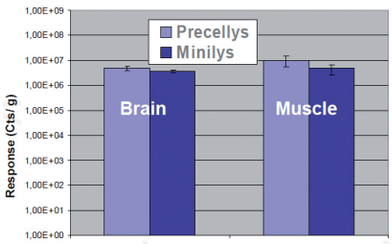
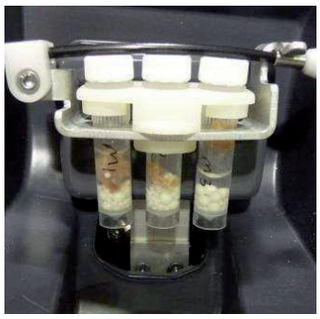


Figure 1: LC-MS/MS analysis from Precellys and Minilys sample.



Muscle tissues in CKmix kit 2mL vial

Minilys processing

CONCLUSION

Precellys Dual and Minilys are suitable and reliable systems to extract Drug from soft tissues for DM&PK testing with a fast process, cross-contamination free, high reproducibility and comparable drug levels extracted.
Minilys homogenizer is a real alternative for standardization of the sample preparation for laboratories with a low or medium throughput of sample.

03712-810-DU057

ASSESSMENT OF *SALMONELLA TYPHIMURIUM* LOAD WITHIN INFECTED NEMATODES

School of Biosciences, University of Birmingham, Birmingham, UK.

CONTEXT

The nematode *Caenorhabditis elegans* is a powerful model system for the study of host-pathogen interactions.
This study looked at innate immune response of *C.elegans* to *Salmonella* Typhimurium. To test whether the altered resistance of lys-7 and abl-1(tyrosine kinase ABL-1) mutant nematodes to *Salmonella* Typhimurium was due to a lower bacterial load within infected animals, wild type (N2), lys-7 and abl-1 animals were exposed to *S. Typhimurium* L1019, and infectious burden quantified over time through viable counts [1].

MATERIAL

- Precellys 24 homogenizer.
- Precellys kit: 03961-1-004 (0.5 mm glass beads)
- Sample: *C.elegans* (Six animals at L4 stage) infected with *S. Typhimurium* strain L1019.
- Buffer: 200 µl M9 with 25 mM levamisole hydrochloride.
- 10 replicates / 5 time points (5 days).

PROTOCOL

- Precellys 24: 6500rpm, 1x10s.
- Analysis: Lysates were serially diluted in M9 and plated onto LB plates containing kanamycin (30 µg/ml) to select for L1019. Colonies were counted by eye and scaled to the original concentration per nematode.

RESULTS

Infection load was assessed through viable counts for 5 days / each 24 hours. lys-7 and abl-1 mutant animals are tolerant of S. Typhimurium infection (Figure 1a). There was no difference between any of the strains (p.0.2) showing that lys-7 and abl-1 single mutants are tolerant to S. Typhimurium infection for at least six days (Figure 1b).

[1] Marsh EK, van den Berg MCW, May RC (2011) A Two- Gene Balance Regulates Salmonella Typhimurium Tolerance in the Nematode Caenorhabditis elegans. PLoS ONE 6(3): e16839. doi:10.1371/journal.pone.0016839

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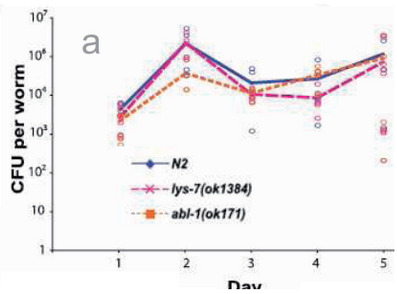
ILLUSTRATIONS



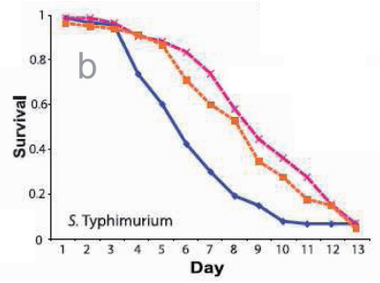


Figure 1: (a) S.Typhimurium load per nematode and (b) nematode survival curve.

CONCLUSION

Lysing nematodes infected with *Salmonella* using the Precellys®24 does not affect the bacteria viability, allowing infection load to be accurately assessed. The potential to process up to 24 individual samples in a short time period with no risk of cross-contamination and reproducibility has considerable benefits over traditional homogenization methods. In this study, **Precellys®24** homogenizer was also used to lyse worm to extract total RNA in order to prepare cDNA and perform an qRT-PCR.

03712-810-DU060

RECOVER LIVE HERPES VIRUS FROM INFECTED MOUSE GANGLIA WITH MINILYS

MVS, LID, National Institute of Allergy and Infectious Diseases, kwang@niaid.nih.gov, Bethesda, Maryland, USA

CONTEXT

The research focus of our laboratory is on human herpes virus pathogenesis and the development of new treatments and vaccines. We often need to test herpes simplex viruses (HSV) and their mutants in a mouse model to study the function of viral genes, evaluate antiviral therapies, and assess the efficacy of vaccine candidates. The titer of infectious HSV in the sensory nerve ganglion innervating the inoculation site is a crucial measurement for these studies.

MATERIAL

- Minilys homogenizer.
- Precellys lysing kit: 03961-1-203 (CK14 - 0.5mL tubes).
- HSV-2 virus stock with known titer.
- Mouse trigeminal ganglia (TG) harvested three days after HSV-2 cornea infection.
- Medium HBSS with 1 % FBS.

PROTOCOL

- Virus stock was diluted to desired concentration and 500 µl was transferred to a homogenizer tube.
- A fresh TG was placed in a homogenizer tube with 500 µl of medium.
- All above samples were kept on ice unless specified.
- Samples were homogenized at RT with settings as indicated (see Figures 1 and 2). Homogenization was paused every 20 seconds and tubes were held on ice for 1 minute then continue homogenization.
- Live virus in homogenates was detected by titration on a Vero cell monolayer in 6-well-plates following standard plaque assay protocol.

RESULTS

The mouse TG tissue required more than 3 k rpm x 45 sec or 4 k rpm x 20 sec to be adequately homogenized (results not shown).
Diluted virus stock (cell free virus) can withstand the homogenization up to 5 k rpm x 60 sec (Fig. 1).
Live virus can be recovered from HSV-2 infected mouse TG (Fig. 2).

ILLUSTRATIONS

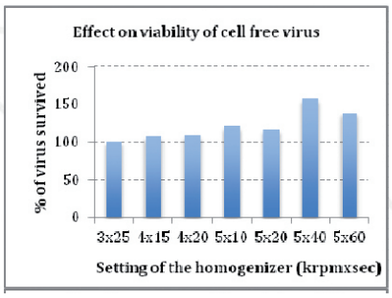


Figure 1

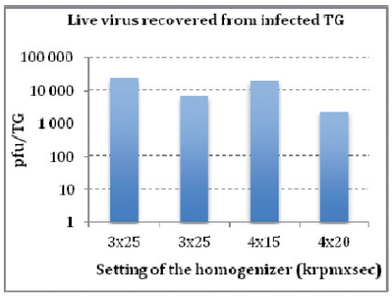


Figure 2

CONCLUSION

Live herpes simplex virus can be effectively recovered from infected mouse tissue with **Minilys** by homogenizing infected tissue in individual, tightly-closed tubes rather than in open tubes with a rotor-stator homogenizer.
This facilitates medium throughput analysis of tissue samples and avoids the possibility of cross-contamination between samples.

03712-810-DU065

DIRECT QUANTITATIVE DETERMINATION OF A NUCLEOTIDE DIPHOSPHATE IN MONKEY AND RABBIT TISSUES USING LC-MS/MS

Pyxant Labs, Inc., Colorado Springs, Colorado, USA

CONTEXT

A sensitive, accurate and precise bioanalytical method was developed for the determination of Cidofovir diphosphate in monkey and rabbit tissues using LCMS/ MS. The method was developed to evaluate concentration levels of the active metabolite in various monkey and rabbit organs and tissues in support of a maximum tolerated dose study [1].

MATERIAL

- Precellys®24.
- Lysing beads were not used to prevent absorption or degradation of the analyte.
- Samples: Monkey and rabbit tissue from liver, lung, spleen, kidney and skin (30 mg each).
- Solvent: 430 µl of 70:30 methanol:water with 30 µl of internal standard.

PROTOCOL

- Precellys®24 parameters: 6500rpm, 1x30 sec.
- The Precellys was used at full capacity (24 samples at a time), followed by centrifugation at 13000 rpm for 5 minutes. The supernatant was filtered through a Sirocco Protein Precipitation plate and transferred to a 96-well collection block for LC-MS/MS analysis.

RESULTS

The Precellys®24 allowed validation of this method over a linear range of 5 to 1000 pg/mg. Full validations were performed for monkey and rabbit liver tissue. Validation experiments included intra- and inter-day precision and accuracy, selectivity/specificity, recovery, matrix effect, lower and upper limit of quantification and stability tests.
The accuracy for monkey liver ranged from 97.7% to 101% and the precision ranged from 4.3% to 8.2% (CV). For rabbit liver, the accuracy range was 101% to 102% and the precision range was 4% to 7.5% (CV).

[1] Foley S, Begley J, Karnik S, Keilholz L, Lambert B. Direct Quantitative Determination of Cidofovir Diphosphate in Monkey and Rabbit Tissues using LC/MSMS. 2012 ASMS meeting abstracts.

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PYXANT LABS

ILLUSTRATIONS

Monkey		Accuracy and Precision (mean, CV)		
Tissue	n	Low 15 pg/mg	Med 250 pg/mg	High 750 pg/mg
Liver	24	97.7% (8.2%)	100% (4.8%)	101% (4.3%)
Spleen	6	94.8% (5.4%)	102% (5.1%)	102% (2.6%)
Kidney	6	88.3% (6.0%)	101% (4.2%)	104% (2.3%)
Lung	6	88.1% (6.1%)	93.1% (5.5%)	105% (6.7%)
Skin	6	88.0% (6.7%)	99.2% (3.1%)	101% (3.8%)

Rabbit		Accuracy and Precision (mean, CV)		
Tissue	n	Low 15 pg/mg	Med 250 pg/mg	High 750 pg/mg
Liver	24	102% (7.5%)	101% (4.3%)	102% (3.8%)
Spleen	6	102% (7.4%)	104% (3.8%)	103% (5.7%)
Kidney	6	105% (8.6%)	102% (3.9%)	103% (3.4%)
Lung	6	91.7% (13%)	99.1% (6.9%)	97.8% (4.9%)
Skin	6	106% (6.1%)	98.2% (5.5%)	101% (4.1%)

CONCLUSION

A trace quantitative method utilizing the Precellys®24 homogenizer was validated and used for the direct determination of Cidofovir diphosphate in animal studies. Stability tests demonstrated that specific handling techniques were necessary to control degradation and absorption of the analyte during tissue processing and for this reason, beads were not used. **For specific applications in which metabolite absorption is an issue, the Precellys still effectively homogenizes tissue samples due to its high speed and multi-directional motion, even without the use of beads.**

03712-810-DU070

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.

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].

MATERIAL

- 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.

[1] L.Amar et al., MicroRNA expression profiling of hypothalamic arcuate and paraventricular nuclei from single rats using Illumina sequencing technology; Journal of Neuroscience Methods; http://dx.doi.org/10.1016/j.jneumeth.2012.0 5.033

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ILLUSTRATIONS

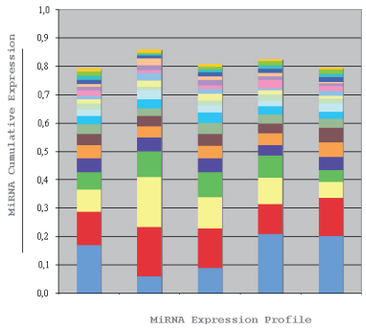

*Figure 1: **MiRNA expression in PVN profiles.** Expression of the 20 most highly expressed miRNAs are shown for the five PVN profiles. Different miRNAs are shown in different colors.*

CONCLUSION

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

DRUG ANALYSIS IN ANIMAL TISSUE

Laboratory of pharmacology, CHU Bordeaux, France

CONTEXT

Within the context of drug analysis in the toxicology field, the responsibility of target molecules in death is investigated. New protocols for lysing matrix are developed to improve the conditions of grinding and the recovery of the molecules regarding constraints of time, cross-contamination, and volume of sample.

See also publication of K.Titier, from CHU Bordeaux, Department of Clinical Pharmacology and Toxicology

MATERIAL AND METHOD

	Brain	Liver	Heart	Lung	Muscle
Weight	0.5g	0.5g	1g	0.5g	0.3g
Water	500µl	500µl	800µl	500µl	600µl
Kit	CK28	CK28	CK28	CK28	MK28
Protocol	6500rpm 1x23 sec.	6500rpm 1x23 sec.	6500rpm 1x23 sec.	6500rpm 1x23 sec.	6500rpm 1x23 sec.

RESULTS

LC-MSMS Analysis were conducted on each of the samples. The graph hereby shows analysis of the liver sample (figure 3) :

- Thioridazine Recovery: 61%.
- Cyamemazine Recovery: 73%

Chromatograms obtained from liver sample spiked with 80 ng/ml of thioridazine and cyamemazine

Figure 1: From left to right: brain, liver, heart, lung, and muscle prior to homogenization.
Figure 2: From left to right: brain, liver, heart, lung, and muscle after homogenization.
Figure 3: Chromatograms obtained from liver sample spiked with 80 ng/ml of thioridazine and cyamemazine.

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ILLUSTRATIONS

Figure 1

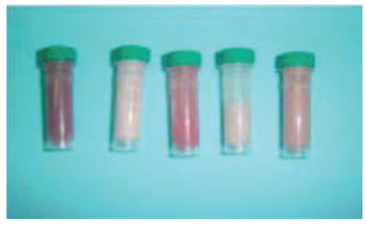

Figure 2

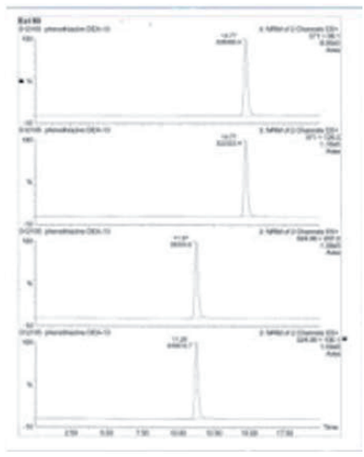

Figure 3

CONCLUSION

The **Precellys®24** is an ideal tool for the lysis of a wide range of samples.
It is not only easy to use but also ensures no cross contamination, and saves time compared to manual grinding.

03961-002-DP006

VIRAL RNA EXTRACTION FROM INFECTED TISSUES

Leuven University, Leuven, Belgium

CONTEXT

In the context of one of the laboratories at the University of Leuven (Belgium), antiviral chemotherapy is studied and focused on.
To evaluate the in vivo activity of the newly discovered antiviral compounds, we treated virus-infected mice with the antiviral compound, and extract the organs in which the virus replicates.

MATERIAL

- Precellys®24
- Precellys® kit CK28 (big ceramic beads)
- Sample : 30-75mg of murine pancreas
- Buffer : volume is adjusted in order to get a 5% w/v homogenate

PROTOCOL

- Precellys®24 parameters: 6500 rpm, 3x5 sec.

RESULTS

After homogenizing the murine pancreas, Leuven University detects :
- Firstly the viral RNA (1)

The homogenization in lysis buffer RLT is performed, and worked further with the supernatant to extract the viral RNA. Graph (1) presents the evolution of viral RNA in the pancreas of viral-induced mice. It is expressed as “copies viral RNA per copy beta actin” (RT-QPCR after total RNA extraction).
- Secondly replicating the infectious virus (2)

In the second try (2) the homogenization in growth medium, (MEM, Gibco), is performed using saline buffer. The supernatant is then used to titrate on a cell culture (96-well plates, and the titer of the virus is determined (expressed as CCID50).

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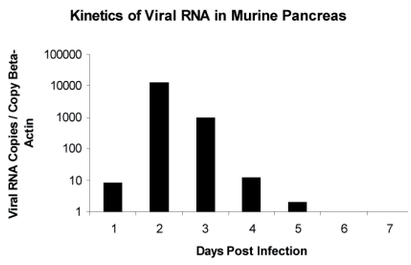
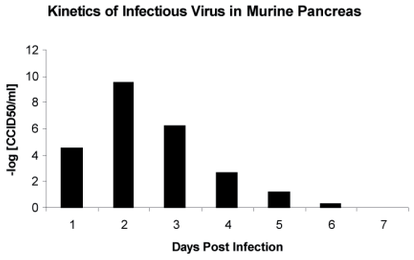
ILLUSTRATIONS



Graph 1



Graph 2

Graph (2) presents the evolution of infectious virus: the titer of infectious virus is expressed as “-log CCID50/ml” where CCID50 stands for “Cell Culture Infective Dose 50%”, which is a measure to express the titer of a virus stock.

CONCLUSION

The **Precellys®24** helped us to evaluate the in vivo activity of the newly discovered antiviral compounds. We extracted RNA virus and infectious virus with Precellys tissue homogenizer.

03961-002-DP016

ISOLATION OF RNA FROM RAT LIVER

Altana Pharma AG, Germany

CONTEXT

In toxicogenomics application field, the molecular biology laboratory of the company Altana Pharma AG is using the Precellys®24 to isolate RNA for subsequent gene expression profiling using micro arrays and quantitative real time PCR.

MATERIAL

- Precellys®24
- Precellys® kit CK28 (big ceramic beads)
- Sample : 30 mg of rat liver previously stored in RNAlater (Ambion) at -20°C
- Buffer : 600µl RTL buffer (RNeasy Mini Kit,Qiagen)

PROTOCOL

- Precellys®24 parameters: 5000rpm, 2x10 sec., 10 sec. break

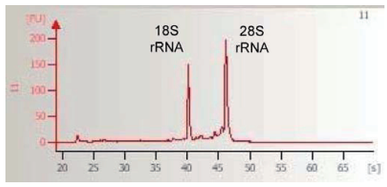
RESULTS

These results were concluded with the collaboration of Altana Pharma AG. Analysis of the subsequently isolated RNA using the Agilent 2100 bioanalyzer.

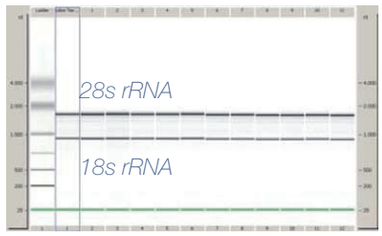
CUSTOMER

ALTANA

ILLUSTRATIONS



Electropherogram of isolated RNA



Gel-like image of RNA's isolated using different Precellys® set-ups

CONCLUSION

The **Precellys®24** is an ideal tool for the lysis of rat liver for subsequently isolating RNA. Particularly for higher numbers the **Precellys®** is superior to the formerly used mechanical blender, regarding time consumption and reproducibility.

03961-002-DP017

POST-MORTEM REDISTRIBUTION OF DRUGS IN RATS

Laboratory of pharmacology, CHU Bordeaux, France

CONTEXT

Drug concentration in tissues and body fluids change between the death and the postmortem specimen collections because of post-mortem redistribution. The aim of this study was to investigate post-mortem redistribution of the 2 cardiotoxic antipsychotic drugs: haloperidol and thioridazine, in order to interpret the postmortem redistribution. The rat has been chosen as the animal model.

MATERIAL

- Precellys®24
- Precellys® kit CK14 (small ceramic beads)
- Sample : rat heart (1/2 v/v)
- Buffer : water

PROTOCOL

- Precellys®24 parameters: 6500 rpm, 2x15 sec.

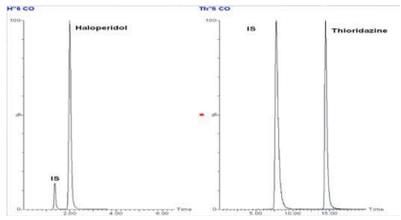
RESULTS

Those chromatograms present liquid-liquid extraction results obtained from heart samples 6 hours after death. At this time the concentration of haloperidol and thioridazine was respectively 472 ng/g and 1435 ng/g (significant variation).

CUSTOMER

C·H·U
Hôpitaux de Bordeaux

ILLUSTRATIONS



Chromatograms of Liquid/ liquid extraction

See also the publication in the journal of Analytical Toxicology, 30(7)/ 419-25, September 2006, Nadege Castaing, Karine Titier, Mireille Canal-Rafin, Nicholas Moore, Mathieu Molimard

CONCLUSION

The **Precellys®24** allows a quick and efficient homogenization on a significant number of samples. The homogenate is perfectly appropriate for drug extraction.

03961-006-DU001.B

TOTAL CATHEPSIN ACTIVITY MEASUREMENTS IN ATLANTIC SALMON MUSCLE

Department of Animal and Aquacultural Sciences (IHA), Norwegian University of Life Sciences (UMB) & Nofima Mat - Norway

CONTEXT

Our research aims at studying quality of fish as food. Enzyme activity measurement allows to evaluate potential fish muscle degradation, both pre- and postmortem. Total cathepsin B activity was measured in salmon muscle homogenates to evaluate the influence of different length of pre-slaughter crowding stress (Non- Stress (NS), Short-term Stress (SS) and Long-term Stress (LS)), on fish fillet quality [1].

MATERIAL

- Precellys®24 homogenizer.
- Precellys® lysing kit: 0810-09 (Mixed ceramics beads).
- Sample: 300 mg of frozen salmon muscle.
- Extraction buffer: 1 mL 100 mM Na-acetate in 0.2% Triton X-100, pH 5.5.
- Centrifugal instrument.

PROTOCOL

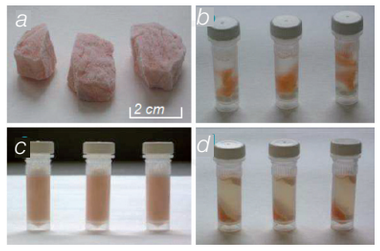
- Precellys®24: 5000rpm, 2x20sec, 10s break.
- Centrifugation: 16 000 g, 30 min.
- Analysis: Cathepsin activity was measured fluorimetrically on the prepared muscle homogenate supernatants (Kirschke,Wood, Roisen and Bird, 1983).

RESULTS

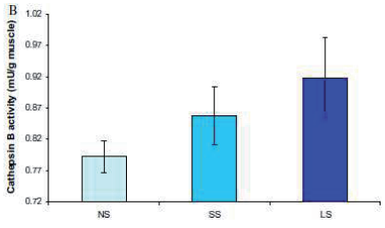
A classical homogenization technique of fish muscle samples implied the use of a manual overhead electric motor and pestles. The samples were homogenized one by one: time consuming technique and perfect homogenization not guarantied.

CUSTOMER

ILLUSTRATIONS



a) Frozen muscle
b) Muscle immerged in buffer in Precellys® tubes
c) Muscle after homogenization with Precellys®24
d) Muscle homogenates after centrifugation



Total cathepsin activity in muscle homogenates

[1] Bahuaud D. et al., Food Chemistry (2009), doi:10.1016 /j.foodchem.2009.05.028

CONCLUSION

Comparing to the former classical method of homogenization of muscle samples, the use of **Precellys®24** allows a perfect homogenization of the samples. **Precellys®24** is **easy to use, simple, efficient and much faster** than the former method.

Precellys®24 brings us **efficiency, modernity, homogeneity** in the results and **time-saving**.

03712-810-DU021

METABOLOMIC AND TRANSCRIPTOMIC STUDY FROM FISH LIVER

Centre for Environment, Fisheries and Aquaculture Science, Cefas Weymouth Laboratory / School of Biosciences, The University of Birmingham, UK

CONTEXT

The goal of this study was to investigate both transcriptomic and metabolomic changes in response to xenoestrogen exposure. Male stickleback fish were used as a model organism for this study as they are environmentally relevant and the stickleback genome draft sequence was available. Key to this study was the ability to carry out both omics techniques upon the same liver tissue homogenate [1], [2].

MATERIAL

- Precellys®24 homogenizer.
- Precellys® kit: 03961-1-003 (ceramic beads 1.4mm).
- Sample: 50 to 150 mg (wet mass) of liver tissue.
- Buffer for omics analyses : 8 ml/g (v/w) methanol and 2.5 ml (v/w) water.
- Qiagen RNEasy RNA kit, DNA-Free (Ambion).

PROTOCOL

- Precellys®24: 6400 rpm, 2x10sec, 30s break.
- Metabolomics analysis: Extraction with methanol, chloroform and water to separate the hydrophilic and hydrophobic metabolites / storage at -80°C > onedimensional ^{1}H NMR spectroscopy.
- Transcriptomics analysis: Total RNA preparation by RNEasy, DNAse treatment, cDNA synthesis and fluorescent labeling for microarraying.

RESULTS

^{1}H-NMR metabolomics gave highly reproducible data and multivariate analyses suggested a concentrationresponse relationship.
Transcriptomic analysis showed induction of hepatic vitellogenins and choriogenins, together with a range of other xenoestrogen-responsive genes. Vitellogenin C was identified as a major responsive isoform, in contrast to other fish species.

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UNIVERSITY OF BIRMINGHAM

Cefas

ILLUSTRATIONS

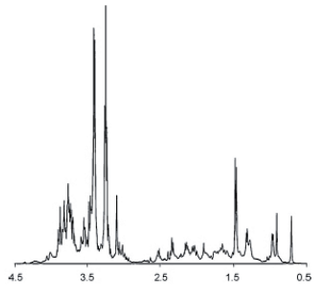

Figure 1: representative one-dimensional 1H NMR metabolite spectrum of stickleback liver

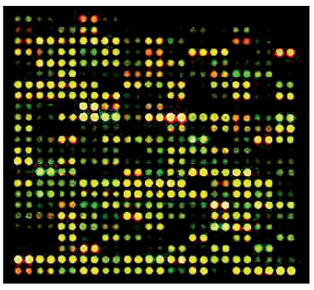

Figure 2: mRNA expression; one of 48 subarrays of the stickleback 15K cDNA array

[1] I. Katsiadaki et al., Aquatic Toxicology doi:10.1016/j.aquatox.2009.07.005
[2] H. Wu et al., Anal. Biochem. 37 (2008) 204–212

CONCLUSION

Application of multiple omics techniques requires a homogenization technique that is fast, to avoid metabolite and RNA degradation, that allows no cross-contamination between samples, and that can produce a homogenate suitable for input into multiple different purification procedures.
Precellys®24 is easy to use, simple, efficient and much faster than former methods.
We have therefore obtained high quality omics data from exactly the same material.

03712-810-DU022

METABOLOMIC STUDY FROM CLAM GILL

Key Laboratory of Coastal Zone Environment Processes, CAS; Shandong Provincial Key Laboratory of Coastal Zone Environment Processes, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, China

CONTEXT

The goal of this study was to assess the sensitivity of three pedigrees (White, Liangdao Red and Zebra) of Manila clam to the acute mercury exposure which is used as the bioindicator for the monitoring of contaminants in marine environments.
The gill of clam was one of target organs for the accumulation of contaminant, therefore the gills tissues were sampled and processed from the acute mercury exposed clams (White, Liangdao Red and Zebra) for the metabolite extraction [1], [2].

MATERIAL

- Precellys®24 homogenizer.
- Precellys® kit: 03961-1-003 (1.4mm ceramic beads).
- Sample: ~100 mg (wet mass) of gill tissue.
- Extraction solvents: 4 ml/g (v/w) methanol and 0.85 ml (v/w) water.

PROTOCOL

- Precellys®24: 6400 rpm, 2x10 sec, 30 s break.
- Metabolomics analysis: Extraction with methanol, chloroform and water to extract the polar metabolites, storage at -80°C, one dimensional 1H NMR spectroscopy (500 M), data analysis.

RESULTS

^{1}H NMR-based metabolomics showed that the White clam (A) was the most sensitive pedigree to the exposure of mercury. However, further studies on the other tissues (digestive gland, muscle, etc.) are necessary to assess their sensitivity of various pedigrees of clam to various types of environmental contaminants.

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ILLUSTRATIONS

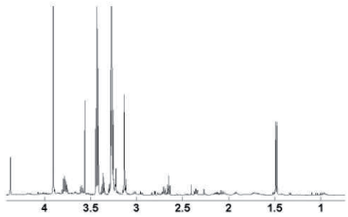

Figure 1: representative one-dimensional 1H NMR spectrum of gill tissue extracts from Manila clam.

[1] Xiaoli Liu, et al., Ecotoxicology DOI 10.1007/s10646-010-0569-x
[2] H. Wu et al., Anal. Biochem. 37 (2008) 204– 212

CONCLUSION

The Metabolomics usually needs to extract the low molecular weight (<1 000 Da) metabolites. Prior to the extraction of metabolites, the homogenization of biological tissues is necessary. **Precellys®24** is a high throughput homogenizer that can process up to 24 biological samples simultaneously. In addition, **Precellys®24** can avoid the degradation of metabolites and cross-contamination between various biological samples. Our work proved the high quality and efficiency of **Precellys®24** in the metabolomics study.

03712-810-DU044

ENZYME ASSAY FROM FROZEN ABDOMINAL KRILL TISSUE

Antarctic Krill Group, Dept. Polar Biological Oceanography Alfred Wegener Institute for Polar and Marine Research in the Helmholtz Association, Am Handelshafen , Bremerhaven, Germany

CONTEXT

The goal of this study was to investigate whether **Antarctic krill** (Fig.1) possesses a circadian clock and whether such a clock controls key physiological processes of krill. To this end, we investigated temporal mRNA expression levels of the canonical clock gene cry2 in individual krill that were maintained both under a light-dark cycle and constant darkness. In addition, we tested whether **gene expression of metabolic key enzymes** in these krill show daily or circadian oscillations, and to what extent transcriptional oscillations of these enzymes also persist at the protein-activity level [1].

MATERIAL

- **Precellys®24** homogenizer with **Cryolys** cooling device to have a constant temperature of 4°C within the homogenization chamber using liquid nitrogen.
- Precellys lysing kit: 013961-1-009 (CKmix).
- Sample: ~100 mg (fresh weight) of frozen abdominal krill tissue.
- Extraction solvents: ice-cold deionized water at a concentration of 100 mg fresh weight (fw) mL^{-1}.

PROTOCOL

- Precellys®24: 5000 rpm, 2x15sec, 10s break.
- Enzyme assay: Centrifugation of homogenate, spectrophotometrically analysis of supernatant in a specific buffer, data analysis.

RESULTS

Overall, our results showed that a **krill endogenous circadian clock** governs metabolic and physiological output rhythms (Fig.2). **Enzyme activity assays** revealed oscillatory patterns with roughly 9-12 h period under both lighting conditions that correlate with the relative changes in transcript abundance. Further studies will be necessary to investigate the contribution of the circadian clock to rhythmic variations in expression and activity of metabolic enzymes in krill and thereby will give a better understanding of how rhythmic physiology and behavior in krill will be regulated.
Time course experiments require **a standardized and equably homogenization of biological tissues** that guarantee a reliable temporal analysis of data. In particular, **enzyme assays require a low temperature during the homogenization process to maintain enzyme activity**.
During this study, **the Cryolys guaranteed a constant temperature of +4°C** within the homogenization chamber and thus avoided an uncontrolled defrosting of the frozen samples.

ILLUSTRATIONS

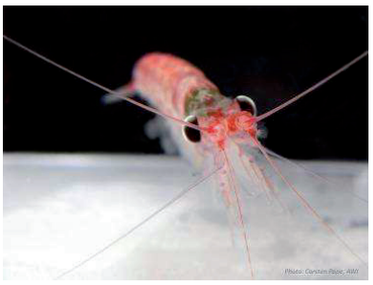

Figure 1: Antarctic the frozen samples. krill Euphausia superba.

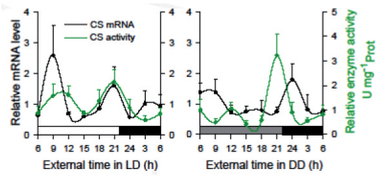

Figure 2: Oscillatory rhythms of the metabolic key enzyme citrate synthase (CS) in light-dark (LD) cycle and in constant darkness (DD).

[1] Teschke, M. et al., PLoS ONE doi:10.1371/journal.pone. 0026090

CONCLUSION

The Metabolomics usually needs to extract the low molecular weight (<1 000 Da) metabolites. Prior to the extraction of metabolites, the homogenization of biological tissues is necessary. **Precellys®24** is a high throughput homogenizer that can process up to 24 biological samples simultaneously. In addition, **Precellys®24** can avoid the degradation of metabolites and cross-contamination between various biological samples. Our work proved the high quality and efficiency of **Precellys®24** in the metabolomics study.

03712-810-DU063

RNA EXTRACTION FROM SUBCUTANEOUS ADIPOSE TISSUE (SCAT) IN HUMAN OBESITY

INSERM UMRS U872 (Eq7) Nutriomic, Nutrition and Endocrinology Department, Pitié-Salpêtrière Hospital, Paris, France. (V. Pelloux)

CONTEXT

Adipose tissue is a critical player in obesity-related metabolic functions. Indeed, adipose tissue is no longer considered to be simply a passive lipid reservoir, but rather an endocrine organ capable of secreting factors that profoundly influence processes such as feeding behavior, energy flux, and immuno-inflammation. As such, obtaining adipose tissue samples are paramount to the understanding of human obesity. Much of our recent understanding regarding the role of **subcutaneous adipose tissue (scAT)** in human obesity is a consequence of using modern molecular biology tools, such as the microarray. [1] These techniques are highly dependent on the quality of total RNA obtained. **Biopsies of scAT are precious and often small amounts**. To improve performance and get a better quality of extracted RNA, we compared two mashing methods.

MATERIAL

- **Precellys®24** vs manual method (Ultra Turrax T8-IKA).
- Precellys kit: 03961-1-009 (CKmix).
- Sample: 10 human scAT samples (about 100 to 400 mg/sample) obtained by needle aspiration.
- Buffer: RLT+ß-mercaptoethanol.

PROTOCOL

- Precellys 24: 6000 rpm, 2x15sec, 20s break.
- Analysis: Total RNA extraction using the RNeasy total RNA Mini kit (Qiagen). Total RNA concentration confirmed using NanoDrop ND-1000 and quality using the Agilent 2100 Bioanalyzer (Agilent Technologies).

RESULTS

Yield for both extractions was determined and shown in figure1. The heterogeneity of the amount of RNA obtained was unequal to the weight of the biopsies. An increase in the amount of extracted RNA is observed with Precellys 24.
Same extracted RNA purity is observed with these two methods (Figure 2a, 2b).
By quantitative PCR, we observed **an amplification factor of the leptin gene using Precellys 24**, which will quantify the expression of genes weakly expressed in this tissue.

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Institut national
de la santé et de la recherche médicale

ILLUSTRATIONS

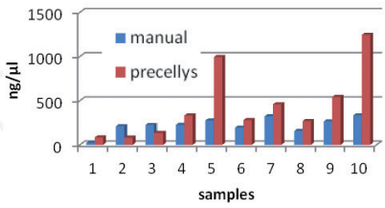

Figure 1: extracted RNA yield obtained with Ultraturax and Precellys methods.

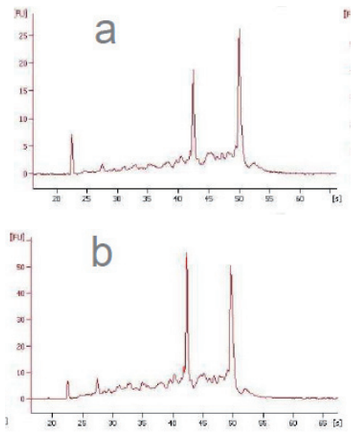

Figure 2: a) sample 7 manual –RIN 7.0; b) sample7 Precellys RIN 7.

[1] Mutch, D. M. & Clement, K. Unraveling the genetics of human obesity. PLoS Genet 2, e188 (2006).

CONCLUSION

Our data demonstrated that Total yield of RNA was higher with **Precellys®24** than manual technique while the purity of RNA remained comparable. Moreover, an amplification factor of the specific gene studied was observed after Precellys®24 homogenization.
Precellys®24 was also more convenient, allowing rapid extraction of multiple samples, while avoiding contamination between each biopsy.

03712-810-DU061

TOTAL RNA EXTRACTION FROM CARTILAGE TUMORS

Quality Control Platform of Nucleic Acids, Programme Carte d'Identité des Tumeurs (CIT), Ligue Nationale Contre le Cancer, Paris, France.

CONTEXT

Our platform is specialized in extraction of nucleic acids from tumoral tissues intended to be analyzed by microarray and sequencing. In this study, nucleic acids have been extracted from important series of **cartilage tumors**. Although DNA extraction from cartilage samples is common, the extraction of high quality RNA is still a challenge. In this way, the Precellys Dual was evaluated to increase quality of extracted RNA from cartilage tumors.

MATERIAL

- Precellys Dual homogenizer.
- Precellys lysing kit: 03961-1-007 (CK28R).
- Sample: 50 to 100 mg cartilage tumors (chondrosarcoma).
- Buffer: 1 ml TRIzol Reagent (Invitrogen) + 200 µl Guanidine thiocyanate 4M (SIGMA).
- miRNeasy Mini kit (Qiagen).

PROTOCOL

- Precellys Dual: 6500 rpm, 3x15sec, 10s break.
- Subsequent RNA extraction and purification was done as described previously [1].
- Verification of RNA quality: Agilent 2100 Bioanalyzer (Agilent Technologies).

RESULTS

Qualification of **RNA and miRNA** is based on some criteria as 28s/18s ratio, RIN and Agilent 2100 Bioanalyzer profiles. Figures 1 and 2 show the results of RNA and miRNA qualifications extracted from a cartilage tumor. Characteristics of total RNA are reported in Table 1.

RNA and miRNA quality and integrity are optimal to microarray and sequencing analysis.

CUSTOMER

ILLUSTRATIONS

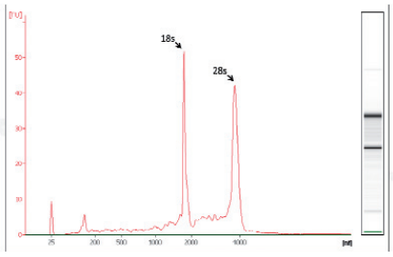

Figure 1: Profile of RNA extracted from a cartilage tumor on Agilent 2100 Bioanalyzer

A260/280	2.07
A260/230	1.9
28s/18s	1.5
%miRNA/RNA	7.88
[RNA] ng/µl	408.3
Quantity (ng)	10819

Table1: Characteristics of total RNA extracted from a cartilage tumor

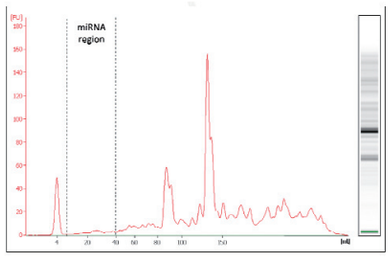

Figure 2: Profile of miRNA extracted from a cartilage tumor on Agilent 2100 Bioanalyzer

[1] Banneau et al., Breast Cancer Research (2010) 12:R63.

CONCLUSION

The **Precellys Dual** is successfully validated for total RNA extraction from cartilage tumors. It provides better homogeneity in grinding of hard tissue as cartilage and so, increases quality and quantity of total RNA extracted. Moreover, the **Precellys Dual** is easy to use, simple, efficient and much faster than former methods.

03712-810-DU062

DNA EXTRACTION FROM FETAL AND NEONATAL PATHOLOGY TISSUES

West Midlands Regional Genetics Laboratory, UK

CONTEXT

The West Midlands Regional Genetics Laboratory (WMRGL) is an NHS diagnostic laboratory providing testing for a wide range of **genetic disorders. Fetal and perinatal pathology tissue samples** are analyzed for chromosome abnormalities by QF-PCR and subtelomere MLPA. Prior to the introduction of the Precellys®24 these tissue samples were finely chopped using a scalpel, digested for >24 hours followed by a manual salting out DNA extraction procedure.

MATERIAL

- Precellys®24 homogenizer vs Proteinase K method.
- Precellys®lysing kit: 03961-1-003 (CK14).
- Sample: 0.5 cm^2 of fetal or neonatal tissue e.g. skin or placental villi.
- Buffer: 190 µl Qiagen EZ1 G2 buffer.
- Proteinase K (600 mAU/ml).

PROTOCOL

- Precellys®24 parameters: 5500rpm, 3x10 sec (15 sec break time).
- Enzymatic method: Incubate overnight at 37°C with 10µl Proteinase K.
- Analysis: DNA extraction by Qiagen EZ1 robot or by manual salting out DNA extraction procedure.

RESULTS

DNA was extracted by Qiagen EZ1 robot using the tissue protocol.
The results are reported in the figure 1.

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West Midlands Regional Genetics Service

ILLUSTRATIONS

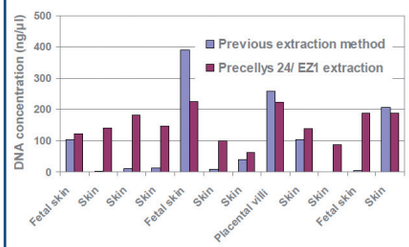


Figure 1: Concentration comparison of previous extraction method to Precellys®/EZ1 extraction.

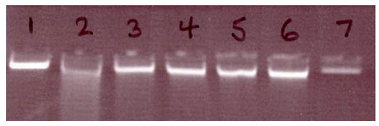


Figure 2: Agarose gel image of DNA extracted by Precellys 24 / EZ1 extraction. Samples showed no or minimal DNA degradation.

CONCLUSION

The **Precellys®24** extraction method has several advantages to the former method used including: speed, reduced staffing time, higher throughput, more uniform results and improved safety.

03712-810-DU064

BINDING OF ONE LIPOPHILIC COMPOUND TO THE PRECELLYS TUBES WITH CERAMIC BEADS DURING TISSUE HOMOGENIZATION

Songmao Zheng, Justina C. Calamia, Departments of Pharmaceutics, University of Washington, Seattle, WA, USA

CONTEXT

To study lipophilic compounds glass vials are widely used in labs. We were aiming to quantify the loss of one **lipophilic compound** in the tissue homogenization process using Precellys tubes with ceramic beads. For that, we selected one compound that shown to bind plastic extensively. The compound info is: small molecule; neural compound; MW>500; experimental / theoretical LogP >3; low water solubility.

MATERIAL

- Precellys®24 homogenizer coupled with Cryolys.
- Precellys®lysing kit: 03961-1-003 (CK14 - 2mL tubes).
- 34 mg of blank human kidney tissue.
- 200µL of buffer (same buffer used in compound extraction, non-organic and contains no protein homogenization reagent) .
- Stock concentration of the lipophilic compound: 0.125µg/mL, 2µg/mL, 20µg/mL and 100µg/mL.

PROTOCOL

- Preparation of a kidney homogenate at 34 mg/2000µL.
- Take 200µL of kidney homogenate into 4 Precellys lysing tubes CK14 and in 4 glass vials as a control group.
- Add 10µL of each stock concentration of the compound to 200µL of kidney homogenate in the Precellys lysing tubes and glass vials.
- Samples were vortexed and let equilibrated at room temperature for 15 min.
- Homogenization using Precellys tubes with 15 seconds of vortexing twice at 6000 rpm with 10 seconds break using Cryolys with dry ice and acetone to cool the unit.
- Take 150µL out from the Precellys tubes or the glass tubes for compound extraction and LC-MS/MS analysis.

RESULTS

Both compound extraction and LC-MS/MS analytical methods were validated and published previously. We assume that the binding of the compound to glass tubes is minimal and thus use glass tubes as controls.

The percentage loss is 19.3 ± 10.9 % (mean ± SD), ranging from 9.3% to 34.5%. The data from the 2µg/mL group may be an outlier due to the big deviation from the other concentration groups. The percentage loss from the other three concentration groups is 14.2 ± 4.8 % (mean ± SD), ranging from 9.3% to 18.9%.

The percentage lost due to Precellys tubes binding does not seem to depend on compound concentrations which is good for calculation that may account for the loss of the drug at an unknown concentration.

The standard curves for the 3 conditions are shown in the figure 1.

ILLUSTRATIONS

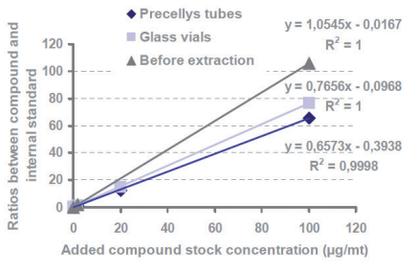


Figure 1: Standards curves

CONCLUSION

We concluded that the combo **Precellys®24 homogenizer / Cryolys** and the **Precellys® lysing kits CK14_2mL** are adequate in the application of tissue homogenization of one **lipophilic compound** in our research given its great consistency in homogenizing different tissue samples and acceptable loss of lipophilic compound (~15% or less) to Precellys tubes with ceramic beads.

03712-810-DU067

DRUG ANALYSIS IN HAIR

The national Institute of Criminalistics and Criminology (NICC), Bruxelles, Belgium

CONTEXT

Within the context of drug analysis in hair new protocols have been developed to establish the toxicological profile of a patient. Those techniques involve constraints as low volumes and concentrations and absence of crosscontamination. Before the analysis by HPLCMS/ MS, the samples had to be reduced in powder form.

MATERIAL

- Precellys® 24 equipment
- Precellys® kit MK28 (metal beads)
- Sample: 1cm hair segments
- Buffer: empty

PROTOCOL

- Precellys®24 parameters: 6500 rpm, 2x50 sec., 15 sec. break

RESULTS

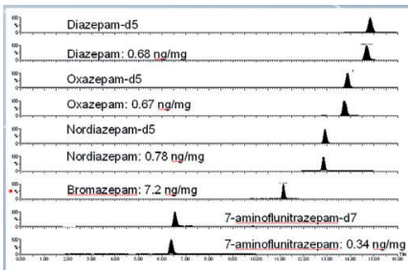
Analysis of an authentic hair sample by HPLC-MS/MS with the following steps :

1. Decontamination (Dichloromethane)
2. Grinding (Precellys®24)
3. Incubation (Methanol)
4. Centrifugation
5. Evaporation
6. Filtration
7. Analysis by HPLC-MS/MS

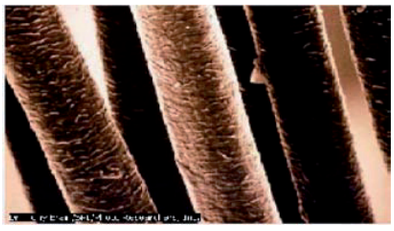
See also the publication
1. Journal of Analytical Toxicology, Vol. 29, Oct 2005
2. Anal Bioanal Chem DOI 10.1007/s00216-007- 1297-9

CUSTOMER

ILLUSTRATIONS



NICC, Belgium January 2005



CONCLUSION

Precellys®24 is successfully evaluated on hair grinding, which is considered a difficult and challenging sample. Results of toxicology tests in crime labs are now faster.

03961-002-DP005

RNA EXTRACTION FROM HUMAN SKIN BIOPSIES

Mouchet N. [1-2.], Galibert M.D. [1] - 1] UMP 6061 CNRS, Rennes, France.
2] Proclaim, Saint-Gregoire, Franceclaim, Saint-Gregoire (France)

CONTEXT

The understanding of molecular mechanisms and gene regulation involved in pigmentation and inflammatory processes, is needed to identify new therapeutic targets. For gene expression quantification, RNA is extracted from skin biopsies exposed and unexposed to UV radiation.

MATERIAL

- Precellys®24 equipment
- Precellys® kit CK14 (small ceramic beads)
- Sample : human skin biopsies (40-70mg)
- Buffer : RA1 lysis buffer (Macherey- Nagel) +1% ß- Mercapto ethanol (350µl)

PROTOCOL

- Precellys®24 parameters:
  6300 rpm, 6x23 sec., 2 min. break (Samples are chilled on ice between each cycle)

Cryolys Option should be used to keep the samples at low temperature.

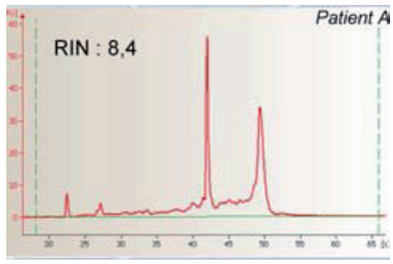
RESULTS

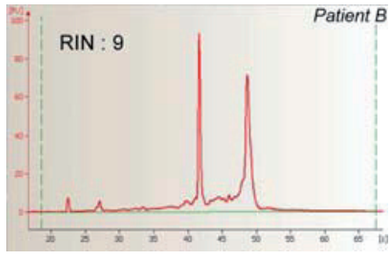
Compared to classical lysis (chaotropics ions + ß- Mercapto ethanol + Vortex), RNA yield increased from 2 to 4 fold (Nanodrop quatification). RNA quality was assessed using a 2100 Bioanalyser. RNA Integrity Number (RIN) is over 8, meaning good quality.

	Classical Lysis	Precellys Lysis
Sample 1	124,6 ng/µl	454,0 ng/µl
Sample 2	160,0 ng/µl	638,0 ng/µl
Sample 1	198,7 ng/µl	801,1 ng/µl

CUSTOMER

ILLUSTRATIONS





Chromatograms from 2 patients A and B

CONCLUSION

RNA extracted in highest quantity using **Precellys®24** allowed us to study transcription modifications of several genes implicated in pigmentation or inflammation after UV-solarspectrum-radiation corresponding to 1 to 2 MED (Minimal Erythema Dose).

03961-006-DU003

ANIMAL TISSUES

LIPID HYDROPEROXIDES (LPO) EXTRACTION FROM TUMOR AND NON-TUMOR RATS TISSUES

Cancer Research Laboratory, University Hospital of Tours, Tours, France.

CONTEXT

The fundamental and clinical project of our research unit is positioned at the junction of the two fields “cancer and nutrition” with a specialization in **lipid biochemistry and breast cancer**. Our research unit has described the potential benefit of the clinical use of lipid nutrients in order to increase the efficiency of cancer treatment. Docosahexaenoic acid (DHA) has the potential to increase tumor sensitivity to chemotherapy with no sensitization of normal tissues. This study [1] was aimed at exploring the mechanism involved in this differential sensitization with a focus on oxidative stress, one of the main determinants involved in DHA enhancement of anthracycline-based chemotherapy.

MATERIAL

- **Precellys 24 homogenizer with Cryolys cooling** device to have a constant temperature of 4°C within the homogenization chamber using liquid nitrogen.
- Precellys lysing kit: 03961-1-009 (CKmix).
- Sample: ~100 mg of frozen tumors, intestine, liver, and heart from rats treated with DHA + epirubicin and from control rats (palm oil, no chemotherapy) .
- Buffer: ice-cold distilled water.

PROTOCOL

- Precellys setting: 6500 rpm, 3x20sec, 50sec break.
- **Lipid hydroperoxides** (LPO) were extracted and assayed with a lipid hydroperoxides assay kit according to the manufacturer’s instructions (Kit no. 705003, Cayman Chemical Company, Ann Arbor, MI, USA).

RESULTS

Overall, our results showed that **supplemental DHA during an anthracycline-based chemotherapy selectively increased tumor level of LPO**. In fact, at baseline (control group) a similar level of LPO was detected in tumors, liver, heart, and intestine. Supplementing animals with DHA during chemotherapy increased the level of LPO in tumors while no change in LPO level was detected in liver, heart, or intestine (figure 1), even though their enrichment with DHA was larger than that of tumors. Enzyme activity assays showed a differential change in antioxidant defenses between tumors and other tissues. This differential handling of oxidative stress between tumors and other tissues might be a mechanism contributing to the absence of toxicity of DHA supplementation to normal tissues during chemotherapy.
During this study, the Cryolys guaranteed a constant cool temperature within the homogenization chamber.

[1] Hajjaji N. et al., Tumor and non-tumor tissues differential oxidative stress response to supplemental DHA and chemotherapy in rats. CCP 2012. DOI10.1007/s00280-012-1884-0

ILLUSTRATIONS

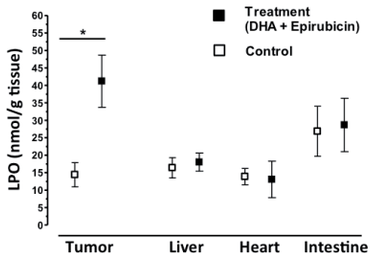


Figure 1: LPO level in tumor and non-tumor tissues at baseline (control rats) and in response to treatment with DHA and epirubicin.

CONCLUSION

The combo Precellys&Cryolys cooling option are suitable and reliable systems to homogenize a large range of rats tissues and tumors to investigate **molecular and cellular mechanisms of action of lipids**. In our rat model, an efficient and equably homogenization of tissues with the **Precellys&Cryolys** was a prerequisite for an optimal subsequent extraction and measure of lipid hydroperoxides in tissues.

03712-810-DU073

ANIMAL TISSUES

PLANT TISSUES

MICRO-ORGANISMS

PRECELLYS RANGE

INDEX

DNA EXTRACTION FROM RICE GRAIN

Kogene Biotech Co – South Korea

CONTEXT

Rice is a key element of the Korean food. Due to Korean regulations, the rice species need to be defined and evaluated. Therefore, DNA qualification and classification are essential.
In this study, a rice grain specie is identified using Precellys®24 vs. mortar.

MATERIAL

- Precellys®24
- Precellys® kit metal bead
- Sample: 1 grain rice - dry
- Without buffer

PROTOCOL

- Precellys®24 parameters: 6000rpm, 2x30s, 20s break

RESULTS

Grinding efficiency:
Rice grains of 3 species were grinded into fine powder following the protocol described

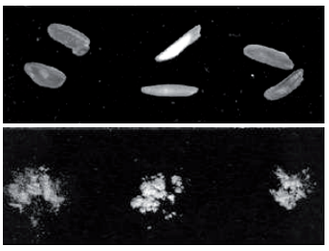
DNA analysis:
Rice grains (*Orysa sartiva L.*) were grinded by Precellys®24 and a mortar.
DNA was extracted and loaded directly onto an agarose gel after PCR amplification. Both Precellys®24 and mortar are efficient to extract a good quality of DNA. The DNA quantity from rice grains grinded by Precellys®24 (P1, P2, P3 and P4) are similar to the result with a mortar (M1, M2, M3 and M4).

CUSTOMER

(주)코젠바이오텍
KOGENEBIOTECH
Global Leader in Molecular Diagnosis

ILLUSTRATIONS

Rice (Orysa sartiva L.)



Precellys®24 reduces into powder any species of rice grain in 1 minute

Precellys®24 gives a faster, easier and more consistent sample preparation with results as efficient as mortar

CONCLUSION

Every rice species on Korean market must be classified via DNA analysis. We compared use of **Precellys®24** vs. mortar and obtained similar PCR results with **Precellys®24**. However, it takes lots of time and labor work with mortar preparation (1 prep in 10 minutes).
We are satisfied with the results of **Precellys®24** in terms of reproducibility, time and labor saving (up to 24 preps per minute).

03712-810-DU013

PLANT TISSUES

PROTEIN EXTRACTION FROM *NICOTIANA BENTHAMIANA* LEAF

Institute of Genetics, Heinrich-Heine University Düsseldorf, Germany

CONTEXT

The laboratory is focused on developmental biology of plants using the model system *Arabidopsis thaliana*.
The aim of this study was to investigate the intracellular localization of protein receptors (CLV1, CLV2, and CRN) in plant cells and their tendencies for protein-protein interactions. To analyze receptor localization and interaction, a transient expression system in Nicotiana benthamiana leaf epidermis cells was used [1].

MATERIAL

- Precellys®24 homogenizer.
- Precellys® kit: 03961-1-003 (ceramic beads 1.4mm)
- Sample: ~0.1 g of *N. benthamiana* leaf tissue.
- Extraction buffer: 750 µL (0.1 M Tris-HCl, pH 8.3, 5 mM dithiothreitol, 5 mM EDTA and protease inhibitor).

PROTOCOL

- Plant tissue homogenized using the Precellys®24: 5500 rpm, 1x20 sec.
- 1h incubation of plant extract at 4°C followed by 10 min denaturation at 95°C.
- Protein separation by SDS-PAGE & Western-blot analysis.

RESULTS

Our transient expression studies of translational fusions with GFP or mCherry now showed that all three receptor proteins can localize to the plasma membrane and have the capacity to undergo multiple interactions.

The Precellys®24 is a fast method for protein isolation. Protein extracts were used to demonstrate fusion protein expression and stability.

[1] A. Bleckmann et al., Plant Physiology, January 2010, Vol. 152, pp. 166–176, www.plantphysiol.org

CUSTOMER

HEINRICH HEINE UNIVERSITÄT DÜSSELDORF

ILLUSTRATIONS

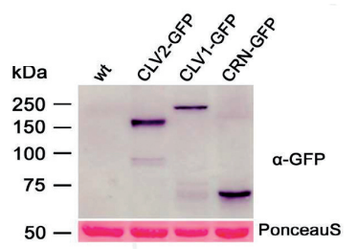


Figure 1: Western-blot analysis of protein extracts from N. benthamiana leaf cells transiently expressing CLV1-GFP, CLV2-GFP, or CRN-GFP. An anti-GFP antibody was used for detection; sizes of protein markers are given in kD. The Ponceau Sstained protein bands of Rubisco are shown as a oading control. wt, wild type.

CONCLUSION

Precellys®24 is well adapted for homogenization of plant tissues to extract and study proteins expression. Sample preparation is not only easy but cross contamination free.

The **Precellys®24** can also be used for efficient isolation of DNA and RNA from plant tissue.

03712-810-DU031

PROTEIN EXTRACTION FROM *ARABIDOPSIS* WITH MINILYS

Institute of Plant Molecular Biology, CNRS, Univ. of Strasbourg, France

CONTEXT

This institute is presently the largest CNRS centre devoted to integrative plant biology. IBMP focus on the molecular and cellular mechanisms of plant growth, differentiation, development and defense reactions against pathogens and environmental stresses. The research programs use functional genomics, genetics, molecular and cell biology and molecular enzymology.

MATERIAL

- Minilys homogenizer.
- Precellys kit: 03961-1-010 (1.4&2.8 ceramic beads mix).
- Samples: 5 *Arabidopsis* seedlings (two weeks old).
- Buffer: 200 µL of 62.5mM Tris-HCl pH8, 4M Urea, 3% SDS, 10% Glycerol, 0.1% Bromophenolblue, 100mM DTT.

PROTOCOL

- Snap-frozen in N2.
- Addition of extraction buffer after (Preparation A) or before homogenization (Preparation B).
- Minilys: 5000 rpm, 20 sec or 5000 rpm, 40s.
- Triplicate by condition.
- Analysis: Western blot.

RESULTS

The total yield of proteins extracted is increasing with buffer extraction and an higher duration of homogenization (Figure 1).
Total proteins were separated on a 8% SDSAcrylamide gel and transfer to a PVDF-membrane.

CUSTOMER

ILLUSTRATIONS

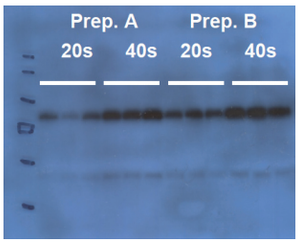
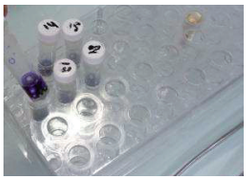


Figure 1: Western blot of Arabidopsis total proteins and detection of HD-Zip transcription factor by anti GFPHRP antibody.

Arabidopsis



Addition buffer

Before homogenization

CONCLUSION

Buffer extraction with an increasing time homogenization (40sec.) allows higher proteins yield and in the same range as with **Precellys®24** tissue homogenizer. **Minilys** provides the optimal balance of efficiency, speed, ease of use with a low throughput. **Minilys** enables cross-contamination free homogenization as opposed grinding with a mortar.

03712-810-DU047

MYCOVIRAL DOUBLE-STRANDED RNA EXTRACTION FROM PLANT LEAF WITH MINILYS

Tokyo University of Agricultural and Technology, Institute of Agriculture, division of Bio-regulation and Bio-interaction, Japan

CONTEXT

This laboratory develops bio-molecular solutions for plant biomass production. Extraction of mycoviral double-stranded RNA (dsRNA) from plant leaves for the investigation of attenuation of *Alternaria* and *Magnaporthe* (*Ascomycete fungi*) infection is a part of the laboratory's daily work.

MATERIAL

- Minilys homogenizer.
- Precellys kit: 03961-1-002 (2.8 mm ceramic beads).
- Samples: 20 mg of green pepper (*Capsicum annum*) leaf and 20 mg of *Malabar spinach* (*Basella alba*) leaf.
- Buffer: 500µL of STE buffer 2X.

PROTOCOL

- Minilys: 5000 rpm, 60 sec x 1, 2 or 3 cycles with no time break on ice between each cycles.
- Addition of SDS ; phenol-chloroform extraction.
- Analysis: Agarose gel electrophoresis stained with EtBr.

RESULTS

A protocol of 5000 rpm x 60 sec with CK28 gave the best results for both green pepper and Malabar spinach leaves homogenization. Extension of the time homogenization attenuated the dsRNA band of mycoviral especially in the Malabar spinach sample.

CUSTOMER

東京農工大学

ILLUSTRATIONS

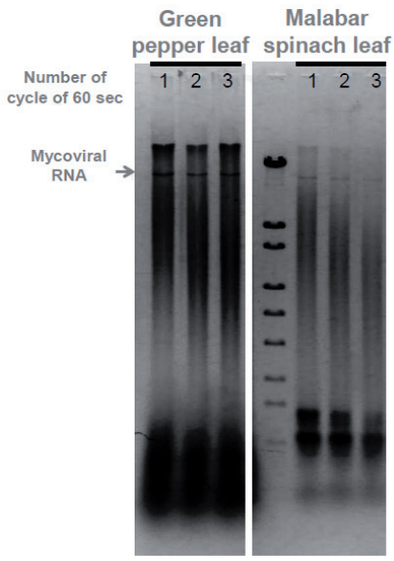


Figure 1: Agarose gel electrophoresis of 5 mg extracted plant

CONCLUSION

A short homogenization allowed to have good dsRNA yield where as a longer one lead to a lower RNA yield.

Minilys is a suitable homogenizer for extracting mycoviral dsRNA from plants. **Minilys** provides a way of standardizing sample preparation for laboratories with a low or medium throughput.

03712-810-DU051

DNA EXTRACTION FROM FRENCH SOILS EVALUATION OF PRECELLYS®24-DUAL 7ML VIAL

Genosol – UMR Microbiology of the soil and environment, Inra Dijon, France

CONTEXT

The Genosol platform is a biological resource centre unique in France and Europe, devoted to the conservation and analysis of the genetic resources of soil microbial communities. The ambition of the GenoSol platform is to centralize the microbial genetic resources of soils and make them available to the scientific community together with the most upto-date technologies for their characterization. In this way, the Precellys®24-Dual was evaluated in comparison with the reference method.

MATERIAL

- Precellys®24-Dual with Precellys® kit 7mL vial with glass and ceramics beads (0907-04 + 03961-1-106).
- Standard method: Mikro-Dismembrator S (Sartorius).
- Samples: Three clayey, silty-clayey, sandy soils (Arceau, Champdôtre, Epoisses).

PROTOCOL

- Precellys®24-Dual protocol : 1g soil + 4mL Buffer / 7mL vial, 5000 rpm 3x15s.
- Electrophoresis gel - DNA extraction yield, qPCR.

RESULTS

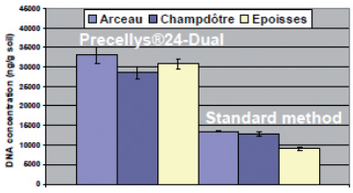
With Precellys®24-Dual, the concentration of DNA extracted is around 2.5 higher than the standard method. The quality of DNA, evaluated by inhibition and restrictive enzyme assays was equivalent with both equipments.

Precellys®24-Dual allows a better cadence: 6 samples could be prepared in 45s versus 120s with standard method (1 sample by run).

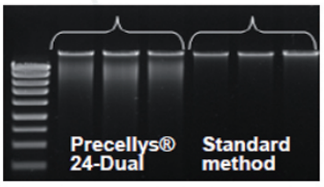
CUSTOMER

Plateforme INRA GenoSol
Exploring the microbial world To better protect our environment

ILLUSTRATIONS



DNA concentration (ng/g soil) for 3 French soils.



Gel electrophoresis analysis, Epoisses soil (triplicate).

CONCLUSION

The **Precellys®24-Dual** is successfully validated for extraction of total DNA with higher yield of extraction. The DNA quality is optimal to study the soil microbial communities. Easy handling, reproducibility and high throughput are appreciated.

03712-810-DU017

DNA EXTRACTION FROM RICE LEAF

National Institute of Agrobiological Sciences - Japan

CONTEXT

The research plan is to isolate the genes of functional proteins and clarify their functions by introducing them into plants.

The lab is studying the gene transfection into a plant seed by electroporation. Our team checks how much volume of DNA is introduced into a seed by a standard molecular biology process.

MATERIAL

- Precellys®24
- Precellys® kit SK38 (Mixed beads)
- Sample: 0,08g of rice leaf cut into pieces
- Buffer: empty

PROTOCOL

Precellys®24 parameters: 5000rpm, 1x15 sec.
Before and after the homogenization, the tube is put in liquid nitrogen (LN).
DNA extraction buffer must be poured into the tube while the sample is frozen.The beads are separated using stainless sieve.
PS: careful of handling LN. Use necessary protective gears.

RESULTS

DNA was extracted with *Nucleon™ PhytoPure™* Genomic DNA Extraction Kits from GE Helthcare.

The migration gives the following results : (Lane No.1 and No.2 are replications)

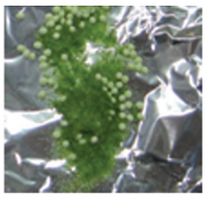
Since the concentration of the λDNA is known, it is used to semi-quantify the amount of extracted DNA by visual observation.

CUSTOMER

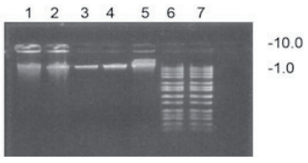
National Institute of Agrobiological Sciences

ILLUSTRATIONS

Fresh leaves, before LN



Frozen leaves after grinding



1. DNA from rice leaf
2. DNA from rice leaf
3. λDNA 0.05µg
4. λDNA 0.1µg
5. λDNA 0.5µg
6. Marker
7. Marker

CONCLUSION

The former method was to grind the leaves manually using mortar and pestle. It was laborious and time consuming. **Precellys®24** gives us a new approach in our sample preparation, combining efficiency and high-throughput.

03712-810-DU014

RNA EXTRACTION FROM *ARABIDOPSIS* WITH MINILYS

Institute of Plant Molecular Biology - CNRS, Univ. of Strasbourg, France

CONTEXT

This institute is presently the largest CNRS centre devoted to integrative plant biology. IBMP focus on the molecular and cellular mechanisms of plant growth, differentiation, development and defense reactions against pathogens and environmental stresses. The research programs use functional genomics, genetics, molecular and cell biology and molecular enzymology.

MATERIAL

- Minilys homogenizer.
- Precellys kit: 03961-1-009 (1.4&2.8 ceramic beads mix).
- Samples: 5 Arabidopsis seedlings (two weeks old).
- Buffer: 1mL TRI Reagent (MRC TR118).

PROTOCOL

- Minilys: 5000 rpm, 20 sec or 5000 rpm, 40s.
- Snap-frozen in N2 or not.
- Triplicate by condition.
- Analysis: total RNA was extracted directly from the homogenized samples using the TRI Reagent protocol before being checked for quality and quantity.

RESULTS

Concentration of RNA obtained from samples after homogenization with Minilys and absorption ratio were determined and shown in the graph 1.
An increasing time homogenization and snap-frozen allow higher RNA yield. High RNA quality is obtained from all extracted samples.

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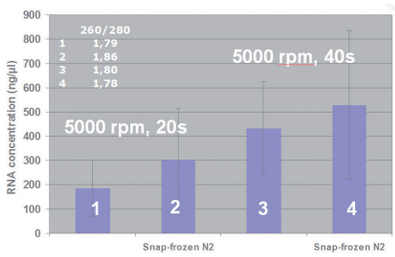
ILLUSTRATIONS



Graph 1: mean concentration of total RNA from 4 extracted pool samples snap frozen before TRI Reagent addition or not and homogenization with Minilys at 5000 rpm 20s or 40s. The corresponding mean absorption ratio (260/280) are also plotted.

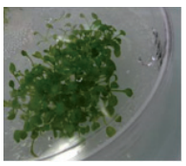


Fig.1: Arabidopsis seedlings

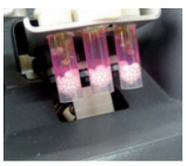


Fig.2: Before homogenization

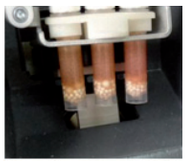


Fig.3: After homogenization

CONCLUSION

An increasing time homogenization (40s) allows higher RNA yield. High RNA quality is obtained from all extracted samples.
Minilys provides the optimal balance of efficiency, speed, ease of use with a low throughput.
Minilys enables cross-contamination free homogenization as opposed grinding with a mortar.

03712-810-DU49

ENVIRONMENTAL BIOMARKERS ANALYSIS

Laboratory of Industrial and Environmental Toxicology ULCO
Dunkerque / ILIS - Lille II

CONTEXT

Mosses are environmental biomarkers used for the long term accumulation and bioaccumulation assessment of atmospheric particles. Organic and inorganic pollutants could be responsible for oxidative stress and biological modifications. The biomarkers are here the MDA concentration by HPLC (MalonDiAldehyde resulting from membranes lipoperoxidation), the 8 hydroxy-2-deoxyguanosine concentration by ELISA (DNA adducts) and the DNA fragmentation (DNA ladder, results shown)

MATERIAL

- Precellys®24
- Precellys® kit MK28 (metal beads)
- Sample: 50 mg of frozen mosses exposed in situ to air pollution
- Buffer: 250 µl of PBS (added after grinding)

PROTOCOL

- Precellys®24 parameters: 6500 rpm, 1x30sec.
- DNA extraction
- DNA agarose gel (1%) in Tris/ Borate
- EDTA buffer

RESULTS

In situ exposures proceed on 2 sites: a site mainly contaminated by industrial emissions (Dunkerque, North of France) and rural site (Montagney, East of France).

During exposures, sampling is carried out every 8 weeks delimiting 3 period exposure : T1, T2, T3 and T0 corresponding to non-exposed mosses.

The “smear” observed indicated the DNA fragmentation. The DNA ladder test seems to reveal DNA fragmentation for samples exposed in industrial site for 16 and 24 months.

ILLUSTRATIONS

Dunkerque

Montagney

CONCLUSION

Only a high contamination could induce the degradation on DNA in *Scleropodium purum*.
The **Precellys® 24** and **Precellys® kit** MK28 allows the grinding of mosses, which are a resistant material to the manual grinding.

03961-006-DU002

PLANT TISSUES

PROTEIN EXTRACTION FROM CYPRESS POLLEN WITH MINILYS

ESPCI, LSABM, Allergy & Environment, France

CONTEXT

The common cypress pollen (*Cupressus sempervirens*) is becoming an increasing cause of respiratory allergy in Europe and some regions worldwide.
Because of its particular structural features and physico-chemical composition, cypress pollen is one of the most pollen difficult to analyze in terms of protein content and therefore allergens.
In aqueous media, the external wall (exine) cracks in a few minutes under the effect of swelling of the intine (inner wall) particularly rich in polysaccharide. Few proteins are then extracted in aqueous conditions.
The dry milling may be a good alternative for the extraction of cypress pollen proteins and to generate fragments of smaller sizes for experiments and ultra- structural analysis of immuno-reactivity [1].

MATERIAL

- Minilys homogenizer.
- Precellys kit: 03961-1-003 (1.4 mm ceramic beads).
- Sample: 100 mg of Cupressus sempervirens pollens.

PROTOCOL

- Minilys: 5000 rpm, 1x30s and 3x30sec (15s break).
- Analysis: Microscopic observation (X40) / 1-D (SDS-PAGE) immunoblotting.

RESULTS

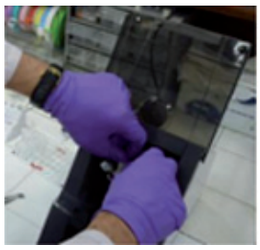
Microscopic observation (Figure 1) confirms that the *Cupressus* pollen dry milling with CK14 beads during 3x30sec seems suited to generate smalls fragments of pollen.
The downstream analysis (results not shown) show that Minilys dry milling is very efficient to extract quickly high concentration of proteins. However, the protein extracted by dry milling were different from the protein extracted by overnight incubation in aqueous media (PBS).

The dry milling is recommended in respect of cypress pollen due to his feature gelling in aqueous media.
Other pollen grains such as birch or grass pollen can be grind in liquid media.

[1] Y. Shahali, J.-P. Sutra, G. Peltre, D. Charpin, H. Sénéchal, P. Poncet. IgE reactivity to Common cypress (C. sempervirens) pollen extracts: evidence for novel allergens. WAO Journal. 2010, 3, 229-234.

CUSTOMER

ILLUSTRATIONS



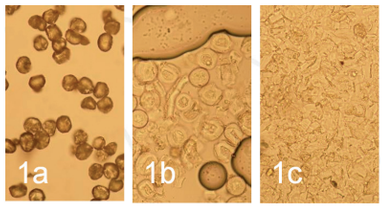


Figure 1: Microscopic observation (X40). Whole Cupressus pollen grain before grinding, dry observation (1a). Cupressus pollen grain after 1x30s dry grinding, observation in water (1b). Cupressus pollen grain after 3x30s dry grinding, observation in water (1c).

CONCLUSION

Minilys is successfully evaluated to extract high levels of proteins from cypress pollen by dry milling and generate fragments of smaller sizes for experiments and ultra structural analysis of immuno-reactivity.
The dry milling with **Minilys** could allow the revelation of different allergens patterns in the *Cupressus* pollen allergy and subsequently be applied to expand the panel of well-defined cypress pollen molecules for a more efficient allergen-based diagnosis and therapy.

03712-810-DU058

ANIMAL TISSUES

PLANT TISSUES

MICRO-ORGANISMS

PRECELLYS RANGE

INDEX

RNA EXTRACTION FROM *STREPTOMYCES (ACTINOBACTERIA)*

Lab. Microbial Metabolism, Shanghai Jiao Tong University, Shanghai, China

CONTEXT

Validamycin is a plant antibiotic that acts against plant fungus that can attack roots. It is effective in protecting seedling and clone cuttings against damping-off disease.
Our lab is focus on Functional Analysis of the Validamycin Biosynthetic Gene Cluster and Engineered Production of Validoxylamine A. This study compares the efficiency of total RNA isolation between Precellys sample preparation and enzymatic lysis.

MATERIAL

- Precellys®24
- Precellys® kit VK01
- Sample : *Streptomyces* (20mg of mycelium)
- Qiagen RNAeasy Mini Kit
- Buffer : Trizol or lysozyme (3mg/mL)

PROTOCOL

Precellys®24 sample preparation is compared with traditional method

1. Precellys®24
Parameters :6500rpm, 2x23 sec., 10 sec. break RNA extraction with trizol method

2. Enzymatic lysis
RNA extraction with Qiagen RNAeasy Mini Kit

Detection of bands by UV illumination

RESULTS

Total extracted RNA is visualized by ethidium bromide staining and UV Illumination. (figure 1)

The quantity of extracted RNA when using the Precellys®24 is almost twice the amount with enzymatic lysis.

ILLUSTRATIONS

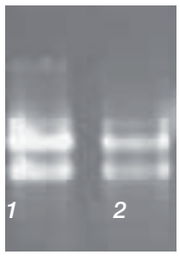
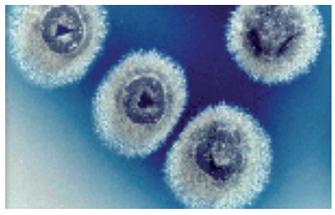


Figure 1: Total RNA extracted from Streptomyces
Lane 1 : total extracted RNA with Precellys®24 homogenization
Lane 2 : total extracted RNA with enzymatic lysis



Mycelium of Streptomyces

CONCLUSION

The quantity of extracted RNA is far higher when using **Precellys®24** than when using enzymatic lysis. In addition to time saving and easy handling, **Precellys®24** increases output of our laboratory activities.

03712-810-DU015

RNA EXTRACTION FROM CYANOBACTERIA WITH BEAD BEATING

Department of Photochemistry and Molecular Science, The Ångström Laboratories,
Uppsala University - Sweden

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 Cyanobacteria[1]).

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.

RESULTS

Yield and absorption ratios for the 3 different extraction methods were determined and shown in the figure 1.
RNA integrity is checked and shown in the figure 2.
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.

CUSTOMER

UPPSALA
UNIVERSITET

ILLUSTRATIONS

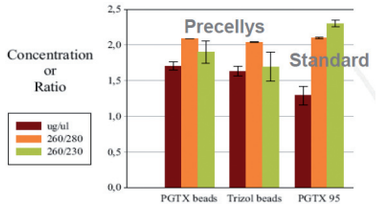


Figure 1: Extracted RNA yield and purity.

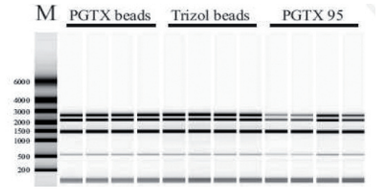


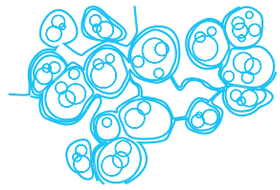
Figure 2: Gel image generated by the automated electrophoresis system.

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.

03712-810-DU023

ENZYMATIC ACTIVITY MEASUREMENTS IN *STREPTOCOCCUS THERMOPHILUS*



Department of Food Science and Microbiology (Di.S.T.A.M),
University of Milan - Italy

CONTEXT

Streptococcus thermophilus is a major component of dairy starter cultures used for the manufacture of yoghurt and cheese.
In this study, ß-galactosidase and lactate dehydrogenase activities were measured in *Streptococcus thermophilus* cells to evaluate the effect of carbon dioxide availability on the homolactic fermentation process[1].

MATERIAL

- Precellys®24
- Precellys® kit: 03961-1-004 (glass beads 0.5 mm)
- Sample: Concentrated *S. thermophilus* cells washed
- Buffer: 50mM tris/HCl buffer, pH7.

PROTOCOL

Precellys®24: 6800 rpm, 3 x 30 sec (30 sec break) in cold room or using Cryolys cooling option.

- Centrifugation: 15.000 xg, 10 min, +4°C.
- Analysis: Protein content within total cell extract by Bradford method / ß-galactosidase and lactate dehydrogenase activities measured spectrophotometrically[1]).

RESULTS

Our results originally show a direct correlation between the distribution of the HPr phosphorylated isoforms and the measured level of ß-galactosidase and lactate dehydrogenase activities (Fig. 1) and its relevance in milk acidification process.

ILLUSTRATIONS

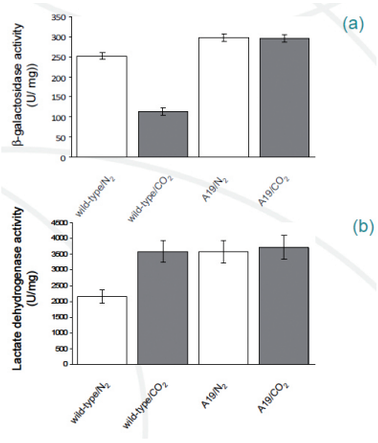


Figure 1: ß-galactosidase (a) and lactate dehydrogenase (b) activities of S. thermophilus wild-type cells (white bars) and A19(Δppc;ΔcarB) mutant cells (grey bars) grown in enriched CO2 or N2 atmosphere.

1) S.Arioli et al., Microbiology 155 (2009), 1953-1965; DOI 10.1099/mic.0.024737-0

CONCLUSION

Comparing to the former classical method of bacterial cell disruption, the use of **Precellys®24** allows a perfect homogenization of the samples. **Precellys®24** is easy to **use, simple, efficient** and much **faster** than the former method. The small dimension of **Precellys®24** allow its location in a cold room thus preserving the activities of the overall enzymatic cell machinery.
Precellys®24 brings us **efficiency, modernity, homogeneity** in the results and **timesaving**.

03712-810-DU028

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 genomewide 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 wholegenome high-resolution tiling array in the soildwelling a-proteobacterium *Rhizobium etli* [1].

MATERIAL

- Precellys®24homogenizer.
- 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.

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.

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.

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

CUSTOMER

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

ILLUSTRATIONS

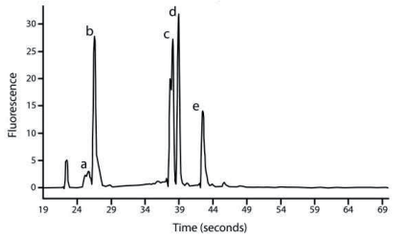


Figure 1: An example of high quality total RNA, illustrating the fragmentation 23S rRNA: (a) small RNA peak including 5S rRNA and the ncRNAs (b) 23S fragment of ~135 bp (c) two 23S fragments of ~1300 bp (d) 16S rRNA (e) intact 23S rRNA.

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.

03712-810-DU032

MECHANICAL DISRUPTION OF MYCOBACTERIAL CELL WALLS

Swiss Tropical and Public Health Institute, Univ. of Basel - Switzerland

CONTEXT

Genomic and proteomic research on mycobacterial diseases requires high quality and quantity preparation of DNA or proteins. Effective lysis of mycobacterial cells for DNA or protein extraction is demanding due to the mycobacteria's robust and waxy cell wall features. Also, many mycobacteria are slow growers, often resulting in small amounts of starting material from a culture. Commercially available kits are not usually applicable to research methods on mycobacterial genomics and proteomics [1] [2].

MATERIAL

- Precellys®24 homogenizer.
- Beads in 2mL tube: 0.1mm zirconia beads.
- Samples: 20 mg (wet wt) pellets of Mycobacteria obtained from cultures re-suspended in phosphatebuffered saline (PBS; pH 7.4), and heat inactivated at 95°C for 60 min.

PROTOCOL

Note that pathogenic mycobacteria must be processed under appropriate biosafety containment.

- Precellys®24: 6800 rpm, 3x30 sec
- DNA extraction: phenol-chloroform extraction and chloroform purification.

RESULTS

Several elements of published DNA extraction protocols were combined and tested to improve DNA yield. Mechanical disruption of the mycobacterial cell wall, after incubation with 4% SDS (final concentration), was found to be crucial for sufficient and satisfactory yields.
DNA yield achieved when testing both combinations of DNA extraction procedures (chemical lysis only vs chemical and mechanical disruption) are shown in the Figure 1.

[1] Kaser, et al. 2009. Optimized method for preparation of DNA from pathogenic and environmental mycobacteria. Appl.Environ.Microbiol. 75, no. 2 (January): 414-418.
[2] Kaser, et al. 2010. Optimized DNA Preparation from Mycobacteria. Cold Spring Harb Protoc 2010, no. 4 (April) :pdb.prot5408. doi:10.1101/pdb.prot5408.

CUSTOMER

Swiss TPH

ILLUSTRATIONS

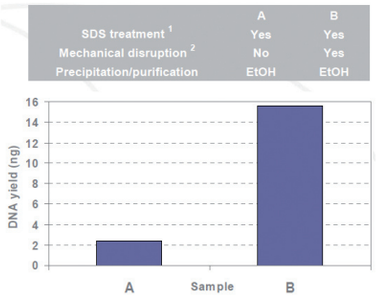


Figure 1. Twenty-milligram (wet weight) pellets of M. ulcerans strain IFIK1066089 were used. (Superscript 1) SDS was applied to a final concentration of 4%. (Superscript 2) Mechanical disruption performed with Precellys 24.

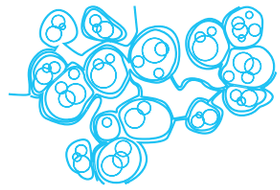
CONCLUSION

Small scale homogenization of samples containing mycobacteria with the **Precellys®24** allows to obtain large amounts of pure genomic DNA as compared to protocols with chemical lysis only.

The additional steps of mechanical disruption and handling are worth it to increase significantly DNA yield.

03712-810-DU034

METABOLITES EXTRACTION FROM *STAPHYLOCOCCUS AUREUS*



Institute of Pharmaceutical Biology, Univ. of Greifswald, Germany

CONTEXT

Staphylococcus aureus is a versatile human pathogen, which causes a wide range of diseases including wound infections, toxic shock syndrome (TSS). In combination with genomics, transcriptomics, and proteomics, the metabolomics approach promises to deliver a global view and a better understanding of regulatory systems, dynamics ranges and the control of metabolic pathways. In this work [1], the goal was to develop a protocol for the investigation of the *S.aureus* metabolome [1]. Microbial metabolomics, requires efficient and reliable methods for sample preparation thereby enabling the design of an optimal sampling protocol for the analysis of intracellular metabolites. For the cell wall disruption a glass beads (Precellys24) as well as a bead mill cell disruption method (Microdismembrator) was tested and compared [1].

MATERIAL

- Precellys®24 vs Micro-dismembrator.
- Precellys kit: 03961-1-005 (0.1mm glass beads).
- Competitor kit: 7mm bead (tungsten carbide).
- Sample: 1 mL of cell suspension from exponential growth phase.

PROTOCOL

- Fast vacuum dependent filtration to separate cells and medium.
- Precellys®24: 6800 rpm, 1-3 cycles x 30s vs competitor: 2 min at maximum speed.
- Extraction of metabolites using water or 60% (w/v) ethanol.
- Analysis of intracellular metabolites by GC/MS and LC/MS using standardized protocols.

RESULTS

For the gram-positive bacterium *S.aureus* a mechanical cell disruption is necessary, since the cell wall will not break up sufficiently using only an organic solution. The comparison of both mechanical disruption, using water as an extraction solution, showed that Precellys is more efficient [1].
In addition the Precellys method after metabolite extraction by 60% (w/v) ethanol was optimized by the addition of several cycles. Two cycles at 6800 rpm for 30s were adopted for the cell disruption part of the protocol for *S.aureus* metabolome analysis (Fig.1), since a higher number of cycles could not obtain higher metabolite concentrations

[1] H. Meyer et al, A protocol for the investigation of the intracellular Staphylococcus aureus metabolome, Analytical Biochemistry, volume 401, issue 2, pages 250-259

ILLUSTRATIONS

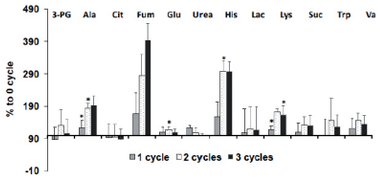


Figure 1: Cell disruption effectiveness dependent on the cycle number of the glass bead method (each cycle is 30s at 6800 rpm in a Precellys homogenizer) Percentage of relative metabolite concentration is shown for no cycle (defined as 100%) (X-axis) and compared to 1,2,3 cycles for each metabolite (Y-axis). Significant difference by two-tailed t test, P<0.1. Cells were harvested in exponential growth phase and metabolites were extracted with 60% (w/v) ethanol.

CONCLUSION

Microbial metabolomics, requires efficient and reliable methods for sample preparation including cell disruption step and an optimal sampling protocol for the analysis of intracellular metabolites. Metabolites extraction was optimized with a high Precellys speed and addition of several cycles.
Precellys®24 is easy to use, simple, more efficient, without cross-contamination.

03712-810-DU0052

MAINTAINING BACTERIAL VIABILITY DURING TISSUE HOMOGENIZATION

Illawarra Health and Medical Research Institute, University of Wollongong, Australia

CONTEXT

In an animal model of invasive infections caused by *Streptococcus pyogenes*, bacteria will colonize various tissues and organs. Bacterial loads present in these tissues is determined by homogenization, serial dilution and plating onto nutrient agar.
This study investigates the use of the Precellys 24 for tissue homogenization.

MATERIAL

- Precellys®24 homogenizer.
- Precellys kits: 03961-1-003 (1.4mm ceramic beads) and 03961-1-002 (2.8mm ceramic beads).
- Samples: skin (122.3 mg) and spleen tissues (38.6 mg).
- Bacteria culture: *S. pyogenes* was grown in Todd Hewitt Broth supplemented with 1% yeast extract at 37°C un til an A600 = 0.5.
- Buffer: 0.7% sterile saline (1000µl) inoculated with *S. pyogenes* (T0 Inoculum).

PROTOCOL

Note that pathogenic S. pyogenes must be processed under appropriate biosafety containment.

- Precellys®24 parameters:
  - Spleen: 6000 rpm, 1x30 sec,
  - Skin: 6000 rpm, 2x30 sec or 6000 rpm, 4x30 sec.
- Analysis: assessment of *S. pyogenes* concentration (CFU/ml) by cultural method.

RESULTS

Following tissue homogenization, the number of viable bacterial cells (CFU/ml) present was determined by serial dilution in sterile 0.7% saline and plating onto nutrient agar. Results (see Figure1) indicate that for all treatment groups the type of bead and the processing time had no significant effect on the viability of *S. pyogenes* (t test p> 0.1).

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University of Wollongong

ILLUSTRATIONS

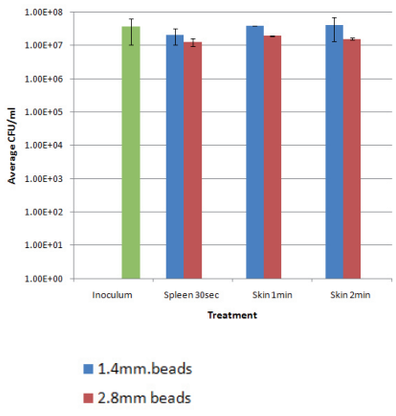


Figure 1: The effect of tissue homogenization on the viability of S. pyogenes. Data represents the mean CFU/ml from triplicate samples with error bars indicating the standard derivation. The inoculum represents the CFU/ml added to each sample.

CONCLUSION

Tissue homogenization (spleen & skin) using the **Precellys®24** with ceramics beads kits (1.4mm or 2.8mm) does not affect the viability of the Gram positive pathogen *S. pyogenes* present in these samples. The bacterial viability is maintained during tissue homogenization.
The potential to process up to 24 individual samples in a short time period with no risk of cross-contamination has considerable benefits over traditional homogenization methods.

03712-810-DU0053

PROTEIN EXTRACTION FROM CANDIDA ALBICANS AFTER INTERACTION WITH *STREPTOCOCCUS GORDONII*

Department of Oral and Dental Science, Univ. of Bristol - UK

CONTEXT

Human mucosal surfaces are colonized by diverse microbial communities. *Candida albicans* colonizes human mucosal surfaces and is a major systemic fungal pathogen. Biofilm formation and virulence are both linked to the ability to transition from the yeast (blastospore) growth form to the filamentous (hyphal) growth form.
The aims of this research were to better understand the interactions between the oral bacteria *Streptococcus gordonii* and the disease associated fungus *C. albicans*.
Specifically, we analyzed the effects of *S. gordonii* DL1 on activation of MAP kinases Cek1 and Mkc1 which impact *C. albicans* morphogenesis from blastospore to the filamentous hyphal form, and the role of H_2O_2 in this interaction[1].

MATERIAL

- Precellys®24 homogenizer.
- Precellys® kit: 03961-1-004 (0.5 mm glass beads).
- Sample: *C. albicans* cells treated with *S. gordonii* DL1 with or without H_2O_2 stimulation.
- Buffer: Lysis buffer Tris-HCl, pH 5, glycerol, TritonX100, SDS, NaCl, NaF, Sodium orthovanadate, glycerol phosphate, sodium pyrophosphate, EDTA, PMSF with 1x protease inhibitor cocktail (Sigma).

PROTOCOL

- Precellys®24: 5000 rpm, 4x30 s, 30 s breaks on ice.
- Centrifugation at 13,000 rpm at +4°C for 10 minute s.
- *C. albicans* cell wall samples were analyzed using SDS-PAGE and Western Blot techniques.

RESULTS

Co-incubation of *C. albicans* with *S. gordonii* DL1 cells led to activation of Cek1. The presence of S. gordonii cells also suppressed the H_2O_2-induced phosphorylation of Mkc1. Thus, the activities of these MAP kinases differentially respond to the presence of, or contact with, *Streptococcus bacteria* in the environment (Fig.1).
These results suggest that filamentation of *C. albicans* may be biochemically promoted by streptococci. In addition, in the presence of streptococci, H2O2 is unlikely to be the main cause of increased hyphal development.

1) C. V. Bamford et al, Infection and Immunity, Sept.2009, p3696-3704. doi:10.1128/IAI.00438-09

CUSTOMER

University of BRISTOL

ILLUSTRATIONS

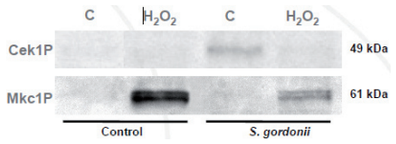


Fig. 1: Western immunoblot analysis of effects of 10mM H202 on phosphorylation of C. albicans MAP kinases after 20 min in the presence or absence of S. gordonii

CONCLUSION

These observations suggest that interactions between *C. albicans* and *S.gordonii* involve physical (adherence) and chemical (diffusible) signals that influence the development of biofilm communities. Thus, bacteria may play a significant role in modulating Candida carriage and infection processes in the oral cavity. **Precellys®24** provided a **simple** and **speedy** method of homogenizing the *C. albicans* cells to produce an array of cell wall samples which could **easily** and **quickly** be analysed at the **same time with confidence** that the samples were comparable.

03712-810-DU025

RNA EXTRACTION FROM MYCOTOXIGENIC FILAMENTOUS FUNGI

Applied Mycology Group, Cranfield Health, Cranfield University, Cranfield, Bedfordshire, UK

CONTEXT

Filamentous fungi cell wall and endogenous RNase activity often hindered the study of the **molecular mechanisms behind secondary metabolites. Toxigenic secondary metabolites** affect human health and represent a risk in the food chain system. The conditions in which **mycotoxins** are produced are still not completely understood especially the molecular mechanism that trigger their production.
The extraction of **high quality RNA in good amounts** was therefore critical to further evaluate the conditions in which mycotoxigenic gene clusters are activated.
Aspergillus flavus NRRL 3357 was used to evaluate the RNA extraction protocol. A manual grinding method using mortar and pestle was compared to different bead beating homogenizers using different beads [1].

MATERIAL

- Precellys®24 homogenizer vs Mortar and pestle.
- Precellys kits: CK14, CK28, VK01, VK05 (2mL vials).
- Sample: ~150 mg of mycelium of *A. flavus* NRRL 3357.
- Lysis buffer: 750 µL, either TRIzol or RLT buffer (provided with Qiagen RNeasy plant mini kit) supplemented with ß-mercaptoethanol.

PROTOCOL

- Precellys®24: 6500 rpm, 2x25 secs (5 sec break).
- The lysate was centrifuged at +4ºC for 5-10 minutes at 16000 g for an initial homogenization.
- RNA purification: Qiagen RNeasy plant mini kit, QIAcube® robotic workstation.

RESULTS

Significant differences were found between the total RNA amounts isolated using the Precellys 24 homogenizer and the manual system (p-value=0.0072). Furthermore the use of glass beads resulted in a yield around 3 times higher than using the traditional method of the mortar and pestle (Figure 1). The high quality of the RNA as also achieved as an example the electropherogram of a high quality RNA sample extracted using the 0.5 mm glass beads (Figure 2).

[1] G.M. Leite, N. Magan, A. Medina. 2012. Comparison of different bead-beating RNA extraction strategies: an optimized method for filamentous fungi. Journal of Microbiological Methods, 88, 413-418.

ILLUSTRATIONS

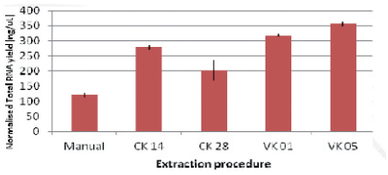


Figure 1: Total RNA yield average per 100 mg of initial biomass and standard deviation comparing different beads with the manual method. Key to treatments: CK – Zirconnium Oxide, VK – Glass; followed by the bead size code 01 – 0.1 mm, 05 – 0.5 mm, 14 – 1.4 mm, 28 – 2.8 mm.

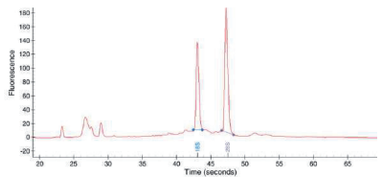


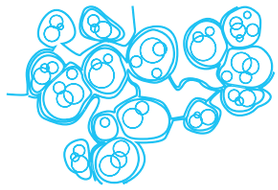
Figure 2: Electropherogram of a good quality RNA sample extracted using Precellys at room temperature. The RQI of this sample is 9.7.

CONCLUSION

The use of the **Precellys®24** with the prefilled **Precellys lysing kit VK05 significantly improved the concentration and the quality of the total RNA** extracted from **mycotoxigenic filamentous fungi** while reducing the required amount of mycelium.
The **ease of use** and the **time reduction** aided in the **molecular studies of mycotoxigenic filamentous fungi**.

03712-810-DU069

MAINTAINING YEAST VIABILITY DURING TISSUE HOMOGENIZATION WITH MINILYS



Lab. Microbiologie Fondamentale et Pathogénicité CNRS UMR- 5234 - Unité Candida et Pathogénicité, Bordeaux, France

CONTEXT

Fungi of medical interest in recent years have become primarily responsible for opportunist parasitic diseases, mainly because of the strong increase of immunodepressed patients. Fungal infections pose a challenge to health professionals who have to face variety of new emerging pathogens, but also manage the problem of resistance to antifungal treatments. The team focus its research on yeasts of medical interest belonging to the genus *Candida*.

MATERIAL

- Minilys homogenizer.
- Precellys kits: CK28R_2mL (03961-1-007) + 2CK50 (03961-1-106); CK28_7mL (03961-1-302) + 4CK50 (03961-1-106);
- Samples: Duplicate of whole tissues of mice (Lung, heart, liver, brain, kidney and spleen).
- Buffer: 1mL water in 2 mL vial or 2mL water in 7mL.
- Candida culture: 1.10^7 CFU; 100µL in 2mL vial or 200µL in 7 mL vial.

PROTOCOL

- Minilys setting: 5000 rpm.

	Lung	Heart	Liver	Brain	Kidney	Spleen
	[0,63g / 0,66g]	[0,110g / 0,02g]	[0,18g / 1,10g]	[0,35g/ 0,41g]	[0,43g/ 0,45g]	[0,33g/ 0,22g]
2mL	2x60s	2x30s				
7mL			2x15s	10s	30s	30s

- Analysis: Assessment *of Candida* concentration (CFU/mL) by cultural method after spreading 100µL of a 100 dilution in YPD plates.

RESULTS

Assessment of yeast concentration was performed on the homogenate samples (Results not shown).
Effect of homogenization on the viability of yeast cells was performed on a pure Candida culture at 1.10^7 CFU. Following Minilys homogenization, either performed in 7 mL or 2mL vial, 100 % of yeast cells survived after the maximum of time set independently the tissue. It's important to limit the temperature rise during long homogenization (>40sec.) by keeping the samples in ice during a few minutes between 40s cycles (Fig.1).

ILLUSTRATIONS

Effect on viability of yeast cells

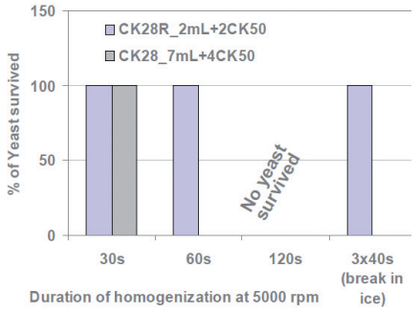


Figure 1: Effect of tissue homogenization on the viability of Candida. Data represents the % of yeast survived (CFU/ml) after the duration of homogenization specified.

CONCLUSION

Homogenization of whole tissues of mice (Lung, heart, liver, brain, kidney and spleen) using the **Minilys** with **Precellys lysing kits** does not affect the viability of the yeast pathogen Candida. The yeast viability is maintained during tissue homogenization up to 2 min limiting excessive temperature rise. The potential to process individual samples in a short time period with no risk of cross-contamination has considerable benefits over traditional homogenization methods.

03712-810-DU072

SAMPLE EXTRACTION FOR QUANTIFICATION OF MOULD ANTIGEN

Institut für Prävention und Arbeitsmedizin, Institut der Ruhr-Universität Bochum (IPA), Bürkle-de-la-Camp Platz, Bochum, Germany

CONTEXT

To assess exposure to mould antigens, **enzyme immunoassays for *Aspergillus fumigatus* and *Penicillium chrysogenum* antigens** had been developed. After different extraction procedures with Precellys homogenization or mixing only, the **antigen yields of airborne** dust samples were compared and one method was defined [1].

MATERIAL

- **Precellys 24 Dual & Cryolys cooling option (+4°C).**
- Precellys lysing kit: SK38_2ml (03961-1-006), SK38_7ml (03961-1-303)
- Samples: Parallel sampled Teflon filters with airborne dust from composting plants.
- Phosphate buffered saline with 0.05% Tween 20.

PROTOCOL

- Precellys 24 Dual: 6000 rpm, 3x20 sec, 30 sec break with cooling vs mixing only.
- Enzyme immunoassays: Aspergillus fumigatus and Penicillium chrysogenum based on polyclonal rabbit antibodies.

RESULTS

For extraction of airborne dust parallel sampled on 10 Teflon filters in composting plants, the SK38 - 6000 rpm homogenization procedures (mixed only, Precellys homogenization, with filter removed or not) were applied. With Precellys SK38 homogenization, *A. fumigatus and P. chrysogenum* antigen yields were higher than by mixing only irrespective of the mode of filter removal. Higher antigen amounts were obtained for both volumes of homogenization: 2mL (Fig. 1) or 7mL kit, with 1mL or 3mL of buffer, respectively.

[1] Sander I, Zahradnik E, van Kampen V, Kespohl S, Stubel H et al. (2012) Development and application of mold antigen-specific enzyme-linked immunosorbent assays (ELISA) to quantify airborne antigen exposure. J Toxicol Environ Health A 75: 1185-1193.

ILLUSTRATIONS

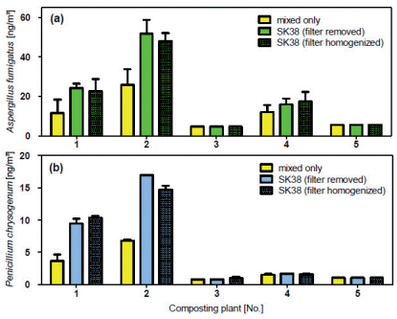


Figure 1: In 5 composting plants, airborne dust was sampled on Teflon filters with a Parallel sampler. The Aspergillus fumigatus (a) or Penicillium chrysogenum (b) antigen yields after extraction in 2 ml Precellys tubes were measured by enzyme immunoassays (mean of 2 filters per procedure).

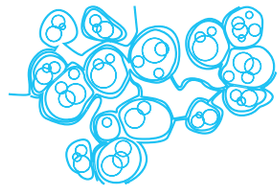
CONCLUSION

The antigen amounts of samples with fungal material were increased after extraction and homogenization with the **combo Precellys®24 Dual & Cryolys and SK38 kit.**

To **assess fungal antigen exposure of airborne dust,** this sample preparation using Precellys homogenization was the method of choice.

03712-810-DU075

DNA EXTRACTION FROM CPGV GRANULES (*BACULOVIRUS*)



Direction Generale de l'Armement, Centre d'Etudes du Bouchet - France

CONTEXT

Research and financial efforts spent on biodefense technologies highlight the current concern for biothreat event preparedness. Nonhazardous but relevant "simulant" microorganisms are typically used to simplify technological developments, testing, and staff training.
With this work, we investigated a new candidate, *Cydia pomonella granulovirus* (CpGV), for simulating large double-stranded DNA virus threat agents such as smallpox. CpGV belongs to the baculovirus family and is currently used as a pesticide. We developed an assay based on real-time PCR to provide a molecular tool to detect and quantify this model virus[1].

MATERIAL

- Precellys®24
- Precellys® kit: 03961-1-005 (glass beads 0.1 mm)
- Sample: occlusion bodies of CpGV named granules
- Buffer: 100 µl of a solution of yeast RNA 0.1mg/ml (to saturate the surface of glass beads)
- Centrifugal instrument
- Real-time PCR

PROTOCOL

- Precellys®24: 6500 rpm, 45sec.
- Centrifugation: 4,000 rpm, 1 min.
- DNA purification.
- Analysis: Real-time PCR assays.

RESULTS

We designed PCR primers and a probe for a specific gene that encodes a structural protein of CpGV. In a preliminary quantitative PCR (qPCR) assay, where no DNA standard was added, the success of this assay was assessed by checking with two different samples of CpGV DNA the presence of an amplicon with the expected size (59 bp) and showed in the figure 1.

The specificity of our qPCR assay against a large panel of potential cross-reactive microorganisms was checked, and the suitability of the assay for environmental samples, especially aerosol studies, was determined.
In conclusion, we suggest using *Cydia pomonella granulovirus* as a simulant of variola virus for biodefense technologies studies.

[1] L. Garnier et al., Applied and environmental microbiology, Mar. 2009, p. 1614–1620 Vol. 75, No. 6

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DGA

ILLUSTRATIONS

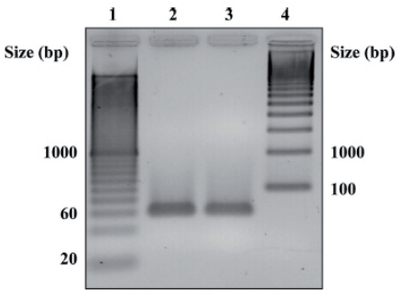


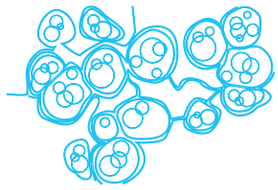
Figure 1: Agarose gel electrophoresis of amplicons produced by the qPCR assay. Lane 1, 20-bp ladder; lanes 2 and 3, PCR products from two different template CpGV DNA samples; lane 4, 100-bp ladder.

CONCLUSION

The use of **Precellys®24** allows the homogenization of the samples leading to efficient DNA extraction from compact microorganisms such as *Cydia pomonella granulovirus* (CpGV) granules. **Precellys®24** was easy to use, simple and fast.

03712-810-DU026

ENZYMATIC ACTIVITY MEASUREMENTS IN *STREPTOCOCCUS THERMOPHILUS*



Department of Food Science and Microbiology (Di.S.T.A.M),
University of Milan - Italy

CONTEXT

Streptococcus thermophilus is a major component of dairy starter cultures used for the manufacture of yoghurt and cheese.
In this study, ß-galactosidase and lactate dehydrogenase activities were measured in *Streptococcus thermophilus* cells to evaluate the effect of carbon dioxide availability on the homolactic fermentation process [1].

MATERIAL

- Precellys®24
- Precellys® kit: 03961-1-004 (glass beads 0.5 mm)
- Sample: Concentrated *S. thermophilus* cells washed
- Buffer: 50mM tris/HCl buffer, pH7.

PROTOCOL

- Precellys®24: 6800 rpm, 3 x 30 sec (30 sec break) in cold room or using Cryolys cooling option.
- Centrifugation: 15.000 xg, 10 min, +4°C.
- Analysis: Protein content within total cell extract by Bradford method / ß-galactosidase and lactate dehydrogenase activities measured spectrophotometrically[1].

RESULTS

Our results originally show a direct correlation between the distribution of the HPr phosphorylated isoforms and the measured level of ß-galactosidase and lactate dehydrogenase activities (Fig. 1) and its relevance in milk acidification process.

ILLUSTRATIONS

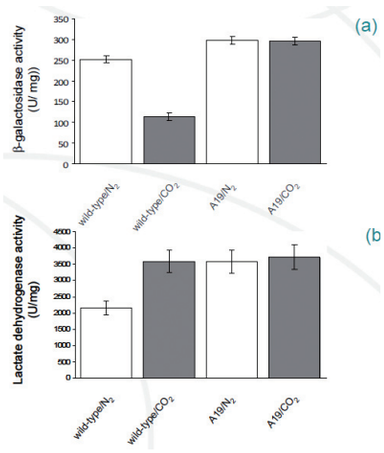


Figure 1: ß-galactosidase (a) and lactate dehydrogenase (b) activities of S. thermophilus wild-type cells (white bars) and A19(Δppc;ΔcarB) mutant cells (grey bars) grown in enriched CO2 or N2 atmosphere.

[1] S.Arioli et al., Microbiology 155 (2009), 1953-1965; DOI 10.1099/mic.0.024737-0

CONCLUSION

Comparing to the former classical method of bacterial cell disruption, the use of **Precellys®24** allows a perfect homogenization of the samples. Precellys®24 is easy to **use, simple, efficient** and much **faster** than the former method. The small dimension of Precellys®24 allow its location in a cold room thus preserving the activities of the overall enzymatic cell machinery.
Precellys®24 brings us **efficiency, modernity, homogeneity** in the results and **timesaving**.

03712-810-DU028

CHIKUNGUNYA VIRAL RNA AND VIRAL TITRATION IN TISSUE SAMPLES IN CYNOMOLGUS MACAQUES AS MODEL

CEA, Division of Immuno-Virology/Institute of Emerging Diseases and Innovative Therapies (iMETI), Fontenay-aux-Roses, France

CONTEXT

The *Chikungunya virus* (CHIKV) is a mosquito-borne alphavirus that induces in humans a disease characterized by fever, rash, and pain in muscles and joints. The aim of this study is to propose a new model for CHIKV infection in adult, immunocompetent cynomolgus macaques. CHIKV infection in these animals recapitulated the viral, clinical, and pathological features observed in human disease[1].

MATERIAL

- Precellys®24 homogenizer.
- Precellys® kit: 03961-1-002 (ceramic beads 2.8mm) for smooth tissues and 03961-1-001 (metals beads 2.8mm) for joint and muscle.
- Samples: Splenic, hepatic, muscular, and joint tissues from cynomolgus macaques inoculated with CHIKV (30 mg /~100mg).
- Buffer: 1 ml lysis buffer (Macherey Nagel) for Viral RNA extraction or 1 ml DMEM supplemented with 10% FCS for Viral titration

PROTOCOL

- Precellys®24: 5000 rpm, 2x20 sec or 2x10 sec
- Tissue viral RNA extraction: Nucleospin 96 RNA kit (Macherey Nagel), Relative quantitative RT-PCR simultaneously with CHIKV and GAPDH primers and probes.
- Determination of viral titers (TCID50/g): on mammalian BHK-21 cell lines based on their TCID50 using 4–5 replicates.

RESULTS

The figure 1 shows the infectious virus titers in spleen, liver, muscle, and joint collected from CHIKV-infected macaques, 6 and 44 dpi.

Our results provide insights into the pathogenesis of CHIKV. We have developed a relevant macaque model of CHIKV infection, in which we demonstrated long-term CHIKV persistence in various tissues and identified macrophages as cellular reservoirs during the late stages of CHIKV infection in vivo.

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ILLUSTRATIONS

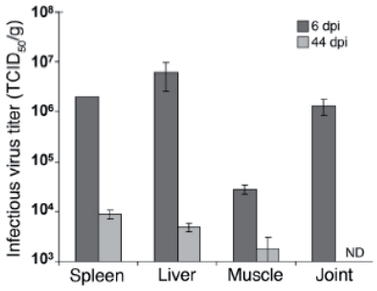

Figure 1: Tissues were collected at 6 dpi from macaques inoculated i.v. with 107 PFU CHIKV, or at 44 dpi from macaques inoculated i.v. with 106 PFU CHIKV, and the amount of infectious virus present in tissues was quantified by TCID50. Data are mean ± SEM of 2 independent virus titrations. The detection threshold was 700 TCID50/g.

[1] K.labadie et al, J Clin Invest. 2010;120(3):894– 906. doi:10.1172/JCI40104. http://www.jci.org

CONCLUSION

The **Precellys®24** is an ideal tool to evaluate both viral RNA and viral titers from various tissues of cynomolgus macaques inoculated with *Chikungunya* virus. **Precellys®24** is very easy to use, it is as simple as using a centrifuge. Comparing to the former method (mortar), we appreciate the cleanness and the cross-contamination free provided by consumables of **Precellys®24** lysing kit. The reproducibility given for various tissues of this study is also a strong advantage of this smart homogenizing equipment.

03712-810-DU033

RNA EXTRACTION FROM CHEESE IN 7ML VIAL

UMR782 Génie et Microbiologie des Procédés Alimentaires,
INRA Thiverval-Grignon, France

CONTEXT

In order to improve our understanding of the activity of the cheese microbial flora (bacteria, yeasts and molds) one promising approach is in situ analysis of the mRNA transcripts. However, even if extraction for DNA from cheese is common, the extraction of RNA is still a challenge. In this way, the Precellys®24-Dual was evaluated to increase the yield of RNA extraction from cheeses.

MATERIAL

- Precellys®24 homogenizer.
- Beads in 7mL kit (empty vial): Ref.03961-1-404
- 0.1 mm and 0.5 mm Zirconium beads.
- Samples: Ripened cheese.

PROTOCOL

- Precellys®24-Dual protocol : 500 mg of ripened cheese + 5 ml Trizol / 7mL vial + 1.6 g zirconium beads of 0.1 mm + 1.6 g zirconium beads of 0.5 mm, 6500 rpm 2x20 s (pause: 15 s).
- Subsequent RNA purification was done as described previously [1].
- Analysis: Bioanalyzer (Agilent).

RESULTS

The following electropherogram (Fig.1) shows the presence of yeast ribosomal RNA (18S and 25S) and bacteria ribosomal RNA (16S and 23S) extracted from ripened cheese after 1 month of aging. The yield is 75 µg of RNA per gram of ripened cheese.

The RNA quality and integrity are optimal to study the cheese microbial flora.

[1] Monnet C, Ulvé V, Sarthou A-S, Irlinger F. (2008) Extraction of RNA from cheese without prior separation of microbial cells. Appl Environ Microbiol 74: 5724-5730

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ILLUSTRATIONS

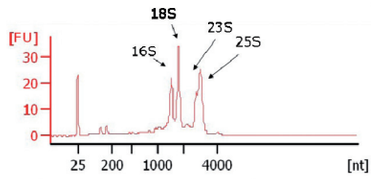

Fig.1: RNA quality assessment with the Agilent bioanalyser: electropherogram of RNA preparation from ripened cheese after 1 month of ageing.

CONCLUSION

Small scale homogenization of samples containing mycobacteria with the **Precellys®24** allows to obtain large amounts of pure genomic DNA as compared to protocols with chemical lysis only.

The additional steps of mechanical disruption and handling are worth it to increase significantly DNA yield.

03712-810-DU036

EXTRACTION OF RNA FROM VARIOUS *CAENORHABDITIS* SPECIES (NEMATODES)

College of Life and Environmental Sciences, School of Biosciences, Univ. of Birmingham, Birmingham, UK.

CONTEXT

Ageing, immunity and stress tolerance are inherent characteristics of all organisms. These traits in *Caenorhabditis elegans* have been shown to be regulated at least in part by the daf-16 gene. Through our research we aimed to establish a correlation between the expression of daf-16 and observed phenotypes (lifespan, immunity and stress tolerance) among four closely related Caenorhabditis species *(C. elegans, C. briggsae, C. remanei and C. brenerri).*
For such studies we required optimal concentration of high quality extracted RNA with minimal genomic DNA contamination. The added complication of using entire organisms for RNA extraction also meant that a very effective method be used to produce homogenized samples for RNA Isolation.

MATERIAL

- Precellys®24 homogenizer.
- Precellys® lysing kit: 03961-1-004 (0.5mm glass beads)
- Sample & buffer: Suspension of M9 buffer with *C.elegans* from a full 9cm petri dish.

PROTOCOL

- Animals (either mixed stage, or staged, depending on requirements) were washed off petri plates using M9 buffer, gently pelleted by centrifugation (1500rpm for 1min) and washed three times with M9 buffer before resuspending in 500µl of M9 buffer.
- Precellys®24: 6400rpm, 2x10secs twice for a total time of 40secs.
- Analysis: RNA was isolated directly from the extract using Qiagen RNeasy kit before being checked for quality and quantity using NanoDrop analysis.

RESULTS

Employing this methodology we were able to successfully isolate required amounts and quality of RNA from four species of the *Ceanorhabditis* genus at various stages of development. On average a yield of 1.5 – 2 µg/µl of RNA was obtained per 9cm petri plate. This RNA was then used for RT-PCR to quantify gene expression studies as shown in Figure 1.

[1] AMRIT, F. R., BOEHNISCH, C. M. & MAY, R. C. (2010) Phenotypic covariance of longevity, immunity and stress resistance in the Caenorhabditis nematodes. PLoS One, 5, e9978.

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ILLUSTRATIONS

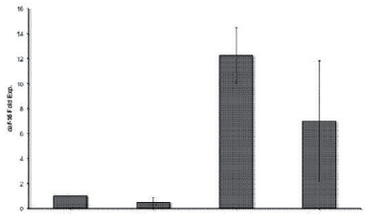


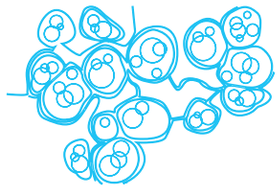
Figure 1: Expression levels of daf-16 (normalized to the reference gene gpd-3) among mixed populations - nematodes at various stages of development adapted from Amrit et al, 2010 [1].

CONCLUSION

The use of the **Precellys®24** to produce homogenized extracts for RNA isolation significantly improved the concentration and quality of extracted RNA as well as improving the speed of the isolation protocol.

03712-810-DU041

PROTEIN EXTRACTION FROM TOUGH MARINE PHYTOPLANKTON



Environmental Proteomics, Sackville, NB, Canada

CONTEXT

We extract total proteins from tough, recalcitrant marine phytoplankton for quantitative analyses of protein composition. We are seeking means to increase the throughput and reliability of our extractions. In this experiment, we compare our standard microprobe sonication/flash freeze protocol to extract total proteins from a single sample at a time, to the Precellys beadbased system to extract total proteins from multiple samples in parallel. [1]

MATERIAL

- Precellys®24 homogenizer.
- Precellys® kit: 03961-1-004 (0.5mm glass beads).
- Samples: culture pellets from *Ostreococcus tauri*, *Thalassiosira pseudonana*, *Synechococcus sp*. WH7803, *Prochlorococcus marinus* PCC 9511 and *Synechococcus sp*. WH8101 re-suspended in buffer.
- Buffer: Protein extraction buffer (Agrisera, Cat. AS08 300) supplemented with the protease inhibitor 4-(2- aminoethyl)-benzene-sulfonyl fluoride HCl (417µM).

PROTOCOL

- Precellys®24: 400µl of suspension in 2mL Precellys tube / 6500 rpm, 3x20 sec (5 sec break) with Cryolys.
- Sonication/flash freeze: 400µl of suspension flashfrozen in liquid nitrogen, thawed and sonicated for 15 sec (amplitude: 30%). This was repeated once.
- Total protein quantification, Immunoblotting with relevant antibodies.

RESULTS

The total yield of proteins extracted from each species was similar using the sonication/ flash freeze protocol or the Precellys® bead beating systems (Table 1). Simultaneous, parallel extractions with the Precellys® system mean sample throughput and reproducibility are higher than with our sonication/flash freeze protocol. The immunoblots show that the quality of the protein extractions was comparable for both a large, soluble protein (ribulose-1,5-bisphosphate carboxylase oxygenase; Figure 1) and for a highly hydrophobic membrane protein (PsbA, D1) (not shown).

[1] Comparison of protein extraction efficacy from phytoplankton using microprobe sonication/flash freeze and PreCellys bead beating protocols for immunoblotting. D.Campbell, A.Cockshutt and R.St-Onge.

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Environmental Proteomics
Protein Detection Services
Quantitative Tools for Photosynthesis, Aquaculture & Environmental Science

ILLUSTRATIONS

Organism	Concentration of proteins (µg µL^{-1})	
	Sonication/ flash freeze	PreCellys 0.5-mm glass beads
Ostreococcus tauri	1.20	1.05
Thalassiosira pseudonana	1.26	1.05
Synechococcus sp. WH7803	3.50	4.44
Prochlorococcus marinus PCC 9511	3.38	5.76
Synechococcus sp. WH8101	5.26	6.02

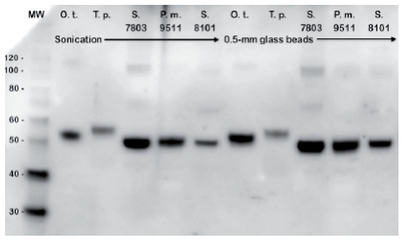

Figure 1: Comparison of ribulose-1,5-bisphosphate carboxylase oxygenase (RbcL) immunodetection from phytoplankton after protein extraction through sonication or Precellys bead beating. RbcL was detected by immunoblotting using a chicken anti-RbcL primary antibody and a horseradish peroxidase-conjugated goat anti-chicken secondary antibody. MW, molecular weight protein ladder (in kDa).

CONCLUSION

The **Precellys®** generated total protein extracts with yields and quality comparable to those of extracts obtained using probe sonication and flash freezing. The extractions with the **Precellys®** greatly increase throughput and improve reproducibility across samples.
Precellys® homogenizer is effective for the protein extraction from tough, recalcitrant marine phytoplankton.

03712-810-DU042

RECOVER LIVE HERPES VIRUS FROM INFECTED MOUSE GANGLIA WITH MINILYS

MVS, LID, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA
kwang@niaid.nih.gov

CONTEXT

The research focus of our laboratory is on human herpes virus pathogenesis and the development of new treatments and vaccines. We often need to test herpes simplex viruses (HSV) and their mutants in a mouse model to study the function of viral genes, evaluate antiviral therapies, and assess the efficacy of vaccine candidates. The titer of infectious HSV in the sensory nerve ganglion innervating the inoculation site is a crucial measurement for these studies.

MATERIAL

- Minilys homogenizer.
- Precellys® lysing kit: 03961-1-203 (CK14 - 0.5mL tubes).
- HSV-2 virus stock with known titer.
- Mouse trigeminal ganglia (TG) harvested three days after HSV-2 cornea infection.
- Medium HBSS with 1 % FBS

PROTOCOL

- Virus stock was diluted to desired concentration and 500 µl was transferred to a homogenizer tube.
- A fresh TG was placed in a homogenizer tube with 500 µl of medium.
- All above samples were kept on ice unless specified.
- Samples were homogenized at RT with settings as indicated (see Figures 1 and 2). Homogenization was paused every 20 seconds and tubes were held on ice for 1 minute then continue homogenization.
- Live virus in homogenates was detected by titration on a Vero cell monolayer in 6-well-plates following standard plaque assay protocol.

RESULTS

The mouse TG tissue required more than 3 k rpm x 45 sec or 4 k rpm x 20 sec to be adequately homogenized (results not shown).
Diluted virus stock (cell free virus) can withstand the homogenization up to 5 k rpm x 60 sec (Fig. 1).
Live virus can be recovered from HSV-2 infected mouse TG (Fig. 2).

ILLUSTRATIONS

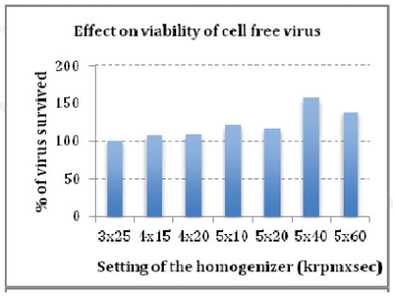

Figure 1

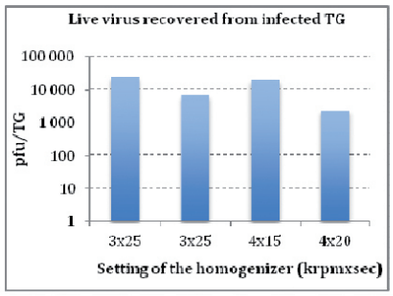

Figure 2

CONCLUSION

Live herpes simplex virus can be effectively recovered from infected mouse tissue with **Minilys** by homogenizing infected tissue in individual, tightly-closed tubes rather than in open tubes with a rotor-stator homogenizer.

This facilitates medium throughput analysis of tissue samples and avoids the possibility of cross-contamination between samples.

03712-810-DU065

METABOLOMIC STUDY OF THE RED ALGAE *CHONDRUS CRISPUS*

MetaboMER, Metabolomic Platform, FR2424 CNRS-UPMC,
Station Biologique de Roscoff, Roscoff, France.

CONTEXT

The goal of this study was to establish the best protocol of extraction of the whole metabolome of algae.
Seaweeds contain a lot of polysaccharides. For instance, in red algae, carrageenans confer a gelling nature to tissue, resulting in difficulties to obtain a reproducible manual grinding. Thus, particular attention has to be paid to grinding and automated techniques to carry out such extraction steps, as cryo-grinding, should be preferred. To avoid metabolite degradation, freeze-drying the sample before grinding is important. Thus, we tested automated cryo-grinding for the metabolite extraction of a red algae, *Chondrus crispus*, submitted to elicitation by methyl jasmonate (MeJA).

MATERIAL

- Precellys® 24 homogenizer with Cryolys cooling device to have a constant temperature of – 20 °C within the homogenization chamber using liquid nitrogen.
- Precellys lysing kit : 03961-1-008 (MK28-R).
- Sample: 3x3 replicates of MeJA elicited & control *Chondrus crispus*.
- Extraction solvent: 1 ml ice-cold [MeOH/H2O (8:2)] for 200mg fresh tissue (added after homogenization)

PROTOCOL

- Each lysing tube was added of 150 mg Fontainebleau sand.
- Precellys® 24: 1x30s 6800rpm - 1x50s 6000rpm - 1x30s 6800rpm - 1x90s 6200rpm (with 5min break between each run).
- Extraction was carried out at +4°C during 1hour with constant agitation. Centrifugation 3000g, 15 min.
- Samples were injected in UHPLC/MS on Dionex U3000 RSLC coupled to a LTQ-Orbitrap Discovery (Thermo, Les Ullis). Data treatment with XCMS and Mev.

RESULTS

Our results clearly demonstrate the elicitation of the red algae *Chondrus crispus* by MeJA (results not shown). Morever, the 9 different replicates display very reproducible metabolomic profiles (Figure 1), thanks to simultaneous grinding with Precellys 24. During this study, the Cryolys guaranteed a constant temperature of -20°C within the homogenization chamber and thus avoided an uncontrolled defrosting of the frozen samples.

CUSTOMER

CNRS UPMC
Station Biologique
Roscoff

ILLUSTRATIONS

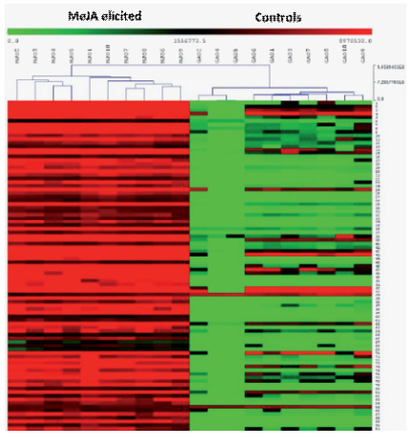

Figure 1: Hierarchical clustering of all detected ions from of control and MeJA elicited Chondrus crispus samples. Samples are in columns; each line represents a detected ion and its variations, from low amount (green) to higher amount (red). Here, only the 91 first ions are represented (2448 detected).

CONCLUSION

Compare to manual grinding, simultaneous cryo-grinding avoided biological material degradation and allowed higher reproducibility, faster grinding and better metabolite extraction.
Cryo-grinding with the combo **Precellys®24** and Cryolys is for our platform a daily necessary tool to perform robust and high-throughput metabolomic analysis.

03712-810-DU068

ANIMAL TISSUES

PLANT TISSUES

MICRO-ORGANISMS

PRECELLYS RANGE

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PRECELLYS RANGE

CHOOSE THE PRECELLYS SYTEM WHICH FITS WITH YOUR NEEDS!

EQUIPMENT	
Precellys®24	EQ03119-200-RD000.0
Precellys®24-Dual	EQ03119-200-RD010.0
Cryolys	EQ05068-200-RD000.0
Minilys	EQ06404-200-RD000.0

ACCESSORIES	
7ml rack	SP03961-810-NC001.0
Centrifuge adapter pack for 7ml tube	SP03961-810-NC002.0

GIVE YOUR PRECELLYS SYSTEM THE BEST LIFE TIME!

SPARE PARTS PRECELLYS®24 AND DUAL	
2mL White blocking plate	SP03119-810-NC002.0
2mL-Dual White blocking plate	SP03119-810-NC009.0
7mL-Dual White blocking plate	SP03119-810-NC010.0
User maintenance kit for Precellys 24	SP03119-810-NC011.0
User maintenance kit for Precellys 24-Dual	SP03119-810-NC012.0
Vacuum joint	SP03119-810-NC003.0
Containment seal	SP03119-810-NC004.0
Fuse	SP03119-810-NC005.0
Anti-rotation tubing kit	SP03119-810-NC006.0
Transport box	SP03119-870-NC005.0
Second year warranty P24	MS03712.900.WE001.0
Second year warranty P24-Dual	MS03712.900.WE002.0

SPARE PARTS FOR MINILYS	
Tube blocking rod with tool	SP06404-810-NC001.0
Adapter set pack (includes 2 x 2mL adapter set and 2 x 7mL adapter set)	SP06404-810-NC002.0
Microtube adapter set	SP06404-810-NC007.0

Precellys 24
for high throughput

Precellys 24 Dual
for flexibility

Minilys
for personal use

Cryolys
for sensitive samples

FIND THE PRECELLYS LYSING KIT WHICH FITS WITH YOUR SAMPLE / ANALYSIS!

0.5mL and 2mL Lysing kits	
Hard tissue grinding MK28	KT03961-1-001.2
Hard tissue homogenizing CK28	KT03961-1-002.2
Soft tissue homogenizing CK14	KT03961-1-003.2
Soft tissue homogenizing CK14_0.5mL	KT03961-1-203.05
Tough micro-organism lysing VK05	KT03961-1-004.2
Tough micro-organism lysing VK05_0.5mL	KT03961-1-204.05
Micro-organism lysing VK01	KT03961-1-005.2
Soil grinding SK38	KT03961-1-006.2
Tissue homogenizing CKMix	KT03961-1-009.2
Bacteria lysing CK01	KT03961-1-010.2

2mL Reinforced Lysing kits	
Hard tissue homogenizing CK28-R	KT03961-1-007.2
Hard tissue grinding MK28-R	KT03961-1-008.2
Tissue grinding CKMix50-R	KT03961-1-013.2
Dry hard tissue grinding CK68-R	KT03961-1-014.2

7mL Lysing kits	
Tissue homogenizing CK28_7mL	KT03961-1-302.7
Soil grinding SK38_7mL	KT03961-1-303.7
Micro-organism lysing VKMix_7mL	KT03961-1-304.7
Bacteria lysing CK01_7mL	KT03961-1-305.7
Tissue grinding CKMix50_7mL	KT03961-1-306.7
Soft tissue homogenizing CK14_7mL	KT03961-1-307.7

Empty tubes and caps	
Precellys 7mL empty tubes and caps (50)	KT03961-1-404.7
Empty 2mL reinforced tubes and caps (200)	KT03961-1-403.2

Beads in bulk	
2.8mm Stainless steel beads (500g)	KT03961-1-101.BK
2.8mm Zirconium oxide beads (325g)	KT03961-1-102.BK
1.4mm Zirconium oxide beads (325g)	KT03961-1-103.BK
0.5mm Glass beads (400g)	KT03961-1-104.BK
0.1mm Glass beads (400g)	KT03961-1-105.BK
5.8mm Zirconium oxide beads (100 beads)	KT03961-1-106.BK
6.8mm Zirconium oxide beads (100 beads)	KT03961-1-107.BK
0.1mm Zirconium oxide beads (450g)	KT03961-1-108.BK

Starter box	
Soft tissue Starter box included 10 tubes of following references: - Hard tissue homogenizing CK28 - Soft tissue homogenizing CK14 - Tissue homogenizing CKMix - Soft tissue homogenizing CK14_0.5mL	KT03961-2-002.SB
Hard tissue Starter box included 10 tubes of following references: - Hard tissue homogenizing CK28-R - Hard tissue grinding MK28-R - Tissue grinding CKMix50-R - Dry hard tissue grinding CK68-R	KT03961-2-003.SB
Large tissue Starter box included 10 tubes of following references: - Hard tissue homogenizing CK28_7mL - Soil grinding SK38_7mL - Tissue grinding CKMix50_7mL - Soft tissue homogenizing CK14_7mL	KT03961-2-004.SB
Micro-organism Starter box included 10 tubes of following references: - Tough micro-organism lysing VK05 - Micro-organism lysing VK01 - Bacteria lysing CK01 - Tough micro-organism lysing VK05_0.5mL * - Micro-organism lysing VKMix_ 7mL *	KT03961-2-005.SB

(included 5 tubes)*

FIND THE PRECELLYS LYSING KIT WHICH FITS WITH YOUR SAMPLE / ANALYSIS!

		LYSE				HOMOGENIZE			GRIND			
		VK01	CK01	VK05	SK38	CK14	CK28	CKMix	MK28R	CK28R	CK50	CK68
Microbial & alga	Algae			√	√	√	√	√	√	√	√	
	Bacteria (Gram +/-)	√	√	√	√							
	Cyanobacteria	√	√	√	√							
	Fungi			√	√							
	Molds			√	√							
	Phytoplankton			√	√							
	Spores	√	√	√	√							
	Viruses	√	√	√	√							
	Yeasts			√	√							
Animal & Human - Fish & insect	Adipose tissue					√	√	√		√		
	Artery					√	√	√		√		
	Bone						√		√	√	√	√
	Brain					√	√	√		√		
	Cartilage					√			√	√	√	
	Colon					√	√	√		√		
	Cornea					√	√	√	√	√	√	
	Ear					√	√	√		√		
	Feces				√	√	√	√		√		
	Fixed Samples					√	√	√		√		
	Hair						√		√	√	√	√
	Heart					√	√	√		√		
	Insects				√	√	√	√	√	√		
	Kidney					√	√	√		√		
	Larvae					√	√	√		√		
	Liver					√	√	√		√		
	Lung					√	√	√		√		
	Mammalian Cells					√		√				
	Muscle					√	√	√		√		
	Skin				√	√	√	√	√	√		
	Spleen					√	√	√		√		
	Tail					√	√	√	√	√		
	Tumors					√	√	√	√	√	√	
	Worm			√		√	√	√		√		
Plant & Soil	Chloroplasts	√	√	√	√							
	Coton						√		√	√	√	√
	Leaves				√	√	√	√		√		
	Mosses						√		√	√	√	
	Pine needles						√			√	√	√
	Plants cells					√		√				
	Roots	√	√	√	√		√			√	√	
	Sediments	√	√	√	√		√			√	√	
	Seeds				√		√		√	√	√	√
	Soils				√		√			√	√	
	Stems						√	√		√	√	√
	Wood						√		√	√	√	√

CONCLUSION

To view our complete sample application database,
see detailed protocols on our application center
or contact *precellys@bertin.fr* for scientific support.

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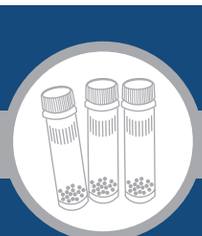

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