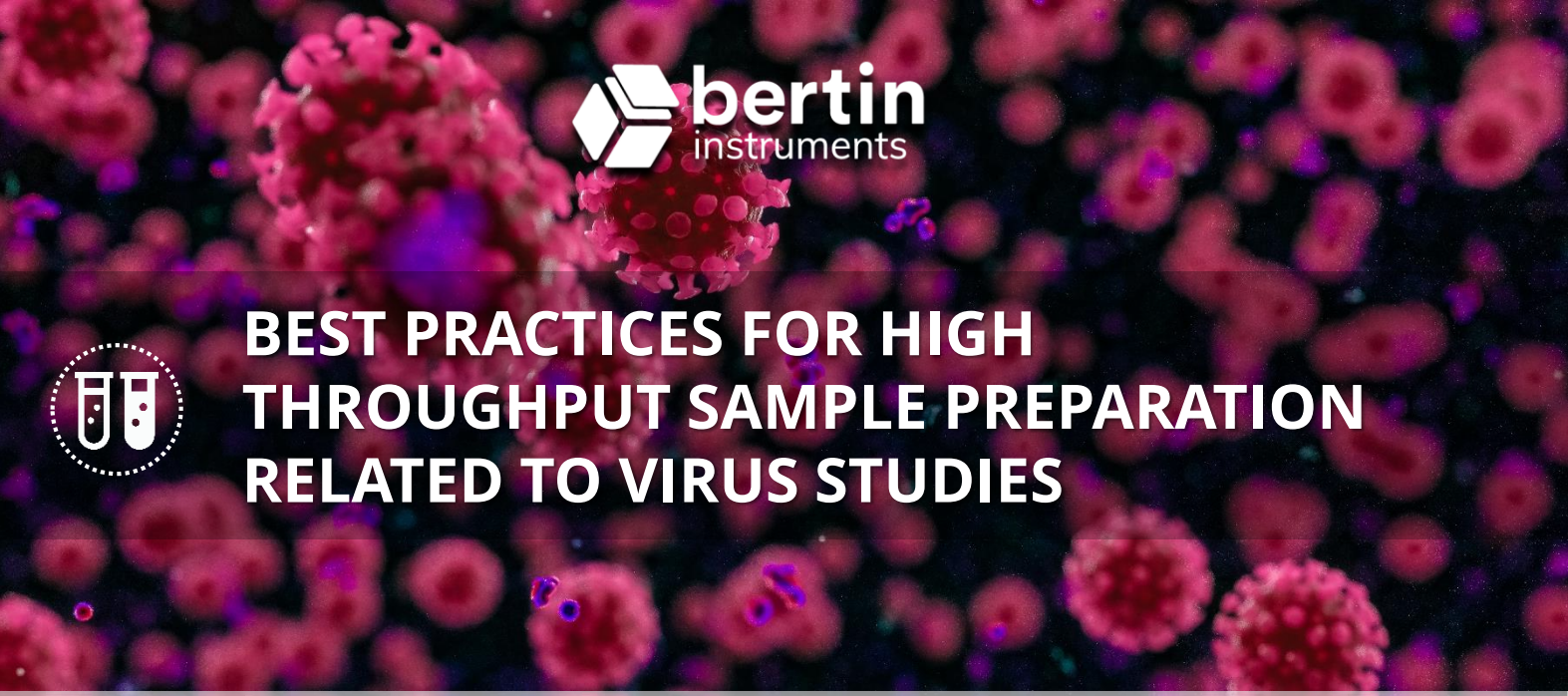

BEST PRACTICES FOR HIGH THROUGHPUT SAMPLE PREPARATION RELATED TO VIRUS STUDIES

Viruses are defined as submicroscopic, parasitic particles of genetic material contained in a protein envelope. To survive, viruses have to infect host cells and use their processes to replicate. The viral replication cycle can lead to dramatic biochemical and structural changes in the host cells, which may lead to cell damage. Most viruses that affect humans cause acute infections, with the development of diseases over a relatively short time period with rapid recovery (influenza, measles, varicella, Covid-19). On the other hand, some viruses lead to persistent infections, where the virus stays continually present in the body throughout the host life. In the case of HIV and HPV, the viral particles slowly increase in number over a long period of time after infection, eventually causing visible symptoms linked to severe immune depression or cancers. It has also been shown that viruses can affect the evolution of species by integrating partially or completely their host genome. For these reasons, **the study of viruses is of vital importance, both to increase our understanding of animal and vegetal life, and to learn how to counteract the dangerous effects of viral infections with vaccines and anti-viral treatments.**

Both anti-viral drug and vaccine development begin with high throughput screening phases. **Virus research workflows therefore need to be designed to be able to process high numbers of samples at the same time while maintaining virus integrity and limiting bias.** Homogenization is the first step of most viral extraction workflows. To obtain reproducible results, a proper homogenization of virus-infected samples is crucial. Mechanical lysis using beads (bead-beating) is the gold standard for standardized approaches to homogenization. **The Precellys tissue homogenizers are the ideal instruments to evaluate viral RNA and viral titer from various tissues thanks to their 3-D bead beating technology.** In this white paper, we present optimized protocols allowing for high throughput and reproducible virology analysis.

EXPLORE THE WORLD OF VIRUSES WITHOUT BIAS WITH THE PRECELLYS® PRODUCT RANGE

SUMMARY

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

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/ 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].

/ MATERIALS

- Precellys®24 homogenizer.
- Precellys® kit: P000911-LYSK0-A (ceramic beads 2.8mm) for smooth tissues and P000910-LYSK0-A (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.

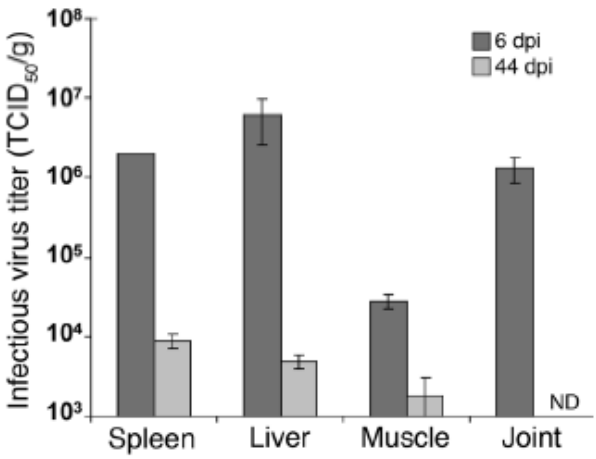

Figure 1. Tissues were collected at 6 dpi from macaques inoculated i.v. with 107 PFU CHIKV, or at 44 dpi from macaques inoculated i.v. with 106 PFU CHIKV, and the amount of infectious virus present in tissues was quantified by TCID50. Data are mean ± SEM of 2 independent virus titrations. The detection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

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.

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

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

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

/ CONTEXT

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

In this study, the quantitative effects of different steps of sample preparation for virome analysis were evaluated with qPCR and next-generation sequencing (NGS). Several procedures were tested for the homogenization, centrifugation, filtration, and random amplification steps, using a mock-virome (including 9 highly diverse viruses among which coronaviruses) and a bacterial mock-community. As a result, an optimized protocol for fecal sample preparation was created, the NetoVIR (Novel enrichment technique of VIRomes). The NetoVIR protocol makes use of the Minilys (Bertin Technologies, France) to efficiently and uniformly homogenize samples. Thanks to Minilys-powered homogenization and other optimized steps (Figure 1), the NetoVIR protocol can recover all viruses present in the mock-virome samples.

/ PROTOCOL

Homogenization :

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

Centrifugation and filtration:

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

Nuclease treatment and DNA/RNA extraction:

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

Random amplification

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

NGS sequencing

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

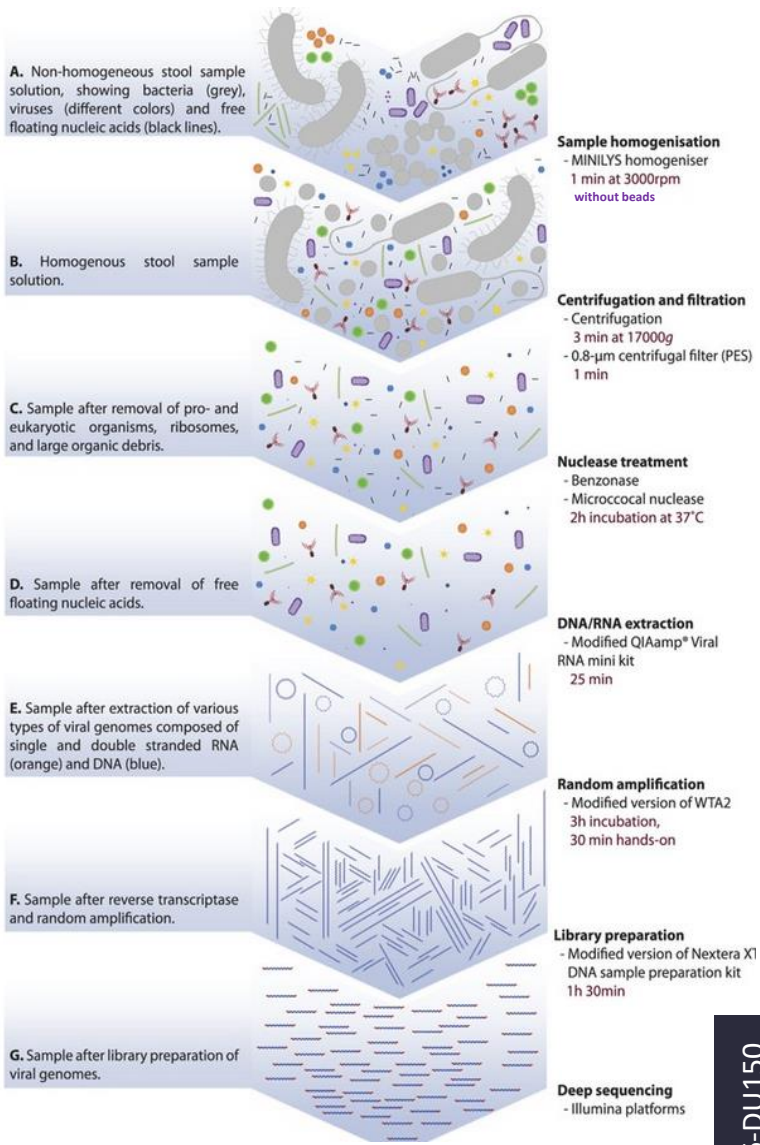

Figure 1: Description of the optimized NetoVIR protocol, On average, the protocol takes 8 h to complete. From [1]

PREC-026-DU150

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

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

/ RESULTS

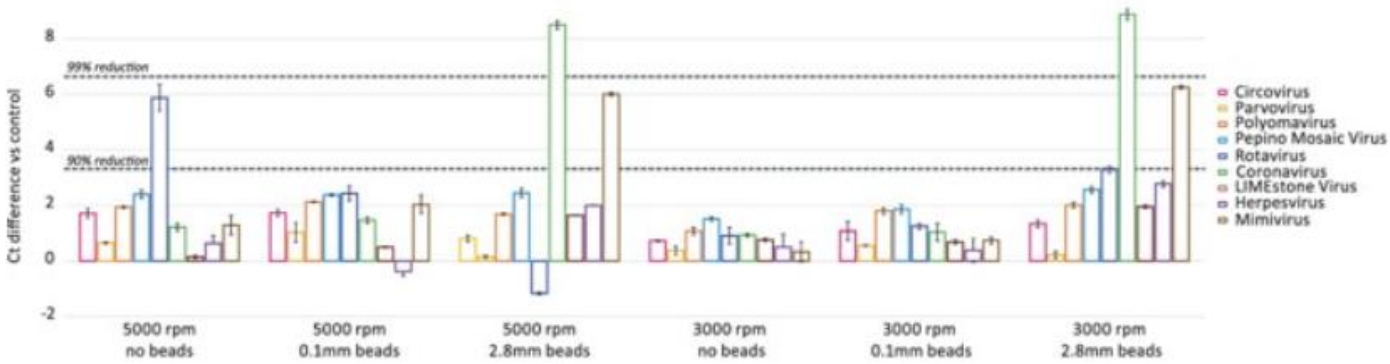

Figure 2: Ct differences vs control for different homogenization experiments performed on the mock-virome community. Standard deviations are based on three qPCR replicates. From [1]. Homogenization with Ø2.8 mm beads led to a destruction of viral particles irrespective of homogenization speed. The reduction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

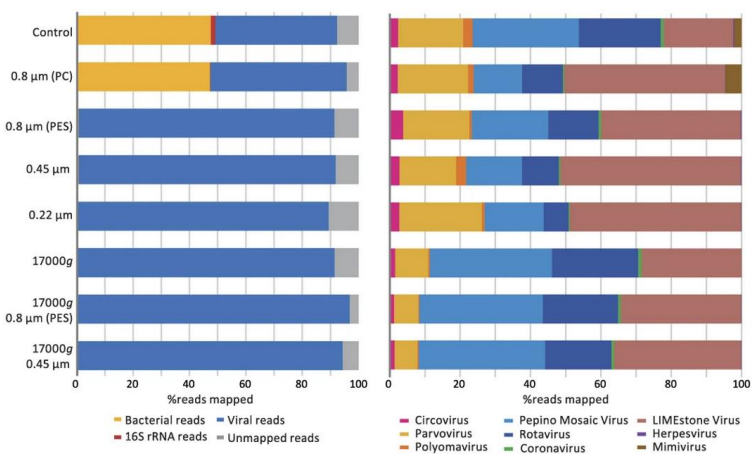

Figure 3: Left: NGS sequencing reads for sample consisting of pooled bacterial and viral communities: percentage of NGS sequencing reads for bacterial, 16S rRNA, viral and unmapped reads for the conditions tested, Right: Distribution of NGS sequencing reads for the mock-virome. From [1].
7 different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

/ CUSTOMER

KU LEUVEN

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

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

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

PREC-026-DU150

RECOVER LIVE HERPES VIRUS FROM INFECTED MOUSE GANGLIA WITH MINILYS

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/ 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.

/ MATERIALS

- Minilys homogenizer.
- Precellys lysing kit: P000912-LYSK0-A (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).

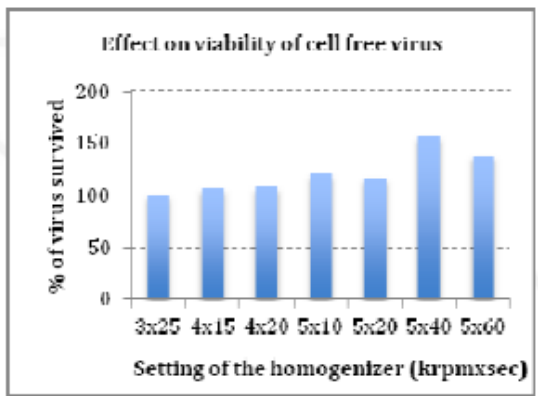


Figure 1: Effect of homogenization parameters on viability of cell free virus

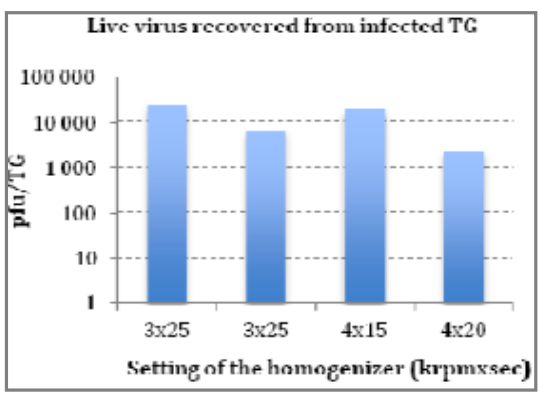


Figure 2: Effect of homogneization parameters on live virus collected from infected TG

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

VIRAL RNA EXTRACTION FROM INFECTED TISSUES

At Leuven University

/ 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.

/ MATERIALS

- Precellys®24
  or alternately Precellys Evolution + Cryolys Evolution
- 2mL Precellys® lysing kit CK28 (2,8mm ceramic beads; ref. P000911-LYSK0-A.0).
- Sample: 30-75mg of murine pancreas.
- Buffer: volume is adjusted in order to get a 5% w/v homogenate.

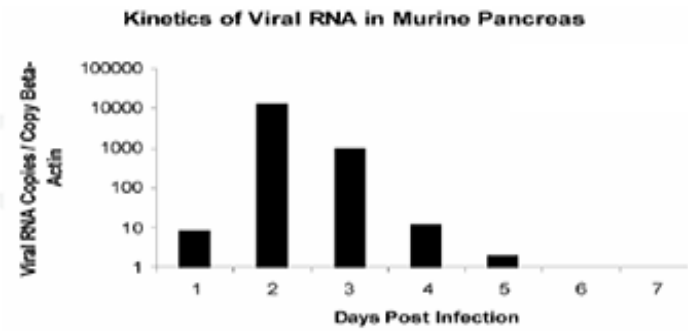
/ PROTOCOL

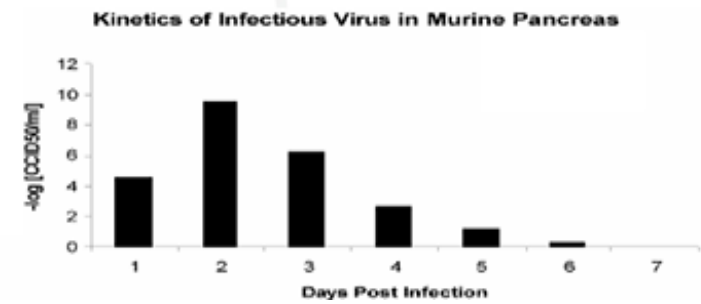
Precellys®24 parameters: 6500 rpm, 3x5 sec pause 10s
Alternately with Precellys Evolution and Cryolys Evolution: 7500rpm, 3 x 5s, pause: 10s at 4°C

/ CUSTOMER

/ 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).





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-B

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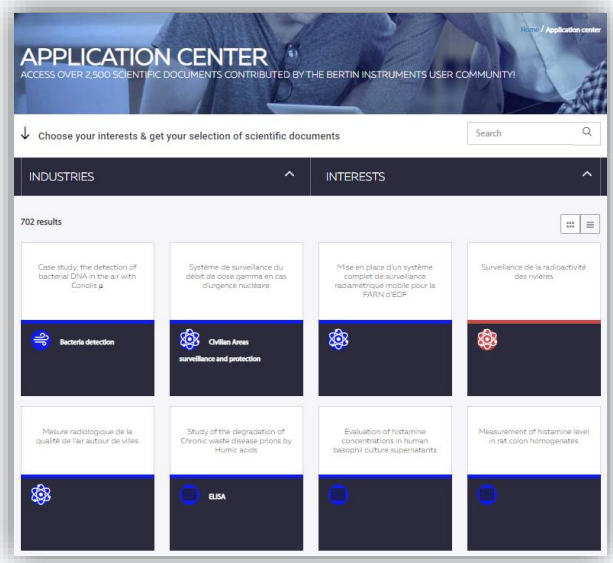

Precellys® Evolution is the most advanced homogenizer gathering high efficiency and versatility for all sample preparation needs:

- **Flexibility:** 24 x 2mL (or 0,5mL), 12 x 7mL, 6 x 15mL and 96 well-plate format
- **Efficiency:** up to 10 000 rpm speed to grind any type of sample
- **Integrity:** protect your molecules with the **Cryolys® Evolution** cooling unit

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