BEST PRACTICES FOR DNA SAMPLE PREPARATION WITH PRECELLYS HOMOGENIZERS

IMPROVE THE EXTRACTION YIELD OF DNA FROM YOUR BIOLOGICAL SAMPLES

DNA extraction is a method to purify DNA by using physical and/or chemical methods from a sample, separating DNA from cell membranes, proteins, and other cellular components. A large number of techniques have been developed since the first DNA extraction was carried out by Friedrich Miescher in 1869. Nonetheless, there are five basic steps of DNA extraction that are consistent across all the possible DNA purification chemistries: 1) cellular lyses, 2) separation of the soluble DNA from cell debris, 3) binding the DNA of interest to a purification matrix, 4) washing proteins and other contaminants away from the matrix and 5) elution of the DNA.

With today's requirements for DNA analyses by multiplex, real-time PCR and NGS, the importance of high-quality, purified DNA cannot be underestimated. Various factors, such as tissue type and DNA integrity, must be considered for choosing the appropriate DNA extraction method. Precellys homogenizers play a key role at the beginning of all the workflows requiring a biological sample homogenization for molecule extraction as a first step. In this White Paper, we have listed some tips and tricks, as well as some case studies of how to use your Precellys homogenizer to optimize the separation of the soluble DNA from your samples.

TIPS & TRICKS FOR DNA SAMPLE PREPARATION

- Make sure that your work surfaces and tools are free of Dnases.
- Pre-cut your samples into smaller pieces, if possible.
- Maintain your samples on ice as much as possible or use Bertin Cryolys cooling systems.
- Use a lysis buffer during the homogenization step in order to increase the yield of DNA. Make sure that the lysis buffer of your choice is compatible with your downstream application.
- Make sure that you have selected the optimal lysing kit and homogenization protocol for your sample type.
- Keep your homogenization cycles short. Give priority to increasing the number of cycles or speed, rather than the duration of each cycle.
- Centrifuge your samples at maximum speed for a few seconds after homogenization. This will facilitate the pipetting of the homogenate (without beads) to a clean tube for your downstream application.

SUMMARY

bertin INSTRUMENTS

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

Molecular Pathology Unit, Liverpool Clinical Laboratories , UK

/ CONTEXT

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

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

/ MATERIALS

Samples: 4mm punch biopsies of frozen specimens

Buffer: Proteinase K buffer

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

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

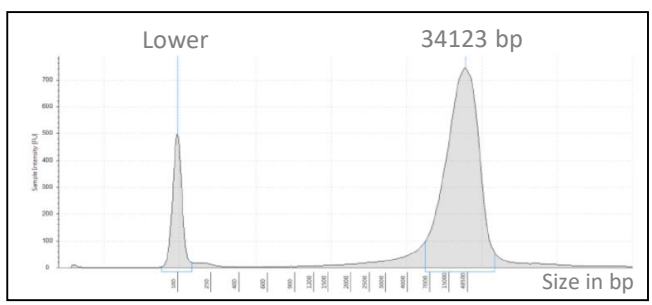
/ PROTOCOL

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

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

/ RESULTS

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



/ CUSTOMER

Liverpool Clinical Laboratories

Dr. Alistair Reid
Consultant Clinical Scientist

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

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

DNA EXTRACTION FROM ARABIDOPSIS LEAVES USING PRECELLYS EVOLUTION VS PRECELLYS 24-DUAL

PEQLAB, Erlangen, Germany

/ CONTEXT

The new Precellys Evolution is even more powerful (up to 10000 rpm) and versatile (0.5, 2.0, 7.0 and 15 ml tubes). Increased grinding power is beneficial for tough or stringy tissues. However, for soft tissues it is known that too much power can lead to degradation of target molecules like DNA.

Therefore the goal was to compare DNA quality (integrity) and quantity (yield) after homogenization of Arabidopsis leaves by Precellys® Evolution or Precellys24-Dual at given rpm.

/ MATERIALS

- Precellys Evolution and Precellys24-Dual
- Precellys lysing kit: CKMix_2mL (KT03961-1-009.2)
- Sample: 100 mg of *Arabidopsis* leaves per prep.
- Buffer: 100 µl TE (pH 8.0)

/ PROTOCOL

Precellys Evolution: 4600, 5900, 7200, 8200, 8800, 10000 rpm; 1 x 20 sec.

Precellys24-Dual: 4000, 5000, 6000, 6500 rpm; 1 x 20 sec.

DNA was isolated using peqGOLD Plant DNA Kit and analyzed by agarose gel electrophoresis for quantity (yield) and quality (integrity/degradation).

/ RESULTS

The gel picture obtained for Precellys Evolution (Fig. 1) shows that the yield and quality is comparable in the range of 4600 – 8800 rpm, whereas degradation was observed at 10000 rpm.

The gel picture obtained for Precellys24-Dual (Fig. 2) confirms that no degradation is observed up to 6500 rpm. Average yield was 2 µg DNA.

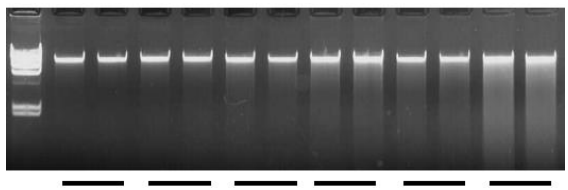


Figure 1: Agarose gel (1.5%, 1xTAE) electrophoresis of DNA isolated from *Arabidopsis* leaves homogenized with Precellys Evolution at given rpm. M = DNA sizer II

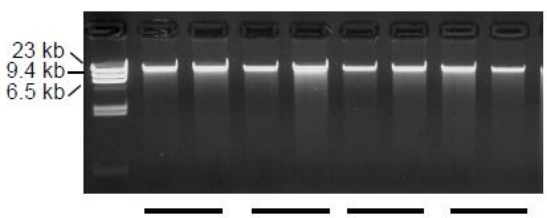


Figure 2: Agarose gel (1.5%, 1xTAE) electrophoresis of DNA isolated from *Arabidopsis* leaves homogenized with Precellys 24-Dual at given rpm. M = DNA sizer II

The homogenizer Precellys Evolution is suitable and convenient for DNA extraction from plant leave tissue. Yield and quality is independent of rpm in the range of 4600 up to 8800 rpm.

Precellys Evolution is an efficient high-throughput homogenizer that is a perfect tool for quickly generating high quality plant extracts for genomic studies.

03712-810-DU089

DNA HUMAN IDENTIFICATION FROM BONE FRAGMENTS

Instituto de Criminalística do Paraná; Universidade Positivo - Brazil

/ CONTEXT

Bone fragment is the most common sample used for DNA human identification from carbonized bodies or corpses, for the lack of any other organic material on these evidences. The scarce DNA in these evidences is the main reason why not always a conclusive result is achieved by traditional methodologies for DNA extraction and purification, such as phenol chloroform with Proteinase K digestion. The new methodology based on Precellys 24 is a new alternative to achieve conclusive results on DNA extraction and purification, in cases where the sample does not have enough organic material.

/ MATERIALS

- Precellys 24 vs conventional method (CM)
- Precellys kit: 03961-1-002(ceramic beads 2.8mm)
- Sample: ~200 mg of bones fragments first grinded in liquid nitrogen on a freezer mill 6750 (Spex, Certiprep)
- Buffer: 400 µl extraction buffer

/ PROTOCOL

Precellys 24: 5900 rpm, 2x30sec, 15s break.

Conventional method: overnight incubation at +56°C with 15µl of Proteinase K.

Phenol-chloroform extraction and DNA purification on Microcon columns.

DNA analysis tests: amplification by PCR (GeneAmp System), visualization on ABI PRISM 310 analyzer.

/ RESULTS

Seven samples were analyzed from bone fragments, from carbonized bodies and corpses. In all cases, DNA extraction by conventional protocol did not give a good result (15 autosomic loci). In these cases, all results were considered inconclusive. The genetic profile was achieved in six out of seven inconclusive tests, giving a genetic profile positive result of 85,7% of the samples processed on Precellys 24 (Tab.1). Only one case was not concluded, due to the high level of sample degradation. When comparing the traditional methodology, this new method with Precellys 24 is faster, more feasible, reliable and more economical. The vacuum system provides security and stability to the samples. The single tubes avoid the risk of cross-contamination.

Case	Type of sample	Genetic relatedness	CM result	P-24 result
01	BF (Drowned)	Brotherhood	Inc.	Conc. (-2 loci)
02	BF (exhumation)	---------	Inc.	Conc. (-2 loci)
03	BF	Object with blood	Inc.	Conc. (-2 loci)
04	BF	Motherhood	Inc.	Inc.
05	BF	Motherhood	Inc.	Conc. (-2 loci)
06	BF	Motherhood	Inc.	Conc. (No loci loss)
07	BF (Cranium)	Fatherhood	Inc.	Conc. (-1 loci)

Tab.1: Result for each samples studied. CM= Conventional Method, P24= Precellys 24 method, BF= bone fragment, Inc= inconclusive result, Conc.= 1) Daphne Manuela Toledo, Hemerson Bertassoni conclusive result

/ CUSTOMER

UNIVERSIDADE POSITIVO

Precellys 24 gave an optimum result, more efficient than the standard extraction protocol on a reduced amount of time, and with a higher yield and the recovery of genetic loci that could not be identified before. Brazilian forensic laboratories can now rely on a unique technique on Precellys 24, and this equipment became a good alternative to DNA extraction from bones in forensic routines.

03712-810-DU024

DNA EXTRACTION FROM ANIMAL TISSUE USING THE PRECELLYS TISSUE DNA EXTRACTION KIT

Bertin Bioreagent, Montigny-Le-Bretonneux, France

/ CONTEXT

DNA extraction from animal tissues and cultured cells is one of the most common techniques used in molecular biology laboratories. It is a critical step for biomolecular and genomic applications.

High quality and high yield DNA can be obtained from any type of tissue sample thanks to an optimal extraction process that relies on 2 steps: tissue homogenization and DNA purification. Finding the appropriate protocols for each one of these steps, and combining them to obtain the best out of any tissue sample is a mandatory milestone for most laboratories.

With its wide range of tissue homogenizers, Bertin Instruments addresses the first challenge of tissue disruption by using a powerful 3D bead-beating technology under the Precellys brand. Bertin Bioreagent has thus developed a DNA extraction kit to complete the sample prep workflow resulting in the Precellys Tissue DNA Extraction Kit, a solution for all of your genomic applications.

/ MATERIALS

- Precellys Tissue DNA Extraction Kit (Cat No. D05701)
- Precellys 24 Touch (Cat No. P002391-P24T0-A.0)
- Precellys Lysing Kit CK14 (Cat No. P000912-LYSK0-A)
- Precellys Lysing Kit CK28-R (Cat No. P000916-LYSK0-A)
- 9 mouse heart and liver samples (25 mg/ sample)
- DNA extraction kit Competitor Q
- DNA extraction kit Competitor M
- Ethanol (100 %)
- Isopropanol (100 %)

/ PROTOCOL

Tissue Homogenization & Lysis

1. Add sample to the lysing kit tube (Lysing kit CK14 for liver samples; Lysing kit CK28-R for heart samples)
2. Add 200 µl Tissue Lysis Buffer and 25 µl Proteinase K. Vortex
3. Homogenize samples (Precellys 24 Touch - Liver samples: 6000 rpm for 1 x 10 sec; Heart samples: 6500 rpm for 3 x 10 sec)
4. Incubate at 55 °C in heat block for 1 hour
5. Centrifuge (≥10,000 x g) for 5 minutes
6. Transfer the supernatant to a sterile 1,5 ml microcentrifuge tube

Bind → Wash → Elution, following the instructions from the Precellys Tissue DNA Extraction Kit user guide.

DNA Quantification and Quality Control

Quantification and quality analysis of DNA by NanoDrop qPCR on housekeeping genes (GAPDH for liver and B2M for heart samples).

DNA EXTRACTION FROM ANIMAL TISSUE USING THE PRECELLYS TISSUE DNA EXTRACTION KIT

Bertin Bioreagent, Montigny-Le-Bretonneux, France

/ RESULTS

Mean DNA concentrations were 2693.27 ± 157 ng of DNA/mg of tissue and 2924.16 ± 219,36 ng of DNA/mg of tissue for mouse heart and liver samples, respectively. DNA yield was significantly higher with the Precellys Tissue DNA Extraction Kit, compared to the competitors.

Table 1.- DNA concentrations (ng of DNA/mg of tissue) of mouse heart and liver samples obtained with the Precellys Tissue DNA Extraction Kit (Bertin Bioreagent).

Sample	DNA (ng/mg)	260/280	260/230
Liver 1	2827,60	2,03	2,02
Liver 2	2717,12	2,06	2,11
Liver 3	3227,76	2,02	2,04
Heart 1	2722,00	2	2,16
Heart 2	2870,00	1,99	1,91
Heart 3	2487,80	2,02	2,12

Mean ΔCq value obtained for liver samples was 3.32 with a SD = 0.02 (Figure 2A), between 10-fold dilutions. The mean ΔCq value obtained for heart samples was 3.30, with a SD = 0.4, between 10-fold dilutions (Figure 2B).

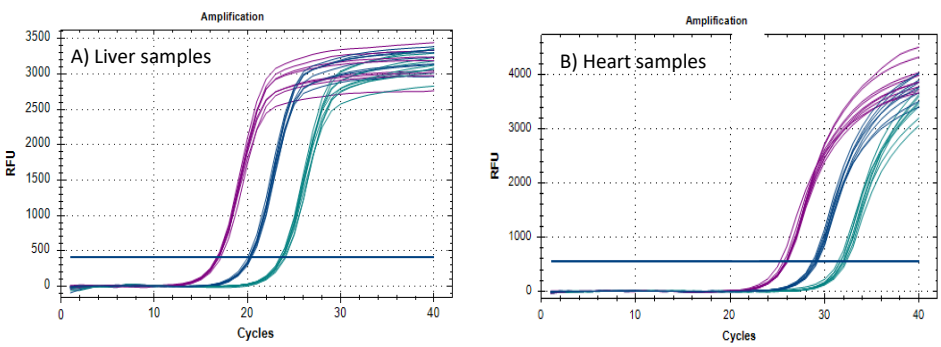


Figure 2.- qPCR results obtained for housekeeping genes (GAPDH for liver and B2M for heart samples) after DNA extraction using the Precellys Tissue DNA Extraction Kit (Bertin Bioreagent).

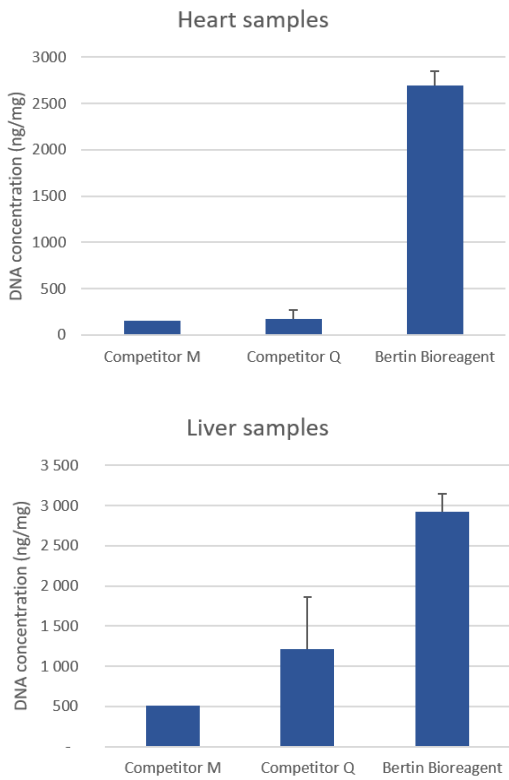


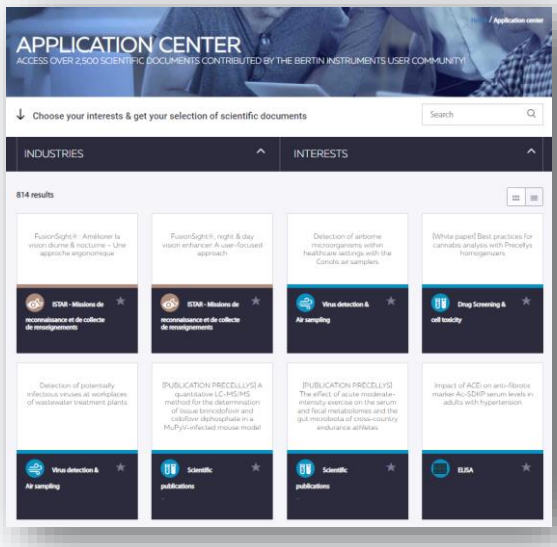
Figure 1.- DNA concentrations (ng of DNA/mg of tissue) of mouse heart and liver samples obtained with the Precellys Tissue DNA Extraction Kit (Bertin Bioreagent), competitor M and Competitor Q.

The Precellys Tissue DNA Extraction Kit from Bertin Bioreagent allows to extract high quality DNA with a very high yield from diverse animal tissues. In conjunction with the power of the 3D bead-beating technology of the Precellys homogenizers, a complete solution is now available for the sample preparation workflow in biomolecular laboratories.

03712-810-DU024

Use the Precellys® Application Center to find the appropriate protocol for your application & optimize it with users feedback!

- **Find scientific documents**
- **Find the appropriate kit**
- **Share with the Precellys® community**



Precellys® Evolution & Cryolys Evolution, the most advanced homogenizer solution 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

PLUS D'INFOS

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OZYME
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