Precellys
The Science of Lysing

BEST PRACTICES IN MOUSE TISSUE SAMPLE PREPARATION FOR RNA EXTRACTION WITH PRECELLYS® EVOLUTION

The lab mouse is the most commonly used mammalian model system for genetic research. Scientists from a wide range of biomedical fields have used the mouse because of its close genetic and physiological similarities to humans, as well as the ease with which its genome can be manipulated and analyzed. Sample preparation is a critical step before proceeding to molecular down-stream analysis. Especially for thermo-sensitive molecules such as RNA. The Cryolys® Evolution cooling unit combined with Precellys® Evolution can be used to maintain a constant low temperature of 4°C within the Precellys sample processing chamber.

IMPROVE EXTRACTION YIELDS FROM MOUSE TISSUE USING THE PRECELLYS® EVOLUTION

SUMMARY

TOTAL RNA EXTRACTION FROM MOUSE KIDNEY BEFORE RNA SEQUENCING

Cell Biology and Modeling Laboratory UMR5239

/ CONTEXT

Gene expression levels in an organ are only reliably estimated if extraction is performed from the whole organ (as smaller parts may not be representative of the whole organ). We seek to extract high quality total RNA from a whole mouse kidney to perform RNA sequencing. The purpose of this application note is to establish the best protocol for RNA extraction and demonstrate that the Precellys® 24 provides a very efficient extraction from either a half or a whole mouse kidney. We compare the RNA quality and integrity after homogenization.

/ MATERIALS

- Precellys® 24
- Precellys® Lysing Kit Soft tissue homogenizing CK14 (KT03961-1-003.2) ; 2ml tubes
- Sample
  - Whole mouse Kidney (~200 mg): 1 sample
  - Whole mouse kidney cut in half (~100 mg): 2 samples
- Buffer Cold Trizol (MRCgene # RT 118) 1ml for all conditions

/ PROTOCOL

Fresh kidneys were cut into pieces (max. 2mm large) in a petridish filled with RNA later and then stored according to manufacturer instructions.

For extraction, small pieces were transferred to 1ml of trizol with forceps, minimizing the quantity of RNA later introduced. They were immediately homogenized with the following set up: 5500 rpm, 20s x 2, 20s

Following homogenization, the homogenate was transferred to a new 2ml tube and beads were washed with 200ul of trizol. The resulting 1.2 ml were submitted to a standard trizol extraction.

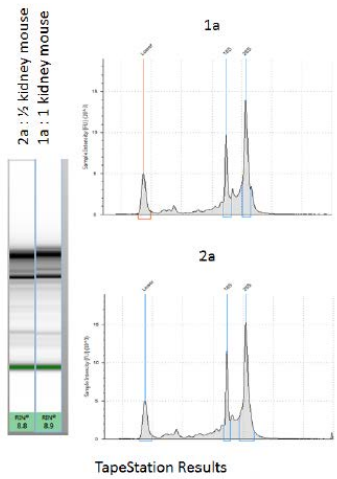
Analysis

Sample purity and RNA integrity were checked respectively with DropSense (Trinean) and TapeStation (Agilent).

/ RESULTS

TapeStation

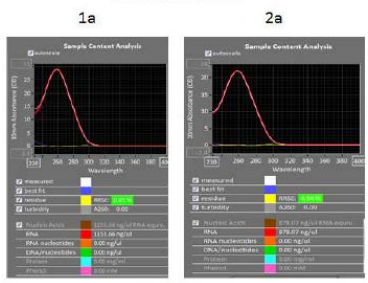
High quality RNA (RIN>8.8) was obtained for all samples



TapeStation Results

DropSense

The Dropsense analysis showed that there was no detectable phenol, protein or DNA in our samples, for both conditions (0ng/ul). As expected, RNA concentration was about twice higher for the 200mg sample as compared to the 100mg sample.



DropSense Results

/ CUSTOMER

/ CONCLUSION

There was no obvious differences in terms of lysis efficiency, sample purity and RNA integrity between the 2 conditions (100 mg or 200 mg of kidney tissue). It is possible to lyse 200mg of kidney tissue with high efficiency. Thanks to the Precellys® 24 and the dedicated lysing kit, we obtain a high quality RNA which is mandatory to perform RNA sequencing.

IMPROVING RNA EXTRACTION FROM MOUSE TISSUES USING THE PRECELLYS® 24

Pharmaceutical Company, San Francisco, California

/ CONTEXT

The time spent on sample processing is often time consuming and arduous when using traditional methods, particularly for large scale animal studies. In our research, mice organs are dissected after small molecule treatment for RNA extraction, followed by cDNA synthesis and gene expression analysis using real time PCR. Our current pulverization method results in poor gene expression results due to RNA degradation during sample extraction. We aim to improve the quality of RNA yield using the Precellys®24, a high throughput sample homogenizer.

/ MATERIALS

- Precellys®24 Homogenizer
- Precellys lysing kits: CK14 2mL (KT03961-1-003.2) for heart, liver and CKMix 2mL (KT03961-1-009.2) for spleen
- Samples: Heart, liver, spleen (10-30mg)
- Buffer: 1mL of Trizol reagent

/ PROTOCOL

- Precellys®24: 5000 rpm, 1x30 sec

- Pulverization: Mortar and pestle, followed by homogenization using a needle and syringe in Trizol.

- Methods: Mouse tissues were removed and snap frozen in liquid nitrogen or dropped into RNAlater prior to homogenization.

/ RESULTS

RNA integrity and yield was evaluated using an Agilent Bioanalyzer, and an average RIN (RNA integrity number) value of 9 (highest score is 10) was observed using the Precellys, with RNA concentration falling between 400-1800 ng/ul. The gel displays clean 18S and 28S rRNA bands with little or no degradation (Figure 1A). RIN values of "N/A" were observed after pulverization, which is indicative of highly degraded RNA as observed on the gel (Figure 1B).

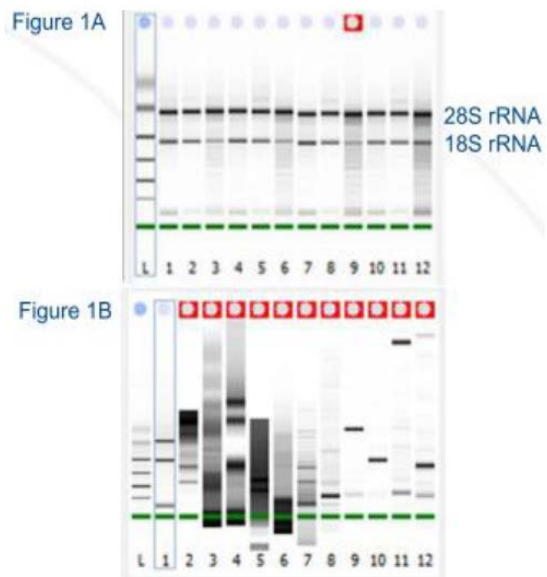


Figure 1. RNA integrity after sample homogenization on the Precellys®24 (A) or using pulverization (B).

/ CONCLUSION

There was no obvious differences in terms of lysis efficiency, sample purity and RNA integrity between the 2 conditions (100 mg or 200 mg of kidney tissue). It is possible to lyse 200mg of kidney tissue with high efficiency. Thanks to the Precellys® 24 and the dedicated lysing kit, we obtain a high quality RNA which is mandatory to perform RNA sequencing.

SPECIFIC PROTOCOL FOR RNA EXTRACTION FROM MOUSE TISSUE

/ RNA EXTRACTION FOR MOUSE EMBRYO (13,5 DAYS)

- SAMPLE TYPE: Animal
- TARGETED MOLECULE£KITS: RNA Extraction
- KITS: CK14_2mL
- QUANTITY: 1
- BUFFER: Lysis Buffer 0,5mL
- PROTOCOL: 5000 rpm – 3 x 30s (30s break)
- INSTRUMENT: Minilys®

/ RNA EXTRACTION FOR MOUSE BONE

- SAMPLE TYPE: Animal
- TARGETED MOLECULE£KITS: RNA Extraction
- KITS: CKMix50_2mL
- QUANTITY: ~1cm
- BUFFER: Trizol 1mL
- PROTOCOL: 6500 rpm – 2 x 20s
- INSTRUMENT: Precellys® 24 + Cryolys®

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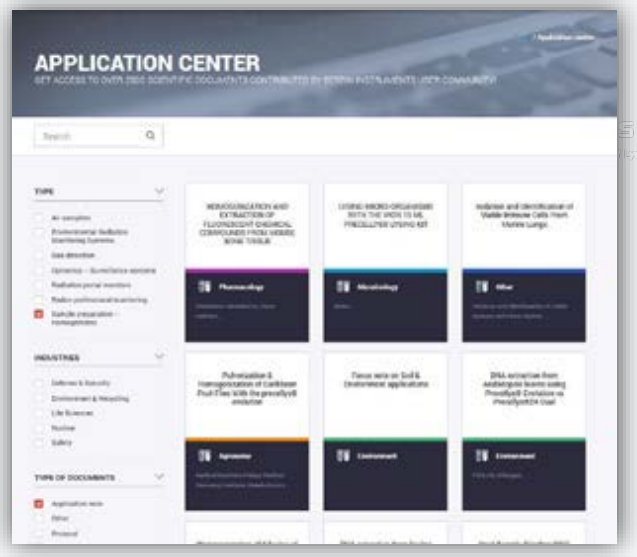

Precellys® Evolution is the most advanced homogenizer gathering high efficiency and versatility for all sample preparation needs:

- **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 Cryolys® Evolution cooling unit

エムエス機器株式会社 www.technosaurus.co.jp
□東京 〒162-0805 東京都新宿区矢来町113番地 TEL(03)3235-0661(代) / FAX(03)3235-0669
□大阪 〒532-0005 大阪市淀川区三国本町2丁目12番4号 TEL(06)6396-0501(代) / FAX(06)6396-0508
□福岡 〒812-0054 福岡市東区馬出1丁目2番23号 TEL(092)631-1012(代) / FAX(092)641-1285