OTHER

# TOTAL RNA EXTRACTION FROM MOUSE KIDNEY BEFORE RNA SEQUENCING

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## / 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 and the Precellys Evolution provide very efficient extractions from either a half or a whole mouse kidney. We compare the RNA quality and integrity after homogenization.

## / MATERIALS

- Precellys 24, or Precellys Evolution + Cryolys Evolution
- 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:

Precellys 24: 5500 rpm, 20s x 2, pause: 20s

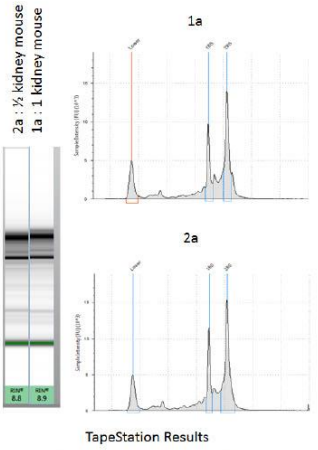
Or with Precellys Evolution: 6500rpm, 20s x 2, pause: 10s at 4°C

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.

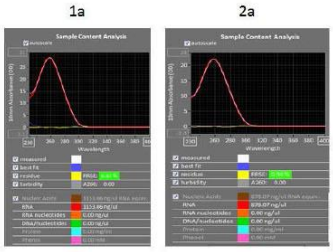
Analysis

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

## / RESULTS




TapeStation Results




DropSense Results

TapeStation

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

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 .

## / CUSTOMER

## / CONCLUSION

There were 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 but also the Precellys Evolution, and the dedicated lysing kit, we obtain a high quality RNA which is mandatory to perform RNA sequencing.

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