OTHER

RNA EXTRACTION FROM NORMAL DOG SKIN BIOPSIES

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/ CONTEXT

Skin is very fibrous and elastic, and efficient RNA extraction from this tissue can be very difficult. We have previously tested several homogenizers to homogenize dog skin samples, with unsatisfactory results, including poor homogenization, poor RNA yield and warming of the samples. To find a better option for RNA extraction from dog skin samples, several types of beads were tested for efficacy in homogenizing frozen skin biopsies using a Precellys® 24 bead homogenizer.

/ MATERIALS

Tissue Samples: Adjacent 4mm full thickness skin biopsies taken from normal 9 week old dog cadaver skin which had been frozen immediately post-mortem and stored at -80°C.

Homogenizer: Precellys® 24.

Lysing Beads tested:

- 1mm zirconium oxide beads (Benchmark Scientific in MP Biomedicals 2ml tubes).
- 2mm zirconium oxide beads (Benchmark Scientific in MP Biomedicals 2ml tubes).
- 2.8mm and 5.0mm zirconium oxide mix beads (CKMix50_2mL, Bertin, Cat #: KT03961-1-013.2).
- 0.7mm garnet flakes and one 6mm zirconium oxide bead (GK60_2mL, Bertin, Cat #: KT03961-1-015.2).
- 6.35mm zirconium oxide beads (Lysing Matrix M, MP Biomedicals).
- One or two 6.35mm steel beads (Lysing Matrix SS, MP Biomedicals).

Lysis Buffers tested: Trizol, RLT+beta mercaptoethanol (Qiagen) or PerfectPure RNA Cell kit Lysis Buffer+TCEP (5Prime).

/ PROTOCOL

Sample preparation: Beads in 2ml tubes were rinsed with 200µl of lysis buffer, then 400µl of lysis buffer was added to the tube and it was placed on dry ice. A 50µl droplet of lysis buffer was frozen in a plastic petri dish placed on a block of dry ice. Frozen 4mm biopsies were placed on the frozen lysis buffer and another 50µl of ice-cold lysis buffer was slowly placed on top and allowed to freeze.

A sterile scalpel blade was used to dice the frozen tissue and lysis buffer. Diced tissue and lysis buffer were added to the frozen beads and lysis buffer in the 2ml tubes, and the tubes were stored at -80°C until homogenization.

Lysis buffer in the tubes was defrosted on ice and the samples homogenized by running 2-3 cycles of 15 sec at 5,000 rpm using a Precellys® 24. Whole frozen biopsies in 500µl of ice-cold lysis buffer were also homogenized for comparison.

RNA Extraction: RNA was purified using a Qiagen RNeasy kit (Trizol and RLT samples) or 5Prime PerfectPure RNA Cell kit, in a final volume of 60µl. RNA quality was analysed using Nanodrop 2000 +/- Agilent TapeStation analysis (RNA integrity number, RIN).

/ RESULTS

Dicing the tissue prior to homogenization was essential as whole 4mm biopsies didn't homogenize well.

There did not appear to be a linear relationship between RNA concentration and bead size or density. It is likely the variation in RNA concentration observed is mostly due to variation in how finely diced the tissue was before homogenization.

Trizol has previously been observed to turn black in combination with steel beads. This didn't occur here, possibly because the sample didn't get warm during homogenization.

Table1. RNA concentrations and purity observed using different lysing kits.

Homogenization (diced tissues)	RNA ng/µl	A 260/280	A 260/230	RIN
Trizol ZrO_2 1mm, BS in MP Bio tubes	293.4	2.04	2.17	5.5
Trizol ZrO_2 2mm, BS in MP Bio tubes	247.3	2	2.07	
Trizol garnet flakes, ZrO_2 6mm, Bertin	110.8	2.01	1.9	
Trizol ZrO_2 6.35mm, MP Bio	263.2	2.03	2.18	
Trizol steel 6.35mm x1, MP Bio	**372.9**	**2.04**	**2.18**	
Trizol steel 6.35mm x2, MP Bio	346.4	2.04	2.19	5.5
RLT ZrO_2 1mm, BS in MP Bio tubes	284.8	2.04	2.14	7.1
RLT ZrO_2 2mm, BS in MP Bio tubes	218.4	2.03	2.04	7.8
RLT ZrO_2 2.8mm & 5mm, Bertin	**371.1**	**2.04**	**2.16**	**8.2**
RLT garnet flakes, ZrO_2 6mm, Bertin	222.9	2.04	2.21	
RLT ZrO_2 6.35mm, MP Bio	198	2.04	2.16	
RLT steel 6.35mm x1, MP Bio	269	2.04	2.16	8.6
PerfectPure ZrO_2 1mm, BS in MP Bio tubes	203	2.09	2.2	7.3
PerfectPure ZrO_2 2.8mm & 5mm, Bertin	**369**	**2.07**	**2.15**	**8.3**
PerfectPure steel 6.35mm, MP Bio	349	2.07	1.86	4.1

/ CONCLUSION

The Precellys® 24 tissue homogenizer efficiently homogenized finely diced dog skin within a short time (30-45 seconds) without warming the samples. Higher concentrations and better quality RNA were purified using the Precellys ® 24 when compared to the bead and rotor-stator homogenizers we have tested previously. Some samples exhibited a degree of RNA degradation, as indicated by a RIN of less than 8 on TapeStation analysis.

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