Application Forum

Homogenization of cartilage tumors to extract total RNA to microarray and sequencing analysis using Precellys bead-beating technology

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Introduction:

High throughput sample preparation of soft tissues has currently become easy thanks to mechanical techniques: bead-beating technologies which is now the most popular and the most practical means for the preparation of tissues for a range of qualitative and quantitative applications whereas manual methods remain a tedious and time-consuming laboratory task.

The sample preparation of hard tissues remains an issue for applications requiring high analytical quality — accuracy, precision and reproducibility — as molecular biology research. The quality of data is directly linked to the consistency of the sample preparation.

The platform of CIT program (“Cartes d'Identité des Tumeurs”, Tumor Identity Cards) is specialized in extraction of nucleic acids from tumoral tissues intended to be analyzed by microarray, RT PCR and sequencing. Although DNA extraction from cartilage samples is common, the extraction of good quality RNA from hard tissues as cartilage tumors is still a challenge. Moreover, microarray and sequencing analysis requires efficient and reliable methods of sample preparation.

In this way, preliminary tests with a Tissue-Lyser (Retsch MM 301, Germany) were performed by the plateforme of CIT program. They showed limits in cartilage samples grinding and RNA quality. Therefore, the Precellys 24 homogenizer was explored in detail (Bertin Technologies, France) (Figure 1) to increase quality of grinding and extracted RNA from cartilage tumors with a specific protocol of RNA extraction. Precellys 24 is a tissue homogenizer using bead-beating technology in 0.5mL, 2mL or 7mL tubes prefilled with beads. A specific figure-8 multidirectional motion gives shaking energy to the beads that grind up to 24 samples in individual sealed tubes which ensures no cross contamination between the tests. Precellys bead-beating homogenization is optimal with Precellys lysing kits with reinforced vials specially designed for hard tissues as the CK28R or MK28R, giving an excellent efficiency with powerful grinding forces.

Figure 1. Precellys 24-Dual homogenizer (Bertin Technologies, France)

Figure 2. Examples of electropherogram profiles of total RNA and miRNA extracted from cartilage tumors on Agilent 2100 Bioanalyzer. (B) and (D) show profiles of total RNA and miRNA extracted from sample CART_003 (“Medium” rank); (C) and (E) show profiles of total RNA and miRNA extracted from sample CART_008 (“Good” rank); (A) show profile of total RNA extracted from sample CART_004 (“Bad” rank, no miRNA analysis was performed on Bioanalyzer for this sample).

Materials and Methods:

In this study, nucleic acids have been extracted from a series of 41 cartilage tumors (chondroma and chondrosarcoma).

Table 1. Description of the RNA quality ranking applied by the platform of CIT program

A

	Rank		
	-	+	++
r 28s/18s	<1.3	1.3 < r < 1.5	1.5 ≤ r
RIN	<6	6 < RIN < 7	7 ≤ RIN
Bioanalyzer profile	Strongly or completely degraded	Profile with a wavy baseline or a small degradation	Intact profile with a flat baseline

B

Quality ranking		This ranking is based on 28s/18s ratio, RIN and Agilent 2100 Bioanalyzer profiles. (A) Each criterion is ranked “-”, “+” or “++” according to the described thresholds. (B) The global quality ranking of a sample is based on an association of the three rank criteria, as explained in table.
Bad	2/3 criteria “-”	
Medium	2/3 criteria “+”	
Good	2/3 criteria “++”	

Table 2. Characteristics and quality ranks of 10 extracted RNA using the Precellys 24-dual homogenizer.

ID sample	A260/A280 nm	r 28s/18s	RIN	Bioanalyzer profile	Rank quality
CART_001	1.74	1	6.6	+	Medium
CART_002	1.64	0.8	5.6	-	Bad
CART_003	1.83	0.6	3.9	-	Bad
CART_004	2.07	1.5	8.6	++	Good
CART_005	2.01	1.3	7.5	++	Good
CART_006	1.74	0	1	-	Bad
CART_007	1.55	1	6.9	+	Medium
CART_008	1.5	1.1	6.9	+	Medium
CART_009	1.55	0	2.5	-	Bad
CART_010	2.02	1	7.1	++	Good

On these 10 samples, 6 present a quality required in microarray analysis and sequencing analysis according to experience of the platform of CIT program (3 samples with “Good” rank and 3 samples with “Medium” rank).

These tissues samples were stored at -80°C until 45 to 75 mg of tissues were transferred into homogenization tubes (Precellys lysing kit CK28R, Bertin Technologies) containing 1 mL TRIzol® Reagent (Invitrogen™) with 200 µl Guanidine thiocyanate 4M (SIGMA ALDRICH™). The cartilage tissues were homogenized with the Precellys 24-Dual homogenizer (Bertin Technologies, France) three times for 15 seconds at 6500 rpm speed with 10 seconds pause between the homogenization steps. Total RNA was then isolated using miRNEasy Mini kit (Qiagen™) according to the manufacturer's instructions. RNA yield was determined by the NanoDrop ND-1000 spectrophotometer at 260/280 nm (Nanodrop Technologies Inc). Quality and integrity of total RNA were further analyzed with an Agilent 2100 Bioanalyzer (Agilent Technologies, UK).

Results and Discussion:

The platform of CIT program has developed a RNA quality ranking (“Bad,” “Limit,” and “Good”) based on three main

criteria: 28s/18s ratio, RIN and Agilent 2100 Bioanalyzer profiles (Table 1A & B). Characteristics and quality ranks of 10 extracted RNA are reported in Table 2 as example. Results show that 30% of extracted RNA are ranked “Good” and 30% ranked “Medium.” Figure 2 shows results of Bioanalyzer profiles of extracted RNA with each quality rank.

Finally, 60% of extracted RNA using the Precellys 24-Dual homogenizer are ranked “Good” or “Medium”, which may be consistent with a RNA quality required in microarray analysis and sequencing analysis according to experience of our platform.

Conclusion:

The Precellys 24-Dual is successfully validated for total RNA extraction from cartilage tumor. It provides better homogeneity in grinding of hard tissue as cartilage and so, increases quality and quantity of total RNA extracted. Moreover, the Precellys 24-Dual is easy to use, simple, efficient and much faster than former methods. The Precellys 24 homogenization will also save hours of work in the sample preparation process so that it is no longer a limiting step, with increased efficiency and reproducibility on particularly challenging samples.

Enlarge the scope of sample preparation with Precellys bead-beating:

The Precellys bead beating technology has first been developed for ESB diagnostic in the 2000s. The scope has then been enlarged to sample preparation for several applications, including R&D Biotech, Pharmacology, Toxicology, Forensic, Medicine, and Food & Environment.

The main advantages of the Precellys technology is the reproducibility, the efficiency on a wide range of samples (from soft to hard tissues) and the diversity of the system specifications enabling low or high throughput process. A patented option Cryolys also enables to overcome the heat limitations of regular grinding and to protect thermo sensitive molecules. A lot of scientist's references are available on the Precellys website where Precellys users can get access to more than 1000 end-users' protocols, publications.

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