APPLICATION NOTE

RNA extraction from cartilage tissue using cryogenic grinding

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Application Note No. D005: DNA/RNA Extraction

Apparatus: Freezer/Mill

Application: PCR

The aim of the study was to find out if there is a connection between the seriousness of arthritic diseases, especially of the knee joint and certain genes. To this end the expression profile of various genes in the tissue of healthy and diseased probands was compared. Total RNA was isolated from cartilage tissue and the activity of certain genes was analyzed via PCR or micro array analysis.

Human Cartilage Tissue

Knee cartilage tissue samples were taken from various patients as well as healthy probands, snap-frozen in liquid nitrogen and stored at -80°C. One to two grams of these samples were taken for RNA extraction and added frozen to the grinding container. With the multi-vial adapter option up to four samples can be homogenized simultaneously in the freezer/mill 6850. According to the adjustment possibilities of the 6850, the samples were held at about -196°C for another two minutes. This assured sufficient cooling and therefore optimum brittleness.

Grinding Procedure / Homogenisation

For technical reasons—due to the large surface area and the small particle size the cartilage powder showed a tendency to thaw quickly, which should be absolutely avoided (RNA degradation) – the phenol-chloroform reagent (peqGold RNAPureTM, peqLAB; Erlangen, Germany) used for further preparation had already been added to the samples (approx. 2 to 2.5ml). After adding the reagent the grinding vials were placed in upright position into liquid nitrogen until the buffer solidified. Then the cartilage samples and the impactor were added to the cooled vial. In order to test for the impactor to not be frozen onto the reagent, the freezer/mill was started briefly to check the movement by listening to the sound of the mill.

The grinding was done in three cycles of two minutes each at maximum frequency. In between these grinding cycles samples were cooled for two minutes to achieve optimum brittleness again. Thus the whole procedure took 12 minutes. The obtained powder was transferred into Falcon tubes and charged with more peqGOLD reagent (approx. 20ml).

Preparation

The RNA was subsequently purified using a phenol-chloroform extraction method according to the producer's protocol. In order to guarantee for optimum RNA clean-up and quality the resulting liquid phase was in addition applied onto RNeasy midi columns.

After photometric examination of the RNA, the samples were analyzed either by quantitative PCR after reverse transcription or alternatively with the Affymetrix GeneChip system.

The grinding vials were cleaned in several steps. First they were cleaned mechanically with tissue paper and 70% alcohol, then with soap suds. After rinsing with water they were rinsed with 70% alcohol. This cleaning process should always be carried out promptly as the thawing phenol-reagent corrodes the vials (the stainless steel accessories are not affected). Prior to the next use the whole grinding set was autoclaved.

Yield of cartilage RNA was between 2 and 20 Ì/g RNA depending on patients samples.