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Friday, 29 September 2015 Analyzing the local changes occurring at the codon level is a way to get an idea about the different functions and processes of a gene. The amounts and relative levels of mRNA coding for two different isoforms of the same protein may be used to compare the relative amounts of the different isoforms of the protein in normal and diseased tissue. At the nucleic acid level, e.g. at the DNA level or at the mRNA level, expression analysis is aimed at uncovering the presence and activity of unique sequences specific for either specific genes or specific cell/tissues. The sequences being examined may be the entire sequence of a gene, an important portion of the sequence or even a region of the sequence not in the coding region of the gene. Starting from a limited amount of sample material, e.g. from a small biopsy specimen, the most important aspect of the analysis is to obtain a minimal amount of highly enriched, presumably pure, RNA. METHODS: 1. The genomic DNA (gDNA) of human, rat or mouse was isolated from frozen tissue. An aliquot of about 50 mg of the frozen tissue was ground to a powder using liquid nitrogen or an appropriate metal tool and the so obtained powder stored at -80°C until use. Typically, about 500 mg of gDNA was isolated from the whole rat or mouse sample using the TRIzol (Invitrogen, Carlsbad, CA) method. Furthermore, approximately 100 mg of gDNA was isolated from human tissues using the TRIzol method. EXAMPLE: 2. RNA was isolated from whole human blood cells using the TRIzol method. Alternatively, total RNA was isolated from human tissue by homogenization in Trizol reagent according to the manufacturer's instructions. A significantly lower amount of RNA is isolated from the entire rat than from the human tissue. EXAMPLE: 3. Alternatively, total RNA was isolated from human tissue using the NucleoSpin RNA II kit according to the manufacturer's instructions. Briefly, flash frozen tissue was homogenized with 0.5 ml of sterile RNase-free water and centrifuged. The aqueous phase was transferred to a clean 15 ml centrifuge tube, mixed with an equal volume of NucleoSpin RNA II solution. A sample of such prepared cell lysate was immediately subjected to isolation of RNA. EXAMPLE: 4

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