PB240

EFFICIENCY OF COFFEE SEEDS RNA EXTRACTION PROTOCO USING RNA INTEGRITY NUMBER ANALYSIS

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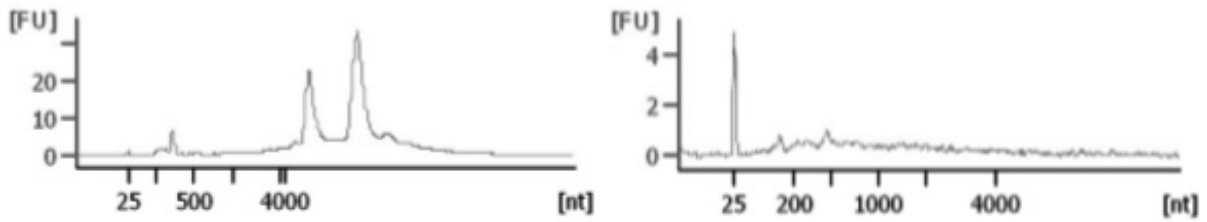
Coffee is one of the most important agricultural products in the world market. The mode of coffee processing, whether wet or dry, determines the flavor characteristic and also the seed quality. Quality RNA isolation is a mandatory requirement for studies of gene expression, including reverse transcriptase (RT), real-time quantitative PCR (RT-qPCR), construction

of cDNA libraries, or microarray analyses. Due to the presence of secondary metabolites, polysaccharides, and polyphenols, standardization of a quality RNA extraction for different coffee plant tissue is very difficult. Also, getting high-quality RNA may be complicated because of RNA susceptibility to RNase degradation. To evaluate purity, the absorbance levels at 280 nm, 260 nm, and 230 nm are use. The A260/A230 absorbance ratio indicates potential contamination with polysaccharides and polyphenols, while the A260/ A280 ratio indicates potential contamination with proteins. When these values are between 1.8 and 2.1, they indicate decontamination of the samples. The RNA Integrity Number (RIN) was developed to remove individual interpretation in RNA quality control, which takes the entire electrophoretic trace into account. This study aimed to identify the most adequate methodology to isolate RNA samples from seed for preparing the cDNA library. Four RNA extraction methods Spectrum®, Direct-zol™ RNA MiniPrep, CTAB protocol, and Pure Link® were tested in coffee seeds processed by three different methods, dry, semi-dry or wet, and dried under shade or dryer conditions. The Spectrum® Plant Total RNA kit method presented samples with the highest RNA quality with RIN up to 8,0 and ideal absorbance relations, indicating samples with little or no contamination with proteins and polysaccharides

A) B)



RIN = 9,20 *RIN = 1,90*

260/280 = 1,86 260/280 = 1,44

Example of electropherogram samples used to train the RIN software: high RIN quality sample (A), and low RIN quality sample (B).