Performance Report of *EasyPure*® Universal Plant Total RNA Kit -ER302

1. Purpose

Evaluate the extraction efficiency of TransGen Biotech's ER302 across varied plant tissues and its suitability for downstream experiments.

2. Materials

2.1 Sample

Type of Sample	Species
Leaf	Tobacco, Wheat, Corn, Tea tree, Lychee, Sugarcane, Cotton, Ilex, Aloe, Pine
Root	Corn roots, Tobacco roots
Tuber	Potato
Flower	Rose
Fruit	Grape, Tomato
Seed	Soybean, Peanut

2.2 Product

Extraction Kits: ER302(*TransGen),* Product from Company T

Downstream Reagents: *TransScript*® II Green One-Step qRT-PCR SuperMix (*TransGen*, AQ311); *TransNGS*® Single Cell Full Length cDNA Synthesis&Amplification Kit (*TransGen*, KC901); *MagicPure*® mRNA Kit (*TransGen* , EC511); *TransNGS*® Fast RNA-Seq Library Prep Kit for Illumina®(*TransGen*. KP701)

2.3 Instrument and Equipment

Qubit Fluorometer, NanoDrop Spectrophotometer, Bio-Rad A600 PCR System, qPCR Instrument, Electrophoresis Apparatus, and Gel Imaging System

3. Methods

Perform RNA extraction and subsequent analyses following the protocols specified for each product. Ensure a minimum of two biological replicates for every sample, utilizing 200mg for fruit samples and 100mg for other sample types. After extraction, elute leaf tissue extracts from plants like aloe, holly, and pine in 50µl of RNase-free water; for other samples, use 100µl of RNase-free water for elution. Assess the A260/280 and A260/230 ratios, Qubit concentrations of the extracts, and proceed with downstream processes including qPCR amplification, full-length cDNA synthesis, and construction of the transcriptome library.

4. Results

4.1 Extraction performance on different type of samples

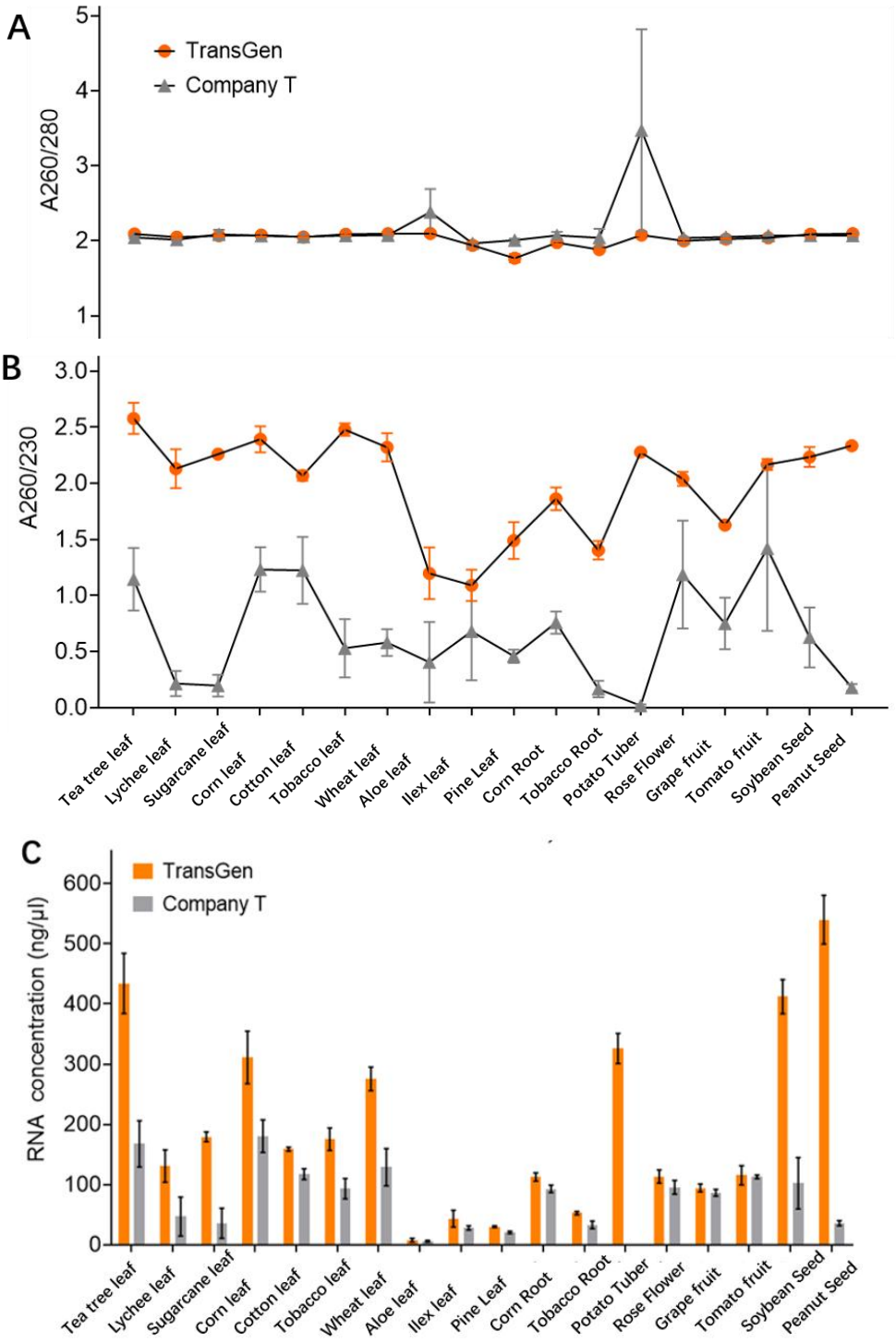


Figure 1. The extraction outcomes of ER302 (TransGen) and competitor Company T for various plant sample types were compared.

A. The A260/280 absorbance ratio of the extracted RNA; B. The A260/230 absorbance ratio of the extracted RNA; C. The concentration of the extracted RNA (Qubit)

Comparative analysis of extraction results against competitor reveals that our ER302 product demonstrates significantly superior overall performance in extraction, notably for leaf, root, and seed samples. Specifically, the competitor was unable to detect RNA concentrations from potato

root samples, whereas our product consistently extracts high-quality RNA exceeding 300 ng/μL. Regarding extraction quality, the competitor's measurements for samples with higher metabolite levels exhibited substantial fluctuations, all showing A260/230 ratios around 1.0. In contrast, our ER302 product consistently produces higher-quality extraction outputs, with A260/280 ratios predominantly around 2.0 and overall superior A260/230 values compared to those of the competitor.

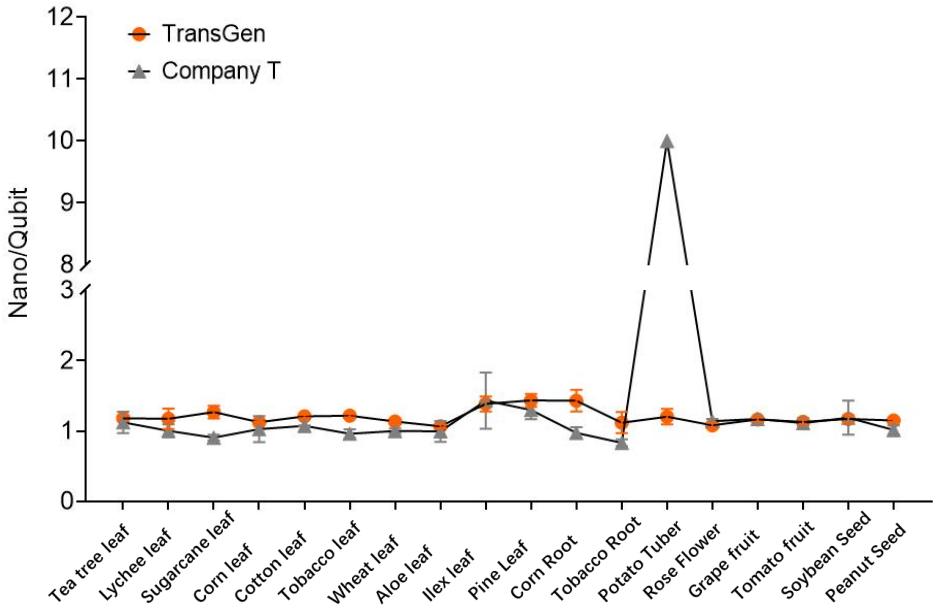


Figure 2. The extraction outcomes of ER302 (TransGen) and competitor Company T by NanoDrop and Qubit were compared.

As Company T's product failed to extract RNA from the potato tuber samples, the Nano/Qubit value neared infinity. For clarity, the results of this sample were set as 10 for illustration purposes.

The NanoDrop nucleic acid detector operates on ultraviolet absorption, with the accuracy of its RNA content detection being notably impacted by impurities in the tested sample. In contrast, the Qubit employs a specific fluorescent dye that selectively binds to RNA, yielding a more precise representation of RNA content in the tested product. Thus, the Nano/Qubit ratio can serve as an indicator of the extraction product purity. The findings reveal that, apart from the elevated reading in the potato sample extracted by Company T, the Nano/Qubit values for RNA extraction from both products exhibit fluctuations within the range of 1.0-1.2.

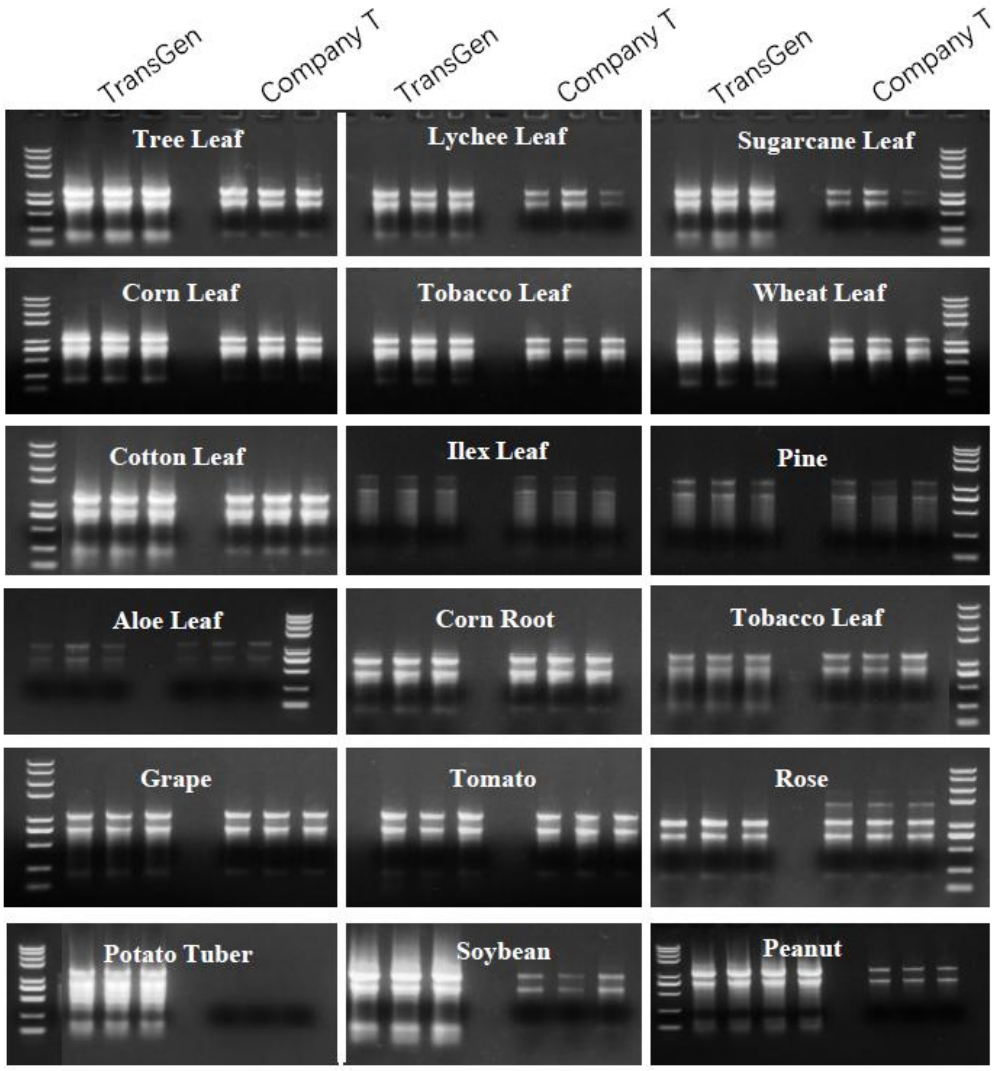


Figure 3. RNA Gel Electrophoresis of ER302 (TransGen) and competitor Company T

Electrophoresis analysis was applied to extracted RNA, the result indicates that the intensity of the bands extracted by ER302 was markedly stronger compared to Company T's product, aligning with the quantification findings (Figure 1). Additionally, the primary bands appeared well-defined and unaltered, exhibiting no apparent signs of degradation or genomic remnants. Notably, even samples with lower extraction outputs like holly leaves, pine needles, and aloe vera leaves displayed distinct primary bands.

4.2 Extraction performance with different sample quantities

4.2.1 Tea leaf (*Camellia sinensis*)

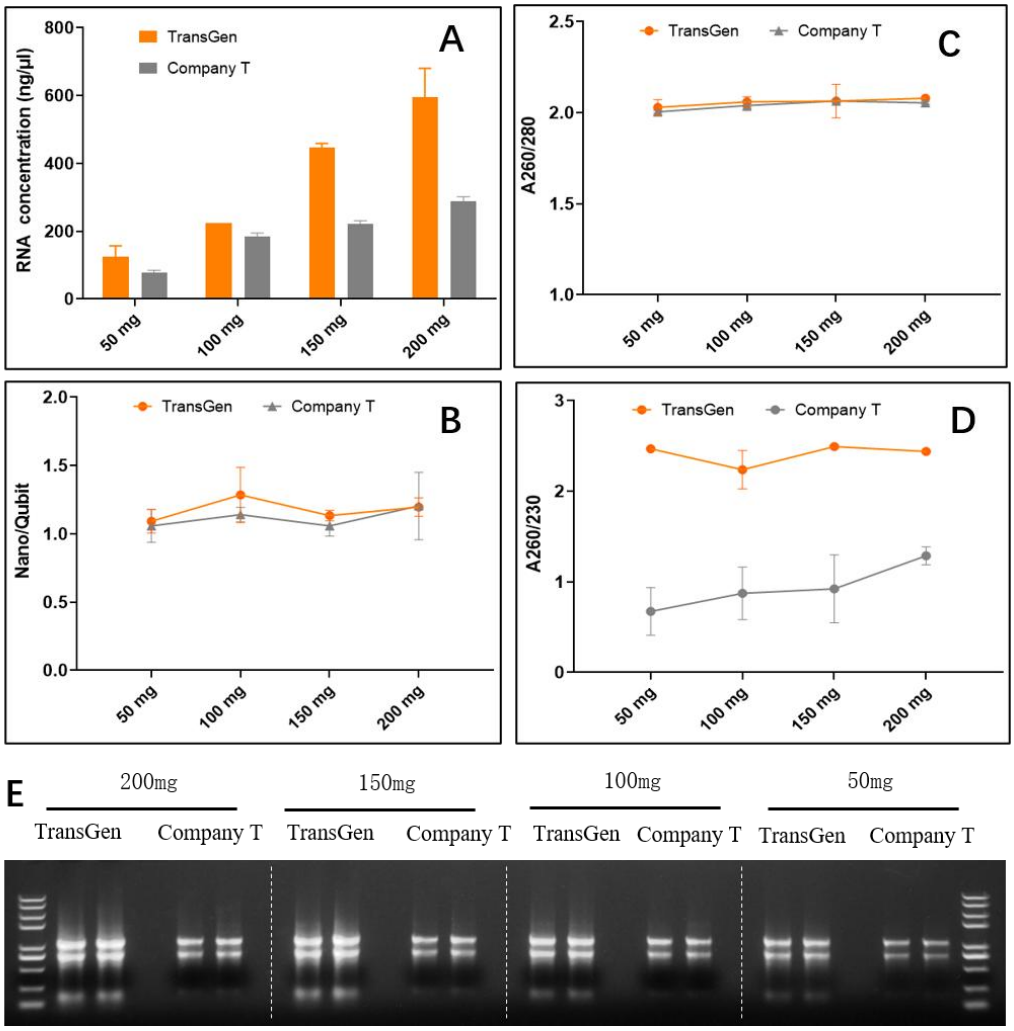


Figure 4. The extracted outcomes of tea leaf samples with varying quantities using ER302 (TransGen) and Company T products.

A. Extracted RNA concentration (Qubit); B. Ratio of NanoDrop to Qubit measurement data; C. A260/280 absorbance ratio; D. A260/230 absorbance ratio; E. Electrophoresis gel image of the extracted RNA.

Tea leaves were chosen as the test material to assess the extraction performance of the products at different sample quantities. The results indicate that ER302 demonstrates superior compatibility across varying sample mass, consistently achieving excellent extraction efficiency, even with a 200mg input, while Company T's product peaks at 150mg. Both products exhibit no notable differences in the Nano/Qubit ratio and A260/280; however, ER302 gives better A260/230 ratio surpassing 2.0. The RNA bands extracted by ER302 retain high integrity, showing no signs of degradation or genomic residues.

4.2.2 Soybean Seed

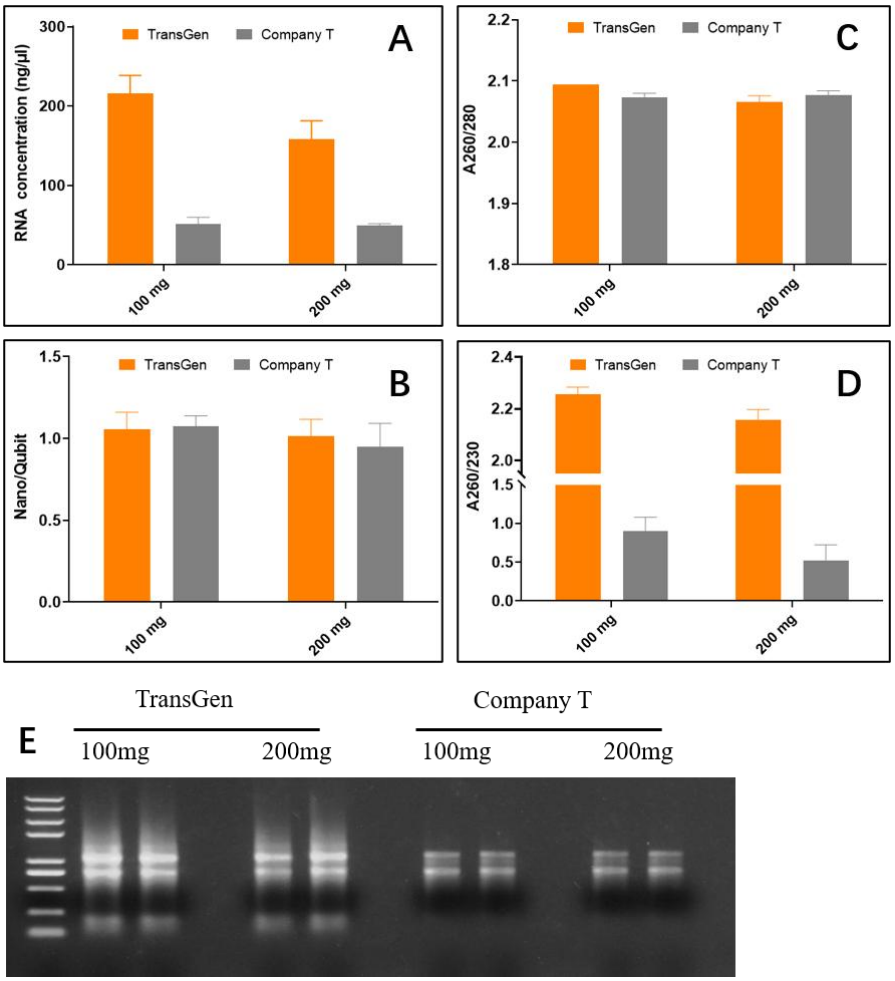
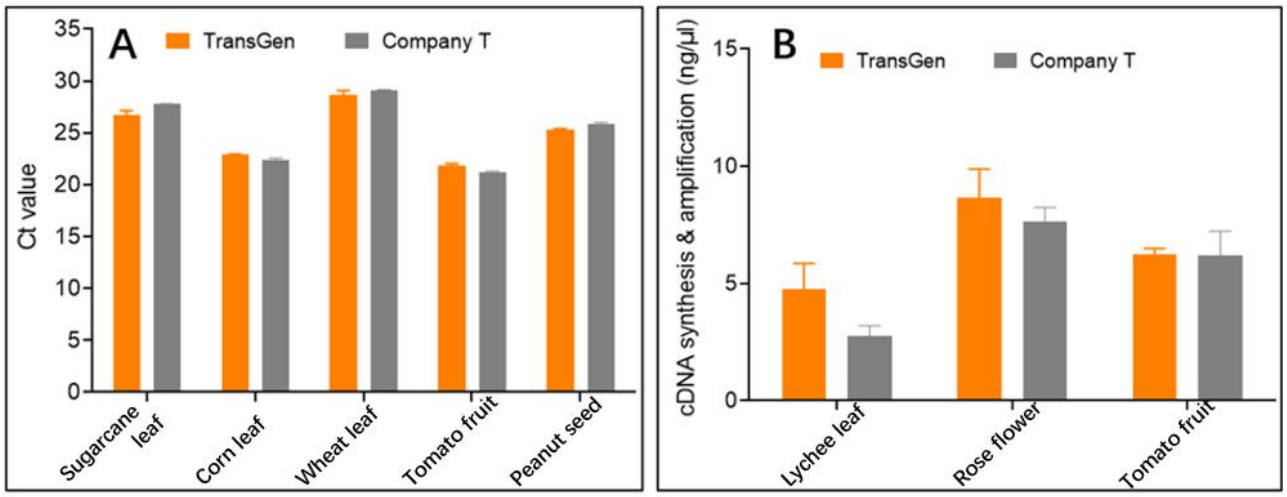


Figure 5. The extraction outcomes of ER302 (TransGen) and Company T products for varying quantities of soybean seeds.

A. Extracted RNA concentration (Qubit); B. Ratio of NanoDrop to Qubit measurement data; C. A260/280 absorbance ratio; D. A260/230 absorbance ratio; E. Electrophoresis gel image of the extracted RNA.

Soybean seeds were chosen as the samples for conducting extraction tests with varying sample quantities. Following the leaf tissue analysis, sample of 100mg and 200mg were selected for the study. The results indicate that both ER302 and Company T products did not exhibit increased extraction yields for seed samples beyond 100mg; instead, there was a decrease in some instances. Therefore, 100mg seems to be the optimal quantity for these products with seed samples, with extraction efficiency being 4.2 times greater than that of the control using ER302. There were no notable differences in the Nano/Qubit ratio and A260/280 values between the products for the extracted samples; however, ER302 consistently demonstrated a marked advantage in A260/230 ratio exceeding 2.0. RNA electrophoresis analysis of the ER302-extracted samples revealed excellent band integrity, indicating no apparent degradation or genomic residues.

4.3 The subsequent analysis of RNA extraction product



Sample Type	Product	Transcriptome library construction conditions					Yield
		Amount of RNA	mRNA Capture	Library Preparation	Method	Amplification Cycle	
Tea Leaf	Company T	500ng	EC511 (TransGen)	KP701 (TransGen)	Stranded Library	12 cycles	696
	TransGen						**834**
Wheat Leaf	Company T	500ng				12 cycles	30.9
	TransGen						**345**
Cotton Leaf	Company T	500ng				12 cycles	624
	TransGen						**684**
Rose Flower	Company T	500ng				12 cycles	864
	TransGen						**954**
Tomato Fruit	Company T	500ng				12 cycles	456
	TransGen						**924**

Figure 6. The subsequent analysis of RNA extraction by ER302 (TransGen) and Company T products.

A. qRT-PCR detection results (template amount 100ng, reaction system 20μl); B. Full-length cDNA synthesis and amplification; C. Transcriptome library construction conditions and yield.

The success of downstream experiments relies on the quality of RNA extraction. Our research incorporated three types of experiments, including qRT-PCR, full-length cDNA synthesis and amplification, and transcriptome library construction, to evaluate the compatibility of the RNA extracted by two different products with downstream reagent kits.

By utilizing our one-step qRT-PCR kit (AQ311) to test the efficiency of reverse transcription and qPCR on the extraction products, the results indicated that both products performed comparably with no significant differences. Subsequent evaluation with our KC901 product to test the full-length cDNA synthesis and amplification capacity of the extraction products revealed a higher cDNA amplification yield with ER302, suggesting superior RNA integrity compared to

Company T's product. Thus, employing ER302-extracted products for targeted gene quantification or gene cDNA fragment cloning would enhance precision and success rates. The RNA extraction, combined with our EC511 and KP701 kits for mRNA capture and library construction, exhibited notably increased library output with ER302-extracted RNA, demonstrating higher mRNA content and purity in ER302-extracted products, which ensures accurate detection of gene expression.

5. Conclusion

(1) ER302 shows exceptional extraction performance, catering to a wide range of plant sample types. Notably, its efficiency in extracting leaf, tuber, and seed tissues surpasses that of competitor significantly. ER302 can extract seed samples up to 14 times more efficiently than competitor. For potato tubers, ER302 yields high-quality RNA exceeding 300 ng/μl, while the competitor fails to produce any measurable yield.

(2) RNA extracted by ER302 demonstrates high integrity and purity, making it particularly suitable for downstream experiments such as full-length cDNA synthesis and transcriptome library construction.

(3) The extraction products exhibit no remnants of genomic DNA (gDNA). The product incorporates a gDNA removal component, effectively eradicating gDNA contamination and minimizing its influence on subsequent analyses.

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