Broad Application of a Single Universal Lysis Buffer for True Total RNA Purification from Challenging Plant Species and Tissues

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INTRODUCTION

RNA extraction from plant tissues presents many more challenges than from animal cells or tissues. This is because plant tissues are quite variable and represent a large number of different species. Plant tissue cell walls are often made up of various compounds and arrangements (celluloses, hemicelluloses, pectin's), therefore finding one protocol that works well with all of these variations is challenging. Furthermore, plant tissues contain various substances that interfere with RNA extractions and/or cause interferences or inhibition in downstream application. Compounds such as phenolics, phytotoxins, polysaccharides, starches, tannins, and other secondary metabolites are present in various amounts and combinations, making it difficult for most RNA and even DNA extraction protocols to yield high quality nucleic acid for downstream applications. Therefore, many plant RNA purification companies have adapted a dual lysis buffer system, which contains two lysis buffers that both have a unique range of plant materials that they are able to work with. The issue with this system is that a process of trial-and-error must be carried out to see which buffer works most effectively on a sample, at the customers cost.

Norgen's Plant/Fungi RNA Purification Kit contains a universal, robust lysis buffer that is very effective over a wide range of plant species. It allows Norgen's Plant/Fungi RNA Purification to target the isolation of RNA from various challenging plant samples, including grape and pine needle, with no need to test different lysis buffers. This saves the customer's time and effort to establish a rapid RNA purification system in the lab.

With Norgen's universal lysis buffer, plant total RNA can be purified from a wide range of fresh or frozen plant tissues, plant cells, or filamentous fungi samples using this kit. Furthermore, the total RNA profile of the sample is purified, from large mRNA and ribosomal RNA, down to microRNA (miRNA) and small interfering RNA (siRNA) using Norgen's proprietary resin as the separation matrix. The procedure is also free of environmentally hazardous compounds such as phenols and chloroform, and yet maintains the integrity of its total RNA profile isolation. The yield and quality of the isolated RNA is of the highest integrity, and is excellent for use in downstream applications such as RNA sequencing, Next Generation sequencing, RT-PCR, qRT-PCR, microarrays, Northern blots for host RNA (miRNA) or for pathogen detection, among many other applications.

In this application note, we show the effectiveness of Norgen's universal lysis buffer to successfully isolate high quality RNA from various plant species, including challenging samples, and we compare it to a popular commercially available kit from Qiagen. Many publications showing the utility of Norgen's universal buffer and kit for various plant species and applications are listed on our website:
https://norgenbiotek.com/product/plantfungi-total-rna-purification-kit

MATERIALS AND METHODS

Plant RNA Isolation

Plant RNA was isolated from 50 mg of plant leaf tissue (equivalent to ~5 x 10^6 plant cells) using Norgen's Plant/Fungi RNA Purification Kit as per the provided protocol **(Figure 1)**. Briefly, the plant leaf tissue (apple, peach, grape, pine needle, strawberry and pear) was ground in a mortar, containing enough liquid nitrogen to cover the sample, with a pestle until the tissue was ground into a fine powder. Next, the universal lysis buffer was added. The lysate was then transferred into a filter column, assembled with an RNAse-free microcentrifuge tube and centrifuged for 1 minute at 14,000 x g (~14,000 rpm) to filter out cellular debris. The flow-through was then transferred to a new RNAse-free microcentrifuge tube and an equal volume of 96-100% ethanol was added and mixed by vortexing. Next, 600 µL of the clarified lysate was then loaded onto an assembled column and centrifuged for 1 minute at 14,000 x g (~14,000 rpm). The flow-through was discarded and the column was reassembled. The remaining lysate was then loaded onto the column and re-centrifuged for 1 minute at 14,000 x g. The column was then washed a total of three times by applying 400 µL of Wash Solution to the column, centrifuging for 1 minute at 14,000 x g (~14,000 rpm) and then discarding the flow-through. Columns were centrifuged for 2 minutes at 14,000 x g (~14,000 rpm) to thoroughly dry the resin. For RNA elution the column was placed into a fresh 1.7 mL elution tube and 50 µL of the elution buffer was applied to the column. Columns were then centrifuged for 2 minutes at 200 x g (~2000 rpm), followed by a 2 minute spin at 14,000 x g. Purified RNA

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was then stored at -20°C for several days or at -70°C for long term storage.
At the same time, RNA was purified from plant leaf tissue using the leading market competitor's plant RNA purification kit according to the manufacturer's protocol and used in comparative experiments.

RNA Gel Electrophoresis

The purified plant or fungal RNA was typically run on 1X MOPS, 1.5% formaldehyde-agarose gels for visual inspection. Generally, 5 µL of each 50 µL elution was run on the gel. The purified plant RNAs (Norgen's and the competitor's) were also resolved on a 1X MOPs, 1.0% formaldehyde-agarose gel for visual comparison.

RT-qPCR Assay

Plant RNA purified from apple, peach and grape leaves, as well as from pine needles, was used as a template for one step RT-qPCR with primers specific for CSVd.

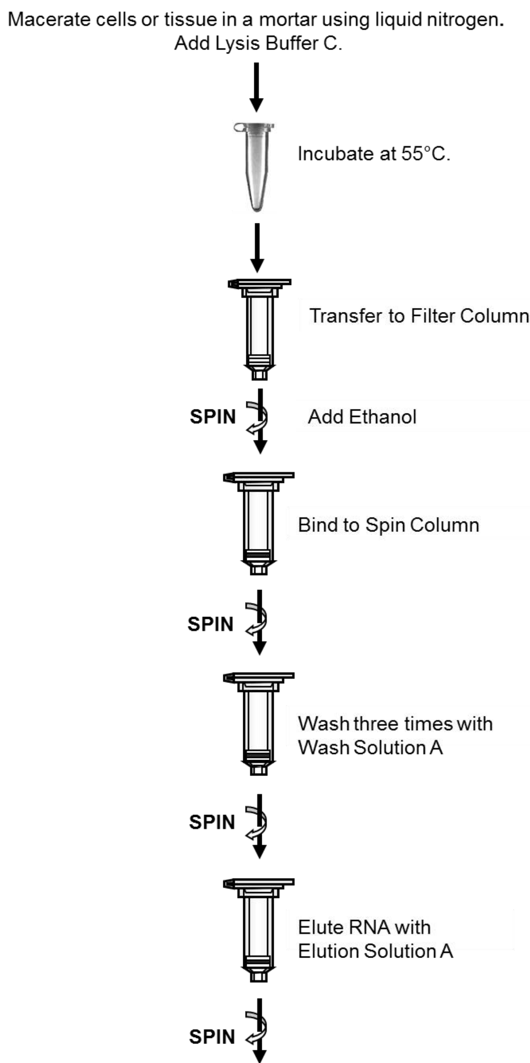

Figure 1. Procedure flowchart for the purification of plant RNA using Norgen's Plant/Fungi RNA Purification Kit.

RESULTS AND DISCUSSION

Sensitive downstream applications of the purified RNA demand that the RNA be of the highest quality. The quality of the purified nucleic acids can be determined through the use of spectrophotometry. Nucleic acids only absorb light that has a wavelength of 260 nm, while contaminants such as phenol, proteins, and other contaminants absorb at 280 nm. Samples with a low 260/280 (below 1.8) have a significant presence of these contaminants that may interfere with downstream processes such as RT-PCR experiments, lowering their efficiency. In order to foster the success of microarrays and gene expression experiments, it is recommended to only use samples with a 260/280 ratio greater than 1.8, optimally greater than 2. Running samples with 260/280 ratios below 1.8 can result in substantially less optimal results.

Figure 2 below demonstrates the effectiveness of Norgen's universal lysis buffer against two lysis buffers from the competitor (Q). Using Norgen's universal lysis buffer, the Plant/Fungi RNA Purification Kit consistently isolates RNA with a high 260/280 ratio (typically above 1.8-2.0) from a variety of plant species while the competitor's two lysis buffers isolated RNA with a 260/280 ratio below 1.3.

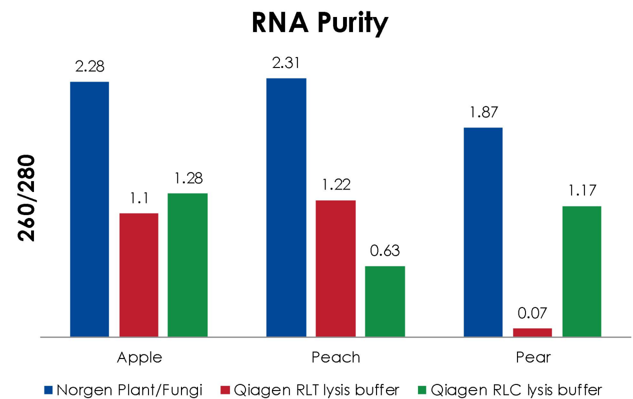

Figure 2. Purity of Plant RNA Samples Isolated from Apple, Peach and Pear. RNA purity was determined spectrophotometrically for RNA samples isolated from apple, peach and pear using Norgen's kit (single lysis buffer) (260/280 = blue bars) and Qiagen's kit (dual RLT & RLC lysis buffers) (260/280 = red & green bars). Norgen's kit consistently isolated pure samples of plant RNA with 260/280 ratios above 2 & 1.8, whereas the competitor's kit had 260/230 ratios below 1.3 & 1.2 for the same samples.

Furthermore, Norgen's Plant/Fungi RNA Purification Kit successfully purified RNA of high quality and quantity from a variety of challenging samples **(Figure 3)**. In contrast, the competitor showed limited compatibility that failed large RNA purification with some plant species as well as small RNA (white box) for all samples tested.

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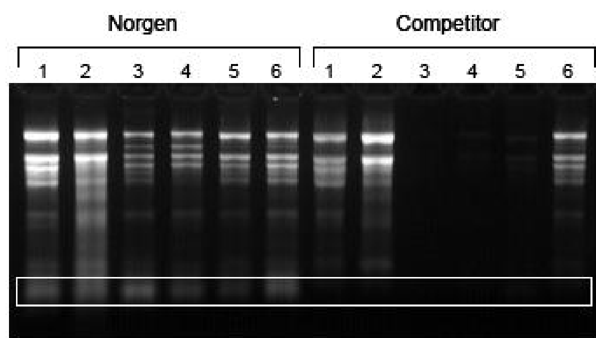

Figure 3. Isolation of High Quality RNA, even from Difficult Samples. RNA was isolated from 50 mg leaf samples of apple (Lanes 1), peach (Lanes 2), grape (Lanes 3), pine needle (Lanes 4), strawberry (Lanes 5) and pear (Lanes 6) using Norgen's kit and a competitors kit. Norgen's kit allowed for the isolation of high quality RNA from all the samples, including the difficult samples, while the competitor failed to isolate RNA from grape, pine needles and strawberry. Furthermore, only Norgen's kit was able to isolate the small RNA species (white box).

In order to assess the ability of Norgen's Plant/Fungi Total RNA Purification Kit to detect sensitive viroids versus the competitor, qRT-PCR was performed with the purified RNA. Figure 3 shows the amplification of the Chrysanthemum stunt viroid (CSVd) transcript from total RNA isolated by Norgen's Plant/Fungi RNA Purification Kit, versus the results of various competitors. Norgen's Plant/Fungi RNA Purification Kit demonstrates its superior ability to detect sensitive viroids compared to the competition.

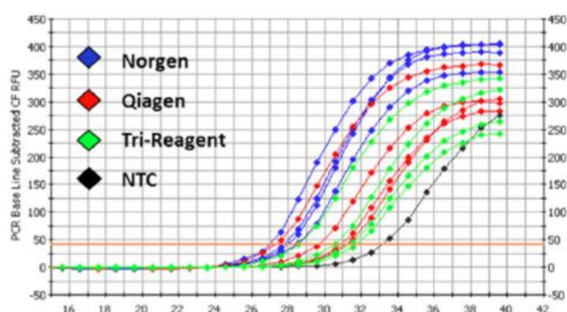

Figure 3. Detection of CSVd in the RNA Yields of Various Kits by qRT-PCR. Norgen's Plant/Fungi RNA Purification Kit yields superior results to that of the competition.

Norgen's Plant/Fungi RNA Purification Kit is second to none in the wide range of plant species and tissues that it is able to isolate high quality RNA from using a single, universal lysis buffer. High quality RNA has been successfully isolated from various plant species and tissues using Norgen's kit either in house, or by customers. These plant species are summarized in Table 1 and Table 2.

Table 1. List of plant species from which high quality and quantities of RNA have been successfully isolated using Norgen's Plant/Fungi Total RNA Purification Kit.

	Plant	Tissue type	Performed by
1	Tobacco (*Nicotiana tabacum*)	Leaf, stem, root	Publication
2	Strawberry	Leaf, fruit, flower	In-house
3	Tomato (*Lycopersicon esculentum*)	Leaf	Publication
4	Blackberry	Leaf, berry	In-house
5	Pepper (*Capsicum annuum*)	Leaf, seed	Publication
6	Herbs	Leaf	In-house
7	Soy bean (legume)	Leaf, stem, root	Publication
8	Persimmon (*Ebenaceae*)	Leaf	Publication
9	Potato (*Solanum tuberosum*)	Leaf, tuber	Publication
10	*Arabidopsis thaliana1*	Leaf, stem,	Publication
11	Plum	Leaf, fruit	Publication
12	Peach (*Prunus persica*)	Leaf	Publication
13	Citrus	Leaf	Publication
14	Apple (*Malus sp.*)	Leaf, flower, pollen	Publication
15	Vanilla bean	Vanilla bean	In-house
16	Pear (*Pyrus sp.*)	Leaf	Publication
17	Cotton (*Gossypium*)	Leaf, cotton	Publication
18	Grape vine (*Vitis sp.*)	Leaf, grape, skin	Publication
19	Mangrove	Leaf	Publication
20	Plum (*Prunus sp.*)	Leaf	Publication
21	Chrysanthemum	Leaf	In-house
22	Palm (*Arecaceae*)	Leaf	Publication
23	Eastern white Red Cedar	Leaf	In-house
24	Pine needle (*Pinaceae*)	Needle	In-house
25	Corn	Leaf, corn	Publication
26	Cucumber	Leaf	Publication
27	*Eucalyptus grandis*	Root	publication
28	*Salvia sclarea*	Root	publication
29	Rice	Leaf	publication
30	*Pseudocyphellaria crocata*	Leaf	publication
31	Palm (*Elaeis guineensis*)	Leaf	publication
32	Sorghum	Leaf	Customer
33	Sugar cane	Leaf	Customer

Table 2. List of fungi and yeast species from which high quality and quantities of RNA have been successfully isolated using Norgen's Plant/Fungi Total RNA Purification Kit.

	Fungi and yeast	Tissue type	Performed by
1	*Hymenoscyphus fraxineus*	culture	Publication
2	*Phanerochaete carnosa*	culture	Publication
3	*Aspergillus niger*	culture	Publication
4	*Saccharomyces cerevisiae*	culture	Publication
5	*Botrytis cinerea.*	culture	Publication
6	*Mucor racemosus*	culture	In-house

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7	*Cladosporium cladosporioides*	culture	In-house
8	*Fusarium oxysporum*	culture	In-house
9	*Penicillium sp.*	culture	In-house
10	*Pichia sp.*	culture	In-house
11	*Rhizopus oryzae*	culture	In-house
12	*Alternaria tenuissima*	culture	In-house

CONCLUSIONS

1. **Universal Lysis Buffer -** Norgen's Plant/Fungi Total RNA Purification Kit provides a universal lysis buffer (as opposed to two lysis buffer system from competitors) that has a wide range of compatibility with a variety of plant species.
2. **Superior Performance -** The universal lysis buffer out performed the two lysis buffer system from Competitor Q, providing higher RNA purity and resulting in better viroid detection using real-time PCR.

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