Isolation of Plant and Fungal RNA from Challenging Samples using Norgen's Plant/Fungi RNA Purification Kit

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INTRODUCTION

Norgen's Plant/Fungi Total RNA Purification Kit provides a rapid method for the isolation and purification of total RNA from a wide range of plant and filamentous fungal species. Total RNA can be purified from fresh or frozen plant tissues, plant cells or filamentous fungi samples using this kit. All sizes of RNA are purified, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The procedure is rapid and convenient, as it does not rely on the use of liquid nitrogen in order to homogenize the samples. The RNA is preferentially purified from other cellular components, such as proteins, without the use of phenol or chloroform. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

Norgen's Plant/Fungi RNA Purification Kit provides an innovative and rapid method for the isolation and purification of total RNA, including small RNA from both plant and fungal cells. The procedure is based on spin column chromatography, using Norgen's proprietary resin as the separation matrix. The purified RNA can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, and expression array analysis.

Despite the current battery of advanced and powerful molecular tools, the isolation of genetic materials from economically important plants remains difficult. Norgen's Plant/Fungi RNA Purification kit has been developed to specifically target the isolation of RNA and DNA from difficult plant materials. In this application note, Norgen's Plant/Fungi RNA Purification Kit is used to isolate total RNA from various challenging plant samples, including grape and pine needles. The purity of the RNA is demonstrated through spectrophotometry, BioAnalyzer and RT-qPCR. The kit is also used to isolate total RNA from plant tissue. In addition, Norgen's Plant/Fungi RNA Purification kit was also used to purify total RNA from various fungal species indicating the broad application diversity of the kit.

METHODS AND MATERIALS

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 600µL of lysis solution with a pestle until the tissue was completely macerated. The lysate was then transferred into an RNAse-free microcentrifuge tube and centrifuged for 2 minutes to remove cellular debris. The supernatant was then transferred to a new RNAse-free microcentrifuge tube and an equal volume of 70% ethanol was added and mixed by vortexing. 600µL 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 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 400µL of Wash Solution to the column, centrifuging for 1 minute and then discarding the flow-through. Columns were centrifuged for 2 minutes to thoroughly dry the resin. For RNA elution the column was placed into a fresh 1.5mL elution tube and 50µL 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 1 minute spin at 14,000 x g. Purified RNA 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.

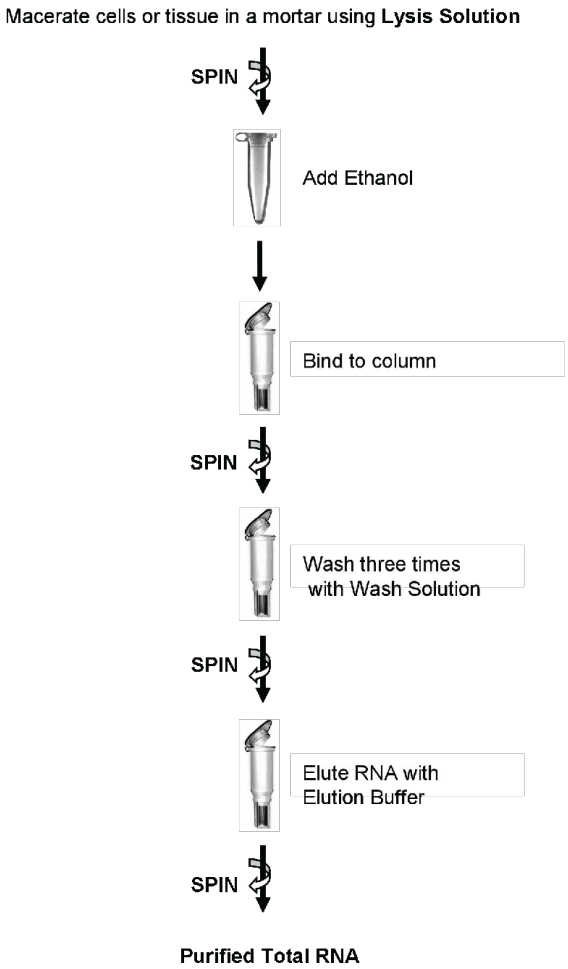

Figure 1. Procedure for the purification of Plant RNA using Norgen's Plant/Fungi RNA Purification kit.

Fungal RNA Isolation

Fungal RNA was isolated from 50 mg of wet fungi samples of Botrytis cinerea, Alternaria tenuissima, Rhizopus oryzae, Penicillium sp. and Fusarium oxysporum. using Norgen's Plant/Fungi RNA Purification kit according to the provided protocol.

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 com 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.

Capillary Electrophoresis

The purified RNAs were loaded onto an Agilent® RNA Nano 6000 chip and resolved on an Agilent® 2100 BioAnalyzer according to the manufacturer's instructions.

RT-qPCR Assay

Plant RNA purified from apple, peach and grape leaves, as well as from pine needles, was used as template for one step RT-qPCR with primers specific for EF1-α. In addition, Plum Pox virus was detected from the total RNA extract of Peach leaves by one step RT-qPCR.

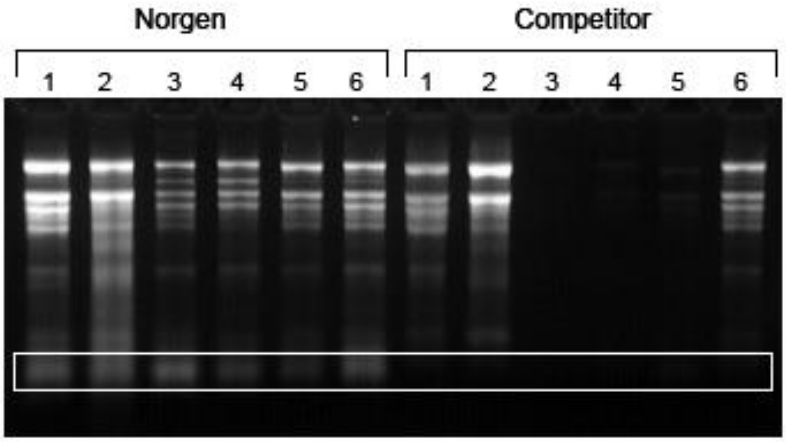

Figure 2. Isolation of High Quality RNA, Even from Difficult Samples. RNA was isolated from 50 mg leaf samples of apple (Lanes 1), peach (Lanes 2), grape (Lanes 3), pine needle (Lanes 4), strawberry (Lanes 5) and pear (Lanes 6) using Norgen's kit and a competitors kit. Norgen's kit allowed for the isolation 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).

RESULTS AND DISCUSSION

Despite the current battery of advanced and powerful molecular tools, the isolation of genetic materials from economically important plants, and in particular RNA remains difficult. Norgen's Plant/Fungi RNA Purification kit has been developed to specifically target difficult plant materials such as Grape, Pine needle and Strawberry. In comparative experiments with a market competitor, the Norgen Plant /Fungi RNA Purification kit successfully purified RNA of a high quality and quantity from a variety of challenging samples **(Figure 2)**.

Downstream applications of the purified RNA demand that the RNA be of the highest quality. Quality of purified nucleic acids can be determined through the use of spectrophotometry. Nucleic acids only absorb light that has a wavelength of 260 nm, while organic contaminants such as phenol, and other aromatic compounds such as TRIzol and or other additional reagents used in RNA extractions absorb light at a wavelength of 230 nm. Samples with a low 260/230 (below 1.8) have a significant presence of these organic 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 260/230 ratio greater than 1.8.

Running samples with 260/230 ratios below 1.8 can result in substantially less optimal results. In Figure 3 it is demonstrated that the Norgen Plant/Fungi RNA purification kit consistently isolates RNA with a high 260/230 ratio (typically above 2.0) from a variety of plant cell types. In contrast, the competitor's kit always isolated RNA with a 260/230 ratio below 1.2. As a result the competitor's kit will isolate RNA which is unsuitable for most downstream applications, whereas the RNA samples purified using Norgen's kit can be used confidently in any downstream applications, including for the highly stringent microarray assays.

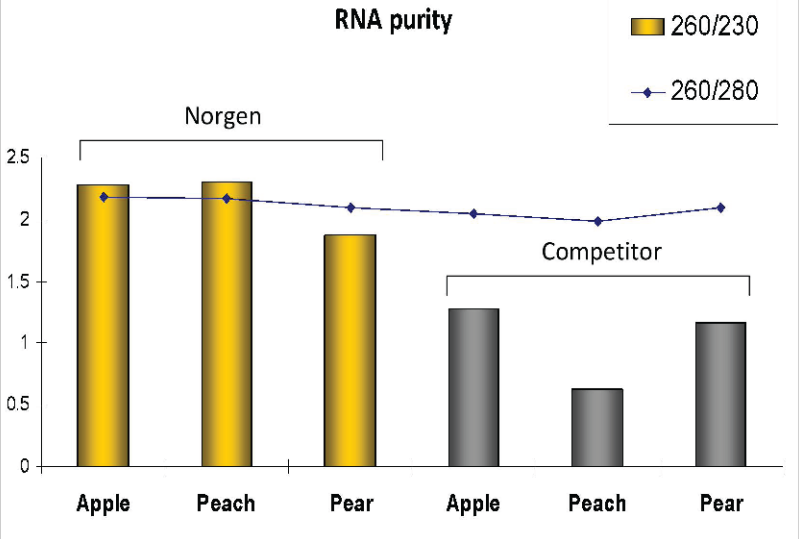

Figure 3. RNA purity of Plant RNA samples isolated from Apple, Peach and Pear. RNA purity was determined spectrophotometrically for RNA samples isolated from apple, peach and pear using Norgen's kit (260/230 = gold bars) and a competitor's kit (260/230 = grey bars). Also shown is the 260/280 ratio for the samples. Norgen's kit consistently isolated pure samples of Plant RNA with 260/230 ratios above 2.0, whereas the competitor's kit had 260/230 ratios below 1.2 for the same samples.

The quality of RNAs isolated by Norgen's kit was further demonstrated by capillary gel electrophoresis **(Figure 4)**. RNA purified from grape was resolved on an Agilent Labon-a-Chip. Figure 4 is the resultant electropherogram of the total RNA isolated from grape leaves. All the RNA species, including microRNA, 18s be observed. Clearly, no microRNA is being lost during the RNA isolation from plant cells.

In order to assess the biological activity of the RNAs isolated from apple, peach, pine needle and grape leaves, RT-qPCR was performed. **Figure 5** shows the amplification of the EF1-α transcript from total RNA isolated by Norgen's Plant/Fungi RNA Purification kit. The PCR product was detected in the RNA samples. This suggests that the RNAisolated from each of the plant species was of a high purity and had retained its biological activity.

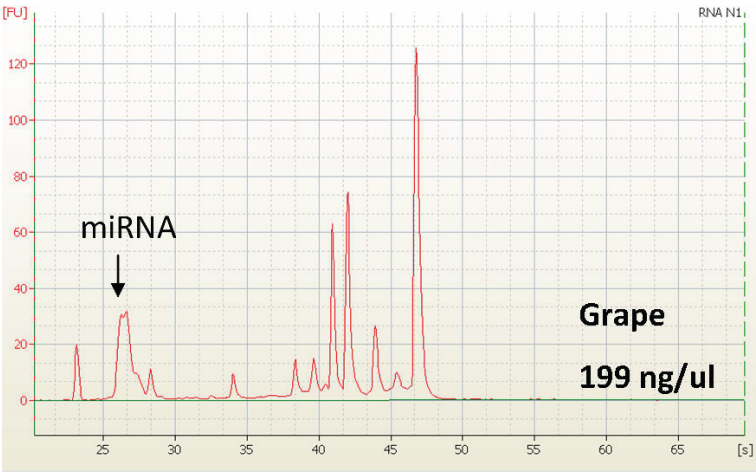

Figure 4. Resolution of Plant RNA on the Agilent BioAnalyzer. Total RNA was isolated with Norgen's Plant/Fungi RNA Purification kit from Grape, resolved on an Agilent Lab-on-a-Chip and an electropherogram was generated. It showed high RNA purity as well as integrity.

In addition to the successful isolation of total RNA from various plant leaf tissues, total RNA was successfully isolated from plum fruit tissue **(Figure 6)**. The RNA was purified using Norgen's Plant/Fungi RNA purification kit from varying input amounts of plum fruit (50mg, 100mg, 200mg and 300mg). RNA was successfully purified from all input levels of fruit tissue cells, demonstrating the sensitivity of the purification method. In addition to plant leaf tissue, total RNA can be isolated from other plant tissues including fruit.

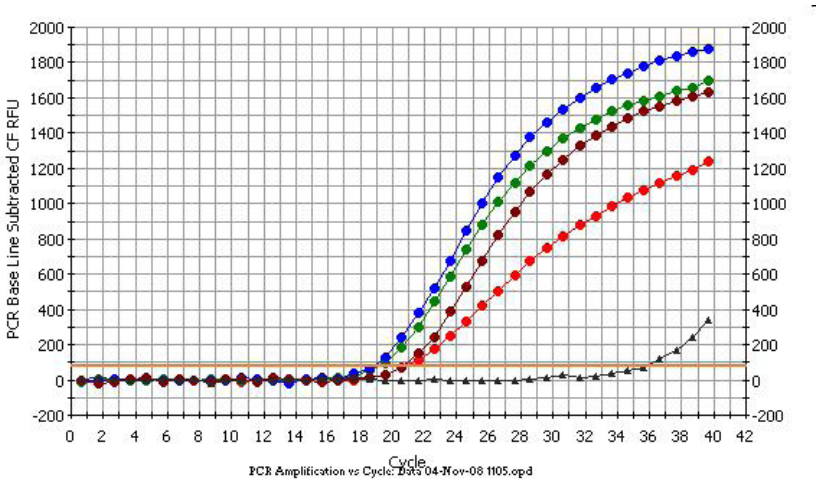

Figure 5. Detection of EF1-α using one step RT-qPCR from purified Plant total RNA. Total RNA was isolated from 50 mg samples of apple (red), peach (green), pine needle (blue) and grape leaves (burgundy) using Norgen's Plant/Fungi Total RNA Purification kit and 3 µL of the RNA was used in an RT-qPCR reaction for the detection of EF1-α. EF1-α was detected from all samples, indicating that the RNA is of high quality and that the Plant/Fungi RNA Purification kit is highly sensitive for total RNA isolation.

It was then determined whether plant pathogenic viruses could be detected from the total RNA purified using Norgen's Plant/Fungi RNA Purification kit. Plum Pox Virus (PPV) is a destructive and economically devastating pathogen of Prunus species. Current containment efforts involve eradication of infected trees based on ELISA surveys, which are labour intensive and less sensitive than PCRbased methods. Recently, an RT-qPCR-based method was developed for its detection.

The detection of PPV in Peach leaf tissue was therefore attempted. Two 50mg samples of infected peach leaf tissue was used as the input and total RNA isolated using Norgen's kit. Purified total RNA was then used as the template for RT-qPCR with primers that detect a Plum Pox virus specific transcript. Two input levels of total RNA (1µL or 3µL) was used as template for the RTqPCR reactions, which were performed in duplicate. The PPV PCR product was detected in all four samples suggesting that the RNA isolated from peach leaf tissueincluded the viral RNA which could be sensitively detected following purification.

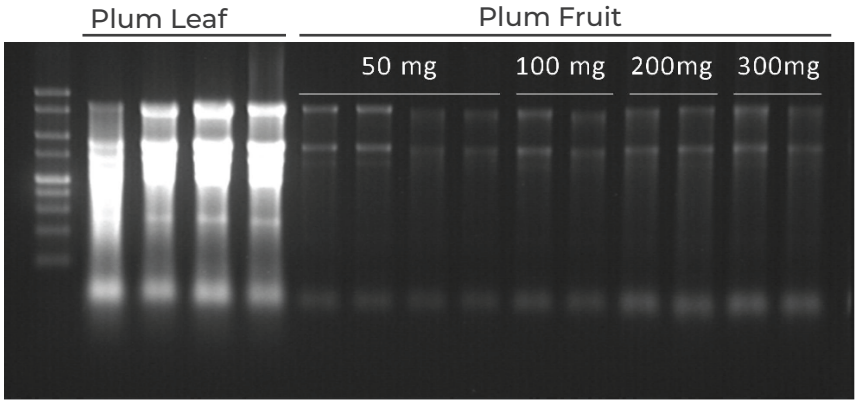

Figure 6. Isolation of total RNA from Plum fruit using the Plant/Fungi RNA Purification kit. In addition to isolating RNA from plant leaf tissue, total RNA was also isolated from plum fruit tissue using Norgen's Plant/Fungi RNA Purification kit. Total RNA was isolated from 50 mg, 100 mg, 200 mg or 300 mg samples of plum. All isolations were performed 4 times for both tissue types. 5 µL of the 50 µL elutions were loaded onto a 1X MOPS, 1.5% formaldehyde-agarose gel.

In addition to the isolation of total RNA from various plant species, Norgen's Plant/Fungi RNA Purification kit was also used to purify total RNA from various fungal species **(Figure 7)**. Total RNA was isolated from 50 mg of wet fungus samples of Botrytis cinerea, Alternaria tenuissima, Rhizopus oryzae, Penicillium sp. and Fusarium oxysporum. The protocol was performed exactly as was stated for the purification of RNA from plant samples. As demonstrated in Figure 7, the RNAs purified by Norgen's protocol forfungi were of a high quality and yield. Furthermore, Norgen's kit again isolated true total RNA, including small RNA species.

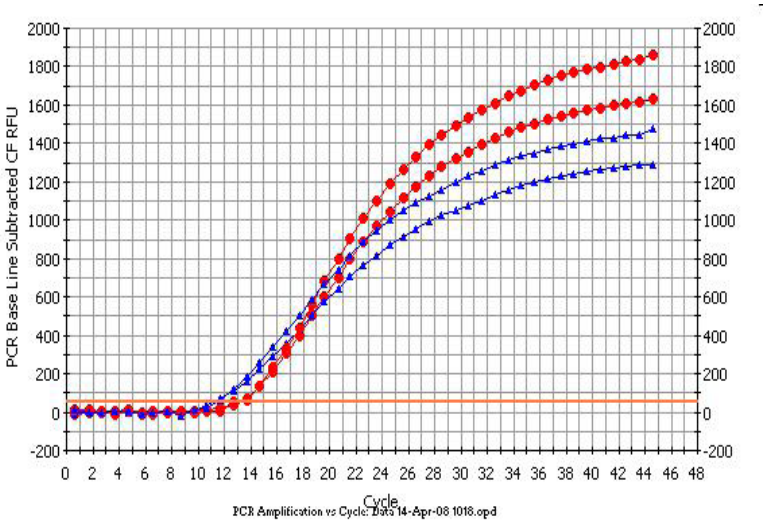

Figure 7. Detection of Plum Pox Virus in Peach Leaves. Total RNA was isolated from two 50 mg samples of peach leaves using Norgen's Plant/Fungi Total RNA purification kit and 1 µL (red) or 3µL (blue) of the RNA from each sample was used in an RT-PCR reaction for the detection of plum pox virus. Plum pox virus was detected from both samples and for both input levels, indicating that the RNA is of a high quality and that the kit is highly sensitive for total RNA isolation.

The quality of RNAs isolated from the fungi by Norgen's RNA purification kit was further demonstrated by capillary gel electrophoresis **(Figure 8)**. RNA purified from Botrytis cinerea, a pathogen of major economic importance in the grape and wine industry, was resolved on the Agilent Lab on-a-Chip. Similar to what was observed for plant RNA, all RNA species, including microRNA, 18s rRNA and 28s rRNA can be observed. This further demonstrates the utility of the Norgen Plant/Fungi RNA Purification kit for the isolation of true total RNA from both plant and fungal species.

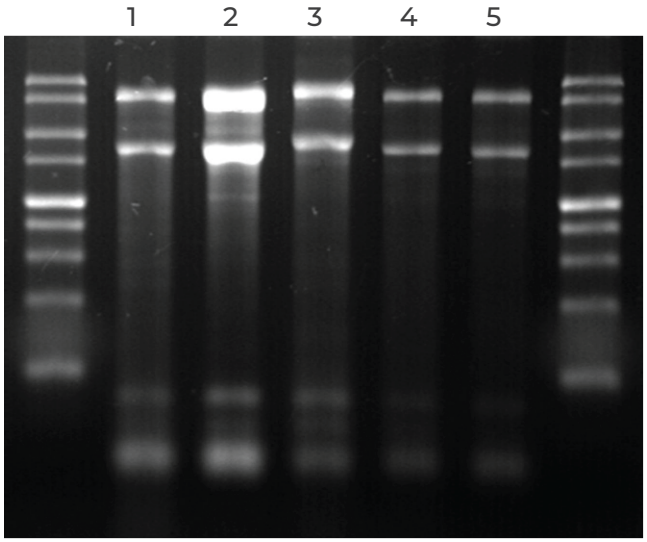

Figure 8. Resolution of total RNA purified from different fungal species on a 1X MOPS, 1.5% formaldehyde-agarose gel. Total RNA was purified from Botrytis cinerea (1), Alternaria tenuissima (2), Rhizopus oryzae (3), Penicillium sp. (4) and Fusarium oxysporum (5) using Norgen's Plant/Fungi RNA Purification kit. Five µL of each 50 µL elution was loaded onto a 1X MOPS, 1.5% formaldehyde-agarose gel. Norgen's kit was able to successfully isolate true total RNA from all fungi tested, including small RNA species.

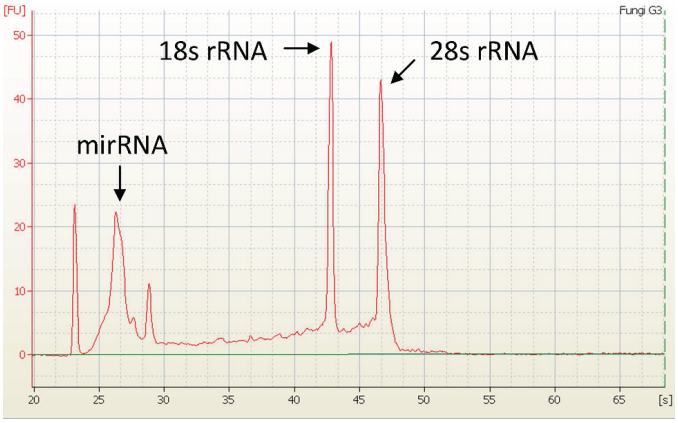

Figure 9. Resolution of fungal RNA on Agilent Bioanalyzer. Total RNA was isolated with Norgen's Plant/Fungi RNA Purification kit from Botrytis cinerea, one of major economically important pathogens in grape industry, resolved on an Agilent Lab-on-a-Chip and electropherograms were generated. It showed high RNA purity as well as integrity.

CONCLUSIONS

Through analysis of the performance of Norgen's Plant/Fungi RNA Purification Kit in the isolation of total RNA from both plants and fungi, a number of conclusions regarding Norgen's kit can be made:

1. Norgen's kit allows for the isolation of high quality total RNA within 30 minutes, and unlike other commercial kits does not require the use of liquid nitrogen for homogenization of samples, making the RNA purification rapid and convenient;
2. Norgen's kit isolates RNAs of high yield, purity and integrity from a wide range of plant and fungal samples. In addition, all sizes of RNA are isolated from large mRNA down to microRNA, without the use of phenol or chloroform. As the RNA is of the highest quality it can be used in a number of downstream applications, including real time PCR;
3. In addition to the purification of total RNA from fresh or frozen plant tissues such as leaves, the successful isolation of total RNA from fruit tissue was demonstrated with as little as 50 mg of input material.
4. It was further demonstrated that viral RNA can be successfully isolated with the total plant RNA and for this reason RNA samples purified using Norgen's kit can be used for the sensitive detection of viral pathogens.

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