Compatibility of DNA and RNA Extraction Methods for Challenging Plant Species

Eric Sonke [3], Nezar Rghei [1], Yousef Haj-Ahmad [1][2] and Won-Sik Kim [1]

(1) Norgen Biotek Corp. 3430 Schmon Parkway, Thorold, Ontario, Canada, L2V 4Y6, (2) Brock University, 500 Glenridge Avenue, St. Catharines, ON, Canada, L2S 3A1, (3) Department of Anatomy and Cell Biology, Schulich School of Medicine and Dentistry, Western University London, ON, Canada.

ABSTRACT

Frequent failure of downstream applications with DNA and RNA is often related to poor sample preparation. In particular, it is often difficult to extract DNA and RNA from samples that contain high levels of phenolic compounds, polysaccharides, volatile compounds and starch in an acceptable quality to be used in sensitive downstream applications such as PCR, RFLP and sequencing. Three different plant DNA and RNA isolation methods were validated to isolate genomic DNA and total RNA from challenging plant species, including raspberries, grapes, pears, pine needles and strawberry leaves. In the results, the relative yield of DNA and RNA, handling time and quality are systemically compared.

DNA Isolation

Grape

Raspberry

Pine needle

Strawberry

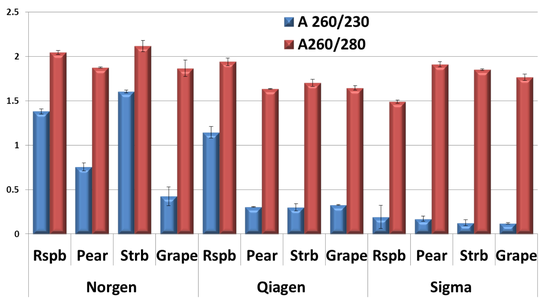
Pear

RNA Isolation

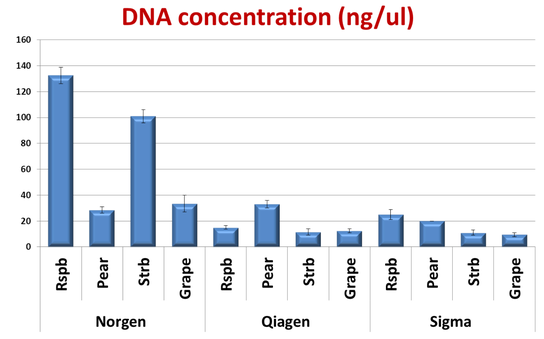
Company	Kit	Input volume	Filtering column	Column activation	Wash solutions	Elution	Handling time (Min.)
Norgen	Plant/Fungi DNA isolation	50 mg-100 mg	Yes	Not required	Wash solution **I** & **II**	100 ul	~30 min/ 10 samples
Qiagen	DNeasy Plant mini	100 mg	Yes	Not required	Wash solution **AP3** and **AW**	100 ul	~ 30 min /10 samples
Sigma	GenElute Plant Genomic DNA miniprep	100 mg	Yes	Yes	Wash solution	**Pre-warmed (at 65˚C)** 100 ul	~ 30 min /10 samples

Company	Norgen Biotek	Qiagen		Sigma
Kit	Plant/fungi total RNA purification kit	RNeasy Plant mini kit	miRNeasy	Tri-Reagent
Resin	Silicon Carbide (SiC) column	Silica column	Silica column	N/A
Phenol	No	No	**Yes**	**Yes**
Processing Time	< 30 min. (10 samples)	< 30 min. (10 samples)	< 30 min. (10 samples)	1 hour

Proven DNA quality and quantity

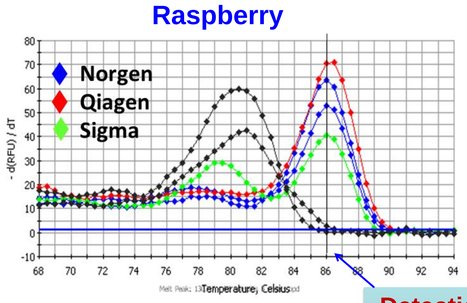


DNA concentration (ng/ul)

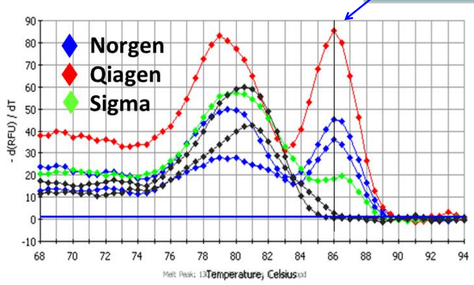


18S rDNA detection in a Real-time PCR (SYBR Green)

Raspberry

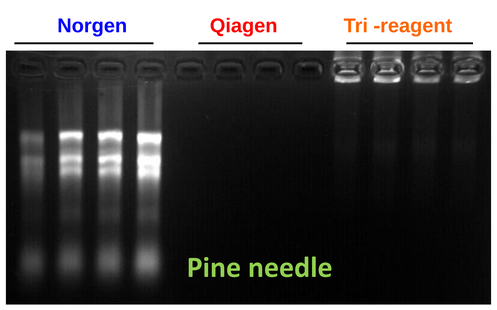


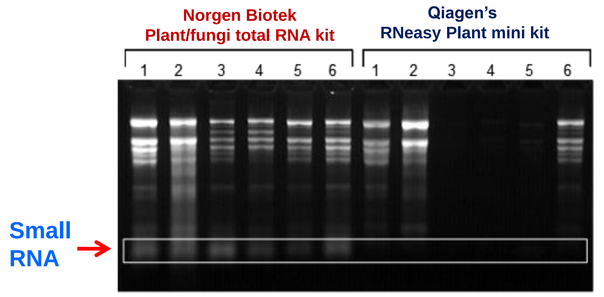
Strawberry



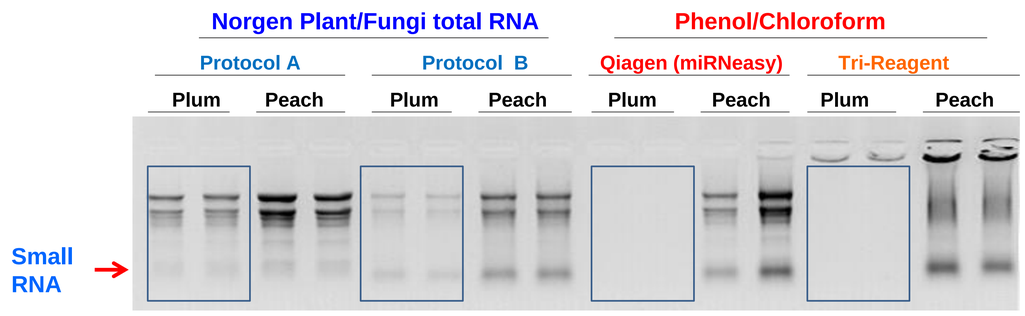
Detection Tm (86˚C)

True compatibility and total RNA profile from challenging plant species

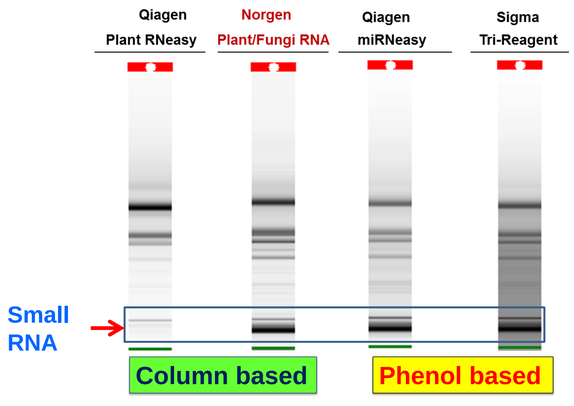




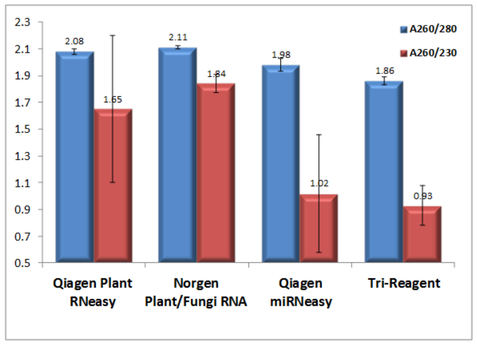
Lane 1 = apple, Lane 2 = peach, Lane 3 = grape, Lane 4 = pine needle, Lane 5 = strawberry, Lane 6 = pear.



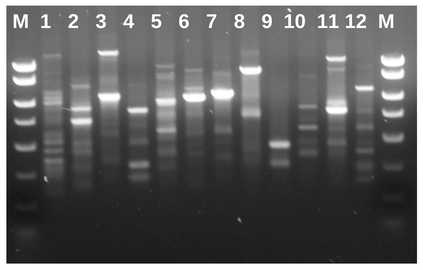
RNA Quality analysis using Bioanalyzer and spectrophotometer



RNA quality (peach)



Application : Random Amplification of Polymorphic DNA(RAPD) analysis



- DNA was isolated from *Prosopis cineraria* using Norgen's Plant/Fungi DNA isolation kit.
- RAPD requires a high DNA quality to generate the band profile that discriminates amplification pattern for the species or sub-specie grouping.
- 12 RAPD primers were successfully amplified the DNA fragments.

3430 Schmon Parkway, Thorold, ON L2V 4Y6 Canada
Phone: (905) 227-8848 | Toll Free: 1-866-NORGENB (667-4362) | Fax: (905) 227-1061 | email: info@norgenbiotek.com