The Importance Of Sample Preparation For Plant miRNA Purification

Won-Sik Kim[1] and Yousef Haj-Ahmad[1,2]

[1]Norgen Biotek Corp, 3430 Schmon Pkwy, Thorold, ON, L2V 4Y6

[2]Brock University, 500 Glenridge Ave., St. Catharines, ON, L2S 3A1

MicroRNAs are endogenous 20 to 24 nucleotide noncoding RNAs that play crucial posttranscriptional regulatory roles in plant and animals. Tremendous efforts are currently being undertaken to understand the profile of the entire miRNA population of a biological sample, which will provide useful information on miRNA activity. Many miRNA discovery tools, including micro arrays and Next-gen-based sequencing, have made it possible to comprehensively and accurately assess the entire miRNA repertoire. This poster deals with the importance of sample preparation on downstream applications. A prerequisite for obtaining successful results from these approaches is an efficient method for total RNA purification without bias. The choice of the method of RNA purification is critical to the outcome of downstream analysis. This is made more significant in variations of the plant specimens and the high phenolics, starch and other inhibitors co-isolating with the RNA. The most popular RNA purification methods (spin columns using Silicon Carbide, spin columns employing silica membrane and phenol/chloroform extraction) are compared in this poster in terms of quality, quantity and recovery of small RNA from difficult and moderately challenging plant samples.

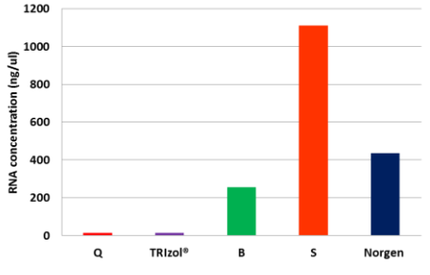
Methods & Materials

To validate the small RNA purification method, five commercially popular kits were compared side by side. Small RNA was purified from *Vitis vinifera Chardonnay*, a challenging plant sample that is economically important for its virus and viroid studies.

	Q	TRIzol®	B	S	Norgen
Input (fresh plant leaves)	50 mg	50 mg	50 mg	50 mg	50 mg
Need B-mercaptoEtOH ?	Yes	Phenol /chloroform extraction	No	Yes	No
Lysate incubation	1-3 min. at 56˚C	N/A	10 min. at RT	3-5 min. at 56˚C	5 min. at 55˚C
Filtration column	Yes	N/A	No	Yes	Yes
Wash I	Yes, 1x		Yes +EtOH, 1x	Yes, 1x	No
Wash II	Yes , 2x		Yes_EtOH 2x	Yes, 2x	3x
Pre-heated (65˚C) elution buffer	No	No	Yes	No	No

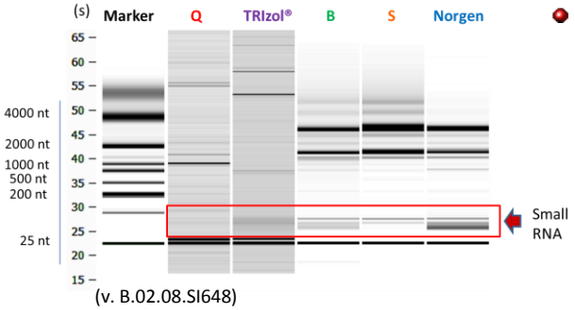
Results & Discussion

- RNA quality and quantity were compared. Kit S gave the best RNA yield, followed by Norgen's kit and Kit B among the compared kits. However Kit Q and even Trizol® (phenol/chloroform) failed to purify RNA from the grape leaves.



RNA Kit	Grape tissue	A260	A280	260/280	260/230
Q	Young leaf	0.01	-0.003	-3.22	0.16
		0.064	0.038	1.66	0.07
TRIzol®	Young leaf	3.49	4.207	0.83	0.2
		9.397	10.088	0.93	0.68
B	Young leaf	7.453	3.185	2.34	1.99
		6.621	2.974	2.23	1.83
S	Young leaf	22.58	10.653	2.12	2.09
		17.333	8.206	2.11	2.21
Norgen	Young leaf	6.051	2.961	2.04	1.71
		10.046	4.873	2.06	1.83

RNA Nano chip



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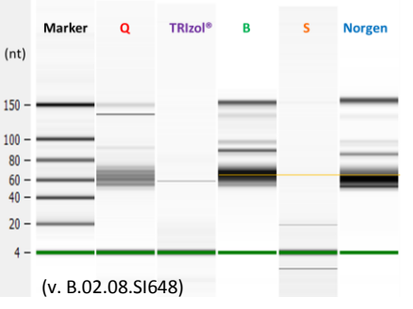
- However, the Agilent Bioanalyzer results revealed that the highest yield of small RNA (<100 nt) was purified using Norgen's Plant/Fungi Total RNA Kit (no phenol/chloroform required).

- Norgen's kit purified the highest yield of small RNA and miRNA based on Agilent Bioanalyzer analysis, compared to the other methods compared.

Small RNA chip



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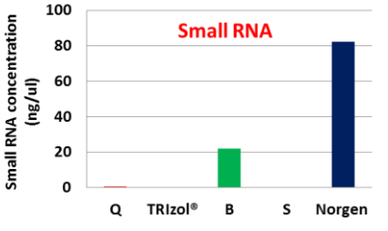
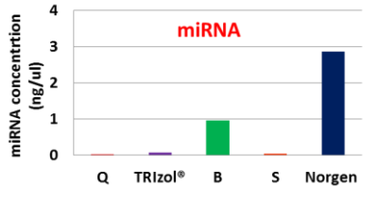
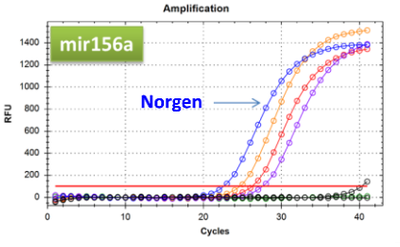
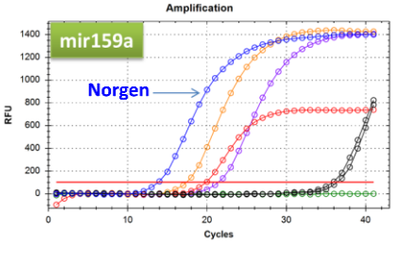




- Two miRNA genes (miR 156a and miR 159a) were sensitively detected from the RNA purified using Norgen's Plant/Fungi Total RNA Purification Kit.

Method	miR 156a	miR 159a
Q	26.26	19.91
TRIzol®	N/A	N/A
B	24.70	17.40
S	27.60	21.41
Norgen	22.61	13.86
NTC	N/A	36.39
	40.29	35.87





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

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