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Full Paper

ILLUMINA®TRUSEQ® VS NEBNEXT® SMALL RNA LIBRARY PREPARATION KIT FOR MIRNA PROFILING IN *PERSICARIA MINOR*: WHICH BETTER?

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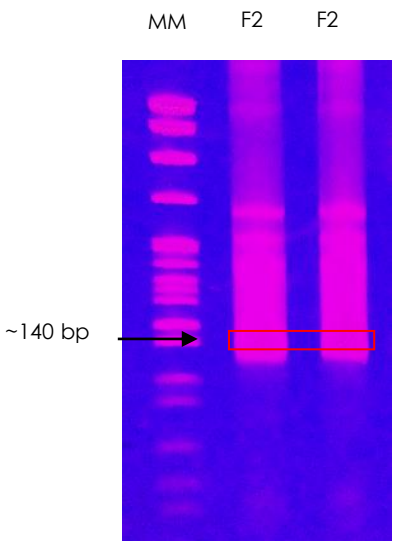
Graphical abstract



Abstract

In plants, a group of non-coding small RNA (sRNA) has been proven to be an important player in regulating gene expression that can govern network of genetic systems. The two major classes of sRNA which are very extensively studied through deep sequencing, microRNA (miRNA) and small-interfering RNA (siRNA) classes, are well documented. However, the isolation method of sRNA differs depending on the type of sample. Here, we demonstrate the miRNA library preparation using two different Small RNA Library preparation kit, Illumina®TruSeq® Small RNA Preparation and NEBNext® Multiplex Small RNA Library Preparation kit on a plant rich in secondary metabolite *Persicaria minor* using recommended protocol. The result show NEBNext® Multiplex Small RNA Library Preparation kit can recover small RNA better than Illumina®TruSeq® Small RNA Preparation kit. Thus, this study recommended NEBNext® Multiplex Small RNA Library Preparation kit for miRNA library preparation on *Persicaria minor*.

Keywords: Persicaria minor, next qeneration sequencing, Illumina®TruSeq® Small RNA Preparation kit, NEBNext® Multiplex Small RNA Library Preparation kit, miRNA

1.0 INTRODUCTION

MicroRNA (miRNA) have been discovered to play an important role in regulating gene expression. Thus, several methods were developed to enable the miRNA discovery from various biological samples. Basically, miRNA have been recognized using three methods: cloning, forward genetics and bioinformatic approaches then validated by experimenting [1]. The most straight forward method for identifying the miRNA is through cloning method by isolating and cloning small RNA. This method is commonly used to discover miRNA in plant [2, 3, 4, 5, 6, 7, 8]. Currently, the most advance technique for miRNA study is Next Generation Sequencing (NGS). These methods can yield the highest miRNA content, which is up to millions read to profile miRNA expression [9]. A technique was introduced in 2005 when the concept sequencing-by-synthesis technology introduced by 454 Life Sciences was published [10]. After the breakthrough, many types of NGS platforms have been developed such as SOLiD™ (Life Technologies Corporation, Carlsbad, CA,

USA), and Illumina Genome Analyzer (Illumina, Inc., San Diego, CA, USA). Subsequently, this approach not only useful to generate a bunch of miRNA data, but also enable to quantify expression levels of miRNA.

Persicaria minor formerly known as *Polygonum minus*, and locally known as kesum is a herb and aromatic plant that commonly available in Malaysia, produces various of secondary metabolites. Conventionally, *P.minor* can be used to cure dandruff and indigestion, and also as a postnatal tonic [11]. The capability of this plant to produce unique flavor and aroma make it suitable to use in local food. This capability may have attributed by the secondary metabolites available in the plant [12]. In addition, the secondary metabolites available in the plant had showed antimicrobial, antioxidant, antifungal, antiviral, and antiulcer activities [13, 14]. Some of the secondary metabolites identified in *P.minor* exist in the form of essential oil with decanal (24.36%) and dodecanal (48.18%) as the two main aldehydes that contribute to the aromatic properties of kesum. Other than the two main aldehyde compound, *P.minor* also contains β-caryophyllene (0.18%), 1-nonanol (0.76%), nonanal (0.86%), 1-undecanol (1.41%), tetradecanal (1.42%), undecanal (1.77%), 1-dodecanol (2.44%), and 1-decanol (2.49%). The abundance of metabolites in *P.minor* make it one of a good candidates in perfume industry and food additive [15]. The abundance of secondary metabolite can interfere with the quality of RNA extracted, thus affect the quality of miRNA [16].
This study is carried out to compare two commercial kit, Illumina®TruSeq® and NEBNext® in constructing miRNA library *for P.minor* since both of the kits work on model plant like *Solanum and Arabidopsis* [17, 18].

2.0 EXPERIMENTAL

Total RNA was isolated using Plant RNA Reagent (Invitrogen) according to protocol recommended for *P.minor* leaves and labelled as F2, C2 and MJ2. Then, the RNA concentration and purity were measured by Qubit and Nanodrop spectrophotometer (ND-1000) respectively. Finally, RNA was subjected to Agilent 2100 Bioanalyzer using RNA 6000 chip to determine the RNA integrity number (RIN).

Only RNA sample, which have RIN over 7, is used in this experiment. Using total RNA as starting material, small RNA library was constructed using, Illumina®TruSeq® Small RNA Preparation kit (Illumina®TruSeq®) and NEBNext® Multiplex Small RNA Library for Illumina (NEBNext®) according to manufacturer's protocols. Both of the workflow of the kit was summarized in Figure 1.

For both kits, small RNA was ligated to 3' adapter and 5' adapter and then 1st strand DNA was synthesized by performing reverse transcription.

Additional step is mentioned by NEBNext® Multiplex Small RNA Library before 5' adaptor ligation to minimize primer dimer formation. The ligated RNA was amplified by PCR and subjected to 6% polyacrylamide gel electrophoresis. Band which showed size in between 145 bp-160 bp for Illumina®TruSeq® Small RNA Preparation kit and 140 bp–150 bp for NEBNext® Multiplex Small RNA Library were sliced and purified. The small RNA libraries retrieved from the gel were analyzed through bioanalyzer using High Sensitivity DNA.

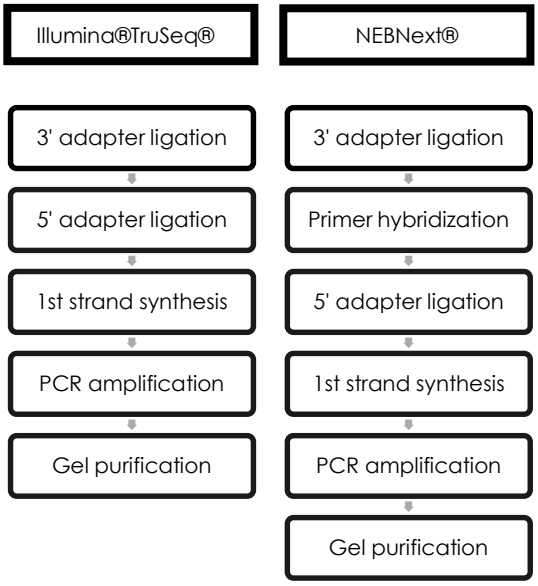


Figure 1 Workflow for Illumina®TruSeq® and NEBNext®

3.0 RESULTS AND DISCUSSION

3.1 Total RNA Extraction

For the purpose of miRNAs study, the first and most important step is to extract high quality total RNA containing miRNA. Choosing an extraction method is very crucial step so that RNA can be extracted efficiently and the degradation of high and low molecular weight RNA can be minimized, because the presence of degraded RNA products in the sample can dilute or mask the miRNAs [16]. Table 1 showed F2 sample have good concentration which is 1860.0 ng/μL measured by Qubit fluorometer. Qubit measurement is more accurate than Nanodrop. Nanodrop measurement is usually higher than Qubit, but Qubit measurement is proven to be highly and consistent with qPCR result prior to NGS [19,20]. For RNA purity, the A260/A280 and A230/A280 ratio value taken from Nanodrop showed the RNA have less protein and phenol contamination as reflected by the reading of 2.01 and 2.06 respectively since those two absorbance reading can detect contamination against protein and organic contaminant like phenol [21].

Figure 2 showed RIN for F2, C2 and MJ2 samples, which are 7.1, 7.3 and 7.6 respectively. According to Illumina®TruSeq® protocol, the starting material must have RIN at least 8. With all the precaution while extracting the RNA for examples working on ice and using RNAse-free material, it's still too difficult to get RIN 8 compared to root or stem part of *P.minor*. So we

decided to use total RNA with RIN above 7 for both Illumina®TruSeq® and NEBNext®.

Table 1 Concentration and purity of RNA sample

Sample	Concentration (ng/µL)	A260/A280	A230/A280
F2	1860.0	2.06	2.09
C2	2168.0	2.00	2.01
MJ2	1685.0	2.00	2.02

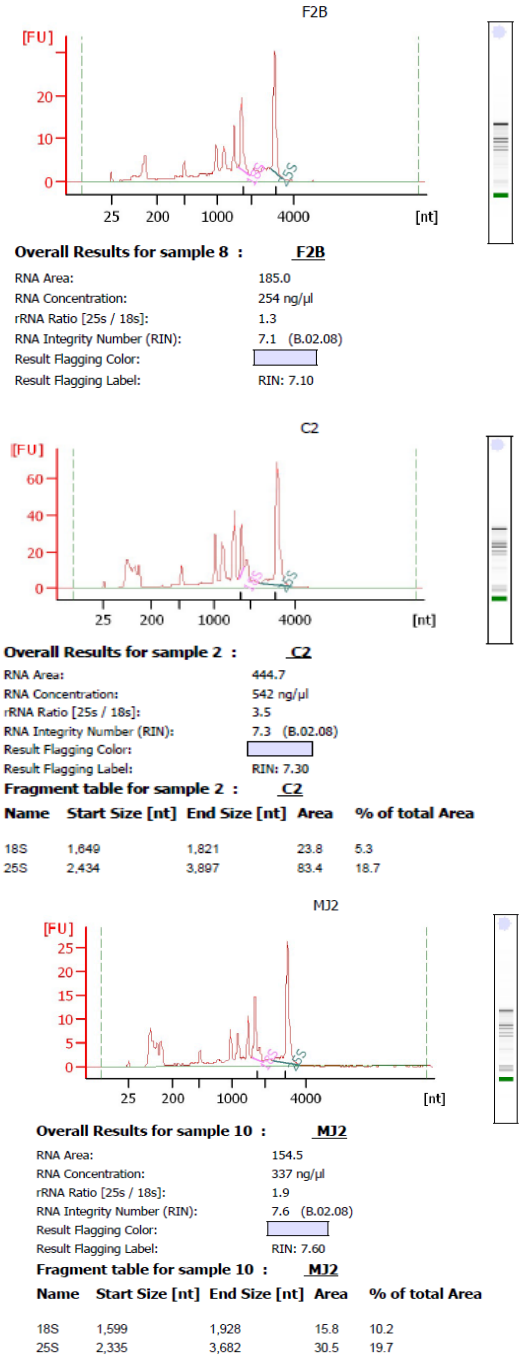


Figure 2 Bianalyzer result for sample F2, C2 and MJ2

3.2 Library Preparation

According to the gel picture in Figure 3 and Figure 4, band with smearing was produced by Illumina®TruSeq® and NEBNext® kits respectively. For Illumina®TruSeq® kit, the gel inside the red box was analyzed through bioanalyzer (not shown) showed that the library was near to the expected size (145 bp-160 bp). However, its concentration was very low, 142.88 pg/µL or 0.142 ng/µL for F2 sample while no peak appear for the rest of the samples. After converting this value into nM, the concentration was only 1.45 nM and not recommended to proceed with sequencing. For NEBNext® kit, the band inside the red box was analyzed and it showed the library peak at 151 bp, so it was considered to be within the expected size range of 140 bp-150 bp and the concentration was 0.673 ng/µL for sample F2, 1.4 ng/µL for sample C2 and 29.61 for sample MJ2. After converting to nM, these samples concentration were 26.64 nM, 12.78 nM and 29.61 nM respectively. Hence it is sufficient to proceed for sequencing since the minimum concentration for sequencing according to Illumina is 4 nM. According to this study, NEBNext® is recommended kit for miRNA library preparation for *P.minor*. This is attributed to the additional step in NEBNext® which is hybridization of excess 3'-adapter after the first ligation reaction. The 3'-adapter hybridization minimizes the 5' and 3' adapter-dimer formation, thus lead to more reads prior to sequencing.

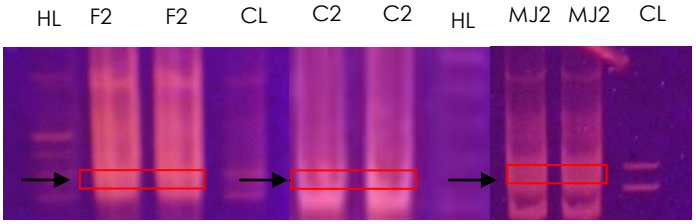


Figure 3 Gel picture showing the product of library preparation using Illumina®TruSeq®. High resolution ladder (HL), miRNA sample (F2, C2 and MJ2), and custom ladder

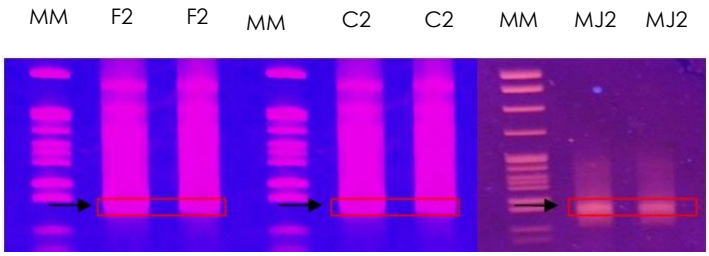


Figure 4 Gel picture showing the product of library preparation using NEB NEBNext®. Molecular marker (MM), and miRNA sample (F2, C2 and MJ2)

4.0 CONCLUSION

Different parameter need to be considered in miRNA Library preparation for NGS. Good quality of starting material need to be prepared so the further step can be proceed confidently. For *P.minor*, good miRNA library can be achieved by using RNA with RIN value of 7 and above. In conclusion, this study recommend NEBNext® Multiplex Small RNA Library Preparation kit for miRNA library preparation of *P.minor*.

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References

[1] Jones-Rhoades, M. W., Bartel, D. P. and Bartel, B. 2006. MicroRNAs and Their Regulatory Roles in Plants. *Annu. Rev. Plant Biol.* 57: 19-53.

[2] Llave, C., Kasschau, K. D., Rector, M. A. and Carrington, J. C. 2002. Endogenous and Silencing-associated Small RNAs in Plants. *Plant Cell*. 14: 1605-1619.

[3] Mette, M. F., van der Winden J., Matzke, M., Matzke, A. J. 2002. Short RNAs Can Identify New Candidate Transposable, Element Families in Arabidopsis. *Plant Physiol*. 130: 6-9.

[4] Park, W., Li, J., Song, R., Messing, J., and Chen, X. 2002. CARPEL FACTORY, a Dicer Homolog, and HEN1, A Novel Protein, Act in MicroRNA Metabolism in *Arabidopsis thaliana. Curr. Biol.* 12: 1484-1495.

[5] Reinhart, B. J., Weinstein E. G., Rhoades, M. W., Bartel, P. and Bartel, D. P. 2002. MicroRNAsin Plants. *Genes Dev*. 16: 16161-1626.

[6] Sunkar, R., Girke, T., Jain, P. K. and Zhu, J. K. 2005. Cloning and Characterization of microRNAs from Rice. *Plant Cell*. 17: 1397-1411.

[7] Sunkar, R. and Zhu, J. K. 2004. Novel and Stress-regulated microRNAs and other smallRNAs from Arabidopsis. *Plant Cell.* 16: 2001-2019.

[8] Xie, Z., Kasschau, K. D., and Carrington, J. C. 2003. Negative Feedback Regulation of Dicer-Like1 in *Arabidopsis* by microRNA-guided mRNA degradation. *Curr. Biol*. 13: 784-789.

[9] Cheng, L., Sun, L., Scicluna, B. J., Coleman, B. M. and Hill, A. F. 2014. Characterization and Deep Sequencing Analysis of Exosomal and Non-Exosomal miRNA in Human Urine. *Kidney International*. 86: 433-444.

[10] Margulies, M., Egholm, M., Altman, W. E., *et al.* 2005. Genome Sequencing in Microfabricated High-Density Picolitre reactors. *Nature*. 437: 376-380.

[11] Baharum, S. N., Bunawan H, Ghani, M. A., Mustapha, W. A., Noor, N. M. 2010. Analysis Of the Chemical Composition of the Essential Oil of Polygonum minus Huds. Using Two-Dimensional Gas Chromatography-Time-Of-Flight Mass Spectrophotometry (GC-TOF MS). *Molecules.* 15(10):7006-7015.

[12] Almey, A. A., Jalal, A., Zahir, S. I., Suleiman, K. M., Aisyah, M. R., and Rahim, K. 2010. Total Phenolic Content and Primary Antioxidant Activity of Methanolic and Ethanolic Extracts Of Aromatic Plants' Leaves. *International Food Research Journal.* 17(4): 1077-1084.

[13] Qader, S. W, Abdulla, M. A. and Hamdan, S. 2012. Potential Biactive Property of Polygonum minus Huds (kesum) Review. *Scientific Research and Essays*. 7: 90-93.

[14] Taiz, L. and Zeiger, E. 2006. *Plant Physiology*. 4th edition. Sinauer Associates, Inc.

[15] Yaakob. 1987. K. B. Kesom oil: A Natural Source of Aliphatic Aldehydes. *Perfumer Flavorist*. 12: 27-30.

[16] Accerbi, M., Schmidt, S. A., De Paoli, E., Park, S., Jeong, D. H. and Green, P. J. 2010. Methods for Isolation of Total RNA to Recover miRNAs and other small RNAs from Diverse Species. *Methods Mol Biol*. 592: 31-50.

[17] Zhang, R., Marshall D., Brayan, GJ., Hornyik, C. 2013. Identification and Characterization of miRNA Transcriptome in potato by High-Throughput Sequencing. *PLoS ONE*. 8(2): e57233.

[18] Creasy, K. M., Khai, J., Borges, F., Van, Ex F., Regulski, M., Meyers, B. C., and Martienssen, R. A. 2014. miRNA Trigger Widespread Epigenetically Activated SiRNAs from Transposons in Arabidopsis. *Nature*. 508: 411-415.

[19] Simbolo, M., Gottardi, M., Corbo, V., Fassan, M., Mafficini, A., Malpeli, G., Lawlor, RT. and Scarpa, A. 2013. DNA Qualification Workflow for Next Generation Sequencing of Histopathological Samples. *PLoS ONE*. 8(6): e62692.

[20] Thermo Fisher Scientific. Qubit Fluorometer® vs. Competitors. [Online]. From: https://www.lifetechnologies.com/my/en/home/life-science/laboratory-instruments/fluorometers/qubit/qubit-fluorometer/qubit-vs-competitors.html. [Acessed on 9 July 2015].

[21] Wolfe, A. R. and Meehan, T. 1994. The Effect of Sodium Ion Concentration on Intrastrand Base-Pairing in Single-Stranded DNA. *Nucleic Acids Res*. 22(15): 223147-223150.