ISSN 1927-5781 (Online)

BioPublisher©

Tree genetics & Molecular Breeding

http://tgmb.biopublisher.ca

2017
TGMB Volume 7

Tree Genetics and Molecular Breeding 2017, Vol.7, No.1, 1-3
http://tgmb.biopublisher.ca

Research Report **Open Access**

The Degree of Purification of mRNA Influences the Fragmentation for Construction Transcriptome Libraries of *Populus*

Lina Wang ✉, Miao He, Yuanxue Zhao
State Key Laboratory of Tree Genetics and Breeding, Northeast Forestry University, 51 Hexing Road, Harbin 150040, China
* These authors contributed equally to this work
✉ Corresponding author Email: wanglina@nefu.edu.cn

Tree Genetics and Molecular Breeding, 2017, Vol.8, No.1 doi: 10.5376/tgmb.2017.07.0001
Received: 25 May, 2017
Accepted: 27 Jun, 2017
Published: 04 Jul, 2017

Preferred citation for this article:
Wang L.N., He M., and Zhao Y.X., 2017, The degree of purification of mRNA influences the fragmentation for construction transcriptome libraries of *Populus*, Tree Genetics and Molecular Breeding, 8(1): 1-3 (doi: 10.5376/tgmb.2017.07.0001)

Abstract The typical workflow of a RNA-seq assay involves the extraction and often further purification of mRNA from tissues; because rRNA reads are not informative it is best to reduce their levels. Fragmentation is essential factors and mostly library preparation protocols use for the detection of libraries, In our experiment the different reagents ratio were used to purify mRNA among those the highly purifies mRNA were used to construct transcriptome libraries. To assess the quality of the mRNA obtained from these methods, the cDNA libraries were analyzed on the Agilent 2100 Bioanalyzer. The option of 2.5 M LiCl binding buffer and 0.1 M LiCl elution buffer combined with 1% of LiDS could thoroughly remove the rRNA and other Impurities to obtain complete, high-purity mRNA molecules. The insights into molecular reactions that our framework allows can be further exploited to improve RNA-seq protocols, as we demonstrate experimentally.

Keywords mRNA; RNA-Seq; Transcriptome library

Introduction

Since Parson (Parsons et al., 1986) confirmed that the poplar can be genetically transformed in 1986, the genetic engineering of trees with poplar as the recipient has made great progress. With the extensive applications and in-depth studies of poplar tissue culture technology, plant cell dedifferentiation and re-differentiation, tissue and organ morphogenesis, various metabolic regulation mechanism, gene expression and regulation have been served in the production practice from multiple sources (Benyó et al., 2016).

1 The Impotant of RNA-seq

RNA-Seq has brought a biological and biomedical revolution by enabling the analysis of genomes, transcriptomes and interactomes to become inexpensive and widespread (Shendure and Ji, 2008, van Dijk, 2013). The cDNA sequencing is considered to be an effective method for functional genomes of non-model species (Metzker, 2010; Yin et al., 2015). Furthermore, cDNA sequencing have the advantages of tissue-specific alternative splicing, identification of new genes and genomic structural variations (Cloonan et al., 2008; Denoeud et al., 2008; Maher et al., 2009). But basically all RNA molecules are jointed with adapters that contain the necessary elements for immobilization on a solid surface and sequencing. In addition, size selection steps are often performed and libraries are usually amplified by PCR.

2 Library Construction is Key Step in RNA-seq

In recent years, seven library construction methods have gradually developed and matured (Head et al., 2014) and RNA sequencing (RNA-seq) has quickly become the standard method for transcriptomics (Wang et al., 2009) and has been further developed into a number of modified protocols that allow detection from tissues (Marine et al., 2011). Several methods have been developed to overcome low concentration and low quality RNA are becoming more and more mature, including RNase (also known as SDRNA) (Sinicropi and Morlan, 2011, Morlan and Sinicropi, 2012), Ribo-Zero (Huang et al., 2011), Duplex-Specific Nuclease (DSN) light normalization (Yi et al, 2011), Ovation RNA-Seq system version 1 (Tariq and Kim, 2011); “NuGEN”, and SMART. Although a great variety of different RNA-seq protocols have been developed, which means to the preparation of nucleic acid into a

form that is math the sequencing platform to be used (Head et al., 2014). Virtually all except for direct RNA sequencing include the basic cDNA production steps of reverse transcription often referred to as first-strand synthesis and second-strand synthesis, which often corresponds to an extended first cycle of the subsequent PCR amplification. The main bottleneck of the transcriptome is library construction, the standard library construction method needs the information of large amount of data, and many obstacles still need to be overcome to apply this technology to woody plants.

3 The Method of Extraction RNA and mRNA

High quality RNA is the basis of biological experiments, However, the extraction of RNA and purification of mRNA are limited e.g. the half-life of mRNA and rRNA were 30s and 20min (Ehretsmann et al., 1992). The content of mRNA in plants accounts for about 5% of total RNA, on the technical level, the construction of transcriptional library is more difficult than that of DNA-seq (Sharma et al., 2012).

In our study, oligo $(dT)_{25}$-coated silica beads were used to extract and purify Mrna (Wang et al., 2007). Although this is a powerful technique, it excludes many non-polyadenylated transcripts other than rRNA (Yang et al., 2011). The main aim of this study is to find out how to extract complete and high-purify mRNA from the leaves of 84k *Populus* and construction a robust library.

4 The Basic Workflow of Library Preparation

The construction of Transcriptome Library: 1) RNA Fragmentation: fragmentation step is necessary to obtain reads covering the entire length of the RNAs. 2) Ligation of 5' adaptor: oligonucleotide adapters are attached to the ends of target fragments. The ligation products are subsequently reverse transcribed. 3) Reverse transcription: reverse transcriptases are that they have a tendency to produce second strand cDNA based on their DNA-dependent DNA polymerase activity. The RNA is converted reverse to cDNA through reverse transcriptase enzyme. 4) PCR amplification: After cDNA synthesis it was further amplified through PCR usually 10 to 12 cycles is often performed to generate sufficient quantities of template DNA to allow accurate quantification and to enrich for successfully adaptered fragments. 5) Size selector: in size section step we removed the free adaptor fragments and also selected the fragments in desirable size. DNase I treatment should be used many times in many parts of the experimental operation throughout the protocol.

5 Conclusion

The well known fact is the quality of sequencing data depends upon on the quality of the sequencing materials. Therefore, the library construction process should guarantee a high molecular recovery of the original fragments in order to achieve the most genomic coverage with the least amount of sequencing. Fragmentation is essential factors and mostly library preparation protocols use for the detection of libraries. The desired library size is determined by the desired insert size during the fragmentation. We compared different purification reagents. The different reagents ratio were used to purify mRNA among those the highly purifies mRNA were used to construct transcriptome libraries. The most suitable reagents for mRNA is shown Wang et al., 2018(doi:gmx130). Testing results of Agilent2100 Bioanalyzer was shown in Figure 1.

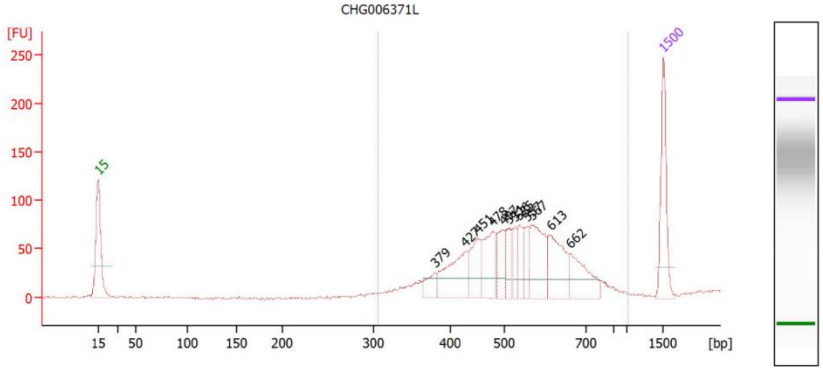

Figure 1 The results of the transcriptome libraries using the suitable purification strategy

Results of the best purification method could thoroughly remove the rRNA, tRNA and other impurities to obtain complete, high-purity mRNA molecules. The discovery of this phenomenon, the summary of the rules and the related purification reagents ratio all these can help us further exploited RNA-seq protocols.

Authors' contributions

L.N.W. conceived, designed and performed the experiments. L.F.W. analyzed the data and supervised the project. M.H. and X.Y.Z. offered statistical analyses. All authors read and approved the final manuscript.

Acknowledgments

This work is supported by Fundamental Research Funds for the Central Universities (No. 2572016BA02), the Harbin applied technology research and development project (Yong Reserve talents Class A) (No. 2016RAQXJ065) and the 111 Project (B16010).

References

Cloonan N, Forrest AR, Kolle G, et al., 2008, Stem cell transcriptome profiling via massive-scale mRNA sequencing, Nature Methods, 5(7): 613-619
https://doi.org/10.1038/nmeth.1223

Denoeud F., Aury J.M., Da Silva C., Noel B., Rogier O., Delledonne M., Morgante M.,Valle G., Wincker P., Scarpelli C., Jaillon O., and Artiguenave F., 2008, Annotating genomes with massive-scale RNA sequencing, Genome Biol., 9: R175
https://doi.org/10.1186/gb-2008-9-12-r175

Ehretsmann C.P., Carpousis A.J., Krisch H.M., 1992, mRNA degradation in prokaryotes, FASEB J., 6: 3186-3192
https://doi.org/10.1096/fasebj.6.13.1397840

Head S.R., Komori H.K., Lamere S.A., et al., 2014, Library construction for next-generation sequencing: overviews and challenges, Biotechniques, 56(2): 61-77
https://doi.org/10.2144/000114133

Huang R., Jaritz M., Guenzl P., et al., 2011, An RNA-Seq strategy to detect the complete coding and non-coding transcriptome including full-length imprinted macro ncRNAs, Plos One, 6(11): e27288
https://doi.org/10.1371/journal.pone.0027288

Marine R., Polson S.W., Ravel J., et al., 2011, Evaluation of a transposase protocol for rapid generation of shotgun high-throughput sequencing libraries from nanogram quantities of DNA, Applied & Environmental Microbiology, 77(22): 8071-8079
https://doi.org/10.1128/AEM.05610-11

Maher C.A., Kumar-Sinha C., Cao X., et al., 2009, Transcriptome sequencing to detect gene fusions in cancer, Nature, 458(7234): 97-101
https://doi.org/10.1038/nature07638

Metzker M.L., 2010, Sequencing technologies-the next generation, Nature Reviews Genetics, 11(1): 1-13
https://doi.org/10.1038/nrg2626

Morlan J.D., Qu K., and Sinicropi D.V., 2012, Selective depletion of rRNA enables whole transcriptome profiling of archival fixed tissue, Plos One, 7(8): e42882
https://doi.org/10.1371/journal.pone.0042882

Sharma S., Mehta R., Gupta R., et al., 2012, Improved protocol for the extraction of bacterial mRNA from soils, Journal of Microbiological Methods, 91(1): 62-64
https://doi.org/10.1016/j.mimet.2012.07.016

Shendure J., and Ji H., 2008, Next-generation DNA sequencing, Nature Biotechnology, 26: 1135-1145
https://doi.org/10.1038/nbt1486

Sinicropi D., and Morlan J., 2015, Methods for depleting RNA from nucleic acid samples: US, US9005891[P]

Tariq M.A., Kim H.J., Jejelowo O., et al., 2011, Whole-transcriptome RNAseq analysis from minute amount of total RNA, Nucleic Acids Research, 39(18): e120
https://doi.org/10.1093/nar/gkr547

Wang C.M., Kim T., Gao D., et al., 2007, Rapid high-yield mRNA extraction for reverse-transcription PCR, Journal of Biochemical & Biophysical Methods, 70(3): 507-509
https://doi.org/10.1016/j.jbbm.2006.10.003

Wang Z., Gerstein M., and Snyder M., 2009, RNA-Seq: a revolutionary tool for transcriptomics, Nature Reviews Genetics, 10(1): 57-63
https://doi.org/10.1038/nrg2484

Yang L., Duff M.O., Graveley B.R., et al., 2011, Genomewide characterization of non-polyadenylated RNAs, Genome Biology, 12(2): R16
https://doi.org/10.1186/gb-2011-12-2-r16

Yi H., Cho Y.J., Won S., et al., 2011, Duplex-specific nuclease efficiently removes rRNA for prokaryotic RNA-seq, Nucleic Acids Research, 39(20): e140
https://doi.org/10.1093/nar/gkr617

Yin D.M., Wang Y., Zhang X.G., et al., 2015, Transcriptome library construction and detection of the peanut seed, Molecular Plant Breeding, 13(2): 431-437