A Method for Isolation of High-quality Total RNA from Small Amounts of Woody Tissue of Scots Pine

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Abstract

Isolation of large amounts of high quality RNA from a limited amount of starting material is particularly difficult if extracting from woody tissues of trees. Often the amount of starting material may be limited, when specific tissues are harvested. There are no commercially available kits specifically developed and intended for isolation of RNA from wood. RNA isolation from wood is difficult in general, and the high amount of resins and other secondary metabolites in wood of Scots pine (*Pinus sylvestris* L.) make the isolation of nucleic acids particularly difficult. Due to the large amounts of resins and other viscous substances in woody tissues of Scots pine, the use of column based extraction techniques is risky because the columns can clog leading to total loss of irreplaceable sample and, in general, the concentration of extracted RNA is low. In our experience, solution-based commercial kits like TRI Reagent (Life Technologies) and cetyltrimethylammonium bromide (CTAB)-based extraction protocols for isolation of RNA from small amounts of wood also did not yield sufficient amounts of RNA for further analysis. Our objective was to develop an improved method for RNA extraction from small amounts of woody tissue of Scots pine (*Pinus sylvestris* L.). We describe a protocol for isolation of RNA using the Thermo Scientific Genomic DNA isolation kit with a modified protocol, which has been used to consistently isolate high quality RNA from small amounts of mature Scots pine phloem and xylem in our laboratory. The extracted RNA is of sufficient quantity and quality for most down-stream applications, including gene expression studies and high-throughput transcriptome sequencing. In addition, the extracted RNA also contained sufficient concentrations of micro RNA and other small RNAs for further analysis.

Key words: RNA isolation, woody tissue, Scots pine, *Pinus sylvestris* L.

Introduction

Extraction of high quality RNA is a prerequisite for determination of gene expression and other functional genetic and genomic studies. Extraction of high quality nucleic acids from plant tissues is more difficult than from animal and human samples due to the tough plant cell wall, and the increased concentrations of secondary metabolites and other compounds in plant cells. These problems are exacerbated, when extracting RNA from recalcitrant material such as woody tissue from mature trees. Often, it is beneficial to collect only a relatively small amount of tissue for RNA extraction in order to minimize the amount of different cell types harvested, and to avoid diluting RNA expression signatures. In our experience, this can lead to use of as little as 30 mg of starting material, and typically less than 100 mg.

Extracted RNA can be described according to concentration, quality and purity. Concentration can be measured by spectrophotometric or spectrofluorometric methods, using RNA-specific binding dyes. The quality can be assessed by gel electrophoresis, or also by RNA Integrity Number (RIN). RIN values range from 1 to 10, with 1 being the most degraded and 10 being the most intact RNA samples. These values are based on the size distribution of RNA molecules determined by microcapillary electrophoresis (Schroeder et al. 2006). Purity of the RNA sample is also important, as various contaminants can inhibit further analysis steps, and DNA contamination can confound PCR and other analyses. DNA contamination is usually assessed by PCR amplification using gene intron-spanning primers that amplify differentially sized fragments from genomic DNA and cDNA. Micro RNAs (miRNA) and other small RNAs have been implicated in the regulation of gene expression, and are increasingly being investigated in plant species (Kruszka et al. 2012). Therefore, an RNA extraction method that enables recovery of both long and small RNAs is beneficial.

Isolation of nucleic acids from mature tree tissues is known to be problematic, and various methods have

been described (Le Provost et al. 2007, Verbylaite et al. 2010, Lorenz et al. 2010, Rai et al. 2010, Siah et al. 2012). In our laboratory, we have utilised methods, which allow the extraction of high quality (RIN > 7) RNA from needles and a large amount (more than one gram) of phloem (Rubio-Piña and Zapata-Pérez 2011). However, commercial kits for RNA isolation and other protocols failed to extract RNA of sufficient concentration and total amount from less than 100 mg of phloem or xylem of mature Scots pine. Protocols employing phenol, for example TriReagent, proved unsuccessful for isolation of RNA from small amounts of woody tissues of Scots pine in our laboratory. Polyphenols can interact with RNA molecules after cell disruption, and the use of phenol in RNA extraction protocols can lead to diminished yields due to these interactions (Schneiderbauer et al. 1991). The best RNA extraction results in our laboratory were achieved using a spin column based Ambion RNAqueous kit. The RNA concentration obtained using this kit can be sufficient but, unfortunately, when extracting RNA from woody tissues, the spin columns can clog, leading to total loss of the sample. In addition, column-based RNA extraction techniques do not efficiently isolate short (<200bp) RNA molecules. Therefore, we developed a high yielding solution-based protocol utilising a Genomic DNA isolation kit (Thermo Fisher Scientific Baltics) with a modified and extended protocol in order to extract RNA of high quality and yield and without DNA contamination from woody tissues of Scots pine. The described method can be used to extract approximately 24 – 48 RNA samples in one day.

Materials and Methods

Fragments of wood (2 × 10 cm) were cut from a mature Scots pine tree at breast height one week after wounding. The samples were placed in liquid nitrogen immediately after collection. Samples of phloem and xylem were cut from the wood fragments for RNA extraction.

For isolation of RNA the Thermo Scientific Genomic DNA purification kit, originally intended for isolation of DNA from 50 – 100 mg of plant tissue, was used. This kit was chosen because in our experience, it delivers constantly good yields of DNA when isolating DNA from xylem of Scots pine as well as other tree species (Lukšienė et al. 2012, Šķipars and Ruņģis 2011, Šķipars et al. 2011). The procedure for isolation of DNA involves sample lysis in lysis solution, phase separation using chloroform, precipitation and wash steps (see manual). The RNA extraction protocol using the Thermo Scientific Genomic DNA purification kit is detailed below, with significant modifications and additions introduced to the original protocol to improve RNA integrity. Each step of the original protocol has been modified and additional steps introduced for the extraction of RNA from small amounts of Scots pine wood tissues. The most significant differences compared to the standard DNA extraction protocol are the use of RNase inhibitor and b-mercaptoethanol. Chloroform was substituted with 1-bromo-3-chloropropane (BCP). BCP is less toxic than chloroform and its use for phase separation decreases the possibility of DNA contamination (Chomczynski and Mackey 1995). In addition, centrifugation times and incubation times have been extended in order to improve the yield.

1. Tissue samples were ground in liquid nitrogen using a Retsch MM 400 ball mill equipped with adapters for 2 ml centrifuge tubes. Samples frozen in liquid nitrogen (LN) were cut into small pieces and placed in 2 ml centrifuge tubes, a 5 mm diameter stainless steel ball was added, the tube closed and placed into LN. The adaptors were chilled in LN prior to the insertion of the tubes into the adaptors. After insertion, the adaptors containing sample tubes were chilled in LN again, and then inserted into the ball mill. The samples were homogenised for two minutes at a frequency of 30 Hz. The adaptors were submerged in LN again and the homogenisation repeated.

2. After homogenisation, 400 ml of lysis solution (provided in the kit) and 200 ml of 1x TE buffer with b-mercaptoethanol (BME) were added. The 1x TE buffer: BME ratio is 1 ml: 40 ml.

3. Samples were vortexed thoroughly and incubated at 65 °C for 30 minutes.

4. After incubation, 600 ml of BCP was added.

5. Samples were vortexed, and centrifuged for 20 minutes at 16,100 g at 4 °C.

6. The supernatant was transferred to new 1.5 ml tubes (it is important to use conical end 1.5 ml tubes otherwise problems could occur in the DNase treatment step when removing supernatant).

7. 800 ml of precipitation solution (provided in kit) was added (prepared by diluting 80 ml of the concentrate with 720 ml of molecular biology grade water).

8. 40 U of RNase inhibitor (Thermo Scientific) were added to the precipitation solution for each sample.

9. Samples were mixed gently, inverting tubes for one minute, and centrifuged for 20 min at > 16,000 g at 4 °C.

10. After discarding the supernatant, the pellet was re-suspended in 100 ml of NaCl solution (provided in kit). It is possible that no pellet is visible at this stage.

11. 10 U of RNase inhibitor were added to each sample.

12. 300 ml of cold (-20 °C) 96% ethanol was added, followed by incubation at -80 °C for one hour. If required, this incubation could also be left overnight.

13.Samples were centrifuged for 20 minutes at 16,100 g at 4 °C.

14. The supernatant was discarded, and 1 ml of cold (-20 °C) 70 % ethanol added.

15. Samples were centrifuged for 20 minutes at 16,100 g at 4 °C.

16. The supernatant was removed, and the pellet was air-dried for two minutes at room temperature.

17. The pellet was re-suspended in 50 ml of 1x TE buffer with 10 U of RNase inhibitor added.

Two methods can be used to remove DNA contamination – DNA digestion by DNase or precipitation of RNA with LiCl. LiCl precipitation is very simple, yet often two or even more precipitation steps could be needed to remove the high amount of DNA, and the concentration of RNA before the precipitation should be at least 200 ng / ml. In addition, the yield of small RNAs will be limited with the use of LiCl precipitation. Treatment with DNase is preferable if there is need to preserve small RNAs or the initial RNA concentration is below 200 ng / ml. Occasionally, the initial level of DNA contamination could be so large that more than one DNase treatment would be necessary. We suggest the use of Thermo Scientific DNase I followed by inactivation by phenol:chloroform:isoamyl alcohol precipitation (25:24:1).

DNase I digestion protocol:

It is preferable to utilise conical ended 1.5 ml tubes because of the small volumes involved, and the use of tubes with a larger diameter at the surface level of the sample could lead to problems in formation of a stable supernatant phase.

1.3 U of DNase I and 5.9 ml of 10x DNase buffer with $MgCl_2$ were added to 50 ml of the RNA sample.

2.4 U of RNase inhibitor were added to the DNase solution for each sample.

3.Samples were incubated for 1 h at 37 °C.

4. 60ml (one volume) of phenol:chloroform:isoamyl alcohol mixture (25:24:1) were added, samples were vortexed, and centrifuged for 20 minutes at 16,100 g at 4 °C.

5.The supernatant was transferred to a new tube.

6.4 U of RNase inhibitor were added to each sample.

7.RNA samples were stored at -80 °C if not used immediately.

The final concentration of RNase inhibitor recommended by the manufacturer is 1 U / ml, however, the final concentrations in the described method were lower, given that the levels of RNases in the solutions were expected to be low.

Initial evaluation of RNA and DNA content in the samples was performed by spectrofluorometry using RNA and DNA specific fluorescent dyes (Qubit instrument and reagents from Life Technologies). DNA and RNA were quantified in the samples before and after treatment with DNAse I. Following DNA removal, the samples were tested for the presence of amplifiable DNA by a PCR assay. The PCR assay for determination of amplifiable DNA contamination (reaction volume 10 ml) was: 1x *Taq* Buffer, 192 nM each primer, 1.5 mM $MgCl_2$, 200 nM dNTP mix, 0.2 U *Taq* DNA polymerase (Thermo Scientific), 1 ml of RNA solution. PCR conditions were: 95 °C 5 min; 40 cycles of 95 °C 30 s, 55 °C 35 s, 72 °C 1 min; 72 °C 5 min. Primers used in this PCR assay were 5'- TGCGCAGGGTCCCTTTG -3' and 5'- CACCAGGGCAGGTGAAGGT -3'. These primers amplify the Scots pine thaumatin-like protein gene (NCBI accession number: JX461338) and span an intron, therefore amplifying different length fragments from genomic DNA and cDNA. PCR products were analysed by agarose gel electrophoresis (1x TAE buffer, 2 % agarose) and visualised using ethidium bromide.

RNA integrity number, RIN (Schroeder et al. 2006) and RNA concentration was determined using the Agilent 2100 Bioanalyzer and the RNA 6000 Nano assay following the manufacturer's protocol. According to manufacturer's instructions the RNA 6000 Nano assay can be used for RNA concentrations ranging from 25 ng / µl – 500 ng / µl, although it is recommended to use at least 50 ng / µl for a meaningful RNA Integrity Number. When using lower concentrations, increased variance of the RIN may be observed between samples. Agilent 2100 Bioanalyzer and the small RNA assay were used to characterise miRNA content in the obtained samples.

Results

RNA concentration

The amount of starting material for RNA extraction, RNA and DNA concentration determined by spectrofluorometry before and after treatment with DNAse I and RNA integrity number and RNA concentration determined using the Agilent 2100 Bioanalyser are shown in Table 1. The RNA samples obtained from phloem (P2-P12) contained higher concentrations of RNA in comparison to the xylem samples. There was a moderate linear correlation between the amount of starting material and RNA concentration in phloem ($r = 0.67$) and xylem ($r = 0.46$) samples. Sample set P2-P6 and X1-X6 was harvested and isolated in November, sample set P7 – 12 and X7 – 12 was harvested and isolated in December following the modified protocol, which includes use of RNase inhibitor. When the correlation of these sample sets is analysed separately, it is clear that either due to the season or other unknown factor the level of correlation between starting amount of material and concentration (thus yield) of obtained RNA sam-

Table 1. Properties of RNA samples obtained in the initial experiment. "conc." is concentration, "nd" is not detected (<1 ng /µl), "nm" is not measurable. Samples P2-P6 and X1-X6 were extracted without the use of RNase inhibitor in the extraction and DNAse treatment workflow

Sample name	Sample mass, mg	RNA conc. before DNAse treatment, ng / µl (Qubit)	RNA conc. after DNAse treatment, ng / µl (Qubit)	RNA conc. after DNAse treatment, ng / µl (Agilent 2100)	RIN	DNA conc. before DNAse treatment, ng / µl (Qubit)	DNA conc. after DNAse treatment, ng / µl (Qubit)
P2	30	156	161	125	4.2	2.82	4.67
P3	60	253	261	221	4.5	4.02	5.59
P4	80	374	358	307	4.3	5.11	6.13
P5	60	304	318	299	4.8	4.58	5.94
P6	100	375	399	374	4.2	13.7	12
X1	40	21.6	22.5	22	4.9	2.65	2.7
X3	20	5.7	9.8	11	3.5	nd	1.67
X4	40	15.3	29.7	31	4.9	1.46	2.69
X5	30	23	27.6	28	4.7	5.34	5.19
X6	100	19	33.7	32	5.6	4.52	4.67
P7	50			134	6.2		
P8	20			73	6.1		
P9	60			67	6.4		
P10	80			166	6.2		
P11	90			190	7.1		
P12	50			200	6.5		
X7	20			3	nm		
X8	50			11	5.7		
X9	60			14	5.9		
X10	70			16	5.3		
X11	100			31	5.8		
X12	90			16	6.2		

ples can vary from moderate (r = 0.58) to very strong (r = 0.94). RNA concentrations in the phloem samples were higher when measured with the Qubit fluorometer compared to the values obtained with the Agilent 2100 Bioanalyser, while in the xylem samples, where RNA concentrations were lower, the concentrations measured by the two methods were more similar (Table 1). One xylem sample (20 mg of starting material) did not yield enough RNA for measurement of RIN (Table 1). All phloem RNA samples, even those extracted from starting material as low as 20 mg, contained RNA concentrations of at least 67 ng / ml after treatment with DNase I. The highest observed RNA concentration was 374 ng/ml (Agilent 2100).

DNA contamination

Using the Qubit dsDNA assay kit, low concentrations of DNA were measured both before and after DNase treatment. In most samples, the measured DNA concentration was in fact higher after DNase treatment. The measured DNA concentrations were also similar for both the phloem and xylem samples, even though the RNA concentrations were much lower in the xylem samples, indicating that the measured DNA concentrations are at the lower limit of detection and are probably a result of background noise. The highest DNA concentration was measured in the sample P6 (13.7 ng / ml). Presence of DNA contamination in the RNA samples was also tested by PCR before and after DNase treatment (Figure 1). Before DNAse treat-

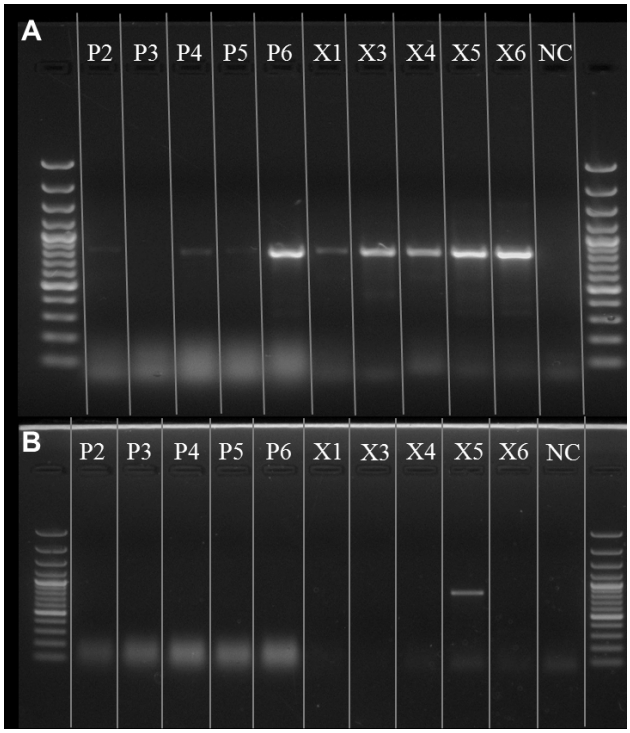

Figure 1. PCR assay of DNA contamination. A is PCR products from untreated RNA samples. B is PCR products from samples treated with DNAse I. NC is no template control, lanes on left and right contain GeneRuler 100 bp Plus DNA ladder (0.5 µg / lane). Presence of a band at ~ 850 bp indicates presence of DNA contamination in PCR template

ment, DNA contamination was detected by PCR in almost all samples, with the exception of sample P3 (possibly due to the presence of PCR inhibitors). The substitution of BCP for chloroform resulted in a smaller amount of DNA contamination (data not shown), which could be due to the formation of a more stable interphase containing the DNA. After DNase treatment, no DNA contamination was detected in any of the samples, with the exception of sample X5. The presence/absence or intensity of PCR fragments was not correlated with the concentration of DNA measured with the Qubit fluorometer. Xylem samples contained comparatively high amounts of contaminating DNA prior to DNase treatment.

RNA quality

Another important parameter of RNA samples besides concentration is the quality of RNA, which determines the range of applications that the extracted RNA can be used for. As RNA is very labile in comparison to DNA, degradation of RNA can occur rapidly, with severe impacts on the quality of the RNA. The integrity of RNA samples can be determined qualitatively by gel electrophoresis, or quantitatively by determination of the RIN using the Agilent 2100 Bioanalyzer. The higher RNA concentrations in the phloem samples can be clearly seen in comparison to the xylem samples (Figure 2). Lanes 11 and 12 represent samples P3 and P4 prior to DNase treatment. When no RNase inhibitor was used, the average RIN value for phloem samples was 4.40 and the maximum difference in RIN values between DNase-treated phloem RNA samples was 0.6. The xylem RNA samples had a lower RNA concentration and a higher variation in RIN values (average = 4.72, max. = 5.6; min. = 3.5). Modification of the RNA isolation protocol by addition of RNase inhibitor to the precipitation solution, NaCl solution, TE buffer and DNAse treatment increased the RIN values of DNase treated phloem RNA samples to an average value of 6.42 (range 6.1-7.1). The average RIN value of xylem RNA samples was increased to 5.78 (range 5.3 - 6.2). The inter-sample variation of RIN values for both phloem and xylem samples was low – the differences between samples with highest and lowest RIN values are 0.9 (X7 - X12) and 1.0 (P7-P12).

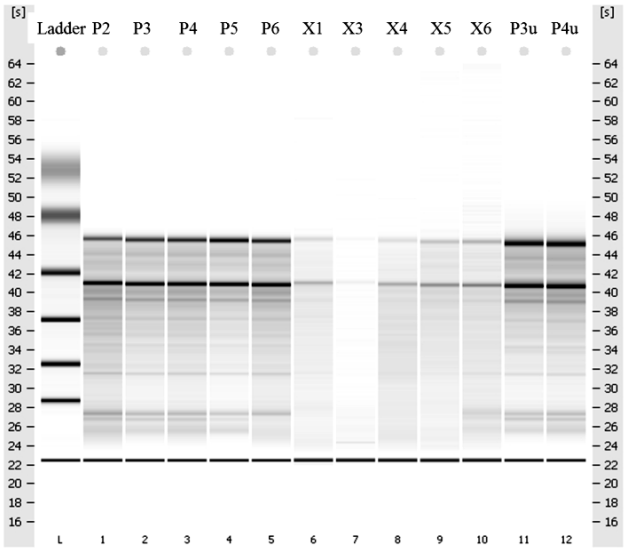

Figure 2. "Gel image" view of electropherograms of microcapillary electrophoresis of RNA samples in the Agilent 210 Bioanalyzer. The lower band present in all samples (at approximately 22 s) is the electrophoresis marker. The marker and the ladder are included in RNA 6000 Nano total RNA kit. The band at approximately 41 s represents 18 S and the band at 46 s represents 25 S ribosomal RNA subunit.

Micro RNA

In addition to analysis of total RNA, small RNA concentrations in the samples (P7 - P12) were analysed with the Agilent 2100 Bioanalyser. The proportion of small RNAs was 8-25 % (average 13.5 %) (Table 2). The minimal proportion of miRNA is 0.5% for downstream use in miRNA sequencing on the Ion Torrent platform without the need for miRNA enrichment, according to the Ion Total RNA-Seq Kit v2 protocol.

Table 2. Results of measurements of miRNA contents and concentration in phloem samples using Agilent 2100 Bioanalyzer and the small RNA assay

Sample name	Concentration of miRNA in sample, pg / µl	miRNA content (%)
P7	2869.90	14
P8	1981.20	25
P9	1161.60	12
P10	2302.40	11
P11	3536.50	8
P12	1651.10	11

Discussion

This report describes a robust method for extraction of RNA of appropriate quality and quantity for downstream applications such as qPCR and transcriptome sequencing from woody tissues of Scots pine. The robustness of this method is illustrated by the correlation between amount of starting material and RNA concentration in obtained samples, the low variation of RIN values between samples. Using the described method it is possible to obtain RNA of utilisable amount and quality from a small starting amount of woody tissues, which are recalcitrant to RNA extraction.

RNA concentration

The amount of extracted RNA from both phloem and xylem should be sufficient for many further applications. The minimal concentration for library construction for RNA sequencing (transcriptome analysis) using the IonTorrent platform is 50 ng / ml using the Ion Total RNA-Seq Kit v2 (Life Technologies). The differences in measurements of RNA concentration using different instruments may have been due to technical differences in the RNA measurement methods. Pipetting errors may also be a factor, as one ml of sample is measured using the Agilent 2100 instrument while two ml of sample were measured using the Qubit instrument.

DNA contamination

DNA contamination was successfully removed by DNase digestion (with the exception of one sample). The reason for DNA contamination remaining in this sample was not immediately obvious. The presence of DNA contamination should be tested using PCR, as this is ultimately more accurate and sensitive than measurement of DNA concentrations in RNA by fluorometric methods.

RNA quality

The RIN values obtained for the RNA samples extracted using the described protocol indicate that the quality of the RNA is sufficient for applications such as gene expression analysis using both intercalating dye- and probe-based qPCR. It has been reported that RIN values of higher than five indicate good total RNA quality and RIN values exceeding eight indicate ideal RNA quality for downstream applications (Fleige et al. 2006). It has also been reported that normalisation of gene expression data using endogenous controls reduces the influence of RNA degradation on results, indicating the significance of normalisation. Caution is still necessary when relying on endogenous controls for normalisation as poor integrity of RNA can increase the variability of qPCR results of these controls (Vermeulen et al. 2011). Yet, to our knowledge, there are no strict criteria stating minimum RIN values for accurate qPCR gene expression analyses. Optiz et al. (2010) suggest that a RIN of 5 and higher can be used successfully in human gene expression profiling by microarray analysis with some specific precautions. In addition, there are reports of RIN-based gene expression data normalisation (Ho-Pun-Cheung et al. 2009, Viljoen and Blackburn 2013). The RIN values obtained using the method described in this report are quite uniform for the phloem RNA samples. RNA concentrations extracted from xylem samples were lower and had a higher variation in RIN values, which could be due to the structural heterogeneity of xylem tissue. Requirements for RNA to be used for preparation of whole transcriptome libraries using the Life Technologies Ion Total RNA-Seq Kit v2 are high RNA quality and RNA concentration of at least 50 ng / ml. Our improved protocol matches or nearly matches these criteria. RNA quality and yield may possibly be further improved by increasing RNase inhibitor concentration in the RNA isolation steps, increasing EDTA concentration during the lysis stage and use of thermo stable RNase inhibitors during the lysis step. Increased temperature and presence of divalent metal ions can facilitate the hydrolysis of phosphodiester bonds between ribonucleotides (AbouHaidar and Ivanov 1999), and usually EDTA is used to remove divalent metal ions. The Lysis solution from the Genomic DNA purification kit – TAE buffer mixture contains EDTA, but the concentration is not given. Another possible source of RNA degradation during the lysis step is activity of heat-tolerant RNases. A heat-tolerant RNase inhibitor like RNasin Plus (Promega) could provide additional inhibition of RNases during the tissues lysis step. However, inhibition of RNA degradation prior to the lysis step is difficult, as woody tissue is extremely hard to cut and some thawing of the samples is inevitable.

Micro RNA

The described RNA extraction protocol also allows for the isolation of small RNAs, suitable for miRNA sequencing. Analysis of miRNAs is often combined with sequencing of messenger RNA, and expression analysis of both types of RNA enables a more thorough insight into molecular genetic processes and interactions. The advantage of the described extraction protocol is that both longer and short RNA fragments are efficiently recovered.

Comparison with other methods

The most direct comparison of this method with previously described extraction methods could be made with the protocol described by Le Provost et al. (2007), which also utilises small amounts (100 – 200 mg) of forest tree species tissue for RNA extraction. Unfortunately RIN values were not utilised in this study and data showing the relationships among sample mass, tissue type and corresponding RNA concentration (or total amount) and presence / absence of DNA are not shown. Siah et al. (2012) compared different methods for isolation of RNA from mature wood tissue, clearly showing that a method well suited for one species might not be appropriate for other species. Kiefer et al. (2000) refered to unsatisfactory results obtained with commercial kits and described a

CTAB-based protocol in conjunction with a commercial kit for extraction of RNA from recalcitrant plant tissues. These reports, describing the efficacy of a particular RNA extraction protocol in a range of species, indicate that the amount and quality of extracted RNA can vary widely between species, and that RNA extraction protocols must be optimised for each species and tissue type.

Conclusion

We describe an RNA extraction protocol based on the Thermo Scientific Genomic DNA purification kit, which enables the isolation of a large amount of RNA without genomic DNA contamination from small amounts of phloem tissue and acceptable amounts of RNA from a small amount of xylem tissue harvested from mature Scots pine trees. The quality of the extracted RNA is sufficient for most down-stream applications, including real-time PCR analysis and high throughput transcriptome sequencing.

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МЕТОД ВЫДЕЛЕНИЯ ВЫСОКОКАЧЕСТВЕННОЙ ОБЩЕЙ РНК ИЗ МАЛОГО КОЛИЧЕСТВА ДРЕВЕСИНЫ СОСНЫ ОБЫКНОВЕННОЙ

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Резюме

Выделение больших количеств высококачественной РНК, используя ограниченное количество исходного материала, особенно затруднительно, если исходный материал – древесина. Часто количество исходного материала ограничено, когда образец должен содержать специфические ткани. Использование коммерческих наборов, основывающихся на использовании проточных колон с фильтрами, рискованно из-за тенденции засорения колон, что может привести к потери незаменимого образца, кроме того, обычно концентрация выделенной таким образом РНК очень мала. Наш опыт работы с наборами для выделения РНК на основе растворов не привёл к желаемому результату по количеству и качеству РНК. Цель нашего исследования – разработать улучшенный метод для выделения РНК из малого количества древесных тканей. Мы использовали комплект „Genomic DNA isolation kit" производства компании "Thermo Scientific" с изменённым протоколом для выделения РНК; этот метод показал стабильные результаты, позволяя выделять высококачественную РНК из тканей флоэмы и ксилемы зрелой сосны обыкновенной. Выделенная РНК характеризуется достаточным качеством и количеством и пригодна для большинства видов применения, в том числе для анализов экспрессии генов и секвенирования транскриптома, используя секвенаторы нового поколения. Кроме того, выделенная РНК также содержит достаточные концентрации микро-РНК и других низкомолекулярных РНК для последующих анализов.

Ключевые слова: выделение РНК, ткани древесины, сосна обыкновенная, *Pinus sylvestris*