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# Time and Temperature Requirements for Effective Removal of High Molecular Weight RNA from Winter Wheat Genomic DNA with LiCl

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## ABSTRACT

Numerous protocols are available for the isolation of plant genomic DNA. Often times, these protocols utilize a wide variety of solutions. The primary purpose of this project was to examine the use of LiCl for the removal of RNA contaminants within genomic DNA samples and its dependency on incubation time and temperature. Our results indicate that LiCl is sufficient for the removal of high molecular weight RNA contaminants from genomic DNA. In addition, our results illustrate varying incubation times with LiCl yield minimal differences in the recovery of genomic DNA and the removal of RNA contaminants. Alternatively, different incubation temperatures produce greater variation in the recovery of genomic DNA and the removal of RNA contaminants. The genomic DNA that was extracted using the appropriate incubation requirements was examined for possible use in downstream applications via polymerase chain reaction (PCR).

## INTRODUCTION

Plant genomic DNA isolations can often be a dirty job in the sense that DNA extractions can also extract RNA, which leads to contaminated DNA samples. One major problem with contaminating RNA is that it can inhibit downstream applications that use genomic DNA (Jobes, Hurley et al. 1995). Currently, laboratories are capable of eliminating contaminating RNA from their genomic DNA by using commercial genomic DNA extraction kits or protocols that utilize RNase (Kaufman 1995; Kang and Yang 2004). RNase is a ribonuclease that degrades RNA (Nichols and Yue 2008). One drawback of using RNase is its ability to contaminate laboratory settings. Therefore, RNase could be problematic for laboratories that are dependent on full length RNA isolation for functional analysis of gene expression. In addition, some laboratories do not use genomic DNA extraction kits because of their high costs.

Since some laboratories cannot use RNase to eliminate RNA contamination from genomic DNA, a variety of protocols have been developed to utilize different chemicals to assist in the removal of contaminating RNA. Such methods include the use of differential precipitation of nucleic acids using salts (Jobes, Hurley et al. 1995) or CsCl gradients

(Ausubel 1992; Foster and Twell 1996). In this study, the use of LiCl for differential precipitation of nucleic acids will be examined in order to develop an efficient method for the removal of RNA contaminants. Jobes, in an attempt to remove RNA from a genomic DNA sample, used a final concentration of 2 M LiCl with incubation requirements ranging from –20°C to 4°C with associated incubation times of 1 h. to overnight respectively (Jobes, Hurley et al. 1995). In an attempt to differentially precipitate nucleic acids, we will evaluate the use of LiCl, final concentration 3 M, with incubation requirements ranging from –20°C to approximately 27°C and incubation times of either 10 min. or 20 min.

## MATERIALS AND METHODS

### *Plant Material*

Winter Wheat; Triticum aestivum (cv. Winoka)

### *Genomic DNA Extraction*

Leaf tissue (0.1-0.2mg) was ground in liquid nitrogen and resuspended in 1mL extraction buffer (100 mM Tris-HCl, pH 8.0; 100 mM EDTA; 250 mM NaCl) and 20% SDS was added to a final concentration of 2.5% SDS. Proteinase K (10 mg/mL) was then added to a final concentration of .05 mg/mL and incubated for 1 h. at 55°C. Samples were centrifuged at 4°C for 10 min. at 20,000 g. The supernatant was transferred to a new tube and the pellet was discarded. The supernatant, which contained the total nucleic acid (TNA) and contaminating proteins, was extracted twice with an equal volume of phenol chloroform/ isoamyl alcohol followed by single extraction with chloroform in order to remove contaminating proteins. In each case, separation of the organic from the aqueous layers was achieved by centrifugation at 4°C for 5 min. at 20,000 g. TNA was then precipitated from the resulting aqueous phase with the addition of 3 M sodium acetate to a final concentration of 0.3 M followed by the addition of 0.6 vol isopropanol and incubation on ice for 10 min. The nucleic acid was precipitated by centrifugation at 4°C for 5 min. at 20,000 g. The supernatant was removed and the pellet, consisting of genomic DNA and RNA, was washed with 200 µL of 70% ethanol and centrifuged at 4°C for 5 min. at 20,000 g. The supernatant was discarded and the pellet was dried under a vacuum. In preparation to test for the removal of RNA, the pellet was resuspended in 200 µL of TE (10 mM Tris; 1 mM EDTA). A sample of 8 µL was set aside and three aliquots of 64 µL each were prepared using the remaining volume.

### *Removal of RNA with the use of LiCl*

In order to determine the optimal conditions for the removal of contaminating RNA in the genomic DNA samples, an equal volume of 6 M LiCl was added to each of the previously made aliquots. Samples were then incubated under the following conditions: I. -20°C for 10 min.; II. Ice for 10 min.; III. Ice for 20 min.; IV. 5°C for 20 min.; and V. Room temperature for 20 min. Samples were centrifuged at 4°C for 10 min. at 20,000 g and the resulting pellets were washed with 70% ethanol. The supernatant containing the remaining nucleic acid was processed by the addition of 3 M sodium acetate to a final concentration of 0.3 M and the addition of 0.6 vol isopropanol with incubation on ice for 10 min. The nucleic

acid was pelleted by centrifugation at 4°C for 5 min. at 20,000 g. The supernatants were removed and both the pellets from the LiCl precipitation and the sodium acetate precipitation were washed with 70% ethanol. All pellets were resuspended in 20μL of TE.

### *Spectrophotometric Analysis*

All resuspended pellets, including the TNA pellet, were analyzed using the NanoDrop ND-1000 Spectrophotometer. Samples were diluted with TE to provide similar concentrations before analysis with gel electrophoresis.

### *Gel Analysis*

Integrity of samples was evaluated using a 0.8% agarose gel, TAE (Tris-acetate-EDTA) Buffer, and 0.5 μg of Lambda DNA/EcoR1 + Hind III Marker (New England Biolabs) which was used as the molecular marker.

### *Image J Analysis*

In order to quantify results from gel analysis, Image J was used to generate plots reflecting nucleic acid levels for each sample. Image J is a software program used to analyze images (http://rsbweb.nih.gov/ij/). Percent composition was determined after drawing a baseline on each plot generated by Image J to obtain the areas under the following curves: genomic DNA, high molecular weight RNA, and low molecular weight.

### *Downstream applications-PCR*

PCR was performed using the PrimeSTAR® HS Premix (Takara Bio Inc.). Samples consisted of genomic DNA (50 ng), dNTP (0.2mM), PCR buffer (1mM Mg2+), and PCR primers (0.2 uM of each forward and reverse primers ordered from Integrated DNA Technologies). The control sample contained zero genomic DNA. Cycling was performed with the Applied Biosystems 2720 Thermal Cycler using the following cycling program: 98°C for 7 min.; 35 cycles of 98°C for 30 sec., 54°C for 5 sec., 72°C for 1 min.; 72°C for 7 min.; 4°C for infinity.

## RESULTS AND DISCUSSION

**Table 1.** Spectrophotometric analysis results for TNA.

| Sample ID | ng/ul | A260 | A280 | 260/280 | 260/230 |
|---|---|---|---|---|---|
| TNA-1 | 996.43 | 19.929 | 9.496 | 2.1 | 1.64 |
| TNA-2 | 328.39 | 6.568 | 3.16 | 2.08 | 1.56 |

As seen in Table 1, TNA was successfully obtained as calculated from the A260 readings. The A260/A280 values reflect possible contamination of nucleic acid with proteins or materials that absorb at A280. As observed, the value is approximately 2 indicating that the removal of proteins was efficient. Our protocol also resulted in minimal contamination

with co-precipitants as is reflected by the high values of the A260/A230. In order to further examine the integrity of the genomic DNA, it was necessary to visualize the sample via gel electrophoresis (Figure 1). Gel electrophoresis was performed using a Lambda marker to indicate sizes of genomic DNA, high molecular weight RNA, and low molecular weight RNA.

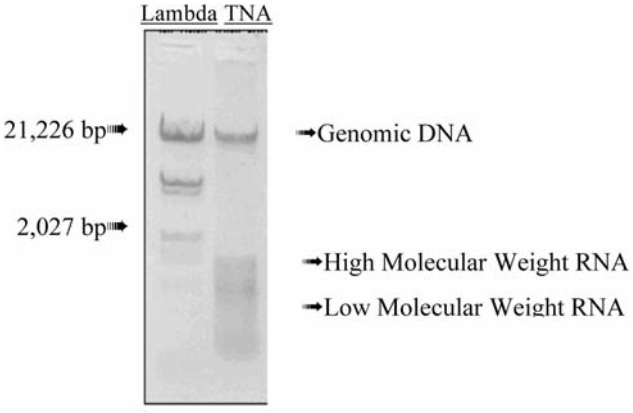


**Figure 1.** Analysis of TNA on a 0.8% agarose gel.

Analysis of the LiCl pellets and resuspended genomic DNA pellets showed numerous implications based on the variables among time and temperature requirements that were tested. Table 2 represents samples after the addition of LiCl that were left on ice for both 10 and 20 minutes along with a sample incubated at -20° for 10 minutes. No major differences could be observed when analyzing the nucleic acid concentrations for the samples left on ice. This implicated that the differences between 10 and 20 min. incubation periods are minimal. Therefore, to improve efficiency in the lab, a 10 min incubation period could be used to achieve genomic DNA isolation.

**Table 2.** Spectrophotometric analysis results of LiCl pellets and final DNA.

| Sample ID | ng/ul | A260 | A280 | 260/280 | 260/230 |
|---|---|---|---|---|---|
| LiCl Pellet-20 min on ice | 933.44 | 18.669 | 8.636 | 2.16 | 2.4 |
| LiCl Pellet-10min on ice | 966.59 | 19.332 | 8.839 | 2.19 | 2.43 |
| LiCl Pellet-10 min at(-20°) | 494.79 | 9.896 | 4.627 | 2.14 | 2.32 |
| DNA Pellet-20 min on ice | 359.18 | 7.184 | 3.681 | 1.95 | 2.35 |
| DNA Pellet-10 min on ice | 280.02 | 5.6 | 2.836 | 1.97 | 2.32 |
| DNA Pellet-10 min at (-20°) | 300.12 | 6.002 | 3.063 | 1.96 | 2.35 |

The sample incubated at -20°C yielded a large amount of genomic DNA. However, incubation at this temperature removed a significantly smaller amount of high molecular weight RNA. In order to improve results in downstream applications, we discourage the use of incubation at –20°C (Table 2). Table 3 demonstrates that incubation on ice for 20 min. removes more nucleic acids after LiCl precipitation, but the resuspended pellet that underwent incubation at 5°C yielded more genomic DNA. This data was also analyzed using gel electrophoresis (Figure 2) and Image J (Figure 4).

**Table 3.** Spectrophotometric analysis results of samples incubated for 20 min. at varying temperatures.

| Sample ID | ng/ul | A260 | A280 | 260/280 | 260/230 |
|---|---|---|---|---|---|
| TNA | 328.39 | 6.568 | 3.16 | 2.08 | 1.56 |
| LiCl pel-ice | 727.64 | 14.553 | 6.646 | 2.19 | 2.29 |
| LiCl pel-5° | 353.49 | 7.07 | 3.247 | 2.18 | 2.33 |
| LiCl pel-room temp | 281.63 | 5.633 | 2.565 | 2.2 | 2.38 |
| Final pel-ice | 163.39 | 3.268 | 1.624 | 2.01 | 2.31 |
| Final pel-5° | 310.12 | 6.202 | 3.127 | 1.98 | 2.26 |
| Final pel-room temp | 34.63 | 0.693 | 0.341 | 2.03 | 2.26 |

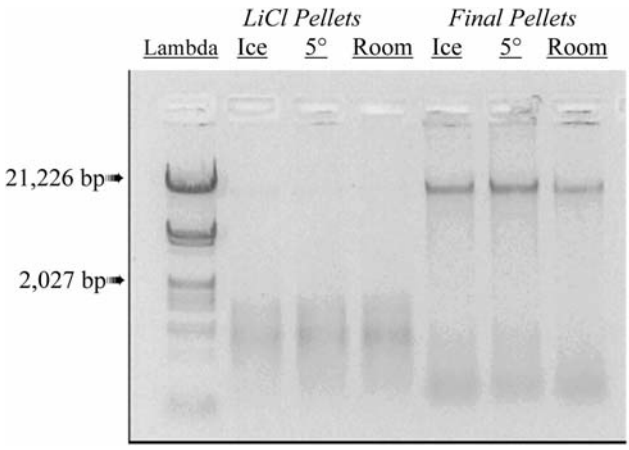


**Figure 2.** 0.8% Agarose gel of samples that underwent incubation on ice, 5°C, and room temperature for 20 min and 0.5µg Lambda DNA marker. Lanes 2-4 are resuspended LiCl pellets. Lanes 5-7 are resuspended genomic DNA.

Based on Figure 2, LiCl does an excellent job at removing high molecular weight RNA and yields samples that contain significant amounts of genomic DNA. Low molecular weight

RNA still remained in many genomic DNA samples (Figure 2). However, incubation at 5°C removed the most low molecular weight RNA.

To quantify results, Image J was used to analyze the nucleic acids for LiCl pellets and resuspended genomic DNA. Figure 3, shows an Image J plot of a TNA sample. A baseline was drawn across each plot to generate composition values. These values were then converted into percentages in order to show their contribution to total nucleic acid concentration (Figure 4).

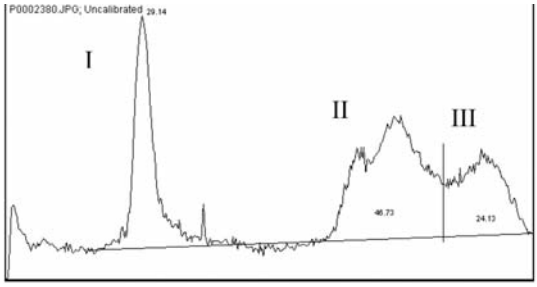


**Figure 3.** Image J plot of TNA composition. Peak I represents genomic DNA. Peak II represents high molecular weight RNA. Peak III represents low molecular weight RNA.

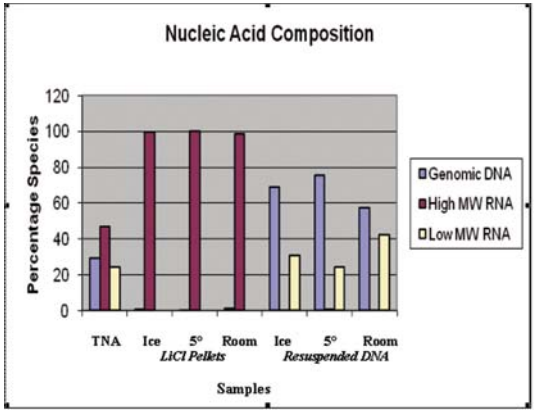


**Figure 4.** Graph showing percentages of nucleic acids yielded from each sample.

To ensure that LiCl treatment can produce genomic DNA that will be useful for downstream applications, PCR was performed using genomic DNA that was achieved using LiCl at 5° for 20 min. It can be seen from these results that the use of LiCl for RNA

precipitation can isolate genomic DNA that can be successfully amplified with the use of PCR (Figure 5).

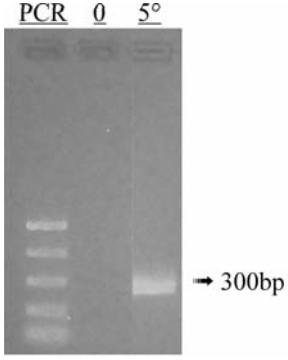


**Figure 5.** Gel analysis of PCR product using 0.15 µg of PCR Marker (New England Biolabs), zero which contained no genomic DNA, and genomic DNA isolation sample from 5°C incubation.

## CONCLUSIONS

A final concentration of 3 M LiCl was successful at differential precipitation of nucleic acids; therefore, allowing one to remove high molecular weight RNA contaminants from plant genomic DNA samples. Based on the results, the most efficient incubation requirements for LiCl treatment consist of 5°C for 10 min. In addition, incubation on ice is also sufficient. Pending the desired quality of genomic DNA, one may not need to incubate at –20°C for 1 h. nor 4°C for overnight (Jobes, Hurley et al. 1995).

## ACKNOWLEDGEMENTS

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