APPLICATION NOTE

Genomic DNA Purification Across Diverse Plant Species

DNA yield, UV absorbance ratios and PCR-based functional validation

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Application Benefits

- **Processing Time**: Less than 60 min.
- **Sample Input**: up to 100 mg fresh tissue or 50 mg dried tissue
- **Typical DNA Yield**: 2–50 µg.
- **Elution Volume**: 100 µL
- **Sample Versatility**: Seeds and fresh leaves from different plant species.
- **Downstream Applications**: PCR, qPCR, DNA digestion and other applications.

Key words
Plant DNA, DNA purification, genomic DNA, seed DNA, plant leaves DNA

Introduction

The isolation of high-quality genomic DNA is a critical step in plant molecular biology, supporting applications such as PCR, genotyping, genetic diversity analysis and DNA sequencing. However, DNA extraction from plant material can be challenging due to the wide variation in tissue composition and the presence of polysaccharides, polyphenols and other compounds that may interfere with DNA purification and downstream enzymatic reactions.

The High-Q™ Spin-Column Plant Genomic DNA Purification Kit from *Tiaris Biosciences* is based on silica membrane technology and is designed to provide a straightforward workflow for isolating genomic DNA from plant samples. Following mechanical disruption and chemical lysis, DNA is purified through selective binding to the silica membrane, followed by washing steps to remove residual contaminants and elution of the purified nucleic acid.

In this application note, the performance of the kit was evaluated using seeds and fresh leaves from different plant species, including Mediterranean tree species. DNA yield and UV absorbance ratios were assessed to characterize the recovered material across different sample types. These results provide an initial assessment of the kit's applicability to diverse plant matrices and its potential to support routine molecular biology workflows.

The protocol begins with mechanical and chemical homogenization of the plant material under denaturing conditions using a specialized lysis buffer (*Fig.1*). This buffer ensures high DNA yield, while the High-Q™ Spin-Columns enable the purification of high-quality DNA suitable for downstream applications.

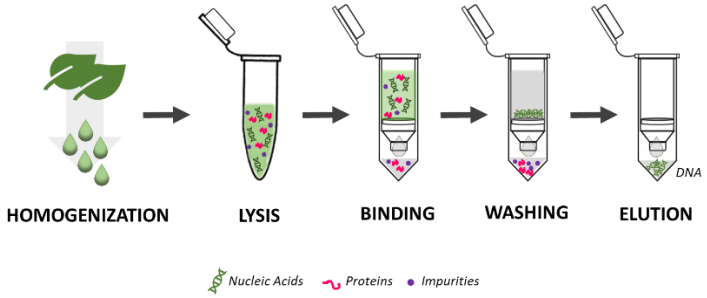

Figure 1. General Workflow of Genomic DNA isolation from plant samples using High-Q™ Spin-Column Plant Genomic DNA Purification Kit.

The kit ensures consistent performance and high reproducibility. The purified genomic DNA is immediately ready to use in molecular biology applications such as PCR, enzymatic digestion, genotyping, sequencing, and more applications.

Optimization Workflow

Plant Material and Genomic DNA Extraction

Genomic DNA was extracted from seeds and fresh leaves representing a diverse range of plant species, including herbaceous plants and Mediterranean tree species (*Table 1*). This selection enabled DNA recovery to be examined across plant materials with different tissue characteristics and potential extraction challenges.

The starting material consisted of up to 100 mg of seeds or 50 mg of fresh leaf tissue per extraction. Samples were processed using the High-Q™ Spin-Column Plant Genomic DNA Purification Kit (*Tiaris Biosciences, Córdoba, Spain*), following the manufacturer's instructions. Purified DNA was eluted in 100 µL of elution buffer. A total of 3 independent DNA extractions were performed per sample type.

Table 1. Plants included as samples for DNA purification

Plants	Scientific name	Seed	Leaves
Wheat (*Triticum aestivum*)		100 mg	50 mg
Asparagus (*Asparagus officinalis*)		100 mg	-
Watermelon (*Citrullus lanatus*)		100 mg	-
Pepper (*Capsicum annuum*)		100 mg	-
Tomato (*Solanum lycopersicum*)		100 mg	50 mg
Spinach (*Spinacia oleracea*)		-	50 mg
Grape (*Vitis vinifera*)		-	50 mg
Carrot (*Daucus carota* ssp *sativus*)		-	50 mg
Holm oak (*Quercus ilex*)		-	50 mg
Almond (*Prunus dulcis*)		-	50 mg
Cork oak (*Quercus suber*)		-	50 mg
Pine (*Pinus* sp.)		-	50 mg
Strawberry tree (*Arbutus unedo*)		-	50 mg
Chestnut (*Castanea sativa*)		-	50 mg

DNA Quantification and Yield Determination

DNA concentration was measured by UV spectrophotometry using NABI microvolume spectrophotometer. DNA yield was calculated from the measured DNA concentration and the final elution volume (100 µL), and expressed as micrograms of DNA per extraction. DNA purity was assessed using the A260/A280 absorbance ratio.

Functional Validation of Purified Plant Genomic DNA

The suitability of the purified genomic DNA for downstream enzymatic amplification was evaluated by PCR targeting the cytochrome c oxidase subunit I (*cox1*) gene. PCR reactions were performed using Stout™ Red PCR Master Mix (*Tiaris Biosciences*), 200 ng of purified genomic DNA per reaction and the primers described by Weller et al, 2000[1]. Primer sequence compatibility with the plant species included in this study was assessed by in silico sequence alignment. Amplification was carried out under the following conditions: 30 cycles with denaturalization at 94ºC, 35 seconds; annealing at 60ºC, 35 seconds and extensión at 72ºC, 12 seconds.

PCR products were separated by agarose gel electrophoresis using a 2% agarose gel and visualized using TiariStain Green Safe (*Tiaris Biosciences*). A DNA molecular weight marker (Tiaris Ruler 50–1000 bp) and a no-template negative control were included in the analysis. The expected amplicon size was 79 bp. Successful amplification was assessed by the presence of a band at the expected size.

Results and Discussion

Genomic DNA Yield isolated from Seeds

High-Q™ Spin-Column Plant Genomic DNA Purification Kit was evaluated using seeds from five plant species. DNA was recovered from all five sample types, although the measured yield varied among species (*Fig. 2*).

The differences in DNA recovery should be interpreted in the context of the plant species. It may reflect variations in tissue composition, cellular characteristics and the accessibility of genomic DNA during sample disruption and lysis.

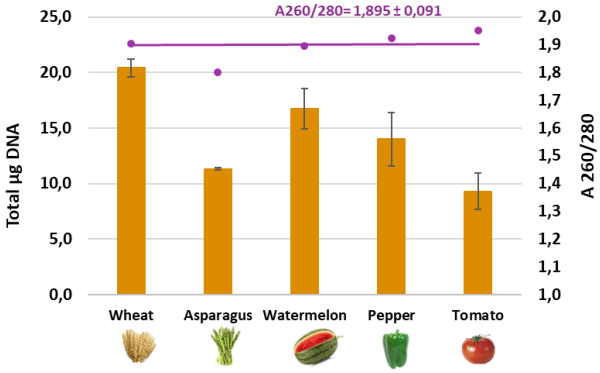

Figure 2. Comparison of Genomic DNA Yield obtained from seeds. *The 260/280 ratio demonstrates the quality of the extracted DNA. The average 260/280 ratio was 1.89.*

Genomic DNA isolated from Fresh Plant Leaves

Genomic DNA was extracted from fresh leaves of wheat, spinach, tomato grape and carrot. DNA recovery was observed for all plant materials, with differences in yield between species (*Fig. 3*).

Leaf tissues can vary substantially in their chemical composition, including their content of polysaccharides, polyphenols and other compounds that may affect DNA isolation[2].

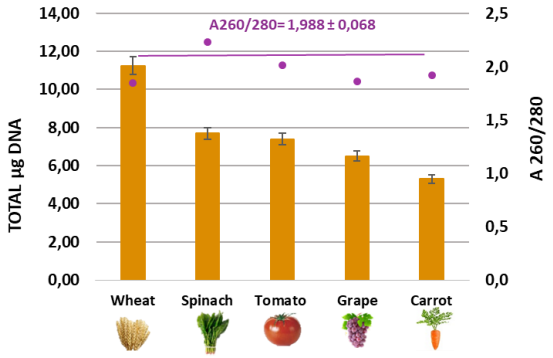

Figure 3. Comparison of Genomic DNA Yield from Leaves. *DNA was extracted from fresh leaves of different plants. The 260/280 ratio demonstrates the quality of the extracted DNA. The average 260/280 ratio was 1.99.*

The observed differences in DNA yield highlight the importance of evaluating purification performance across multiple plant matrices rather than relying on a single model species.

Purification Of Plant Genomic DNA From Different Trees

The versatility of the High-Q™ Spin-Column Plant Genomic DNA Purification Kit was further evaluated using fresh leaves from six Mediterranean tree species.

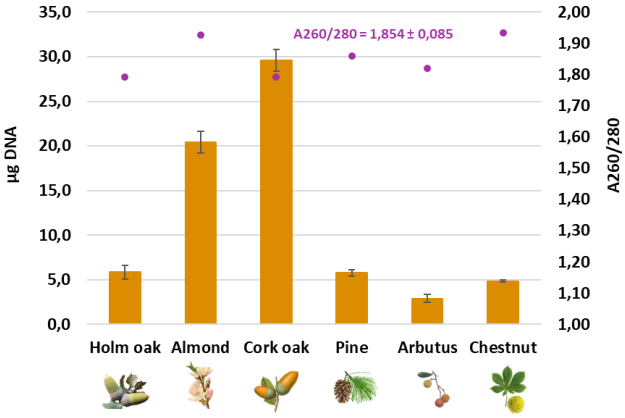

Figure 4. Performance of Genomic DNA Extraction from Leaves of Different Tree Species. *The genomic DNA yield depended on the tree species used. The average 260/280 ratio was 1.85.*

The mean A260/A280 ratio across the tree samples was approximately 1.85. The yield measurements and absorbance ratios provide an initial characterization of DNA recovery from a range of woody plant tissues and support the evaluation of the kit across diverse botanical matrices.

PCR-Based Functional Validation of Purified Plant Genomic DNA

DNA yield and A260/A280 absorbance ratios were assessed, and the suitability of the recovered DNA for PCR amplification was evaluated in selected samples.Successful amplification across the different plant samples provides functional evidence that the DNA purified with the High-Q™ Spin-Column Plant Genomic DNA Purification Kit is suitable for the *cox1* PCR assay under the condition evaluated.

Agarose gel electrophoresis confirmed the presence of the expected-size amplification product in all samples tested (*Fig 5 and data not shown*), demonstrating the suitability of the purified DNA for the PCR assay under the conditions evaluated.

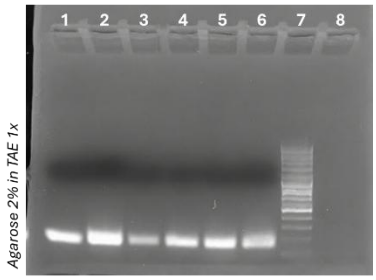

Figure 5. PCR amplification of the cox1 gene using purified plant genomic DNA. *Lanes:* ***1-4****, genomic DNA from seeds:* ***1.*** *Pepper;* ***2.*** *Tomato;* ***3.*** *Wheat;* ***4.*** *Asparagus:* ***5-6****, genomic DNA from leaves:* ***5.*** *Almond;* ***6.*** *Spinach.* ***7.*** *Tiaris Ruler 50-1000 bp;* ***8.*** *Negative Control.*

This result complements the spectrophotometric measurements by demonstrating that the recovered DNA can be used as a template for enzymatic amplification.

Conclusion

The High-Q™ Spin-Column Plant Genomic DNA Purification Kit from *Tiaris Biosciences* enabled the recovery of genomic DNA from a range of plant materials, including seeds, fresh leaves and leaves from different Mediterranean tree species.

DNA yield and A260/A280 absorbance ratios provided an initial characterization of the recovered material. Importantly, PCR amplification of the *cox1* gene produced the expected-size amplicon in all samples tested, providing functional evidence of the suitability of the purified DNA for the PCR assay evaluated.

These results support the applicability of the silica membrane-based purification workflow to diverse plant matrices and its use in plant molecular biology workflows requiring PCR-compatible genomic DNA.

References

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2. Varma A, Padh H, Shrivastava N. (2007) Plant genomic DNA isolation: an art or a science. Biotechnol. J. 2 (3): 386–392.