Promega

Product Application

DNA Purification from Plant Seeds and Leaves on the KingFisher™ Flex

Purify DNA from ground plant seeds and leaves using the Maxwell® HT 96 gDNA Blood Isolation System on the KingFisher™ Flex Purification System.

Kit: Maxwell® HT 96 gDNA Blood Isolation System (Cat.# A2671)

Analyses:

- UV absorbance
- Dye-based quantitation
- qPCR

Sample Type: Ground plant seeds and leaves

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

The method described in this report can be provided upon request, please contact TechServ: **techserv@promega.com** for additional details.

Materials Required:

- KingFisher™ Flex Purification System (Thermo Fisher Scientific, Cat.# 24074431)
- KingFisher™ Deep-well 96 Plate (Thermo Fisher Scientific, Cat.# 95040450)
- KingFisher™ 96 tip comb for DW magnets (Thermo Fisher Scientific, Cat.# 97002534)
- KingFisher™ Flex Run Protocol (Maxwell_HT_gDNA_Seed_Leaf_96_V1.0.bdz)
- Maxwell® HT 96 gDNA Blood Isolation System (Cat.# A2671)
- CTAB Buffer (Cat.# MC1411)
- 95-100% Ethanol, molecular biology-grade
- 100% Isopropanol, molecular biology-grade
- Heat block or incubator set to 70°C

Protocol:

1. Grind seeds and leaves according to standard laboratory procedures (in plate or tubes).
2. Add 1ml of CTAB buffer, 40µl of Proteinase K and 20µl RNase A to each sample and vortex. Optional: A master mix of the above reagents (plus at least 10% overage) can be prepared immediately before sample addition and 1.06ml added to each well.
3. Incubate at 70°C for 30 minutes.
4. Prepare KingFisher™ Deep-well plates during the incubation time, as indicated below:
    a. Plate 1/ Tip Plate: Add KingFisher™ 96 Tip Comb for DW Magnets.
    b. Plate 2/ Elution: Add 110µl of Elution Buffer per well.
    c. Plate 3/ Ethanol Wash: Add 450µl 50% EtOH per well (make fresh daily).
    d. Plate 4 / Wash 3: Add 400µl Wash Buffer (WBA) and 50µl 50% EtOH per well.
    e. Plate 5 / Wash 2: Add 900µl Wash Buffer (WBA) and 100µl 50% EtOH per well.
    f. Plate 6 / Wash 1: Add 900µl Wash Buffer (WBA) and 100µl 50% EtOH per well.
    g. Plate 7/ Bind Plate: Add 365µl of 100% Isopropanol, 300µl Cell Lysis Buffer (CLD), and 25µl of Resin per well. Note: Mix resin thoroughly to resuspend before addition.

5. After the heated incubation step, transfer 300µl of each lysate into Plate7/Bind Plate. Avoid transferring solid material and oil as these materials can inhibit downstream assays. Optional: following incubation, lysates can be vortexed and centrifuged.
6. Start the method "Maxwell_HT_gDNA_Seed_Leaf_96_V1.0.bdz" and load the plates onto the KingFisher™ Flex Purification System.
7. Continue the KingFisher™ Flex Run Protocol until complete (total run time is 1 hour 6 minutes).

Results:

DNA was purified from single ground seeds, 3 or 4 x 3.5mm ground leaf punches or 30mg of wheat flour in a 96 deep-well plate. The 30 minutes heated incubation was done in an Eppendorf ThermoMixer® C using a plate adaptor without shaking. After heated incubation, lysates were homogenized by tip mixes before loading into Plate7/Bind Plate.

DNA was successfully purified from all sample types. Purified DNA was detectable with both absorbance and fluorescence quantitations. All purified DNA was amplifiable with no or low amplification inhibition. The variability over the 8 purification replicates may be due to variability in samples and/or the grinding step.

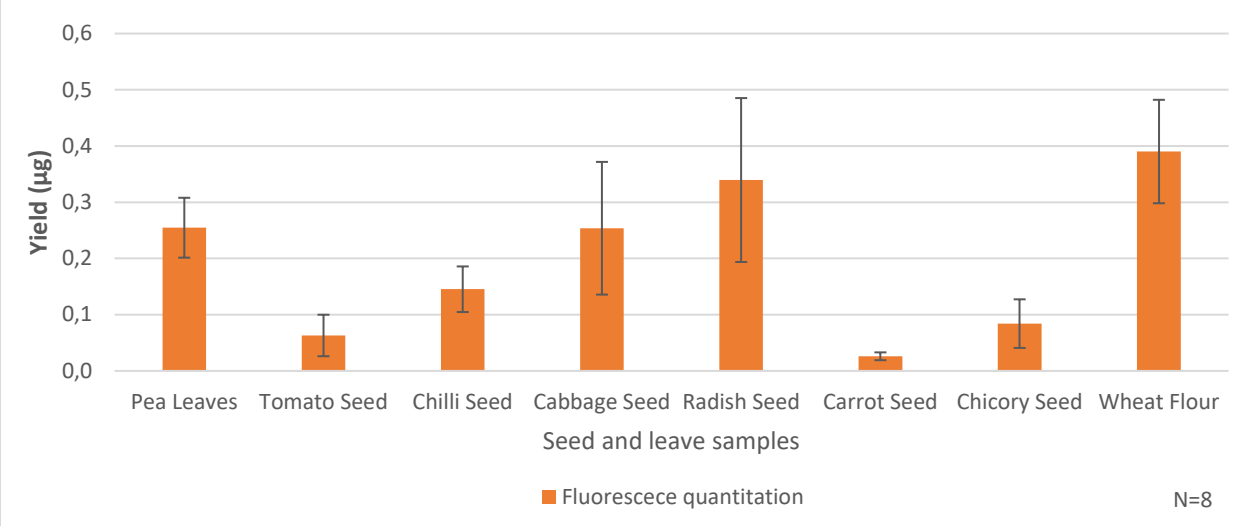

Figure 1. Fluorescence quantitation of DNA purified from plant seeds and leaves using the Maxwell® HT 96 gDNA Blood Isolation System on the KingFisher™Flex instrument. The yields of purified DNA were determined using fluorescence quantitation with the QuantiFluor® dsDNA System (Cat.# E2670) using Lambda DNA as a standard on the GloMax® Discover Microplate Reader (Cat.# GM3000). Average yields were calculated based on concentrations and the recovered volume in the elution plate (~95µl). Average yields ± standard deviation for N=8 are shown.

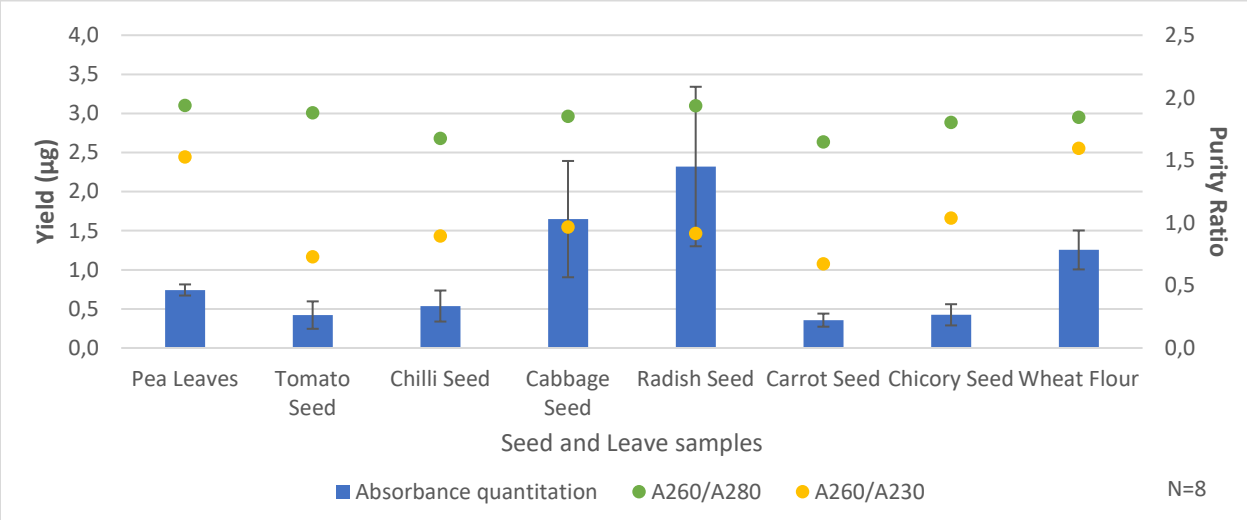

Figure 2: Absorbance quantitation and purity ratios of DNA purified from plant seeds and leaves with using the Maxwell® HT 96 gDNA Blood Isolation System on the KingFisher™Flex instrument. The yields and purity ratios of purified DNA were determined using absorbance quantitation (Tecan Spark® multimode reader). Average yields were calculated based on concentrations and the recovered volume in the elution plate (~95µl). Average yields ± standard deviation for N=8 are shown.

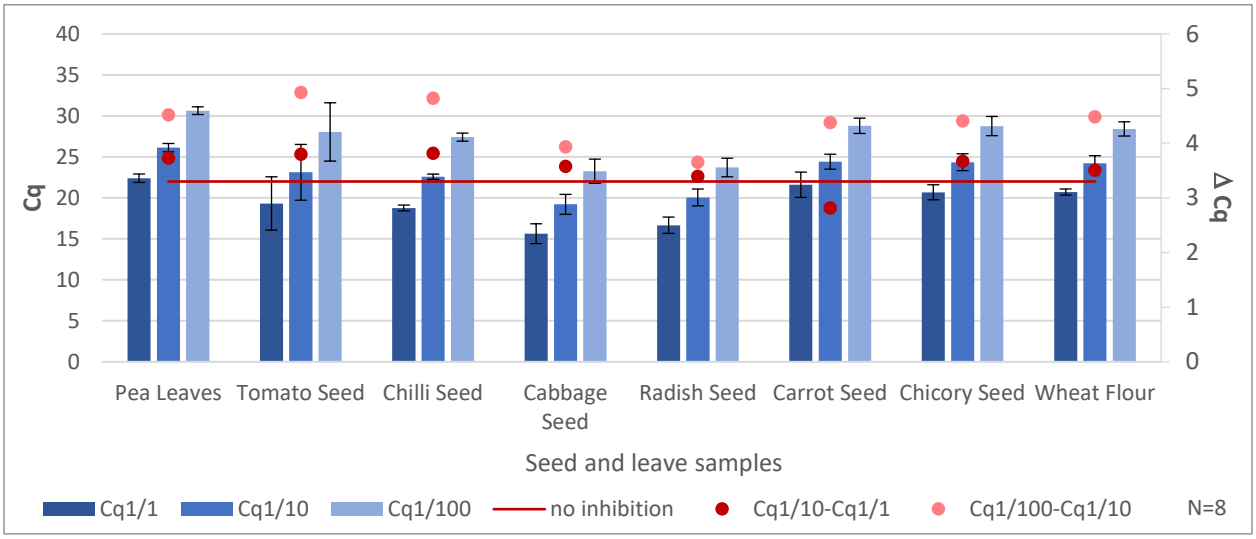

Figure 3. qPCR amplification and inhibition test of DNA purified from plant seeds and leaves using Universal Plant Primers[1]. DNA was purified from seeds and leaves on the KingFisher™Flex instrument. Purified gDNA were diluted (1/10 and 1/100) and amplified by qPCR using Universal Plant Primers[1] and GoTaq® qPCR Master Mix (Cat.# A6001) following TM#318 on a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems™) or a CFX96 Touch™ Real-Time PCR Detection System (BioRad). ΔCq were calculated between Cq values obtained from undiluted eluates (1/1) and the 1/10 dilution and between Cq values obtained from 1/10 and 1/100 dilutions to analyze qPCR inhibition. A theoretical value of ΔCq=3.3 obtained for 10-fold template dilutions indicates no amplification inhibition, represented as a line. Shown are the average values for N=8 ± standard deviation.

References:

1. Wang J, et al., 2011. Universal endogenous gene controls for bisulphite conversion in analysis of plant DNA methylation, *Plant Methods*, **7:39**.