Purification of Plant DNA using QuickPick™ SML Plant DNA kit

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ABSTRACT

The QuickPick™ SML Plant DNA purification kit provides a fast and simple means of purifying genomic DNA from a variety of plants or their organelles. The technique does not require any organic solvents and eliminates the need for repeated centrifugation, vacuum filtration or column separation.

The reagent volumes of the QuickPick™ SML Plant DNA purification kit can be scaled up or down for different sample amounts either with the PickPen® manual tools or with the MagRo™ robotic workstation. The purified genomic DNA is typically at least 30 kbp. DNA fragments of this length denature completely during thermal cycling and can be used downstream applications such as PCR amplifications.

INTRODUCTION

The DNA content varies widely between different plant materials. DNA content depends on the haploid genome size and the ploidy of the sample. For example, *Arabidopsis thaliana* has a small diploid genome and correspondingly lower DNA yields. Nucleic acid yields from young plant tissues are often higher than from old plant tissue, because young plant tissue generally contains more cells than the same amount of older plant tissue. Young plant tissue of the same weight contains fewer metabolites (such as polyphenolics) which can affect the performance of downstream applications. Below is described the purification of genomic DNA from different plant species using various sample amounts and PickPen® 1-M tool. The purifications were carried out following the kit instructions.

PRINCIPLE OF THE QuickPick™ SML PLANT DNA KIT

DNA in the plant tissue sample is released using Proteinase K and Lysis Buffer. Released DNA is bound to the Magnetic Particles in the presence of Binding Buffer. Magnetic Particles with the bound DNA are washed three times with the Wash Buffer. The DNA is then eluted from Magnetic Particles with the Elution Buffer.

MATERIALS & METHODS

Genomic DNA was purified from lettuce (*Lactuca sativa* L. cv. Grand Rapids), barley (*Hordeum vulgare*), potato tuber (*Solanum*) and basilica (*Ocimum basilicum*). Sample amounts were 25 mg, 50 mg and 100 mg. Potato tubers were homogenized with liquid nitrogen using mortar and pestle. Leaves from basilica, lettuce and barley were homogenized with liquid nitrogen using a pellet pestle homogenizer. After homogenization Lysis Buffer, Proteinase K and RNase A (20 mg/ml) were added into homogenized samples according to Table 1.

Samples were lysed at +65^{0}C for 20 minutes and centrifuged for 5 minutes at 18 000 x *g*. The supernatants of the samples were transferred into the tubes containing Binding Buffer and Magnetic Particles and incubated for 10 minutes using end-over-end rotator. Magnetic Particles were collected and washed three times in Wash Buffer using PickPen® 1-M tool. Magnetic Particles were released into Elution Buffer and incubated for 5 minutes. After elution Magnetic Particles were collected from Elution Buffer and discarded. The QuickPick™ SML Plant DNA purification kit reagent scaling volumes for different sample amounts are presented in the Table 1.

Table 1. QuickPick™ SML Plant DNA kit reagent scaling volumes

	25 mg	50 mg	100 mg
Lysis Buffer	37,5 µl	75 µl	150 µl
Proteinase K	2,5 µl	5 µl	10 µl
RNase A	2,5 µl	5 µl	10 µl
Binding Buffer	62,5 µl	125 µl	250 µl
Magnetic Particles	2,5 µl	5 µl	10 µl
Wash Buffer	3 x 100 µl	3 x 200 µl	3 x 500 µl
Elution Buffer	25 µl	50 µl	100 µl

RESULTS

Genomic DNA was purified from potato tuber, leaves of lettuce, barley and basilica using QuickPick™ SML Plant DNA kit and analyzed by spectrophotometry and agarose gel electrophoresis.

A yield comparison from different plant species using various amounts of sample materials is presented in Table 2.

Table 2: Yields of the purified DNA from different plant species using various sample amounts.

Sample	Amount (mg)	Yield (µg)
Lettuce	25	0.5
	50	1.6
	100	3.0
Barley	25	0.5
	50	1.3
	100	3.4
Potato tuber	25	0.2
	50	0.6
	100	1.2
Basilica	25	0.5
	50	1.1
	100	2.5

The overall yield varied between different plant species ranging from 1.2 – 3.4 µg (sample amount 100 mg). An increase of the sample amount from 25 mg to 100 mg resulted in linear increase in the yields of the purified DNA for every plant species (Fig. 1). The purities for all of measured DNA samples were ≥ 1.8 (A_{260}/A_{280}) regardless of the species of plant (data not shown).

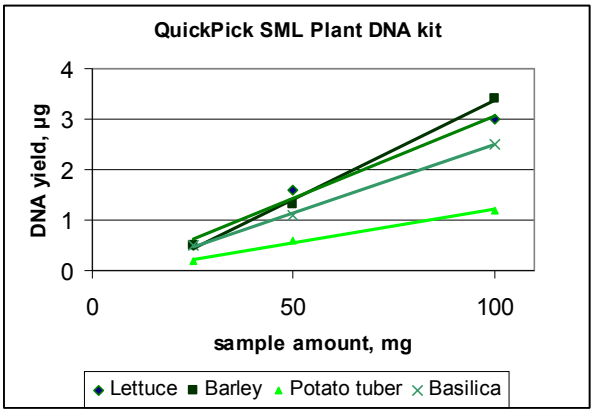

Figure 1. Total yields of the purified DNA from various sample amounts.

The quality of purified DNA was analyzed by agarose gel electrophoresis (Fig. 2).

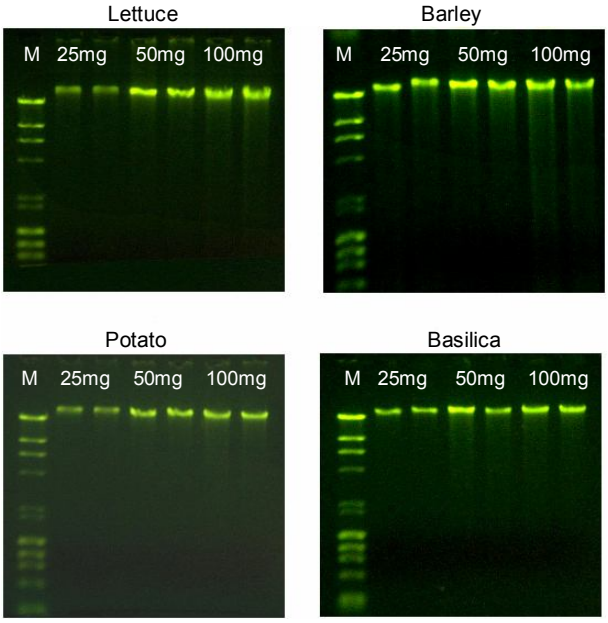

Figure 2. The quality of genomic DNA from different plant species. The eluates were applied into 1% agarose gel using amounts proportional to the original sample sizes. M = molecular size standard (λ DNA / *Hin*dIII).

The purified DNA from all plant species appeared as clear and solid bands on agarose gel confirming that the products are composed of high-quality DNA.

CONCLUSION

According to the results, the QuickPick™ SML Plant DNA kit generates high-quality DNA as purified from different plant species. It is possible to scale the QuickPick™ SML Plant DNA kit up-and-down by using various amounts of samples with different kit reagent volumes.

The QuickPick™ SML Plant DNA kit provides a fast and simple genomic DNA purification method from plant and can be applied to both manual PickPen® tools and the automated MagRo 8-M robotic workstation.