Application note

QuickExtract – Rapid and efficient extraction of PCR-ready genomic DNA from plant and seed samples

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Introduction

The ability to rapidly screen large populations is vital for breeding and characterisation of transgenic plants. Genotyping by methods based on BHQ™ Probes or KASP™ are widespread but generally require nucleic acid purification. A more cost-effective and less time-consuming approach is needed. The QuickExtract™ Plant DNA Extraction Solution provides a simple, rapid DNA extraction method to prepare genomic DNA for high-throughput processing. It is used for leaf or seed samples. The extracted DNA is ready for amplification-based analyses.

The extraction requires less than ten minutes to prepare PCR-quality DNA with two simple heating steps (see Figure 1). The procedure is convenient and can easily be scaled to process hundreds of samples in multi-well robotic automation systems.

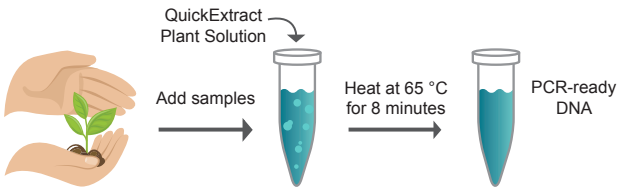

Figure 1. Overview of the QuickExtract workflow. After the two heating steps, the released DNA can be used directly for amplification-based analysis, stored at +4 °C for four weeks, or transferred to -20 °C for archival purposes.

This application note demonstrates the suitability of using the QuickExtract Plant DNA Extraction Solution for multiple plant species. The requirement for grinding is also investigated. Genotyping results are shown for KASP and BHQ Probes. In addition, the effect of storage on the quality of the extracted DNA was determined.

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Materials, methods and results

a) Determination of the impact of grinding

Whole seeds from wheat, tomato, pepper and hulled sunflower were ground or incubated whole with QuickExtract Plant DNA Extraction Solution – 100 µL of QuickExtract Solution was added to tomato and pepper seeds, 200 µL was added to wheat seeds, and 300 µL was used for sunflower seeds. The processed samples were diluted 1:4 or 1:16 prior to PCR amplification with KASP on the IntelliQube™. Genotyping results of the four plant species are shown in Figure 2. Grinding had little to no effect on cluster plot analysis for tomato and sunflower seeds. However, grinding was required for amplification of QuickExtracted DNA from wheat seeds and inhibitory for PCR with pepper seeds. The decision whether to grind a seed sample or perform the extraction on whole seeds must be determined empirically.

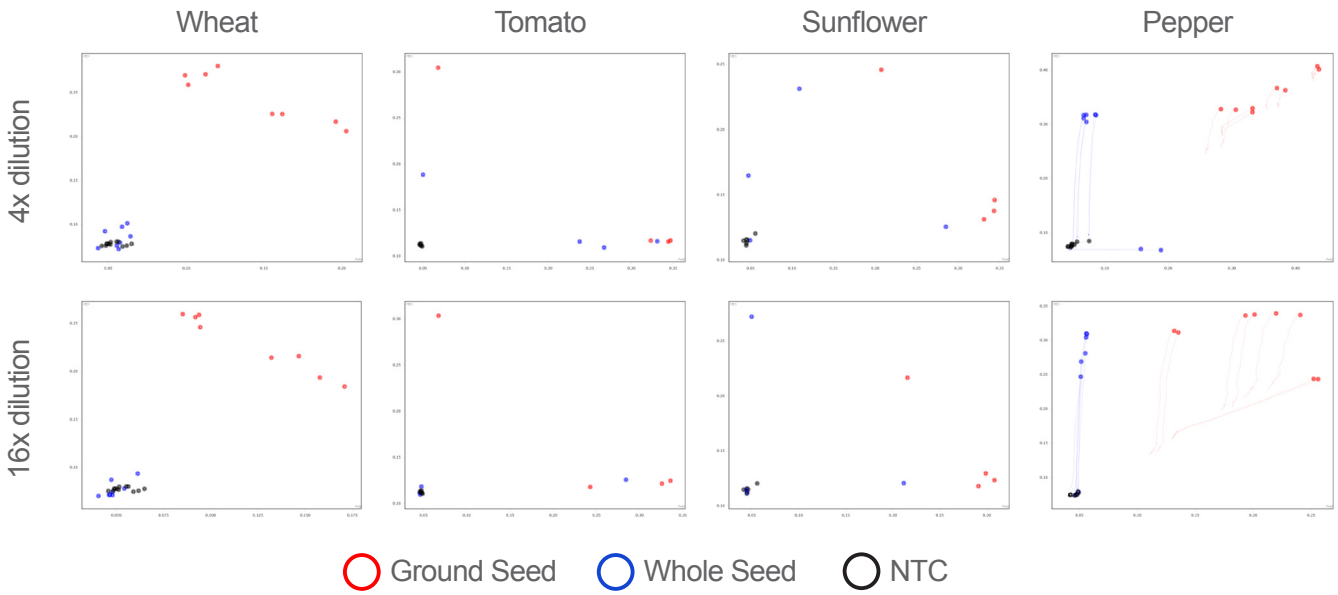

Figure 2. The effect of grinding seed material. Wheat, Tomato, Sunflower and Pepper seeds were ground (red circles) or extracted whole (blue circles) with QuickExtract Plant DNA Extraction Solution. Samples were diluted 4-fold (top) or 16-fold (bottom) prior to PCR amplification with KASP on the IntelliQube. Water was used as a negative control (NTC).

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b) Evaluation of BHQ and KASP chemistries for eight commercially important crop types

QuickExtract Plant DNA Extraction Solution was added to samples of seed or leaf material of eight crop types – Corn, Wheat, Rapeseed, Soy, Tomato, Pepper, Cotton, and Sunflower.

Extracts were incubated as in Figure 1, diluted 2- to 8-fold, and PCR amplified on the IntelliQube with KASP and BHQ chemistries. Table 1 lists the crops tested, sample type, grinding requirements, starting weight of the sample and volume of QuickExtract Plant DNA Extraction Solution used.

Crop	Tissue type	Processing	Grinding required	Mass (mg)	QuickExtract Buffer added (µL)
Corn	Seed	Chipped	Optional	~20 mg	200
				~100 mg	400
	Leaf	Punches	Optional	1-2 punches	100
				5 punches	200
				20 punches	400
Wheat	Seed	Whole	Required	~35 mg	200
	Leaf	Cut	Required	~5-10 mg	100
				~20 mg	200
				~45 mg	400
Rapeseed	Seed	Whole	Optional (Grinding slighly better)	~5 mg	100
	Leaf	Punches	Grinding not tested	~5-10 mg	100
Soy	Seed	Half	Grinding not tested	~80 mg	200
Tomato	Seed	Whole	Optional	~3 mg	100
	Leaf	Punches	Optional	5 punches	200
				15 punches	400
Pepper	Seed	Whole	No Grinding	~8 mg	100
	Leaf	Punches	No Grinding	5 punches	200
				15 punches	400
Cotton	Seed	Whole	Required	~100 mg	200
		Hulled	Required	~50 mg	200
	Leaf	Punches	Not tested	2 punches	200
Sunflower	Seed	Whole	Required	~100 mg	200
		Hulled	Optional	~50 mg	200-300
	Leaf	Punches	Not tested	2 punches	200

Table 1. Sample and pre-treatment of validated crops, seeds or leaves, grinding requirement conditions, starting weight and volume of required QuickExtract solution. The guidelines show how much tissue to use, and whether grinding the sample must be determined empirically for each plant species and tissue type. For reference, one punch is 6 mm in diameter. Additional optimisation may be required.

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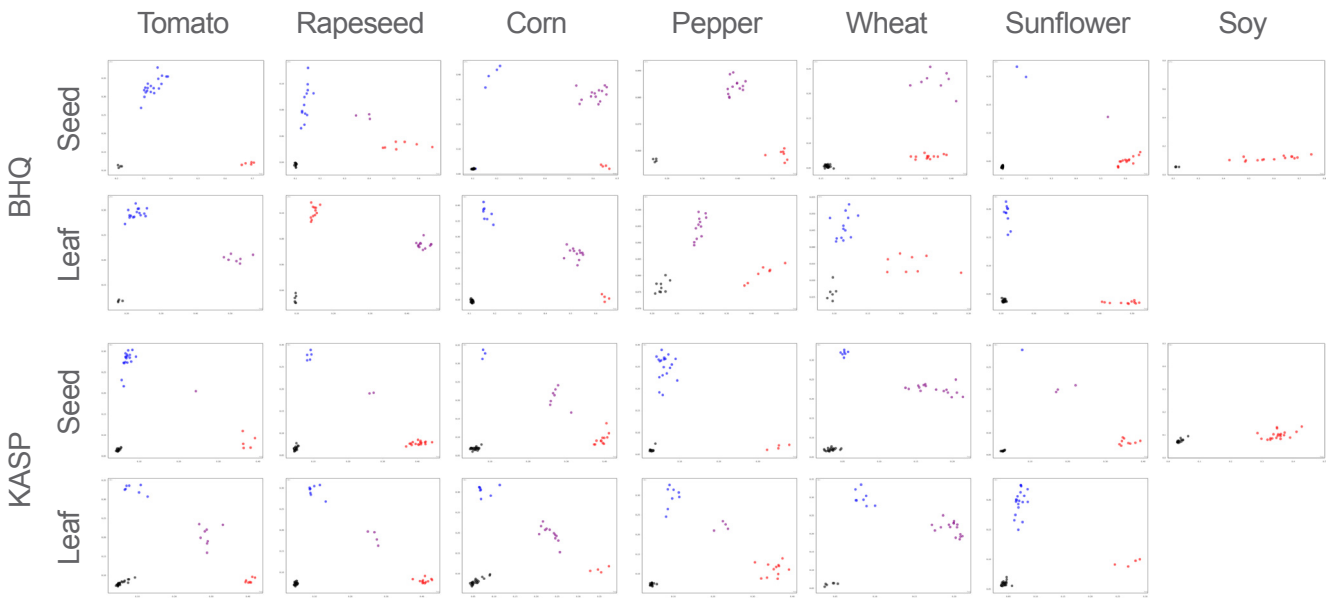

Figure 3. Cluster plots for KASP and BHQ chemistries. Results are shown using seven different crops – Tomato, Rapeseed, Corn, Pepper, Wheat, Sunflower, and Soy. Analyses were performed using KASP and BHQ Probes.

c) Stability of extracted DNA for at least 4 weeks at +4 ˚C

We assessed the stability of the extracted DNA. Corn and tomato leaf samples were processed with the QuickExtract Plant DNA Extraction Solution. The DNA was PCR amplified using two different KASP assays for each crop type. Amplifications were performed immediately after sample processing. Sample plates were stored at +4 °C. After one month of storage at +4 °C the amplifications were repeated. Figure 4 shows a comparison of the genotyping data, using the original QuickExtract Plant DNA Extraction Solution lysate, and the same QuickExtract lysate one month later. No significant difference in endpoint signal or cluster quality was observed.

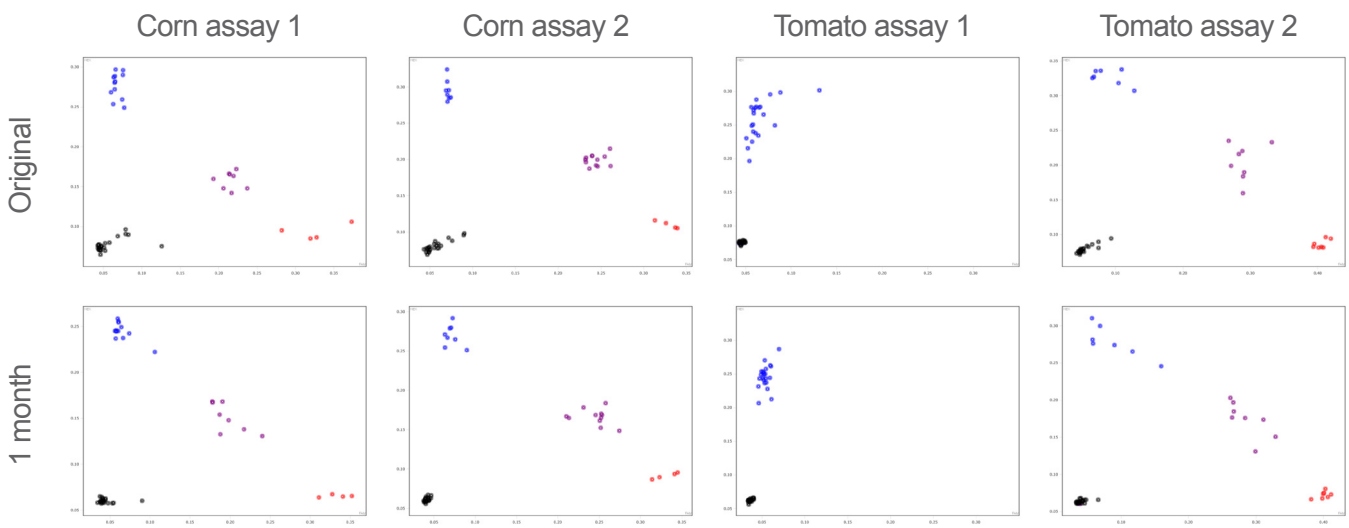

Figure 4. Stability of DNA stored at 4 ˚C. DNA extracted from corn and tomato with QuickExtract was PCR amplified (original) with two KASP assays and then stored at 4 °C for 1 month. The samples were then again PCR amplified against the same two assays (1 month).

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Conclusion

The QuickExtract Plant DNA Extraction Solution allows for fast and simple genotyping of plant samples. Tomato and sunflower seeds allow genotyping without grinding. Wheat seeds required grinding for amplification, whereas grinding of pepper seeds was inhibitory for PCR. Leaf material for these crops showed similar results for the seed material (data not shown). We recommend that the necessity or requirement for grinding be determined for each sample type. Also, performing serial dilutions of the QuickExtract extract after sample processing is advised, for example 1:4, 1:8, 1:16, to determine the optimal amount of processed sample volume for your downstream application. Finally, it is important to emphasise that seeds must be cleaned efficiently if genotyping, since interpretation of results may be complicated by any non-plant material adhering to the seed coat.

Results show that the QuickExtract Plant DNA Extraction Solution may be used to extract DNA from leaf or seed material from multiple types of plants for endpoint PCR applications. Good genotyping results and clusters, using KASP and BHQ Probes for different plant species, were generated. Parameters to be considered for each crop and sample (leaf or seed) are: initial sample mass, whether grinding is necessary, volume of the QuickExtract Plant DNA Extraction Solution required and dilution of the QuickExtracted sample to use for the assay.

In addition, it was shown that very good genotyping results can be achieved even after storing the original lysate four weeks at +4 °C. This application note shows that QuickExtract Plant DNA Extraction Solution provides a fast and simple method to prepare genomic DNA for KASP genotyping, or use of BHQ Probes – all in a single tube, without the use of toxic chemicals, columns or precipitation and resuspension steps. The method is ideally suited to high-throughput applications employing a liquid handler combined with the use of a programmable heating block or water bath.

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