Using the phytoGEM® DNA extraction method with the Hydrocycler™: a new high-throughput approach to plant molecular SNP screening.

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Introduction

Since Mendel studied the basic principle of genetics in peas, the use of genetic-driven decision in plant breeding has been growing constantly. Nowadays, genetic markers are largely brought into play to avoid the need of phenotyping. The so-called marker-assisted selection (MAS) applies diagnostic markers to forecast the phenotype and optimize the selection of new crops. Since the comprehensive sequencing of plants, such as maize, has become readily available, single nucleotide polymorphisms (SNPs) have been widely used in MAS.

However, the extraction of DNA from plants is generally difficult and time-consuming, due to the presence of a tough cell wall and several contaminants, such as polysaccharides, polyphenols, tannins, and alkaloids. Overcoming this issue in DNA extraction will set the springboard for more efficient plant molecular breeding.

In this application note, we show a novel workflow combining different technologies to reduce time, plastic consumption, and costs of plant DNA extraction, as well as increase the analysis throughput. The workflow suggested here includes:

- MicroGEM's *phyto*GEM® chemistry (MicroGEM® #XPP1000) for rapid temperature-driven extraction (TDE) of DNA from seeds, leaves, and roots of rapeseed plants (*Brassica Napus*);
- Hydrocycler™ (LGC) to run high-throughput TDE and Kompetitive Allele Specific PCR for the genotyping.

Methods and Results

Genomic DNA was extracted from different parts of rapeseed plants (*Brassica Napus*), specifically leaves, roots, and seeds. The collection of samples was performed as follow:

1) **Dried leaves:** A 5x5 mm piece of rapeseed leaf was directly cut off the plant, put into a 96 well plate, and then dried at 60°C overnight.

2) **Roots:** Rapeseed roots were cut off 5 mm below the hypocotyl 10 days after the beginning of germination and then transferred into a 96 well plate.

3) **Seeds:** One dry rapeseed seed was transferred to each well of a 96 well plate.

Afterwards, two 4 mm glass balls were added to each well and the samples were shattered for 30 seconds with a frequency of 30 Hz.

The extraction was carried out using *phyto*GEM chemistry, which exploits the power of the *prep*GEM® thermophilic proteinase combined with a cocktail of mesophilic enzymes called *Histosolv*, aimed to help degrade the plant cell wall prior to extraction. The *prep*GEM proteinase differs from proteinase K in that it is able to work at an optimal temperature of 75°C degrees, eliminating the need for ionic detergents as support for the protein denaturation and purifications steps for its removal. In addition, the *prep*GEM proteinase can be heat inactivated with a 95°C step.

The extraction reaction mix was prepared using *phyto*GEM as follows:

Reagents	Amount/sample
Enhancer	10 µl
Histosolv	10 µl
10X **GREEN** Buffer	10 µl
*prep*GEM proteinase	1 µl
Nuclease-free water	69 µl

The extraction mix was added to the 96 well plate and incubated in a Hydrocycler as follows:

Temperature	Time
52ºC	5 min
75ºC	10 min
95ºC	5 min

Before the 95 °C step (used to inactivate the *prep*GEM proteinase), the amount of dsDNA was quantified, using the QuantiFluor® dsDNA dye (Promega).

DNA was successfully extracted from all samples using *phyto*GEM chemistry (#XPP1000) in a Hydrocycler (Figure 1).

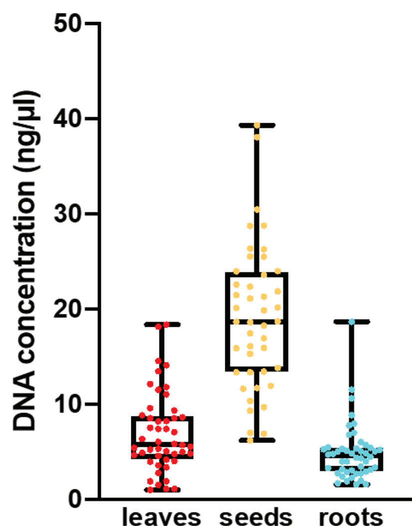


Figure 1: DNA was successfully extracted from all the samples. For dried leaves and roots, n=48, for seeds, n=44. Every dot represents a sample. Data is shown as a box plot where the centre line represents the median value, the limits represent the 25th and 75th percentile, and the whiskers extend from the box to the largest and lowest value.

As expected, seeds produced the highest concentration of DNA (19.120 ± 1.138 ng/µl), followed by dried leaves and roots (6.946 ± 0.603 and 4.869 ± 0.429 ng/µl, respectively).

Moreover, the amount of DNA extracted showed a significant positive correlation with the amount of starting material (Figure 2, data shown for roots, $p < 0.0001$ and $R^2 = 0.426$).

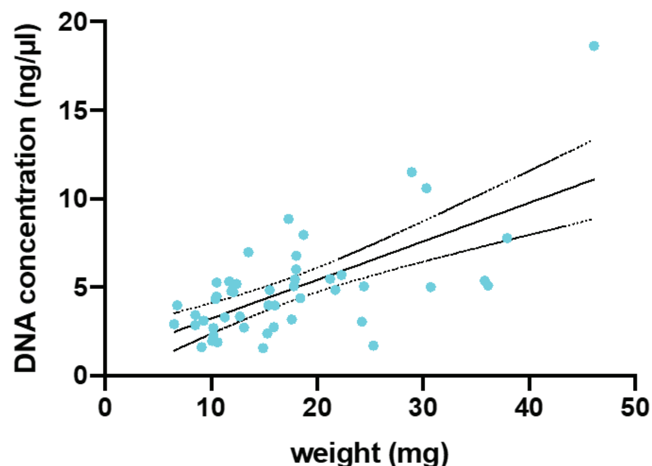


Figure 2: Simple linear regression of amount of starting material and concentration of extracted DNA (n=48). Data is shown as a scatter plot, where the dashed lines represent the 95% confidence interval for the linear regression.

Undiluted and diluted extracted DNA was used to identify specific SNPs and their zygosity via Kompetitive Allele Specific PCR (KASP) assay and to evaluate the compatibility of the *phyto*GEM chemistry with this downstream analysis.

The KASP™ reaction mix (LGC, #KBS-1050) was prepared for all the samples (125x), as follows:

Reagents	Amount
KASP mix	500 µl
BSA (20 mg/mL)	20 µl
Primer for mutation 1 (100 µM)	1.6 µl
Primer for mutation 2 (100 µM)	1.6 µl
Primer common (100 µM)	4 µl
Nuclease-free water	500 µl

Extracts were mixed with 8 µl of KASP reaction mix and loaded on a 96 well plate together with positive controls for homozygous and heterozygous, as well as no template control. The amplification program was run on the Hydrocycler following the manufacturer's instructions.

The DNA diluted 1:5 was the most suitable for all samples, given that the KASP graphs showed clear separation between groups, enabling the discrimination between haplotypes (Figure 3).

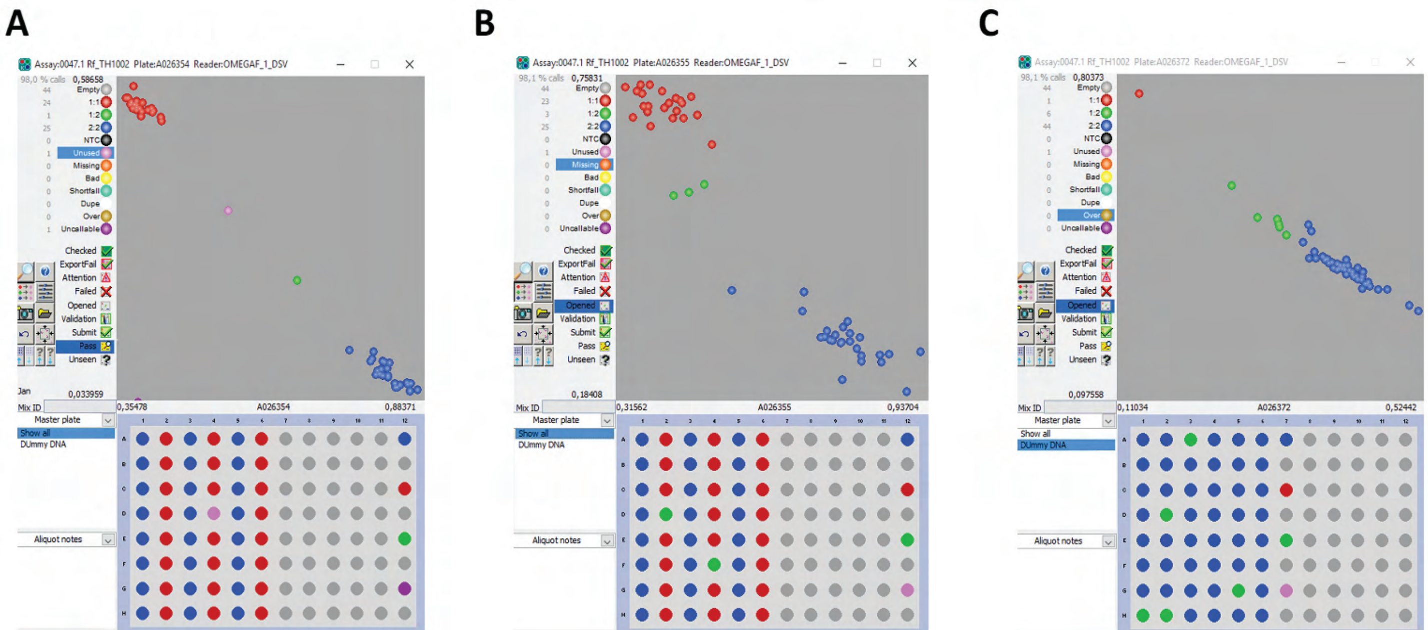
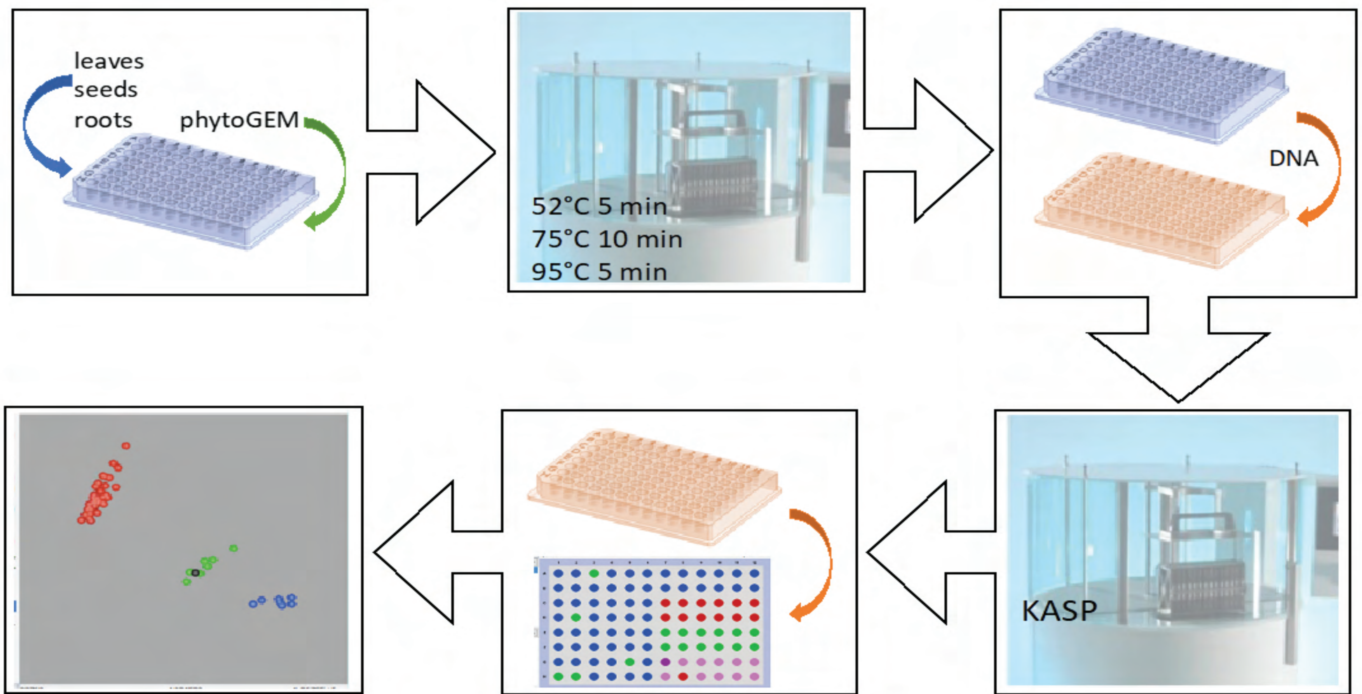


Figure 3: Output images of the software (FLUOstar® Omega SNP device) used for data analysis. DNA was used in 1:5 dilution for all samples (A, leaves; B, seeds; C, roots). Red and blue represents homozygous genotype for the mutation of interest, identified by primer 1 and 2, respectively. Green represents heterozygosity and light pink unclassifiable samples (including the no template control).

Conclusion

The extraction of DNA from plants has represented, so far, the rate-limiting factor in plant molecular breeding, due to the presence of a tough plant cell wall and different contaminants and inhibitors present in the starting material.

This application note shows how the combination of *phyto*GEM chemistry with the Hydrocycler provides a rapid solution to perform high-throughput temperature-driven plant DNA extraction suitable for KASP-based genotyping. In fact, with the Hydrocycler, it is possible to process up to 960 samples (using 96 well plates) or up to 6144 samples (using 384 well plates) simultaneously, making it suitable for high-throughput settings.



In conclusion, the combination of *phyto*GEM chemistry with a Hydrocycler provides:

- Plant DNA extraction in less than 30 minutes
- Up to 6144 samples processed simultaneously
- Reduced costs for laboratory personnel, plastic consumables (tubes, plates, tips etc.)
- A reduction of plastic waste, no chemical waste
- A more sustainable workflow.

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At MicroGEM, our goal is to democratize molecular biology, enabling a broader spectrum of users to both employ and benefit from molecular techniques. The first step is the simplification of sample preparation. Our temperature-driven, single-tube process simplifies and reduces the number of steps for traditional nucleic acid extraction, resulting in high-quality extracts with reduced contamination and high yields - all in minutes, not hours.

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