sbeadex™ plant kit for seed extractions

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Introduction

Reliable, standardised and high quality DNA preparations, delivered by chemistry-adapted robotic platforms are key elements of many genomics based plant breeding programs. Global breeding cycles and the need to test an ever increasing number of samples are driving the need for automated extraction platforms which combine high throughput with the delivery of highly purified DNA.

The sbeadex plant kit

The sbeadex™ mini plant kit (Cat. No. 41601 and 41610 - Appendix 2) has been developed to extract genomic DNA from a wide variety of plant materials (leaves, seeds, fruits, etc.) which can be used for a wide range of plant types without adaptation. The magnetic particle based DNA extraction protocol can be easily automated using the oKtopure™ automated robot developed by the instrumentation team at LGC.

Figure 1 below shows the sbeadex coated magnetic particles novel two-step binding mechanism in the presence of detergents and salts. After binding and washing steps, the purified DNA is released in the elution buffer without any ethanol evaporation or DNA drying step.

Figure 1: sbeadex unique technology includes a two step binding mechanism enables a second wash step using pure water. This results in higher yields, higher DNA purity and efficiently removes all inhibiting salts and alcohols. As no alcohol is used, drying and evaporation of the DNA is not necessary resulting in a time, money and waste minimising extraction.

Validated protocols for a broad range of plant sample species and types

As sample materials differ in age, starting weight and a range of other potential biological or project specific challenges, the ability to develop specific extraction solutions is often key to the delivery of successful DNA extraction project outcomes. A key advantage of the sbeadex chemistry is the capacity for the development of customised protocols to deliver DNA of suitable quality for any downstream processing requirement. In applications where the standard protocol is not able to deliver DNA preparations as required, our application team can establish protocols and optimised reagents to generate DNA for more difficult species or specif project requirements.

The table below provides examples of previously validated plant materials using the oKtopure / sbeadex system.

Table 1: Overview of plant species and sample types for which optimised extraction protocols have been validated and established; as plant samples might differ in composition and DNA yields, optimised protocols are available for most of the plant species using improved lysis conditions and other changes in the standard protocol.

Plant species	Leaves	Seeds
Apricot (*Prunus armeniaca*)	✓	
Barley (*Hordeum vulgare*)	✓	✓
Beet, sugar (*Beta vulgaris*)	✓	
Canola / Oilseed (*Brassica napus*)	✓	✓
Chicory (*Cichorium intybus*)	✓	
Corn (*Zea mays*)	✓	✓
Cotton (*Gossypium*)	✓	✓
Cucumber (*Cucumis sativus*)	✓	✓
Flax (*Linum usitatissimum*)	✓	
Grape (*Vitis vinifera*)	✓	✓
Lettuce (*Lactuca sativa*)	✓	
Muskmelon (*Cucumis melo*)	✓	✓

Onion (*Allium cepa*)	✓	
Parsley (*Petroselinum crispum*)	✓	✓
Peach (*Prunus persica*)	✓	
Pepper (*Capsicum annuum*)	✓	✓
Potato (*Solanum tuberosum*)	✓	
Rice, Asian (*Oryza sativa*)	✓	✓
Rubber (*Hevea brasiliensis*)	✓	✓
Soybean (*Aphis glycines*)	✓	✓
Sunflower (*Helianthus annuus*)	✓	
Tobacco leaves (*Nicotiana tabacum*)	✓	✓
Tomato (*Solanum lycopersicum*)	✓	
Wheat (*Triticum L.*)	✓	✓

Materials and methods

Extraction protocol summary

As described above, high quality DNA preparations can be delivered using the standarised protocols through the combination of sbeadex chemistry and oKtopure automation from more than 90% of plant species.
To demonstrate the utility of the standard protocol DNA was extracted from seed samples as described below. Further details of the protocol are provide in Appendix 1.

Table 2: Short protocol for sbeadex extractions from seeds.

Extraction step	Incubation time (mins)	Homogeneous RNAse treated rice lysate
Binding	10	520 µL binding buffer 60 µL sbeadex beads 200 µL lysate
Wash 1	10	400 µL
Wash 2	10	400 µL
Wash aqua dest	10	400 µL
Elution - AMP buffer	10	100 µL

Results and discussion

DNA quality overview

In total, 10 seeds (5 different plant species) were extracted using the standard sbeadex protocol. To check the DNA quality and integrity we compared the DNA extractions by gel electrophoresis and quantified the DNA yields using UV measurement by NanoDrop measurement (Table 3). It is shown that the UV measurement has been successful as DNA was detected in all cases. The differences between the DNA concentrations from difference samples are due to the size of seeds, starting material and the genome size of the different species. 260 / 280 and 260 / 230 measurements, an indicator for potential contaminations with proteins and other biomolecules, also demonstrate high quality DNA preparations.

Table 3: The DNA yields of the samples following extraction using sbeadex magnetic beads were measured by UV measurement / NanoDrop. Two seed samples of each plant species were extracted to compare yields and integrities of the DNAs. UV measurement demonstratres that DNA can be detected in all cases.

Sample #	NanoDrop	260 / 280	260 / 230
A	17,01	1,61	0,71
A	13,16	1,59	0,75
B	52,96	1,78	0,94
B	48,34	1,71	0,81
C	11,68	1,80	0,90
C	12,16	1,76	0,54
D	62,40	1,78	1,06
D	81,93	1,86	1,19
E	15,52	1,64	0,66
E	17,90	1,66	0,69

Gel electrophoresis

As NanoDrop measurements are not always a reliable indicator of DNA integrity gel electrophoresis of the samples was under taken to demonstrate the ability of the sbeadex chemistry to extract high molecular DNA extraction. Using lambda DNA as a reference for high molecular weight, the gel demonstrates that sbeadex is able to extract DNA with a molecular weight of >10 kb in combination with a high DNA stability is no smear occurs.

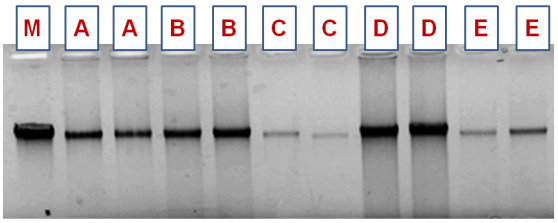

Figure 2: 10 µL of DNA extract were used for a 1% agarose gel electrophoresis to check DNA integrity and compare the DNA yields. As UV measurements are often very approximate as contaminations and RNA are absorbing at the measured wavelength, gel electrophoresis is a better method to compare the final DNA yields. The gel shows that there are no significant differences between the seed samples extracted by the oKtopure and the competitor technology. **M:** Lambda DNA marker; **A:** plant species A; **B:** plant species B; **C:** plant species C; **D:** plant species D; **E:** plant species E;

PCR

Along with successful DNA extraction of required yield and quality, it is important that the purified DNA is fully functional for further down-stream applications like PCR, NextGen sequencing or SNP genotyping. Therefore, for all plant seeds samples, were checked using PCR by amplifying a plastic gene coding for tRNAses to demonstrate if the DNA is suitable for ongoing processing. The picture demonstrates samples provide DNA usable for PCR.

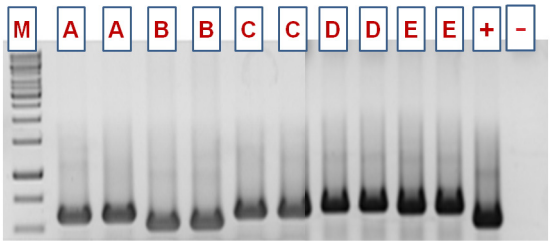

Figure 3: 10 µL PCR product (C+D chloroplast universal primer, end point PCR) were loaded on a 1.2% Agarose Gel to check for PCR inhibitors. Both platforms extracted PCRable DNA with no signs of inhibition. **M:** 100bp marker; **A:** plant species A; **B:** plant species B; **C:** plant species C; **D:** plant species D; **E:** plant species E; **+** Positive control; **-** Negative control

Automation of extraction with oKtopure

oKtopure overview

sbeadex has been optimised for the oKtopure: an instrument from LGC which delivers increased throughput. The system provides nucleic acid preparations suitable for downstream processing applications such as SNP genotyping or sequencing. It is a small footprint robotic platform which utilises 8 magnetic “underdeck” stations to hold beads in place during nucleic acid extraction while the 96 tip head automatically transfers lysing solutions and washing buffers. Holding the beads while moving liquids allows a significantly increases in DNA yield and quality in comparison to other system. Additionally, cost efficiencies are realized through reduced consumable requirements delivered by an offline wash station which enables the reuse of tips up to 40 times, saving up to 50% over alternative platforms.

Appendix 1: Standard seed extraction protocol

1.) Add 250 µL of Lysis buffer BL to each sample and grind disruption and homogenization of plant material and incubate at 65°C incl RNase digestion for at least 10 minutes.
2.) Centrifuge at 2 500 g for 10 minutes.
3.) Transfer of 200 µL lysate to prefilled 520 µL of Binding buffer PN and 60 µL sbeadex particles (please take care that sbeadex beads are fully re-suspended before using).
4.) Mix thoroughly by pipetting up and down several times. Incubate for 10 minutes at room temperature to allow sufficient time for binding to occur.
5.) Bring magnet into contact with the sample tubes and wait for 1 minute at room temperature to allow the sbeadex particles to form a pellet.
6.) The oKtopure removes the supernatant and discards the buffers. Ensure as much of the supernatant is removed as is possible without dislodging the particle pellet.
7.) Move the magnet away from the sample tubes and add 400 µL of Wash buffer PN 1 and re-suspend the pellet.
8.) Mix thoroughly by pipetting up and down 5 times or until pellet is fully re-suspended.
9.) Incubate at room temperature for 1 minute, agitating the sample during the time period. Bring magnet into contact with the sample tubes and wait for 10 minutes at room temperature to allow the sbeadex particles to form a pellet.
10.) Remove the supernatant and discard. Ensure as much of the supernatant is removed as is possible without dislodging the particle pellet. Move the magnet away from the sample tubes.
11.) Repeat steps with 400 µL of Wash buffer PN 2 and repeat steps with 400 µL of pure water.
12.) Add 100 µL of Elution buffer PN and re-suspend the pellet.
13.) Mix thoroughly and pipette up and down 5 times or until pellet is fully re-suspended. Vortex periodically and bring magnet into contact with the sample tubes. Wait for 1 minutes at room temperature to allow the sbeadex particles to form a pellet.
14.) Remove the eluate and place into a new sample tube. To avoid particle transfer it is recommended to transfer only 80 µL of the eluate.

Appendix 2: Catalogue information

Table 4: Catalogue numbers for oKtopure, sbeadex and related products. The kits are available in different bulk formats of 960, 2500, 5000, 10000 and 40000 extractions per kit.

Catalogue number	Description	Units
NAP41601	sbeadex mini plant	96 tests*
NAP41602	sbeadex maxi plant	96 tests*
NAP41610	sbeadex mini plant	960 tests*
NAP41620	sbeadex maxi plant	960 tests*
KBS-0009-001	oKtopure high throughput DNA extraction robot	1
KBS-0009-002	oKtowash™, concentrated wash buffer (500 mL)	1
KBS-0009-003	oKtopure off line tip wash option	1
KBS-0009-004	oKtopure mix plates (Thermo 1.2 mL deep well plate)	1
KBS-0009-005	Wash buffer bulk reservoirs (pack of 4)	1
KBS-0009-999	Extended 12 month on-site fully inclusive service contract	1

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