LGC Genomics

The oKtopure™ and sbeadex® plant nucleic acid extraction kit

Dr Heiko Hauser, Dr Dietrich Köster and Dr Frank Schubert,
LGC Genomics, Ostendstrasse 25, TGS Haus 8, 12459 Berlin, Germany

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 oKtopure instrument from LGC Genomics, delivers increased throughput with extraction protocols optimised for the magnetic bead based sbeadex® chemistry. The system provides nucleic acid preparations suitable for downstream processing applications such as SNP genotyping or sequencing. Cost efficiencies are realised through reduced consumable requirements delivered by an offline wash station which enables the re-use of tips up to 40 times, saving as much as 50% over alternative platforms.

oKtopure overview

The oKtopure is a small footprint robotic platform which utilizes 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 significant increase in DNA yield and quality in comparison to other systems.

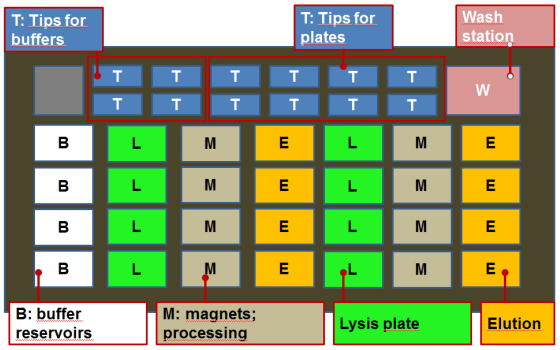

Figure 1: Desk overview of the oKtopure system; 8 magnetic devices provide 8, in parallel processing sites. Plant cell lysis and homogenisation is done offline, after incubation and spinning down of cell debris the supernatant is transferred to the process site (L➞M). Here, the main extraction steps take place including initial binding to the sbeadex beads, washing, second binding, pure water wash step and final elution. The pure DNA is transferred finally to the Elution Plate (M➞E).

The table 1 provides a specification overview for the oKtopure instrument used in combination with the sbeadex® chemistry.

Table 1: Specification overview - oKtopure and sbeadex® plant

Key features of the oKtopure / sbeadex® system	Specification
Elution volume	100 µL
Format / robotic platform	oKtopure
Sample type	Plant samples (leaves, seeds, flour etc.)
Chemistry	sbeadex® plant
Final wash	2 binding steps; final wash with pure water
Grade of automation	Full walk away automation
Nucleic acid purification type	DNA
Average yield sbeadex® mini	1 - 15 µg
Average yield sbeadex® maxi	20 - 80 µg

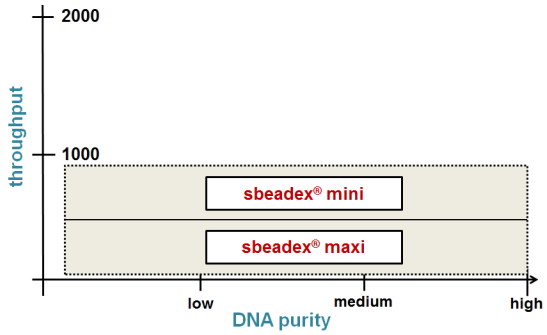

Figure 2: The flexibility of the oKtopure is driven by the ability to adapt the sbeadex chemistry on any type of plant sample. Typically, a manual protocol is developed and transferred to the robot including technical configuration to guarantee optimised extraction.

Example plant species validated with oKtopure and or sbeadex® for nucleic acid extraction

A key advantage of the oKtopure / sbeadex® combination 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 of required quality or yield, our application team can establish unique protocols and buffer / lysis reagent combinations. The table below provides examples of plant materials previously validated using the system:

Table 2: 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.)	✓	✓

The oKtopure and sbeadex® - an example study with rice leaves

The sbeadex® plant kit (Cat. No. 41601, 41602, 41610 and 41620) has been developed to extract genomic DNA from a wide variety of plant materials (leaves, seeds, fruits, etc.) and can be used for a wide range of plant types without customisation. The magnetic particle based DNA extraction protocol can be easily automated using the oKtopure.

Figure 3 demonstrates the key step in sbeadex® coated magnetic particles binding DNA sbeadex® uses a 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. The sbeadex® mini and maxi plant kit is supplied with ready-to-use buffers.

Figure 3: sbeadex® unique technology includes a two step binding mechanism enables users 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,

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 a set of rice leaf samples as described below. Further details of the protocol are provide in Appendix 1.

Table 3: Short protocol for sbeadex® extractions from rice leaves.

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

To check the DNA quality and integrity we compared the DNA extractions on the oKtopure platform using the sbeadex® plant kit by gel electrophoresis and compared it to DNA extracted using an alternative automated extraction technology. Further assessment of DNA was made using UV quantification by NanoDrop measurement and PicoGreen to verify the quantification results.

UV quantification

Table 4: In total 32 samples were extracted using the oKtopure and another robotic platform; the DNA yields of the samples following extraction using sbeadex® magnetic beads have been measured by UV measurement / NanoDrop. To calculate the total DNA yields, the average yields were multiplied with the final elution volume.

oKtopure	NanoDrop	oKtopure	NanoDrop
O1	6.026	O9	5.253
O2	4.812	O10	4.860
O3	6.215	O11	5.908
O4	5.258	O12	5.185
O5	5.808	O13	4.989
O6	4.786	O14	5.318
O7	5.456	O15	5.649
O8	4.513	O16	5.684
Median conc.		5.358	
Alternative platforms	**NanoDrop**	**Alternative platforms**	**NanoDrop**
C1	16.18	C9	17.85
C2	15.45	C10	11.43
C3	13.79	C11	10.51
C4	12.83	C12	10.44
C5	12.38	C13	16.23
C6	13.61	C14	10.25
C7	7.678	C15	9.974
C8	12.42	C16	10.9
Median conc.		10,8812	

Gel electrophoresis

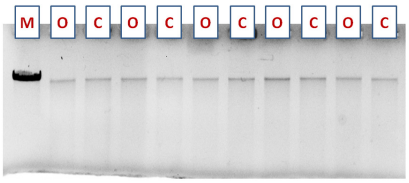

Figure 3: 10 µL of DNA extract were used for a 1% agarose gel electrophoresis to check DNA integrity and compare the DNA yields. UV measurements are often approximations as a result of contaminations and RNA which absorb light at the specified. wavelength. Therefore, gel electrophoresis is used to further compare the final DNA yields. The gel shows that there are no significant differences between the rice leaf samples extracted by the oKtopure and the competitor technology. **M:** Lambda DNA marker; **O:** oKtopure:10 µL eluate on gel; **C:** competitor: 10 µL eluate on gel;

PicoGreen quantification

Table 5: In total 32 samples have been extracted using the oKtopure and an alternative robotic platform; the DNA yields of the samples following extraction using sbeadex® magnetic beads have been measured by PicoGreen measurement. To calculate the total DNA yields, the average yields has been multiplied with the final elution volume. Finally, the relationship between the value averages of NanoDrop to PicoGreen measurement (which was adapated to be the gold standard here) has been calculated. The oKtopure / sbeadex® demonstrates higher final DNA yields in combination with a lower overestimation ratio.

oKtopure	PicoGreen	oKtopure	PicoGreen
O1	1.777854	O9	1.80181
O2	1.645073	O10	1.903612
O3	1.861183	O11	1.923925
O4	1.791884	O12	2.027322
O5	1.883279	O13	1.766547
O6	1.762134	O14	2.03261
O7	1.785307	O15	1.860785
O8	1.809242	O16	2.008495
Median conc.		1.852.566	
Median total (100 µL)		185.256.638	
Median Nanodrop		5.358	
Standard deviation		0.10737	
Over-estimation by NanoDrop		3 x	
Alternative platforms	**PicoGreen**	**Alternative platforms**	**PicoGreen**
C1	2.2009	C9	2.258941
C2	2.274683	C10	2.110072
C3	2.296872	C11	1.871759
C4	2.067473	C12	2.264135
C5	2.223487	C13	2.291074
C6	2.274683	C14	1.942433
C7	2.296872	C15	2.100731
C8	2.067473	C16	1.980573
Median conc.		2.154.751	
Median total (100 µL)		140.058.798	
Median NanoDrop		10,8812	
Standard deviation		0.139747	
Over-estimation by NanoDrop		5 x	

Summary

oKtopure provides significant savings in processing time and consumables costs with no loss of DNA quality or yield.

Appendix 1: Standard rice leaf extraction protocol

1. Add 250 µL of Lysis buffer PN to each sample and grind disruption and homogenisation 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. The oKtopure transfers 200 µL lysate (oKtopure deck: position L → M) 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 10 minutes 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 10 minutes, 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 10 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: Cost saving available with oKotpure and sbeadex®

Costs per extraction is a crucial factor for high throughput applications. Depending on the robotic technology and the throughput of the project, there are costs for plastics between 20-30 € cents / extraction which add to the costs for each extraction. The oKtopure delivers a significant reduction in costs through an offline wash station which allows the re-use of tips up 30-40 times. Table 6 shows a direct comparison between the consumable costs of the oKtopure and other extraction platforms.

Table 6: Overview of costs for consumables for the different systems. The oKtopure can be ordered with and without the offline washing system. Other technologies including liquid handling systems and magnetic particle systems require increased consumables per extraction as there is no re-use of tips.

Feature	oKtopure with washing	oKtopure without washing	Other technologies
Tips / sample	No tips	1 tip	4-6 tips
Grinding plate	1	1	1
Processing plate	1	1	1-6
Elution plate	1	1	1

Appendix 3: Catalogue information

Table 7: 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
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
NAP41610	sbeadex® mini plant	960 tests*
NAP41620	sbeadex® maxi plant	960 tests*

(*) sbeadex® plant kit is also available in different format for higher throughput customers, please contact customer service.

LGC Genomics

www.lgcgenomics.com

Germany
Ostendstr. 25 • TGS Haus 8
12459 Berlin

Tel: +49 (0) 30 5304 2200
Fax: +49 (0) 30 5304 2201
Email: info.de@lgcgenomics.com

United Kingdom
Unit 1-2 Trident Industrial Estate • Pindar Road
Hoddesdon • Herts • EN11 0WZ

Tel: +44 (0) 1992 470 757
Fax +44 (0) 1438 900 670
Email: info.uk@lgcgenomics.com

USA
100 Cummings Center • Suite 420H
Beverly • MA 01915

Tel: +1 (978) 232 9430
Fax: +1 (978) 232 9435
Email: info.us@lgcgenomics.com

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