Application note

Automation of sbeadex Lightning using the Hamilton STAR

Introduction

sbeadex™ Lightning utilises a revolutionary, magnetic bead-based nucleic acid purification chemistry to provide an innovative solution to rapid isolation of highly pure and high-quality nucleic acids. It combines exceptional speed and simplicity (3 protocol steps, typically 5 minutes) with the highest nucleic acid quality standards, whilst significantly reducing liquid and hazardous waste as well as plastic consumption and energy for transportation and instrumentation. Designed for both high-throughput and automation, the purification system can be readily adapted and optimised to suit a broad range of workflows and tissue types.

Here we demonstrate ease of automation of the sbeadex Lightning chemistry for a range of plant and livestock sample types. Optimisation of the protocols is illustrated using different lysis conditions and protocol adaptations, and protocols are applied to the Hamilton® Microlab™ STAR system (Hamilton Company) for automated liquid handling. As protocol optimisation is essential for good sample processing, the supplied method provides an optimisation control window allowing rapid protocol optimisation for different sample types including control of liquid volumes, timings for wash steps and waiting times on and off the magnet.

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In addition, we compare the sbeadex Lightning protocols on the Hamilton STAR to processing of the same lysates using our original sbeadex chemistry for both livestock and plant samples to illustrate the savings in time, plastics and reagents. More information about automation using the Hamilton STAR is available on our website.

Methods

Protocol development on the Hamilton Microlab STAR system

All sample-specific protocols for the Hamilton Microlab STAR system (hereafter Hamilton STAR) were developed using a system with the specifications detailed in the sbeadex Lightning on the Hamilton STAR system manual. Hamilton STAR protocols for automation of the sbeadex Maxi Plant DNA Purification Kit and the sbeadex Livestock DNA Purification Kit were created based on the recommended volumes with no further adaptation. For sbeadex Lightning, some testing of lysate conditions was performed to demonstrate routes of optimisation for specific sample types.

The Hamilton STAR protocols were developed using a 96-well head with liquid sensing, allowing for control of how liquids are aspirated. This is an important step for lysis transfer, especially when there is a requirement to avoid cellular debris, and the protocols use electrical conductivity tips to achieve this. To allow further control and rapid optimisation, the user can control almost all aspects of the protocol from the Protocol Optimization Menu (POM). The POM allows for a user to control shaking time, washing time and magnet on and off time. For further information, please see the sbeadex Lightning DNA purification manual for the Hamilton STAR.

Sample types and lysis

In this study, six different species were selected to represent sample matrices commonly used with the original sbeadex chemistry. These comprised three plant species (canola, maize and soybean) and three livestock species (cattle/bovine, pig/porcine, and rainbow trout). Table 1 details sample types and collection method.

All purification protocols used a fixed input quantity of tissue known to function with the

Species	Sample type	Quantity used per purification
Canola (*Brassica napus*, black rape)	Seed	35 mg (+/- 10 mg) Approx 4-5 seeds*
Maize (*Zea mays*, var. Swift)	Seed	35 mg (+/- 10 mg) Approx 0.2 seeds*
Soybean (*Gycine max*, var. Edame and Frisky V)	Seed	35 mg (+/- 10 mg) Approx 0.5 seeds*
Bovine (*Bos taurus*)	Muscle biopsy	50 mg (+/- 5 mg) collected using 3 x 3 mm biopsy core punch
Porcine (*Sus domesticus*)	Ear punch (frozen)	50 mg (+/- 10 mg) collected using Allflex® collection device (Merck & Co., Inc., Rahway, NJ, USA)
Rainbow trout (*Oncorhynchus mykiss*)	Muscle biopsy	30 mg (+/- 5 mg) collected using 1 x 3 mm biopsy collection device

Table 1. Sample types and quantities used per purification. *Calculated using average seed weight.

original sbeadex methodology. Before lysis, all seed samples were ground to a fine flour using a coffee grinder.

For animal samples, 110 µL of lysate per bovine and trout sample or 25 µL of lysate per porcine sample was transferred to the purification protocol. For all plant samples, 500 µL lysis buffer containing 1 µL Protease K solution and 20 µL Debris capture beads was used per purification. Lysates were vortexed for 5 minutes at 1,600 rpm and then incubated at 65 ºC for 30 minutes.

sbeadex Lightning and sbeadex reagents
Tables 2 and 3 detail the suggested reagents required to perform sbeadex Lightning purification using the Hamilton STAR. Ultrapure

	Core Kit A			Core Kit B			
Component	**NAP40-030-01**	**NAP40-030-02**	**NAP40-030-03**	**NAP40-031-01**	**NAP40-031-02**	**NAP40-031-03**	**Storage conditions**
Binding buffer LP	22 mL	220 mL	2,200 mL	-	-	-	Room temperature*
Binding buffer LU	-	-	-	22 mL	220 mL	2,200 mL	Room temperature*
sbeadex particle suspension	2.2 mL	22 mL	220 mL	2.2 mL	22	220 mL	Room temperature
Elution buffer AMP	11 mL	110 mL	1,100 mL	11 mL	110	1,100 mL	Room temperature

Table 2. Components supplied in the sbeadex Lightning core kits. *Store in the dark for long-term preservation.

	Part codes	Volume	Storage conditions
sbeadex™ Lightning Nucleic Acid Purification Starter Kit (20 purifications)	NAP40-032-00		
Lysis buffer PN	NAP10-006-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer PVP	NAP10-010-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer UR	NAP10-004-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer BL	NAP10-002-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer H	NAP10-003-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer LI	NAP10-005-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Protease K solution	NAP30-002-01/...-02/...-03/...-04/...- 05/...-06	1-1,000 mL	Room temperature
RNase A solution	NAP30-003-01/...-02/...-03	1-100 mL	4 °C
Debris capture beads	NAP20-005-00/...-01/...-02/...-03/...-04/...- 05	1-1,000 mL	Room temperature
Ultra pure water	customer supplied		Room temperature

Table 3. Lysis buffers and supplements for sbeadex Lightning (purchased separately).

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Component	Part codes	Number of purifications
sbeadex Livestock Purification Kit (96 purifications)	NAP44701	96 purifications
sbeadex Maxi Plant Purification Kit (96 purifications)	NAP41602	96 purifications

Table 4. sbeadex kits used in this study.

water at pH ≤6.5 is required for washing. Table 4 details the sbeadex kits utilised in this study. Please see our website for a complete list of available kit sizes.

sbeadex Lightning protocol optimisation

For the sbeadex Lightning protocols, a range of lysis conditions were tested per sample type, adjusting either lysis time or the specific lysis buffer used. For each sample type, binding was tested with both Binding buffer LP (Core Kit A) and Binding buffer LU (Core Kit B) to illustrate the importance of optimising both lysis and binding steps for individual sample types. Lysis buffers used for each sample type are detailed in table 5. For all livestock samples, Protease K was added during lysis (10 µL Protease K per sample for porcine and trout, 20 µL Protease K solution per sample for bovine).

For all sample types, the relevant original sbeadex chemistry (livestock or plant) was run in parallel with the sbeadex Lightning protocol as a comparator. Samples were lysed for both 24 and 48 hours to determine impact of lysis time on DNA yield and quality.

During manual optimisation of lysis conditions, all physical conditions for lysis remained constant including shaking time (5 minutes at 1,600 rpm) and lysis temperature (65 ºC).

Protocol automation

For purification from each sample type, the Hamilton STAR template (.pkg) was modified via the POM, using a manually tested baseline protocol. Adaptations involved adjustments

Species	Sample type	sbeadex chemistry	sbeadex Lightning chemistry	Lysis buffer					
				PVP	PN	LI	UR	H	Tris EDTA + Protease K
Canola	Seed	sbeadex Maxi Plant Nucleic Acid Purification Kit	Core Kit A, Core Kit B	✓	✓	✓	✓	✓	X
Maize	Seed	sbeadex Maxi Plant Nucleic Acid Purification Kit	Core Kit A, Core Kit B	✓	✓	✓	✓	✓	X
Soybean	Seed	sbeadex Maxi Plant Nucleic Acid Purification Kit	Core Kit A, Core Kit B	✓	✓	✓	✓	✓	X
Bovine	Muscle biopsy	sbeadex Livestock Nucleic Acid Purification Kit	Core Kit A, Core Kit B	✓	X	✓	✓	✓	X
Bovine	Ear punch	sbeadex Livestock Nucleic Acid Purification Kit	Core Kit A, Core Kit B	✓	X	✓	✓	✓	✓
Rainbow trout	Muscle biopsy	sbeadex Livestock Nucleic Acid Purification Kit	Core Kit A, Core Kit B	✓	X	✓	✓	✓	X

Table 5. Summary of the reagents tested with each sample type.

to lysate transfer volumes, shaking duration, duration of time on magnet and lysate aspiration heights. Pre-developed protocol adaptations for automation of the original sbeadex kits for plant and for livestock samples were used for comparison.

Post-purification DNA analysis

Following DNA purification on the Hamilton STAR, all nucleic acids were quantified via UV spectrophotometry using a QuantiFluor® dsDNA system (Promega, United Kingdom) using a BMG PHERAstar® spectrophotometer (BMG, Switzerland) to obtain yield and purity values. To generate gel images, Midori Green Direct DNA stain (Nippon Technology, Japan) was added to normalised DNA samples (300 ng per sample) as per manufacturer's instructions, and samples run on 1% agarose gels.

For each DNA sample, a dilution series was prepared (neat, 1 in 5, 1 in 10 and 1 in 20). These dilution series were used as template in KASP genotyping reactions (three KASP assays per sample type) to ensure no downstream inhibition of PCR.

For bovine and maize samples, DNA libraries were prepared for Amp-Seq, Biosearch Technologies' targeted genotyping by sequencing chemistry, using SNPline™ instrumentation. Libraries were processed through the Amp-Seq workflow using panels containing 199 loci (bovine) and 768 loci (maize). DNA was normalised to 8 ng/μL using QuantiFluor, and Amp-Seq Master Mix prepared with 5 μM stage 1 and stage 2 primer. Prepared libraries were compared to known, sequenced and functional libraries for yield and fragment distribution using an Agilent Bioanalyzer (Agilent, UK) to demonstrate compatibility of DNA purified using the Hamilton STAR sbeadex Lightning protocol with high-throughput downstream genetic analysis.

Results and discussion

Optimisation of the sbeadex Lightning lysis step

The sbeadex Lightning chemistry is designed to facilitate sample-specific optimisation of the lysis step to ensure the highest possible yields and quality of DNA are obtained. For all sample types in this study, lysis optimisation was performed against the appropriate standard original sbeadex protocol (either plant or livestock kit). Lysis optimisation was performed manually using a range of lysis buffers (table 5), and then the optimal protocol transferred to the Hamilton STAR.

Figure 1 illustrates the DNA quality and quantity obtained from porcine samples during initial sample-specific manual optimisation of the protocol. Comparable DNA yields and quality were obtained with the sbeadex Lightning lysis buffers as for original sbeadex chemistry, although values did vary between the different lysis buffers with Lysis buffer LI and Tris EDTA performing most comparably. This variability highlights the benefits of performing sample-specific protocol optimisation prior to automation of sbeadex Lightning chemistry. The extended lysis time (48 hours) had limited effect on either DNA yield or quality. Comparable results were obtained for all other species tested in terms of yield and quality, and observed variability in these values for the different lysis buffers used (data not shown).

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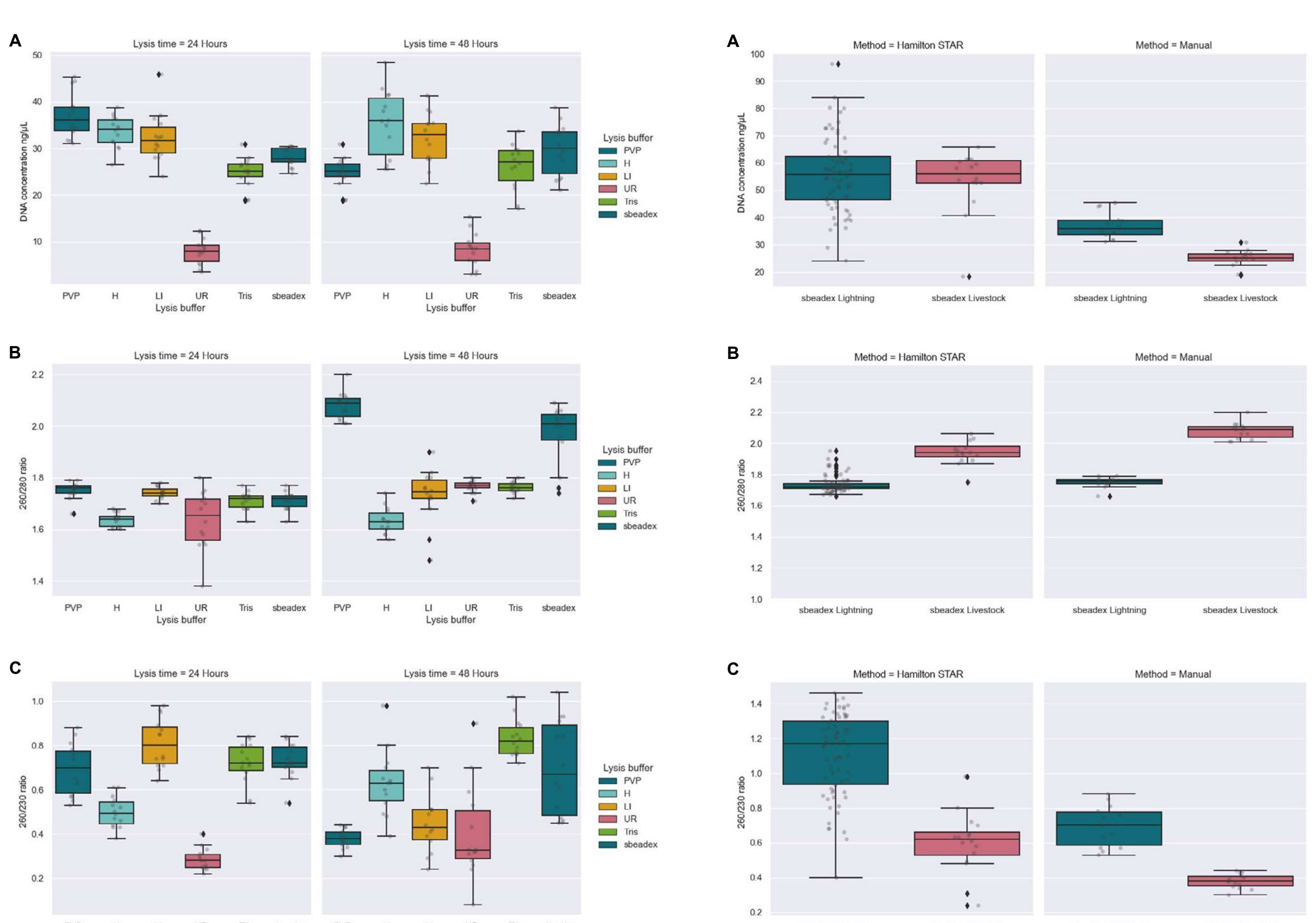

Figure 1. DNA yield and quality values for manual purification from porcine tissue using a range of sbeadex Lightning lysis buffers (with Core Kit A) and using the sbeadex Livestock Nucleic Acid Purification Kit. DNA yield values (ng/µL) and DNA quality values (260/280 and 260/230 ratios) are shown for both 24 hour and 48 hour lysis in figures 1A, 1B and 1C respectively.

Figure 2. Comparison of DNA yield and quality between the automated Hamilton STAR protocol and the manual protocol. Results are shown for both sbeadex Lightning chemistry (using Lysis buffer PVP and Core Kit A, teal bars) and for the sbeadex Livestock Nucleic Acid Purification Kit (red bars). For sbeadex Lightning results, n=80 for automated purification and n=14 for manual purification. For original sbeadex, n=14 for both automated purification and for manual purification.

Comparison of manual and automated DNA purification

Once sbeadex Lightning sample-specific lysis and binding conditions were optimised manually, protocols for all sample types were transferred to the Hamilton STAR for automation. Protocols for original sbeadex were generated for the Hamilton STAR to mimic conditions used for automation using the KingFisher™ Flex (Thermo Fisher Scientific). All protocols transferred easily from manual to the Hamilton STAR, highlighting the ease of automation of sbeadex Lightning chemistry.

Figure 2 demonstrates the DNA quantity and quality obtained from porcine samples, comparing automated purification using the Hamilton STAR with manual purification. Automation shows distinct improvements in DNA yield and quality for both sbeadex Lightning and original sbeadex chemistries. In addition, the automated sbeadex Lightning protocol generated less plastic waste than the original sbeadex equivalent, requiring 192 fewer tips (2 x 96 racks) and two fewer plastic troughs.

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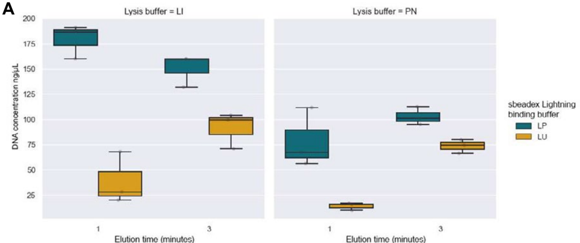
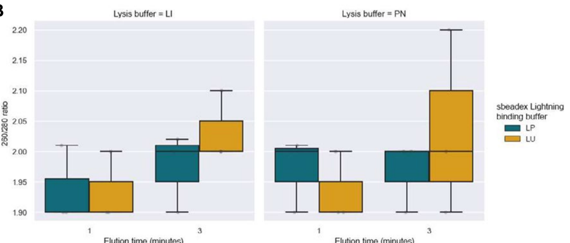
Optimisation of the Hamilton protocol using the POM screen

Following automation of manual protocols on the Hamilton STAR, any further optimisation of the purification protocol is made easier using the POM screen. Within a single block experiment, multiple chemical factors can be verified quickly while physical factors can be performed consistently across independent runs.

Figure 3 demonstrates results for automated purification of DNA from lysed maize seed samples, for which a range of factors were easily adjusted using the POM screen. Shaking time was adjusted using the POM (1 minute or 3 minutes), and results are shown for two different lysis buffers (LI and PN), and for both binding buffers (LP and LU). For this automated protocol, time on the magnet was increased to 60 seconds to ensure sbeadex particles were captured prior to supernatant removal. For maize samples, DNA yield and purity values were best when Binding buffer LP was used. Lysis buffer LI generated the highest DNA yields, although increasing the shaking time for the elution step to 3 minutes significantly increased DNA purity across all samples.

Using 520 µL lysate for plant seed samples allowed for 200 µL of lysate to be safety transferred without risk of disrupting pelleted material or Debris capture beads. For Hamilton STAR automation of all protocols, a -3 mm offset was used for transfer of lysate, using liquid sensing. This ensured that any debris on the surface, either plant materials or fatty layers formed during livestock sample lysis, was avoided during transfer. The POM can be utilised to easily adjust aspiration height up to +5 mm from the bottom of the plate or -5 mm from the bottom of the sample, which is crucial if tip blockages are to be avoided.





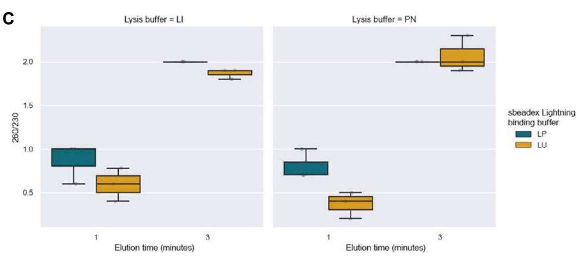


Figure 3. Comparison of DNA yield (3A) and quality (3B, 3C) when using the POM screen to adjust protocol parameters. Data shown is for automated purification from maize seed using the Hamilton STAR platform. Lysis was performed pre-automation, using either Lysis buffer LI or PN. Both sbeadex Lightning binding buffers were used (LP = teal bars, LU = orange bars). The POM screen was used to adjust shaking time from 1 minute to 3 minutes. n=48.

Figure 4 shows two examples of the POM, one used for bovine and trout samples (4A) and the other for all plant seed samples (4B).

Comparison of automated sbeadex Lightning and original sbeadex protocols

Following successful automation of the sbeadex Lightning protocols using the Hamilton STAR, downstream analysis of the purified DNA was performed confirming equivalent yields and purity for both sbeadex Lightning and original sbeadex chemistries.

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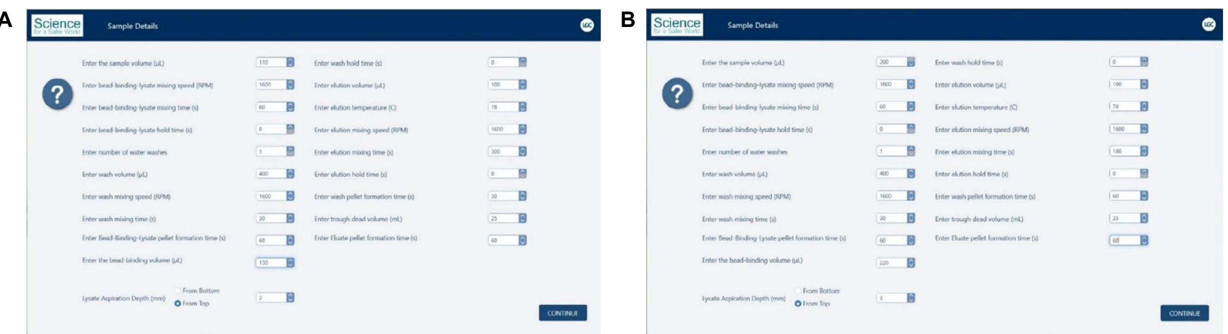

Figure 4. The POM menu detailing protocol details for automation of the sbeadex Lightning chemistry for bovine and trout samples (4A) and for plant seed samples (4B).

Figure 5 illustrates the DNA yields (quantified using QuantiFluor and through UV spectrophotometry) for automated DNA purification using both sbeadex Lightning and original sbeadex chemistries. DNA yields are high and are equivalent between the chemistries when purification is automated, across a range of different sample types. To illustrate DNA quantity and quality further, purified DNA was visualised via gel electrophoresis, as shown in figure 6. This further confirms the high quantities of high molecular weight DNA obtained through automation, which are suitable for downstream analysis.

Whilst performance is comparable for original sbeadex and sbeadex Lightning, a range of benefits are offered through the use of sbeadex Lightning. Table 6 illustrates the savings in time, consumables, and waste for sbeadex Lightning compared to original sbeadex when automating on the Hamilton. These include 80% time savings, a 60% reduction in liquid waste, 45% fewer tips required, and an average reduction of 65% in solvent waste.

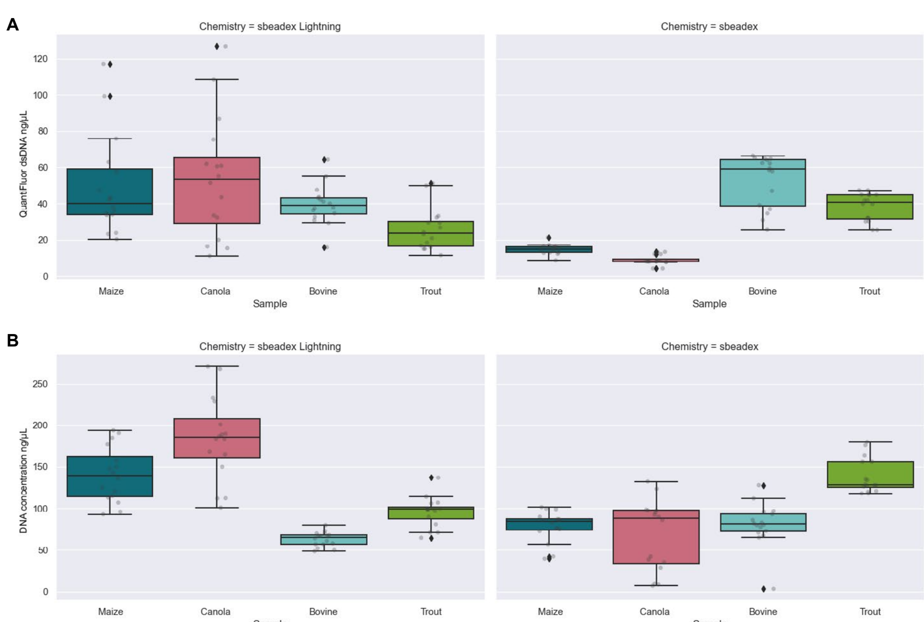

Figure 5. DNA yields for a range of sample types following automated DNA purification on the Hamilton STAR using both sbeadex Lightning and original sbeadex chemistries. DNA yields were quantified using QuantiFluor (5A) and via UV spectrophotometry (5B).

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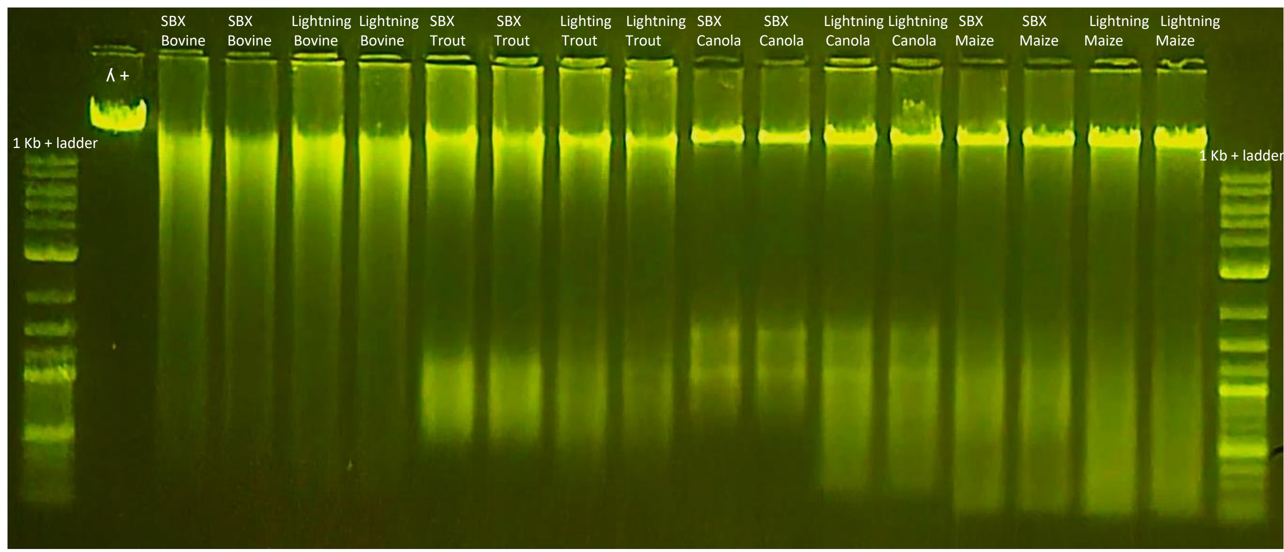

Figure 6. Gel electrophoresis visualisation of duplicate samples of DNA purified on the Hamilton STAR using sbeadex Lightning (Lightning) and original sbeadex (SBX) chemistries. DNA samples were normalised to 300 ng/µL. Results for four species are shown; bovine, trout, canola and maize.

Purification chemistry	Protocol time per 96-well plate	Tips required per 96-well plate	Liquid waste per 96-well plate	Solvent waste per 96-well plate	Guanidine salts utilised
sbeadex	90 min	288	1.58 mL	580 mL*	Yes
sbeadex Lightning	18 min	192	0.61 mL	200 mL	No

Table 6. A summary of the key savings for sbeadex Lightning compared to original sbeadex. This table summaries the savings in protocol time, plastic tips required, liquid waste and waste solvents/ethanol. All values refer to standard protocols post-lysis.
*Value for sbeadex Livestock Nucleic Acid Purification Kit. Value for sbeadex Maxi Plant Nucleic Acid Purification KIt are lower due to use of water in some steps.

Downstream DNA applications

KASP

DNA purified using the Hamilton STAR for automation, for both sbeadex Lightning and original sbeadex chemistries, was used as template in KASP end-point genotyping reactions. Figure 7 illustrates the genotyping cluster plot for one representative KASP assay, specific to soybean. All DNA dilutions generated good genotyping results with distinct clusters. Overall, a 98% call rate was achieved for soybean samples and there was 100% concordance of genotyping calls across the DNA dilutions. These results confirm that automation of DNA purification using sbeadex Lightning and original sbeadex does not result in any downstream PCR inhibition.

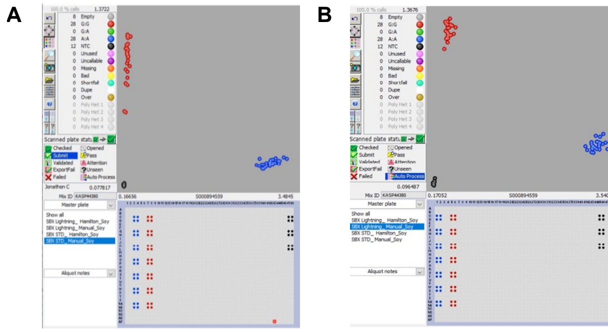

Figure 7. End-point genotyping cluster plots for soybean DNA (all dilutions) purified using sbeadex (7A) and sbeadex Lightning (7B) on the Hamilton STAR system. No PCR inhibition was observed in these samples.

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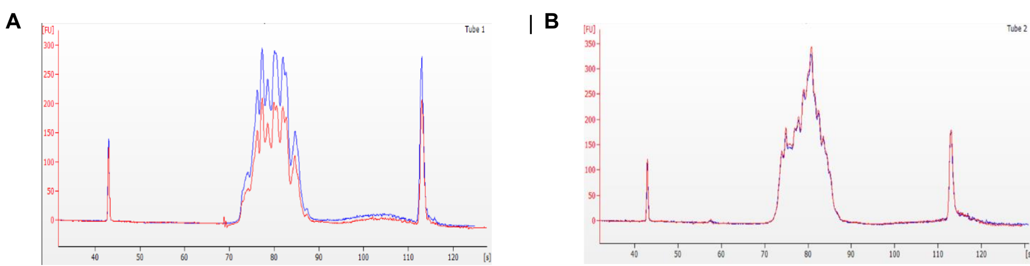

Figure 8. Bioanalyzer traces for Amp-Seq libraries generated from bovine DNA (8A) and maize DNA (8B), comparing results for sbeadex Lightning (red traces) and sbeadex (blue traces) purification of DNA. Comparable yields and library distributions were observed for each library.

Amp-Seq

DNA purified using sbeadex Lightning on the Hamilton STAR were successfully used to generate Amp-Seq libraries. Generated libraries were of equivalent yield and distribution as previously generated libraries for both bovine and maize DNA purified using original sbeadex (figure 8).

Summary

Our new sbeadex Lightning chemistry protocol is easily automatable using the Hamilton STAR platform. Once your manual sample-specific protocol is optimised, it can be transferred to the Hamilton STAR, with the POM window facilitating easy adjustments to physical protocol parameters.

Results presented demonstrate that sbeadex Lightning generates DNA of comparable yield and quality to original sbeadex, and that protocol automation improves these parameters for both chemistries. The automated sbeadex Lightning protocol requires just 18 minutes post lysis compared against the 1.5 hours needed for original sbeadex, with added benefits of reduced plasticware and reduced liquid waste. DNA purified using the automated sbeadex Lightning protocol is shown to be suitable for a broad range of downstream applications including end-point genotyping and NGS library preparation, making it an excellent choice for high-throughput automated workflows.

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