Application note

Automation of the sbeadex Blood DNA Purification Kit using Hamilton robotics

Introduction

The sbeadex™ Blood DNA Purification Kit is optimised for the isolation of highly purified nucleic acids from blood samples. The purification protocol can be easily automated and is compatible with many robotics platforms. LGC Biosearch Technologies have previously demonstrated automation using the KingFisher Flex (Thermo Fisher Scientific).

Here we have developed and verified a protocol for the automation of the sbeadex Blood DNA Purification Kit on the Hamilton Microlab STAR system (Hamilton Robotics), a platform routinely used in molecular diagnostics laboratories. The Hamilton Microlab STAR system's accessories mean that it can perform required heat steps without the need to offload plates, and that it does not require reagent plates to be pre-filled; both features result in enhanced automation of nucleic acid purification protocols as we detail here.

Methods

Protocol development

Biosearch Technologies worked to develop and optimise a protocol for automation of the sbeadex Blood DNA Purification Kit on the Hamilton Microlab STAR system. Specifications of the Hamilton instrument set up are detailed in table 1 and additional Hamilton equipment used is detailed in table 2. Protocol development involved investigation into liquid classes, methods for handling of the specific sbeadex Blood DNA Purification Kit reagents, appropriate shaking times, temperature optimisation and guard-banding. Laboratory consumables used in this study are detailed in table 3.

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Item	Units	Supplier	Part number	Notes
Hamilton STAR baseline	1	Hamilton	870101	Instrument with only 8-channel head can be used with protocol adjustment
Modular Arm for 4 / 8 / 12 Ch. / MPH	1	Hamilton	173051	
MPH 96 TADM, 1000 µL, CO-RE II, STAR	1	Hamilton	10120001	
2 channels 1000 µL, CO-RE II, RPC, STAR	4	Hamilton	10140943	
CO-RE GRIPPER 1000 µL on Waste Block	1	Hamilton	184089	

Table 1. Hamilton instrument specification used in this study.

Item	Units	Supplier	Part number	Notes
MFX Gravity Waste Module	1	Hamilton	10102492	
96-well magnetic block	1	Alpaqua	10103443	
Heater shaker	2	Hamilton	188319	
Landscape Shaker Carrier Base	1	Hamilton	187001	
Tip carrier (TIP_CAR_480_A00)	2	Hamilton	182085	
Plate carrier (PLT_CAR_L5AC_A00)	2	Hamilton	182090	
96 deep-well block heater shaker adapter	1	Hamilton		May be a custom order

Table 2. Additional Hamilton equipment used in this study.

Item	Units	Supplier	Part number	Notes
1000 µL filter tips	5 racks	Hamilton	235905	
300 µL filter tips	1 rack	Hamilton	235903	
sbeadex blood DNA purification kit	1	LGC Biosearch Technologies	NAP44410	
96-well 2.2 mL deep-well plate	1	Fisher	11594754	Can be changed subject to optimisation. Minimum well volume recommended: 2.2 mL
96-well 0.8 mL storage plate	1	Greiner	12194162	Can be changed subject to re-teaching. Minimum plate volume recommended: 0.2 mL
96-well 0.2 mL PCR plate	1	Fisher	12719458	

Table 3. Laboratory consumables used in this study.

Application note

Automation of the sbeadex Blood DNA Purification Kit using Hamilton robotics

The protocol was developed using a 96-channel head for all liquid handling to ensure DNA quality, quantity, and minimal contamination. A 96-channel head is ideal for full plates although it is possible to load partial tip racks to reduce tip waste if not working with full plates. Protocols for working with an 8-channel head can be developed upon request.

The specific plateware to be used and the positions chosen by users for consumables need to be taught to the Hamilton prior to performing the developed protocol. The protocol assumes that all troughs contain identical dead volumes.

Protocol verification

Once the Hamilton protocol was finalised, verification was performed to assess performance of the protocol. For this, automation of the sbeadex Blood DNA Purification Kit on the KingFisher Flex was used as the comparator as this automation protocol is well established.

For DNA purification, pooled blood samples from three donors (stored in EDTA tubes) were used as starting material. Working in a BSL-2 hood, 12 replicates of 200 µL thawed blood were supplemented with Proteinase K prior to Lysis Buffer SB addition. The lysis plates for each instrument (Hamilton and KingFisher) were manually assembled side-by-side using the same reagents. Using identical lot numbers of the sbeadex Blood DNA Purification Kit, the lysed samples were processed simultaneously on the Hamilton and the KingFisher Flex.

Following DNA purification, absorbance values were measured for all samples at 230 nm, 260 nm and 280 nm using a 2 µL droplet Nanophotometer (Implen) to enable DNA yield and quality to be assessed.

Tables 4 and 5 contain details of qPCR set up. Eluted DNA (50 µL) from each instrument was serially diluted (log10) and amplified using qPCR and an in-house RNase P assay. All PCR plate preparation was performed using Bioseach Technologies' liquid handlers and qPCR was performed on the CFX-384 (BioRad). RNAse P was detected by CFO labelled probes. End-point fluorescence and sample C_q values were measured.

Two Hamilton-purified samples underwent *Eco*RI digestion (1 µg) and T4 ligase-mediated DNA ligation (200 ng) with no post-digestion clean up, using in-house protocols. Plasmid pSF-CMV was used as a spike-in control. The products from each reaction were analysed by gel electrophoresis.

Reagent	Quantity	Supplier	Part number	Final concentration
RapiDxFire qPCR 5 × Master Mix GF	1	Biosearch Technologies	30050-2	1 ×
SuperROX, concentration 15 µM	1		SR-1000-1	75 nM
RNase P assay (100 ×)	1		Not available to purchase*	1 ×

Table 4. Composition of the qPCR master mix, including final concentrations. Individual reactions consisted of 1.2 µL of this master mix with 3.8 µL of sample to give a total reaction volume of 5 µL.
* Individual oligonucleotides targeting RNase P can be purchased as individual reagents. Please see our website for more information.

Application note

Automation of the sbeadex Blood DNA Purification Kit using Hamilton robotics

Step	Temperature (°C)	Time (minutes:seconds)	Number of cycles
1	95	02:00	1
2	95	00:03	50
	60	00:30	

Table 5. Thermal cycling conditions used for the qPCR.

Operating procedure
Once samples were plated and supplemented with lysis buffer and Proteinase-K, the plate was loaded onto the deck of the Hamilton along with tips, reagent troughs and a destination plate, as instructed by the on-screen loading guide. Following the on-deck heated lysis step, binding buffer bead mix was manually added to each well before resuming the protocol on the Hamilton which concludes with eluted sample being transferred into the destination plate for downstream processing.

Results and discussion

Hamilton protocol
Table 6 details the protocol that has been developed for automation of the sbeadex Blood DNA Purification Kit on the Hamilton. The Hamilton system specifications used are detailed in table 1. The protocol can be modified to accommodate system specifications that differ to those detailed in table 1, such as the use of an 8-channel head or a smaller base instrument.

Lysis buffer SB is manually added to the sample following addition of Proteinase K. The required heating and shaking steps for sample lysis are automated on the Hamilton. Following this, Binding buffer SB and sbeadex bead mix is added manually before proceeding to automated downstream steps on the Hamilton. In our experiments all heat steps were performed using the Hamilton rather than off-deck. The protocol has a total run time of 85 minutes on deck per 96-well plate.

The run file (.pkg) for the Hamilton system can be accessed here.

Step	Ratio of reagent to sample	Volume (µL)	Time (minutes)	Temperature (°C)	Shaking	Speed (rpm)	Notes
Lysis	1:1	200	30	95	Constant	1500	No cooldown
Binding	1.8:1	360	4	RT	Constant	1350	Binding buffer bead mix comprised of 9:1 ratio of Binding buffer SB to sbeadex beads. Manually added to each well.
Buffer BN1	4:1	800	5	RT	Constant	1000	
Buffer TN1	4:1	800	5	RT	Constant	1350	
Buffer TN2 × 2	2:1	400	2.5	RT	Constant	1700	
Buffer AMP	N/A	50	10	76	Constant	1750	Variable

Table 6. Summary of the sbeadex Blood DNA Purification Kit protocol used when automating on the Hamilton Microlab STAR system. Ratio of reagent to sample is provided for general use. A ratio is not supplied for elution buffer AMP as this will depend on customer requirements. Volumes stated refer to those used throughout the development and verification of the protocol. RT denotes room temperature.

Application note

Automation of the sbeadex Blood DNA Purification Kit using Hamilton robotics

Verification

DNA yield from 200 µL blood samples was equivalent between Hamilton-purified samples and KingFisher-purified samples (figure 1A, table 7; Student's T-test, df = 15.8, P = 0.0967). Measured absorbance values were used to calculate 260/230 and 260/280 ratios. Both ratios were significantly higher for Hamilton-purified samples than for KingFisher purified samples (figures 1B and 1C, table 7; Student's T-test, df = 21, P<0.0001).

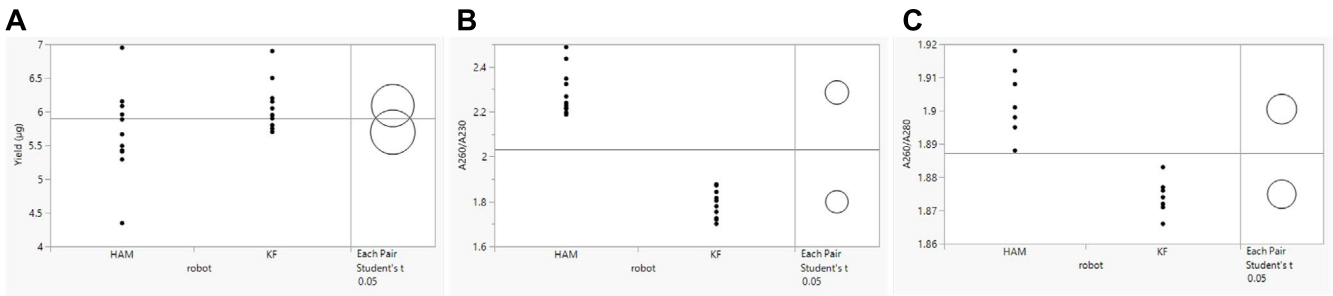

Figure 1. A) Scatter plot showing equivalent yield (µg) between samples purified on the Hamilton robot and on the KingFisher robot. B) Scatter plot illustrating higher A260/230 ratios for samples purified on the Hamilton than on KingFisher. C) Scatter plot showing higher A260/280 ratio for samples purified on the Hamilton (HAM) than on the KingFisher (KF).

	Concentration		Adjusted yield		A260/230		A2260/280	
Robot	Mean	Std dev	Mean	Std dev	Mean	Std dev	Mean	Std dev
Hamilton	115.273	9.819	5.696	0.650	2.286	0.101	1.900	0.009
KingFisher	121.833	7.554	6.092	0.378	1.799	0.063	1.875	0.004

Table 7. Means and standard deviations of concentration, adjusted yield, A260/230 and A260/280 ratios for Hamilton- and KingFisher-purified DNA. Adjusted yield accounts for differences in eluate volume between wells, which were measured by reverse pipetting. Std dev = standard deviation.

In the qPCR experiments, C_q values for Hamilton-purified samples and KingFisher-purified samples were comparable as shown in figure 2. Neat Hamilton-purified samples amplified on average 0.7 cycles before KingFisher-purified samples and demonstrated comparable variation. qPCR efficiency calculated from Hamilton-purified samples fell within the acceptable range (90-110%) for an inhibitor-free reaction, and these samples performed better than the KingFisher-purified samples (table 8).

Application note

Automation of the sbeadex Blood DNA Purification Kit using Hamilton robotics

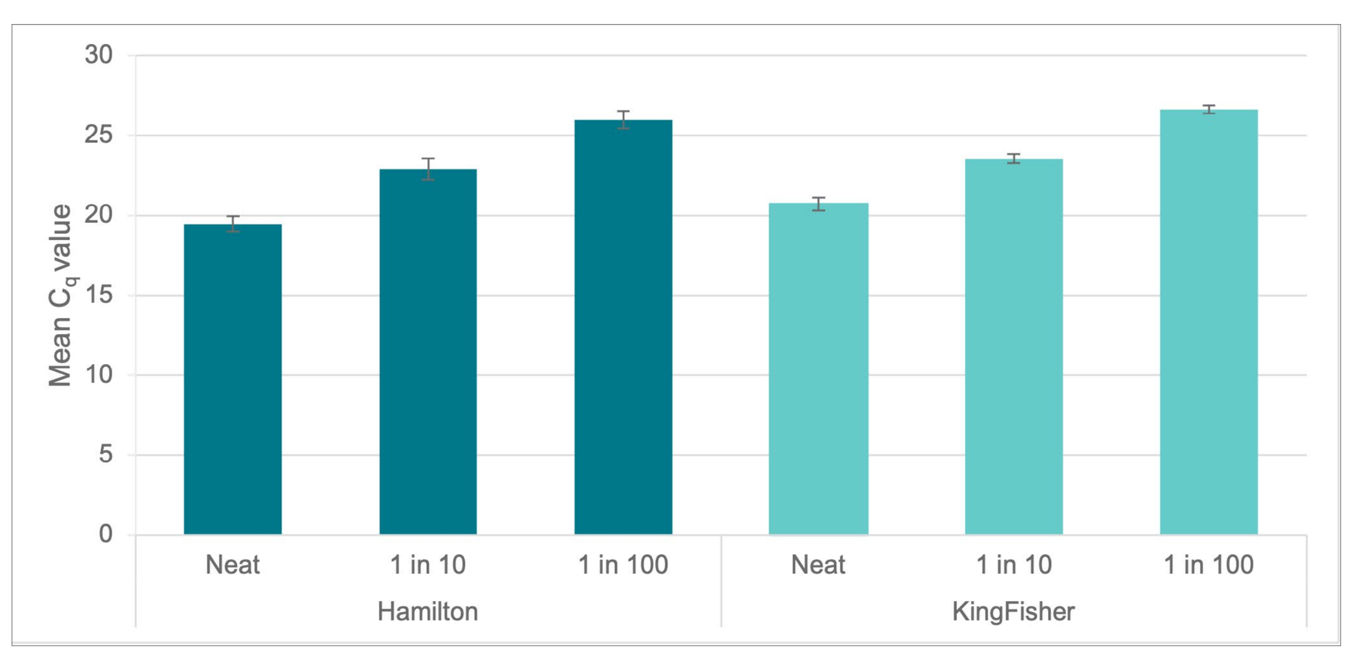

Figure 2. Mean C_q values for RNase P for both Hamilton-purified and KingFisher-purified samples. Purified DNA was used neat (n=24), diluted 1 in 10 (n=12) and 1 in 100 (n=12). Error bars represent standard deviation.

Robot	PCR Efficiency (%)	Rsq
Hamilton	101.44	0.989
KingFisher	118.55	0.98

Table 8. RNaseP qPCR efficiency values for DNA purified on the Hamilton and on the KingFisher.

To understand the compatibility of purified DNA with common Next Generation Sequencing (NGS) enzymatic reactions, representative Hamilton-purified DNA from blood samples underwent both restriction digestion and ligation and were analysed by gel electrophoresis (figure 3). *Eco*RI was functional in gDNA-negative controls and in gDNA in the presence and absence of plasmid (lanes 3 and 4, and 10 and 11). Ligation of digested plasmid resulted in a similar band pattern to undigested control (lanes 5-7), indicating likely concatemers of plasmid running in higher bands. This was also seen when spiked into a gDNA background, with re-ligation of genomic material and larger novel ligation products running near the top of the gel (lanes 12 and 13). Overall, this indicates that purification of DNA from blood samples on the Hamilton does not inhibit enzymatic reactions commonly used in NGS library preparations.

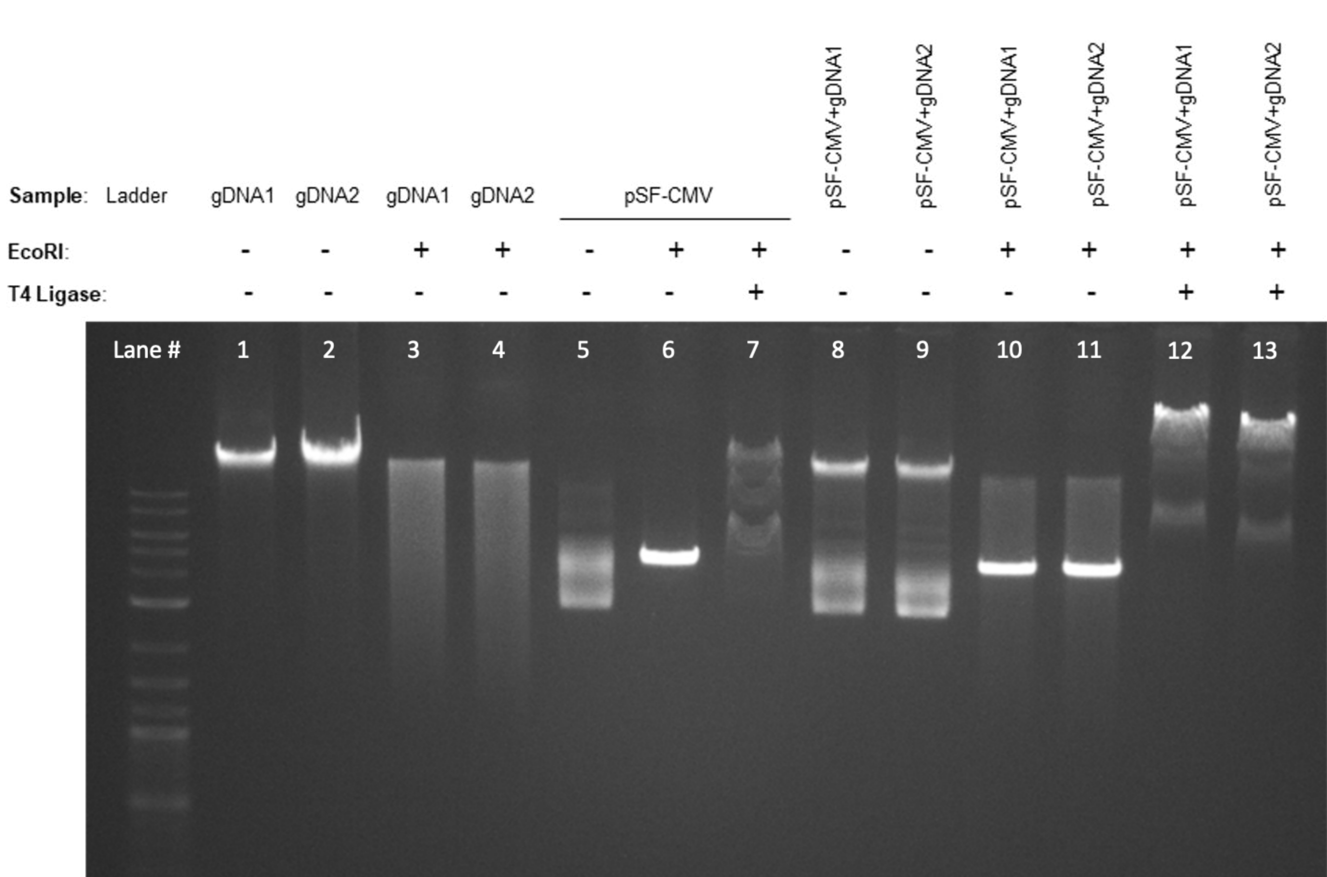

Figure 3. Agarose (1%) gel electrophoresis of digested Hamilton-purified gDNA (lanes 3-4), plasmid control DNA digested and ligated (lanes 5-7), or both Hamilton-purified DNA and plasmid digested and ligated (lanes 8-13), indicating broad enzyme functionality across both reactions. Lanes 1 and 2 contain untreated Hamilton-purified DNA.

Guard banding

Within the protocol development process, guard-banding of certain parameters of the sbeadex Blood DNA Purification Kit protocol was performed.

Within the dispensing steps, it was demonstrated that fewer tips can be employed during the protocol, i.e. one set for supernatant aspiration, one column per reagent dispensed, but that this increases the risk of contamination. Dispensing reagents from above the wells, or using all clean tips, gave the best qPCR efficiencies. When supernatant tips were re-used, this resulted in higher levels of contamination within the samples.

Work was also carried out to investigate adjustments to the volumes (and combinations of volumes) of BN1 and TN1 wash buffers used. None of the adjustments had a significant effect and the target 260/230 ratio was not reached indicating that the volumes used in the standard method are already optimal. There was a small but significant improvement in downstream C_q value with increasing TN1 volumes which may assist to guide protocol optimisation if lower C_q values are desired.

Application note

Automation of the sbeadex Blood DNA Purification Kit using Hamilton robotics

Optimisation

It is possible to adjust both the input blood volume and/or the elution volume used in these experiments to accommodate limiting blood volumes or total yield requirements. Volumes of between 50 and 200 µL can be used for input blood and for elution without compromising the outputs significantly; for all combinations a minimum total yield of 2 µg DNA is still possible.

Summary

To summarise, Biosearch Technologies have achieved the goal of developing a standard protocol for automation of the sbeadex Blood DNA Purification Kit on the Hamilton Microlab STAR system. With this protocol, we can achieve equivalent or improved performance metrics (DNA quality, C_q values and PCR efficiencies) to those for the well-established KingFisher Flex protocol for automation of the same kit. We also demonstrate use of Hamilton-purified samples in downstream enzymatic reactions typical of NGS library preparation workflows.

The run file (.pkg) for the Hamilton protocol outlined in table 4 is available for customers to use. There are opportunities to optimise this further depending on your requirements, such as lower blood input volume, reduced plastic consumption or use of an 8-channel head. The Global Workflow Solutions Team at Biosearch Technologies welcomes your enquiries and would love to assist in the optimisation of your workflow. Please contact them at IST.UK@lgcgroup.com.

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