Application note

Automation of the sbeadex Pathogen Nucleic Acid Purification Kit using Hamilton robotics

Introduction

The sbeadex™ Pathogen Nucleic Acid Purification Kit is optimised for the isolation of highly purified nucleic acids from viral, bacterial and yeast samples. The purification protocol is well suited to automation and is compatible with many robotics platforms. LGC Biosearch Technologies have previously demonstrated automation using the oKtopure and the KingFisher Flex (ThermoFisher Scientific).

Here we have developed and verified a protocol for automation of the sbeadex Pathogen Nucleic Acid Purification Kit on the Hamilton Microlab STAR system (Hamilton Robotics), a platform routinely used in molecular diagnostics laboratories. The Hamilton Microlab STAR system's onboard accessories mean that it can perform required heat steps (lysis and elution) 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. We directly compared performance of the sbeadex Pathogen Nucleic Acid Purification Kit using the Hamilton Microlab STAR with that of ThermoFisher Scientific's popular KingFisher Flex.

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

Application note

Automation of the sbeadex Pathogen Nucleic Acid Purification Kit using Hamilton robotics

Methods

Protocol development

Biosearch Technologies worked to develop and optimise a protocol for automation of the sbeadex Pathogen Nucleic Acid Purification Kit on the Hamilton Microlab STAR system (hereafter Hamilton). 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 Pathogen Nucleic Acid Purification Kit reagents, appropriate shaking times, temperature optmisation and guard-banding. Laboratory consumables used in this study are detailed in table 3.

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 Microlab STAR instrument specifications.

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		Hamilton	187001	
Tip carrier (TIP_CAR_480_A00)	1	Hamilton	182085	
Plate carrier (PLT_CAR_L5AC_A00)	1	Hamilton	182090	
60 mL trough carrier (RGT_CAR_5R60_A00)	1	Hamilton	194057	
120 mL trough carrier (RGT_CAR_3R120_B00)	1	Hamilton	194058	
96 deep-well block heater shaker adapter	1	Hamilton		May be custom order

Table 2. Additional Hamilton equipment used in this study.

Application note

Automation of the sbeadex Pathogen Nucleic Acid Purification Kit using Hamilton robotics

Item	Units	Supplier	Part number	Notes
1000 µL filter tips	2 racks	Hamilton	235905	
300 µL filter tips	1 rack	Hamilton	235903	
60 mL troughs	1	Hamilton	194051	
120 mL troughs	3	Hamilton	194052	
sbeadex Pathogen Nucleic Acid Purification Kit	1	Biosearch Technologies	NAP40-024-04	
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

Table 3. Consumables used in this study.

Protocol verification

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

AccuPlex SARS-CoV-2 controls (LGC Clinical Diagnostics formerly SeraCare, part number 0505-0168) were diluted to 20, 12, 10, 8, 4 and 0 copies per reaction in human nasopharyngeal swabs. Each swab was incubated in 12 mL of Te buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0). Each sample dilution was lysed before separating into the Hamilton and the KingFisher Flex blocks for purification. Human swab diluent was supplemented with carrier RNA at 1 µg/mL.

Using identical lot numbers of the sbeadex Pathogen Nucleic Acid Purification Kit, the Hamilton and the KingFisher Flex were run simultaneously. Sixteen extraction replications per concentration were amplified in duplicate giving 32 technical PCR replications per concentration, per plate. One qPCR plate was run, along with two end-point PCR (ePCR) plates used as independent measures of amplification.

Tables 4 and 5 contain details of PCR set up. N1 and N2 targets were detected by FAM-labelled probes and RNase P (RP, human control) was detected by CFO-labelled probes. All PCR plate preparation was performed using Biosearch Technologies' liquid handlers and qPCR was performed on the CFX-384 (BioRad). End-point fluorescence, sample C_q values and the Limit of Detection (LOD) were all measured.

Concentrations of control material reported were verified using an in-house digital PCR method. Samples extracted on the KingFisher Flex were used as standards.

The verification method was performed in duplicate, with one run executed by an untrained operator to assess the reproducibility of the protocol.

Application note

Automation of the sbeadex Pathogen Nucleic Acid Purification Kit using Hamilton robotics

Reagent	Quantity	Supplier	Part number	Final concentration
RapiDxFire qPCR 5X Master Mix GF	1	Biosearch Technologies	30050-2	1X
EpiScript RNase H- Reverse Transcriptase, 200 U/µL	1		ERT12925K-ENZ	5 U/µL
SuperROX, concentration 15 µM	1		SR-1000-1	75 nM
2019-nCoV N12/RnP Blend (100X)*	1		not available to purchase	1X

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.
* This blended product is no longer available. Individual oligonucleotides targeting the same sequences used in these reactions can be purchased as individual reagents. Please see our website for more information.

Step	Temperature (°C)	Time (minutes:seconds)	Number of cycles
1	50	15:00	1
2	95	02:00	1
3	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, 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

Hamilton protocol

Table 6 details the protocol that has been developed for automation of the sbeadex Pathogen Nucleic Acid 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 absence of a multi-pipette head or a smaller base instrument.

Viral inactivation is performed off-deck with the manual addition of Lysis buffer SB to the sample. 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 94 minutes on deck per 96-well plate.

Application note

Automation of the sbeadex Pathogen Nucleic Acid Purification Kit using Hamilton robotics

Step	Volume (µL)	Time (minutes)	Temperature (°C)	Shaking	Speed (rpm)	Notes
Lysis of sample	200	30	95-55 ramp	Constant	1500	
Binding	340	4	RT	Constant	1250	Binding 320 µL; Beads 20 µL – both added manually
Buffer BN1	400	5	RT	Constant	1500	
Buffer TN1	400	5	RT	Constant	1500	
Buffer TN2	400	5	RT	Constant	1500	
Buffer AMP	60	10	90	Constant	1750	

Table 6. Summary of the sbeadex Pathogen Nucleic Acid Purification Kit protocol used when automating on the Hamilton Microlab STAR system. RT denotes room temperature.

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

Verification

No significant differences were observed between Hamilton-purified samples and KingFisher Flex-purified samples for all experimental parameters measured including total fluorescence, C_q values, probability of sample detection and the predicted LOD for each purification protocol. This provides confidence in the Hamilton protocol for automation of the sbeadex Pathogen Nucleic Acid Purification Kit and confirms equivalent performance to the well-established KingFisher Flex automation protocol.

Samples purified on the Hamilton system generated equivalent or higher ROX-normalised fluorescence values than identical samples purified on the KingFisher Flex. Figure 1 illustrates the higher fluorescence values for Hamilton-purified samples observed in qPCR (figure 1A), and the equivalent fluorescence values of Hamilton-purified and KingFisher Flex purified samples in ePCR (figure 1B).

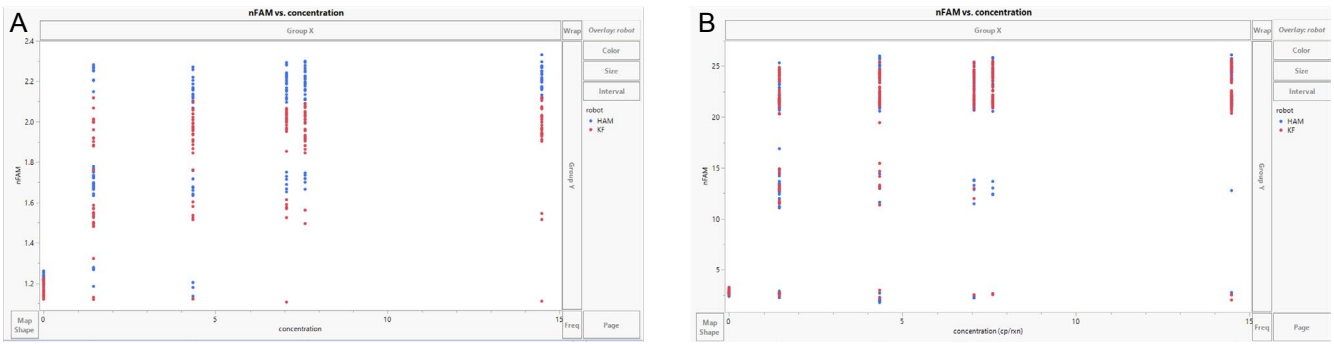

Figure 1A and B. ROX-normalised endpoint fluorescence of diluted samples purified on the Hamilton instrument (HAM, blue data points) and the KingFisher Flex instrument (KF, red data points). Figure A details qPCR-derived fluorescence and figure B details ePCR-derived fluorescence. Samples purified on the Hamilton showed higher fluorescence across nucleic acid concentrations and within qPCR calling clusters compared to those purified on the KingFisher Flex (1A), although fluorescence values overlapped in ePCR (1B).

Application note

Automation of the sbeadex Pathogen Nucleic Acid Purification Kit using Hamilton robotics

No significant differences in C_q values were observed between the Hamilton-purified samples and KingFisher Flex-purified samples across all RNA concentrations tested (figure 2).

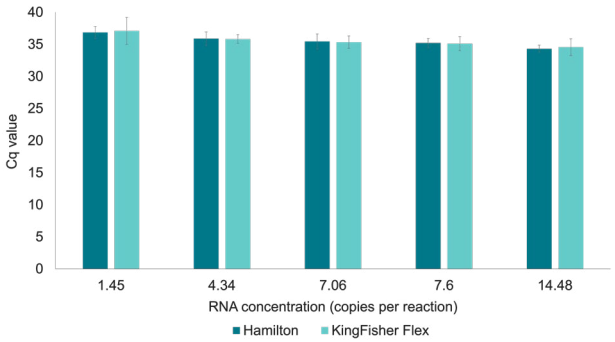

Figure 2. SARS-CoV-2 sample average C_q values for sample dilution series on both robots based on qPCR. No significant different in the C_q value for the nCoV assays was observed between the two robots at any concentration (paired t-test, P_{range} = 0.30-0.74).

Probability of sample detection for both robots was calculated from logistic regression based on ePCR. As shown in table 7, the probability of detection of amplified material overlapped between the two robots.

Robot		
	Hamilton	KingFisher Flex
	Probability of detection	Probability of detection
Concentration of RNA (copies/reaction)	Mean	Mean
0	0.18	0.27
1.45	0.33	0.44
4.34	0.71	0.78
7.06	0.92	0.94
7.6	0.94	0.95
14.48	1.00	1.00

Table 7. Probability of sample detection at various concentrations on each instrument from logistic regression based on ePCR. Values for probability of detection of amplified material overlapped between the two robots.

In this study LOD is defined as the concentration at which ≥95% of samples amplified into the positive cluster (ePCR), and the point at which positive samples did not exceed a C_q of 40 cycles (qPCR). LOD was predicted using logistic regression models based on generated data for samples purified using either robot. For both methods (qPCR and ePCR), the LOD for the N1 and N2 targets for Hamilton-purified samples was lower than for KingFisher Flex-purified samples (table 8). The same pattern was observed for the RNase P assay (data not shown).

Application note

Automation of the sbeadex Pathogen Nucleic Acid Purification Kit using Hamilton robotics

Method	Robot	Predicted LOD	Lower CI95	Upper CI95
qPCR	Hamilton	9.46	8.15	11.60
	KingFisher	10.29	8.59	13.30
ePCR	Hamilton	8.08	7.12	9.45
	KingFisher	7.53	6.53	9.02

Table 8. Predicted LOD and 95% confidence intervals (CI95) based on logit regression models using data generated on ePCR and qPCR for samples extracted on either robot. The LOD for Hamilton-purified samples was lower and with a smaller confidence interval than for KingFisher-purified samples.

The LOD from both robots was in line with the established assay LOD (approximately 10-12 copies per reaction).

To determine reproducibility of the protocol for automation of the sbeadex Pathogen Nucleic Acid purification kit, both the KingFisher Flex purification and the Hamilton purification were performed by two independent operators. Figure 3 details normalised fluorescence values for both instruments and illustrates the broadly overlapping clusters between the two independent runs thus reinforcing the reproducibility of the protocol. Figure 4 illustrates the overlapping C_q values obtained for both instruments across the two independent runs, and hence demonstrates the equivalent performance of the protocol regardless of operator. In addition, more variation is observed between C_q values of samples of similar concentration than between the two independent runs.

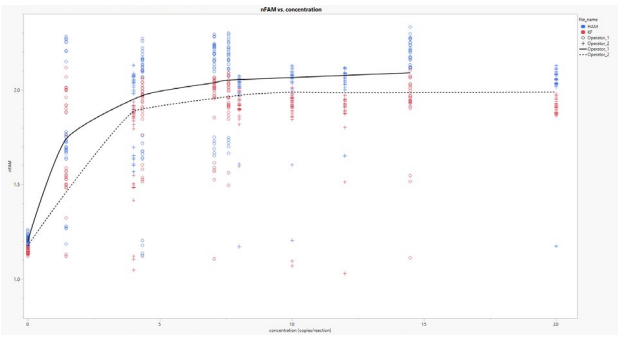

Figure 3. Normalised nCoV fluorescence of diluted samples purified on the Hamilton instrument (HAM, blue data points) and the KingFisher Flex instrument (KF, red data points) across two independent runs. Circles denote operator 1 and crosses denote operator 2. Trendlines represent each run. nFAM clusters broadly overlap between the two independent runs, reinforcing reproducibility of the protocol.

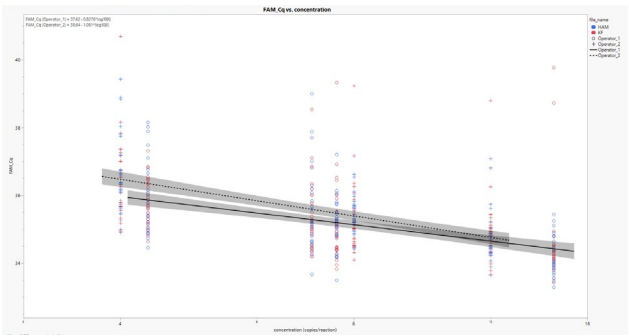

Figure 4. Alignment of C_q values obtained from diluted samples purified on the Hamilton instrument (HAM, blue data points) and the KingFisher Flex instrument (KF, red data points) across two independent runs. Circles denote operator 1 and crosses denote operator 2. Trendlines represent each run. C_q values overlap between runs when accounting for concentration differences, indicating equivalent performance. Indeed, slopes close to 1 indicate good assay linearity. In addition, more variation is observed between C_q values of samples of similar concentration than between the two independent runs.

Application note

Automation of the sbeadex Pathogen Nucleic Acid Purification Kit using Hamilton robotics

Guard banding

Within the protocol development process, guard-banding of certain parameters of the sbeadex Pathogen Nucleic Acid Purification Kit protocol was performed. Key observations include:

- The TN2 wash step (important for removal of guanidine from extracts): a mis-dispense during this step of up to 50% resulted in only a 0.5-1 C_q difference in end results.
- Lysis temperature: a range of lysis temperatures were tested and compared to the KingFisher Flex purified samples. There was no significant difference between samples lysed at 65 °C, 95 °C, those lysed following the KingFisher Flex protocol and a concentration-matched non-extracted control with regards to C_q values (Wilcoxon pairs, df = 23, P_{range} 0.19-0.58) or normalised fluorescence (Wilcoxon pairs, df = 23, P_{range} 0.06-0.68). A lysis temperature of 95 °C was selected to match the KingFisher Flex protocol most closely.
- Lysis time: a range of lysis times were tested (3, 15 and 30 minutes) and compared to the KingFisher Flex purified samples along with a concentration-matched non-extracted control. No significant difference in C_q values was observed between samples lysed for the different lysis times and those lysed following the KingFisher Flex protocol (Wilcoxon pairs, df = 23, P_{range} 0.10-0.65). A lysis time of 30 minutes was selected to match the KingFisher Flex protocol and non-extracted control most closely.
- Inclusion of Protease K: for both Hamilton-purified and KingFisher Flex-purified samples, the inclusion or Protease K in the purification process significantly improved C_q values compared to the no-Protease K controls for each robot (Oneway ANOVA, n = 71, P<0.001). The spread of total fluorescence values was also substantially reduced for samples purified with the inclusion of Protease K.

These results illustrate the robustness of the sbeadex Pathogen Nucleic Acid Purification Kit and have identified several key protocol parameters that can be adjusted and optimised if required.

Troubleshooting

Based on our experience of automating the sbeadex Pathogen Nucleic Acid Purification Kit on the Hamilton robot, there are a few troubleshooting tips to be aware of that we have outlined in table 9.

Error	Issue	Resolution
Insufficient liquid detected at eluate transfer stage	Relates to liquid detection or the taught position of the head	• Automatic error handling: Tip aspirate from bottom • Verify taught position for eluate aspiration • Ensure cLLD is disabled in the aspiration settings and/or liquid class
TN2 overage – post-dispense error	Error suggests TN2 has run out, but this is not the case	• Can increase the post-aliquot until total volume aspirated reaches 1000 µL (maximum tip capacity)
Insufficient emptying of gravity waste trough	Can result in waste carryover on supernatant aspiration tips	• Remove any kinks or elevation in tubing • Increase the dispense height for supernatant waste

Table 9. Troubleshooting tips for automation of the sbeadex Pathogen Nucleic Acid Purification Kit on the Hamilton Microlab STAR system.

Application note

Automation of the sbeadex Pathogen Nucleic Acid Purification Kit using Hamilton robotics

Optimisation

There are plenty of opportunities for further optimisation of the protocol developed at Biosearch Technologies for automation of the sbeadex Pathogen Nucleic Acid Purification Kit on the Hamilton Microlab STAR system. It is possible to run certain steps in parallel to optimise run time. Protocol adjustments can be made to improve yield, and throughput can also be increased by using a 96-head and associated troughs. The purification protocol is robust, and the guard-banding section outlines key protocol areas where adjustments can be made if desired such as lysis time and lysis temperature.

Summary

To summarise, Biosearch Technologies have achieved the goal of developing a standard protocol for automation of the sbeadex Pathogen Nucleic Acid Purification Kit on the Hamilton Microlab STAR system. With this protocol, we can achieve equivalent or improved performance metrics (LOD, C_q values and total fluorescence) to those for the well-established KingFisher Flex protocol for automation of the same kit. We also demonstrated good reproducibility and robustness of the sbeadex Pathogen Nucleic Acid Purification Kit protocol on the Hamilton Microlab STAR system.

The run file for the Hamilton protocol outlined in table 6 is available for customers to use. There are opportunities to optimise this further to improve the parameters that are most important to your laboratory, be that total yield, run time, throughput or plastic consumption. 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.

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC