Science & Justice 59 (2019) 411–417

Contents lists available at ScienceDirect

Science & Justice

journal homepage: www.elsevier.com/locate/scijus

Development of enhanced sensitivity protocols on the RapidHIT™ 200 with a view to processing casework material

David Shackleton[a,*], Jenny Pagram[b], Nicholas Andrews[a], Simon Malsom[a], Lesley Ives[a], Des Vanhinsbergh[a]

[a] Key Forensic Services, 4 Penfold Drive, Wymondham, Norfolk NR18 0WZ, UK
[b] Key Forensic Services, 207c Cavendish Place, Birchwood Park, Warrington, Cheshire WA3 6WU, UK

ABSTRACT

The RapidHIT™ 200 device from IntegenX® provides a sample-to-profile platform that is capable of processing a variety of sample types. In this study we review the sensitivity of the 'Run Other' protocol for processing crime stain type samples containing various input quantities of DNA using the AmpFℓSTR® NGMSElect™ Express PCR Amplification Kit cartridges available from IntegenX®. The range of DNA inputs which achieved useable results were not as desired and therefore various enhancements to the instruments extraction processes were investigated. These studies showed an improvement in the range of DNA input templates that could by processed on the RapidHIT™ 200 by using the enhanced methods and resulted in three new run protocols.

1. Introduction

The use of short tandem repeats (STR) is a central tool within the United Kingdom (UK) Criminal Justice System with DNA being sourced from a wide variety of materials. The RapidHIT® 200 (IntegenX®, Pleasanton, CA, USA) integrates the sample handling steps starting from buccal swabs or crime stain samples through DNA extraction, normalisation, amplification, separation and detection [1–4]. This instrument has been validated for use on buccal samples for loading to the UK National DNA Database (NDNAD) by Key Forensic Services, UK [5]. This validation was carried out to the appropriate standards for DNA forensic work in the UK [6–8] and includes the use of the AmpFℓSTR® NGMSElect™ Express PCR Amplification Kit from Thermo-Fisher Scientific (Waltham, MA, USA) [9].

Previous studies published on the use of the RapidHIT™ 200 device with crime stain material [10–14] have focused on the AmpFℓSTR® GlobalFiler™ Express PCR Amplification Kit (Thermo-Fisher Scientific, Waltham, MA, USA) and are not, therefore, directly related to the use of the instrument on these sample types in the UK. A literature search revealed no publications on the use of NGMSElect™ Express PCR kit with casework type samples on the RapidHIT™ 200.

In order to examine the suitability of the RapidHIT™ 200 for processing crime stain type materials using the NGMSElect™ Express cartridges available from IntegenX® a series of tests have been undertaken using the standard 'Run Other' protocol. This protocol was designed by IntegenX® to facilitate the processing of samples with lower input DNA templates than seen with typical buccal samples and is based on their protocols for GlobalFiler™ Express [11]. It is selected during instrument run setup and is different to the 'Buccal' protocol previously validated [5]. After the results from the initial NGMSElect™ Express study, co-operation from IntegenX® was sought to devise and test a selection of variants on the instrument extraction parameters and attempt to improve the range of input templates that yield useful DNA profiles.

In this paper we present the results from the studies on both the 'Run Other' and enhanced protocols.

2. Materials and methods

2.1. Sample preparation

Positive control samples were created by adding 100 µl of whole blood from a male donor with a known DNA profile, directly to a 3" Forensic Woodstick Mini-Swab (WA Products, Essex, UK).

In order to mimic basic casework samples from blood spots on non-porous surfaces, blood samples were prepared on acetate sheets (WA Products, Essex, UK) using whole blood from the same donor as used for the positive samples. Blood spots were created to cover the range of spots routinely encountered in casework: 10 mm, 7 mm, 5 mm, 2 .5 mm & 1 .5 mm diameters. Each sample was allowed to dry at room temperature. Samples were swabbed from the acetate using wet 3" Forensic Woodstick Mini-Swab (WA Products, Essex, UK) to remove all visible blood. Each stain and swab was photographed to produce a visual reference set for future operators.

In order to test a range of input DNA than achieved with the blood

* Corresponding author.
E-mail address: david.shackleton@keyforensic.co.uk (D. Shackleton).

https://doi.org/10.1016/j.scijus.2019.03.002
Received 20 September 2018; Received in revised form 25 February 2019; Accepted 3 March 2019

Table 1
Expected and measured amounts of DNA (± 1× SD) on both blood spots and cell-line samples.

Blood spot diameter/Expected total DNA		Average total DNA from 'Bench Top' process:
Positive		233.40 ng ± 74.9
Blood spots	10 mm	307.45 ng ± 49.6
	7 mm	152.82 ng ± 43.4
	5 mm	73.09 ng ± 27.5
	2.5 mm	3.48 ng ± 0.8
	1.5 mm	1.26 ng ± 0.4
Cell-line sample	15 ng	13.90 ng ± 0.9
	1 ng	798.8 pg ± 173.2
	100 pg	110.0 pg ± 24.7
	25 pg	23.1 pg ± 20.0
	6 pg	5.6 pg ± 11.2

samples (≤15 ng), the human embryonic palatal mesenchymal cell-line, designated 1000 M (ATCC, Manassas, Virginia, USA) was used. Samples were prepared to give DNA yields in the 15 ng to 6 pg range, based on 6 pg DNA per diploid cell using PBS buffer to create the dilutions. In each case 30 µl of cells/PBS buffer was added to OmniSwabs™ (GE Healthcare/Whatman, Maidstone, UK) [11].

Table 1 shows the expected concentration of the DNA on each swab based on 6 pg DNA per diploid cell and measured concentration from the Bench Top processes.

2.2. RapidHIT™ 200 protocols

Two RapidHIT™ 200 instruments (RH200-0021 and RH200-0024) were used. These instruments were operating with version 1.3.3 of the RapidHIT™ 200 software suite, which incorporates GeneMarker® HID Auto v.2.6.13 (SoftGenetics®, State College, PA, USA) [15].

The bead concentration, LysisSoakTime and DNAToBeadCaptureCycles parameters control the stages of the extraction process which affect DNA extraction efficiency. Each parameter change is described as follows:

x**A**/**B**/**C** where **A** = bead concentration, **B**
= LysisSoakTime (in minutes), **C**
= DNAToBeadCaptureCycles (in seconds)

The bead concentration is the concentration of paramagnetic beads in the sample cartridge relative to IntegenX® standard concentration,[1] the LysisSoakTime is the length of time which samples are held in lysis buffer with the heaters switched on and the DNAToBeadCaptureCycles is the length of time allowed for the DNA to be bound to the paramagnetic beads before the magnets are inserted into the sample cartridge to immobilise the beads prior to washing.

The initial testing was carried out using the standard 'Run Other' protocol; using the nomenclature defined above this protocol would be described as x1/6/50.

In order to test the use of increased paramagnetic bead concentrations, IntegenX® manufactured one-off custom NGMSElect™ Express cartridges with x2, x3 & x4 the standard concentration at the request of the authors. For the other tested parameters the 'Run Other' protocol script within the v.1.3.3 software was modified by the authors. Parameter sets (Table 2) were tested individually and then in combination, combinations were based on the results from the single parameter tests.

[1] The precise concentration of beads is not known to the authors as it is IntegenX® propriety information

Table 2
Parameter sets tested.

Run parameter	Setting (bead conc/lysis time (mins)/ bead capture (s))
Run other protocol	x1/6/50
Increased DNAToBeadCaptureCycles	x1/6/100, x1/6/200
IncreasedLysis soaktime	x1/30/50, x1/60/50
Combinations	x1/30/100
	x2/6/100, x3/6/100, x4/6/100

2.3. RapidHIT processing and data analysis

Each blood spot, positive control and cell-line dilution was tested twice on each parameter; positive controls were included in all runs. In all cases sample was introduced to the RapidHIT™ 200 sample cartridge using sterile tweezers. These samples were processed using 2 × 3-sample NGMSElect™ Express cartridges.

Profile analysis was carried out using the GeneMarker® HID Software v2.6.13 (SoftGenetics®, State College, PA, USA). A limit of detection of 35 rfu was used throughout; this value was derived during the validation of the RapidHIT™ 200 using NGMSElect™ Express cartridges for buccal samples [5]. Data analysis was carried using the Microsoft® Excel software.

2.4. Manual processing and data analysis

In order to provide comparison to the current 'Bench Top' processes in place at Key Forensic Services for the processing of casework samples two sets of each blood spot, positive control and cell-line dilution were profiled. DNA extraction was carried out using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) and concentrated using Microcon® (Merck Millipore, Burlington, MA, USA) giving a final extract volume of 20 µl. Each sample was quantified, in duplicate, using Plexor® HY real-time PCR quantification kit (Promega, Maddison, WI, USA). Allele amplification was carried out using the PowerPlex® ESI17 Fast PCR amplification kit (Promega, Maddison, WI, USA) and electrophoretic separation carried out on the Life Technologies™ 3130xl (Thermo Fisher Scientific, Waltham, MA, USA). Profile analysis was carried out using GeneMapper ID-X v1.3 (Thermo Fisher Scientific, Waltham, MA, USA). A limit of detection of 25 rfu was used throughout; this value was derived during the validation of the 'Bench Top' methods at Key Forensic Services. Data analysis was carried out in the Microsoft® Excel software.

3. Results & discussions

3.1. Sample preparation

Blood samples were set up as described – see Supplementary Information for photographs of samples. Table 1 shows the total amount of DNA present in each sample processed via the 'Bench Top' process and, therefore, an estimate of the amount of DNA introduced to the sample cartridge for each type tested. This range covers the typical range of sample input template seen from crime stain materials [Key Forensic Services – Internal Data, April 2018].

3.2. Comparison between 'run other' and 'bench top' process

All samples processed with both the 'Bench Top' and RapidHIT™ 200 'Run Other' protocols were concordant with the expected donor alleles and no incidences of additional 'drop-in' peaks were observed.

A greater number of donor alleles were detected over a wider range of input template amounts when using the 'Bench Top' process compared to the run other protocol (Fig. 1). The percentage of expected donor alleles is calculated for all samples in each DNA input template category using the following formula, homozygote peaks > 500 rfu are

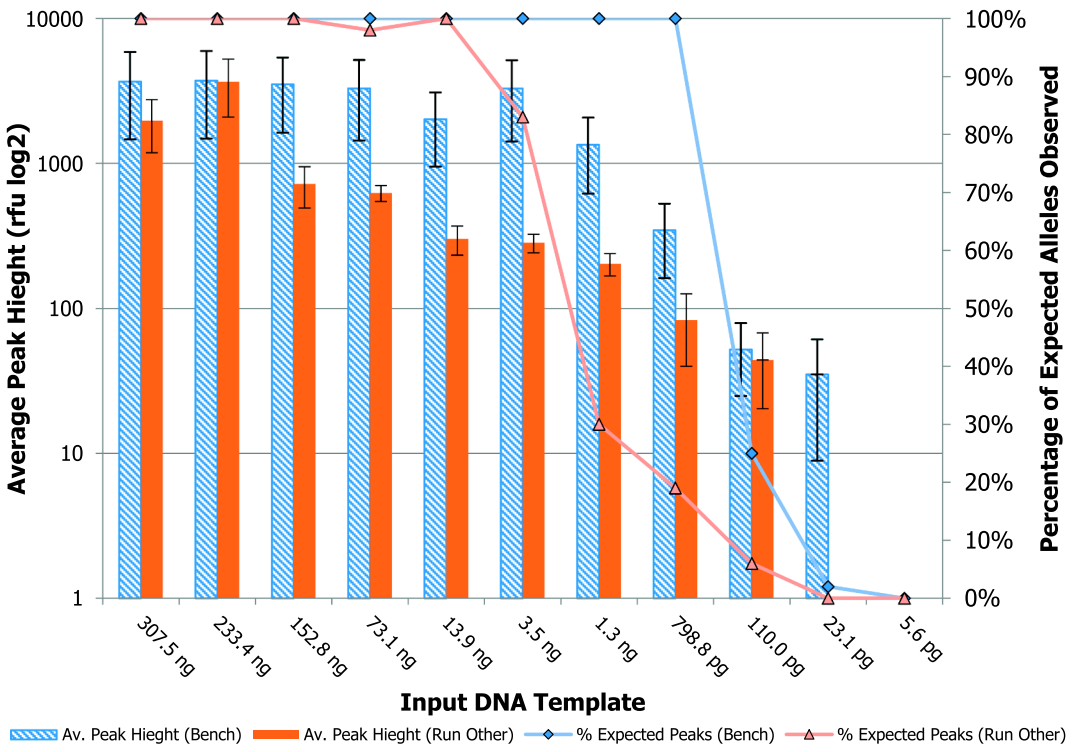

Fig. 1. Comparison of the performance of the RapidHIT™ 200 'Run Other' protocol compared to the current 'Bench Top' method. (n = 4 per template; averages ± SD).

counted as two peaks:

$$\left(\frac{\text{"Number of alleles observed in all profiles in category"}}{\text{"Number of alleles in PCR kit"} \times \text{"Number of samples processed in category"}} \right) \times 100$$

This calculation gives an overall percentage of donor peaks, as opposed to an average of the repeats or maximum observed.

As the DNA input template is decreased there is an associated decrease in average peak height.

Whilst this trend is expected, it can be seen that the average peak height is lower with the RapidHIT™ 200 protocols than the 'Bench Top' methods.

It can be seen that there is more profile information gained from the bench top methods in the input template range between 110 pg and

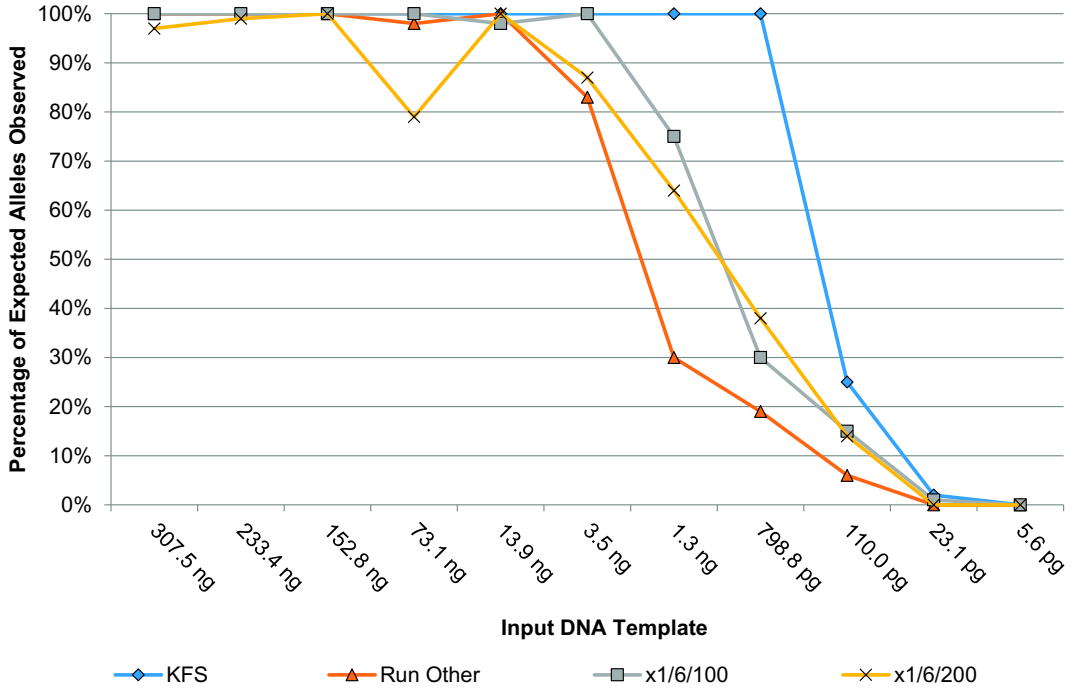

Fig. 2. Comparison of performance of increased DNAToBeadCaptureCycles times (n = 4 per DNA Input Template).

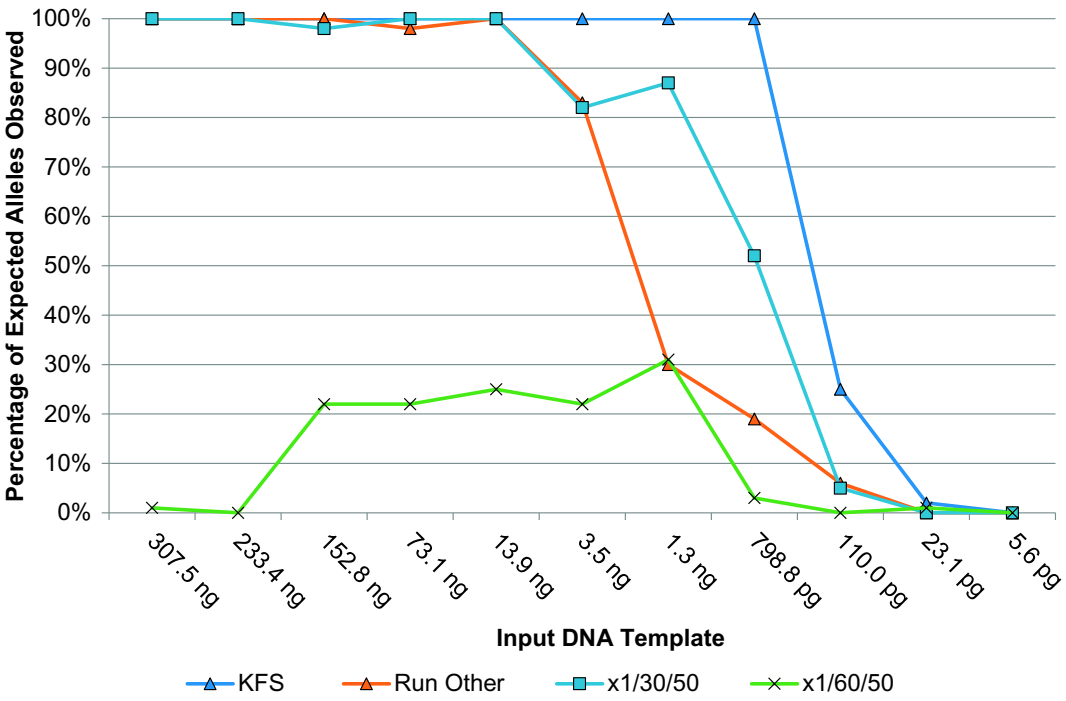

Fig. 3. Comparison of performance of increased lysis times to 'Run Other' Protocol (n = 4 per DNA Input Template).

13.9ng; this is the range for a large number of crime stain samples [Key Forensic Services – Internal Data, April 2018]. Examining the average peak heights it is clear that this difference in performance is not due to the differences between the limit of detection used on the two systems. The average peak height from the 'Bench Top' method is in excess of the limit of detection down to 110 pg. Therefore, the enhanced protocols have been assessed to attempt to improve the range of DNA input templates which can be processed on the RapidHIT™ 200.

3.3. Assessment of enhanced protocols

Initially the 'DNAToBeadCaptureCycles' time was extended. Fig. 2 shows the effect of increasing the time allowed for DNA to bind to the beads in terms of the percentage of expected donor alleles observed.

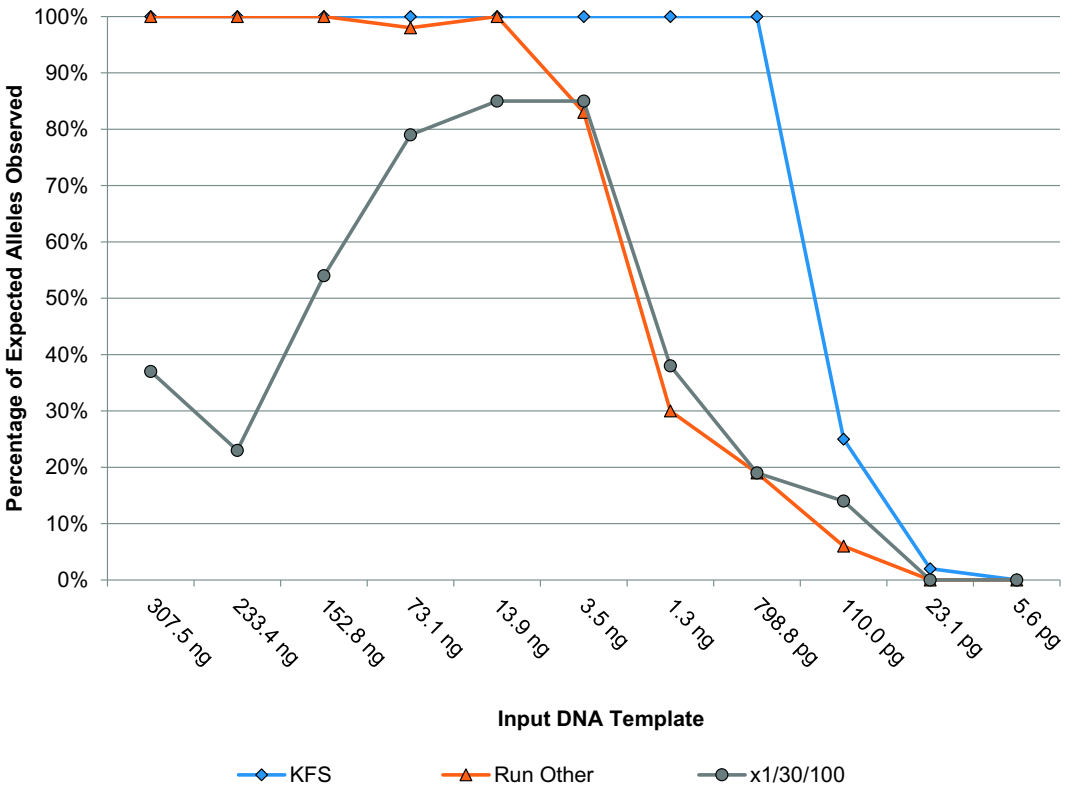

Fig. 4. Comparison of combined increased of DNAToBeadCapture and Lysis Times to 'Run Other' Protocol (n = 4 per DNA Input Template).

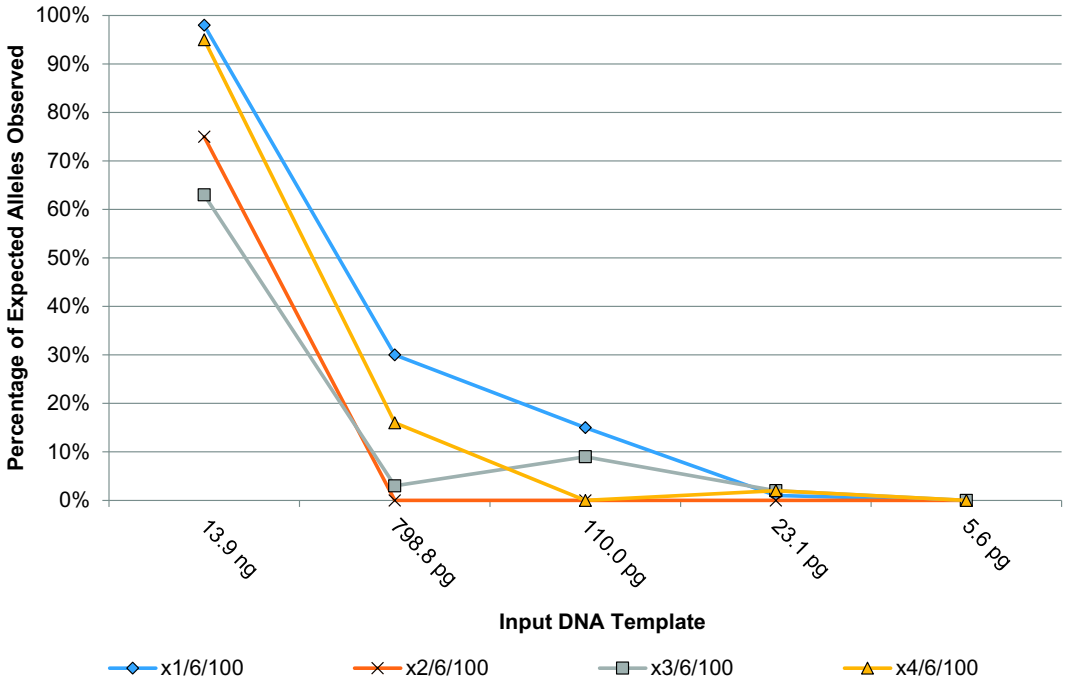

Fig. 5. Comparison of performance of increased bead concentrations to the x1/6/100 protocol (n = 4 per DNA Input Template).

DNAToBeadCaptureCycles times of both 100 s and 200 s show improvement over the original 'Run Other' protocol with full profiles being generated from all samples processed under the x1/6/100 condition at an input DNA template of 3 .5ng.

Altering the lysis time was also seen to show improvement with the x1/30/50 conditions, in terms of the percentage of expected donor alleles observed (Fig. 3). However, the use of x1/60/50 caused a severe reduction in the number of observed alleles. It is believed that this is due to over-loading of the PCR reaction with template DNA or due to the extended period for which the samples are held at 120 °C in the lysis buffer, causing denaturation and possibly degradation of the DNA.

Combining the 30 min lysis time with the 100 s DNAToBeadCaptureCycles setting also showed a decreased percentage of observed alleles at the higher DNA input templates (Fig. 4). As this is similar to the response seen with the x1/60/50 setting it is expected that this is due to overloading over the PCR reaction; preventing the amplification.

3.4. Assessment of increasing bead concentrations

In order to assess the effect of increasing the concentration of the para-magnetic beads the 6/100 parameter set was used with x2, x3, and x4 bead concentration. The study focused on the low-template, cell-line samples. As it can be seen (Fig. 5) the increased bead concentrations did not improve the performance of the RapidHIT™ 200 system to process low inputs of DNA and a reduction in performance was observed at most DNA concentrations compared with the x1/6/100.

3.5. Protocol selection

Based on the data presented here three new protocols are proposed; x1/6/100, x1/6/200 and x1/30/50. Fig. 6 shows the effect of these protocols in terms of average peak height when compared to the 'Run Other' protocol. It can clearly be seen that the x1/6/100 protocol has improved peak heights when compared to the 'Run Other' protocol and the other new protocols at the majority of DNA input templates. Therefore, this protocol is recommended for stain types similar to those tested in this study. Whilst the x1/6/200 and the x1/30/50 protocols are not as efficient as the x1/6/100 protocol these were retained at this stage to allow for testing of a wider range of DNA substrate materials during future studies.

Fig. 6 also shows the average peak height ratio (%, smallest/largest peak height) for each dataset for heterozygotes. It can be seen that as the profile heights decrease the peak height ratios also decrease, as expected. With the exception of one locus at the x1/30/50 parameter, profiles from DNA input templates of 798.8pg or less contained only single peaks due to allelic drop out.

4. Conclusion

Even though this is a limited study in terms of sample numbers it can be seen from the data collected that it is possible to improve the range of DNA input templates that can be processed on the RapidHIT™ 200. The greatest affect was seen by increasing the time allowed for the DNA to bind to the beads in the wash chamber before applying the magnets ('DNAToBeadCaptureCycles') or by extending the lysis time up to 30 min. However, it must be made clear that the range of DNA input templates is still not as wide as possible with the traditional 'Bench Top' processes.

Using these modified parameters in combination also results in improved allele detection at lower input DNA templates. There is, however, a detrimental effect when higher input templates are used or lysis time is extended beyond 30 min. The use of increased concentrations of paramagnetic beads within the sample cartridges was also shown to be slightly detrimental to the overall number of alleles detected without demonstrating a significant improvement in performance. In all cases where a reduction in detected alleles was observed in higher DNA templates it is believed that this is as a result of overloading of the PCR reaction. This may also have been compounded by the increased lysis time causing some level of DNA denaturation or degradation.

Based on these results three new protocols have been proposed to replace the 'Run Other' protocol for the AmpFℓSTR® NGMSElect™ Express PCR Amplification Kit cartridges on the RapidHIT™ 200 for sample types similar to those tested in this study. The use of x1/6/100, x1/6/200 and 1x/30/50 protocols will require further studies to

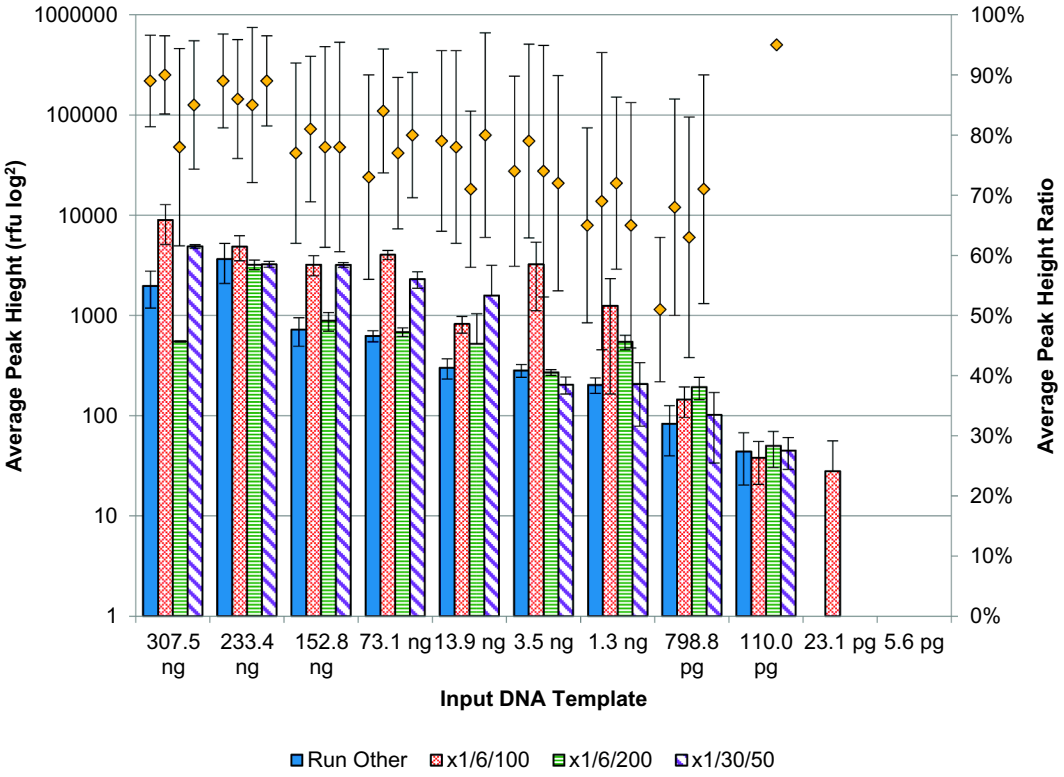

Fig. 6. Average peaks heights and average peal height ratio ◆ observed with new run protocols (n = 4 per template; averages ± SD).

determine which protocol is optimal dependant on the DNA input template and substrate for a range of different crime stain types. All of these options extend the run time of the instrument, from 1 h 40 min (Run Other) up to 2 h 35 min (x1/30/50 protocol). This is compared to an approximate time of 7 h for the Bench Top process with a similar sample number. Whilst this extended run time is unfortunate this is offset by the benefit of increasing the sensitivity of the instrument and the range of crime stains that yield useful results. The process is still much faster than traditional 'Bench Top' methods by several hours and requires far less operator intervention, reducing operator time and contamination risk. Following the outcome of this study IntegenX® will release these protocols in version 2.1 of the RapidHIT™ 200 instrument software [16]. They will be available as an optional extra alongside the 'Run Other' and 'Buccal' protocols on the instruments potentially further extending the range of materials that can be processed with lower DNA input templates.

Funding sources

All work was funded by Key Forensic Services.

Acknowledgements

This work would not have been possible without the help of the instrument manufacturer, IntegenX®; in particular David King, Stefanie Gangano and Charles Troup.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scijus.2019.03.002.

References

[1] L.K. Hennessy, H. Franklin, Y. Li, J. Buscaino, K. Chear, J. Gass, N. Mehendale, S. Williams, S. Jovanovich, D. Harris, K. Elliott, W. Nielsen, Developmental validation studies on the RapidHIT™ human DNA identification system, Forensic Sci. Int. Genet. Suppl. Ser. 4 (2013), https://doi.org/10.1016/j.fsigss.2013.10.003.

[2] S. Verheij, L. Clarisse, M. van den Berge, T. Sijen, RapidHIT™ 200, a promising system for rapid DNA analysis, Forensic Sci. Int. Genet. Suppl. Ser. 4 (2013), https://doi.org/10.1016/j.fsigss.2013.10.130.

[3] M. Holland, F. Wendt, Evaluation of the RapidHIT™ 200, an automated human identification system for STR analysis of single source samples, Forensic Sci. Int. Genet. 14 (2015) 76–85, https://doi.org/10.1016/j.fsigen.2014.08.010.

[4] S. Jovanovich, G. Bogdan, R. Belcinski, J. Buscaino, D. Burgi, E.L.R. Butts, K. Chear, B. Ciopyk, D. Eberhart, O. El-Sissi, H. Franklin, S. Gangano, J. Gass, D. Harris, L. Hennessy, A. Kindwall, D. King, J. Klevenberg, Y. Li, N. Mehendale, R. McIntosh, B. Nielsen, C. Park, F. Pearson, R. Schueren, N. Stainton, C. Troup, P.M. Vallone, M. Vangbo, T. Woudenberg, D. Wyrick, S. Williams, Developmental validation of a fully integrated sample-to-profile rapid human identification system for processing single-source reference buccal samples, Forensic Sci. Int. Genet. 16 (2015) 181–194, https://doi.org/10.1016/j.fsigen.2014.12.004.

[5] D. Shackleton, J. Pagram, L. Ives, D. Vanhinsbergh, Development and validation of the RapidHIT™ 200 utilising NGMSElect™ express for the processing of buccal swabs, Forensic Sci. Int. 289 (2018), https://doi.org/10.1016/j.forsciint.2018.05.041.

[6] International Standard Organization, ISO17025-2005, Guidance, 2005 (2005), pp. 1–36.

[7] The United Kingdom Accreditation Service (UKAS), Accreditation for Suppliers to the UK National DNA Database, (2012).

[8] International Laboratory Accreditation Cooperation, Guidelines for Forensic Science Laboratories (G19), (2002).

[9] R.L. Green, R.E. Lagacé, N.J. Oldroyd, L.K. Hennessy, J.J. Mulero, Developmental validation of the AmpFℓSTR®NGM SElect™ PCR amplification kit: a next-generation STR multiplex with the SE33 locus, Forensic Sci. Int. Genet. 7 (2013) 41–51, https://doi.org/10.1016/j.fsigen.2012.05.012.

[10] Z. Thong, Y.H. Phua, E.S. Loo, B.H. Shue, C.K.C. Syn, Investigative leads from DNA: casework experience from the IntegenX RapidHIT™ 200 system, Forensic Sci. Int. Genet. Suppl. Ser. 5 (2015) e69–e70, https://doi.org/10.1016/j.fsigss.2015.09.028.

[11] L.K. Hennessy, N. Mehendale, K. Chear, S. Jovanovich, S. Williams, C. Park, S. Gangano, Developmental validation of the GlobalFiler® express kit, a 24-marker STR assay, on the RapidHIT® system, Forensic Sci. Int. Genet. 13 (2014) 247–258, https://doi.org/10.1016/j.fsigen.2014.08.011.

[12] Z. Thong, Y.H. Phua, E.S. Loo, S.K. Goh, J. Ang, W.F. Looi, C.K.C. Syn, Evaluation of the RapidHIT™ 200 system: a comparative study of its performance with Maxwell® DNA IQ™/Identifiler® plus/ABI 3500xL workflow, Forensic Sci. Int. Genet. 19 (2015) 22–27, https://doi.org/10.1016/j.fsigen.2015.05.006.
[13] S. Gangano, K. Elliott, K. Anoruo, J. Gass, J. Buscaino, S. Jovanovich, D. Harris, DNA investigative lead development from blood and saliva samples in less than two hours using the RapidHIT™ human DNA identification system, Forensic Sci. Int. Genet. Suppl. Ser. 4 (2013), https://doi.org/10.1016/j.fsigss.2013.10.022.
[14] A.A. Reena Roy, Generating rapid DNA profiles from crime scene samples commonly encountered in the United Arab Emirates, J. Forensic Res. 06 (2015), https://doi.org/10.4172/2157-7145.1000296.
[15] M.M. Holland, W. Parson, GeneMarker® HID: a reliable software tool for the analysis of forensic STR data, J. Forensic Sci. 56 (2011) 29–35, https://doi.org/10.1111/j.1556-4029.2010.01565.x.
[16] IntegenX, User Bulletin: RapidHIT System Software Version 2.1, (2015).