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Assessment of QIAGEN™ Investigator® 24plex GO! kit workflow for autosomal STR profiling of forensic reference samples

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Abstract

Background: DNA profiling has proven to be a valuable technique for identification of individuals in crime. Currently, the technique targets several short tandem repeat (STR) regions in human genome. However, increasing number of samples submitted for STR analysis may lead to delays due to the limited number of experienced analysts who might be available at any given moment and the time taken to complete lengthy DNA profiling procedures. This study was conducted to test the specificity, repeatability, reproducibility and robustness of Investigator® 24plex GO! kit for genotyping of reference samples submitted to the Royal Malaysian Police Forensic DNA Laboratory for DNA database.

Material and methods: In this study, Investigator® 24plex GO! kit was used to directly amplify STR loci from buccal swab cell of reference samples that had previously been STR typed using GlobalFiler™ Express kit. Capillary electrophoresis was carried out on a 3500xL Genetic Analyser using POP-4® Polymer. Amplified products were assigned to particular STR alleles using the GeneMapper ID-X version 1.4 software.

Results: Our study shows that STR profiles generated using Investigator® 24plex GO! gave concordance results with those previously obtained using the GlobalFiler™ Express kit. In addition, quality sensors included in the kit are of particular importance for determining the effectiveness of the PCR reaction and help to indicate the nature and quantity of DNA template for PCR amplification.

Conclusion: The Investigator® 24plex GO! kit is reliable for STR typing of reference samples.

Keywords: Buccal swabs cell, Investigator® 24plex GO!, GlobalFiler™ Express, Reference samples, DNA database, Autosomal STR, DNA typing

Background

DNA profiling has proven to be a valuable technique for identification of individuals involved in criminal investigations (Johnson and Williams 2004; Williams and Johnson 2005; Phillips 2008; Butler 2015). Currently, the technique targets several short tandem repeat (STR) regions of the human genome. Repeat numbers across a combination of STR loci are highly variable and effectively unique to particular individuals (Perlin 2000; Ruitberg et al. 2001; Kowalczyk et al. 2018). Thus, STR data from a suspect or an offender can be compared with those generated from biological materials collected from scene of a crime, the victim, or from those profiles stored in a databank (Perlin 2000; Ruitberg et al. 2001). However, the currently increasing number of samples submitted for STR analysis may lead to delays due to a limited supply of experienced analysts and the time

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taken to obtain DNA profiles (Wallace et al. 2014; Liu 2014; Butler 2015).

In a typical forensic DNA laboratory, direct polymerase chain reaction (PCR) amplification is commonly employed to overcome these issues (Myers et al. 2012; Tucker et al. 2012). Direct amplification of STR loci reduces the time required for sample preparation and potentially helps laboratories to process increased number of samples (Wang et al. 2011; Oostdik et al. 2013; Hall and Roy 2014). In addition, the direct PCR amplification technique may also reduce risks of cross-over contamination (Caputo et al. 2017; Ambers et al. 2018). Several commercial direct amplification STR kits such as GlobalFiler™ (Ludeman et al. 2018), GlobalFiler™ Express (Wang et al. 2015), PowerPlex® ESI 16/17 Fast and PowerPlex® ESX 16/17 Fast Systems (McLaren et al. 2014), Yfiler ® Plus (Gopinath et al. 2016), PowerPlex® Y23 System (Thompson et al. 2013) and PowerPlex™ 18D (Myers et al. 2012) have been developed and reported to give high-quality STR profiles. Nevertheless, analysts have no control over amount of DNA input or any measurement of DNA template quality in the direct amplification STR workflow scheme. Insufficient DNA template will cause stochastic effects such as allelic dropout and heterozygote imbalance, whilst too much input DNA may lead to nonspecific amplification, stutter products and an increased baseline for STR allele calls (Cavanaugh and Bathrick 2018).

Investigator® 24plex GO! kit (Qiagen, Hilden, Germany) was designed to enable direct STR profiling of reference (blood and buccal cell) samples (Hares 2015; Habib et al. 2017; Kraemer et al. 2017). This 6-dye megaplex kit contains PCR assays targeting 22 polymorphic STR loci and amelogenin, a sex-determining region (Hares 2012; Hares 2015). Unlike other direct amplification kits, the Investigator® 24plex GO! is also supplied with two pairs of primers targeting artificial templates as quality sensors (QS 1 and QS 2). The inclusion of these QS primer mixes helps to determine the effectiveness of the PCR reaction and to indicate the nature and quantity of the DNA template (Zahra and Goodwin 2016; QIAGEN 2018). Here, QS 1 will show stable amplification even in the presence of extremely high inhibitor concentrations. In contrast, QS 2 is more sensitive to inhibitors and the relationship between QS 1 and QS 2 signal heights can indicate the level of inhibition [30]. If both QS signals are unaffected, then low-quality STR profiles might be attributed to degradation of the DNA template (Scherer et al. 2015).

In this study, we used Investigator® 24plex GO! kit (QIAGEN, Hilden, Germany) to characterised reference samples that were previously STR typed using GlobalFiler™ Express kit (Thermo Fisher Scientific, USA). This study was conducted to test the robustness of Investigator® 24plex GO! kit for genotyping of reference samples submitted to the Royal Malaysian Police Forensic DNA Laboratory for DNA database (Hakim et al. 2019).

Materials and methods

Sample preparation

Twenty-four buccal cell samples (single-source human DNAs) collected using Bode Buccal DNA Collector™ (Bode Technology, Virginia, USA) were used in this validation study. These buccal cell samples were all previously characterised for STR loci using GlobalFiler™ Express kit (Thermo Fisher Scientific, USA). Samples (1.2 mm) were punched into a 96-well plate using BSD600 DUET (Microelectronic Systems Pty Ltd, Australia) and 2 μl of Investigator® 24plex GO! kit lysis buffer was added directly on to each punched sample. The plate was then briefly centrifuged followed by incubation at 95 °C for 5 min. It is important to note that all 24 samples were used for PCR optimisation and cross-contamination studies whilst only 22 samples were included for reproducibility studies. Two samples were excluded from the reproducibility studies and were replaced with allelic ladder and positive control.

PCR optimisation and cross-contamination study

A total of 20 μl of PCR reaction mixture was used for STR profiling using Investigator® 24plex GO! kit (QIAGEN, Hilden, Germany) and consisted of 7.5 μl of Fast Reaction Mix 2.0 and 12.5 μl Primer Mix with 1.2-mm punched samples. As part of the validation study, PCR was first optimised using either 24 or 25 cycles of amplification. A total 19 negative controls (NC) were also included in the amplification plates to check for possible contamination of the reagents or inadvertent transfer between samples (Additional file 1: Table S1). STR profiles from the NCs must contain no more than two peaks in allelic positions above the laboratory's threshold value. The PCR amplification was performed on the GeneAmp® PCR System 9700 Thermal Cycler (Life Technologies, Foster City, CA) using a gold-plated sample block and set in 'max ramp' mode.

Capillary electrophoresis

Sample mixtures contained 12 μl Hi-Di Formamide, 0.5 μL DNA Size Standard 550 (BTO) and 1 μl of amplified PCR products were prepared for capillary electrophoresis. The mixtures were heated to 95 °C for 3 min prior to quick chilling in a cold block for another 3 min. Capillary electrophoresis was carried out on a 3500xL Genetic Analyser (Applied Biosystems, USA) using POP-4® Polymer (Life Technologies, CA, USA). The machine was set up according to procedures described by the manufacturers with one minor modification; injection voltage was set at 1.2 kV for three injection times (20 s,

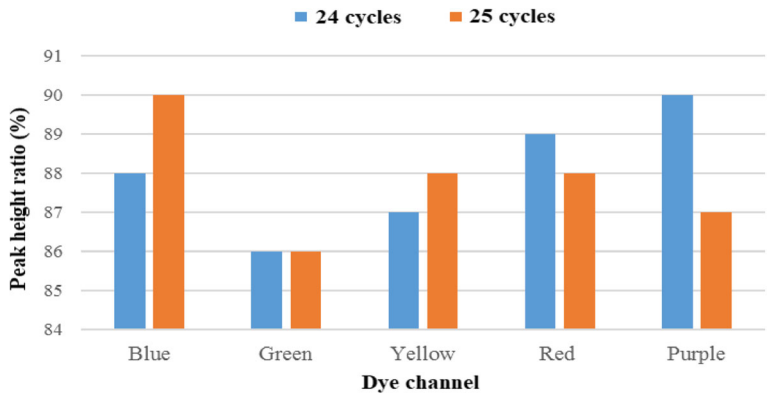

Fig. 1 Graph depicts a heterozygous percentage of peak height balance (%) for samples amplified at 24 cycles and 25 cycles as well as the average peak height balance for each dye channel. The peak height balance (%) for the quality sensors was calculated separately and not included in the results for the purple channel or overall average

33 s and 40 s). Amplified products were assigned to particular STR alleles using the GeneMapper ID-X version 1.4 software (Life Technologies, USA) and a value of 100 relative fluorescence units (RFU) was set as the minimum peak detection threshold for STR allele calls.

Reproducibility study

Twenty-two samples were punched according to the PCR plate layout using 25 cycles of amplification (Additional file 1: Table S2). The PCR mixture, optimal values for cycle number (25 cycles), injection time (33 s) and loading voltage (1.2 kV) as determined from the optimisation tests described above were used for PCR amplification; see later in optimisation and cross-contamination assessment results. One PCR reaction plate was prepared by Operator A and capillary electrophoresis was run on Genetic Analyser 1 (GA 1) and Genetic Analyser 2 (GA 2) on day one for reproducibility assessment between Genetic Analysers. The same plate was also run by operator A on the next day using GA 1 to study reproducibility across time. Another PCR reaction plate was prepared by operator B on the next day and was run on GA 1 to study reproducibility between operator A and operator B. Average peak height variation between experimental work conducted by operator A (day 1 vs. 2), instruments (GA 1 vs. GA 2) and operators (Operator A vs. Operator B) were analysed for assessing reproducibility.

STR data analysis

The panels, bins and stutter data settings for the Investigator® 24plex GO! kit were provided online by Qiagen

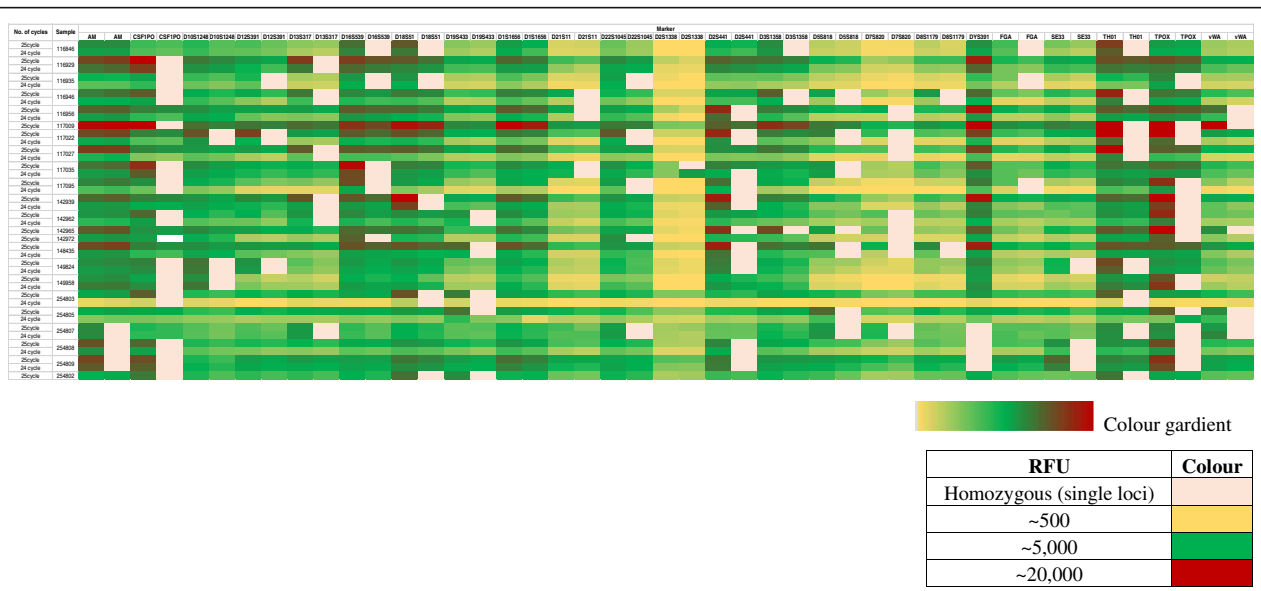

Fig. 2 Heat map of the sample peak heights. Samples 117009, 142965, 142972 and 254802 from 24 cycle set and sample 148857 from both 24 and 25 cycle sets were not included due to a failed amplification (as indicated by the quality sensors) and pull up peaks respectively

Table 1 Analytical threshold (AT) calculation

Sample control ID	•Threshold channel				
	Blue	Green	Yellow	Red	Purple
NC 1	39	37	38	57	31
NC 2	23	21	18	30	64
NC 3	21	22	20	33	36
NC 4	23	25	19	26	34
NC 5	29	36	23	29	31
NC 7	58	34	25	22	24
NC 8	31	23	18	24	23
NC 9	31	22	21	23	22
NC 10	24	23	22	25	26
NC 11	47	38	23	38	38
NC 12	22	22	22	38	36
NC 13	43	37	25	29	24
NC 14	42	34	17	52	33
NC 15	31	24	24	31	27
NC 16	31	16	17	30	29
NC 17	41	28	29	55	43
NC 18	40	32	20	44	32
NC 19	39	24	21	70	29
Highest RFU	58	38	38	70	64
Average	34.68	28.00	22.58	37.11	32.84
Std. Dev.	10.09	6.84	4.99	13.67	9.70
[a]LOD	64.95	48.52	37.56	78.13	61.95
[b]LOQ	135.57	96.39	72.50	173.85	129.87

[a]Average + 3SD
[b]Average + 10SD
•Analytical threshold for size marker was set to 50 RFU and AT = 3 × LOQ

(*24plex_Panels_v3*). Data analysis was performed using the GeneMapper ID-X, version 1.4 software. Negative control samples from the Investigator® 24plex GO! optimisation study were analysed at 1 RFU to determine the analytical threshold. Highest peak height for every channel was recorded and analytical threshold, limit of detection (LOD) and limit of quantification (LOQ) were determined. All other Investigator® 24plex GO! studies were analysed using an analytical threshold of 100 RFU, except for the orange dye channel which was set at 50 RFU. Data compilation and calculations were performed using Microsoft Office Excel 2016.

Results and discussion

PCR cycle number and capillary electrophoresis conditions

The heterozygote peak height balance for each of the two cycling condition tested (24 vs. 25) and is shown in Fig. 1. Overall, the samples amplified using 25 cycles yielded much better results compared with those amplified using 24 cycles in this study. Two out of the 24 samples amplified for 24 cycles gave only partial profiles (sample 142965 and 142972), a further one had multiple alleles (sample 254802) whilst another produced no sizing data (sample 117009). Thus in total, only 20 samples from the 24 cycle's amplification plate produced data that could be analysed. Whilst 23 samples from all samples amplified for 25 cycles generated almost full profiles, and only one sample (148857) had multiple alleles. The optimal amplification cycle number should generate profiles with heterozygous peak heights with minimal occurrences of artefacts or allelic drop-out events. In general, peak height balance for both the 24 and 25 cycles tests were the same (88%), but samples amplified using the 25 cycles procedure had a better chance for generating full STR profiles.

This is supported by the heat map of the RFU peak height shown in Fig. 2 where samples amplified using 25 cycles gave profiles with greater peak height (overall mean 6344 RFU) compared with samples amplified using 24 cycles (3714 RFU). However, both the 24 and 25 cycles PCR amplification procedure generated peak heights in the acceptable range of 500 RFU to 17,000 RFU. The number of alleles with peak heights greater than 25,000 RFU was determined for each set of samples. These can often be difficult to identify correctly due to the presence of stutter and pull-up peaks. Only one profile (sample 148857) amplified using the 25 cycles protocol had a peak height above 25,000 RFU which resulted in a pull-up peak, whereas none for sample amplified using 24 cycles.

Because the peak heights recorded for the QS 1 and QS 2 markers may vary slightly between different experiments, it was important for us to define a regular quality sensor balance range for analysing samples on Bode Buccal DNA Collector™ (Bode Technology, Virginia, USA). In our study, the mean quality sensor balance of QS 1/QS 2 was 64% and 73% for 25 cycle and 24 cycle amplifications (respectively). DNA profiling runs with a peak height balance between 60 and 70% should give high-quality STR allele calls (QIAGEN 2018). In general, 20% lower QS 2 peak height values as compared with QS 1 values indicate PCR inhibition (QIAGEN 2018). None of the samples we tested during this study showed PCR inhibition. Based on the available data above, the 25 PCR cycle method was used for genotyping our reference samples using the Investigator® 24plex GO! kit. All three injection times tested (20 s, 33 s and 40 s) gave similar results (data not shown) and the 33 s injection time was chosen for capillary electrophoresis.

Analytical threshold calculations

The negative controls run in the optimisation step of the abovementioned were analysed at 1 RFU and any peak

Table 2 Observed STR profiles the Investigator® 24plex GO! for 22 reference samples[a]. The profiles generated by GlobalFiler™ Express kit for 22 reference samples[b]

(a)

Allelic specificity		254805	254802	254803	254807	254808	254809	117095	142939	142962	142965	142972
D3S1358	*A*	16, 17	15, 16	15, 16	15, 16	16, 18	16, 18	14, 15	15, 16	15, 17	17, 17	16, 17
	B	16, 17	15, 16	15, 16	15, 16	16, 18	16, 18	14, 15	15, 16	15, 17	17, 17	16, 17
vWA	*A*	17, 17	16, 19	16, 19	17, 17	17, 18	17, 18	14, 17	14, 18	18, 19	17, 17	17, 18
	B	17, 17	16, 19	16, 19	17, 17	17, 18	17, 18	14, 17	14, 18	18, 19	17, 17	17, 18
D16S539	*A*	10, 12	11, 12	11, 12	10, 14	11, 13	11, 13	11, 11	9, 12	10, 14	10, 11	9, 9
	B	10, 12	11, 12	11, 12	10, 14	11, 13	11, 13	11, 11	9, 12	10, 14	10, 11	9, 9
CSF1PO	*A*	10, 11	11, 11	11, 11	11, 13	12, 12	12, 12	12, 12	10, 11	12, 12	10, 11	12, 12
	B	10, 11	11, 11	11, 11	11, 13	12, 12	12, 12	12, 12	10, 11	12, 12	10, 11	12, 12
TPOX	*A*	8, 8	8.9	8, 9	8, 8	8, 8	8, 8	11, 11	8, 8	8, 8	8, 8	9, 9
	B	8, 8	8.9	8, 9	8, 8	8, 8	8, 8	11, 11	8, 8	8, 8	8, 8	9, 9
Amelogenin	*A*	X, Y	X, Y	X, Y	X, X	X, X	X, X	X, Y	X, Y	X, Y	X, Y	X, Y
	B	X, Y	X, Y	X, Y	X, X	X, X	X, X	X, Y	X, Y	X, Y	X, Y	X, Y
D8S1179	*A*	12, 14	12, 15	12, 15	12, 15	13, 16	13, 16	10, 13	13, 16	13, 14	13, 15	13, 16
	B	12, 14	12, 15	12, 15	12, 15	13, 16	13, 16	10, 13	13, 16	13, 14	13, 15	13, 16
D21S11	*A*	31, 32.2	28, 31	28, 31	28, 31	30, 32.2	30, 32.2	29, 32	29, 31.2	29, 32.2	27, 29	28, 31
	B	31, 32.2	28, 31	28, 31	28, 31	30, 32.2	30, 32.2	29, 32	29, 31.2	29, 32.2	27, 29	28, 31
D18S51	*A*	14, 17	15, 15	15, 15	12, 16	14, 16	14, 16	14, 18	15, 15	14, 18	15, 17	14, 17
	B	14, 17	15, 15	15, 15	12, 16	14, 16	14, 16	14, 18	15, 15	14, 18	15, 17	14, 17
DYS391	*A*	10, 10	10, 10	10, 10				10, 10	9, 9	10, 10	9, 9	10, 10
	B	10, 10	10, 10	10, 10				10, 10	9, 9	10, 10	9, 9	10, 10
D2S441	*A*	11, 14	11.3, 12	11.3, 12	11, 14	11, 11	11, 11	12, 12	10, 10	10, 11	11, 11	12, 14
	B	11, 14	11.3, 12	11.3, 12	11, 14	11, 11	11, 11	12, 12	10, 10	10, 11	11, 11	12, 14
D19S433	*A*	13, 13	12, 13	12, 13	14, 13	11, 13	11, 13	14, 16	12.2, 14	14, 14	14.2, 15	13, 14.2
	B	13, 13	12, 13	12, 13	14, 13	11, 13	11, 13	14, 16	12.2, 14	14, 14	14.2, 15	13, 14.2
THO1	*A*	7, 8	9, 9	9, 9	9, 9	7, 8	7, 8	7, 9.3	8, 9	7, 8	6, 9	7, 9
	B	7, 8	9, 9	9, 9	9, 9	7, 8	7, 8	7, 9.3	8, 9	7, 8	6, 9	7, 9
FGA	*A*	21, 25	21, 25	21, 25	21, 22	23, 24	23, 24	19, 19	19, 23	19, 21	23, 26	22, 23
	B	21, 25	21, 25	21, 25	21, 22	23, 24	23, 24	19, 19	19, 23	19, 21	23, 26	22, 23
D22S1045	*A*	11, 17	11, 15	11, 15	15, 15	11, 15	11, 15	15, 15	11, 15	11, 15	15, 16	11, 11
	B	11, 17	11, 15	11, 15	15, 15	11, 15	11, 15	15, 15	11, 15	11, 15	15, 16	11, 11
D5S818	*A*	11, 11	11, 12	11, 12	10, 10	12, 13	12, 13	10, 13	11, 12	10, 11	10, 10	11, 12
	B	11, 11	11, 12	11, 12	10, 10	12, 13	12, 13	10, 13	11, 12	10, 11	10, 10	11, 12
D13S317	*A*	11, 12	11, 12	11, 12	12, 12	10, 11	10, 11	8, 10	8, 8	12, 12	8, 11	11, 12
	B	11, 12	11, 12	11, 12	12, 12	10, 11	10, 11	8, 10	8, 8	12, 12	8, 11	11, 12

Table 2 Observed STR profiles the Investigator® 24plex GO! for 22 reference samples[a]. The profiles generated by GlobalFiler™ Express kit for 22 reference samples[b] *(Continued)*

D7S820	*A*	10, 12	11, 13	11, 13	12, 12	10, 11	10, 11	8, 10	11, 13	11, 11	10, 10	11, 11
	B	10, 12	11, 13	11, 13	12, 12	10, 11	10, 11	8, 10	11, 13	11, 11	10, 10	11, 11
SE33	*A*	22.2, 27.2	26.2, 28	26.2, 28	18, 27.2	30.2, 30.2	30.2, 30.2	25.2, 29.2	23.2, 26.2	24.2, 26.2	19, 20	20, 26.2
	B	22.2, 27.2	26.2, 28	26.2, 28	18, 27.2	30.2, 30.2	30.2, 30.2	25.2, 29.2	23.2, 26.2	24.2, 26.2	19, 20	20, 26.2
D10S1248	*A*	13, 15	15, 16	15, 16	14, 15	13, 15	13, 15	14, 15	13, 15	15, 17	12, 15	12, 15
	B	13, 15	15, 16	15, 16	14, 15	13, 15	13, 15	14, 15	13, 15	15, 17	12, 15	12, 15
D1S1656	*A*	15, 16	15, 16.3	15, 16.3	12, 16	11, 15	11, 15	15, 17	15, 16	11, 16	11, 14	14, 15
	B	15, 16	15, 16.3	15, 16.3	12, 16	11, 15	11, 15	15, 17	15, 16	11, 16	11, 14	14, 15
D12S391	*A*	18, 20	18, 20	18, 20	19, 22	19, 21	19, 21	20, 22	17, 20	17, 22	18, 19	23, 25
	B	18, 20	18, 20	18, 20	19, 22	19, 21	19, 21	20, 22	17, 20	17, 22	18, 19	23, 25
D2S1338	*A*	20, 21	19, 22	19, 22	21, 23	22, 24	22, 24	17, 24	18, 19	23, 24	20, 24	20, 24
	B	20, 21	19, 22	19, 22	21, 23	22, 24	22, 24	17, 24	18, 19	23, 24	20, 24	20, 24
(b)												
Allelic specificity		148435	149824	149958	116846	116929	116935	116946	116956	117009	117022	117027
D3S1358	*A*	16, 17	15, 18	15, 16	17, 17	16, 16	17, 18	18, 18	14, 17	16, 17	17, 18	16, 17
	B	16, 17	15, 18	15, 16	17, 17	16, 16	17, 18	18, 18	14, 17	16, 17	17, 18	16, 17
vWA	*A*	15, 19	17, 18	14, 17	16, 18	16, 19	14, 16	17, 18	16, 16	17, 17	14, 17	14, 17
	B	15, 19	17, 18	14, 17	16, 18	16, 19	14, 16	17, 18	16, 16	17, 17	14, 17	14, 17
D16S539	*A*	9, 10	10, 13	11, 13	12, 12	9, 11	11, 11	12, 13	10, 12	12, 13	10, 12	10, 12
	B	9, 10	10, 13	11, 13	12, 12	9, 11	11, 11	12, 13	10, 12	12, 13	10, 12	10, 12
CSF1PO	*A*	10, 12	12, 12	10, 10	11, 12	9, 9	12, 12	10, 10	10, 12	12, 12	10, 12	10, 11
	B	10, 12	12, 12	10, 10	11, 12	9, 9	12, 12	10, 10	10, 12	12, 12	10, 12	10, 11
TPOX	*A*	8, 9	8, 11	8, 8	9, 11	8, 10	8, 8	8, 9	8, 9	8, 8	8, 8	8, 10
	B	8, 9	8, 11	8, 8	9, 11	8, 10	8, 8	8, 9	8, 9	8, 8	8, 8	8, 10
Amelogenin	*A*	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y
	B	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y
D8S1179	*A*	13, 13	10, 11	10, 15	11, 15	12, 13	13, 16	13, 13	11, 12	13, 15	14, 15	10, 13
	B	13, 13	10, 11	10, 15	11, 15	12, 13	13, 16	13, 13	11, 12	13, 15	14, 15	10, 13
D21S11	*A*	30, 31	31, 32.2	29, 31	32.2, 33	29, 32.2	29, 31	29, 29	32.2, 32.2	31.2, 33.2	30, 31	28, 32.2
	B	30, 31	31, 32.2	29, 31	32.2, 33	29, 32.2	29, 31	29, 29	32.2, 32.2	31.2, 33.2	30, 31	28, 32.2
D18S51	*A*	15, 17	13, 17	15, 23	14, 14	16, 20	14, 14	14, 16	19, 22	16, 17	15, 16	14, 19
	B	15, 17	13, 17	15, 23	14, 14	16, 20	14, 14	14, 16	19, 22	16, 17	15, 16	14, 19
DY391	*A*	10, 10	10, 10	10, 10	11, 11	10, 10	11, 11	11, 11	10, 10	10, 10	10, 10	10, 10
	B		10, 10	10, 10	11, 11	10, 10	11, 11	11, 11	10, 10	10, 10	10, 10	10, 10
D2S441	*A*	10, 10	11, 11	10, 14	10, 11	11.3, 14	11, 11.3	11, 11.3	11, 11	11, 12	12, 12	12, 14

Table 2 Observed STR profiles the Investigator® 24plex GO! for 22 reference samples[a]. The profiles generated by GlobalFiler™ Express kit for 22 reference samples[b] *(Continued)*

	B	10, 10	11, 11	10, 14	10, 11	11.3, 14	11, 11.3	11, 11.3	11, 11	11, 12	12, 12	12, 14
D19S433	*A*	13, 13	14, 15.2	13, 14.2	13, 15.2	13.2, 15	14, 15.2	15, 9	14, 15.2	13, 13.2	13.2, 15.2	12, 13
	B	13, 13	14, 15.2	13, 14.2	13, 15.2	13.2, 15	14, 15.2	15, 9	14, 15.2	13, 13.2	13.2, 15.2	12, 13
THO1	*A*	7, 8	9.9	6, 9	7, 7	8, 9	8, 9	9, 9	7, 10	7, 7	7, 7	7, 7
	B	7, 8	9.9	6, 9	7, 7	8, 9	8, 9	9, 9	7, 10	7, 7	7, 7	7, 7
FGA	*A*	21, 22	19, 22	20, 22	19, 19	19, 24	19, 19	[c]**OL**	22, 23	22, 24	23, 26	19, 23
	B	21, 22	19, 22	20, 22	19, 19	19, 24	19, 19	**27.2**	22, 23	22, 24	23, 26	19, 23
D22S1045	*A*	15, 16	11, 16	15, 16	15, 19	15, 17	11, 11	15, 17	15, 17	15, 16	17, 17	15, 16
	B	15, 16	11, 16	15, 16	15, 19	15, 17	11, 11	15, 17	15, 17	15, 16	17, 17	15, 16
D5S818	*A*	10, 10	13, 14	10, 11	10, 12	10, 12	7, 9	12, 12	10, 12	9, 10	10, 10	11, 12
	B	10, 10	13, 14	10, 11	10, 12	10, 12	7, 9	12, 12	10, 12	9, 10	10, 10	11, 12
D13S317	*A*	8, 12	9, 12	9, [a]**OL**	9, 12	8.8	10, 11	8, 8	10, 11	8, 11	8, 10	8, 8
	B	8, 12	9, 12	9, [c]**OL**	9, 12	8.8	10, 11	8, 8	10, 11	8, 11	8, 10	8, 8
D7S820	*A*	11, 11	8, 10	11, 13	10, 12	10.1, 11	10, 13	8, 11	10, 10	10, 11	10, 10	11, 11
	B	11, 11	8, 10	11, 13	10, 12	10.1, 11	10, 13	8, 11	10, 10	10, 11	10, 10	11, 11
SE33	*A*	21, 30.2	27.2, 27.2	26.2, 31.2	19, 27.2	20, 22.2	21, 26.2	22.2, 25.2	20, 30.2	25.2, 26.2	20, 22.2	21, 30.2
	B	21, 30.2	27.2, 27.2	26.2, 31.2	19, 27.2	20, 22.2	21, 26.2	22.2, 25.2	20, 30.2	25.2, 26.2	20, 22.2	21, 30.2
D10S1248	*A*	13, 14	13, 13	15, 15	13, 15	14, 15	13, 14	13, 15	13, 15	13, 14	16, 16	14, 16
	B	13, 14	13, 13	15, 15	13, 15	14, 15	13, 14	13, 15	13, 15	13, 14	16, 16	14, 16
D1S1656	*A*	12, 16	16, 17	13, 15	11, 16.3	15, 17	12, 16	13, 15	14, 17.3	12, 13	15, 17	14, 15
	B	12, 16	16, 17	13, 15	11, 16.3	15, 17	12, 16	13, 15	14, 17.3	12, 13	15, 17	14, 15
D12S391	*A*	18, 20	19, 19	19, 24	18, 19	**10, 10**	19, 19	17, 23	21, 23	19, 22	18, 18	17, 21
	B	18, 20	19, 19	19, 24	18,19	**20, 23**	19, 19	17, 23	21, 23	19, 22	18, 18	17, 21
D2S1338	*A*	22, 23	16, 22	17, 19	23, 25	20, 23	16, 18	23, 24	17, 24	20, 23	20, 22	20, 23
	B	22, 23	16, 22	17, 19	23, 25	20, 23	16, 18	23, 24	17, 24	20, 23	20, 22	20, 23

[c]*OL* Off ladder

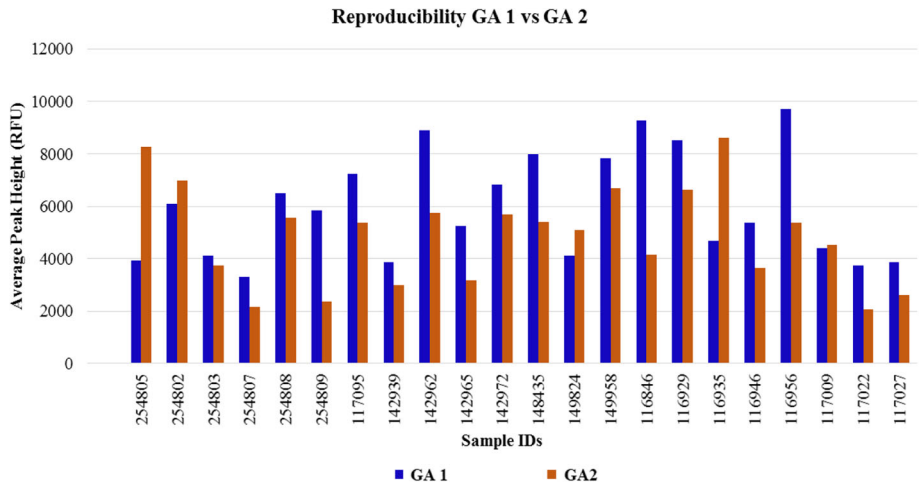

Fig. 3 Average peak heights (RFU) of 22 reference samples run on Genetic Analyser 1 (GA 1) and Genetic Analyser 2 (GA 2) by same operator (operator A) for reproducibility study between Genetic Analysers

that could be attributed to an artefact, pull-up or spike was removed. The mean, standard deviation, LOD and LOQ were calculated for each dye colour across all the remaining peaks (Table 1).

The most conservative value observed was the LOQ for the red channel (173.85 RFU). Even though, a 170 RFU cut off should be theoretically be taken as the suitable analytical threshold, during this study we found that a 100 RFU worked best as the analytical threshold for runs from both Genetic Analysers, as STR alleles can be fully called at this RFU value. For the orange dye channel, which detects the size marker, the analytical threshold was set to 50 RFU. The rest of the settings followed the default setting values obtained from QIAGEN's Investigator template for GeneMapper ID-X version 1.4 (QIAGEN 2018).

Cross contamination

Nineteen negative controls (NC) were processed together with reference samples to monitor cross contamination (refer PCR optimisation and cross-contamination study mentioned in the "Materials and methods" section for details). The contamination studied demonstrated the absence of contamination events that would compromise the integrity of results. Some artefacts were noted in all dye channels when analysed at 1 RFU. However, no alleles were detected above the analytical threshold of 100 RFU. These artefacts are possibly dye-labeled primer by-products and were either present prior to amplification or generated in part during the thermal cycling process and do not interfere with the kit results.

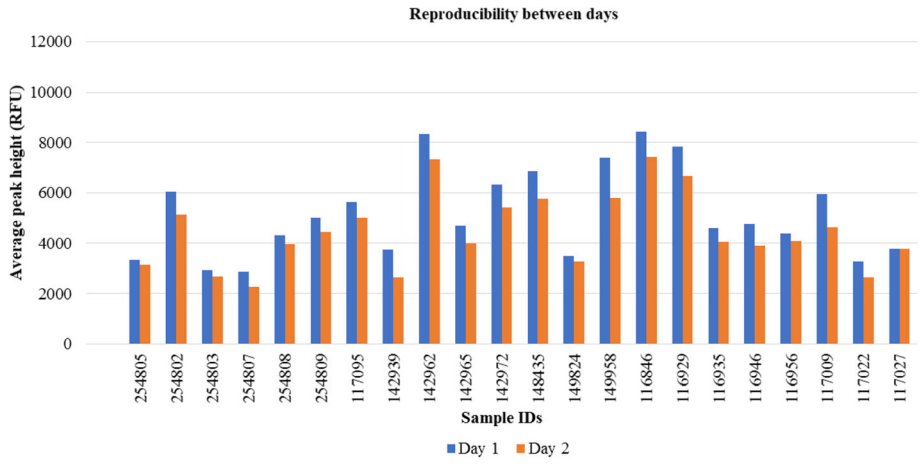

Fig. 4 Average peak heights (RFU) of 22 reference samples between two different runs on Genetic Analyser 1 (GA1) by operator A (same operator and machine) to study reproducibility across time

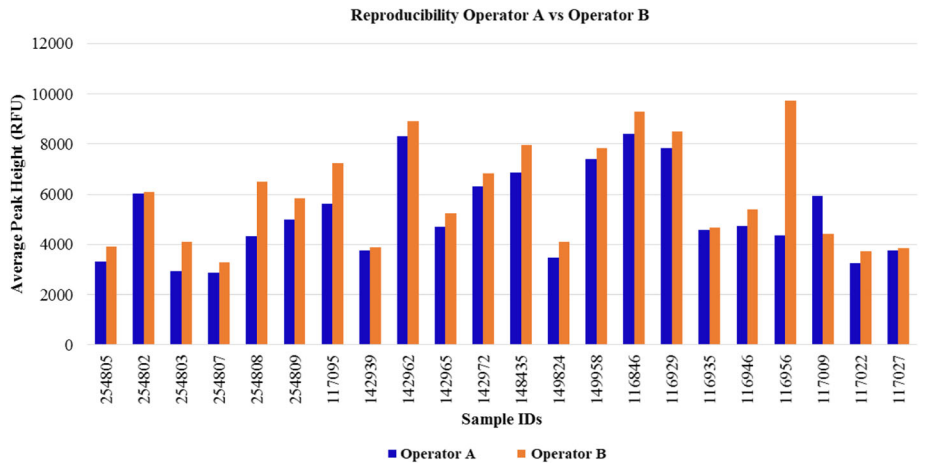

Fig. 5 Average peak heights (RFU) of 22 reference samples between two different runs on Genetic Analyser 1 (GA 1) by operator A and operator B for reproducibility study between operators

Concordance

The STR profiles generated using Investigator® 24plex GO! gave concordance results with those previously obtained using the GlobalFiler™ Express kit except FGA locus for sample 116946 (off-ladder peak for Investigator® 24plex GO! vs. allele 27.2 in GlobalFiler™ Express kit) and D12S391 locus in sample 116929 (homozygote for allele 10 using Investigator® 24plex GO! and allele 20 and 23 using GlobalFiler™ Express). The peak for sample 116946 at the FGA locus is in a position that one would expect to find a 27.2 allele. Therefore, this was not really a discordance, but relates to allelic ladder precision. The discordant results between Investigator® 24plex GO! and GlobalFiler™ Express kit for sample 116929 at locus D12S391 need to be investigated further, preferably by sequencing the D12S391 locus.

Reproducibility

Alleles at all loci showed no discrepancies between various experimental runs. However, there were only slight differences in allele peak heights between runs, different operators and different Genetic Analyser (see Table 2a and b). Generally, Genetic Analyser 1 (GA 1), showed slightly higher peak heights for all samples suggesting a higher sensitivity for this machine (Figs. 3, 4 and 5). In this context, GA 1 might be preferred for analysing samples with low DNA template.

Allelic ladder precision

Proper sizing of the separated amplified products is crucial for accurate assignment of STR alleles and was also evaluated in this study. The average base-pair size, standard deviation and percent coefficient of variation for five representative STR loci (Additional file 1: Table S3). The percent coefficient of variation (% CV) was below 0.06% for every allele in the allelic ladder (Additional file 1: Table S4) which suggests minimal variability between alleles. The highest standard deviation (+/−0.15 bp) was observed for SE33 allele 26.2.

Generally, a sizing bin of +/−0.5 bp is used around each allele in the STR allelic ladder (Butler 2012). The standard deviation of allele size estimates between different runs in our experimental works is well inside the required value above indicating that one can be confidant regarding accurate size calling.

Conclusion

Overall, our study shows that the Investigator® 24plex GO! kit is reliable for STR typing of reference samples. In addition, quality sensors included in the kit are of particular importance for determining the effectiveness of the PCR reaction and help to indicate the nature and quantity of DNA template for PCR amplification.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s41935-020-00203-5.

'**Additional file 1: Table S1.** PCR plate layout for the Investigator 24plex GO! Kit optimization and cross contamination study using 25 cycles of amplification. **Table S2.** PCR plate layout for the Investigator 24plex GO! Kit on repeatability and reproducibility study using 25 cycles of amplification. **Table S3.** Average of peak height, standard deviation and percentage coefficient of variation (%CV) for 5 locus (vWA, THO1, SE33, D2S441 and TPOX) of the 24plex GO! **Table S4**. Maximum standard deviation (Std. Dev.) and percentage of coefficient of variation (%CV) for each locus of the 24plex GO! ladder by fluorescence-labelled dye / matrix standard.

Abbreviations

DNA: Deoxyribonucleic acid;; STR: Short tandem repeat; PCR: Polymerase chain reaction; NC: Negative control; PC: Positive control; NTC: No-template control; QS: Quality sensor; RFU: Relative fluorescence unit; GA: Genetic analyser machine; AL: Allelic ladder; LOD: Limit of detection; LOQ: Limit of quantitation; CV: Coefficient of variation; PHR: Peak height ratio

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Adherence to national and international regulations
Not applicable.

Authors' contributions
HMH collected, analysed and interpreted the data and wrote the manuscript. HOK, SAI and NHML helped to collect and analyse the data. JL and AEK contributed to the drafting of the manuscript and contributed to data interpretation. GKC and HAE designed the research and edited the manuscript. All authors have read and approved the final version of this manuscript.

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Availability of data and materials
Not applicable

Ethics approval and consent to participate
This study was carried out in accordance with Malaysian DNA Identification Act 2009 and DNA Identification Regulations Act 2012 and all experimental protocols were approved by DNA Databank Division (D13), Criminal Investigation Department, Royal Malaysia Police.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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