Advancing forensic RNA profiling

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Chapter 5

DNA transfer and cell type inference to assist activity level reporting: Post-activity background samples as a control in dragging scenario

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Abstract

In an earlier study we examined the composition of human cell material prevailing on trousers in an activity-related scenario (controlled dragging-by-ankles experiment) van den Berge et al. (2016). This included DNA and RNA profiling of samples taken from the location of interest prior to the activity, and DNA profiling of similar samples after the activity. Here, we present results of a third sample set that was simultaneously collected post-dragging, which involves an area of the trousers that was not touched by the dragger. Results of this study increase our understanding regarding the importance of including control region samples when investigating activity-related scenarios.

Introduction

The prevalence, transfer, persistence and recovery of human cell material has increasingly gained attention, for instance because of the importance in addressing activity-level questions [2]. In a previous study we described the analysis of samples taken from the exterior surface of the ankle area of the trousers prior (background/control samples) and post (activity samples) a controlled dragging-by-ankle experiment [1]. Results showed that samples taken prior to an activity resulted in STR profiles where a major contributor could be deduced that corresponded to the owner of the trousers, despite the occurrence of often many non-donor alleles. The majority of samples collected after the dragging scenario resulted in at least partial DNA profiles where both the victim and perpetrator could be retrieved with the perpetrator representing a deducible major in approximately a quarter of the samples. Unlike this study [1], forensic casework scenarios do not allow for the analysis of pre-activity background samples from the exact same location as post-activity samples. We now describe the analysis of post-activity samples taken in the proximity of the grabbed area, that was however not touched by the dragger. Analysis of these samples provide insight in the suitability of including background control regions.

Materials and methods

Samples were collected by tape lifting 26 knee areas of trousers after performing dragging experiments as described in Ref. [1]. The tape lifts were processed in its entire and DNA was extracted using the QIAamp DNA Mini Blood kit (QIAGEN) according to manufacturer's instructions. DNA quantification and ethanol precipitation was performed as described in Ref. [1]. DNA profiles were generated using the AmpFlSTR® NGM™ PCR Amplification Kit (Life Technologies). Amplification, separation and interpretation was performed as described in Ref. [1].

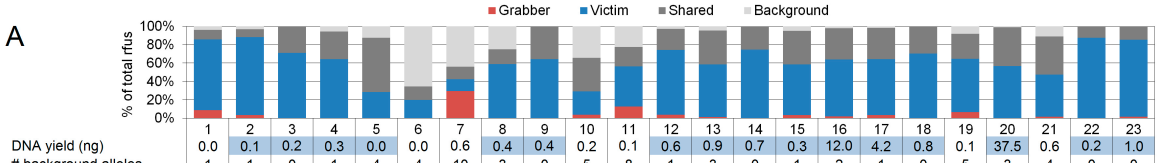
Donors with known genotypes (and informed consent) were used, so that the percentage of total rfus in the STR profile for each donor or for unknown contributors could be determined. Shared alleles were considered as a separate class. Profile interpretation was performed using the 15 STR loci in the NGM kit (Amelogenin was not included). Maximum allele count was used to determine the minimum number of contributors in a profile. Alike in Ref. [1], the LoCIM-tool was used to deduce the alleles of the most prominent component in the STR profile and to determine whether the "victim" or the "grabber" represents the major contributor in the DNA profile. STR profiles with ≤7 alleles were not subjected to further analysis.

Results and discussion

Three of the 26 samples were not subjected to further analysis, as these samples showed ≤7 alleles. An overview of DNA profiling results of the remaining 23 knee-area-of-trouser samples is shown in Figure 1A. Shown in Figure 1B and C are the results that had previously been obtained for the same trouser leg pre-, and post-dragging, respectively [1]. Alike for the ankle pre-dragging samples (Figure 1B), the majority of post-activity knee samples (Figure 1A) tend to show lower DNA yields compared to the ankle samples taken after the dragging activity (Figure 1C). Alleles corresponding to the victim (owner) were detected in the 100% of the knee samples (Figure 1A). Non-owner alleles are observed in 71% of the knee samples, but generally the owner (victim) alleles are substantially higher than non-owner alleles (peak height ratio victim/background). Hence, for 70% of the samples a major contributor could be deduced that corresponded to the owner of the trousers, while for the remaining samples no major contributor was deduced. Difficulty in deducing a major for these samples is most likely caused by the presence of some relatively high background alleles, preventing the possibility to deduce a major contributor in the STR profile. The estimated minimum number of contributors for the knee samples ranged up to two, which occurred in 61% of the samples. Grabber alleles are detected in 57% of the samples, with a maximum percentage of detected grabber alleles of 23% (Figure 1A, sample 7). An increase in detected grabber alleles can be observed especially in samples that show low numbers of owner alleles and high numbers of background alleles. These grabber alleles cannot be distinguished from background alleles, as can be seen by the low grabber/background peak height ratios. These results present a similar view as the results presented in Figure 1B and are in contrast to the post-activity samples (Figure 1C) in which the grabber stands out more clearly from the background alleles with an increased peak height ratio between grabber to background alleles.

Concluding remarks

With the increased interest in activity-related scenarios, the use of appropriate control regions for STR-profiling of this type of samples is highly relevant. We showed that in post-activity samples, a mixture of the owner of a private object and a perpetrator can be distinguished from background signals. In both pre- and post-activity control region samples, on the contrary, alleles matching a perpetrator may appear that are indistinguishable from background signals.



A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
DNA yield (ng)	0.0	0.1	0.2	0.3	0.0	0.0	0.6	0.4	0.4	0.2	0.1	0.6	0.9	0.7	0.3	12.0	4.2	0.8	0.1	37.5	0.6	0.2	1.0
# background alleles	1	1	0	1	4	4	10	3	0	5	8	1	3	0	1	2	1	0	5	3	4	0	0
Min. # contributors	1	2	1	2	2	1	2	1	1	2	2	2	2	1	2	2	2	1	2	2	2	1	1
% detected alleles victim	30	55	23	53	16	5	9	32	33	9	59	68	68	45	36	74	53	53	53	100	65	30	75
% detected alleles grabber	11	5	0	0	0	0	23	0	0	5	18	6	6	0	5	5	5	0	15	0	5	0	5
PH ratio victim/backgr.	2.5	1.7	-	0.9	3.0	1.2	1.4	0.9	-	1.2	1.1	1.6	2.3	-	1.2	3.4	3.0	-	2.7	7.7	1.2	-	-
PH ratio grabber/backgr.	0.0	0.5	-	-	-	-	0.2	-	-	0.6	0.4	0.7	0.0	-	0.0	2.0	2.0	-	0.3	-	0.3	-	-

B	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
DNA yield (ng)	8.2	0.2	0.6	0.4	0.9	0.1	0.1	0.2	0.5	0.6	0.3	12.5	10.5	4.4	29.0	6.9	2.6	0.7	0.4	4.9	2.3	1.5	1.3
# background alleles	9	3	13	25	10	1	8	14	5	20	14	24	19	17	2	6	2	19	14	10	10	17	4
Min. # contributors	2	2	2	3	2	2	3	3	2	3	3	3	3	2	2	3	2	3	2	2	2	3	2
% detected alleles victim	85	27	100	100	100	59	55	82	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
% detected alleles grabber	16	20	40	65	29	10	18	36	16	50	32	33	33	26	0	23	5	25	45	11	11	41	9
PH ratio victim/backgr.	0.9	1.2	4.5	3.0	1.9	1.8	1.5	1.4	3.4	1.0	1.6	2.4	3.0	5.9	7.5	10.4	2.7	3.7	2.8	7.4	3.5	6.4	7.8
PH ratio grabber/backgr.	0.0	0.2	0.2	0.3	0.0	0.0	0.4	0.2	0.9	0.4	0.8	0.3	0.4	0.3	-	0.4	0.0	0.1	0.3	0.4	0.9	0.2	0

C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
DNA yield (ng)	8.7	0.4	0.9	2.8	1.4	0.3	0.4	0.1	0.6	1.3	2.0	10.0	6.2	2.0	2.5	2.0	2.0	0.6	0.6	0.7	0.7	3.9	5.6
# background alleles	4	3	3	5	13	19	22	11	20	15	16	20	35	8	2	5	21	10	10	19	21	9	2
Min. # contributors	3	2	2	2	3	3	3	3	3	2	3	3	3	3	2	3	3	3	3	3	3	3	2
% detected alleles victim	50	63	63	60	87	67	97	83	97	67	100	100	100	100	93	100	97	100	100	97	100	100	100
% detected alleles grabber	100	93	100	100	100	100	97	47	83	80	80	100	87	93	63	97	90	93	93	97	83	97	97
PH ratio victim/backgr.	0.7	1.5	1.2	0.8	1.4	1.4	1.6	1.5	7.3	1.0	6.2	2.3	2.2	2.6	2.9	5.3	2.0	4.1	3.2	2.1	3.4	10.2	10.8
PH ratio grabber/backgr.	12.5	3.3	3.2	3.7	3.2	1.9	2.6	1.3	1.7	1.0	1.0	2.7	0.7	1.4	1.2	2.2	1.1	1.8	1.6	1.6	1.1	2.9	2.0

Figure 1. Detailed information for A) samples from the knee area of the trousers that were collected after a dragging activity (this area was not touched by the grabber during the activity and are therefore regarded as background/control samples); B) corresponding background samples taken from the trouser leg ankle (dragging location) prior to performing the dragging scenario; C) corresponding samples from the trouser leg ankles collected after a dragging activity. Indicated per sample are the percentages of total rfus in the STR profile belonging to the grabber (red bars), victim (blue bars), shared alleles (dark grey bars) and background (light grey bars). Markings in the DNA yield section indicate samples for which the grabber (red) or victim (blue) was deduced as major contributor in the STR profile. Absence of colour indicates samples for which the deduced major does not match the grabber or victim.

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