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Is an increased drop-in rate appropriate with enhanced DNA profiling?

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ABSTRACT

With enhanced capillary electrophoresis (CE) injection the fold increase in peak heights relates to the fold increase in sensitivity. As a result, DNA profiles appear similar when the amount of DNA in the PCR was adjusted according to the sensitivity of the analysis. With enhanced CE injection, however, more drop-in alleles were observed. In (probabilistic) DNA profile interpretation, it seems opportune to account for an amount of drop-in that is in accordance with the sensitivity of the method.

1. Introduction

Increased CE injection settings are known to result in increased peak heights (PHs), decreased drop-out and increased drop-in [1]. The fold increase in PHs follows the enhancement of CE injection. Thus, PHs should remain similar when three or six-fold lower DNA amounts are analysed with three or six-fold higher injection settings. We examined similarity of a set of 480 DNA profiles generated according to this idea.

2. Materials and methods

NGM profiles were prepared according to Table 1. Each DNA extract was amplified in fourfold using 29 cycles and PCR products were analyzed using (1) 3kV5s, (2) 3kV15s or (3) 9kV10s CE injection settings on a ABI3130XL. PCR and CE were performed as described in [2], except that 9kV10s analysis was not preceded by purification of the PCR product.

3. Results and discussion

Adjusting the input amount of pristine DNA in the PCR according to the sensitivity of the analysis resulted in similar profiles (Fig. 1). However, higher sensitivity resulted in more drop-ins, especially with low amounts of DNA (set 1) and even though the amount of DNA was adjusted according to the sensitivity of the analysis (Table 2). Drop-in alleles resided on stutter position, except for 20% of the drop-in alleles observed with 9kV10s CE injection settings.

4. Conclusion

Adjusting the amount of pristine DNA to the sensitivity of the method resulted in DNA profiles with similar appearance except that with increased sensitivity, a slightly larger proportion of the alleles represent drop-ins. Since pristine DNA was used, this effect is solely due to the analyses conditions. It is expected that with casework samples, relatively more background DNA may occur when samples carry less donor material and enhanced interrogation methods are applied. This may further increase the level of non-donor alleles that can be considered drop-in alleles (or an extra contributor).

Probabilistic interpretation of DNA profiles using likelihood ratio (LR) principles requires defined hypotheses. These hypotheses include assumptions regarding the composition of the DNA sample. It is desired that these assumptions are as close to the true composition as possible, because incorrect assumptions may affect the LR. Often, an overestimated drop-in rate of 0.05 is used as this will lead to a conservative interpretation under the prosecution hypothesis [2]. The drop-in rate is dependent on the settings for profiling, and a rate of 0.05 may not always be an overestimate. Therefore, it seems opportune to calculate LRs with drop-in rates that relate to the profiling settings. A suggested drop-in rate for NGM 29 cycles 9kV10s DNA profiles would be around 0.10. When PCR products are, for example, filtrated using DTR columns prior to CE injection or when increased cycling is performed, it may be opportune to further increase the drop-in rate [2].

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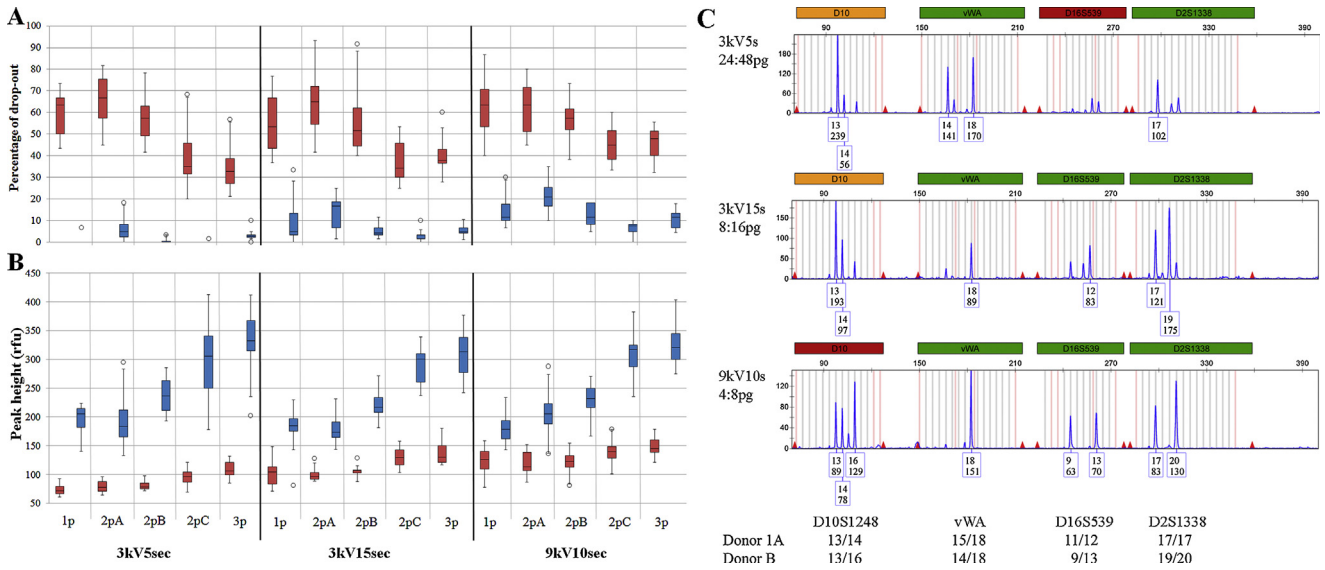

Fig. 1. Overview of percentages of drop-out (A), peak heights (B) and exemplar electropherograms with genotypes of the contributors (C) for samples described in Table 1. A & B: Set 1 is in red; set 2 in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Overview of samples for which the amount of DNA in the PCR was adjusted according to the sensitivity of the analysis.

				Amount of DNA in the PCR (pg)[a]					
	Mixture proportion			Set 1			Set 2		
Name	Donor A, B, C or D	Donor E	Donor F	3kV5s	3kV15s	9kV10s	3kV5s	3kV15s	9kV10s
1p	1	0	0	24	8	4	120	40	20
2pA	1	0.5	0	24:12	8:4	4:2	120:60	40:20	20:10
2pB	1	1	0	24:24	8:8	4:4	120:120	40:40	20:20
2pC	1	2	0	24:48	8:16	4:8	120:240	40:80	20:40
3p	1	0.5	2	24:12:48	8:4:16	4:2:8	120:60:240	40:20:80	20:10:40

[a] The amount of DNA in the PCR reversely followed the sensitivity: 3kV15s and 9kV10s used 1/3 and 1/6 compared to 3kV5s, respectively.

Table 2
Overview of drop-in alleles detected in profiles with adjusted amounts of DNA with varying CE settings.

	CE settings	Total # drop-ins	Avg. # drop-ins/ profile	Avg. % drop-in[a]	# profiles drop-in rate >0.05
Set 1	3kV5s	0	0.0	0.0%	0
	3kV15s	4	0.1	4.6%	1
	9kV10s	17[b]	0.2	7.0%	8
Set 2	3kV5s	4	0.1	2.0%	0
	3kV15s	12	0.2	2.7%	0
	9kV10s	34[b]	0.4	2.6%	0

[a] # drop-ins/total # of alleles per profile.
[b] Of which 11(set 1) and 30 (set 2) at −1 and/or +1 repeat unit stutter position.

Conflict of interest

None.

Acknowledgement

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