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Ratios and distances of pull-up peaks observed in GlobalFiler kit data

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ABSTRACT

Short tandem repeat (STR) analysis is widely used for forensic examinations with a capillary electrophoresis instrument such as the 3500xL Genetic Analyzer. This instrument adapts multi-locus STR kits to examine up to 27 loci using a 6-fluorescent dye system and corrects the spectral overlap between each dye. However, inaccurate spectral correction can cause pull-up peaks. Here, we examined the pull-up peaks observed in GlobalFiler kit data in terms of their peak height ratios and distances from their parent allele peaks when using the 3500xL and the 3130xl Genetic Analyzers. With the 3500xL, 546 pull-up peaks were observed, and their pull-up ratios averaged 1.03 ± 0.32% (range 0.260–2.80%). Of the 546 pull-up peaks, 534 peaks (97.8%) were within ± 1 bp from their parent allele peaks. Overall, the pull-up peaks toward adjacent shorter wavelength channels (e.g., from yellow to green) tended to be observed in the left side (shorter bp) of the corresponding parent allele peaks, and the opposite side tendency was observed for those pull-up peaks toward adjacent longer wavelength channels. These tendencies were also observed in the GlobalFiler data generated with the 3130xl and in the data obtained by injecting a J6 matrix standard with LIZ 500 or 600 v2 size standard into the 3500xL and 3130xl. Inspection of raw data revealed that the shift of pull-up peaks from their parent allele peaks was derived from sigmoid, pull-down, or slightly shifted pull-up shapes. Based on the obtained data, we propose a standard for assessment of questionable pull-up peaks.

1. Introduction

Short tandem repeat (STR) analysis is widely used for forensic examinations, as well as for population genetics and kinship analyses. In STR analyses, peak detection is performed with a capillary electrophoresis instrument such as the Applied Biosystems 3500xL Genetic Analyzer (Life Technologies/Thermo Fisher Scientific, South San Francisco, CA, USA). This instrument adapts multi-locus STR kits to examine up to 27 loci using a 6-fluorescent dye system [1–5]. Since there is some overlap in the emission spectra between each dye, this overlap is corrected by the instrument in the "multi-component analysis" [6–8]. When the spectral correction is not well performed as a result of changing experimental conditions such as temperature drift or degradation of buffers or polymers, spectral pull-up is likely to be observed [7,8]. Also, when too much DNA is added to the PCR reaction, the increased amount of PCR product can saturate the CCD camera input (off-scaled), which makes the spectral correction inaccurate and causes spectral pull-up [7–9].

Gill et al. [10] reported that pull-up peaks derived from off-scale samples were observed directly below their parent allele peaks (only within three scan units using the ABD 377 gel sequencer). Since this study, the observed positions of pull-up peaks have not been well studied, although pull-up peak height ratios were examined by Gill et al. [11] and Life Technologies [6]. Even at present, pull-up peaks are treated as being observed directly under very high parent allele peaks or within very close proximity to them [12,13].

Recently, in order to utilize the GlobalFiler kit with the 3500xL Genetic Analyzer in Japanese police laboratories, we have examined this STR kit through validation, population, and concordance studies [3,14–17]. While the 3500xL Genetic Analyzer along with the GlobalFiler kit (threshold value: 175 RFU) was sensitive enough to detect small amounts of DNA down to 0.125 ng [3], pull-up peaks (≧175 RFU) were observed, even from not off-scaled peaks [3]. Because pull-up peaks are artifact peaks [7,8] and complicate the STR profile comparisons between the casework and reference samples, pull-up peaks should be assessed, and deleted in the data analysis process [12,13]. In this study, to construct a standard for assessing questionable pull-up peaks, we examined the pull-up ratios and distances of the pull-up

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peaks that were observed in the GlobalFiler kit mainly with the 3500xL Genetic Analyzer but also with the Applied Biosystems 3130xl Genetic Analyzer (Life Technologies/Thermo Fisher Scientific) for comparison.

2. Materials and methods

2.1. Electrophoresis data from the GlobalFiler kit and J6 matrix standard

2.1.1. GlobalFiler data

The GlobalFiler kit electrophoresis data generated by our internal validation study [3] were used in this study. Briefly, the data were generated as follows: the stored DNA samples of 95 unrelated Japanese individuals were amplified using 1 ng of DNA with the GlobalFiler kit, and electrophoresis was performed once for each sample with the 3500xL instrument (named 3500xL #01) and the 3130xl instrument (named 3130xl #01) according to the supplier's instructions [9]. The data acquisition in previous internal validation study [3] and the data analysis in this study were both performed with the approval of the Ethics Committee of the National Research Institute of Police Science.

2.1.2. J6 matrix standard data

To confirm the above GlobalFiler data in terms of the distances of pull-up peaks, an experiment using the J6 matrix standard was also performed. In this experiment, the 3500xL #01, two other 3500xL instruments (named 3500xL #02 and #03), and the 3130xl #01 were used by injecting the DS-36 matrix standard (Dye set J6; Life Technologies/Thermo Fisher Scientific) along with the GeneScan 600 LIZ dye size standard v2.0 (Life Technologies/Thermo Fisher Scientific) or GeneScan 500 LIZ dye size standard (Life Technologies/Thermo Fisher Scientific). The details of the reagent solutions are shown in Table S1. For the 3500xL #01–03, 10 μl of each solution was deposited into 24 wells and injected twice to yield 48 electrophoresis data for each size standard. For the 3130xl, 10 μl of each solution was deposited into 16 wells and injected twice to yield 32 electrophoresis data for each size standard.

2.2. Pull-up ratio and distance of pull-up peaks

The electrophoresis data were analyzed using GeneMapper ID-X 1.4 (Life Technologies/Thermo Fisher Scientific). The threshold values for peak detection were set to 40 RFU for the 3500xL data and to 20 RFU for the 3130xl data. These values were calculated as the analytical threshold by the formula (mean of noise peak heights in baseline + 10 × standard deviation of them) [3]. Pull-up peaks were extracted from the exported CSV file using our original Excel macro files according to the criteria shown below. Our macro files also calculated a pull-up ratio (%) [pull-up peak height (RFU)/parent allele peak height (RFU) × 100] and distance (bp) [pull-up peak position (bp) − parent allele peak position (bp)].

2.2.1. Criteria for extracting pull-up peaks from the GlobalFiler data

- The candidate pull-up peak is not an allele peak.
- The candidate pull-up peak is located within ± 2 bp of only one parent allele peak in a different color channel.
- The candidate pull-up peak is not located between the positions of the minus two-repeat stutter and the plus one-repeat stutter of an STR allele in the same color channel (i.e., not in the range of −8.75 bp to +4.75 bp for a 4-nucleotide STR locus and not in the range of −6.75 to +3.75 bp for a 3-nucleotide STR locus. Note that we set these ranges by following the minus or plus one stutter ranges specified in the "GlobalFiler_stutter_v1.txt" file for the GeneMapper ID-X software [9].).
- The candidate pull-up peak is not located within ± 1 bp of an Amelogenin or Yindel allele in the same color channel.
- The candidate pull-up peak is not located within ± 1 bp of size standard peaks.
- The parent allele peak is not off-scaled. (Note that 1-ng amplified GlobalFiler data with the 3500xL #01 did not show any off-scaled peaks, but those with the 3130xl #01 did.)

2.2.2. Criteria for extracting pull-up peaks from the J6 matrix standard data

- The candidate pull-up peak is not a matrix standard peak.
- The candidate pull-up peak is located within ± 2 bp of the average position of each of the 6-color matrix standard peaks. The average position of each color peak was calculated separately for the 600 LIZ or 500 LIZ size standard using all electrophoresis data from the 3500xL #01–03 or the 3130xl #01.
- The parent matrix standard peak is not located within ± 1 bp of the size standard peaks.
- The parent matrix standard peak is not off-scaled.

3. Results and discussion

3.1. Pull-up ratios observed in GlobalFiler kit data

With the 3500xL #01, 546 pull-up peaks were observed, and their pull-up ratios averaged 1.03 ± 0.32%, with a range of 0.260–2.80% (Table 1, Fig. 1). The electropherograms of the highest pull-up peak and the highest pull-up ratio are shown in Fig. 2. For the 3130xl #01, as many as 2242 pull-up peaks were generated, and their pull-up ratios averaged 1.36 ± 0.54%, with a range of 0.318–4.49% (Table 1, Fig. 1). The range of the pull-up peaks with the 3130xl #01 was larger than that of the 3500xL #01 in terms of the observed number and the pull-up ratios.

3.2. Distance of pull-up peaks

In the GlobalFiler kit data produced with the 3500xL #01, of the 546 pull-up peaks observed, 534 peaks (97.8%) were within ± 1 bp from their parent allele peaks. Overall, the pull-up peaks toward adjacent shorter wavelength channels (e.g., from yellow to green) tended to be observed in the left side of the corresponding parent allele peaks (i.e., with shorter bp; Fig. 3). In addition, the pull-up peaks toward adjacent longer wavelength channels tended to be observed in the right side (i.e., with longer bp; Fig. 3). These tendencies were also observed with the 3130xl #01 in analyzing the GlobalFiler data (Fig. S1), and in

Table 1
Observed pull-up peaks with GlobalFiler kit.

	3500xL #01	3130xl #01
Observed number	546 (≥40 RFU[a]) 1 (≥175 RFU[b])	2242 (≥20 RFU[a]) 364 (≥50 RFU[c]) 2 (≥150 RFU[b])
Pull-up ratio		
Mean ± Std	1.03 ± 0.32%	1.36 ± 0.54%
Median	1.03%	1.28%
Min	0.260%	0.318%
Max	2.80%	4.49%

[a] This value was determined as the analytical threshold in our validation study [3], and also used for detecting pull-up peaks in this study.
[b] This value was used as the threshold for peak detection in our validation study [3].
[c] This value is possibly used as another threshold value for the GlobalFiler kit along with the 3130xl, since this value was adopted in the validation study of the GlobalFiler Express kit for the 3130xl [1].

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