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Large-scale concordance study for 16 autosomal STRs analyzed with PowerPlex ESI17 and NGM SElect

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ABSTRACT

This study aimed at identifying the frequency of kit-dependent discordances and genetic specialties in STR DNA typing, which is particularly important for the interpretation of DNA profiles as well as national and international database searches. We analyzed a total of 27′510 buccal swabs with the PowerPlex ESI 17 and NGM SElect amplification kits and documented discordances and other anomalies. We found kit-dependent full dropouts to be the most frequent events, most of which were associated with the NGM SElect kit. The total kit-dependent discordance rate determined in this study amounts to 0.74%. With this dataset, we also provide dropout rates, which can be used to estimate silent allele frequencies for 16 common STR loci amplified with the PowerPlex ESI17 and NGM SElect amplification kits.

1. Introduction

In Switzerland, DNA profiles stored in the national DNA database originate from various cantonal laboratories employing different STR kits. Since most of the amplification kits available for forensic STR typing use different primers, discordances in the resulting profiles can occur e.g. due to primer binding site mutations or other genetic variants. By creating DNA profiles with one kit only, potential errors might go undetected and wrongly typed markers might enter the database, thereby leading to false positive or false negative searches. In order to estimate and potentially prevent these errors, it is important to know the frequency and types of discordances that can occur.

2. Study design

In routine buccal swab analysis performed by our lab, two swabs are collected, extracted separately and amplified with both the PowerPlex ESI17 and the NGM SElect amplification kits. DNA fragment analysis is achieved by capillary electrophoresis and the raw data is interpreted using the Genemapper software. In June 2014, the previously used standard kit PowerPlex ESI17 Pro was replaced by PowerPlex ESI17 Fast and NGM SElect was replaced by NGM SElect Express. However, since the primers used in the newer kits are identical to the old ones, this change of kits should not be relevant to the outcome of this study.

Between January 2014 and June 2019, 27′510 buccal swabs were analyzed with both kits and the resulting profiles were screened for null alleles, partial dropouts, discordant allele calls and tri-allelic patterns.

3. Results

In a total of 27′510 analyzed samples, 304 kit-specific events or genetic specialties were documented and sorted into 7 categories (Table 1).

3.1. Null alleles

With 142 incidences, the most common events detected were full dropouts, the majority of which were associated with the NGM SElect kit (122 out of 142). In two of those cases, full dropouts were assumed to have happened at the same locus with both kits, judging by the height of the neighboring peaks.

Using the NGM SElect kit, null alleles were found to occur most frequently in markers SE33 and vWA. When using ESI17, the markers most often affected by null alleles were TH01, SE33 and FGA. We listed the number of null alleles observed per locus for each of the two amplification kits separately and calculated the null allele frequencies with respect to the total number of alleles analyzed (Table 2).

Four samples were categorized as dubious, because it was unclear whether the observed difference in the profiles should be interpreted as a discordance (marker in one kit homozygous, while heterozygous in the other kit) or as a dropout.

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Table 1
Table containing the number of observed events of each category and their percentage proportion with respect to the total number of observed events (n = 304).

Category	Number of observed events	Frequency of each category with respect to total number of observed events [%]
Null allele	140	46.05
Tri-allelic pattern	66	21.71
Partial dropout	54	17.76
Discordance	17	5.59
SE33 38.2/3	11	3.62
Other	8	2.63
Dubious	8	2.63
Total	304	100.00

Table 2
Table listing the number of null alleles per locus and corresponding null allele frequencies with respect to the total number of alleles analyzed (n = 27,510*2) per amplification kit.

	NGM SElect amplification kit		PowerPlex ESI 17 amplification kit	
Locus	Number of null alleles	Null allele frequency [%]	Number of null alleles	Null allele frequency [%]
FGA	1	0.0018	3	0.0055
TH01	4	0.0073	6	0.0109
D16S539	0	0.0000	1	0.0018
D1S1656	2	0.0036	0	0.0000
D21S11	1	0.0018	0	0.0000
D22S1045	1	0.0018	0	0.0000
D10S1248	2	0.0036	1	0.0018
D18S51	6	0.0109	0	0.0000
D2S1338	1	0.0018	1	0.0018
D2S441	2	0.0036	1	0.0018
D12S391	3	0.0055	0	0.0000
D19S433	9	0.0164	2	0.0036
vWA	34	0.0618	0	0.0000
SE33	56	0.1018	4	0.0073
D3S1358	0	0.0000	0	0.0000
D8S1179	0	0.0000	1	0.0018
Total	122		20	

3.2. Partial dropouts

We reported 54 cases of partial dropouts or imbalances between peaks. 56% of those were observed in profiles resulting from NGM amplification, 33% were found in ESI17 profiles and 11% showed imbalances when amplified with both kits. In four cases, it was unclear whether to document them as partial dropouts/imbalances or tri-allelic patterns – they were therefore categorized as dubious.

3.3. Discordances

We observed a total of 17 discordant allele calls, 8 of which were also filed as partial ESI17 dropouts, since one of the peaks was found to be significantly lower than the other one. There were 7 cases where a peak 24.1 in marker FGA obtained with NGM SElect was in discordance with a significantly lower peak 24 obtained with the ESI17 amplification kit.

3.4. Tri-allelic patterns

We observed 66 tri-allelic patters. Most of the markers displaying this pattern (61 out of 66 cases) showed three alleles of uneven intensity and therefore were thought to be the result of a somatic mutation [1], while in 5 cases, the peaks were of equal height. The loci with the most frequent occurrence of tri-allelic patterns were SE33 (21 out of 66 cases), D18S51 (9 out of 66 cases) and D3S1358 (7 out of 66 cases).

In marker SE33, a repeatedly occurring tri-allelic pattern was observed: In 11 cases, profiles amplified with PowerPlex ESI17 showed an additional allele 38.2 or 38.3. For three of those cases, peak heights of all three peaks were found to be in a similar range, while in all the other cases, peak heights for allele 38.2 or 38.3 were significantly lower. Due to unusual peak morphology and missing stutter, this additional allele might be attributed to an artifact.

3.5. Others

8 other events such as high stutter peaks, possible artifacts, peak shifts and (partial) dropouts of the amelogenin alleles were documented.

4. Conclusion

We found discordant allele calls in 0.06%, discordance due to null alleles in 0.51% and due to partial dropouts in 0.17% of all cases analyzed. The total discordance of 0.74% lies in the range of previously reported discordance rates [2,3]. The results presented here might help kit providers adjust their primers accordingly. Also, they serve as an argument to employ two kits with different primers amplifying the same set of markers and/or allow at least one mismatch in database searches. With a discordance rate close to 1% and no mismatch allowed in the search parameters, every 100th hit might potentially go undetected. With this large-scale study, we provide dropout rates for 16 common STR loci that can be used to estimate silent allele frequencies for probability calculations with Western European allele frequency data.

Declaration of Competing Interest

None.

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