B45 A Performance Evaluation of the VeriFiler™ Plus Polymerase Chain Reaction (PCR) Amplification Kit for Single Cell Forensics

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Learning Overview: The goal of this presentation is to inform attendees about how a standard, commercial Short Tandem Repeat (STR) profiling kit performs when utilized to genotype single cells and how single cells can be used to resolve mixed samples in forensic science.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by showing that single cell analysis can be used to resolve complex mixed samples using regular commercial STR profiling kits. The analysis goes deep into the performance of each locus, evidencing behaviors that are not visible when working on bulk DNA.

This presentation will show that single cells are a unique invaluable resource of clean and unique profiles of each contributor in a mixed sample and that isolation and profiling of pure single cells can lead to the solution of cases with multiple contributors. For single cell forensics to be practically usable, it's necessary to have an appropriate digital cell-sorting device and high-performance commercial kits for STR profiling. This work evaluated the performance of the Verifiler Plus™ PCR amplification kit with 25 multiplexed markers. The analysis of performance goes deep into each locus, evidencing behaviors that are not visible when working on bulk DNA, and it is finally demonstrated how forensic single cell analysis enables statistical confirmation of true loci vs. drop in peaks by double cell allele calling.

Mixed samples are one of the last unresolved challenges in forensic genetics. Solutions to overcome this problem have been adopted or proposed both on the sample preparation and on the data analysis side. Yet none has reached the sensitivity and non-ambiguity required for expert witness reporting in court. Furthermore, complex mixtures where two or more contributors share the same type of body fluid (sperm, blood, or epithelial) represent a higher level of complication since there is no available technique to phenotypically distinguish nor isolate cells of the same type from different individuals.[1] Single cell forensics enables us to resolve the problem of complex mixtures, allowing us to deduce each contributor's profile through a collection of single cells representing each, in purity, a single contributor to the mixed sample. Existing STR PCR amplification kits are not officially validated for DNA input below 0.5ng and, although most of them have the capability to produce detectable amplicons from single cells (~3–6pg), a performance evaluation is needed in order to adopt optimized protocols when attempting single cell analysis.

A simulated mixed forensic evidence, containing White Blood Cells (WBCs) and Sperm Cells (SCs) was generated in the laboratory. Cells from the dried swab were resuspended, fixed, and stained according to the DEPArray™ Forensic SamplePrep kit protocol and isolated on a DEPArray™ NxT digital sorter. A total of 30 single WBCs and 14 SCs were recovered, individually lysed, and PCR amplified using 29, 30, and 32 cycles with the Verifiler™ Plus™ kit using half-volume reaction (12.5µl). Capillary Electrophoresis (CE) separation was performed for all samples on an ABI® 3500 Genetic Analyzer and GeneMapper® ID-X (v1.5) was used for data analysis.

PCR performance was evaluated in terms of Completeness and Concordance for the three cycling conditions in order to identify the optimal one. Overall, results show that with increasing PCR cycle numbers, the allele detection rate grows and the system reaches levels of completeness higher than 80% at both 30 and 32 cycles on both WBC and SC, in agreement with previous findings on different kits.[2,3] The appearance of a drop in peaks and unconventional height of stutter bands are the most common artifacts that could impact single cell STR profiling accuracy.[3,4] In this evaluation, at 29 cycles no detectable unspecific peaks were noticed, producing clean profiles with 100% concordance. One additional cycle introduced a minimal number of drop in peaks (1%) and a few more unconventional stutter bands (4.1%). Reaching 32 cycles, a more-than-proportional increase in both drop-ins and stutters was observed. Considering these trends, 30 cycles seems to be the best compromise to maximize the number of informative alleles while still maintaining a very low level of non-specific peaks. Utilizing Double Cell Allele Calling to confirm real positive, only those found in at least two cells, it was found that with just six cells belonging to the same contributor, it's possible to match maximum completeness with very high concordance.

Reference(s):

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Mixed Samples, Single Cell, Digital Sorting

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