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Research paper

# Revealing the challenges of low template DNA analysis with the prototype Ion AmpliSeq™ Identity panel v2.3 on the PGM™ Sequencer

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ABSTRACT

Forensic scientists frequently have to deal with the analysis of challenging sources of DNA such as degraded and low template DNA (LtDNA). The capacity to genotype difficult biological traces has been facilitated by emerging technologies. Massive parallel sequencing (MPS) on microchip among other technologies promises high sensitivity and discrimination power. In this study we evaluated the combined use of the Quantifiler® Trio DNA Quantification Kit with the prototype Ion AmpliSeq™ Identity panel v2.3 and PGM™ platform in LtDNA samples. Coverage, allele balance, allele drop-out/in, consistency and variance were assessed. Overall, the results showed a great level of performance and consistency in terms of genotyping capability even under the most challenging conditions, making it possible to obtain consistent SNP profiles with 31 pg of DNA and partial informative profiles with as little as 5 pg or with severely degraded DNA. In addition, we demonstrated that the stochastic effects observed in some samples are due to the amplification of the library rather than sequencing.

Based on our data, we proposed general recommendations for the analysis of casework samples starting from the use of quantification data, which proved to be critical in deciding whether to process the samples via STR (short tandem repeat) analysis or SNP MPS.

In our experience, the use of the prototype Ion AmpliSeq™ Identity panel v2.3 has revealed a new applicable solution for processing LtDNAs. This approach provides users with an additional tool for analysis of traces that either would not give informative results with conventional STR-based techniques.
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## 1. Introduction

The need for alternative markers for human identification purposes has been discussed in the last 2 decades [1]. Currently, short tandem repeat (STR) markers have been the most used targets due to high level of discriminatory capacity even in the most challenging situations. The DNA degradation process, due to bacterial activities or heat and chemical stresses, leads to very short fragments [2] that only fall within the lower end of STR amplicons size range. In cases of severely degraded DNA, STRs analysis is limited due to restrictions in the length of the amplicons that can be generated. Mini-STR analysis has been implemented as an alternative [3,4] but single nucleotide polymorphism (SNP) analysis provides a greater guarantee of genotyping severely fragmented DNA as the variation of interest is much shorter [5]. In order to reach the same level of discrimination as commercially available STR kits, it is necessary to generate panels with a sufficient number of informative SNPs [1]. The selection of SNPs to include in these types of panels has been debated and many alternative options have been released in the last 10–15 years to be used on capillary electrophoresis or Real-time PCR platforms [6–9]. Total number of markers that can be analyzed in a single experiment, as well as cost and time to results were the main limitations that prevented routine adoption of a SNP multiplex kit in forensic laboratories. Furthermore, the analysis of mixed tracks appears to be a challenge due to the fact that a single SNP marker is associated with only two allelic variants. This makes it difficult to interpret the analysis of complex mixtures especially when contributors mixture ratios are very unbalanced. Massive parallel sequencing (MPS) is currently the most promising technology for analyzing genotypes at a sequencing level, where several samples and a high number of markers can be processed simultaneously [10,11]. The Personal Genome Machine®

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(PGM™ instrument) by Thermo Fisher Scientific allows MPS to occur in a non-fluorescence approach, providing users with a very flexible, rapid and scalable tool for genomic investigation. Results can be obtained in less than 2 days and experiments can be designed quite easily by adapting the number of samples and the number of target regions of interest to the microchip size. Reliability of this method has been proved by comparative analysis versus other MPS approaches [12–15] and it is now an accepted technology for the analysis of genetic disorders, pathogen and diseases diagnosis [16,17] and cancer profiling [18,19].

Increasing demand for analysis of severely degraded and very low template DNA samples [20–22], (LtDNA) is prompting forensic scientists to make critical decisions on the optimal sample processing workflow in order to gather the highest level of information per sample. Many guidelines have been proposed in the recent years on the best way to analyze LtDNA [20,23,24], suggesting assembly of a consensus profile by putting together results from different STR amplification replicates [25,26]. Stochastic effects such as allele drop-in/out should be considered when approaching the analysis of LtDNA samples [27]. In most cases, the limited amount of sample availability makes it impossible to run multiple different PCR reactions. In these cases the forensic analysts have to decide whether to process the sample by STR or SNP analysis. In most cases, this decision is taken on the basis of the quantification data or the availability of specific instrumentation in the laboratory. Recently, the Quantifiler® Trio DNA Quantifiction Kit has been released by Thermo Fisher Scientific. By using this kit, it is now possible to not only quantitate the DNA at a sub-picograms level but also to predict the level of degradation of the sample by the calculation of a Degradation Index (DI) [28]. When the DI detects a severe level of degradation, this could be a key predictor that partial non-informative profiles would be obtained with STR kits [36]. In these cases SNP analysis may provide additional information to identify an individual.

In our study we compared STR fragments obtained with the NGM SElect™ Kit and SNPs using a prototype version of the Ion AmpliSeq™ Identity panel (v2.3) with MPS on LtDNA. This SNP panel is composed of a total of 119 SNPs that include 90 autosomal and 29 Y-chromosome SNPs. Among the 90 autosomal markers, 86 are unlinked comprising a subset of Kenneth Kidd's 45 IISNPs-plex and the ii52 SNPforID52-plex [7–9]. We first ran a sensitivity study from 1 ng to 5 pg with 007 control-DNA and then developed a controlled thermal degradation protocol to generate fragmented DNA (from 007 DNA and blood sample) in a fast and easy way. We modified published protocols [29,2] and adapted to our degradation DNA target in order to obtain a range of degradation levels, including the amount at which STR analysis does not give informative results. To test sensitivity, several replicates of the dilution series were analyzed for the rate of allele drop-out/in out of 2261 independent genotyping events. A total of 1900 independent genotypes were analyzed in the 007 DNA and blood degradation study. Allele balance and consistency in the obtained genotypes were evaluated. The outcome of the sensitivity and degradation study assisted in defining general indications for analyzing LtDNA. We applied these last indications to the analysis

**Table 1**
Experiment summary and quantification.

| Sample type | Sample ID | Sample description | SA quantity[a] (pg/μl) | DI[b] | ODI[b] | Tot. input (pg)[c] | Mean read length (bp) | Mean depth on target read/SNP |
|---|---|---|---|---|---|---|---|---|
| 007 | 1 ng | Control male DNA from cell line | 174 | 0.7 | 0.8 | 1044 | 102 | 739 |
| 007 | 250 pg | Control male DNA from cell line | 43 | 0.7 | 0.8 | 258 | 102 | 806 |
| 007 | 125 pg | Control male DNA from cell line | 19 | 1.1 | 0.9 | 114 | 101 | 571 |
| 007 | 62 pg | Control male DNA from cell line | 9.6 | 0.7 | 0.8 | 57.4 | 101 | 640 |
| 007 | 31 pg | Control male DNA from cell line | 5.3 | 0.7 | 0.9 | 31.8 | 100 | 614 |
| 007 | 15 pg | Control male DNA from cell line | 3.2 | 1.2 | 0.6 | 19.1 | 104 | 795 |
| 007 | 5 pg | Control male DNA from cell line | 0.7 | 0.5 | 0.6 | 4.08 | 102 | 658 |
| 007 | 1H | Control male DNA from cell line | 648 | 2 | 1.9 | 200 | 90 | 718 |
| 007 | 2H | Control male DNA from cell line | 340 | 7 | 4.4 | 200 | 82 | 589 |
| 007 | 3H | Control male DNA from cell line | 74 | 386 | 24.4 | 222 | 69 | 438 |
| 007 | 4H | Control male DNA from cell line | 40 | 497 | 55.6 | 238 | 65 | 279 |
| 007 | 5H | Control male DNA from cell line | 22 | 571 | 85.1 | 132 | 62 | 333 |
| Blood | 0H | Whole male blood sample | 563 | 0.8 | 1.0 | 100 | 98 | 479 |
| Blood | 2H | Whole male blood sample | 378 | 2.7 | 3.1 | 100 | 80 | 533 |
| Blood | 3H | Whole male blood sample | 211 | 4.9 | 3.2 | 100 | 82 | 490 |
| Blood | 4H | Whole male blood sample | 74 | 17 | 4.8 | 74 | 77 | 564 |
| Blood | 5H | Whole male blood sample | 8 | 74 | 6.8 | 48 | 75 | 385 |
| Bone | Bone 1 | Bone powder from real casework | 2.9 | 2 | 1.7 | 27.7 | 112 | 89 |
| Bone | Bones 1–2 | Bone powder from real casework | 4.3 | 1 | 1 | 36.9 | 114 | 108 |
| Bone | 155 | Bone powder, 70 years old bone | 3.3 | 40 | – | 20 | 88 | 0 |
| Bone | 155-1 | Bone powder, 70 years old bone | 3.1 | 4 | – | 18.6 | 85 | 148 |
| Bone | 98 | Bone powder, 70 years old bone | 6.3 | 79 | – | 37.9 | 129 | 26 |
| Bone | 98-1 | Bone powder, 70 years old bone | 1.6 | 2.6 | – | 9.8 | 131 | 52 |
| Bone | 264 | Bone powder, 70 years old bone | 3.5 | 4.9 | – | 21.4 | 81 | 0 |
| Bone | 264-1 | Bone powder, 70 years old bone | 0.7 | 4.9 | – | 4.02 | 69 | 15 |
| Blood | Drop-1 | Dried blood samples from casework | 5.6 | 13 | – | 33.5 | 76 | 482 |
| Blood | Drop-2 | Dried blood samples from casework | 0.3 | NA | – | 1.8 | 73 | 24 |
| Tooth | Tooth | Tooth sample from a remain | 713 | 1.4 | 0.9 | 200 | 96 | 346 |

[a] SA Quantity refers to the average quantifications obtained by the Quantifiler® Trio Kit small amplicon replicate signals.
[b] Degradation index (DI) was calculated as the ratio between the quantifications obtained with the Quantifiler® Trio Kit by the small and large amplicons respectively. Observed degradation index (ODI) was calculated as the ratio of the average coverage from the 10 smallest to the 10 largest amplicons in the SNP panel.
[c] 6 μl of total DNA input were used for the library multiplex PCR.

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