Chapter 4

Degraded DNA sample analysis using DNA repair enzymes, mini-STRs and (tri-allelic) SNPs

Forensic Science International: Genetics Supplement Series (2009) 2:505–507

Antoinette A. Westen
Titia Sijen

Abstract

DNA degradation may cause the loss of the longer short tandem repeat (STR) markers, resulting in DNA profiles with lower discrimination power. We compared standard STR profiling with DNA repair enzyme incubation, and genotyping with mini-STRs or (tri-allelic) single nucleotide polymorphisms (SNPs) in progressively degraded, UV-irradiated DNA samples. In highly degraded DNA samples, most of the standard STR markers fail to amplify, while mini-STRs and especially (tri-allelic) SNPs still provide valuable information.

Introduction

The degradation of DNA may hinder successful human identification. The amplicon size of the STR markers that are used for DNA profiling usually ranges between 100 and 450 base pairs (bp). Due to DNA degradation, the longer fragments often cannot be amplified resulting in partial DNA profiles with lower discrimination power. To cope with degraded DNA, most strategies aim at shorter amplicon sizes, like with mini-STRs or SNPs [1,2]. Another possibility is to repair the DNA before amplification by the means of DNA repair enzymes [3,4].

In this study we compare these alternatives to standard STR typing (AmpF*l*STR® SGM Plus™, Applied Biosystems (AB)) for progressively UV-degraded DNA samples. We evaluated two commercially available DNA repair enzyme cocktails, PreCR™ (New England Biolabs) and Restorase™ (Sigma), against mini-STRs (AmpF*l*STR® MiniFiler™, AB), bi-allelic SNPs (GenPlex™, AB) and tri-allelic SNPs (as described by Westen et al. [5]).

Material and methods

All measurements were performed in dedicated laboratories (ISO 17025 accredited). In order to obtain artificially degraded DNA, pristine DNA (Quantifiler™ human DNA standard, AB) of 200 ng/µL was denatured for 5 min at 95 °C, placed on ice and irradiated with 254 nm UV-light in a CL-1000 UV CrossLinker (UVP, Inc.) at 0.9 J/cm2 for 0, 10, 30 and 120 min.

The incubations with DNA repair enzymes, PreCR™ and Restorase™, were performed according to the manufacturer's protocols in a 50 µL volume with a DNA input of 5 µL 200-fold diluted sample. 10 µL of the enzyme-incubated sample was used as input for the SGM Plus™ reactions.

Genotyping with the MiniFiler™ and SGM Plus™ kits was performed according to the manufacturer's protocols in a volume of 25 µL. GenPlex™ SNP genotyping was performed according to protocol v2.0.3 and the tri-allelic SNPs were analysed as described by Westen et al. [5] using SNaPshot™ single base extension (AB). The UV-irradiated samples of 200 ng/µL were diluted 200-fold and 1 µL was used as input for the SGM Plus™, GenPlex™ and tri-allelic SNP PCRs. For MiniFiler™ 1 µL of 800-fold diluted sample was used, since this kit uses 30 cycles PCR instead of 28 cycles.

All samples were analysed on an ABI Prism 3130xl Genetic Analyzer (AB). Analysis of the results was done with GeneMapper® v4.0 for GenPlex™ and GeneMapper® ID v3.2.1 for the other methods (AB).

Table 1
Comparison of analysis methods for degraded DNA samples.

	0 min UV	10 min UV	30 min UV	120 min UV	# Loci tested	RMP[a]	DNA input (ng)
SGM+							
Avg.[b]	100.00%	65.15%	27.27%	13.07%	11	3.0×10^{-13}	1
s.d.[c]	0.00%	3.71%	11.34%	8.22%			
n[d]	10	6	10	8			
PreCR & SGM+							
Avg.	100.00%	–	32.95%	14.77%	11	3.0×10^{-13}	5
s.d.	0.00%	–	25.27%	17.16%			
n	4	0	4	4			
Restorase & SGM+							
Avg.	100.00%	–	32.95%	22.73%	11	3.0×10^{-13}	5
s.d.	0.00%	–	28.11%	26.24%			
n	4	0	4	4			
MiniFiler							
Avg.	100.00%	95.83%	76.39%	59.72%	9	8.2×10^{-11}	0.25
s.d.	0.00%	5.32%	8.33%	5.32%			
n	4	4	4	4			
GenPlex							
Avg.	100.00%	98.40%	98.40%	87.77%	47[e]	9.6×10^{-18}	1
s.d.	0.00%	0.75%	0.75%	3.76%			
n	2	2	2	2			
Tri-allelic SNPs							
Avg.	100.00%	100.00%	100.00%	73.33%	15[f]	3.2×10^{-6}	3[g]
s.d.	0.00%	0.00%	0.00%	0.00%			
n	2	2	2	2			

[a] Random match probability for all loci in the kit determined in US Caucasians for (mini-)STRs and in Europeans for (tri-allelic) SNPs.
[b] Average percentage of detected alleles per profile.
[c] Standard deviation over the average percentage of detected alleles per profile.
[d] Number of measurements per method and treatment.
[e] Only 47 of the 49 GenPlex™ SNPs are analyzed due to background peaks.
[f] Determined in 153 Dutch samples, the 15 loci contain 8 tri-allelic and 5 bi-allelic SNPs; 2 presumed SNPs appeared to be fixed.
[g] The tri-allelic SNPs are measured in three multiplexes with 1 ng DNA input each.

Results and discussion

In order to determine which method performs best in examining degraded DNA, a series of UV-irradiated DNA samples was analysed with SGM Plus™ and evaluated against PreCR™, Restorase™, MiniFiler™, GenPlex™ and tri-allelic SNPs. Increasing UV-irradiation time results in progressive DNA degradation as demonstrated by detection of about 13 % of the alleles for SGM Plus™ after 120 min (Table 1, Fig. 1).

The SGM Plus™ results after incubation with PreCR™ or Restorase™ seem to show a slight enhancement in the average percentage of detected alleles compared to standard SGM Plus™ analysis (Table 1, Fig. 1). However, they also show very large standard deviations due to non-consistent results. In contrast, the mini-STRs show reproducible results with an average of about 60 % of detected alleles after 120 min of UV-irradiation (Table 1, Fig. 1). In addition, MiniFiler™ uses only 250 pg of DNA, while the PreCR™ and Restorase™ incubation reactions were performed with 5 ng DNA, whereas at least 50 ng DNA was recommended by the manufacturers. Furthermore, the hands-on and total processing time is the shortest for MiniFiler™, especially when compared to GenPlex™ and the tri-allelic SNPs.

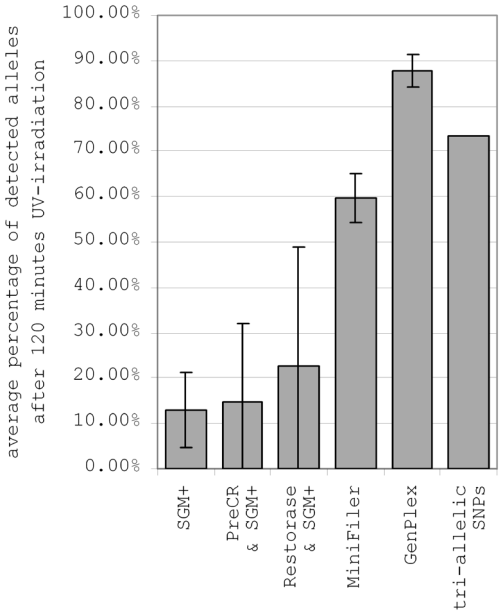

Fig. 1 The results for six analysis methods are plotted against the average percentage of detected alleles per profile after 120 min UV-irradiation. The error bars represent the standard deviation; when no error bars are displayed, all measurements were equal.

Both GenPlex™ and the tri-allelic SNPs showed an average percentage of detected alleles that was higher than for MiniFiler™; namely 88 % and 73 % respectively after 120 min UV-irradiation (Table 1, Fig. 1). This is probably due to the smaller amplicon sizes, being 59–115 bp for GenPlex™ and 58–100 bp for tri-allelic SNPs, compared to 70–283 bp for MiniFiler™. GenPlex™ has a very small random match probability compared to the other methods (Table 1). On the other hand, with GenPlex™ it is much more difficult to detect mixtures or contamination than with (mini-)STRs or tri-allelic SNPs.

The best choice for a certain method depends on the degradation level of the DNA sample and the type of investigation. When the DNA is highly degraded, SNPs perform better than (mini-)STRs. Unfortunately, no SNP information is stored in the (Dutch) national DNA databases and SNPs can therefore only be used in one-to-one comparisons, like for example with ante-mortem and post-mortem DNA evidence. In contrast, with a very small chance on discordance [6], MiniFiler™ results can be hold against the national DNA databases.

Conclusion

When the larger amplicons from a standard STR kit fail to amplify due to DNA degradation, MiniFiler™ can be used to complement the STR results, since it comprises of the longer amplicons from the AmpF*l*STR® SGM Plus™, Profiler™ and Identifiler™ kits. In our study, MiniFiler™ shows more reproducible results and a higher average percentage of detected alleles than standard STR analysis after incubation with DNA repair enzymes from PreCR™ or Restorase™. The SNP genotyping results

from GenPlex™ and the tri-allelic SNPs showed an even higher percentage of detected alleles than MiniFiler™ and are very suitable for one-to-one comparisons, like in human identification cases.

Role of funding

This study was supported by a grant from the Netherlands Genomics Initiative/ Netherlands Organization for Scientific Research (NWO) within the framework of the Forensic Genomics Consortium Netherlands. They had no involvement in the development of this paper.

Conflict of interest

None.

Acknowledgements

The authors would like to thank B. Erkamp, D. Mourik and C. Vervat for setting up the initial DNA repair experiments and A. Matai for technical assistance.

References

1. L.A. Dixon, et al., Analysis of artificially degraded DNA using STRs and SNPs—results of a collaborative European (EDNAP) exercise, Forensic Sci. Int. 164 (2006) 33–44.
2. M. Fondevila, C. Phillips, N. Naveran, M. Cerezo, A. Rodriguez, R. Calvo, L.M. Fernandez, A. Carracedo, M.V. Lareu, Challenging DNA: assessment of a range of genotyping approaches for highly degraded forensic samples, Forensic Sci. Int. Genet. Supplement Series 1 (2008) 26–28.
3. J. Nelson, Repair of damaged DNA for forensic analysis. http://www.ncjrs.gov/pdffiles1/nij/grants/227498.pdf (2009).
4. J. Ballantyne, Assessment and in vitro repair of damaged DNA templates. http://www.ncjrs.gov/pdffiles1/nij/grants/214166.pdf (2006).
5. A.A. Westen, A.S. Matai, J.F.J. Laros, H.C. Meiland, M. Jasper, W.J.F. de Leeuw, P. de Knijff, T. Sijen, Tri-allelic SNP markers enable analysis of mixed and degraded DNA samples. Forensic Sci. Int. Genet. (2009), doi:10.1016/j.fsigen.2009.02.003.
6. C.R. Hill, M.C. Kline, J.J. Mulero, R.E. Lagace, C.W. Chang, L.K. Hennessy, J.M. Butler, Concordance study between the AmpF*l*STR MiniFiler PCR amplification kit and conventional STR typing kits, J. Forensic Sci. 52 (2007) 870–873.