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Degraded DNA samples made informative by using superprimers

Martin E. Mautner[a,*], Mariela Caputo[b], Daniel Corach[b]

[a] Biodynamics SRL, Buenos Aires, Argentina
[b] SHDG-FFYB, Universidad de Buenos Aires, Buenos Aires, Argentina

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ABSTRACT

The use of long ssDNA polynucleotides in PCR of degraded DNA samples can discriminate Short Tandem Repeat (STR) genotypes otherwise missed by current commercial kits.

1. Introduction

The multiplex amplification of Short Tandem Repeats (STRs) is a reliable and widespread method used for obtaining individual genetic profiles from several types of DNA samples [1,2]. Unfortunately, the currently available kits are not always well suited for amplifying degraded DNA found in most archaeological, aged and forensic samples [3]. This shortfall arises from the need to amplify non-specific sequences [4] in order to obtain non-overlapping amplicons for size discrimination in capillary electrophoresis (CE). We have found that this downside may be overcome by using long ssDNA polynucleotides as primers [5,6]. By annealing closer to the target repeat sequences, this long primers reduce the actual length of intact DNA required for polymerization, while at the same time they yield amplicons of larger sizes suitable for CE detection in multiplex assays. They behave in a similar fashion to mini-STRs [7], but at the same time render larger amplicons covering the full CE range.We also show that only the 3' priming region of the long primers is important for a correct annealing, thus allowing for the design of practically any primer sequence of choice.

2. Materials and methods

DNA samples had been extracted 23 years ago from decomposed corpse tissues using a proteinase K/SDS incubation, followed by organic solvent extraction [8], and stored at −20 °C.

Primer sequences were designed based on the information provided by the STRbase website [4] and the GenBank. Fluorescently labeled primers were purified by HPLC. Long ssDNA polynucleotides of 200 nucleotides were purified by PAGE. All other primers were desalted.

The nonaplex mixture was prepared with 1X Colorless GoTaq® reaction buffer (containing 1.5 mM $MgCl_2$) (Promega, Madison, WI, USA), 0.625 mM of additionally supplemented $MgCl_2$, 200uM of each dNTP, 2.5 units of GoTaq® Hot Start DNA Polymerase (Promega) and a set of nine primers (IDT, Corelville, IA, USA) ranging from 62.5 nM to 250 nM.

PowerPlex® Fusion 6C (Promega) reactions were performed as specified by the manufacturer [9], except that the final reaction volume was 12.5 µL.

Nonaplex PCR amplifications were performed in a 20 µL reaction volume. Cycling conditions included an initial denaturation step of 2 min at 94 °C, followed by 35 cycles of 10 s at 94 °C, 60 s at 59 °C and 45 s at 72 °C and 30 min soaking at 60 °C.

Cadaveric DNA samples were split in halves and 7.5uL applied to each reaction. The input mass ranged between 50 pg and 500 pg.

Sample dilutions of the PCR products were run in an ABI 3500 sequencer (Applied Biosystems) with a 50 cm-capillary array and POP7 polymer (Applied Biosystems). Results were analyzed with GeneMapper® ID-X 1.2 (Applied Biosystems).

3. Results

A nonaplex PCR mixture was designed to encompass eight long pair of primers (60nt and 200nt each) that amplify the STR markers CSF1PO, Penta E, D5S818, D13S317, Penta D, TPOX, SE33 and D22S1045 plus one pair of short primers that amplifies the gender marker Amelogenin. The nonaplex mixture produced clean CE profiles with the expected genotype for gDNA 2800 M (Promega) (Fig. 1).

The standard primers used for amplifying the Amelogenin marker showed to be fully compatible with the longer primers.

* Corresponding author.
E-mail address: martin@biodynamics.com.ar (M.E. Mautner).

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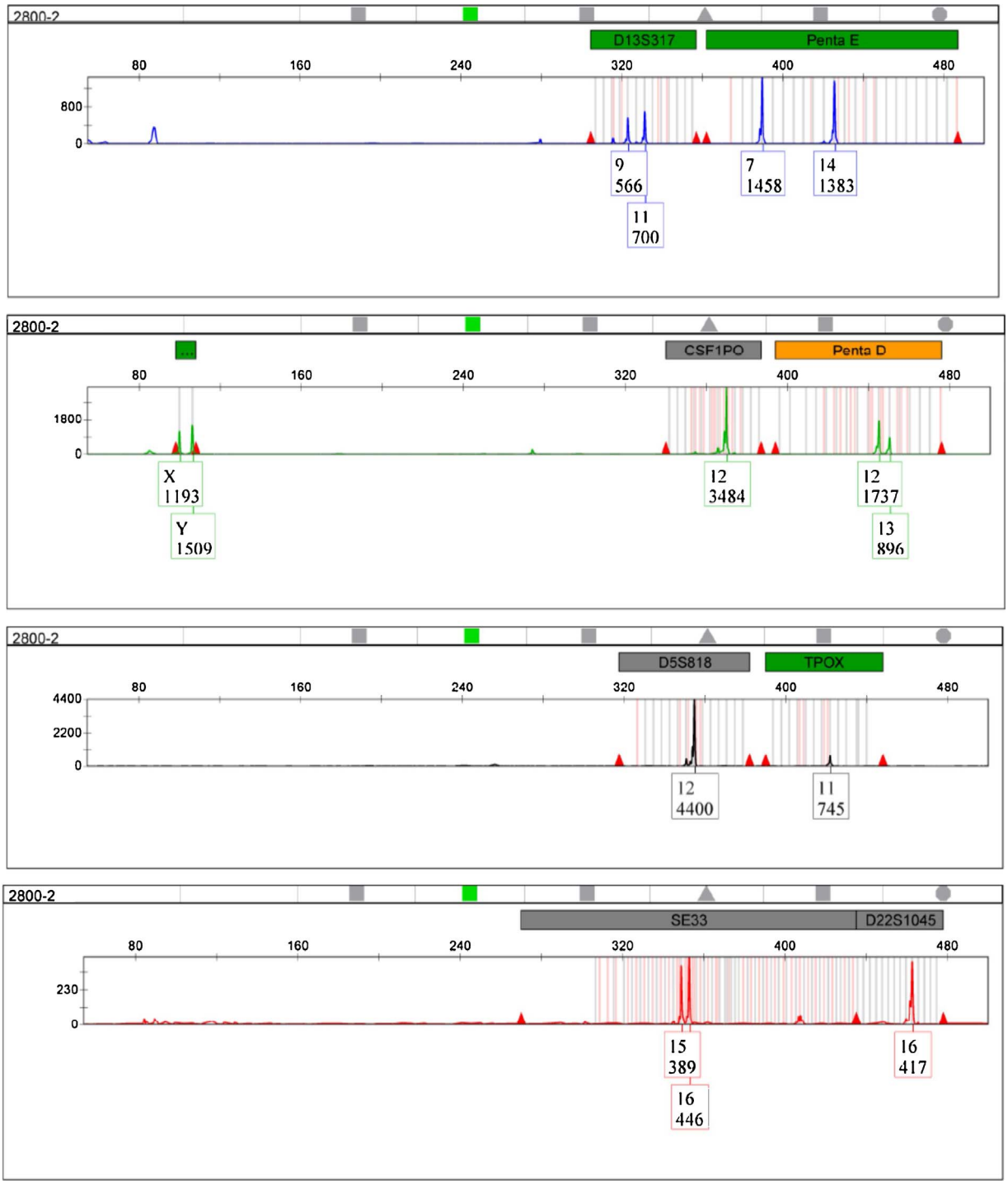

Fig. 1. Nonaplex CE profile.

D13S317, Penta E and Penta D markers were successfully amplified with primers bearing M13 non-homologous sequences at 5′ (Fig. 1).

The nonaplex PCR mixture described above was used to amplify degraded DNA from cadaveric samples and compared to PowerPlex® Fusion 6C (Promega). Equal volumes of DNA samples (7.5 μL) were used as input for both assays. In all cases the nonaplex mixture provided more information for the long amplicon markers (Table 1).

4. Discussion

We have shown the advantage of using long ssDNA polynucleotides as primer surrogates for obtaining STR profiles in degraded DNA samples. By annealing closer to the repeats, these *superprimers* extend much shorter DNA sequences than the standard primers, working in a similar way to the mini-STRs. Differently from the mini-STRs, the incorporation of the long primer sequences into the amplicons make them suitable for obtaining the more diverse CE lengths required in multiplex assays.

We have employed regular PCR solutions and conditions without any further adjustments, making the reaction fully compatible with standard, short primers. More complex multiplex assays (e.g., the 20 expanded CODIS markers) may be readily designed by the addition of more primers of different sequences and sizes.

We have also shown that long primers only complementary at the

Table 1
Nonaplex vs PowerPlex® Fusion 6C.

		STR Marker								
Sample	Multiplex	D13S317	Penta E	Amelogenin	CSF1PO	Penta D	D5S818	TPOX	SE33	D22S1045
A5000	Fusion 6C	–	–	X,Y	–	–	–	–	–	–
	Nonaplex	**14**[a]	**11,22**	X,Y	**10,13**	**11**	**9,11**	–	–	–
A5001	Fusion 6C	–	–	X,Y	–	–	–	–	–	–
	Nonaplex	**10**	**19**	X,Y	**10,11,12**	**9**	**11**?	–	–	–
A5004	Fusion 6C	*11*[b]		X,Y	13	–	–	8	–	–
	Nonaplex	**14**	**11,22**	X,Y	**10**,13	**11,13**	**9,11**	8	**19,31.2**	**15,16?**
A5163	Fusion 6C		–	X,Y		–	–	–	–	–
	Nonaplex	**9,12**	**12,16**	X,Y	**10,12**	**12**	**8,11**	**8**	**20,28.2**	**15**
A5165	Fusion 6C	–	–	X,Y	–	–	–	–	–	–
	Nonaplex	**9,12**	–	X,Y	**10,12**	–	**8,11**	–	**20**	–
A5523	Fusion 6C	–	–	X,Y	–	–	–	–	–	–
	Nonaplex	**9,12**	**12**	X,Y	**11,12**	**10**	**11**	**8,10**	**17,26.2**	–
A5570	Fusion 6C	–	–	X,Y	–	–	–	–	–	–
	Nonaplex	**8,12**	**7,10**	X,Y	**10,12**		**12,13**		**22,27.2**	
A5616	Fusion 6C	–	–	X,Y	–	–	–	–	–	–
	Nonaplex	**11**	**7,13**	X,Y	**11**	**9,11**	**11,13**	–	**16,23.2**	–

[a] Alleles only obtained using long primers are in bold.
[b] Alleles only obtained using standard primers are in italics.

3′-priming region can be successfully employed, providing flexibility in designing multiplex reactions that require several primers.

While we have designed the long primer sequences to anneal closer to the repeats, these results could be further improved with primers longer than 200 nucleotides, capable to anneal exactly at the extremes of the repeat regions. They could be prepared by synthetic chemistry or by PCR amplification followed by strand separation.

5. Conclusion

We have demonstrated that genotyping information present in degraded DNA samples, otherwise not detected by the commercial kits, can be readily obtained by using long ssDNA polynucleotides.

The incorporation of long primers in multiplex STR assays may also be used in reference DNA samples to prevent the incorporation into the final PCR products of deletions or insertions that may be present in the outlying sequence.

Additionally, long primers may also be employed in applications involving other types of fragmented DNA sequences, for instance in oncogenic and pregnancy testing of circulating cell-free DNA (ccfDNA).

Conflict of interest statement

MEM is the inventor of the pending patent applications USPTO 15/283,851 and PCT EP2017/067189 related to using long ssDNA polynucleotides in PCR.

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References

[1] C.T. Caskey, A.O. Edwards, DNA typing with short tandem repeat polymorphisms and identification of polymorphic short tandem repeats. US Patent No. 5, 364, 759. Houston, 1994.
[2] J.W. Schumm, C.J. Sprecher, Multiplex amplification of short tandem repeat loci. US Patent No. 6, 479, 235 B1. Madison, 2002.
[3] J.M. Butler, Forensic DNA typing, biology, Technology and Genetics of STR Markers, 2nd ed., Elsevier Academic Press, Burlington, 2005.
[4] J.M. Butler, D.J. Reeder, NIST short tandem repeat DNA internet database. http://www.cstl.nist.gov/strbase/index.htm Last date Accessed: April 10th, 2017.
[5] Mautner, ME: USPTO Application 15/283, 851, 2016.
[6] Mautner, ME: PCT EP2017/067189; 2017.
[7] J.M. Butler, Y. Shen, B.R. McCord, The development of reduced size STR amplicons as tools for analysis of degraded DNA, J. Forensic Sci. 48 (5) (2003) 1054–1064.
[8] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: a Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
[9] Technical Manual TMD045, Promega Corp, Madison, Wisconsin, USA. Revision 04;2017.