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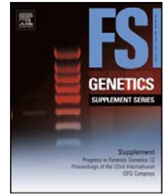

Research article

Selective enrichment of human DNA from non-human DNAs for DNA typing of decomposed skeletal remains

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ABSTRACT

The degraded or decomposed body remains pose serious challenges for successful short tandem repeat (STR)-based DNA typing for human identification because of contamination with non-human DNAs and environment-induced changes. Owing to their tropical environment, which results in robust microbial infestation, obtaining good quality and quantity of human DNA for body identification by DNA profiling has not been very successful in those regions. To address this issue, we attempted to selectively recover the human DNA from the mixture of non-human DNAs by employing the biotinylated oligonucleotides, designed complementary to the regions flanking the STR loci to selectively capture the respective STR loci that are used for DNA profiling. Initially, the fragmented human DNA was hybridized with the biotinylated oligos, followed by conjugation of biotin with streptavidin-coated paramagnetic beads. After extensive washing to remove non-specific binding, repeat-containing DNA fragments were eluted employing a magnetic particle concentrator. Such repeat-enriched DNA fragments were subjected to PCR amplification to analyze STRs employing commercial human DNA typing kits. Our preliminary analysis has shown that there was selective recovery of STR-containing human DNAs when biotinylated oligos were used.

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1. Introduction

The presence of highly polymorphic short tandem repeats (STRs) or microsatellite-containing regions in the human genome serves as highly reliable forensic markers for human identification (ID). Being polymorphic and thus unique, these microsatellite regions provide a very high probability of establishing the identity of an individual. Although forensic DNA profiling has become important evidence in civil and criminal justice delivery systems for human ID purposes, DNA profiling in heavily degraded or fragmented DNA is an extremely challenging exercise; and not often a successful profile is obtained from such forensic remains. Especially, in the regions where tropical climatic conditions prevail, the forensic skeletal remains are more vulnerable to environmental insults, resulting in scanty availability of good quality and quantity human DNA, which ultimately results in unsuccessful DNA typing. However, over the recent years, several novel approaches were developed, of which, mini-STR genotyping became helpful in testing degraded DNA [1,2]. In spite of these developments, the forensic DNA profiling of degraded skeletal samples remains a great challenge because unsuccessful DNA profiling can not only be attributed to degraded/fragmented DNA, but also to heavy microbial infestation and the presence of PCR inhibitors in the skeletal remains that prevent successful forensic identification. Thus, our focus in this study was to develop a strategy to selectively enrich human DNA from a mixture of non-human DNAs prior to PCR amplification to analyze STR repeats using commercial human DNA typing kits. Here, we employed a biotinylated oligonucleotide-mediated capture approach—wherein human-specific biotinylated oligonucleotide probes were designed to the regions flanking the STR-containing regions in the human genome to specifically recover the target loci of interest using biotin–streptavidin affinity.

2. Materials and methods

For the purpose of enriching human DNA from mixtures containing non-human DNAs by biotinylated oligonucleotide-mediated capture approach, 16 biotinylated oligonucleotides were designed (Table 1) in the genomic regions flanking the STR regions in the human genome by extending few additional bases upstream of the actual STR amplification primers reported elsewhere [3]. At the 3′-end of such extended primers, a biotin tag was added with an aim to 'pull-down' the biotinylated oligo-genomic DNA hybrid complexes using the streptavidin-coated paramagnetic beads.

In order to test whether such an approach would be successful, our initial attempts to selectively enrich human DNA was carried out

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Table 1
List of biotinylated oligonucleotide probes designed for this study.

Locus	Biotinylated oligonucleotide probe (5′–3′)
FGA	TTCTATGACTTTGCGCTTCAGGACTTCAAT
D18S51	TTCTTGAGCCCAGAAGGTTAAGGCTGCAGT
THO1	TCCTGTGGGCTGAAAAGCTCCCGATTATCC
CSF1PO	ATTTCCTGTGTCAGACCCTGTTCTAAGTAC
D7S820	GATTCCACATTTATCCTCATTGACAGAATT
D13S317	ACAGAAGTCTGGGATGTGGAGGAGAGTTCA
D8S1179	ATGTATTTTTGTATTTCATGTGTACATTCG
D3S1358	ATGAAATCAACAGAGGCTTGCATGTATCTA
D2S1338	CCAGTGGATTTGGAAACAGAAATGGCTTGG
D19S433	CTGCACTCCAGCCTGGGCAACAGAATAAGAT
vWA	CCCTAGTGGATGATAAGAATAATCAGTATG
D19S1338	CTCCTGCAATCCTTTAACTTACTGAATAACTC
D16S539	GGGGGTCTAAGAGCTTGTAAAAAGTGTACA
D5S818	AGCCACAGTTTACAACATTTGTATCTTTAT
D21S11	ATATGTGAGTCAATTCCCCAAGTGAATTGC
AMEL	GCCAACCATCAGAGCTTAAACTGGGAAGCTG

on sonicated sheared human DNA (to simulate natural conditions of DNA fragmentation), where the efficiency of recovery was tested for 15 different microsatellite loci and the sex determining locus Amelogenin (AMEL) using the respective oligonucleotide probes. This process, based on the modification of the microsatellite capture protocol reported elsewhere [4], involved hybridization of biotinylated oligonucleotides to the target regions in the human genome. The hybridization mix containing the biotinylated oligos and the human DNA was initially denatured at 95 °C for 5 min and then the temperature was decreased slowly allowing the oligos to hybridize to the complementary regions in the human DNA. This was followed by conjugation of biotin with streptavidin-coated paramagnetic beads (Dynabeads: Dynal, Oslo, Norway) and stringent washing of the resultant complex with buffer containing 1× SSC (0.015 M sodium citrate and 0.15 M sodium chloride) and 0.1% sodium dodecyl sulphate, for three times, to remove unbound human and non-human DNAs in order to enrich the target molecule of interest. The bound fraction was then eluted in TE (10 mM Tris–HCl [pH 8.0] and 1 mM EDTA) buffer after disrupting the biotin–streptavidin interactions by boiling at 95 °C for 5 min. To test the efficiency of enrichment, the 'pulled-down' fragments were subjected to PCR amplification using Amp*Fl*STR® Identifiler® kit (Applied Biosystems, USA) using a GeneAmp® PCR system 9700 (Applied Biosystems, USA) according to the manufacturer's instructions. Fragment analysis was carried out employing ABI PRISM® 3100 Genetic Analyzer and GeneMapper™ ID Software (Applied Biosystems, USA).

3. Results and discussion

The genotyping data showed that all the 15 STR loci and the AMEL locus were successfully 'pulled-down' when specific oligonucleotide probes were used during biotin-mediated hybridization-capture. In the control experiments, when the same capture was performed with non-biotinylated oligonucleotides, we found that none of the 15 microsatellite loci plus AMEL were recovered, suggesting that the capture using biotinylated oligonucleotides was indeed specific. Our preliminary analysis of the selective enrichment of human DNA regions using the biotinylated oligonucleotide-based approach demonstrates the successful capture of the target regions of interest. Although, in the preliminary studies, we could show selective capture of 15 STR loci and AMEL locus, using biotinylated oligonucleotide probes in the artificially degraded human DNA, in future experiments we aim to apply this method to actual forensic samples containing mixtures of human and non-human DNAs to test its efficiency. Once standardized, these alternative approaches to selectively enrich loci of interest in human DNA prior to PCR amplification using commercial human DNA typing kits would certainly be of great significance to the forensic community to unambiguously establish the identity of an individual, especially when compromised and challenging DNA samples are used for forensic genetic analysis.

Conflict of interest

None.

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