Human Leukocyte Antigen alleles as an aid to STR in complex forensic DNA samples

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1. Issues with challenging DNA samples

Advancements in DNA recovery and processing together with the introduction of analysis methods designed for low template DNA samples has made obtaining DNA profiles easier, quicker, and more efficient. However, those advancements have introduced new issues. Analysis of low template samples has revealed many problems with relying on STR in human identification since STRs are very prone to stochastic effects, particularly when low copy samples are involved. Stochastic effects are random fluctuations occurring during early cycles of PCR amplification that manifest as variations in the results from repeated analyses of the same sample.

Currently there are no methods to eliminate the issues with STR profiling of low template DNA source material.

The other major issue with STRs is difficulty in interpretation of the results from mixed samples containing DNA from several contributors in various proportions. In cases with highly unbalanced mixtures, the STR markers will not be able to differentiate between the alleles from the same locus. PCR-based analytical method generally allows for the detection of a minor DNA contributor in an unbalanced mixture only when its contribution is more than 10% of the total DNA content. However, to be able to detect all the minor alleles, the minor contributor has to be at least 20@ of the total DNA.

2. Current methods of sample deconvolution

Investigation focus has shifted into exploring other markers as a potential aid to STR in complex mixtures. An example is deletion/insertion polymorphism (DIP), which is part of the human DNA length polymorphism group based on indels. Indels are responsible for approximately 16-25% of human DNA variation.

In order for DIPs and STRs to form a marker, the two loci cannot be independent form each other, have to be less than 500 bp apart and close enough together to make recombination events highly unlikely [29].

The DIP-STR markers are not only highly polymorphic and located throughout the whole genome [15], bur are also very easy to genotype (IDEM as STR profiling [30]). Markers have been successfully applied to resolve extremely unbalanced DNA mixtures with 2 contributors, producing a high resolution profile from a donor with only 0.1% of contribution to the analysed DNA mixture [28].

Other potentially useful markers can be found in single nucleotide polymorphisms (SNP) substitutions. There are several characteristics of SNPs that make them useful markers for forensic analysis:

- When amplified, their PCR products are less than 100 base pairs in length, perfect size markers for analysing highly degraded DNA samples. STR amplifications are 300-400 bp long.
- SNPs have lower mutation rate over STR, making them valuable markers for paternity tests and other analyses.

Unfortunately, the majority of SNPs are unable to detect more than contributor in a mixed sample [39]. Fortunately, as in case of DIP markers, the forensic potential of SNPs can be increased by linking them with STRs. SNP-STR typing does not require any additional genotyping techniques and can be analysed using methods routine for STR profiling.

3. Human leukocyte antigen

The Human Leukocyte Antigen (HLA) system known also as the Human major Histocompatibility Complex (MHC) consists or more than 20,000 identified alleles [50] located on chromosome 6p21. This complex has been found to be the most polymorphic gene system discovered in the human genome [50]. It's highly polymorphic and it's also characterised by a high linkage disequilibrium and high density of genes [52]. The role of HLA system is to help the body's immune system distinguishing between its own protein and the foreign proteins made by viruses and bacteria.

Genes of the HLA system are closely linked together and as a result, the whole complex in inherited as a haplotype. However, there are possibilities for random recombination within the haplotype. Generally, the inheritance is ruled by Mendelian law, with one HLA haplotype coming from each parent. In case of unrelated individuals, the possibilities of random combinations of HLA antigens on a haplotype are immense.

4. Application of HLA complex in the field of forensic science

5. Potential of massively parallel sequencing in forensic analysis of the HLA complex

6. Conclusions

Despite their limitations, STRs remain the most reliable and widespread markers used in forensic identification. Taking into account all of the past and successful application of both HLA and MPS in forensic science, it is safe to assume, that

when combined, they could create a very powerful method for future implementations in forensic analysis. With such high polymorphism and discriminatory power, addition of even a single HLA marker to any standard STR-baed analysis can significantly increase chances of positive idenfitication.