SNP detection by hyperplex digital PCR for patient sample identification

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Background & objectives

Background:
Clinical hospitals need to check sample identity and quality frequently to avoid processing the wrong sample or contaminated samples. The gold standard for sample identification consists in short tandem repeat (STR) quantification due to its high discrimination power. This is notably the method of choice for forensic applications.[1]
Single nucleotide polymorphism (SNP) detection is a potential alternative to STR analysis for sample identification with new features. First, SNP detection works better than STR analysis on degraded or altered DNA, as it works on shorter targets and since SNP loci have lower mutation rates, for instance.[2] Second, SNP detection allows to validate variant signatures by direct comparison to sequencing which is not possible with STR analysis. In particular, detecting 5 or more tri-allelic SNP yields discrimination powers approaching that obtained by STR analysis which makes it a good candidate for field applications. This requires a highly sensitive multiplexed readout such as digital PCR.
Objective:
The objective of this work is to develop a highly multiplexed digital PCR assay up to 16-plex enabling the simultaneous analysis of 5 tri-allelic SNPs for routine sample identification testing.

[1] Butler, J.M. et al.; STRs vs. SNPs: thoughts on the future of forensic DNA testing. *Forensic Sci Med Pathol* **2007**; 3:200-205.

[2] Westen, A. A. et al.; Tri-allelic SNP markers enable analysis of mixed and degraded DNA samples. *Forensic Sci. Int.***2009**; 3: 233-241.

Methods

Methods:
This work was done on the naica® digital PCR system (Stilla Technologies) using a color combination approach consisting of detecting each targets with two different fluorophores instead of having one fluorophore per target classically. 5 tri-allelic SNPs were selected with a minor allele frequency of at least 22%. The final assay enables the quantification of all 15 alleles from these 5 SNPs as well as the quantification of chromosome Y to define the gender resulting in a discrimination power of 1.4/10000.

Results

Results:
We first demonstrated that this 16-plex assay accurately determines the correct signature of the 5 SNPs in cell samples, in correlation with sequencing analysis. Then the compatibility of this assay was assessed on various clinical samples (blood, frozen tissues and fixed tissues) with good concordance.

Conclusion

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The use of color combinations with the naica® system enables to reach high multiplexing level (16-plex here). This approach was successfully applied to tri-allelic SNPs detection allowing to develop an assay for routine sample identification.