OLISA™ Micro Arrays : Fast, Reliable and Competitive Method for Genotyping

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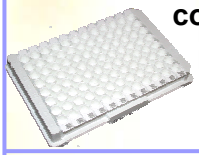
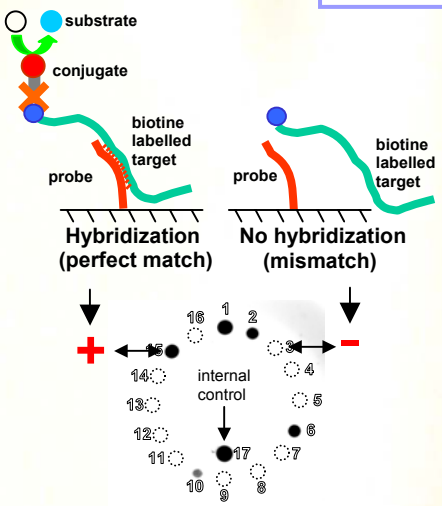
OLISA™ (OLIgo Sorbent Arrays) are micro arrays of up to 17 probes arrayed on the well bottom of a 96-well microtiter plate using a proprietary surface chemistry.
It has been used to characterize two human SNPs, mutation of Factor V 'Leiden' (G1691->A) and mutation of factor II (prothrombin G20210->A), both associated with an increase in Venous Thrombosis[1,2]. Short oligonucleotide probes were designed to hybridize specifically to each allele of amplified DNA targets, during a single temperature process. Detection was performed using a colorimetric method. This system allowed to accurately characterize genotypes.



[1] Bertina *et al.*, *Nature* 369: 64-67, 1994. [2] Poort *et al.*, *Blood* 88: 3698-3703, 1996

Principle, Material and Methods

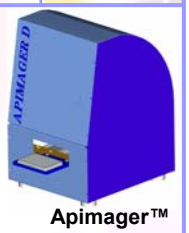


OLISA™ Principle

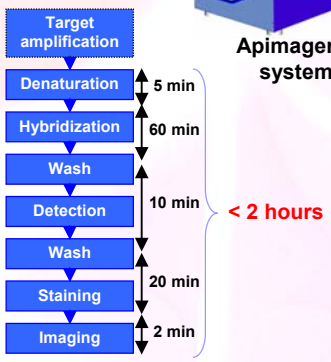
Targets:
Genomic DNA was multiplex PCR amplified to generate biotin-labeled fragments, each containing a SNP **(G-A substitutions).**

Probes:
Short oligonucleotides designed to ensure **high specificity of hybridization**.

Result Imaging:
Arrays were read using a proprietary densitometry reading system (Apimager™) which converts color levels into numerical data.



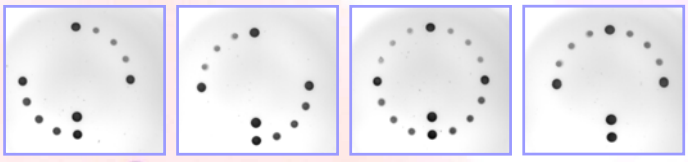
Apimager™ system



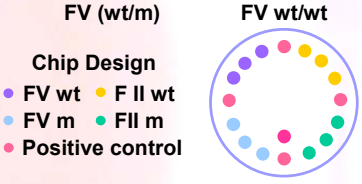
OLISA™ protocol

Results

-**Four probes were designed**, to detect wt and mutant alleles for Factor V and Factor II mutations.
-. **The genotypes were easily characterized by spot patterns**, allowing accurate identification of homozygous and heterozygous samples for each allele.
- ***Sensitivity*** **:** OLISA™ micro arrays can detect amplified targets from the femtomole level, (10^8 to 10^{12} PCR molecules / well).
- ***Specificity*** **:** Single base mutations and SNPs can be distinguished at a signal to noise level greater than 2.
- ***Reproducibility*** **:** CV is lower than 10 % for the whole process.



Sample 1 FII (wt/wt) FV (m/m)
Sample 2 FII (m/m) FV (wt/wt)
Sample 3 FII (wt/m) FV (wt/m)
Sample 4 FII wt/wt FV wt/wt



Factor II / Factor V genotyping using OLISA™

Conclusion

OLISA™ provides rapid and accurate results to characterize genotypes (up to 16 alleles per well), in less than 2 hours in a standard lab. Format 96-well microtiter plate allows integration in automated systems. The colorimetric detection method allows sensitive and cost-competitive multi-detection of mutations. This OLISA™ system can easily be customized to dedicated applications to fulfill customer's needs. Other applications, such as GMO's detection, biological species identification, or genetic diseases prognosis have been validated using this flexible technology.

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