

*Discovery and Scoring of Sequence Variations  
with MALDI-TOF Mass Spectrometry*

SEQUENOM<sup>®</sup>



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Sr. Scientist Mol. Biol.  
SEQUENOM GmbH

Meeting on  
New and Developing Technologies for  
Genetic Diagnostics  
Salisbury, July/05-06/2004

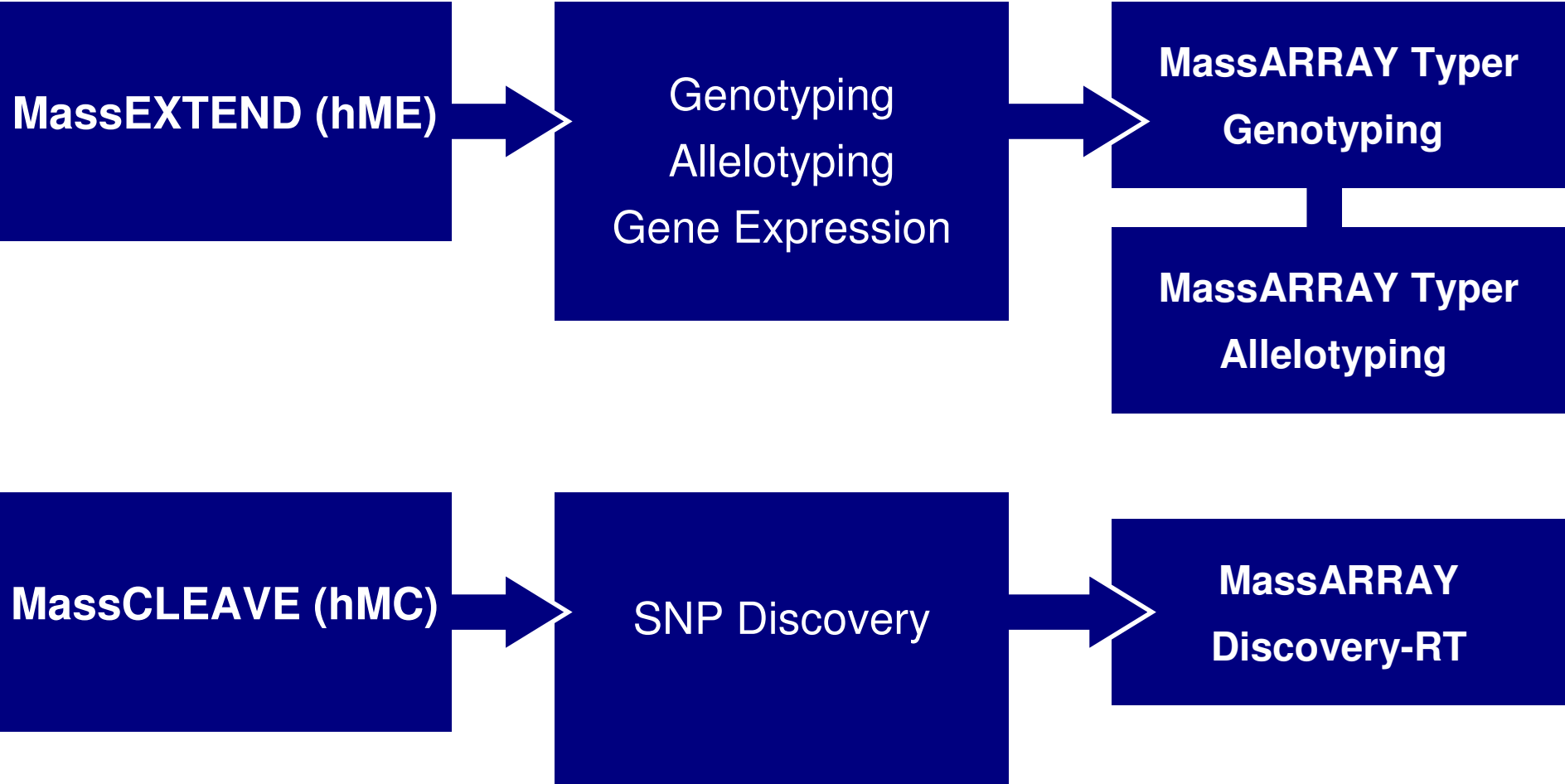
# MassARRAY™ – Applications

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## Chemistries

## Applications

## Software





5  $\mu$ l

PCR

+ 2  $\mu$ l

dephosphorylation

+ 2  $\mu$ l

cycled MassEXTEND reaction

extended Primer

NNddX

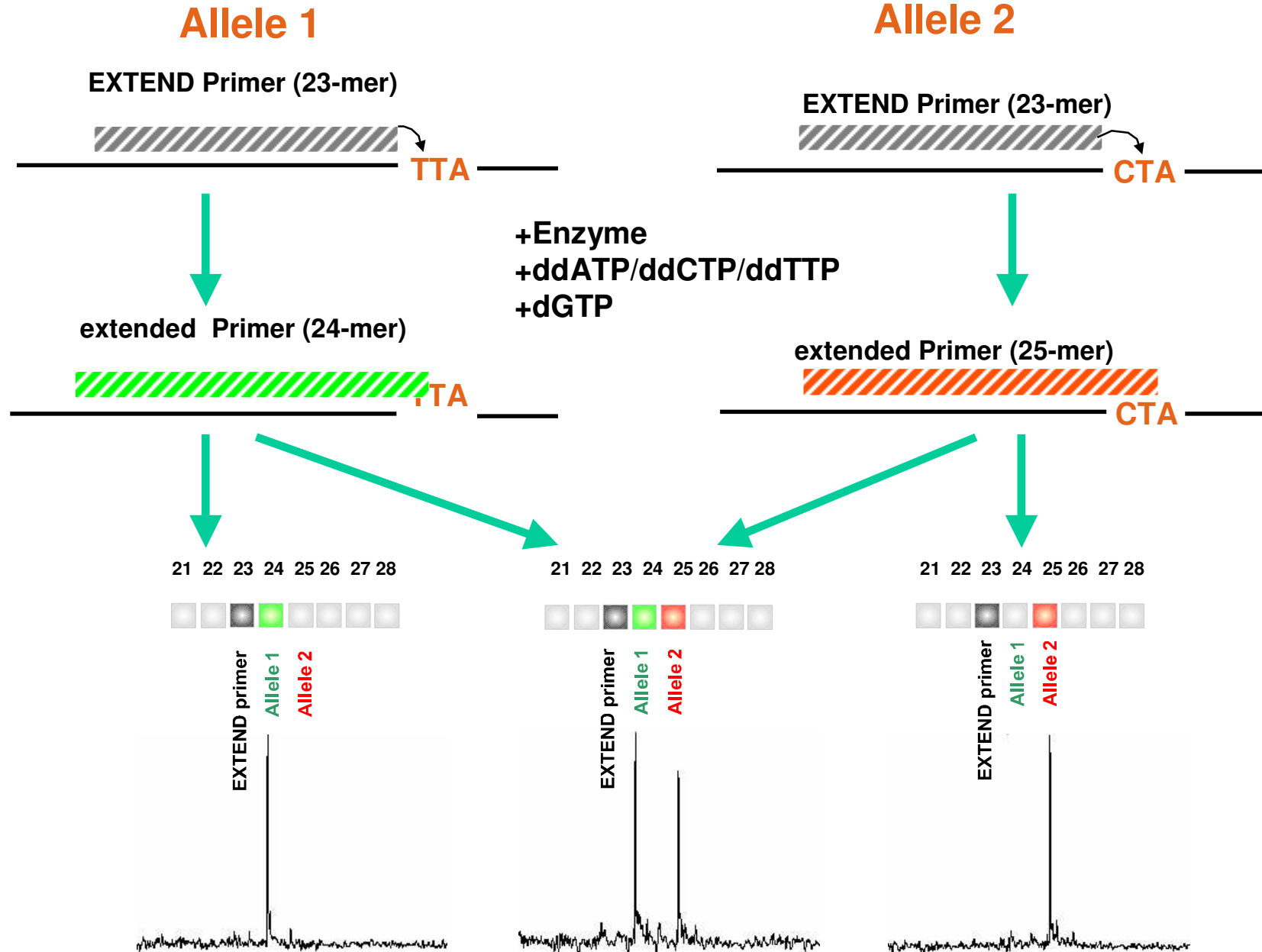
+ 16  $\mu$ l

sample conditioning

15 nl

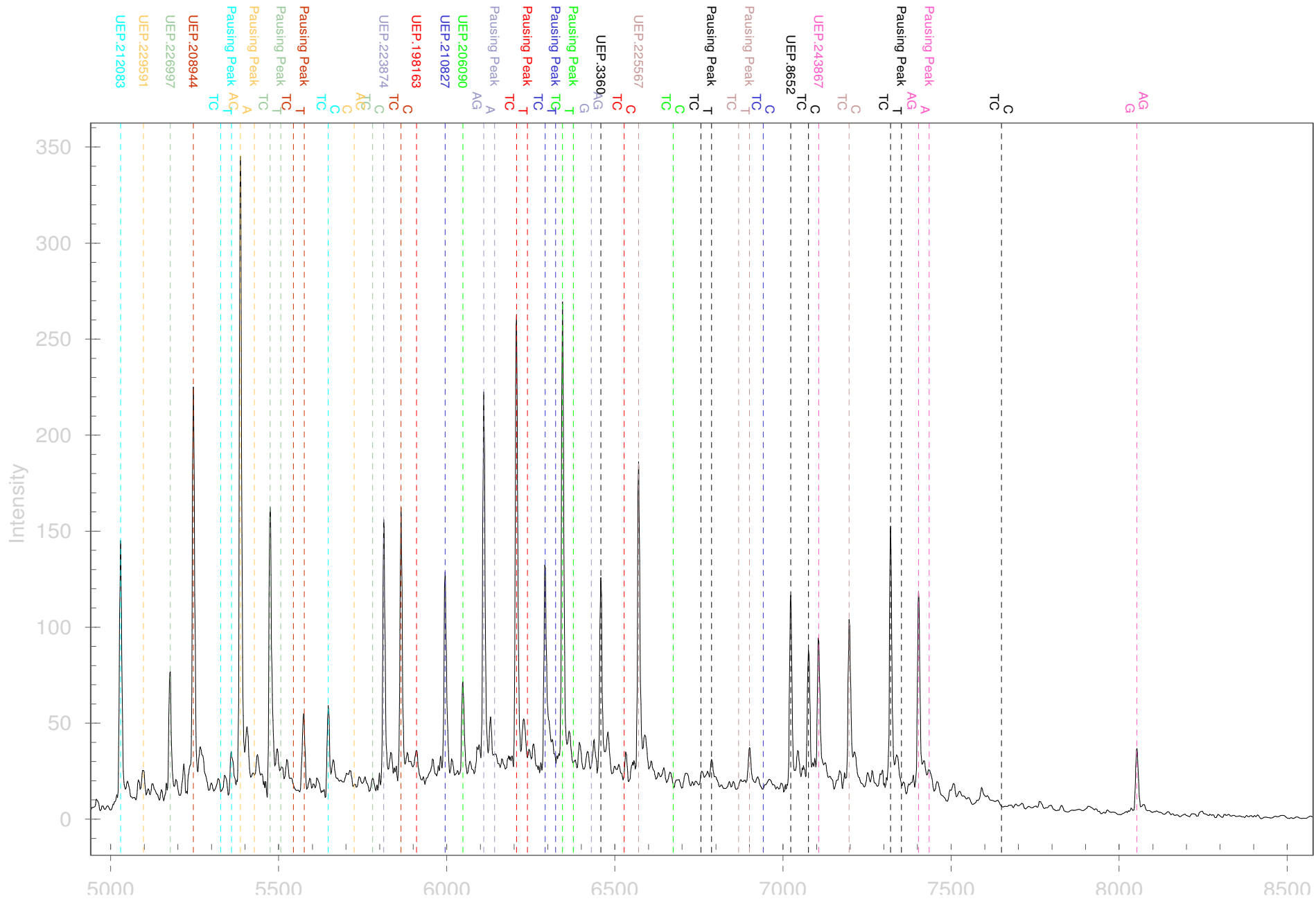
MALDI-TOF MS analysis

## Reaction Principle

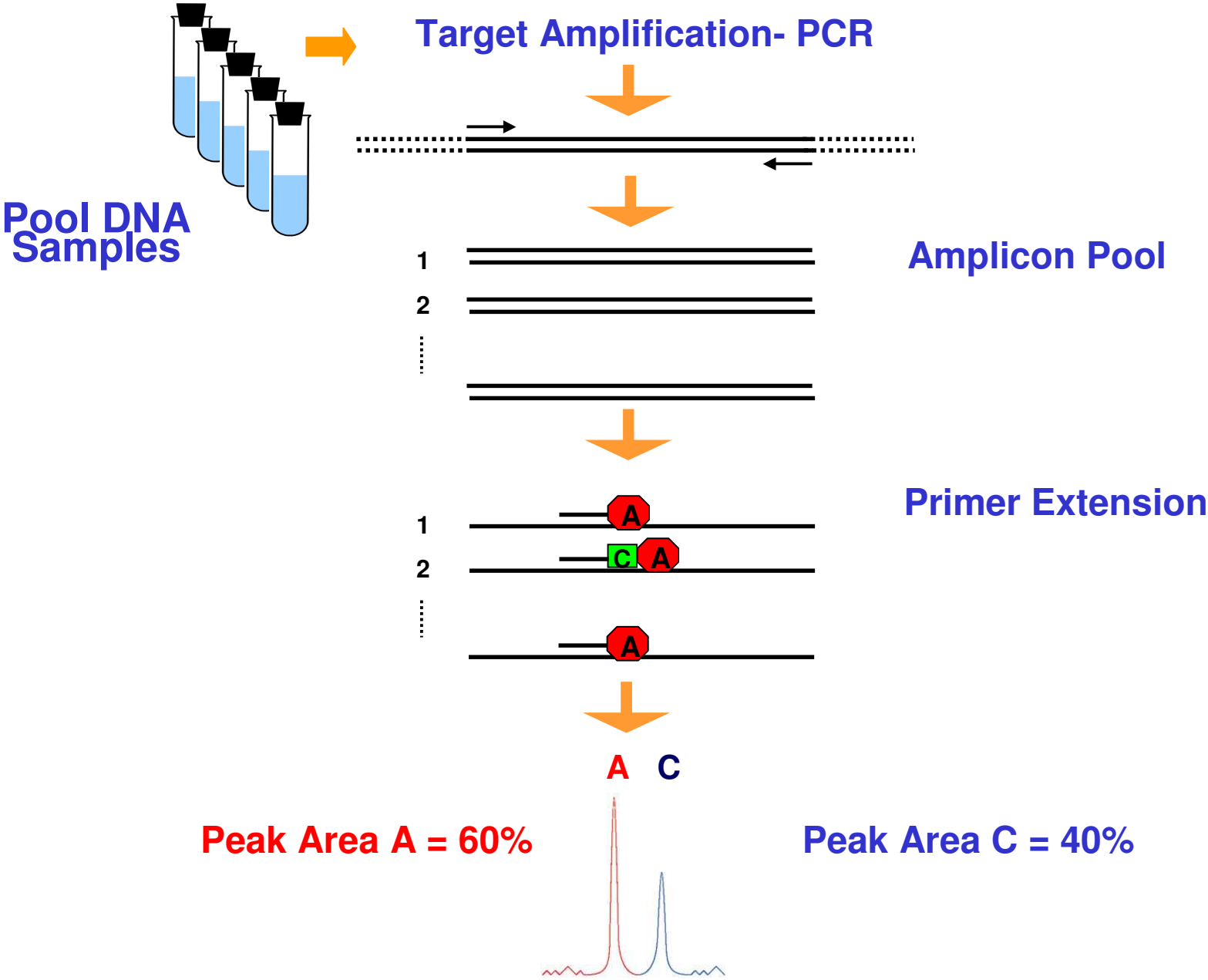


# high level Multiplexing

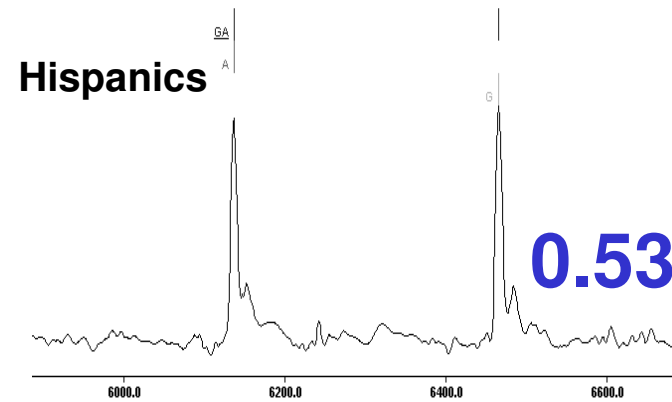
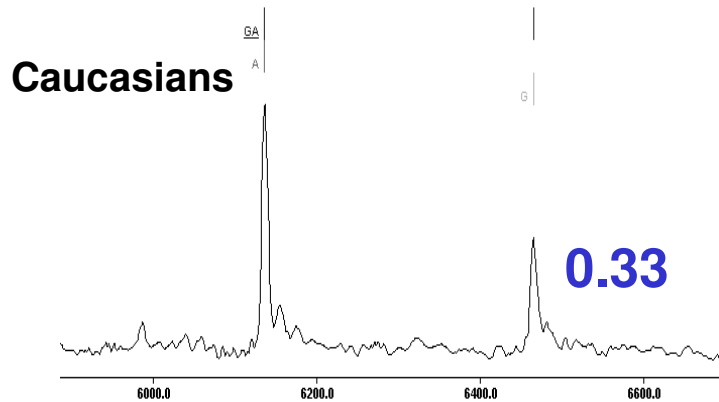
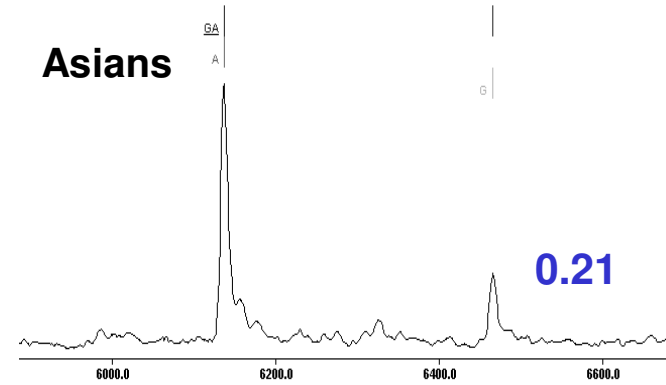
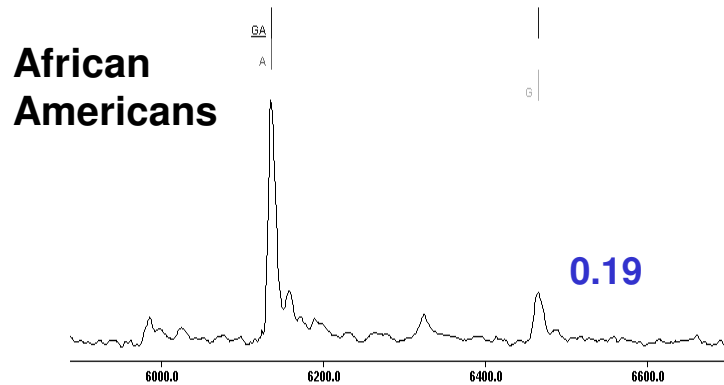
## 12-plex at PCR and hME level



# Allelotyping with MassARRAY™



## Different ICAM-1 Frequencies in 4 Ethnic Groups (minor allele frequencies indicated)

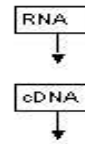




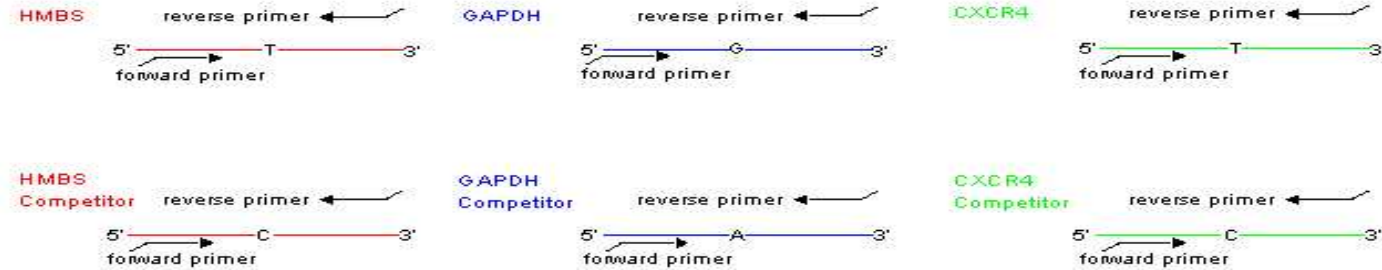
# Gene Expression Analysis

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## Reverse Transcription

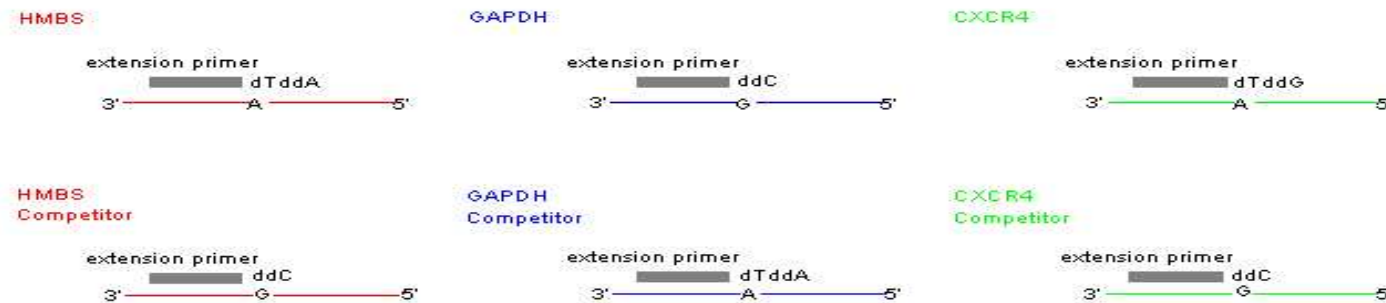


## PCR



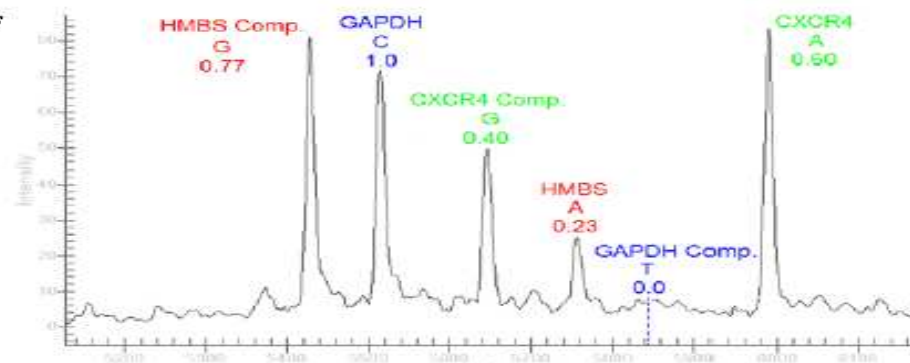
## SAP Treatment

## hME Reaction



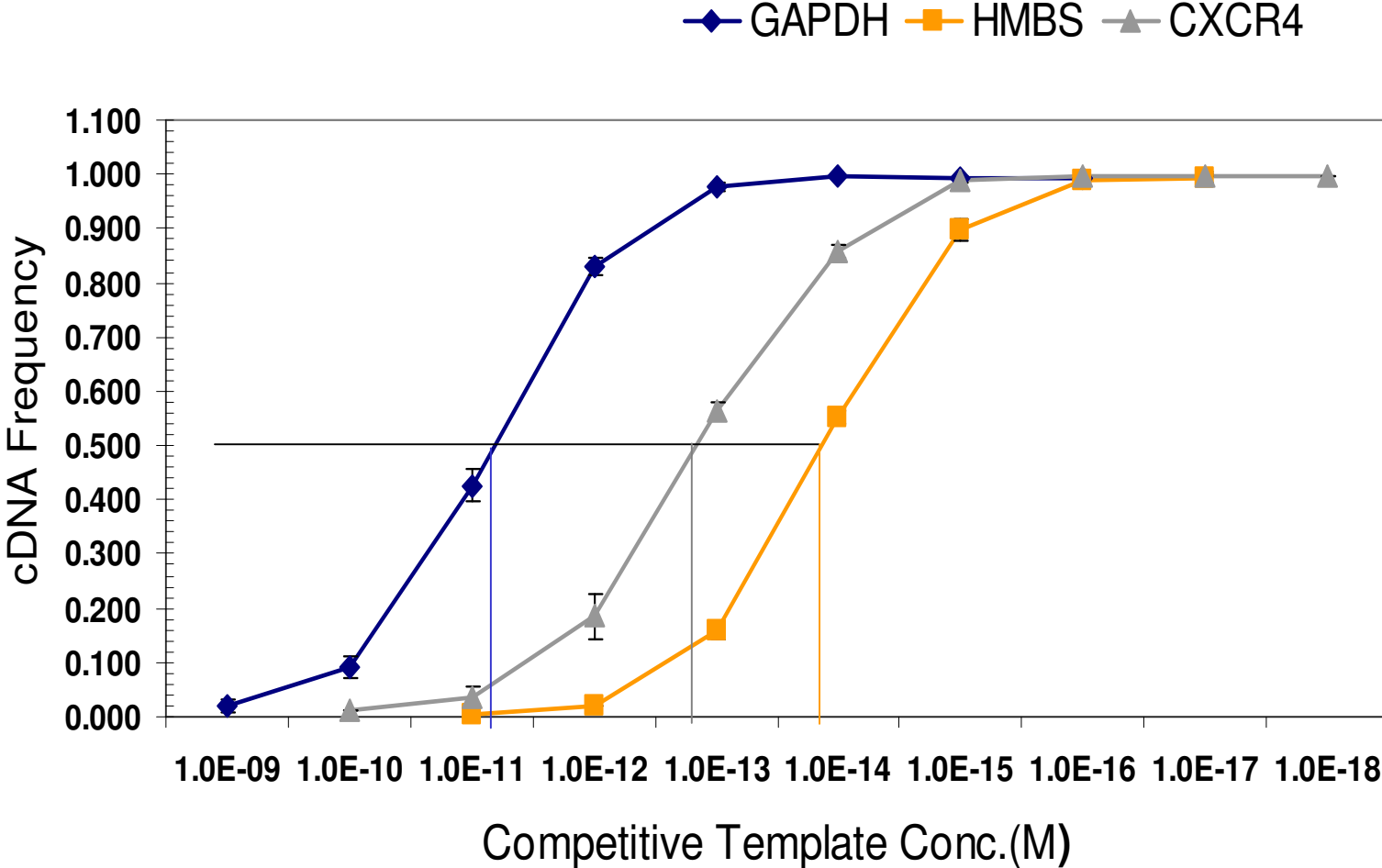
## Sample Conditioning, Nanodispersing, and Mass Spectrometry

## Mass Spectrometry Results





## Competitive Template Titration



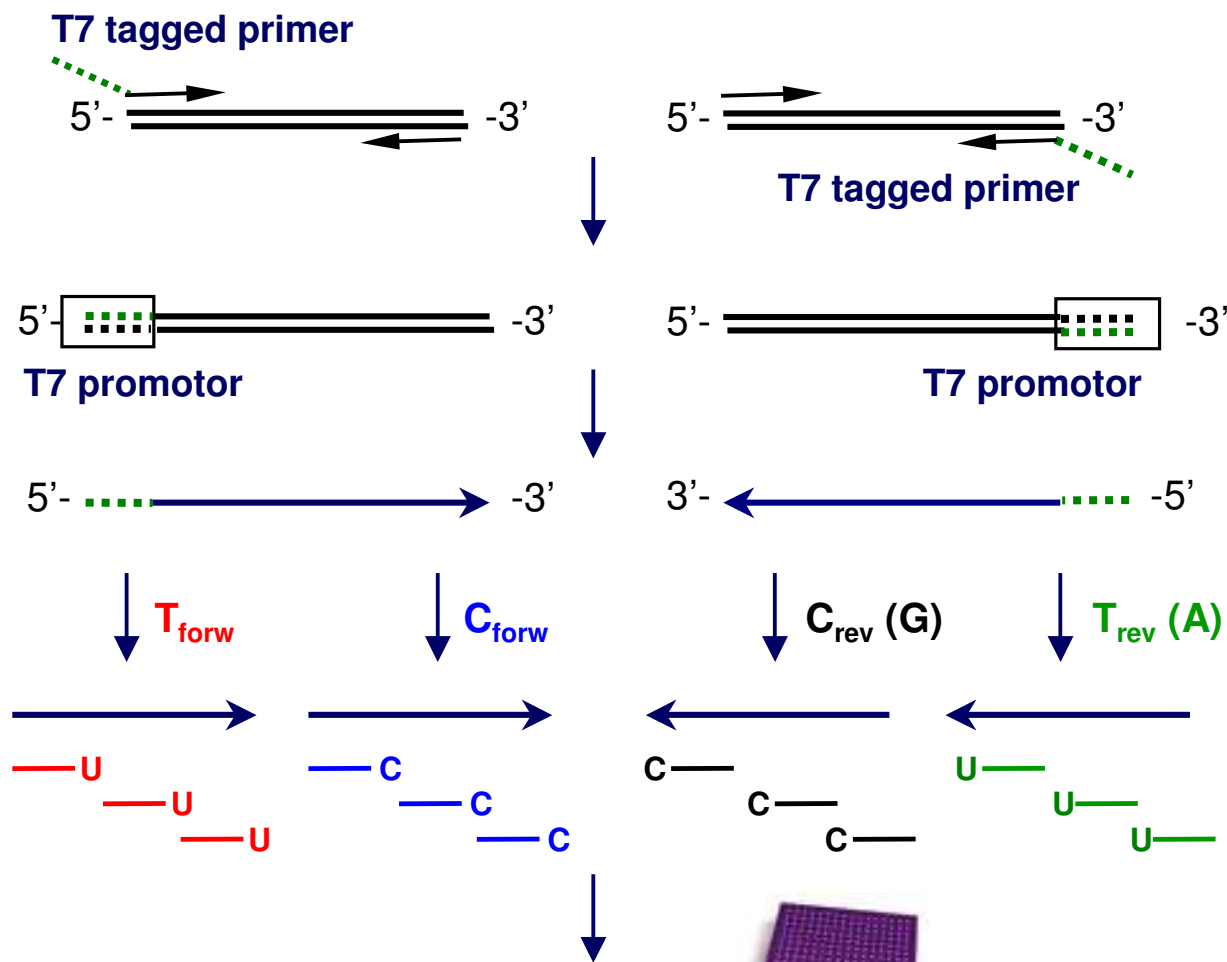


# MassCLEAVE™ Assay

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forward strand

reverse strand



PCR from genomic DNA

SAP treatment

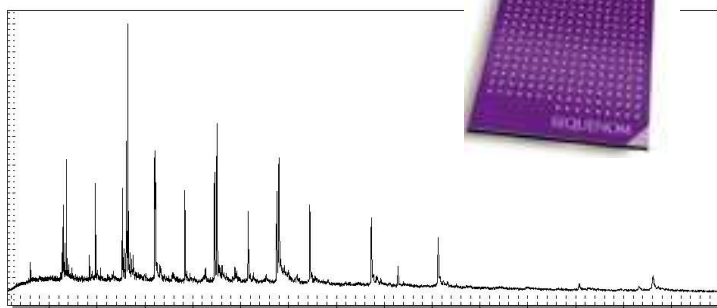
*In vitro* transcription

4 base-specific cleavage reactions

Conditioning

Dispensing

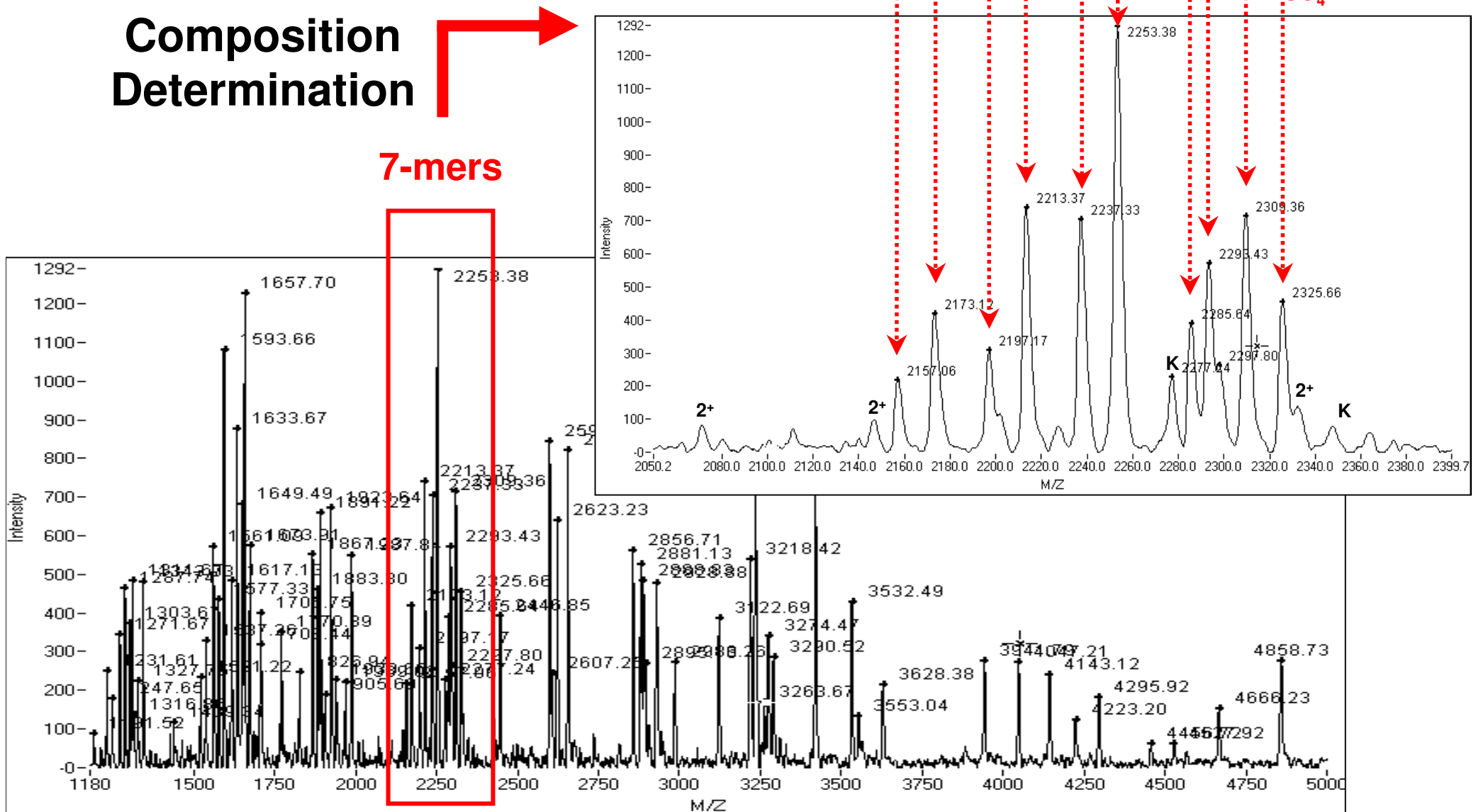
MALDI-TOF MS



# Peak Pattern and Identity

Composition Determination

7-mers

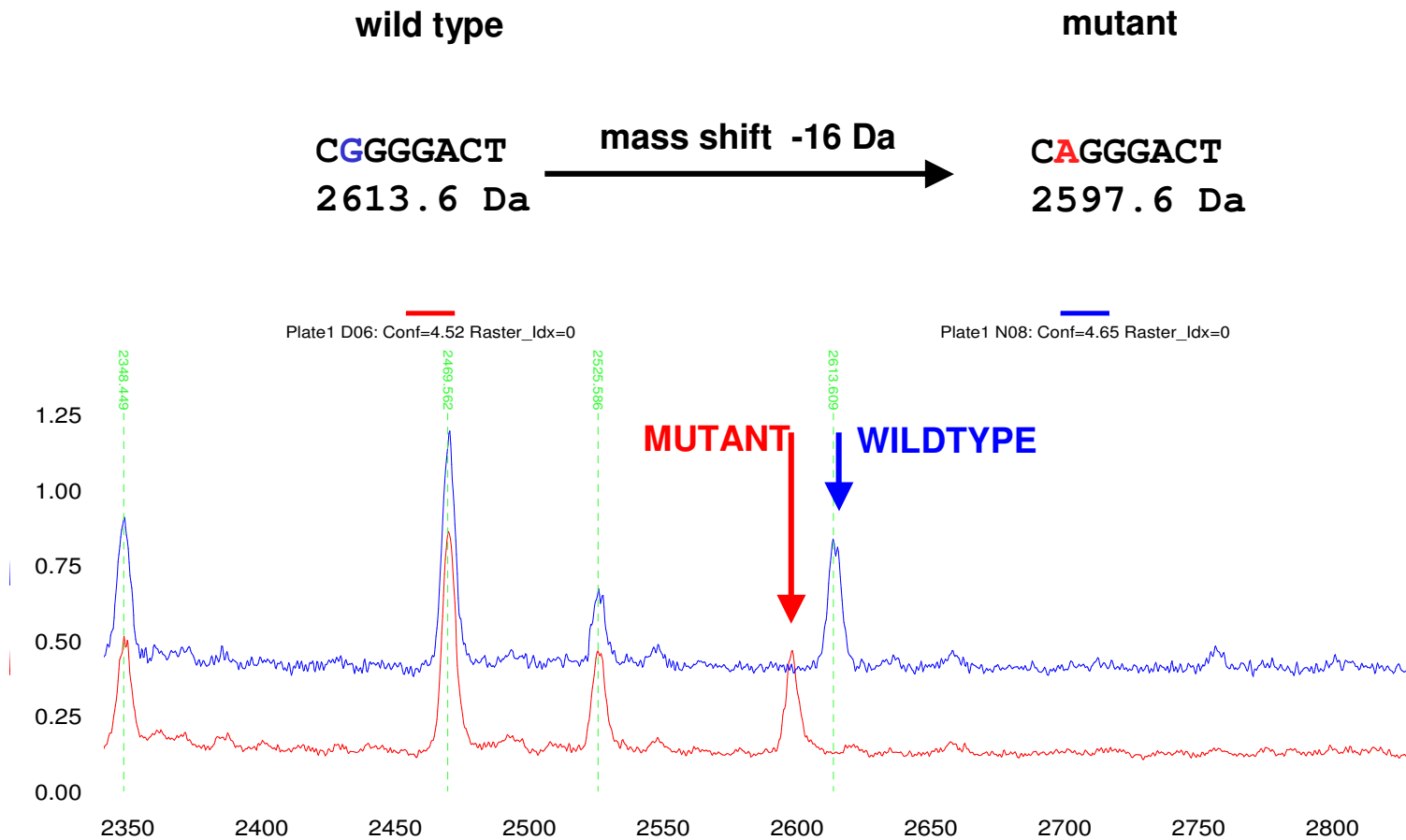


mass spectrum of a ~1000 bp amplicon after cleavage reaction

# Impact of Sequence Changes I



## T-specific cleavage, forward strand



# Impact of Sequence Changes II

5' ~CACAGCTACTTCTC [G/A] GGGACTGC ~3'  
3' ~GTGTCGATGAAGAG [C/T] CCCTGACG ~5'

## C-specific cleavage, forward strand

wild type

mutant

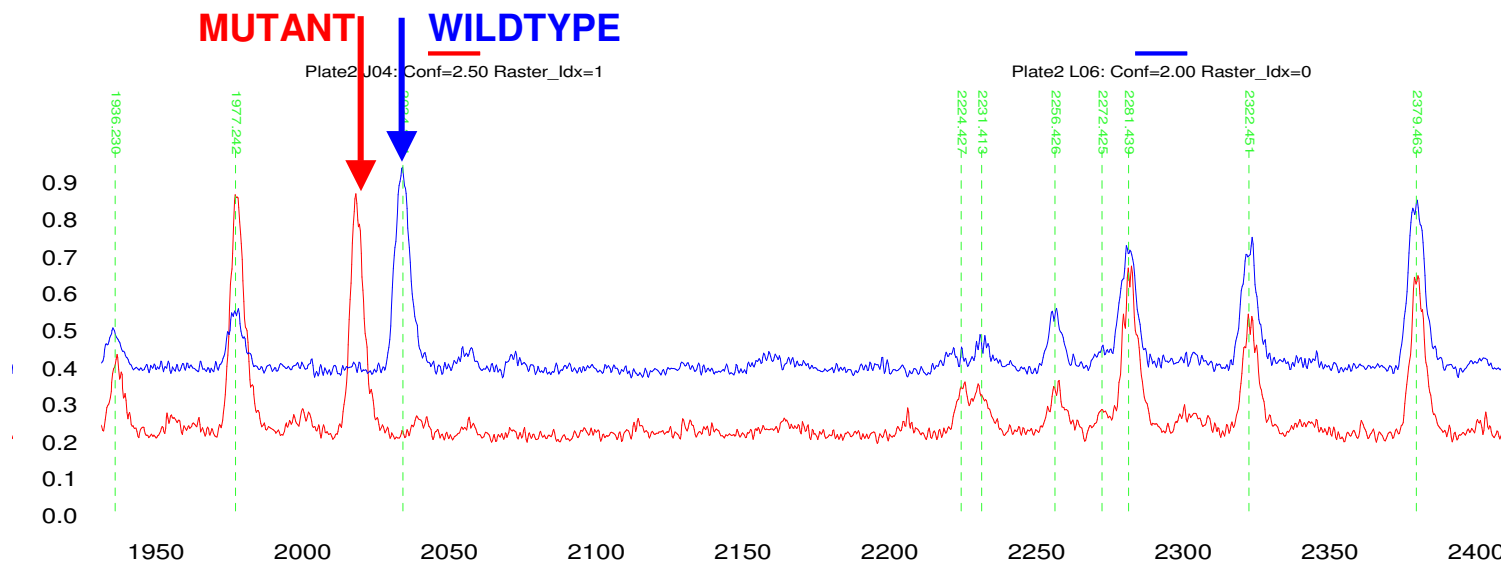
GGGGAC

mass shift -16 Da

GGGGAC

2034.3 Da

2018.3 Da

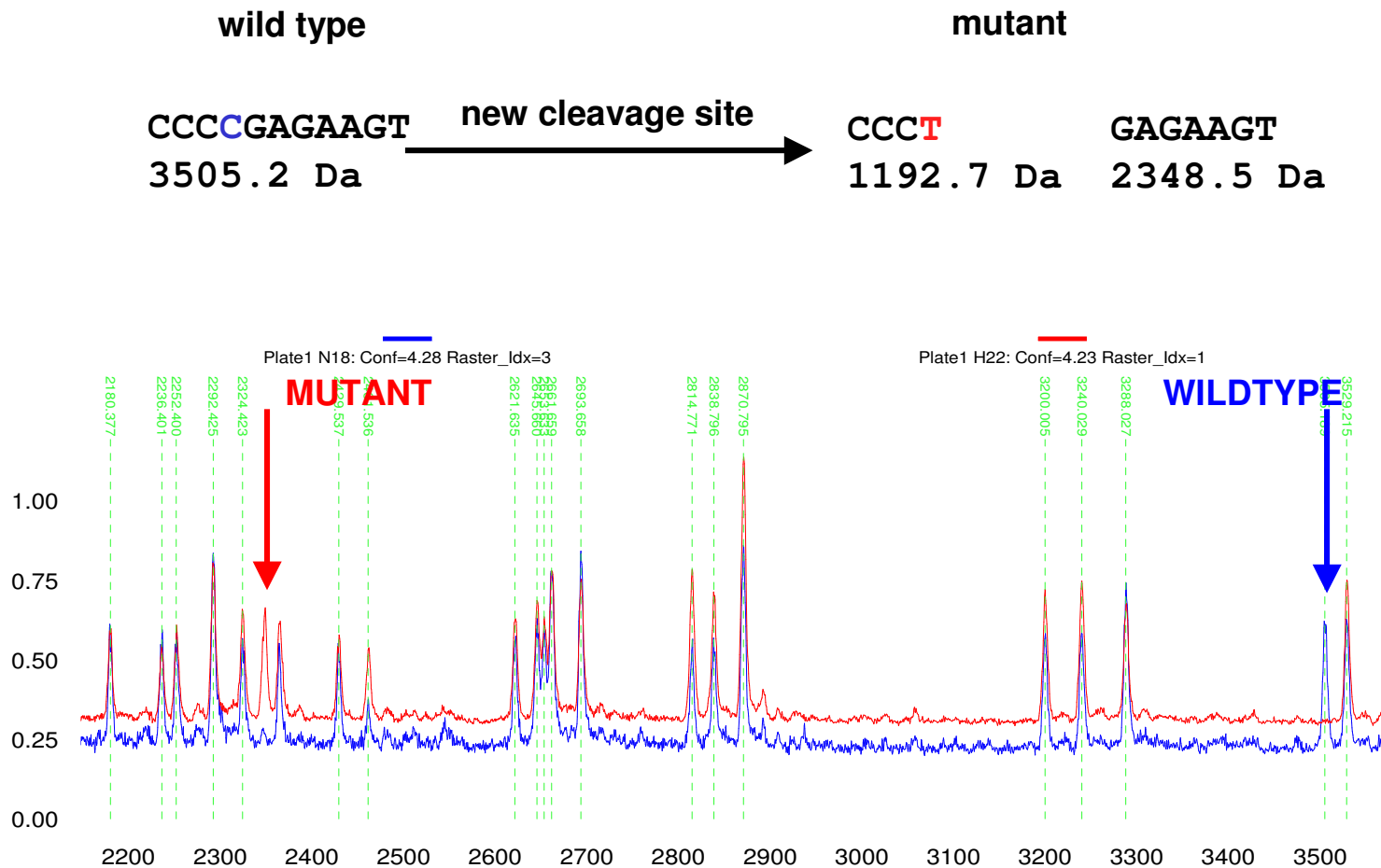


# Impact of Sequence Changes III

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5' ~CACAGCTACTTCTC [G/A] GGGACTGC~3'  
3' ~GTGTCGATGAAGAG [C/T] CCCTGACG~5'

## T-specific cleavage, reverse strand

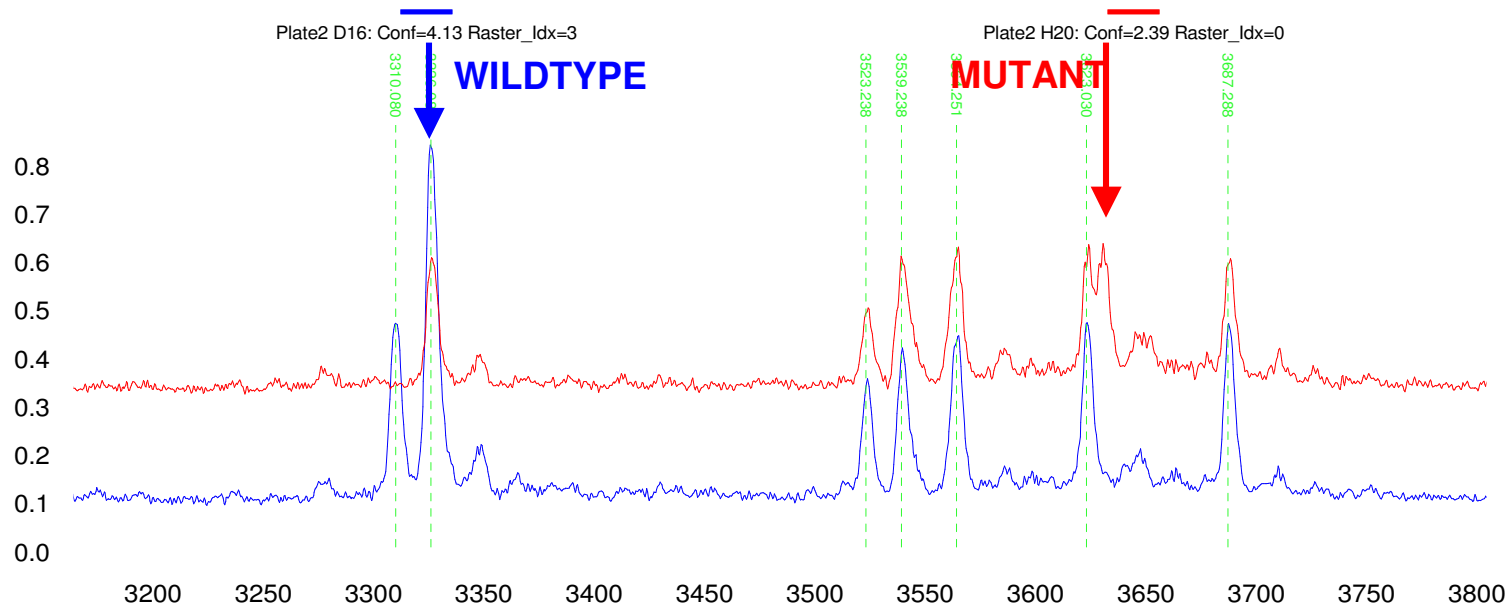




# Impact of Sequence Changes IV

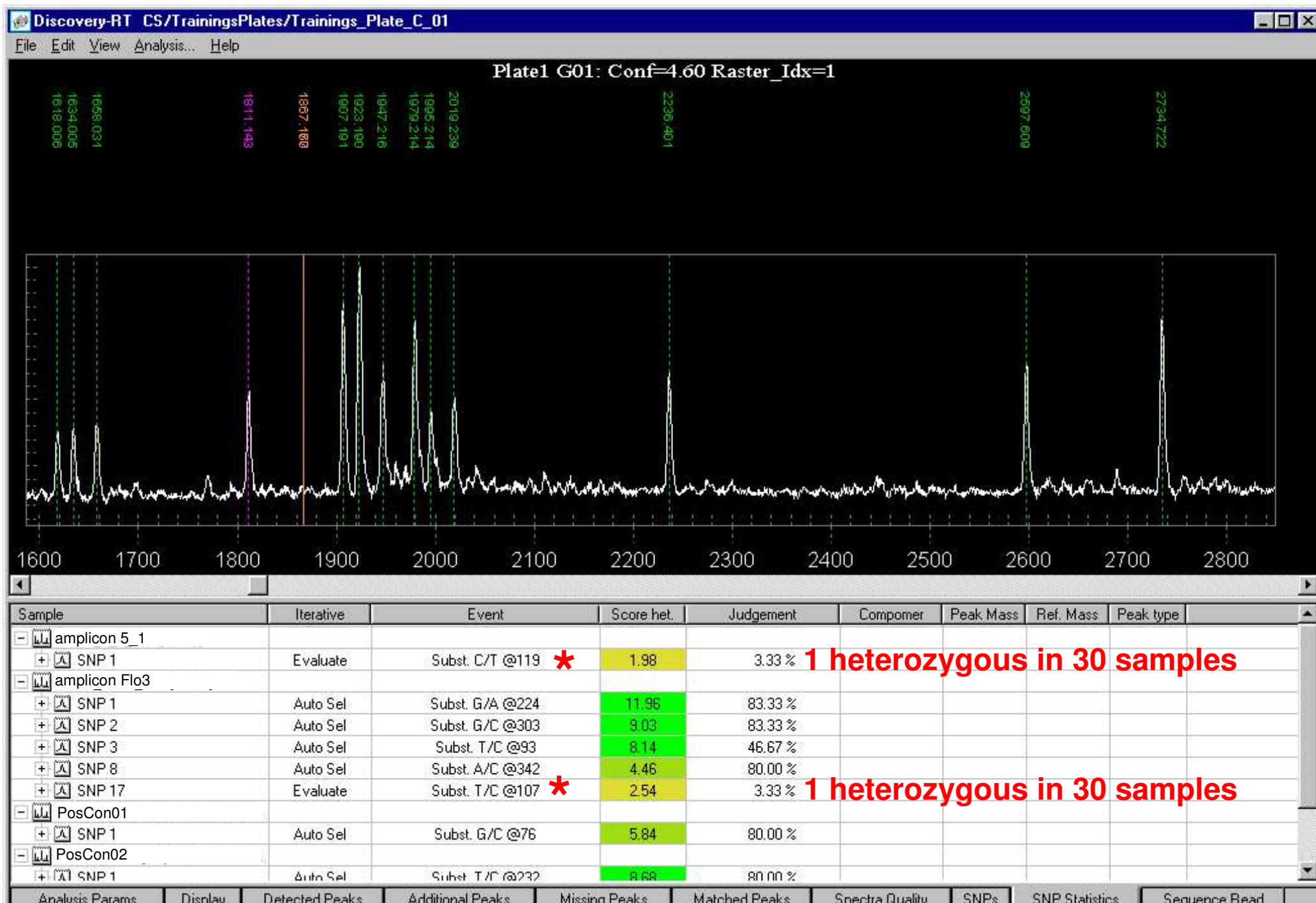


## C-specific cleavage, reverse strand



# SNP Discovery - Typical Results

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## Targeted SNP Discovery

vs.

## Diagnostic Mutation Detection

mainly single base events

multiple sequence changes in  
narrow regions  
detection of long insertions deletions

focused on target sequence  
'summary over analyzed sample set'

sample in the center of interest  
genotype per sample required

requires accurate identification of  
nature and position of variation

screening tool:

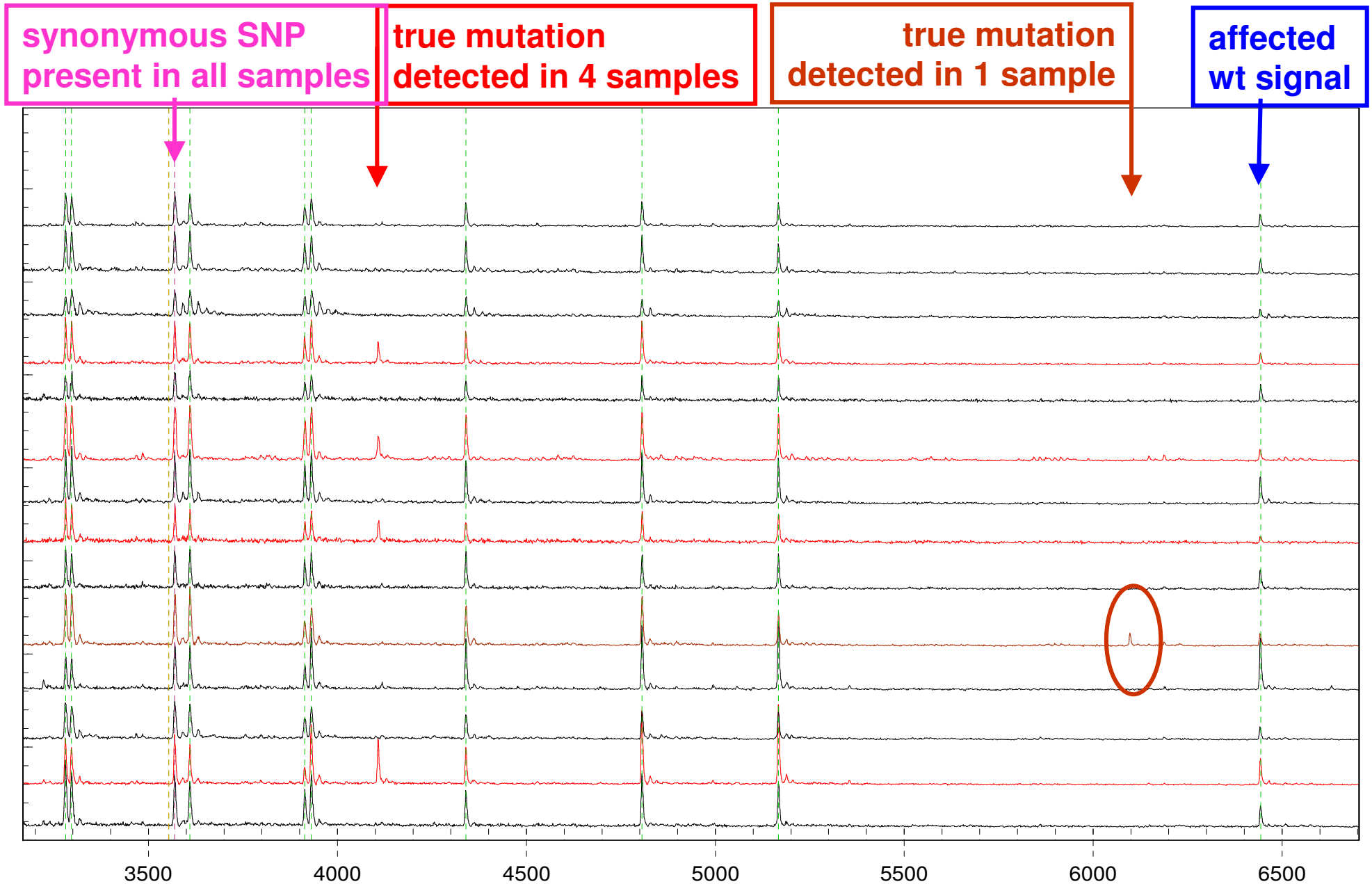
- discrimination of SNP-like pathogenic and non-pathogenic variants
- minimization of false negative and false positive results
- accurate position is beneficial, but not essential (confirmatorial identification)

# Mutation Detection

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detection of multiple variation affecting one fragment (T-forward reaction)

~ TCCAGCACAGAA [A/T] AAAAG [G/-] T ~ TAA [A/G] CAGAACTG ~ TCC [-/A] TA ~



# Methylation Analysis

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## Bisulphite Treatment

Cytosine



Uracil

Methylated-Cytosine



Cytosine

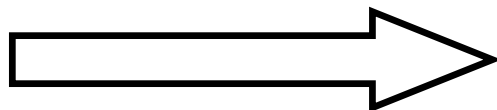
ATCT<sup>CH3</sup>GTACTGTC<sup>CH3</sup>GA  
TAGAGC<sup>CH3</sup>ATGACAGC<sup>CH3</sup>T

Bisulphite  
treatment

ATUT<sup>CH3</sup>GTAUTGTC<sup>CH3</sup>GA

loss of strand  
complementarity

TAGAGC<sup>CH3</sup>ATGAUAGC<sup>CH3</sup>T

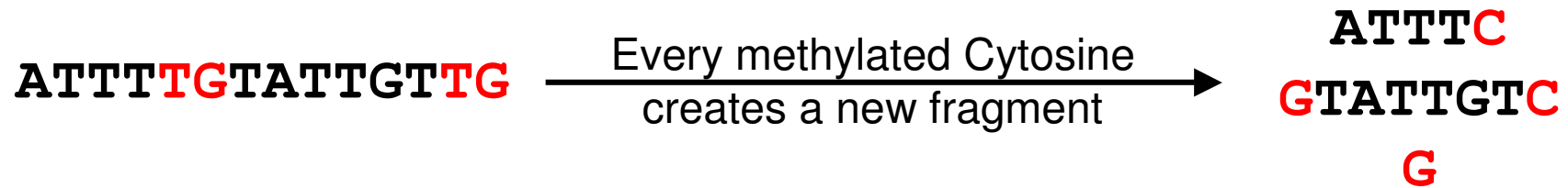


Single Strand Amplification

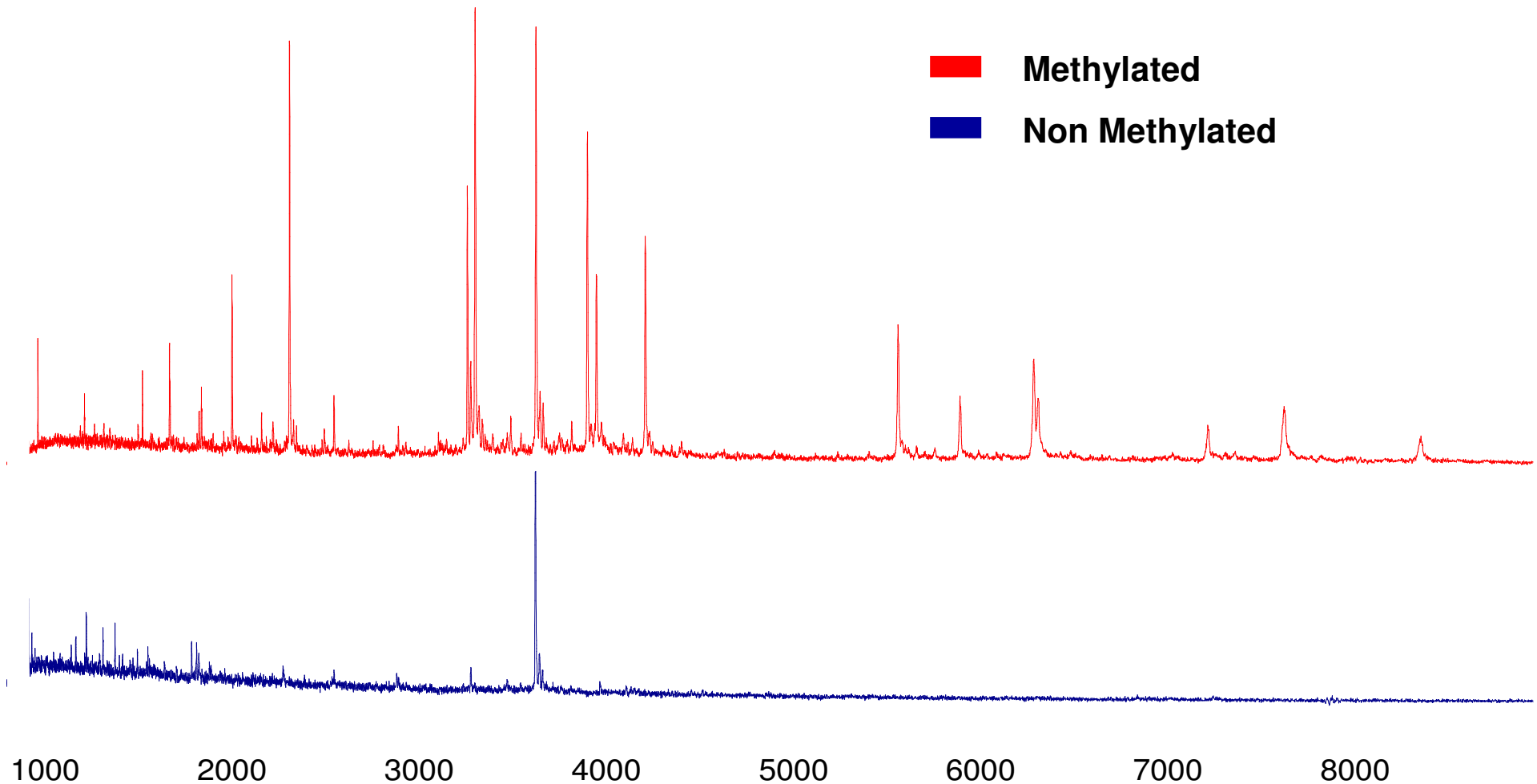
# Methylation Analysis

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C-specific forward reaction



**■ Methylated**  
**■ Non Methylated**

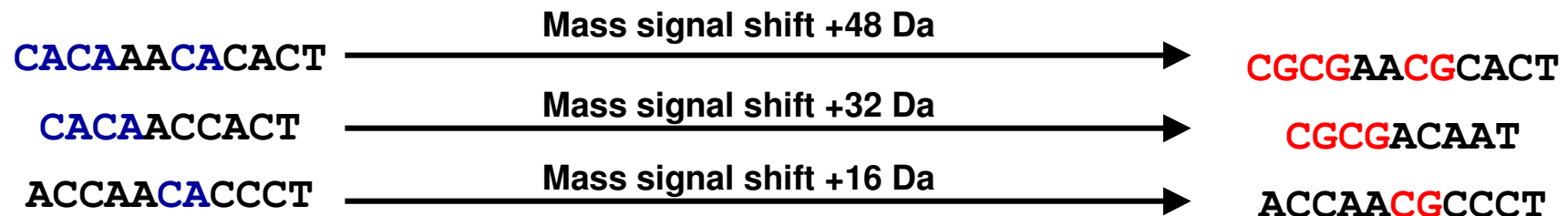
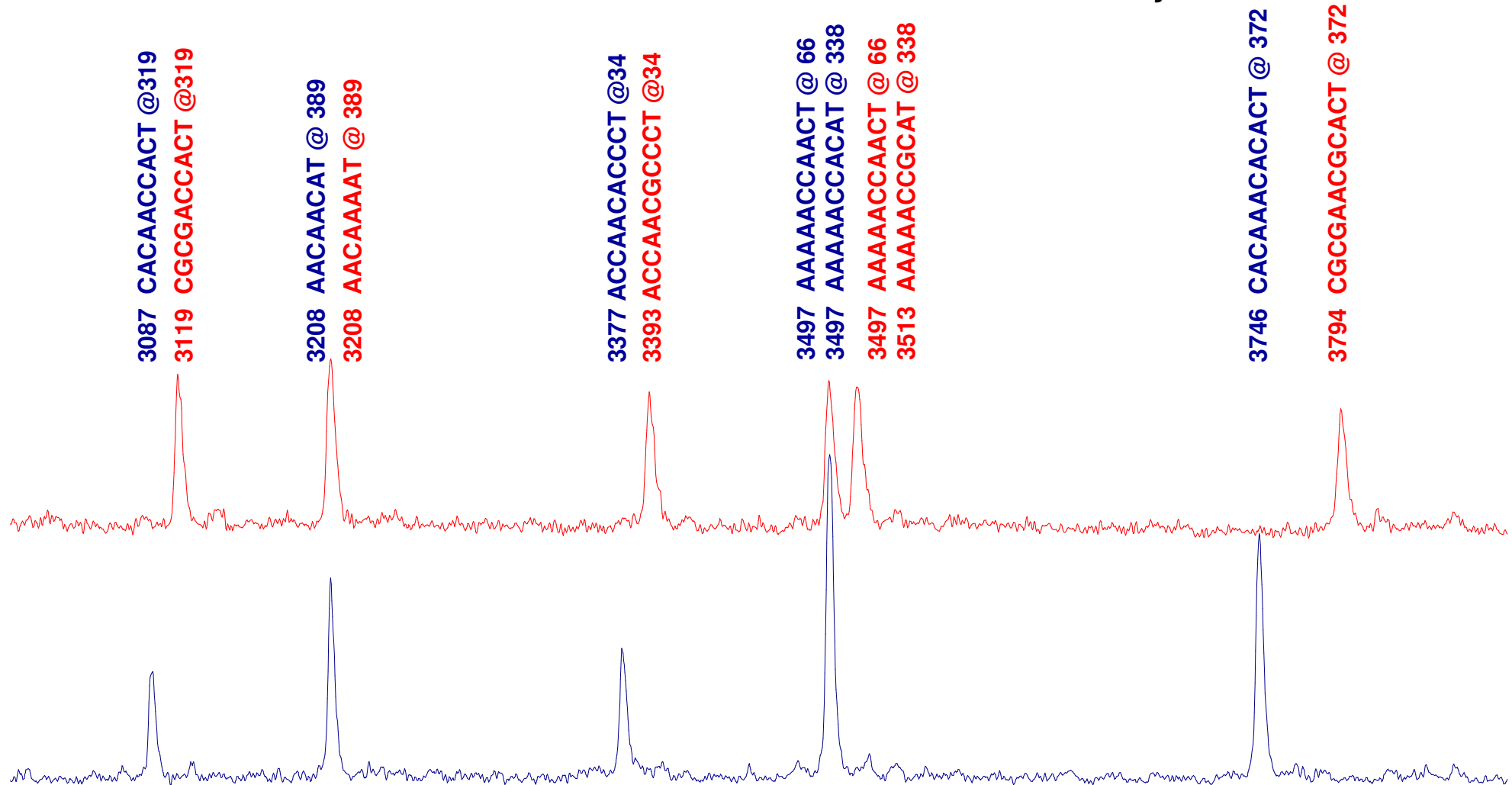


# Methylation Analysis

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T-specific cleavage reverse strand

■ methylated  
■ non-methylated





# Identification and Typing

e.g. MYCOBACTERIUM



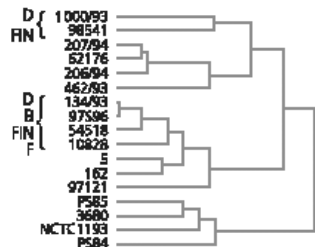
*M. tuberculosis* R+/R-

D  
N  
A

Marker specific  
PCR



in hypervariable  
region(s)



**Result:**

- Identification
- Confidence
- (SNP @pos.x)



## Identification and Typing Tool:

PCR-product

(T7-Polymerase Promotor Tag)

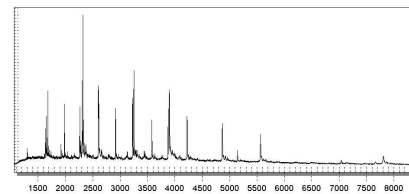
*in vitro* Transcription

RNA

base specific cleavage

Sequence specific  
cleavage pattern

M  
a  
s  
s  
C  
L  
E  
A  
V  
E



Match and compare peak  
pattern with database  
(SNP discovery)

S  
o  
f  
t  
w  
a  
r  
e

E.coli pos.	411	451
a_MLA2_Mtuberculosis	CTCGGGTT	GTAAACCTCT
a_MLB6_Mscrofulaceum	CTCGGGTT	GTAAACCTCT
a_MLC2_Mxenopi	CTCGGGTT	GTAAACCTCT
a_MLD1_Mmarinum	CTCGGGTT	GTAAACCTCT
a_MLE1_Mkansasii	CTCGGGTT	GTAAACCTCT
a_MLF2_Mintracell	CTCGGGTT	GTAAACCTCT
a_MLG4_Mavium	CTCGGGTT	GTAAACCTCT
a_MLH1_Mabscessus	CTCGGGTT	GTAAACCTCT
a_MLI1_Mfortuitum	CTCGGGTT	GTAAACCTCT
a_MLK5_Mcelatum	CTCGGGTT	GTAAACCTCT
a_MLL1_Msmegmatis	CTCGGGTT	GTAAACCTCT

## MassARRAY™ – A High Performance DNA Analysis Tool Box

Genotyping	Quantitative Gene Analysis	Re-Sequencing
<ul style="list-style-type: none"><li>• Individual Genotyping</li><li>• Multiplex Genotyping</li><li>• Oligo QC</li></ul>	<ul style="list-style-type: none"><li>• Allele Frequency (Allelotyping)</li><li>• Gene Expression Analysis</li><li>• Viral Load Determination*</li><li>• Vaccine QC*</li><li>• Loss of Heterozygosity*</li><li>• Gene Copy Number*</li></ul>	<ul style="list-style-type: none"><li>• SNP Discovery</li><li>• Pathogen Identification*</li><li>• Pathogen Typing*</li><li>• Methylation analysis*</li><li>• Mutation Detection*</li></ul>

\*R&D Projects

**MassARRAY™ includes virtually any high performance DNA analysis application on one platform**

## **SEQUENOM®**

- Dirk van den Boom
- Sebastian Böcker  
(current at University of Bielefeld)
- Mathias Ehrich
- Christiane Honisch

## **Methexis Genomics, Ghent Belgium**

- Marc Zabeau
- Patrick Stanssens

## **National Genetics Reference Laboratory, Wessex UK** study on BRCA 1, hMLH 1, MSH 2

- Chris Mattocks
- Helen White