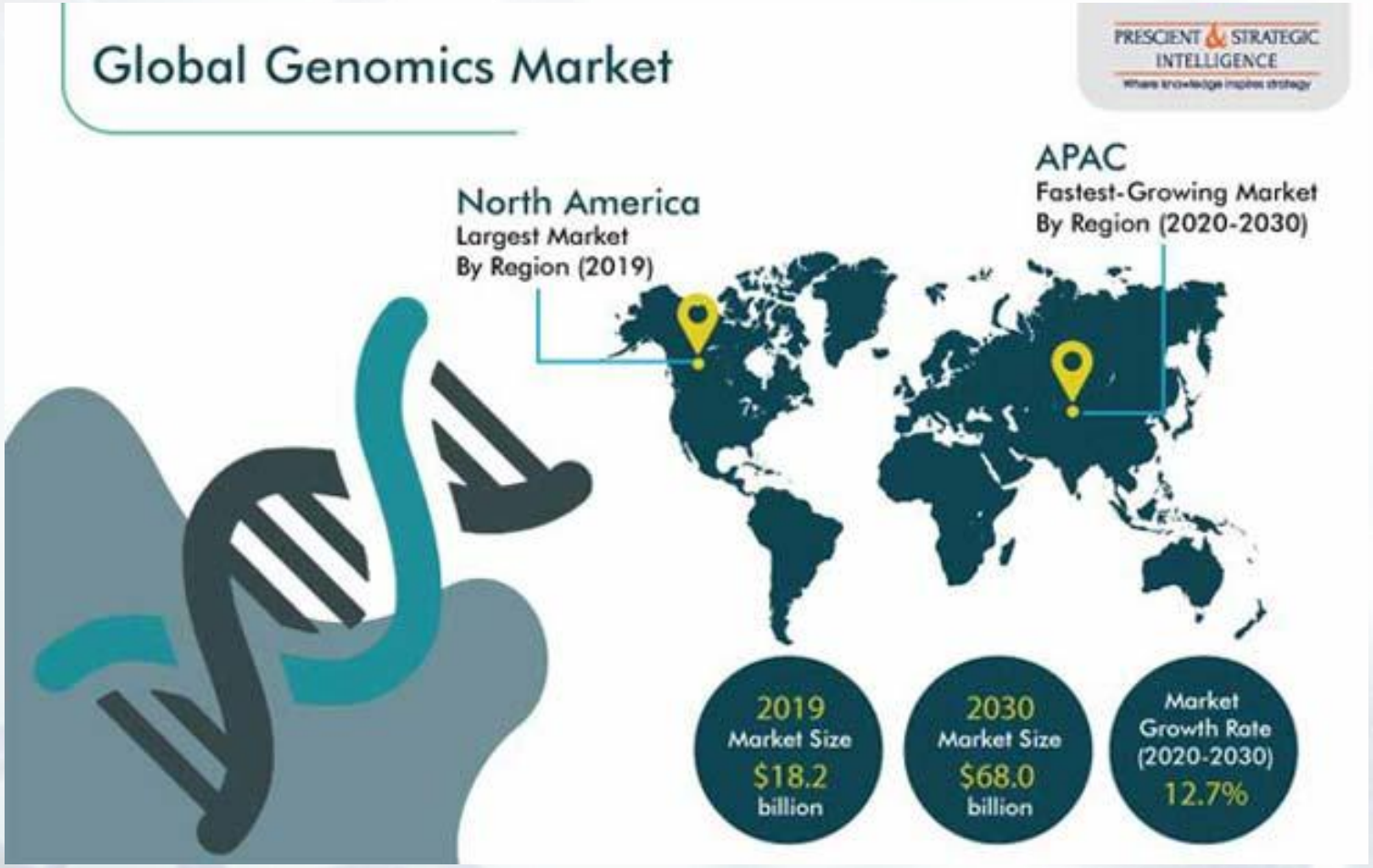
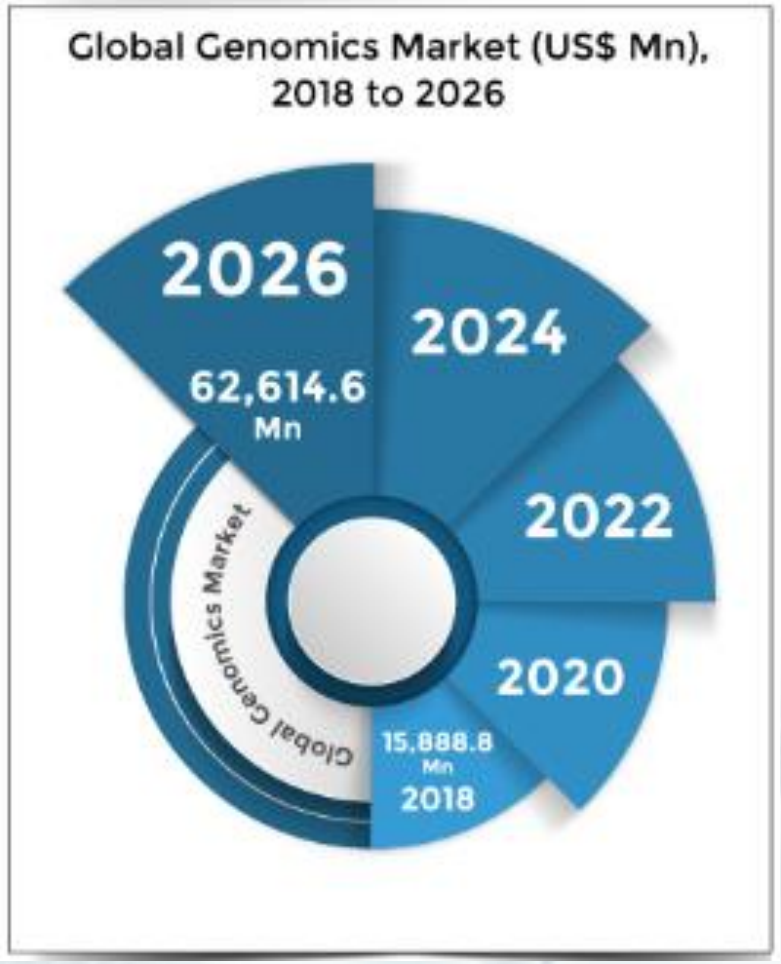
Introduction of Agena Bioscience's

MassARRAY Overview

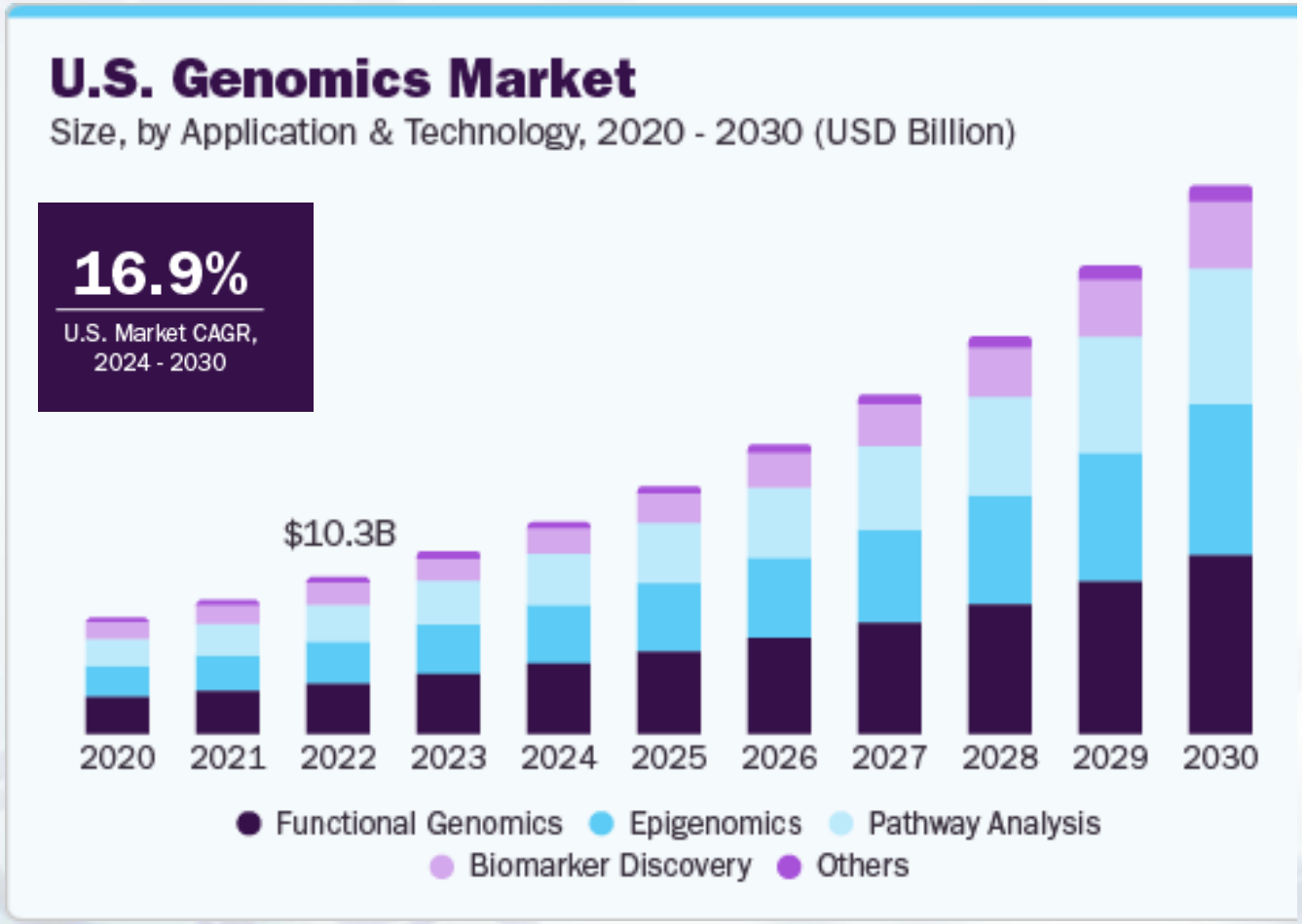
20 March, 2025

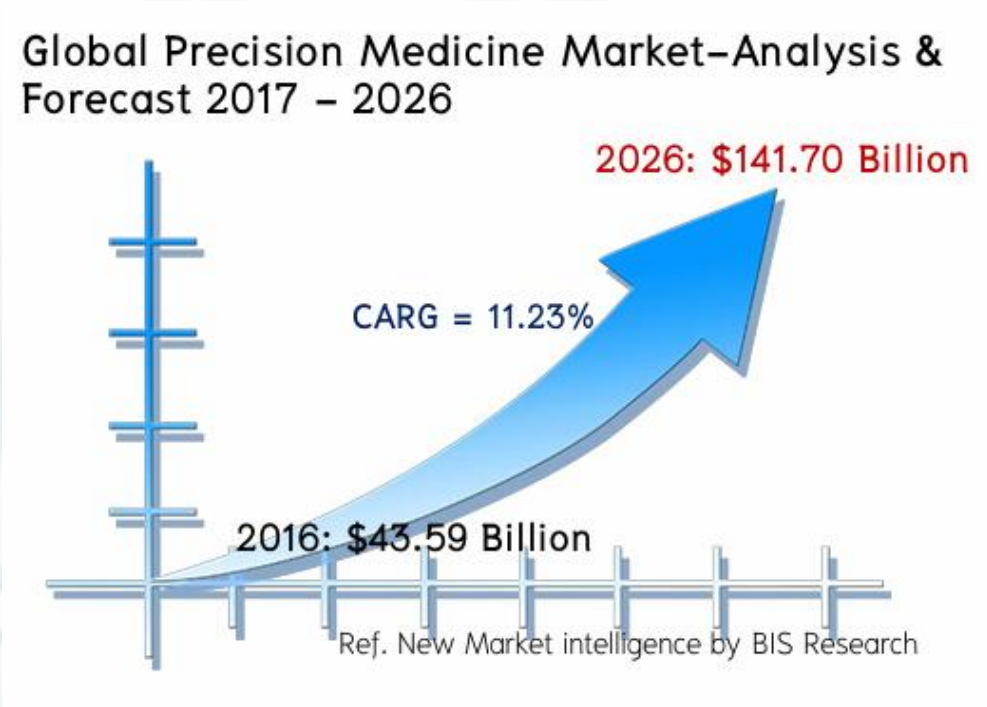
Genomic Market





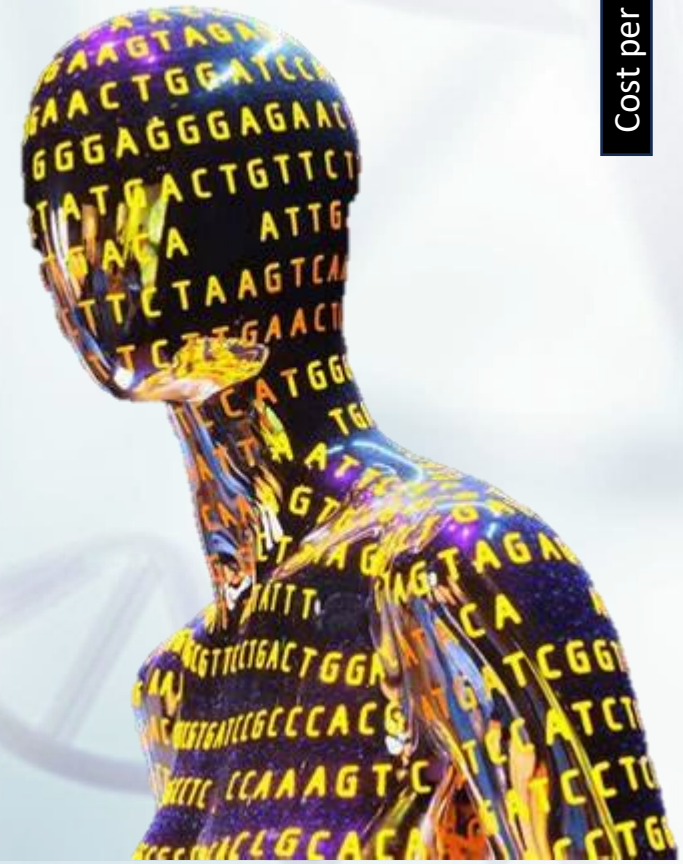
Genomic Market

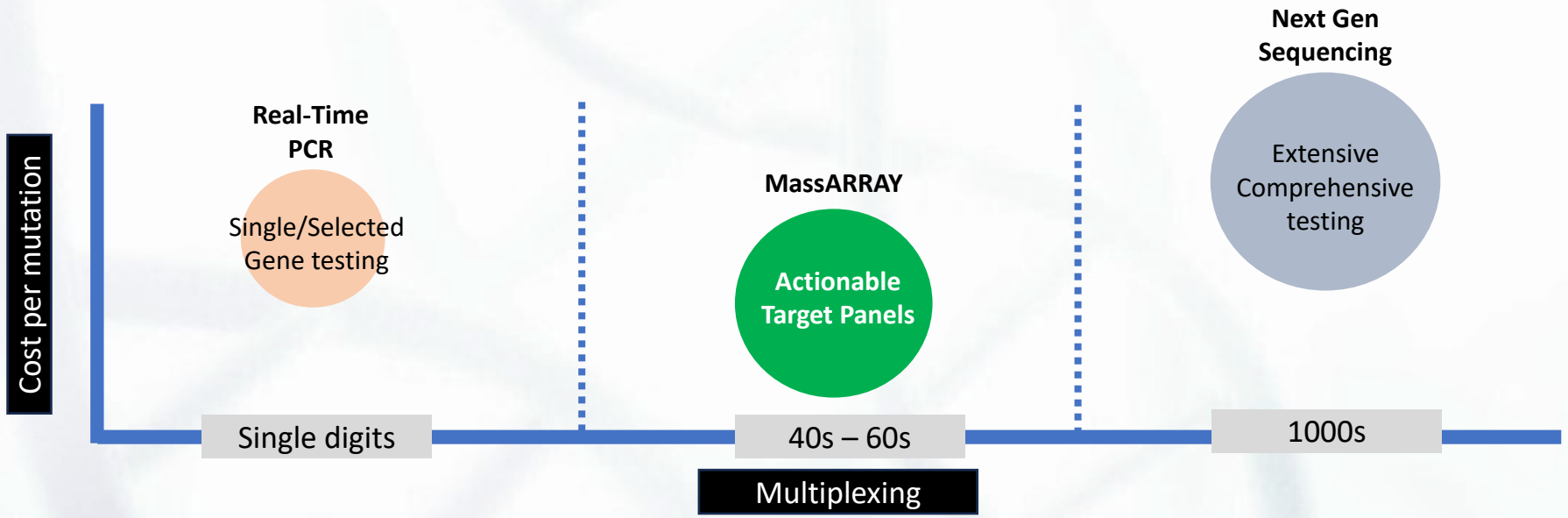




Market Segmentation

Robust, Flexible, and High Throughput





• De-Novo • Single SNPs	• De-Novo • Multiplexing • Local validation • Local Primer Production • LDT develops test – Patent • Cost efficiency due to multiplexing SNPs per well	• Novo • Comprehensive testing • Import from Principle • BioIT requirements

Kockum, I., Huang, J., & Stridh, P. (2023). Overview of Genotyping Technologies and Methods. *Current Protocols*, *3*(4), e727.

CURRENT PROTOCOLS
A Wiley Brand

Overview of Genotyping Technologies and Methods

Ingrid Kockum,[1] Jesse Huang,[1] and Pernilla Stridh[1,2]

[1]Center for Molecular Medicine, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden
[2]Corresponding author: *pernilla.strid@ki.se*

Published in the Essential Lab Techniques section

Genetics is a cornerstone of molecular biology, and there have been significant developments in genotyping technologies during the last decades. Genotyping can be used for a wide range of applications, such as genealogy, assessing risks and causes for common diseases and health conditions, animal and human research, and forensic investigations. So how do you perform a genetic study? This overview covers key concepts in genetics, the development of genotyping methods, and a comparison of several techniques, including PCR, microarrays, and sequencing. A general process of the steps involved in genotyping, from DNA preparation to quality control, is described with relevant protocols referenced. Different types of DNA variants are illustrated, including mutations, SNP, insertions, deletions, microsatellites, and copy number variations, with examples of their involvement in disease. We discuss the utilities of genotyping, such as medical genetics, genome-wide association studies (GWAS), and forensic science. We also provide tips for quality control, analysis, and results interpretation to help the reader design and perform a genetic study or scrutinize such studies from the literature.

Keywords: genetics • genotyping • GWAS • methodology • microarray • NGS • PCR

How to cite this article:
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INTRODUCTION

Genetics is a keystone of molecular biology vide a foundation for academics (undergraduates/graduates) and industry professionals.

Table 2 Overview of Genotyping Technologies

Name	Cost	# markers	Pro	Con	Ref.
PCR-RFLP	+	1	Easy to run in any lab, fast, flexible	Time consuming, manual inspection	(Saiki et al., 1985)
Allele-specific PCR	+	1	Easy to run in any lab, fast, flexible	Time consuming, manual inspection	(Gaudet et al., 2009)
TaqMan PCR	+	1	Standardized, more accurate	Manual inspection, requires specific equipment	(Hui et al., 2008)
Microsatellite	+	1	Robust, do not require specific equipment, flexible	Low resolution, manual inspection, time consuming	(Weber & May, 1989)
Pyrosequencing	++	1	Captures all potential alleles	Time consuming, manual inspection, requires specific equipment	(Kreutz et al., 2013)
MassARRAY system					
iPLEX	++	60 ++	Multiplex assay	Manual inspection, requires specific equipment	(Gabriel et al., 2009; Tang et al., 1999)
Multiplex TaqMan	++	up to 100	Multiplex assay	Requires specific equipment	(Martínez-Cruz et al., 2011)
Genotyping arrays	+++	50k-2 mil	Multiplex assay	Requires specific equipment and expertise	(Verlouw et al., 2021)
NGS-Exome	++++	25000	captures all coding variants	Requires specific equipment and expertise, demanding processing	(Seaby et al., 2016)
NGS-Whole Genome	+++++	up to 40 mil	captures all variants	Requires specific equipment and expertise, demanding processing	(Slatko et al., 2018)

Rough relative cost per genotype is indicated by +, since prices vary over time.

Agena Bioscience®

- Headquarters in San Diego, California, USA
- Agena markets our products in over 30 countries worldwide through direct sales offices in Germany, China and Australia,

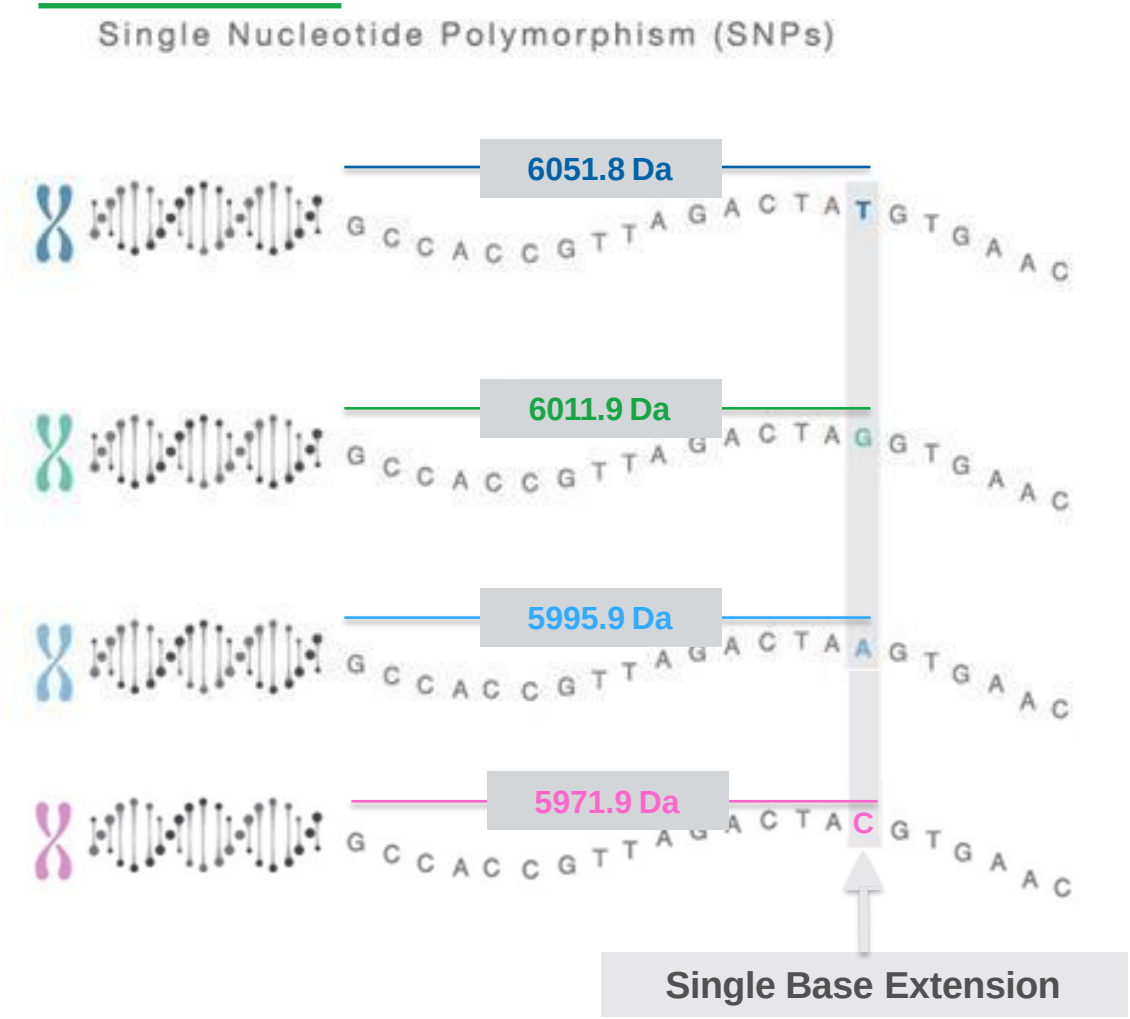
SEQUENOM®

(BEFORE 2014)

Agena BIOSCIENCE

MassARRAY system

Mass-Detection With The MassARRAY



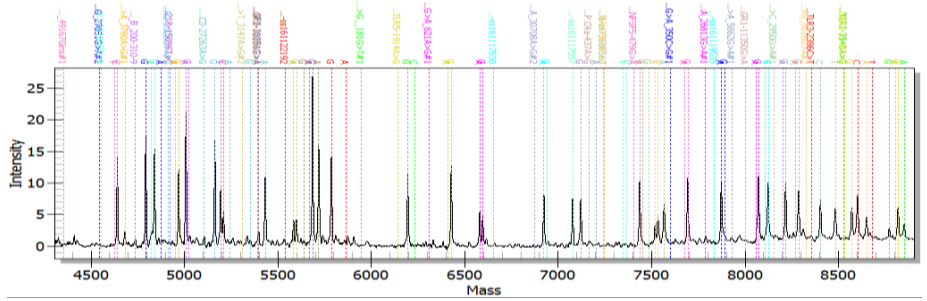
The MassARRAY is unique technology to detect DNA mutation SNP , Insertion, Deletion

Dalton

Unit of Mass

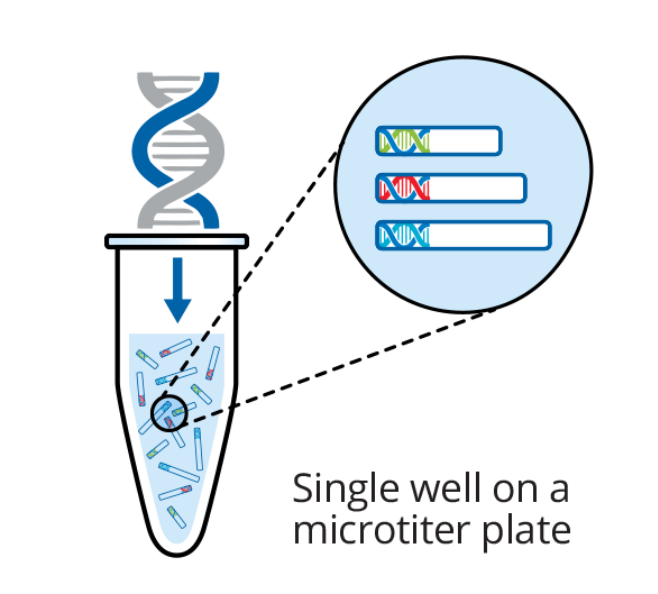
The Dalton (Da) is a unit of mass widely used in physics and chemistry.

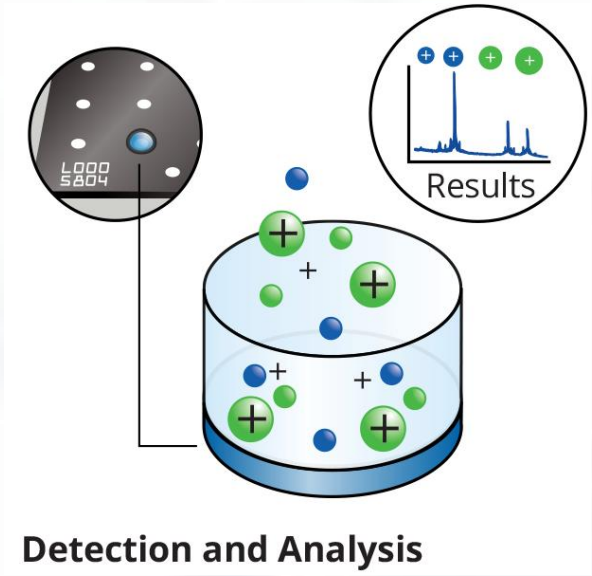
Cytosine (C)	Adenine (A)	Guanine (G)	Thymine (T)
247.2 Da	271.2 Da	287.2 Da	327.1 Da



MassARRAY Detection Range
4000 – 9000 Daltons

MassARRAY Technology





Multiplex PCR

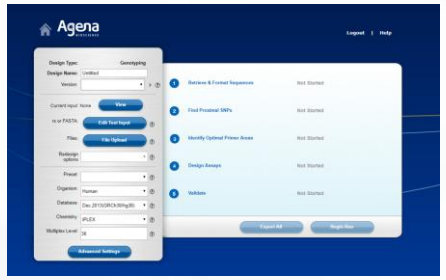
MALDI-TOF Mass Spectrometry

MassARRAY

Next Generation Sequencing

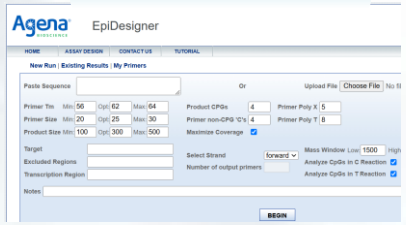
Genetics

AGENA Cx

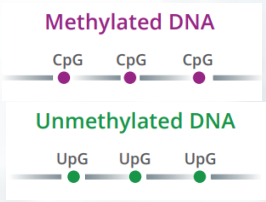


Epigenetics

EpiDesigner

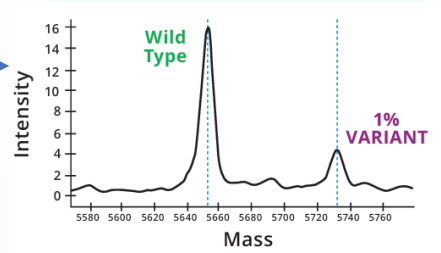


Bisulfile

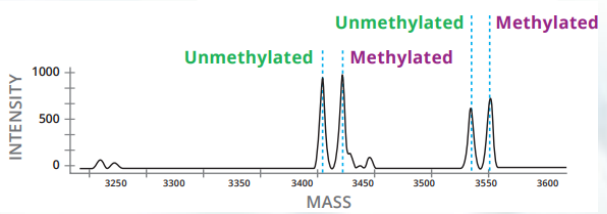


Detection

Analysis by TYPER

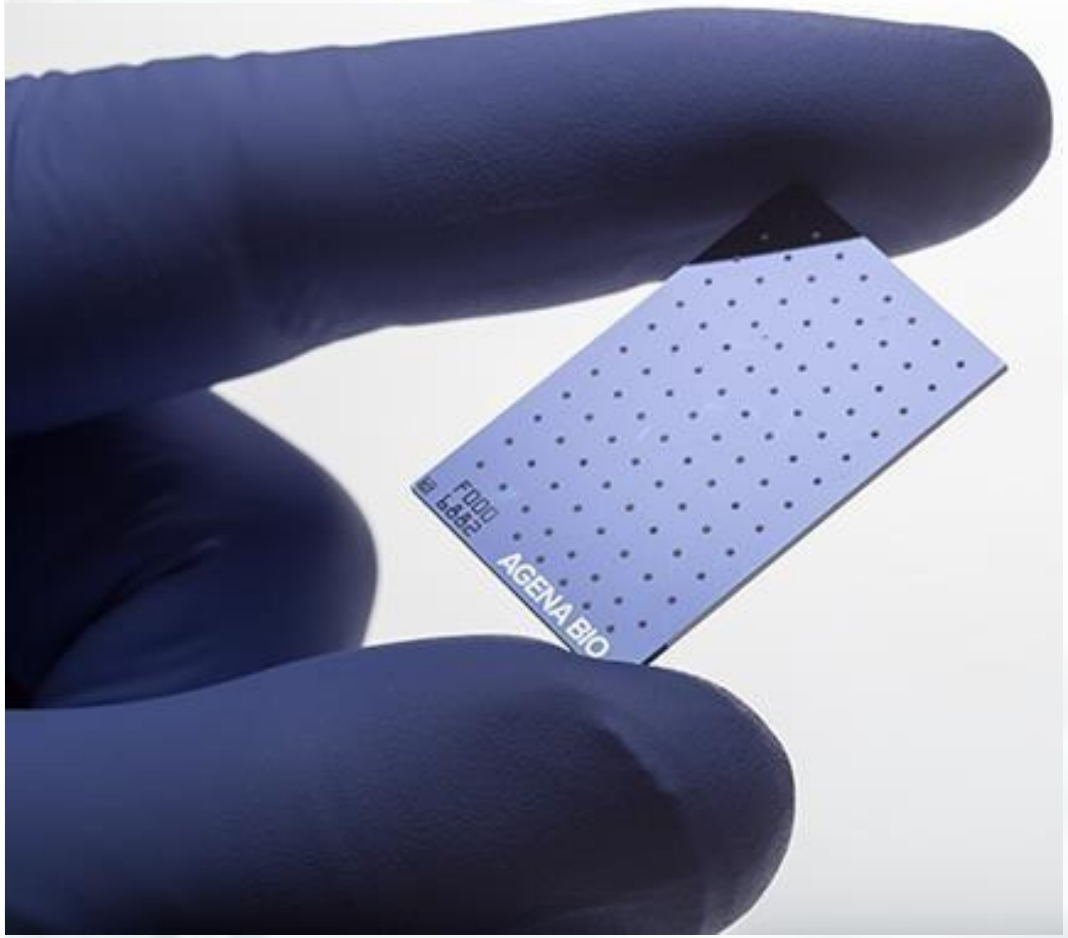


EpiTYPER



SpectroCHIP Arrays

A Powerful, Open Source Array for Custom Multiplex Assay Designs



Composed of 96 or 384 inert pads evenly spaced across a matrix.

- Each pad binds to any DNA analyte mixture
- Enables side-by-side testing of different assays
- Utilizes nanoliters of sample

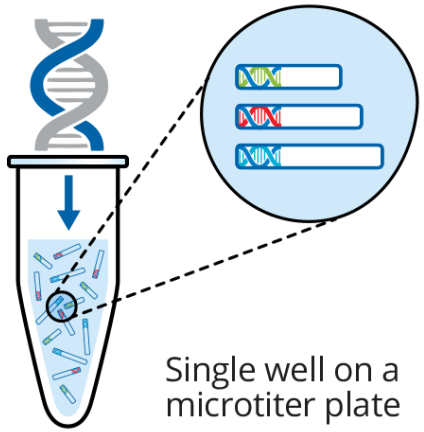
Genotype up to 50* (usually around 30) genetic variants on each SpectroCHIP Array pad

Flexible biomarker detection.

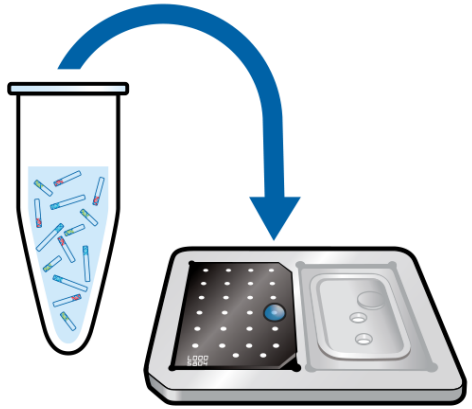
SNPs Insertions Deletions Translocations CNV

***actual number may vary depending on chemistry and application**

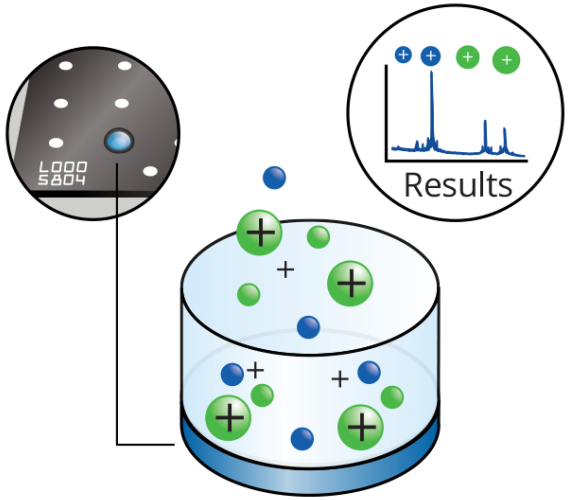
SAMPLE PROCESS JOURNEY



Endpoint PCR
Amplify and extend up to 40 target-specific DNA fragments in a single reaction.



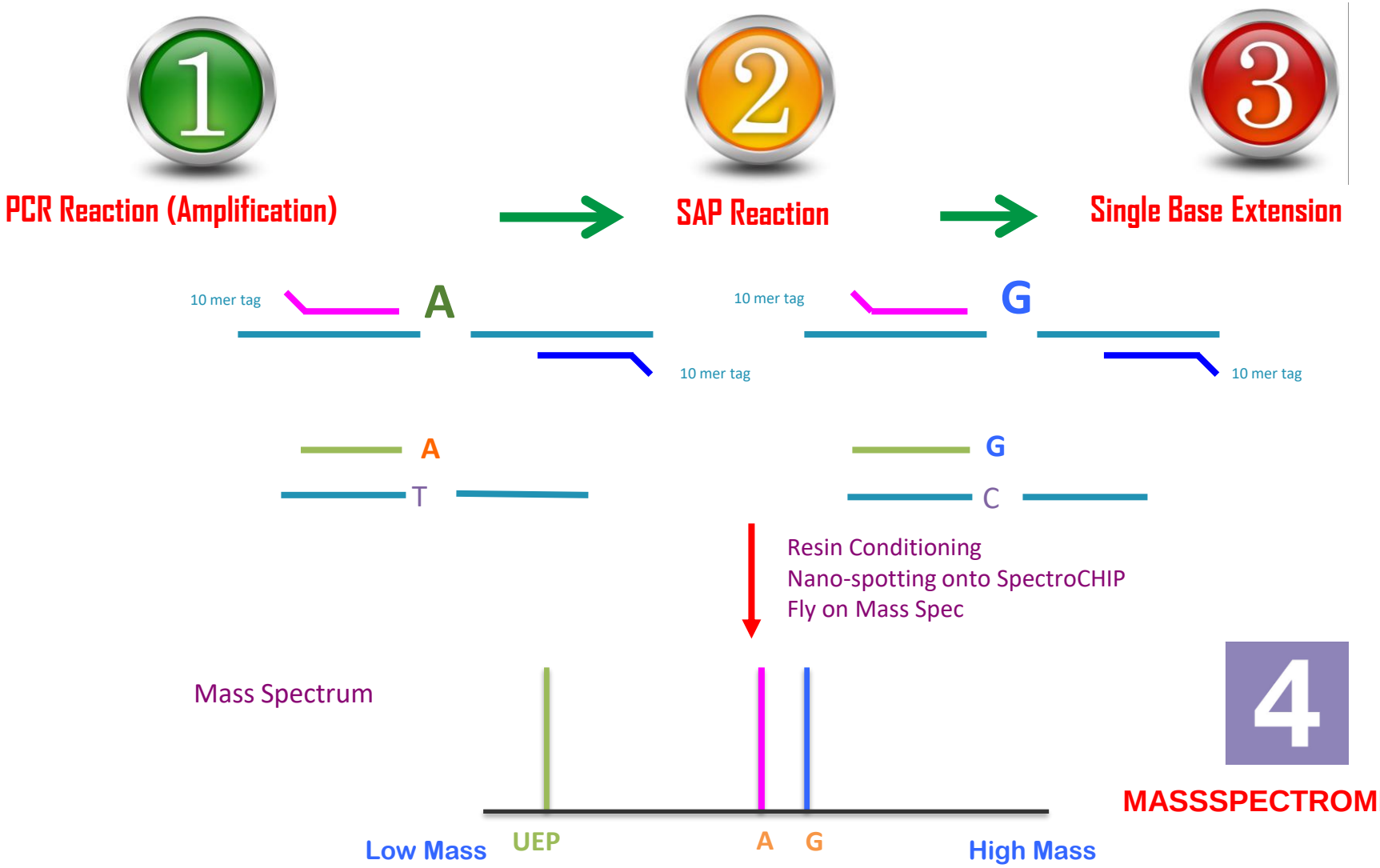
Transfer Analyte
Transfer a small amount of sample to a single pad on the SpectroCHIP® Array.



Detection and Analysis
Multiple tests can be run on a single SpectroCHIP Array. Hundreds of mutations can be tested per sample.

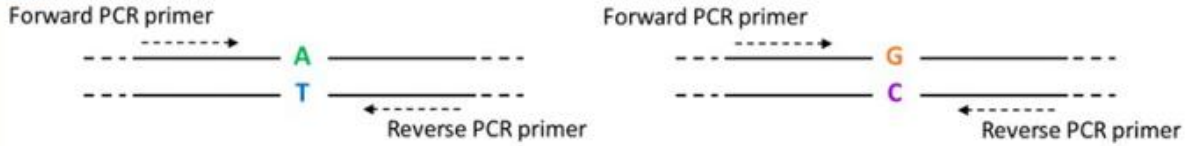
* Use multiple reactions for >40 targets if required.

GENOTYPING REACTION : SNP (A/G)

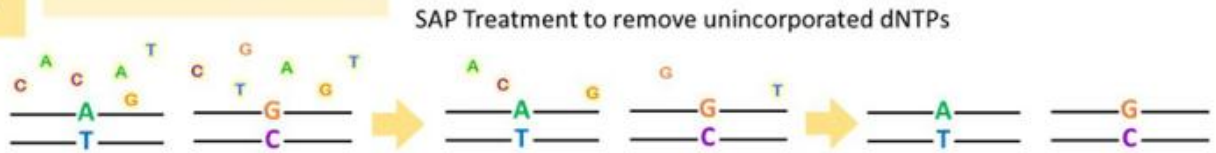


PCR : Polymerase Chain Reaction SAP : Shrimp Alkaline Phosphatase UEP : Unextension Primer

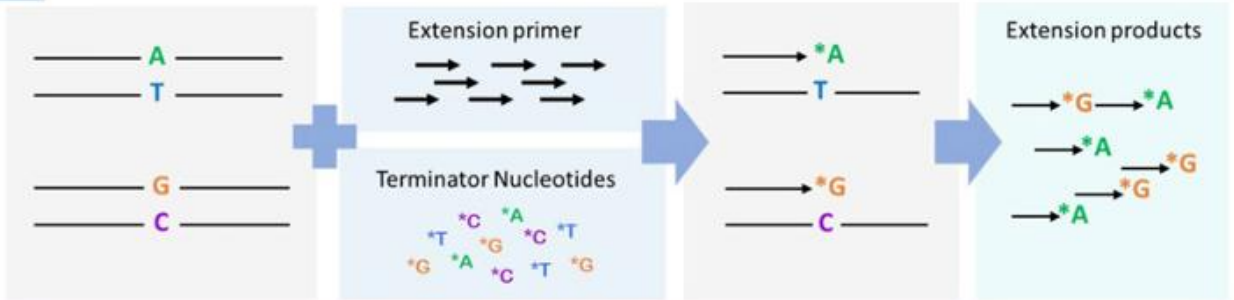
1 PCR Reaction



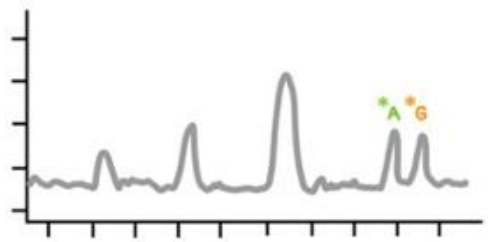
2 SAP Reaction



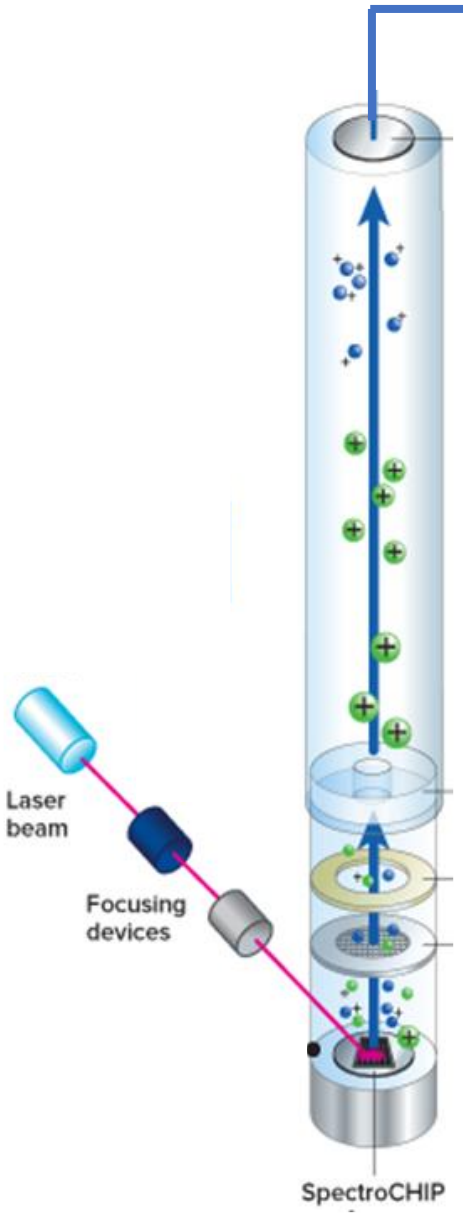
3 Single Base Extension



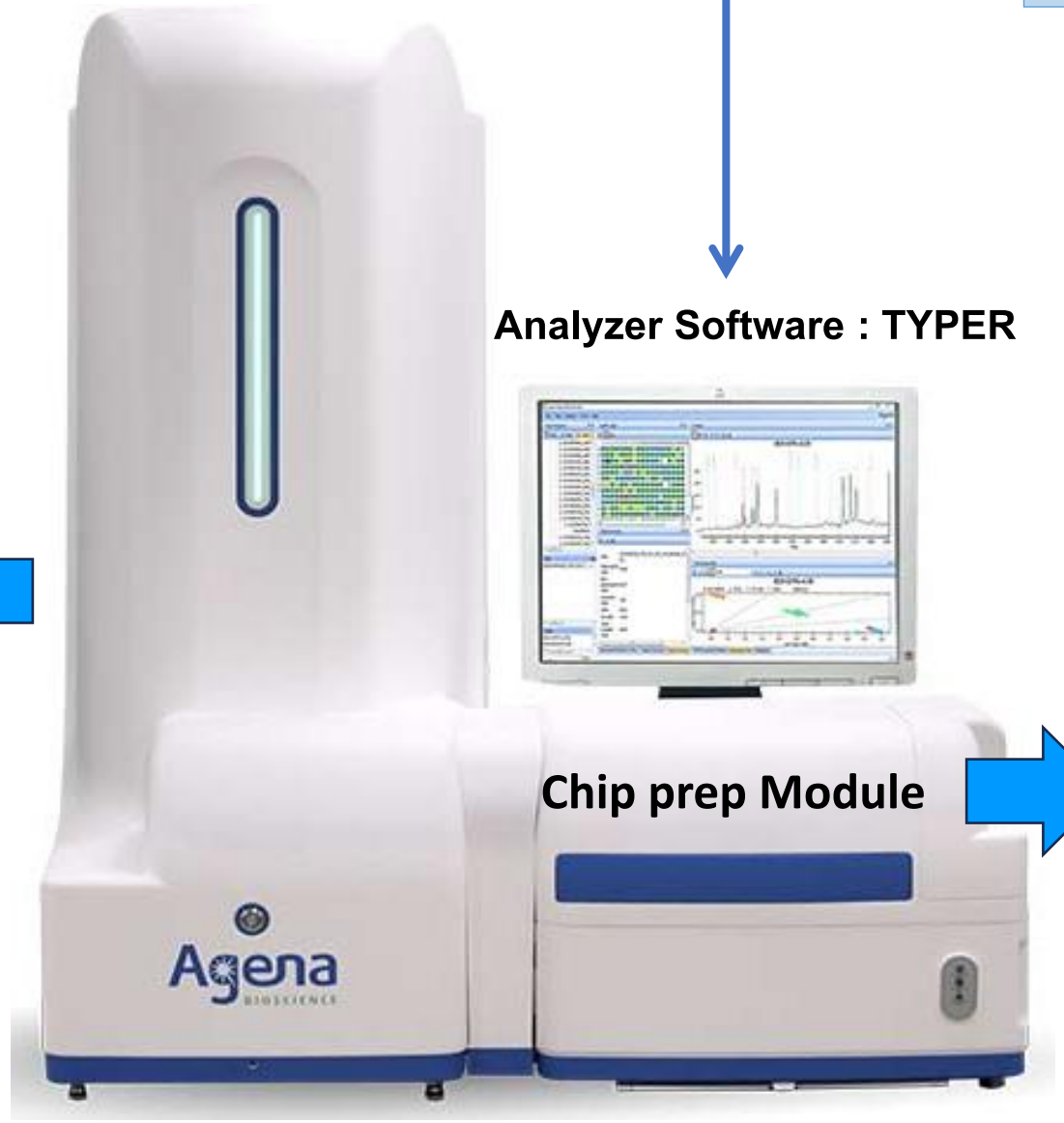
4 MASS SPECTROMETRY



MassARRAY platform

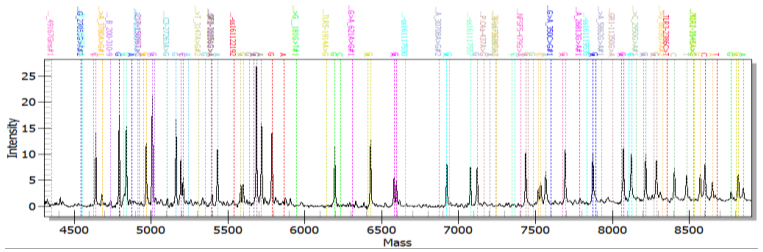


Mass Analyzer

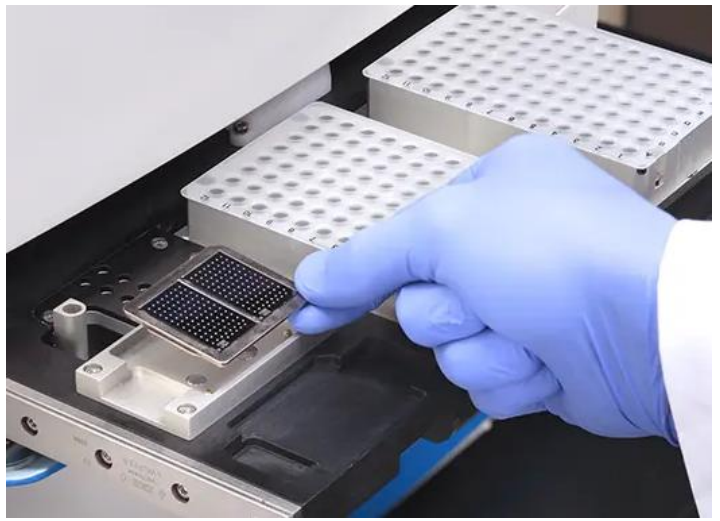


Analyzer Software : TYPER

Chip prep Module

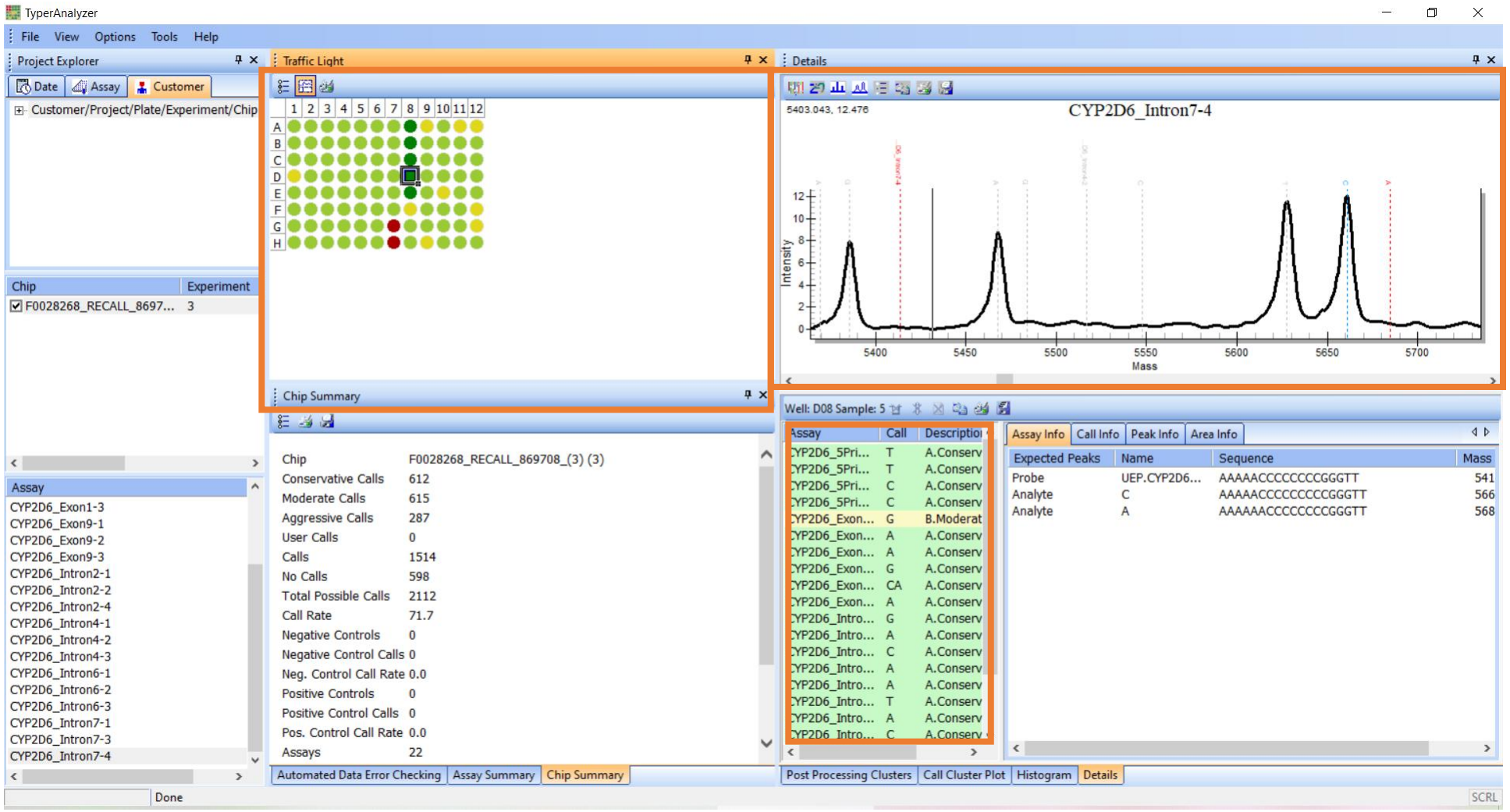


Flatform: 96 wells x 2 plates

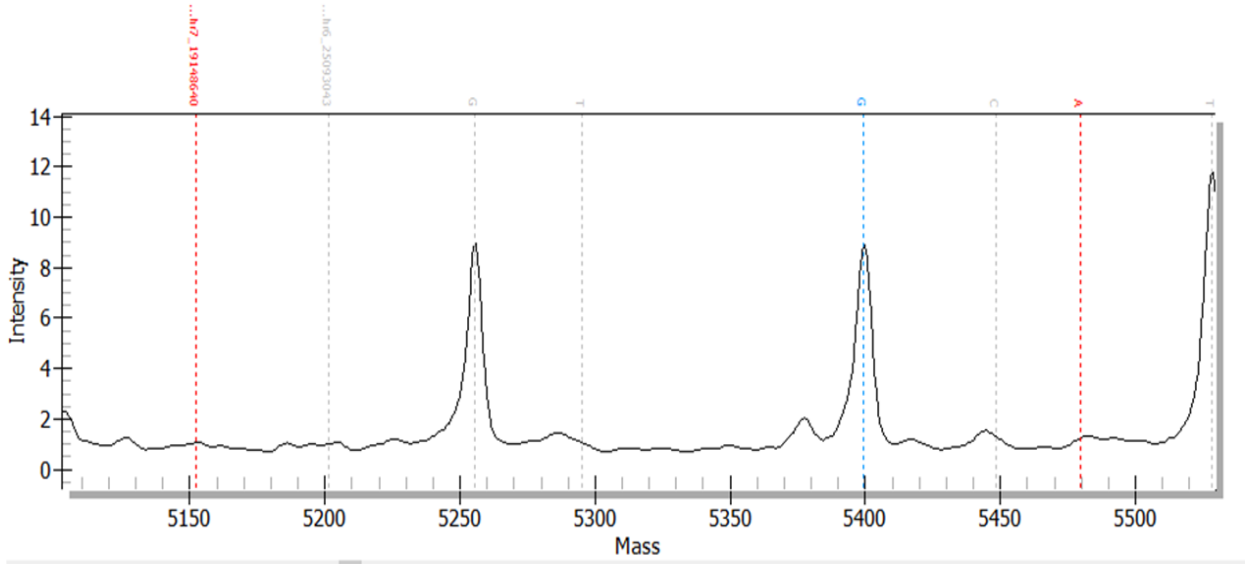


PATENTED “SPECTROCHIP”

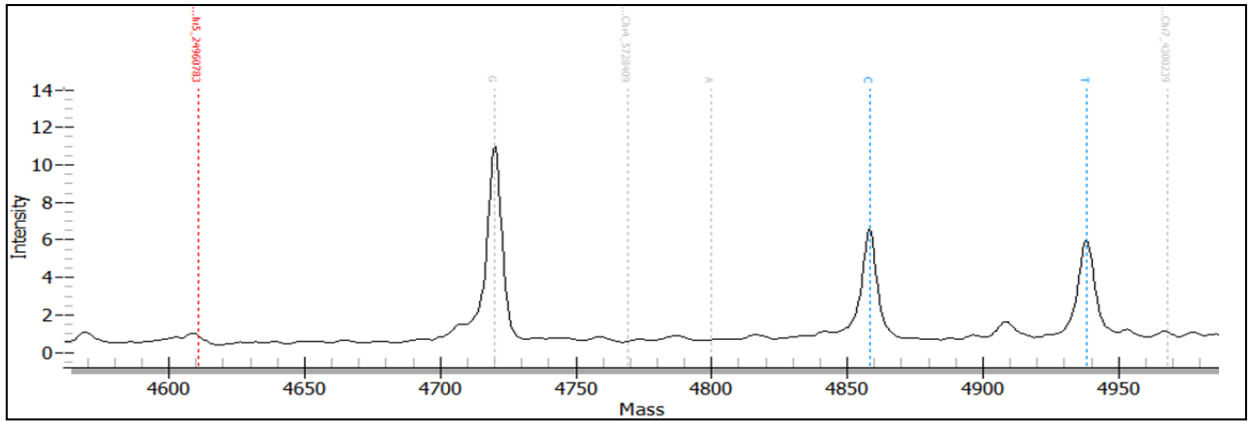
REPORT: EASE INTERPRETATION



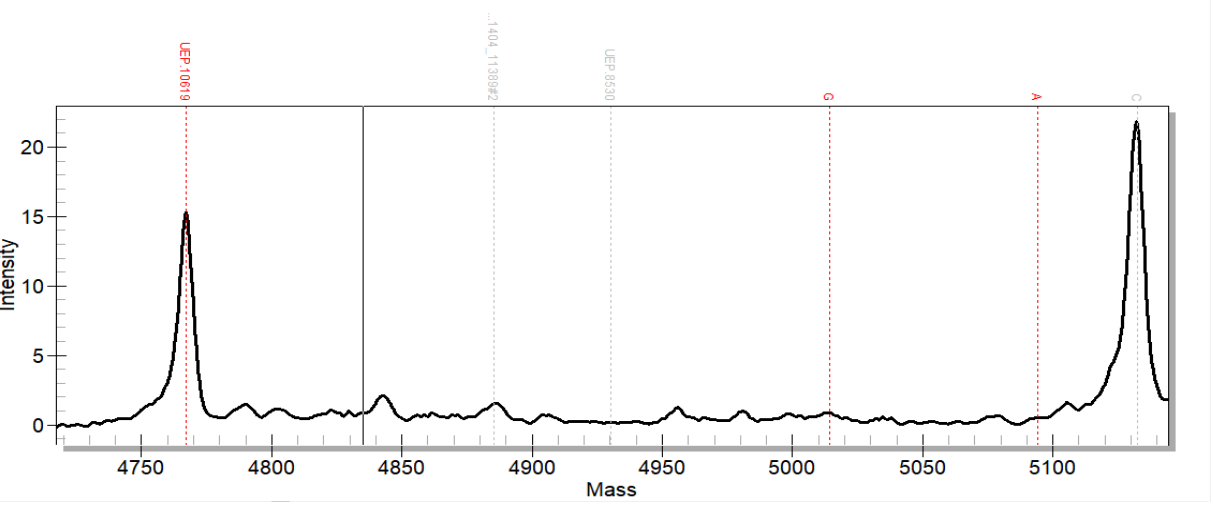
REPORT: Spectrum peak



Call: Homozygous GG



Call: Heterozygous CT



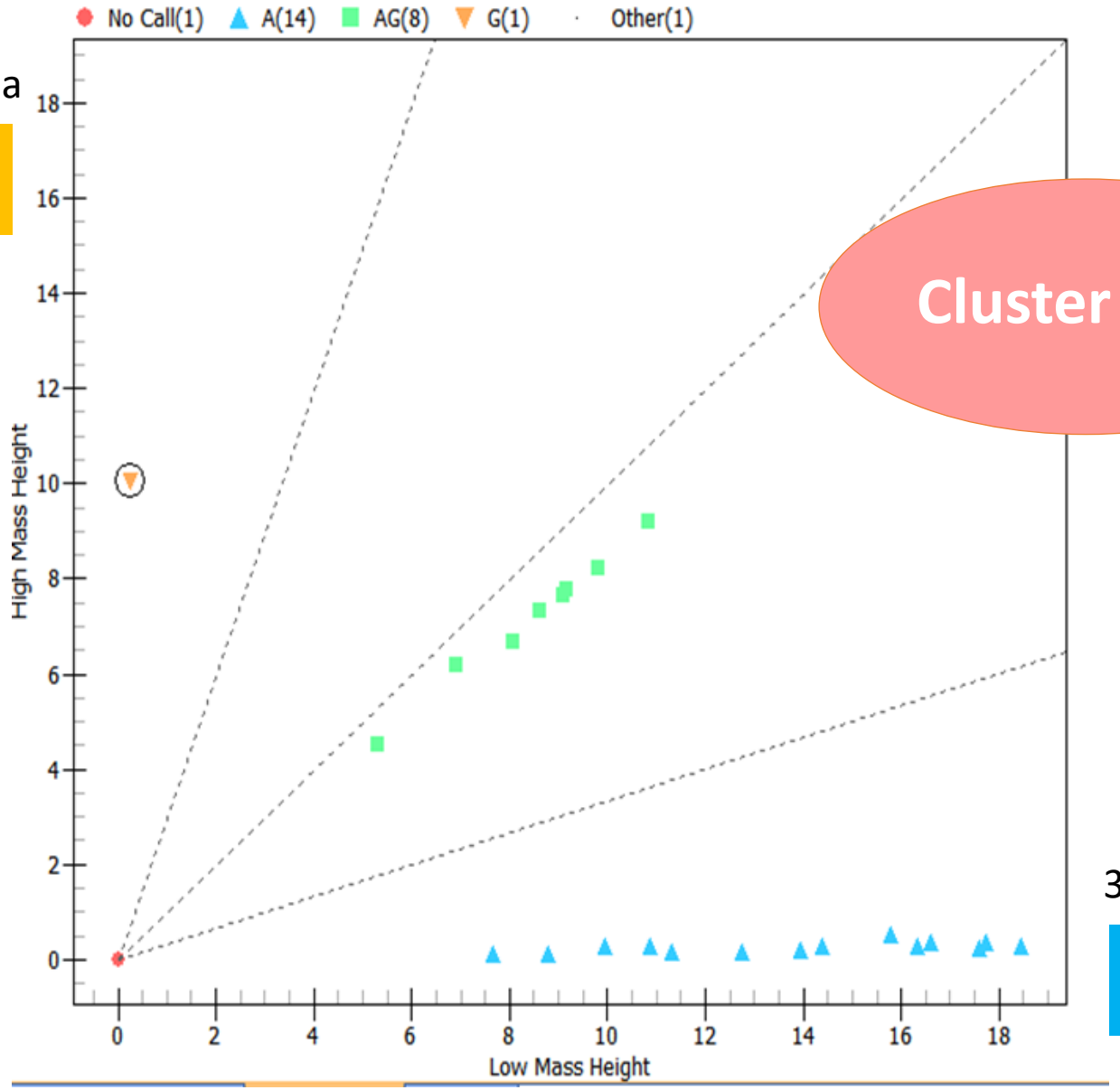
Only UEP Peak

No Call

REPORT: EASE INTERPRETATION

329.2 Da

G



Cluster Plot

313.2 Da

A

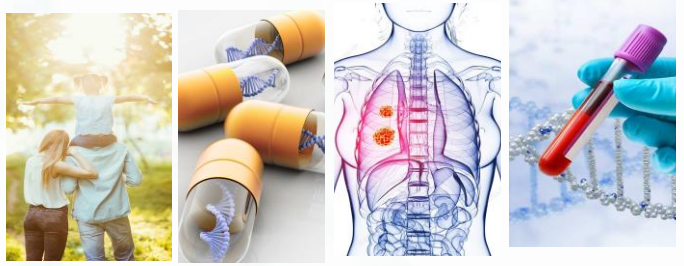
REPORT: EASE INTERPRETATION

EXCEL

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O			
1	SAMPLE_	WELL	Cum_Chr1	Cum_Chr1	Cum_Chr1	Cum_Chr1	Cum_Chr1	Cum_Chr2	Cum_Chr2	Cum_Chr3	Cum_Chr3	Cum_Chr3	Cum_Chr4	Cum_Chr4	Cum_Chr4	Cu		
2	16016	D04	G	T	C	A	C	A	T	G	G	G	G	T	T	A		
3	16017	D05	G	T	C	A	C	A	T	G	G	G	G	T	T	A		
4	C-588 F	C04	A	T	T	A	C	A	T	G	G	A	A	T	T	G		
5	C-588 F1-1	C06	A	T	T	A	C	A	T	G	G	A	A	T	T	G	C	C	A
6	C-588 M	C05	G	G	C	A	C	A	A	G	G	G	A	T	T	G	C	T	G
7	C-662 F	C01	G	G	C	A	C	G	T	G	T	A	G	T	T	A	T	T	A
8	C-662 F1-1	C03	AG	G	C	G	C	AG	T	G	T	A	G	TA	T	GA	T	T	A
9	C-662 M	C02	A	G	C	G	C	A	T	G	T	A	G	A	T	G	T	T	A
10	C-665 F	B04	G	G	C		C	G	T	G	T	A	G	T	T	A	T	T	A
11	C-665 F1-1	B06			C												T		
12	C-665 M	B05	A	G	C	AG	C	G	T	G	A	G	G	T	T	G	C	T	A
13	C-685 F	D01	G	G	T	A	C	A	T	G	G	G	G	T	T	A	T	T	G
14	C-685 F1-1	D03	AG	GT	T	A	C	A	T	G	G	GA	GA	T	T	GA	CT	TC	AG
15	C-685 M	D02	A	T	T	A	C	A	T	G	G	A	A	T	T	G	C	C	A
16	CU023 F	B01	G	G	G	A	C	A	A	G	T	A	G	A	T	G	T	T	G
17	CU065 M	A06	G	G	C	A	C	A	T	G	G	G	G	T	T	A	C	C	G

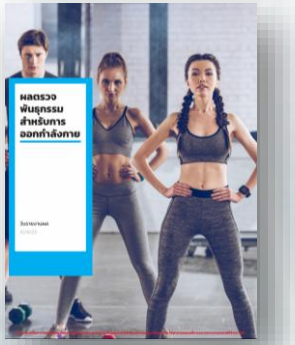
Applications

- **Sickness**
  - **Pharmacogenetic**
  - **Cancer panels** — Highly Sensitive, Liquid biopsy
  - **Genetic disorder**
  - **Infection screeing**
  - **Methylation**



MassARRAY

- **Wellness**
  - **Thailand : Kin Yoo Dee**
  - **Korea Panel**



SICKNESS

ONCOLOGY (CANCER)

- EMPHASIZE ADVANCEMENT
- PREDICTIVE FACTORS
- PROGNOSIS FACTORS
- TARGET THERAPIES SELECTION

Tissue specimen
Liquid Biopsy Panels

PHARMACOGENOMICS (PRECISION MED)

- SCAN ADME GENES
- PREDICT DRUG RESPONSE
- SIDE EFFECT, ADRs
- DRUG SURVEILLANCE FOR SENSITIVE PATIENTS

VeriDose® CYP2D6 CNV Panel
VeriDose® DPYD Panel

GENETIC DISORDERS

- ULTRASENSITIVE MATERNAL OR PRENATAL SCREENING
- LESS INVASION SCREENING

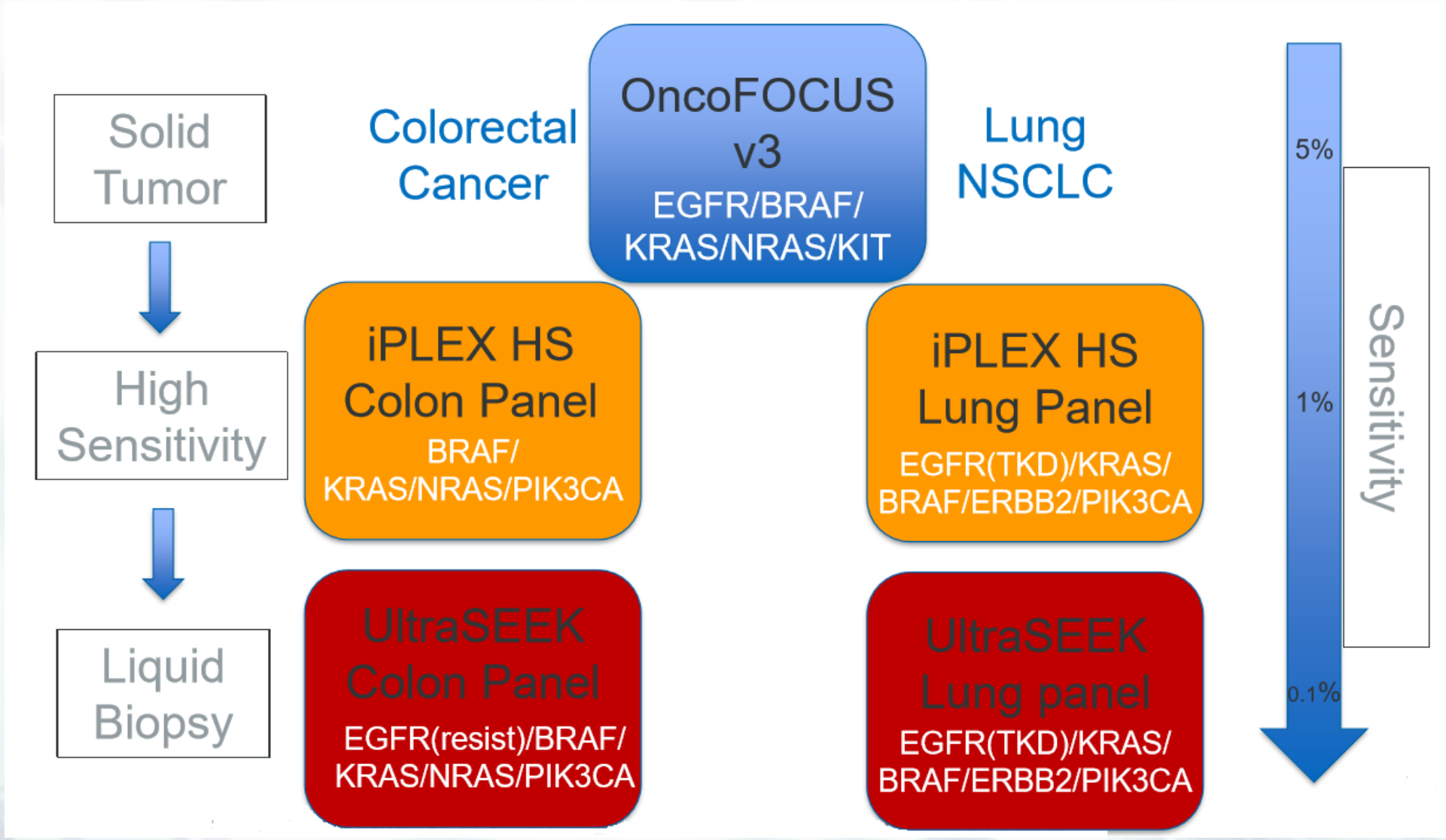
Hearing loss
Wilson's disease
Cystic Fibrosis Mutation

INFECTIOUS APPLICATIONS

- FAST SCREENING
- BACTERIA
- VIRUSES
- MYCOPLASMA
- NON CULTURE

Sexually transmitted disease
Human Papilloma Virus
Mycobacterium

MASSARRAY : Targeted Somatic Mutation Panels



PHARMACOGENETICS

VeriDose® CYP2D6 CNV Panel

CYP2D6 copy number variation (CNV)

CYP2D6/CYP2D7 “hybrid alleles” including exon 9 exchanges, *13 and *68.

22 points in 7 regions

VeriDose® Core Panel

85 SNPs/INDELs across 16 genes

Designed to robustly analyze low quality DNA

Genes Analyzed with the VeriDose Core Panel			
• CYP2B6	• CYP3A4	• HLA-A	• TPMT
• CYP2C19	• CYP3A5	• HLA-B	• UGT1A1
• CYP2C9	• CYP4F2	• NUDT15	• VKORC1
• CYP2D6	• DPYD	• SLCO1B1	• 2C cluster

Patient Genetic Results

Gene	Genotype	Phenotype	Phenotype adjusted for concomitant medications*	Additional Comments
CYP2B6	*1/*1	normal	normal	Notes appear here.
CYP2C19	*1/*1	normal	poor	
CYP2C9	*1/*2	intermediate	intermediate	
CYP2D6	*1/*2	normal	normal	
CYP3A5	*3/*3	poor	poor	
HLA-A*31:01	-	positive	positive	
HLA-B*15:02	-	negative	negative	
HLA-B*57:01	-	negative	negative	
HLA-B*58:01	-	negative	negative	
NUDT15	*1/*1	normal	normal	
SLCO1B1	*1A/*1A	normal	normal	
TPMT	*1/*3A	intermediate	intermediate	
VKORC1	*1/*1	normal	normal	

*Phenotype adjusted based on the concomitant use of inhibitors or inducers. See the Regarding Phenotype Adjustment section at the end of the report for full details.

Gene	Drugs
CYP2B6	Efavirenz
CYP2C19	Amitriptyline, citalopram, clobazam, clomipramine, clopidogrel, doxepin, escitalopram, esomeprazole, imipramine, lansoprazole, omeprazole, pantoprazole, prasugrel, sertraline, ticagrelor, trimipramine, voriconazole
CYP2C9	Aspirin, celecoxib, flurbiprofen, ibuprofen, lornoxicam, meloxicam, naproxen, phenytoin, piroxicam, tenoxicam, warfarin
CYP2D6	Amiodarone, amitriptyline, amphetamine, aripiprazole, atenolol, atomoxetine, bisoprolol, brexpiprazole, cavedilol, clomipramine, clonidine, clozapine, codeine, desipramine, doxepin, duloxetine, eliglustat, flecainide, fluoxetine, fluphenazine, fluvoxamine, haloperidol, hydrocodone, iloperidone, imipramine, methylphenidate, metoprolol, mirtazapine, moclobemide, nebivolol, nortriptyline, odansetron, olanzapine, oxycodone, paroxetine, perphenazine, pimozide, propafenone, propranolol, quetiapine, risperidone, tamoxifen, tetrabenazine, tramadol, tropisetron, trimipramine, venlafaxine, vortioxetine, zuclopenthixol
CYP3A5	Tacrolimus
HLA-A	Carbamazepine (*31:01)
HLA-B	Abacavir (*57:01), allopurinol (*58:01), carbamazepine (*15:02), oxcarbazepine (*15:02), phenytoin (*15:02)
NUDT15	Azathioprine, mercaptopurine, thioguanine
SLCO1B1	Atorvastatin, fluvastatin, rosuvastatin, simvastatin
TPMT	Azathioprine, mercaptopurine, thioguanine
VKORC1	Acenocoumarol, warfarin

Current Medications

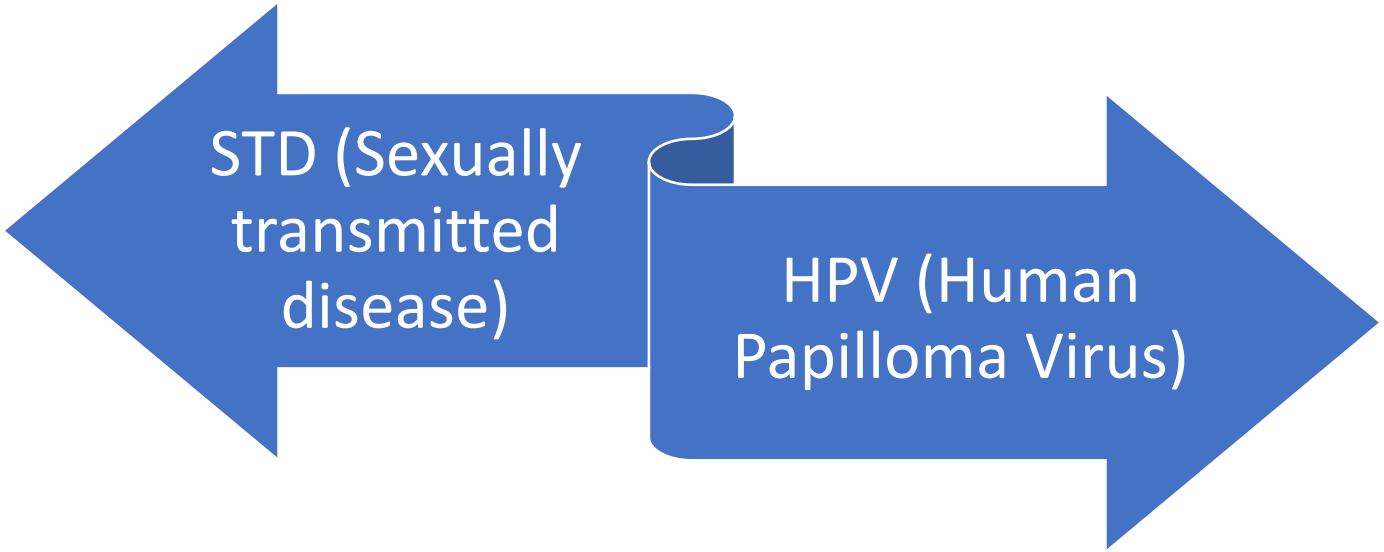
Medication name	Description
esomeprazole	CYP3A5 Weak Inhibitor, Substrate CYP2C19 Strong Inhibitor, Substrate

*Note: Inhibitor and inducer information was based on the Drug Interactions Flockhart Table.
If a current medication is linked to a guideline supported by this tool, it will appear in the 'Future Medications' table of this report.

Pathogen detection

Pathogen detection for infectious disease

Target pathogens	CT (Chlamydia trachomatis)
	MG (Mycoplasma genitalium)
	UU (Ureaplasma urealyticum)
	UP (Ureaplasma parvum)
	NG (Neisseria gonorrhoea)
	MH (Mycoplasma hominis)
	TV (Trichomonas vaginalis)
	GV (Gardnerella Vaginalis)
	CA (Candida Albicans)
	TP (Treponema pallidum)
	HSV1
	HSV2
Internal control	hARF3



- Low risk

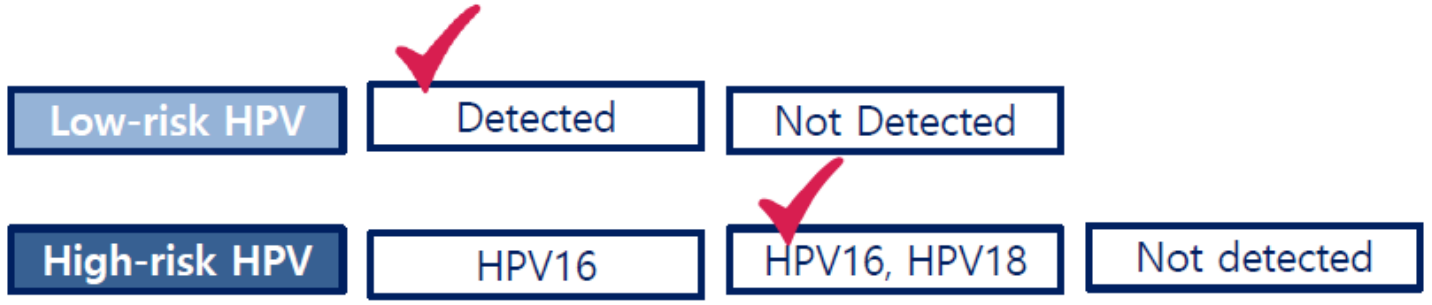
HPV 6, 11

- High risk

HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68

HPV (Human Papilloma Virus)

- **Targets; Low-risk HPVs, High-risk HPVs**
  - Low risk: HPV 6, 11
  - High risk: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
- **Multiplexing in 1 well (total 17 targets+1 internal control)**
- **Screening program for Health care check-up purpose**



Pathogen detection

Mycobacterium strains identification & Drug-resistant tuberculosis mutation detection

W1 identified strains		W2 identified strains	
Mycobacterium tuberculosis complex		M. mucogenicum	M. lentiflavum
Mycobacterium avium complex		M. Terrae	M. marinum
M. Abcessus	M. Malmoense	M. peregrinum	M. simiae
M. intracellulare	M. Scrofulaceum	M. chimaera	M. gastri
M. asiaticum	M. smegmatis	M. gordonae	M. phlei
M. fortuituminfection	M. szulgai	M. genavense	M. septicum
M. haemophilum	M. xenopi	M. Triviale	M. massiliense
M. kansasii	M. celatum		
M. stutzeri	M. chelonae		
M. Ulcerans			

Catalog	Drug name	Resistance gene
First-line drug	Isoniazid	katG, inhA
	Rifampicin	rpoB
	Streptomycin	rpsL, rrs
	Ethambutol	embB
	Pyrazinamide	pncA
Second line drug	Fluoroquinolone	gyrA, gyrB
	Ethionamide Prothionamide	inhA
	Para-amino salicylic acid	thyA
	Clofazimine	Rv0678 (mmpR)
	amikacin kanamycin	rrs, eis
	Capreomycin	rrs
	Cycloserine	alr
	Linezolid	rplC, rrl
	rifape ntine rifab utin	rpoB

SCIENTIFIC REPORTS

OPEN

Rapid Sputum Multiplex Detection of the *M. tuberculosis* Complex (MTBC) and Resistance Mutations for Eight Antibiotics by Nucleotide MALDI-TOF MS

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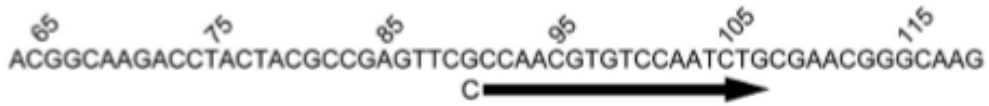
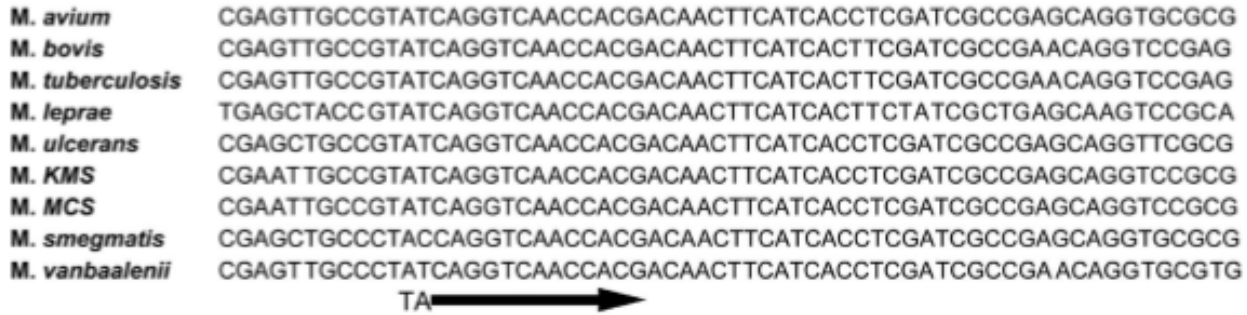
Kang-Yi Su[1,2], Bo-Shiun Yan[3], Hao-Chieh Chiu[1,2], Chong-Jen Yu[4], So-Yi Chang[3], Ruwen Jou[5], Jia-Long Liu[2], Po-Ren Hsueh[2,4,*] & Sung-Liang Yu[1,2,6,7,8,*]

The increasing incidence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Mycobacterium tuberculosis* (MTB) adds further urgency for rapid and multiplex molecular testing to identify the MTB complex and drug susceptibility directly from sputum for disease control. A nucleotide matrix-assisted-laser-desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS)-based assay was developed to identify MTB (MTBID panel) and 45 chromosomal mutations for resistance to eight antibiotics (MTBDR panel). We conducted a 300 case trial from outpatients to evaluate this platform. An MTBID panel specifically identified MTB with as few as 10 chromosome DNA copies. The panel was 100% consistent with an acid-fast stain and culture for MTB, nontuberculous mycobacteria, and non-mycobacteria bacteria. The MTBDR panel was validated using 20 known MDR-MTB isolates. In a 64-case double-blind clinical isolates test, the sensitivity and specificity were 83% and 100%, respectively. In a 300-case raw sputum trial, the MTB identification sensitivity in smear-negative cases using MALDI-TOF MS was better than the COBAS assay (61.9% vs. 46.6%). Importantly, the failure rate of MALDI-TOF MS was better than COBAS (11.3% vs. 26.3%). To the best of our knowledge, the test described herein is the only multiplex test that predicts resistance for up to eight antibiotics with both sensitivity and flexibility.

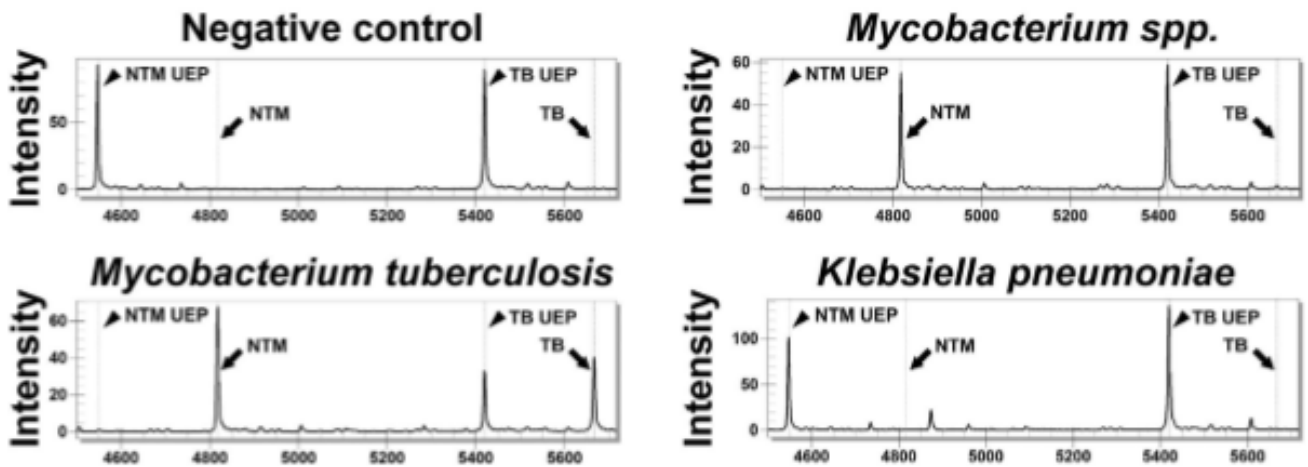
A


MCE3B
65
75
85
95
105
115
ACGGCAAGACCTACTACGCCGAGTTCGCCAACGTGTCCAATCTGCGAACGGGCAAG
C

B


gyrA
M. avium CGAGTTGCCGTATCAGGTCAACCACGACAACTTCATCACCTCGATCGCCGAGCAGGTGCGCG
M. bovis CGAGTTGCCGTATCAGGTCAACCACGACAACTTCATCACTTCGATCGCCGAACAGGTCCGAG
M. tuberculosis CGAGTTGCCGTATCAGGTCAACCACGACAACTTCATCACTTCGATCGCCGAACAGGTCCGAG
M. leprae TGAGCTACCGTATCAGGTCAACCACGACAACTTCATCACTTCTATCGCTGAGCAAGTCCGCA
M. ulcerans CGAGCTGCCGTATCAGGTCAACCACGACAACTTCATCACCTCGATCGCCGAGCAGGTTCGCG
M. KMS CGAATTGCCGTATCAGGTCAACCACGACAACTTCATCACCTCGATCGCCGAGCAGGTCCGCG
M. MCS CGAATTGCCGTATCAGGTCAACCACGACAACTTCATCACCTCGATCGCCGAGCAGGTCCGCG
M. smegmatis CGAGCTGCCCTACCAGGTCAACCACGACAACTTCATCACCTCGATCGCCGAGCAGGTGCGCG
M. vanbaalenii CGAGTTGCCCTATCAGGTCAACCACGACAACTTCATCACCTCGATCGCCGAACAGGTGCGTG
TA

C


Negative control
Mycobacterium spp.
Mycobacterium tuberculosis
Klebsiella pneumoniae
Intensity
NTM UEP
NTM
TB UEP
TB
4600
4800
5000
5200
5400
5600

Publications

Design and development of MassARRAY-based bacteriological assay 10 BACTERIAL FOODBORNE PATHOGENS IN A SINGLE REACTION

Primer ID	Bacterial Targets (species)
Bac16_ bac1/2	Bacteria
Eco001N	*E.coli/Shigella spp.*
Ent001	*Enterococcus faecalis*
Ent003	*Enterococcus faecium*
Clos001	*Clostridium perfringens*
Cmp002	*Campylobacter jujuni*
Cmp005 Cmp006	*Campylobacter coli*
Lis001	*Listeria monocytogenes*
Stp001	*Staphylococcus aureus*
Sal002	*Salmonella spp.*

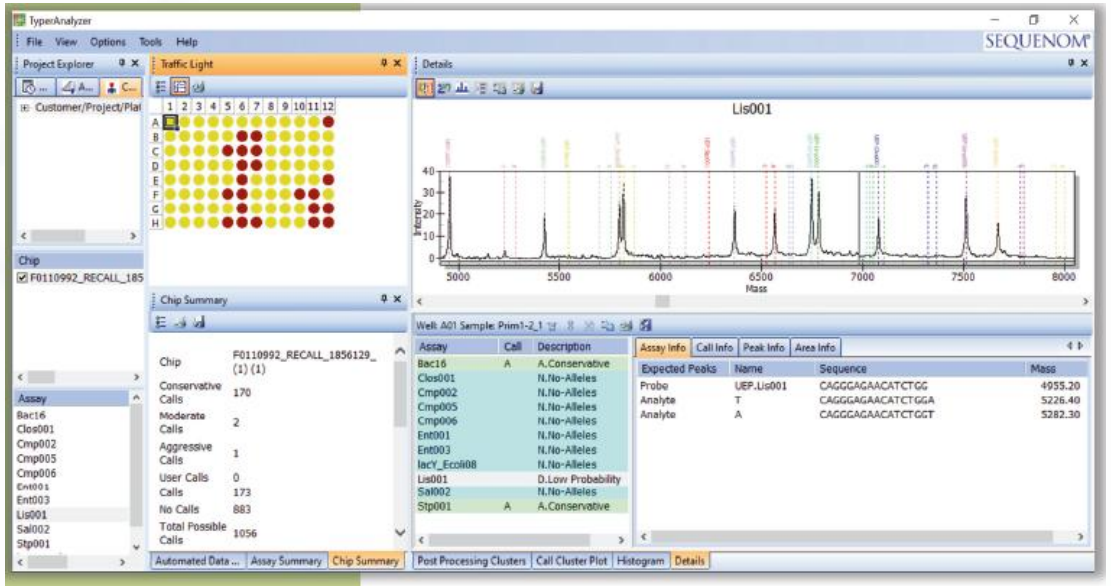


Figure 4 : TyperAnalyzer software for analysis of multiplexing reaction correlated to specific well on SpectroChip.

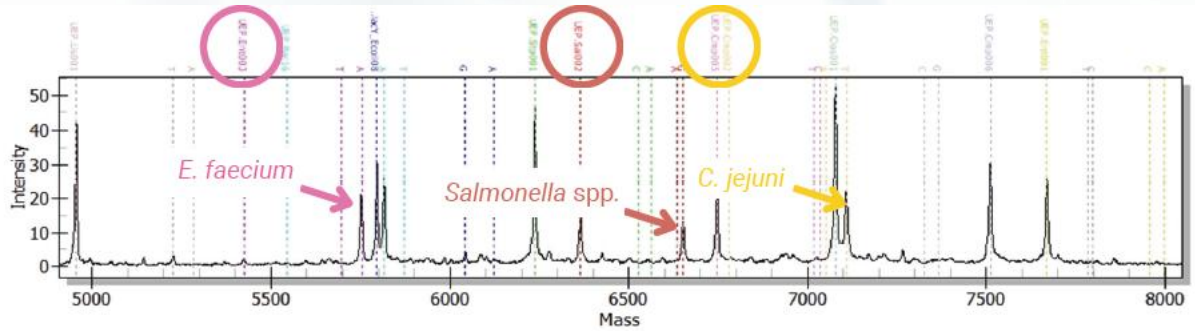
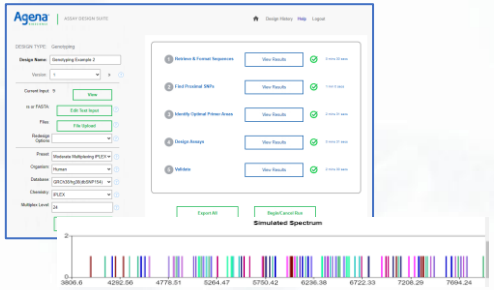


Figure 5 : *Enterococcus faecium*, *Salmonella* spp., and *Campylobacter jejuni* were identified in a single assay. The circles represent the mass-to-charge ratio (m/z) of unextended primers (UEP), while the arrows indicate the mass spectral peak corresponding to the extended base, facilitating bacterial identification.

BIOTEC
a member of NSTDA

MassARRAY System workflow

AgenaCx Account



1 Select Target Maker

2 Design primer

3 Optimize & validation

4 Sample run

5 Analyze data & Report

Customized

- Assay By AGENA
- Partner
- Own panel

Disease

SMA
Thalassemia
Hearing loss
TB
STD
Encephalitis

Wellness

Thailand : Kin Yoo Dee
Nutrigenomics
Exercise

Korea Panel
Epi-clock (Epigenetic)
Cancer Disease Susceptibility
Gut Microbiome
Skin & Hair
chronic disease

MASSCLEAVE
iPLEX Pro methylation

Methylation
Epigenetics

iPLEX Pro

SNP, Insertion, Deletion
Translocation
copy number variant,
somatic mutation