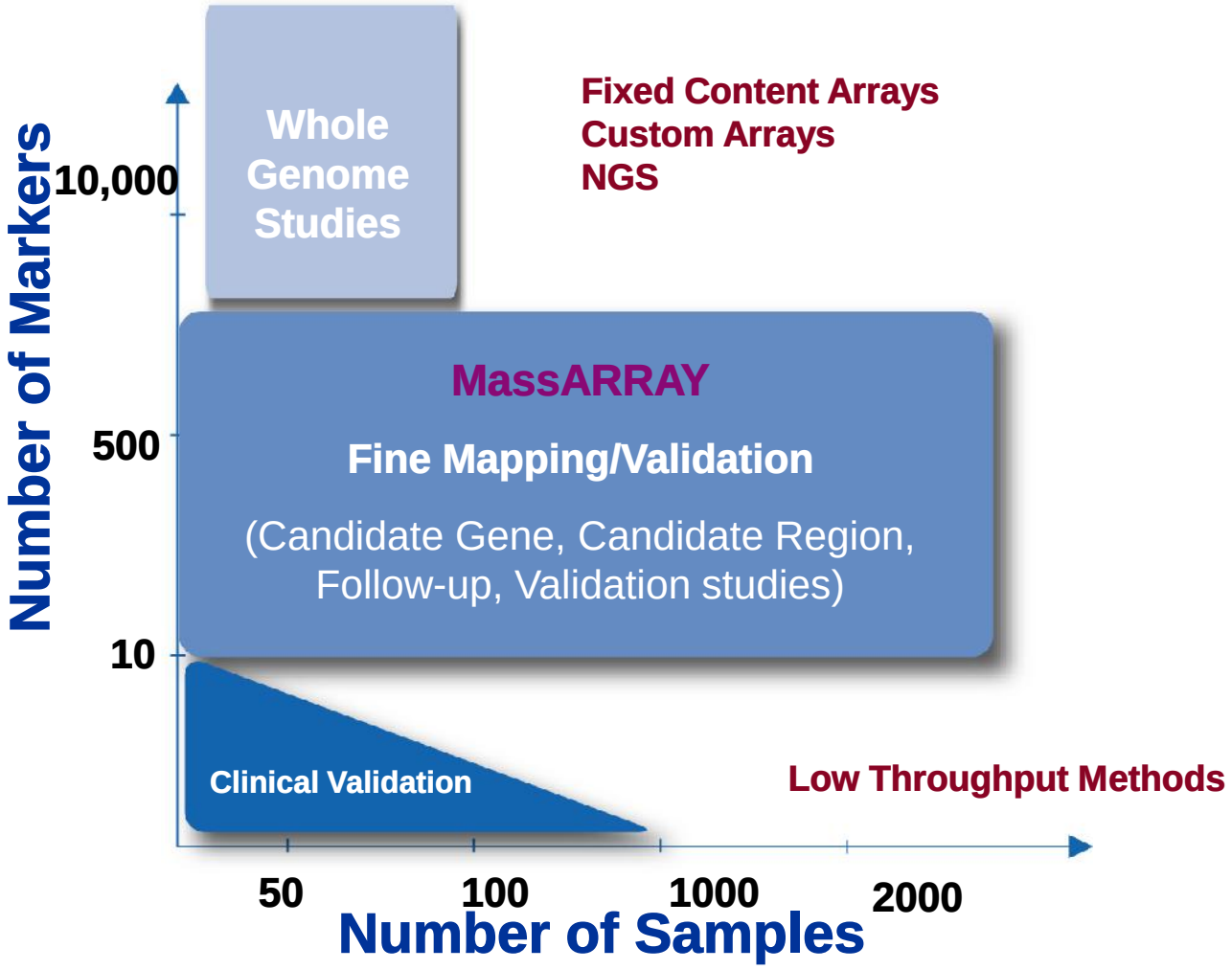
MassARRAY® : The Powerful Technology for Multi-gene Analysis

Pornsiri Aswapairin

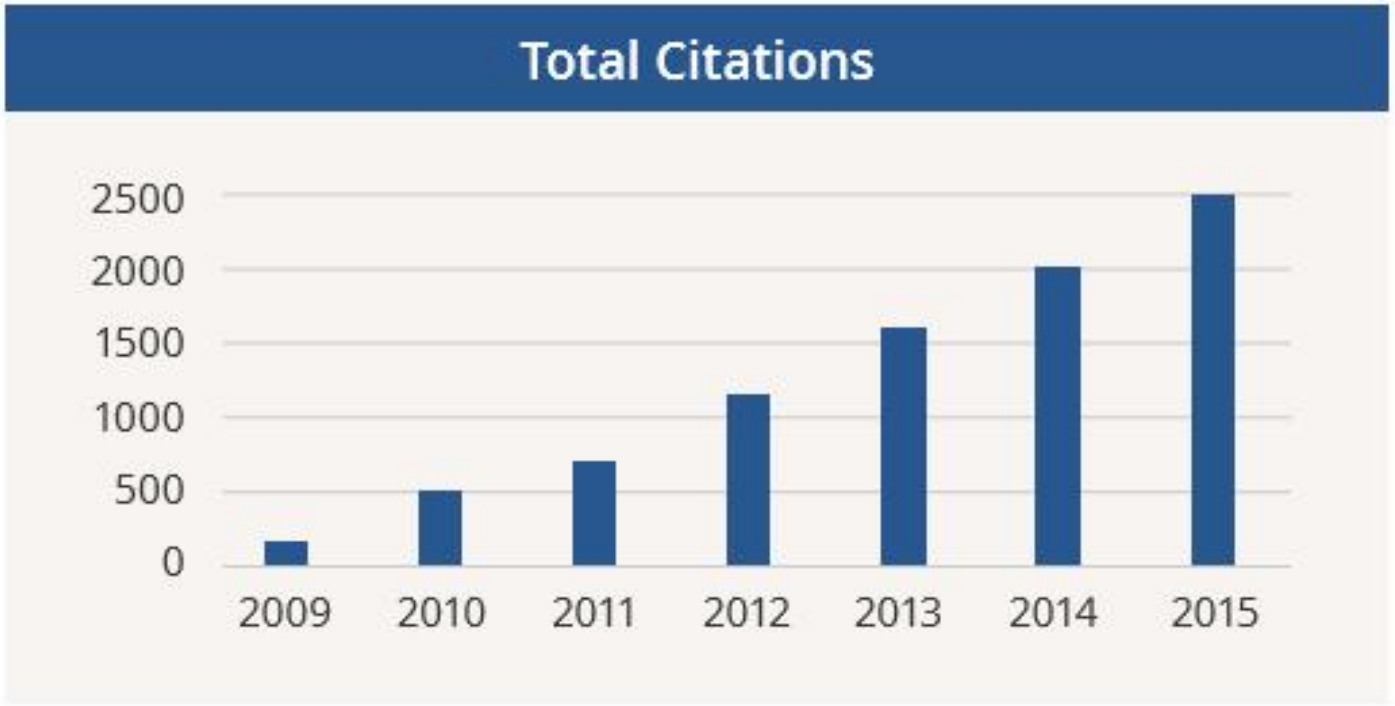
Agenda

- Introduction
- Principal of Technology
- Data Analysis
- Assay Design
- Applications
- Summary

Which fields using MassARRAY



Over 2500 published studies



PLOS ONE

RESEARCH ARTICLE

Assessment of common somatic mutations of *EGFR*, *KRAS*, *BRAF*, *NRAS* in pulmonary non-small cell carcinoma using iPLEX® HS, a new highly sensitive assay for the MassARRAY® System

Abstract

Increased early detection and personalized therapy for lung cancer have coincided with greater use of minimally invasive sampling techniques such as endobronchial ultrasound-guided biopsy (EBUS), endoscopic ultrasound-guided biopsy (EUS), and navigational biopsy, as well as thin needle core biopsies. As many lung cancer patients have late stage disease and other comorbidities that make open surgical procedures hazardous, the least invasive biopsy technique with the highest potential specimen yield is now the preferred first diagnostic study. However, use of these less invasive procedures generates significant analytical challenges for the laboratory, such as a requirement for robust detection of low level somatic mutations, particularly when the starting sample is very small or demonstrates few intact tumor cells. In this study, we assessed 179 clinical cases of non-small cell lung carcinoma (NSCLC) that had been previously tested for *EGFR, KRAS, NRAS, and BRAF* mutations using a novel multiplexed analytic approach that reduces wild-type signal and allows for detection of low mutation load approaching 1%, iPLEX® HS panel for the MassARRAY® System (Agena Bioscience, San Diego, CA). This highly sensitive system identified approximately 10% more *KRAS*, *NRAS*, *EGFR* and *BRAF* mutations than were detected by the original test platform, which had a sensitivity range of 5–10% variant allele frequency (VAF).

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Multiplexed transcriptome analysis to detect *ALK*, *ROS1* and *RET* rearrangements in lung cancer

Received: 03 October 2016
Accepted: 28 December 2016
Published: 09 February 2017

Toni-Maree Rogers[1,*], Gisela Mir Arnau[1,*], Georgina L. Ryland[1,*], Stephen Huang[2], Maruja E. Lira[2], Yvette Emmanuel[3], Omar D. Perez[4], Darryl Irwin[3], Andrew P. Fellowes[1,†], Stephen Q. Wong[1,†] & Stephen B. Fox[1,†]

ALK, *ROS1* and *RET* gene fusions are important predictive biomarkers for tyrosine kinase inhibitors in lung cancer. Currently, the gold standard method for gene fusion detection is Fluorescence *In Situ* Hybridization (FISH) and while highly sensitive and specific, it is also labour intensive, subjective in analysis, and unable to screen a large numbers of gene fusions. Recent developments in high-throughput transcriptome-based methods may provide a suitable alternative to FISH as they are compatible with multiplexing and diagnostic workflows. However, the concordance between these different methods compared with FISH has not been evaluated. In this study we compared the results from three transcriptome-based platforms (Nanostring Elements, Agena LungFusion panel and ThermoFisher NGS fusion panel) to those obtained from *ALK*, *ROS1* and *RET* FISH on 51 clinical specimens. Overall agreement of results ranged from 86–96% depending on the platform used. While all platforms were highly sensitive, both the Agena panel and Thermo Fisher NGS fusion panel reported minor fusions that were not detectable by FISH. Our proof–of–principle study illustrates that transcriptome-based analyses are sensitive and robust methods for detecting actionable gene fusions in lung cancer and could provide a robust alternative to FISH testing in the diagnostic setting.

J Antimicrob Chemother 2014; **69**: 1526–1532
doi:10.1093/jac/dkt544 Advance Access publication 26 January 2014

Journal of Antimicrobial Chemotherapy

High-throughput informative single nucleotide polymorphism-based typing of *Neisseria gonorrhoeae* using the Sequenom MassARRAY iPLEX platform

Objectives: *Neisseria gonorrhoeae* antimicrobial resistance (AMR) is a global problem heightened by emerging resistance to ceftriaxone. Appropriate molecular typing methods are important for understanding the emergence and spread of *N. gonorrhoeae* AMR. We report on the development, validation and testing of a Sequenom MassARRAY iPLEX method for multilocus sequence typing (MLST)-style genotyping of *N. gonorrhoeae* isolates.

Methods: An iPLEX MassARRAY method (iPLEX14SNP) was developed targeting 14 informative gonococcal single nucleotide polymorphisms (SNPs) previously shown to predict MLST types. The method was initially validated using 24 *N. gonorrhoeae* control isolates and was then applied to 397 test isolates collected throughout Queensland, Australia in the first half of 2012.

Results: The iPLEX14SNP method provided 100% accuracy for the control isolates, correctly identifying all 14 SNPs for all 24 isolates (336/336). For the 397 test isolates, the iPLEX14SNP assigned results for 5461 of the possible 5558 SNPs (SNP call rate 98.25%), with complete 14 SNP profiles obtained for 364 isolates. Based on the complete SNP profile data, there were 49 different sequence types identified in Queensland, with 11 of the 49 SNP profiles accounting for the majority ($n=280$; 77%) of isolates. AMR was dominated by several geographically clustered sequence types. Using the iPLEX14SNP method, up to 384 isolates could be tested within 1 working day for less than Aus$10 per isolate.

Conclusions: The iPLEX14SNP offers an accurate and high-throughput method for the MLST-style genotyping of *N. gonorrhoeae* and may prove particularly useful for large-scale studies investigating the emergence and spread of gonococcal AMR.

SCIENTIFIC REPORTS

OPEN

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

Received: 22 July 2016
Accepted: 21 December 2016
Published: 30 January 2017

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.

ORIGINAL ARTICLE BACTERIOLOGY

Comparison of a multiplexed MassARRAY system with real-time allele-specific PCR technology for genotyping of methicillin-resistant *Staphylococcus aureus*

M. W. Syrmis[1,2,3], R. J. Moser[4], D. M. Whiley[1,2,3], V. Vaska[5], G. W. Coombs[6], M. D. Nissen[1,2,3], T. P. Sloots[1,2,3] and G. R. Nimmo[5,7]

1) Queensland Paediatric Infectious Diseases Laboratory, Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital and Health Service District, Brisbane, 2) Clinical Medical Virology Centre, The University of Queensland, Brisbane, 3) Queensland Children's Medical Research Institute, The University of Queensland, Brisbane, 4) Sequenom Inc., Sequenom Asia Pacific, Brisbane, 5) Microbiology Division, Pathology Queensland, Central Laboratory, Brisbane, 6) Department of Microbiology and Infectious Diseases, PathWest Laboratory Medicine-WA, Royal Perth Hospital, Perth, Western Australia and 7) School of Medicine, Griffith University, Gold coast, Queensland, Australia

Abstract

The Sequenom MassARRAY iPLEX single-nucleotide polymorphism (SNP) typing platform uses matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) coupled with single-base extension PCR for high-throughput multiplex SNP detection. In this study, we investigated the use of iPLEX MassARRAY technology for methicillin-resistant *Staphylococcus aureus* (MRSA) genotyping. A 16-plex MassARRAY iPLEX GOLD assay (MRSA-iPLEX) was developed that targets a set of informative SNPs and binary genes for MRSA characterization. The method was evaluated with 147 MRSA isolates, and the results were compared with those of an established SYBR Green-based real-time PCR system utilizing the same SNP–binary markers. A total of 2352 markers belonging to 44 SNP–binary profiles were analysed by both real-time PCR and MRSA-iPLEX. With real-time PCR as the reference standard, MRSA-iPLEX correctly assigned 2298 of the 2352 (97.7%) markers. Sequence variation in the MRSA-iPLEX primer targets accounted for the majority of MRSA-iPLEX erroneous results, highlighting the importance of primer target selection. MRSA-iPLEX provided optimal throughput for MRSA genotyping, and was, on a reagent basis, more cost-effective than the real-time PCR methods. The 16-plex MRSA-iPLEX is a suitable alternative to SYBR Green-based real-time PCR typing of major sequence types and clonal complexes of MRSA.

MassARRAY Platform

96-well Platform

384-well Platform

MassARRAY™ Applications

One System – Many Applications

- Genotyping
- Somatic Mutation
- Copy Number Variation
- Methylation
- Comparative Sequence Analysis

SpectroCHIP™ → MassARRAY™ System → Data Analysis

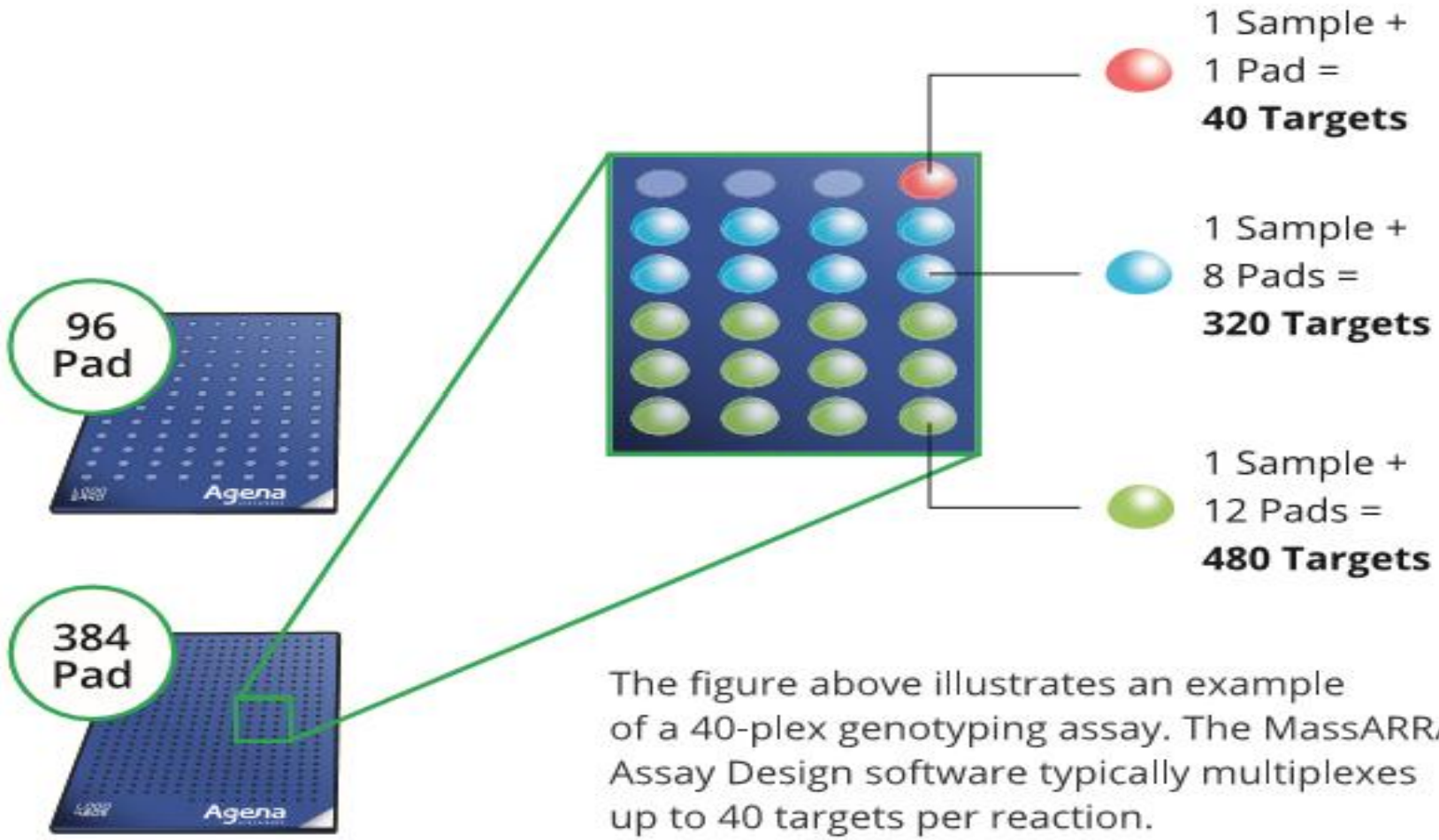
Agena BIOSCIENCE

Your Scientific Specialist

Flexibility and Cost Efficiency in a Single Workflow

- Minimal input DNA : 5-10 ng per well
- Flexible sample types : FFPE, Blood, Biopsy, Sputum, Saliva, Tissue, Seed, Leaf, etc.
- Efficient multiplexing : 40 targets per well

Multiplex up to 40 targets per well



The figure above illustrates an example of a 40-plex genotyping assay. The MassARRAY Assay Design software typically multiplexes up to 40 targets per reaction.

DNA Analysis Based on MassARRAY

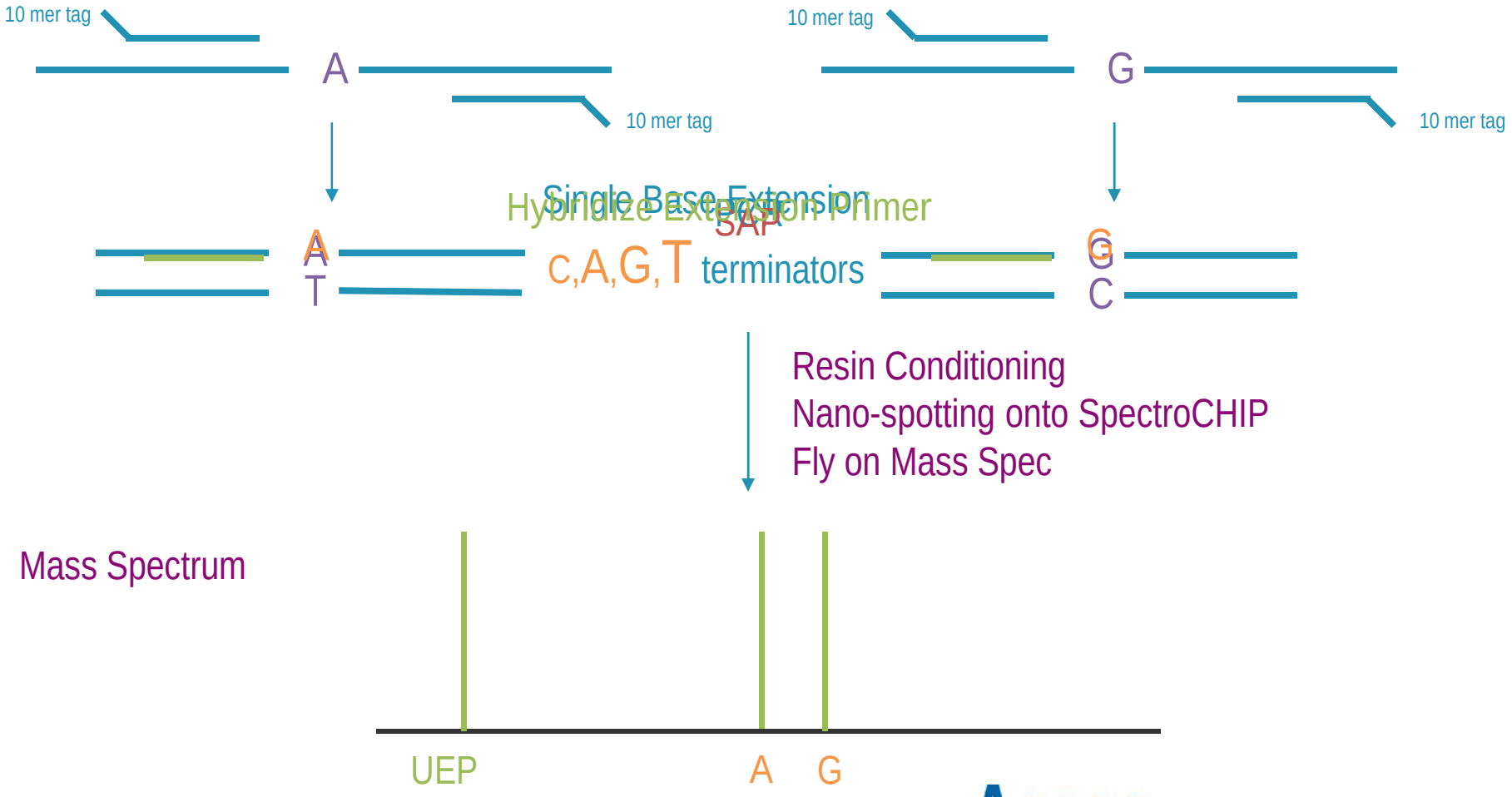
DNA consists of a four-letter alphabet:
A, C, G, T

Each base within a DNA strand has defined molecular mass:

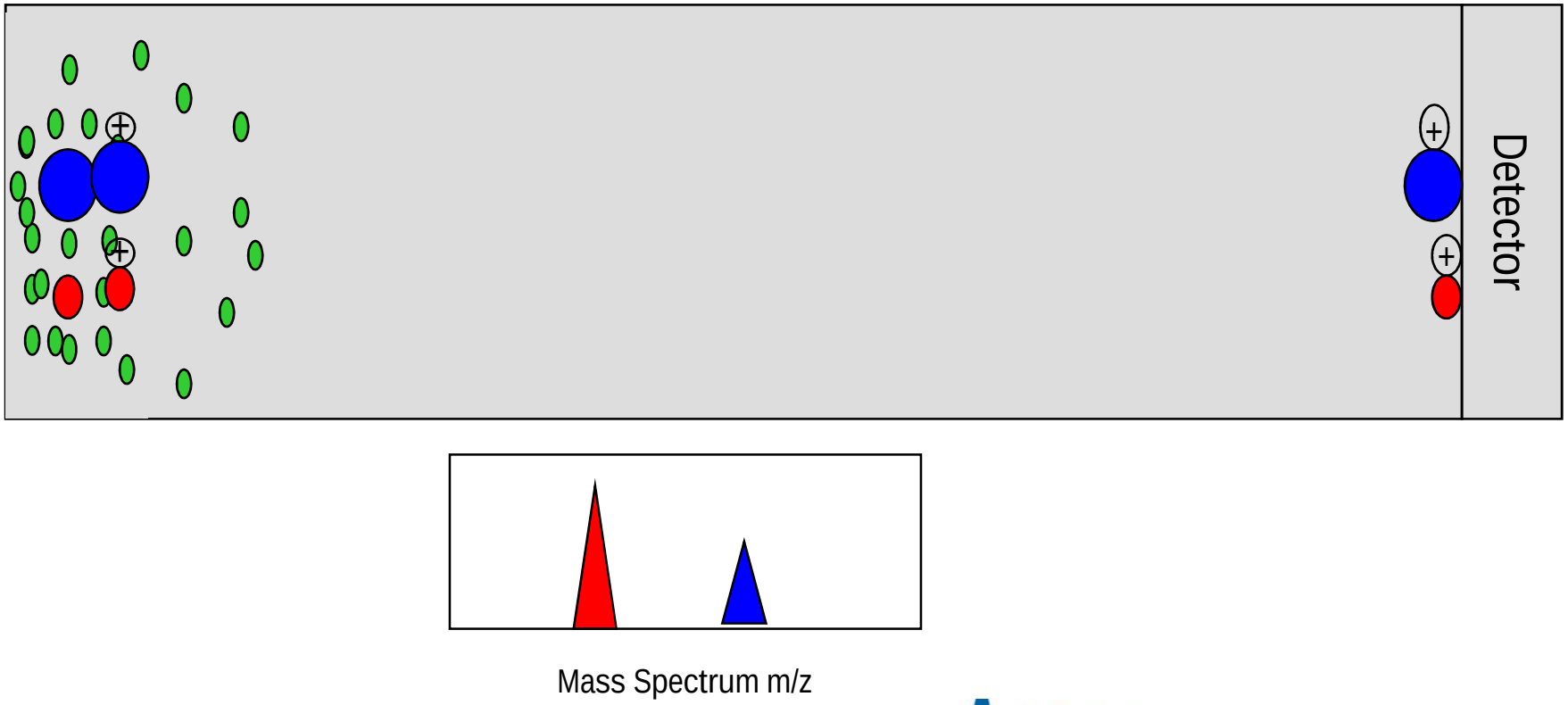
dCMP = 289.2 Da
dTMP = 304.2 Da
dAMP = 313.2 Da
dGMP = 329.2 Da

How to Detect Genotyping

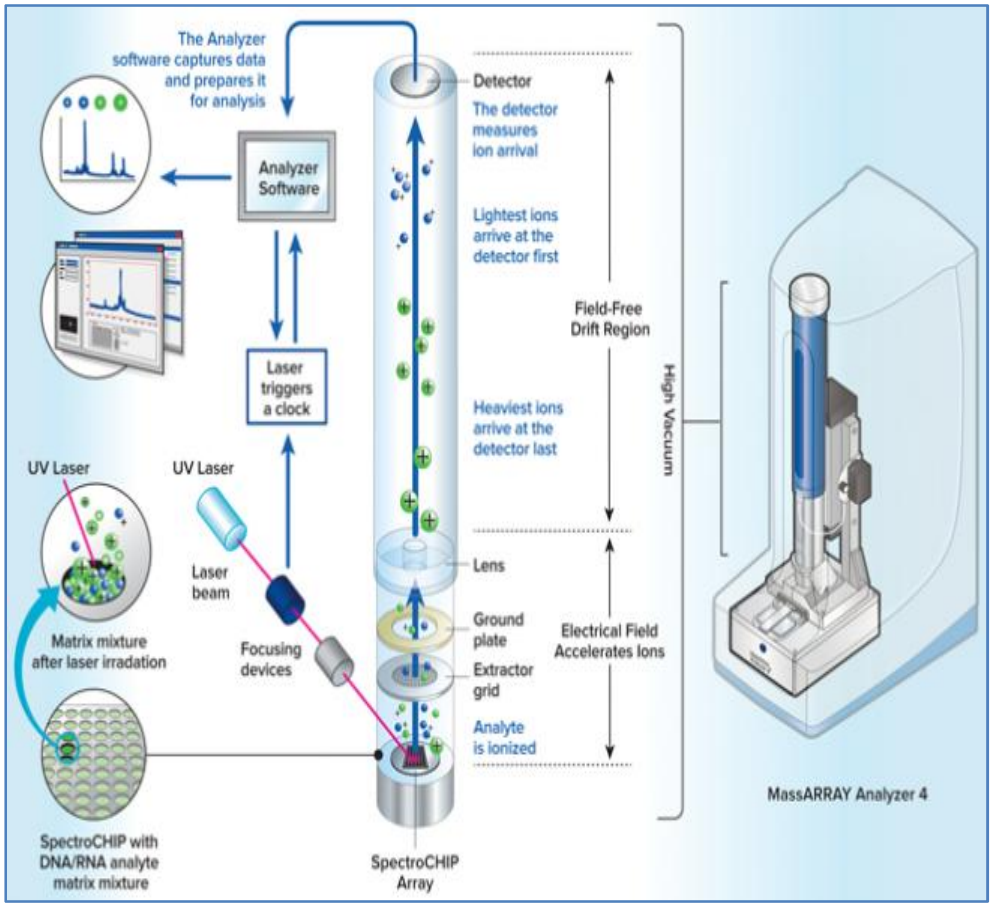
Example of an [A/G] SNP



Analysis of DNA Based on Mass

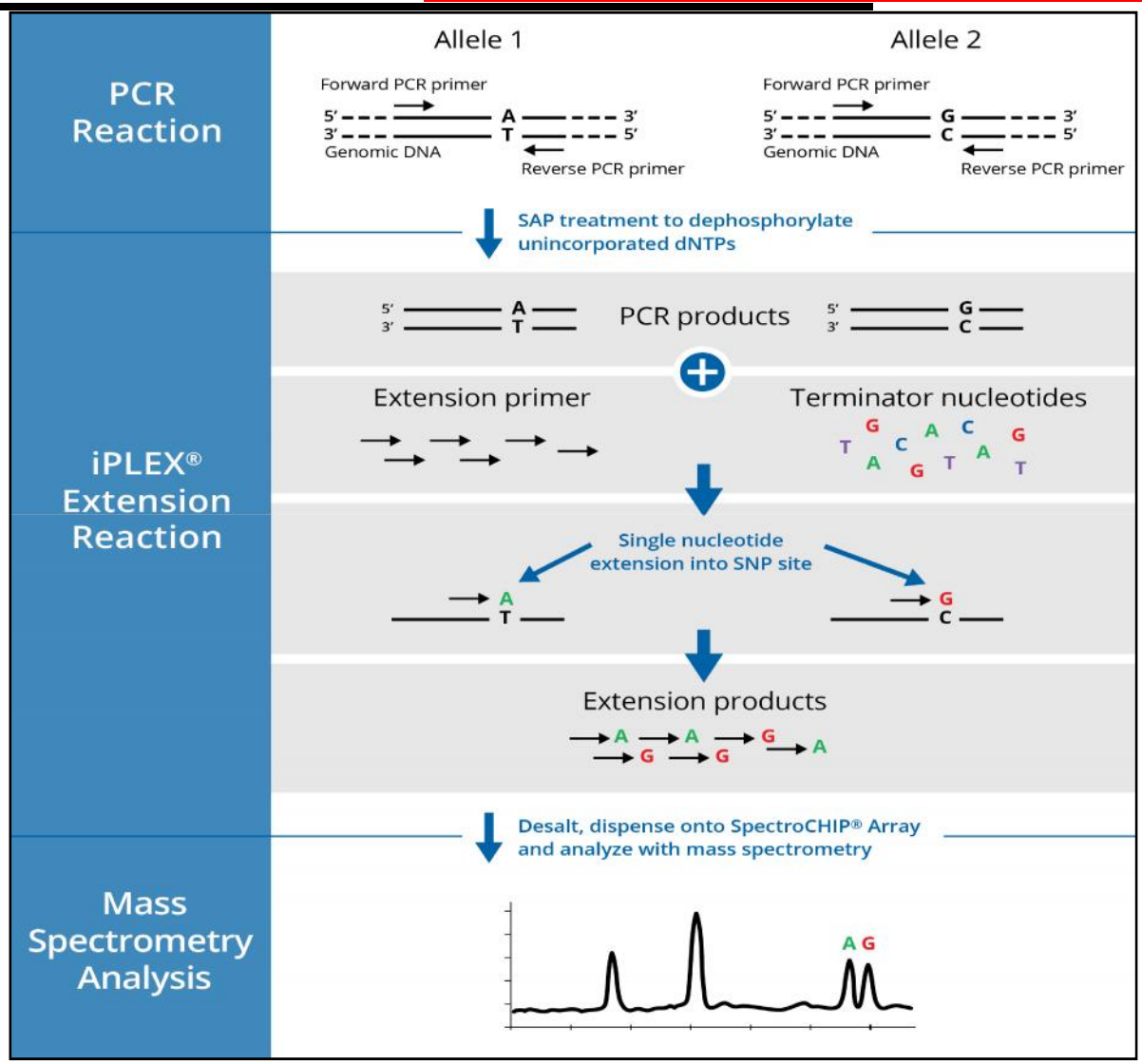


MALDI-TOF Mass Spectrometry with the MassARRAY®

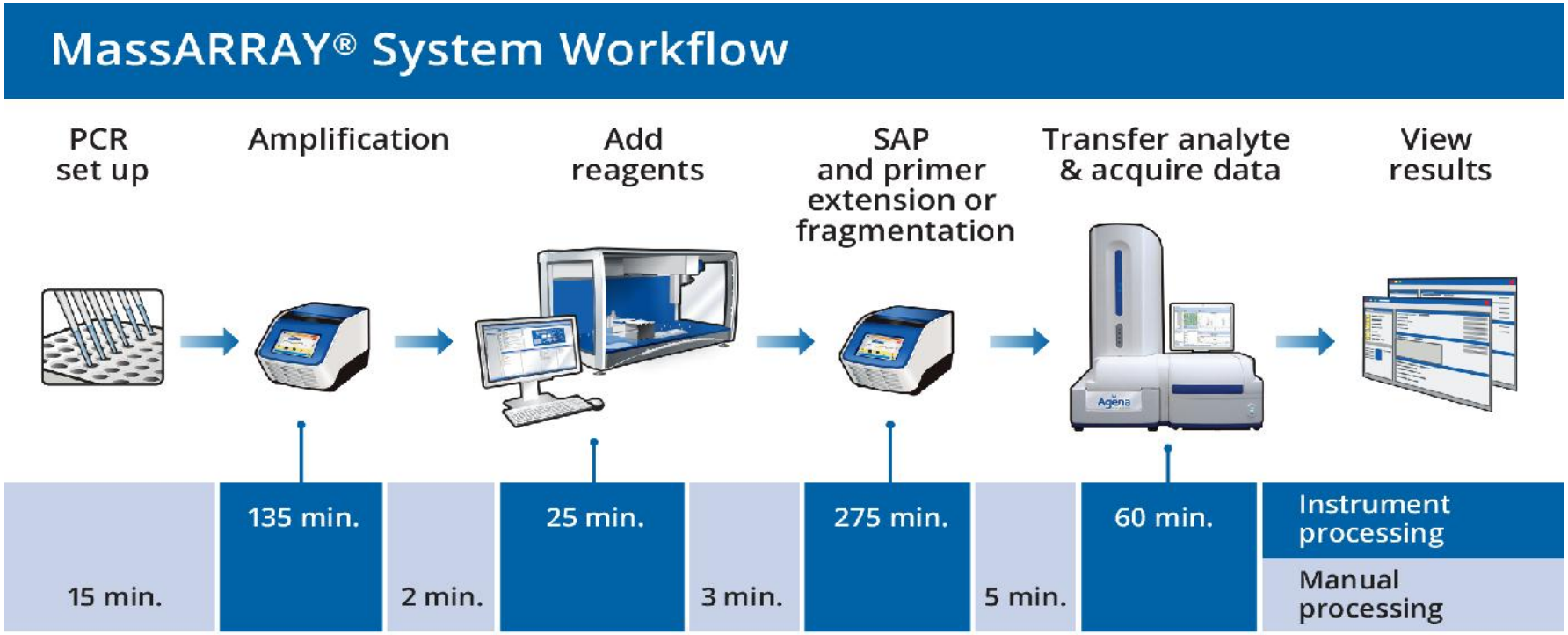


- Molecular mass is a unique intrinsic property.
- The mass of a nucleic acid string is determined by its length and composition (ATCG).

Overview of MassARRAY Assay Chemistry

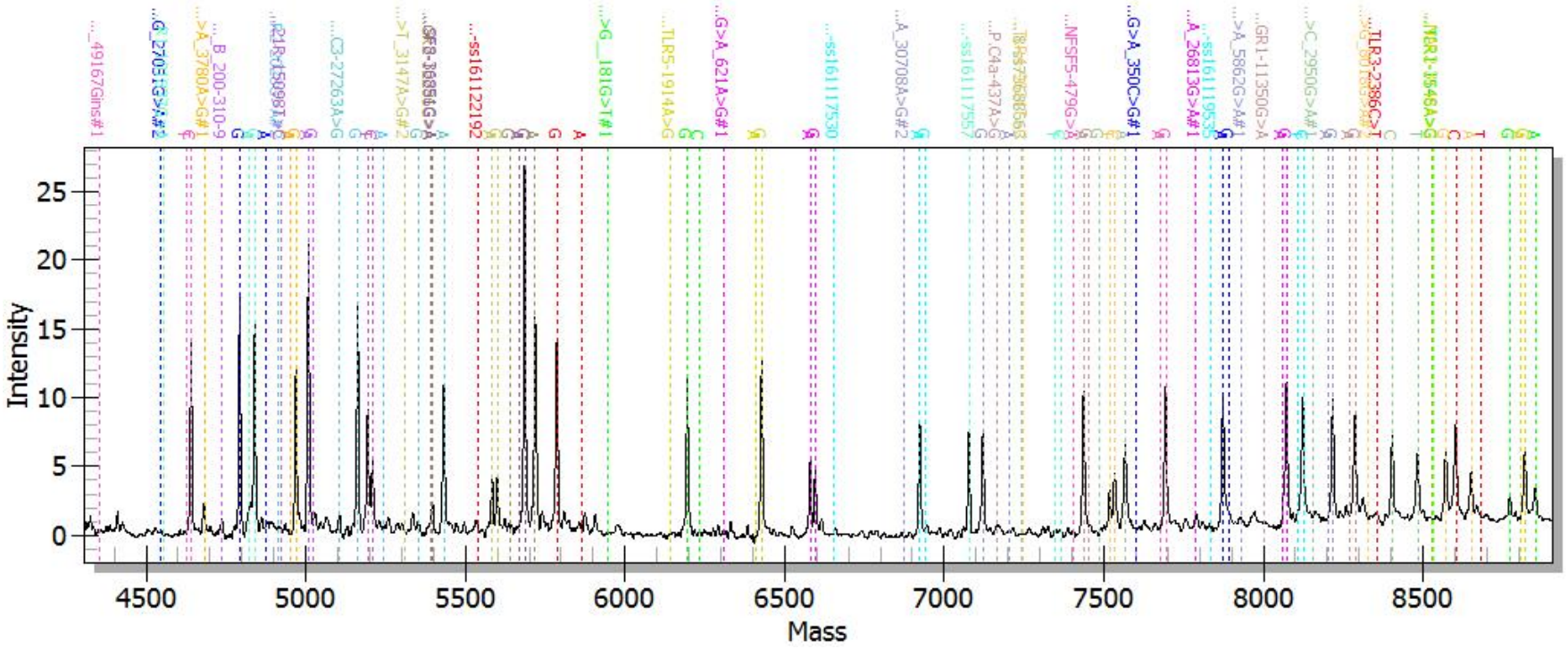


Workflow



10-30 seconds per well

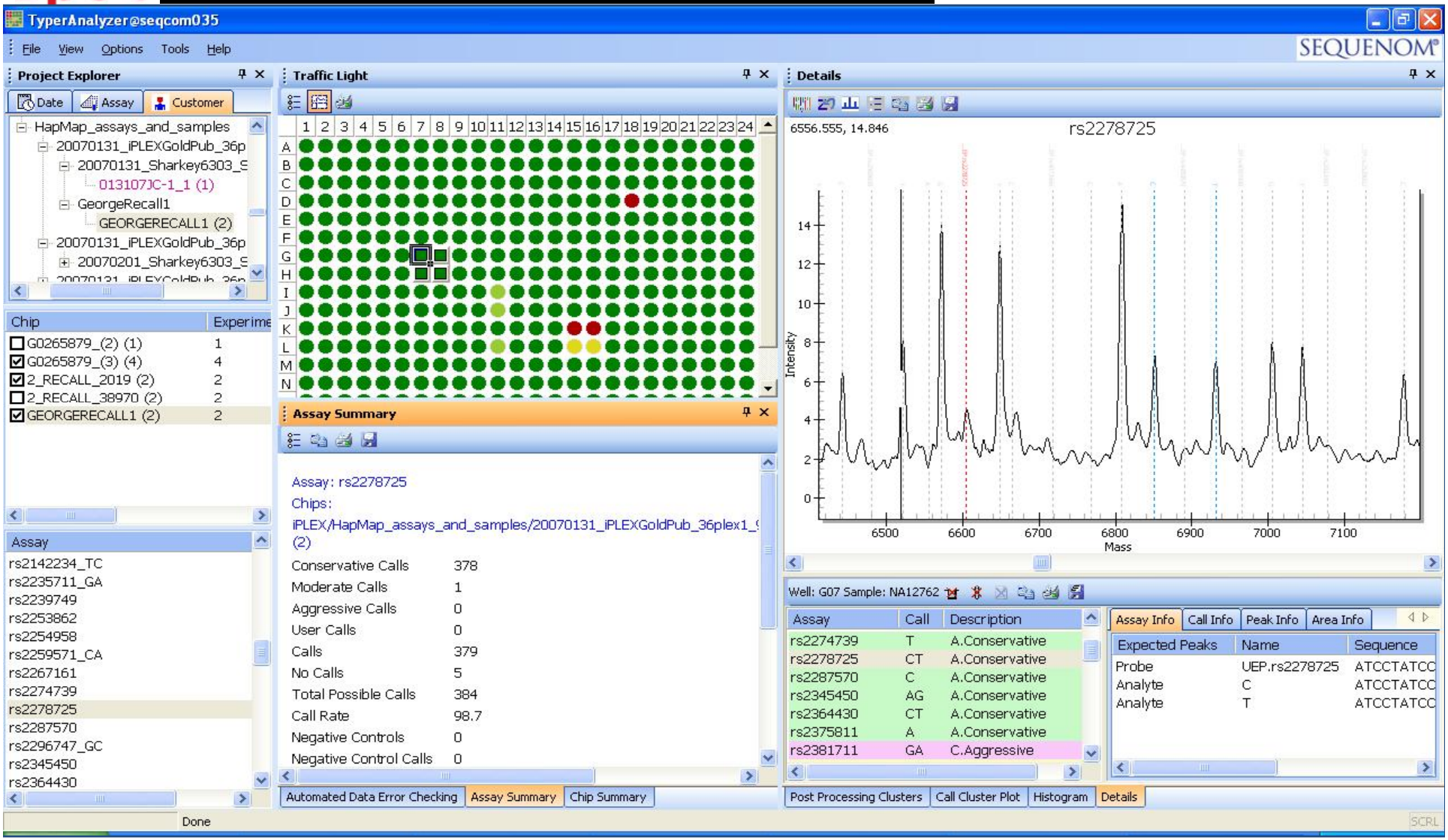
Typical SNP Panel (31-plex)



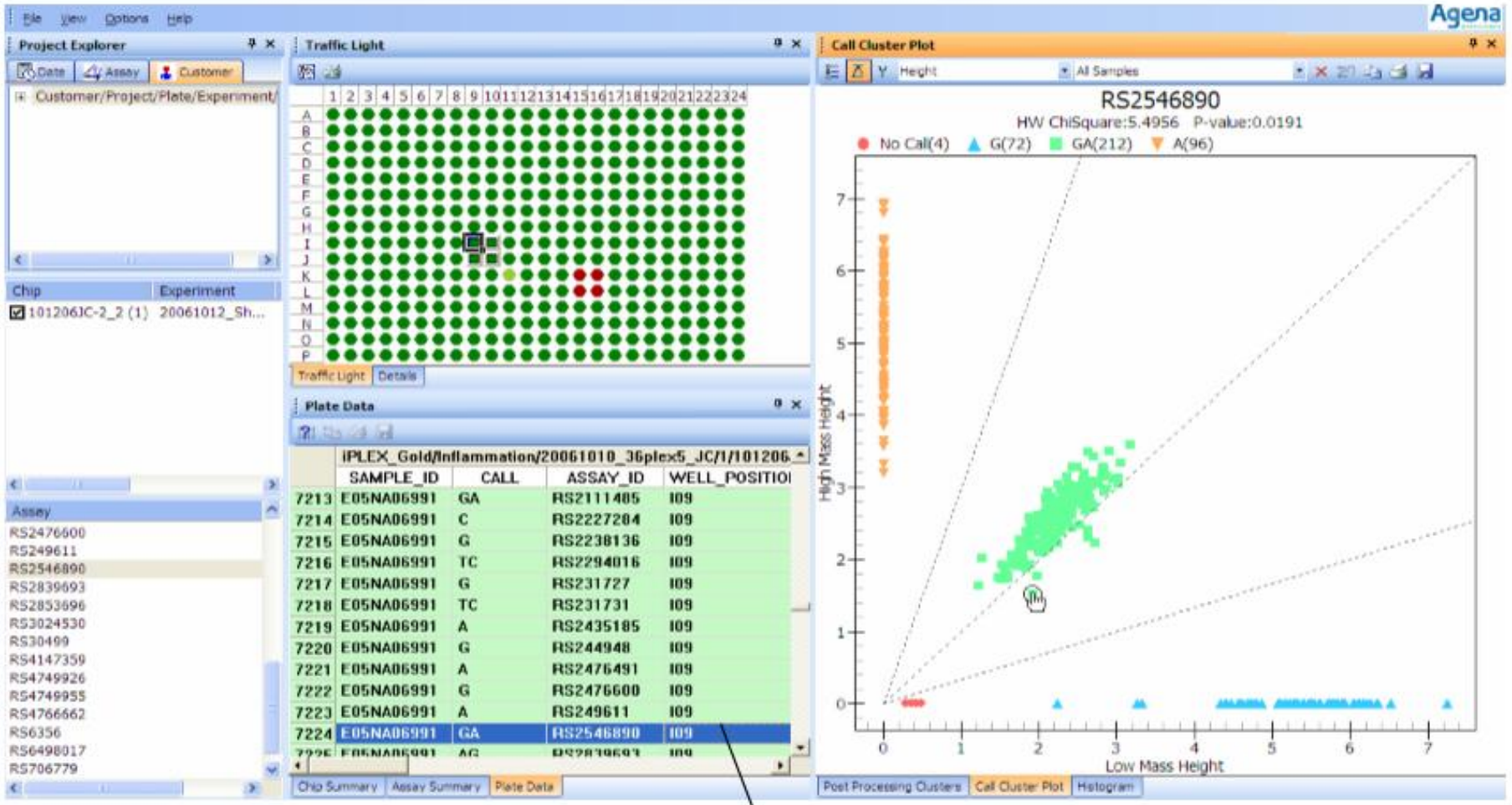
Your Scientific Specialist

Data Analysis

Window of Typer Software

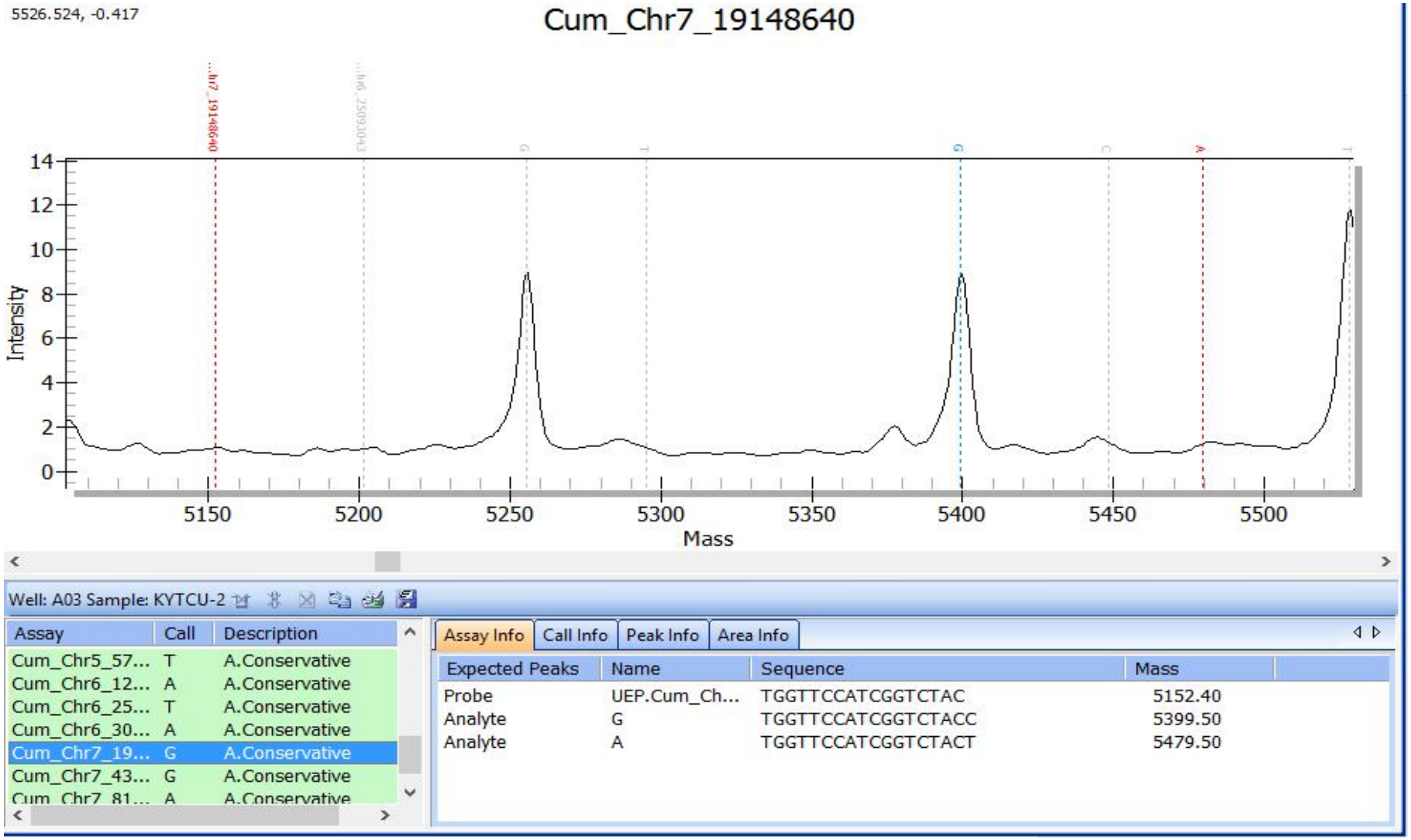


Call Cluster Plot

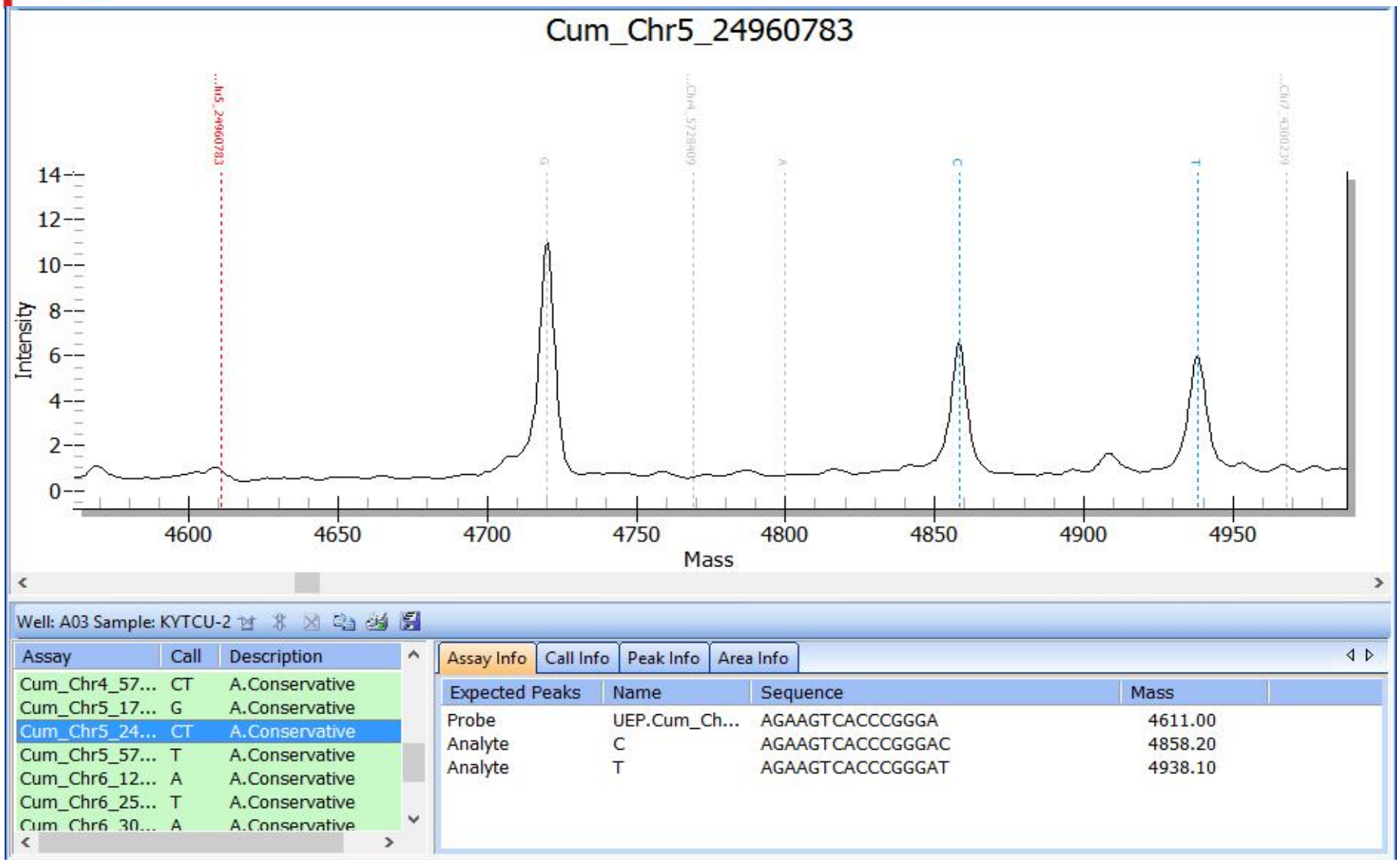


Result selected in the plate data pane.

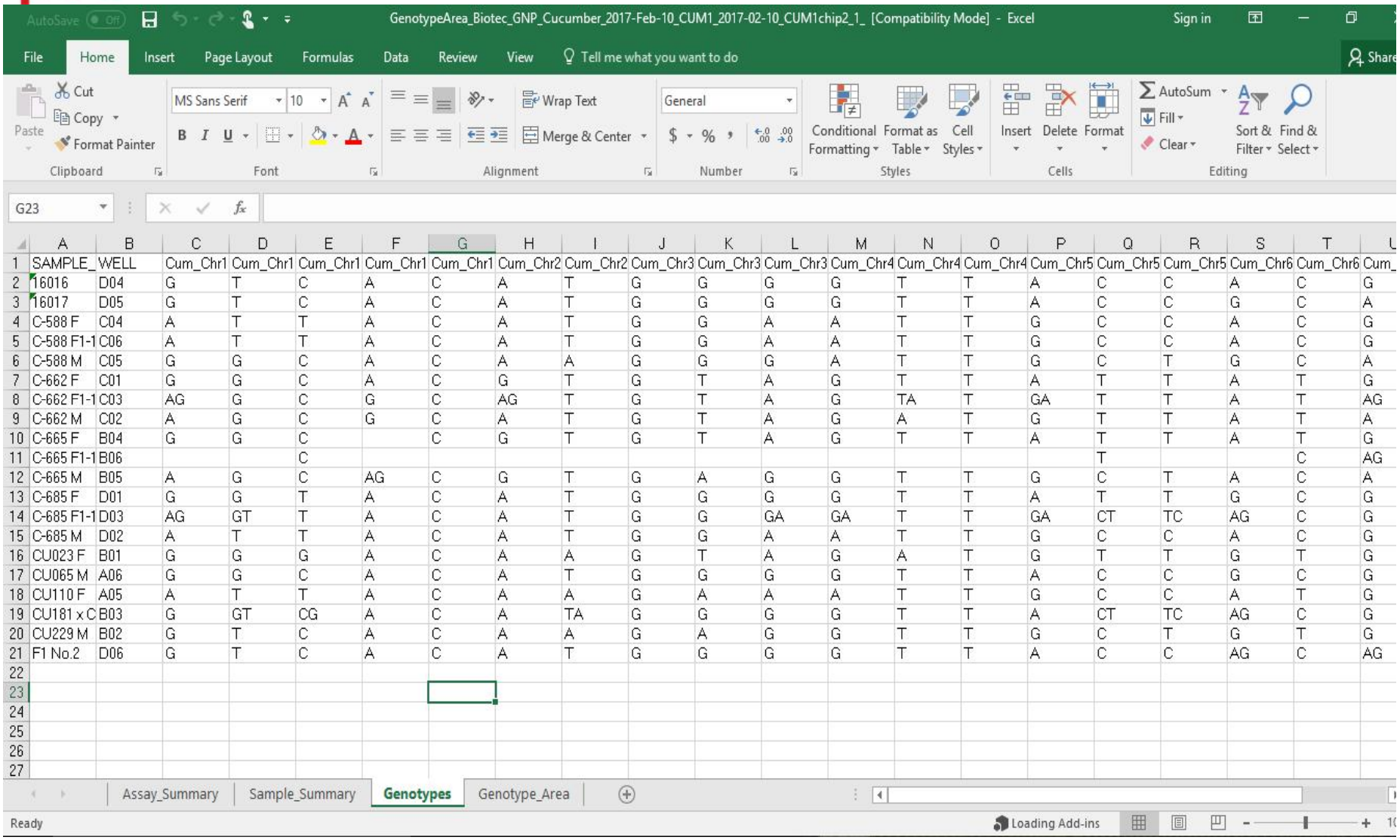
Spectrum of Homozygous Call



Spectrum of Heterozygous Call



Exel file of Genotyping Report



GenotypeArea_Biotec_GNP_Cucumber_2017-Feb-10_CUM1_2017-02-10_CUM1chip2_1_ [Compatibility Mode] - Excel

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
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	Cum_Chr5	Cum_Chr5	Cum_Chr5	Cum_Chr6	Cum_Chr6	Cum_
2	16016	D04	G	T	C	A	C	A	T	G	G	G	G	T	T	A	C	C	A	C	G
3	16017	D05	G	T	C	A	C	A	T	G	G	G	G	T	T	A	C	C	G	C	A
4	C-588 F	C04	A	T	T	A	C	A	T	G	G	A	A	T	T	G	C	C	A	C	G
5	C-588 F1-1	C06	A	T	T	A	C	A	T	G	G	A	A	T	T	G	C	C	A	C	G
6	C-588 M	C05	G	G	C	A	C	A	A	G	G	G	A	T	T	G	C	T	G	C	A
7	C-662 F	C01	G	G	C	A	C	G	T	G	T	A	G	T	T	A	T	T	A	T	G
8	C-662 F1-1	C03	AG	G	C	G	C	AG	T	G	T	A	G	TA	T	GA	T	T	A	T	AG
9	C-662 M	C02	A	G	C	G	C	A	T	G	T	A	G	A	T	G	T	T	A	T	A
10	C-665 F	B04	G	G	C		C	G	T	G	T	A	G	T	T	A	T	T	A	T	G
11	C-665 F1-1	B06			C												T			C	AG
12	C-665 M	B05	A	G	C	AG	C	G	T	G	A	G	G	T	T	G	C	T	A	C	A
13	C-685 F	D01	G	G	T	A	C	A	T	G	G	G	G	T	T	A	T	T	G	C	G
14	C-685 F1-1	D03	AG	GT	T	A	C	A	T	G	G	GA	GA	T	T	GA	CT	TC	AG	C	G
15	C-685 M	D02	A	T	T	A	C	A	T	G	G	A	A	T	T	G	C	C	A	C	G
16	CU023 F	B01	G	G	G	A	C	A	A	G	T	A	G	A	T	G	T	T	G	T	G
17	CU065 M	A06	G	G	C	A	C	A	T	G	G	G	G	T	T	A	C	C	G	C	G
18	CU110 F	A05	A	T	T	A	C	A	A	G	A	A	A	T	T	G	C	C	A	T	G
19	CU181 x C	B03	G	GT	CG	A	C	A	TA	G	G	G	G	T	T	A	CT	TC	AG	C	G
20	CU229 M	B02	G	T	C	A	C	A	A	G	A	G	G	T	T	G	C	T	G	T	G
21	F1 No.2	D06	G	T	C	A	C	A	T	G	G	G	G	T	T	A	C	C	AG	C	AG
22																					
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25																					
26																					
27																					

Assay_Summary | Sample_Summary | Genotypes | Genotype_Area

Ready

Assay Design

Agena BIOSCIENCE

Assay Design Options

- Fully customized options

Outsource

- Assays by Agena
- Certified Service Providers

- Agena or a CSP can design a custom panel for you

In-house

- Assay Design Suite (ADS 2.0 Software)

- Patient-specific panels for ultrasensitive detection
- Disease-specific panels for tumor profiling
- Gene-specific panels for therapy selection
- Mutation-specific panels for validation

Assay Design Suite Software

Online Tools - AgenaCx

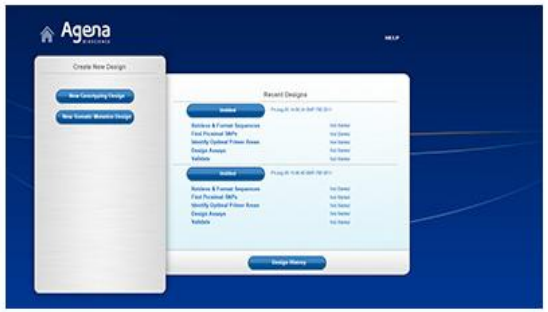
https://agenacx.com/online-tools/

AgenaCx

HI PORNSIRI ASSAWA !

ONLINE TOOLS

ASSAY DESIGN SUITE V2.0



LAUNCH

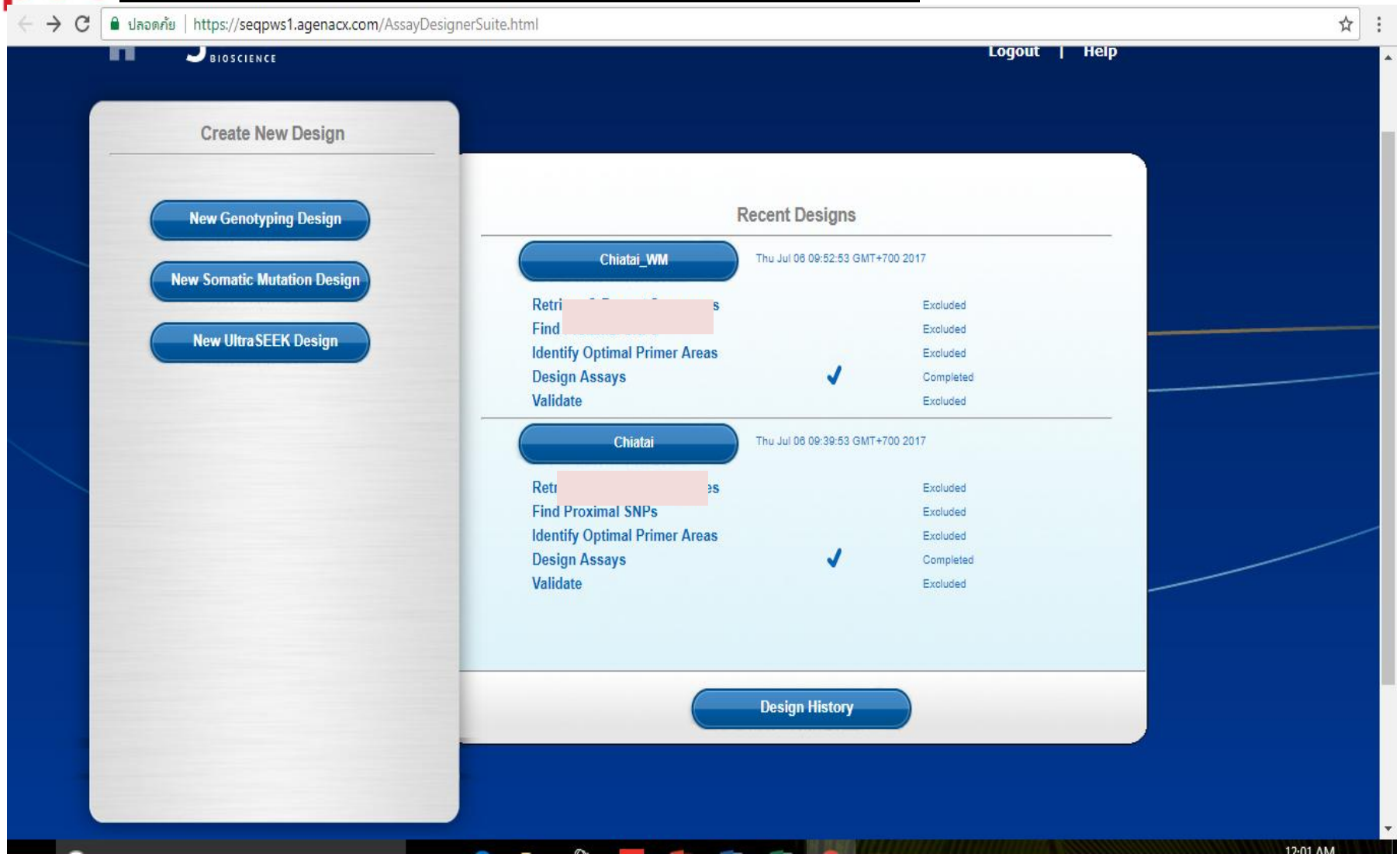
Assay Design Suite is a comprehensive and powerful tool for designing genotyping, somatic mutation, and ultra sensitive assays. The user-friendly interface integrates the design steps of importing RS numbers or sequences, retrieving and formatting

ATTENTION! - Epi Designer Access

We are experiencing difficulties with EpiDesigner and are working to repair the problem. We apologize for any inconvenience, and will soon restore access.

EPIDESIGNER

Assay Design Tool



Application

Applications

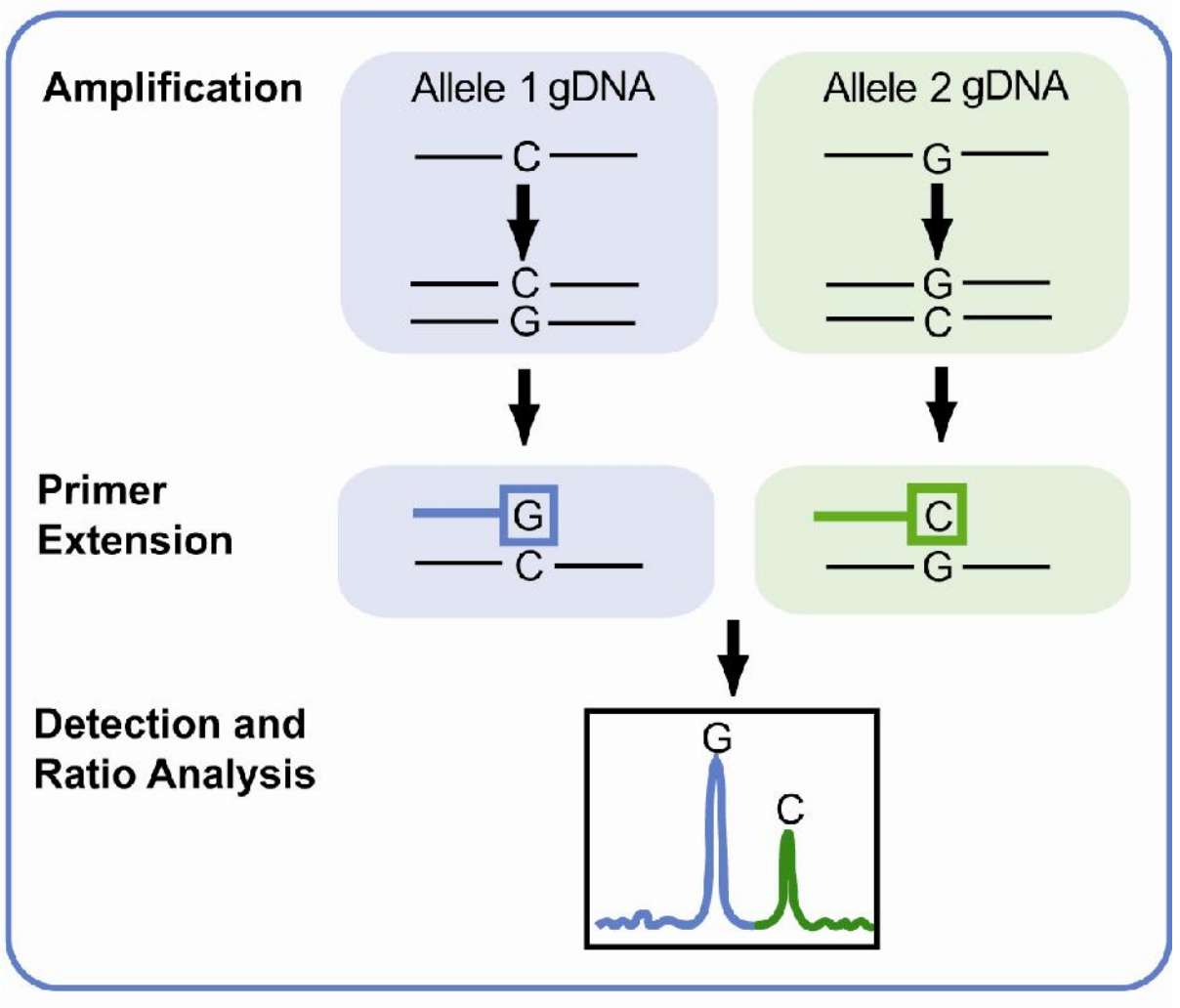
- Oncology :
  - ➞ Lung, Colon Cancer
- Pharmacogenetic
  - ➞ CYP2D6, PGx74
- Carrier Screening and Inherited Disease :
  - ➞ HLA sensitivity, B-Thalassemia
- Infectious Disease :
  - ➞ Pathogen Identification, AMR, HPV Typing
- Agricultural Genetics :
  - ➞ Breeding, QTL Analysis, Crop Strain Validation

Summary

Advantage

- Non-fluorescence based: no background noise
- Multiplexing, Multi-gene testing :
  - ➞ up to 40 markers per well
- High accuracy 99.7%, High call rate > 99%
- Quantitative analysis
- Cost Effective
- High Throughput
- Flexible

Principal of MassARRAY Technology

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BIOSCIENCE