

MassARRAY: The Powerful Technology for Multi-gene Analysis



Present By :
MissChutipat Sriaimsard

Agena
BIOSCIENCE

12 January 2018

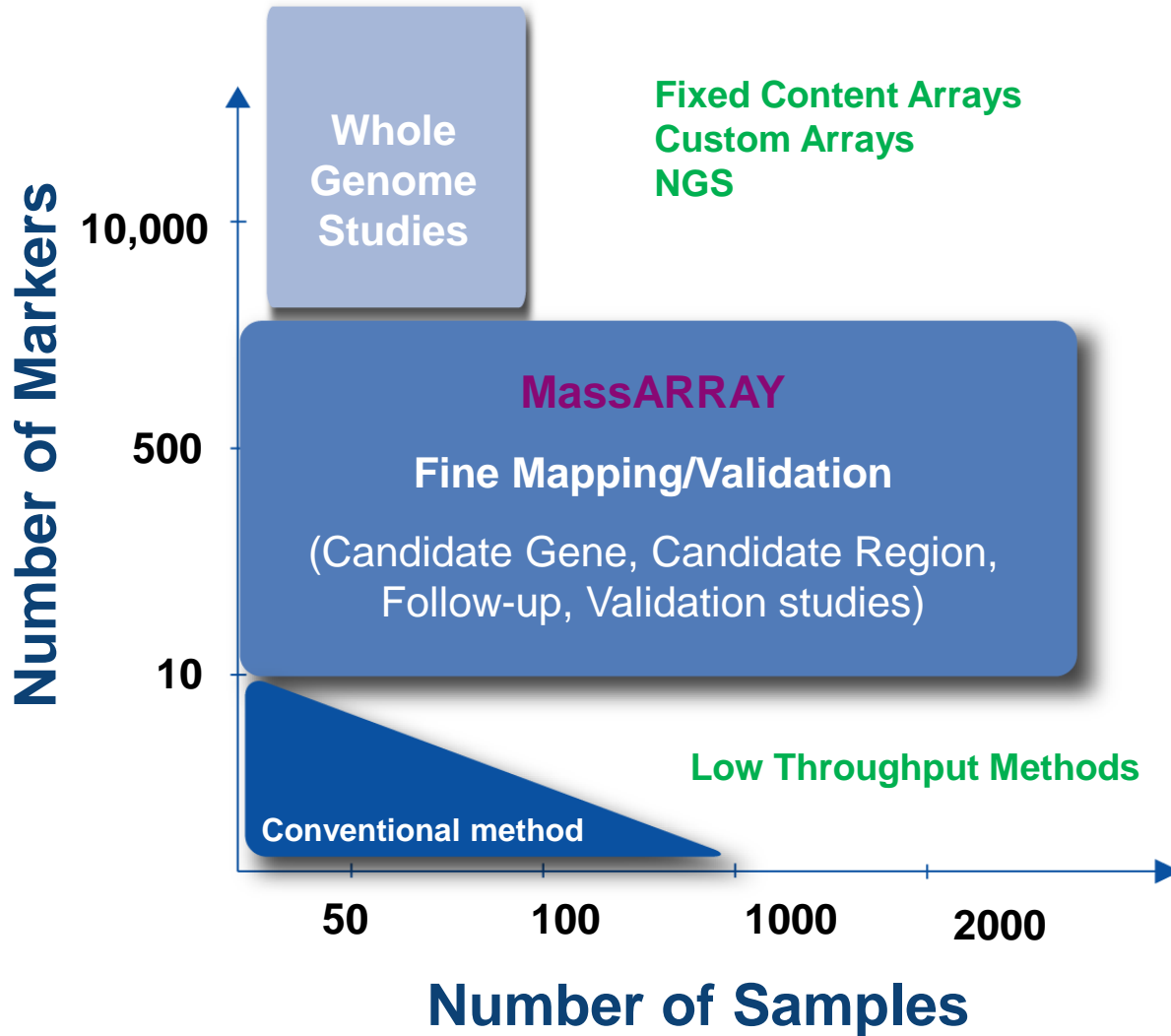
Sci
Spec Your Scientific Specialist

Overview

- Introduction to MassARRAY
 - Principal
 - Data analysis
 - Assay design
- Application of MassARRAY[®]
 - Agricultural Genomics

Genome analysis

Whole genome studies are conducted and followed by fine mapping



MassARRAY[®] System



**Chip prep module
96 well plate**

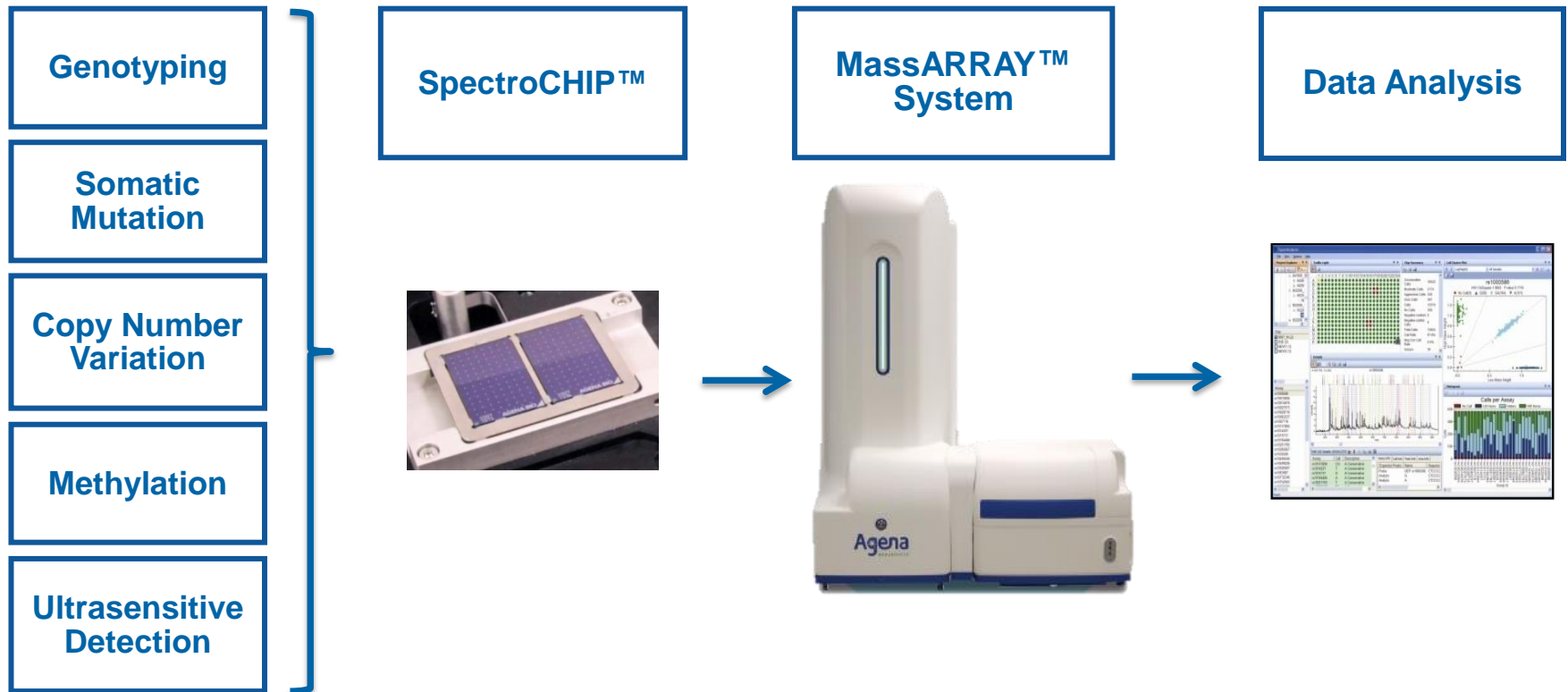


384-well plate platform

Proven Versatility of the MassARRAY® System



MassARRAY™ Applications



The MassARRAY® System for Genotyping

Flexibility and Cost Efficiency in a Single Workflow

- Minimal input DNA → 5-10 ng required per well
- Flexible sample types

✓ Blood plasma

✓ Biopsy

✓ Buccal cells

✓ Serum

✓ Formalin-fixed tissue

✓ Ear punches

✓ Fresh frozen tissue

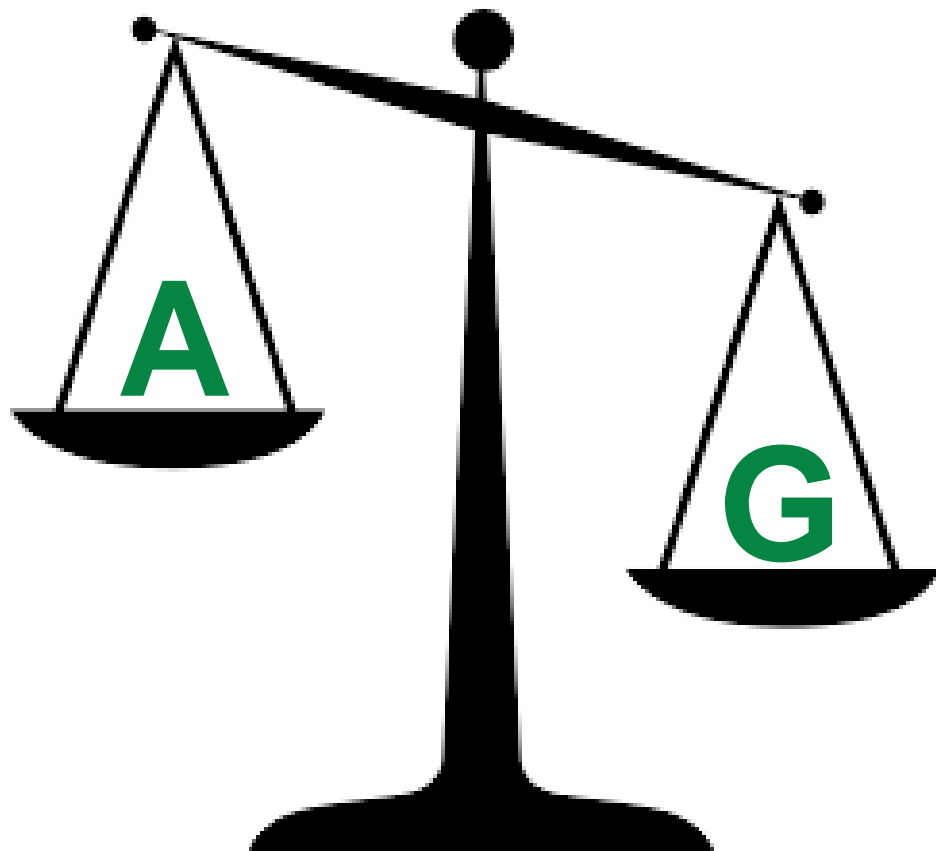
✓ Micro-dissected cells

✓ Hair follicles

– root, seeds, leaf, etc

- Efficient multiplexing → 40+ assays per well

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

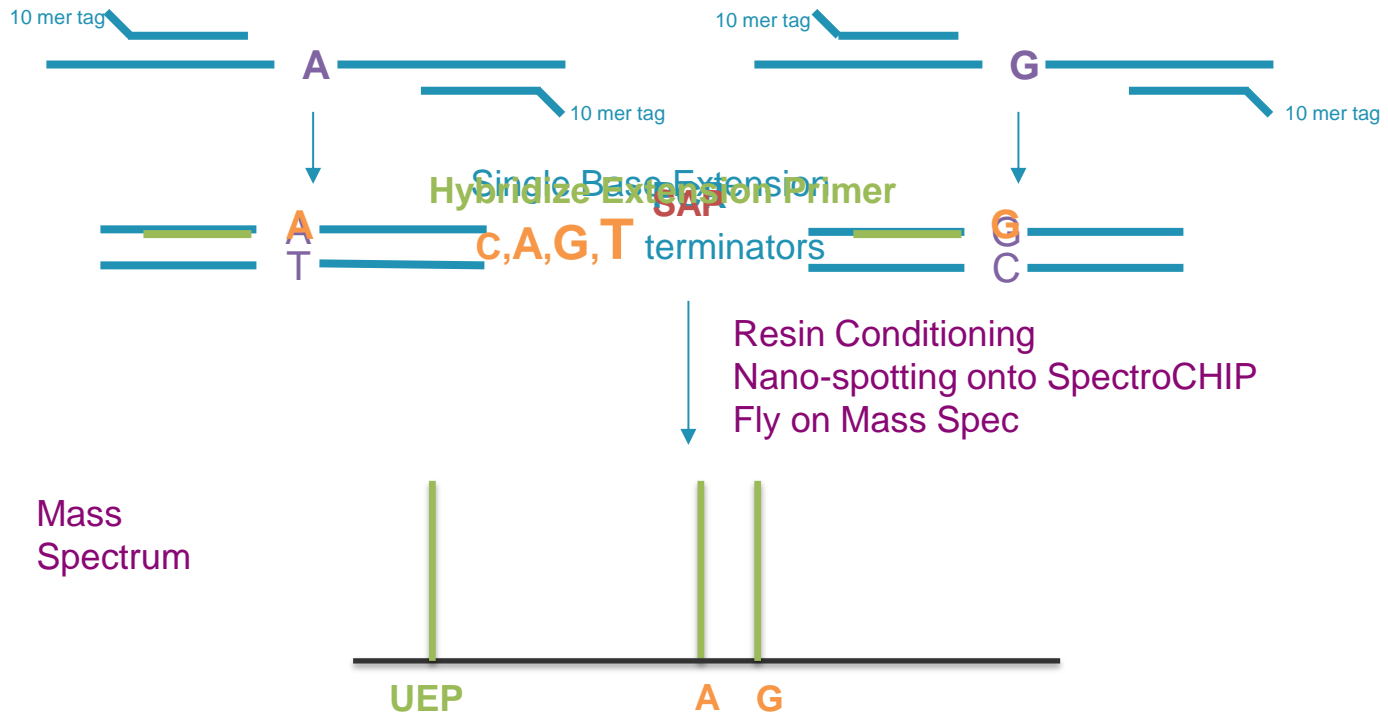
dAMP = 313.2 Da

dGMP = 329.2 Da

GENOTYPING

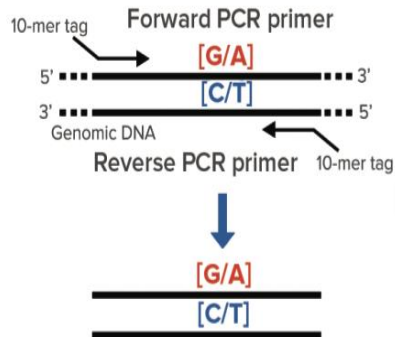
Example of an [A/G] SNP

PCR Reaction (Amplification) → SAP Reaction → Extension Reaction



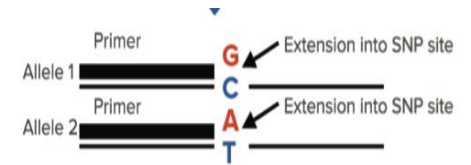
MassARRAY® Process

Amplification

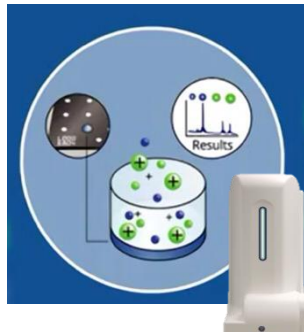


SAP

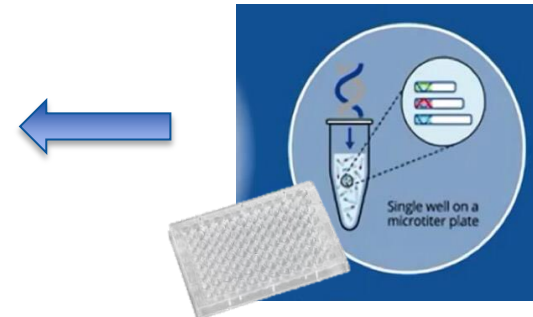
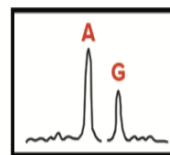
iPLEX®
Extension
Reaction

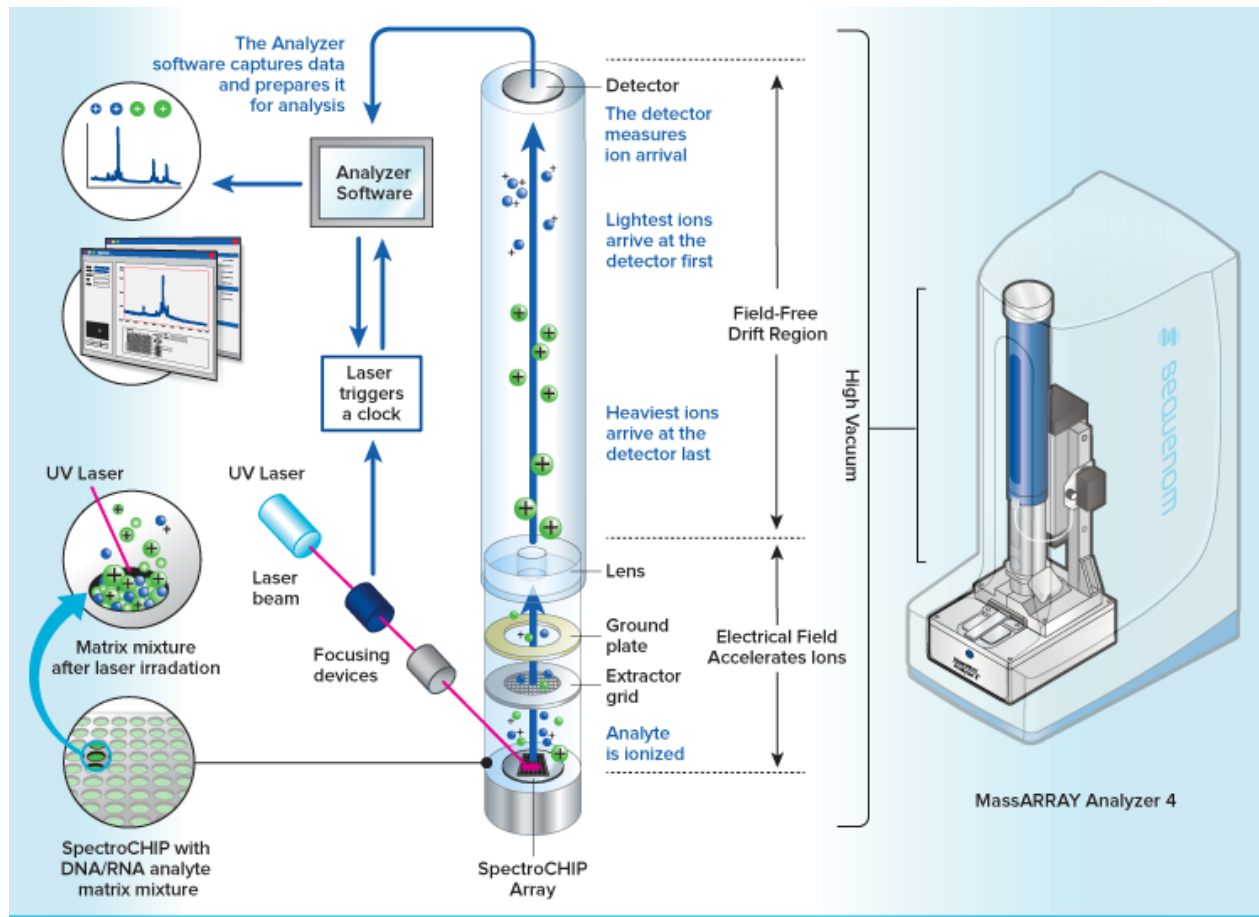


PCR Product



MALDI-TOF Mass
Spectrometry
Analysis

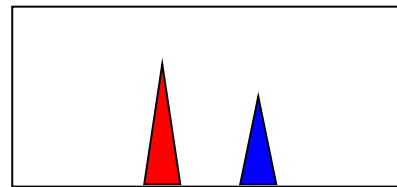
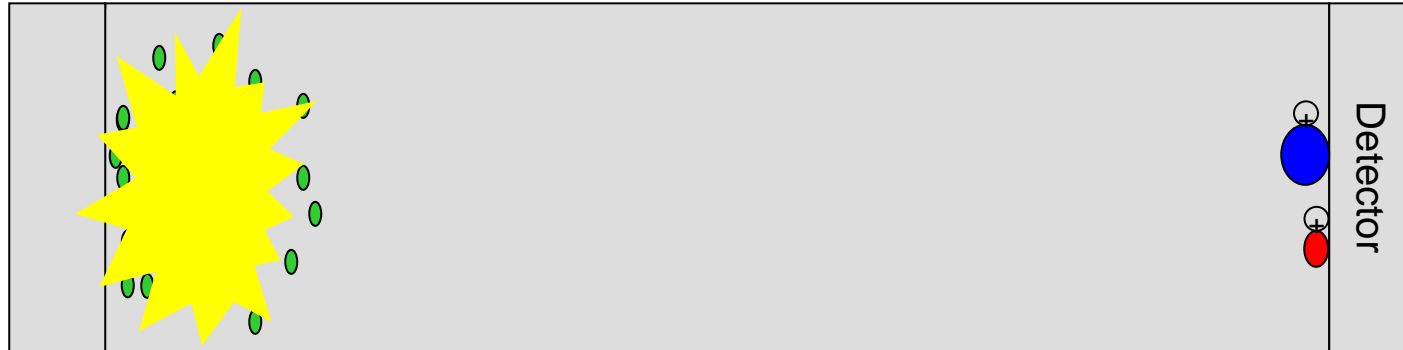




- Molecular mass is a unique intrinsic property.
- The mass of a nucleic acid string is determined by its length and composition (ATCG).

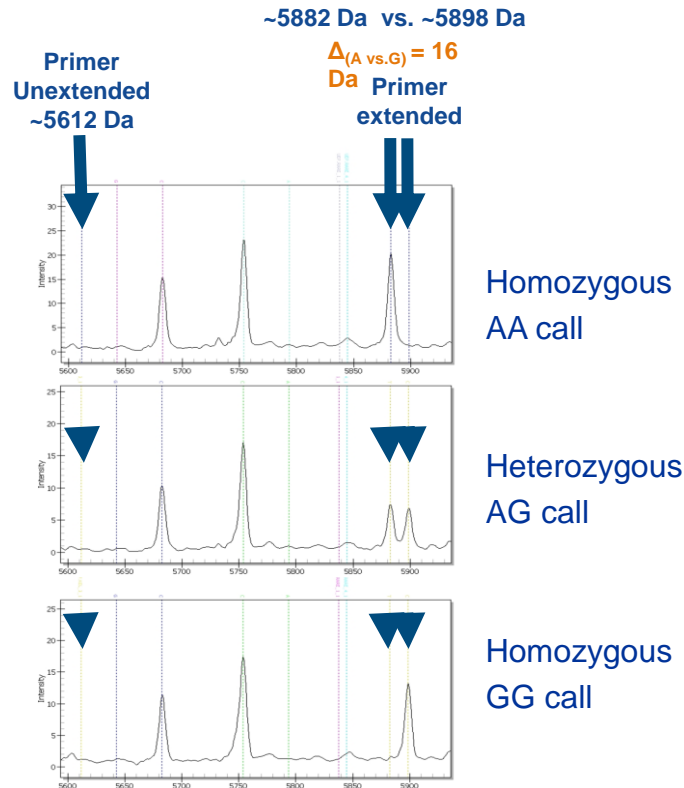
Matrix Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry (MALDI-TOF MS)

Laser Desorption/Ionization



Mass Spectrum m/z

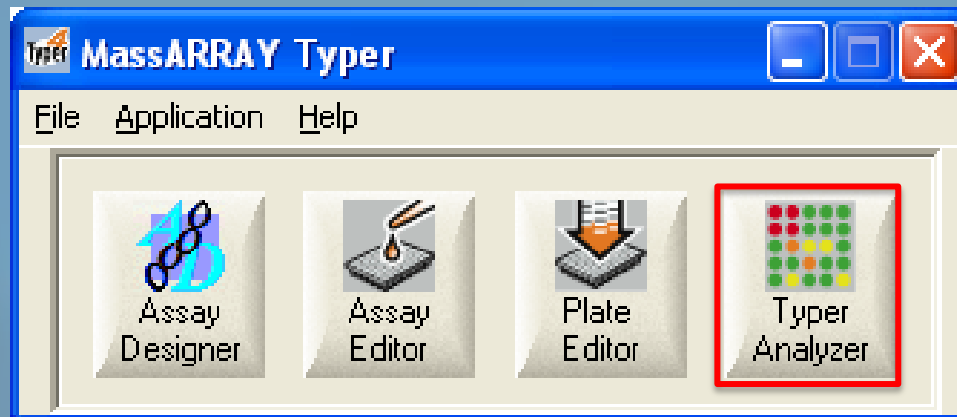
Mass spectra of 3 different SNP [A/G] samples



- MALDI-TOF MS of iPLEX reaction products shows peaks for the unextended primer (5612 Da) and extended primers (1 nucleotide)
- Heterozygous sample show two peaks, one at 5882 Da and the other at 5898 Da
- First two peaks are from other SNP assays in the same multiplex reaction.

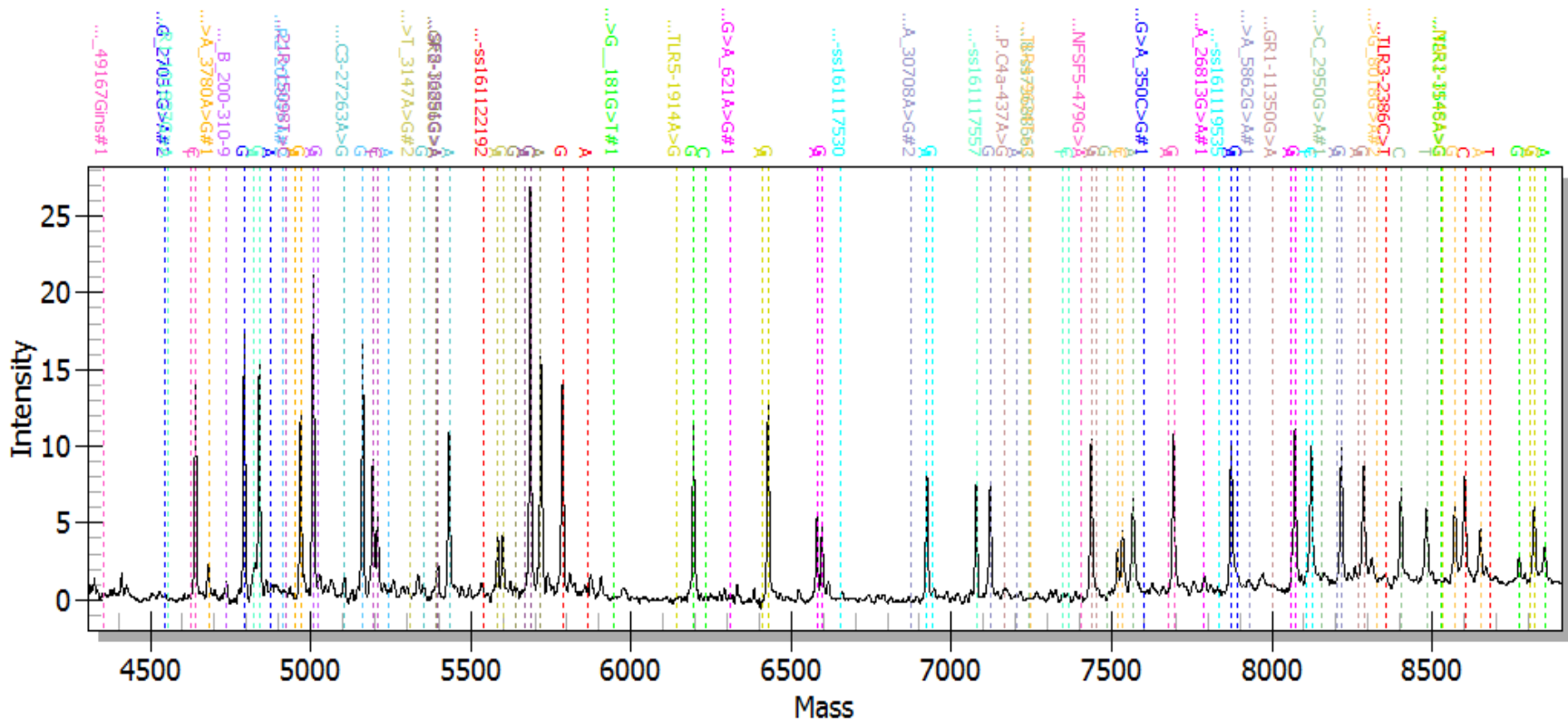
Data Analyzer

Software application suite for Genotyping
& Somatic Mutation Analysis



Typical SNP Panel (31-plex)

The Power of Multiplexing Multiple Markers in a Single Well



Generating Report

The screenshot displays the TyperAnalyzer@seqcom035 software interface. The main window is divided into several panes:

- Project Explorer:** Shows a hierarchical tree of assays and samples. A yellow box labeled "CHIP LIST" highlights a selection of assays.
- Traffic Light:** A grid of colored circles representing assay results. A yellow box labeled "Assay LIST" highlights a specific assay in the list below.
- Assay Summary:** Provides detailed statistics for the selected assay (rs2278725), including call rates and error counts.
- Details:** Displays a mass spectrum plot for the assay, showing intensity versus mass. A red dashed line indicates the expected peak at 6556.555, 14.846.

Assay Summary Data:

Assay:	rs2278725
Chips:	IPLEX/HapMap_assays_and_samples/20070131_IPLXGoldPub_36plex1_(2)
Conservative Calls	378
Moderate Calls	1
Aggressive Calls	0
User Calls	0
Calls	379
No Calls	5
Total Possible Calls	384
Call Rate	98.7
Negative Controls	0
Negative Control Calls	0

Assay List Data:

Assay	Call	Description
rs2274739	T	A.Conservative
rs2278725	CT	A.Conservative
rs2287570	C	A.Conservative
rs2345450	AG	A.Conservative
rs2364430	CT	A.Conservative
rs2375811	A	A.Conservative
rs2381711	GA	C.Aggressive

Mass Spectrum Data:

Mass	Intensity
6556.555, 14.846	~14
6600	~12
6800	~10
7000	~8

Data Analysis

TypewriterAnalyzer

File View Options Tools Help

Project Explorer Traffic Light Details

Customer/Project/Plate/Experiment/Chip

Chip: L0017691_(1) Experiment: 1

Chip Summary

Chip: L0017691_(1)

Conservative Calls: 444

Moderate Calls: 56

Aggressive Calls: 4

User Calls: 0

Calls: 504

No Calls: 24

Total Possible Calls: 528

Call Rate: 95.5

Negative Controls: 0

Assay

Cum_Chr1_1143447

Cum_Chr1_17305528

Cum_Chr1_28662279

Cum_Chr1_28667990

Cum_Chr1_28999369

Cum_Chr2_19978357

Cum_Chr2_8104400

Cum_Chr3_18775

Cum_Chr3_35005861

Cum_Chr3_37762964

Cum_Chr4_17847401

Well: A02 Sample: KYTCU-1

Assay Call Description

Cum_Chr1_11... G A.Conservative

Cum_Chr1_17... T A.Conservative

Cum_Chr1_28... T A.Conservative

Cum_Chr1_28... A A.Conservative

Cum_Chr1_28... C B.Moderate

Cum_Chr2_19... A A.Conservative

Cum_Chr2_81... T A.Conservative

Intensity

Cum_Chr1_28662279

Mass

Expected Peaks Name Sequence Mass

Probe UEP.Cum_Ch... CACACTTAAGCTTTGTTTCATC 6331.10

Analyte C CACACTTAAGCTTTGTTTCATCC 6578.30

Analyte G CACACTTAAGCTTTGTTTCATCG 6618.30

Analyte T CACACTTAAGCTTTGTTTCATCT 6658.20

Automate... Assay Su... Chip Sum... Post Processing Clusters Call Cluster Plot Histogram D:Details

Data Analysis

TypewriterAnalyzer

File View Options Tools Help

Project Explorer Traffic Light Details

Customer/Project/Plate/Experiment

Chip L0017691_(1) (1)

Chip Summary

Chip L0017691_(1)
 Conservative Calls 444
 Moderate Calls 56
 Aggressive Calls 4
 User Calls 0
 Calls 504
 No Calls 24
 Total Possible Calls 528
 Call Rate 95.5
 Negative Controls 0
 Negative Control Calls 0

Cum_Chr1_28662279

Well: A03 Sample: KYTCU-2

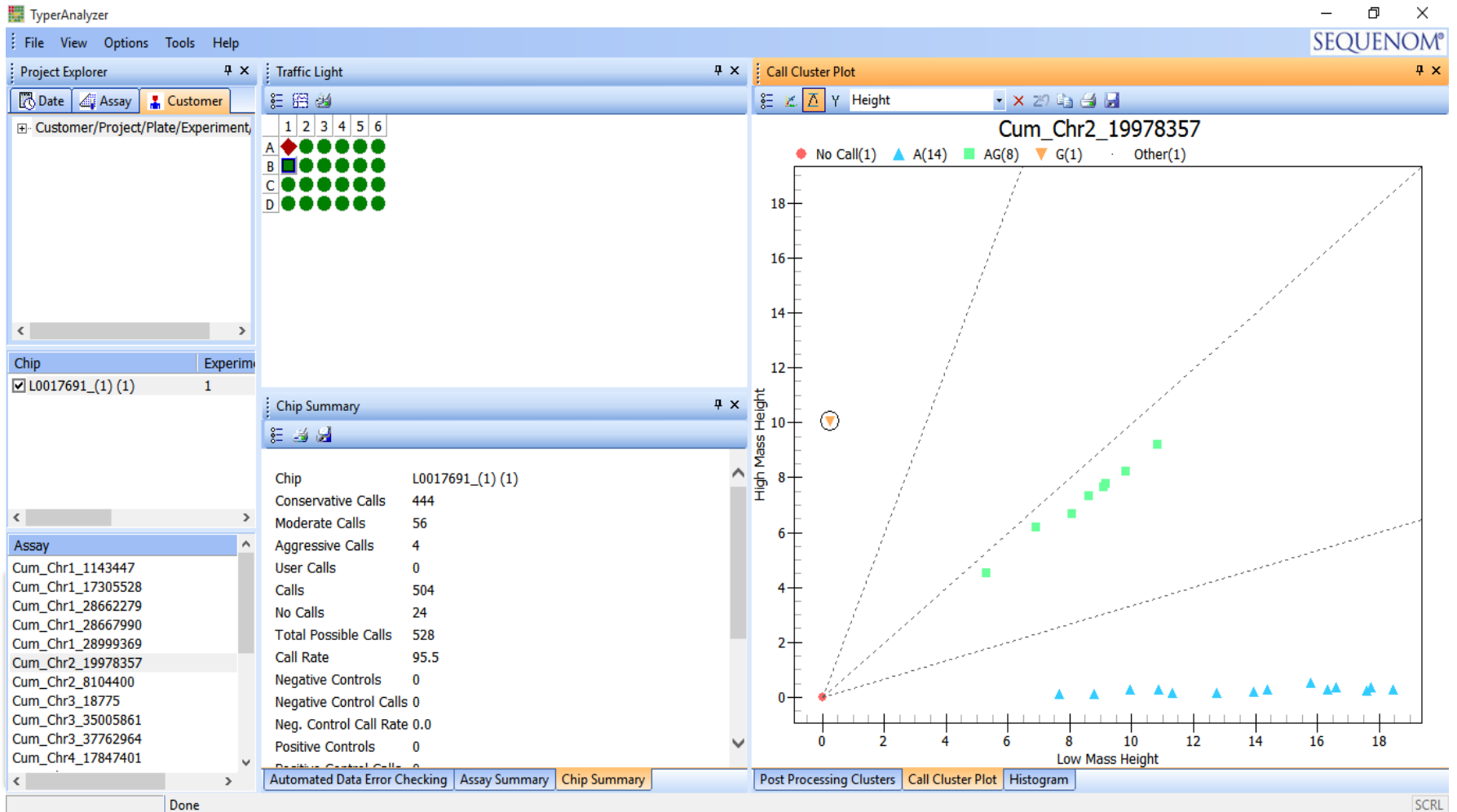
Assay	Call	Description
Cum_Chr1_11...	G	A.Conservative
Cum_Chr1_17...	T	A.Conservative
Cum_Chr1_28...	GT	A.Conservative
Cum_Chr1_28...	A	A.Conservative

Expected Peaks	Name	Sequence	Mass
Probe	UEP.Cum_Ch...	CACACTTAAGCTTTGTTTCATC	6331.10
Analyte	C	CACACTTAAGCTTTGTTTCATCC	6578.30
Analyte	G	CACACTTAAGCTTTGTTTCATCG	6618.30

Automated... Assay Sum... Chip Sum... Post Processing Clusters Call Cluster Plot Histogram Details

Ready

Data Analysis



Data Analysis

B [Compatibility Mode] - Excel

FILE HOME INSERT PAGE LAYOUT FORMULAS DATA REVIEW VIEW

Clipboard Font Alignment Number Styles Cells Editing

E2

	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1	rs1228662	rs1228667	rs1228999	rs2219978	rs2281044	rs3218775	rs3235005	rs3237762	rs4217847	rs4220081	rs4257284	rs5217301	rs5224960	rs5257581	rs6212587	rs6225093	rs6230700	rs7219148	rs7243002	rs728138860	
2	T	A	C	A	T	G	G	G	G	A	T	G	C	C	G	C	A	A	T	A	
3	GT	A	C	A	A	G	G	G	G	A	CT	G	CT	T	A	T	A	G	G	A	
4	T	A	C	A	TA	G	G	G	G	A	CT	G	CT	TC	AG	CT	A	GA	GT	A	
5	T	A	C	A	TA	G	G	G	G	A	T	G	C	TC	AG	CT	A	GA	GT	A	
6	GT	A	C	A	TA	G	G	G	G	A	CT	G	CT	TC	AG	CT	A	GA	GT	A	
7	T	A	C	G	T	G	G	A	G	T	T	A	C	T	A	C	A	G	G	A	
8	T	A	C	A	T	G	G	G	G	A	T	G	C	C	G	C	A	A	T	A	
9	T	A	C	A	T	G	G	GA	G	TA	T	GA	C	TC	AG	C	A	GA	GT	A	
10	T	A	C	AG	T	G	G	GA	G	TA	T	GA	C	TC	AG	C	A	GA	GT	A	
11	T	A	C	AG	T	G	G	GA	G	TA	T	GA	C	TC	AG	C	A	GA	GT	A	
12	T	A	C	AG	T	G	G	GA	G	TA	T	GA	C	TC	AG	C	A	GA	GT	A	
13	T	A	C	AG	T	G	G	GA	G	TA	T	GA	C	TC	AG	C	A	GA	GT	A	
14	T	A	C	A	T	G	G	GA	G	TA	T	GA	C	TC	AG	C	A	GA	GT	A	
15	T	A	C	AG	T	G	G	GA	G	TA	T	GA	C	TC	AG	C	A	GA	GT	A	
16	T	A	C	A	T	G	G	GA	G	T	T	GA	C	TC	AG	C	A	GA	GT	A	
17	T	A	C	A	T	G	G	GA	G	TA	T	GA	C	TC	AG	C	A	GA	GT	A	
18	T	A	C	AG	T	G	G	GA	G	TA	T	GA	C	TC	AG	C	A	GA	GT	A	
19	T	A	C	AG	T	G	G	GA	G	TA	T	GA	C	TC	AG	C	A	GA	GT	A	
20	T	A	C	AG	T	G	G	GA	G	TA	T	GA	C	TC	AG	C	A	GA	GT	A	
21	T	A	C	A	T	G	G	GA	G	TA	T	GA	C	TC	AG	C	A	GA	GT	A	
22	T	A	C	A	T	G	G	GA	G	TA	T	GA	C	TC	AG	C	A	GA	GT	A	
23	T	A	C	A	T	G	G	GA	G	T	T	GA	C	TC	AG	C	A	GA	GT	A	
24	T	A	C	A	T	G	G	GA	G	TA	T	GA	C	TC	AG	C	A	GA	GT	A	
25																					
26																					
27																					
28																					

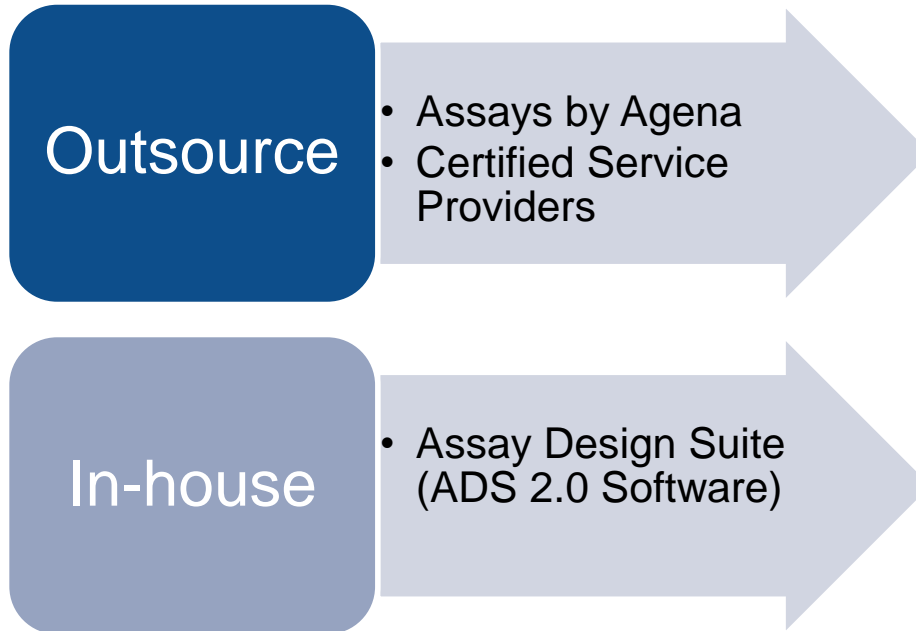
Genotypes

READY 100%

Assay Design

Assay Design Services

Fully customized options



- Assays by Agena
- Certified Service Providers

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



- 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

AgenaCx Account

<https://www.agenacx.com/Home>

HOME

AgenaCx

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If you are a returning user from the old AgenaCx Site, and this is your first visit to our new site, please [update your password here](#) before logging in.

EMAIL

PASSWORD

REMEMBER ME

LOGIN

[Register](#) | [Lost your password?](#)

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AgenaCx provides you with the ability to:

- Access Agena Bioscience's online resources
- View and download the latest AgenaCx data
- Exchange data files securely
- Contact your local Agena Bioscience representative
- View tutorials and training videos
- Log a support request

Our goal is to ensure your success with AgenaCx.

Welcome to the new AgenaCx
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AgenaCx Account

PRODUCT SUPPORT

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SUPPORT

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 sequences, finding proximal SNPs, identifying optimal areas for PCR primers, designing, and verifying multiplexed assays into a single software package. Assay Design Suite also enables users to design control SNPs into the panel, specify high priority SNPs, and use multiple design iterations for increased multiplexing efficiency. The enhanced exporting functionality generates output files for easy oligo ordering.

ATTENTION! - EpiDesigner Access

We are experiencing difficulties with access to EpiDesigner from this page; and are working to repair the problem. Temporarily, you may access EpiDesigner at <http://www.epidesigner.com>. We apologize for any inconvenience.

EPIDESIGNER

LAUNCH

EpiDesigner is a tool for designing quantitative methylation assays for

AgenaCx Account

The screenshot displays the Agena Bioscience web interface. On the left, a 'Create New Design' sidebar contains three buttons: 'New Genotyping Design' (highlighted with a red box), 'New Somatic Mutation Design', and 'New UltraSEEK Design'. A red arrow points from this button to a central modal window. The modal window is titled 'Design Type: Genotyping' and contains the following fields and buttons:

- Design Name: Untitled
- Version: [dropdown]
- Current input: None [View]
- rs or FASTA: [Edit Text Input]
- Files: [File Upload]
- Redesign options: [dropdown]
- Preset: [dropdown]
- Organism: Human
- Database: Dec.2013(GRCh38/hg38)
- Chemistry: iPLEX
- Multiplex Level: 36
- [Advanced Settings]

On the right, a workflow table shows the following steps:

Step	Task	Status
1	Retrieve & Format Sequences	Not Started
2	Find Proximal SNPs	Not Started
3	Identify Optimal Primer Areas	Not Started
4	Design Assays	Not Started
5	Validate	Not Started

Below the table are 'Export All' and 'Begin Run' buttons. At the bottom of the main interface, there is a 'Design Assays' section with a 'Validate' button, a 'Rejects Completed' status with a checkmark, and a 'Design History' button.

Oligo order excel file

Oligo Order - Excel

SNP ID	Forward Primer ID	Forward Primer Sequence	Reverse Primer ID	Reverse Primer Sequence	Extended Primer ID	Extended Primer Sequence
rs1_W1	rs1_W1_F	ACGTTGGATGATGAGACAAAGGTAGGTATC	rs1_W1_R	ACGTTGGATGTCTGTCAAAGACATGTGCC	rs1_W1_E	GCCAAACCCTCCCTC
rs2_W1	rs2_W1_F	ACGTTGGATGGCTGAAAGTTTCAAACCTCG	rs2_W1_R	ACGTTGGATGCTCCTTCTATCACCACAATG	rs2_W1_E	ACGTCACGACAAGT
rs3_W1	rs3_W1_F	ACGTTGGATGTAACGCCTCATAGATCCCAG	rs3_W1_R	ACGTTGGATGGGTTGATTTGGTTACCGGTG	rs3_W1_E	cGAACCGCCGATCGGTA
rs4_W1	rs4_W1_F	ACGTTGGATGTAATACTCCACCACCTCCAG	rs4_W1_R	ACGTTGGATGTTGCCTGTAGCTGATGTTGG	rs4_W1_E	GAAGAGTTAAACGACGC
rs5_W1	rs5_W1_F	ACGTTGGATGTTGGAAGCTGAATCTGGAGG	rs5_W1_R	ACGTTGGATGCTTTTCGATTGTACGCTGG	rs5_W1_E	TCAATCTTGATCGCCTT
rs6_W1	rs6_W1_F	ACGTTGGATGTTGGTCTGCTTTAGTCTCCC	rs6_W1_R	ACGTTGGATGTTACAGGGCTCCAACATC	rs6_W1_E	GCCGGAGGTTATCAATTA
rs7_W1	rs7_W1_F	ACGTTGGATGTTGGCAAACGAACAACAGGC	rs7_W1_R	ACGTTGGATGCTTCATGATAGCAAGTGGTC	rs7_W1_E	GCAAAGAGCTTGAGGATG
rs8_W1	rs8_W1_F	ACGTTGGATGACCGGAACCAAACTCATC	rs8_W1_R	ACGTTGGATGGCGTACAGATCCTTCAATC	rs8_W1_E	TCAATTCTTGCTCTAGAGA
rs9_W1	rs9_W1_F	ACGTTGGATGATGCTGAAGCTTCCAGAAC	rs9_W1_R	ACGTTGGATGAAACAAGTCGAGAAAAAGG	rs9_W1_E	tTTTACTCCCTTAGTCCTT
rs10_W1	rs10_W1_F	ACGTTGGATGTA AAAAGCCCCAGGATAAAG	rs10_W1_R	ACGTTGGATGTCAGGTATGAGTGTCCATGC	rs10_W1_E	GCTTCAA AAAATTCATCGAGT
rs11_W1	rs11_W1_F	ACGTTGGATGCGTAGAGGATAACTACGACC	rs11_W1_R	ACGTTGGATGCCAAGATGGCAAACACTACC	rs11_W1_E	ccccACCTGATGCTTCAAAC
rs12_W1	rs12_W1_F	ACGTTGGATGCAGTGACTCGTATACTAAAGC	rs12_W1_R	ACGTTGGATGCCAAAAC TAGCGGAAGAAC	rs12_W1_E	ATAAACTTCTACGAGACCAA

Distinct advantages

Of MassARRAY for Nucleic Acid Analysis

1. Don't use fluorescence

- Mass of the actual bioanalyte is detected - 4 decimal place accuracy
- No non-specific background issues – background is a different mass

2. System is quantitative

- Many biological phenomena need to be accurately quantified
- Allele ratios, gene copy number, methylation

3. Multiplexed assays

- Provides high throughput
- Cost effectiveness

4. Very sensitive and flexible

- High accuracy – published 99.7%
- High genotyping call rates (+98%)
- Numbers of samples and markers are easily scaled
- Simple and flexible assay design with little optimization required
- Comprehensive Genetic Analysis >> Somatic mutation, Rare mutation, SNP, Epigenetic

Application of MassARRAY® in Agricultural Genomics

Applications in Agricultural Genomics

- Parentage verification for plant breeding
- Crop strain validation
- Marker assisted breeding
- Candidate genetic marker evaluation
- Genetic Mapping and QTL analysis
- Phenotype selection
- SNP validation
- Etc.

MassARRAY- SNPs detection in Oil palm

Ting et al. *BMC Genomics* (2016) 17:289
DOI 10.1186/s12864-016-2607-4

BMC Genomics

RESEARCH ARTICLE

Open Access



Fine-mapping and cross-validation of QTLs linked to fatty acid composition in multiple independent interspecific crosses of oil palm

[Ting NC](#)^{1,2}, [Yaakub Z](#)¹, [Kamaruddin K](#)¹, [Mayes S](#)³, [Massawe F](#)², [Sambanthamurthi R](#)¹, [Jansen J](#)⁴, [Low LE](#)¹, [Ithnin M](#)¹, [Kushairi A](#)¹, [Arulandoo X](#)⁵, [Rosli R](#)¹, [Chan KL](#)¹, [Amiruddin N](#)¹, [Sriharan K](#)⁵, [Lim CC](#)⁵, [Nookiah R](#)¹, [Amiruddin MD](#)¹, [Singh R](#)⁶.

Candidate SNP markers (designated SNPE) flanking various genes associated with FA and oil biosynthesis were mined from the P5 genome build. The oil palm SNP assay design and genotyping were performed by a service provider, Agena Bioscience, Inc. (San Deigo, California) using the iPLEX[®] biochemistry on MassArray[®] system [23]. A custom two-multiplexed genotyping assay was designed and optimized for a panel of 40 SNPs using the Assay Design Suite 1.0 software (Agena Bioscience, Inc. San Deigo, California)

MassARRAY- SNPs detection in Oil palm



HHS Public Access

Author manuscript

Nature. Author manuscript; available in PMC 2014 October 26.

Published in final edited form as:

Nature. 2013 August 15; 500(7462): 340–344. doi:10.1038/nature12356.

The oil palm *Shell* gene controls oil yield and encodes a homologue of SEEDSTICK

Rajinder Singh¹, Eng-Ti Leslie Low¹, Leslie Cheng-Li Ooi¹, Meilina Ong-Abdullah¹, Ting Ngoot Chin¹, Jayanthi Nagappan¹, Rajanaidu Nookiah¹, Mohd Din Amiruddin¹, Rozana Rosli¹, Mohamad Arif Abdul Manaf¹, Kuang-Lim Chan¹, Mohd Amin Halim¹, Norazah Azizi¹, Nathan Lakey², Steven W Smith², Muhammad A Budiman², Michael Hogan², Blaire Bacher², Andrew Van Brunt², Chunyan Wang², Jared M Ordway², Ravigadevi Sambanthamurthi^{1,4}, and Robert A Martienssen^{3,4}

These 80 SNP markers (designated as SNPE) were genotyped in the T128 selfed population using the Sequenom MassArray® iPlex platform.

Rice Genotyping - Cornell Rice Panel

Accessions



Donor parent

- *O. rufipogon* – IRGC 105491
- Aus-like ancestor
- No agronomic traits of interest
- Crosses well with both *indica* and *japonica* varieties

Recurrent parent

- *O. sativa* – Jefferson
- Tropical japonica
- High-yield
- Semi-dwarf plant type
- High level of disease resistance
- Early maturity



Pure seed plot of NIL 43_1-2 in Beaumont, Texas

Fig 1. Parent accessions: *O. rufipogon* inflorescences (a) and seed (b), Jefferson inflorescences (c) and seed (d)

Imai, I., McCouch, S. R., and McClung, A. M. (2011) Plant and Animal Genomics, San Diego, California.

- Collaborative project among IRRI, JIRCAS, U. of Calcutta, U. of Aberbeen, Arkansas, Duke
- 18,760 SNPs discovered on Affymetrix 44K rice chip
- 84 SNPs for higher resolutions of key traits for MassARRAY panel
- Currently designing MassARRAY panels arranged by chromosome

8 SNP Marker Analysis for Quality and Agronomic Traits (rice)

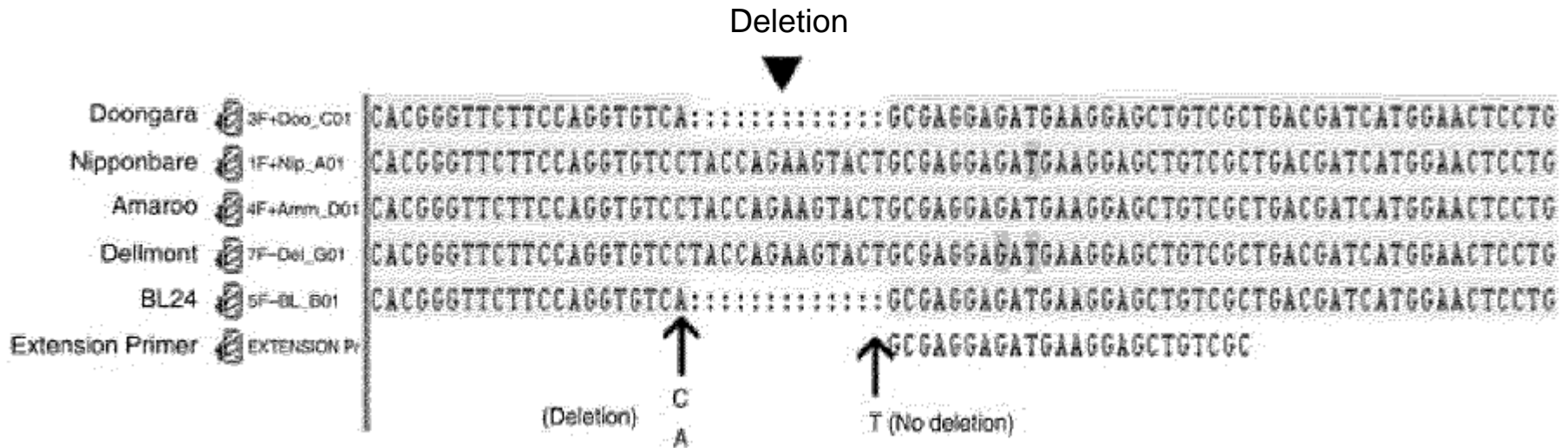
Loci	Functions	Mutation and traits
<i>sd-1</i>	gibberellin 20-oxidase	C/T mutation reduces plant height and increases yield
<i>sd-del</i>		380-383bp deletion increases yield
<i>Pi-ta</i>	928-aa polypeptide	G: blast resistant; T: susceptible
<i>waxyIN1</i>	granule-bound starch synthase	Starch quality. A: low amylose; G: high amylose
<i>waxyEX6</i>		Starch quality. A: low amylose; T: high amylose
<i>alk3</i>	Starch synthase	Cooking and eating quality. alk3 'G'+alk4"GC": high gelatinization temperature and low alkali spreading
<i>alk4</i>		
<i>fgr</i>	BADH2	8bp deletion results in fragrant rice

Conclusion: MassARRAY is a powerful tool for SNP detection and confirmation in rice and can be applied to segregating populations in breeding programs for traits selection.

Masouleh, A. K., et al. *Plant Biotech J.* 2009. 7:355-63

Rice SNP Marker Analysis: Ability to Detect Deletions

MassARRAY iPLEX Gold assays are capable to detect large or small deletions.



Masouleh, A. K., et al. *Plant Biotech J.* 2009. 7:355-63

SNP Identification and Panel Development for *BADH1* Haplotype Association Study

Haplotypes of the *BADH1* Gene in 92 Diverse Rice Varieties

Variety	S-1	S-2	S-3	S-4	S-5	S-6	S-8	S-9	S-11	S-14	S-15	S-16	S-17	S-18	S-19	SNP Haplotype	Protein Haplotype	Frequency
Jaya	G	C	G	T	T	A	A	C	C	T	T	T	T	A	T	SH1	PH1	38
ADT43	A	C	G	T	T	A	A	G	C	T	T	C	T	A	T	SH2	PH1	19
Basmati 370	G	C	A	A	C	T	G	G	T	T	C	C	C	C	T	SH3	PH2	17
Taraori Basmati	G	A	A	A	T	T	G	G	T	T	C	C	C	C	T	SH4	PH2	6
Kalanamak 3119	G	C	A	T	T	A	A	G	C	A	T	C	C	A	C	SH5	PH3	2
Taipai 309	G	C	A	A	T	T	G	G	T	T	C	C	C	C	T	SH6	PH2	1
Jyothi	G	C	G	T	C	A	G	C	C	T	T	T	T	A	T	SH7	PH1	1
Pusa 44	G	C	G	T	T	A	A	G	C	T	T	T	T	A	T	SH8	PH1	1
SKR 126	G	C	G	T	T	A	A	G	C	T	T	C	T	A	T	SH9	PH1	1
CSR 10	G	C	G	T	T	T	G	G	T	T	T	C	T	A	T	SH10	PH4	1
IR 64	G	C	G	T	T	A	A	C	C	T	T	T	C	A	T	SH11	PH1	1
Pusa 1266	G	C	A	A	C	T	G	C	T	T	C	C	C	C	T	SH12	PH2	1
Kasturi	G	C	A	T	C	T	G	G	T	T	C	C	C	C	T	SH13	PH2	1
Pusa 1121	A	C	G	T	T	A	A	C	C	T	T	C	T	A	T	SH14	PH1	1
Pant Dhan 4	G	C	G	T	T	A	A	C	C	T	T	T	T	C	T	SH15	PH5	1

Conclusion: There is no association between salt tolerance and the *BADH1* haplotypes

A high-throughput assay for rapid and simultaneous analysis of perfect markers for important quality and agronomic traits in rice using multiplexed MALDI-TOF mass spectrometry

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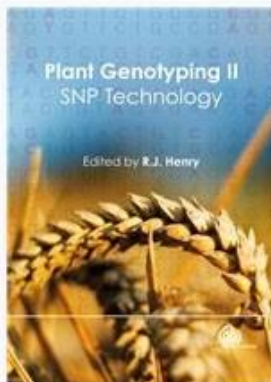
Summary

The application of single nucleotide polymorphisms (SNPs) in plant breeding involves the analysis of a large number of samples, and therefore requires rapid, inexpensive and highly automated multiplex methods to genotype the sequence variants. We have optimized a high-throughput multiplexed SNP assay for eight polymorphisms which explain two agronomic and three grain quality traits in rice. Gene fragments coding for the agronomic traits plant height (semi-dwarf, *sd-1*) and blast disease resistance (*Pi-ta*) and the quality traits amylose content (*waxy*), gelatinization temperature (*alk*) and fragrance (*fgr*) were amplified in a multiplex polymerase chain reaction. A single base extension reaction carried out at the polymorphism responsible for each of these phenotypes within these genes generated extension products which were quantified by a matrix-assisted laser desorption/ionization-time of flight system. The assay detects both SNPs and indels and is co-dominant, simultaneously detecting both homozygous and heterozygous samples in a multiplex system. This assay analyses eight functional polymorphisms in one 5 µL reaction, demonstrating the high-throughput and cost-effective capability of this system. At this conservative level of multiplexing, 3072 assays can be performed in a single 384-well microtitre plate, allowing the rapid production of valuable information for selection in rice breeding.

Keywords: matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF), multiplex perfect markers, rice, single nucleotide polymorphism.

CABI Book Info

Plant genotyping II: SNP technology



Description

This book aims to describe some of the important recent developments in plant genotyping. It is based upon a second workshop held recently to review progress in this area. Recent developments focus on high-throughput methods and generally target single nucleotide polymorphism (SNP) discovery and analysis. The topics covered include: SNP discovery in plants; SNPs and their use in maize; rare SNP discovery with endonucleases; sequence polymorphisms in the flanking regions of microsatellite markers; SNP discovery by ecotilling using capillary electrophoresis; genotyping by allele-specific PCR; the MassARRAY system for plant genomics; mutation screening; nanotechnology (the future of cost-effective plant genotyping); functionally associated molecular genetic markers for temperate pasture plant improvement; genotyping for rice eating qualities; towards universal loci for plant genotyping; DNA banks as a resource for SNP genotyping; DNA extraction from plant tissue; future prospects for plant genotyping.

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Descriptor(s)

[alleles](#)

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Book Chapters

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Author(s): Edward, K. J. Poole, R. L. Barker, G. L.
- Chapter: 2 (Page no: 30) **SNPs and their use in maize.**
Author(s): Rafalski, A. Tingey, S.
- Chapter: 3 (Page no: 44) **Rare SNP discovery with endonucleases.**
Author(s): Cross, M. J.
- Chapter: 4 (Page no: 68) **Sequence polymorphisms in the flanking regions of microsatellite markers.**
Author(s): Ablett, G. Henry, R. J.
- Chapter: 5 (Page no: 78) **Snp discovery by ecotiling using capillary electrophoresis.**
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- Chapter: 6 (Page no: 88) **Genotyping by allele-specific PCR.**
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- Chapter: 8 (Page no: 114) **Mutation screening.**
Author(s): Izquierdo, L.
- Chapter: 9 (Page no: 133) **Nanotechnology: the future of cost-effective plant genotyping.**
Author(s): Pattemore, J. A. Trau, M. Henry, R. J.
- Chapter: 10 (Page no: 154) **Functionally associated molecular genetic markers for temperate pasture plant improvement.**
Author(s): Forster, J. W. Cogan, N. O. I. Dobrowolski, M. P. Francki, M. G. Spangenberg, G. C. Smith, K. F.
- Chapter: 11 (Page no: 187) **Genotyping for rice eating qualities.**
Author(s): Bradbury, L. M. T. Waters, D. L. E. Henry, R. J.
- Chapter: 12 (Page no: 195) **Towards universal loci for plant genotyping.**
Author(s): Pacey-Miller, T.

polymerase chain reaction
reviews

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Livestock Genotyping

Assays by Agena: Bovine Panels

- U.S. Department of Agriculture (USDA) Markers selected from HapMap project
- Genome wide SNPs with high MAF >0.41 selected for discrimination of inbred dairy herds
- 2 well panels for parentage verification with 100-121 SNPs per International Society for Animal Genetics (ISAG) recommendations
- Additional SNPs/wells for disease susceptibility and trait identification



Pig Identification and Parentage Exclusion



Rohrer, G. A. et al. *Animal Genetics*. 2007.
38 (3): 253–258

Comparison of 60 SNPs & 10 microsatellite markers

- Designed panel with 80 SNPs
 - 155 boars that represent four US purebred populations tested representative of US purebred Duroc, Hampshire, Landrace and Yorkshire populations
 - 60 SNPs w/ MAF >0.15 selected for the final panel of 60 markers
- Parentage Exclusion Probability
 - One parent: 0.9974 (all data)
 - Ranged from 0.9594 to 0.9963 within breeds.
- Sire Exclusion Probability
 - When dam's genotype is known: 0.99998 (all data)
 - Ranged from 0.99868 to 0.99997 within breeds

Parental exclusion probabilities for SNP and microsatellite marker panels were similar, but genotyping was more sensitive for individual identification.



Relationships among *calpastatin* single nucleotide polymorphisms, *calpastatin* expression and tenderness in pork longissimus¹

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Summary

Genome scans in the pig have identified a region on chromosome 2 (SSC2) associated with tenderness. *Calpastatin* is a likely positional candidate gene in this region because of its inhibitory role in the calpain system that is involved in postmortem tenderization. Novel single nucleotide polymorphisms (SNP) in *calpastatin* were identified and used to genotype a population ($n = 1042$) of Duroc–Landrace–Yorkshire swine for association with longissimus lumborum slice shear force (SSF) measured at days 7 and 14 postmortem. Three genetic markers residing in the *calpastatin* gene were significantly associated with SSF ($P < 0.0005$). Haplotypes constructed from markers in the *calpastatin* gene were significantly associated with SSF (F -ratio = 3.93; P -value = 0.002). The levels of normalized mRNA expression of *calpastatin* in the longissimus lumborum of 162 animals also were evaluated by real-time RT-PCR and were associated with the genotype of the most significant marker for SSF ($P < 0.02$). This evidence suggests that the causative variation alters expression of *calpastatin*, thus affecting tenderness. In summary, these data provide evidence of several significant, publicly available SNP markers associated with SSF that may be useful to the swine industry for marker assisted selection of animals that have more tender meat.

Keywords *calpastatin*, gene expression, meat quality, pig, single nucleotide polymorphism.

Predictive SNPs in Calpastatin for Pork Tenderness



Nonneman, D. et al. J. Anim. Sci. 2011: 2010-3556

- 194 SNPs in calpastatin locus on pig chr 2 were identified by re-sequencing
- SNP Genotyping on MassARRAY using 40 pigs
- 37 SNPs significantly associated with slice shear force in USMARC population
- 4 SNPs significantly associated with tenderness, juiciness, chewiness, and other flavor scores in all 4 populations

SNP markers identified by the association study should be predictive of pork tenderness in industry populations

MassARRAY™ Customers List

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- Kansas State University
- etc





Questions

