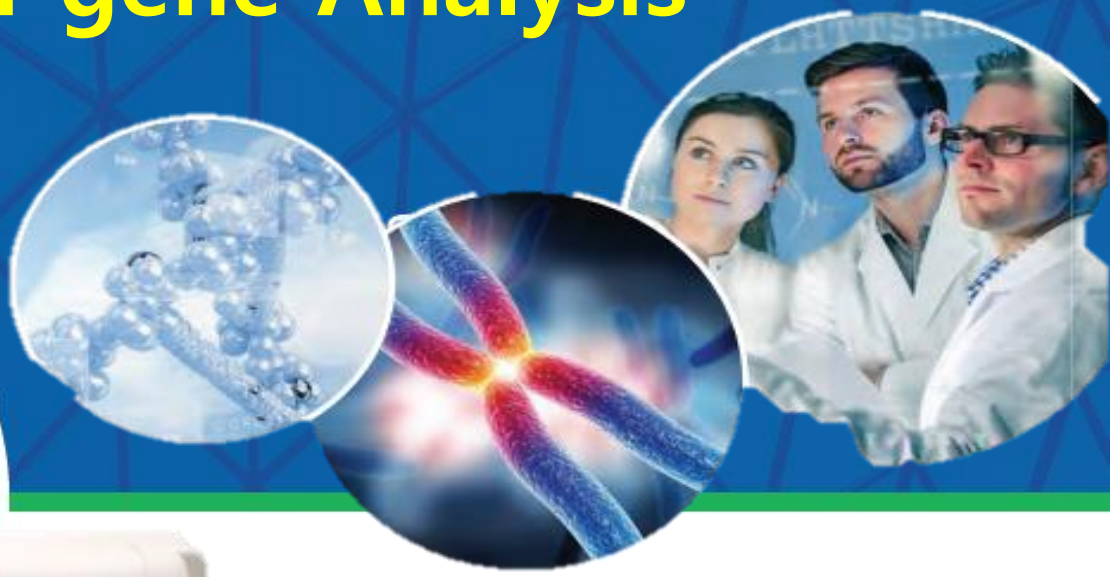
MassARRAY: The Powerful Technology for Multi-gene Analysis



Present By :
MissChutipat Sriaimsaard

Agena BIOSCIENCE

12 January 2018

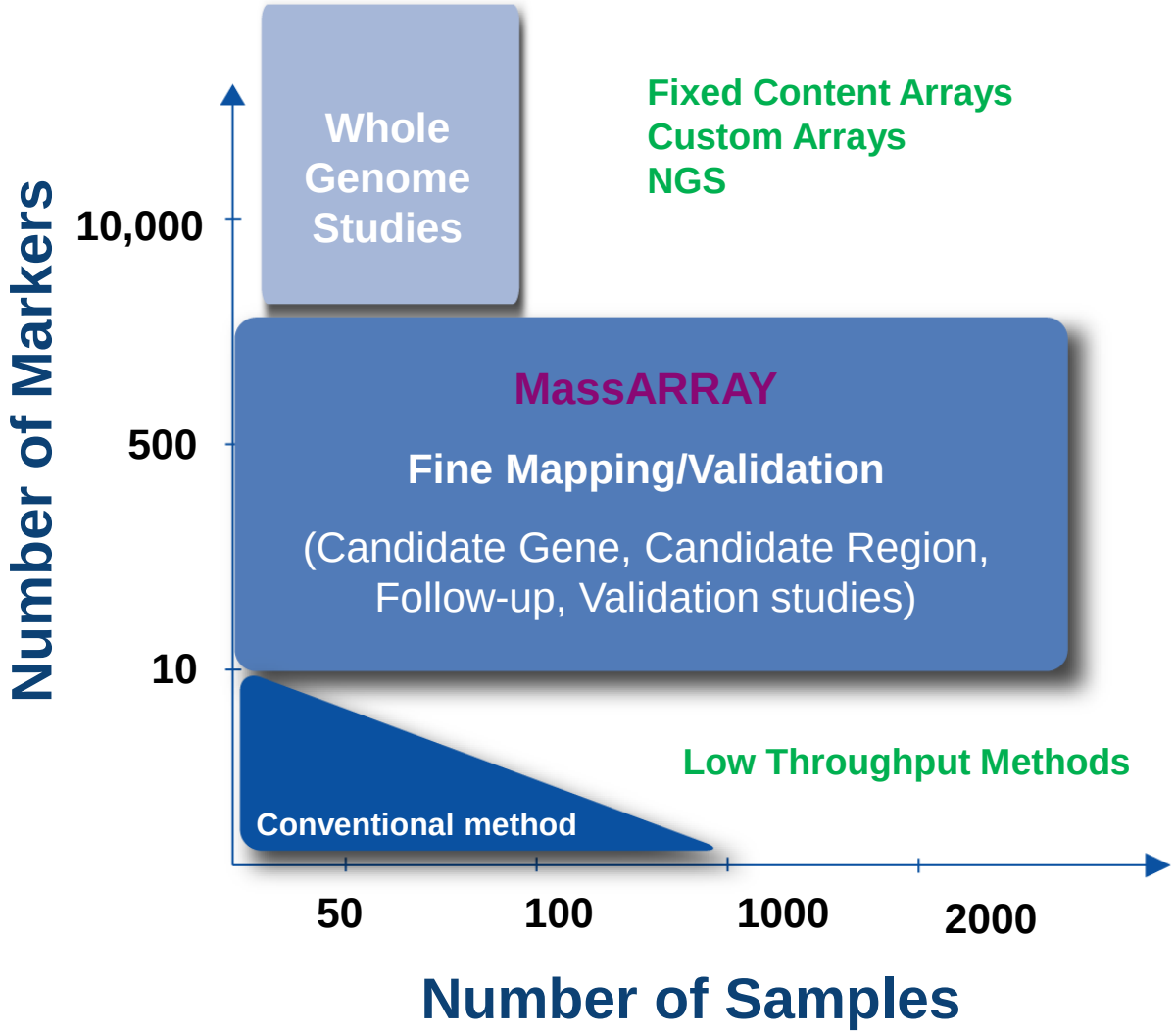
SciSpec Your Scientific Specialist

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

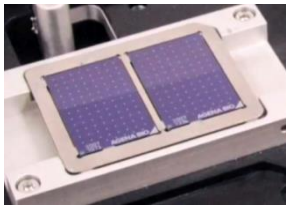
384-well plate platform

Proven Versatility of the MassARRAY® System

MassARRAY™ Applications

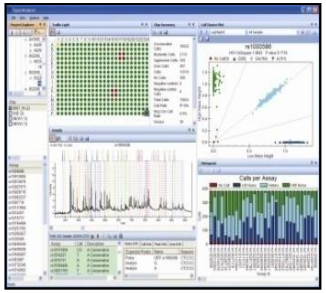
- Genotyping
- Somatic Mutation
- Copy Number Variation
- Methylation
- Ultrasensitive Detection

SpectroCHIP™



MassARRAY™ System

Data Analysis



The MassARRAY® System for Genotyping

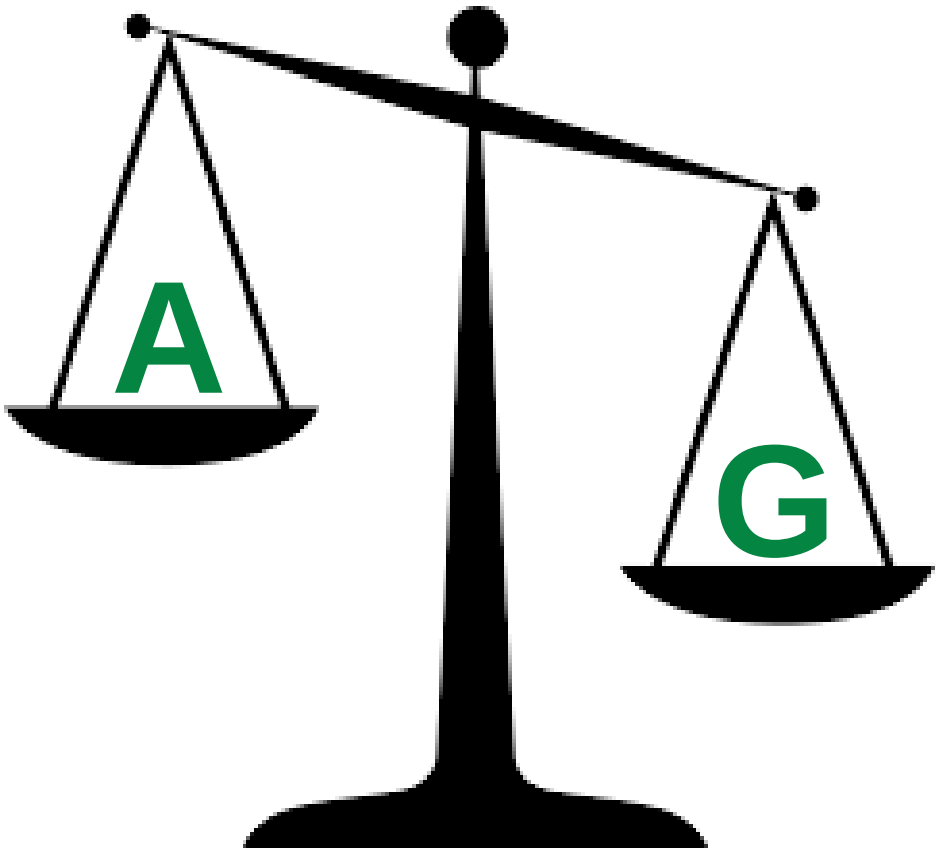
Flexibility and Cost Efficiency in a Single Workflow

- 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

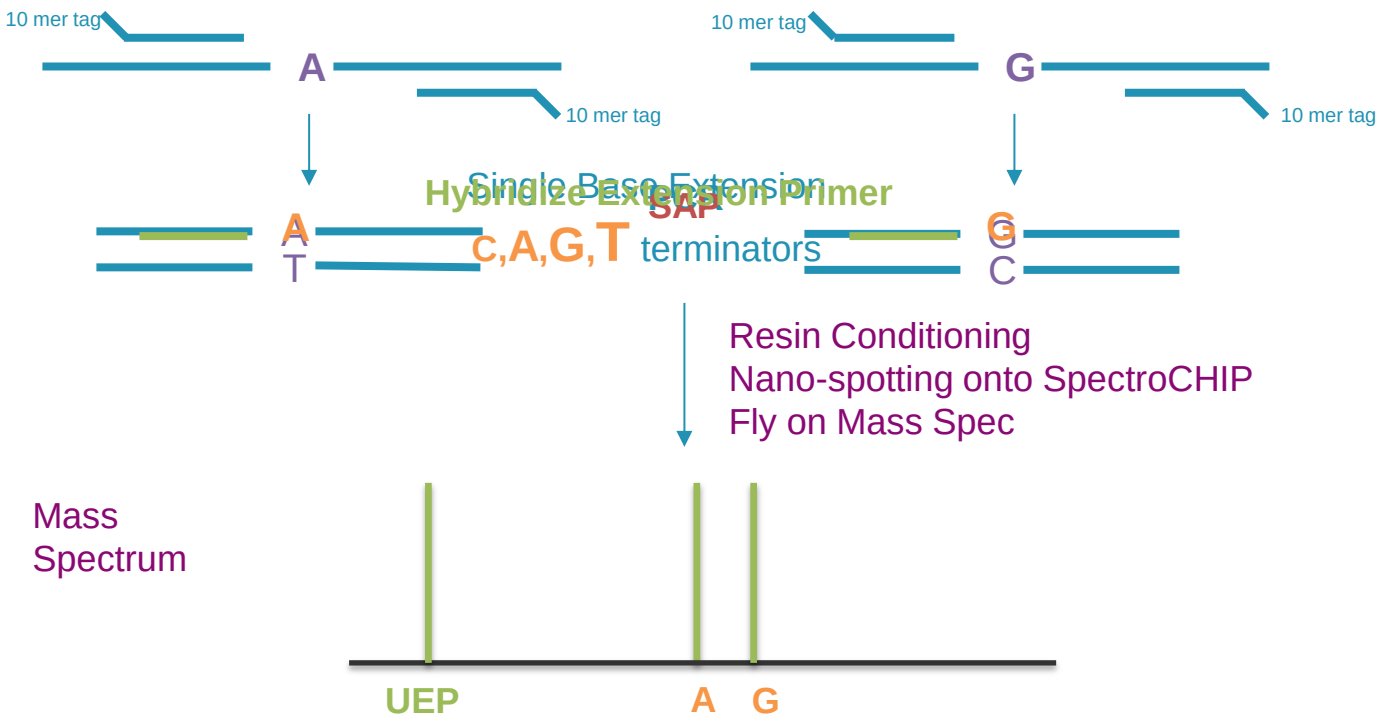
Each base within a DNA strand has defined molecular mass:

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

GENOTYPING

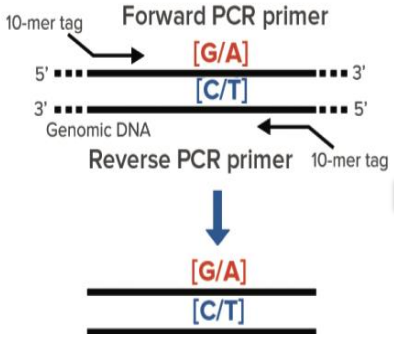
Example of an [A/G] SNP

PCR Reaction (Amplification) → **SAP Reaction** → **Extension Reaction**



MassARRAY® Process

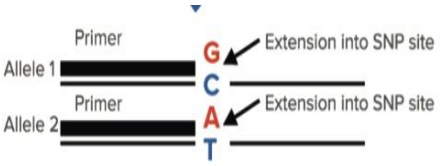
Amplification

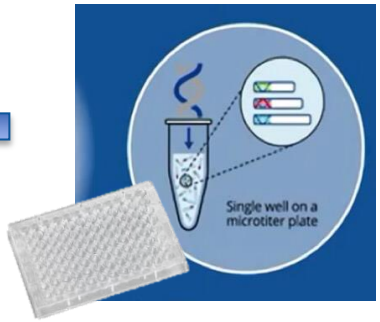


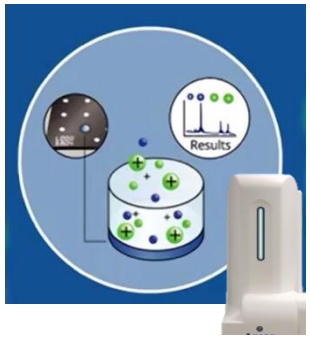
PCR Product

SAP

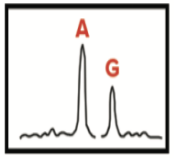
iPLEX® Extension Reaction

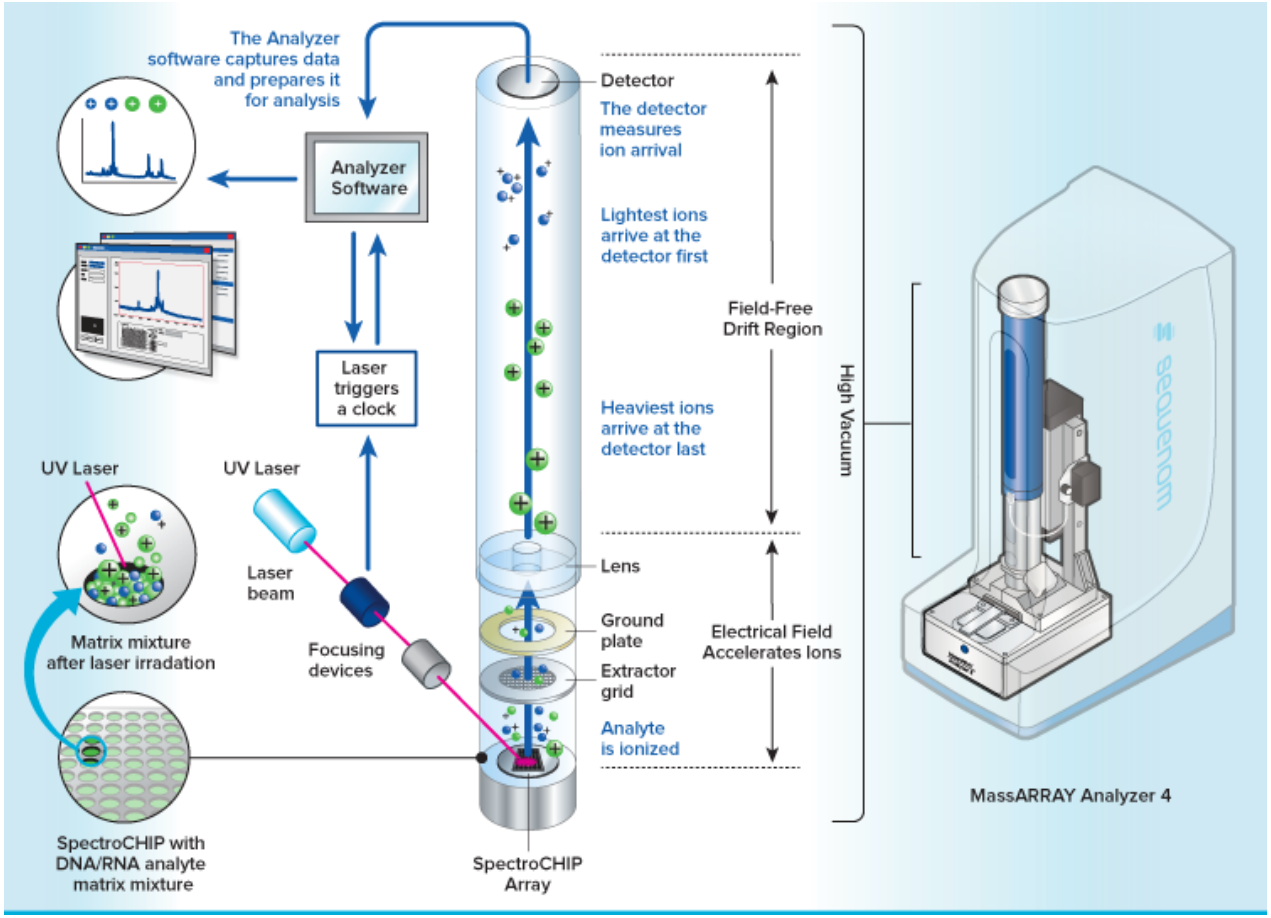






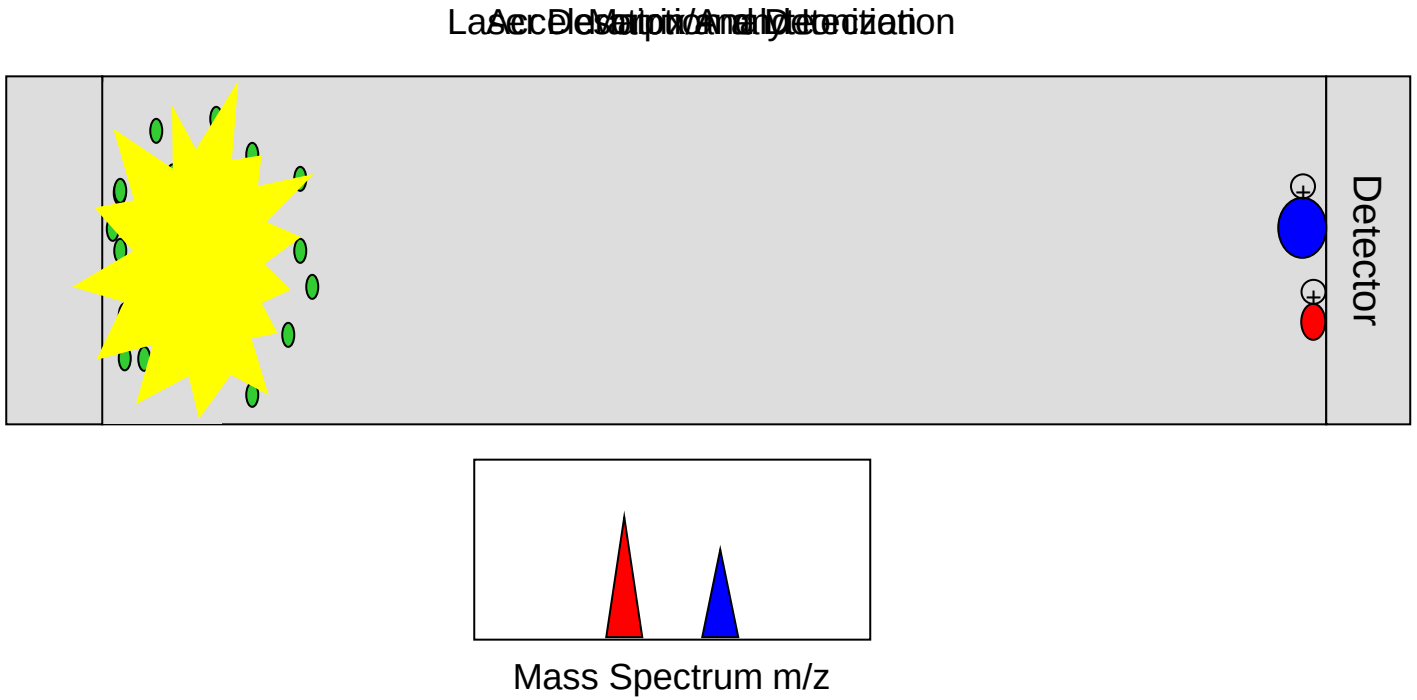
MALDI-TOF Mass Spectrometry Analysis



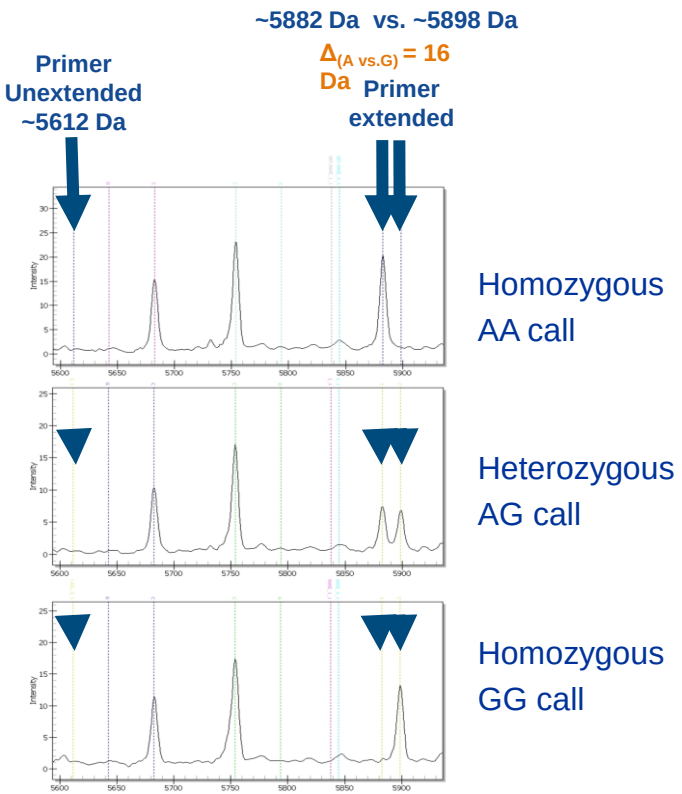


- **Molecular mass is a unique intrinsic property.**
- **The mass of a nucleic acid string is determined by its length and composition (ATCG).**

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



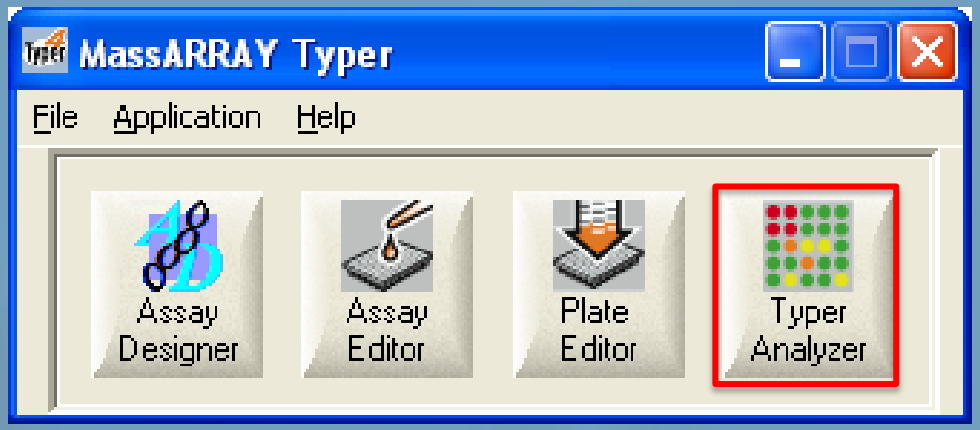
Mass spectra of 3 different SNP [A/G] samples



- 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.

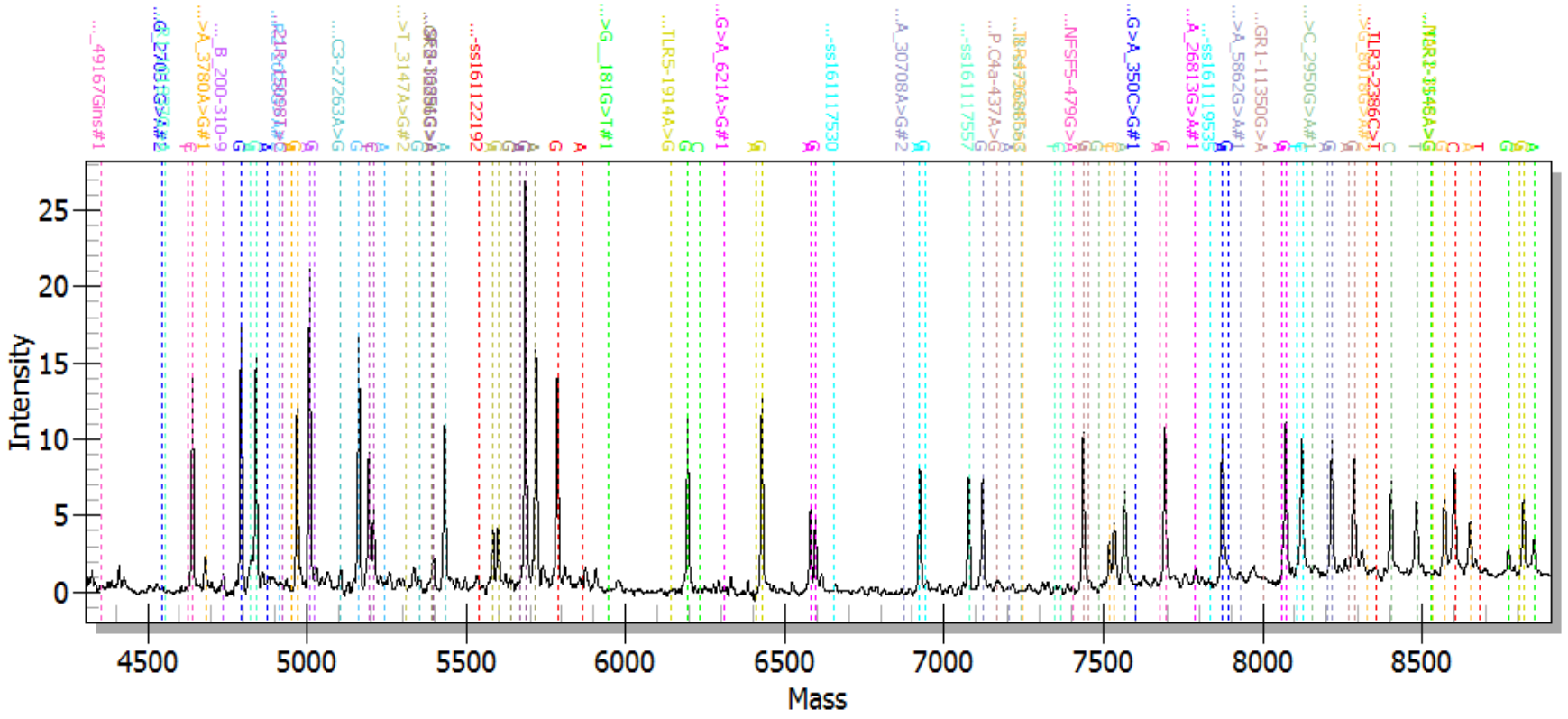
Data Analyzer

Software application suite for Genotyping & Somatic Mutation Analysis

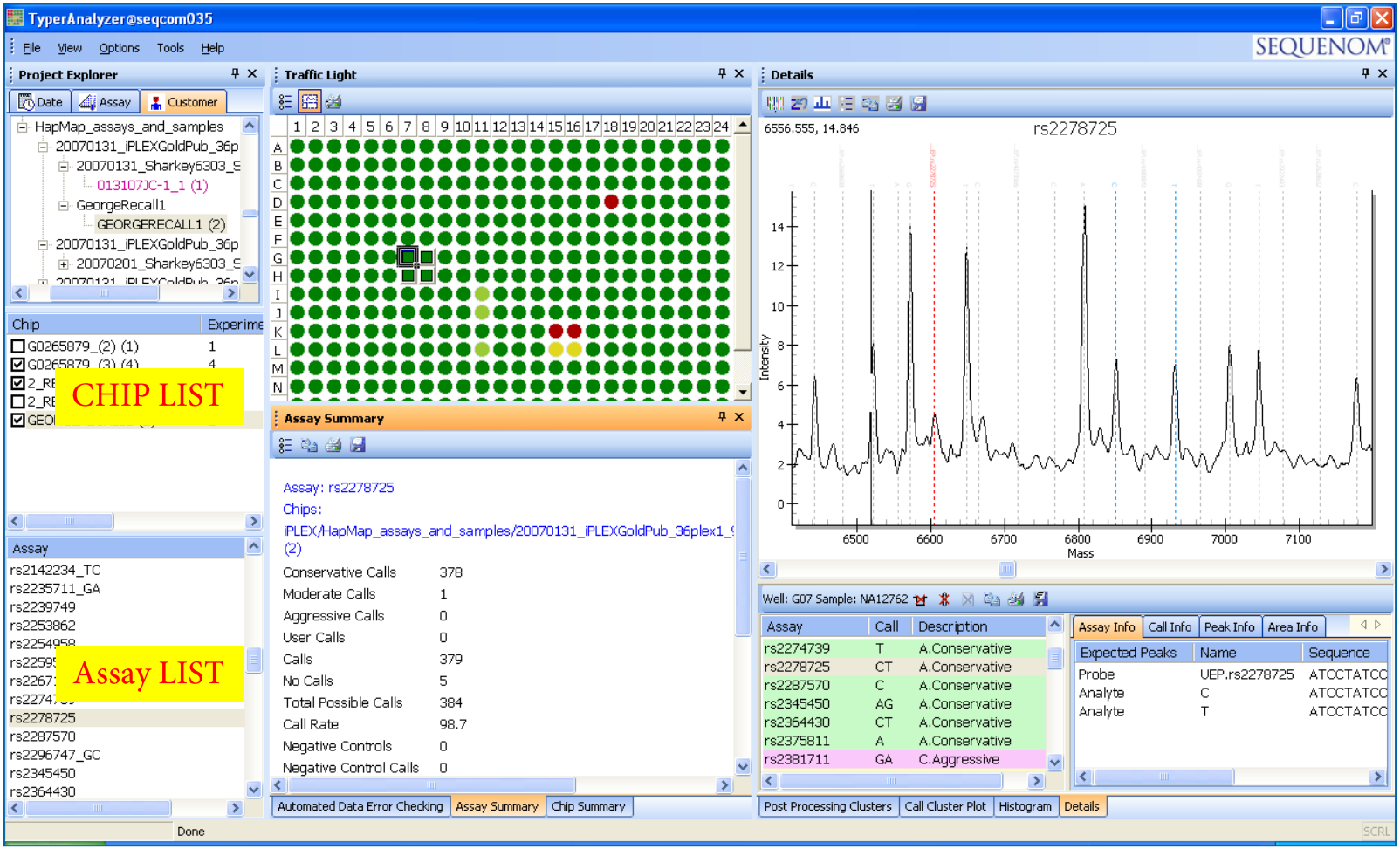


Typical SNP Panel (31-plex)

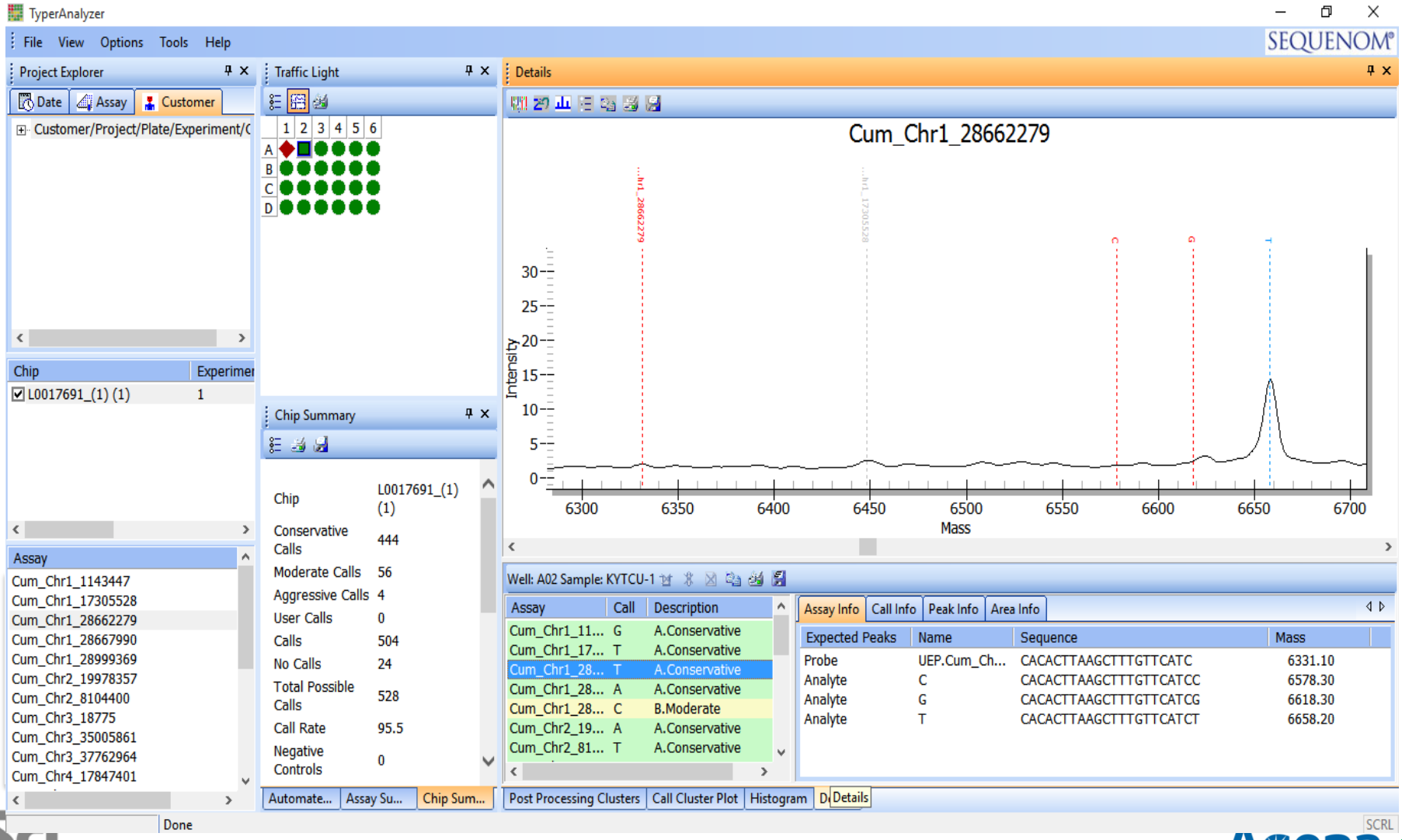
The Power of Multiplexing Multiple Markers in a Single Well



Generating Report



Data Analysis



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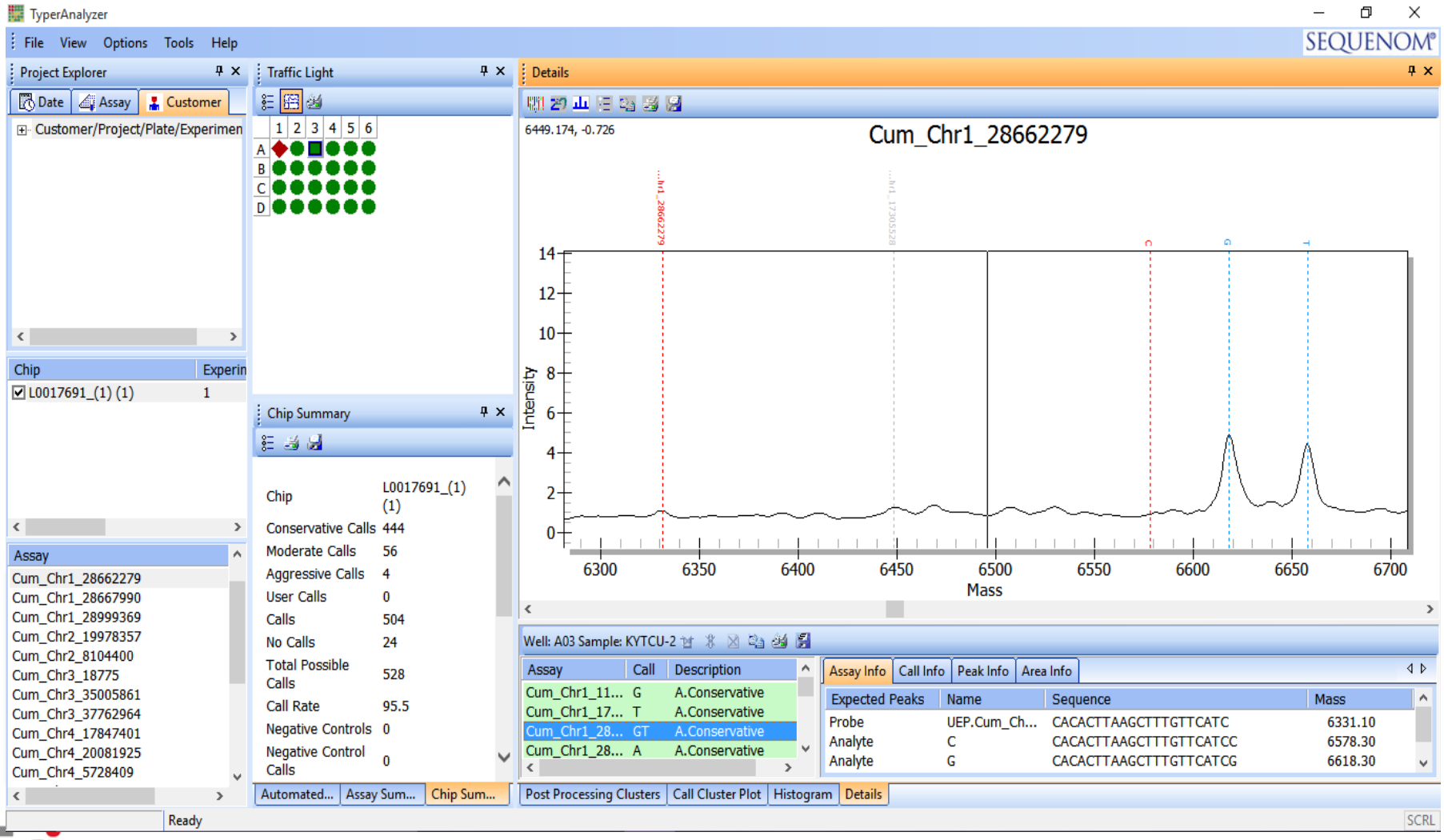

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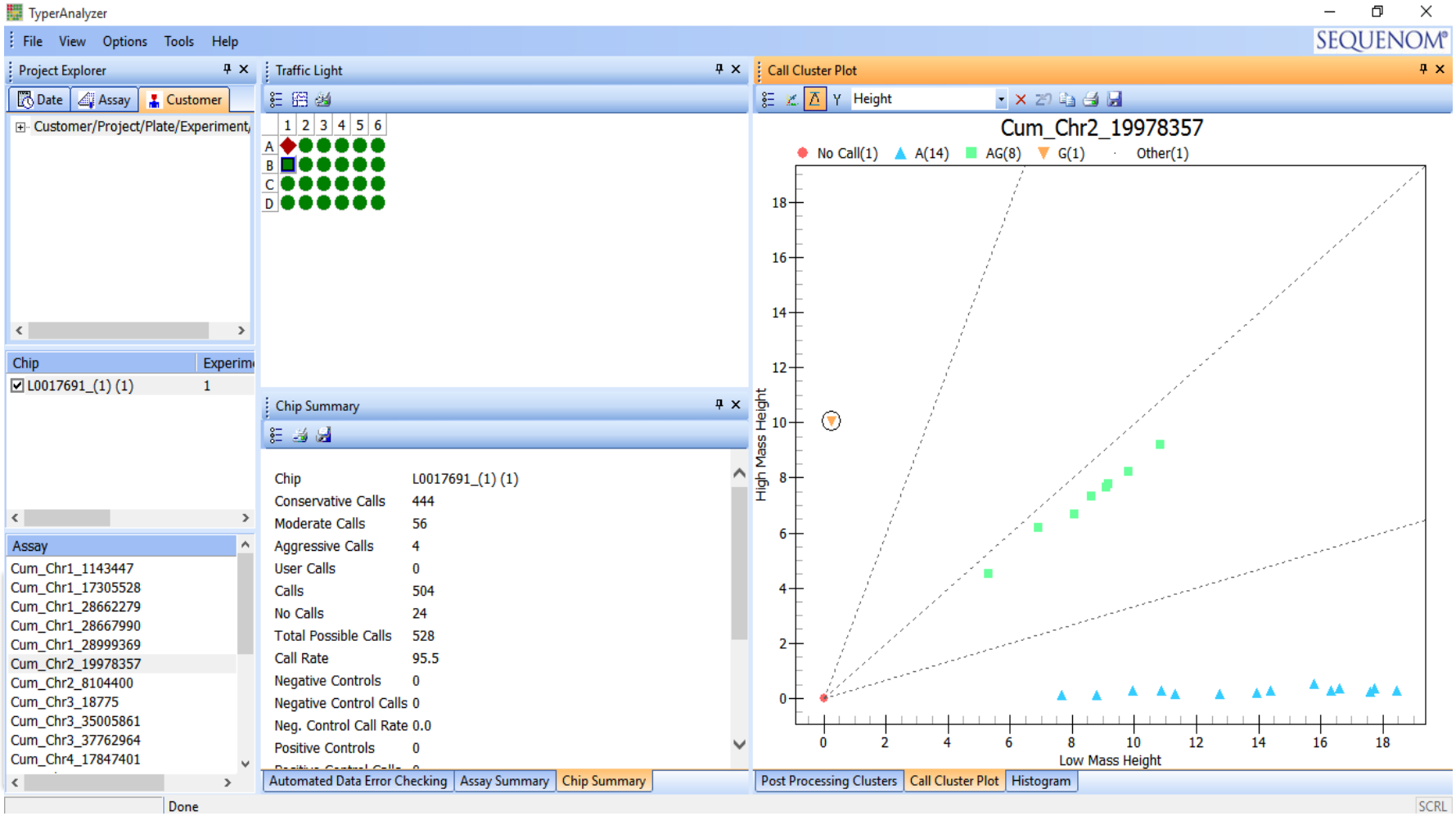

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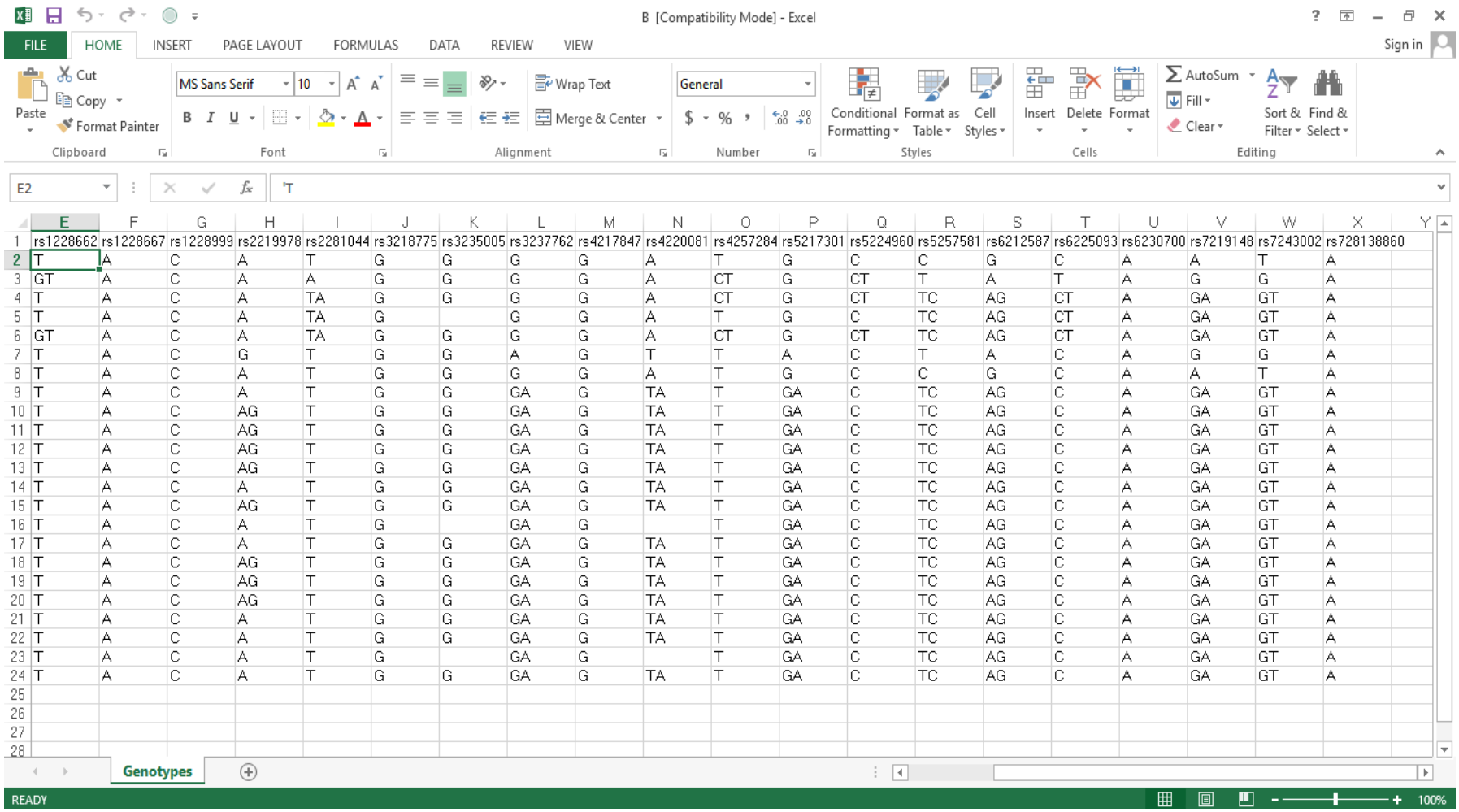

B [Compatibility Mode] - Excel

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AutoSum Fill Clear Sort & Filter Find & Select — Editing

E2 'T

	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	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	
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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

Outsource

- Assays by Agena
- Certified Service Providers

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

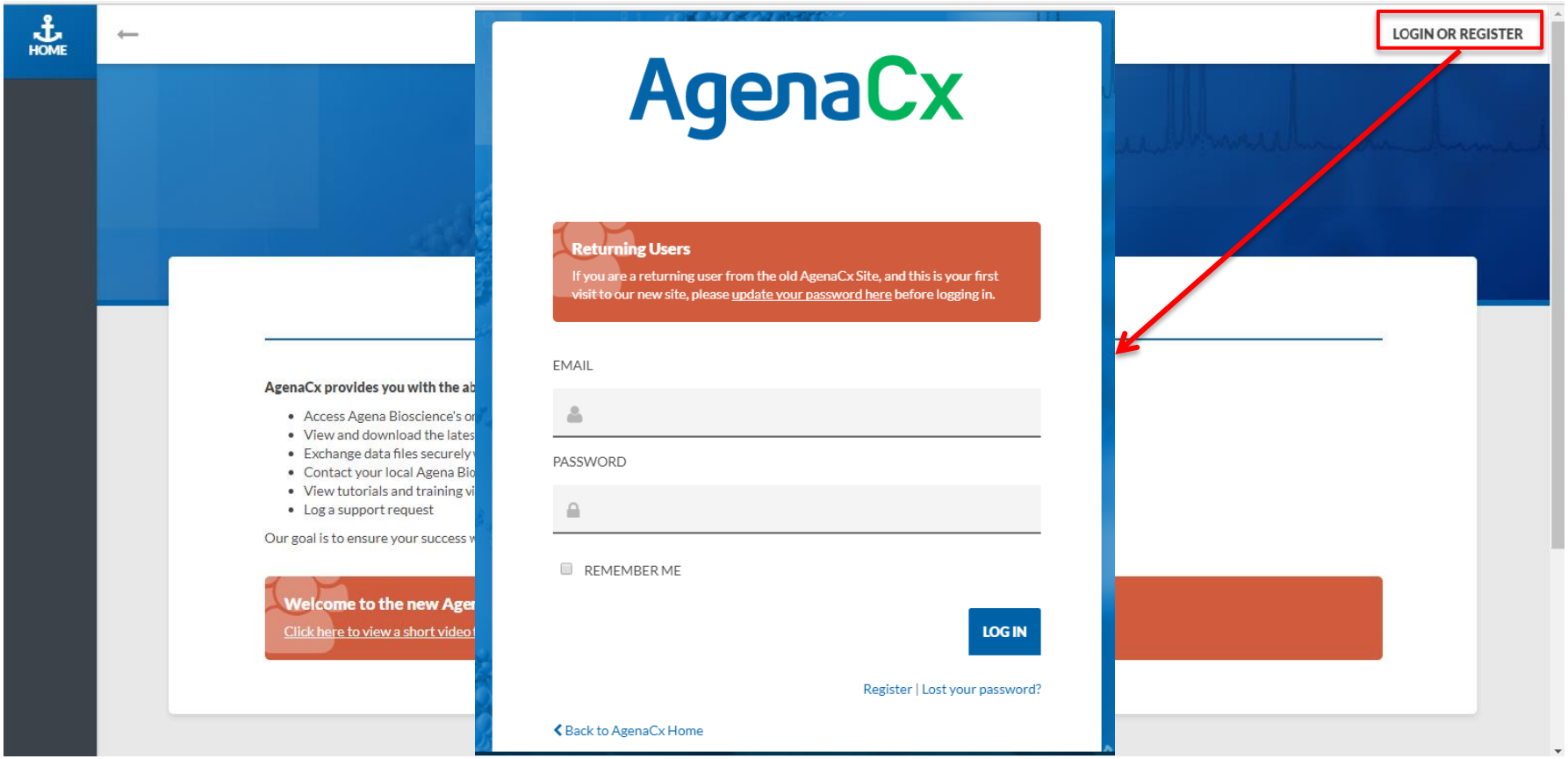
In-house

- 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

AgenaCx Account

https://www.agenacx.com/Home



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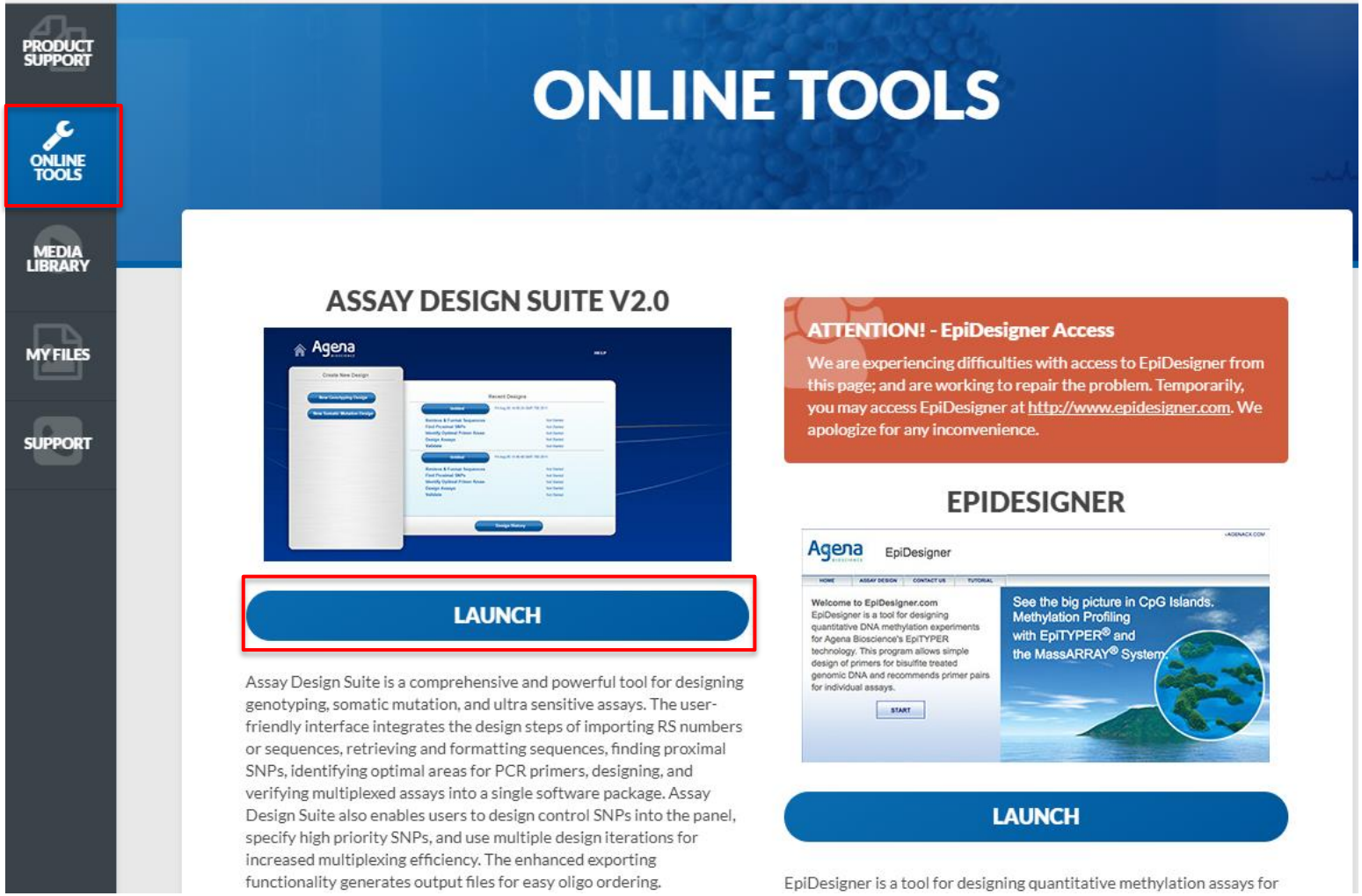

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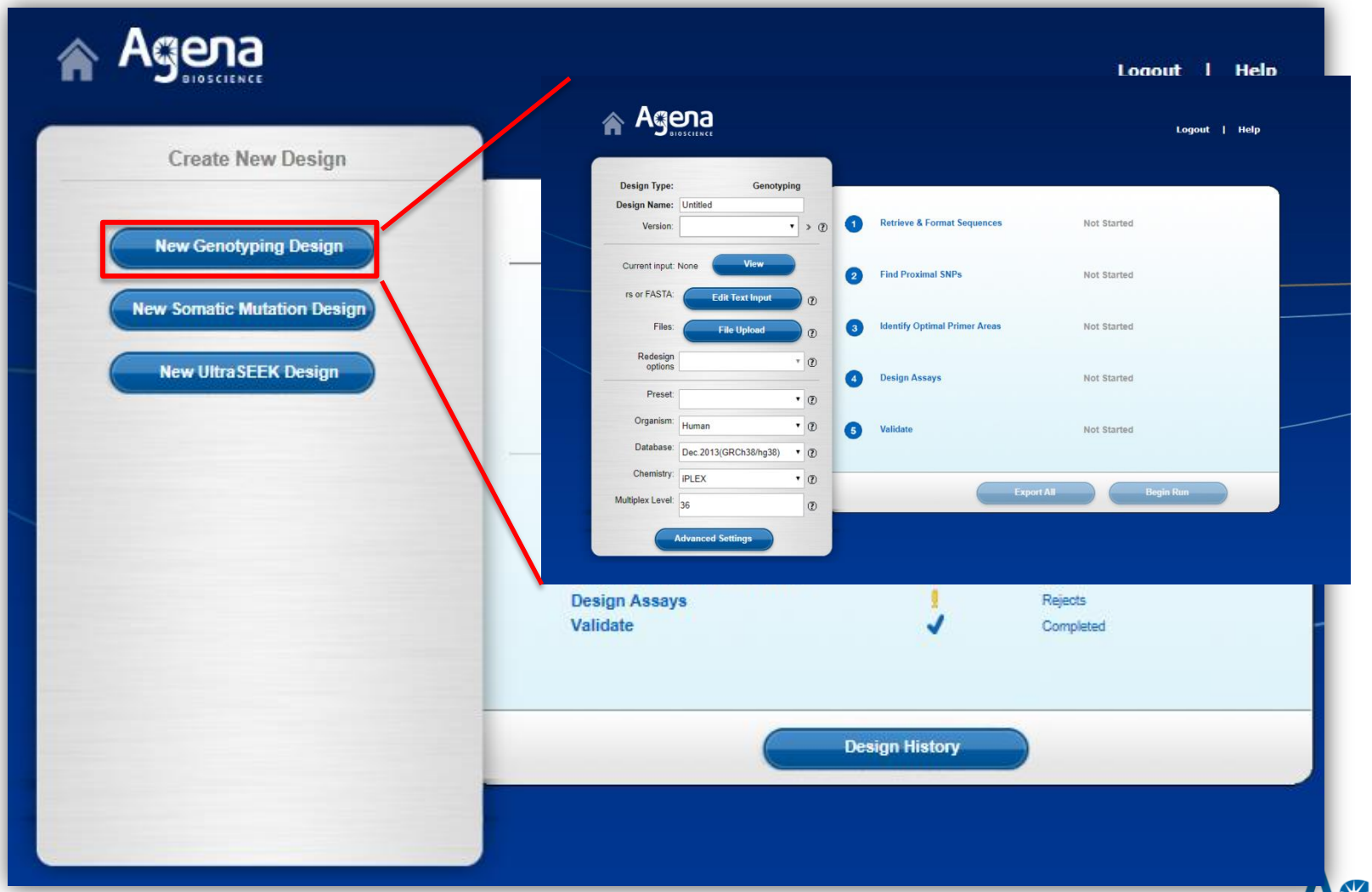
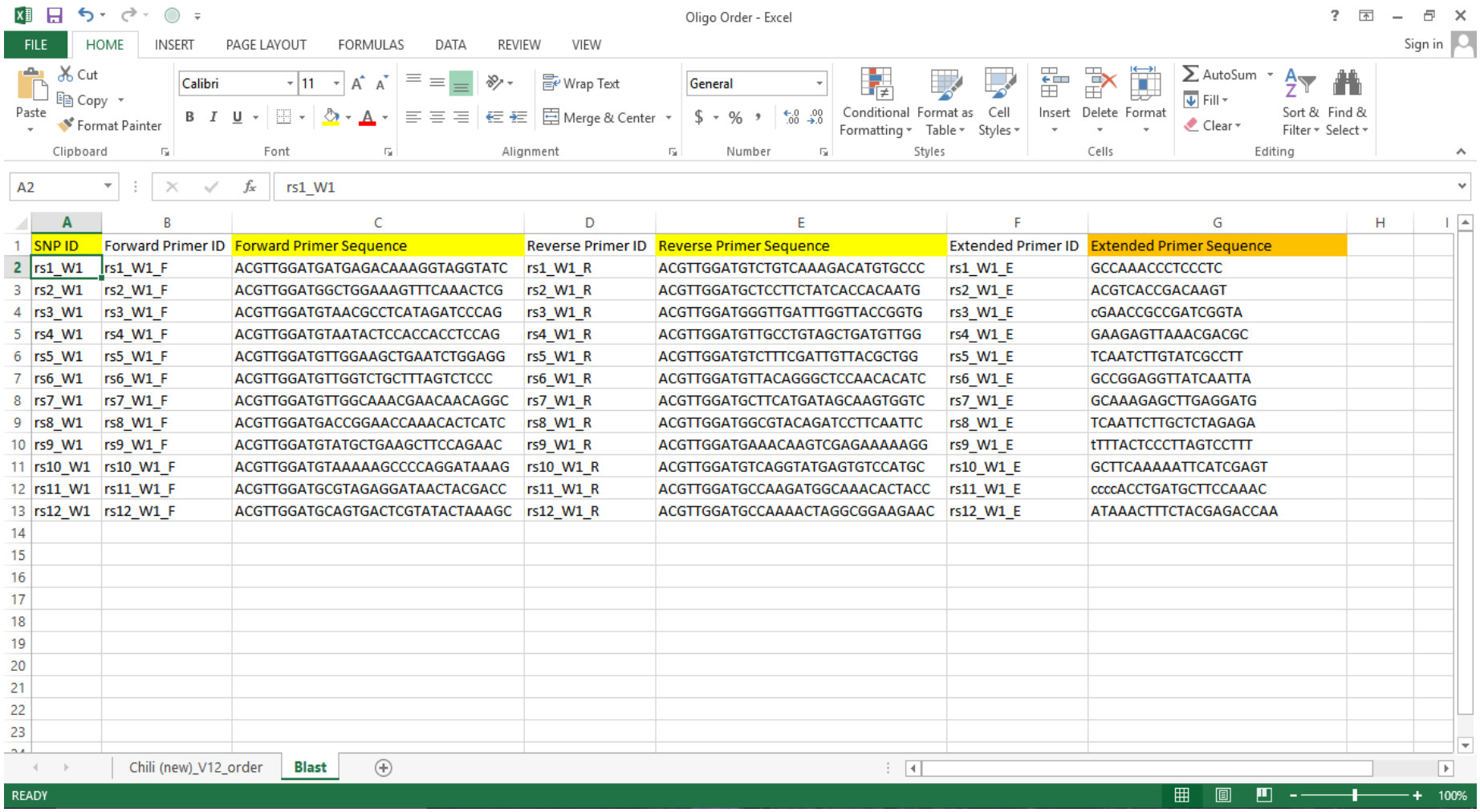


Oligo order excel file



Oligo Order - Excel

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AutoSum Fill Clear Sort & Filter Find & Select — Editing

A2 rs1_W1

	A	B	C	D	E	F	G
1	SNP ID	Forward Primer ID	Forward Primer Sequence	Reverse Primer ID	Reverse Primer Sequence	Extended Primer ID	Extended Primer Sequence
2	rs1_W1	rs1_W1_F	ACGTTGGATGATGAGACAAAGGTAGGTATC	rs1_W1_R	ACGTTGGATGTCTGTCAAAGACATGTGCCC	rs1_W1_E	GCCAAACCCTCCCTC
3	rs2_W1	rs2_W1_F	ACGTTGGATGGCTGGAAAGTTTCAAACTCG	rs2_W1_R	ACGTTGGATGCTCCTTCTATCACCACAATG	rs2_W1_E	ACGTCACCGACAAGT
4	rs3_W1	rs3_W1_F	ACGTTGGATGTAACGCCTCATAGATCCCAG	rs3_W1_R	ACGTTGGATGGGTTGATTTGGTTACCGGTG	rs3_W1_E	cGAACCGCCGATCGGTA
5	rs4_W1	rs4_W1_F	ACGTTGGATGTAATACTCCACCACCTCCAG	rs4_W1_R	ACGTTGGATGTTGCCTGTAGCTGATGTTGG	rs4_W1_E	GAAGAGTTAAACGACGC
6	rs5_W1	rs5_W1_F	ACGTTGGATGTTGGAAGCTGAATCTGGAGG	rs5_W1_R	ACGTTGGATGTCTTTCGATTGTTACGCTGG	rs5_W1_E	TCAATCTTGTATCGCCTT
7	rs6_W1	rs6_W1_F	ACGTTGGATGTTGGTCTGCTTTAGTCTCCC	rs6_W1_R	ACGTTGGATGTTACAGGGCTCCAACACATC	rs6_W1_E	GCCGGAGGTTATCAATTA
8	rs7_W1	rs7_W1_F	ACGTTGGATGTTGGCAAACGAACAACAGGC	rs7_W1_R	ACGTTGGATGCTTCATGATAGCAAGTGGTC	rs7_W1_E	GCAAAGAGCTTGAGGATG
9	rs8_W1	rs8_W1_F	ACGTTGGATGACCGGAACCAAACACTCATC	rs8_W1_R	ACGTTGGATGGCGTACAGATCCTTCAATTC	rs8_W1_E	TCAATTCTTGCTCTAGAGA
10	rs9_W1	rs9_W1_F	ACGTTGGATGTATGCTGAAGCTTCCAGAAC	rs9_W1_R	ACGTTGGATGAAACAAGTCGAGAAAAAGG	rs9_W1_E	tTTTACTCCCTTAGTCCTTT
11	rs10_W1	rs10_W1_F	ACGTTGGATGTAAAAAGCCCCAGGATAAAG	rs10_W1_R	ACGTTGGATGTCAGGTATGAGTGTCCATGC	rs10_W1_E	GCTTCAAAAATTCATCGAGT
12	rs11_W1	rs11_W1_F	ACGTTGGATGCGTAGAGGATAACTACGACC	rs11_W1_R	ACGTTGGATGCCAAGATGGCAAACACTACC	rs11_W1_E	ccccACCTGATGCTTCCAAAC
13	rs12_W1	rs12_W1_F	ACGTTGGATGCAGTGACTCGTATACTAAAGC	rs12_W1_R	ACGTTGGATGCCAAAACTAGGCGGAAGAAC	rs12_W1_E	ATAAACTTTCTACGAGACCAA

Chili (new)_V12_order | Blast

READY 100%

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], Sritharan 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

Published in final edited form as:

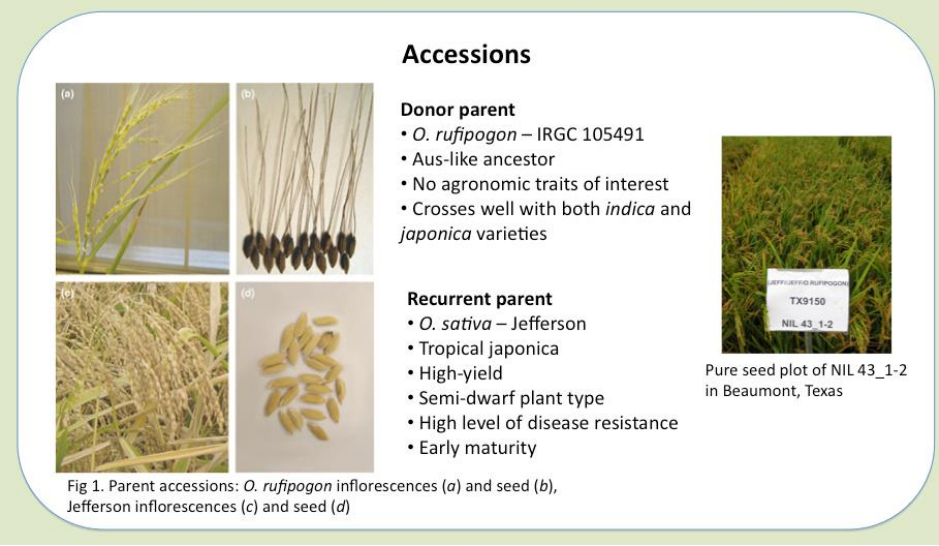
Nature. 2013 August 15; 500(7462): 340–344. doi:10.1038/nature12356.

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

Rajinder Singh[1], Eng-Ti Leslie Low[1], Leslie Cheng-Li Ooi[1], Meilina Ong-Abdullah[1], Ting Ngoot Chin[1], Jayanthi Nagappan[1], Rajanaidu Nookiah[1], Mohd Din Amiruddin[1], Rozana Rosli[1], Mohamad Arif Abdul Manaf[1], Kuang-Lim Chan[1], Mohd Amin Halim[1], Norazah Azizi[1], Nathan Lakey[2], Steven W Smith[2], Muhammad A Budiman[2], Michael Hogan[2], Blaire Bacher[2], Andrew Van Brunt[2], Chunyan Wang[2], Jared M Ordway[2], 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



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)

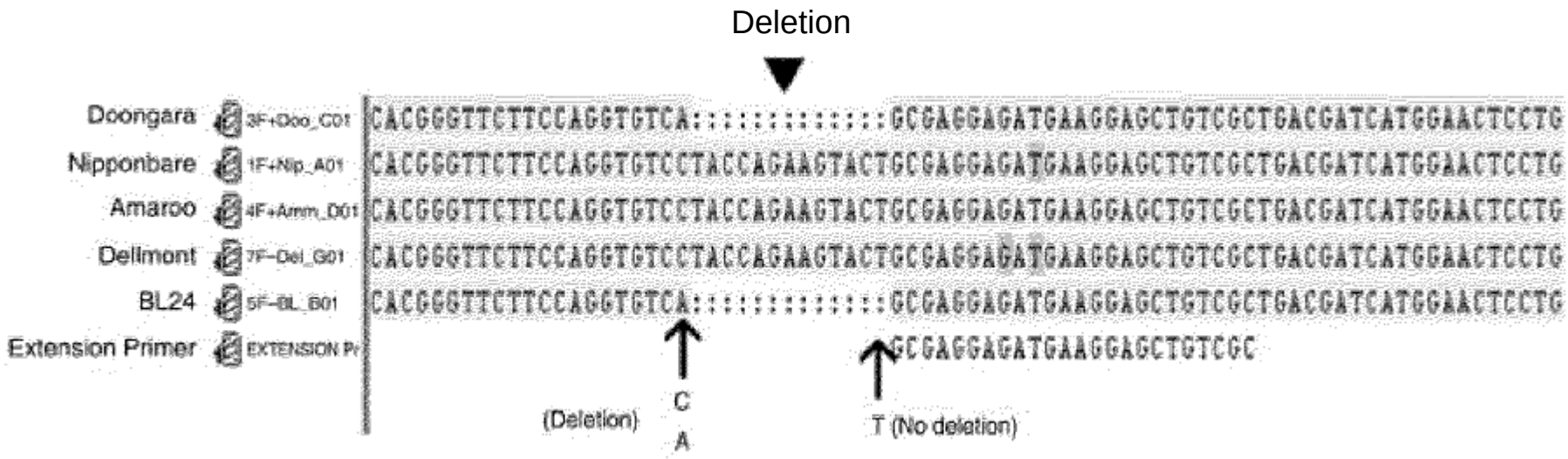
Loci	Functions	Mutation and traits
sd-1	gibberellin 20-oxidase	C/T mutation reduces plant height and increases yield
sd-del		380-383bp deletion increases yield
Pi-ta	928-aa polypeptide	G: blast resistant; T: susceptible
waxyIN1	granule-bound starch synthase	Starch quality. A: low amylose; G: high amylose
waxyEX 6		Startch quality. A: low amylose; T: high amylose
alk3	Starch synthase	Cooking and eating quality. alk3 'G'+alk4"GC": high gelatinization temperature and low alkali spreading
alk4		
fgr	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

Singh, A. *et al.* 2010 *Mol. Breeding* 26,(2): 325-338,

Plant Biotechnology Journal

Plant Biotechnology Journal (2009) 7, pp. 355–363 doi: 10.1111/j.1467-7652.2009.00411.x

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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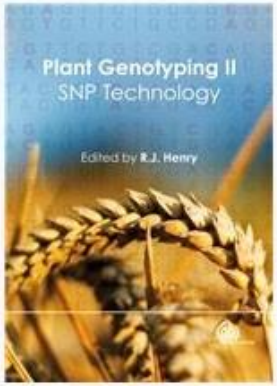
Summary

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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genotypes

Book Chapters

Chapter: 1 (Page no: 1)	**SNP discovery in plants.** 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 ecotilling using capillary electrophoresis.** Author(s): Eliott, F. Cordeiro, G. Bundock, P. C. Henry, R. J.
Chapter: 6 (Page no: 88)	**Genotyping by allele-specific PCR.** Author(s): Waters, D. L. E. Bundock, P. C. Henry, R. J.
Chapter: 7 (Page no: 98)	**The MassARRAY system for plant genomics.** Author(s): Irwin, D.
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
rice
single nucleotide polymorphism

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Livestock Genotyping

Agena BIOSCIENCE

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 diary 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.

ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

doi:10.1111/j.1365-2052.2009.01903.x

Relationships among *calpastatin* single nucleotide polymorphisms, *calpastatin* expression and tenderness in pork longissimus[1]

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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

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- Washington State University
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Questions