

Introduction of MassARRAY platform and their applications of SNPs genotyping in plant breeding

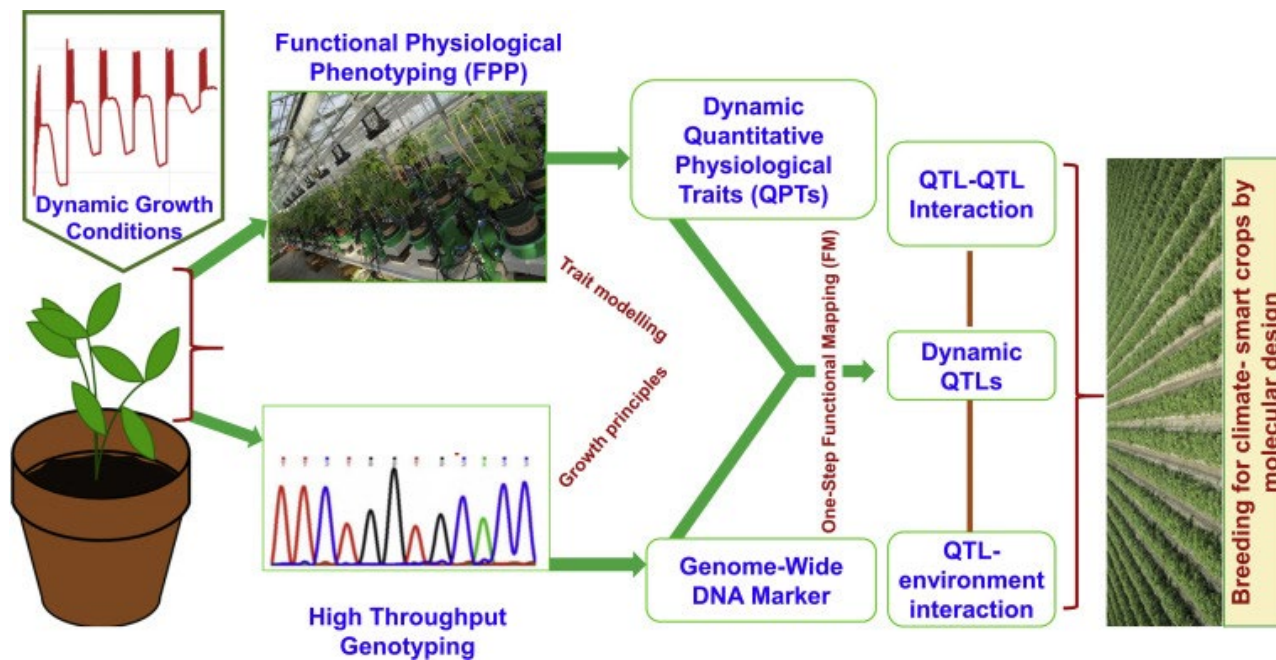
Monthathip Thongkum, PhD

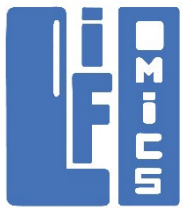
Product specialist, Lifomics Co., LTD



Genotype

- การตรวจสอบรูปแบบดีเอ็นเอ (genotyping)
- การค้นหาความหลากหลายทางพันธุกรรมในบริเวณที่สนใจ (variation analysis)
- รูปแบบความหลากหลายทางพันธุกรรมในปัจจุบัน
 - substitution (SNP), Insertion, Deletion.





Causes for sequence (dis)similarity in biological meaning

GCAGCGG-----AGCGGGTTGA	Seq1
...	
GCTGCGCCGTGAGCGGGTTGA	Seq2
++-++++-----+++++	Score

mutation: a nucleotide at a certain location is replaced by ***another*** nucleotide (e.g.: ATA → AGA)

insertion: at a certain location one new nucleotide is inserted in between two existing nucleotides (e.g.: AA → A**G**A)

deletion: at a certain location one existing nucleotide is deleted (e.g.: ACTG → AC-G)

indel: an **insertion** or a **deletion**

Plant/ animal/ microorganism

Genome/ DNA extraction

Base calling

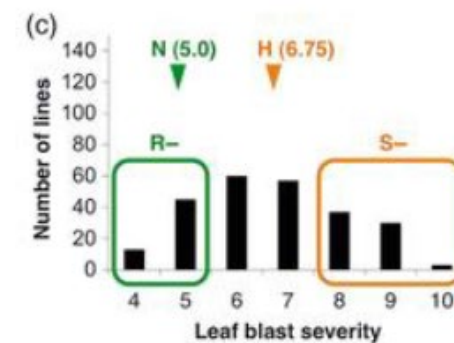
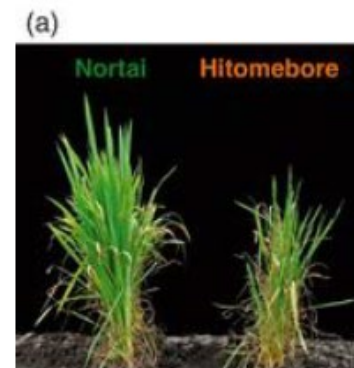
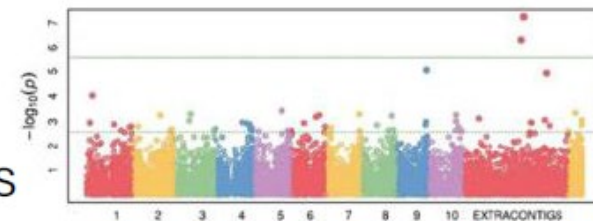
High throughput
sequencing technology

Genotype of population

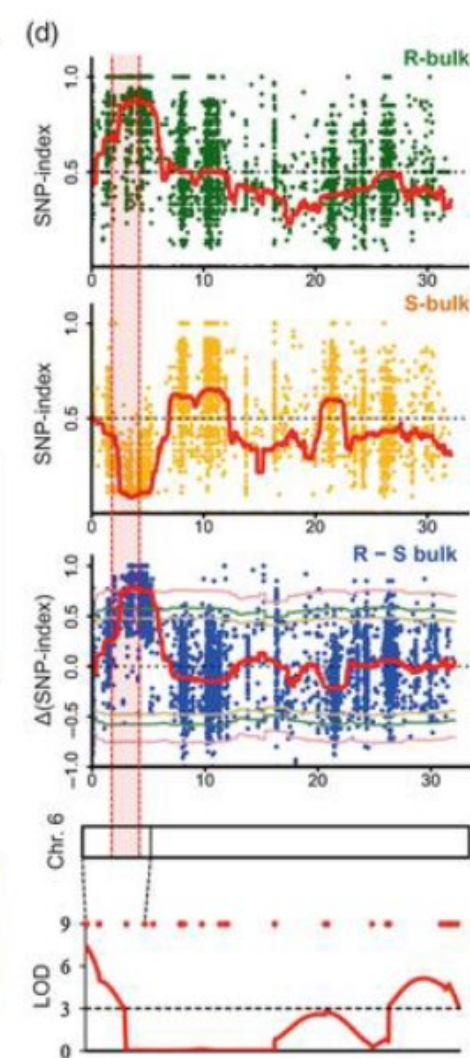
GWAS, QTL

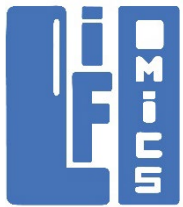
Phenotype

GWAS



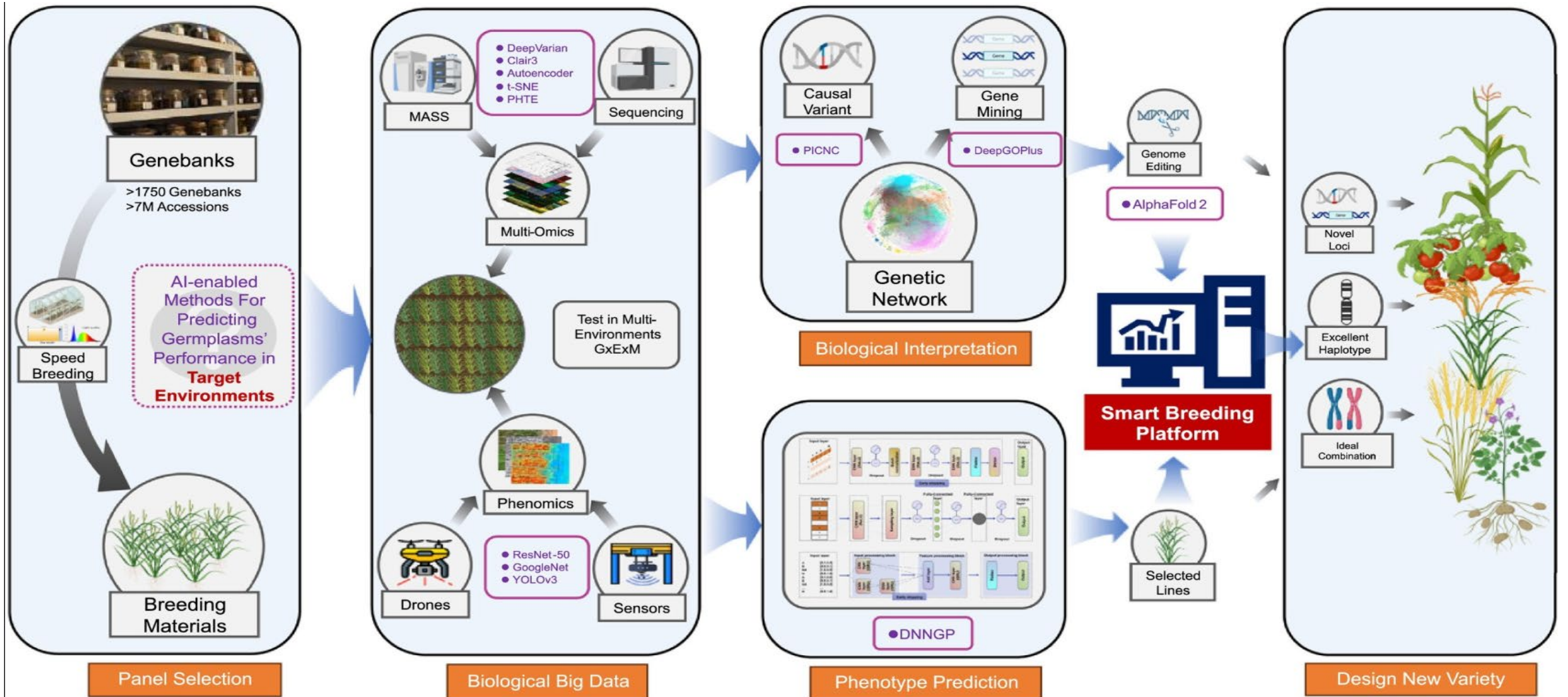
QTL



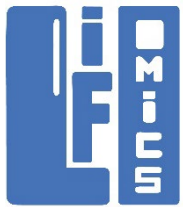


Advantages of SNP Markers

- Abundant in the genome: SNPs are highly polymorphic, providing numerous markers for analysis.
- High throughput: Modern genotyping technologies allow for efficient and cost-effective SNP analysis.
- Co-dominant inheritance: Both alleles can be detected, providing more precise information.
- Stable: SNPs are generally stable genetic markers.

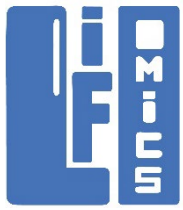


Trends in Genetics



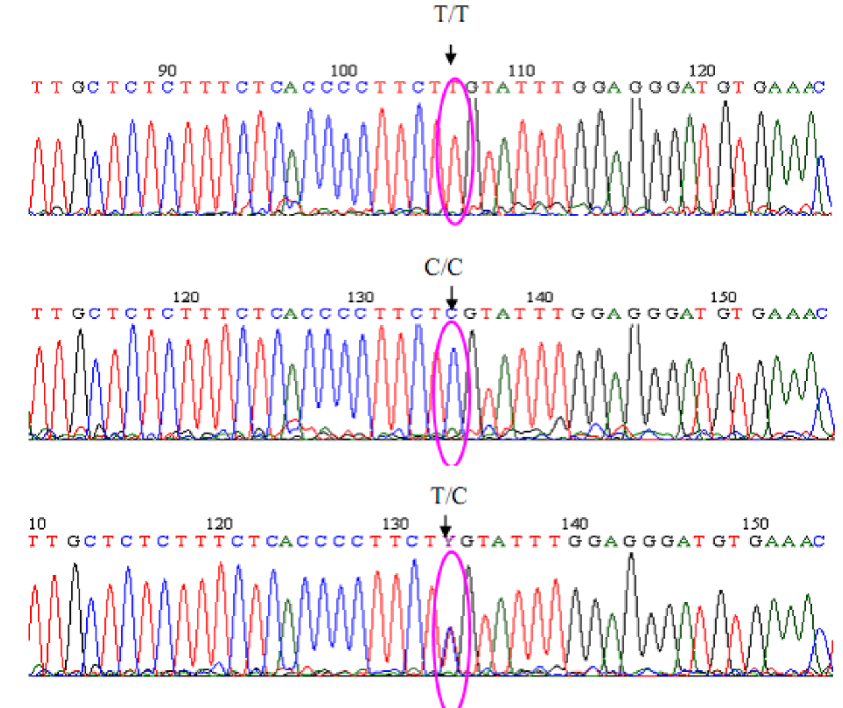
SNP based marker applications

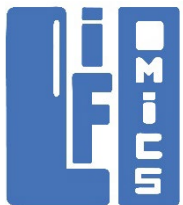
- Cultivar Identification: Hybridity Testing
- Seed Purity Testing
- Germplasm Purity Testing
- Molecular Breeding: Diversity Analysis: Germplasm Fingerprinting
 - Marker-Assisted Selection (MAS): Accelerating and precision in breeding
 - Marker Assisted Backcross (MABC)
- Genetic/QTL Mapping: Identifying gene locations
- Genome-Wide Association Studies (GWAS): Identifying trait associated genetic variants
- Genomic Selection (GS)



Molecular marker (SNP based)

- CAPS : Cleaved Amplified Polymorphic Sequences
- KASP (KASPar) : Kompetitive Allele Specific PCR
- PACE : PCR Allele Competitive Extension
- HRM (High Resolution Melting)
- MassArray
- DNA Sequencing





MASSARRAY TECHNOLOGY

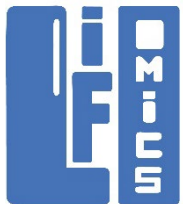


Introduction

MassARRAY debrief

Applications

Discussion



MASSARRAY TECHNOLOGY

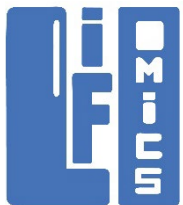


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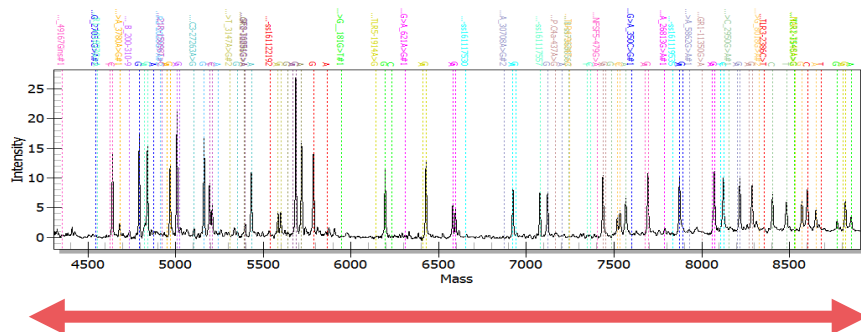
Mass-Detection With The MassARRAY



The **Mass**ARRAY is unique technology to detect DNA mutation SNP , Insertion, Deletion

Dalton
Unit of Mass

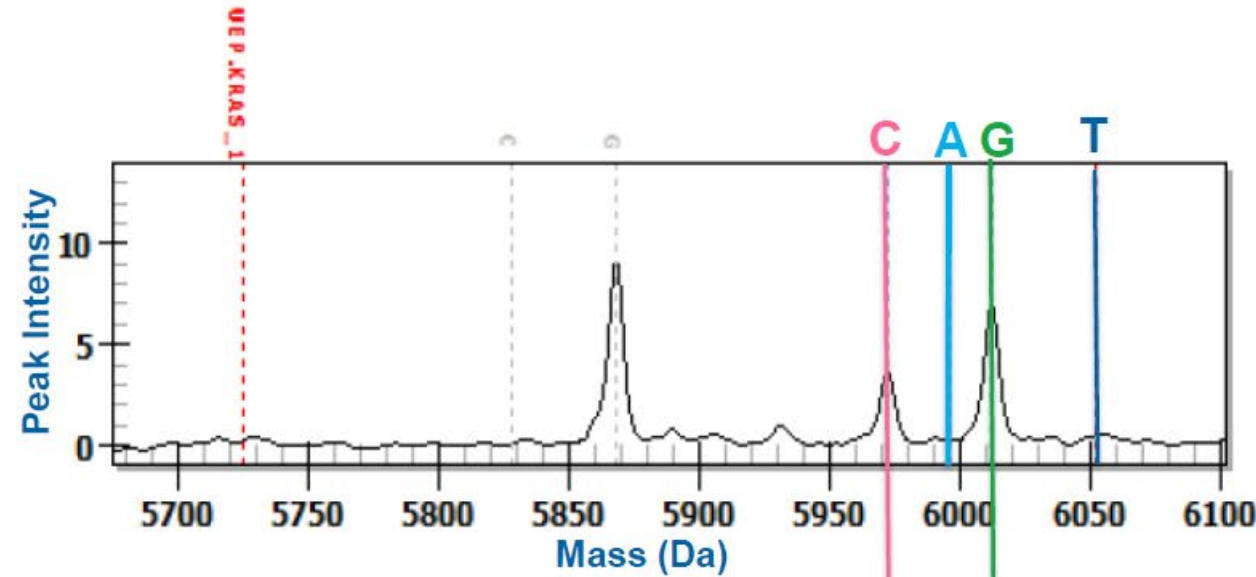
The Dalton (Da) is a unit of mass widely used in physics and chemistry.



MassARRAY Detection Range **4000 – 9000 Daltons**

Mass-Detection With The MassARRAY

Single Nucleotide Polymorphism (SNPs)



Heterozygous G/C

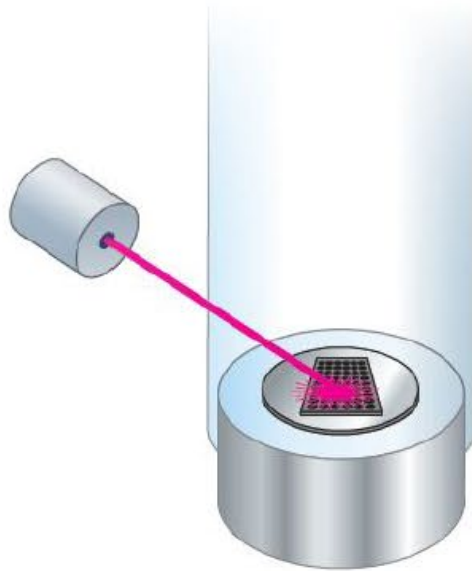
Cytosine (C)	Adenine (A)	Guanine (G)	Thymine (T)
247.2 Da	271.2 Da	287.2 Da	327.1 Da

Technology Overview

Matrix-Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF)

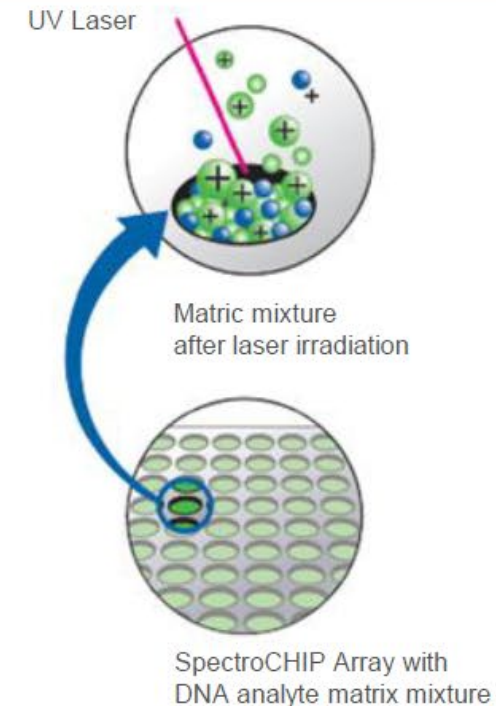
1 DNA Irradiation/Ionization

SpectroCHIP Arrays are placed in the MassARRAY analyzer and the samples are irradiated by a laser.



2 DNA Separation

Samples become ionized (positively charged) and begin to migrate through the vacuum tube



Technology Overview

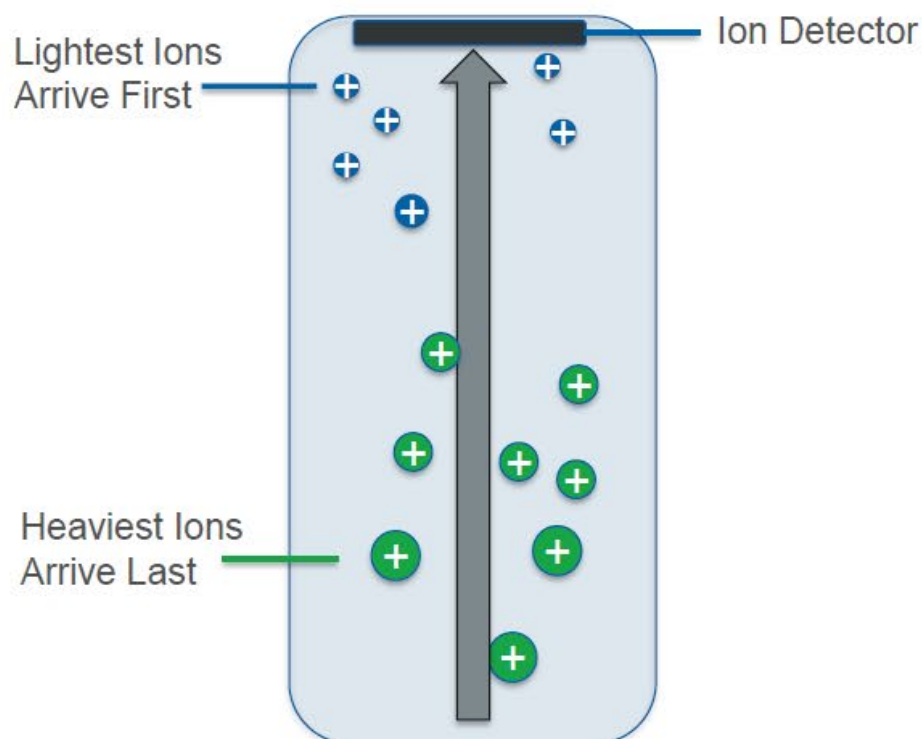
SYSTEMS



Matrix-Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF)

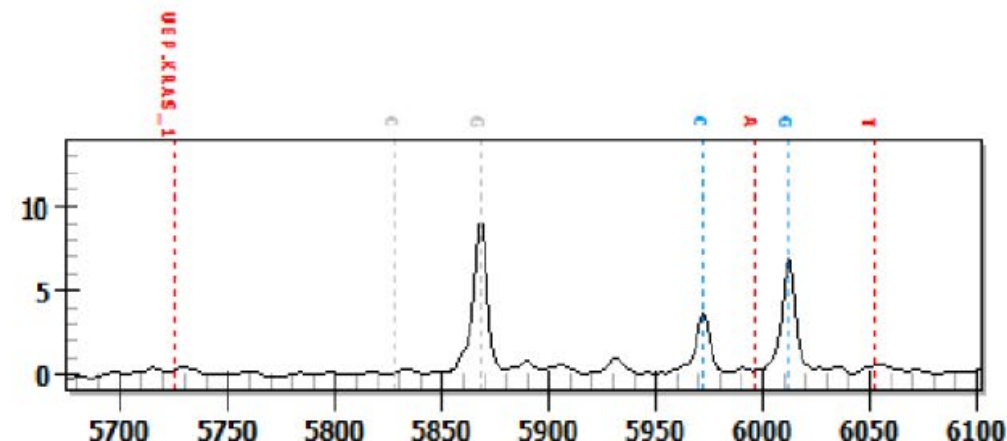
3 Time of Flight

Mass dependent, the DNA analyte ions arrive to the detector at different speeds leading to different arrival times (or times of flight).



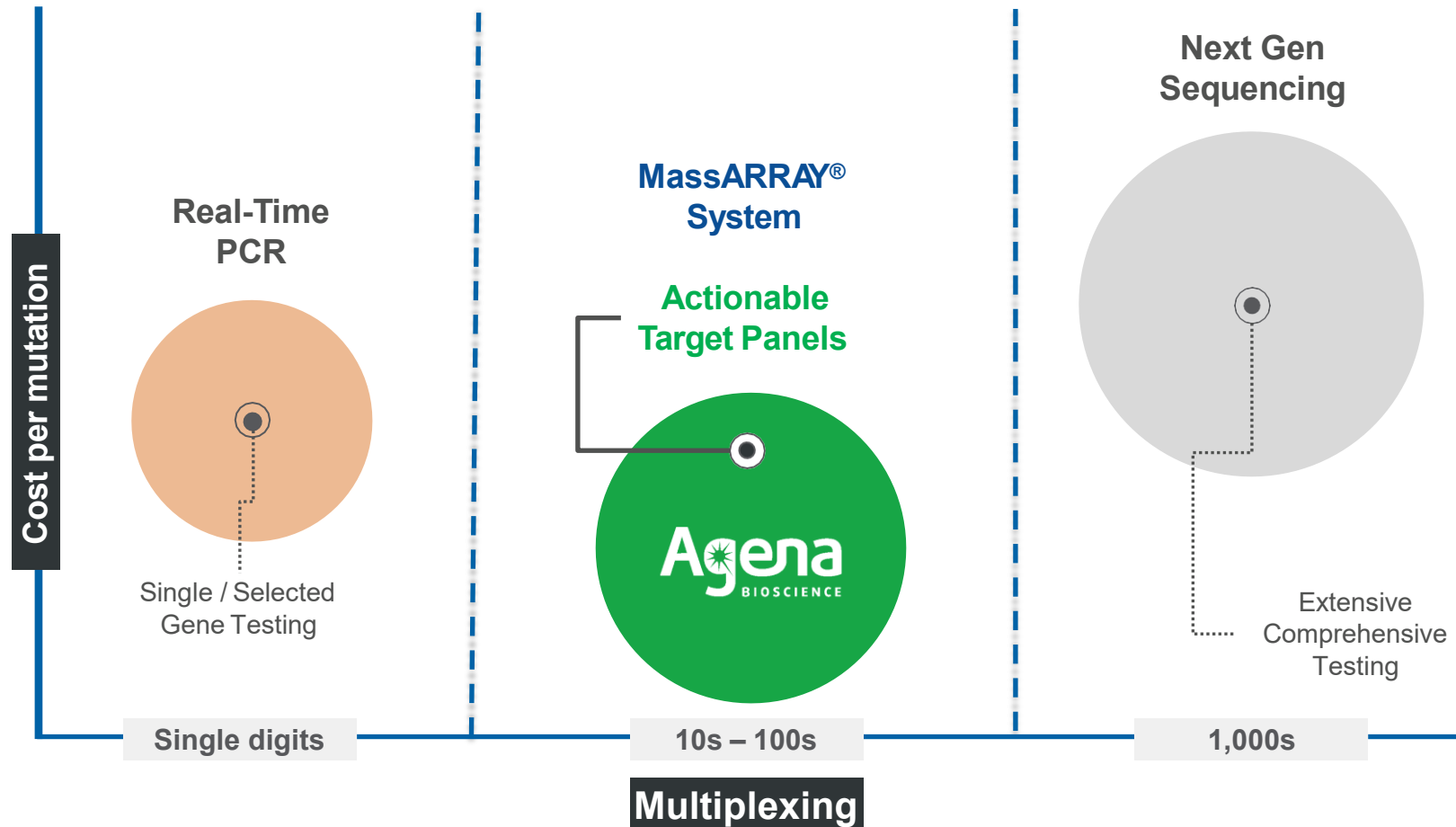
4 DNA Analysis

Using arrival times, the software determines the mass of each DNA ion and generates a mass spectrum to distinguish among the different genetic targets.



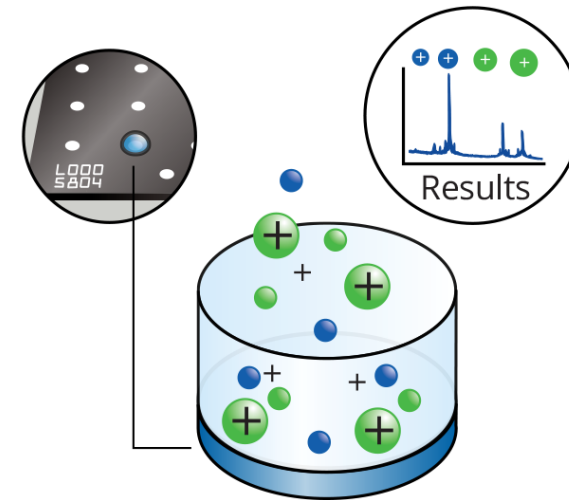
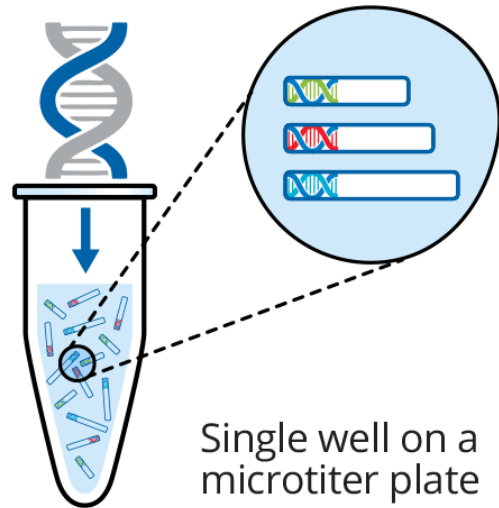
Cost-Effective, Targeted Genetic Analysis

Robust, Flexible, and High Throughput



Technology Overview

Matrix-Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF)



Detection and Analysis



Multiplex PCR

**MALDI-TOF
Mass**

Technology Overview

Matrix-Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF)



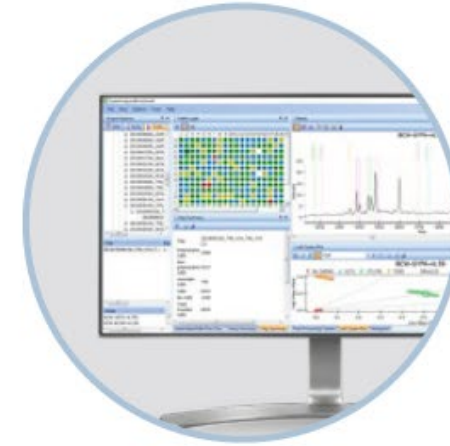
PCR/SAP/Extension

Multiplex end-point PCR followed by a single base extension reaction



Target Sequence Detection

Automated analyte transfer and data acquisition



Data Analysis

Data display and report generation

96 FORMAT



Run Time: 7 Hrs*
Hands-on Time: 23 Min



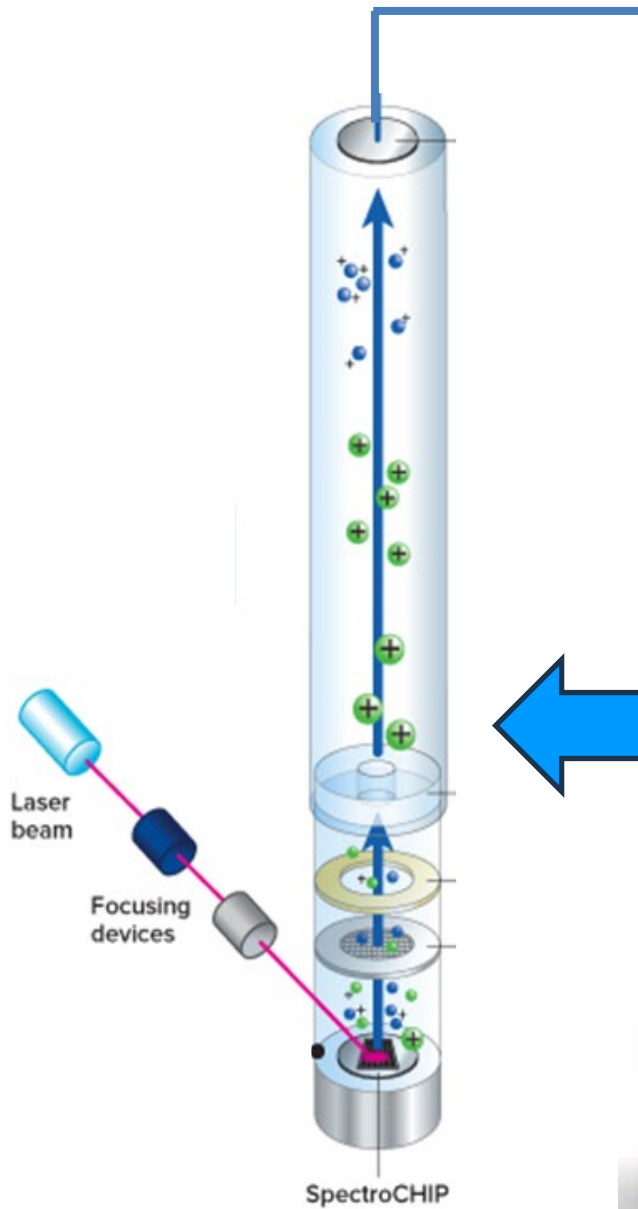
Run Time: 90 Mins*
Hands-on Time: 5 Min



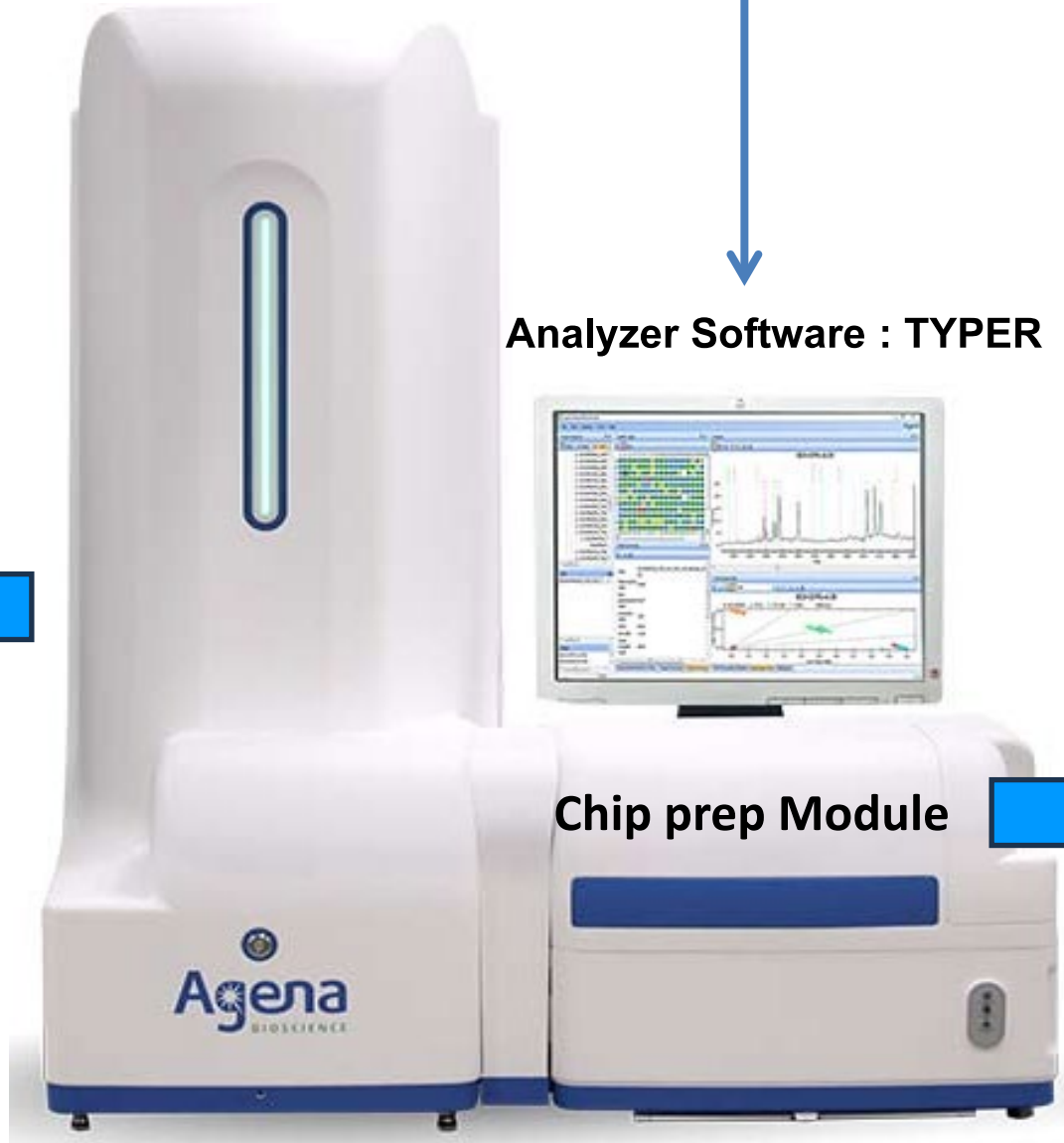
Hands-on Time:
5-10 Min

Technology Overview

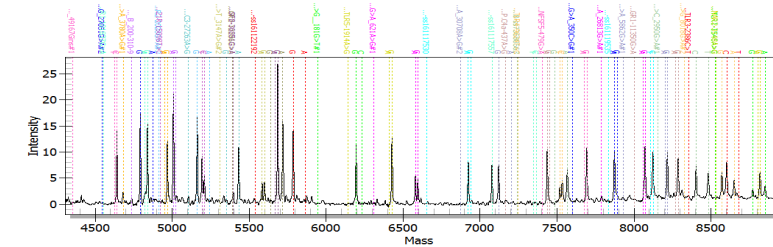
Matrix-Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF)



Mass Analyzer



Analyzer Software : TYPER



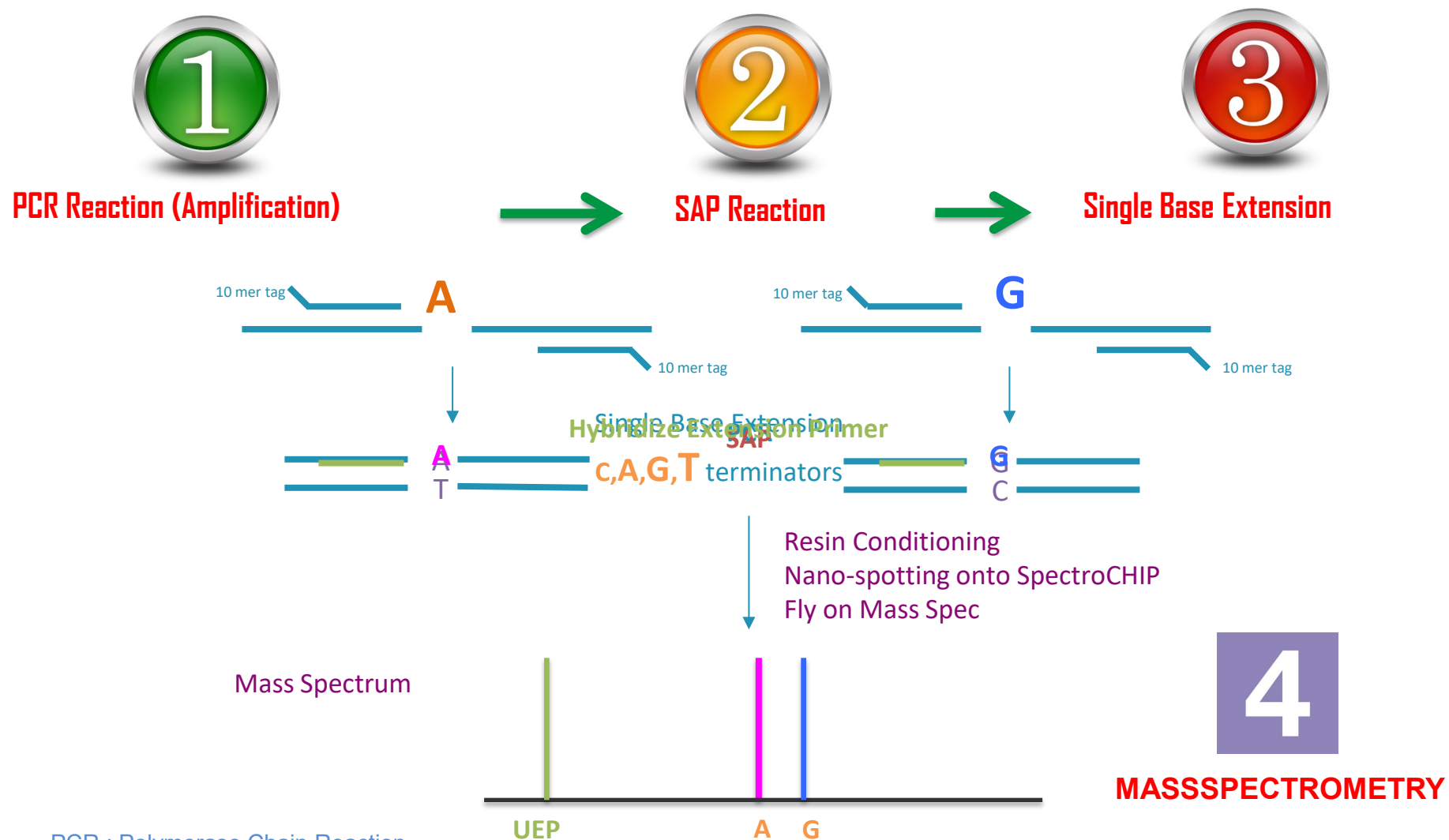
Platform: 96 wells x 2 plates



Chip prep Module

PATENTED "SPECTROCHIP"

GENOTYPING REACTION : SNP (A/G)



PCR : Polymerase Chain Reaction

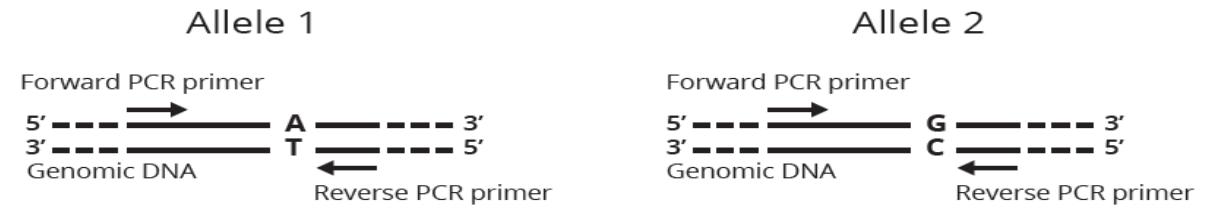
SAP : Shrimp Alkaline Phosphatase

UEP : Unextension Primer



1

PCR REACTION



2

SAP REACTION

SAP treatment to dephosphorylate unincorporated dNTPs

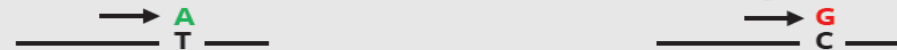


3

SINGLE BASE EXTENSION



Single nucleotide extension into SNP site



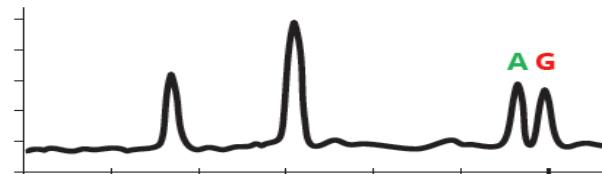
Extension products



4

MASSSPEC ANALYSIS

Desalt, dispense onto SpectroCHIP® Array and analyze with MALDI-TOF MS



Key Highlight of MassARRAY

“MULTIPLEXING”

Up to 60 Targets per
a Single Reaction...

Simple Workflow



PCR/SAP/Extension



10 Secs / Well (96 Chip)

96 Sample $\geq$ 15 Mins



PATENTED “SPECTROCHIP”

5-10 ng DNA/ Reaction

High accuracy “99.7%”

270laser shots

(30 x 9 random areas) in a
well to optimize Ionization

“MULTIPLE USAGE”

shelf life 1 month
in desiccator/ dry cabinet

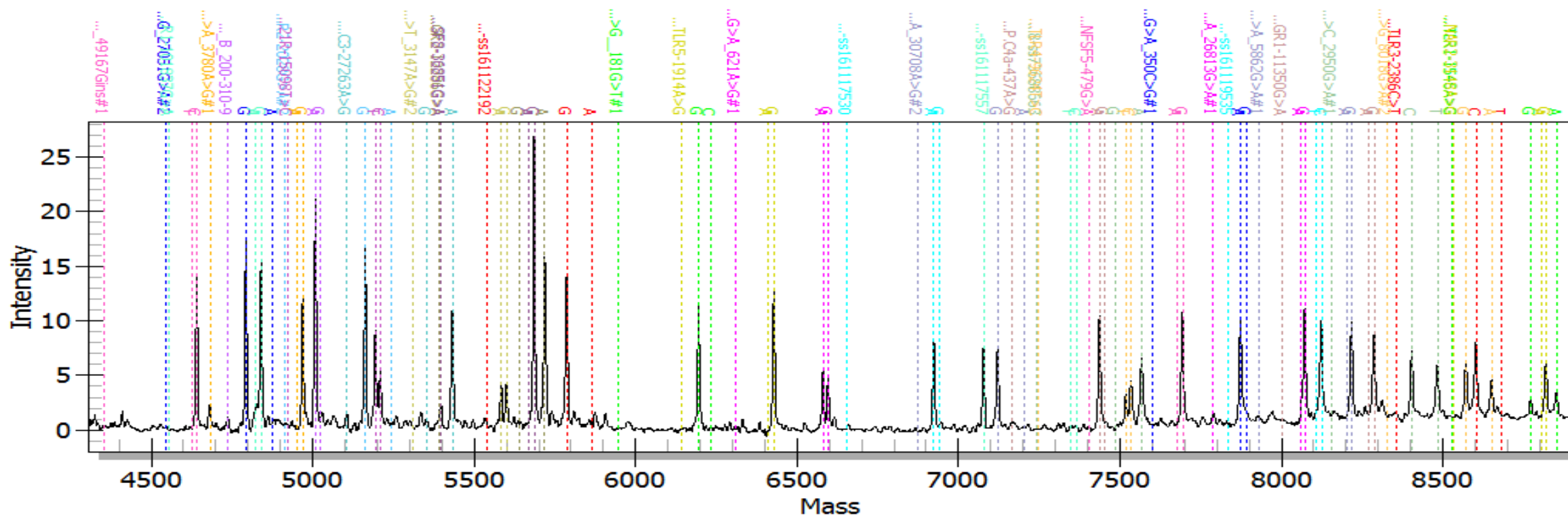
Separate run

not require batch run

ADVANCED SOFTWARE : TYPER

Minimal Bioinformatics Analysis

Simplified data analysis and reporting. No complex bioinformatics required.



CUSTOM PANEL

FREE ONLINE ASSISTANT : CUSTOMIZATION

The image displays two side-by-side screenshots of the Agena Bioscience online tools interface.

Left Screenshot: Customer Support Portal

- Logo: **Agena BIOSCIENCE**
- Section: **CUSTOMER SUPPORT PORTAL**
- Input fields: Email (`monthathip@lifomics.com`) and Password (masked with dots).
- Buttons: **Log in**, [Forgot your password?](#), [Need Agena Customer Portal Assistance?](#), and [Agena Employee Access](#).

Right Screenshot: Assay Design Suite

Top navigation: **Agena BIOSCIENCE** | ASSAY DESIGN SUITE | [Home](#) | [Design History](#) | [Help](#) | [Logout](#)

DESIGN TYPE: Genotyping

Design Name: Genotyping Example 2

Version: 1

Current Input: 9 [View](#)

rs or FASTA: [Edit Text Input](#)

Files: [File Upload](#)

Redesign Options: [Dropdown]

Preset: Moderate Multiplexing iPLEX

Organism: Human

Database: GRCh38/hg38(dbSNP154)

Chemistry: iPLEX

Multiplex Level: 24

[Advanced Settings](#)

Workflow Progress:

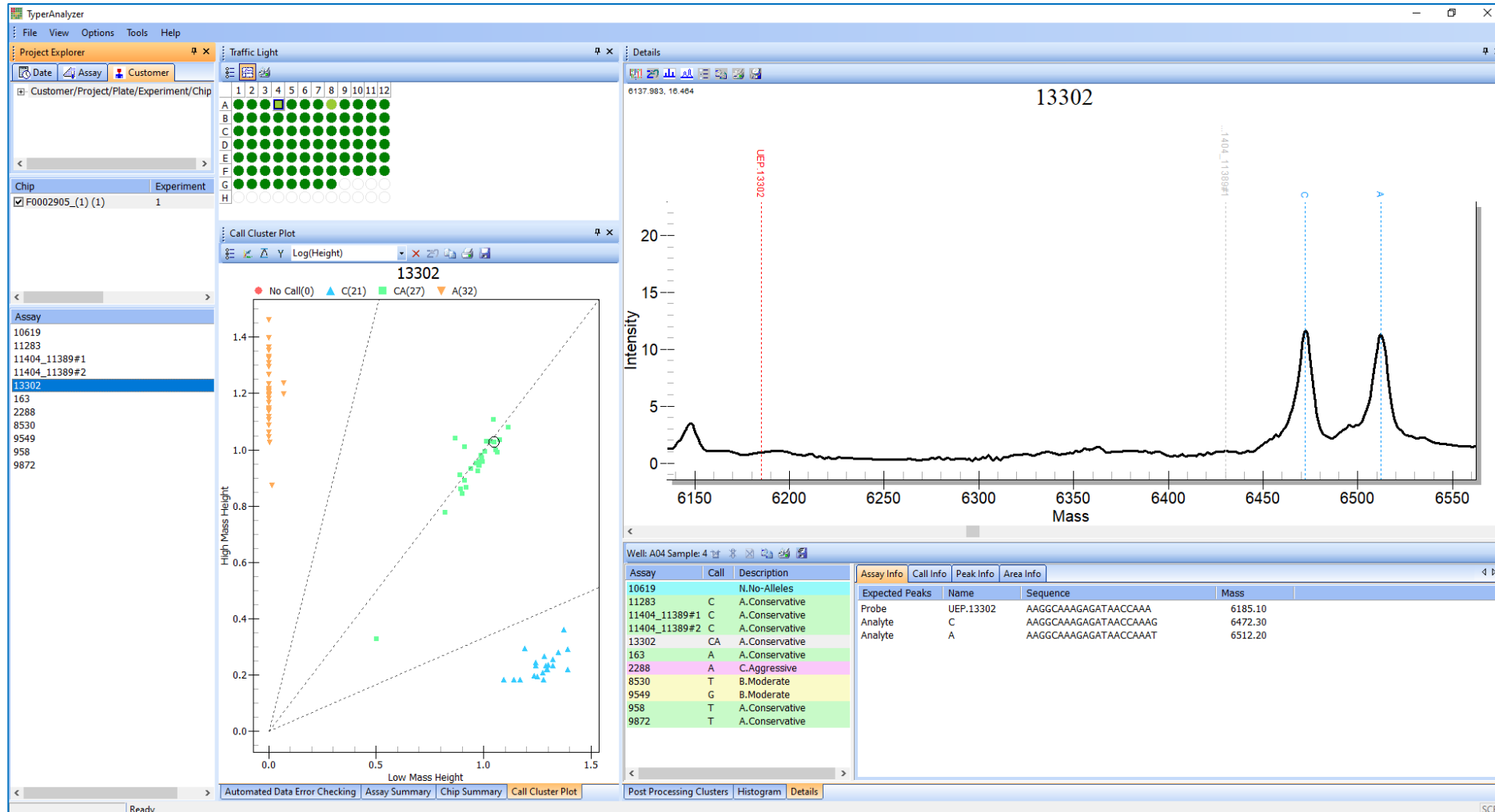
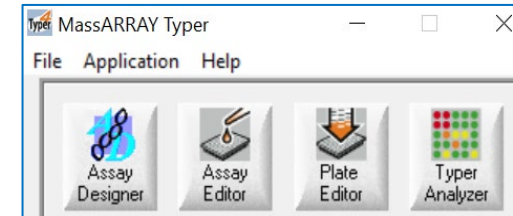
Step	Action	Status	Time
1	Retrieve & Format Sequences	View Results	0 mins 30 secs
2	Find Proximal SNPs	View Results	1 min 0 secs
3	Identify Optimal Primer Areas	View Results	2 mins 31 secs
4	Design Assays	View Results	0 mins 31 secs
5	Validate	View Results	2 mins 30 secs

[Export All](#) [Begin/Cancel Run](#)

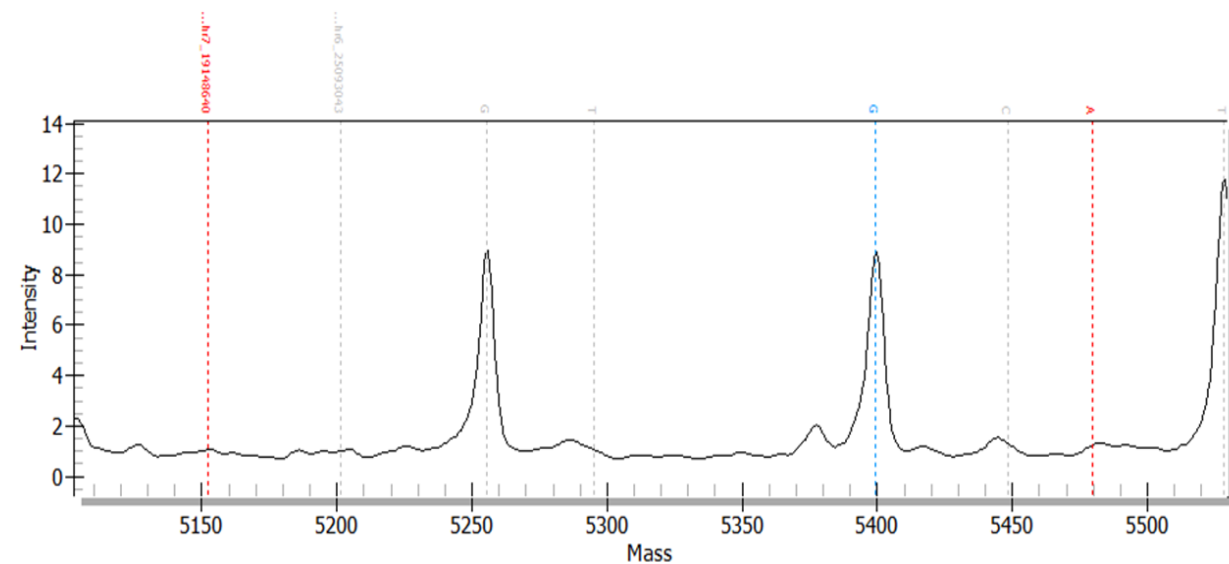
<https://agenacx.com/online-tools/>

REPORT: EASE INTERPRETATION

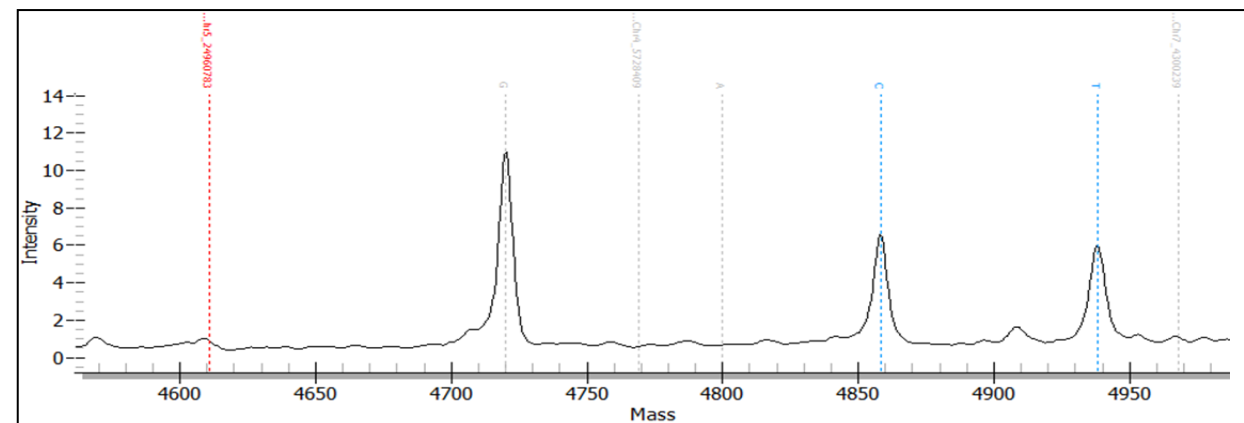
TYPER software > Typer Analyzer



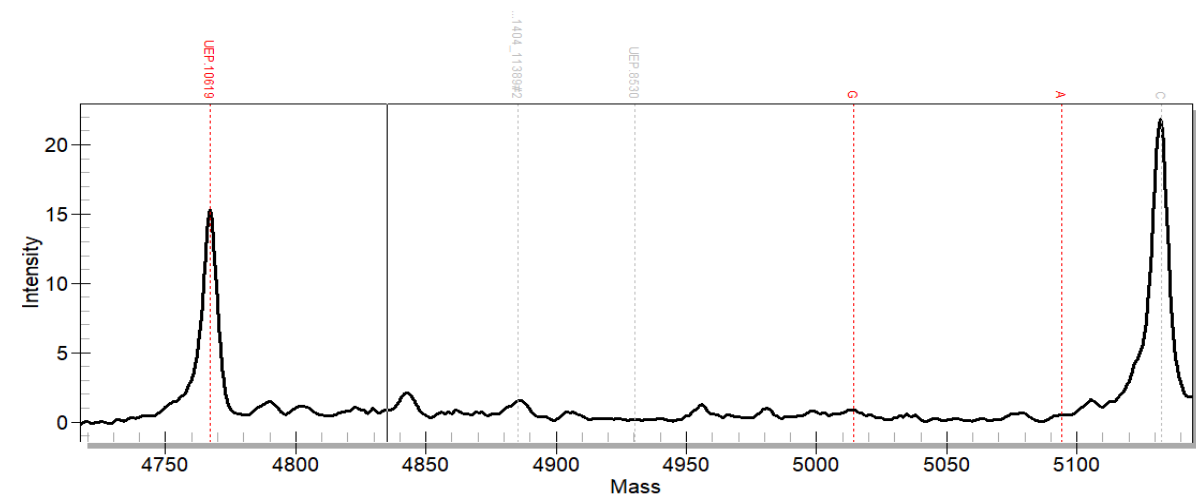
REPORT: Spectrum peak



Call: Homozygous GG



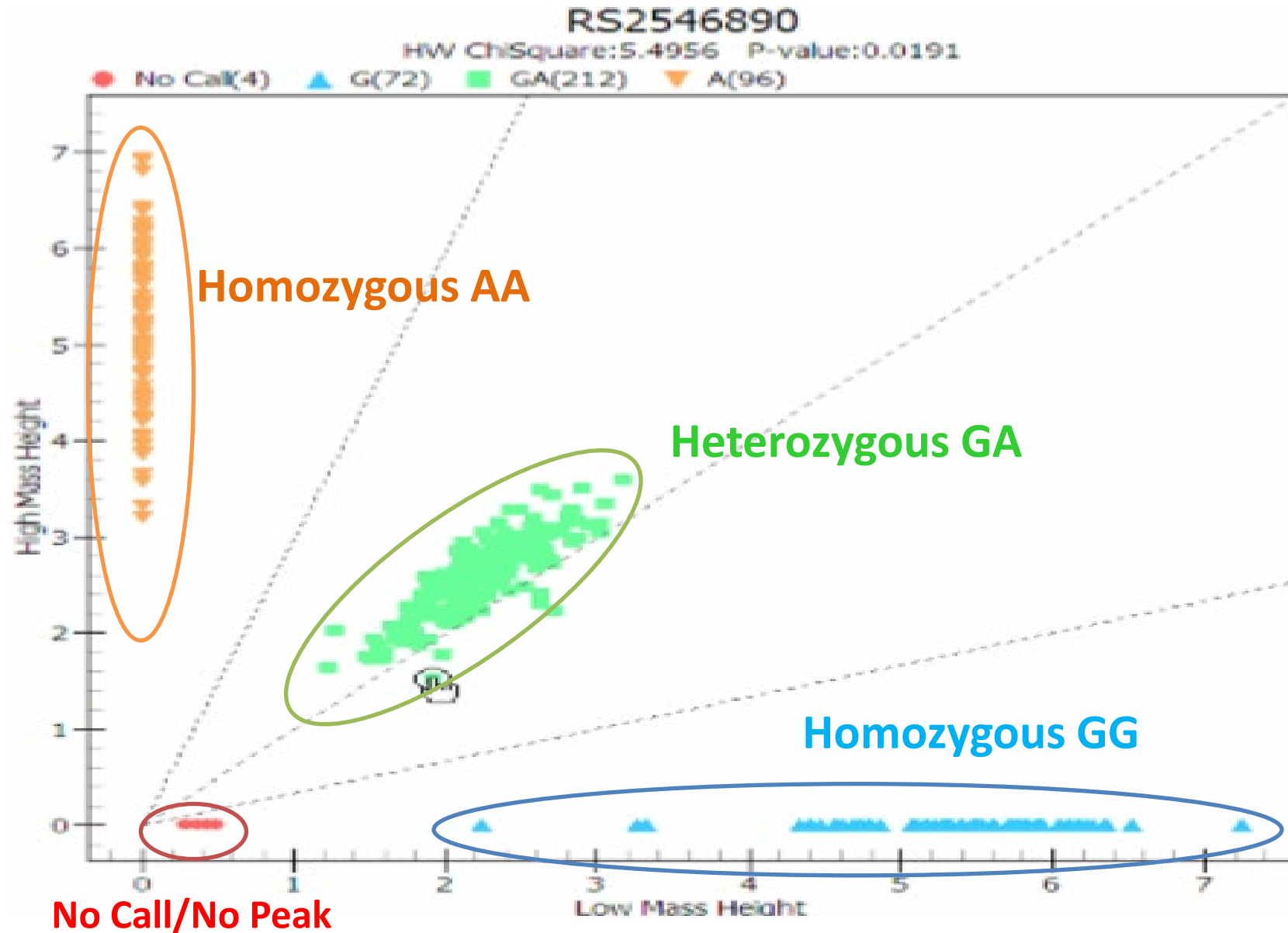
Call: Heterozygous CT



Only UEP Peak

No Call

CALL CLUSTER PLOT



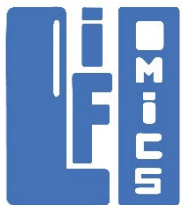
REPORT OPTION: EXPORT FORMAT



Sample Name

Assay Name

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
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_C
2	16016	D04	G	T	C	A	C	A	T	G	G	G	G	T	T	A	C	C	A
3	16017	D05	G	T	C	A	C	A	T	G	G	G	G	T	T	A	C	C	G
4	C-588 F	C04	A	T	T	A	C	A	T	G	G	A	A	T	T	G	C	C	A
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



MASSARRAY TECHNOLOGY



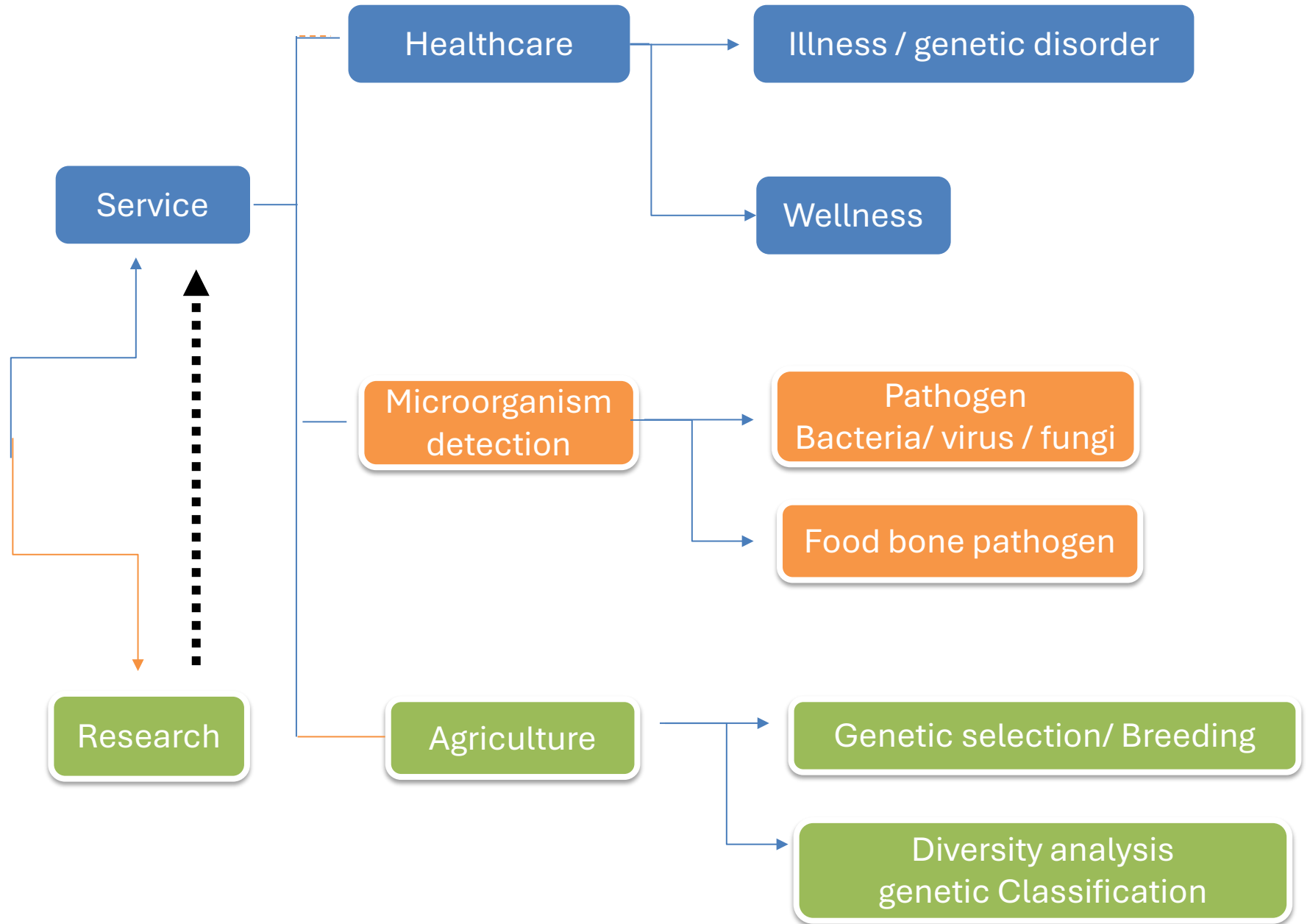
Introduction

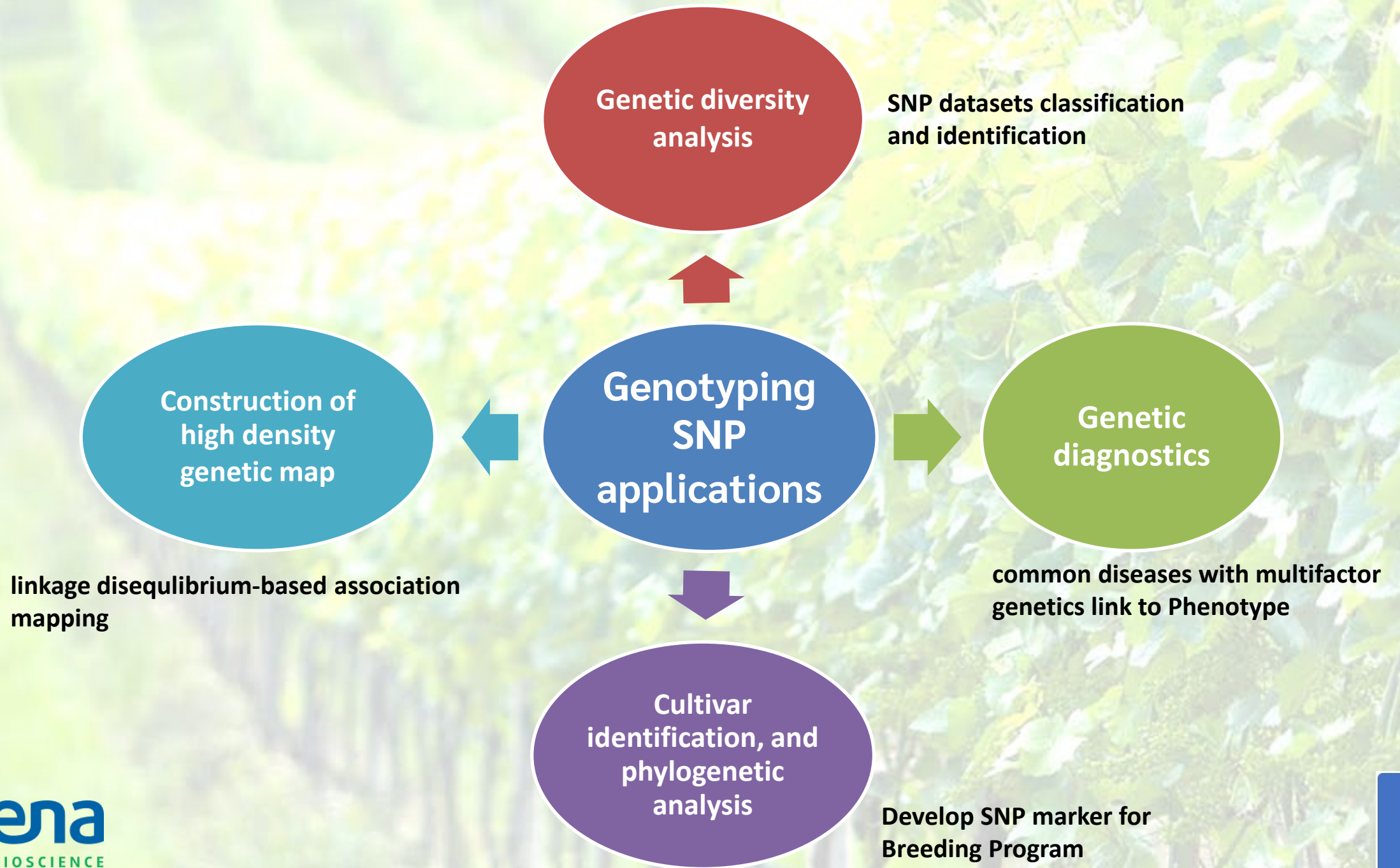
MassARRAY debrief

Applications

Discussion

Applications





เทคนิคการตรวจกล้าปาล์มน้ำมันลูกผสมอย่างรวดเร็วโดยใช้เครื่องหมายโมเลกุลสลับ Rapid

Detection Technique of Oil Palm Seedlings Using SNPs Molecular Markers

ภรณี สว่างศรี และคณะ 2560

ผลงานวิจัยดีเด่น กรมวิชาการเกษตร ปี 2560

สำนักวิจัยพัฒนาเทคโนโลยีชีวภาพ (Biotechnology Research and Development Office) และ ศูนย์วิจัยปาล์มน้ำมันสุราษฎร์ธานี

เทคนิคการตรวจกล้าปาล์มน้ำมันลูกผสมอย่างรวดเร็วโดยใช้เครื่องหมายโมเลกุลสลับ

Rapid Detection Technique of Oil Palm Seedlings Using SNPs Molecular Markers

ภรณี สว่างศรี¹ รุ่งภา พิทักษ์สินกุล¹ อรรัตน์ วงศ์ศรี¹ สุวัฒน์ กอศักดิ์¹ ชัยอนิจ ดิสฐบรรจง¹

ดนัย นาคประเสริฐ¹ พิชัยรัตน์ ผู้เริงศรี¹

Paranee Sawangsi¹ Rungrapa Pitakansakul¹ Oratt Worngsi¹ Suwimon Kolasuk²

Chayanit Distabanjong¹ Danai Nakprasert¹ Hathairat Urairong¹

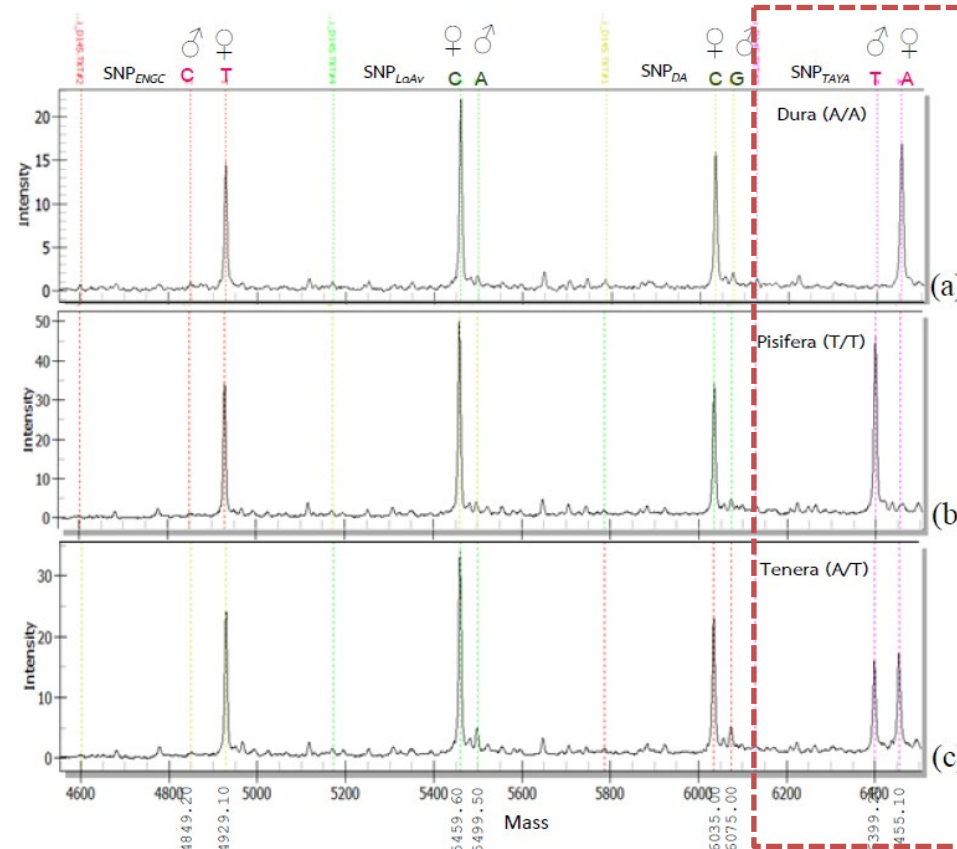
ABSTRACT

Mass production of oil palm seedlings may be contaminated due to the presence of dura type resulting in reduction of seedling quality. Inspection of dura type in nursery is necessary for maintain high quality of tenera oil palm. The application of real time PCR technique is employed for this detection technique is rapid, low cost and precise. The research was conducted at the Biotechnology Research and Development Office and the Suratthani Center during July 2015 to June 2017. The research work was carried out by bulk sample using SNPs molecular markers in Surat-Thani 7, tenera group). Five percent of seedlings (500 seedlings out of 10,000 seedlings) for bulk DNA extraction (10 seedlings/sample), those contaminated standard value (0 - 100 percent). The result showed that 50 of the samples were contaminated with dura type whereas 1 sample showed no contamination. Among 26 samples, all single seedlings confirmed the previous result. There were 3 dura seedlings four groups that equivalent to 0.6 percent of false result (from technique reveals the acceptable and practicable for oil palm). In addition, the application of MassARRAY technique was employed for markers analysis for 4 locations of SNPs markers simultaneously distinguished oil palm group and tenera type by a single test.

Keywords : oil palm seedling, rapid detection, bulk sample, SNP

¹ สำนักวิจัยพัฒนาเทคโนโลยีชีวภาพ (Biotechnology Research and Development Office)

² ศูนย์วิจัยปาล์มน้ำมันสุราษฎร์ธานี (Suratthani Oil Palm Research Center)



SNP_{TAYA}



A/A dura Sh/Sh pisifera sh/sh T/T



A/T tenera Sh/sh

ใช้ MassARRAY ตรวจสลับทั้ง 4 ตำแหน่ง ได้แก่ SNP_{ENG} SNP_{LaAv} SNP_{DA} และ SNP_{TAYA} พบว่าตำแหน่ง SNP_{TAYA} แสดง Genotype ที่เป็น polymorphism สอดคล้องกับลักษณะ Phenotype ของผลปาล์ม ที่เมื่อ Genotype เป็น Heterozygous AT แสดงลักษณะผลเปลือกหนากะลาบางซึ่งเป็นที่ต้องการของตลาด ซึ่งสามารถคัดเลือกต้นลูกผสมที่มีลักษณะดังนี้ได้ตั้งแต่ระยะต้นกล้า

Identification of gene associated with sweetness in corn (*Zea mays* L.) by genome-wide association study (GWAS) and development of a functional SNP marker for predicting sweet corn.

RUANJAICHON, Vinitchan, et al. 2021, Plants,10.6: 1239.



Article

Identification of Gene Associated with Sweetness in Corn (*Zea mays* L.) by Genome-Wide Association Study (GWAS) and Development of a Functional SNP Marker for Predicting Sweet Corn

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Abstract: Sweetness is an economically important nating quality trait for sweet-corn breeding. To investigate the genetic control of the sweetness trait, we conducted a genome-wide association study (GWAS) in an association panel consisting of 250 sweet corn and waxy corn inbred and recombinant inbred lines (RILs), together with the genotypes obtained from the high-density 600K maize genotyping single-nucleotide polymorphism (SNP) array. GWAS results identified 12 significantly associated SNPs on chromosomes 3, 4, 5, and 7. The most associated SNP, AX_91849634, was found on chromosome 3 with a highly significant p-value of $<1.53 \times 10^{-14}$. The candidate gene identified within the linkage disequilibrium (LD) of this marker was *shrunken2* (*Zm00001d04122*; *sh2*), which encodes ADP-glucose pyrophosphorylase (AGPase), a 60 kDa subunit enzyme that affects starch metabolism in the maize endosperm. Several SNP markers specific to variants in *sh2* were developed and validated. According to the validation in a set of 81 inbred, RIL, and popular corn varieties, marker *Sh2_rs844805326*, which was developed on the basis of the SNP at the position 154 of exon 1, was highly efficient in classifying *sh2*-based sweet corn from other types of corn. This functional marker is extremely useful for marker-assisted breeding in *sh2*-sweet corn improvement and marketable seed production.

Keywords: maize; kernel sweetness; *shrunken2*; genome-wide association study (GWAS); single-nucleotide polymorphism (SNP)

1. Introduction

Sweet corn (*Zea mays* L. var. *rugosa* Bonaf.) is globally an economically important crop, and it is widely used for human consumption from both immature and mature kernels as fresh, canned, and frozen due to its high sugar content [1–3]. The demand for sweet corn has been rising on a yearly basis due to enhanced consumption and the increasing

check for updates

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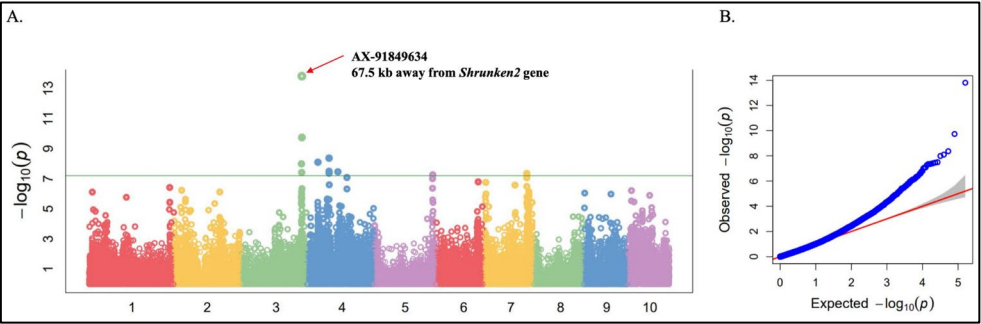
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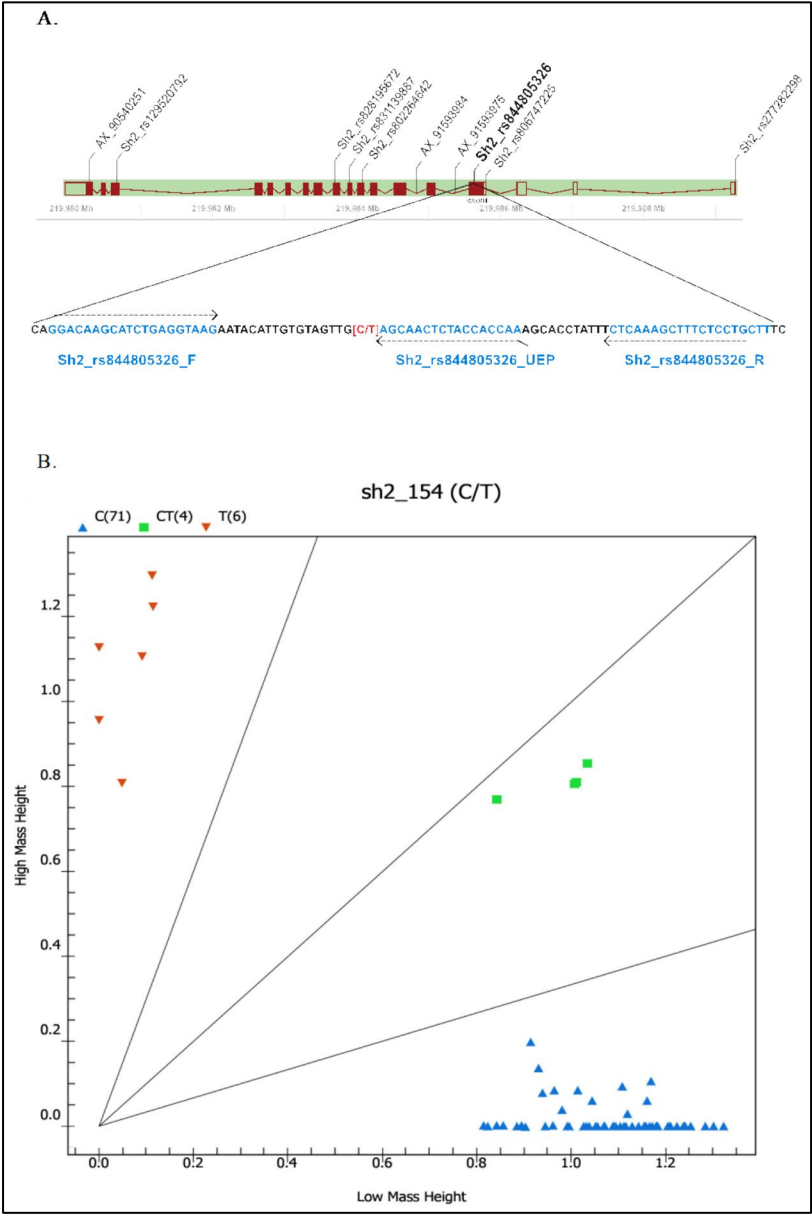
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Plants 2021, 10, 1239; <https://doi.org/10.3390/plants10061239>



การใช้เทคนิค MassARRAY ในงาน SNP detection เพื่อคัดเลือกรหัส functional marker ที่เกี่ยวข้องกับยีนที่ควบคุมการสร้างน้ำตาล (shrunken2) ในเมล็ดข้าวโพดหวาน (sweet corn) จากการค้นหา SNP และศึกษา GWAS พบ SNP ตำแหน่ง sh2_154 บนโครโมโซม 3 มีความสัมพันธ์กับความหวานในเมล็ด จึงสามารถใช้ตำแหน่งนี้เพื่อค้นหาต้นที่มีลักษณะที่ต้องการในงานปรับปรุงพันธุ์ต่อไป





A													B													C																
	Person A	Person B	Person C	Person D	Person E	Person F	Person G	Person H	Person I	Person J	Person K	Person L		Person M	Person N	Person O	Person P	Person Q	Person R	Person S	Person T	Person U	Person V	Person W		Person X	Person Y	Person Z	Person AA	Person AB	Person AC	Person AD	Person AE	Person AF	Person AG	Person AH	Person AI	Person AJ	Person AK	Person AL		
Person A	1	2	3	4	5	6	7	8	9	10	11	12		13	14	15	16	17	18	19	20	21	22	23		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Person B	1	2	3	4	5	6	7	8	9	10	11	12		13	14	15	16	17	18	19	20	21	22	23		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Person C	1	2	3	4	5	6	7	8	9	10	11	12		13	14	15	16	17	18	19	20	21	22	23		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Person D	1	2	3	4	5	6	7	8	9	10	11	12		13	14	15	16	17	18	19	20	21	22	23		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Person E	1	2	3	4	5	6	7	8	9	10	11	12		13	14	15	16	17	18	19	20	21	22	23		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Person F	1	2	3	4	5	6	7	8	9	10	11	12		13	14	15	16	17	18	19	20	21	22	23		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Person G	1	2	3	4	5	6	7	8	9	10	11	12		13	14	15	16	17	18	19	20	21	22	23		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Person H	1	2	3	4	5	6	7	8	9	10	11	12		13	14	15	16	17	18	19	20	21	22	23		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Person I	1	2	3	4	5	6	7	8	9	10	11	12		13	14	15	16	17	18	19	20	21	22	23		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Person J	1	2	3	4	5	6	7	8	9	10	11	12		13	14	15	16	17	18	19	20	21	22	23		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Person K	1	2	3	4	5	6	7	8	9	10	11	12		13	14	15	16	17	18	19	20	21	22	23		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Person L	1	2	3	4	5	6	7	8	9	10	11	12		13	14	15	16	17	18	19	20	21	22	23		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Person M	1	2	3	4	5	6	7	8	9	10	11	12		13	14	15	16	17	18	19	20	21	22	23		24	25	26	27													

Article

Towards Heat Tolerant Runner Bean (*Phaseolus coccineus* L.) by Utilizing Plant Genetic Resources

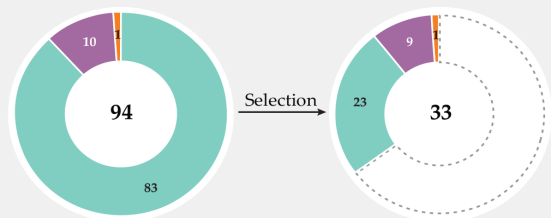
Svenja Bomers ^{1,†}, Eva M. Sehr ^{2,†}, Eveline Adam ³, Philipp von Gehren ¹, Karin Hansel-Hohl ², Noémie Prat ¹ and Alexandra Ribarits ^{1,*}

MATERIAL

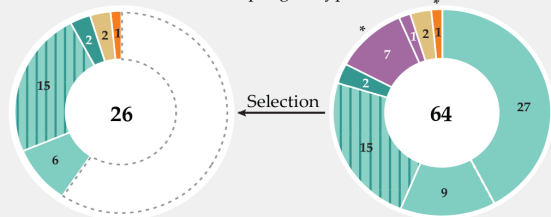
GENOTYPING

PHENOTYPING

2018
n = 8 per genotype



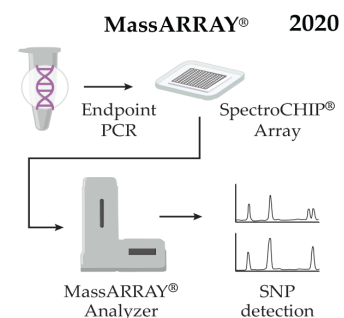
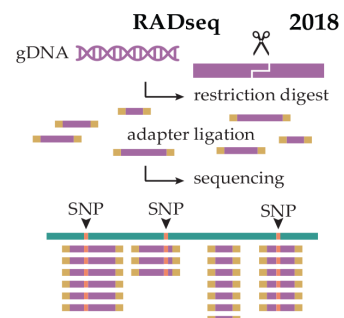
2020
n = 6 per genotype



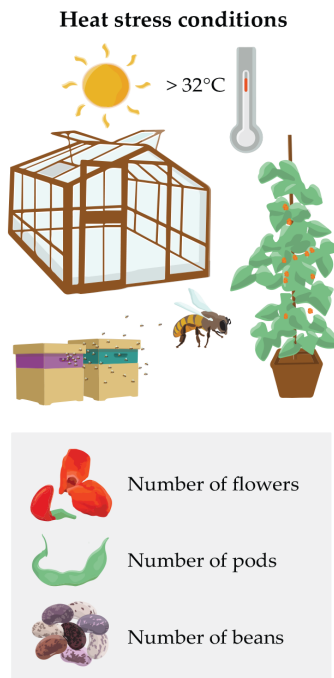
Accession 2018
 Accession 2020
 Spanish accession
 * Double phenotyped
 Variety
 Breeding line
 Bonela

METHODS

GENOTYPING



PHENOTYPING



GWAS I

260 $G_{18} \sim P_{18}$

7 SNPs

GWAS II

33 $G_{18} \sim P_{18}$

148 SNPs

45 $G_{18} \sim P_{20}$

182 SNPs

65 SNPs

in total **82 SNPs**

Literature

10 SNPs

Blast result

72

MassARRAY® result

52

Monomorphic

40

Favorable allele identification

18 SNPs



การพัฒนาเครื่องหมายโมเลกุลเพื่อจำแนกเพศในหม่อนผลสด

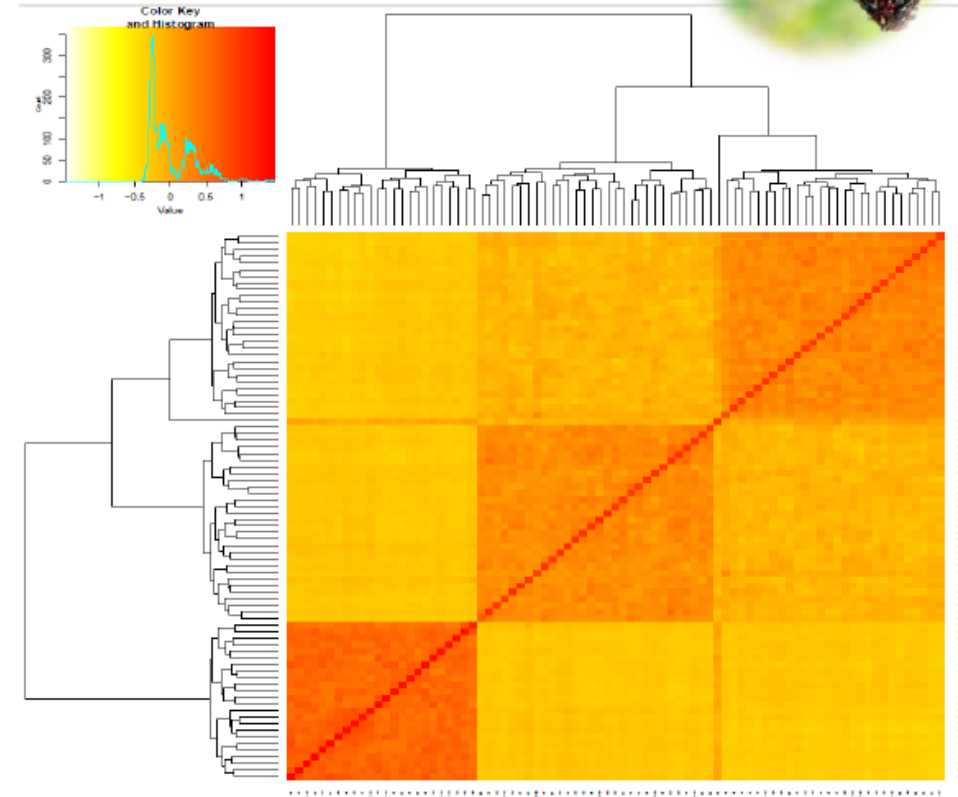
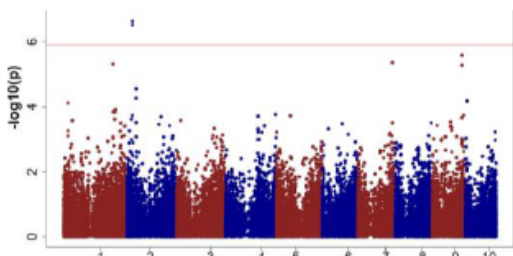
(DNA marker development for sex classification in *Morus* spp.)



ต้นหม่อน F1



ประชากร	จำนวน SNP	Polymorphic SNP
1. SRC650 x African 1	23,050	2,203
2. SK2802 x น้ำพรม 2	24,041	2,416
3. ขนกลาง 3 x น้ำพรม 2	23,741	1,802



Population diversity illustrated with Kinship

20 highest associated SNP as markers

MassARRAY markers were designed for sex determination in mulberry

SNP ID	Forward Primer ID	Forward Primer Sequence	Reverse Primer ID	Reverse Primer Sequence	Extended Primer ID	Extended Primer Sequence
9872_W1	9872_W1_F	ACGTTGGATGATTGCAGGTACAAGAAGC	9872_W1_R	ACGTTGGATGCATTTCACATAGCGAGG	9872_W1_E	TGCAGATTGCTTGGG
10619_W1	10619_W1_F	ACGTTGGATGCACAGAATGGCTTGAGAG	10619_W1_R	ACGTTGGATGGTCCGACCTATTAGCATC	10619_W1_E	ACACCATTGTTCCT
8530_W1	8530_W1_F	ACGTTGGATGAAGCTTGGGAGCAATCTG	8530_W1_R	ACGTTGGATGTCAAGAGCTCGAAGGAAG	8530_W1_E	GAGCAATCTGCAGGTA
163_W1	163_W1_F	ACGTTGGATGTCCCTGCTCTGTTAAGTG	163_W1_R	ACGTTGGATGAACATTTGGGCTCTCTCC	163_W1_E	CTCTCCCGCATCTCT
9549_W1	9549_W1_F	ACGTTGGATGAGTCTGGTCCACCATTG	9549_W1_R	ACGTTGGATGAATTGCGTGCA		
13302_W1	13302_W1_F	ACGTTGGATGGGCTTCTTTTTTCGTTT	13302_W1_R	ACGTTGGATGGAACAAGCTGC		
2288_W1	2288_W1_F	ACGTTGGTGCCTTGAGTAGGAAAGGTG	2288_W1_R	ACGTTGGATGTGGGAACCTGG		
11283_W1	11283_W1_F	ACGTTGGATGAATCACCAAGTGAACCC	11283_W1_R	ACGTTGGATGCAACCGTAAATC		
958_W1	958_W1_F	ACGTTGGATGAGGTTTGGTTGACTGCG	958_W1_R	ACGTTGGATGAGCGGACATGC		
11404_11389 #2_W1	11404_11389 #2_W1_F	ACGTTGGATGATTTCGACAAGCGTAGCC	11404_11389 #2_W1_R	ACGTTGGATGGAATCATGTCA		
11404_11389 #1_W2	11404_11389 #1_W2_F	ACGTTGGATGATTTCGACAAGCGTAGCC	11404_11389 #1_W2_R	ACGTTGGATGGAATCATGTCA		

ลำดับเบสของดีเอ็นเอที่มีความสัมพันธ์กับลักษณะเพศ

ทดสอบในประชากร 80 ต้น

- ตำแหน่งดีเอ็นเอที่เครื่องหมาย 10619_W1

GCTTGAGAGTTTCTGCAGTA[G/A]AGGGAACAAATGGTGTCTTT

ลักษณะพันธุกรรมสอดคล้องกับลักษณะเพศโดย เบส G มีแนวโน้มในการกำหนดลักษณะเพศต้นตัวเมีย เบส A กำหนดลักษณะเพศผู้ 90.70%

- ตำแหน่งดีเอ็นเอที่เครื่องหมาย 9872_W1

GAAGCTGCAGATTGCTTGGG[T/C]GCCGACTGTGTGCCTCGCTA

ลักษณะพันธุกรรมสอดคล้องกับลักษณะเพศโดย เบส T มีแนวโน้มในการกำหนดลักษณะเพศต้นตัวเมีย เบส C กำหนดลักษณะเพศผู้ 73.47%





Rubber tree application

- 376 individuals for DArTseq (2016-2017)  **DArT** | diversity arrays technology

- GWAS analysis in Leaf Fall Disease

- Highly significant in GWAS
- Literature review

- 280 samples with 30 markers

minimizing off-target with Sanger

Total SNPs	Average	Min	Max
% Total SNPs	91.88	55.71	100.00
Homo ref. freq	0.57	0.02	0.99
Homo alt. freq	0.29	0.00	0.97
Hetro. Freq	0.15	0.00	0.45



th.wikipedia.org

Applications for medical plant

- จำแนก และระบุสายพันธุ์พืชสมุนไพร เช่น จำแนกสายพันธุ์ กัญชา กลุ่มหมามู่ย กลุ่มเจตมูลเพลิง ขมิ้นชัน
- ระบุสายพันธุ์พืชปลอมปนในยาผง เช่น การปนพืชสะเดา คະน้ำลงในแคปซูลของฟ้าทะลายโจร
- จำแนกเพศ เช่น พืชกัญชา แยกตัวผู้ ตัวเมีย เป็นต้น
- งานปรับปรุงพันธุ์ ตรวจหา **Marker** ที่สัมพันธ์กับการผลิต

สารสำคัญ

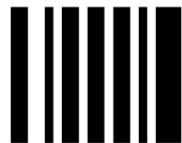
Species



DNA



Barcode

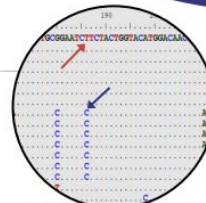




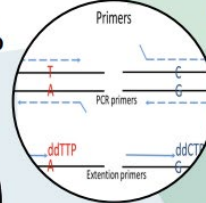
สถาบันวิจัยสมุนไพร
กรมวิทยาศาสตร์การแพทย์
Department of Medical Sciences



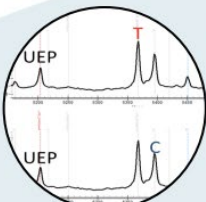
เปิดบริการ
การตรวจวิเคราะห์สารพันธุกรรมใน
ตำแหน่งโมเลกุลสั้น หรือตำแหน่ง InDel



1. เลือกตำแหน่งที่สนใจ



2. ออกแบบไพรเมอร์



3. วิเคราะห์มวลโมเลกุล

เป็นการวิเคราะห์สารพันธุกรรมในตำแหน่งโมเลกุลสั้น หรือตำแหน่ง InDel ด้วยเทคนิค MALDI-TOF Mass Spectrometry โดยใช้หลักการวิเคราะห์มวลโมเลกุลของสารพันธุกรรม เป็นเทคนิคที่แม่นยำ รวดเร็ว และตรวจได้หลายตัวอย่างพร้อมกัน

ชนิดตัวอย่าง

- DNA
- RNA

* จากพืช สัตว์ หรือสิ่งมีชีวิตที่ไม่ก่อโรคในคน

การนำไปใช้ประโยชน์

- การระบุชนิดพืชสมุนไพร
- การตรวจพืชดัดแปรพันธุกรรม (GMOs)
- การคัดเลือกพันธุ์พืช
- การปรับปรุงพันธุ์พืชและสัตว์
- การตรวจยีนก่อโรคต่างๆ
- การตรวจการปนปลอมของพืชสมุนไพร

สอบถามรายละเอียดได้ที่

โทรศัพท์: 02-9510000 ต่อ 99390
Email: mpri_dmssc@dmssc.mail.go.th
<https://mpri.dmssc.moph.go.th>
Facebook: MedPlant Dmssc

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	<i>E.coli/Shigella spp.</i>
Ent001	<i>Enterococcus faecalis</i>
Ent003	<i>Enterococcus faecium</i>
Clos001	<i>Clostridium perfringens</i>
Cmp002	<i>Campylobacter jejuni</i>
Cmp005	<i>Campylobacter coli</i>
Cmp006	<i>Campylobacter coli</i>
Lis001	<i>Listeria monocytogenes</i>
Stp001	<i>Staphylococcus aureus</i>
Sal002	<i>Salmonella spp.</i>



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

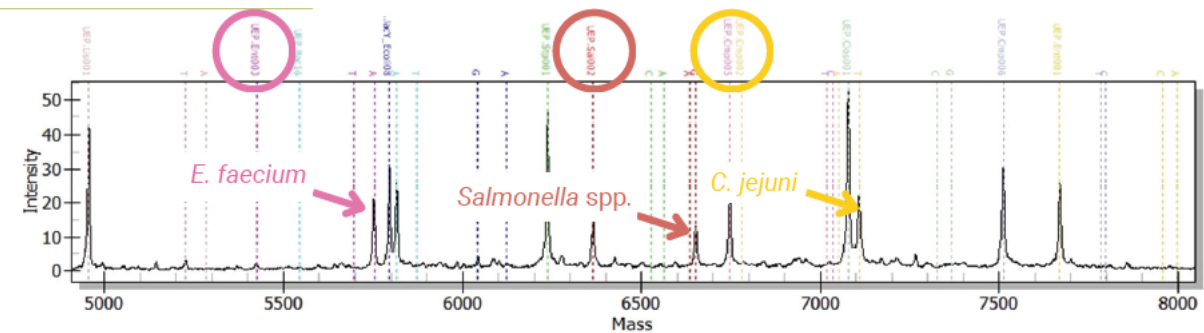
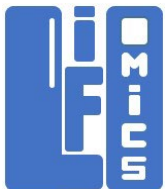


Figure 5 : *Enterococcus faecium*, *Salmonella spp.*, and *Campylobacter jejuni* were identified in a single assay. The circles represent the mass-to-charge ratio (m/z) of unextended primers (UEP), while the arrows indicate the mass spectral peak corresponding to the extended base, facilitating bacterial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



BIOTEC
a member of NSTDA

MassARRAY System workflow



1

2

3

4

5

Select Target
Markers

Design primer

Optimize &
validation

Sample run

Analyze data &
Report

1-4 week

1 week



Run Time: 7 Hrs*
Hands-on Time: 23 Min



Run Time: 90 Mins*
Hands-on Time: 5 Min



Hands-on Time:
5-10 Min

MassARRAY system in Thailand



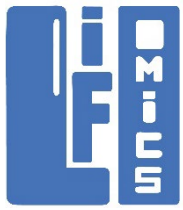
ศูนย์โอมิกส์แห่งชาติ
(National Omics Center)



กรมวิทยาศาสตร์การแพทย์
Department of Medical Sciences



ศูนย์วิทยาศาสตร์ข้าว
ทีมวิจัยนวัตกรรมด้านเทคโนโลยีชีวภาพพืชและการเกษตร
แบบแม่นยำ



THANK YOU



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[lifomics](https://www.instagram.com/lifomics)