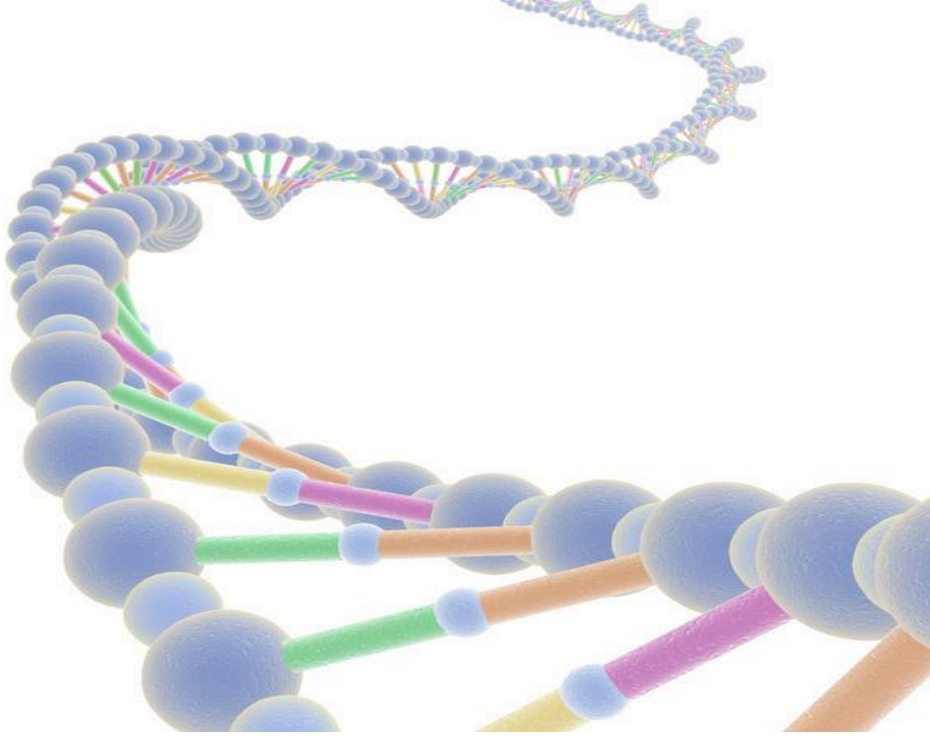


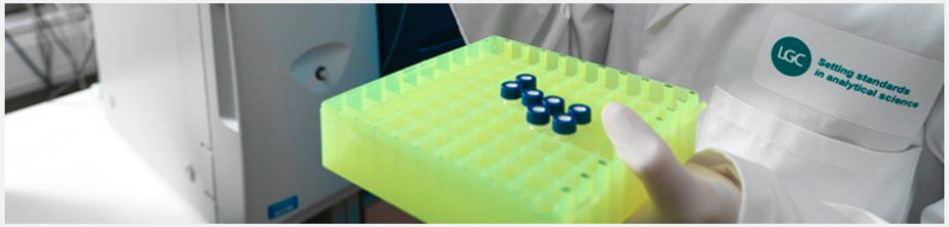
The Powerful Technology for Genomic Analysis

27th March 2018, PSU

- INTRODUCTION
- **Sbeadex** : DNA extraction kit
- **KASP** : method for genotyping
- **MASS ARRAY** : Multi-gene Analysis

- INTRODUCTION
- Sbeadex : DNA extraction kit
- KASP : method for genotyping
- MASS ARRAY : Multi-gene Analysis

About LGC



LGC is an international leader in the extended life sciences sector, including human healthcare, agri-food & the environment.

We provide a comprehensive range of reference materials, proficiency testing schemes, genomics reagents and instrumentation, as well as research and measurement services. Our scientific tools and solutions enable organisations to advance research, develop new products and form an essential part of their quality and compliance procedures.

Our 2,300 employees include internationally-recognised scientists who are experts in their field. LGC is headquartered in London and operates out of 19 countries worldwide. We are extensively accredited to quality standards such as GMP, GLP, ISO 13485, ISO 17034, ISO 17043, ISO/IEC 17025 and ISO 9001.

LGC has been home to the UK Government Chemist for more than 100 years and is the UK National Measurement Laboratory and Designated Institute for chemical and bio measurement.

LGC has been privately-owned since 1996 and has diversified through internal investment and acquisition to be an international leader in its chosen niche markets. LGC is now owned by funds affiliated with KKR.

We help customers conform to international statutory and regulatory standards. Science is at the heart of all we do - for a safer world.

LGC has been home to the UK Government Chemist for more than 100 years and is the UK National Measurement Laboratory and Designated Institute for chemical and bio measurement… Privately-Owned since 1996.

AGENA BIOSCIENCE : COMPANY AT A GLANCE

Agena BIOSCIENCE

SEQUENOM®

(BEFORE 2014)

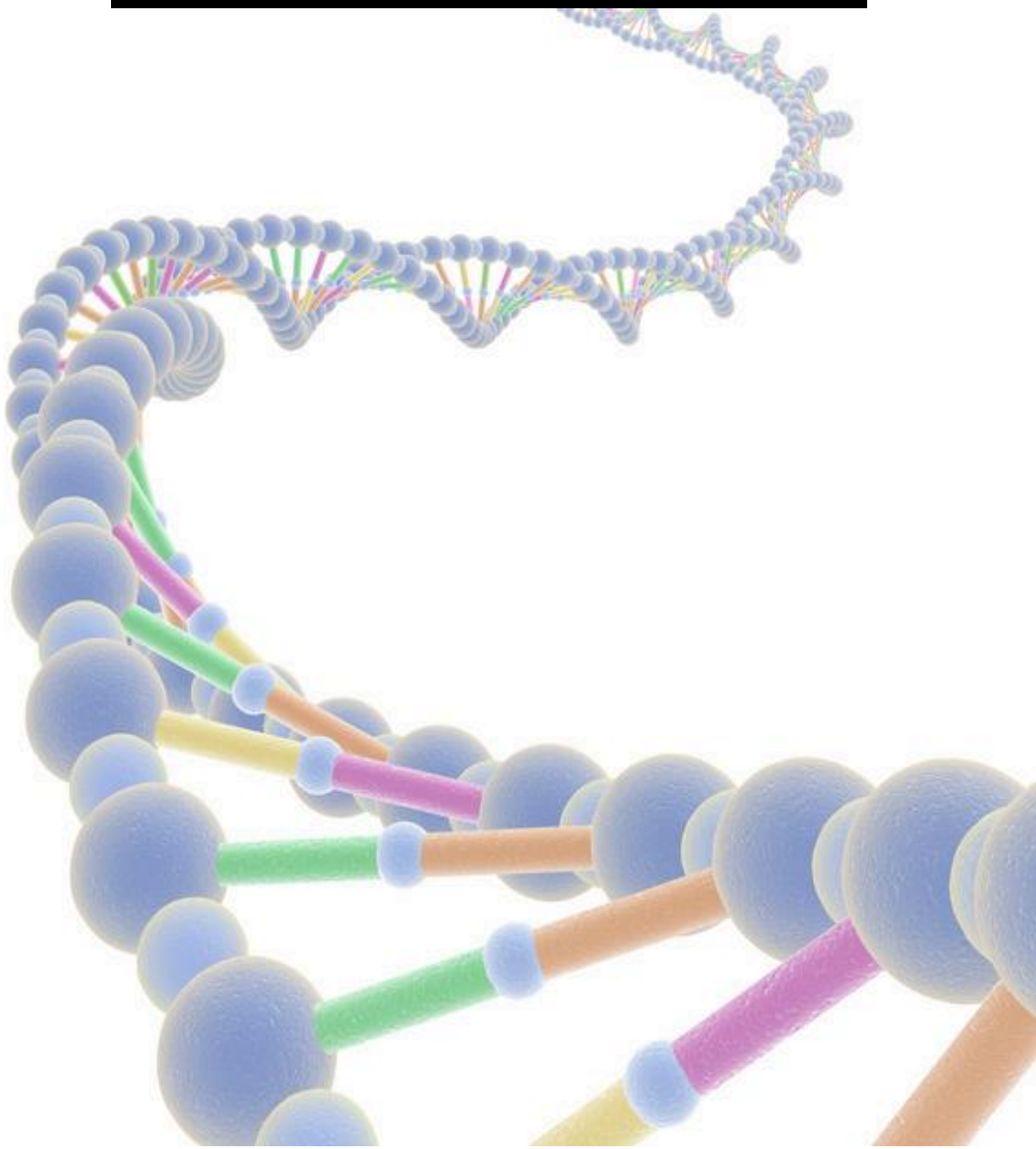
HQs : San Diego, CA

- INTRODUCTION
- **Sbeadex** : DNA extraction kit
- KASP : method for genotyping
- MASS ARRAY : Multi-gene Analysis

Sbeadex： DNA extraction kit for whom seeking both high yield and purified DNA.

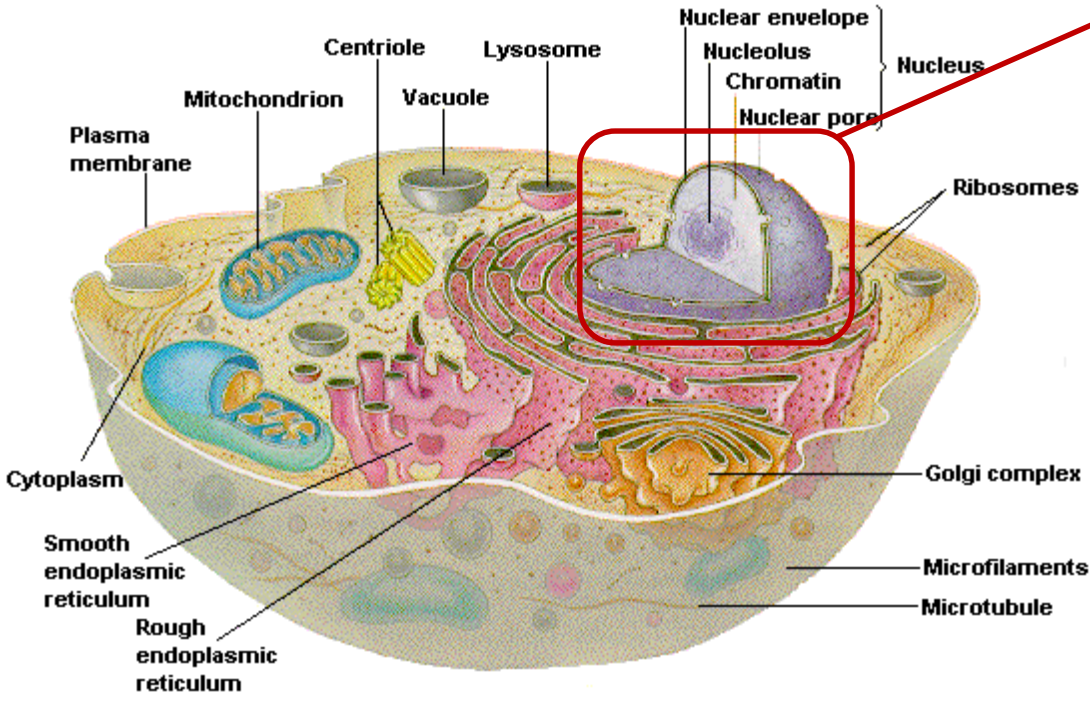
OUTLINE

- INTRO TO DNA
- Workflow overview
- Summary



To get pure DNA: an underestimated challenge

DNA is part of a complex, multicomponent cell.



Goal: pure DNA

Compounds to be removed

High molecular weight compounds

- Polysaccharides
- Polypeptides
- Polyphenols
- Lipids
- Others

Low molecular weight compounds

- Dye stuff
- Secondary metabolites (alcaloides, terpenes)
- Bivalent metal ions
- Preservatives
- Others

→ Storage of samples:

frozen, fresh & storage buffers (ie. Oragene)

→ Anti-Coagulants (blood):

EDTA; Citrate; Heparine

→ Environmental: young, old, illness, etc.

→ Time & throughput

→ Sample weight & volume

→ Human impact

OUTLINE

- INTRO TO DNA
- Workflow overview
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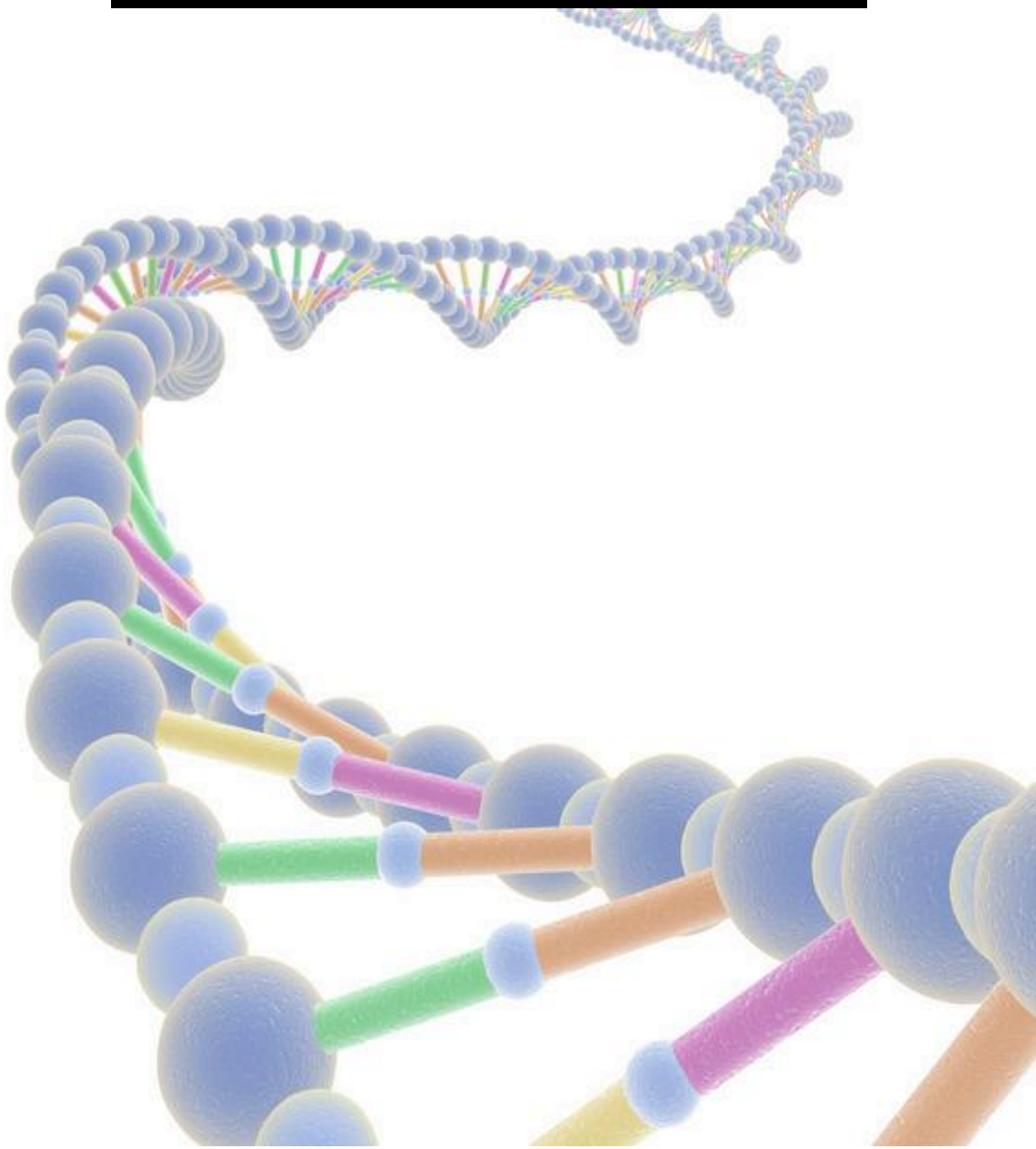
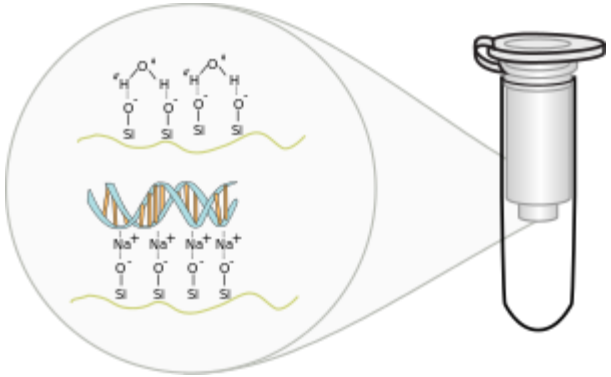


DNA extraction technologies



Columns

Magnetic beads

Common workflow:

Lyse → Bind → Wash → Elute

Lysis conditions – Key for success

I **Homogenization**

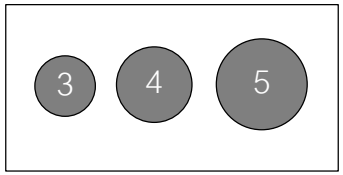
II **Lysis buffer**

III **Incubation**

IV **Centrifugation**

Steal balls:

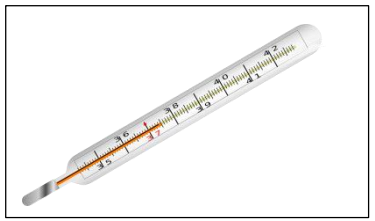
3, 4, 5 mm



CTAB, PVP, SDS

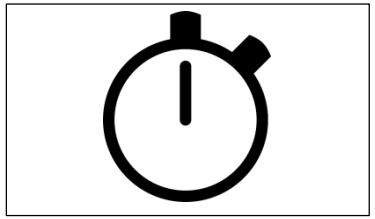
+/- 65°C

>/= 10 min

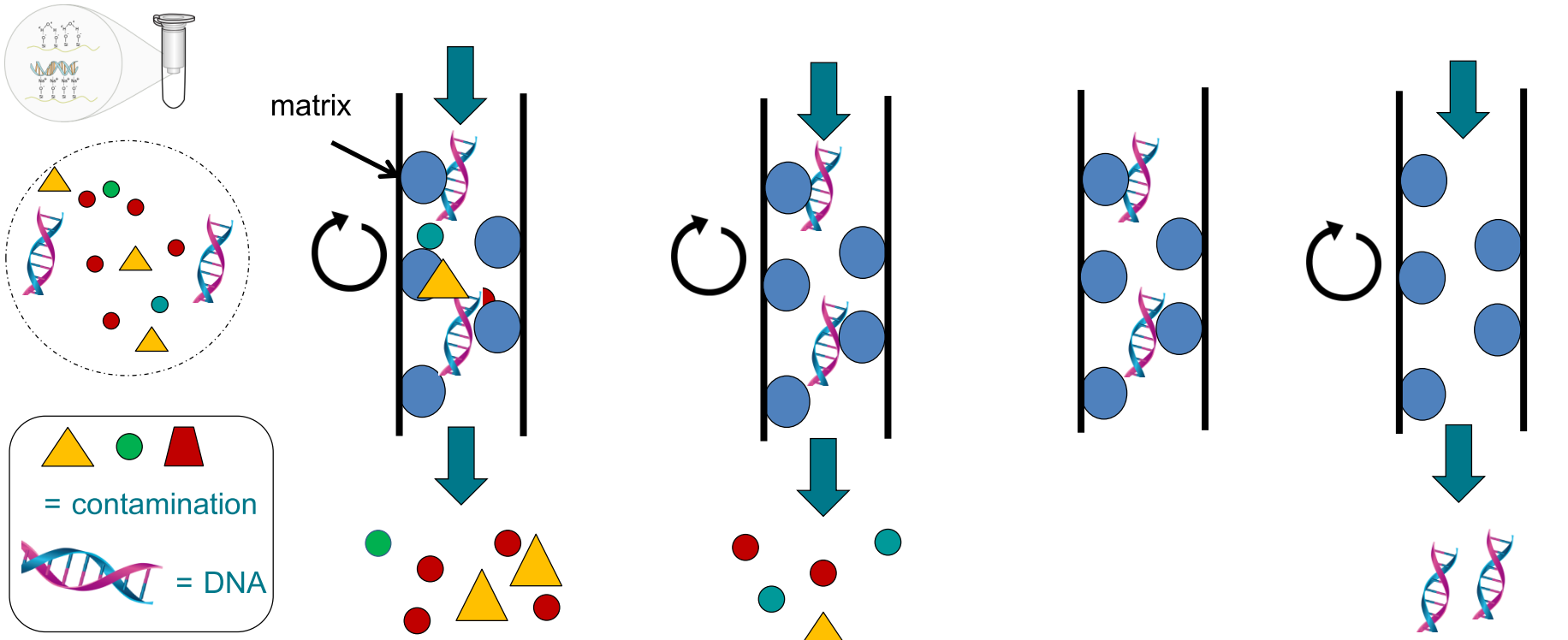


10 min.;

2500 x g



Kleargene spin plates: Columns



1. Lysis of cells → 2. Binding of DNA → 3. Washing of DNA → 4. Drying → 5. Elution of DNA

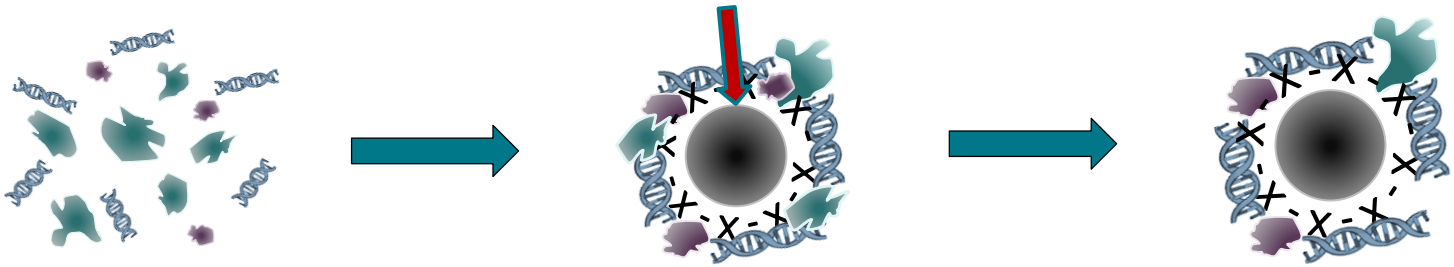
Total time: ~1 h

Kleargene spin plates: Features

Kleargene feature	Specification
Extraction technology	Spin columns
Binding material & technology	Silica → Polarity binding
Final extraction wash buffer	(70%) Ethanol
Extraction format	96 & 384
Starting lysate volume	96 format : 300 µl 384 format: 200 µl
Automation	Semi-automated; i.e. Genespin
Sample material	Plant samples & Rodent tails
Application	Genotyping

sbeadex: Backbone technology

sbeadex magnetic particle



Lysis

Bind 1

Wash 1

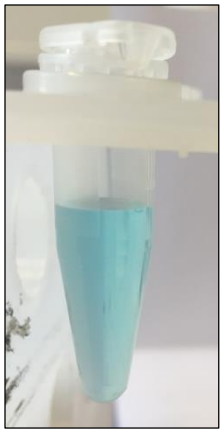
Bind 2

USP

Wash 2 (water)

Elution

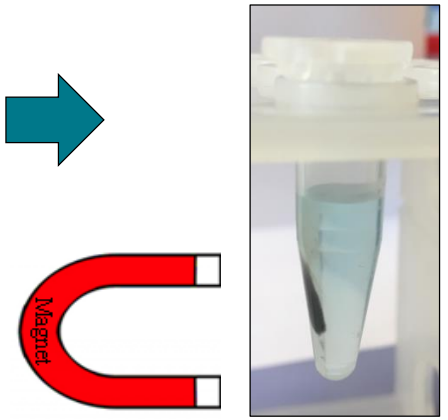
sbeadex: single vials



Lysis

Add beads to lysis

Bind DNA to beads

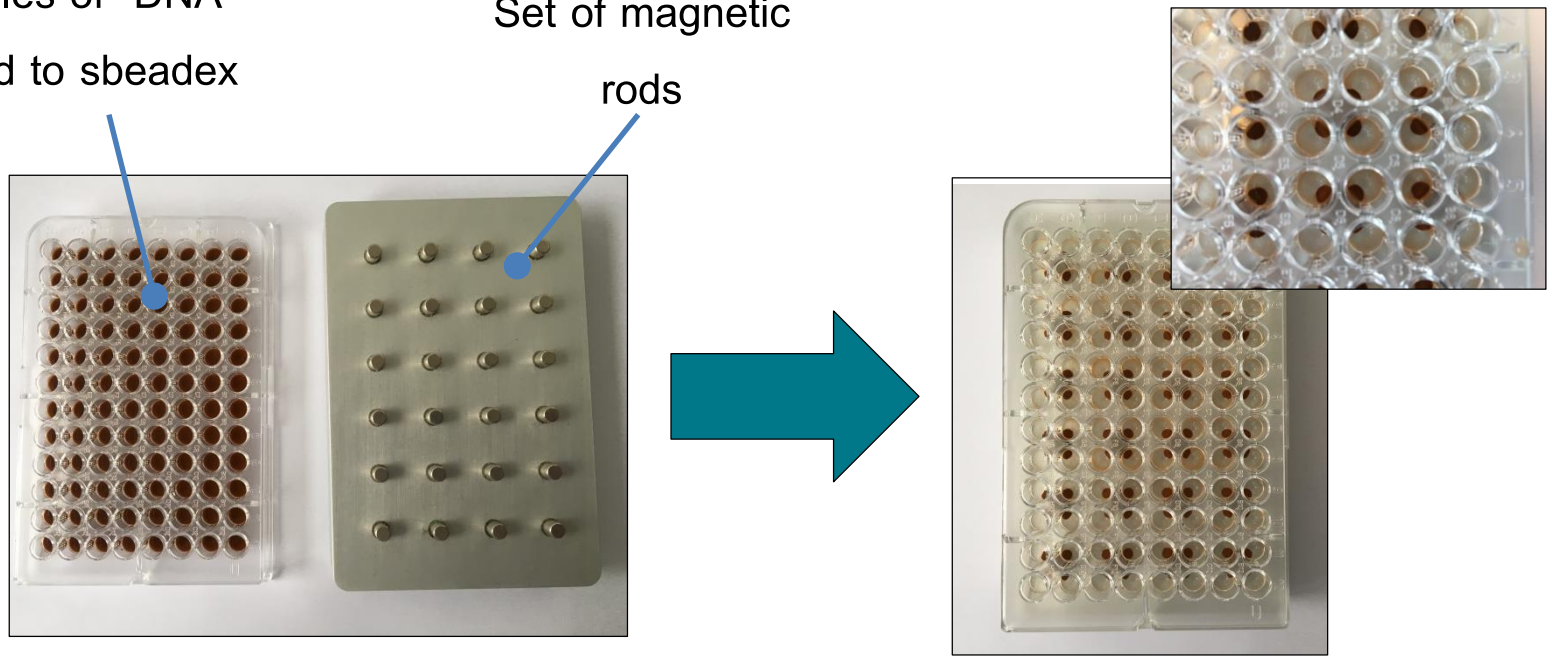


Collect DNA on beads

sbeadex: 96 format (plates)

Microplate with 96 samples of DNA bound to sbeadex

Set of magnetic rods



Manual extractions possible.

>96 Samples?

Automation.

sbeadex: Features

sbeadex feature	Specification
Extraction technology	Proprietory magnetic beads
Binding material & technology	2-step Binding mechanism; Silica & DNA adaptor
Final extraction wash buffer	Pure water
Extraction format	96
Starting lysate volume	sbeadex mini: 20 -30 mg sbeadex maxi: 80 -100 mg
Automation	Fully-automated: oKtopure; KingFisher; open liquid handlers
Sample material	Plant, Livestock, Forensics, Plasmids
Application	All: KASP; Sequencing; arrays; PCR…

Product	Lysis	Bind 1	Wash 1	Bind 2	Wash 2	Wash 3	Dry	Elution
sbeadex	→	→	→	→	Water			
Kleargene	→	→	→		EtOH	→	→	
Qiagen	→	→	→		EtOH		→	

→ Unique 2-step binding mechanism

→ Final water wash

→ No Drying step

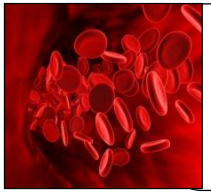
LGC: DNA extraction portfolio

Plant samples

- Grains
- Vegetables
- Oilplants
- Fruits & trees
- Sample types:
  - Leaves
  - Seeds
  - Flowers
  - Roots, etc.

Livestock samples

- Blood & tissue
- Hair
- Semen & others
- Species:
  - Cattle, pig, sheep, goat, horses
  - Horses, insects etc
  - Chicken & other birds
  - Fish



Human samples

- Low volume blood (up to 2 ml)
- High volume blood (up to 10 ml)
- Tissue
- Special applications
  - Forensics
  - Hair
  - Stool

NAP – Business overview

85%

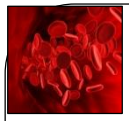
AgBio-

-Plants- & livestock-

Forensic

5%

- Police and special applications-



Human

5%

- Blood, cohorts & diagnostics-

Others

5%

- (Soil) bacteria, plasmids, etc. -

sbeadex: Validated protocols for

Plant species	Leaves	Seeds
Apricot (Prunus armeniaca)	✓	
Barley (Hordeum vulgare)	✓	✓
Beet, sugar (Beta vulgaris)	✓	
Canola / Oilseed (Brassica napus)	✓	✓
Chicory (Cichorium intybus)	✓	
Corn (Zea mays)	✓	✓
Cotton (Gossypium)	✓	✓
Cucumber (Cucumis sativus)	✓	✓
Flax (Linum usitatissimum)	✓	
Grape (Vitis vinifera)	✓	✓
Lettuce (Lactuca sativa)	✓	
Muskmelon (Cucumis melo)	✓	✓

Plant species	Leaves	Seeds
Onion (Allium cepa)	✓	✓
Parsley (Petroselinum crispum)	✓	✓
Peach (Prunus persica)	✓	
Pepper (Capsicum annuum)	✓	✓
Potato (Solanum tuberosum)	✓	
Rice, Asian (Oryza sativa)	✓	✓
Rubber (Hevea brasiliensis)	✓	✓
Soybean (Aphis glycines)	✓	✓
Sunflower (Helianthus annuus)	✓	✓
Tobacco leaves (Nicotiana tabacum)	✓	✓
Tomato (Solanum lycopersicum)	✓	✓
Wheat (Triticum L.)	✓	✓

sbeadex livestock

Tissue

Blood FTA cards

Plant samples

Hair samples

Sperm / semen

sbeadex livestock

One protocol – all livestock samples

USP

OUTLINE

- INTRO TO DNA
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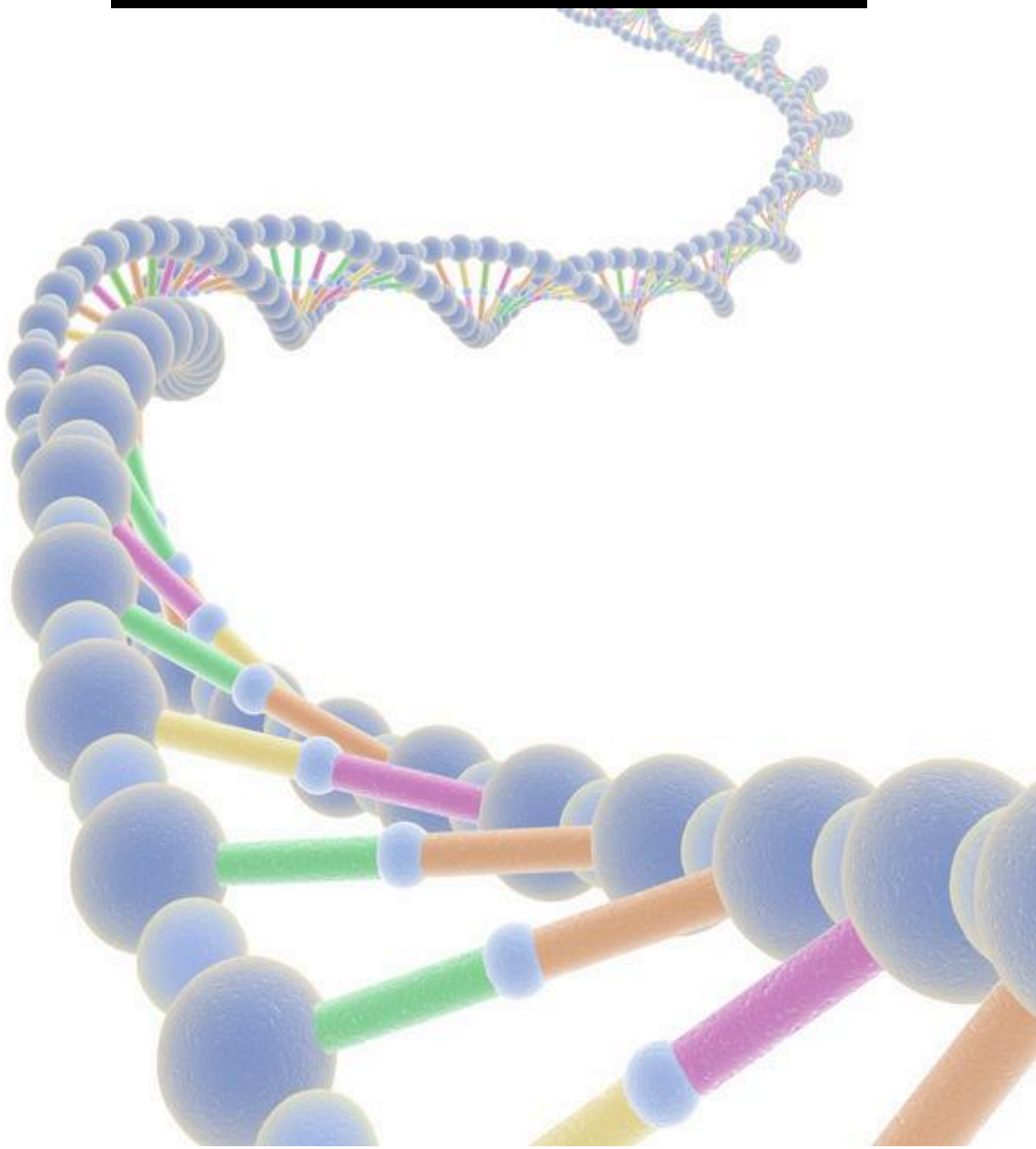
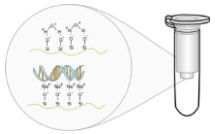


Summary

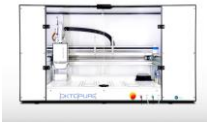
Sbeadex

- Targets: plant & livestock
- High DNA quality
- All down-stream applications
- Flexibility: allows protocol optimizations
- oKtopure



Kleargene

- Targets: plant & rodent tails
- Highest throuputs: up to 20 k extr./ day
- 96/ 384 format
- Genotyping/ PCR
- Genespin



Instru-mentation

- oKtopure & Genespin
- Workhorses for DNA extraction
- Full and semi-automated extractions
- Low costs for plastics (tips and plates)

- INTRODUCTION
- Sbeadex : DNA extraction kit
- **KASP : method for genotyping**
- MASS ARRAY : Multi-gene Analysis

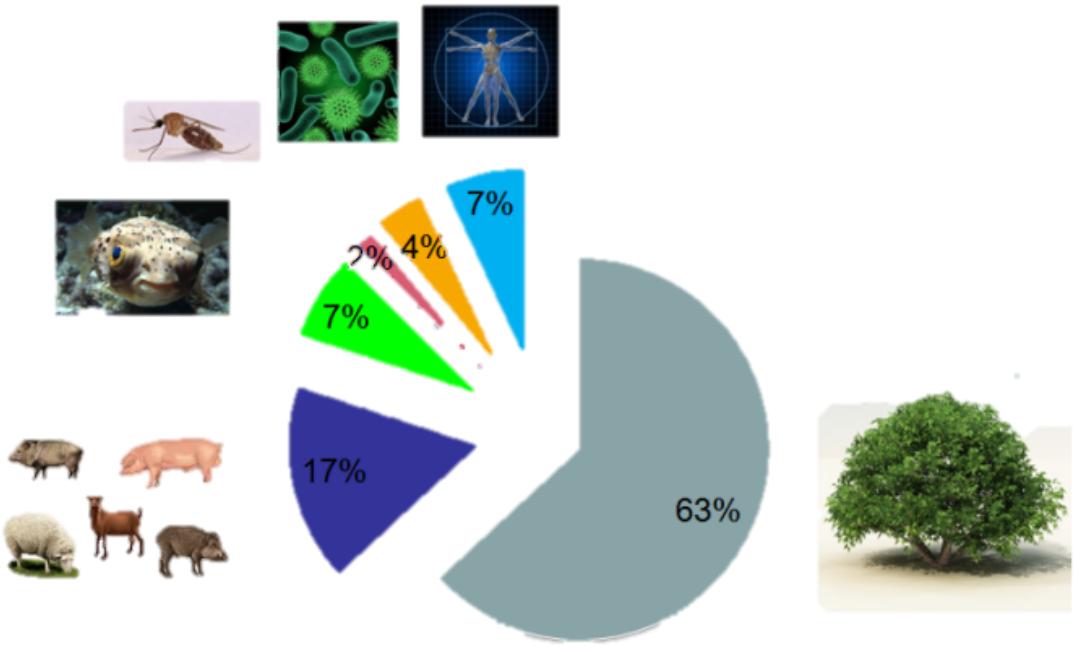
KASP： A new era of KASP method for genotyping

Science

for a safer world

- Introduction to KASP
- Components for KASP
- KASP assay design
- Summary

Over 360,000 unique KASP assays developed in our laboratories



- >25,000 Validated Human assays.
- Multiple assays to key genetic diseases such as Cystic Fibrosis and Breast & Ovarian Cancer
- Extensive Panels for key field crops (~13,600 assays)

Over **2000** published papers referencing KASP (Google scholar)

Crop Science

Title : QTL Mapping and Molecular Breeding for Developing Stress Resilient Maize for Sub-Saharan Africa (Oct 2014)

- KASP good at fingerprinting due to simplicity of data and low drop out rate

- CIMMYT routinely uses KASP, generating in excess of a million data points annually for different purposes

-The KASP average genotyping error rate in positive control DNA samples varied between 0.7 to 1.6%, which is lower than that observed in the Illumina GoldenGate (2.0-2.4 %)

- Because of the continuous change in SNP genotyping technology and high upfront costs, large-scale projects could benefit from exploiting genotyping service providers and avoid significant investment in setting up in-house SNP genotyping platforms, such as the KASP assay

Electronic Journal of Biotechnology

One-step, codominant detection of imidazolinone resistance mutations in weedy rice (*Oryza sativa* L.)

KASP has low cost, high throughput, and high specificity and sensitivity as has been demonstrated in massive SNP genotyping studies in biomedical research [23], genome-wide SNP platforms for rice genotyping [24] and molecular markers–assisted wheat breeding

Thus, the method (KASP) validated here for timely and accurate detection of RWR is a valuable and cost-effective tool for decision making in Clearfield rice management and regional surveillance of RWR in the framework of a sustainable use of this production system.

For ALSPAC, the entire cohort (10,145 participants, including 38 carriers of the rare A allele) was genotyped using KASP with a genotyping accuracy of 100%

nature COMMUNICATIONS

Received 7 Mar 2014 | Accepted 30 Jul 2014 | Published 16 Sep 2014 DOI: 10.1038/ncomms5871 OPEN

A rare variant in *APOC3* is associated with plasma triglyceride and VLDL levels in Europeans

Recherche uO Thèses uOttawa

Molecular Marker Applications in Oat (Avena Sativa L.) Breeding and Germplasm Diagnostics

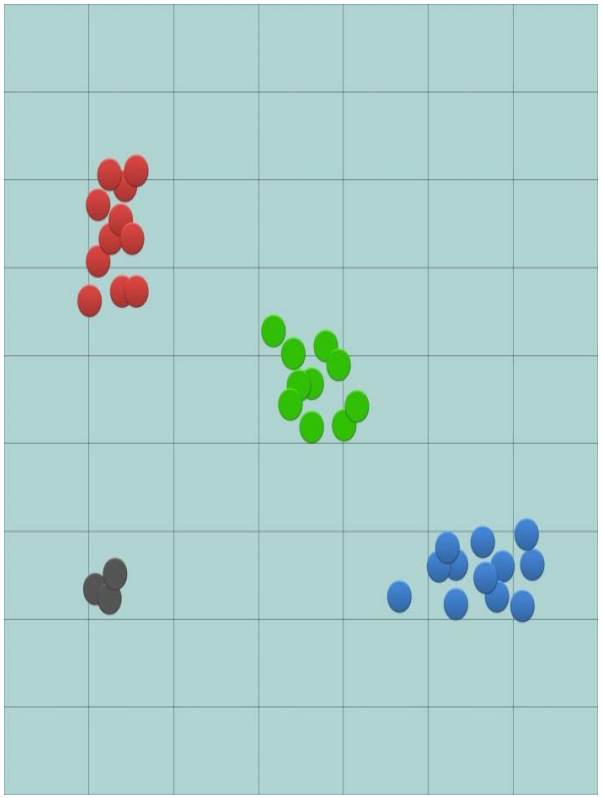
GBS will likely provide a good source for future KASP™ marker design

Therefore, we recommend KASP™ for rapid cultivar identification and GBS for more conclusive identification and/or for purity assessment"

The present work can also be considered as a resource for breeders. Firstly, KASP™ and GBS have been developed for rapid cultivar identification and purity assessments

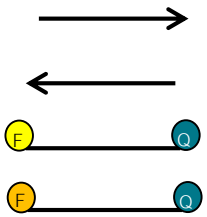
Introduction to KASP

- Kompetitive Allele Specific PCR
- Endpoint PCR-based genotyping chemistry
- Suitable for single nucleotide polymorphisms (SNPs) and Insertions / Deletions (InDels).
- Fluorescent signal is generated during the PCR - read at the end of the thermal cycle.
- Signal plotted on a cluster plot.



Taqman® vs KASP

Taqman® components:



1 x Forward primer

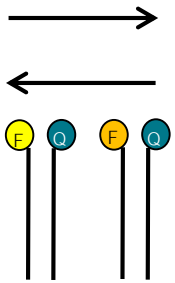
1x Reverse primer

Dual labelled probe 1

Dual labelled probe 2

Reaction mix

KASP Components



2 x Allele-specific forward primer

1x Reverse primer

Reaction mix (containing fluorescent quencher cassettes)

Taqman®	KASP
Detect single nucleotide polymorphisms (SNPs)	
Fluor-Quencher Probe	KASP Master MIX
1 Forward Primer	2 Forward Primer
2 dual labelled probes	No probes
complex regions/Gene expression	complex regions
	large insertion/deletion

- Introduction to KASP
- Components for KASP
- KASP assay design
- Summary

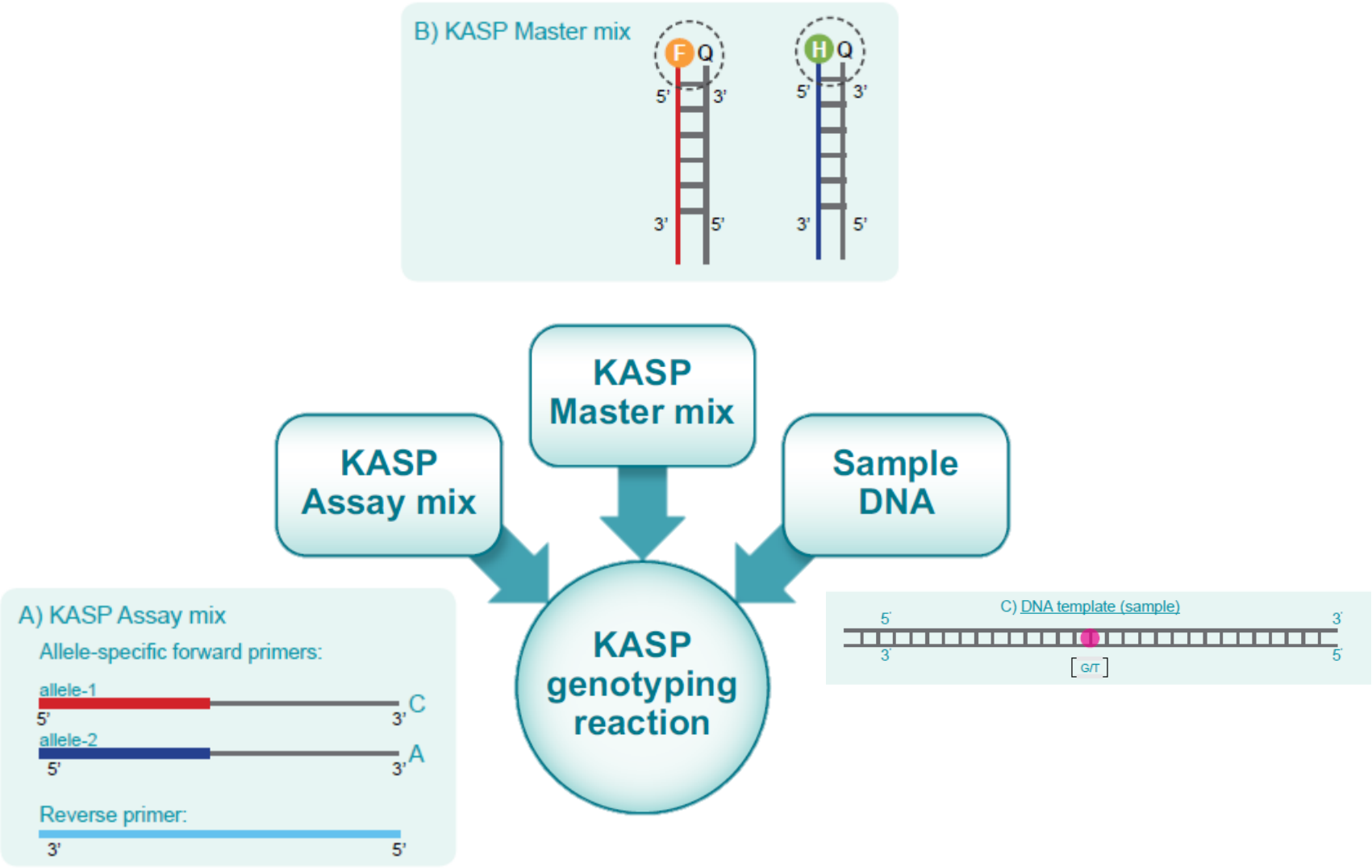
KASP: components of the reaction

- The three key components for the KASP reaction are discussed in detail in the first video in the KASP series:

LGC's KASP Genotyping Chemistry Explained:
LGC Group video
2,734 views

- The primers are contained within KASP Assay mix, and run with KASP Master mix
- Please note: KASP Assay mix works exclusively with KASP Master mix, and cannot be used with any other mix.

Assay components:



How does KASP work?

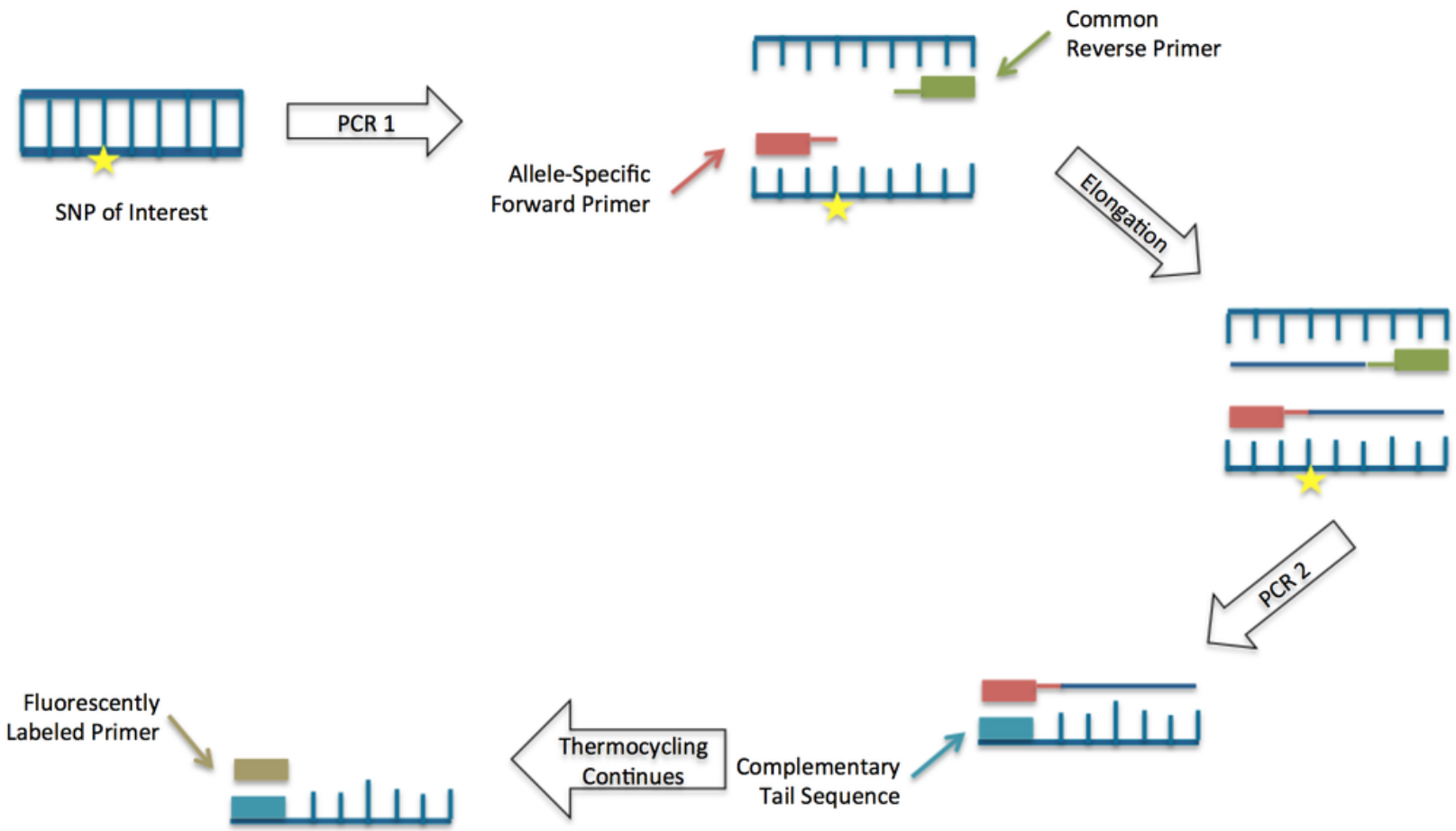
- Now that you have an understanding of the components in a KASP reaction, it is time to delve deeper into how KASP chemistry actually works
- The second video in the KASP series explains the details of this:

LGC's KASP Genotyping Chemistry Explained:

LGC Group video

6,472 views

Schematic drawing of the KASP method (Kompetitive Allele Specific PCR)



OUTLINE

- Introduction to KASP
- Components for KASP
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KASP Assay design

- The main types of polymorphisms that can be genotyped using KASP are:

Different types of polymorphisms

- SNP differentiation ____A____ or ____G____ ?
- Single base insertion or deletion ____A____ or ____-____ ?
- Small insertions or deletions

  (2-26 bases) ____AA____ or ____AGCTGGTCA____ ?
- Large insertions or deletions

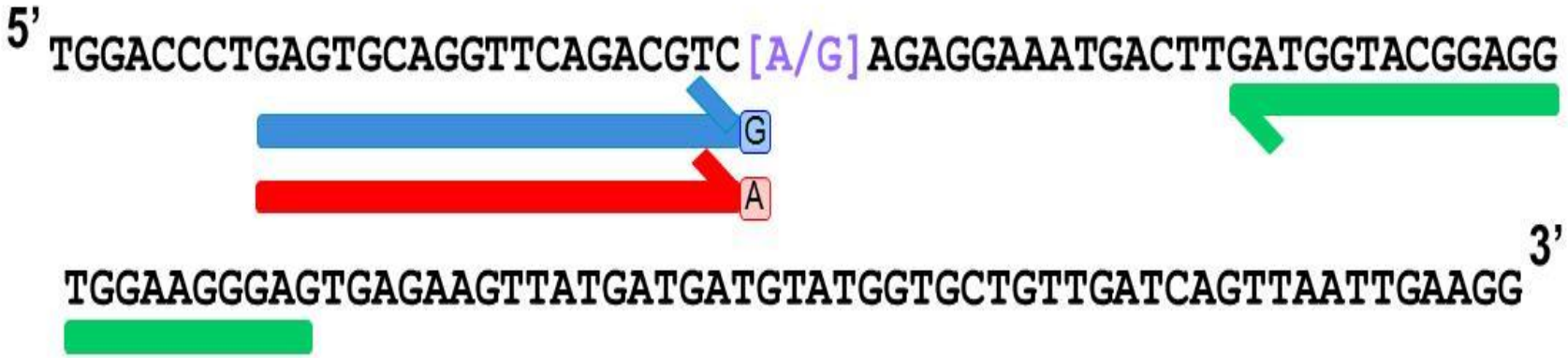
  (>26 bases) ____AA____ to ____A - - - - - - - - A - - - - ____?

  n = 26 – 200,000 bases

KASP Assay design

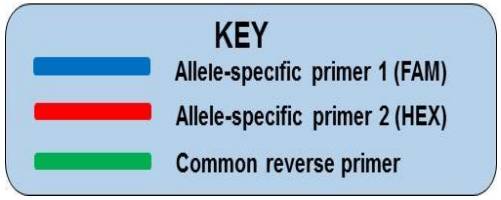
Standard KASP assay

- Atypical KASP assay designed to a SNP
- The SNP is an A/G
  - One allele-specific primer is designed to the G base (detected with FAM)
  - One allele-specific primer is designed to the A base (detected with HEX)
  - A common reverse primer is also designed that will work with both allele-specific primers.

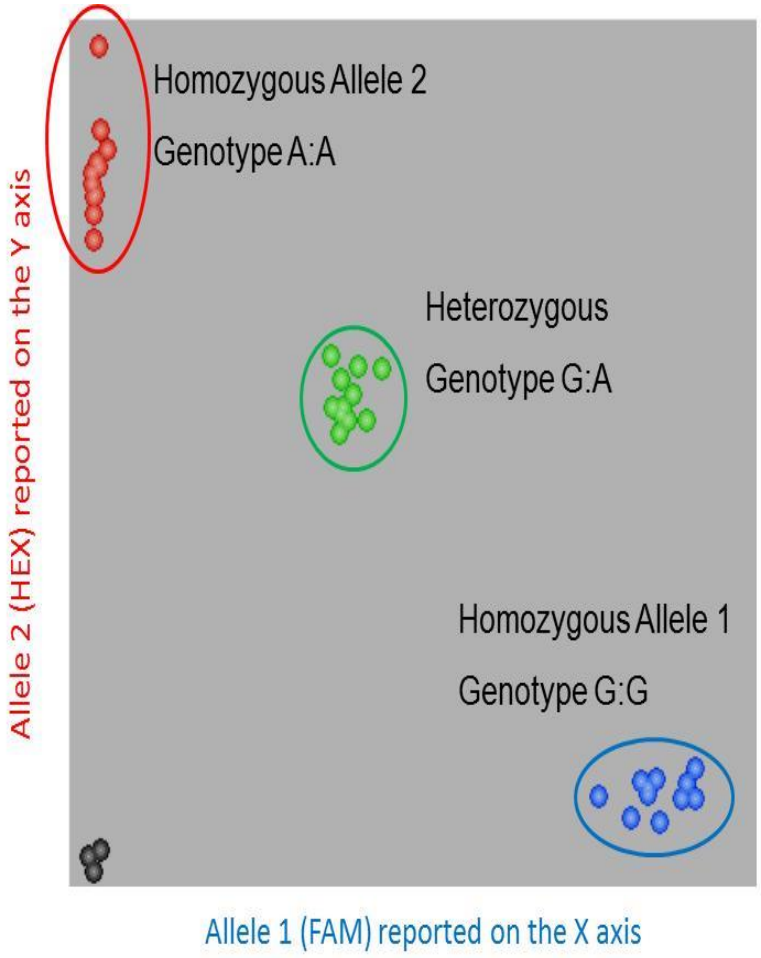


Note: despite the example the first letter inside the bracket is generally labelled with FAM and the one after the / symbol is labelled with Hex.

Please specify if the design requires specific labelling of each allele.



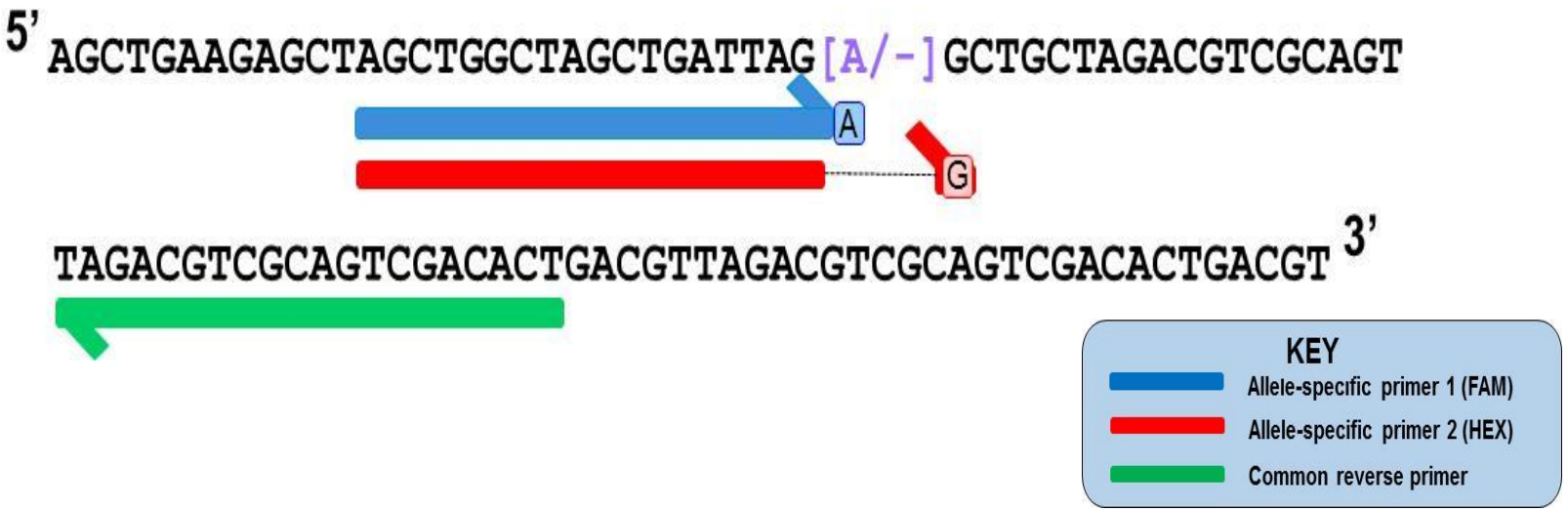
KASP Assay design



Typical results from a KASP SNP assay

Single base insertion / deletion

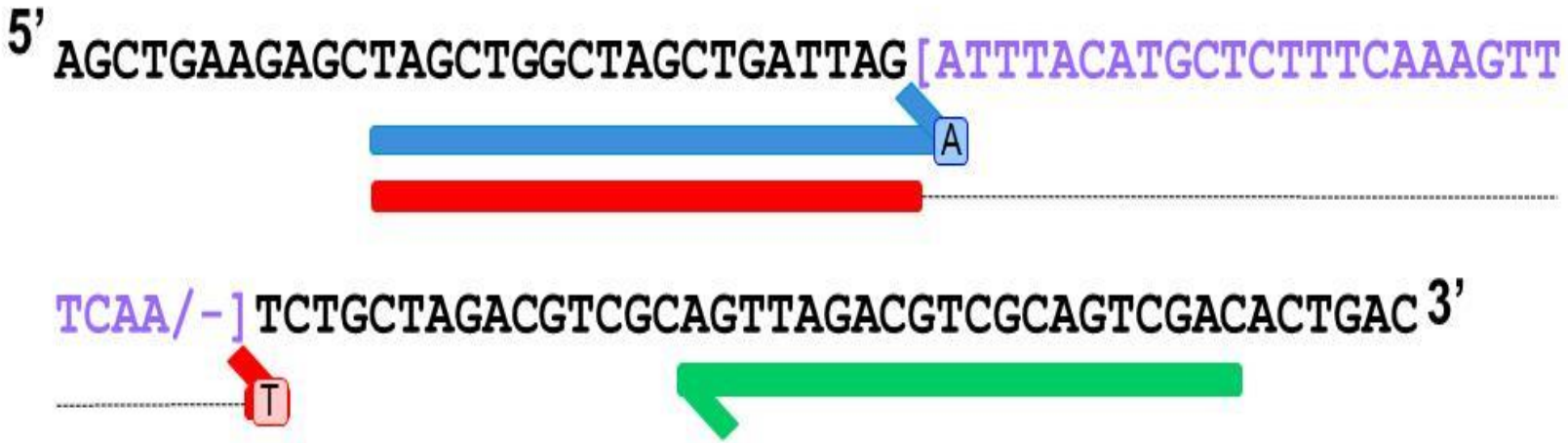
- KASP assays can be designed to detect single base insertions or deletions
  - One allele-specific primer is designed to incorporate the indel sequence (detected with FAM)
  - One allele-specific primer is designed to the standard sequence (detected with HEX)
  - A common reverse primer is also designed that will work with both allele-specific primers.



KASP Assay design

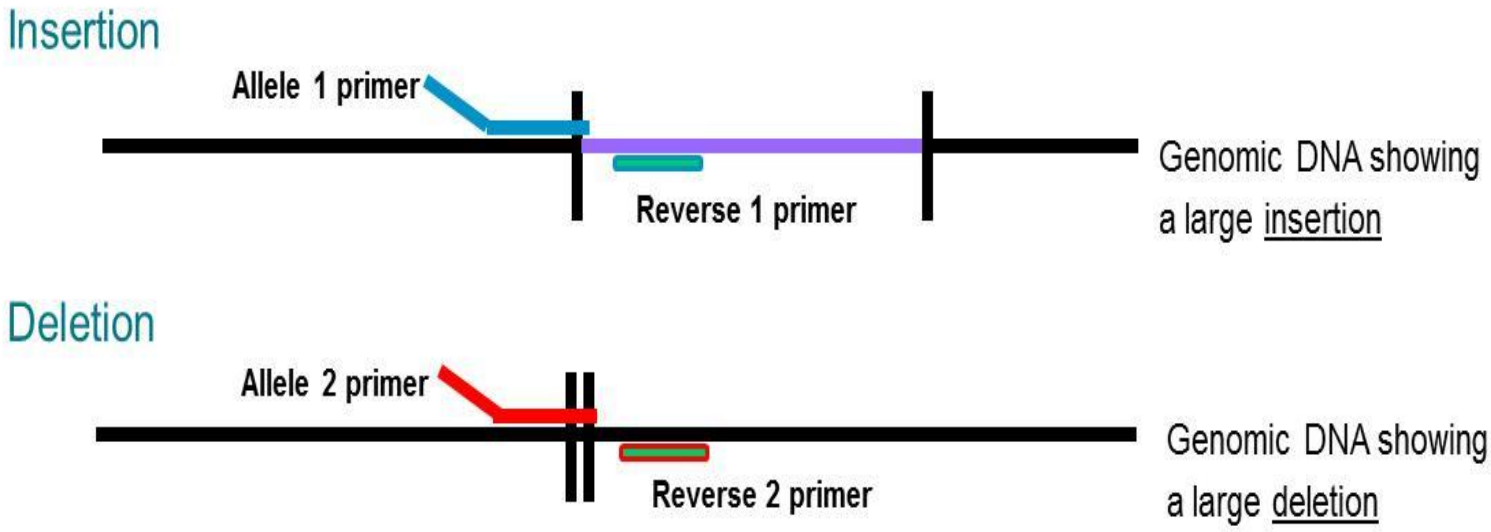
Insertion / deletion of 2-26 bases

- Standard KASP assays (3 primers) can be designed to detect insertions or deletions of up to 26 bases
  - One allele-specific primer is designed to incorporate the InDel sequence (detected with FAM)
  - One allele-specific primer is designed to the standard sequence (detected with HEX)
  - A common reverse primer is also designed that will work with both allele-specific primers.



KASP Assay design

Large insertion or deletion: overview

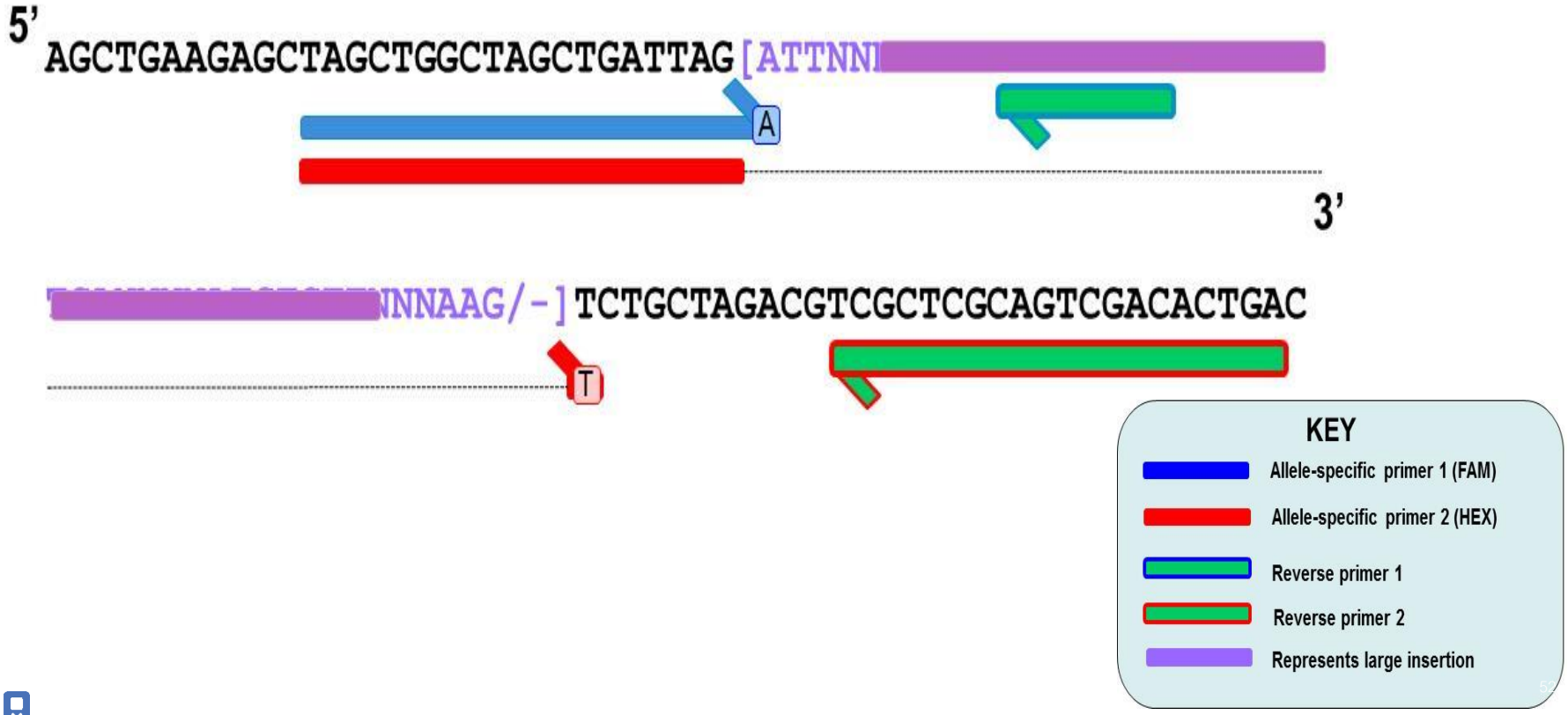


- Assays for large insertions / deletions require four primers rather than the typical three:
  - The first allele-specific primer is designed to incorporate part of the insertion sequence (detected with FAM), with a reverse primer located within the inserted sequence
  - The second allele-specific primer is designed across the region where the insertion would be (detected with HEX), with a reverse primer downstream of the insertion point.

KASP Assay design

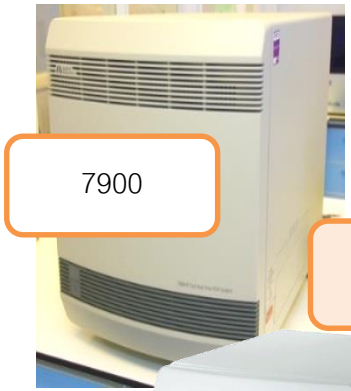
Large insertion or deletion: assay design

- There is no upper size limit for the InDel aside from the requirement for the sequence to all be present on the same strand of DNA.



KASP on qPCR instruments

- We know that KASP works well on most qPCR machines
- These include:



7900

Viia7

ABI

7500

Agilent

Mx3000

BIO-RAD

CFX

Roche

LC480

OUTLINE

- Introduction to KASP
- Components for KASP
- KASP assay design
- Summary

Summary

- KASP real-time mix is suitable for detect : agriculture animal and human targets
- assays are correctly designed : highly specific and sensitive detection
- analysis for detection : gene expression and copy number variation
- Use of KASP real-time for SNP genotyping and pathogen/species detection

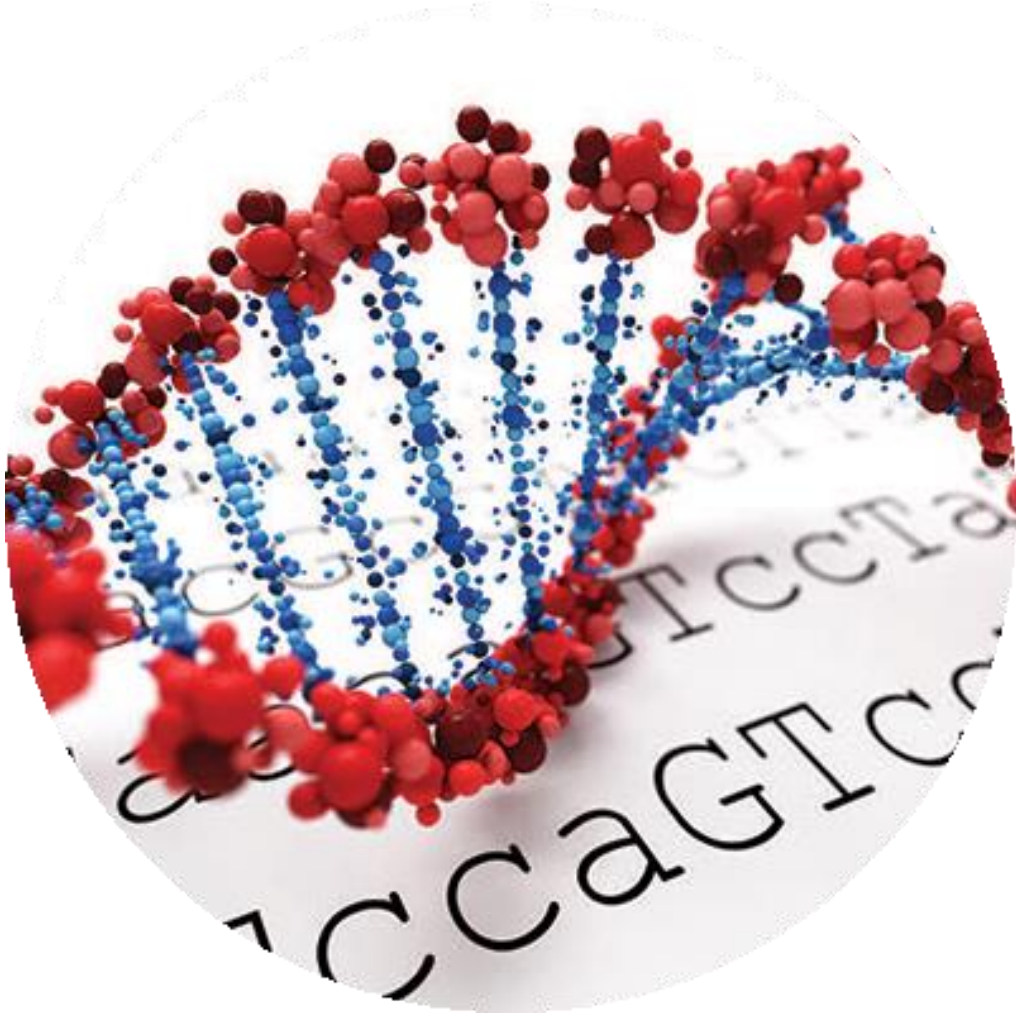
- INTRODUCTION
- Sbeadex : DNA extraction kit
- KASP : method for genotyping
- **MASS ARRAY : Multi-gene Analysis**

MassARRAY: The Powerful Technology for Multi-gene Analysis

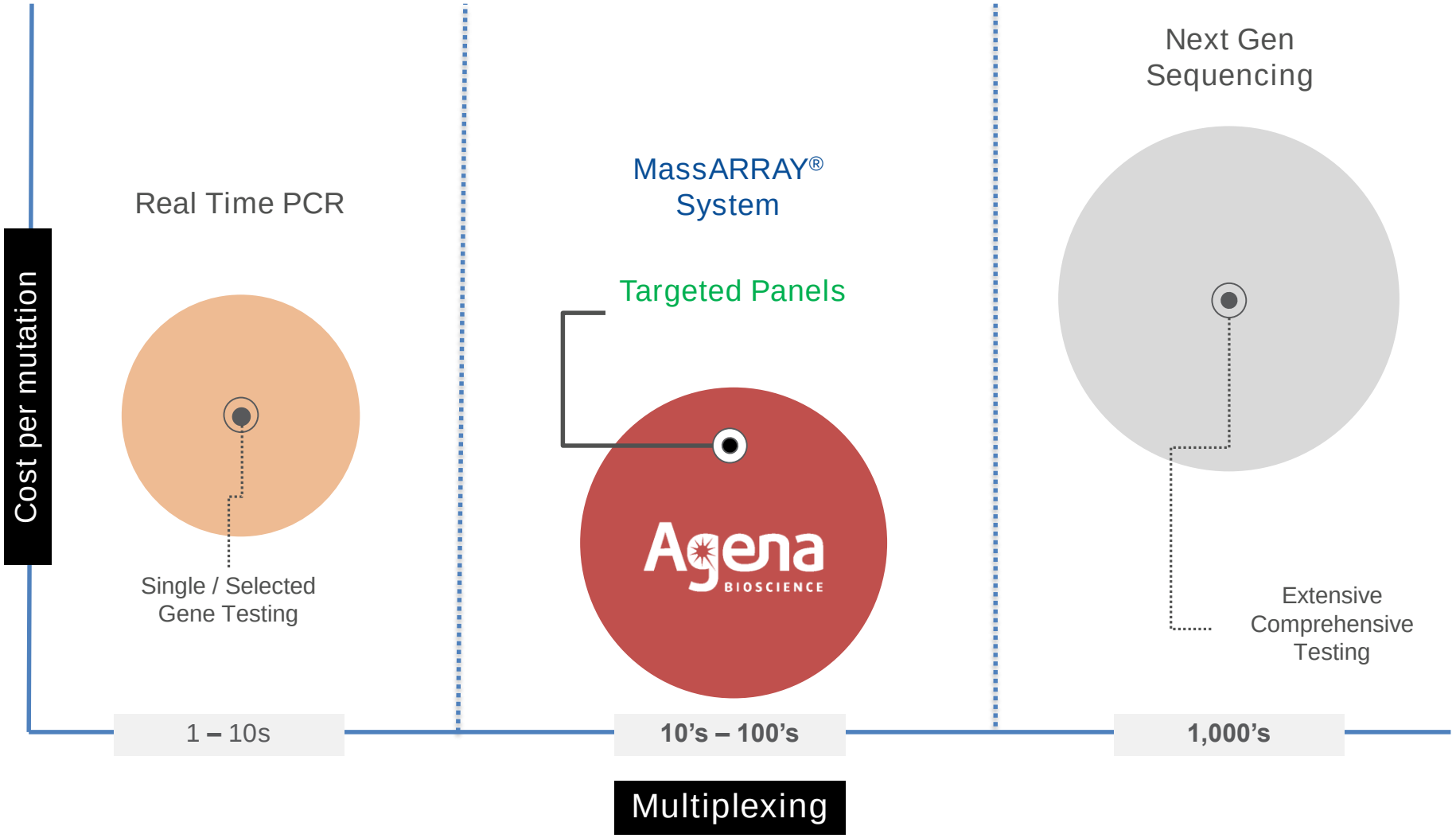
Agena BIOSCIENCE

OUTLINE

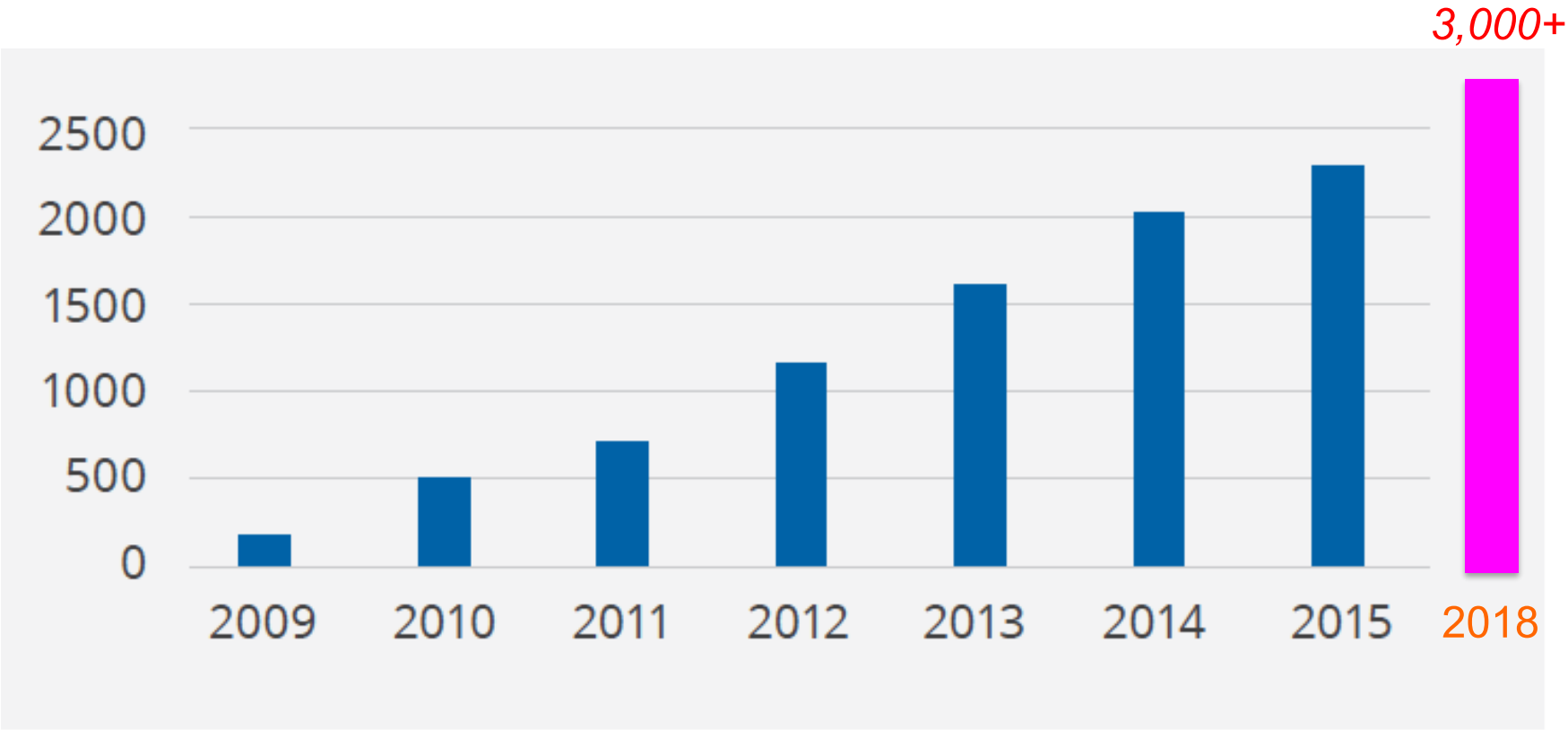
- INTRO
- MASS ARRAY DEBRIEF
- APPLICATIONS



Cost-Effective, Targeted Genetic Analysis Robust, Flexible, and High Throughput

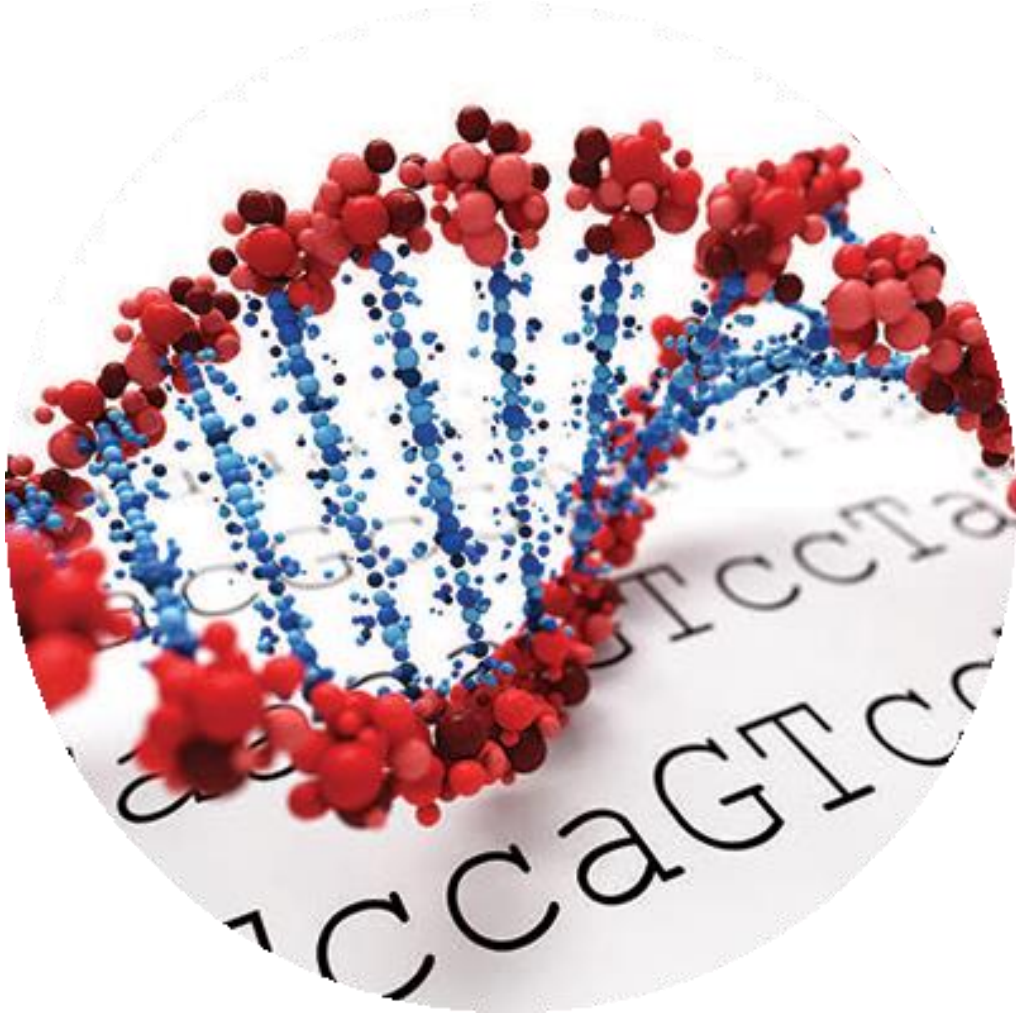


SKYROCKET PUBLICATION OF MASSARRAY



OUTLINE

- INTRO
- MASS ARRAY DEBRIEF
- APPLICATIONS



MassARRAY® System

Chip prep module

96 well plate

384-well plate platform

Proven Versatility of the MassARRAY® System

- Proprietary mass spectrometry-based detection system with robust chemistries and advanced data analysis software for sensitive, accurate analyses

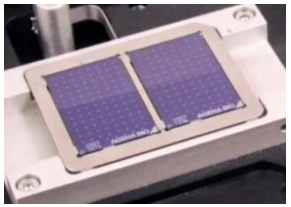
MULTI-PURPOSES

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

MassARRAY™ Applications

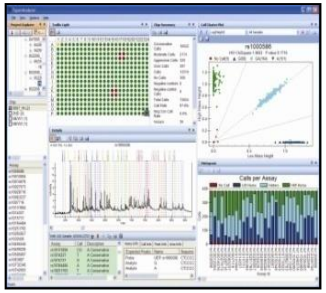
- Genotyping
- Somatic Mutation
- Copy Number Variation
- Methylation
- Ultrasensitive Detection

SpectroCHIP™

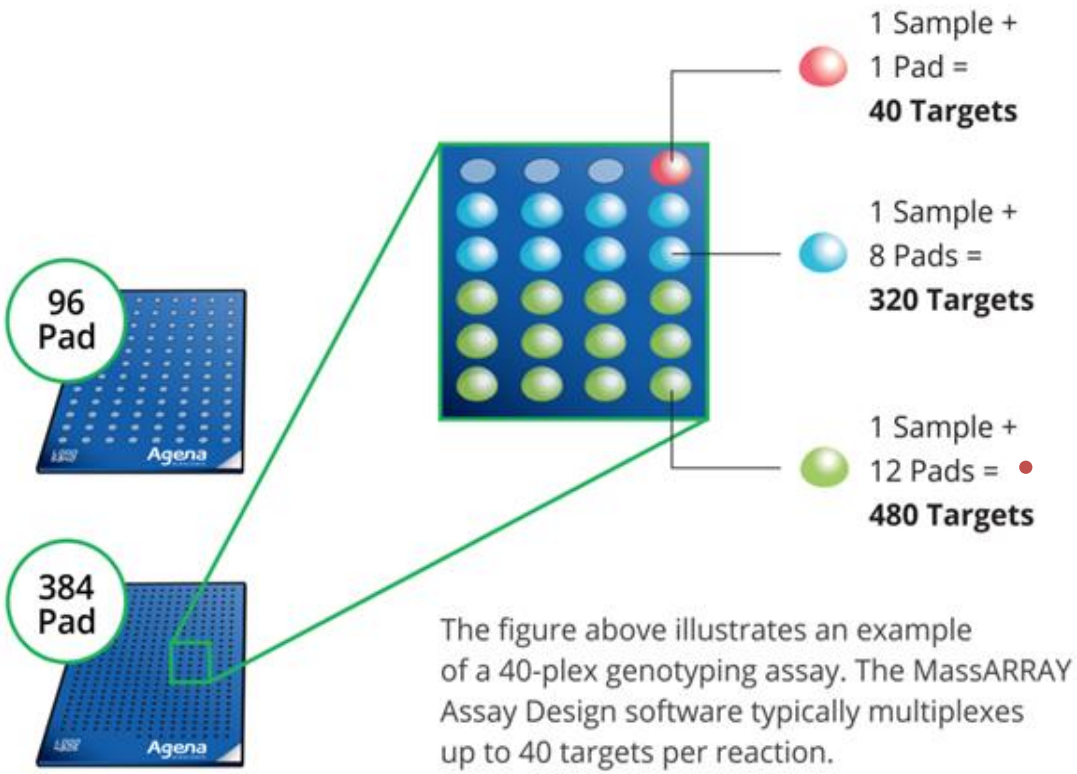


MassARRAY™ System

Data Analysis



A Dynamic and Flexible Platform *A Single Platform with Modular Formats*

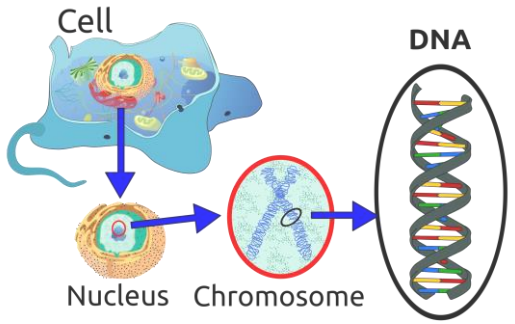


The figure above illustrates an example of a 40-plex genotyping assay. The MassARRAY Assay Design software typically multiplexes up to 40 targets per reaction.

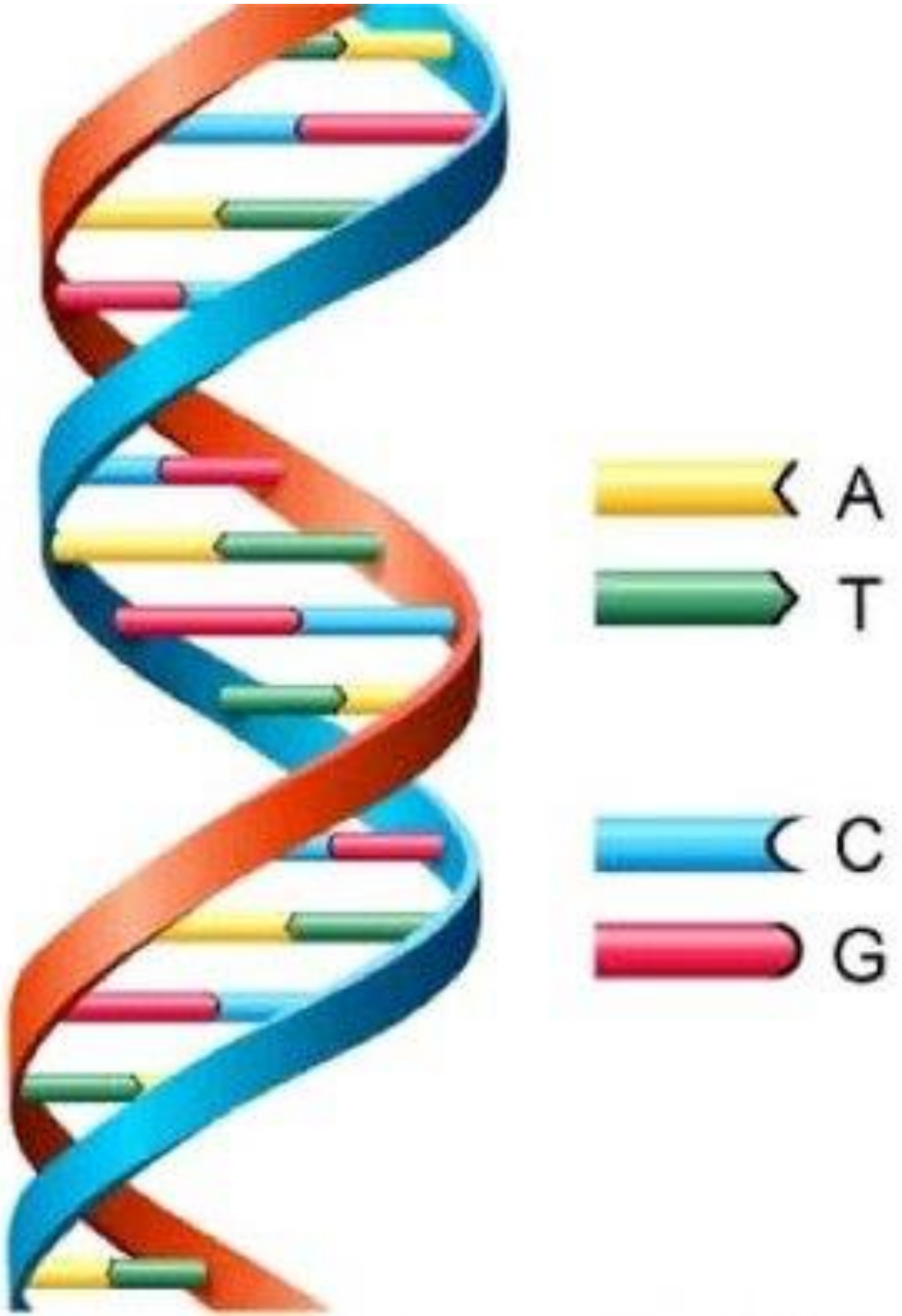
- **Highly scalable throughput**
  - 96 or 384-pad SpectroCHIP® Arrays*
  - From 1 to 10 arrays per day
  - 10s – 100s – 1000s samples per day
- **Marker x Sample flexibility**
  - Multiplex 5 - 40 targets per well
  - Run 1 to 24 reactions per sample
  - Screen 10s to 100s mutations per sample

FLEXIBLE DNA SAMPLING

- Just 5-10 ng DNA per well (reaction)
- FFPE, fresh and frozen tissue samples
- Liquid Biopsy
- Blood plasma and serum
- FTA cards
- Whole genome amplified DNA
- Buccal cells
- Ear punches
- Semen
- Hair pulls
- Micro-dissected cells



DNA FORMS OF NUCLEOBASES “A, C, G, T”



DIFFER IN MASS

dAMP = 313.2 Da

dTMP = 304.2 Da

dCMP = 289.2 Da

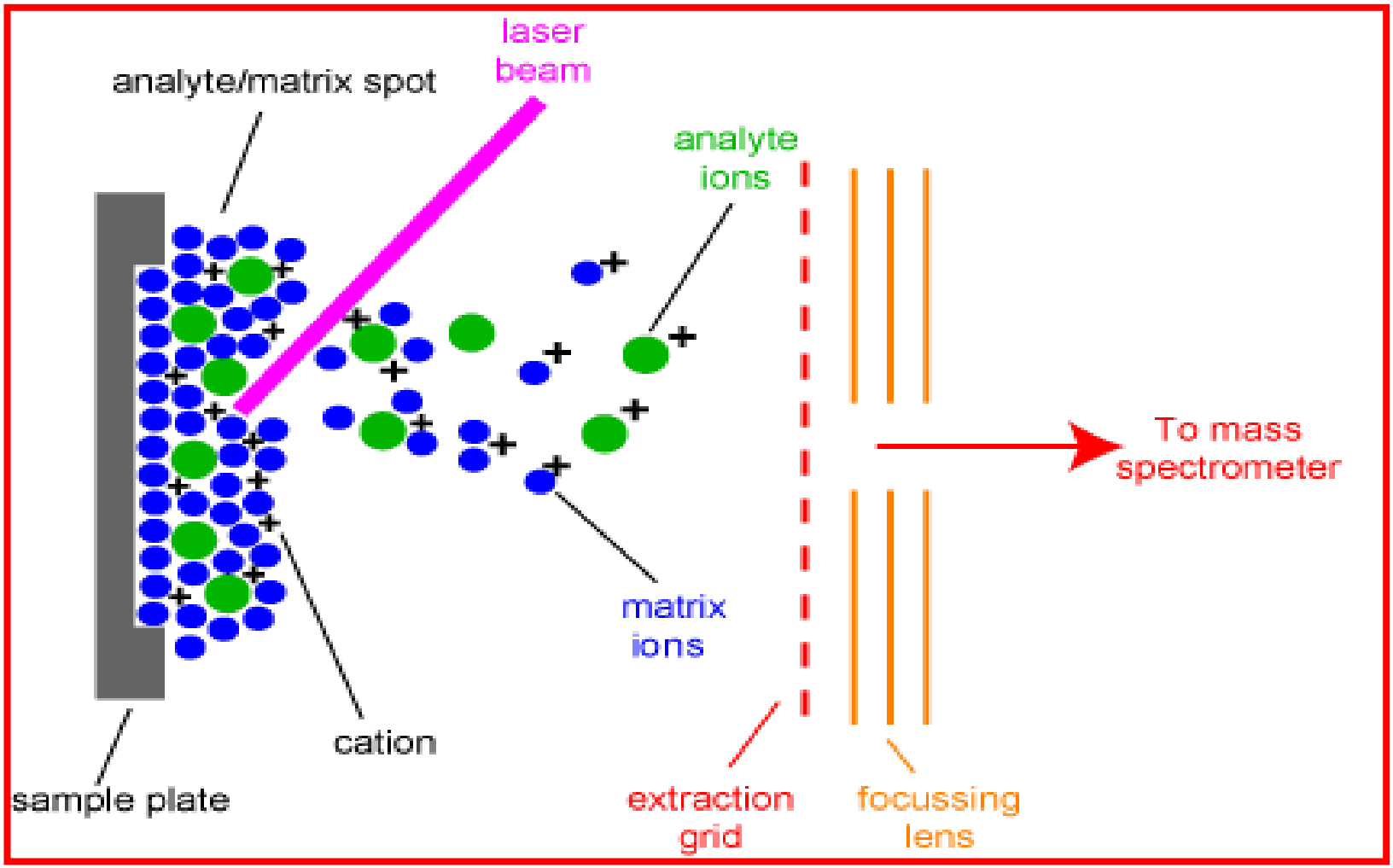
dGMP = 329.2 Da

MASSARRAY : INTUITIVE & STRAIGHTFORWARD

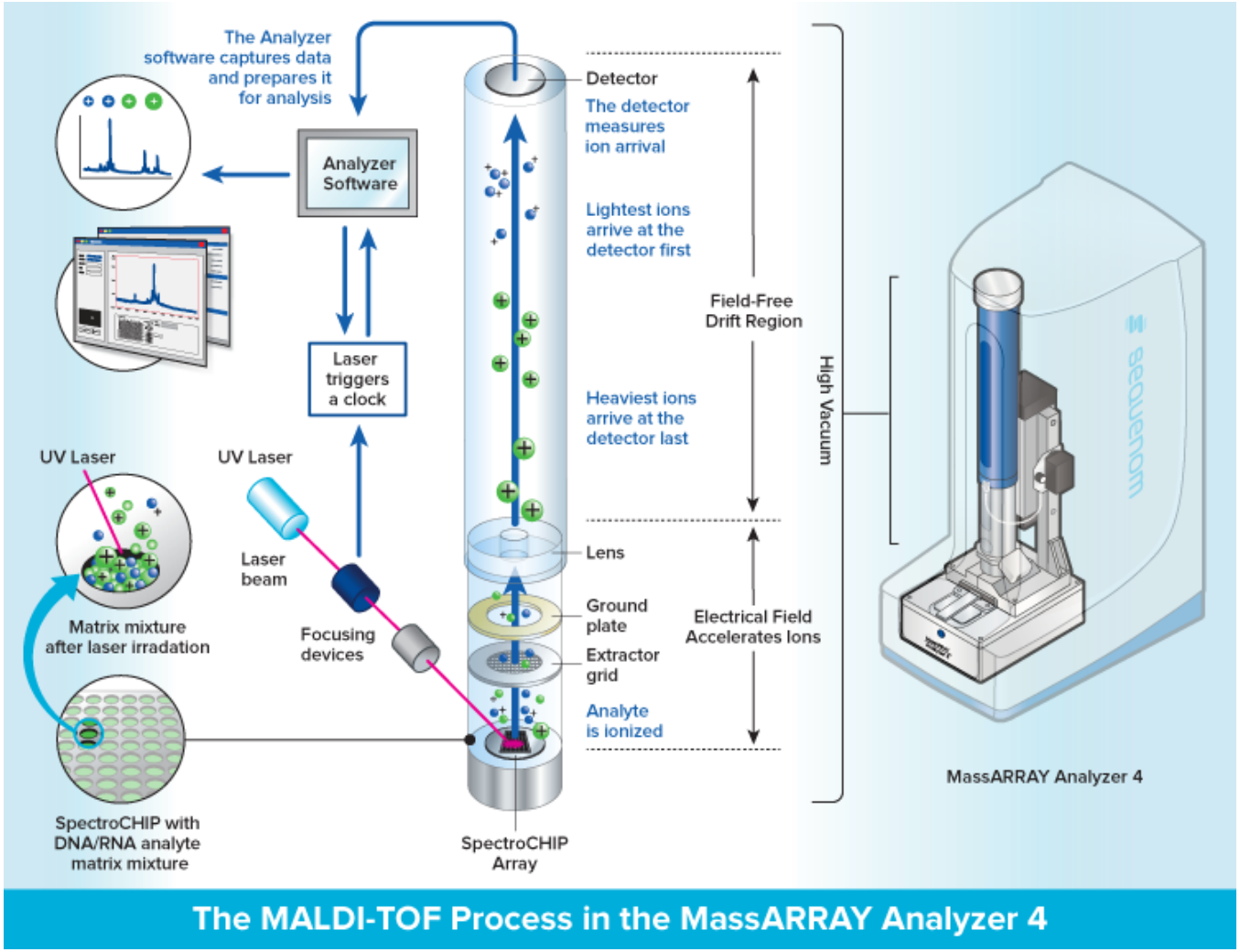
MALDI-TOF

“Mass as Signature”

Matrix Assisted Laser Desorption/Ionization (MALDI)



Processes



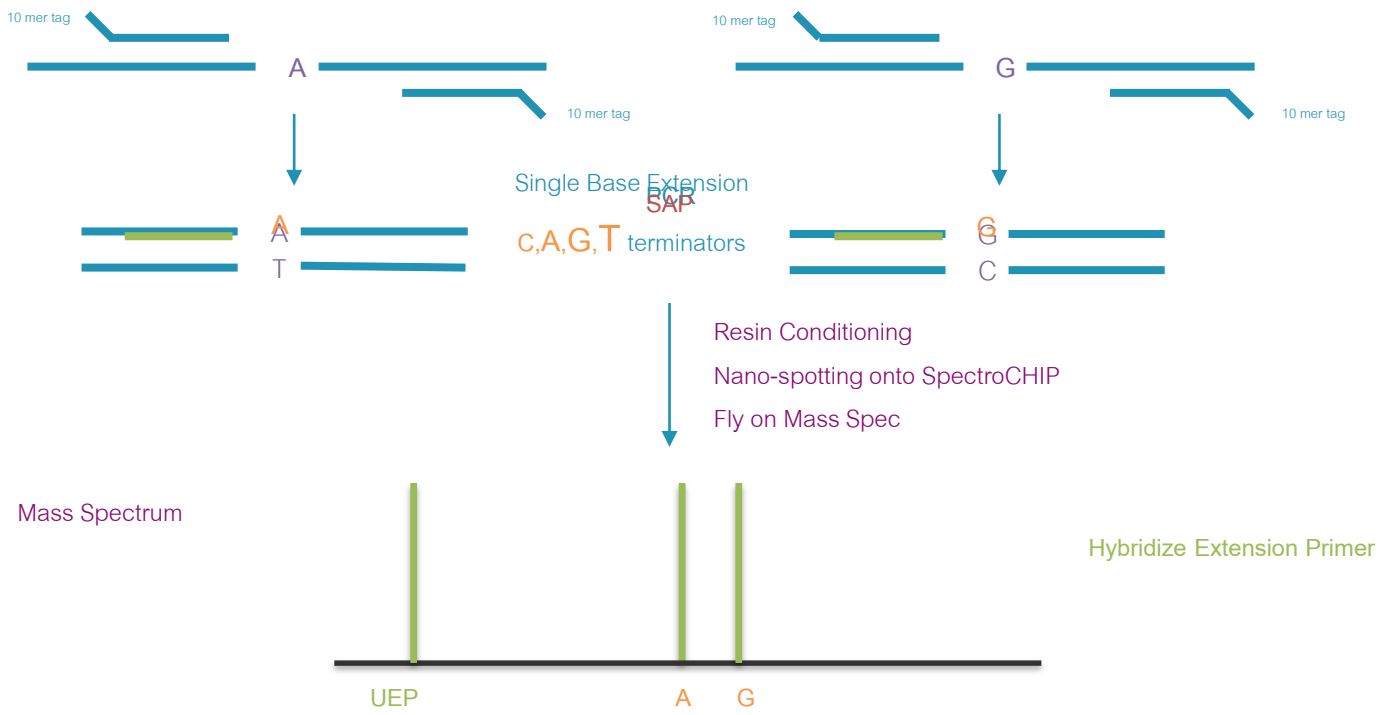
The MALDI-TOF Process in the MassARRAY Analyzer 4

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

GENOTYPING

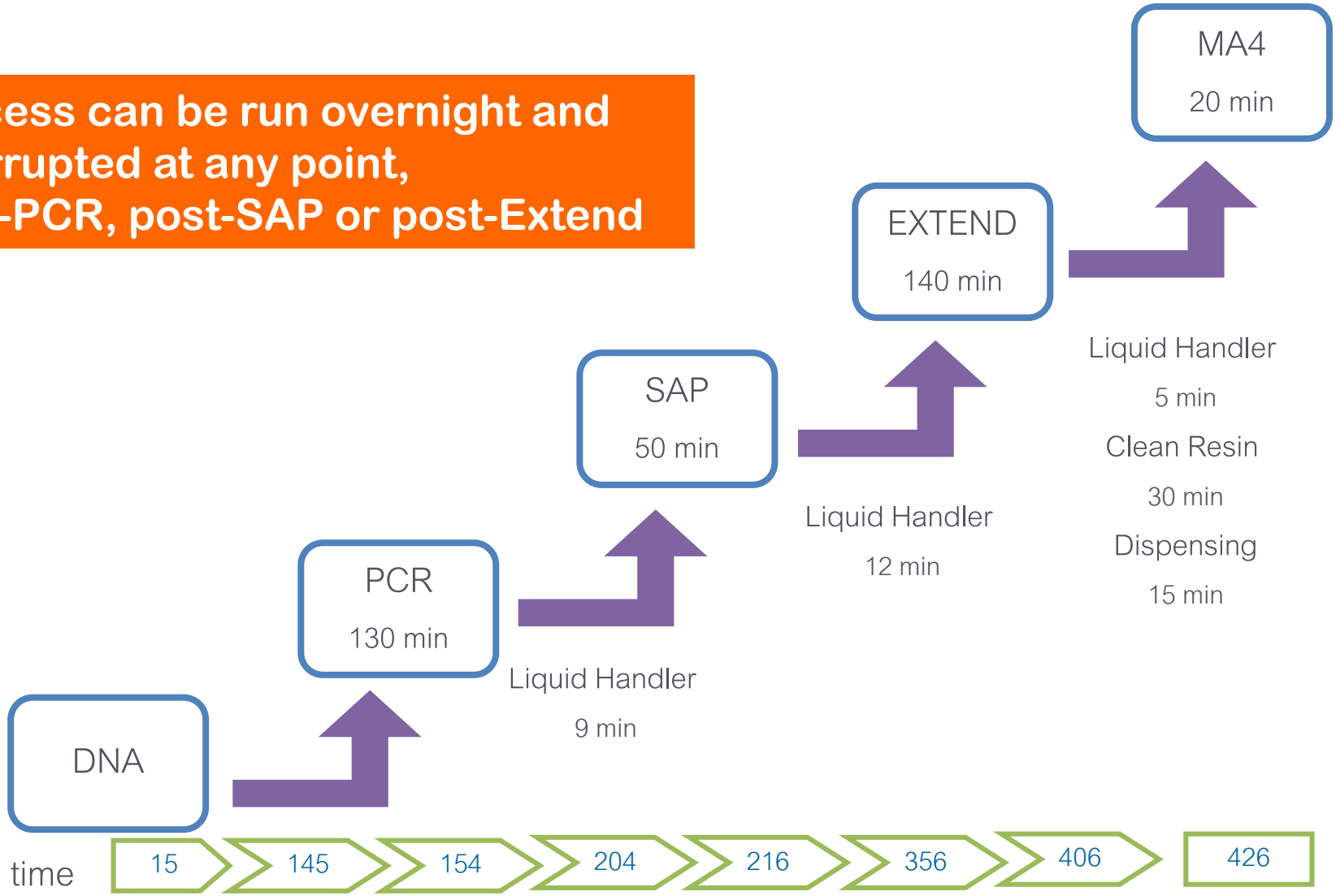
- Example of an [A/G] SNP

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



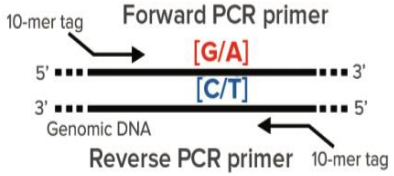
Process Workflow : 7-8 hours

Process can be run overnight and interrupted at any point, post-PCR, post-SAP or post-Extend



MassARRAY® Process

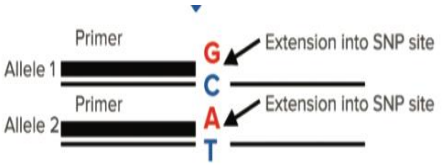
Amplification

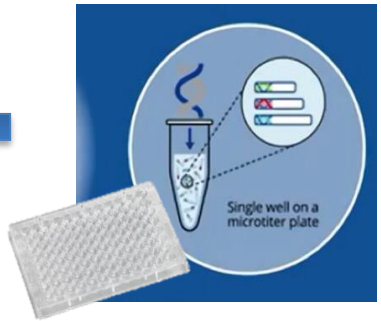


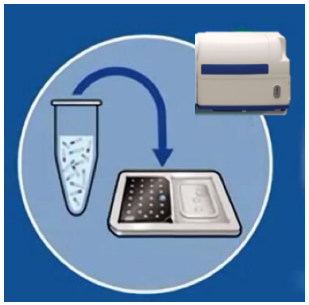
PCR Product

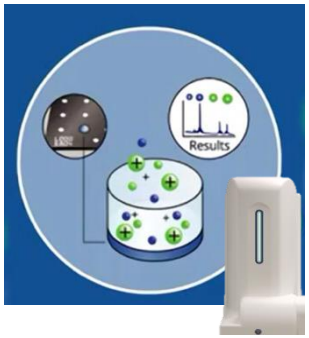
SAP

iPLEX® Extension Reaction

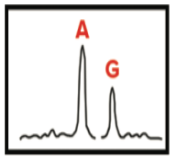




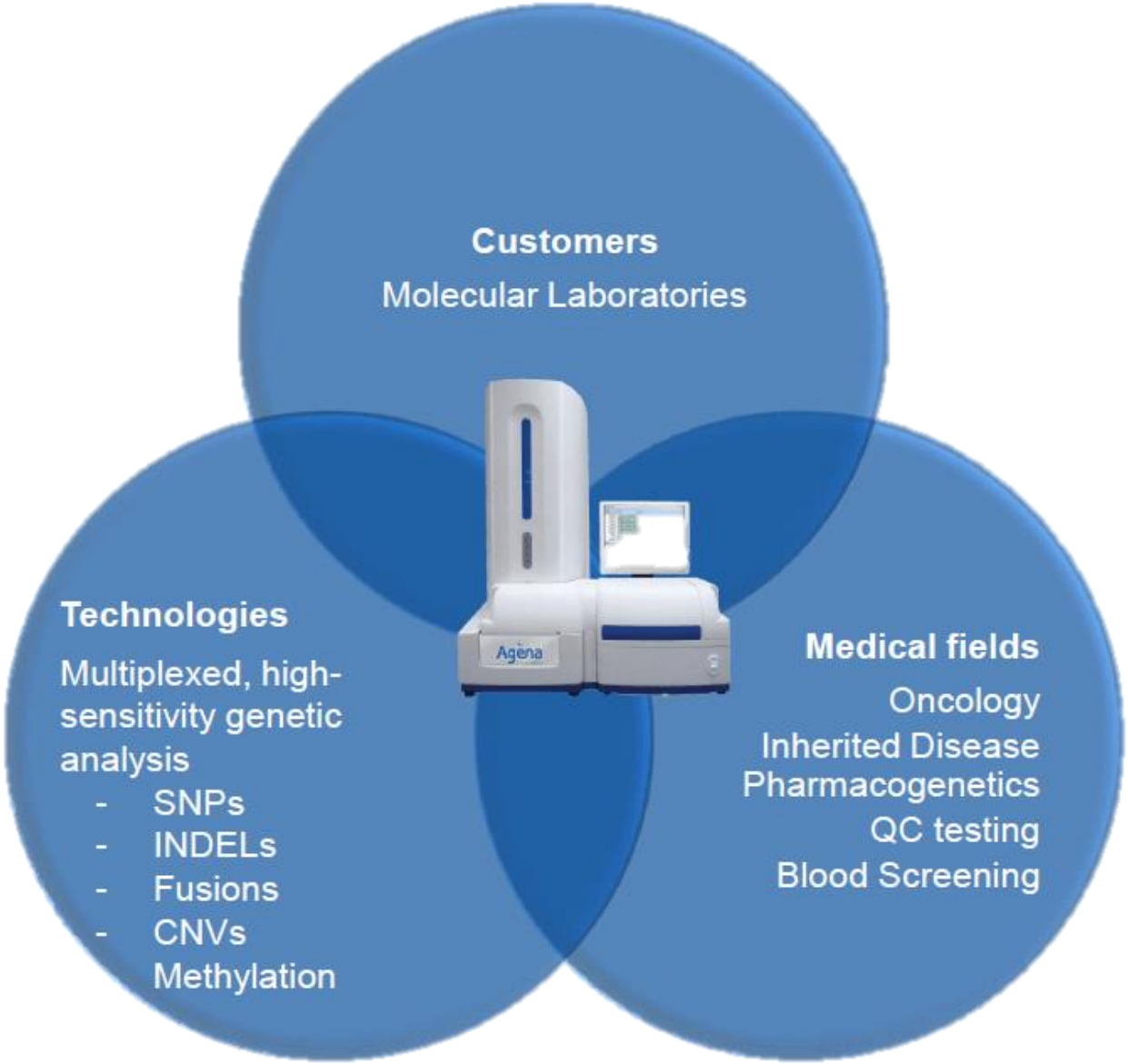




MALDI-TOF Mass Spectrometry Analysis



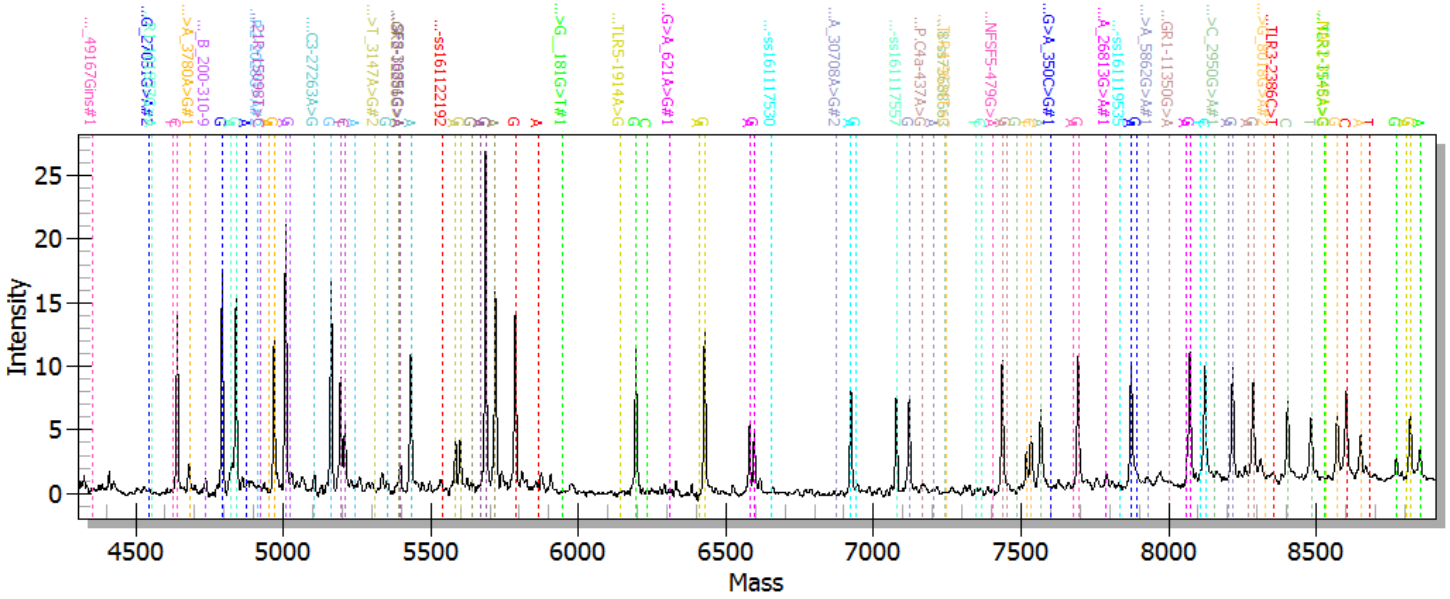
ASSAYS BY AGENA: MAIN APPLICATIONS



- The Power of Multiplexing Multiple Markers in a Single Well

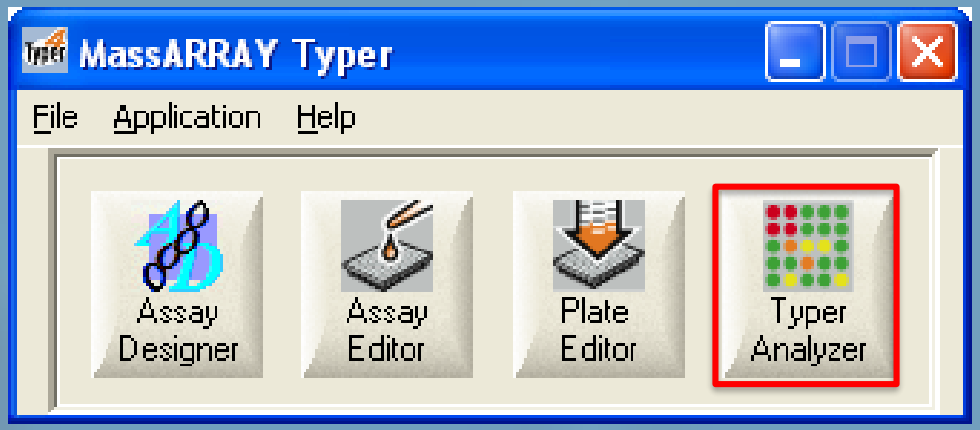
ADVANCE SOFTWARE PROCESSING

BASED ON "MASS"



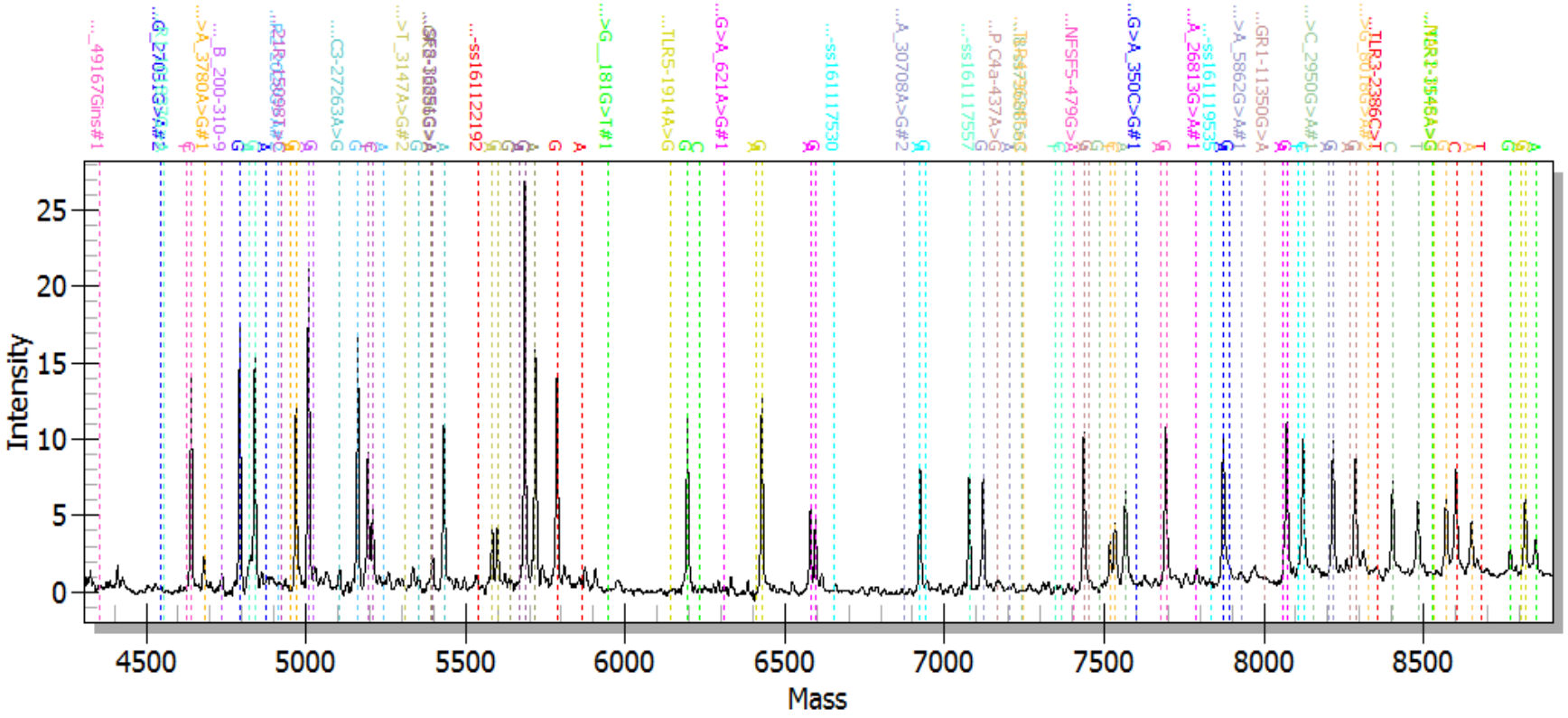
Data Analyzer

Software application suite for Genotyping & Somatic Mutation Analysis

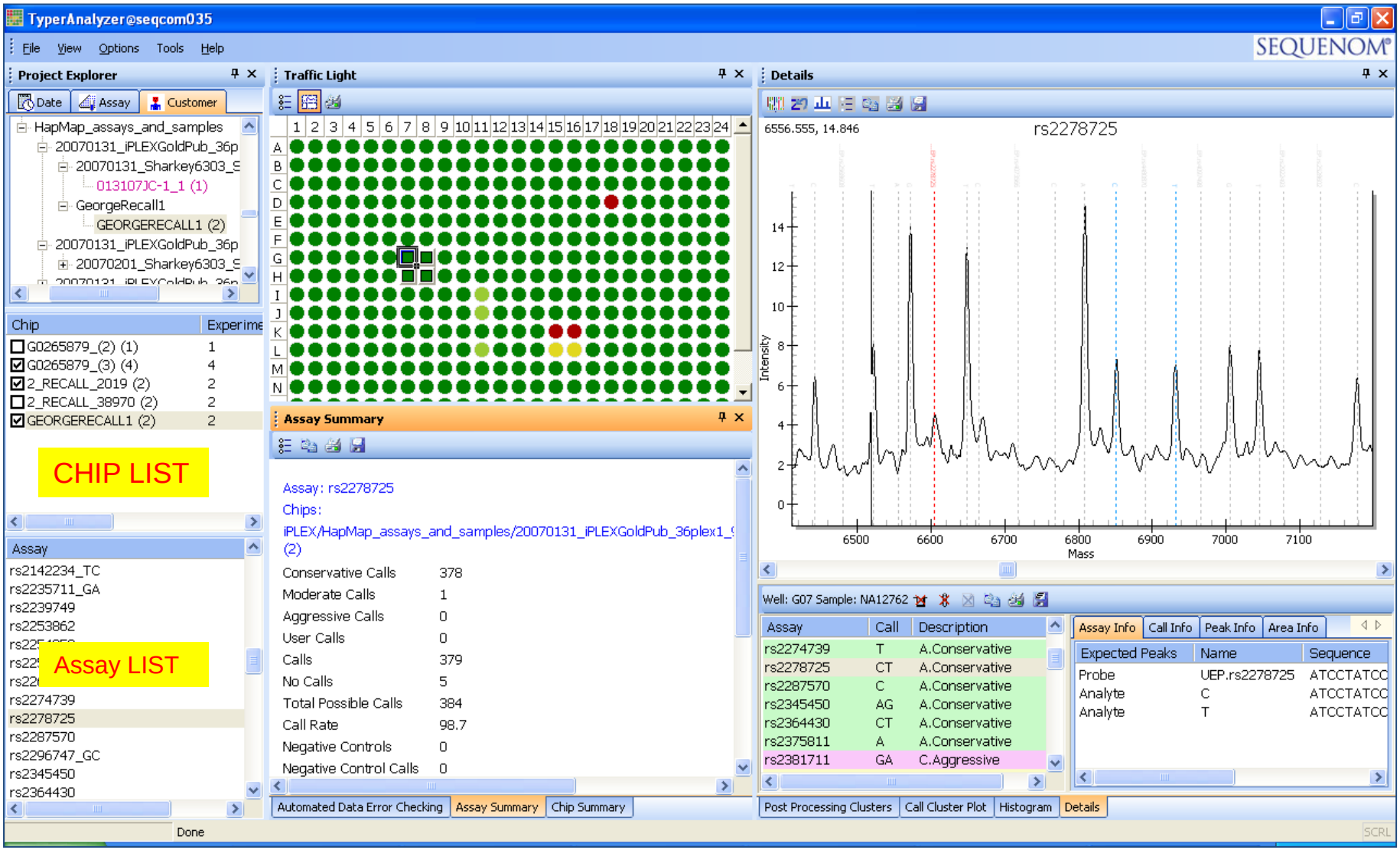


Typical SNP Panel (31-plex)

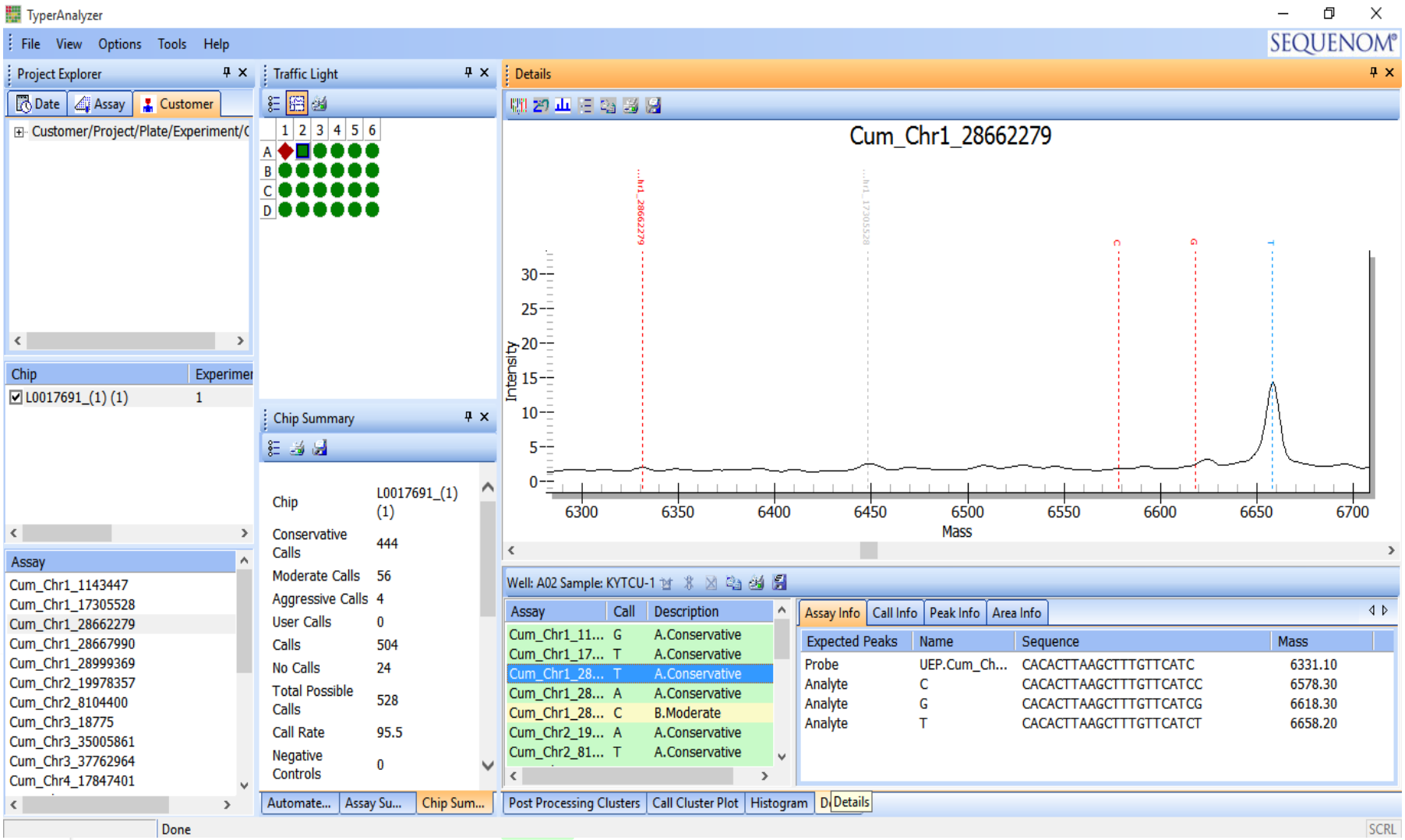
- The Power of Multiplexing Multiple Markers in a Single Well



Generating Report



Data Analysis



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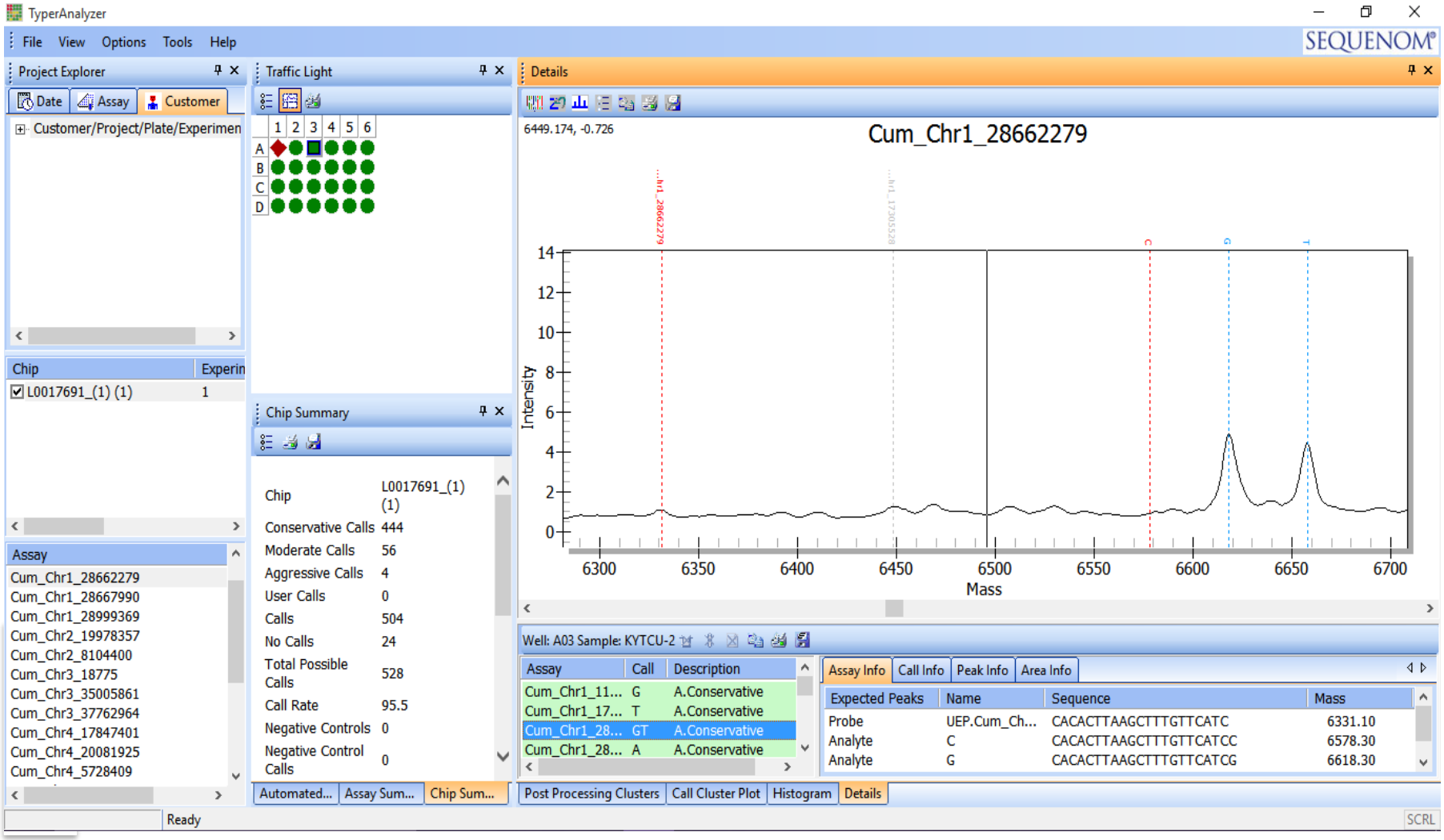

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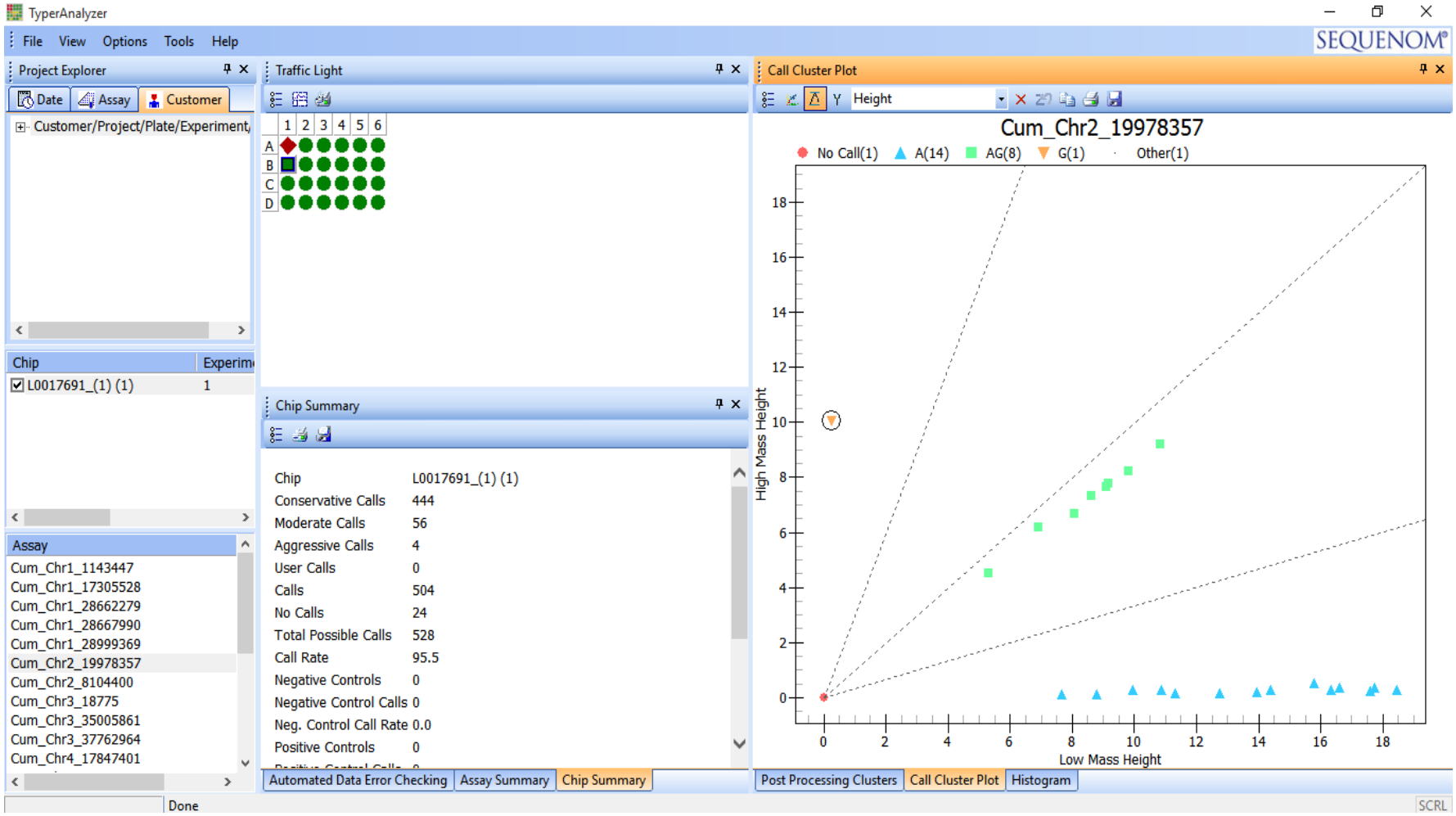

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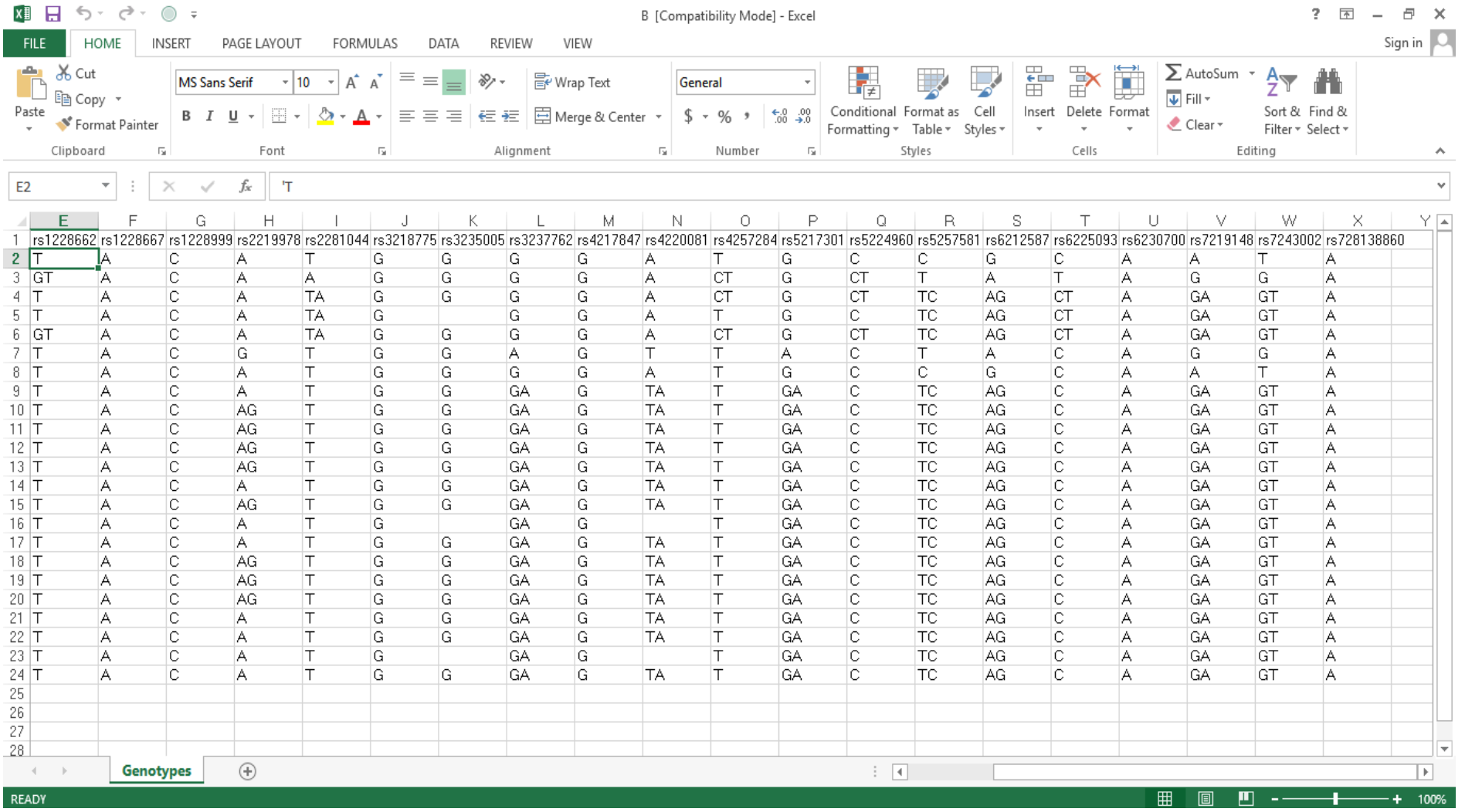

B [Compatibility Mode] - Excel

FILE HOME INSERT PAGE LAYOUT FORMULAS DATA REVIEW VIEW

Sign in

Paste Cut Copy Format Painter

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Conditional Formatting Format as Table Cell Styles Insert Delete Format AutoSum Fill Clear Sort & Filter Find & Select

Clipboard Font Alignment Number Styles Cells Editing

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2	T	A	C	A	T	G	G	G	G	A	T	G	C	C	G	C	A	A	T	A	
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25																					
26																					
27																					
28																					

Genotypes

READY 100%

Assay Design

Assay Design Services

- Fully customized options

Outsource

- Assays by Agena
- Certified Service Providers

In-house

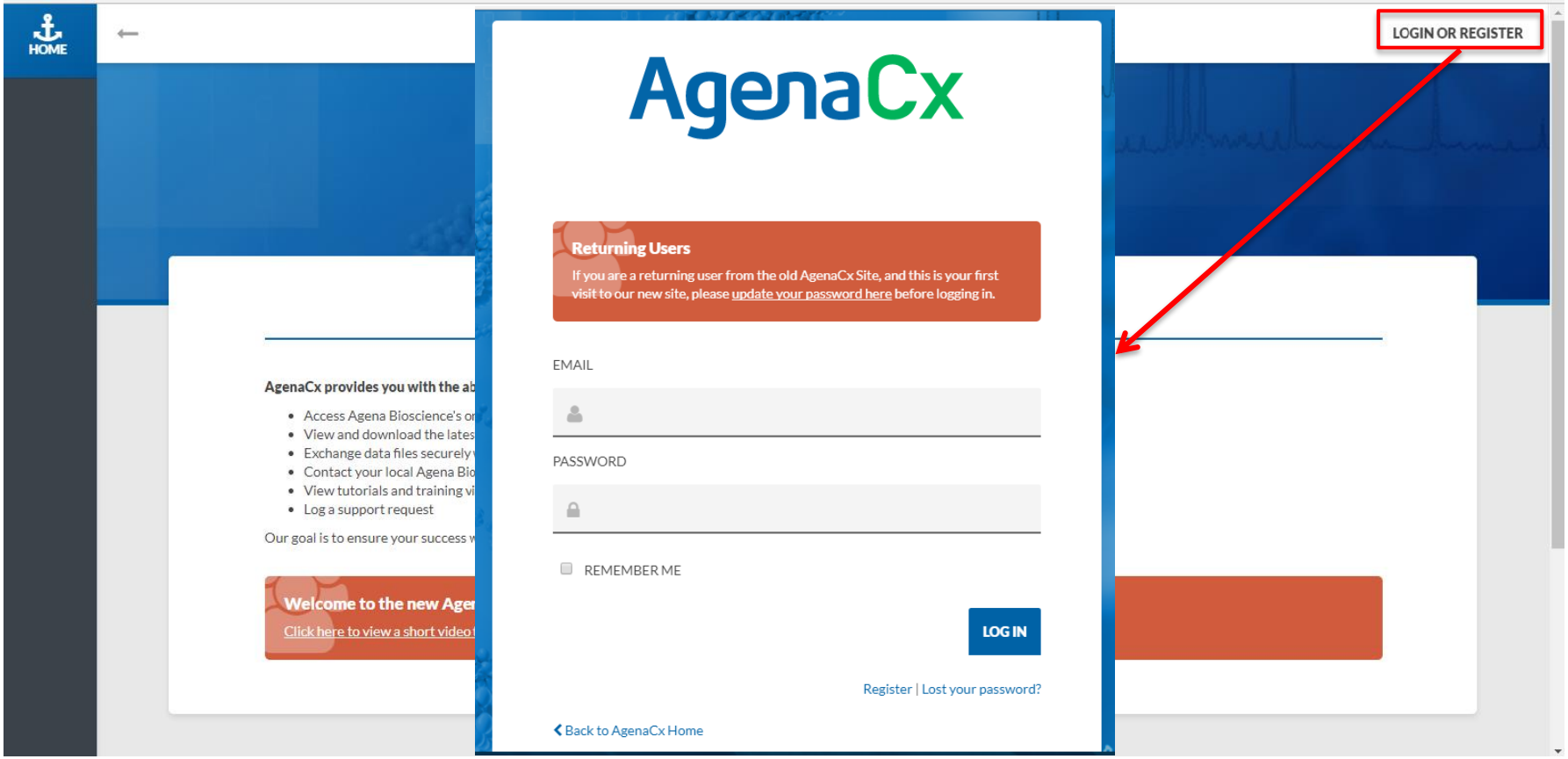
- Assay Design Suite (ADS 2.0 Software)

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

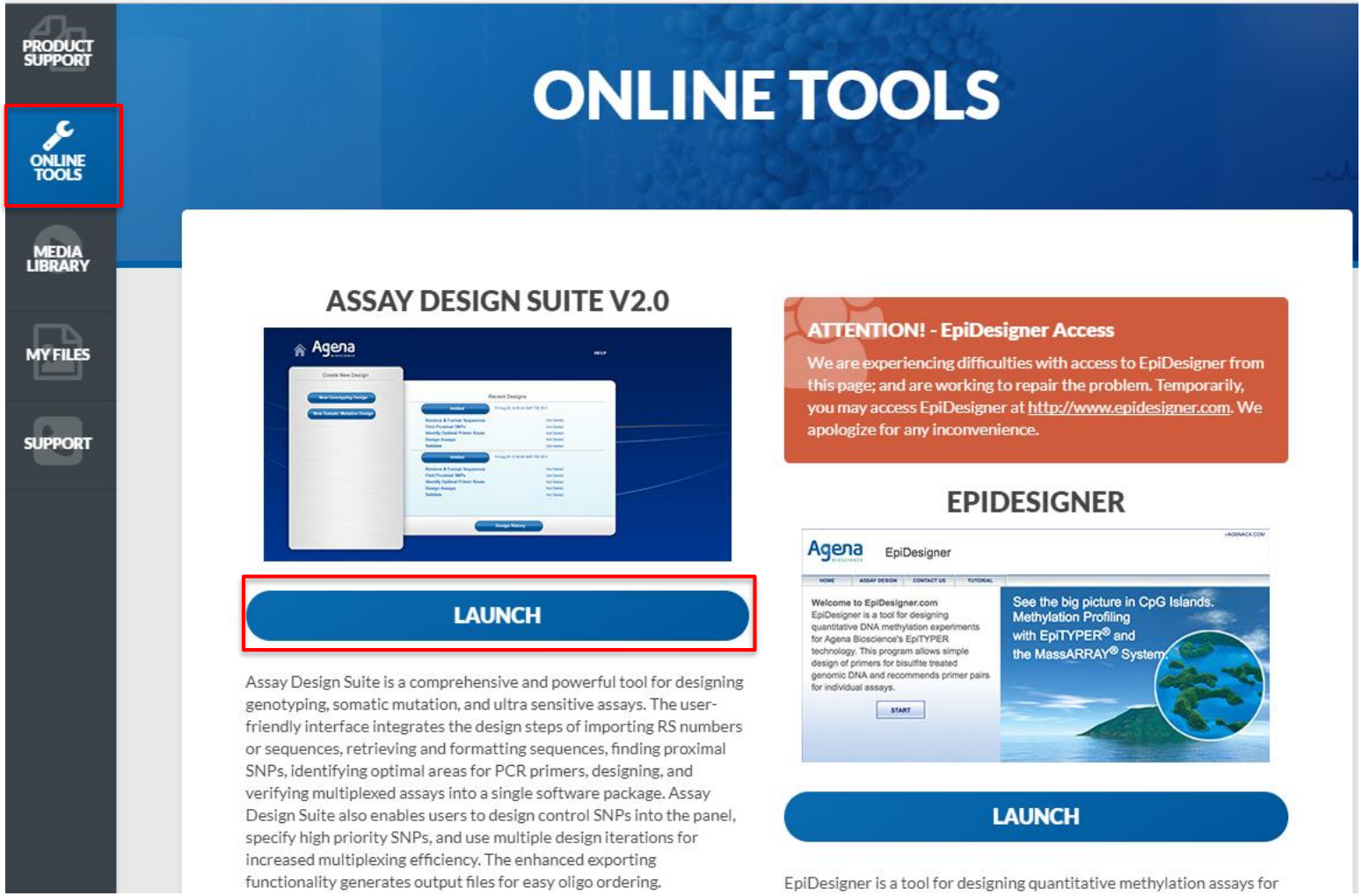
- Patient-specific panels for ultrasensitive detection
- Disease-specific panels for tumor profiling
- Gene-specific panels for therapy selection
- Mutation-specific panels for validation

AgenaCx Account

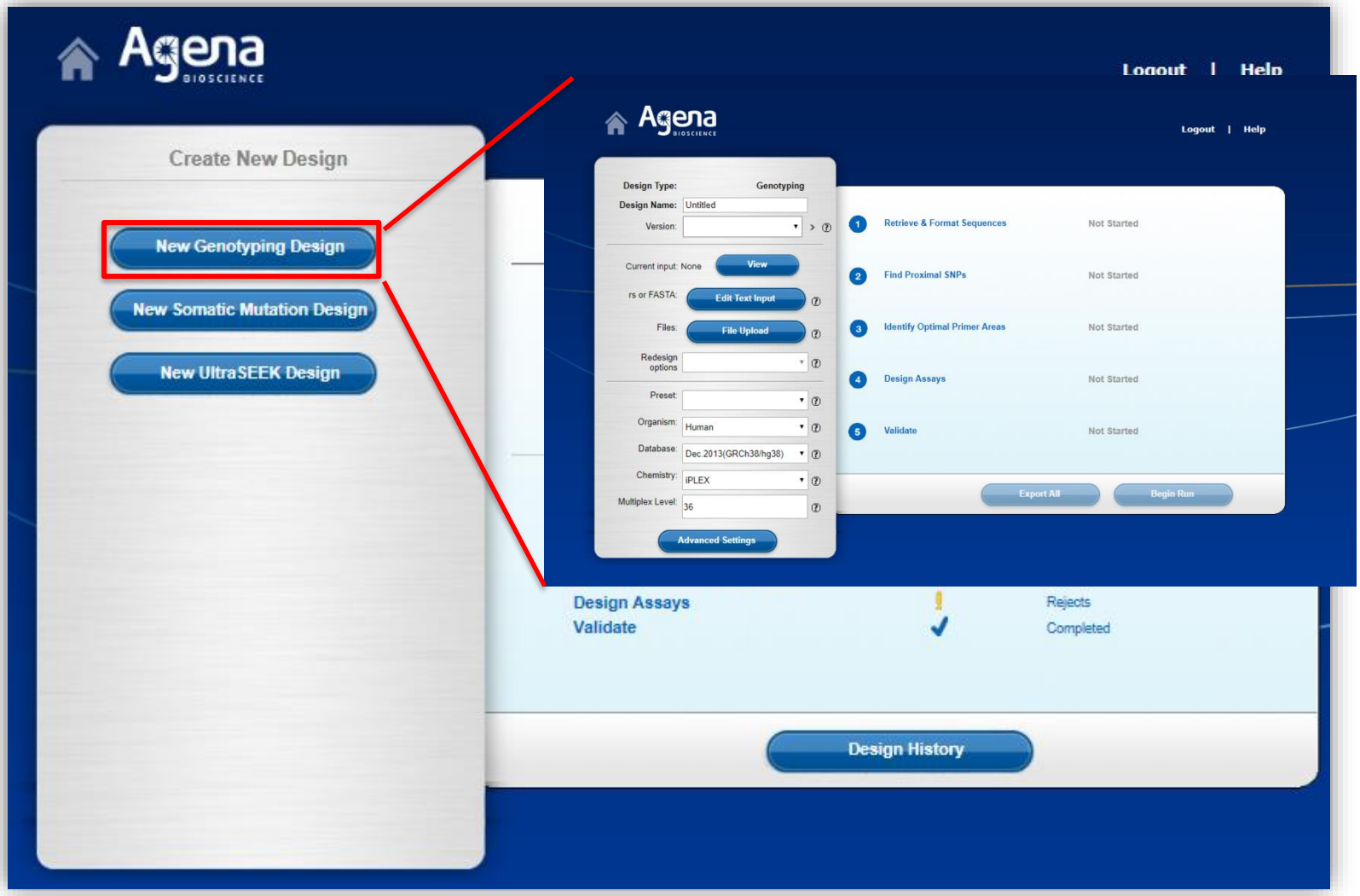
https://www.agenacx.com/Home



AgenaCx Account



AgenaCx Account



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

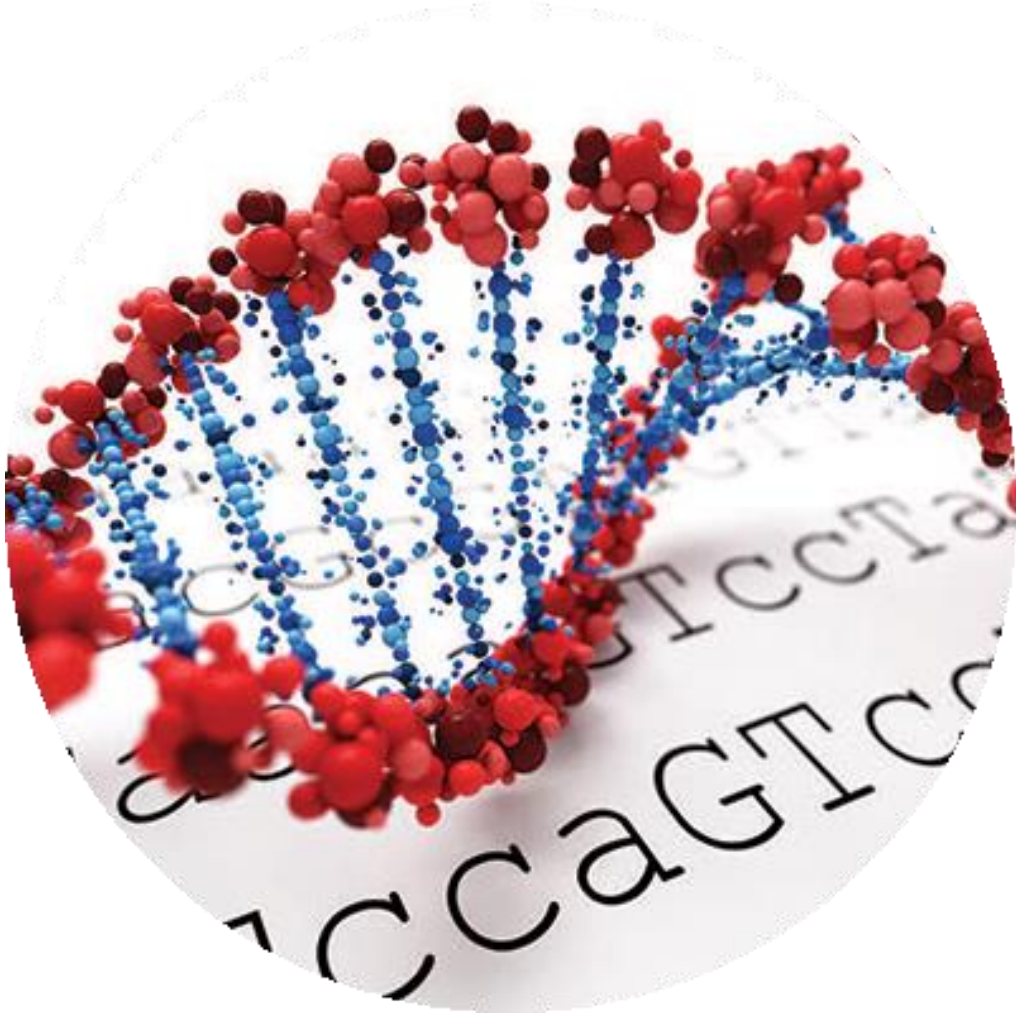
2. System is quantitative
   - Many biological phenomena need to be accurately quantified
   - Allele ratios, gene copy number, methylation

3. Multiplexed assays
   - Provides high throughput
   - Cost effectiveness

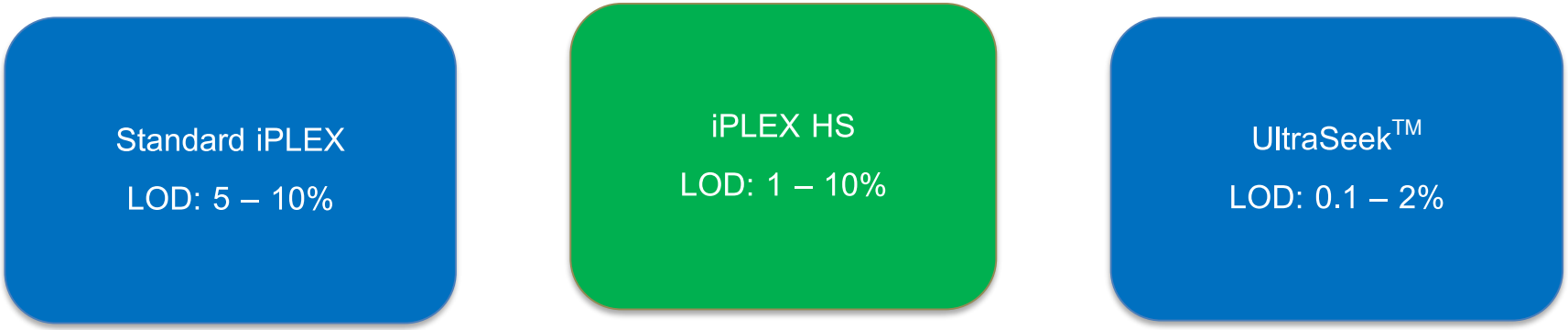
4. Very sensitive and flexible
   - High accuracy – published 99.7%
   - High genotyping call rates (+98%)
   - Numbers of samples and markers are easily scaled
   - Simple and flexible assay design with little optimization required
   - Comprehensive Genetic Analysis >> Somatic mutation, Rare mutation,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

OUTLINE

- INTRO
- MASS ARRAY DEBRIEF
- APPLICATIONS



Sensitivity Across the Spectrum



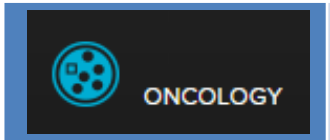
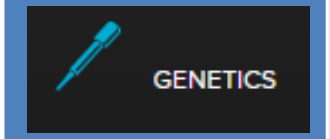
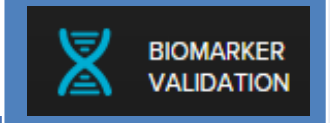
Germline
Inherited mutations

Somatic
Standard biopsy

Somatic
Liquid biopsy

Application of MassARRAY® in Humen Genetics

Clinical Research Solutions : Targeted Biomarker Panels

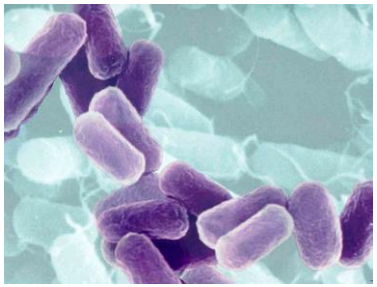
	Application	Panel	Content
	Tumor Profiling	**OncoCarta™ v.1** **OncoFOCUS™ v.3**	19 oncogenes with 238 hot spot mutations EGFR, KRAS, NRAS, BRAF and KIT with 300+ mutations
	Lung Adenocarcinoma	**LungCarta™** **LungFusion™**	26-gene with 200+ mutations ROS1, RET, ALK translocations
	Melanoma	**MelaCarta™**	20-gene panel with 70 mutations
	Colon	**ColoCarta™**	6-gene panel with 32 mutations
	Gynecological	**GyneCarta™**	13-gene panel with 168 mutations
	ccfDNA/CTC	**UltraSEEK™**	12-gene panel with 26 mutations
	Carrier Screening	**Cystic Fibrosis**	CFTR with 23+ mutations
	Blood Group Typing	**HemoID™**	16 blood group systems, 23 platelet and neutrophil antigens
	Drug Metabolism	**ADME PGx** **ADME CYP2D6** **ADME CYP2C19** **ADME CYP2C9/VKORC1**	36-gene panel with 191 SNPs CYP2D6 panel with 35 SNPs CYP2C19 with 31 SNPs CYP2C9 (36 SNPs), VKORC1 (9 SNPs)
		**iPLEX® Pro Sample ID** **iPLEX® Pro Exome ID**	44 SNP panel with gender ID, DNA QC 44-SNPs spanning intron-exon junctions with gender ID, DNA QC

oncology solutions

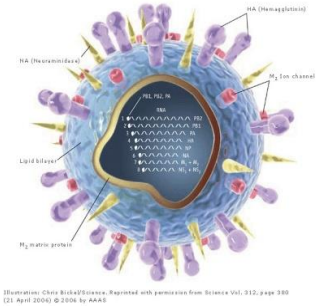
Application	Panel	Content
Colon	IPLEX HS COLON	KRAS, NRAS, BRAF, EGFR, and PIK3CA with 86 mutations
Lung	OncoFOCUS™ v.3	EGFR, KRAS, BRAF, NRAS and KIT with 200+ mutations
	LungFUSION™	ALK, ROS1 and RET translocations
	IPLEX HS LUNG™	5-genesEGFR with 70 mutations
Melanoma	MelaCarta™	20-gene panel with 70 mutations
Gynecological	GyneCarta™	13-gene panel with 168 mutations
ccfDNA/CTC	UltraSEEK™	12-gene panel with 26 mutations

- Molecular Sequence Detection & Typing

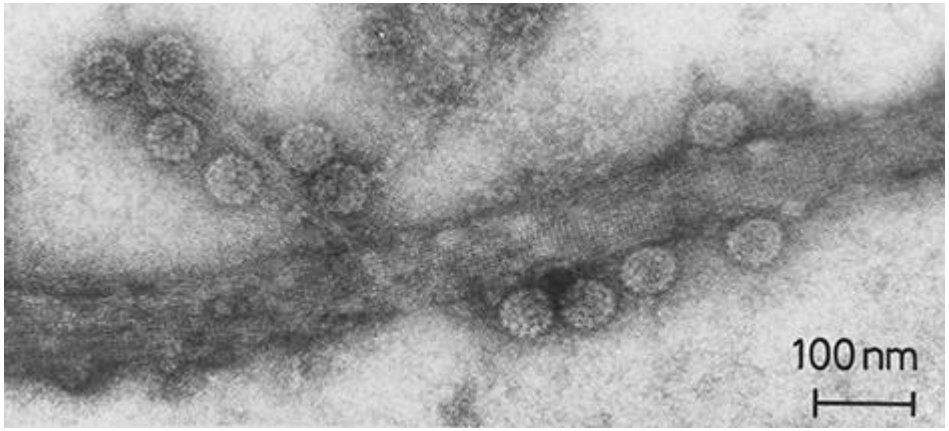
  - Ability to detect and type microbes at the molecular level
    - Epidemiology & transmission studies
    - Bacterial/viral strain and sequence typing
    - Viral detection and typing
    - Outbreak monitoring and tracking



  - Ability to detect, type and quantify pathogens with the one system
    - High sensitivity & specificity
    - Multiplexing – create panels



- Human papillomavirus



- More than 120 different HPV types known
- DNA virus
- High risk types known to cause cancer

Multiplexed Detection of MDR MTB

SCIENTIFIC REPORTS

OPEN

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

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

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.

Many Examples – Microbial Detection &Typing

Detection of HPV subtypes by mass spectrometry in FFPE tissue specimens: a reliable tool for routine diagnostics

Mark Kriegsmann,[1] Petra Wandernoth,[2] Katharina Lisenko,[3] Rita Casadonte,[4] Rémi Longuespée,[4] Norbert Arens,[2] Jörg Kriegsmann[2,5]

OPEN ACCESS Freely available online

PLOS ONE

Simultaneous Detection and Identification of Enteric Viruses by PCR-Mass Assay

Jingzi Piao[1,2☯], Jun Jiang[3☯], Bianli Xu[4☯], Xiaohong Wang[2], Yanfang Guan[5], Weili Wu[3], Licheng Liu[5], Yuan Zhang[3], Xueyong Huang[4], Pengzhi Wang[5], Jinyin Zhao[3], Xiaoping Kang[2], Hua Jiang[2], Yuanyin Cao[1], Yuling Zheng[2], Yongqiang Jiang[2]*, Yan Li[6]*, Yinhui Yang[2]*, Weijun Chen[2,3,5]*

1 College of Plant Protection, Shenyang Agricultural University, Shenyang, China, **2** State Key Laboratory of Pathogen and Biosecurity, Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing, China, **3** Key Laboratory of Genome Sciences and Information, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China, **4** Center for Disease Control and Prevention of Henan Province, Zhengzhou, China, **5** Beijing Genomics Institute in Wuhan, Wuhan, China, **6** Affiliated Hospital of Academy of Military Medical Sciences, Beijing, China

Syrmis *et al. BMC Infectious Diseases* 2014, **14**:307
http://www.biomedcentral.com/1471-2334/14/307

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

A comparison of two informative SNP-based strategies for typing *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis

Melanie W Syrmis[1,2], Timothy J Kidd[1,2], Ralf J Moser[3], Kay A Ramsay[1,2], Kristen M Gibson[1], Snehal Anuj[1], Scott C Bell[1,4], Claire E Wainwright[1,5], Keith Grimwood[1,2], Michael Nissen[1,2,6], Theo P Sloots[1,2,6] and David M Whiley[1,2*]

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

ELSEVIER

Mass spectrometry-based comparative sequencing to detect ganciclovir resistance in the UL97 gene of human cytomegalovirus

Clara C. Posthuma[a,*,1], Martha T. van der Beek[a,1], Caroline S. van der Blij-de Brouwer[a], Pim L.J. van der Heiden[b], Erik W.A. Marijt[b], Willy J.M. Spaan[a], Eric C.J. Claas[a], Christa Nederstigt[a], Ann C.T.M. Vossen[a], Eric J. Snijder[a], Aloys C.M. Kroes[a]

[a] Department of Medical Microbiology, Center of Infectious Diseases, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands
[b] Department of Hematology Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands

MULTI-PURPOSES

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

Mutations can be detected in circulating plasma DNA with the MassARRAY® System

OPEN ACCESS Freely available online

PLOS | ONE

Multi-Purpose Utility of Circulating Plasma DNA Testing in Patients with Advanced Cancers

Geraldine Perkins[1], Timothy A. Yap[1,2], Lorna Pope[1], Amy M. Cassidy[1], Juliet P. Dukes[1], Ruth Riisnaes[1], Christophe Massard[1,2], Philippe A. Cassier[2], Susana Miranda[1], Jeremy Clark[1], Katie A. Denholm[2], Khin Thway[1], David Gonzalez De Castro[1], Gerhardt Attard[1,2], L. Rhoda Molife[2], Stan B. Kaye[1,2], Udai Banerji[1,2], Johann S. de Bono[1,2]*

1 Division of Clinical Studies, The Institute of Cancer Research, Sutton, Surrey, United Kingdom, **2** Drug Development Unit, Royal Marsden NHS Foundation Trust, Sutton, Surrey, United Kingdom

Abstract

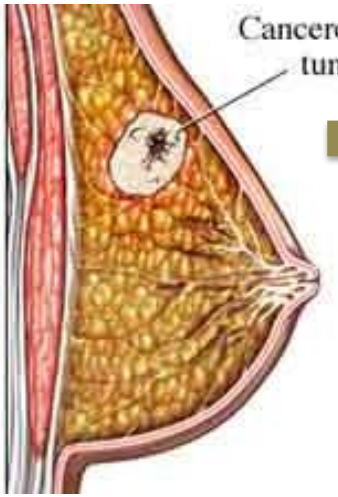
Tumor genomic instability and selective treatment pressures result in clonal disease evolution; molecular stratification for molecularly targeted drug administration requires repeated access to tumor DNA. We hypothesized that circulating plasma DNA (cpDNA) in advanced cancer patients is largely derived from tumor, has prognostic utility, and can be utilized for multiplex tumor mutation sequencing when repeat biopsy is not feasible. We utilized the Sequenom MassArray System and OncoCarta panel for somatic mutation profiling. Matched samples, acquired from the same patient but at different time points were evaluated; these comprised formalin-fixed paraffin-embedded (FFPE) archival tumor tissue (primary and/or metastatic) and cpDNA. The feasibility, sensitivity, and specificity of this high-throughput, multiplex mutation detection approach was tested utilizing specimens acquired from 105 patients with solid tumors referred for participation in Phase I trials of molecularly targeted drugs. The median cpDNA concentration was 17 ng/ml (range: 0.5–1600); this was 3-fold higher than in healthy volunteers. Moreover, higher cpDNA concentrations associated with worse overall survival; there was an overall survival (OS) hazard ratio of 2.4 (95% CI 1.4, 4.2) for each 10-fold increase in cpDNA concentration and in multivariate analyses, cpDNA concentration, albumin, and performance status remained independent predictors of OS. These data suggest that plasma DNA in these cancer patients is largely derived from tumor. We also observed high detection concordance for critical 'hot-spot' mutations (*KRAS*, *BRAF*, *PIK3CA*) in matched cpDNA and archival tumor tissue, and important differences between archival tumor and cpDNA. This multiplex sequencing assay can be utilized to detect somatic mutations from plasma in advanced cancer patients, when safe repeat tumor biopsy is not feasible and genomic analysis of archival tumor is deemed insufficient. Overall, circulating nucleic acid biomarker studies have clinically important multi-purpose utility in advanced cancer patients and further studies to pursue their incorporation into the standard of care are warranted.

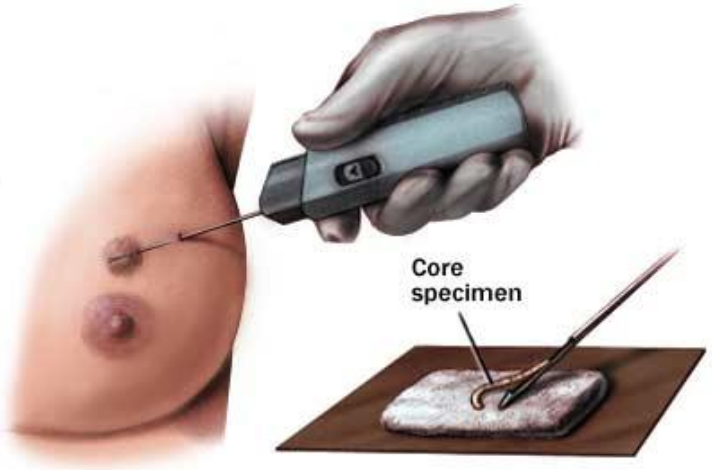
Citation: Perkins G, Yap TA, Pope L, Cassidy AM, Dukes JP, et al. (2012) Multi-Purpose Utility of Circulating Plasma DNA Testing in Patients with Advanced Cancers. PLoS ONE 7(11): e47020. doi:10.1371/journal.pone.0047020

Editor: Jose Luis Perez-Gracia, University Clinic of Navarra, Spain

Received May 15, 2012; **Accepted** September 7, 2012; **Published** November 7, 2012

MASSARRAY EARYLY BREAST CANCER SCHEMA

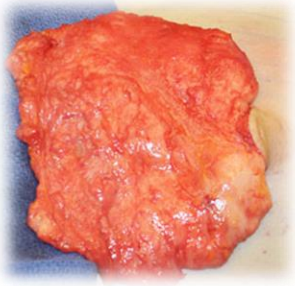




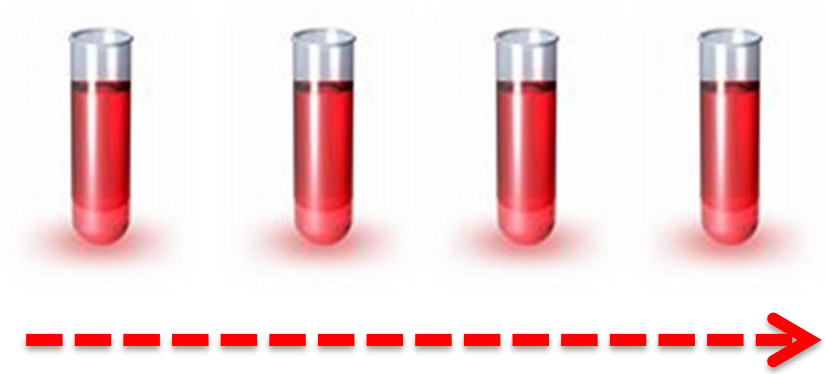
CURATIVE SURGERY + ADJUVANT

1 CTC PROGNOSIS

2 Cancer DNA profiling



FOLLOW UP



cancer DNA detected

3

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:

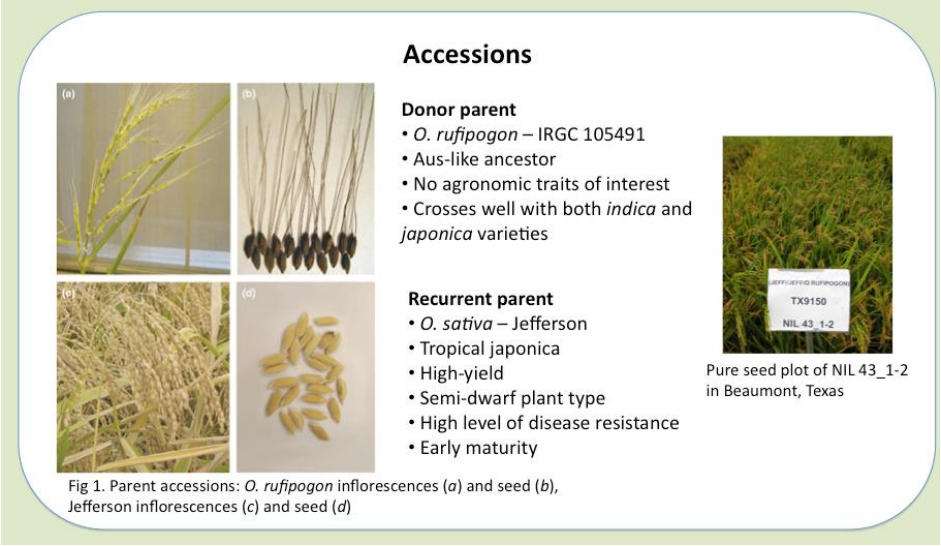
Nature. 2013 August 15; 500(7462): 340–344. doi:10.1038/nature12356.

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

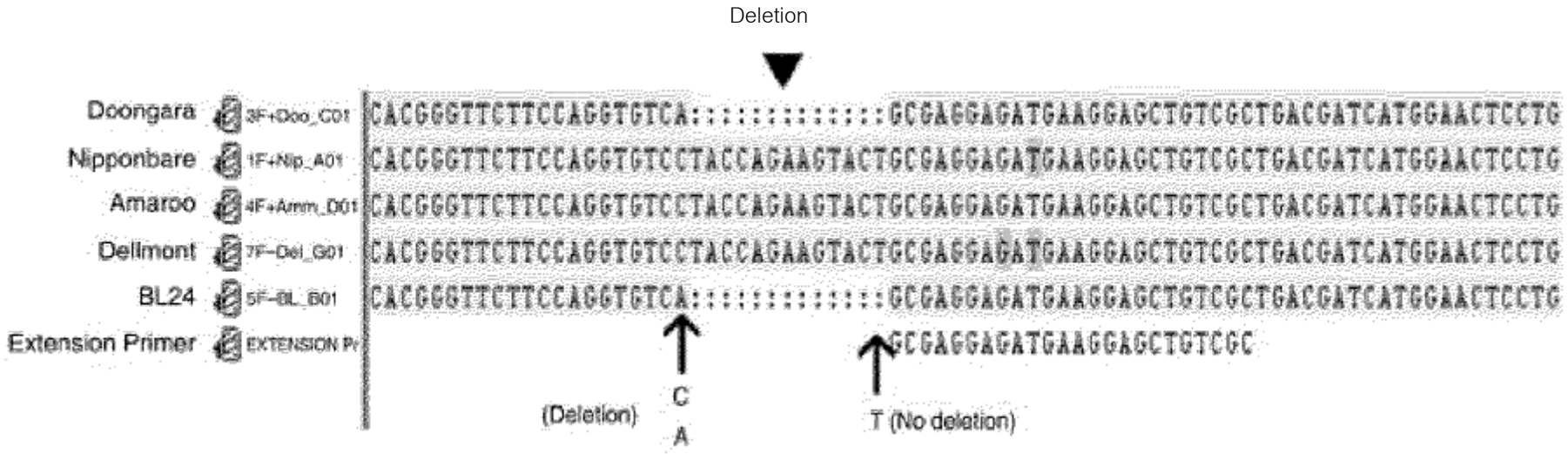
8 SNP Marker Analysis for Quality and Agronomic Traits (rice)

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.

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

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

Ardashir K. Masouleh[1], Daniel L. E. Waters[1], Russel F. Reinke[2] and Robert J. Henry[1,*]

[1]Centre for Plant Conservation Genetics, Southern Cross University, Lismore, NSW 2480, Australia

[2]Yanco Agricultural Institute, Yanco, NSW 2703, Australia

Received 19 December 2008;
revised 3 February 2009;
accepted 4 February 2009
*Correspondence (fax +61 266222080;
e-mail robert.henry@scu.edu.au)

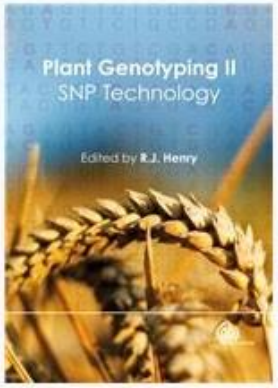
Keywords: matrix-assisted laser desorption ionization-time of flight (MALDI-TOF), multiplex perfect markers, rice, single nucleotide polymorphism.

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.

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.

Available In Print →

Book details

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Henry, R. J.

Author Affiliation
Centre for Plant Conservation Genetics Southern Cross University Lismore, New South Wales, Australia.

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genetic markers
genetic polymorphism
genetic variation
genotypes

Book Chapters

polymerase chain reaction
reviews
rice
single nucleotide polymorphism

Subject Code(s)
FF005 - Field Crops. (New March 2000)
FF020 - Plant Breeding and Genetics
ZZ360 - Molecular Biology and Molecular Genetics. (Discontinued March 2000. Reinstated and Revised June 2002)

Record Number
20083134963

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 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

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

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

ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

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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

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

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

Questions

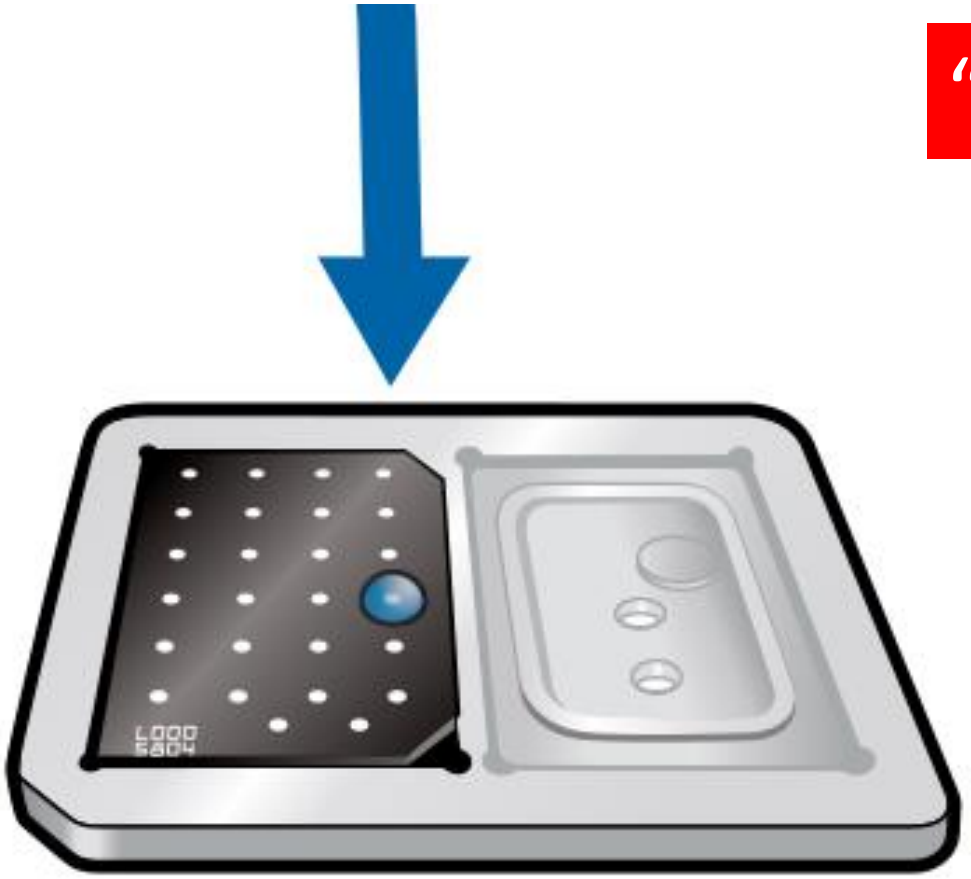
PRECISION & MINIMIZE

HUMAN ERROR

“SPECTRO-CHIP”


PATENTED “SPECTROCHIP”

MASSARRAY: KEY HIGHLIGHT



"MULTIPLEXING"

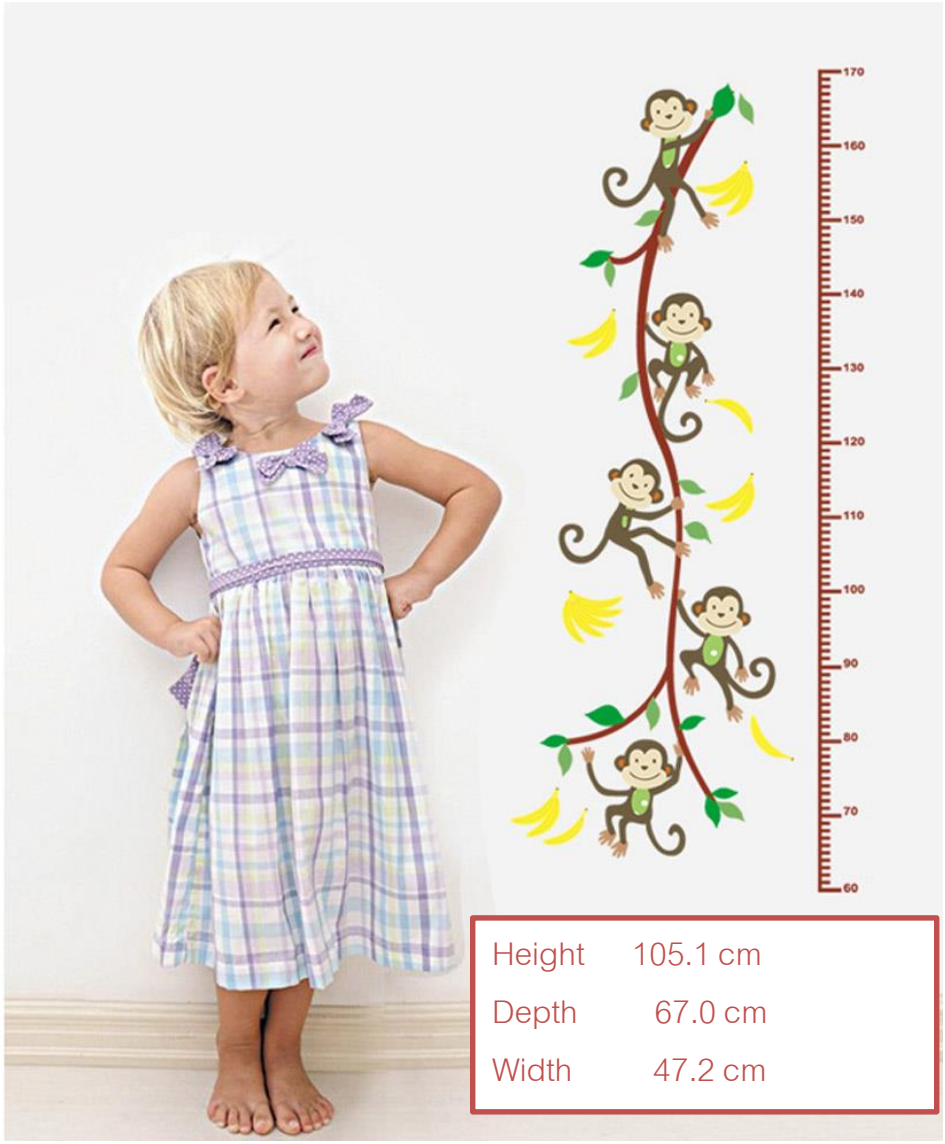
40 Target Specific DNA Fragments per a Single Reaction…

9 Laser shots per sample

Agena BIOSCIENCE

COMPACT


MassARRAY
Agena
BIOSCIENCE


170
160
150
140
130
120
110
100
90
80
70
60
Height 105.1 cm
Depth 67.0 cm
Width 47.2 cm