Nanosphere

Advancing Diagnostics Through the Power of Nanotechnology

Contents

- The Verigene System
- CYP 2C19 Genotyping

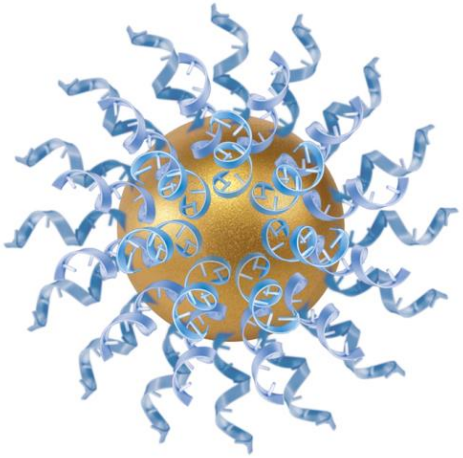
Company Overview

International Institute for Nanotechnology
Northwestern University

- Nanosphere, Inc. (Nanosphere) is a publicly traded, molecular diagnostics company located in Northbrook, IL close to Chicago.
- Founded in 2000 based upon nanotechnology discoveries at Northwestern University by Dr. Robert Letsinger and Dr. Chad Mirkin.
- 149 issued patents; over 56 pending
- On-going, exclusive relationship to advance technology in biodiagnostics

Company Overview

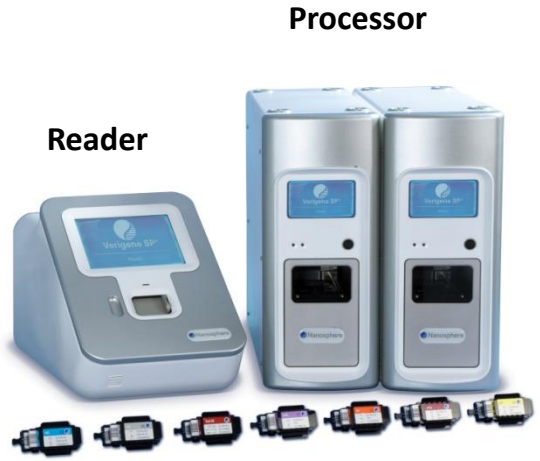
- Genetics
- Pharmacogenetics
- Infectious Disease



The Verigene® System

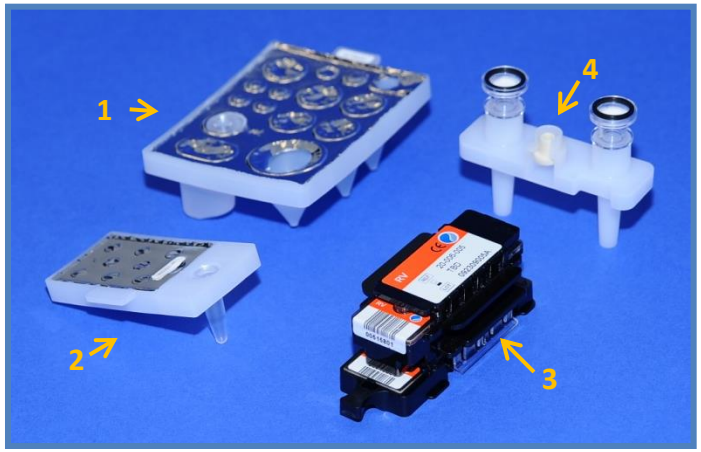
INSTRUMENTS

- Verigene® Reader
  (cleared USA, Europe, and South Korea)
- Verigene® Processor *SP*
  (cleared USA, Europe, and South Korea)



LAB CONSUMABLES

1. Verigene® Extraction Tray
2. Verigene® Amplification Tray
3. Verigene® Test Cartridge
4. Verigene® Tip Holder Assembly



Pharmacogenomic Test

- Verigene® Clopidogrel Metabolism Plus(CLO+) Nucleic Acid Test (CE-IVD)
  - Multiplex testing of two *CYP2C19* loss-of-function alleles (**2, *3),* and one *CYP2C19* gain-of-function allele *(*17)*
- Verigene® Warfarin Metabolism Nucleic Acid Test (CE-IVD)
  - Multiplex testing of two *CYP2C9* alleles (**2, *3),* and VKORC1 gene

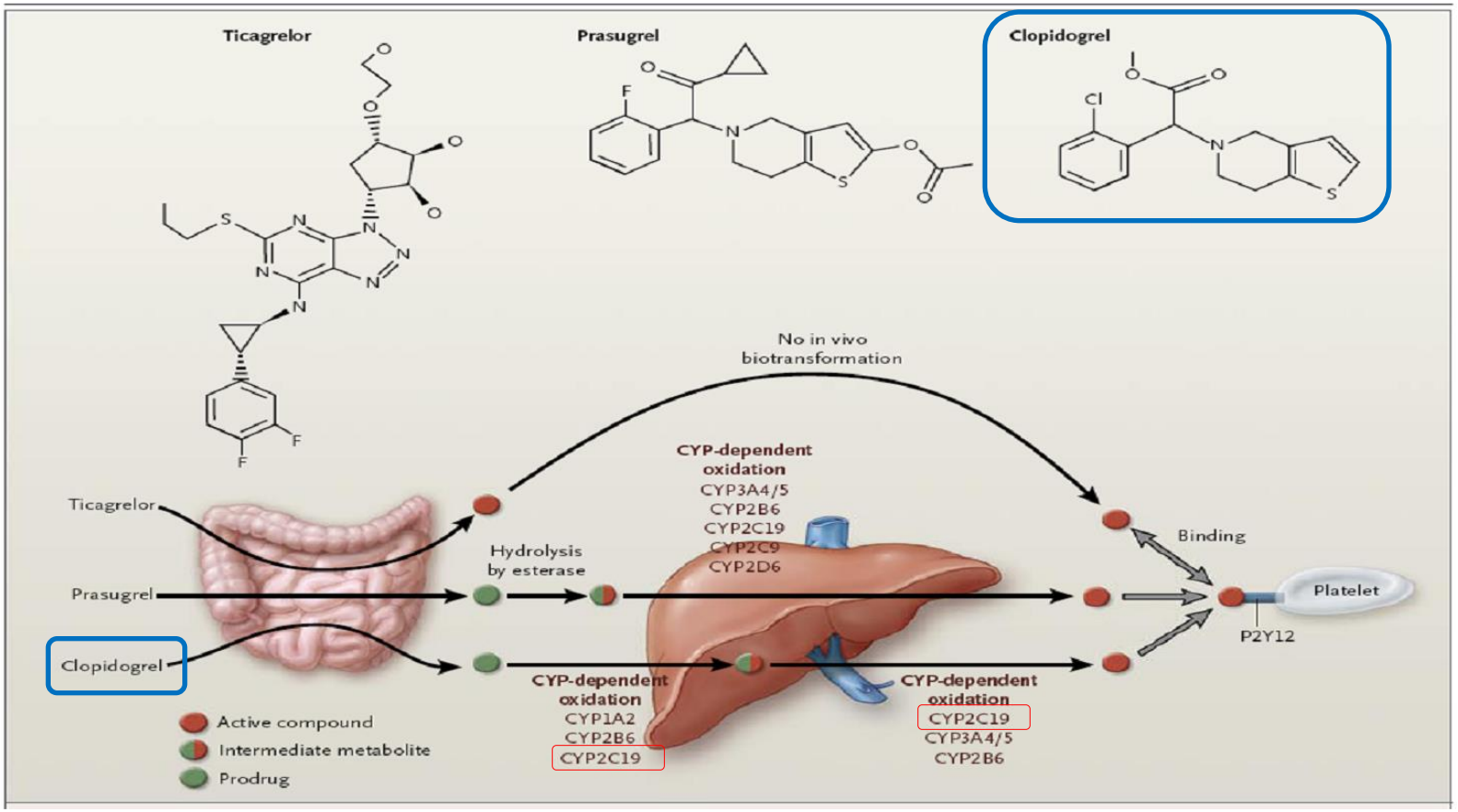
Infectious disease Test

- Verigene® Respiratory Virus Plus Nucleic Acid Test (CE-IVD)
  - Multiplex testing of Influenza(A, B) and RSV(A, B), Influenza A subtype(2009H1N1, H1, H3), Olsetamivir Resistance(2009-H1 H275Y, H1 H275Y)

CYP2C19 Genotyping

Antiplatelet Therapy - Clopidogrel

CLOPIDOGREL METABOLISM



GENETIC VARIATIONS OF THE *CYP2C19* GENE

- ✓ ***17** = Gain-of-function SNP
  (18% Americans, 16% Africans, 2% East Asians)
- ✓ ***2** = Loss-of-function SNP
  (~12% Americans, 15% Africans, 29% East Asians)
- ✓ ***3** = Loss-of-Function SNP
  (.02% Americans, .5% Africans, 9% East Asians)
- ✓ ***1** = Wild type

SNP, single-nucleotide polymorphism.
Scott SA et al, *Clin Pharmacol Ther*. 2011 Jun 29. doi: 10.1038/clpt.2011.132. [Epub ahead of print]

GENETIC VARIATIONS OF THE *CYP2C19* GENE

- Genetic variations of CYP2C19 gene result in a spectrum of metabolic phenotypes:

Metabolic Phenotype		Genotype
Ultra-rapid Metabolizer	**UM**	*17/*17; *1/*17
Extensive Metabolizer	**EM**	*1/*1;
Intermediate Metabolizer	**IM**	*1/*2; *1/*3;
Poor Metabolizer	**PM**	*2/*2; *3/*3; *2/*3

- The *CYP2C19**2 and *3 polymorphisms are seen in
  - **more than 55% of Asians**
  - approximately 40% of African-Americans
  - approximately 30% of Caucasians

PHARMACOGENETIC INFORMATION

- **Plavix - 2010 FDA: Black Box Warning**

WARNING: DIMINISHED EFFECTIVENESS IN POOR METABOLIZERS

The effectiveness of Plavix is dependent on its activation to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19 [see Warnings and Precautions (5.1)]. Plavix at recommended doses forms less of that metabolite and has a smaller effect on platelet function in patients who are CYP2C19 poor metabolizers. Poor metabolizers with acute coronary syndrome or undergoing percutaneous coronary intervention treated with Plavix at recommended doses exhibit higher cardiovascular event rates than do patients with normal CYP2C19 function. Tests are available to identify a patient's CYP2C19 genotype; these tests can be used as an aid in determining therapeutic strategy [see Clinical Pharmacology (12.5)]. Consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers [see Dosage and Administration (2.3)].

Plavix PI, Bristol Meyers Squibb / Sanofi Pharmaceuticals, March 2010

PHARMACOGENETIC INFORMATION

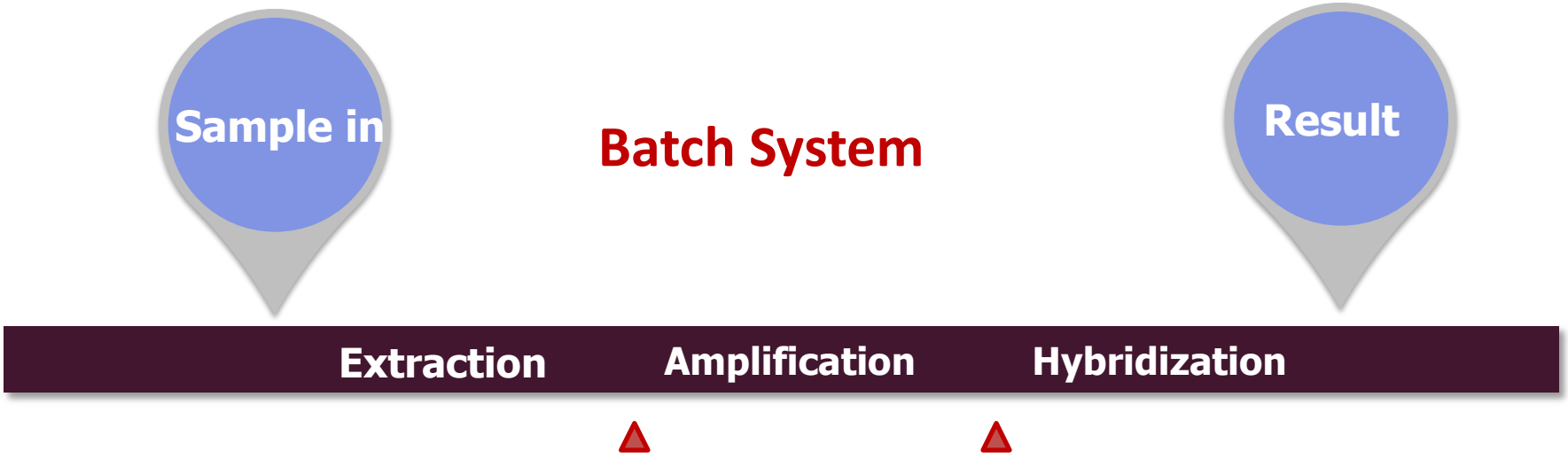
➢ 2011 ACCF/AHA Update on Management of patients with UA/NSTEMI

2011 Focused Update Recommendations – Table 3	
Class IIb	**Platelet function testing** to determine platelet inhibitory response in patients with UA/NSTEMI (or, after ACVS and PCI) on thienopyridine therapy may be considered if results of testing may alter management (Level of Evidence: B)
Class IIb	**Genotyping** for CYP2C19 loss-of-function variant in patients with UA/NSTEMI (or, after ACVS and PCI) on thienopyridine therapy might be considered if results of testing may alter management (Level of Evidence: C)

A report of the American college of Cardiology foundation/
American heart association Task force on Practice guideline

Genotyping method

- Gene Sequencing
- PCR



1

Sample in

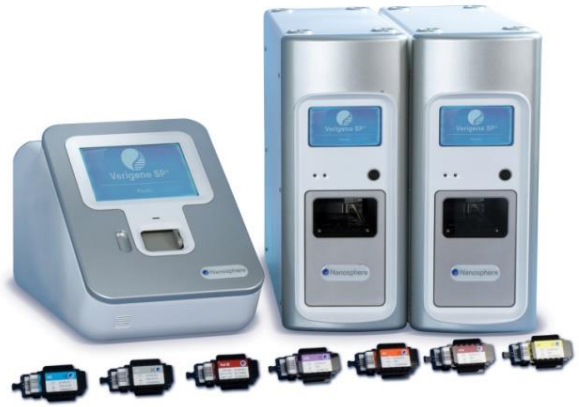
2.5 hours

3

Result

Extraction Amplification Hybridization

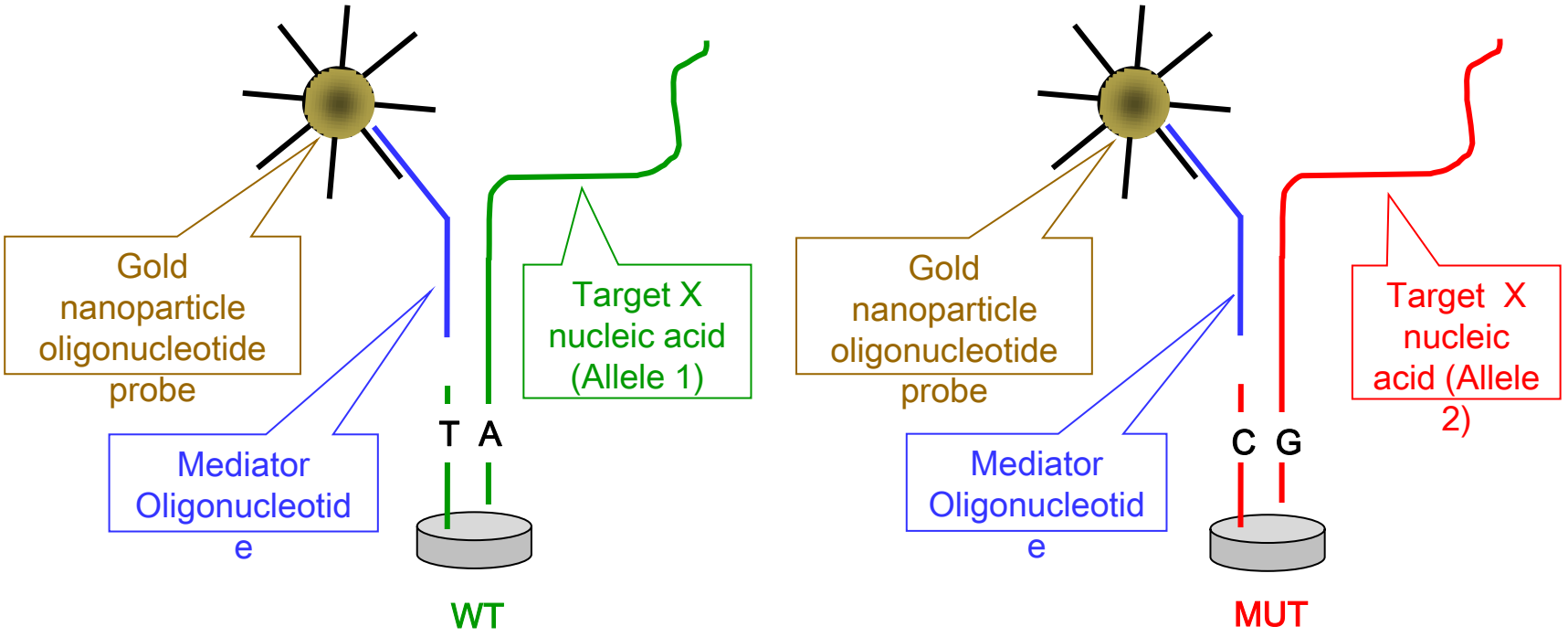
2



Sample in

Result

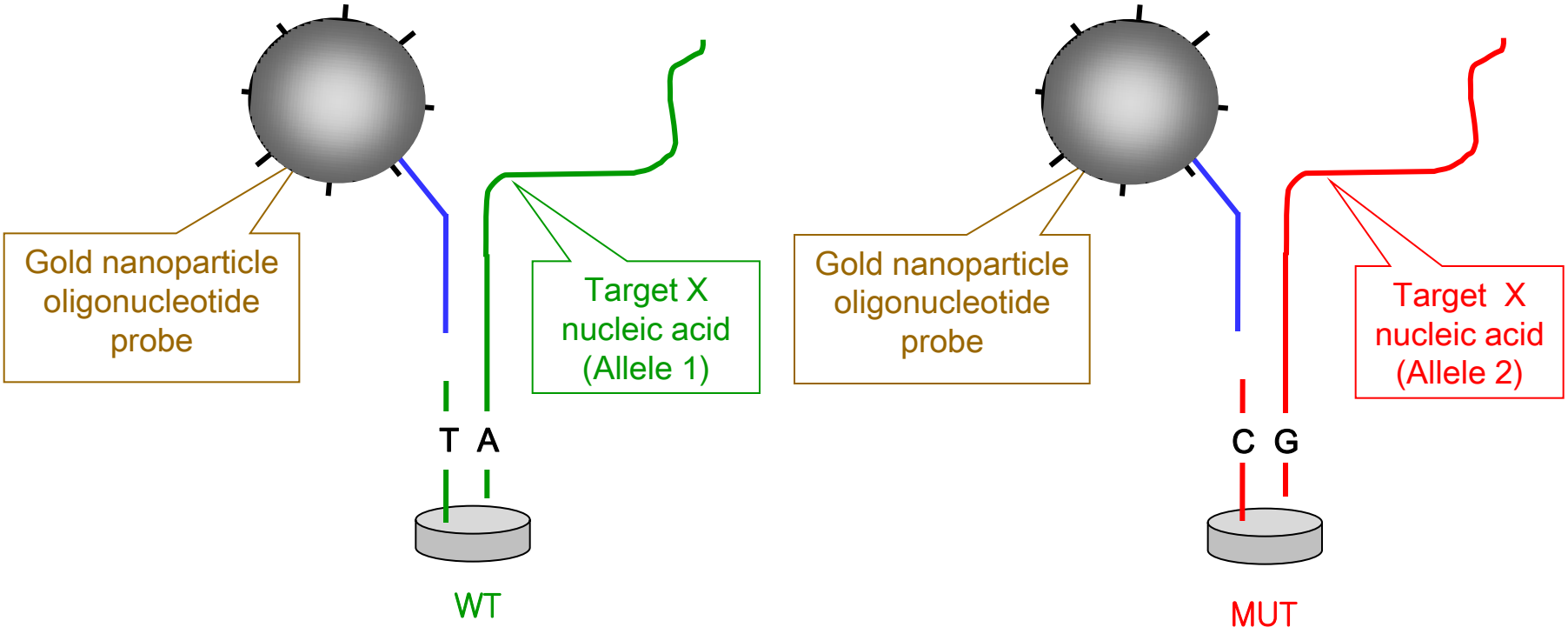
Extraction Amplification **Hybridization**



Sample in

Result

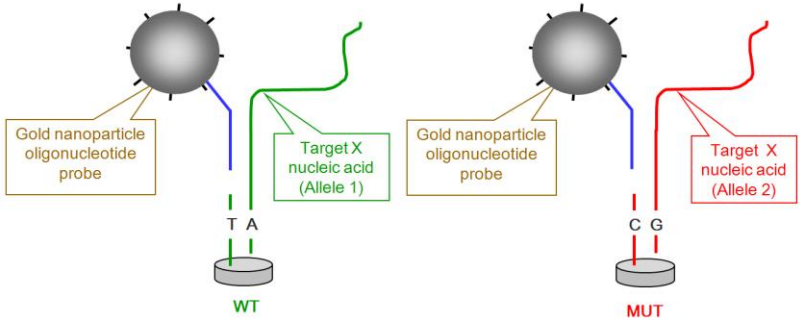
Extraction Amplification **Hybridization**

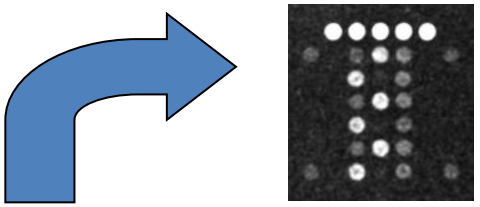


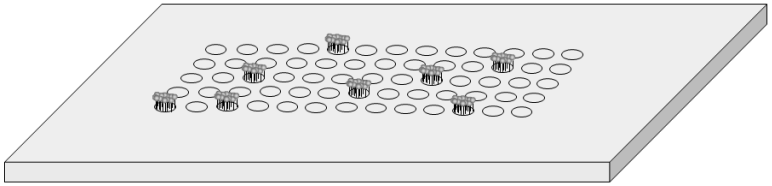
Sample in

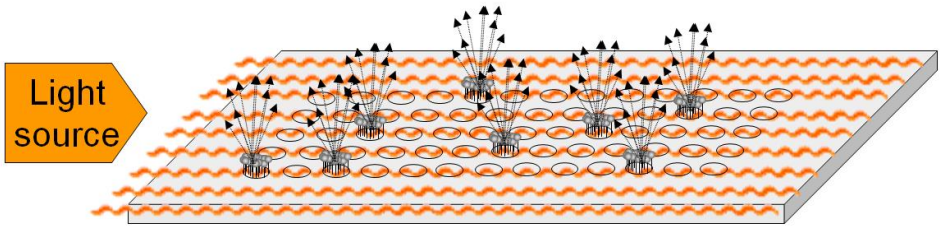
Result

Extraction Amplification Hybridization





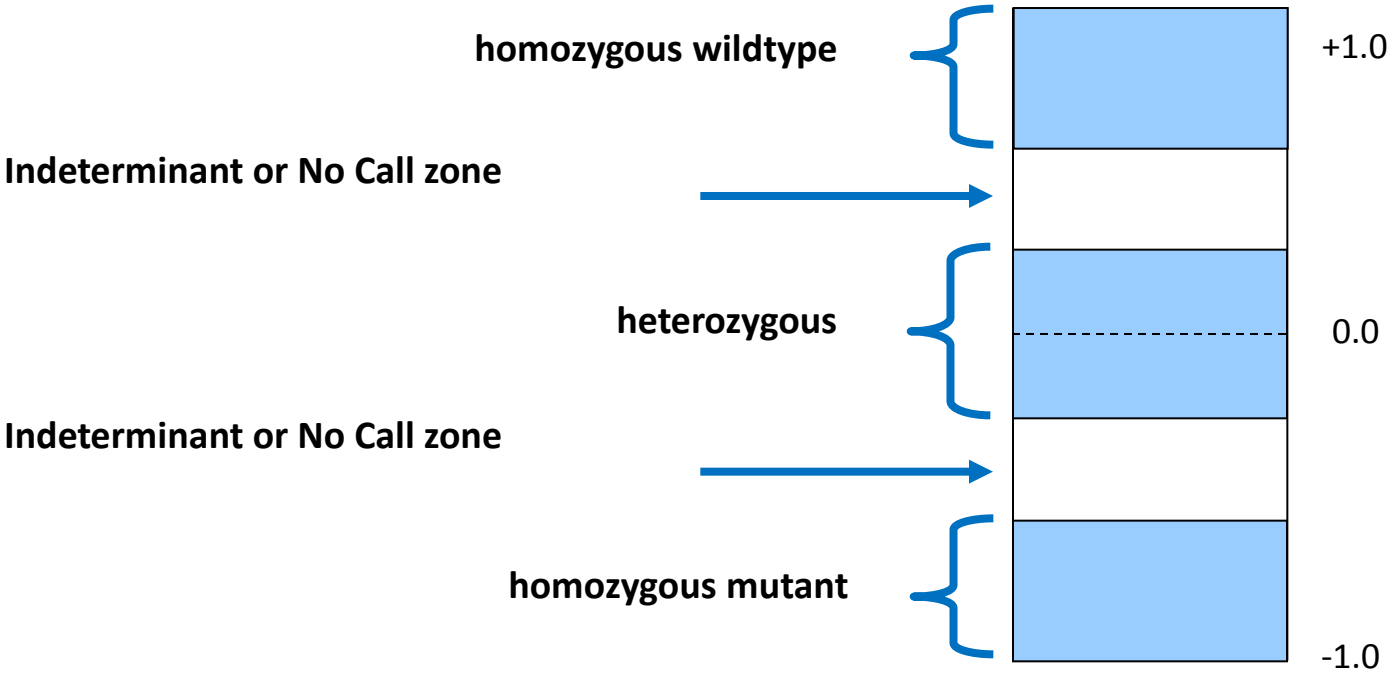




The Verigene® System

Technology Overview

Genotype number = $\frac{\text{net WT signal} - \text{net MUT signal}}{\text{net WT signal} + \text{net MUT signal}}$



The Verigene® System

Technology Overview

- Example of a CLO+ test report – *CYP2C19* *2 Mutant

P2 Lab

Detail Report
01·11·11 07:17 p.m.
Operator ID: administrator
S/N: 08092057
Session ID: lipemic_samples_CLOplus_011111

For Investigational Use Only. The performance characteristics of this product have not been established.

Sample	8470032208–049		
Test	CLO+	**Processing completed**	01·11·11, 07:10 p.m.
Cartridge	01902866	**Analysis completed**	01·11·11, 07:17 p.m.

Summary

✻ *1	Mut Detected	✻ *2 [1]	–0.51 Mutant

Detail

✻ *1	Mut Detected	✻ *2 [1]	–0.51 Mutant
*3 [2]	0.94 Wild Type	*17 [3]	0.99 Wild Type

Genotype Score Ranges

[1] – Mutant (–1.00 to –0.50)
[2] – Wild Type (0.60 to 1.00)
[3] – Wild Type (0.61 to 1.00)

Processor Quality Control Status

Processor Module	B:4
Processing Time	149 minutes
Occlusions Corrected	0
Seal Pressure	Status not available
Seal Pressure Decay	Status not available
Processing Temperature	40.0 C
Failure Status	no failure

PERFORMANCE DATA (cont.)

- Method Comparison – Genotype Distribution
  - The following tables represent the genotype distribution tested in the methods comparison study and the call accuracy.
  - There was a 100% agreement between the Processor SP and the comparative method.

Method Comparison Results – Genotype Distribution				
CYP2C19*2		**Verigene® System**		
		Wild Type	**Heterozygous**	**Mutant**
Sequencing	**Wild Type**	218	0	0
	Heterozygous	0	154	0
	Mutant	0	0	30

Source: Verigene® *CYP2C19* (CLO+) Nucleic Acid Test – Package Insert

PERFORMANCE DATA (cont.)

- Method Comparison – Genotype Distribution (cont.)

Method Comparison Results – Genotype Distribution				
*CYP2C19*3*		Verigene® System		
		Wild Type	Heterozygous	Mutant
Sequencing	Wild Type	373	0	1[a]
	Heterozygous	0	26	0
	Mutant	0	0	2

Method Comparison Results – Genotype Distribution				
*CYP2C19*17*		Verigene® System		
		Wild Type	Heterozygous	Mutant
Sequencing	Wild Type	327	0	0
	Heterozygous	0	70	0
	Mutant	0	0	5

[a] One sample was incorrectly genotyped by bi-directional sequencing. Repeat sequencing of this sample confirmed the Verigene System call of *3/*3.

Source: Verigene® *CYP2C19* (CLO+) Nucleic Acid Test – Package Insert

PERFORMANCE DATA (cont.)

Blinded Methods Comparison Study		Bi-Directional DNA Sequencing							
		*1/*1	*2/*1	*2/*2	*8/*1	*9/*1	*10/*1	*17/*1	*17/*17
Verigene® Test (*2-*10, *13, *17 alleles)	*1/*1	38							
	*2/*1		26						
	*2/*2			2					
	*8/*1				1				
	*9/*1					1			
	*10/*1						1		
	*17/*1							29	
	*17/*17								2

100% concordance, 100% sensitivity, 100% specificity

Gurbel PA et al., Presented at ACC 2011, New Orleans

SUMMARY

- **Nanosphere Verigene system**
  - ✓ Full automation system
  - ✓ On-demand Random access system
  - ✓ 2.5 h Test result report
  - ✓ Multiplex platform (CYP 2C19 *2, *3, *17)
  - ✓ Precised result

Thank You