Bringing Personalized Medicine to the Clinic

cMET: Simultaneous and Quantitative Analysis of Copy Number Variation and Gene Expression in a Single Reaction

Molecular Med Tri-Con
Feb., 13, 2013

PrimeraDx

Lilly Kong, DVM
CSO, PrimeraDx

PrimeraDx – Simplifying Molecular Diagnostics with a Proprietary Platform

Only technology available to enable Multi-Modal Multiplex testing

- Ability to detect and quantify DNA, RNA, mRNA and miRNA all in a single-well reaction
- Proprietary technology with large IP estate
- Automated platform that dramatically improves lab workflow, reduce costs, and enables new markets

Technology advantages provide opportunity for differentiated content; several assays in development

- cMET/EGFR and other oncology assays in partnership with Pharma
- KRAS/NRAS/BRAF, EGFR and several ID products currently in development

Technology is validated and in the market now

- Eli Lilly companion diagnostics relationship, with multiple other opportunities in progress
- Initial open platform placements ramping up

Technology has unique and large commercial opportunity

- “Open platform” approach
- Cleared IVD kit sales
- Companion diagnostic partnerships

Regulatory path is clear and in process

Unique Technology Addresses Current Problems While Enabling New Markets and Applications

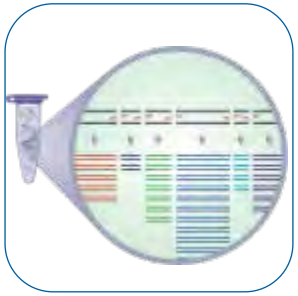
The Marriage of PCR and Capillary Electrophoresis

Integrated PCR, CE, fluidics, detection and analysis software

Real-time detection of PCR products separates targets by size

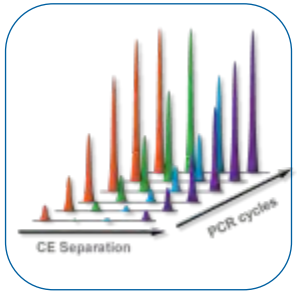
Sampling as the reaction progresses allows quantitation

Multiplex, mulitmodal, quantitative results provide unique, clinically actionable data



PCR

+



CE

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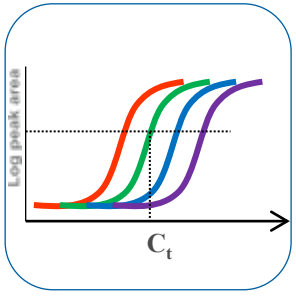

Quantification

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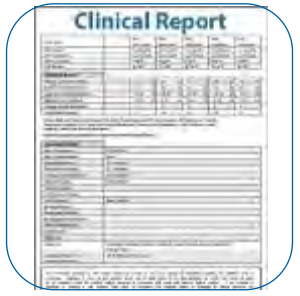


Clinically Actionable Results

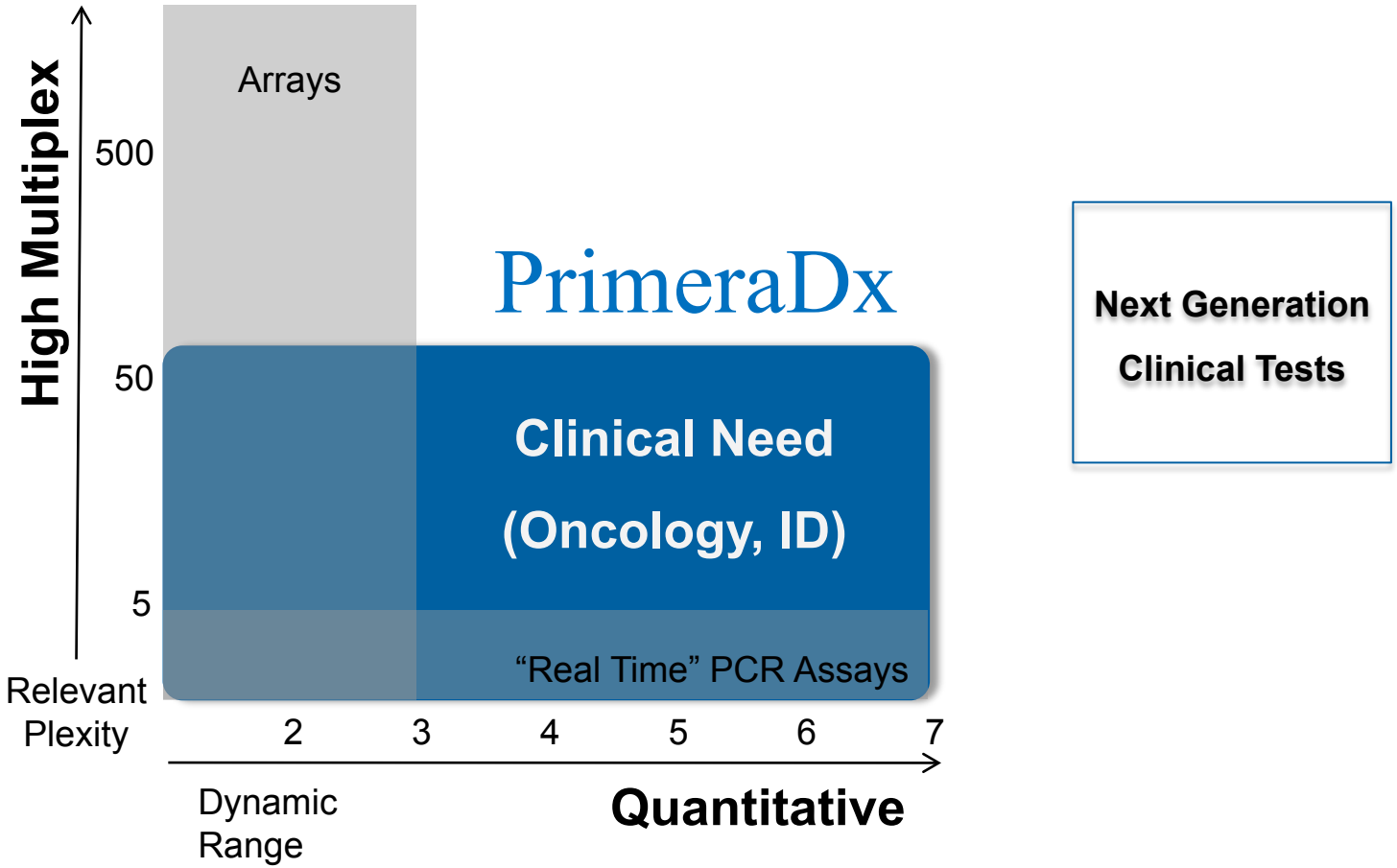
The ICEPlex System – Fully Automated, Real-Time, Multiplex qPCR

Walk-away Workflow with Automated Reporting of Assay Results

- **Dual lasers, multiple dye sets, dozens of targets per dye**
- **Assay dynamic range (and simultaneous detection) of 10 – 10,000,000 copies of multiple targets in a single sample**
- **Innovative software to track, analyze and report results**
- **Proven reliability – customer experience**
- **Manufactured under QSR, ISO and GMP standards**
- **Flexible software: User-definable assay conditions for LDT capabilities, company-developed assay design software speeds assay/product development**

There is No Comparable Technology Available

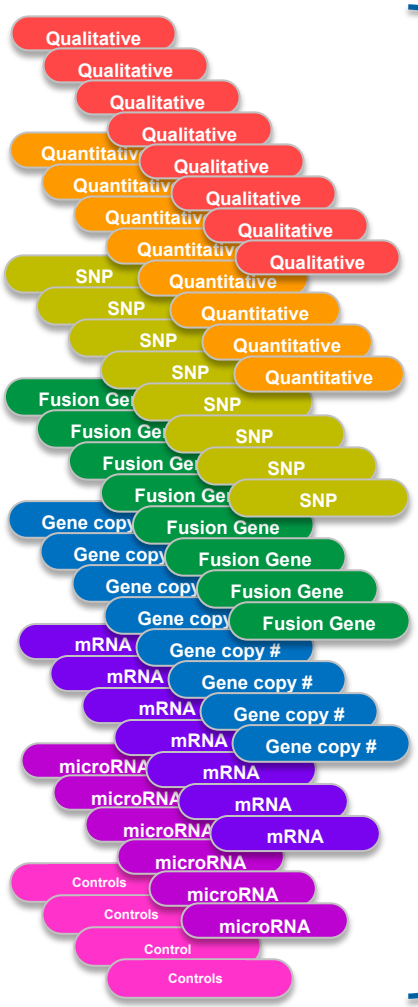
ICEPlex Enables Real-time Multi-modal, Multiplex, Quantitative Tests



Multi-Modal Testing is the Future of MDx – Breakthrough for Labs and Clinicians

Diagnostics Now Require Next Generation "All in one Well" Capabilities

- End-point Qualitative Detection
- Real-time Quantification
- SNP
- Fusion Gene
- Gene copy #
- mRNA
- microRNA
- Controls

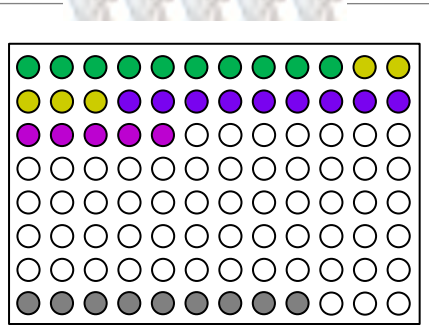
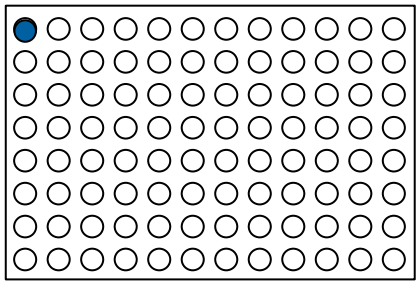


A real-time, multiplex, multi-modal "All in one well" MDx solution

Only PrimeraDx Can Do This

Unique, Critical Capability in the Molecular Dx Space

Many Tests, One Reaction/One Platform

		Current practice: many platforms, many techs	PrimeraDx ICEPlex
Oncology Multi-Modal Panel: Fusion gene variants + SNPs + Gene Expression Signature + MicroRNAs + Standards and controls	29 Targets plus 9 Standards and Controls		
	96 Patient Samples	**6 days**	**1 morning**

Regulatory Plan and Quality Systems Enable PDx in the Market

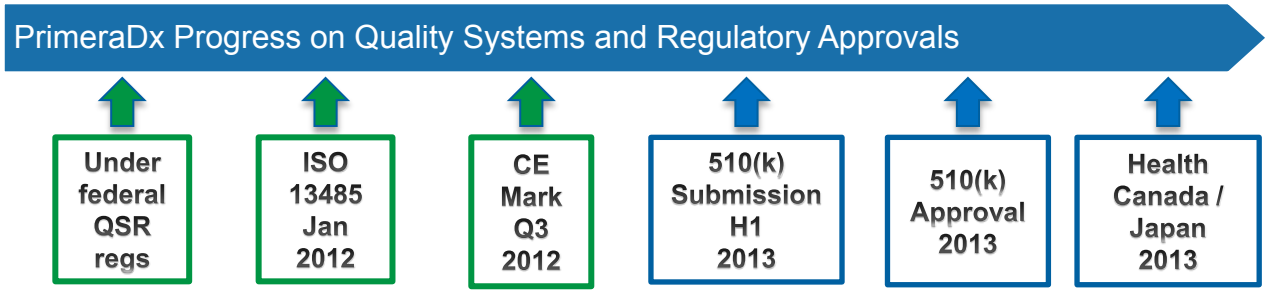
Market Needs Drive Approach

Achieving Regulatory Clearances

- IVD kit sales are a major portion of market
- CDx Partners need a Cleared Platform
  - Will be a major differentiator for PrimeraDx

Regulatory Milestones

- Operate under QSRs ✓
- ISO 13485 ✓
- CE marks ✓
- FDA *de novo* 510(k) 2013



PrimeraDx's Open Platform Product: Next Generation qPCR

Users Design Complex Multiplex Assays with Ease….

In Silico Multiplex Assay Design SW Tools Enable Customers

Automated Workflow Simplifies Testing Procedures

…..and PrimeraDx Provides the Tools Needed to Diagnose Disease

PrimeraDx Open Platform Products

Instrument

ICEPlex enables CLIA labs to run laboratory developed tests *and* PrimeraDx IVDs

Consumables

Four cartridge sizes
- 8 and 24 well
- 48 well
- 96 well

Universal Reagent Kit
Capillary Electrophoresis plates
All on-board consumables

Products

Open Platform Product

Instrument, software and consumables needed for CLIA labs to run LDTs

PrimeraDx Technology Has Very Broad Clinical IVD Utility

Proven Capabilities in All Major Diagnostic Areas….

Oncology
- ❑ Expression panels
- ❑ Copy number variation
- ❑ SNP panels
- ❑ Polysomy
- ❑ Insertions
- ❑ Deletions
- ❑ Fusion products (replaces FISH)
- ❑ Methylation
- ❑ Combinations of **any and all** of the above

More in Development

ID
- ❑ Quantitative, multi-pathogen detection
- ❑ Mixed pathogen panels
- ❑ Viral load
- ❑ Resistance detection
- ❑ Hospital acquired infections
- ❑ Multiple sample/swab types
- ❑ Panels that represent physician ordering patterns

More in Development

Others
- ❑ Food Safety Testing
- ❑ Pharma QC
- ❑ Drug Metab
- ❑ Genetic Dis

More in Development

Panels for demonstration purposes only. Not for clinical diagnostic use.

PrimeraDx Products Have Very Broad Clinical IVD Utility

Proven Capabilities in All Major Diagnostic Areas….

Oncology
- ❑ KRAS/NRAS/BRAF
- ❑ EGFR
- ❑ cMET/EGFR CNV with cMET Expression
- ❑ cMET Mutation Panel
- ❑ EML4-ALK
- ❑ Lymphoma
- ❑ BCR-ABL
- ❑ Methylation
- ❑ microRNA/mRNA/ gDNA Panel

More in Development

ID
- ❑ Fungal Panel (direct blood detection)
- ❑ Transplant Panel (multiple viral loads)
- ❑ *C. difficile*
- ❑ STI Panel (yeast, virus, bacteria, parasites)
- ❑ Ultra-Sensitive JCV/BKV (differentiation, viral loads)
- ❑ Respiratory Panel (pathogens including drug-resistant strains)

More in Development

Others
- ❑ Food Safety Testing
- ❑ Pharma QC
- ❑ Drug Metab
- ❑ Genetic Dis

More in Development

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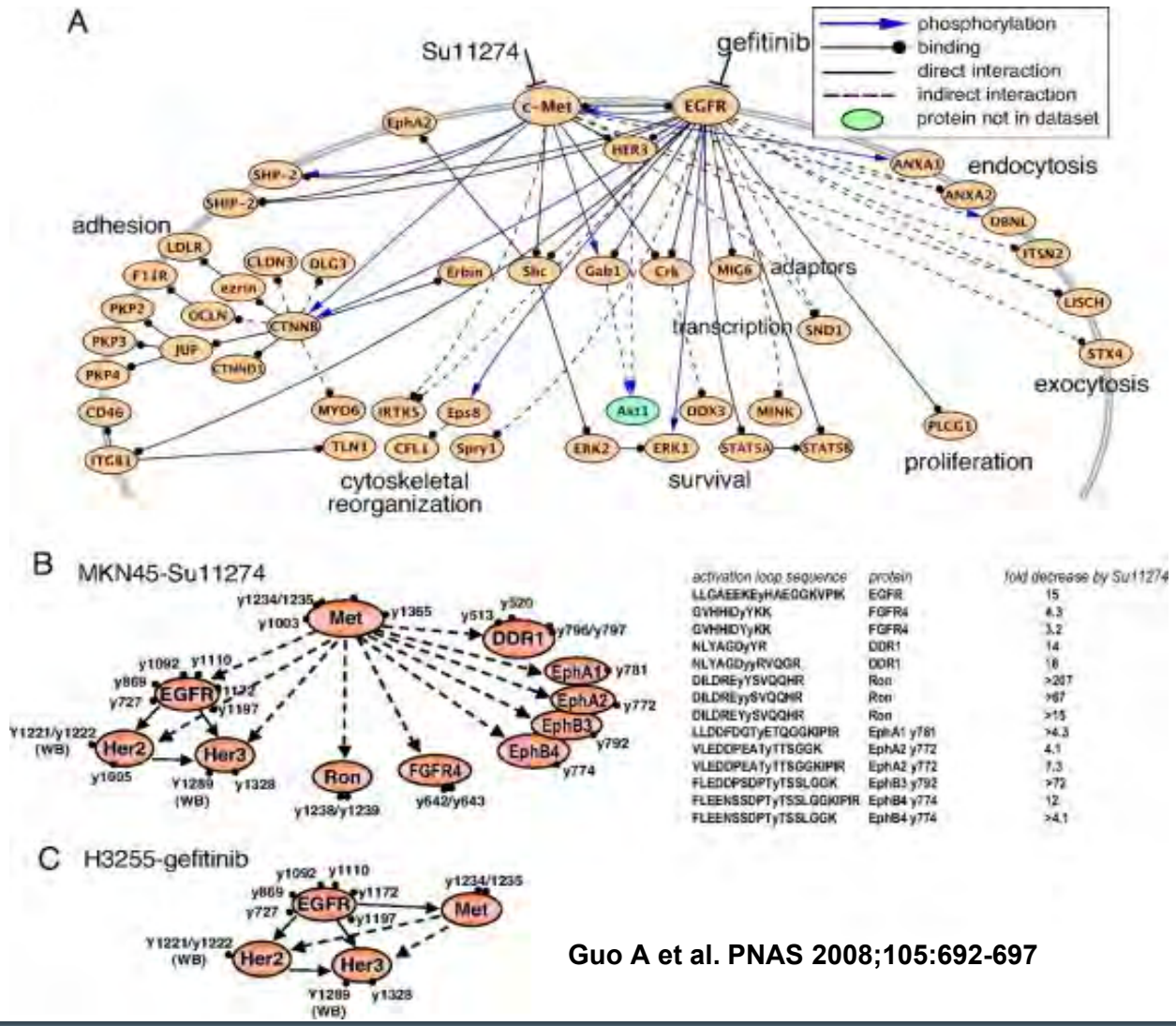
c-MET/EGFR Copy Number Variation and cMET Gene Expression Single-Tube Assay

c-MET and EGFR – Critical in Many Pathways, Targeted by Many Drugs



Guo A et al. PNAS 2008;105:692-697

Why c-MET???

cMET is a receptor tyrosine kinase that, after binding with its ligand, hepatocyte growth factor (HGF), activates many signaling pathways, driving proliferation, motility, migration and invasion.

Although c-MET is important in the control of tissue homeostasis under normal physiological conditions, it has also been found to be aberrantly activated in human cancers via mutation, amplification or protein overexpression. Dysregulation and constitutive activation of c-MET leads to cell proliferation, cell survival, angiogenesis, invasion and metastasis.

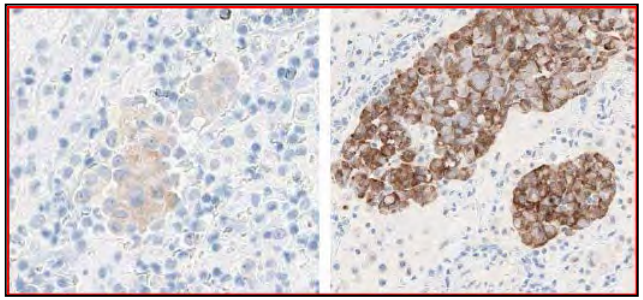
Overexpression/amplifcation of c-MET has been observed in various carcinomas, including gastric, NSCLC, colorectal, kidney tumors.

Amplification of c-MET has been identified as one mechanism to confer resistance to EGFR-specific tyrosine kinase inhibitors in lung cancers.

Challenges of Current Platforms & Multi-modality

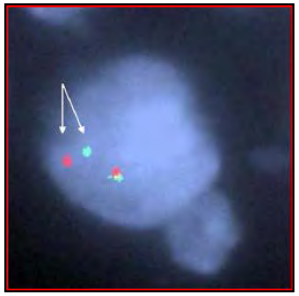
IHC: Protein Expression/phosphorylation

- Low sensitivity of antibodies
- strong background staining, weak target antigen staining and autofluorescence



FISH: Gene/Chromosomal CNV

- Inter-lab discordance 20%+
- 5-7 day turn-around time



Real-Time PCR: Gene Expression / SNP/CNV

- Multiplex limitations on most other PCR platforms

Many biomarkers in many complex pathways targeted by many drugs:

- Creates a need for single tube, multiplex and multi-modal tests

A "Killer App" For a High Unmet Need – Only PrimeraDx Can Do This

cMET & EGFR COPY NUMBER VARIATION

gDNA cMET, EGFR and Ref Gene #1 on Chromosome 7

gDNA Ref Gene #2

gDNA Ref Gene #3

Primers located in introns selective for gDNA

QUANTITATIVE cMET GENE EXPRESSION PROFILE

mRNA cMET and Ref Gene #1 on Chromosome 7

mRNA Ref Gene #2

mRNA Ref Gene #3

Primers spanning exon/exon junctions selective for mRNA

ONE TUBE ASSAY

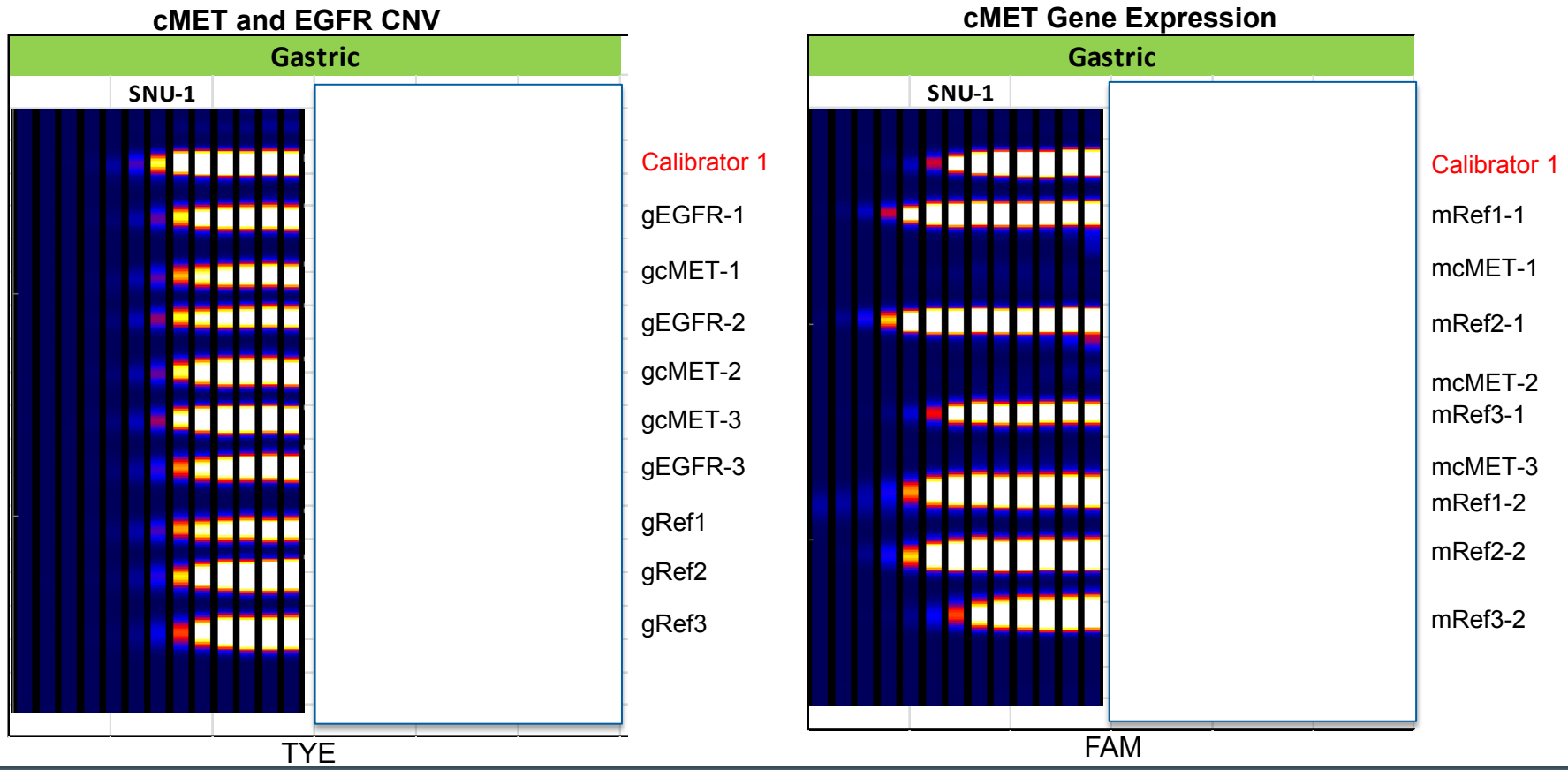
- ❑ Multi-modal
- ❑ Multiplex (18+6 plex)
- ❑ Quantitative
- ❑ FFPE
- ❑ Whole process controls

Detects and Measures

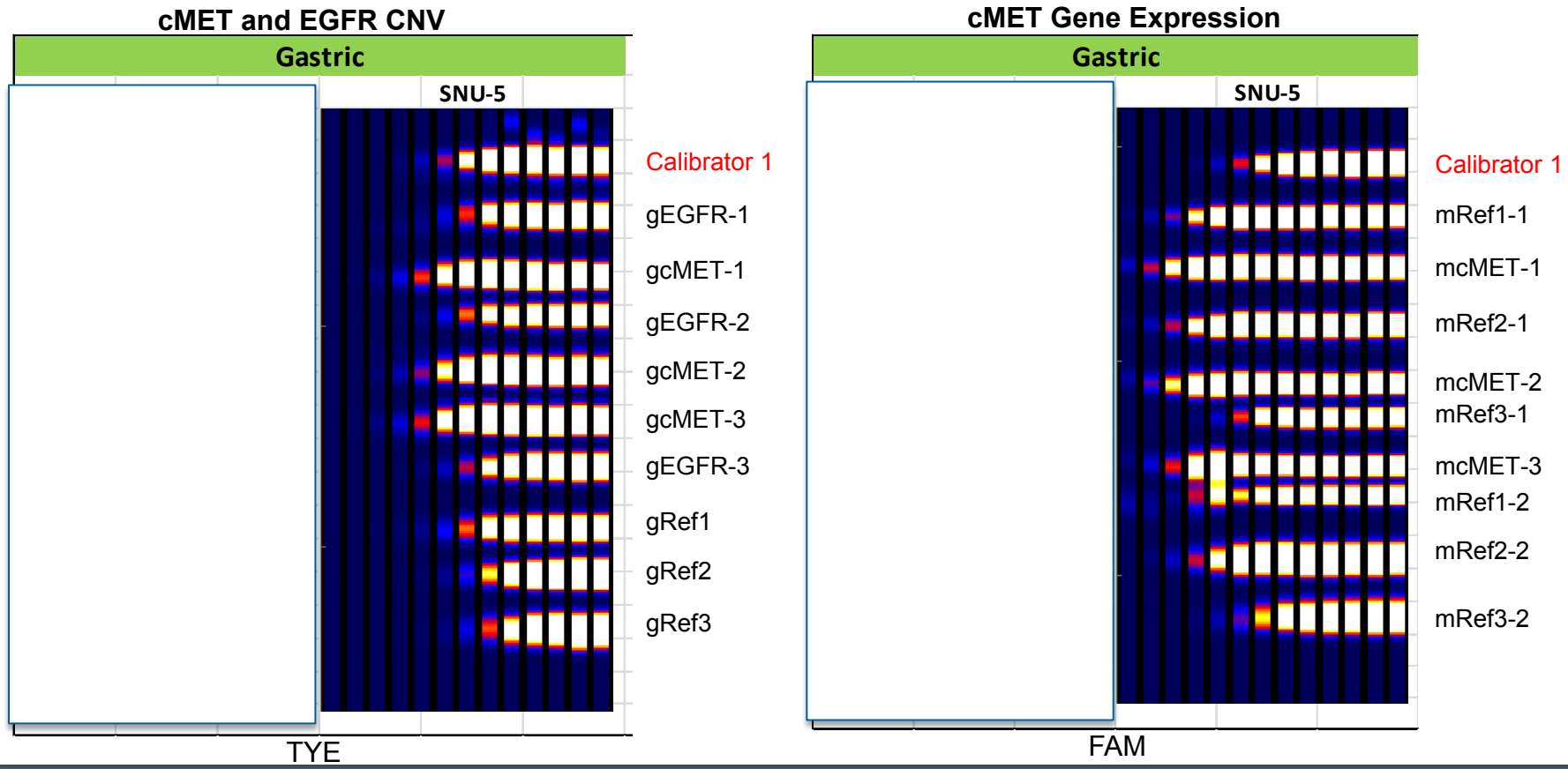
- ❑ CNV
- ❑ Gene Expression
- ❑ Polysomy
- ❑ Reference genes

Panels are designed for demonstration purposes only. Not for clinical diagnostic use.

Single Tube CNV and Gene Expression Analysis – Gastric

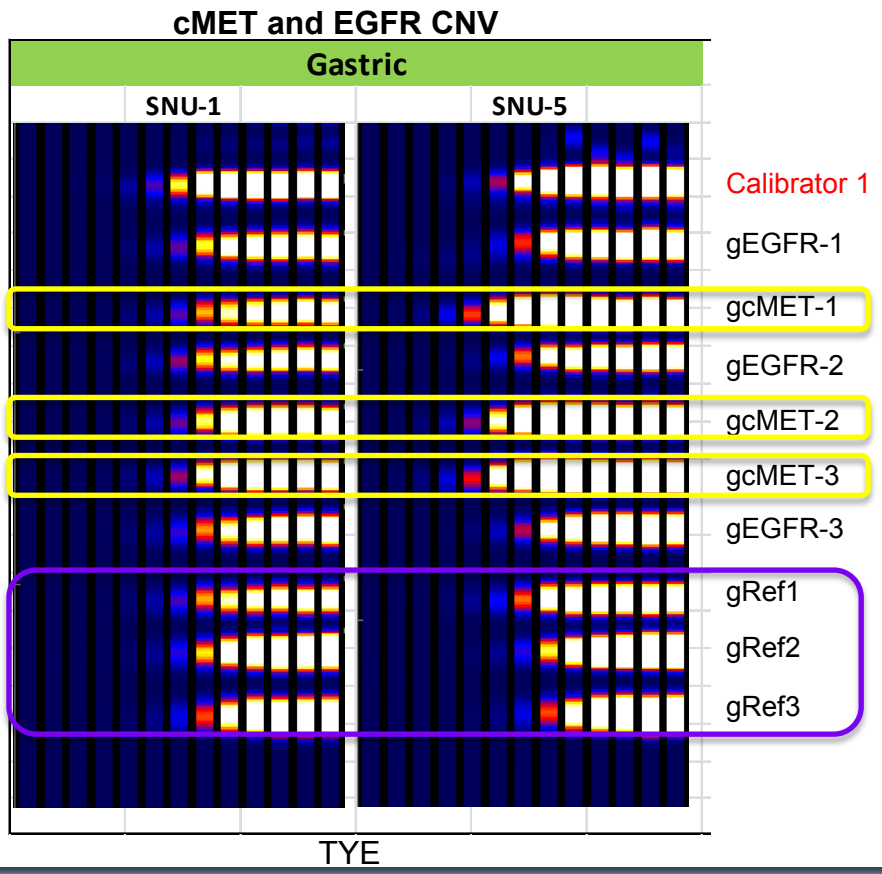


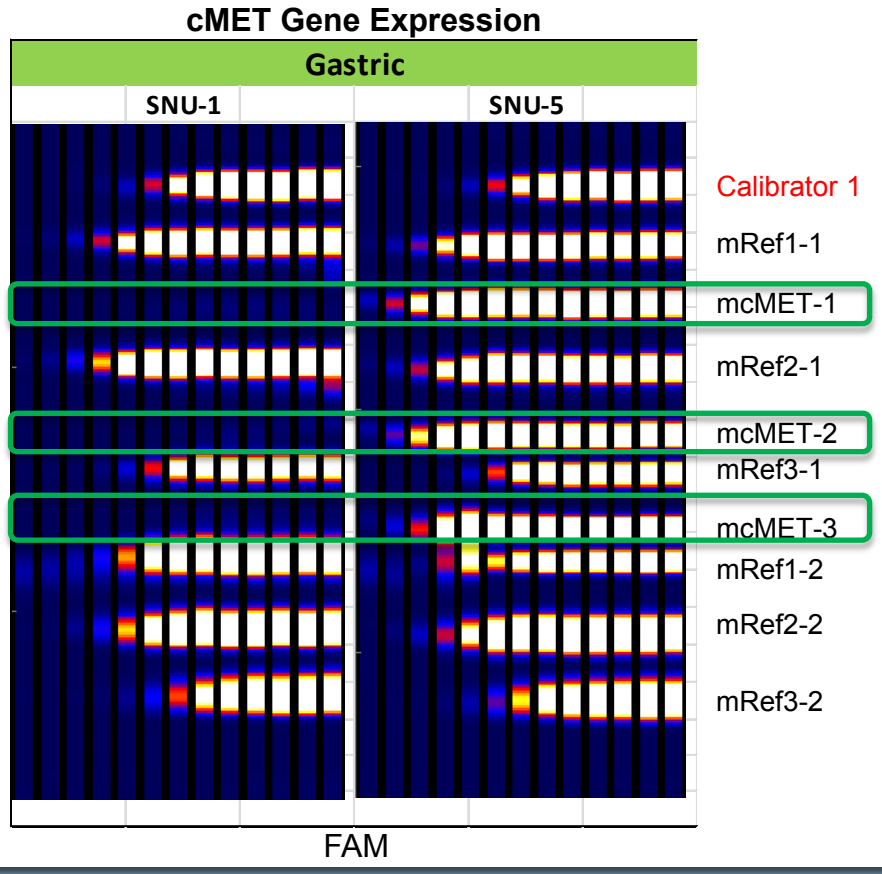
Single Tube CNV and Gene Expression Analysis – Gastric



Single Tube CNV and Gene Expression Analysis – Gastric

Sample	Tissue Source	cMET Copy #	cMET Expression	Ch 7 Polysomy
SNU-1	Gastric	2	No	No
SNU-5	Gastric	>10	High	Yes





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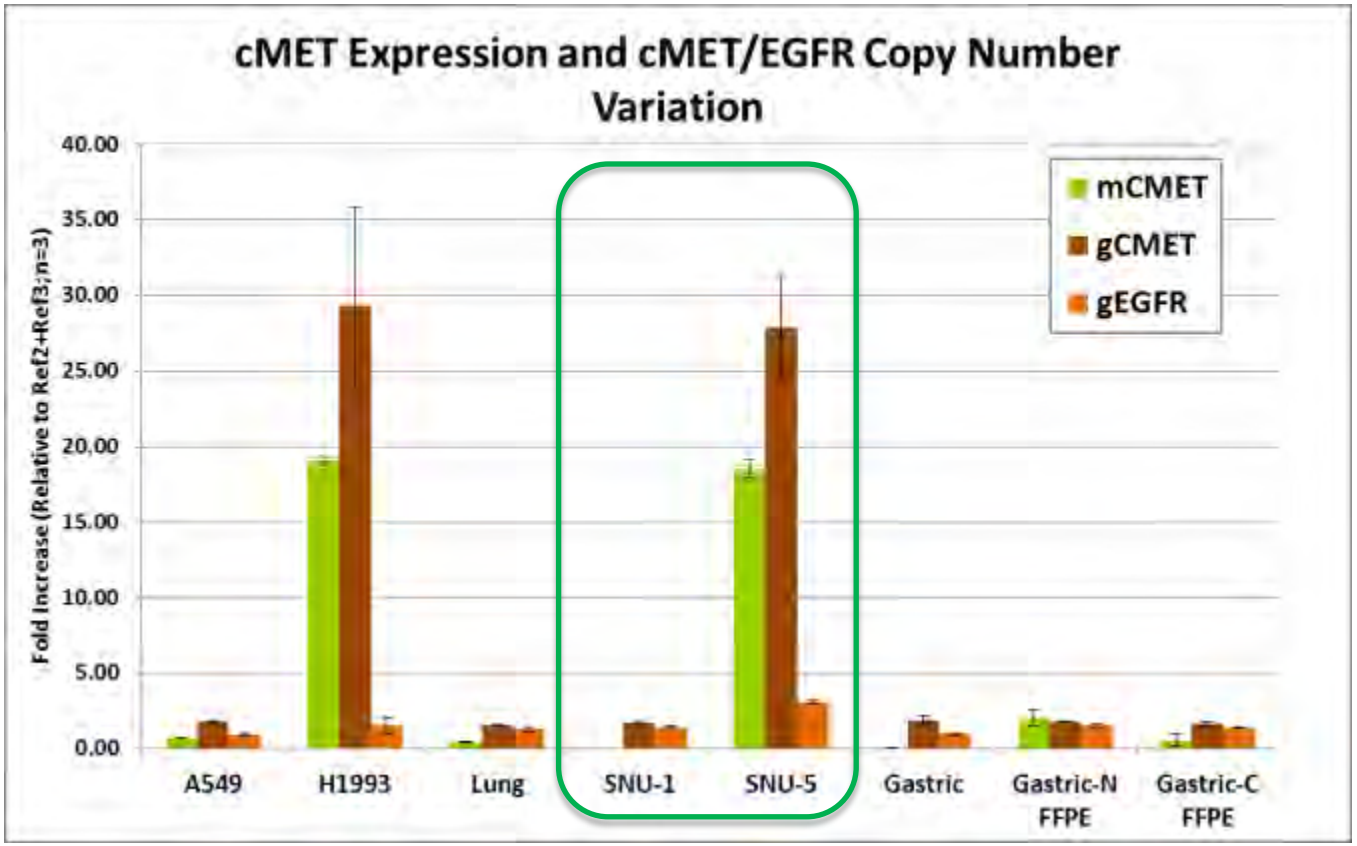
PrimeraDx

cMET Gene Expression and CNV Analysis



- Data were normalized to reference genes Ref2 and Ref3 and fold-change calculated
- No cMET amplification or expression in SNU-1
- SNU-5 shows significant cMET amplification and overexpression

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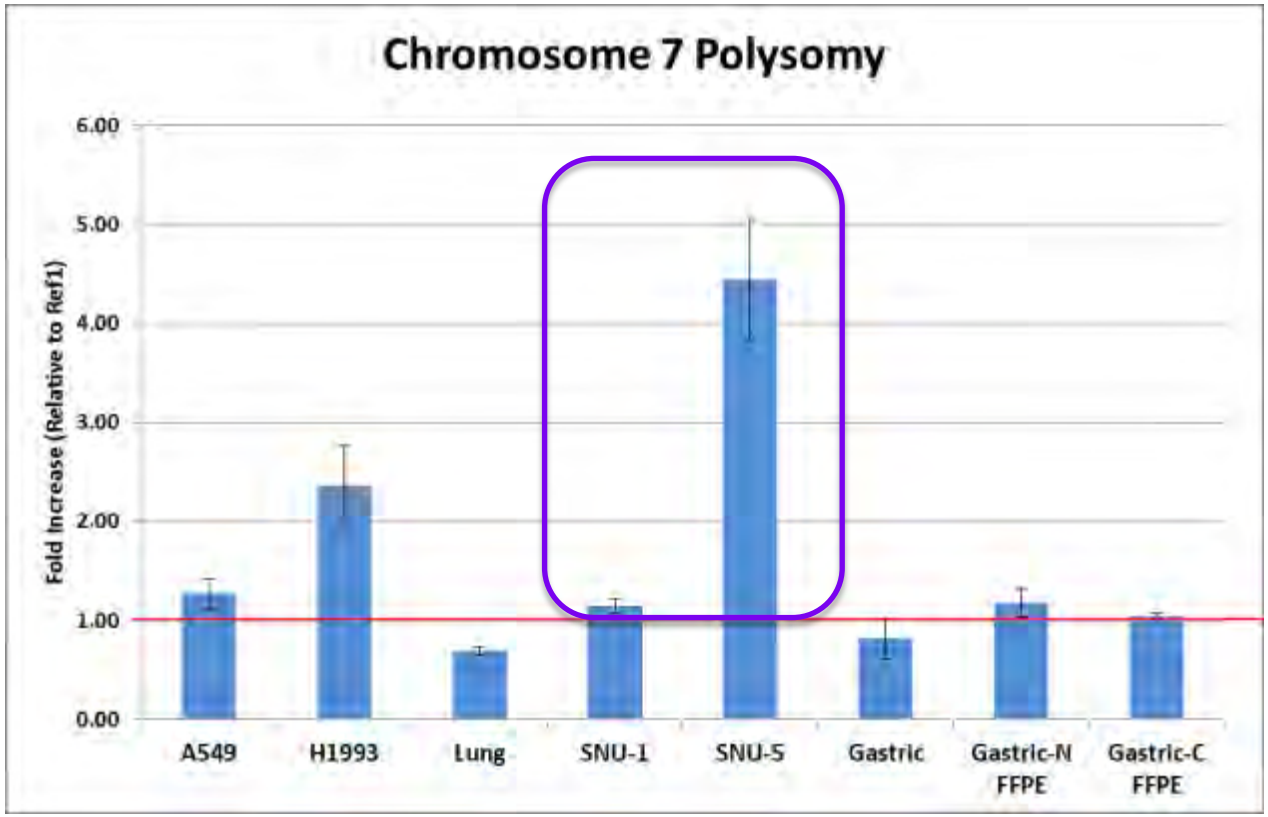
Chromosome 7 Polysomy Status



No Change

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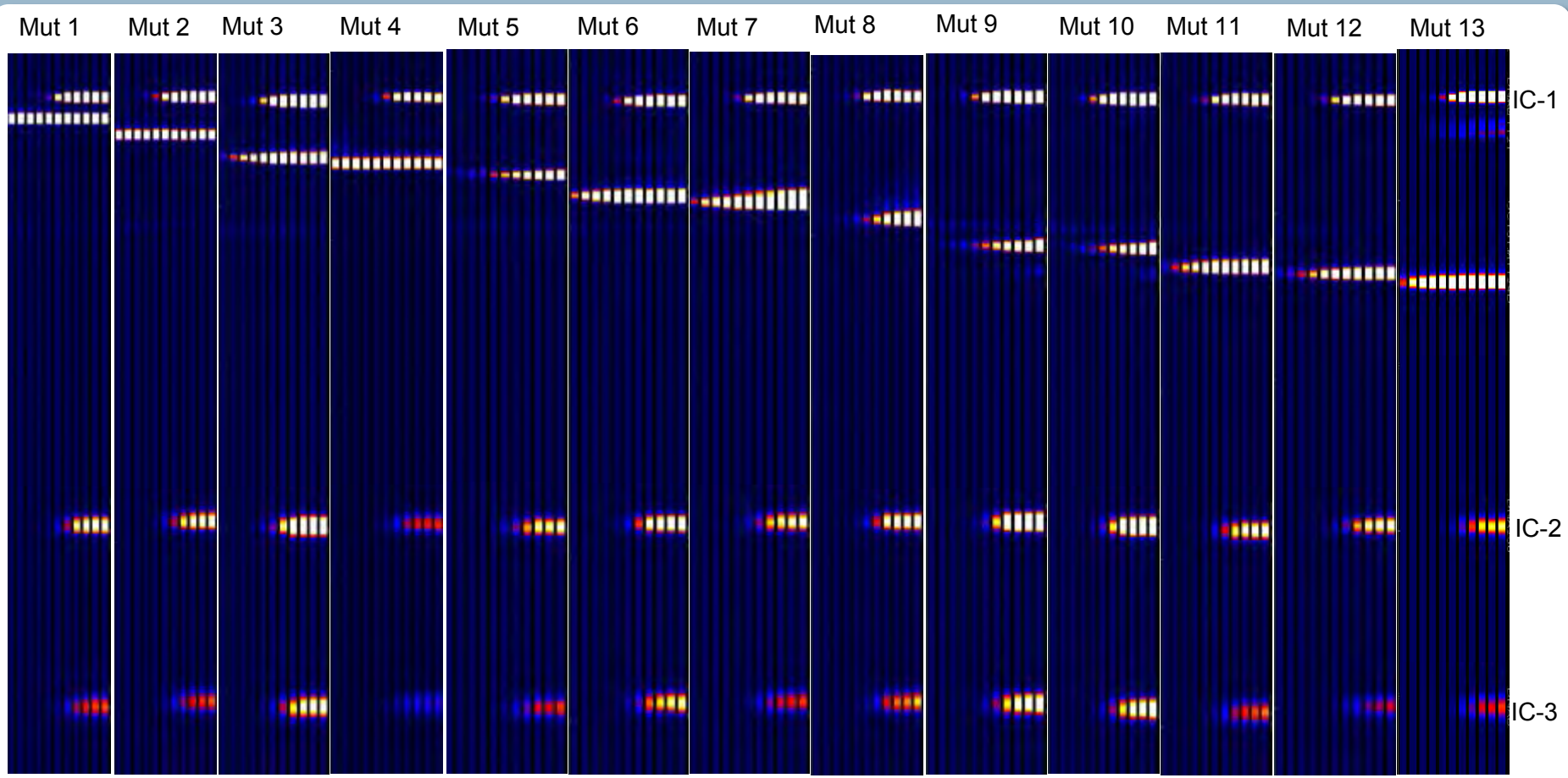
cMET 13-Target Single-tube Mutation Panel



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Summary

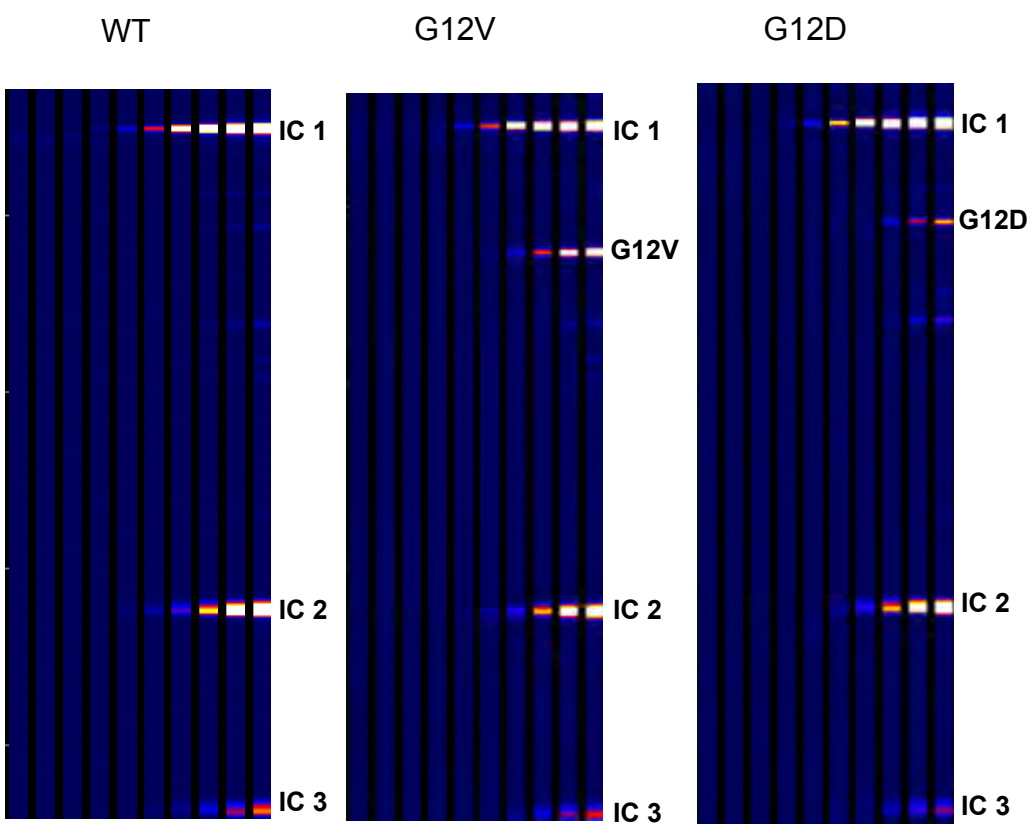
- An 18-target, 24-plex, single-tube multimodal assay designed to detected amplification of cMET and EGFR genes, expression of cMET and polysomy of chromosome 7, as well as a 13-target single-tube cMET mutation panel have been developed on the ICEPlex system
- Quality result: Built-in controls and redundancy assure quality and precision
- Quicker TAT: Impacts patient care
- All-in-one assay: Simplifies lab operation and improves lab economics
- Minimum nucleic acid input: Resolves specimen size issue
- Versatile sample types: FFPE blocks, Fresh-frozen tissues, Cell lines, Blood

PDx Makes "Liquid Biopsy" Possible – Massive Potential Market in Cancer Screening

KRAS/BRAF Panel: Detects DNA in Serum/Plasma, and Also Works in FFPE

One-tube KRAS / BRAF Panel

- KRAS G12R
- KRAS G12C
- KRAS G12S
- KRAS G12D
- KRAS G12A
- KRAS G12V
- KRAS G13R
- KRAS G13C
- KRAS G13S
- KRAS G13D
- KRAS G13A
- KRAS G13V
- BRAF V600E/D



Screening and disease recurrence monitoring require repeated testing

1% selectivity - highly sensitive serum/plasma assay

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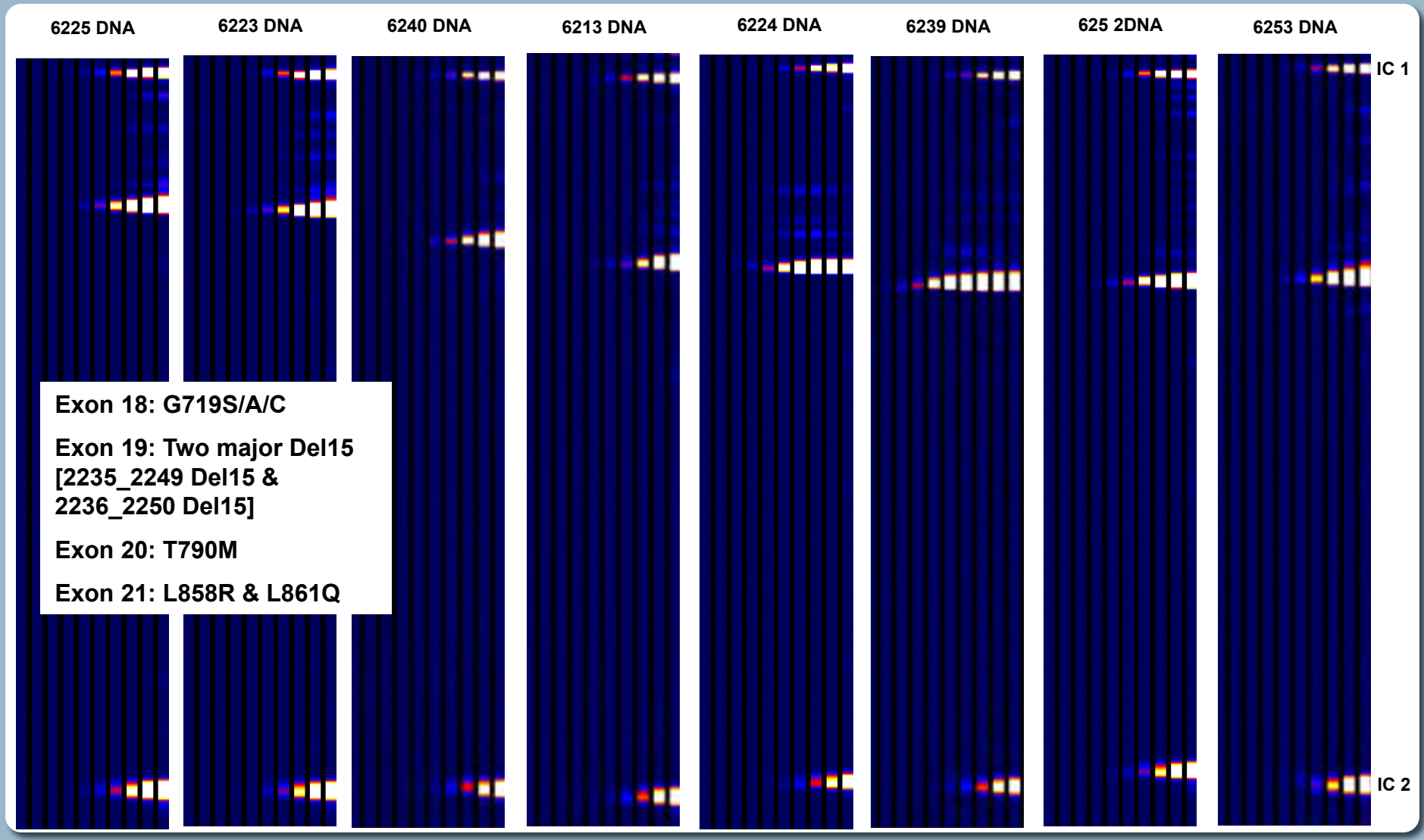
Multiplex Detection of EGFR Mutations in One Single Reaction



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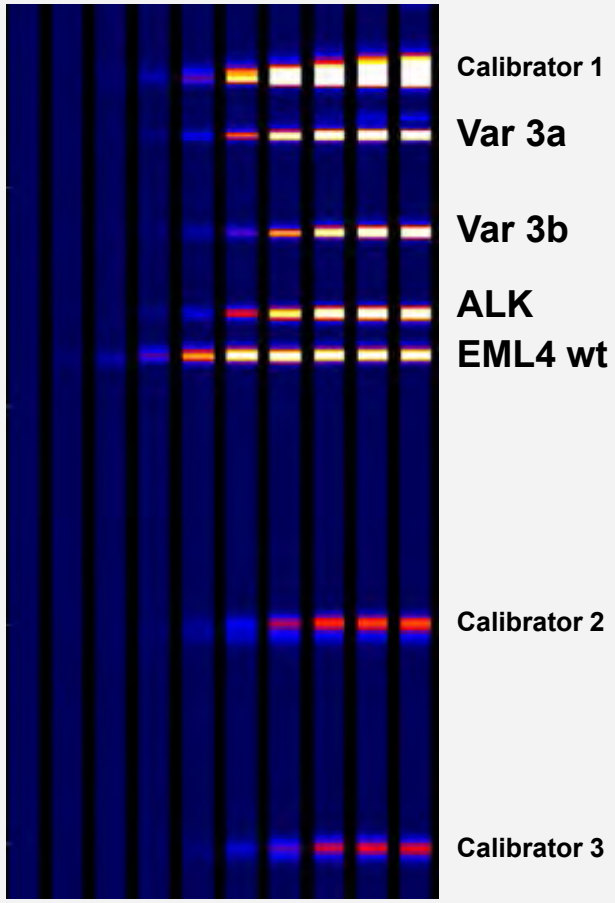
EML4-ALK: Fusion Gene Assay Detects Eight Fusion Variants

Fusion Detection Will Displace FISH in the Marketplace – Huge Potential



Target	Amplicon size
Var 1	161
Var 2	166
Var 3a	128
Var 3b	154
Var 4a	143
Var 4b	124
Var 5a	133
Var 5b	118
ALK	173
EML4 wt	185

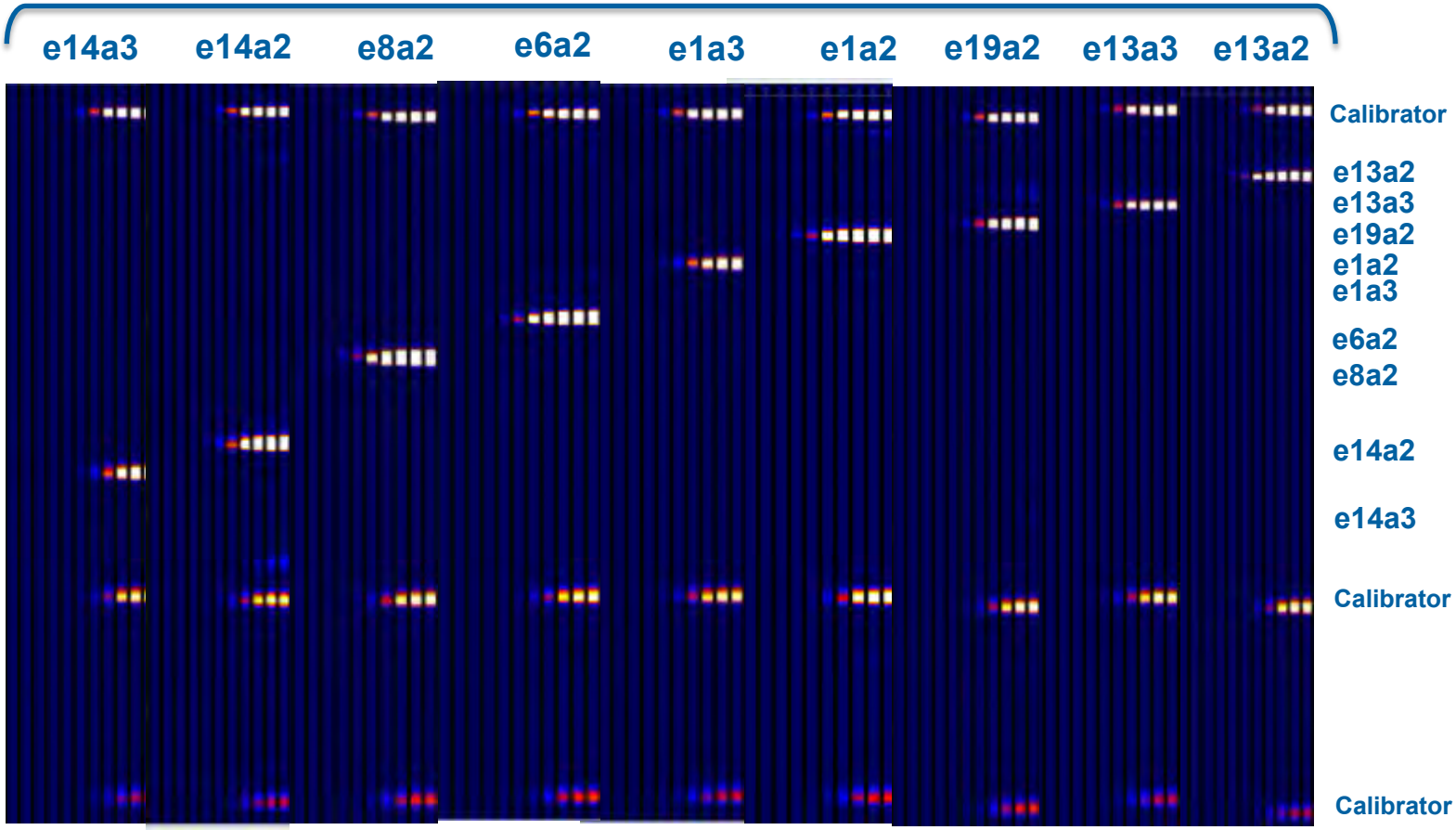
Fusion Gene Variant Detection on the ICEPlex is simple, and provides definitive answers in just a few hours.

Panels for demonstration purposes only. Not for clinical diagnostic use.

BCR-ABL Fusion Gene Assay Detects Nine Fusion Variants

PrimeraDx Panel Represents the Best Test Available

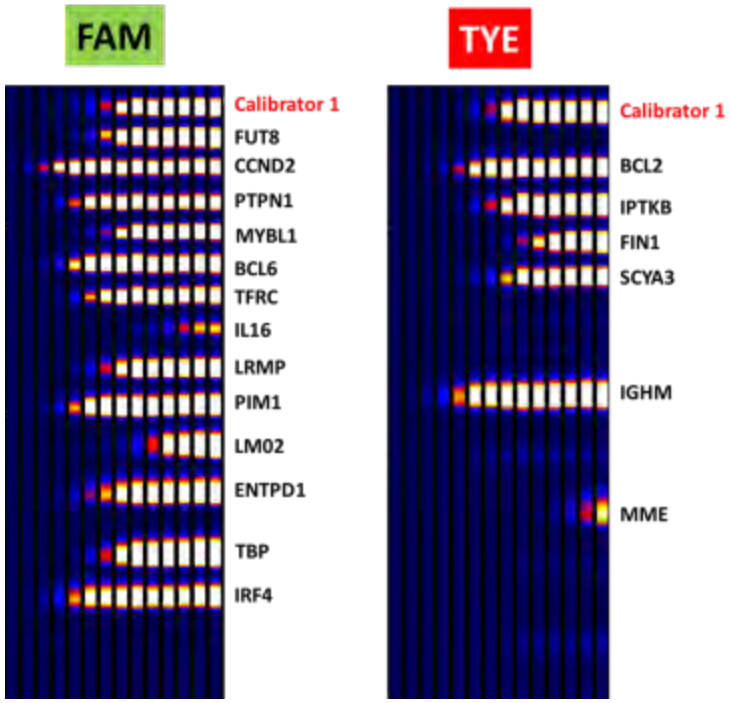
One-tube BCR-ABL SNP Panel

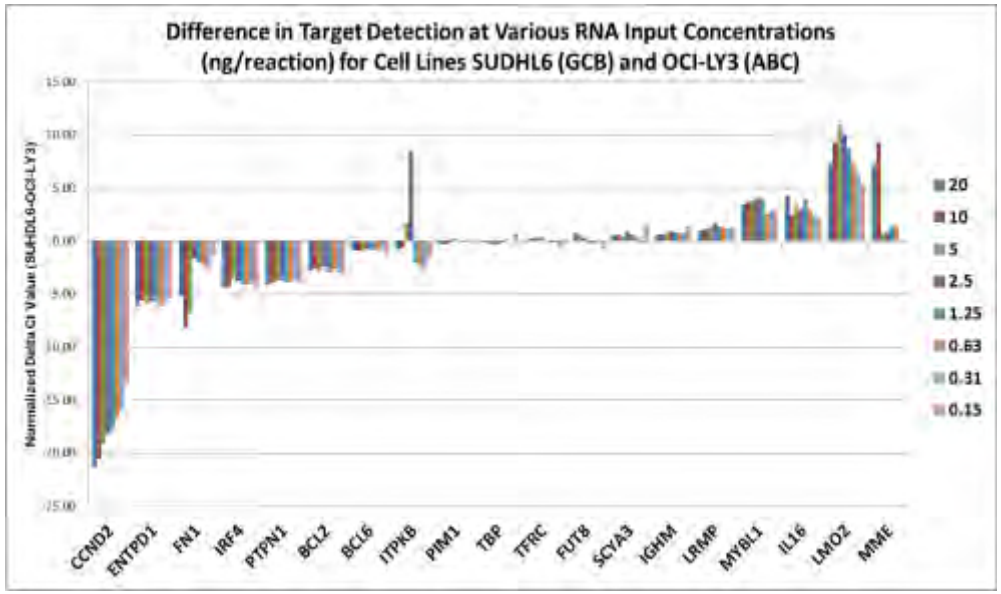


Individual plasmid target templates were detected by the single tube BCR-ABL Assay

Panels for demonstration purposes only. Not for clinical diagnostic use.

Lymphoma Gene Expression Panel Differentiates GCB from ABC





- A novel quantitative 19-plex mRNA expression profiling assay designed to allow DLBCL tumor classification on FFPE specimens in a single tube PCR reaction was developed on the ICEPlex system.
- The ICEPlex DLBCL assay allowed discrimination of ABC and GCB cell lines based on specific target expression patterns generated from < 1ng of RNA from FFPE-isolated material.

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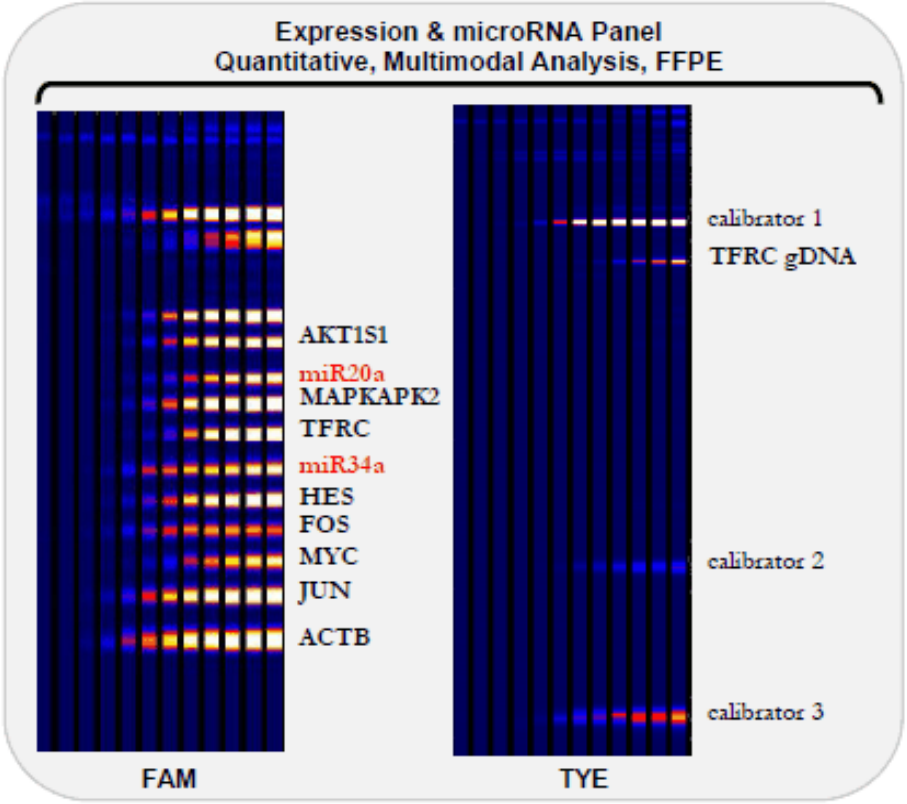
Simultaneous Quantification of mRNA and miRNA expression



Currently being investigated for complex molecular assays (multimodal multiplex qPCR) supporting drug development with expectation that taking a single assay through regulatory approval will be more feasible

Platform selection is important:
Robust assay performance
Ease of development
Regulatory path
Clinical laboratory accessibility
Meeting the need of the CDx effort

Courtesy of Eli Lilly

CDx Partnership for IVD Development

Multiyear, Multiproduct Deal with Eli Lilly; Others in Process

Features	• Assay design • Assay development • Under Design Control • Tissue-specific	• Final robustness testing • Transfer to manufacturing • cGMP production lots • IDE status • CE Mark	• Trial support to CLIA CROs • V&V studies • Concordance analyses • PMA prep & submission • XUS registrations	• Pre-launch conversion from CROs to clinical labs • Facilitate lab partner reimbursement • Support post-approval surveillance trial
Deliverables	IVD-track assay for use in CLIA or R&D setting	IVD-track IUO for use in registrational trial	• PMA submission • Global registrations	Global distribution

Partner funds product development, expands pipeline of “killer-apps.”

PrimeraDx retains all commercial rights.

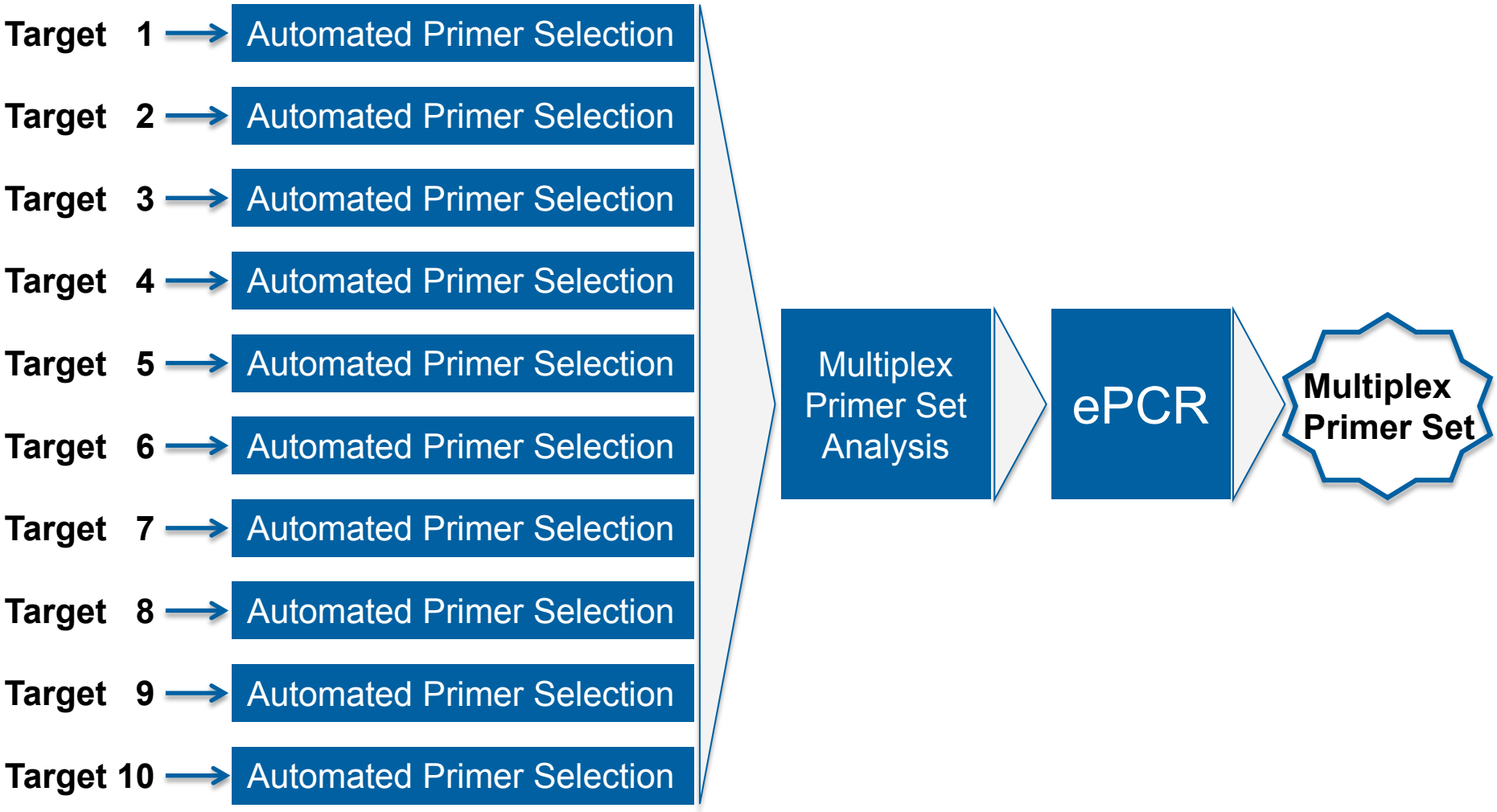
PrimeraDx – Providing Solutions for Today's Dx Needs

- The only **Fully Quantitative, High Multiplex** qPCR Platform
- A completely **Automated Workflow**
- Allows for **Multi-modal** assay configurations
- The ideal platform for **Companion Diagnostics**
- Numerous complex products in **Diverse Clinical Areas**

Primer Design Use Case: BCR-ABL Panel

ICEPlex system and assays have not been approved by the FDA for IVD. This information is for demonstration purpose only

Assay Design Workflow – Automated Wizard-like User Interface



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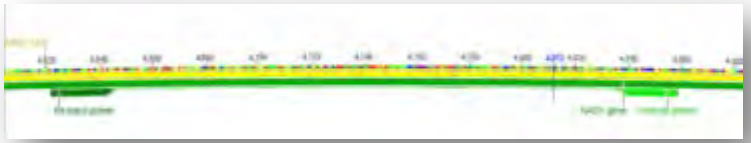
Primer Design for Target Region



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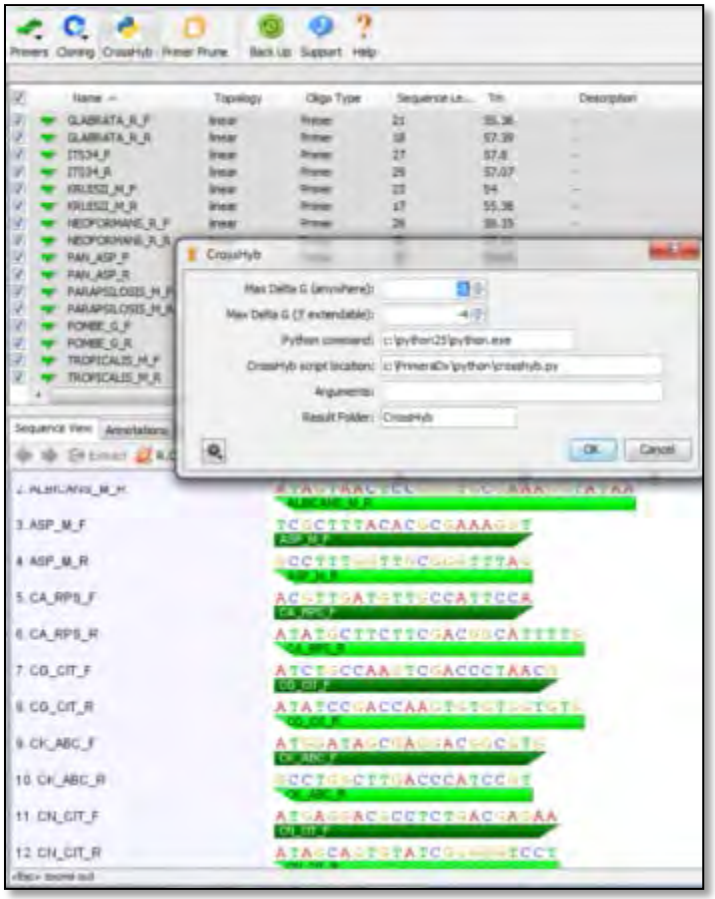
Automated Primer Filtering & ePCR

In silico prediction of off-target amplifications



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The Finalized BCR-ABL Panel – RT Steps

Simple and Painless High Multiplex Assay Design

RT Reaction

	Stock	[Final]	per run
5x RT Buffer	5x	1x	4
dNTP Mix	10mM	0.5mM	1
DTT	100mM	5mM	1
RT Primer Mix	20x	1x	1
SuperScript III	200U/ul	20U/ul	0.1
Template			10
H2O			2.9
	Total	Total	20

RT Primer mix

	uM in 20x mix	Sequence
ABLa2 RT5	1	TCCAACGAGCGGCTT
ABLa3 RT2	1	TTTGGTTTGGGCTTCAC
ABLwt RT	1	TCTCGGAGGAGACGT

Cycling Protocol

RT	**50C**	**30min**
RT Inactivation	**90C**	**5 min**
Cool-down	**4C**	**5 min**

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The Finalized BCR-ABL Panel – PCR Steps

Simple and Painless High Multiplex Assay Design

PCR Reaction			
	Stock	[Final]	
FastStart buffer	10x	1x	5
MgCl2	25mM	0.5 mM	1
dNTPs	10mM	0.3 mM	1.5
FastStartTaq	5 U/ul	5 U	1
Calibrators mix	25x	0.3x	0.6
Primer mix	30x	1x	2
Template (cDNA)			14
H2O			24.9
		Total	50

PCR Protocol			
Intial Denaturation	1x	96C	600 sec
Pre-CE Cycling	17x	64C	40 sec
		72C	40 sec
		96C	10 sec
CE Cycling	24x	64C	40 sec
		72C	190 sec
		96C	10 sec
CE Sampling	11x	11 separations	
		18, 20...38	
Separation Time		350 sec	
Total Run Time		3hrs 29min	

PCR Primer	uM in 25x n	Sequence
e13	5	GCTGACCAACTCGTGTGTGAAACTCC
e19	5	TCATGGAGGAGGTGGGCATCTACC
e6	5	ATATTGCTGTCAGGCCAATGCTCAGTTTGC
e8	5	CCACCCTGACCACCCCTTGCTG
e1	5	TGTCCGAGGCCACCATCGTG
ABLwt	3.75	ACATCACGCCAGTCAACAGTCTG
ABLa2	7.5	/56-FAM/ACCAACGAGCGGCTTCACTCAGA
ABLa3	2.5	/56-FAM/ATAAAAATTAATTTTGGTTTGGGCTTCACACCATTCC
ABLwt	3.75	/56-FAM/TCTCGGAGGAGACGTAGAGCTTG

ICEPlex system and assays have not been approved by the FDA for IVD. This information is for demonstration purpose only

The Finalized BCR-ABL Panel – Corresponding Sizes for Each Targets

Simple and Painless High Multiplex Assay Design

Target	Amp Size	Forward		Reverse	
e13a2	128	e13	GCTGACCAACTCGTGTGTGAAACTCC	ABLa2	/56-FAM/ACCAACGAGCGGCTTCACTCAGA
e13a3	136	e13	GCTGACCAACTCGTGTGTGAAACTCC	ABLa3	/56-FAM/ATAAAAATTAATTTTGGTTTGGGCTTCACACCATTCC
e19a2	140	e19	TCATGGAGGAGGTGGGCATCTACC	ABLa2	/56-FAM/ACCAACGAGCGGCTTCACTCAGA
e6a2	168	e6	ATATTGCTGTCAGGCCAATGCTCAGTTTGC	ABLa2	/56-FAM/ACCAACGAGCGGCTTCACTCAGA
e8a2	179	e8	CCACCCTGACCACCCCTTGCTG	ABLa2	/56-FAM/ACCAACGAGCGGCTTCACTCAGA
e14a2	204	e13	GCTGACCAACTCGTGTGTGAAACTCC	ABLa2	/56-FAM/ACCAACGAGCGGCTTCACTCAGA
e14a3	212	e13	GCTGACCAACTCGTGTGTGAAACTCC	ABLa3	/56-FAM/ATAAAAATTAATTTTGGTTTGGGCTTCACACCATTCC
e1a2	144	e1	TGTCCGAGGCCACCATCGTG	ABLa2	/56-FAM/ACCAACGAGCGGCTTCACTCAGA
e1a3	153	e1	TGTCCGAGGCCACCATCGTG	ABLa3	/56-FAM/ATAAAAATTAATTTTGGTTTGGGCTTCACACCATTCC
ABLwt	223	ABLwt	ACATCACGCCAGTCAACAGTCTG	ABLwt	/56-FAM/TCTCGGAGGAGACGTAGAGCTTG

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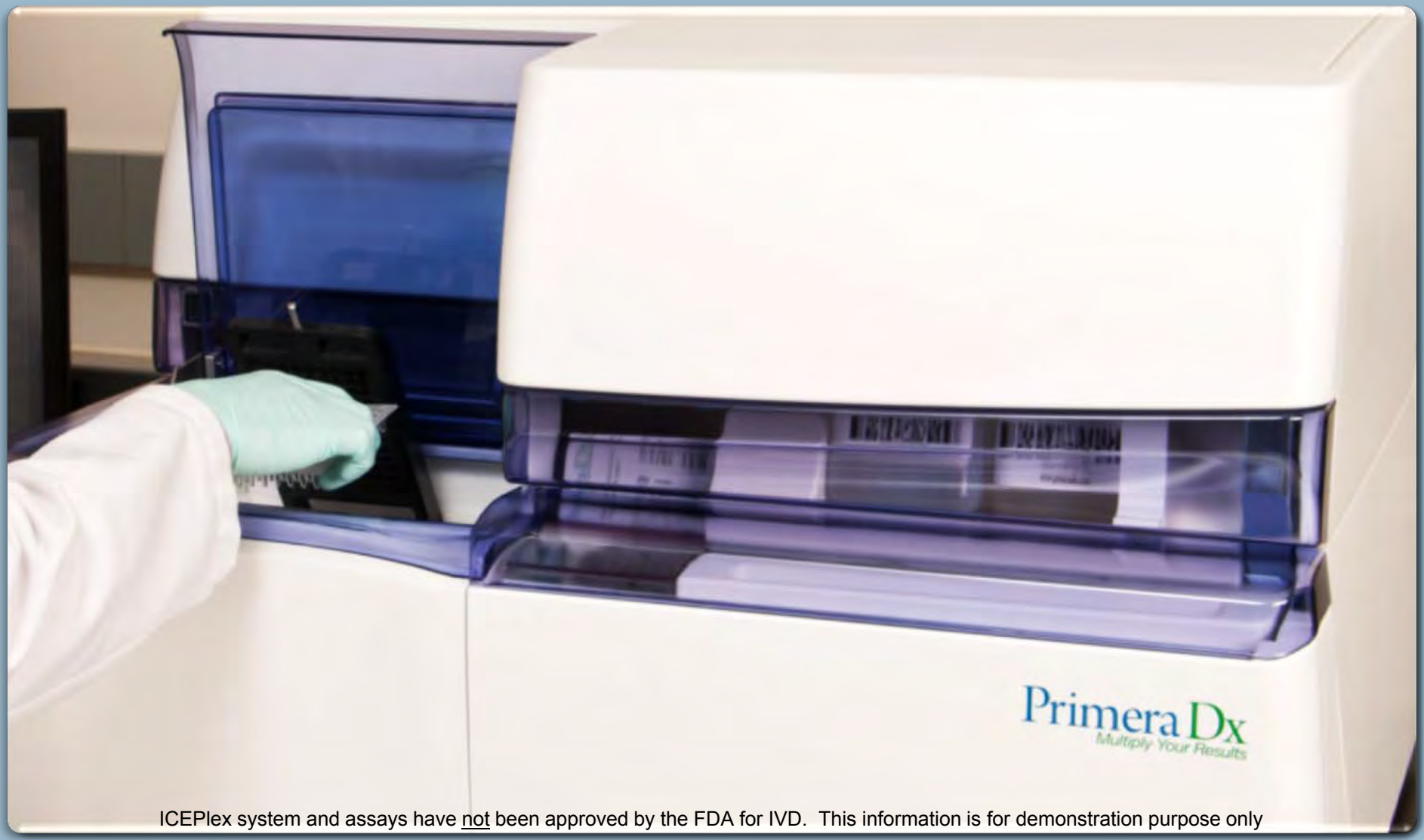
Insert the PCR Plate into the Thermal Block



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Insert the PCR Plate into the Thermal Block

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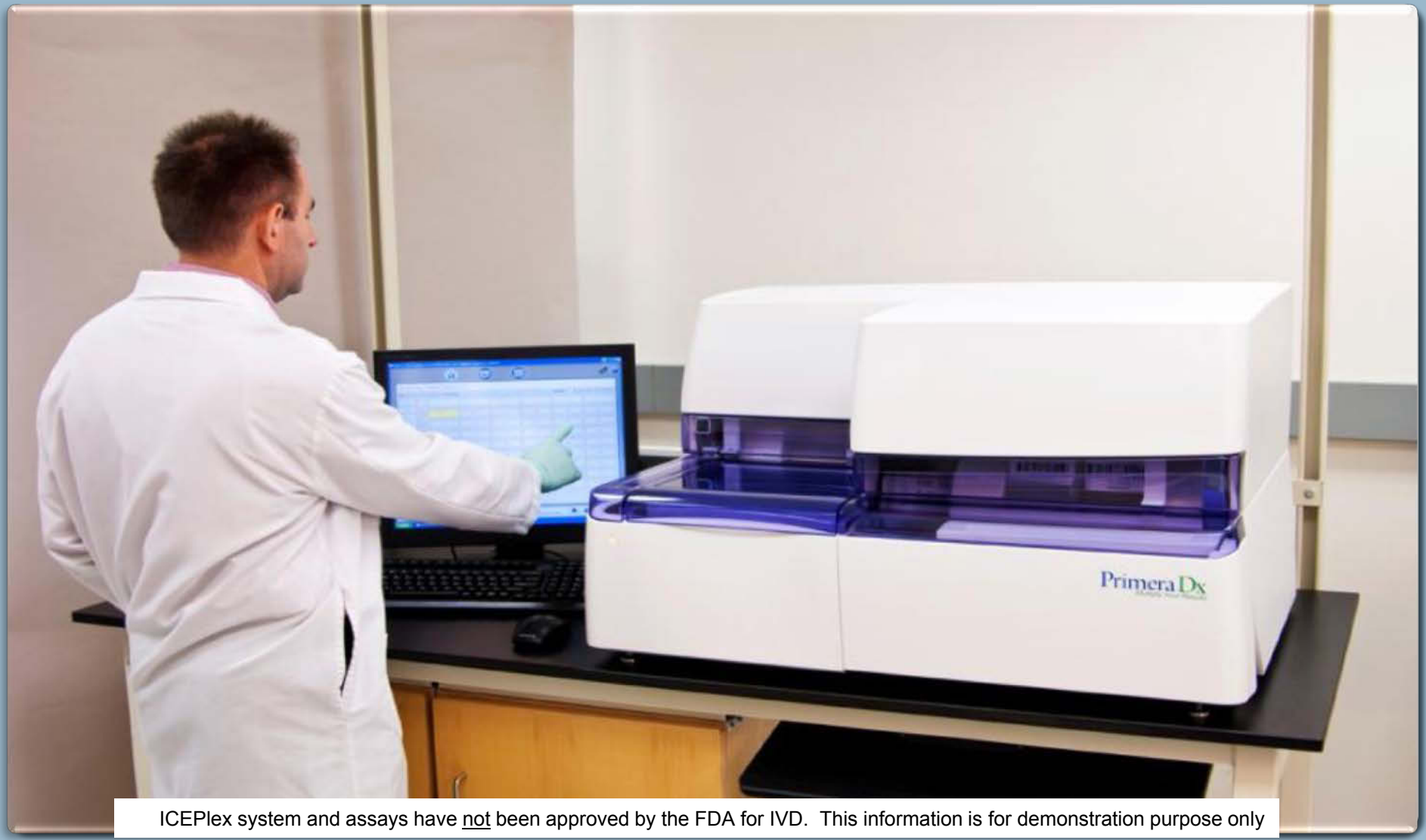
Input the Platemap and Run Protocol



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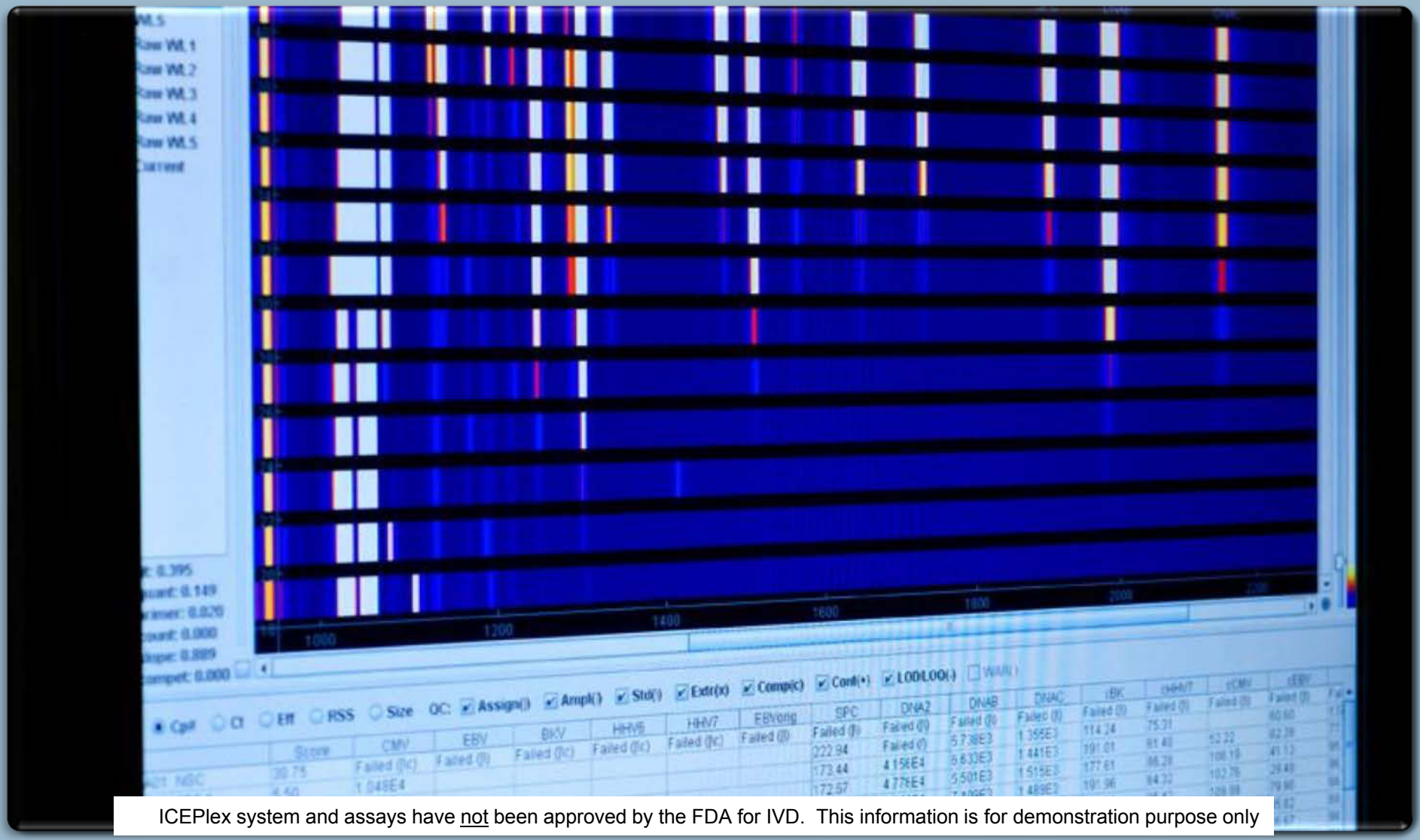
Results!



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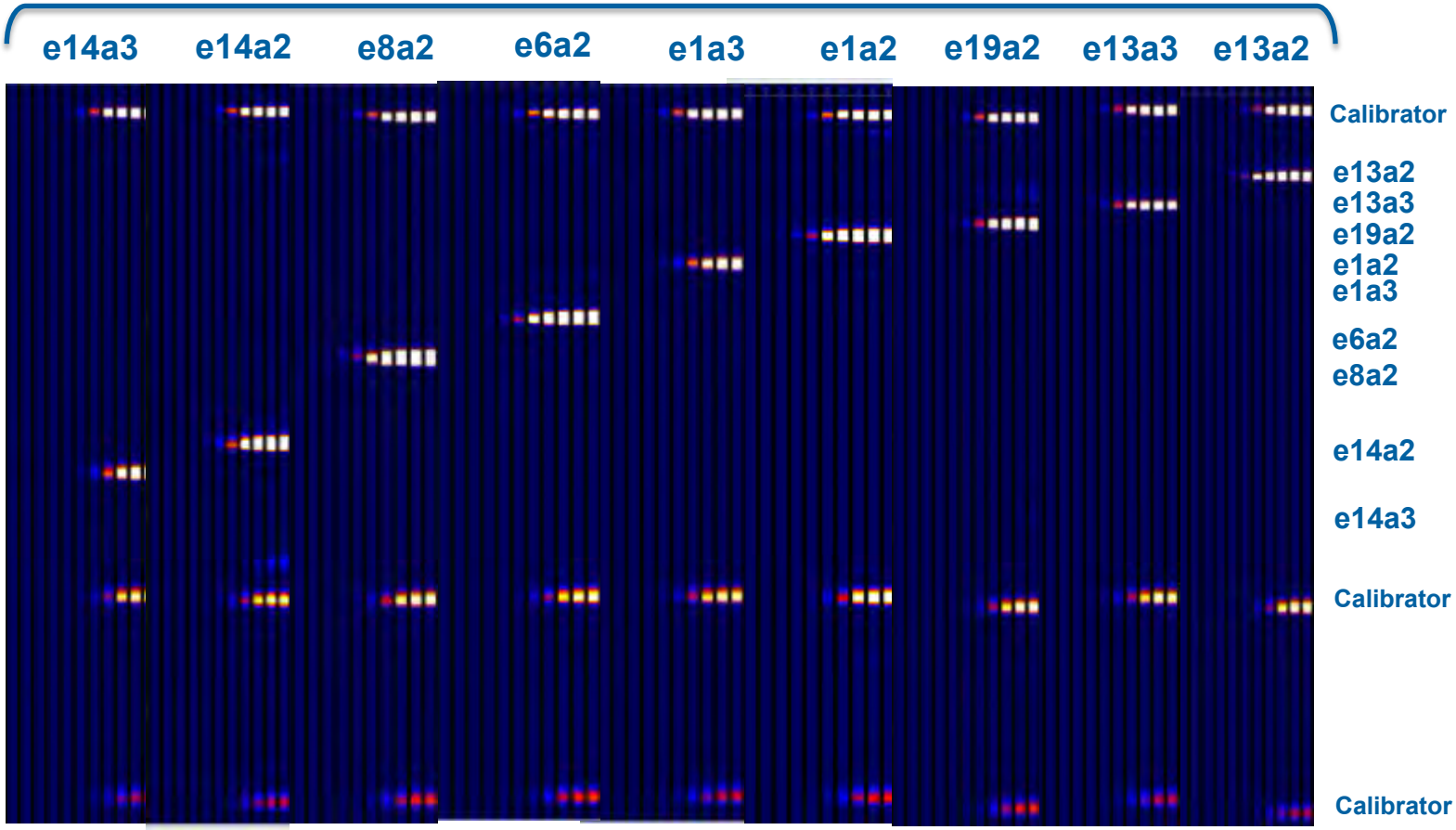
BCR-ABL Fusion Gene Assay Detects Nine Fusion Variants

PrimeraDx Panel Represents the Best Test Available

One-tube BCR-ABL SNP Panel



Individual plasmid target templates were detected by the single tube BCR-ABL Assay

Panels for demonstration purposes only. Not for clinical diagnostic use.

PrimeraDx

The Multiplex PCR Company