

## Bringing Personalized Medicine to the Clinic



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

**WDD-2013, Nanjing, China**

Lilly Kong, DVM, CSO

**PrimeradX**

April 25, 2013

# 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

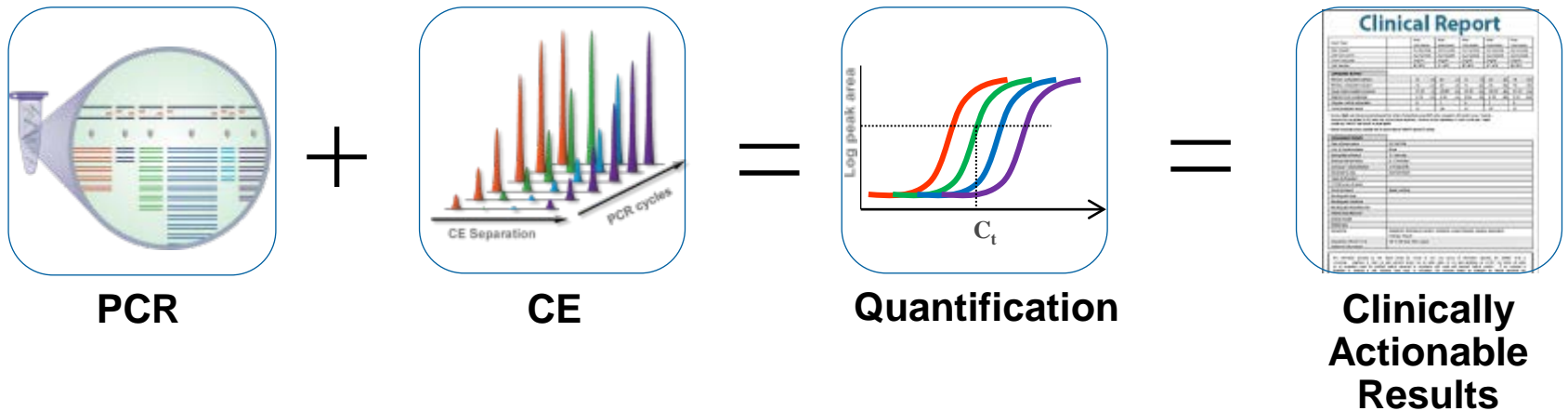
## The Marriage of PCR and Capillary Electrophoresis

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, multimodal, quantitative results provide unique, clinically actionable data



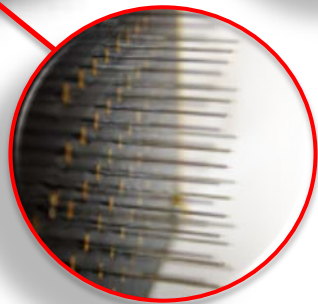
# The ICEPlex System – Fully Automated, Real-Time, Multiplex qPCR

## Walk-away Workflow with Automated Reporting of Assay Results

Thermal  
Cycler



On-board Reagents

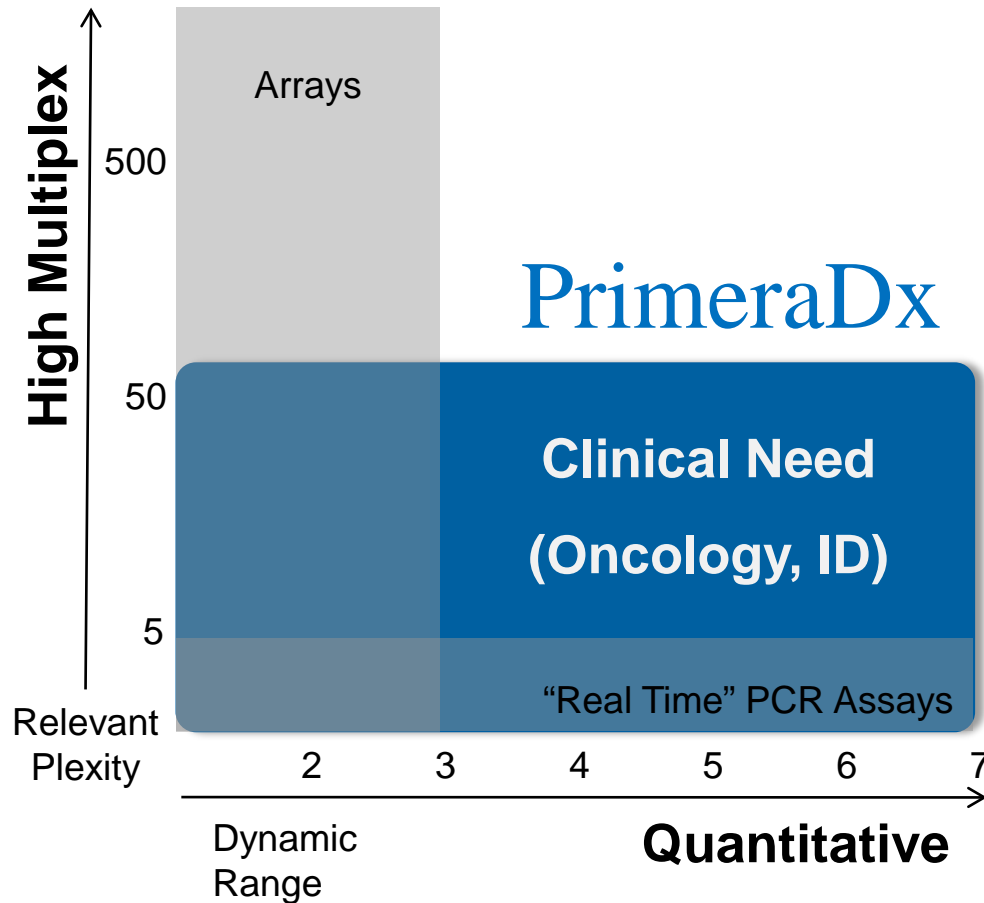


Capillary  
Cartridge

- 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

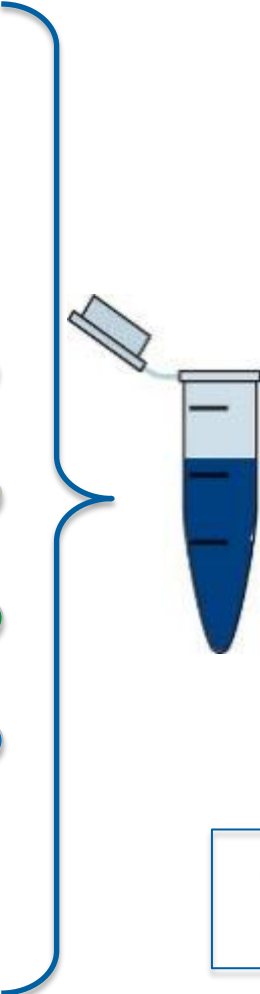
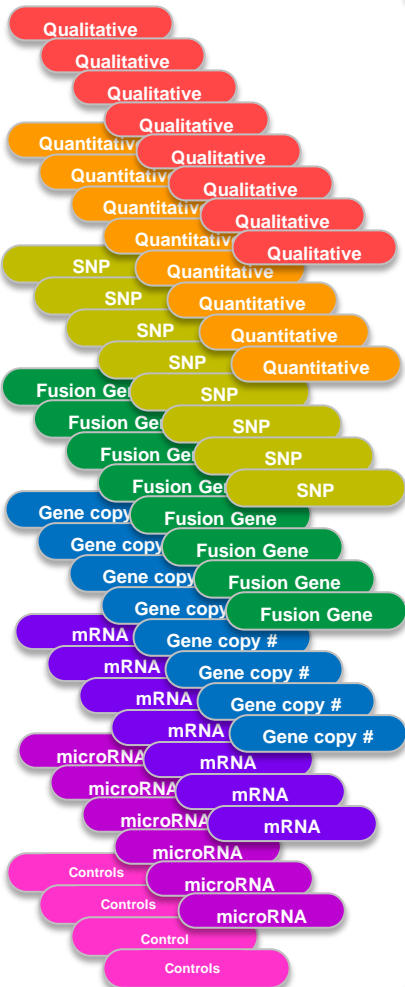


**Next Generation  
Clinical Tests**

# 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

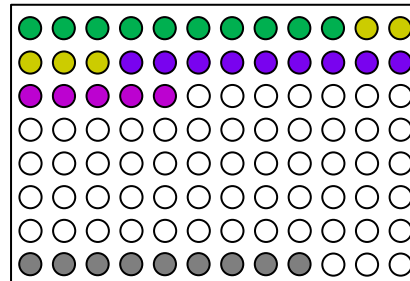
+

MicroRNAs

+

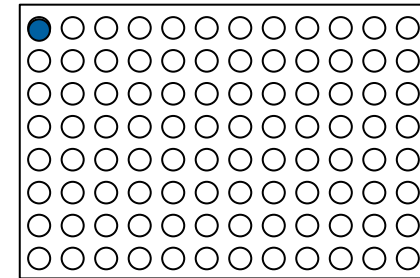
Standards and controls

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

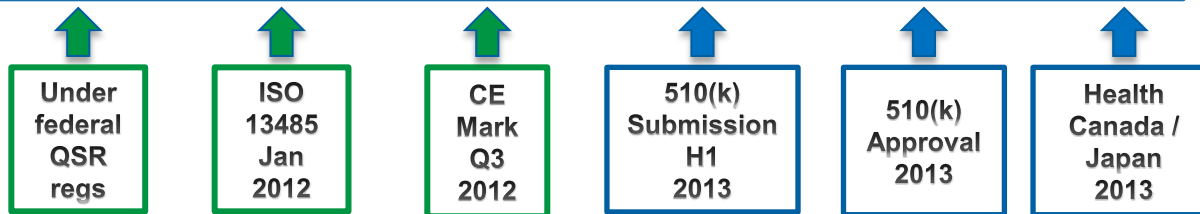
- IVD kit sales are a major portion of market
- CDx Partners need a Cleared Platform
  - Will be a major differentiator for PrimeraDx



## Regulatory Milestones

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

### PrimeraDx Progress on Quality Systems and Regulatory Approvals

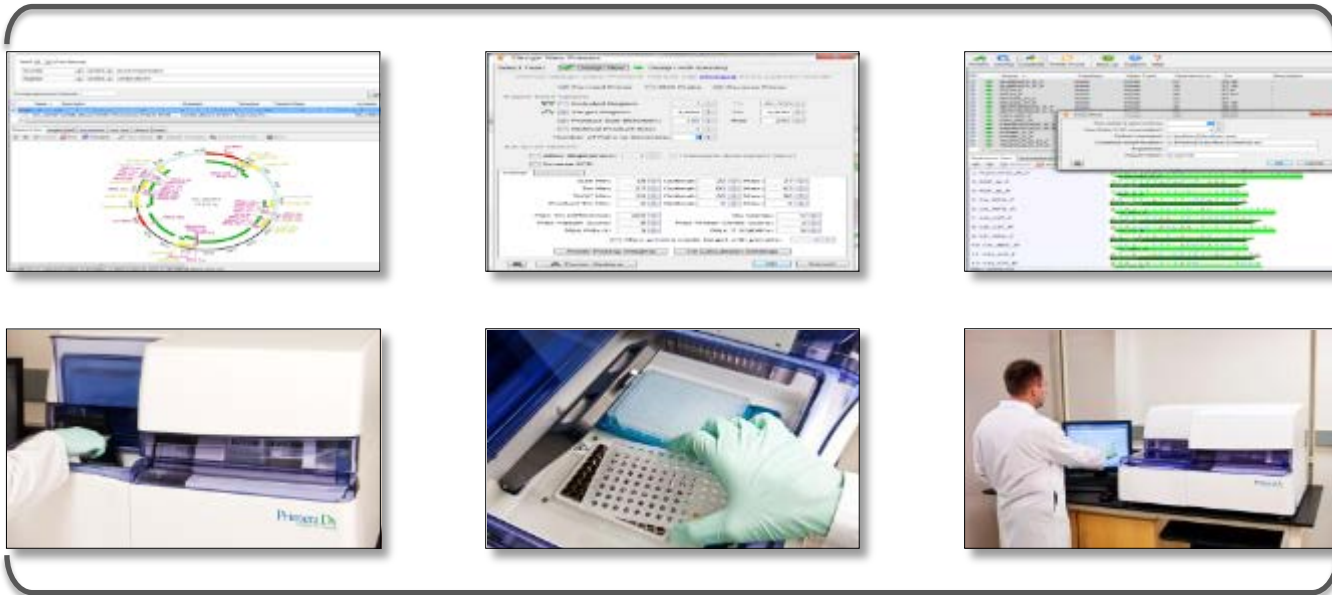




# PrimerDx's Open Platform Product: Next Generation qPCR

Users Design Complex Multiplex Assays with Ease....

In Silico Multiplex Assay Design SW Tools Enable Customers



Automated Workflow Simplifies Testing Procedures

.....and PrimerDx Provides the Tools Needed to Diagnose Disease

# 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



## Others

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

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

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



## Others

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

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

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

## Bringing Personalized Medicine to the Clinic



## PrimeraDx Multimodal Lung Cancer Panels

PrimeraDx

CONFIDENTIAL

# Most Sophisticated yet Economical Way to Perform Quality Non-Small Cell Lung Cancer Testing

One Platform, Multiple Target Types – Only PrimeraDx Can Do It!

Target of Interest for NSCLC	Current Testing Methodology	PrimeraDx
cMET/EGFR Copy Number	FISH	✓
cMET Gene Expression	IHC	✓
cMET Mutation Analysis	PCR, Sequencing, Microarray	✓
KRAS Mutation Analysis	PCR, Sequencing, Microarray	✓
NRAS Mutation Analysis	PCR, Sequencing, Microarray	✓
EGFR Mutation Analysis	PCR, Sequencing, Microarray	✓
EML4-ALK	PCR/Fragment analysis	✓

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



# 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/amplification 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.

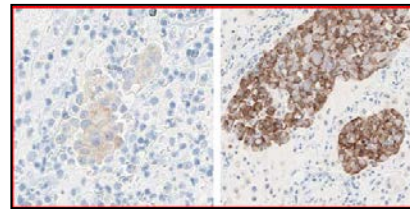
# Challenges of Current Platforms & Multi-modality

Many biomarkers in many complex pathways targeted by many drugs creates a need for single tube, multiplex and multi-modal tests

## IHC:

### Protein Expression / phosphorylation

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



## FISH:

### Gene Copy Number

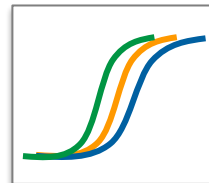
- Inter-lab discordance 20%+
- 5-7 day turn-around time



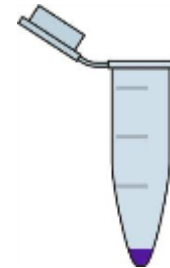
## qPCR:

### Gene Expression / Mutations / Gene Copy Number

- Plexity limitations on most other PCR platforms



## Single-tube Assay

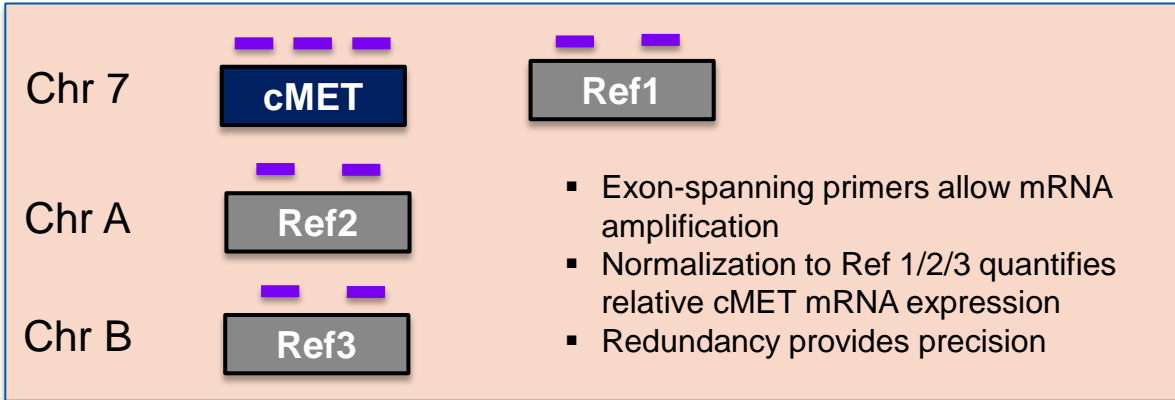


- Quantitative
- Qualitative
- Inexpensive
- Sample conservative

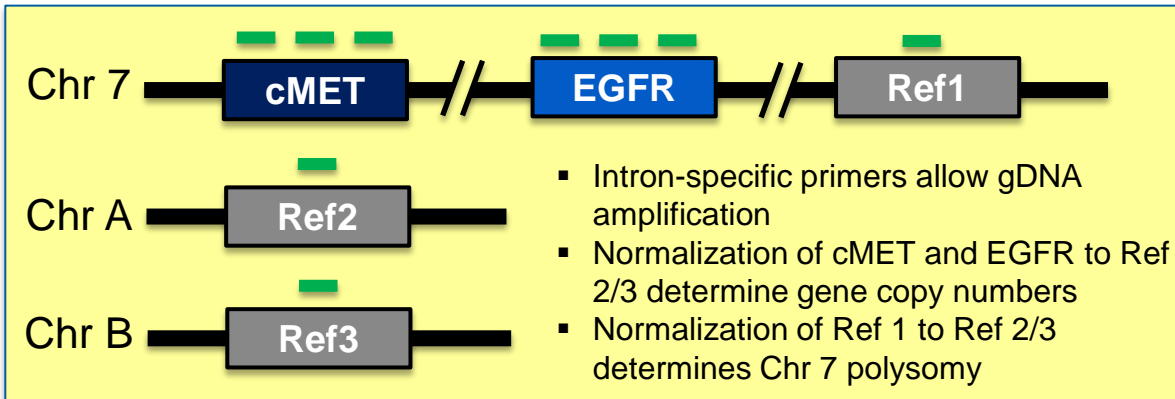


# A “Killer App” For a High Unmet Need – Only PrimeraDx Can Do This

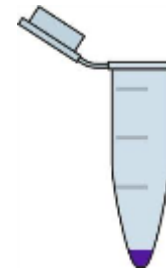
## cMET Gene Expression Assessment



## cMET / EGFR Gene Copy Number Assessment



Single-tube  
Quantitative  
21-plex reaction



- 9 mRNA targets
- 9 gDNA targets
- Quantitation standards
- External process controls for all steps to assure quality of the assay

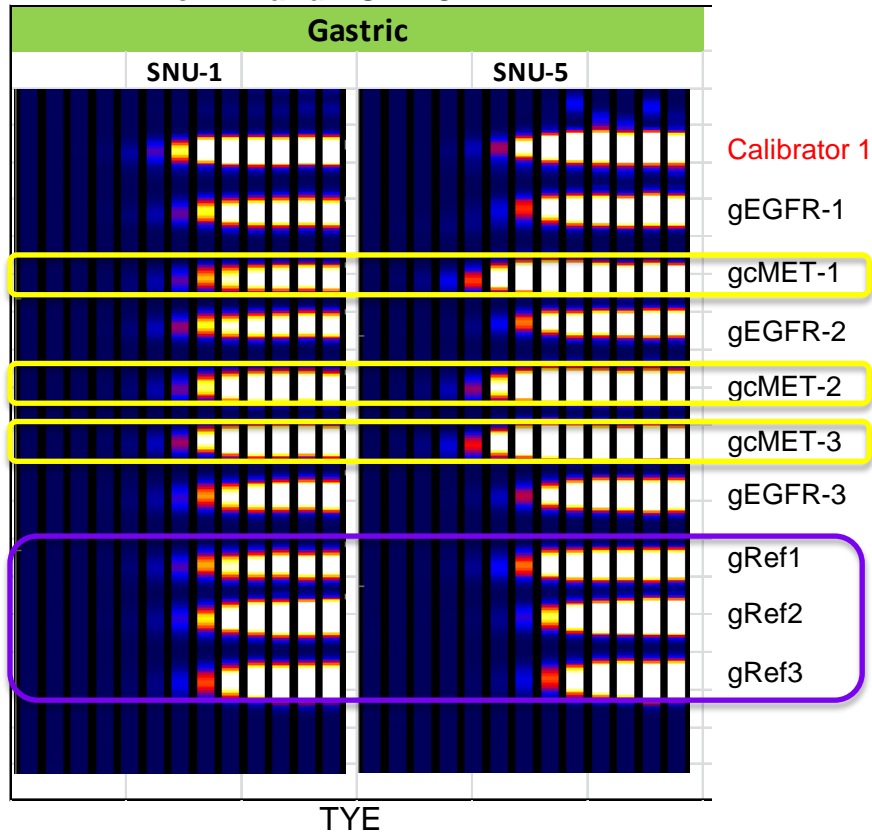
Replaces IHC, FISH  
and qPCR  
with a single reaction

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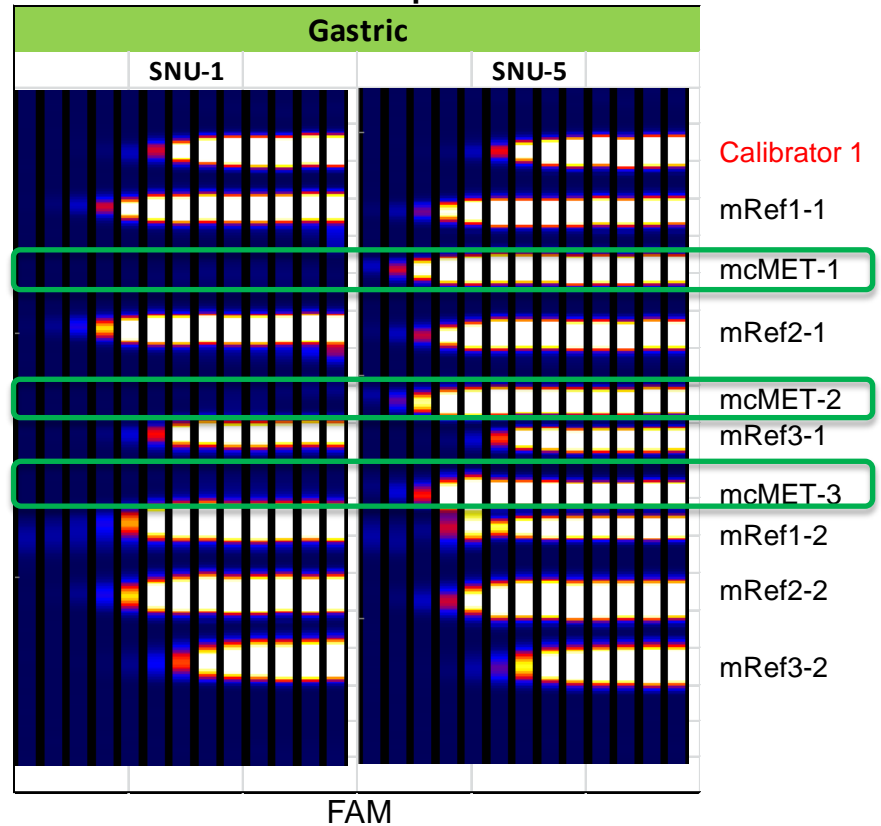
# Single Tube CNV and Gene Expression Analysis – Gastric

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

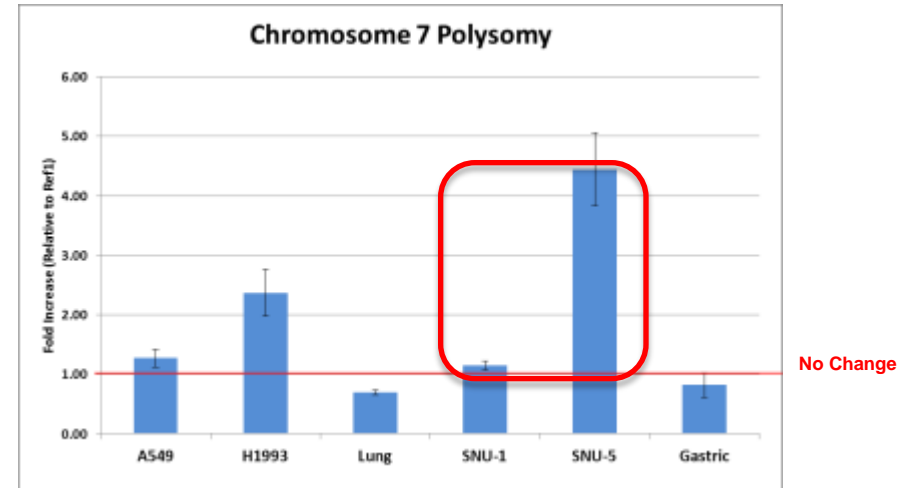
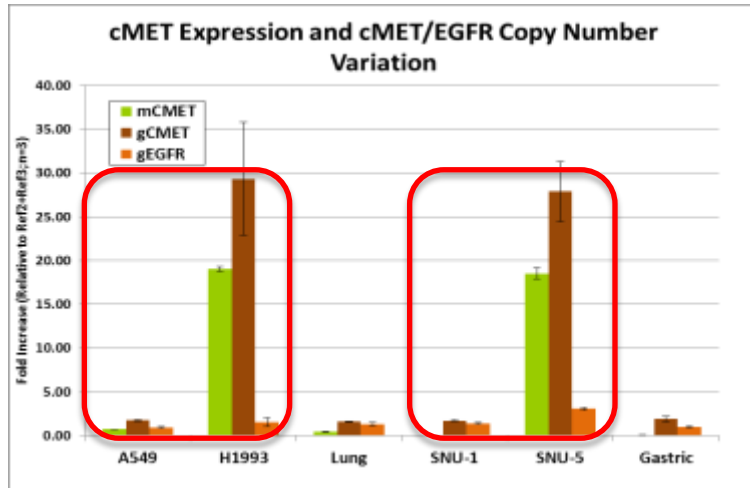
cMET and EGFR CNV



cMET Gene Expression



# cMET/EGFR: Copy Number, Expression and Polysomy in ONE TUBE



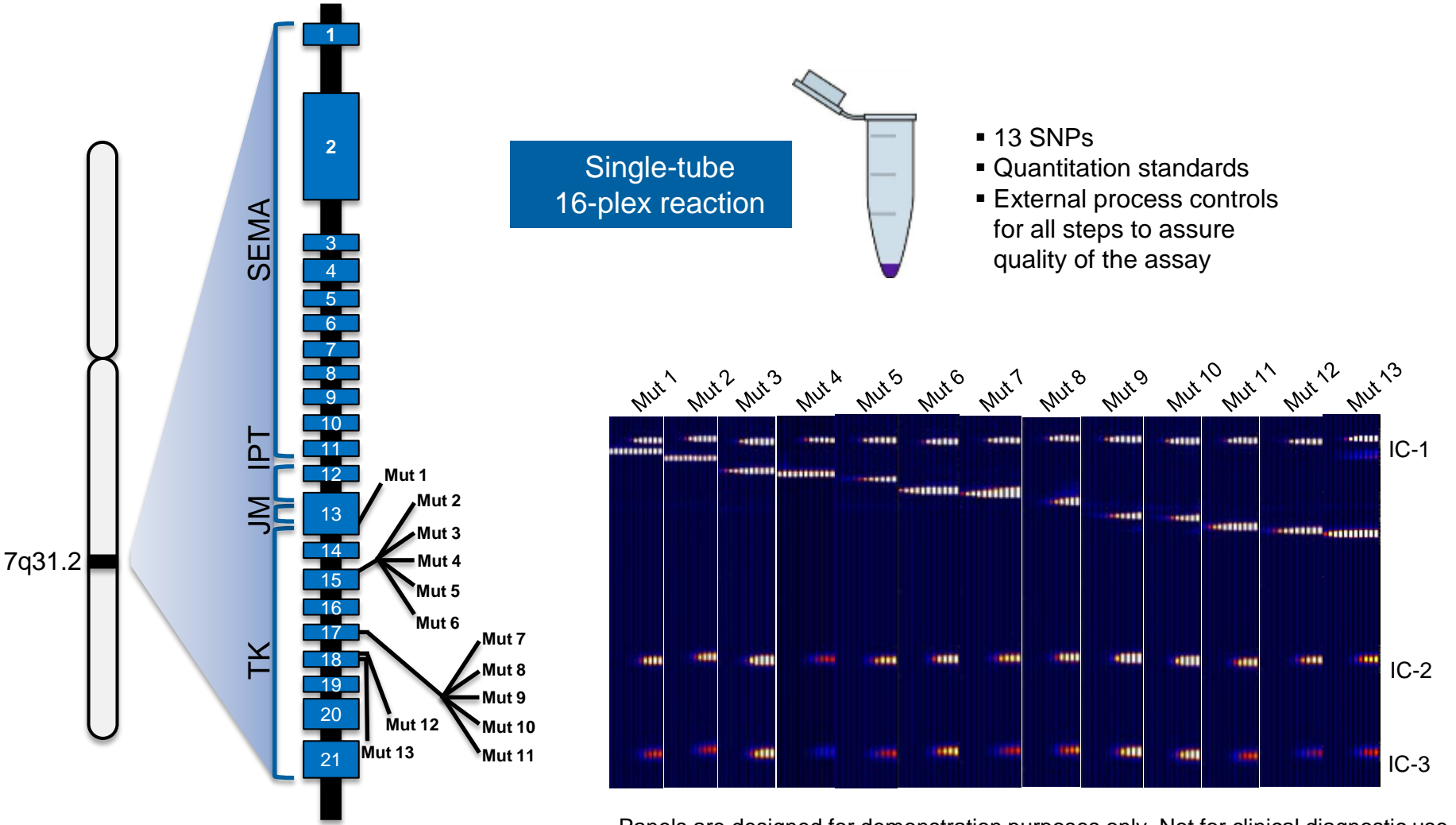
Sample	Source	Tissue Origin	Matrix	MET Copy Number	MET Expression	Chromosome 7 Polysomy	Reference*	Confirmation of Published Data
A549	Cell Line	Lung	Fresh Frozen	2	Low	Unknown	3	✓
H1993	Cell Line	Lung	Fresh Frozen	>10	High	Unknown	3	✓
SNU-1	Cell Line	Gastric	Fresh Frozen	2	No	No	1, 2	✓
SNU-5	Cell Line	Gastric	Fresh Frozen	>10	High	Yes	1, 2	✓

\*(1) Catenacci D, Cancer BioTher, 2011, 12(1): 9-46  
 (2) Smolen G, PNAS, 2006 103(7): 2316-2321  
 (3) Lutterbach B, Cancer Res, 2007, 67: 2081

Assay under development – preliminary data only

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# cMET Mutation Panel Completes cMET Franchise



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# Summary of cMET Assays

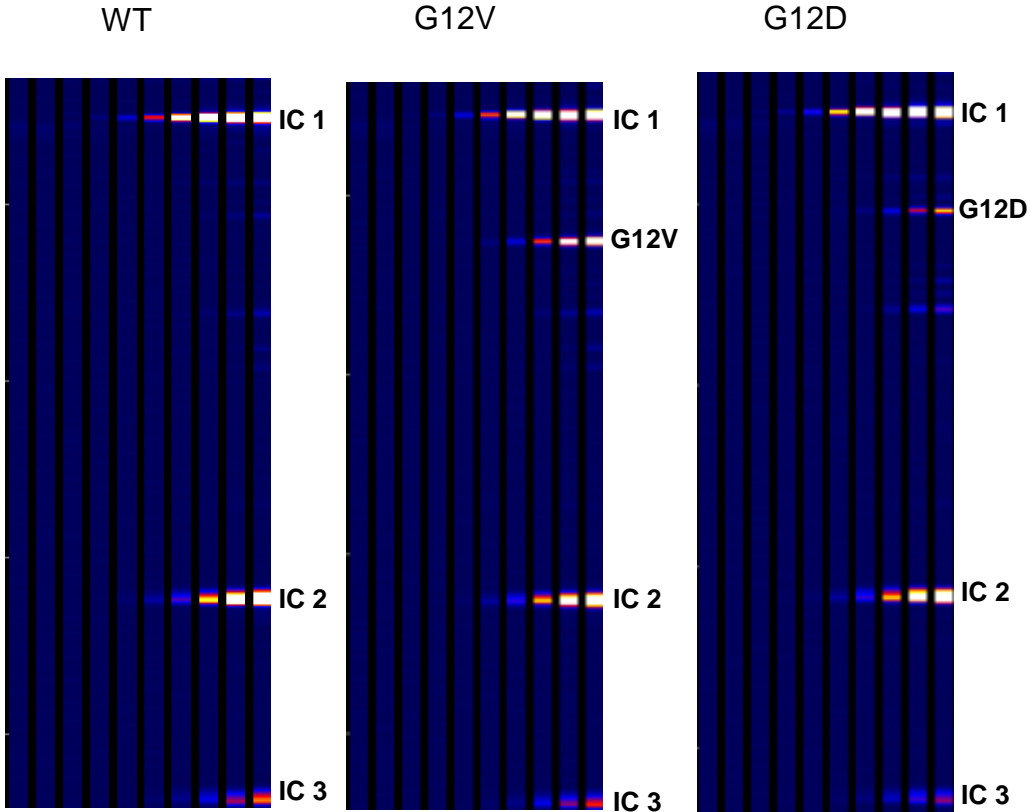
- An 18-target, 21-plex, single-tube multimodal assay designed to detect amplification of cMET and EGFR genes, expression of cMET and polysomy of chromosome 7, as well as a 13-target single-tube cMET mutation panel have been developed on the ICEPlex system
- Target quantification showed good reproducibility with tight %CV values well below the CSLI-recommended value
- LOQ values for target quantification showed reliable discrimination of < 2 fold change input for all targets
- All-in-one assay: Simplifies lab operation and improves lab economics
- Quicker TAT: Impacts patient care
- Minimum nucleic acid input: Resolves specimen size issue
- Versatile sample types: FFPE blocks, Fresh-frozen tissues, Cell lines, Blood
- Quality result: Built-in controls and redundancy assure quality and precision

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# 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

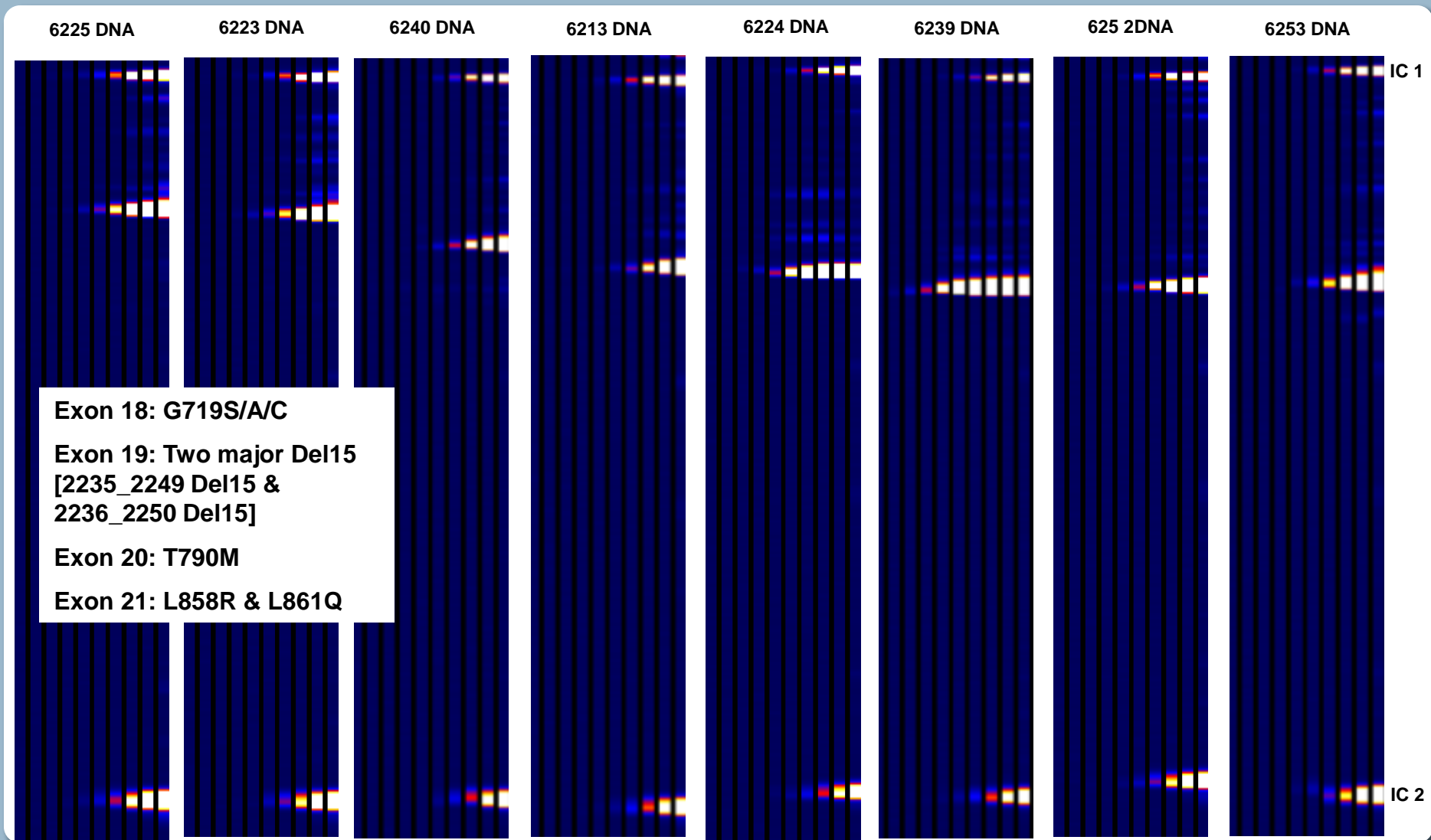


Screening and disease recurrence monitoring require repeated testing

1% selectivity - highly sensitive serum/plasma assay

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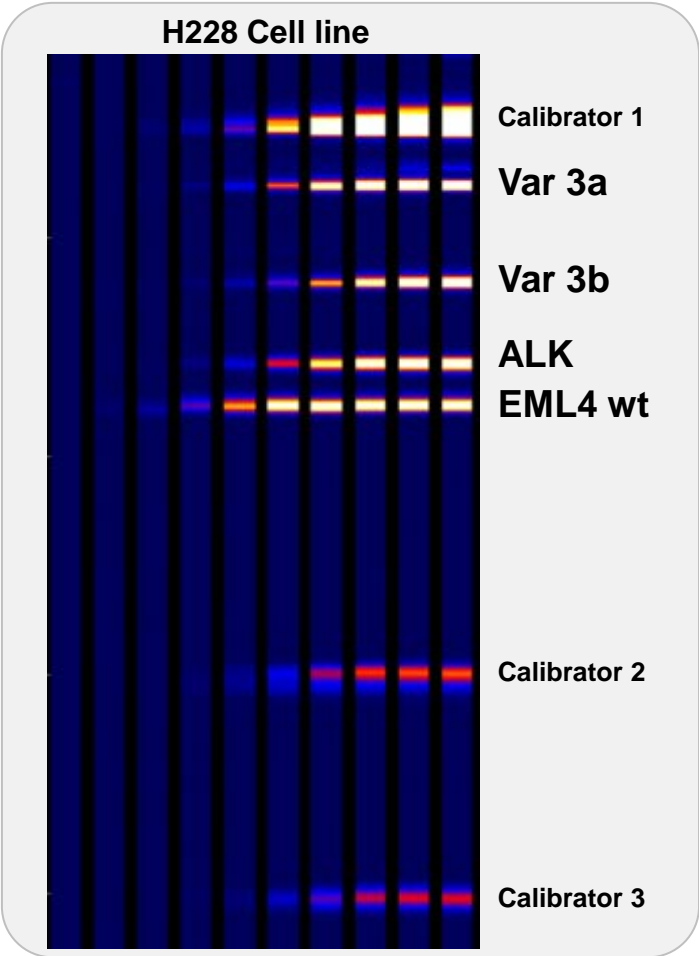
# Multiplex Detection of EGFR Mutations in One Single Reaction



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# EML4-ALK: Fusion Gene Assay Detects Eight Fusion Variants

Fusion Detection Will Displace FISH in the Marketplace – Huge Potential



<u>Target</u>	<u>Amplicon size</u>
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

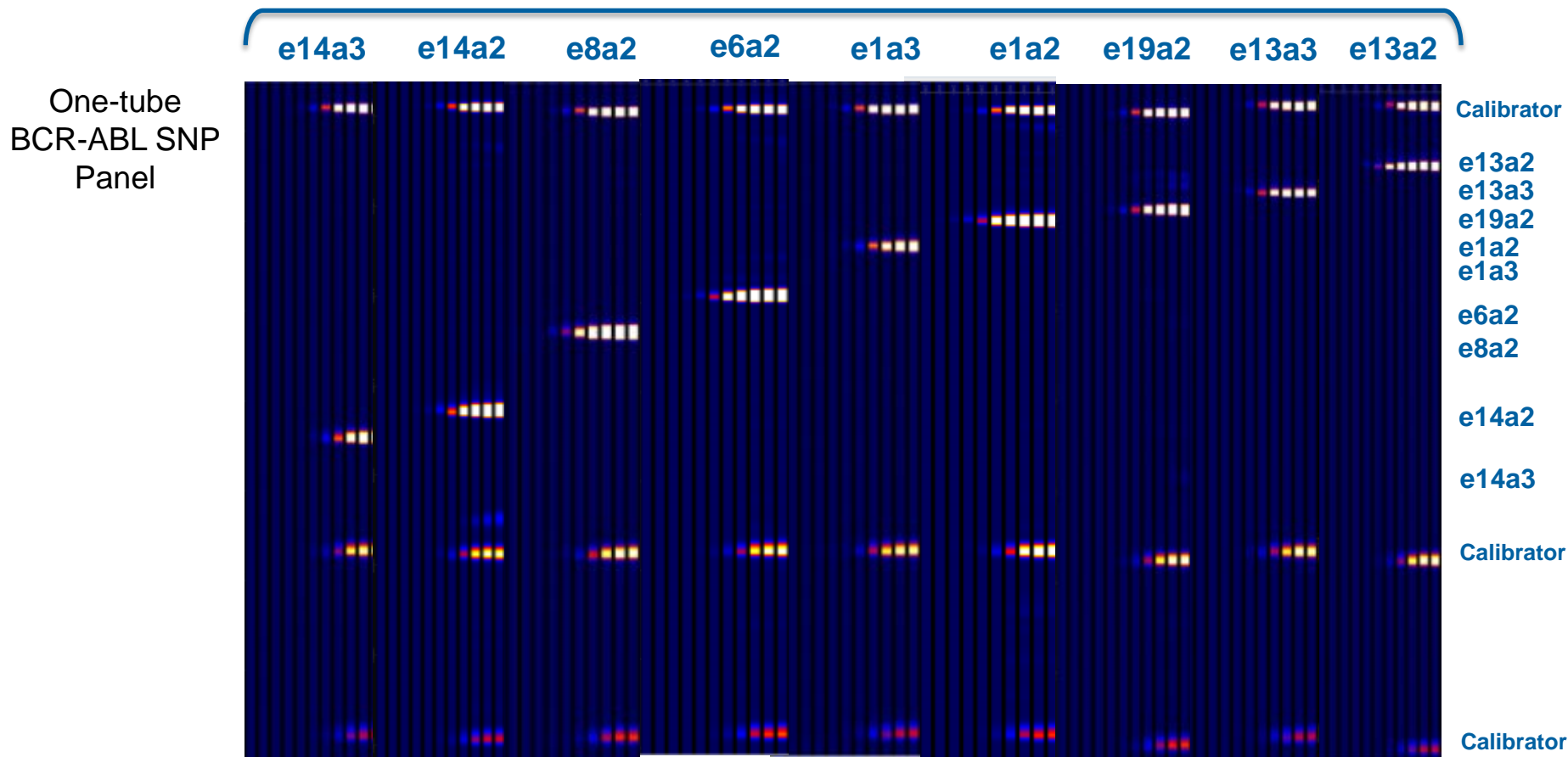
**Fusion Gene Variant Detection on the ICEPlex is simple, and provides definitive answers in just a few hours.**

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# BCR-ABL Fusion Gene Assay Detects Nine Fusion Variants

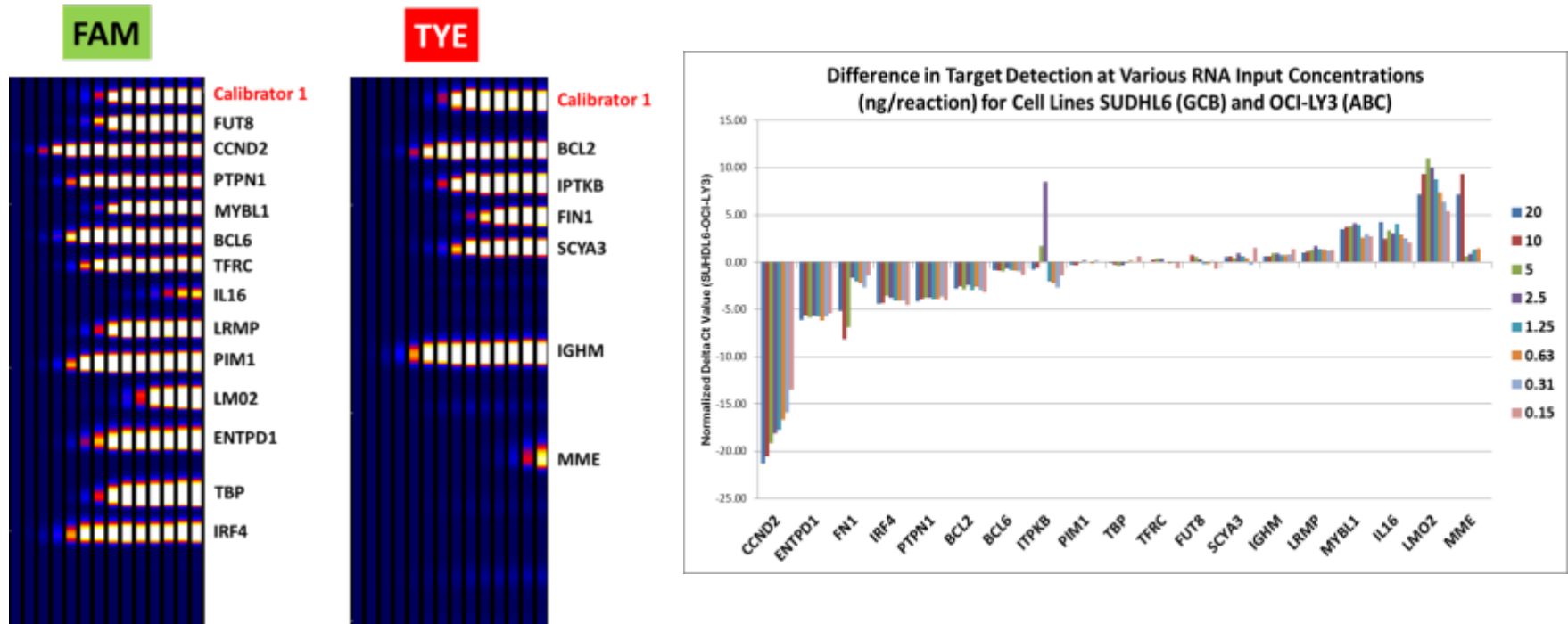
PrimeradX Panel Represents the Best Test Available



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

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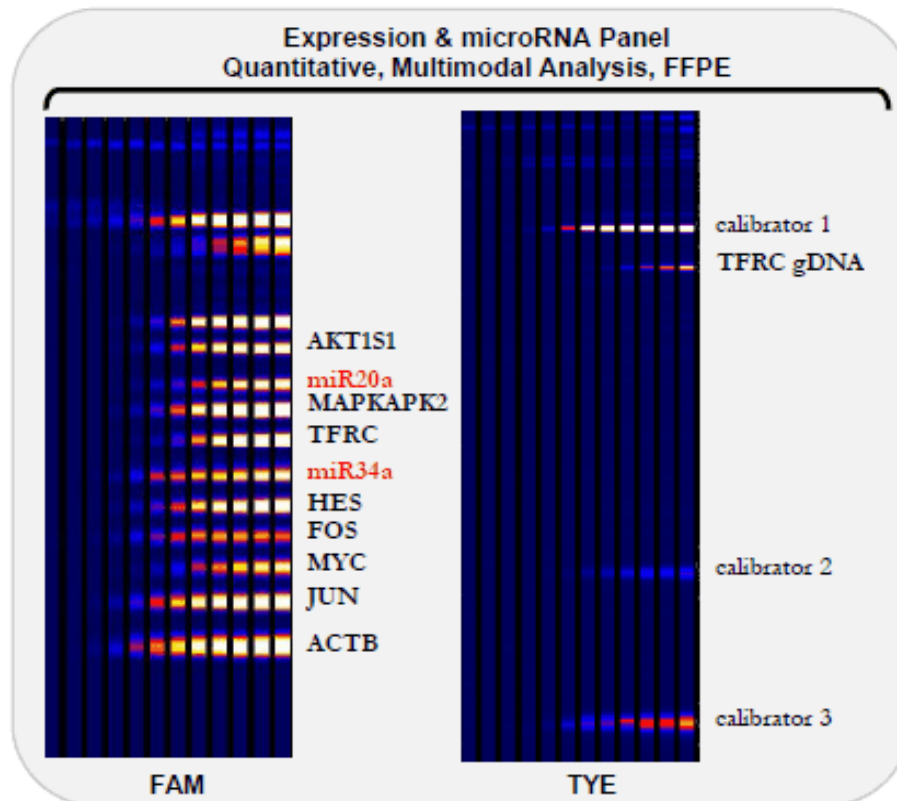
# Lymphoma Gene Expression Panel Differentiates GCB from ABC



- 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

# CDx Partnership for IVD Development

## Multiyear, Multiproduct Deal with Eli Lilly; Others in Process



<b>Features</b>	<ul style="list-style-type: none"> <li>• Assay design</li> <li>• Assay development</li> <li>• Under Design Control</li> <li>• Tissue-specific</li> </ul>	<ul style="list-style-type: none"> <li>• Final robustness testing</li> <li>• Transfer to manufacturing</li> <li>• cGMP production lots</li> <li>• IDE status</li> <li>• CE Mark</li> </ul>	<ul style="list-style-type: none"> <li>• Trial support to CLIA CROs</li> <li>• V&amp;V studies</li> <li>• Concordance analyses</li> <li>• PMA prep &amp; submission</li> <li>• XUS registrations</li> </ul>	<ul style="list-style-type: none"> <li>• Pre-launch conversion from CROs to clinical labs</li> <li>• Facilitate lab partner reimbursement</li> <li>• Support post-approval surveillance trial</li> </ul>
<b>Deliverables</b>	IVD-track assay for use in CLIA or R&D setting	IVD-track IUO for use in registrational trial	<ul style="list-style-type: none"> <li>• PMA submission</li> <li>• Global registrations</li> </ul>	Global distribution

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

PrimeraDx retains all commercial rights.

# PrimerADx – Providing Solutions for Today's Dx Needs

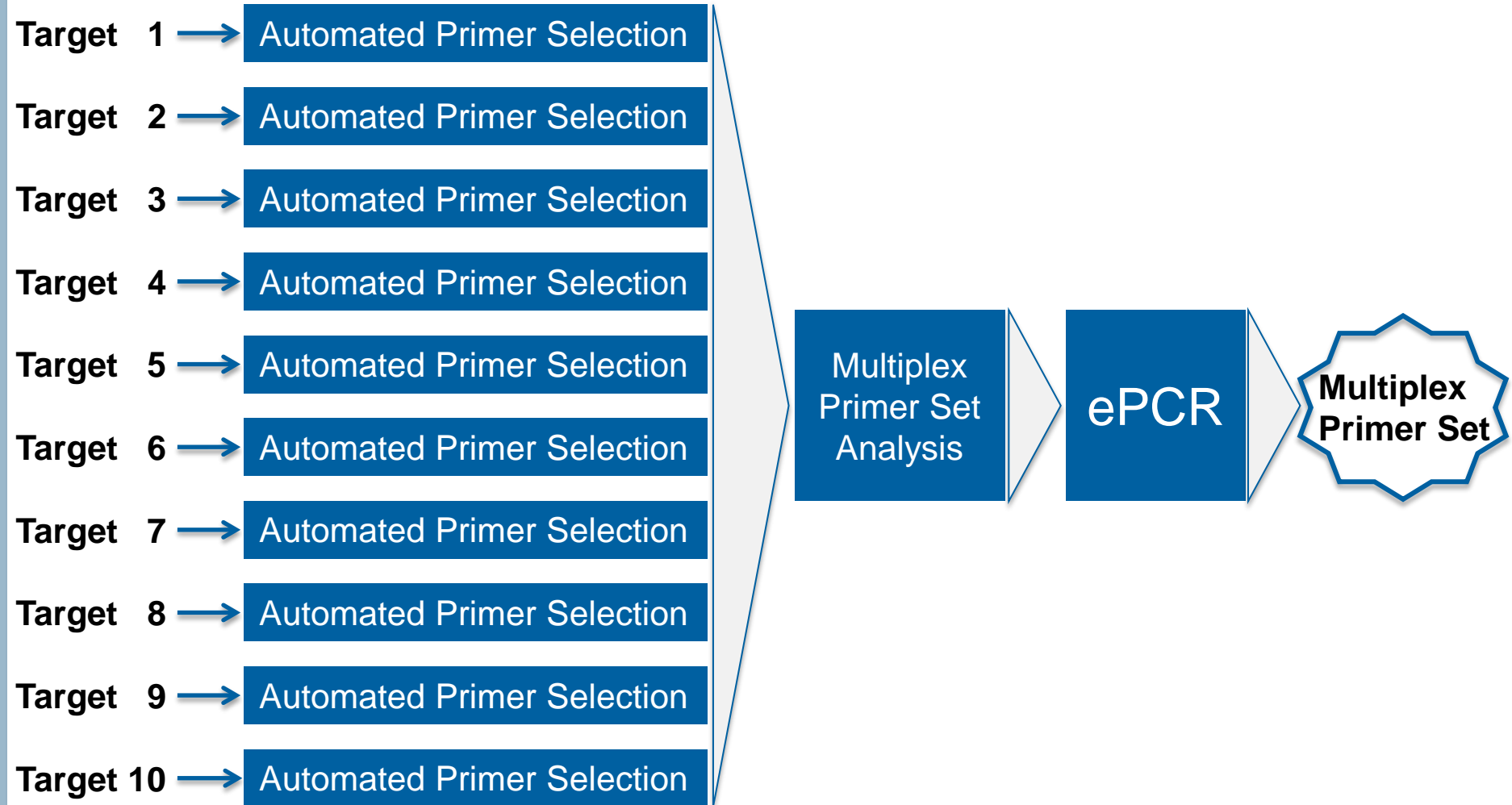
- The only **Fully Quantitative, High Multiplex** qPCR Platform
- A completely **Automated Workflow**
- Allows for **Multi-modal** assay configurations
- The ideal platform for **Companion Diagnostics**
- Numerous complex products in **Diverse Clinical Areas**

# Primer Design Demonstration Case: Transplant 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



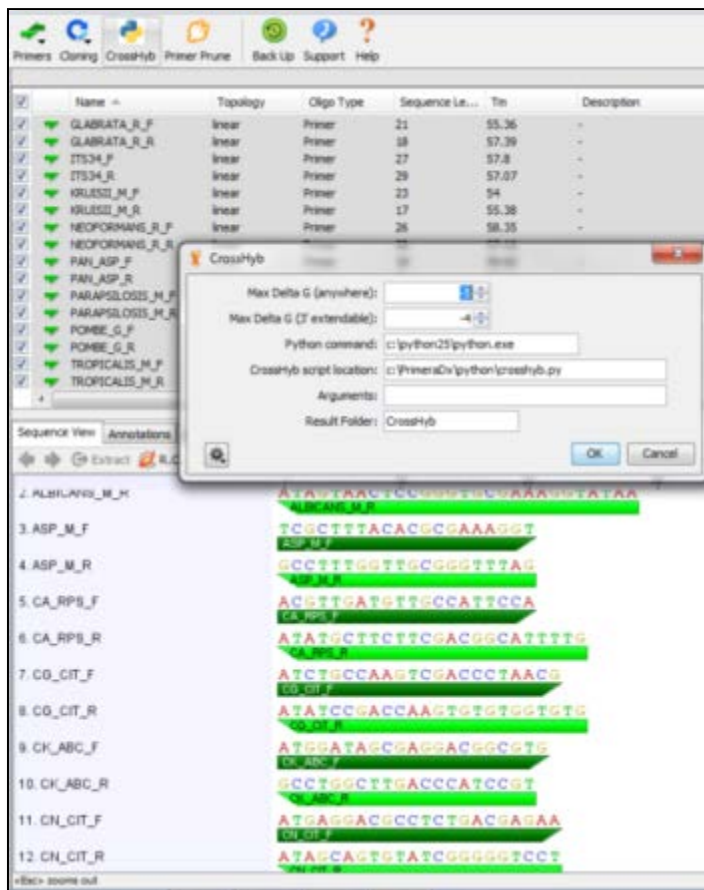
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# Automated Primer Filtering & ePCR

## *In silico* prediction of off-target amplifications



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# ICEPrimer Multiplex Primer Set

Target	Size (bp)	Forward Primer	Reverse primer
BKV	135	FAM-5'-	5'-
scBKV	121	GCTTGATCCATGTCC AGAGTCTTCA	GGAAGGAAAGGCTG GATTCTGAGAT
CMV	172	FAM-5'-	5'-
scCMV	187	TCCGGCGATGTTTAC TTTATCAACC	CCGTGATAAAACAC AAACTGGCAAA
EBV-q (single copy gene)	214	FAM-5'- AATGACTCCAACACC TCCGTCTCTC	5'- ATAAATTATTATGGT AGTGGCTGGGATAA CAGTAAGATG
scEBV	199		
EBV-s (multicopy gene)	153	FAM-5'- AGAACCCAGACGAGT CCGTAGAAGG	5'- CTACCCAGGTTCC TGTGAAAAGCAAGA
HHV6b	193	FAM-5'-	5'-
scHHV6b	177	TCCCAATTGTCTAGC ATGTTCTCCA	TATTTAACAAGTTGA GAGGCATGCGGAT
HHV7	146	FAM-5'-	5'-
scHHV7	161	ATATTGTGCCTTGCA GCTCTATGTTTCTC	ACCGAGATGCGGCT TTTATAGTTGA
Extraction control	283	FAM-5'- TGCTTTTGTAATTGG CTTCTGACCA	5' - CGCAATCCAATAAC TTGGAACGAAT

Target	Concentration in 25X mix	Final Concentration in reaction
BK (forward)	6 µM	240 nM
BK (reverse)	6 µM	240 nM
HHV7 (forward)	7 µM	280 nM
HHV7 (reverse)	7 µM	280 nM
CMV (forward)	4.2 µM	168 nM
CMV (reverse)	4.2 µM	168 nM
EBV-q (forward)	7 µM	280 nM
EBV-q (reverse)	7 µM	280 nM
HHV6 (forward)	7 µM	280 nM
HHV6 (reverse)	7 µM	280 nM
Sample Prep Control (forward)	4.2 µM	168 nM
Sample Prep Control (reverse)	4.2 µM	168 nM
EBV-s (forward)	7 µM	280 nM
EBV-s (reverse)	7 µM	280 nM

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# Different Enzymes/Chemistries Can Be Applied

PCR kit or reagent	Manufacturer	Part#
PCR buffer	PrimeruDx proprietary buffer	n/a
PCR primer mix	PrimeruDx	n/a
Sensitivity controls mix	PrimeruDx	n/a
Universal Calibrator #1	PrimeruDx	510350
AptaTaq Δexo DNA polymerase(with Glycerol)	Roche	05 458 030
dNTP mix	New England Biolabs	N0447L
MgCl <sub>2</sub>	Roche	11 600 770 001
Water, PCR-grade	Qiagen	129112

PCR kit or reagent	Manufacturer	Part#
Multiplex PCR kit	Qiagen	206143
Multiplex PCR Master Mix	Qiagen	1022829
Water, PCR-grade	Qiagen	1012888
HotStarTaq DNA Polymerase	Qiagen	203207
Transplant Panel primer mix	PrimeruDx	n/a
Sensitivity controls mix	PrimeruDx	n/a
Universal Calibrator #1	PrimeruDx	510350

Reagent	Starting concentration	Final concentration	Amount to add, μL
Water	n/a	n/a	13.1
PCR Reaction Buffer	5X	1X (contains some dNTP)	10
MgCl <sub>2</sub>	25 mM	2.5 mM	5
dNTP mix	10 mM	additional 240 μM	1.2
PCR primer mix	25X	1X	2
Sensitivity controls mix	25X	1X	2
Universal Calibrator	25X	0.25X	0.5
Δexo AptaTaq	5 U/μL	6U	1.2
Extracted DNA template	n/a	n/a	15
Total volume			50 μL

Reagent	Starting concentration	Final concentration	Amount to add, μL
Water	n/a	n/a	4.5
Multiplex PCR Master Mix	2X	1X	25
Transplant Panel primer mix	25X	1X	2
Sensitivity Controls mix	25X	1X	2
Universal Calibrator	25X	0.25X	0.5
HotStarTaq DNA Polymerase	5 U/μL	5U	1
Extracted DNA template	n/a	n/a	15
Total volume			50 μL

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# ICEPlex Automated Amplification and Detection Process

Step	Temperature, °C	Duration, seconds	Number of cycles
Initial denaturation	98	900	1
Amplification	64	90	18
	72	60	
	96	20	
Amplification with Injection	64	90	28
	72	150	
	96	10	

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# Insert the PCR Plate into the Thermal Block



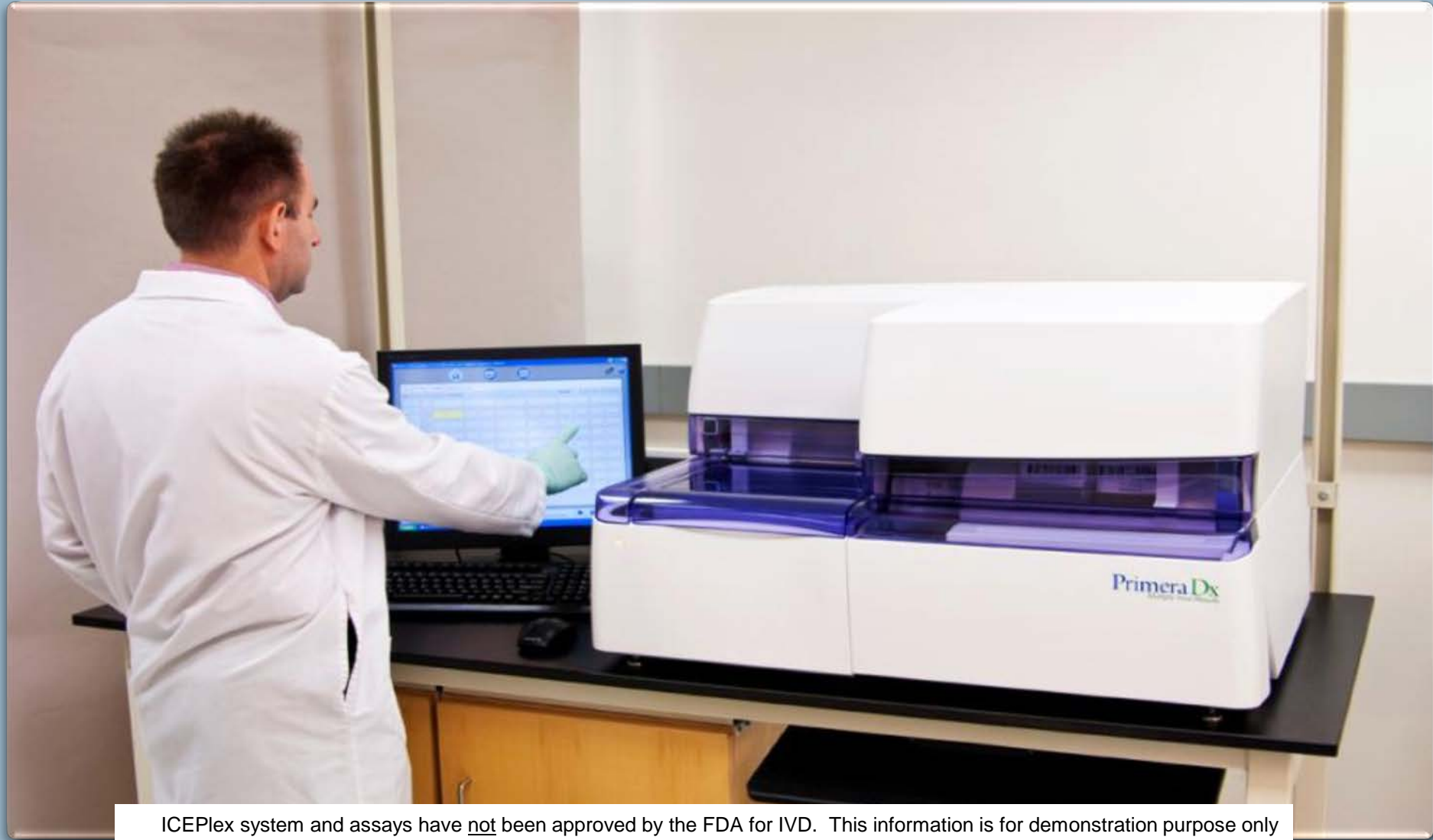
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# Insert the PCR Plate into the Thermal Block



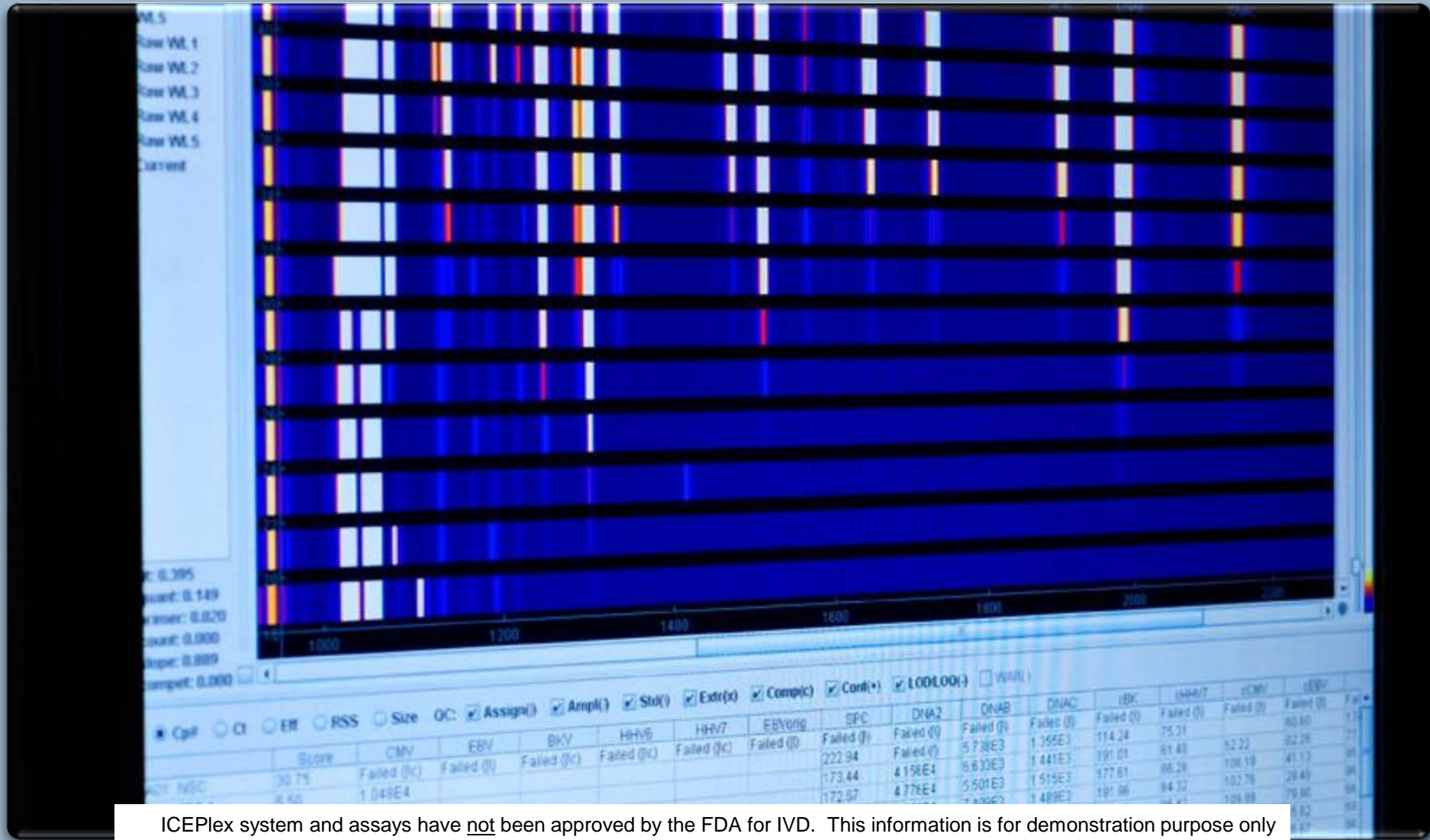
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# Input the Platemap and Run Protocol



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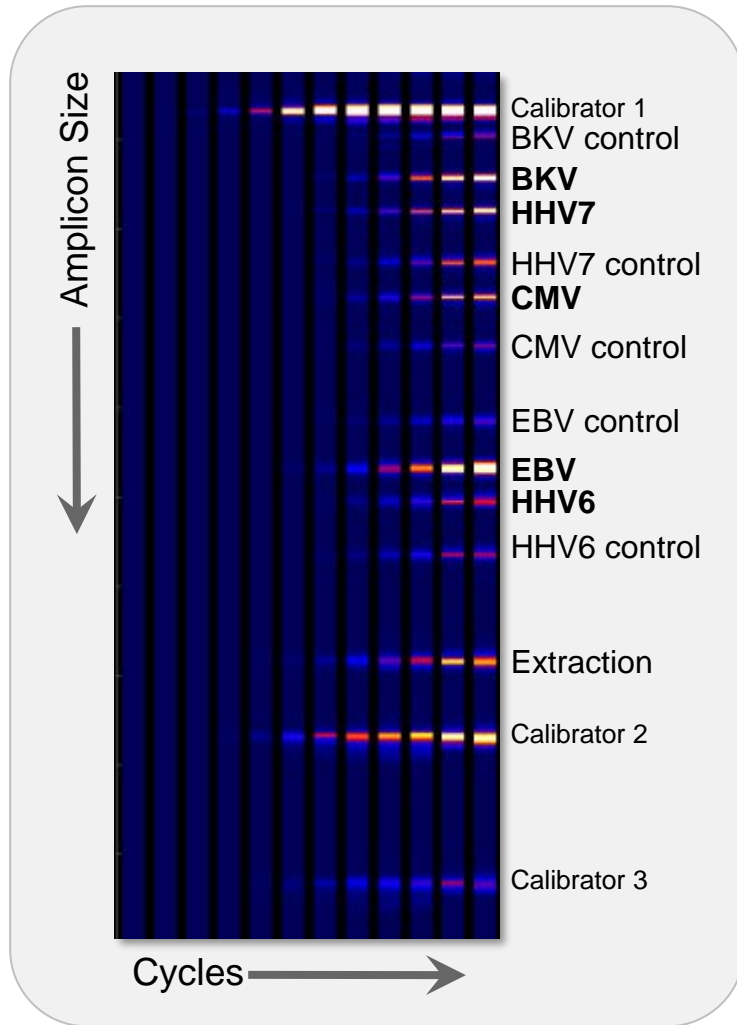
# Results!



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

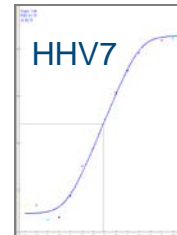
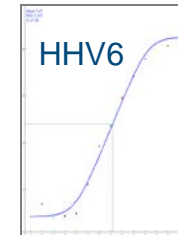
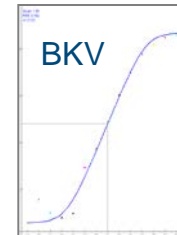
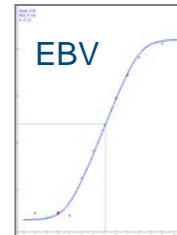
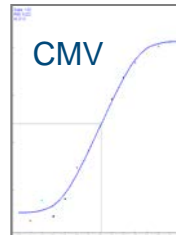


# Transplant Panel – Automated Multiplex Results



## Result Report

Viral load	CMV	EBV	BKV	HHV6	HHV7
<b>Log<sub>10</sub> cps/ml</b>	4.5	4.6	4.5	4.5	4.7



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

PrimeraDx

*The Multiplex PCR Company*