

**Multiplexed ICE COLD-PCR (MX-ICP)
for Detection of Low-Level Mutations
in Any Liquid Biopsy Sample
using Sanger Sequencing,
NGS, or ddPCR**

**Katherine Richardson, Ph.D.
V.P., Research & Development**

What is your FAVORITE?

Sample Type

Non-invasive samples

- Plasma/Serum
- Exosomes
- CTCs
- Urine
- Saliva
- Etc.

Tissue Samples

- FFPE
- FNAs
- Fresh/Frozen
- Etc.

Platform

- Sanger
- NGS
- Digital PCR
- Pyrosequencing
- BEAMing
- COBAS
- HRM

How to Enhance Assay & Platform the Performance?

✓ Improve hardware of the platform

- Innovation in platform design and operation
- Have to resubmit to regulatory agencies

✓ Improve the bioinformatics software

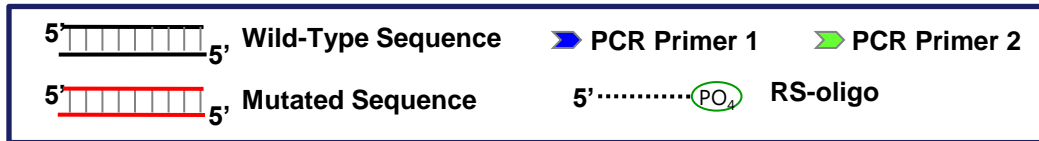
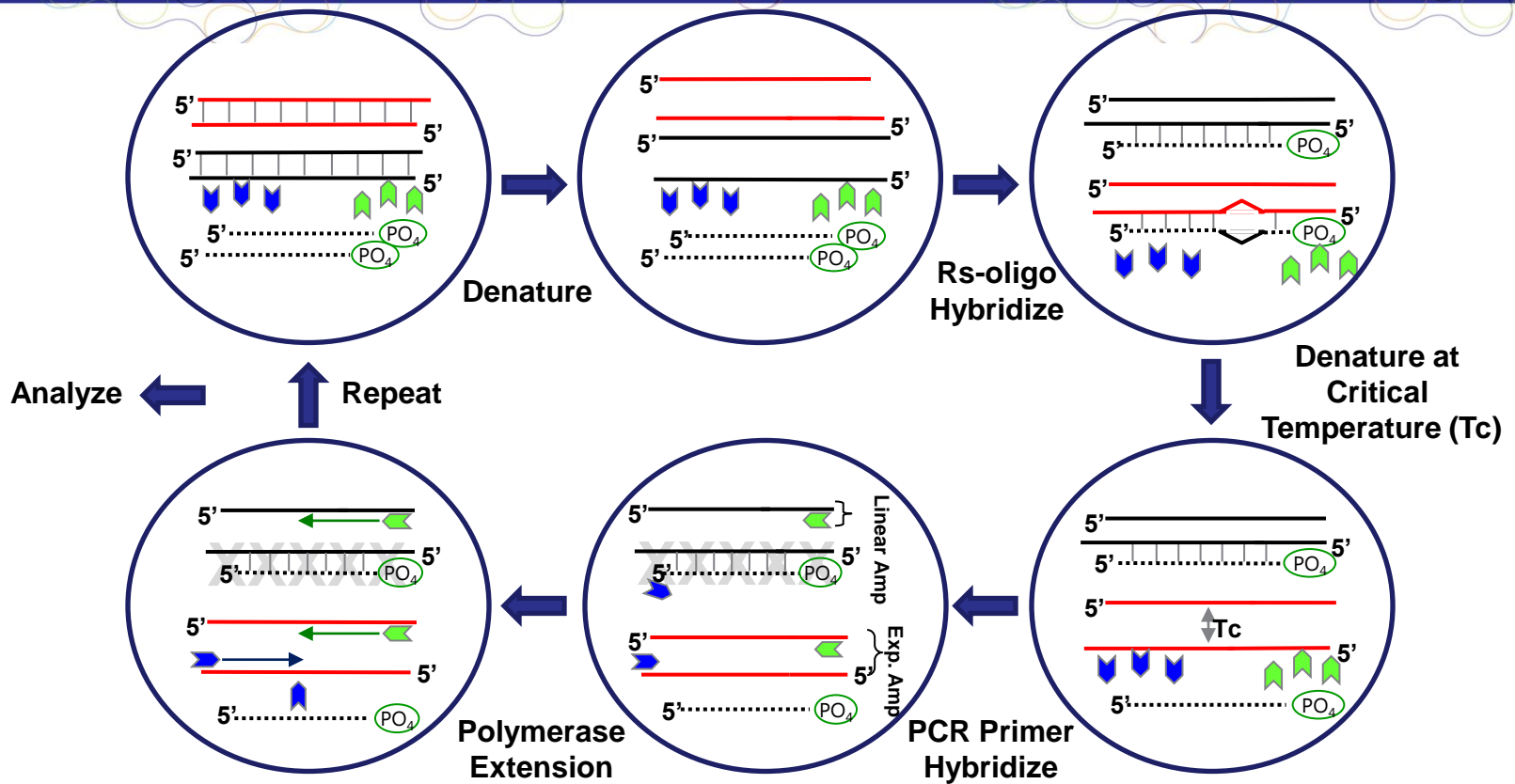
- Only so far you can go to reduce digital noise

✓ Enhance the capabilities of your assay

- Detecting specific mutations
 - Allele-specific PCR and detection systems

- Preferentially enriching all mutations in a specified region

ICE COLD-PCR Assay

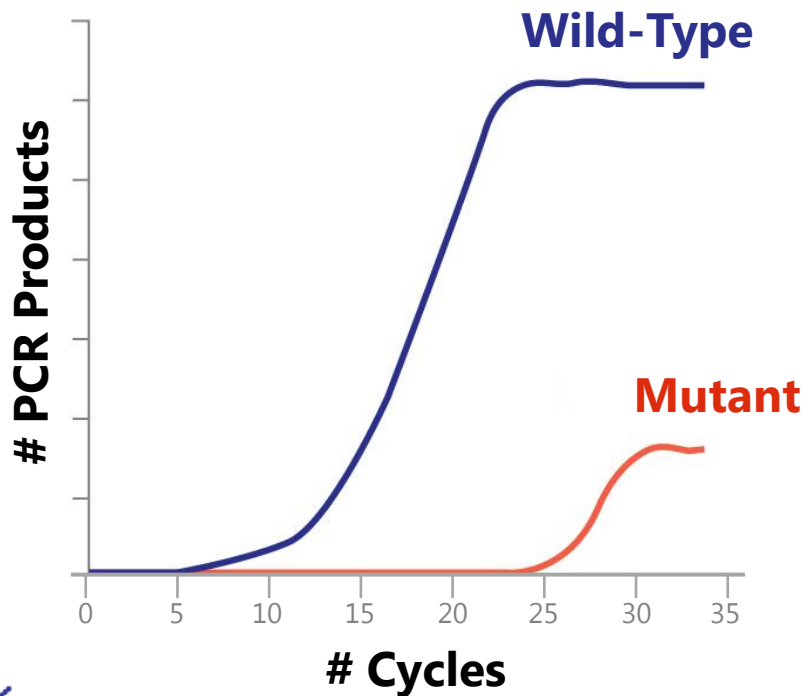


PCR Process that Enriches for ANY Mutation in the Region Interrogated by the RS-oligo.

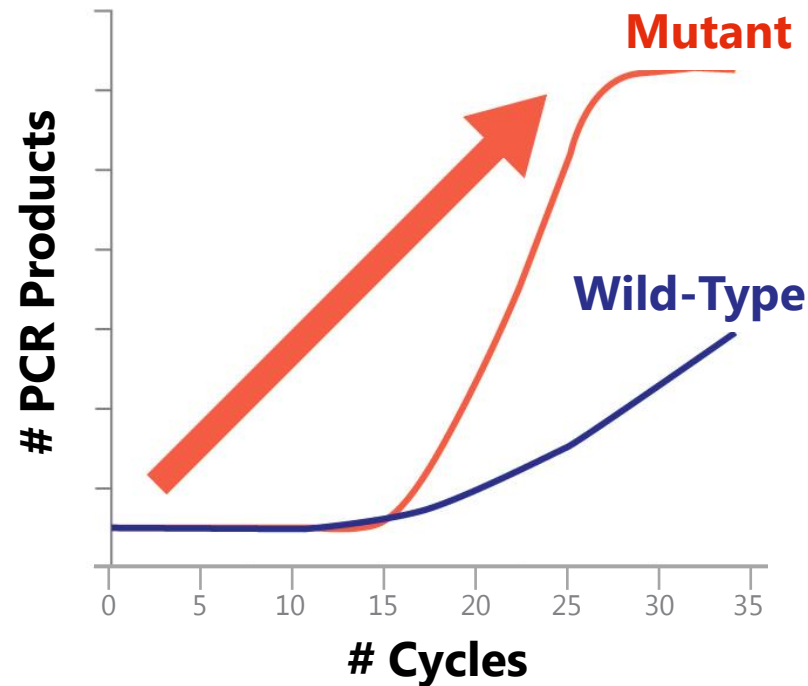
MX-ICP Enables Improved Sensitivity for Mutation Detection

Unique technology allows samples to be preferentially enriched for Mutations in DNA through selective amplification

Traditional PCR

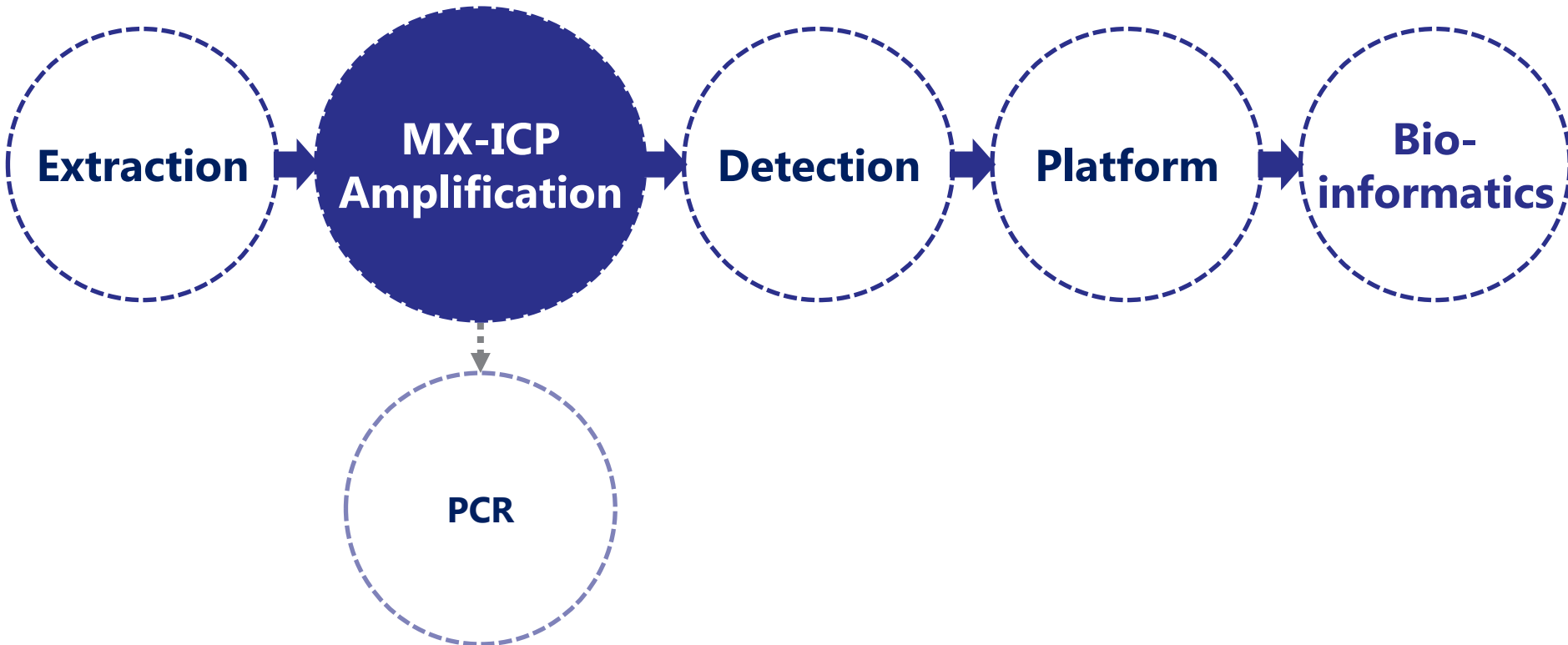


MX-ICP



The MX-ICP Enables Easy Integration into Laboratory Workflow

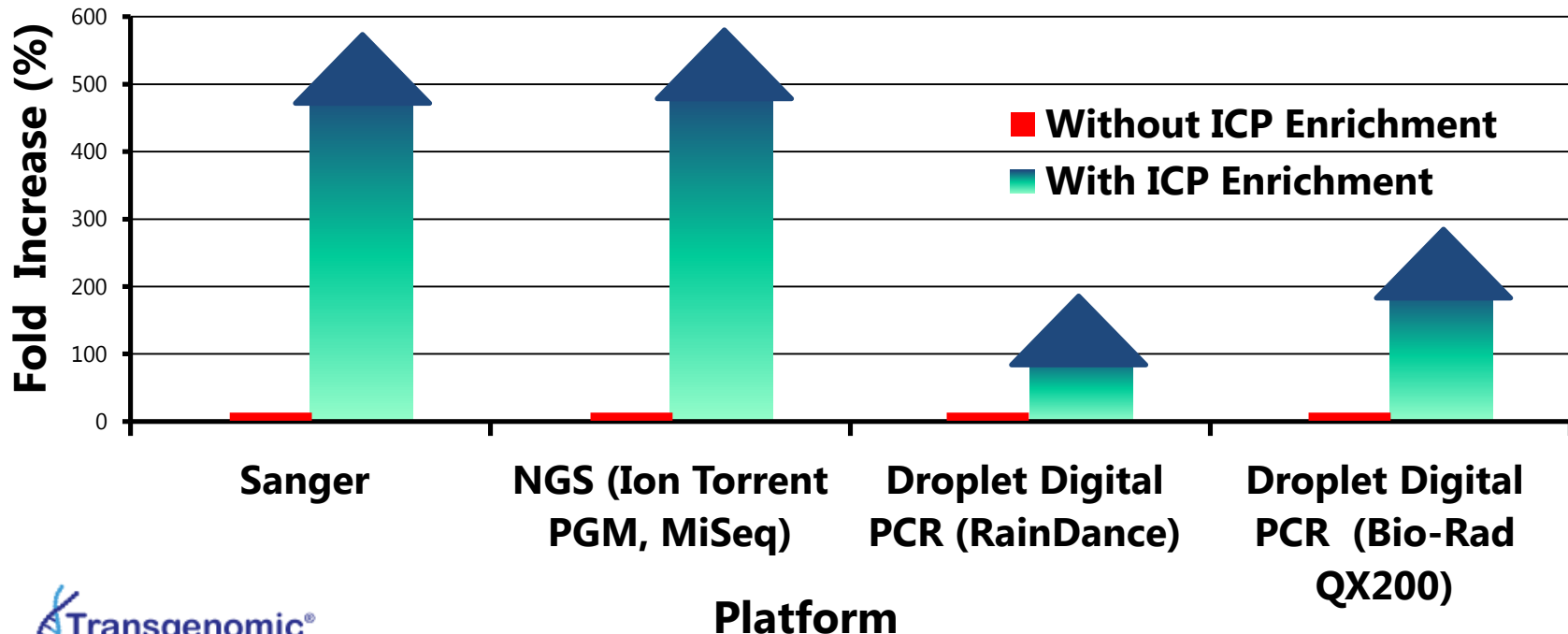
- Easily integrates on any platform
- Replaces traditional PCR



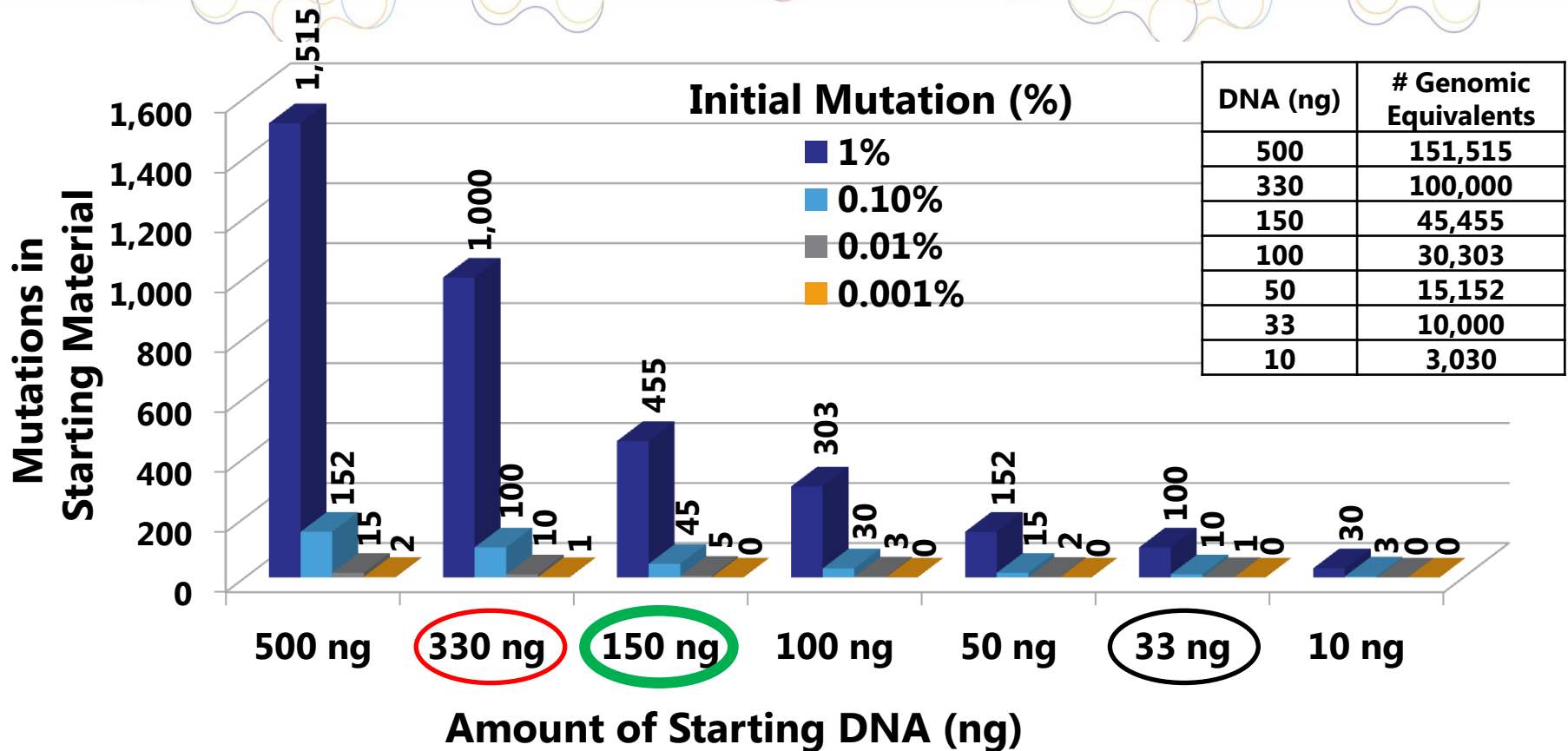
MX-ICP Enhances Mutation Detection

- ✓ Not Allele-Specific
- ✓ All Alterations Detected
- ✓ Custom Design of Assays for Your Targets

Enhancement for Detection of 0.01% Mutation in
in 150 ng starting DNA



Number of Genomic Copies and Mutations Present in Starting Material



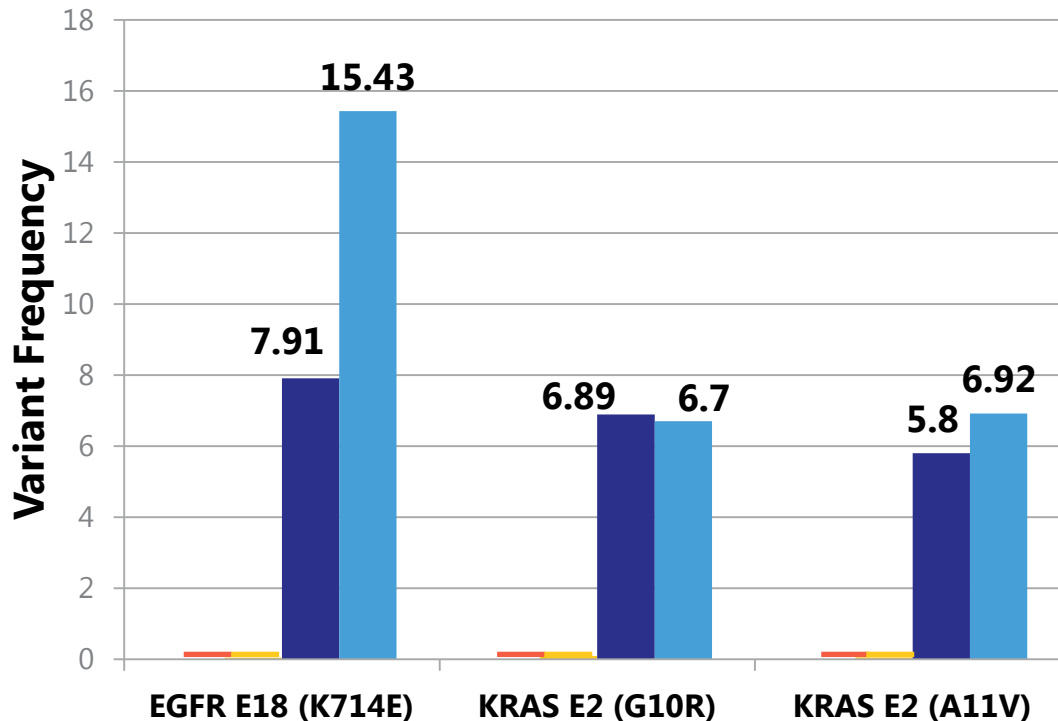
33 ng: Theoretical Limit of Detection for 0.01%

150 ng: Robust detection of 0.01% mutation using MX-ICP

330 ng: Robust detection of 0.01% mutation using ddPCR

MX-ICP plus Next-Gen Sequencing (NGS): Ion Torrent and MiSeq

Enhances Mutation Detection by NGS Platforms



■ Ion Torrent without ICP

■ MiSeq without ICP

■ Ion Torrent with ICP

■ MiSeq with ICP

— Zero or insufficient detection of mutation on NGS

- **cfDNA samples with known mutations in FFPE**
- **Multiple targets, all mutations enriched with MX-ICP**
 - EGFR Exon 18
 - KRAS Exon 2

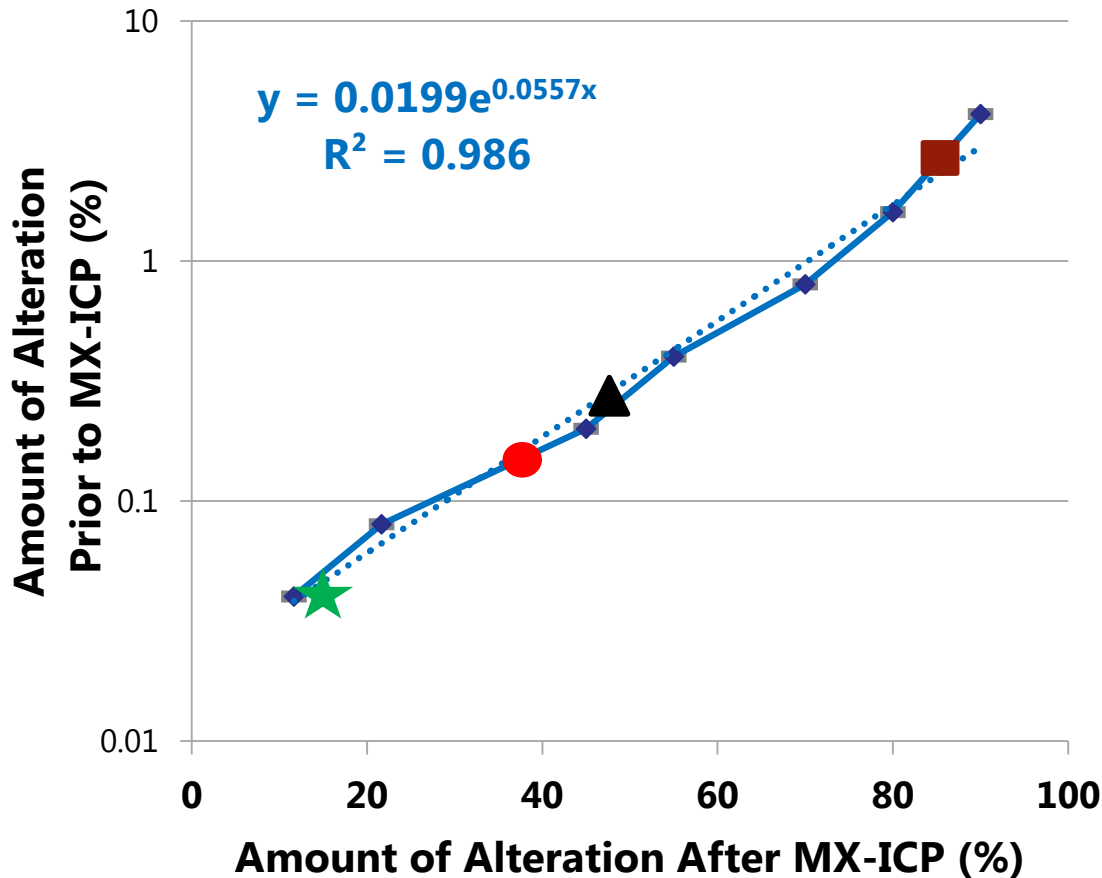
Level of Detection of MX-ICP: VariantCaller - EGFR Exons 18 and 20; IGV- EGFR Exon 19 Deletion

**MX-ICP Detects Low Level Mutations
(150 ng of DNA in starting sample)**

Mutation Proportion of Initial Sample for MX-ICP Enrichment	Percent Mutation Detected After NGS Analysis of MX-ICP		
	EGFR Exon 18: c.2155G>A p.G719S	EGFR Exon 19: c.2235_2249del15, p.E746_A750del	EGFR Exon 20: c.2369C>T p.T790M
20% mutation	92.6%	98.9%	94.4%
5% mutation	88.9%	98.1%	87.3%
1% mutation	74.3%	97.6%	58.1%
0.5% mutation	58.1%	95.6%	49.0%
0.1% mutation	33.7%	95.5%	19.2%
0.05% mutation	22.6%	94.5%	11.1%
0.01% mutation	7.5%	79.7%	4%
0.005% mutation	Below LoQ	Below LoQ	Below LoQ

MX-ICP Enables Quantification of Amount of Mutation in Starting Sample:

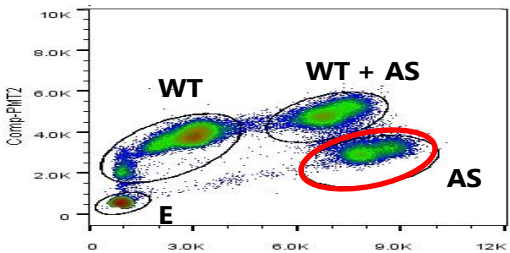
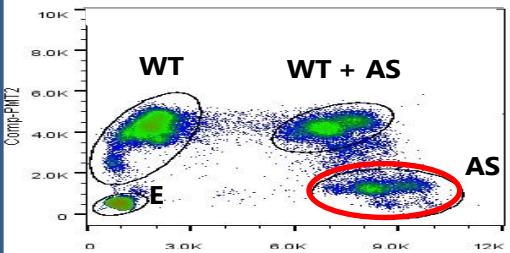
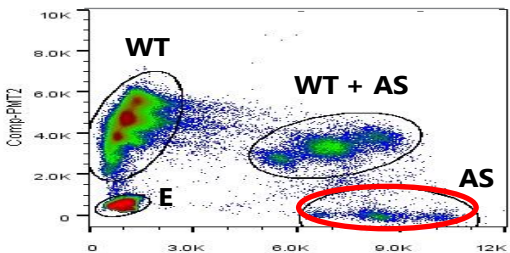
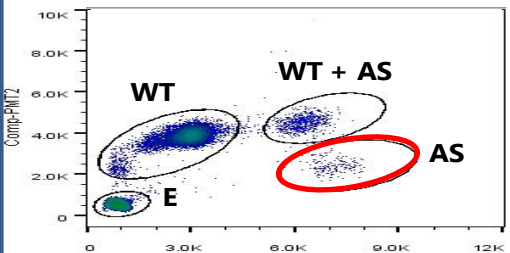
LoD for EGFR S492R (3 reps)



- ◆ Average LOD
- Digitally verified Sample
4.1% before MX-ICP
90% after MX-ICP
- ▲ Sample Dilution (Rep 4)
0.04 before MX-ICP
15% after MX-ICP
- CRC: Sample
?? before MX-ICP
40% after MX-ICP
Calculated 0.2% Before MX-ICP
- ★ CRC: Sample
?? before MX-ICP
10% after MX-ICP
Calculated 0.03% Before MX-ICP

Droplet Digital PCR and MX-ICP of EGFR T790M: RainDance Data

150 ng of starting DNA containing 0.1 or 0.01% EGFR Exon 20 T790M Mutation Enriched using MX-ICP prior to ddPCR

Amount of T790M Mutation in Starting Sample	Dilution of Starting MX-ICP product Necessary	
	1 / 1 Million	1/ 10 Million
0.1% T790M	 <p>14% Mutation</p>	 <p>13% Mutation</p>
0.01% T790M	 <p>1% Mutation</p>	 <p>2% Mutation</p>

AS = Mutant Droplets

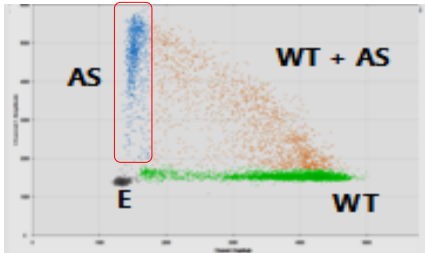
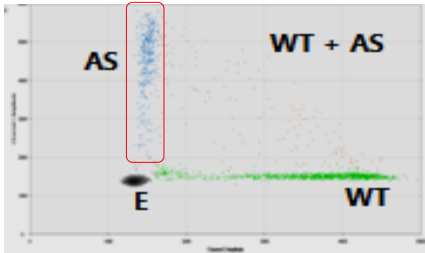
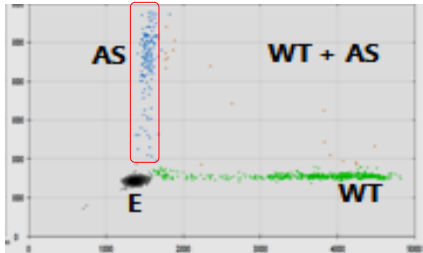
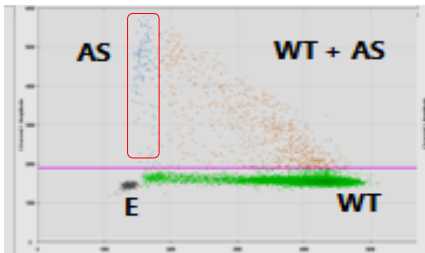
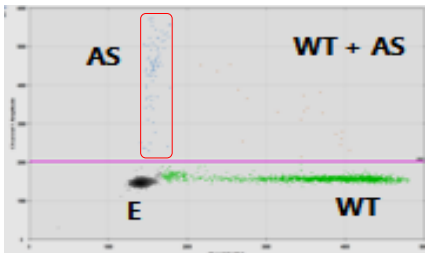
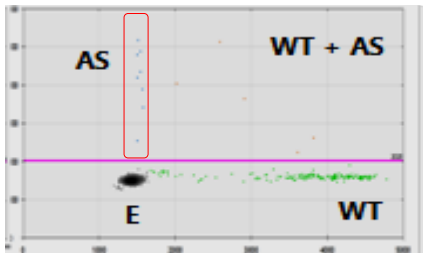
WT = Wild-Type Droplets

WT + AS = Dual Occupancy Droplets

• Less DNA Needed = Less Plasma = Less Blood

Droplet Digital PCR and MX-ICP of EGFR T790M. Bio-Rad QX200

150 ng of starting DNA containing 0.1 or 0.01% EGFR Exon 20 T790M Mutation Enriched using MX-ICP prior to ddPCR

Amount of Mutation in Starting Sample	Dilution of Starting MX-ICP product Necessary		
	1 / 100,000	1 / 1 Million	1/ 10 Million
0.1% T790M	 <p>22% Mutation</p>	 <p>25% Mutation</p>	 <p>22% Mutation</p>
0.01% T790M	 <p>3% Mutation</p>	 <p>5% Mutation</p>	 <p>7% Mutation</p>

AS = Droplets containing Mutations

WT = Wild-Type Droplets

WT + AS = Dual Occupancy Droplets

Compelling Data on MX-ICP

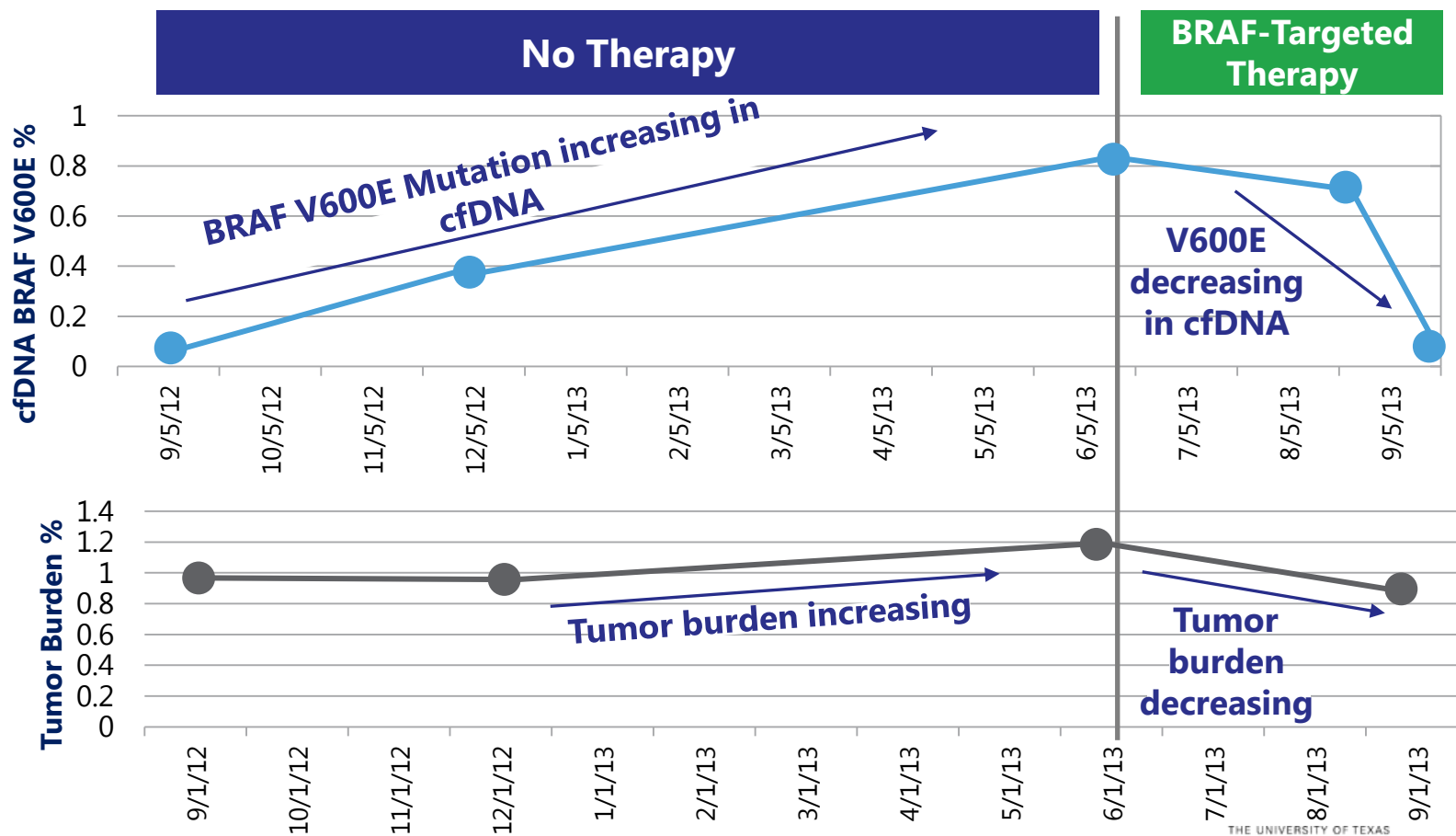
MX-ICP significantly ENHANCES the sensitivity of detection of ALL DOWNSTREAM SEQUENCING TECHNOLOGIES tested

Detection of Alterations in DNA							
Platform	0.05% Mutation in 150 ng DNA			0.01% Mutation in 150 ng DNA			Tested with Transgenomic ICE COLD-PCR Samples
	Without MX-ICP	With MX-ICP	Fold Increase in Sensitivity	Without MX-ICP	With MX-ICP	Fold Increase in Sensitivity	
Sanger	No	Yes	≥400	No	Yes	≥400	Yes
NGS	No	Yes	≥400	No	Yes	≥400	Yes
ddPCR RainDance	Yes	Yes	≥100	No	Yes	≥100	Yes
ddPCR Bio-Rad QX200	Yes	Yes	≥100	No	Yes	≥100	Yes
Pyro – sequencing	No	Yes	≥200	No	Yes	≥200	No¹

¹How Kit et al. Sensitive Detection of KRAS Mutations Using Enhanced-ice-COLD-PCR Mutation Enrichment and Direct Sequence Identification. (2013) *Human Mutation* 34:1568

Monitoring Mutations in cfDNA from a Patient: Example of Effective and Improved Treatment

Detects actionable Mutations in DNA from plasma of patients prior to tumor growth
Supports determination of when to start treatment and monitoring of response



MX-ICP Fits into YOUR Process



- ✓ **Enhanced sensitivity of mutation detection necessary liquid biopsies**
 - **Any Biofluid or Tissue**
- ✓ **Enhanced detection of:**
 - **ALL DNA/RNA mutations present in sample enriched in a single assay :**
 - **MX-ICP for KRAS Exon 2: > 42 possible mutations enriched in codons 12 &13**
 - **MX-ICP for EGFR Exon 19: > 60 possible deletions enriched**
- ✓ **Enhanced performance for all widely used genetic testing platforms:**
 - **NGS, Sanger, Digital PCR**
- ✓ **Simple implementation at minimal cost.**

Questions/Challenges

- ✓ **What levels of mutations are relevant for the patient?**
 - **In Tissues**
 - **In Liquid Biopsies**

- ✓ **Are these levels the same for all mutations?**
 - **Suspect different mutations will have different cut points**
 - **Maybe both mutation and patient dependent**

- ✓ **Are these levels the same for all cancers?**
 - **Suspect different cancers will have same mutation but different cut point**
 - **Maybe cancer and patient dependent**

Answers?

- ✓ **Need clinical trials to answer the above**
 - **Need sufficient plasma**
 - **Need longitudinal time points**

- ✓ **Implement MX-ICP into your favorite process/platform**

- ✓ **Transgenomic Offers:**
 - **MX-PCR + MX-ICP analysis of clinical trial samples**
 - **Licensing ICP technology for your in-house development program**
 - **MX-ICP as a CLIA test for clinical trial enrollment/patient monitoring**
 - **MX-ICP RUO kits**

Thank You