The Use of Multiplexed ICE COLD-PCR Coupled to Multiple Downstream Analysis Platforms

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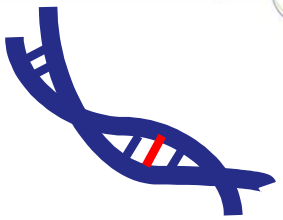
ICE COLD-PCR Advantages



Non-Allele Specific – Enriches ANY & ALL Variants

Improves clinical outcome by use of non-invasive liquid biopsies for treatment & monitoring

Works on any sample – tissue, plasma, cells, exosomes, FFPE, CTC's

Works on any current detection platform – NGS, ddPCR, Sanger. Easily integrates into existing workflow

Significantly improves sensitivity – 100 to 500 fold increase

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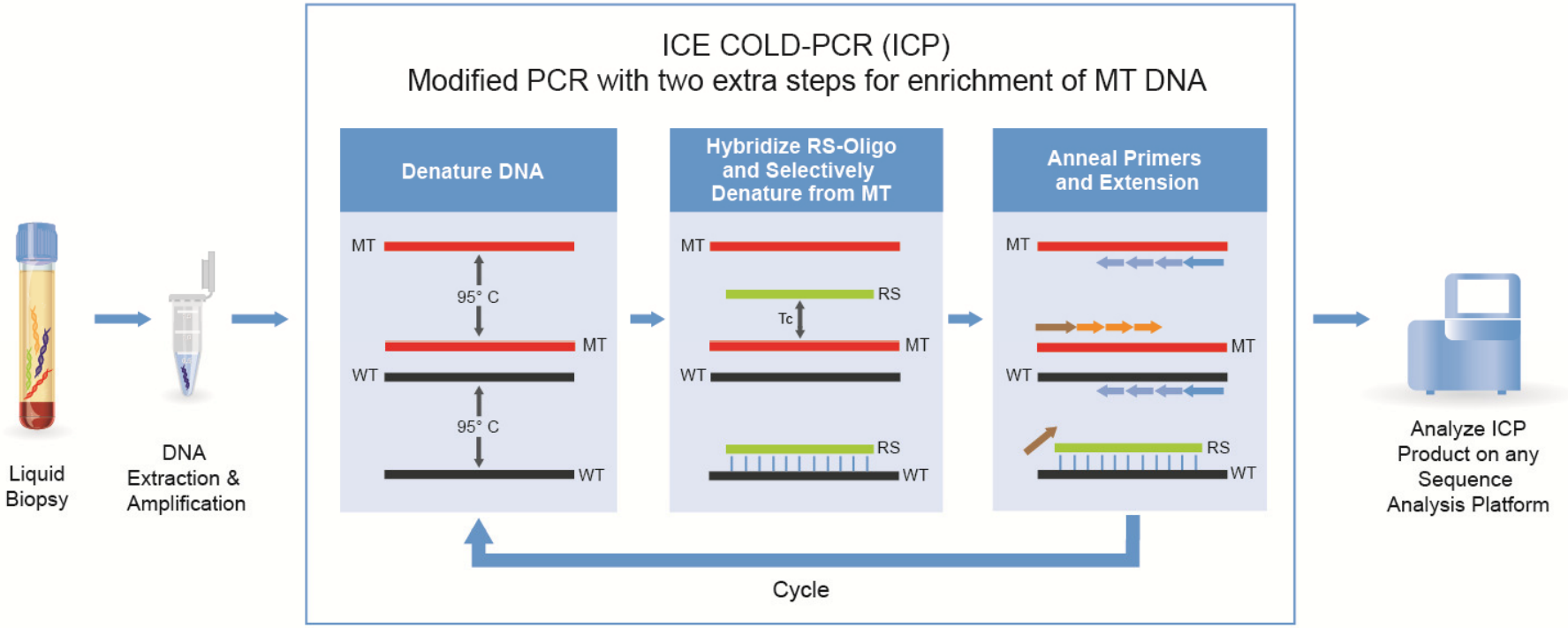
Exclusive license from Dana Farber Cancer Institute

ICE COLD-PCR Methodology



- ✓ Enriches variant alleles from a mixture of wild type (WT) and mutant (MT) DNA
- ✓ The RS (Reference Sequence)-Oligo binds one strand of the WT and MT DNA
- ✓ At the critical temperature (Tc), the RS-Oligo:MT heteroduplex is denatured
- ✓ Resulting in selective amplification of MT DNA.

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ICE COLD-PCR
Flexible Workflow

Patient Sample

DNA Isolation & Amplification

Mutation Enrichment ICE COLD-PCR

NGS

ddPCR

Sanger

Comprehensive Genomic Analysis

- Mutations
- Indels
- Etc.

Compatible with existing commercial solutions

QIAGEN

life technologies

ThermoFisher SCIENTIFIC

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multiplexed ICE COLD-PCR

QIAGEN

life technologies

ion torrent by Thermo Fisher Scientific

illumina

BIO-RAD

QIAGEN

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RainDance Technologies

ICE COLD-PCR Increases Sensitivity of ALL Platforms

Platform	Sensitivity	Mutation type	Quantitative	Fold Increase/ Advantage
Sanger	10 - 20%	ALL	Yes	
ICP + Sanger	≥0.01%	ALL	Yes	>1000
NGS	3-5%	ALL	Yes	
ICP + NGS	≥0.01%	ALL	Yes	>500
ddPCR	≥0.01%	Hotspot	Yes	
ICP + ddPCR	≥0.01%	Hotspot	Yes	Lower detection limits with less input DNA
Pyrosequencing	5%	Site	Yes	
ICP + Pyrosequencing[1]	≥0.05%	Site	Yes	>100

ALL: Includes insertions, deletions, and point mutations

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

ICE COLD-PCR Validation Summary

ICE COLD-PCR	Details
Technology Development	✓ ICE COLD-PCR developed at Dana Farber Cancer Institute ✓ TBIO has exclusive license (IP) ✓ Recent NIH Grant awarded to TBIO to Augment Multiplexing Capabilities of ICE COLD-PCR Technology in Collaboration with Dana-Farber Cancer Institute
Technology Publications	✓ 150+ Publications on COLD-PCR and ICE COLD-PCR
Technology Validation	✓ Pilot Studies ongoing with major Pharma / Biotech ✓ Studies ongoing with major academic institutes (MD Anderson, University of Melbourne etc.) ✓ In-house longitudinal studies with patient samples (FFPE and Liquid Biopsy) acquired by TBIO ✓Ongoing concordance testing of tumor and time-matched plasma

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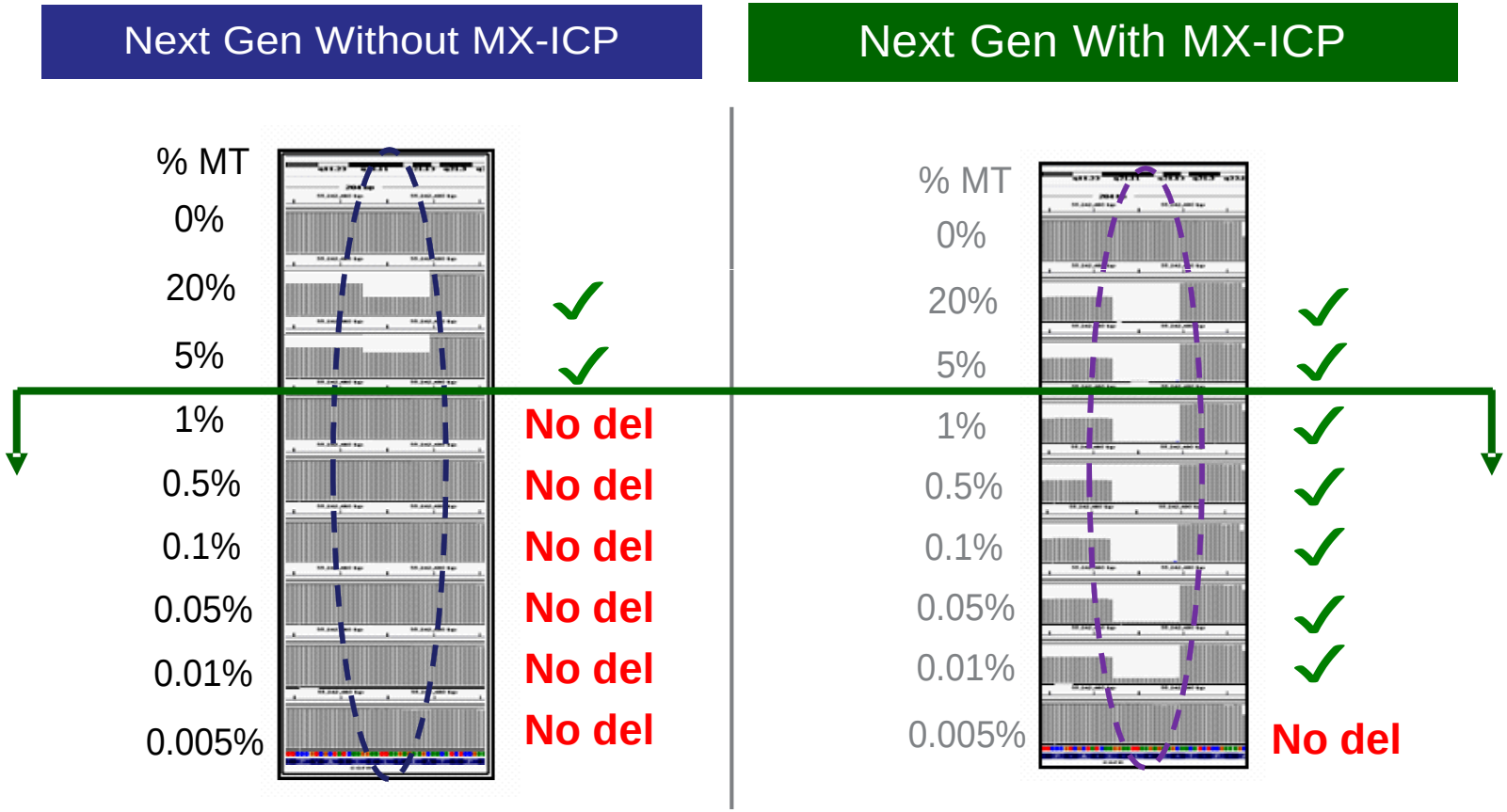
THE UNIVERSITY OF MELBOURNE

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

MX-ICP Enables Detection of More EGFR Mutations in Plasma than NGS Alone: Better Diagnosis

- EGFR is one of the most common mutations present in up to 15-20% of all lung cancer patients*
- % MT (sensitivity) refers to the concentration of mutant DNA in the total DNA sample



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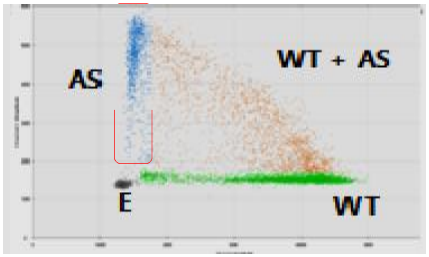
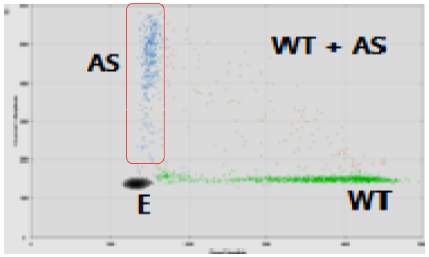
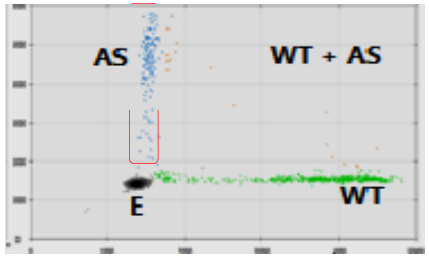
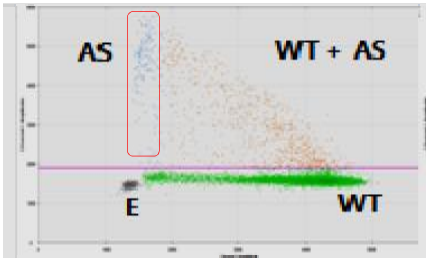
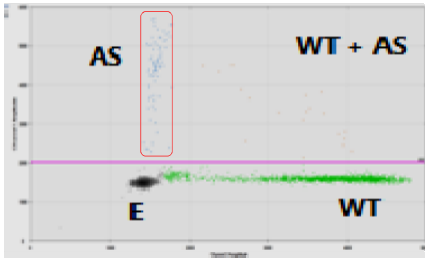
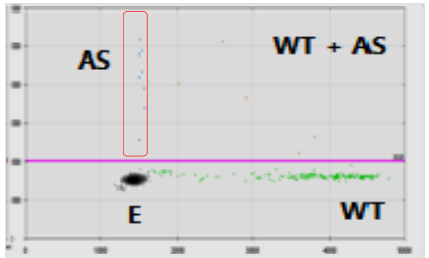
EGFR * Present is 14-20% of lung cancer in Caucasians & 40-50% in Asian

ICE COLD-PCR plus ddPCR: EGFR T790M Detection by 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	 **22% Mutation**	 **25% Mutation**	 **22% Mutation**
0.01% T790M	 **3% Mutation**	 **5% Mutation**	 **7% Mutation**

AS = Droplets containing Mutations

WT = Wild-Type Droplets

WT + AS = Dual Occupancy Droplets

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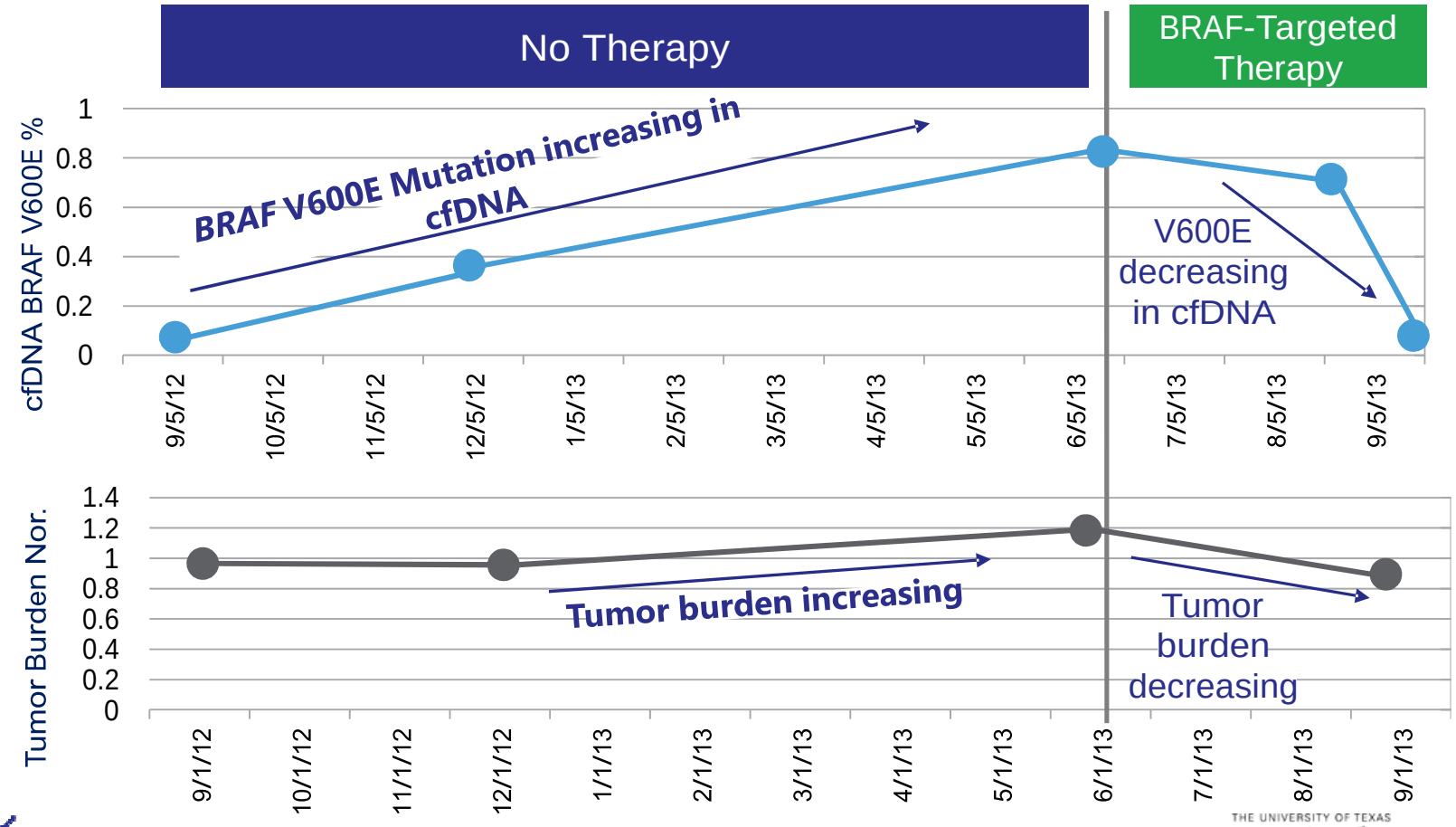
•Less DNA Needed = Less Plasma = Less Blood

cfDNA, ICE COLD-PCR and Patient Monitoring: 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.



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Thank You

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