Enhanced Detection of Low-Level DNA Alterations Using Multiplexed ICE COLD-PCR (MX-ICP) Coupled to NGS or ddPCR
Outline

- Tantalizing Tidbits of Data
- How ICE COLD-PCR Works
- MX-ICP Data
- Real Samples
Do You Want More for Less?

- More sensitivity for detecting sequence alterations?
- More potential for your **platforms**?
- More enrichment of ALL alterations in one reaction?
- Less amount of **starting material** to detect 0.01% alterations?
- Less amount of **sample** needed for analysis?
- Less cost for analysis of multiple alterations?

If you answered “YES,” You Need Transgenomic’s ICE COLD-PCR!

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

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**Fold Increase in Sensitivity of Platform for Detection of 0.01% Sequence Alteration in 150 ng starting DNA**

<table>
<thead>
<tr>
<th>Platform</th>
<th>Without ICP Enrichment</th>
<th>With ICP Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanger</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NGS (Ion Torrent PGM, MiSeq)</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td>Droplet Digital PCR (RainDance)</td>
<td>300</td>
<td>600</td>
</tr>
<tr>
<td>Droplet Digital PCR (Bio-Rad QX200)</td>
<td>400</td>
<td>600</td>
</tr>
</tbody>
</table>

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*Transgenomic®*

Advancing Personalized Medicine
## Compelling Data on MX-ICP

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

<table>
<thead>
<tr>
<th>Platform</th>
<th>0.05% Alteration in 150 ng DNA</th>
<th>0.01% Alteration in 150 ng DNA</th>
<th>Tested with Transgenomic ICE COLD-PCR Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without MX-ICP</td>
<td>With MX-ICP</td>
<td>Fold Increase in Sensitivity</td>
</tr>
<tr>
<td>Sanger</td>
<td>No</td>
<td>Yes</td>
<td>≥400</td>
</tr>
<tr>
<td>NGS</td>
<td>No</td>
<td>Yes</td>
<td>≥100</td>
</tr>
<tr>
<td>ddPCR RainDance</td>
<td>Yes</td>
<td>Yes</td>
<td>≥100</td>
</tr>
<tr>
<td>ddPCR Bio-Rad QX200</td>
<td>Yes</td>
<td>Yes</td>
<td>≥100</td>
</tr>
<tr>
<td>Pyro – sequencing</td>
<td>No</td>
<td>Yes</td>
<td>≥200</td>
</tr>
</tbody>
</table>

MX-ICP Enables Improved Sensitivity for Detection of Alterations in Nucleic Acids

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

Traditional PCR

MX-ICP

![Graph showing Traditional PCR and MX-ICP](image)
How MX-ICP Works

- MX-ICP technology preferentially enriches DNA sequences that contain nucleotide alterations.
- Enriched amplification of altered sequences is achieved by the use of an oligonucleotide complementary to the wild-type sequence (RS-oligo).
- This RS-oligo inhibits PCR amplification of wild-type sequences while allowing amplification of DNA containing an alteration in the RS-oligo region.

**Flowchart of MX-ICP Process**

1. **Denature**
   - AS → WT
   - 95°C

2. **Reduce Temperature: Cross-Hybridize**
   - AS → WT
   - RS → RS

3. **Selectively Denature Mismatched Sequences**
   - WT → WT
   - RS → RS

4. **Reduce Temperature: Primer Annealing to Both Strands of AS But Only One Strand of WT**
   - WT → WT
   - RS → RS
   - Tc

5. **Amplification**
   - AS → AS
   - AS → AS

**Legend**

- **AS** – Altered Sequence
- **WT** – Wild-Type
- **RS** – Reference Sequence Oligo
- **Tc** – Critical Temperature

**Complete MX-ICP Cycles & transfer to DNA Analysis Platform**
The MX-ICP Laboratory Workflow Integration

- Easily integrates on any platform
- Replaces traditional PCR
MX-ICP is Flexible

Multiple Sample Types

Non-invasive samples
- Blood
  - cfDNA
  - CTCs
- Urine
- Sputum
- Bronchial lavage

Tissue Samples
- FFPE
- FNAs
- Fresh/Frozen

Any Platform
- PCR
- DHPLC
- Digital PCR
- Pyrosequencing
- Sanger
- NGS
- NGS
- COBAS
- BEAMing
- HRM
MX-PCR Reproducible Detection Using Digitally Verified Mixes of Cell lines

Two groups of cell line mixtures from Horizon Discovery
Group 1 (G1) contains 5 alterations plus PIK3CA H1047R and EGFR G719S
Group 2 (G2) contains 5 alterations not in Group 1 plus PIK3CA H1047R and EGFR G719S

- Consistent detection of alterations from digitally verified DNA mixed containing multiple alterations
- Reproducible pre-amplification using Transgenomic’s MX-PCR
Standard Curves: Calculation of Percent Alteration of Input DNA

LOD Average for EGFR Exon 18 G719S Cell line Mixtures:
SW48 (G719S) + K562 (wild-type)

LOD Average for EGFR Exon 12 S492R Cell line Mixtures:
Horizon Discovery + K562 (wild-type)

The linear regression equations for the amplicons can be used to determine the amount of the alteration present in the starting sample.
### MX-ICP plus Sanger Produces Mutation Detection at Sensitivities <0.01% EGFR Exon 19 Deletions

<table>
<thead>
<tr>
<th>Input Mut %</th>
<th>Standard PCR, M13-Based Sequencing</th>
<th>Output</th>
<th>Input Mut %</th>
<th>ICE COLD-PCR w/Sanger</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% del</td>
<td><img src="image1.png" alt="Graph" /></td>
<td>WT</td>
<td>1% del</td>
<td><img src="image2.png" alt="Graph" /></td>
<td>100% del</td>
</tr>
<tr>
<td>0.1% del</td>
<td><img src="image3.png" alt="Graph" /></td>
<td>WT</td>
<td>0.1% del</td>
<td><img src="image4.png" alt="Graph" /></td>
<td>95% del</td>
</tr>
<tr>
<td>0.01% del</td>
<td><img src="image5.png" alt="Graph" /></td>
<td>WT</td>
<td>0.01% del</td>
<td><img src="image6.png" alt="Graph" /></td>
<td>60% del</td>
</tr>
<tr>
<td>WT</td>
<td><img src="image7.png" alt="Graph" /></td>
<td>WT</td>
<td>WT</td>
<td><img src="image8.png" alt="Graph" /></td>
<td>WT</td>
</tr>
</tbody>
</table>
Level of Detection of MX-ICP: VariantCaller - EGFR Exons 18 and 20; IGV- EGFR Exon 19 Deletion

MX-ICP Easily Detects Nucleotide Alterations at 0.01% (150 ng of DNA in starting sample)

<table>
<thead>
<tr>
<th>Mutation Proportion of Initial Sample for MX-ICP Enrichment</th>
<th>Percent Alteration Detected After NGS Analysis of MX-ICP</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% alteration</td>
<td>92.6%</td>
</tr>
<tr>
<td>5% alteration</td>
<td>88.9%</td>
</tr>
<tr>
<td>1% alteration</td>
<td>74.3%</td>
</tr>
<tr>
<td>0.5% alteration</td>
<td>58.1%</td>
</tr>
<tr>
<td>0.1% alteration</td>
<td>33.7%</td>
</tr>
<tr>
<td>0.05% alteration</td>
<td>22.6%</td>
</tr>
<tr>
<td>0.01% alteration</td>
<td>7.5%</td>
</tr>
<tr>
<td>0.005% alteration</td>
<td>2.0%</td>
</tr>
</tbody>
</table>
MX-ICP plus Next-Gen Sequencing (NGS): Ion Torrent and MiSeq

Detects Mutations that Are Not Detected by NGS Alone

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

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

- 0.1% T790M
  - 500,000 copies input: 14% Altered Sequence
  - 50,000 copies input: 13% Altered Sequence

- 0.01% T790M
  - 500,000 copies input: 1% Altered Sequence
  - 50,000 copies input: 2% Altered Sequence

WT = Wild-Type droplets; AS = Droplets containing Altered Sequences; WT+AS = dual occupancy droplets; E = Empty droplets

- ≤150 ng of input DNA + MX-ICP facilitates ddPCR analysis using RainDance
- MX-ICP + RainDance makes feasible analysis of low-level alterations in cfDNA
ddPCR Data: Bio-Rad QX200

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

<table>
<thead>
<tr>
<th>Input Copies</th>
<th>0.1% T790M</th>
<th>0.01% T790M</th>
</tr>
</thead>
<tbody>
<tr>
<td>86,500</td>
<td>AS</td>
<td>WT + AS</td>
</tr>
<tr>
<td>8,650</td>
<td>AS</td>
<td>WT + AS</td>
</tr>
<tr>
<td>865</td>
<td>AS</td>
<td>WT + AS</td>
</tr>
<tr>
<td>22% Altered Sequence</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Input Copies</th>
<th>109,000</th>
<th>10,900</th>
<th>1,090</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AS</td>
<td>WT + AS</td>
<td>AS</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>WT</td>
<td>E</td>
</tr>
<tr>
<td>25% Altered Sequence</td>
<td>7% Altered Sequence</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

WT = Wild-Type droplets; AS = Droplets containing Altered Sequences; WT + AS = dual occupancy droplets; E = Empty droplets

• ≤150 ng of input DNA + MX-ICP facilitates ddPCR analysis using the BioRad QX200
• MX-ICP + QX200 makes feasible analysis of low-level alterations in cfDNA
MX-ICP: Transformative Technology

Enables comprehensive tumor analysis of **ALL** alterations in multiple genes from **PLASMA** samples or **TISSUES**, at the levels of sensitivity that make possible detection and monitoring of cancer tumor markers.
Examples of Longitudinal Assessment of BRAF Mutations in cfDNA from Patient Plasma

ICE COLD-PCR: A Valid Assay for Patient Monitoring and Surveillance
Phase I: cfDNA samples = tumor tissue surrogate

<table>
<thead>
<tr>
<th>Tissue Surrogate</th>
<th>Patient</th>
<th>Disease</th>
<th>Tissue Mutation</th>
<th>Treatment Prior to Baseline cfDNA</th>
<th>Baseline cfDNA Mutations</th>
<th>Follow-up cfDNA Mutation</th>
<th>Treatment Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Papillary Thyroid Cancer</td>
<td>BRAF V600E</td>
<td>No</td>
<td>BRAF V600E</td>
<td>Wild Type</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Melanoma</td>
<td>BRAF V600E</td>
<td>No</td>
<td>BRAF V600E</td>
<td>Wild Type</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Melanoma</td>
<td>BRAF V600E</td>
<td>Yes</td>
<td>Wild Type</td>
<td>BRAF V600E</td>
<td>PD</td>
<td></td>
</tr>
</tbody>
</table>
Monitoring Nucleotide Alterations in cfDNA from a Patient: Example of Effective and Improved Treatment

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

No Therapy

BRAF-Targeted Therapy

BRAF V600E alteration increasing in cfDNA

V600E decreasing in cfDNA

Tumor burden increasing

Tumor burden decreasing
MX-ICP Enhances Cancer Detection & Monitoring

Delivers Unique Non Allele-Specific Amplification

- Detection sensitivity in ranges necessary for non-invasive cancer testing by liquid biopsies
  - Any Biofluid or Tissue

- Allows detection of:
  - ALL DNA/RNA alterations (not allele-specific)

- Improves detection when utilized with all widely used genetic testing platforms (e.g. NGS, Sanger, Digital PCR)

- Simple implementation at minimal cost.
MX-ICP: Wide Range of Uses

Pharma/biotech
- Tailored services to:
  - Detect DNA alterations at preclinical stage
  - Correlation of low-level DNA alterations with response
  - Development of companion diagnostics

Clinical Diagnostic
- Customized:
  - Development of diagnostics
  - Enhanced sensitivity
  - Add into current workflow

Academics
- Delivery of:
  - Rapid detection of all alterations using current platforms
  - Identification of all low-level DNA alterations
  - Acceleration of research that enables advances in oncology and patient care

Clinical Oncologists
- Rapid development of:
  - Improvements in patient care
  - Identification of all relevant DNA alterations using currently validated platforms,
  - Implementation of plasma/serum oncology assays

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**Horizon Discovery**

**RainDance**

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Questions?