The BioTrove OpenArray™ NT Cycler

‘A system to banish the curses of too many genes & too few samples’
3072 through-holes per OpenArray plate are arranged in 48 subarrays.

Each subarray consists of 64 through-holes in an 8x8 pattern.
Coatings enable reagents to load into and stay within the bottomless through-holes via passive capillary action.
BioTrove deposits your assays in the OpenArrays

BioTrove sends a kit with OpenArray loading materials and Arrays to your lab

Load your samples with Mix onto the OpenArrays

Cycle and image OpenArrays

Results!
OpenArray Process Overview

AutoLoader for Sample Loading
OpenArray Process Overview

Path of pipette tip over the subarray
OpenArray Process Overview

Placing loaded OpenArray into cycling case

- Fill with Immersion Fluid
- Insert Loaded OpenArray into Case
- UV cure glue

Ready for thermal cycling
BioTrove OpenArray™ NT Cycler

Cycling and Imaging

- SYBER
- Hydrolysis probes
- Hybridisation probes

- Absolute quantitation
- Relative quantitation
- Comparative quantitation
Efficient PCR

- 6 logs dynamic range
- Primers pre-loaded
- 56 replicates

- TFR amplicon
- >98% amplification efficiency

Performance against biological relevant sample

Morrison et al; NAR, 34(18) 2006.
Precision

Morrison et al; NAR, 34(18) 2006.

70% Assays have precision <0.5 delta Ct
Correlation with conventional platforms

Morrison et al; NAR, 34(18) 2006

PCR Ar (ΔΔC_T Heart - Liver)

ABI (ΔΔC_T Heart - Liver)

R^2 = 0.98
Nucleic Acids Research Advance Access published online on September 25, 2006
Nucleic Acids Research, doi:10.1093/nar/gkl039
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Methods Online

Nanoliter high throughput quantitative PCR

Tom Morrison, James Hulsey, Javier Garcia, Karl Yoder, Aviv Katz, Douglas Roberts, Jamie Cho, Tanya Kanigan, Sergey E. Iljin1, Daniel Horowitz1, James M. Dixon1 and Colin J.H. Erenman1

BioTrove Inc. 12 Gil Street, Suite 4000, Woburn, MA 01801, USA. 1Johnson & Johnson Pharmaceutical Research & Development LLC, Spring House, PA 19477, USA.

Development and Experimental Validation of a Predictive Threshold Cycle Equation for Quantification of Virulence and Marker Genes by High-Throughput Nanoliter-Volume PCR on the OpenArray Platform†

Robert D. Stedfield1, Samuel W. Baitske1, Dieter M. Touhofou1, Sarah M. Miller1, Tiffany M. Stedfield1, Erdogan Gulbin1, James M. Tioljo2,3 and Syed A. Husheen1,3

Department of Civil and Environmental Engineering,1 Center for Membranal Ecology,1 and Department of Civil and Soil Systems Engineering,2 Michigan State University, East Lansing, Michigan 48824, and Department of Chemical Engineering, University of Michigan, Ann Arbor, Michigan 48109.

Received 5 December 2007; Accepted 12 April 2008.

Toxicogenomics screening of small molecules using high-density, nanocapillary real-time PCR

LAURA VASS1, JÁNOS Z. KELEMEN1, LILIÁNA Z. FEHÉR2, ZSOLT LÖRINCZ3, SÁNDOR KULIN, SÁNDOR CSEH3, GYÖRGY DORMÁN4 and LÁSZLÓ G. PUSKÁS1,2

1Laboratory of Functional Genomics, Biological Research Center of the Hungarian Academy of Sciences, Temesvári krt. 62., 6726 Szeged; 2Avidin Ltd., Közép fasor 52., 6726 Szeged; 3TargetEx Ltd., Kápolna köz 4A., 2120 Dunakeszi; 4Innobios LP, Kondorosi út 80., 1119 Budapest, Hungary.
Real-Time PCR Throughput Example

Labor and Time Savings

• 24,192 real-time PCR reactions
  – 56 real-time PCR tests against 432 samples = 9 OpenArray™ Plates
  – Equivalent to 72 x 384-well microplates or 252 x 96-well microplates

Reagent and Sample Savings

• Compared to 5 uL qPCR reactions (384 well microplate)
  – 64-fold savings on Master Mix
  – 7-20 fold savings on TaqMan® or SYBR Green® reagents
  – 2-4 fold increase in qPCR tests per sample
• Compared to 25 uL qPCR reactions (96 well microplate)
  – 320-fold savings on Master Mix
  – 30-100 fold savings on TaqMan® or SYBR Green® reagents
OpenArray Opportunity Segmentation

Number of Genetic Determinants

- Whole Genome Discovery
  - Hypothesis free
- Hypothesis-driven Discovery
- Validation
- Commercialization
  - Molecular Dx

Number of Specimens

- Custom OpenArrays
- OpenArray Pathways

BioTrove
Flexible OpenArray Layouts

<table>
<thead>
<tr>
<th>Subarray Location of each assay</th>
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<tbody>
<tr>
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<tr>
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</table>

56 assays in each subarray
48 samples on each OpenArray
Flexible feature/sample formats

112 assays
24 samples

168 assays
16 samples

224 assays
12 samples
OpenArray Pathways Products
For Life Science Research

- Comprehensive coverage
  - >550 key genes in each panel
- Simple and convenient to use
  - Add sample and run
- Validated assays from BioTrove
  - RefSeq library reference
- High Throughput
  - 12 specimens per run
  - 9216 real time-PCR answers in 3 hr
- Embedded controls for high precision
  - Each panel has no assay
Pathway and Disease Panels

Signal Transduction: JAK-STAT, NFKB, AKT, GPCR, cAMP and MAP kinase

Cancer: DNA repair, angiogenesis, cell adhesion and ECM, apoptosis, cell cycle and many cancer-specific kinases and transcription factors

Cardiovascular Disease: ECM, NO and oxidative stress pathways, hypoxia, lipid metabolism, platelet activation and apoptosis

ADME: Genes for Phase I and Phase II metabolizing enzymes plus ABC and SLC transporters

Apoptosis: BCL, caspase, TNF, p53 and genes encoding DNA damage proteins

Inflammation: Genes involved in chronic and acute inflammation including chemokines, interleukins, BMP, TNF and B- and T-cell activation proteins

Kinome: Kinase genes, proteins in kinase complexes, and kinase binding molecules
Kinome

Kinase genes, proteins in kinase complexes, and kinase binding molecules

Assays for Reference genes are included allowing easy $\Delta C_T$ and $\Delta \Delta C_T$ calculation
Kinase Cancer Gene Discovery Experiment

- Samples: Matched normal/cancer prostate fresh frozen tissue specimens from 5 males; 10 samples in all.

- BI Chief Pathologist selected tissue section and RNA extracted with Qiagen kit.

- cDNA generated and analyzed on Kinome OpenArray per BioTrove protocol.

- Same samples run on Affy GeneChips U133 A&B at Beth Israel Hospital core lab with no pre-amplification.
For each kinase measurement from a single tumor sample, the plot correlates the microarray normalized fluorescent signal with the OpenArray ΔCT.

Many OpenArray hits (triangles) fall in microarray noise (box).
Conclusion-Take Home

• With same sample prep (no pre amp) on clinical specimens, the OpenArray allows researchers to discover and measure the activity of over 40% more kinase genes than is possible on an Affy microarray.

• This capability could significantly impact our current understanding of disease processes by providing insight into the biological relevance of those genes whose *de novo* expression levels are below the limit of detection of microarray systems.

• The OpenArray is a tool for *de novo* discovery of gene functionality.

“Imagine what you’re missing…”
**TaqMan PCR**
Performance similar to SYBR Green real-time PCR

- **Gene target** = EDNRB
- **Ct precision** = 0.25
- **Amplification efficiency** = 95%
- **Amplicon target**
- Similar precision for cDNA sample

- **Gene target** = CDK4
- **48 replicates**
- **Ct precision** = 0.25
- **200 amplicon copies**
## miRBase Targets Version 5

### List of Genomes Currently Available

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<tr>
<th>Species</th>
<th>Taxonomy</th>
<th>No. miRNAs</th>
<th>No. Transcripts</th>
<th>No. Targets</th>
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Dynamic range
Discrimination of close family members
Unique OpenArray Capability

~900 miR assays in each human miR panel
3 samples per OpenArray
Like a miR microarray
But Quantitative Real-Time Data
Summary

- Precision and accuracy of PCR in a high-density format
- High productivity
- Reliable, Flexible and Simple Workflow
- Cost-Effective with Familiar PCR chemistries
- Multiple Applications
- Pre-validated or customer-specified assay panels
Thank you!