OpenArray® Real-time PCR System

Genotyping
Gene Expression
Digital PCR

Kris Ridley
Senior Technical Sales Specialist
Asia-Pacific

life technologies™
QPCR is changing...
Overview

1. OpenArray Instrument
2. Genotyping and Gene Expression
3. Digital PCR
4. Single-cell gene expression – in development
5. Global miRNA expression profiling – in development
1. OpenArray is:

Designed around MID-DENSITY Realtime PCR:

• Ideal for VALIDATING SNPs or genes of interest identified by Next Gen Seq or microarray studies

• Ideal for LARGE SCALE gene expression studies: throughput beyond 7900/ViiA7 capabilities

<table>
<thead>
<tr>
<th>Benefits of Realtime PCR</th>
<th>Advantages over Microarrays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed</td>
<td>Lower investment</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Less labour</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Higher sample throughput</td>
</tr>
</tbody>
</table>

CONCEPT = REACTION MINIATURISATION...
OpenArray® Plates

- 3072 throughholes: Equivalent to 32 x 96-well plates or 8 x 384-well microplates

### OpenArray Plate Layout

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Subarrays</td>
<td>48 (12 x 4)</td>
</tr>
<tr>
<td>Through-holes</td>
<td>33nl</td>
</tr>
<tr>
<td></td>
<td>64 per subarray (8 x 8)</td>
</tr>
</tbody>
</table>

### Data Points per day with OpenArray

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotyping</td>
<td>➢ 70,000</td>
</tr>
<tr>
<td>Gene Expression</td>
<td>➢ 32,000</td>
</tr>
</tbody>
</table>
Advantages of single-plex, high density QPCR:

QPCR reactions are *isolated* and *independent*

- OpenArray enables multiple, physically separate parallel reactions:
  - No interference
  - Flexibility to add or remove assays without needing to re-optimise
  - Small reaction volume = major cost saving over 384-well QPCR
OpenArray Realtime PCR Platform

NT Cycler
(Realtime instrument)

AccuFill
(automated sample loading)

Computer & Analysis Software

OpenArray® plates & Accessories

OpenArray® Sealing Station
OpenArray® AccuFill™

• **CONVENIENCE**: The user *only* needs to combine samples and master mix then Accufill loads OpenArray plates

• **SPEED**: Loads each plate in less than 2mins

• **CONSISTENCY**: Very little pipetting, less potential for human error
At Life Technologies

Select/design assays

Assays printed to plate

Load samples with Accufill

Seal plate

Shipping

Cycle and Image

Collect Results!
2. The Primary Applications for OpenArray are in Mid-Density

SNP Genotyping  Gene Expression
What really is Mid-Density GT/GX?

Data points =

- Prospective projects should be at least **25,000 - 30,000** data points (genotypes or expression amplification curves) to be an appropriate ‘fit’ for the OpenArray® system.

- Ideal for profiling 10s-100s pre-defined target genes or SNPs from >1000 samples

- **Mid-density also makes Digital PCR commercially viable due to multiple miniaturised reactions (more later...)**
The Life Technologies Genomics Workflow Solution

NGS

Mid-density array

Validation

Standard QPCR

Screening

Capillary sequencing

CE/SOLiD™

Ion Torrent™

OpenArray™

ViiA™7

3500Dx

Discovery

Molecular Medicine

Number of Samples

Small

100s

1000s

Projects

16

Large

Number of SNPs or Genes

Small

Large

Scalable
TaqMan® OpenArray® SNP Genotyping

For validating SNPs discovered by Next-Gen sequencing or hybridisation arrays:

Major cost benefits realised at 10s-100s SNPs vs > 1000 samples

TaqMan SNP Genotyping Assays:

• *Two differentially-labelled, allele-specific probes*
  
  – Probes compete for target SNP during realtime PCR
  – Relative abundance of homozygotes:heterozygotes determined by plotting VIC vs FAM

Over 73,000 genotypes per day
**TaqMan® SNP Genotyping Assays**

**Pre-designed**

- **4.5 million pre-designed HUMAN SNP assays**, for high density genome-wide coverage (70% HapMap), >160,000 wet chemistry validated
- > 70,000 coding SNP assays, including many putative functional SNPs
- > 2,600 Drug Metabolism SNP assays for 220 drug metabolism and transporter genes
- >10,000 MOUSE SNP Assays (mapping, QC breeding, speed congenics etc)

**Custom**

- Ideal for uncommon organisms
- Simple online tool
- Stringent design algorithm
- Instant feedback pass/fail
Suitable Genotyping Projects

• Bad Fit:
  – < 16 SNPs
  – TaqMan® single tube assays
  – Small studies (< 1000 samples)
  – Not looking to increase #SNPs or samples

  Better stick to Conventional (96/384) QPCR

• TaqMan® OpenArray® SNP Genotyping
  – 16 – 256 SNPs (or more if spread over multiple arrays)
  – 1000 to > x1000’s of samples
  – Larger validation studies – Great for follow-up on Genome-wide Association Study data
Example: OpenArray SNP Genotyping in cancer study

Interleukin and interleukin receptor gene polymorphisms and susceptibility to melanoma

Fangyi Gu\textsuperscript{1,2,*}, Abrar A. Qureshi\textsuperscript{3,4}, Tianhua Niu\textsuperscript{1,2}, Peter Kraft\textsuperscript{1,2,5}, Qun Guo\textsuperscript{2,3}, David J. Hunter\textsuperscript{1,2,3}, and Jiali Han\textsuperscript{2,3}


- Used OpenArray to validate SNPs associated with melanoma susceptibility
- 25 SNPs from 5 interleukin/interleukin receptor genes vs 438 samples (219 melanoma, 219 control)
- Identified 4 SNPs within IL-6R associated with melanoma
  - 3 intron SNPs – one located in intron 1 at a predicted TF binding site
  - 1 non-synonymous SNP in exon 9 – predicted to cause protein structure change

\textit{OpenArray validated 25 targets – 2 were considered for further study!}
OpenArray Genotyping Application: Summary

• Main advantages:
  – Greater GT throughput than conventional QPCR/CE
  – More accurate and sensitive than hybridisation arrays

• Simple workflow: Load sample > Cycle > Image

• Reliable chemistry from TaqMan MGB probes and powerful data analysis

• Flexible plate formats for project diversity (sample:SNP ratio)

• Easy assay design and ordering with no hidden costs
  – Just add sample and master mix
Recent Genotyping References

2010

OpenArray® Gene Expression

• High throughput Realtime PCR
• Ideal for VERIFICATION of NGS transcriptome studies
• >32,000 expression profiles per day
• 10X faster than 384-well Fast qPCR
OpenArray User-defined Gene Expression Assays

*Researcher defines the content of assays on the OpenArray plate*

Inventoried TaqMan Gene Expression Assays:

<table>
<thead>
<tr>
<th>Species</th>
<th>Assay Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human (&gt;99% genome)</td>
<td>29,222</td>
</tr>
<tr>
<td>Mouse</td>
<td>19,100</td>
</tr>
<tr>
<td>Rat</td>
<td>10,862</td>
</tr>
<tr>
<td>Other model species (15)</td>
<td>2,118</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>61,302</strong></td>
</tr>
</tbody>
</table>

Or **Custom Design** an assay for **ANY GENE** from **ANY ORGANISM**
Example: Custom SYBR Green Gene Expression for Toxicogenomics Screening

**The OpenArray Plate**
- Custom OpenArray Plate containing user-defined SYBR® Green assays
- 56 toxicogenomic marker genes selected
- 48 samples screened per plate

**The Study**
- Samples were HepG2 cells exposed to 625 distinct compounds
- Hepatotoxic response quantified by gene expression changes in 56 toxicogenomic biomarkers for every sample
- Single screen involved nearly 35,000 Real-Time PCR measurements
- Sample prep took 4 weeks
- **14 OpenArray Plates run in just 4 days** (110 x 384-well plates, 240 hours estimate)
**Toxicogenomics Screening**

**Results**

- Authors identified compounds that were *structurally similar* but induced *different* hepatoxic responses, and compounds that were *structurally dissimilar*, but produced *similar* hepatoxic responses – **targets for further study!**
Authors reported key advantages of OpenArray

- **OpenArray** enables a large number of samples to be run against a large number of genes, thus avoiding the “two culprits of toxicogenomics: ‘the curse of dimensionality’ (too many genes), and ‘the curse of dataset sparsity’ (too few samples)”

- **Superior** analytic performance compared to microarrays where noisy data leads to false results.

- Short time to results

OpenArray enables large scale Gene Expression projects to be run in a fraction of the time and at a fraction of the cost of conventional real-time PCR
OpenArray for Gene Expression - Summary

More samples & More assays in Less time & with Less cost

• Simple workflow
  – preloaded with assays—just add sample and cycle

• Format flexibility for Genomics
  From Discovery (many assays, few samples)...
  To Screening (few assays, many samples)

• Detection sensitivity comparable to 7900/ViiA7

• Only difference is lower DYNAMIC RANGE (6 vs 9 logs) due to effect of reaction volume. Still, 2 logs better than hybridisation arrays
Recent Gene Expression references

2010


2009


2008

Current Applications

Gene Expression  Toxicogenomics
SNP Genotyping  Pathogen detection
Target Validation  Biomarker Discovery

Micro RNA profiling...
Digital PCR (now)

Single-cell analysis...

New Applications for 2011
3. Digital PCR
What is Digital PCR (dPCR)??!

- **PCR**: Semi-quantitative
  - **qPCR**: Relatively-quantitative
    - **dPCR**: ABSOLUTELY QUANTIATIVE!
The quantitative discriminatory power of dPCR

How many beans are in the jar?

Analog (qPCR)

Answer: same # as in reference jar (relative)

Digital

Answer: 23 yellow, 45 red, 16 green (discriminatory and absolute)
What is Digital PCR (dPCR)?

Digital PCR takes QPCR and gives it **ABSOLUTE QUANTIFICATION**

<table>
<thead>
<tr>
<th>qPCR</th>
<th>Digital PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Answer: Ct, D Ct, or DDCt,</td>
<td>Answer: copies / uL</td>
</tr>
<tr>
<td>Relative</td>
<td>Absolute</td>
</tr>
<tr>
<td>Depends on endogenous control used</td>
<td>Absolute – no controls needed*</td>
</tr>
<tr>
<td>Depends on detection chemistry (TaqMan, SYBR)</td>
<td>Absolute – independent of chemistry</td>
</tr>
<tr>
<td>Depends on instrument used (vendor, model, S/N, light source, detection filter, cycling mode)</td>
<td>Absolute – independent of instrument</td>
</tr>
<tr>
<td>Depends on assay efficiency</td>
<td>Absolute – independent of assay efficiency</td>
</tr>
</tbody>
</table>

* For some applications, controls to normalize for sample loading may be desired
What can you do with Digital PCR?

• Single-cell studies of transcript copy number
• Rare transcript/allele detection
• Low-fold (n>4) Copy Number Variation (as compared to duplex qPCR)
• Isolating heterogenous mixtures of alleles
  • Template absolute quantification prior to Next-Generation Sequencing
  • Quantifying low level pathogens (ie. plasma viral load)
• Nucleic acid standard measurements
Digital PCR Video...
Digital PCR Overview

Based on PCR amplification of **single** template molecules/reaction

Count number of positive reactions

**Sample Prep** → **Dilution** → **Distribution** → **Reaction** → **Readout**

**Description:**
- Isolate nucleic acid starting material for analysis
- Dilute DNA to achieve a single copy of template per reaction once distributed
- Distribute DNA into multiple reaction vessels
- Perform PCR reactions to amplify single template molecules
- Determine the number of template molecules present

Illustration:
- Blood/tissue/cell sample → DNA → Dilute DNA → Distribute DNA → Target DNA Molecule → Reaction vessel → Positive/ Negative PCR Reaction → Readout
OpenArray® Digital PCR Workflow

Customer sample

New Digital PCR Products

dPCR Master Mix

TaqMan® assay

OpenArray® Digital PCR Plates

OA Real-Time PCR System

Primary analysis

AccuFill (or AutoLoader I)

OpenArray® Digital PCR Software

Digital PCR answer: # copies / uL
## OpenArray Digital PCR Customer Test Sites

<table>
<thead>
<tr>
<th>Customer Test Site</th>
<th>Application</th>
<th>Statement of Work Summary</th>
<th>Test Site Agreement complete</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>(US) National Institute of Standards and Technology</td>
<td>Nucleic acid standards for viral load &amp; GMO quantitation</td>
<td>NIST will quantify # copies for cytomegalovirus (CMV), a certified reference plasmid, and maize reference material; will compare results to Fluidigm</td>
<td>Y</td>
<td>Generating data</td>
</tr>
<tr>
<td>TATAA Biocentre</td>
<td>Single cell (circulating tumor cells) &amp; copy number variation</td>
<td>TATAA will quantify expression from each allele of single astrocyte cells from brain; will measure CNV of HER2 oncogene in circulating tumor cells from patient blood</td>
<td>Y</td>
<td>Generating data</td>
</tr>
<tr>
<td>U Miami</td>
<td>NGS quantitation (SOLiD)</td>
<td>U Miami will compare performance, workflow, and cost to existing solution (qPCR with Roche UPL probes) for NGS targeted sequencing and RNAseq</td>
<td>In process</td>
<td>Pending customer legal sign-off</td>
</tr>
<tr>
<td>U Georgia</td>
<td>NGS quantitation (454)</td>
<td>UGA will compare performance, workflow, and cost to existing solution (nanodrop + 4 dilutions and small-scale emulsion PCR preps)</td>
<td>Y</td>
<td>Generating data</td>
</tr>
<tr>
<td>U Calgary</td>
<td>Heterogeneous expression from single stem cells</td>
<td>Calgary will measure expression of two targets across cell types at different stages of differentiation (undifferentiated, Erythroid differentiated, and Myeloid differentiated), repeated in 5-10 cells of each differentiation state.</td>
<td>Y</td>
<td>Generating data</td>
</tr>
</tbody>
</table>
Think of OpenArray as having **3 Applications**

1. SNP Genotyping

   ![SNP Genotyping Graph]

2. Gene Expression

   ![Gene Expression Graph]

3. Digital PCR

   ![Digital PCR Image]
Summary

• Higher throughput research is enabled by higher throughput technologies

• QCR is also heading towards higher throughput – OpenArray is the beginning...

• OpenArray applications are driven by lowering the cost per reaction due to nanolitre volumes in a convenient, easy to use system

• OpenArray enables QPCR studies to provide the BIGGER picture (GT/GX) or FINER detail (dPCR)
Questions?

Kris Ridley
Sr Technical Sales Specialist – OpenArray
Asia-Pacific

kristian.ridley@lifetech.com