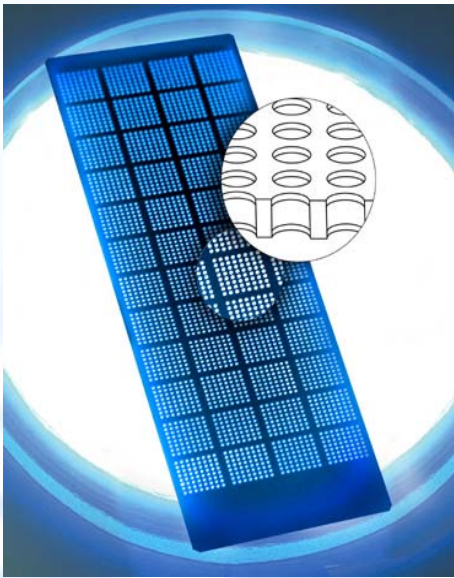


# Massively Parallel Nanofluidic Systems and their Applications

Colin J.H. Brenan, Ph.D.  
Chief Technology Officer  
BioTrove Inc.



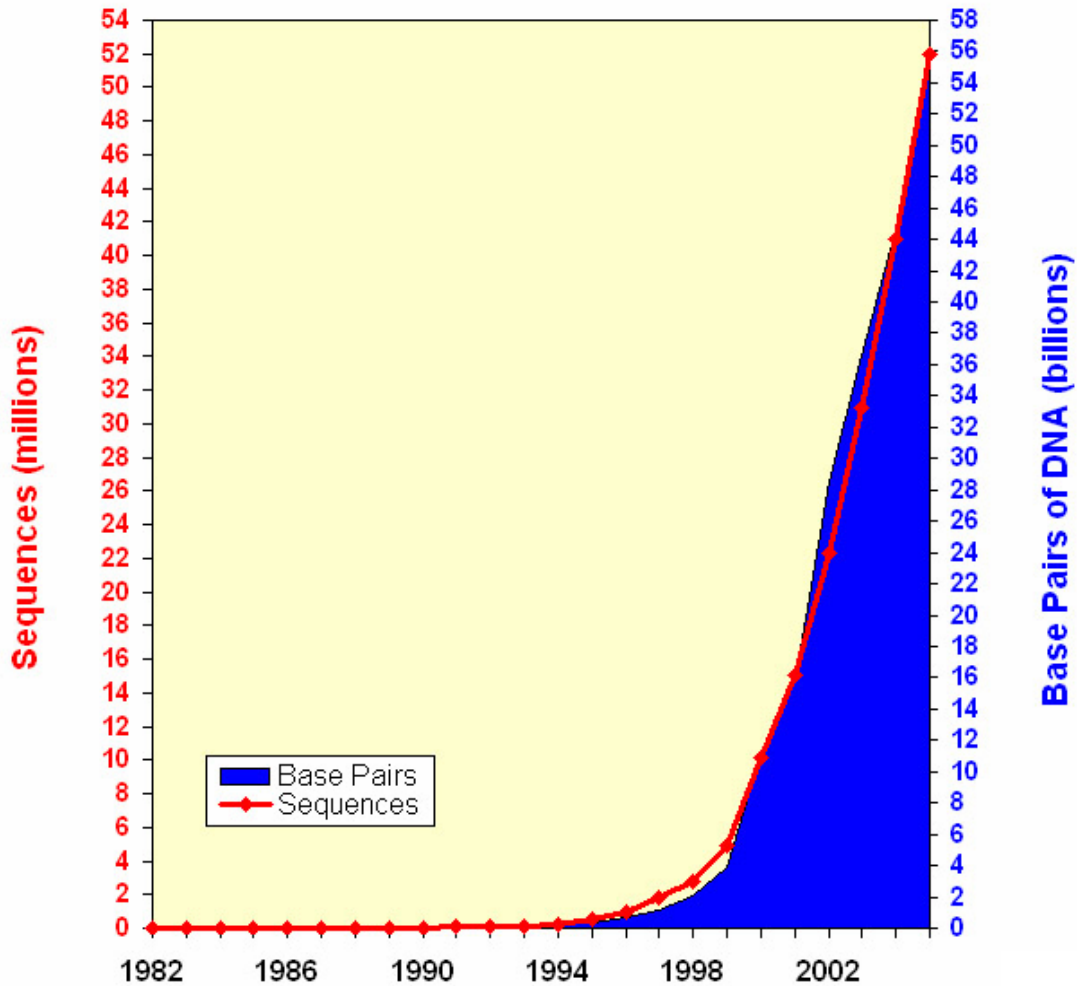
3<sup>rd</sup> qPCR Symposium  
Freising, Germany  
March 28, 2007

# Outline

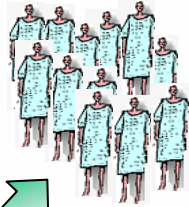
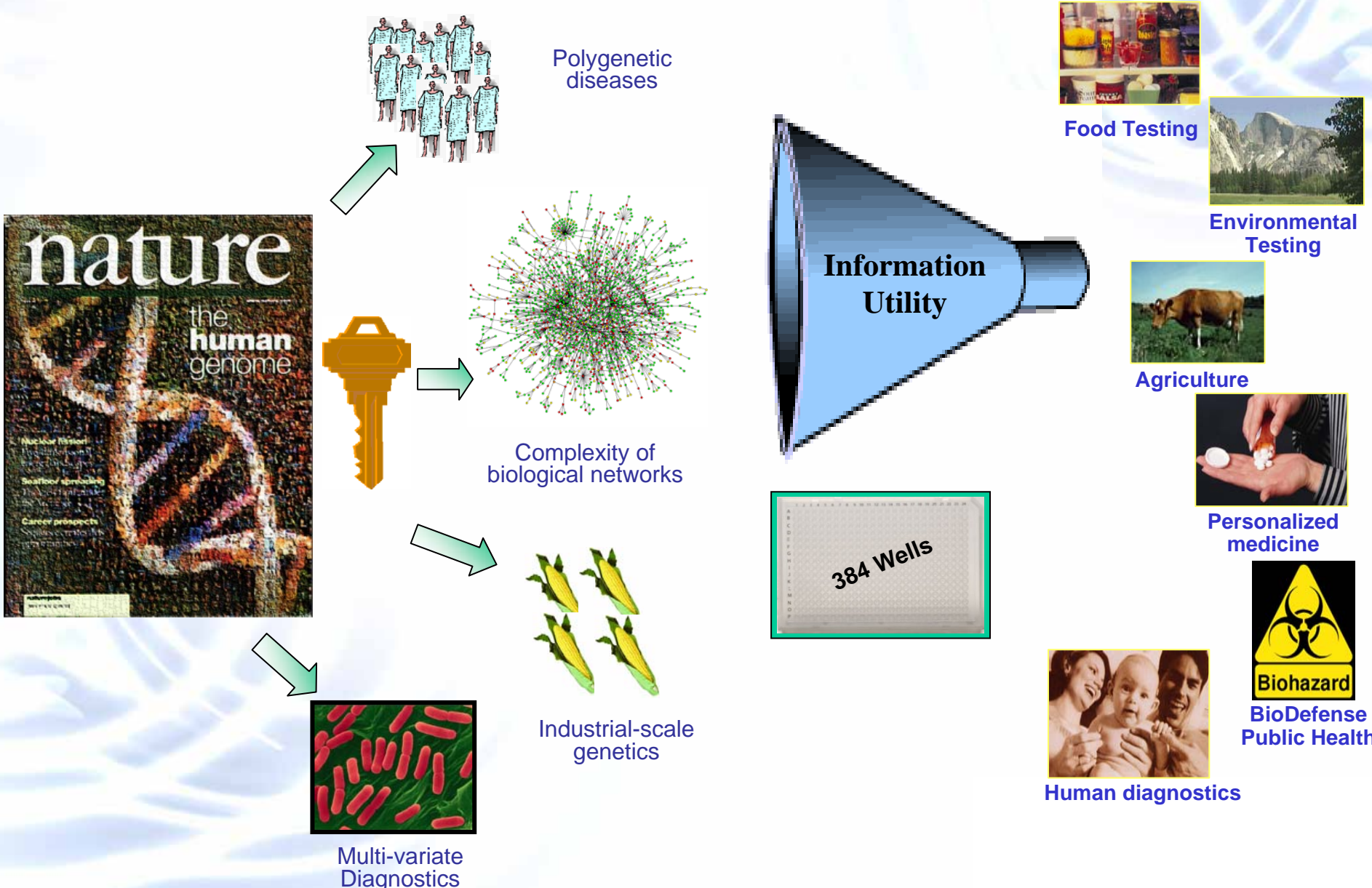
- Motivation and Opportunity
- Technology overview
- Applications
  - End point PCR
    - SNP Genotyping
    - Pathogen Detection
  - Real-time PCR
    - Gene expression
- Summary

# The explosive growth in genomic information...

## Growth of GenBank (1982 - 2005)



# Has greatly exceeded our capacity to utilize it



Polygenetic diseases



Food Testing



Environmental Testing



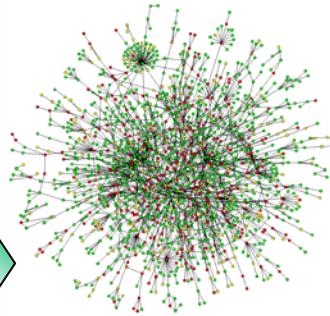
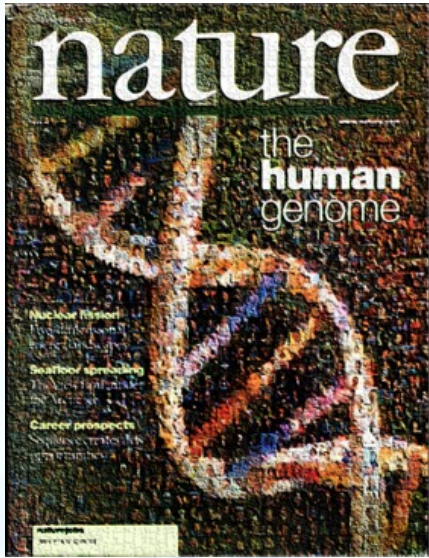
Agriculture



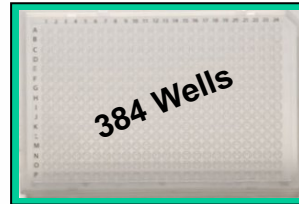
Personalized medicine



BioDefense Public Health



Complexity of biological networks



384 Wells



Industrial-scale genetics

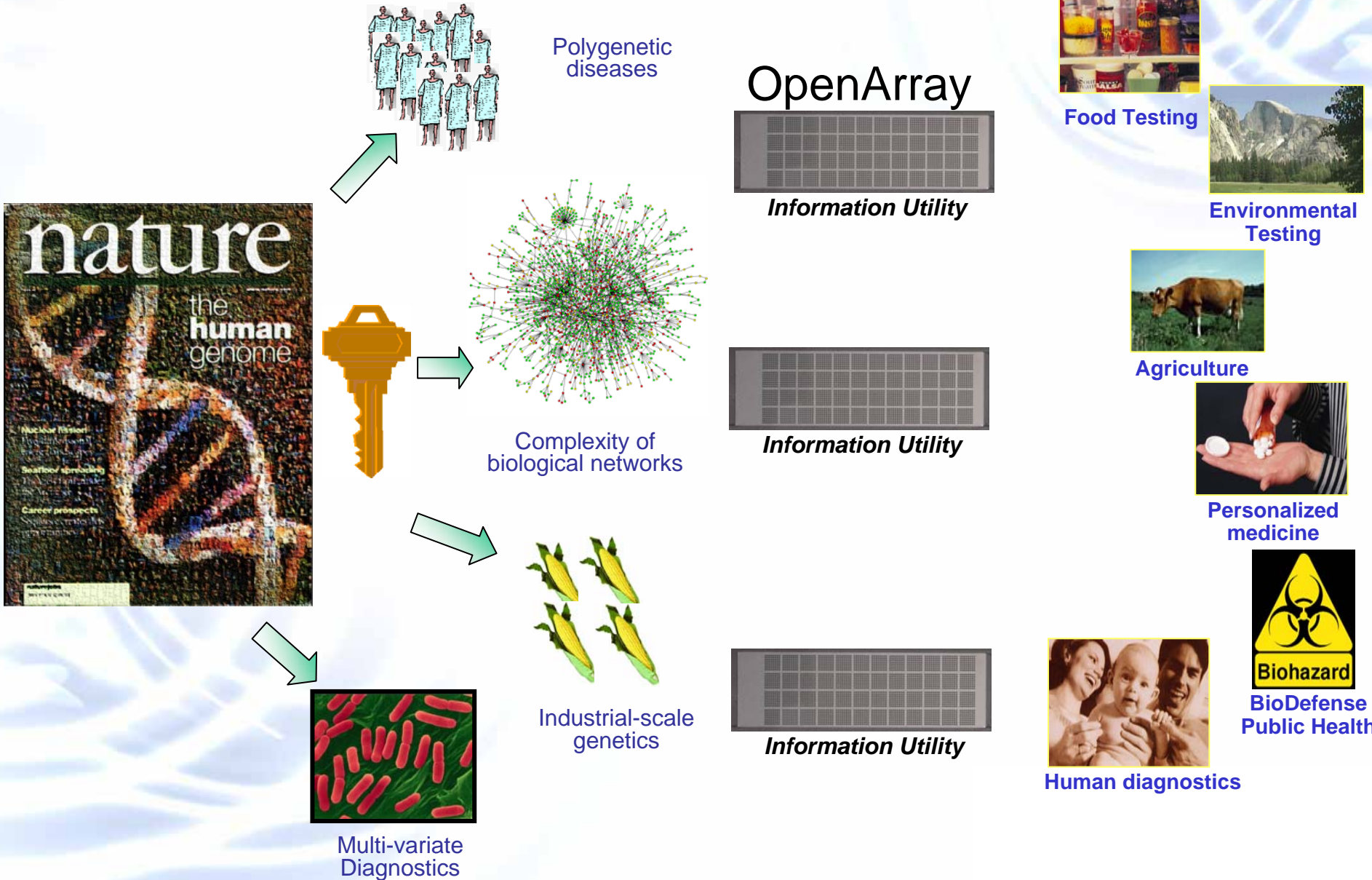


Multi-variate Diagnostics



Human diagnostics

# Has greatly exceeded our capacity to utilize it



# Opportunities for Nanofluidic Systems

- Enable complex multivariate analyses to be run in a simple and affordable manner
  - Solution phase reactions
- Benefits
  - Higher analytical throughput
  - Lower costs
  - Application Flexibility
  - Simple to use
    - Fits existing lab workflow
  - High quality information
- Time value of information
  - Decreased time to decision
  - Faster time to market

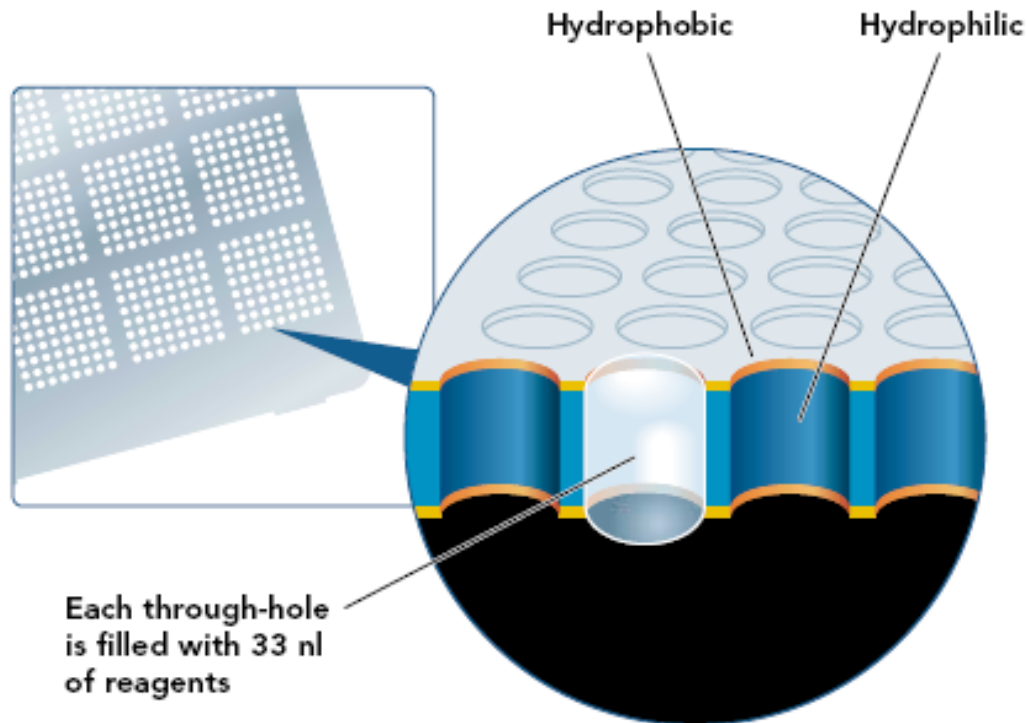
# Nanofluidic Design Challenges

- **Interface with “outside world” (e.g. microplates)**
- **Equivalence to macroscale performance**
- Evaporation at small scales.
- Loss of assay sensitivity.
- Bio-incompatibility of materials.
- Fouling and degradation of coatings.
- Dispensing Problems – gas bubbles, vapor lock.
- Clogging of needles.
- Dead dispensing volumes (e.g. Piezo)
- Complex, unreliable robotics.
- Integration with macroscale fluidic systems.

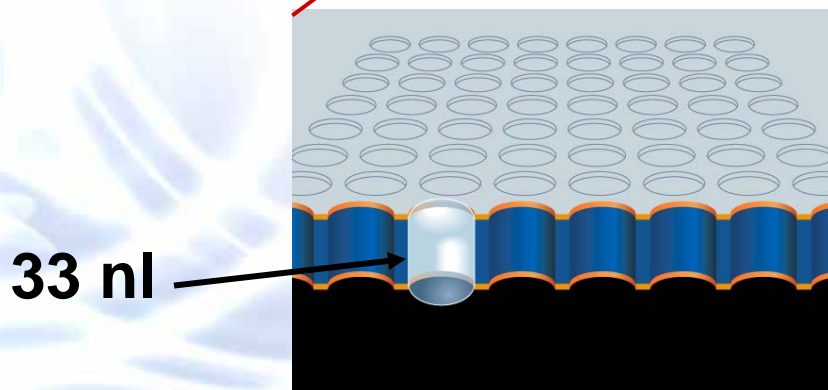
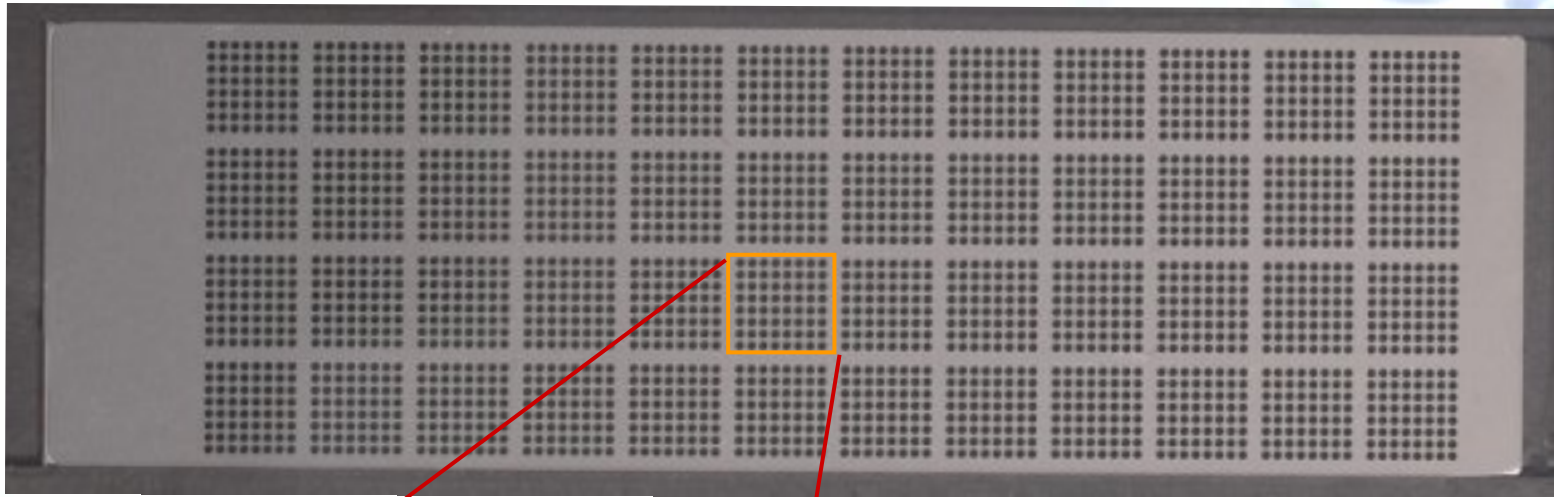


# OpenArray™ Nanotiter Plates

A nanotiter plate is a **differentially coated self-metering** through-hole array for containing and manipulating nanoliter-scale volumes of fluids



# OpenArray Anatomy



**Cross-section**

- **3,072 through-holes**
- **48 X 64 subarrays**
- **33 nL/reaction**

# OpenArray Benefits

- High throughput. Each OpenArray can run 3072 assays in parallel.
- Low volume. Each through-hole requires only 33-nL.
- Flexible format. Numerous sample vs. assay combinations possible
- Simple workflow. OpenArray™ plates are preloaded with assay reagents. Researchers load samples using easy techniques.
- Quick time-to-results. Samples can be loaded, cycled, imaged, analyzed single day



5' Exonuclease assays for endpoint applications  
or  
Primer sequences for Real-time qPCR

Customer →



→ BioTrove

1	A	1	Assay A	TGGAGCATCCCTGCACAAGT	Desalt
1	B	1	Assay B	GCACTCAACGGAGCCTTCT	Desalt
1	C	1	Assay C	ACCCGAGAGAAAAGCTCTGG	Desalt
1	D	1	Assay D	GATGGAGCGCAGCATAACCA	Desalt
1	E	1	Assay E	TGGGTGTGGCATCTCACAAA	Desalt
1	F	1	Assay F	AGCTCGATGGTGGTCATGGA	Desalt
1	G	1	Assay G	TCCTCTCCACCCCATCCTC	Desalt
1	H	1	Assay H	CCAGACTGTGGAGGGGTCT	Desalt
1	A	2	Assay I	CTCCGAAATTGCTGTGGAC	Desalt
1	B	2	Assay J	CCAAAACAACCCCCATCA	Desalt
1	C	2	Assay M	ATGATTGGCAGGGAGCCTA	Desalt
1	D	2	Assay N	TTCCAGCTGTCTGCAGTC	Desalt
1	E	2	Assay O	GCCTGGGACCTGCAATAA	Desalt
1	F	2	Assay P	TTCTGCCACACAGGGAATG	Desalt
1	G	2	Assay Q	CCTCCAGACCAGGGCATAGG	Desalt
1	H	2	Assay R	CCAGCCTCCAGGGAAGAGAC	Desalt
1	A	3	Assay S	GCGACTGTGCTCTCCAC	Desalt
1	B	3	Assay T	GGCAGGGTCTGAAGCAGAT	Desalt

## Pre-loaded OpenArray Plates



# Customer

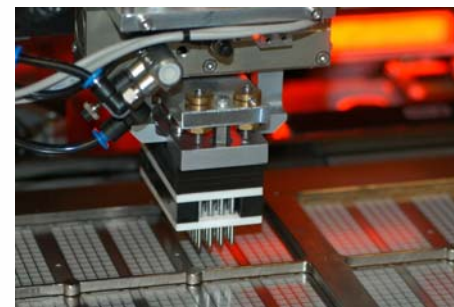
Store at -20°C



## Consumable Kit

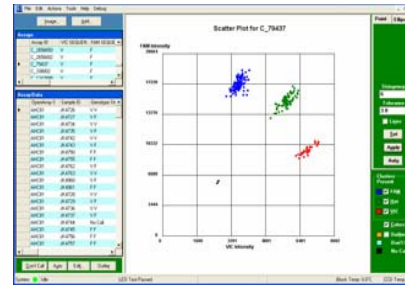
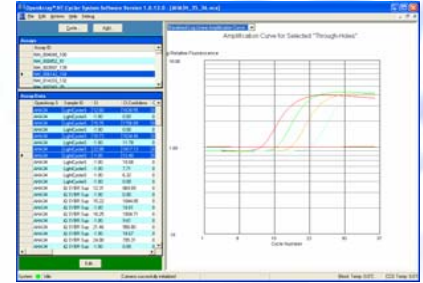
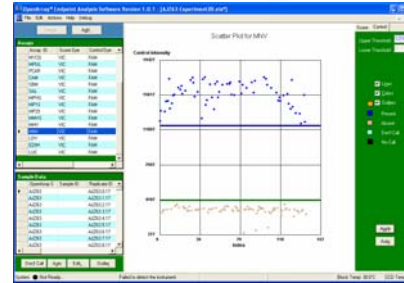
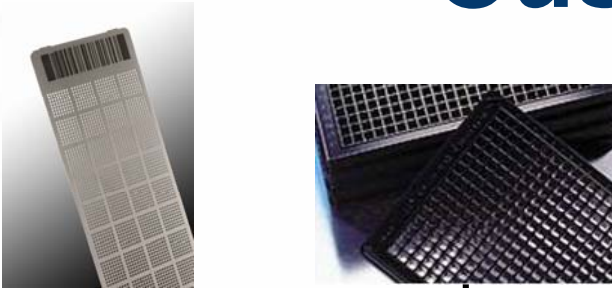


# BioTrove



# BioTrove

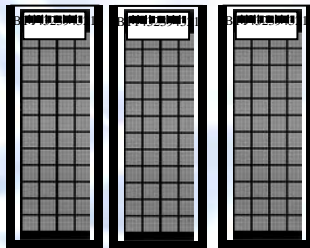
# Customer Workflow



Data analysis  
in OpenArray  
Software



OpenArray plate loading



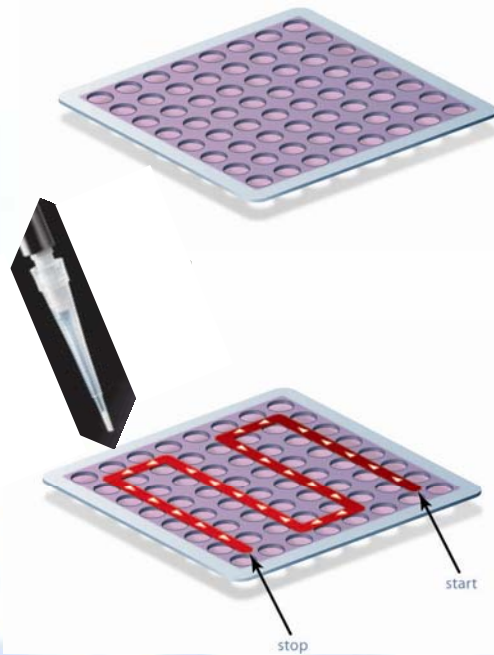
Insert and seal in a  
transparent cassette



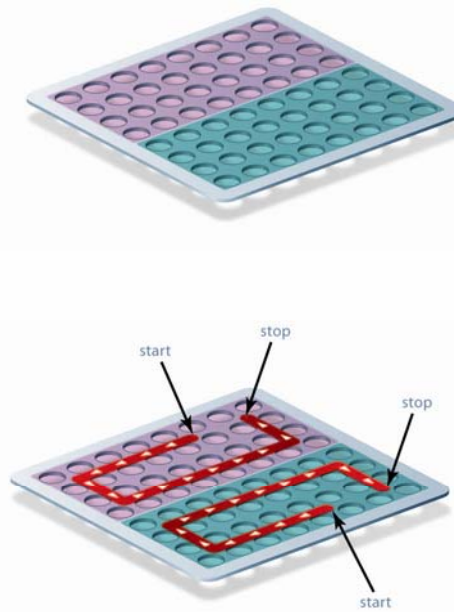
Cycling and Imaging

# Sample Loading

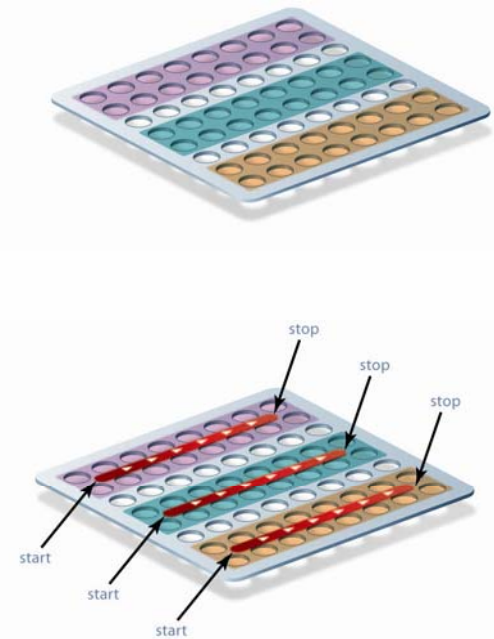
1 Template/SA



2 Templates/SA



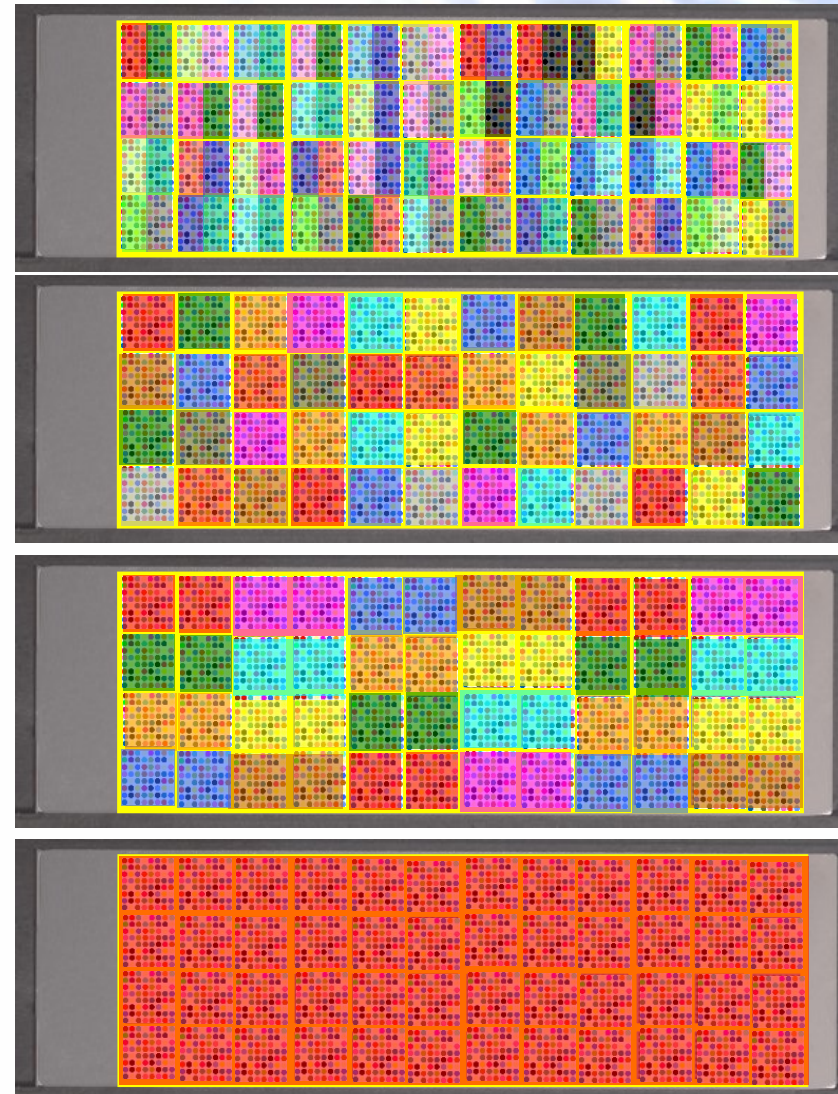
3 Templates/SA



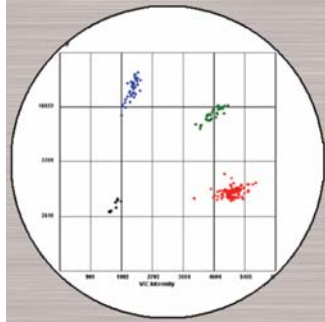
# Versatile Sample & Assay Layouts

Analyze different combinations of samples per plate & assays per sample.

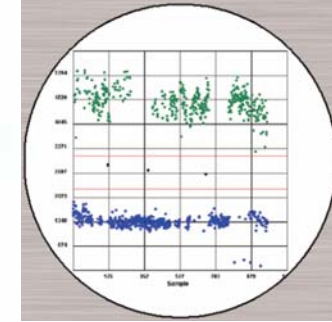
Samples/plate	Assays/sample
144	16
96	32
48	64
24	128
12	256
6	512
3	1024
1	3072



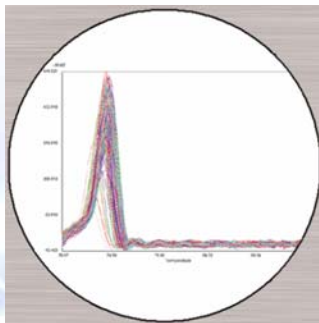
# Applications



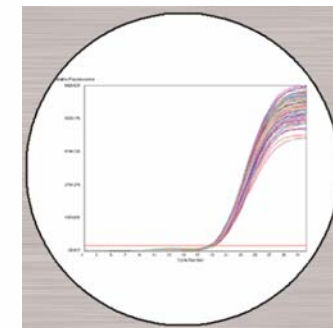
SNP Genotyping



Endpoint Detection

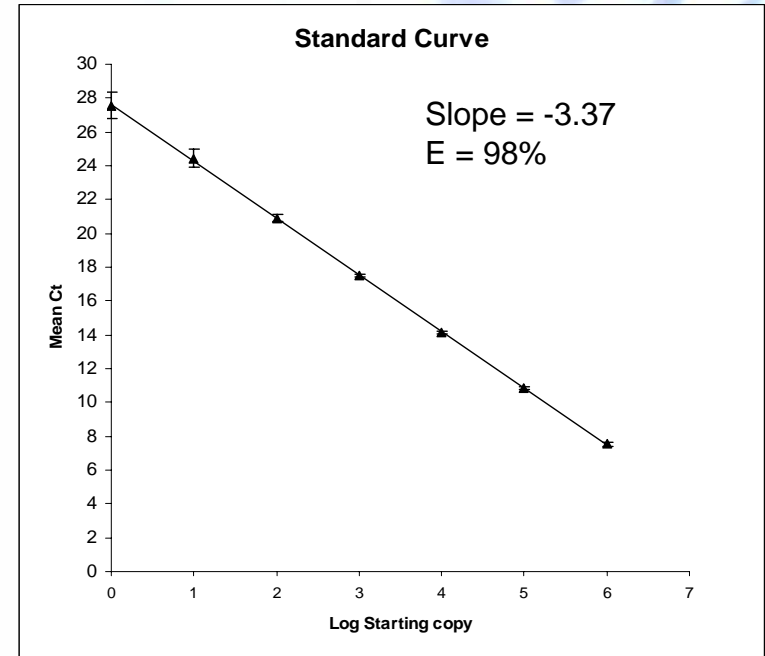
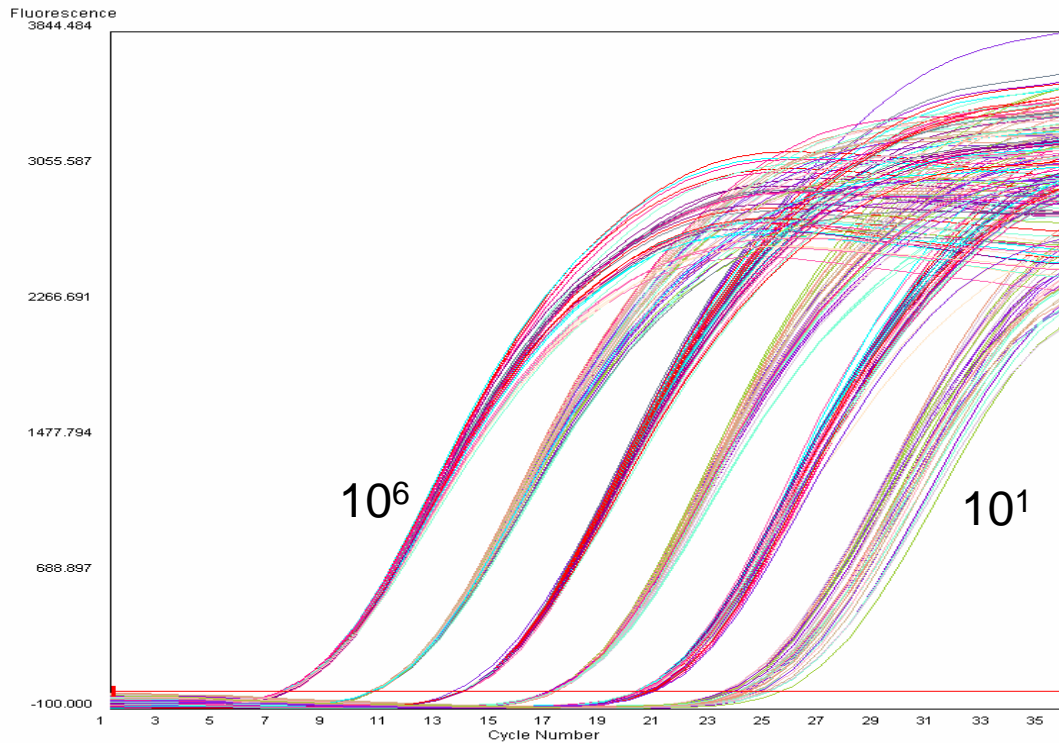


Melt Curve Profiles



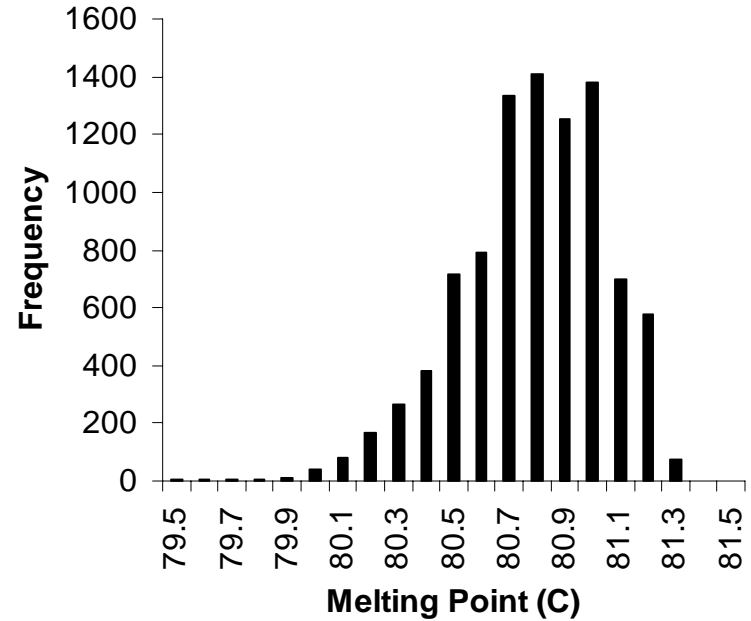
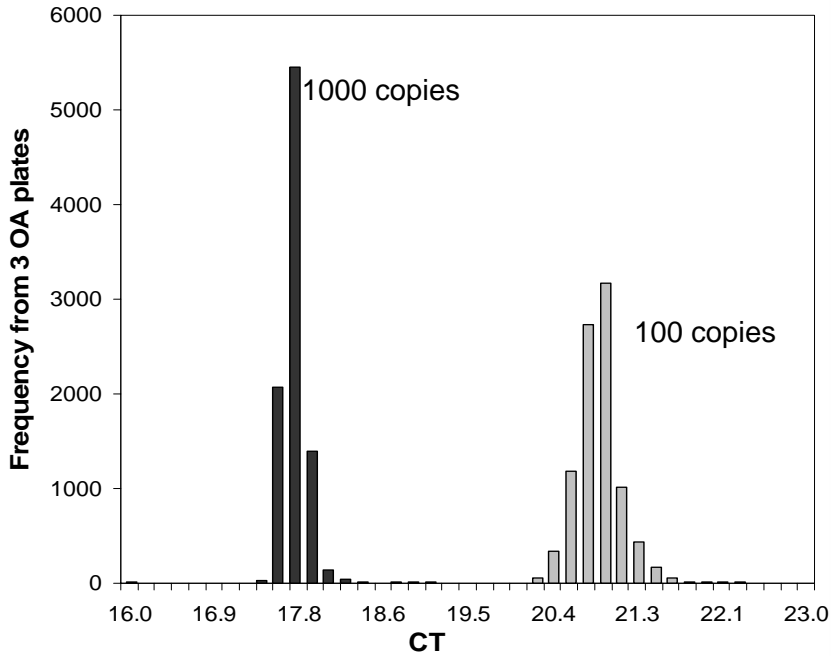
Real-Time qPCR

# High Efficiency PCR



- 6 logs dynamic range
- Primers pre-loaded
- TFR amplicon
- >98% amplification efficiency

# C<sub>T</sub> & T<sub>M</sub> Precision



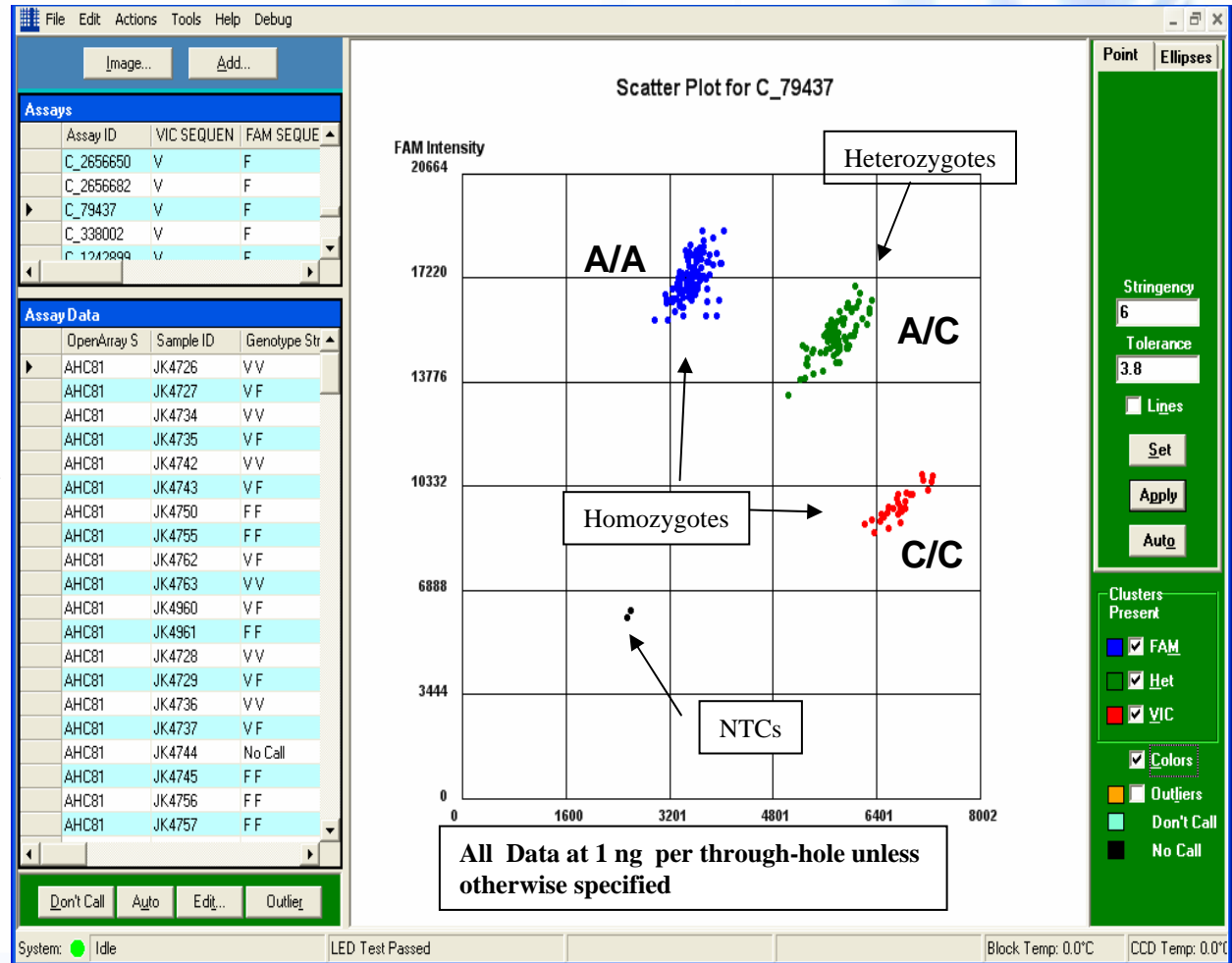
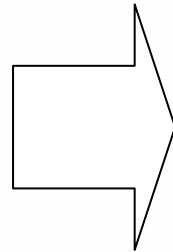
	1000 copies				100 copies			
	OA1	OA2	OA3	All 3	OA1	OA2	OA3	All 3
Median Ct	17.67	17.62	17.66	17.65	20.76	20.71	20.8	20.76
STD Ct	0.14	0.10	0.19	0.15	0.26	0.22	0.24	0.24
Outliers (>3 STD Ct)	1.0%	0.8%	0.7%	2.2%	2.2%	0.2%	1.2%	2.1%

	Arrays 1-3	Array 1	Array 2	Array 3
<b>TM Average</b>	80.7	80.8	80.8	80.7
<b>Tm STD</b>	0.28	0.26	0.32	0.23

- Uniform amplification
- Arrays are from different mfg lots

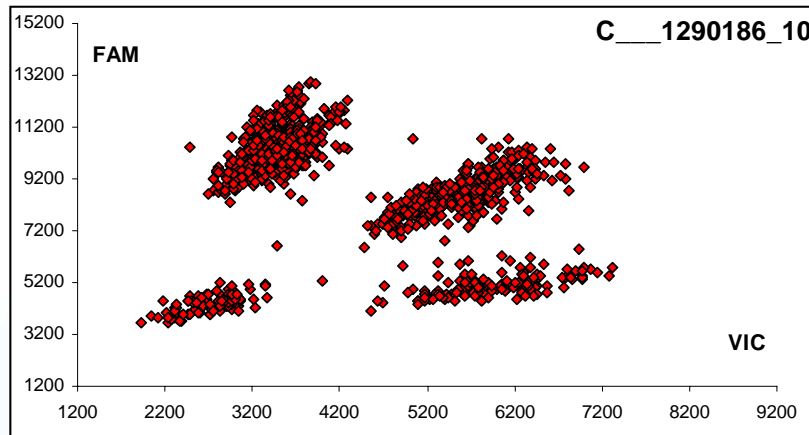
- $\Delta T_{\text{STEP}} = 0.25\text{C}$
- $\text{STD } T_{\text{M}} \sim \Delta T_{\text{STEP}}$

# TaqMan SNP Genotyping

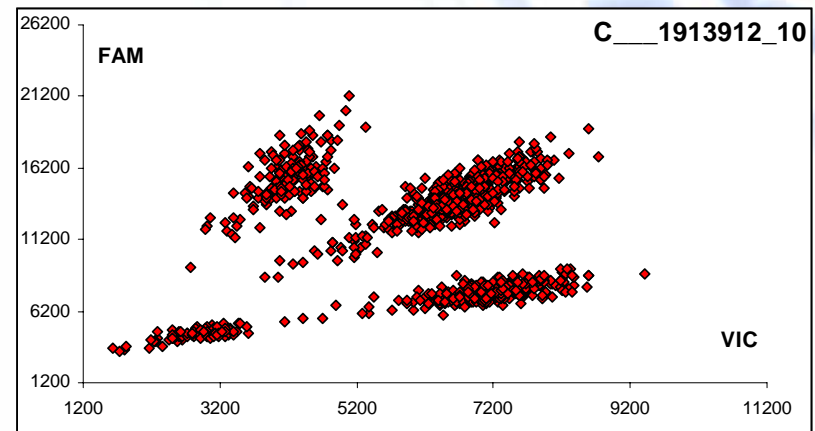


Duplex Taqman endpoint assay  
>99.5% Accuracy >95% Call rate

# Reproducible Performance



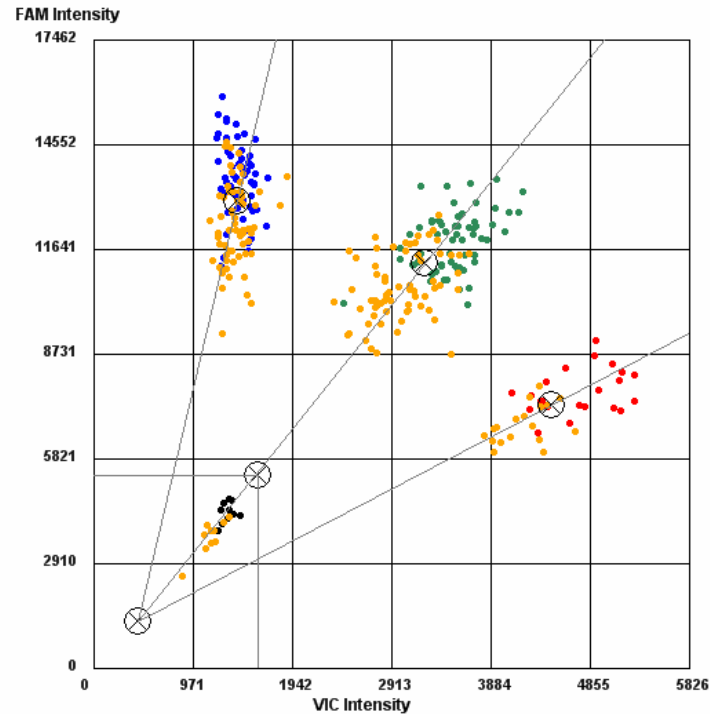
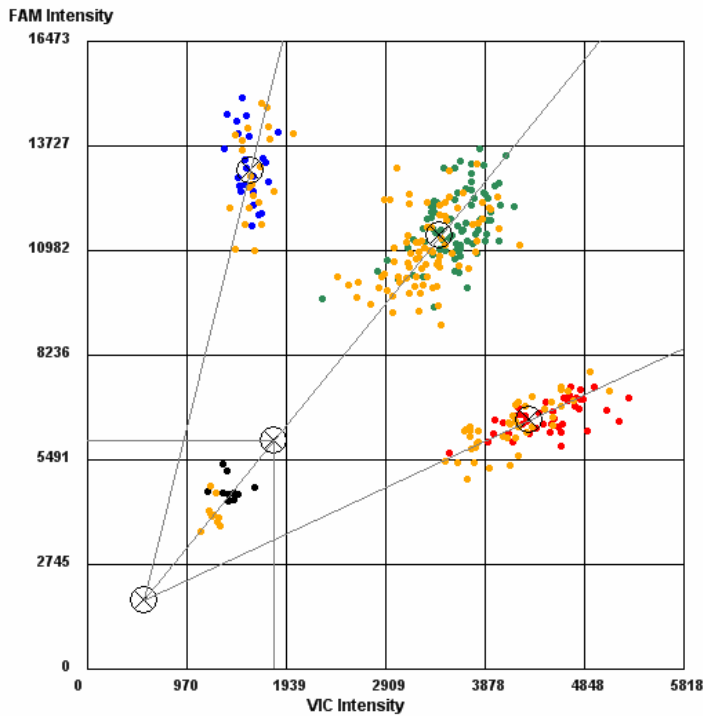
Call rate = 98.88%  
Accuracy = 99.9%



Call rate = 99.9%  
Accuracy = 99.9%

- Overlay of data from 19 OpenArrays from 4 different mfg runs
- Overlay of 1216 samples per assay
- 90 CEPH DNAs & 6 NTCs
- 1 ng DNA/rxn

# Pre-amplification (if needed)



- 96 CEPH DNA were split into two separate pools of 30ng/uL.
- First pool – dilute by 10-fold, amplified with GenomiPhi™ V2 DNA Amplification Kit yielding 448 ng/uL concentration
- Second pool – No change
- Genotype both pools with a panel of 32 TaqMan SNP assays in duplicate (two assays shown).
- WGA samples in orange
- Call rate = 99%, Accuracy = 100%

# Multivariate Mouse Pathogen Detection

- Economic value in maintaining healthy mice
  - Infected mice = economic loss
- Current detection methods slow and expensive
  - Cell culture w/ morphology or PCR confirmation
- Time to answer critical to prevent spread of infection and minimize financial impact



# Experiment

- 21 TaqMan PCR end point assays for each mouse pathogen and controls arrayed in triplicate in each subarray.
- All reactions validated in a microplate.
- DNA and RNA targets (RT for RNA targets)
- Four experiments
  - Absolute limit of detection for single control targets.
  - Sensitivity and dynamic range for a poly-target control (21 template mix).
  - Determination of inhibiting amounts of DNA on a static background of 21 template mix.
  - Detection of pathogen DNA/cDNA spiked into samples (fecal pellet, nasal aspirate, and mesenteric lymph node) from healthy mice

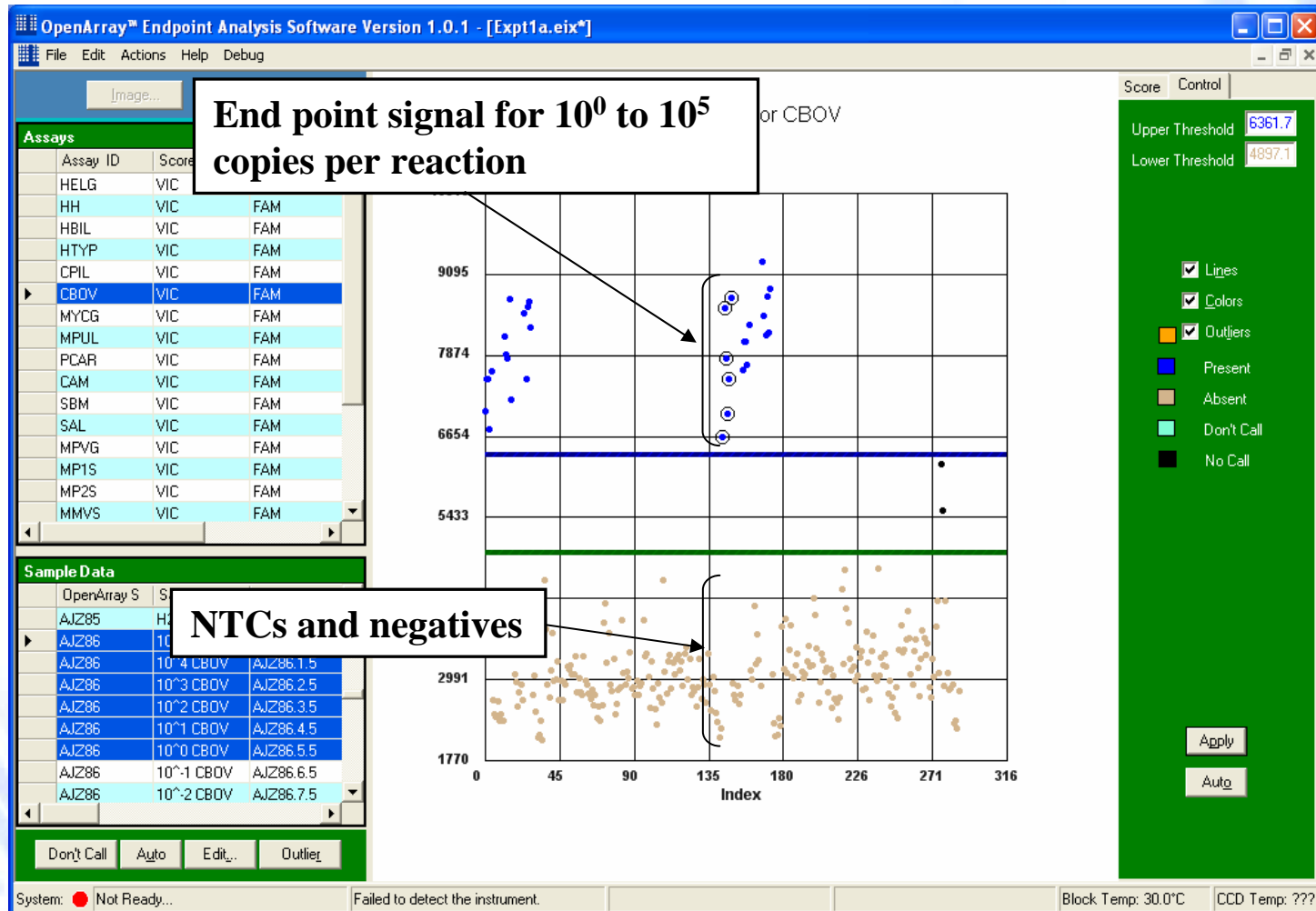


# Mouse Pathogen panel

Assay #	Assay Abreviation	Assay
1	HELG	Helicobacter - Genus
2	HH	Helicobacter hepaticus
3	HBIL	Helicobacter bilis
4	HTYP	Helicobacter typhlonius
5	CPIL	Clostridium piliforme
6	CBOV	Cornybacterium bovis
7	MYCG	Mycoplasma - Genus/Acholeplasma laidlawii
8	MPUL	Mycoplasma pulmonis
9	PCAR	Pneumocystis carinii
10	CAM	Campylobacter - Genus
11	SBM	Streptobacillus moniliformes
12	SAL	Salmonella - Genus
13	MPVG	Mouse Parvovirus - Genus
14	MP1S	Mouse Parvovirus group 1 (MPV-1)
15	MP2S	Mouse Parvovirus group 2 (MPV-2)
16	MMVS	Minute Virus of Mice (MVM)
17	MHV	Mouse Hepatis Virus (MHV)
18	MNV	Murine Norovirus (MNV)
19	LDV	Lactic Dehydrogenase Elevating virus (LDV)
20	EDIM	Mouse Rotavirus Group A (EDIM)
21	LUC	Luciferase (NARC/SPIKE Control)

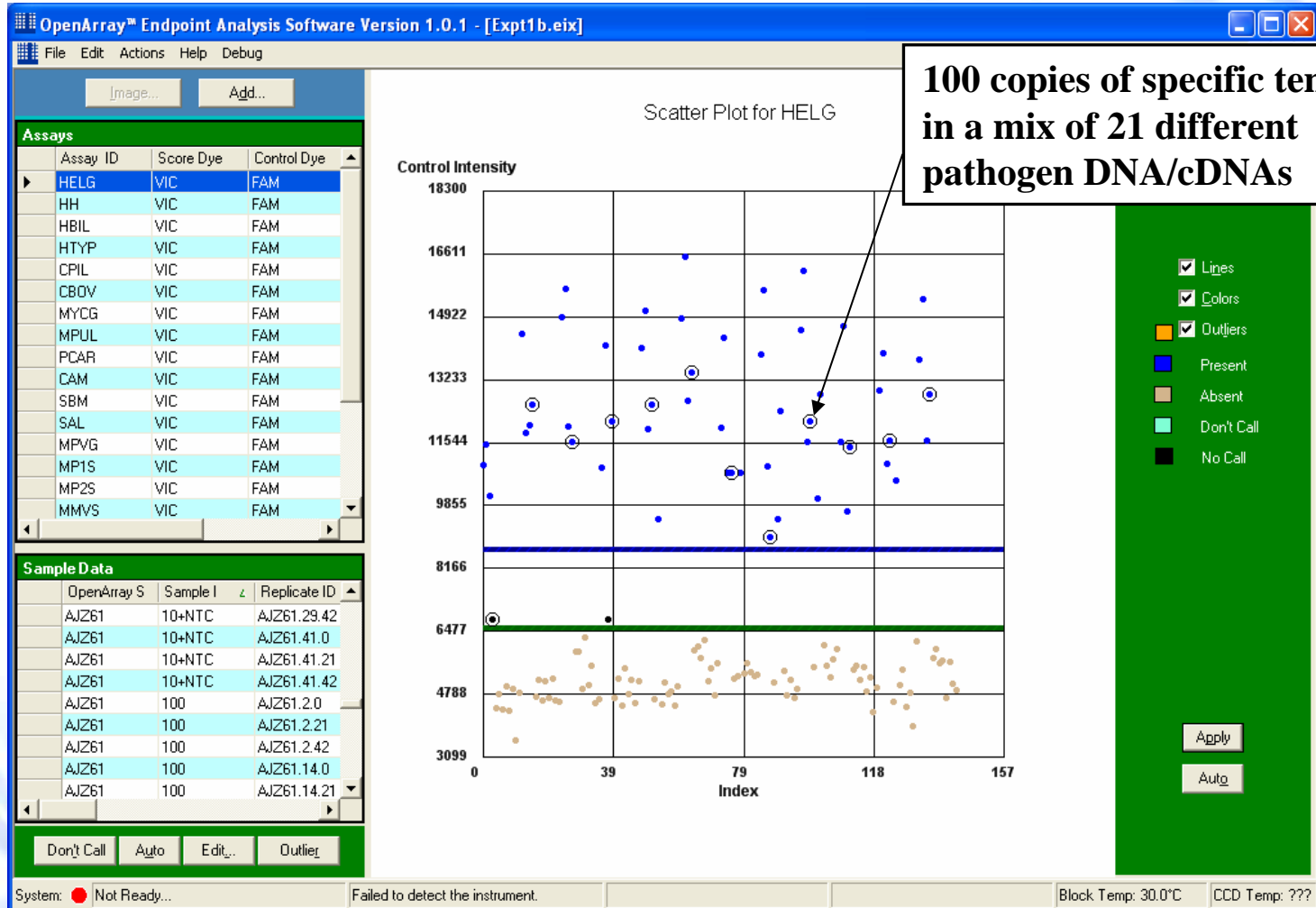
DNA and RNA Targets

# Single target Dynamic Range and LOD



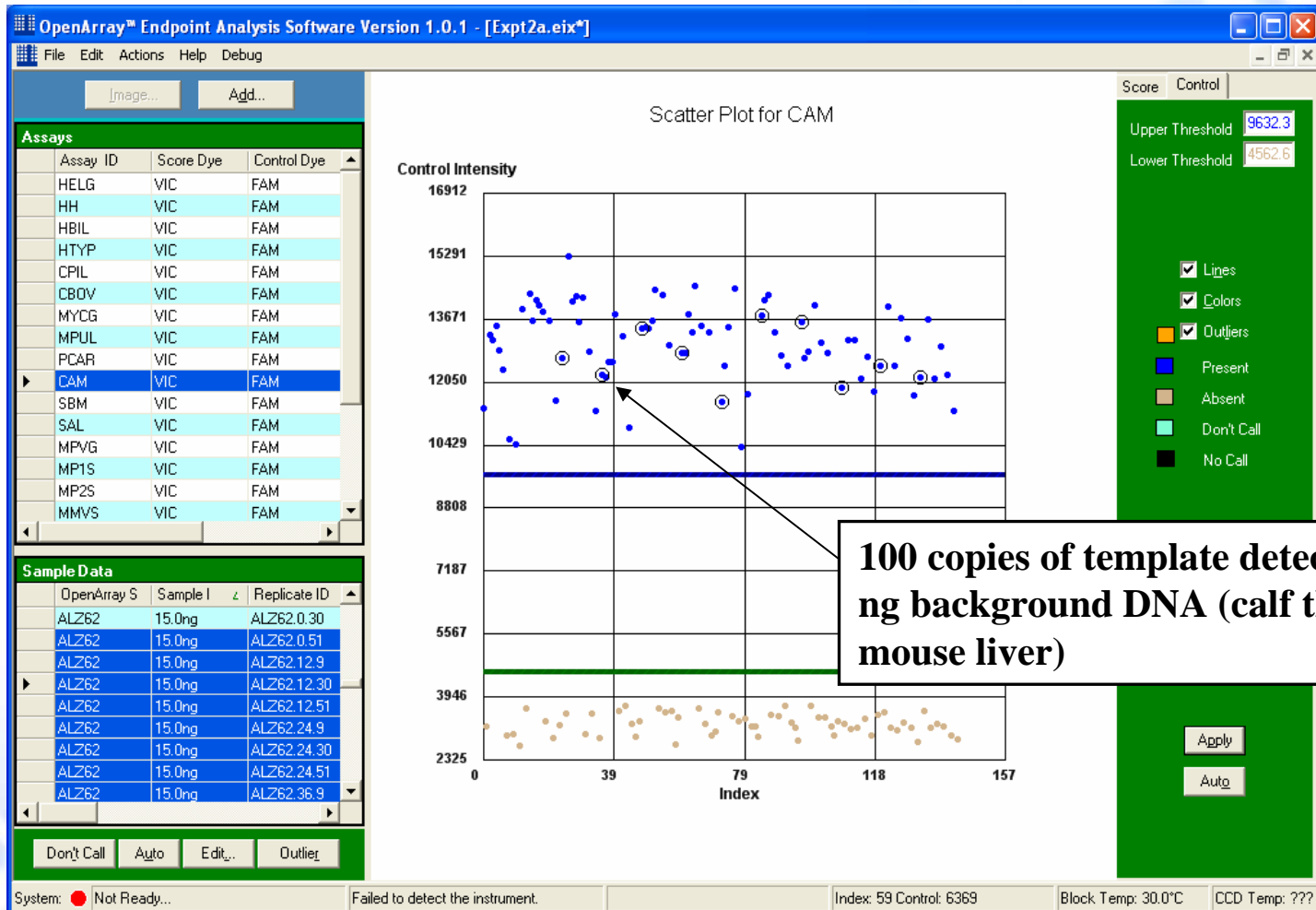
- *Corynebacterium bovis* (COBV) bacteria
- LOD: 30 copies/uL
- Target: purified DNA plasmid

# Reproducible detection of 100 copies/rxn



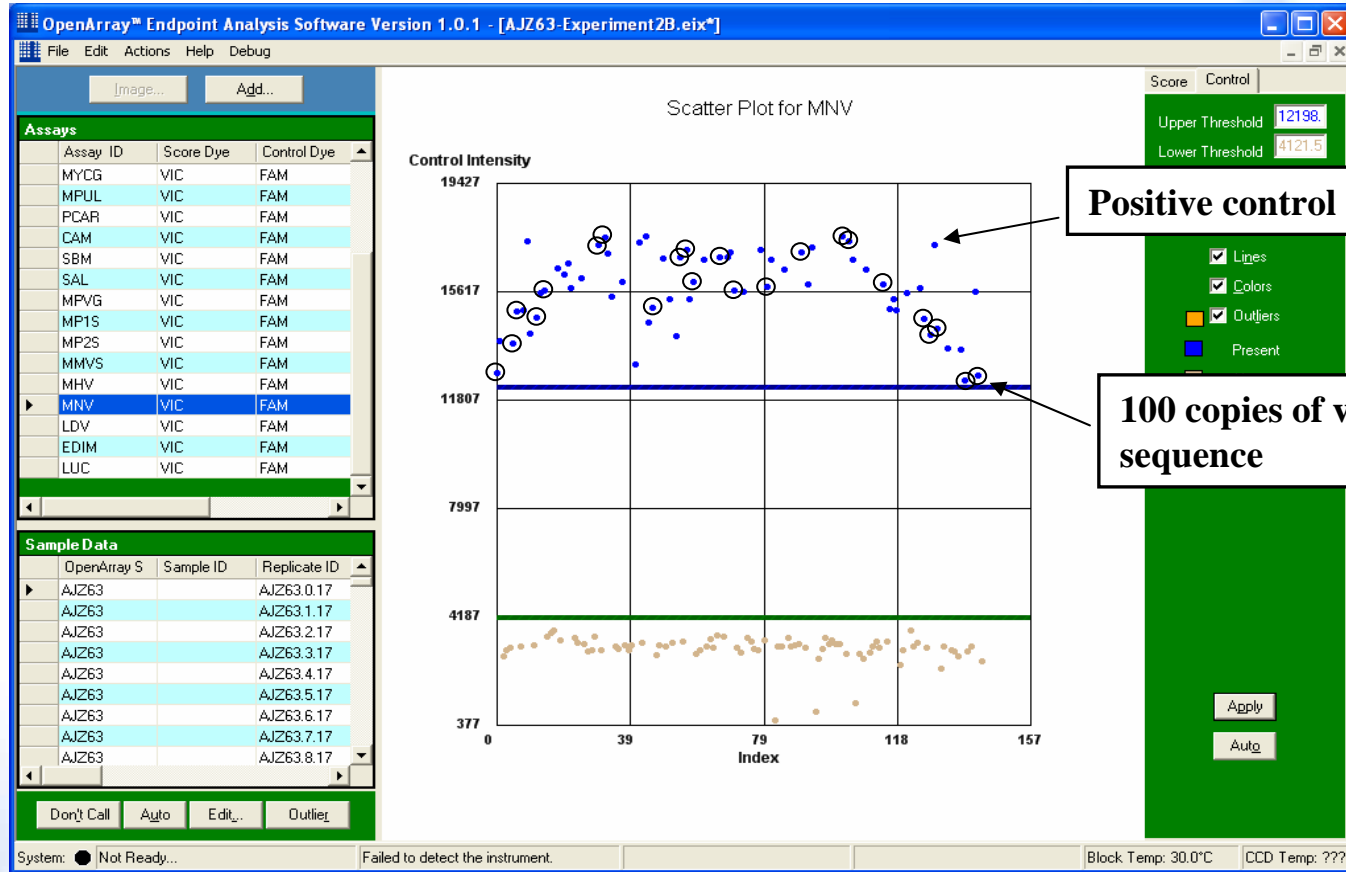
- *Cornybacterium bovis* (COBV)
- Target: purified DNA template

# In insensitive to high DNA background levels



- *Cornybacterium bovis* (COBV) bacteria

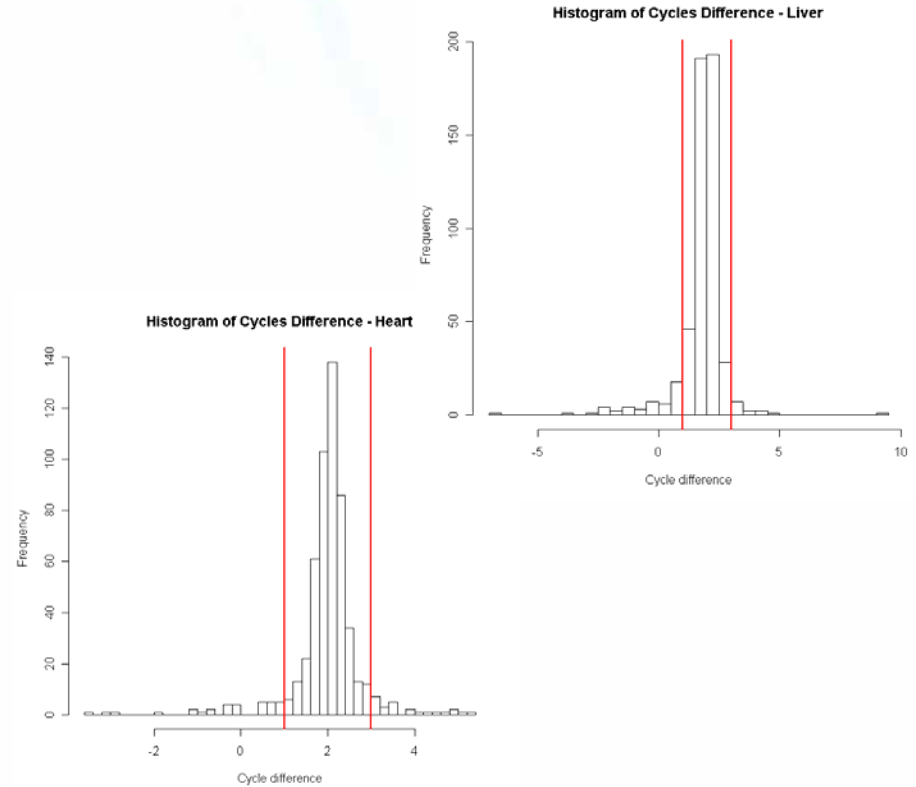
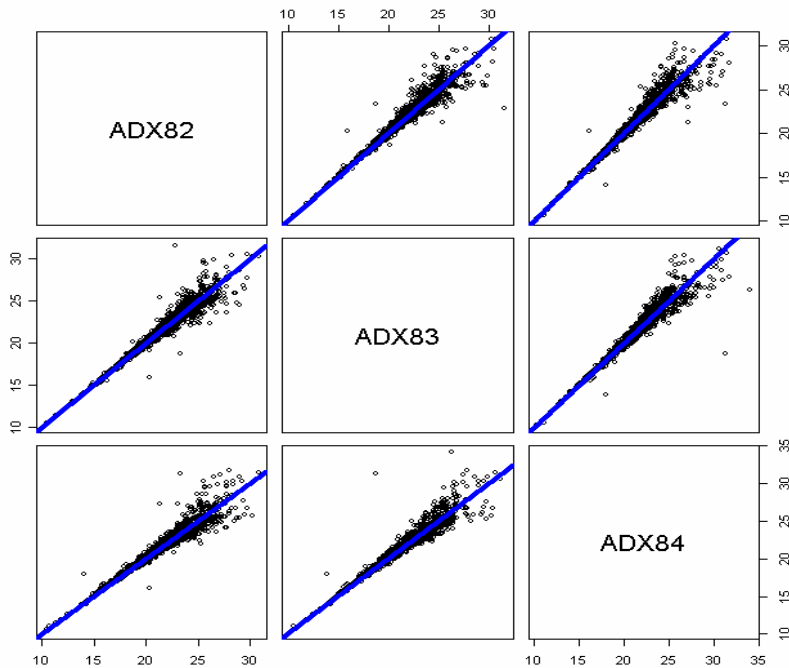
# Robust detection with field-collected samples



- Target: Murine norovirus
- Sample sources: Fecal pellet, nasal aspirates and mesenteric lymph node
- LOD: 100 copies/rxn in DNA background
- Equivalent or better than a microplate

# SYBR Green I Real-time PCR

## Reproducible and Precise



### High plate-to-plate reproducibility

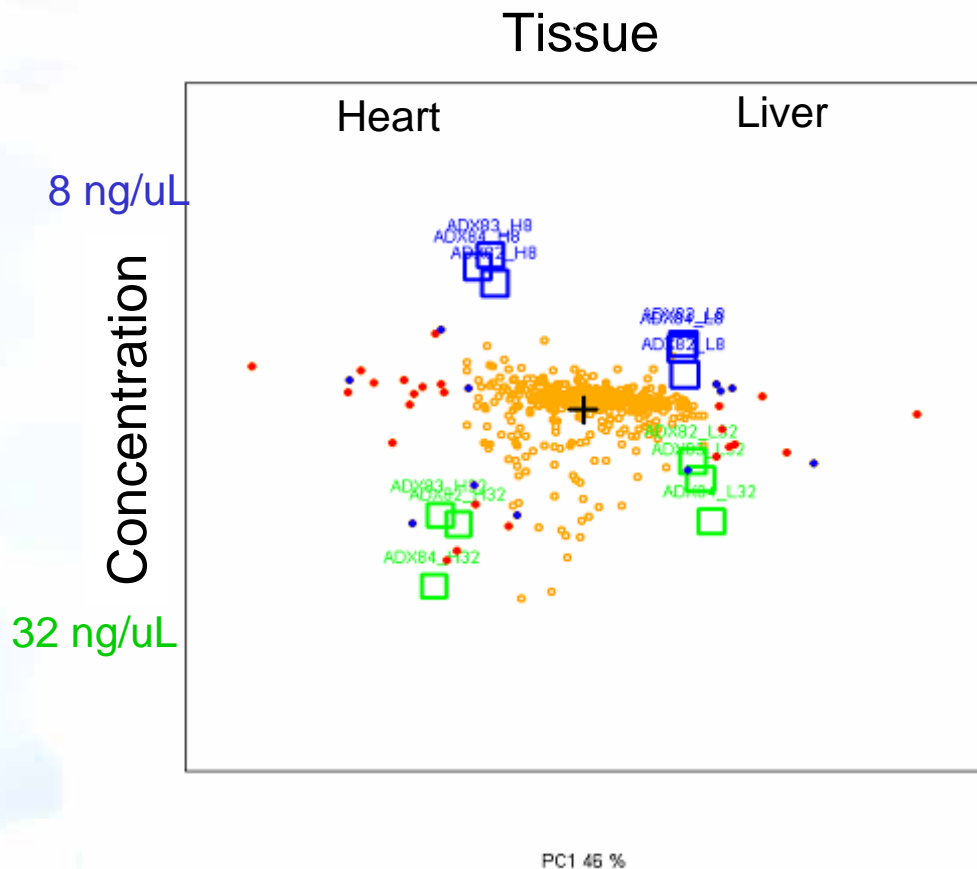
- 3 OA plates
- 508 kinase transcripts + 13 endogenous controls
- Pairwise spearman  $R^2 = 0.985$
- BD Normal Adult Human Heart mRNA
- 8 ng/ul and 32 ng/ul

### High precision

- 4-fold change (8-32 ng)
- $\Delta CT = 2$

Dixon et al., "An exercise in evaluating a novel genomics technology: Analyzing data from a pilot, high density RT-PCR experiment", submitted.

# Differential Kinase Gene Expression



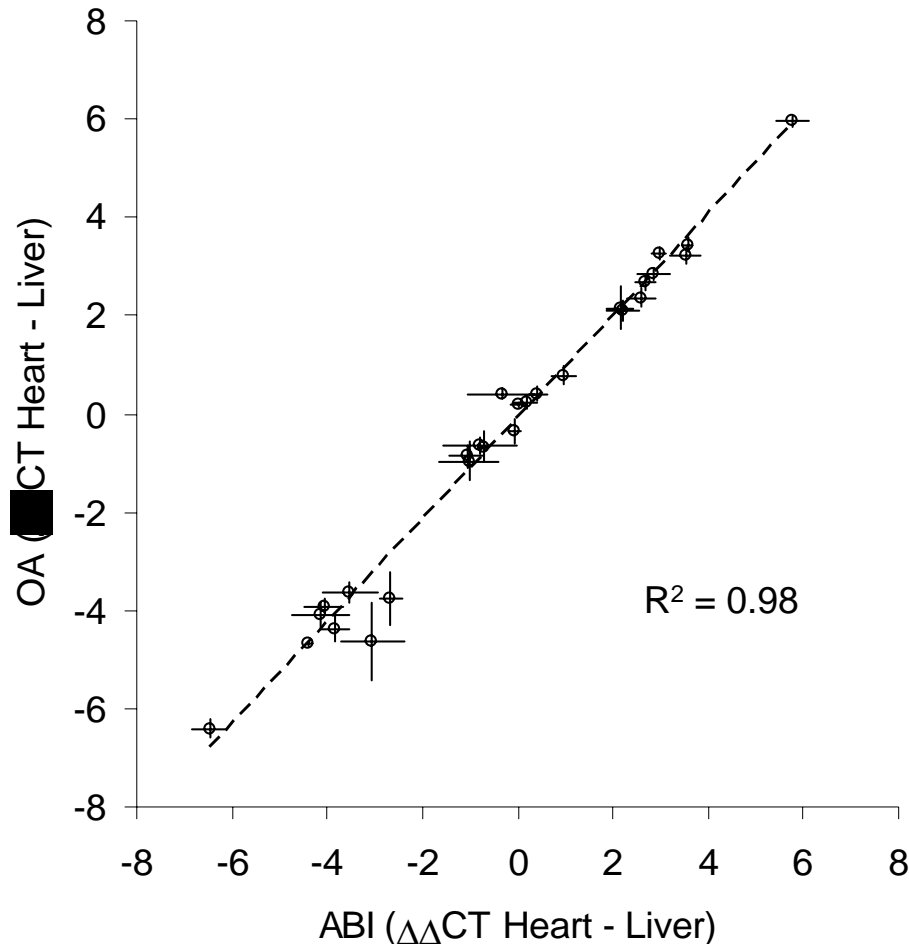
## Tissue-specific kinase expression

NM_001715*	B Lymphoid Tyrosine Kinase (BLK)
NM_006549*	Calcium/Calmodulin-dependent Protein Kinase 2, beta (CAMKK2), transcript variant 2
NM_000730*	Cholecystokinin A Receptor (CCKAR)
NM_001825*	Creatine Kinase, , Mitochondrial 2 (CKMT2)
NM_152247*	Carnitine Palmitoyltransferase 1B (CPT1B)
NM_004119*	FMS-related Tyrosine Kinase 3 (FLT3)
NM_053029*	Myosin, Light Polypeptide Kinase (MYLK), transcript variant 4
NM_004563*	Phosphoenolpyruvate Carboxykinase 2 (mitochondrial) (PCK2), Nuclear Gene Encoding Mitochondrial Protein
NM_000289*	Phosphofructokinase, muscle (PFKM)
NM_002627*	Phosphofructokinase, platelet (PFKP)
NM_006201*	PCTAIRE Protein Kinase 1 (PCTK1), transcript variant 1

- Spectral map based on principal component analysis
- First principal component separates tissue type; second separates concentration
- 35 genes found highly correlated with tissue type and concentration (red & blue)
- 11 are known to be differentially expressed between the two tissues (blue)

Dixon et al., "An exercise in evaluating a novel genomics technology: Analyzing data from a pilot, high density RT-PCR experiment", submitted.

# Concordance with Microplates



28 BT SYBR assays run in ABI 7900 @ 1 ng total RNA equivalence per well

- Excellent cross platform correlation in precision & accuracy

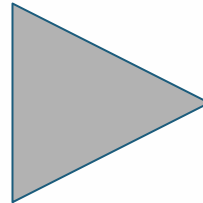
# OpenArray Impact

## Miniaturization and parallel high throughput

### Existing Genomic Laboratory



### Nanofluidic Laboratory



- 98,000 SNP genotypes or
- 27,650 qPCR analyses per FTE/day

# OpenArray Value

## CapEx Savings

**X 144**

**X 24**

**=**

**BioTrove NT Cyclor**

## Material Savings

**X 1/64 =**

## Time to Answer

5000 samples @ 32 SNPs in duplicate

Taqman PCR Microplate  
(28 days)

**x 1/17**

Hybridization Microarray  
(104 days)

**x 1/65**

**= OA Plate  
(1.6 days)**



**BioTrove**

# Summary

- OpenArray miniaturization and parallelism leads to substantial savings in cost, time, lab space, and labor to complete a project.
- The OpenArray provides a high degree of experimental flexibility by enabling different combinations of PCR assays and samples to be analyzed quickly and efficiently.

# Acknowledgments

## **BioTrove**

James Hurley, Ph.D.  
Tom Morrison, Ph.D.  
Kevin Munnelly  
Elen Ortenberg  
Jamie Cho  
Douglas Roberts, Ph.D.  
Javier Garcia, Ph.D.  
Tanya Kanigan, Ph.D.

## **Johnson & Johnson**

Sergey Ilyin, Ph.D.  
Daniel Horowitz  
James Dixon, Ph.D.