WAFERGEN BIOSYSTEMS

SmartChip: A Flexible, High-Density, High-Throughput Real-Time PCR System

Advancing the science of tomorrow today.

WaferGen at a Glance

Background

Founded in 2003

Commercialize next generation, 5,000+ feature real-time PCR system

Products

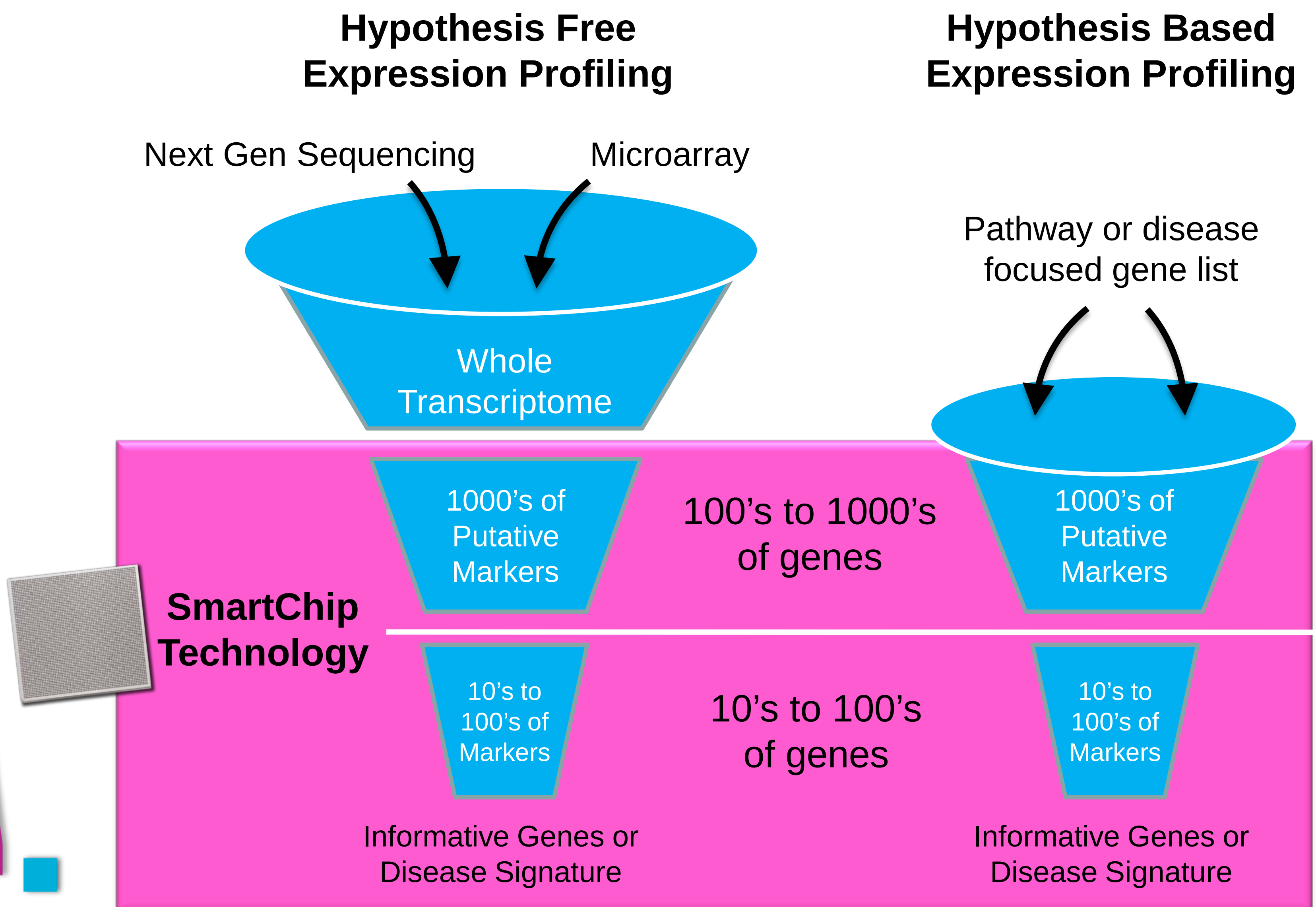
The SmartChip System combines discovery and validation with a single technology

- Real-time PCR
- High-density SmartChip Panels
- High-throughput analyzer
- Nano volume dispenser

Content-ready and custom SmartChip Panels

Custom assay development with rapid turnaround

SmartChip: One Platform, Multiple Phases

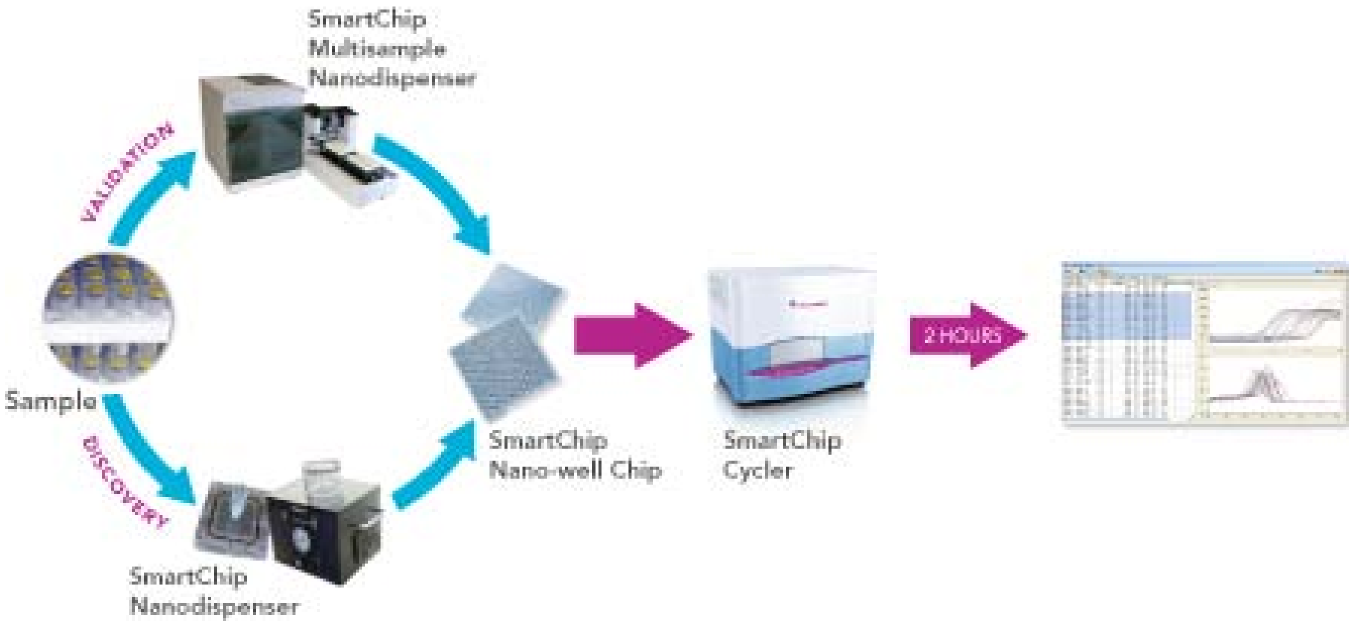


The SmartChip Complete System is…

- SmartChip Nanodispenser
- SmartChip Cycler
- SmartChip Multisample Nanodispenser

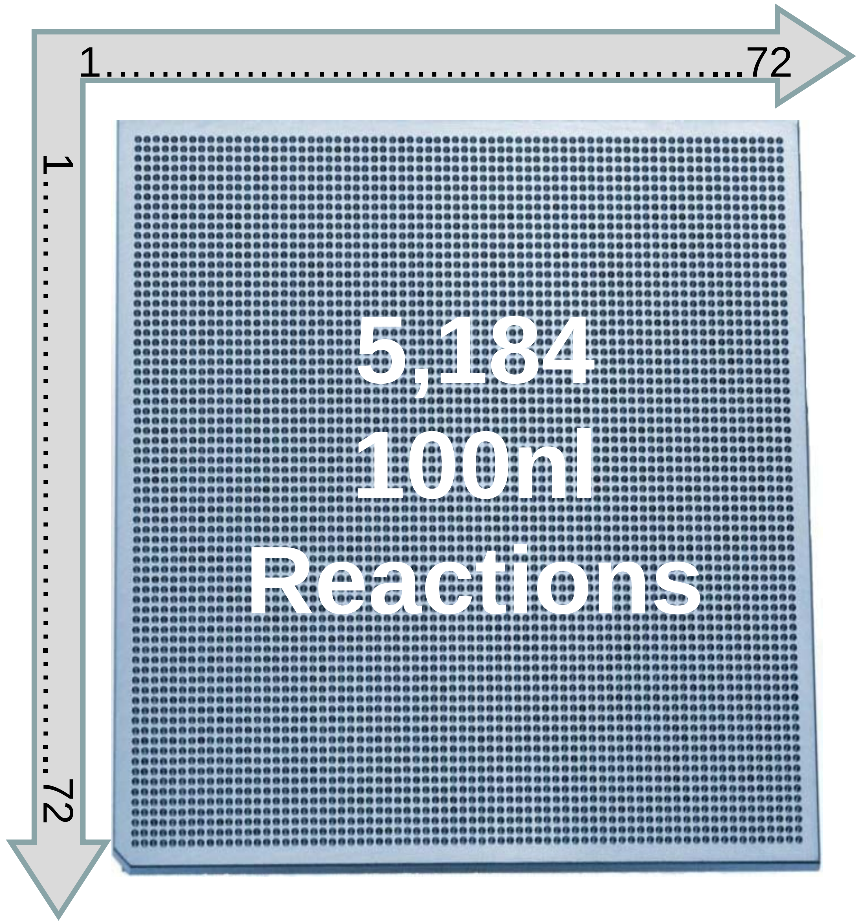
The SmartChip System

Discovery & Validation in one system



- Discovery: profile 1000’s of genes with a single sample
  - Identify a broader set of more informative biomarkers or targets for further studies
- Validation: screen 10’s to 100’s of genes on multiple samples
  - Integrated solution for simple nanoliter scale real-time PCR

The SmartChip System is….



SmartChip Technology Advantage

1 SmartChip = 13 384 well plates

Minimal pipetting steps

Time to Data = 2.25 hr

Only hours away from medium to large sample study results

“Smart” use of reagents

=

Sample Workflow and Timing

Cell lines or Tissue (Frozen or FFPE)

↓

Isolate total RNA and perform Reverse Transcription
(0.5-1.0μg total RNA/5,000 assays)

↓

Dispense Master Mix and cDNA across the SmartChip

↓

Perform Quantitative RT-PCR

↓

Data analysis and QC

Customer Protocol Dependent

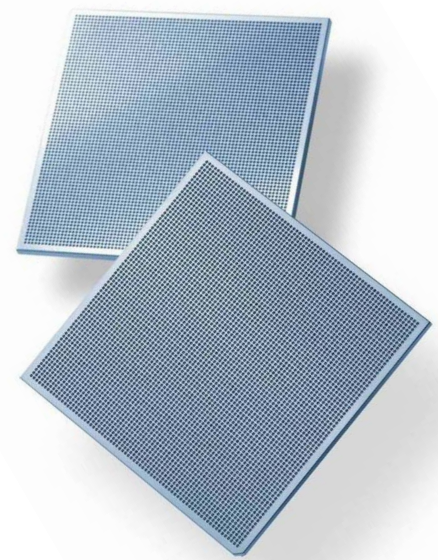
2.5 hrs

0.5 hr

Advancing the science of tomorrow today.

Performance Specifications

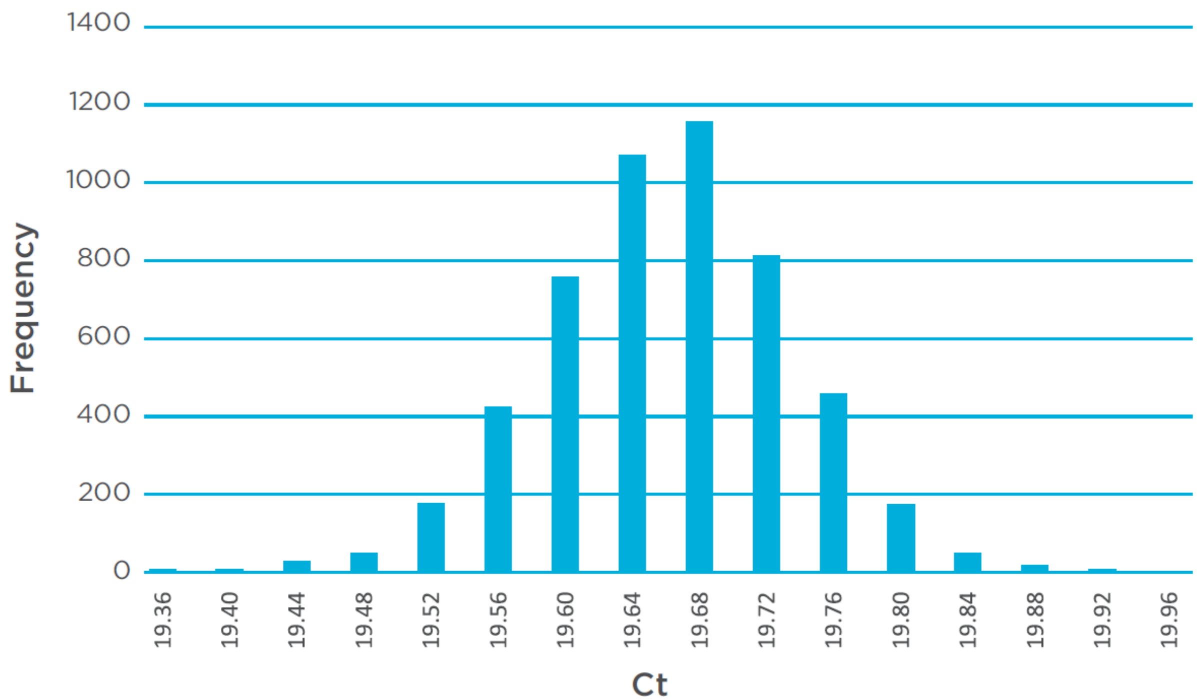
- Sensitivity:
  - ~10copies cDNA
- Precision:
  - Standard Deviation <0.10 Ct between replicates at 650 copies/well (>150 runs)
- Reproducibility (chip to chip):
  - R-square value between chips >0.95



Advancing the science of tomorrow today.

Performance Specifications

- Precision:
  - Standard Deviation <0.10 Ct between replicates at 650 copies/well (>150 runs)



SmartChip Panels

Pre-optimized and custom content

- The SmartChip Human Oncology Panel
  - 968 gene-specific assays covering 16 functional groups tested in quadruplicate
  - Includes 11 endogenous and 6 exogenous controls
- The SmartChip Human microRNA Panel
  - ~800 microRNA specific assays designed to the human miRBase version 14.0 tested in quadruplicate
  - Includes 12 endogenous and 6 exogenous controls
- SmartChip Custom Panel
  - Choose from a library of validated gene expression assays or have our assay development team optimize specific assays for your research needs.

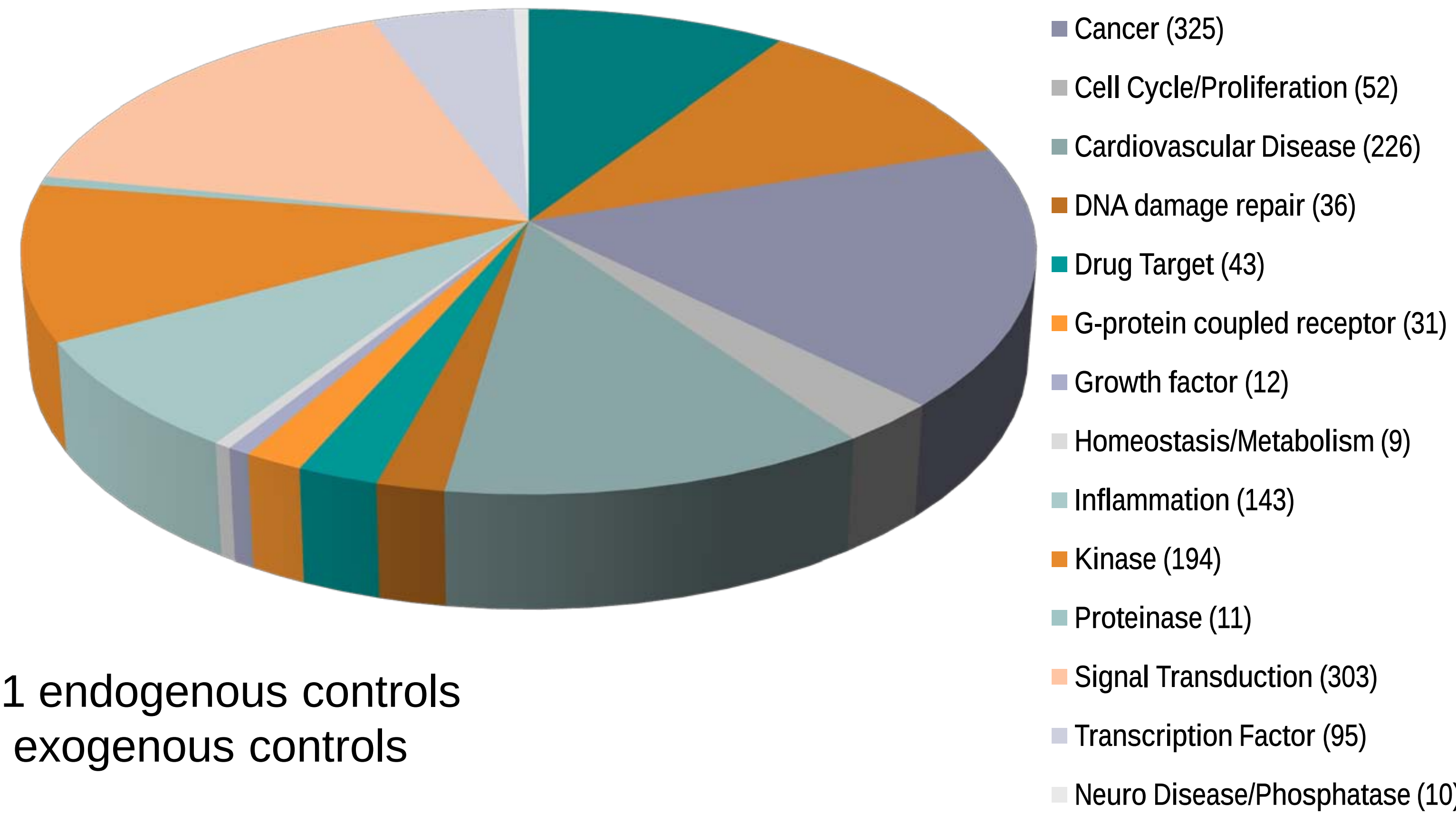
Assay Design and QC Criteria

Quality in Assay Development

- Each assay designed under stringent criteria
  - Specificity
  - Screened for primer dimers
  - Primer screened for secondary structure
  - Amplicon screened for secondary structure
  - SNP screened
  - Splice variant inclusivity
- Each assay subjected to performance criteria in a functional QC test using SYBR real-time PCR analysis
  - Amplicon sequencing
  - Dynamic range testing
  - Sensitivity
  - Specificity
  - Melt curve analysis
  - Gel analysis (single band)

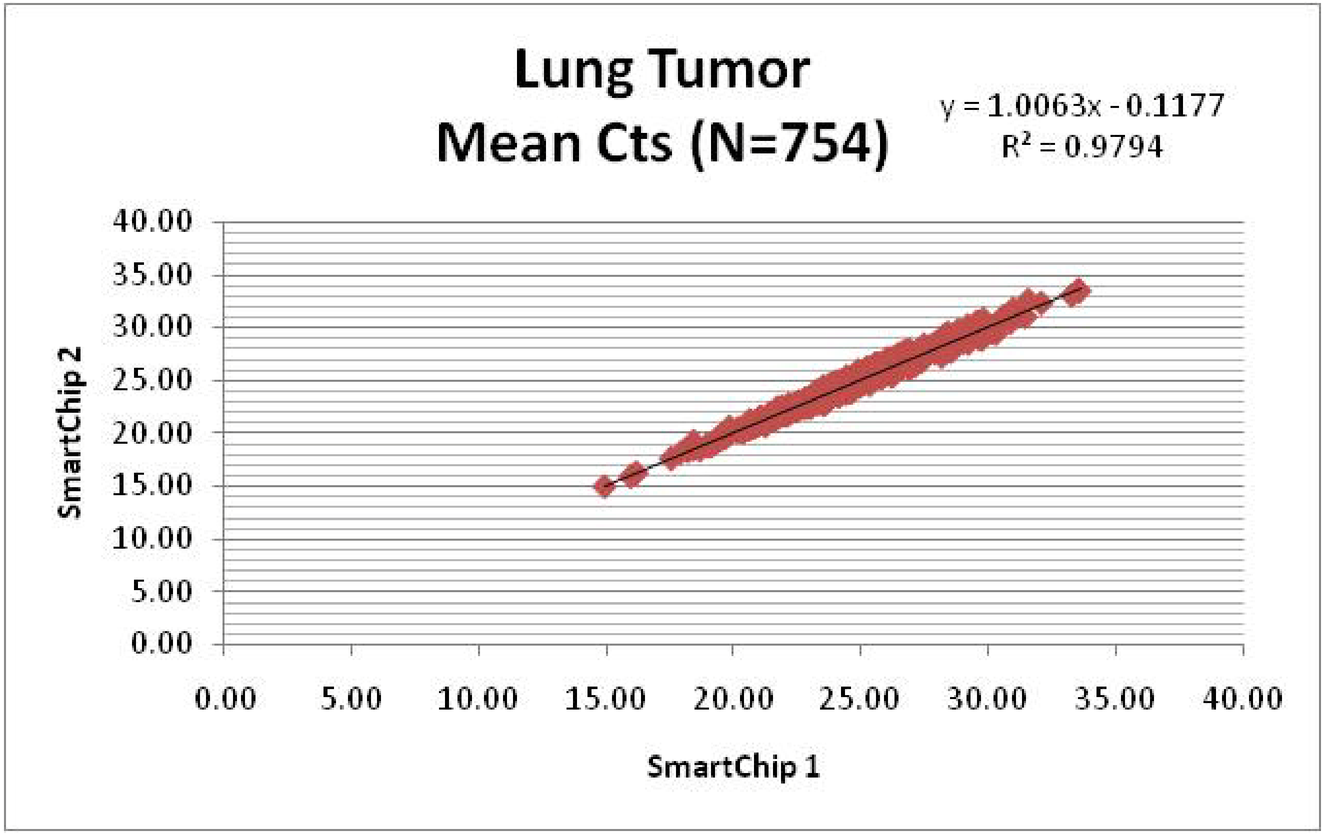
SmartChip Human Oncology Panel

~1000 genes in quadruplicate
Covering the following gene families:



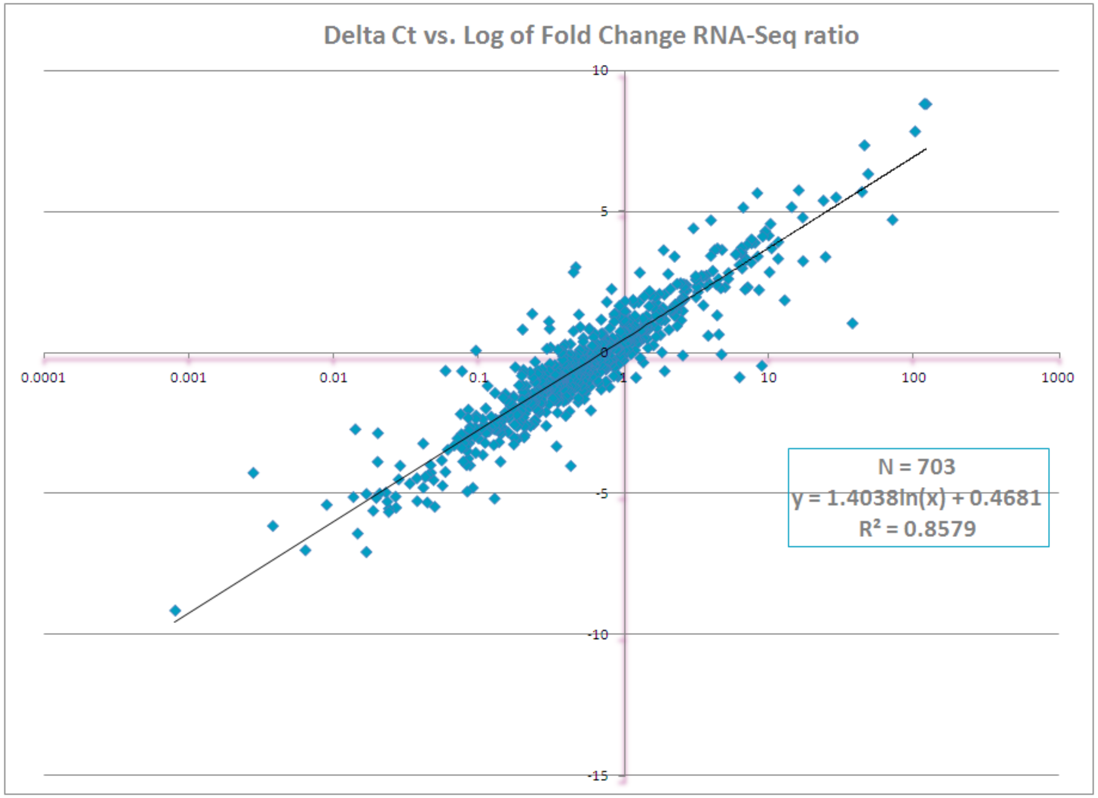
21 endogenous controls
6 exogenous controls

Chip-to-Chip Reproducibility



Advancing the science of tomorrow today.

Comparison Against Ref-Seq



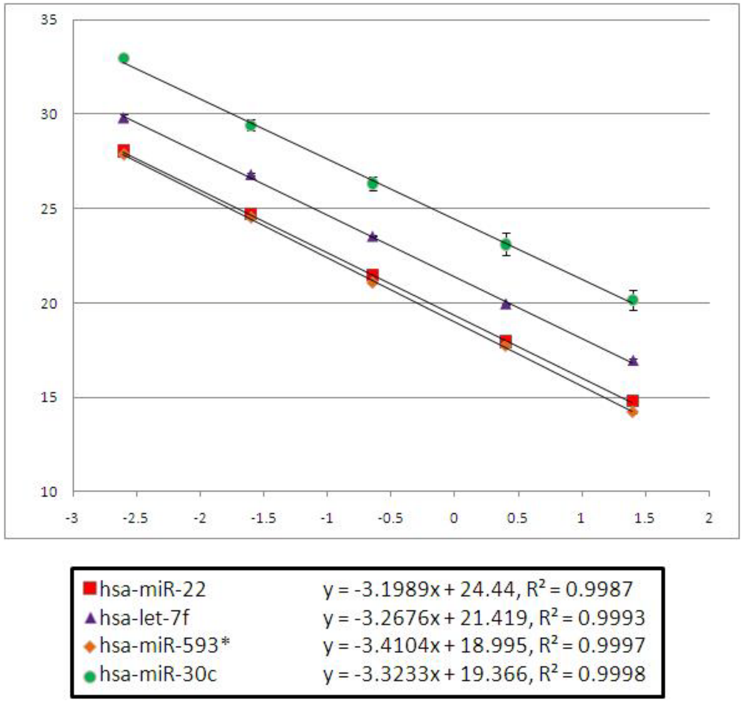
- Data from the publically available SEQC project for the same samples are compared for the same genes as a log of the fold change determined for the RNA-Seq data

Advancing the science of tomorrow today.

SmartChip Human microRNA Panel

A comprehensive microRNA profiling panel

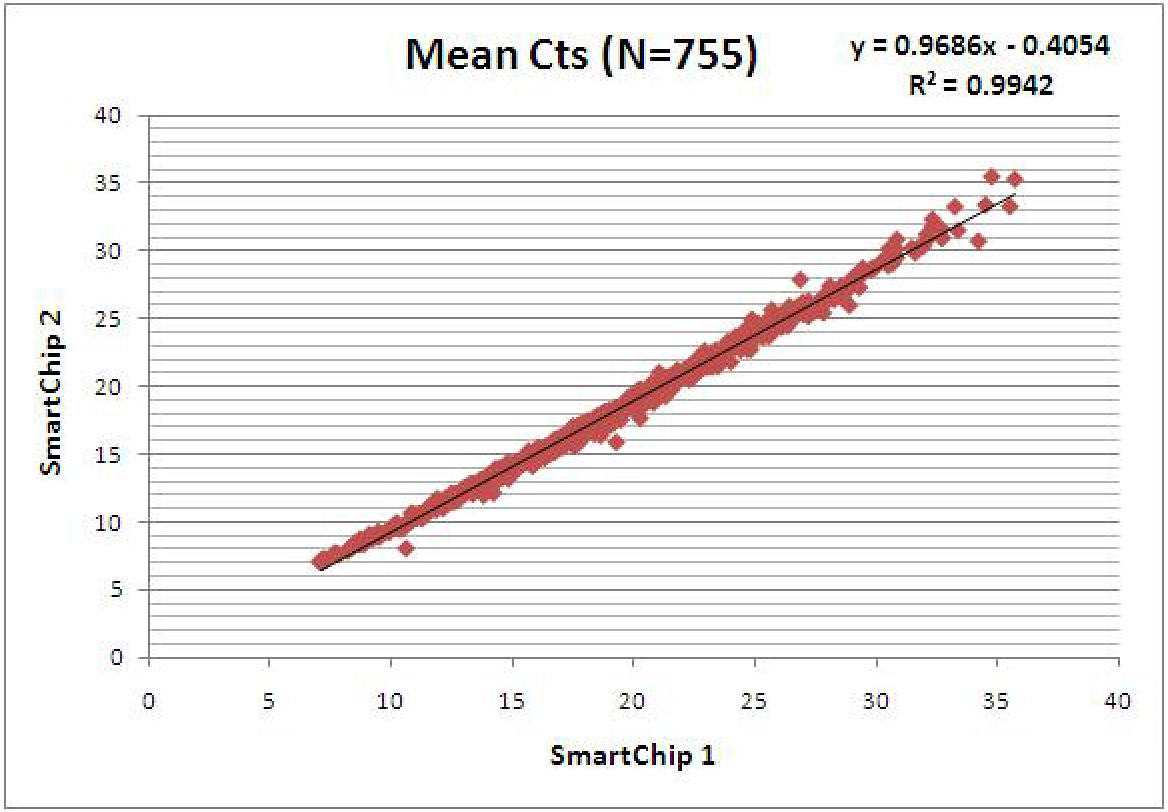
- ~ 800 microRNA targets, in quadruplicate
  - microRNA specific RT-primers and process to increase specificity
  - Assays passed strict QC criteria; specificity & dynamic range
- 11 Endogenous Controls (small nucleolar RNAs)
  - SNORA 66 & 74A
  - SNORD 7, 24, 38B, 43, 44, 48, 49A, 54 & 58A
- 6 Exogenous Yeast Controls



Advancing the science of tomorrow today.

Chip-to-Chip Reproducibility

SmartChip Human microRNA Panel



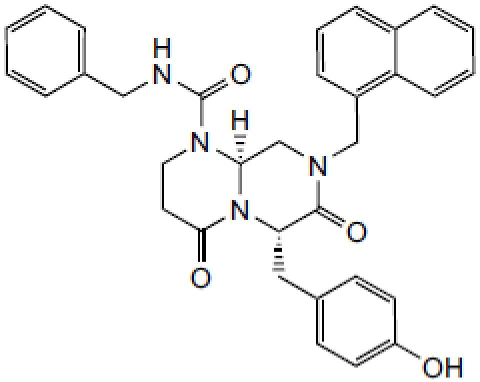
Advancing the science of tomorrow today.

USC Study - Dr. Michael Kahn

- Evaluate oncogene and miRNA signatures in two carcinoma cells lines following drug treatment
  - AsPC-1 (Human pancreatic adenocarcinoma)
  - HCT-116 (Human colorectal carcinoma)
- A total of 6 samples were tested on both the oncology and miRNA SmartChips
  - HCT-116 DMSO 24 hours (control)
  - HCT-116 DMSO 96 hours (control)
  - HCT-116 Drug Treatment 24 hours
  - HCT-116 Drug Treatment 96 hours
  - AsPC-1 DMSO 96 hours (control)
  - AsPC-1 Drug Treatment 96 hours

Wnt/β-catenin Signaling Pathway

- The Wnt-β-catenin pathway is critical to normal development and also to the initiation and progression to cancer
- "The switch from β-catenin-CBP to β-catenin-p300 controls one of the most fundamental cell switch points…"
- ICG-001 is a selective inhibitor of β-catenin-CBP interaction



Chemical structure of ICG-001

Michael McMillan and Michael Kahn. (2005) Investigating Wnt signaling: a chemogenomic safari. *Drug Discovery Today, 10(21): 1467 – 1474.*

Wnt/β-catenin Signaling Pathway

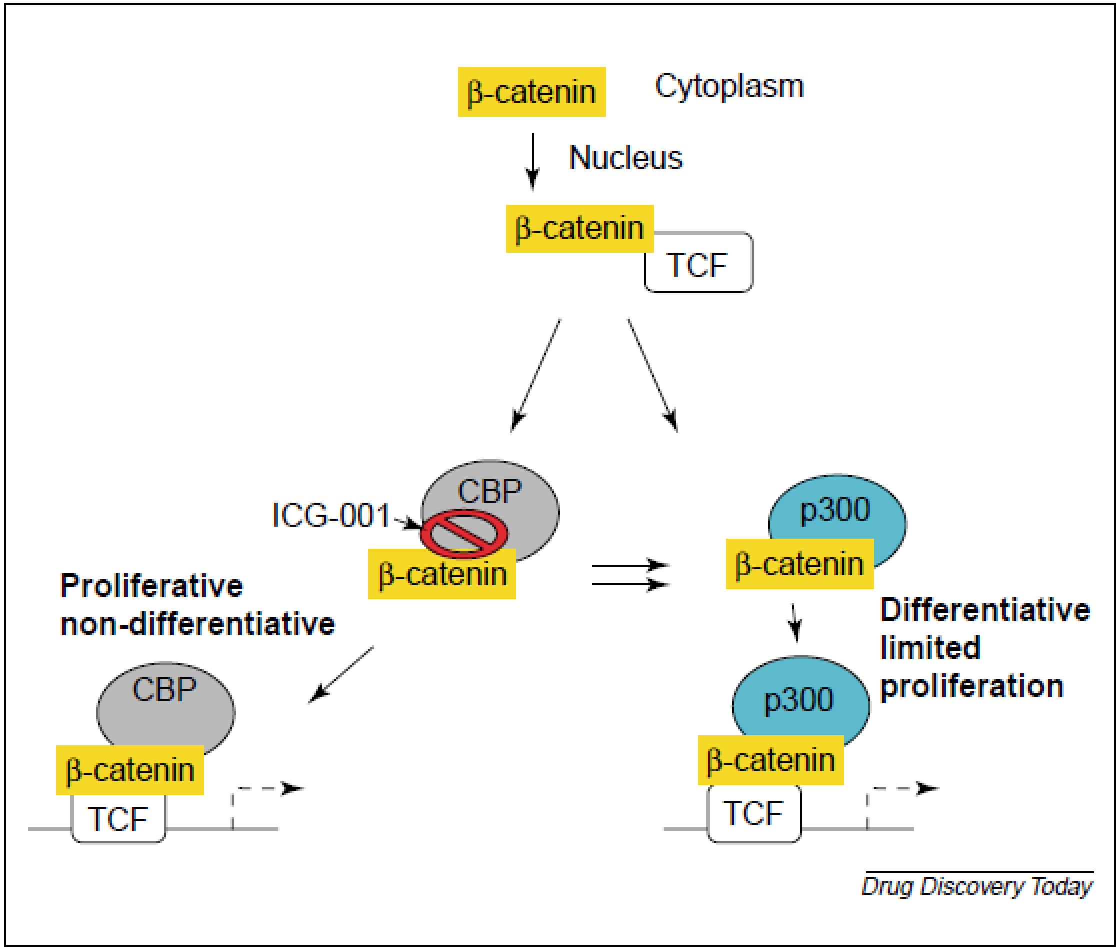
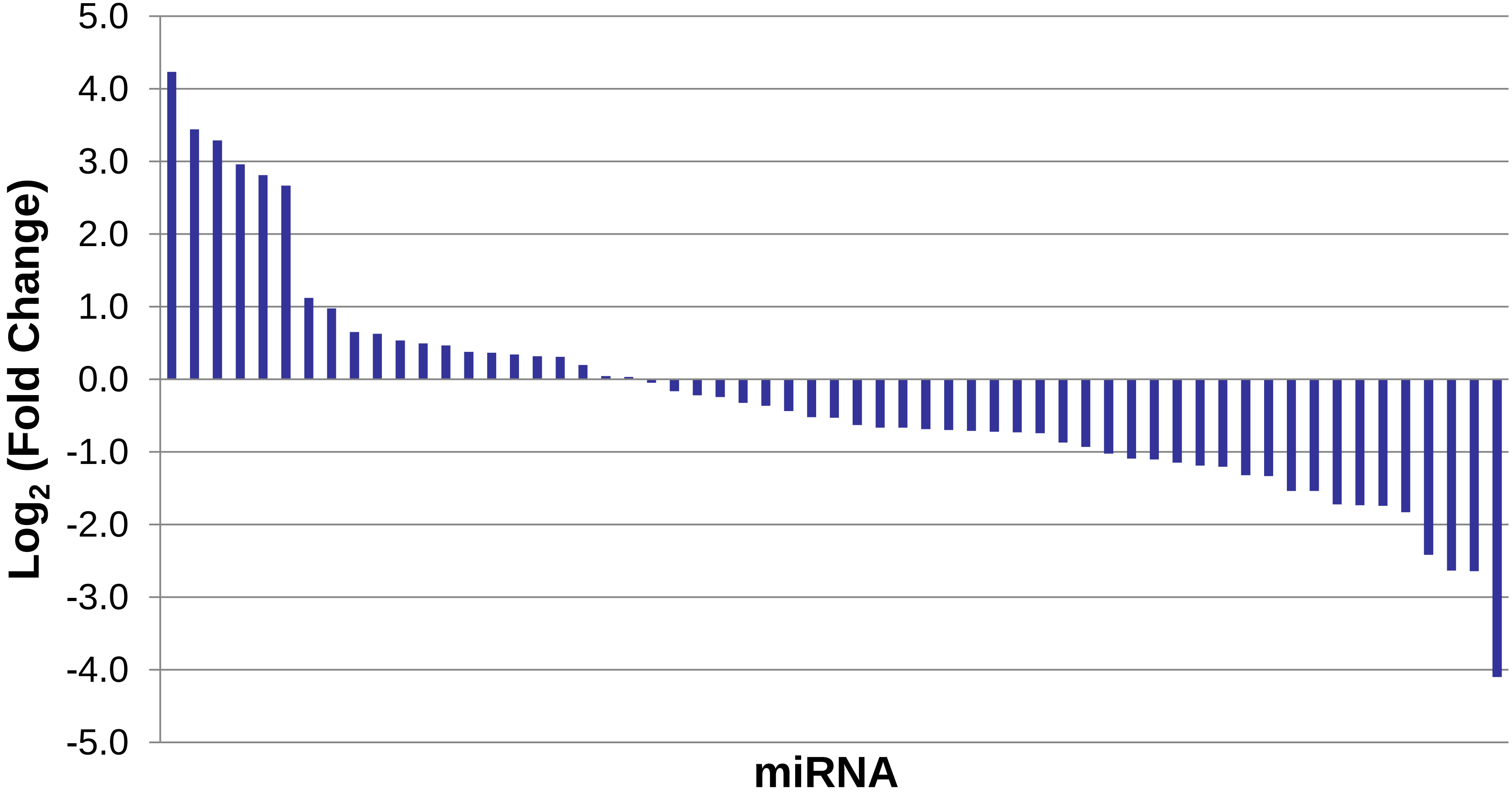


FIGURE 4

Model for differential coactivator usage in Wnt–β-catenin transcription and its role in regulating proliferation versus differentiation.

Michael McMillan and Michael Kahn. (*2005)* Investigating Wnt signaling: a chemogenomic safari. *Drug Discovery Today, 10(21): 1467 – 1474.*

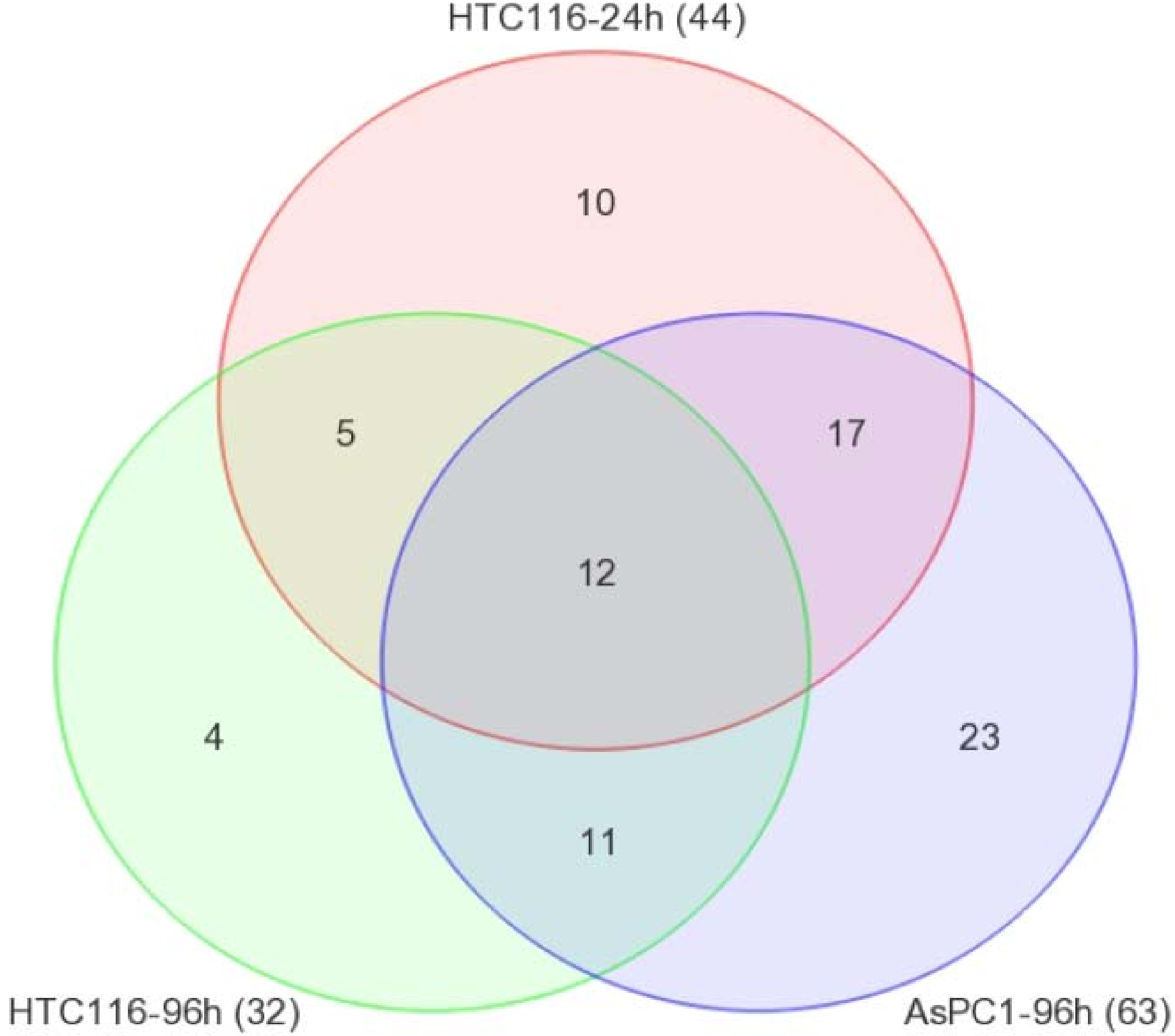
Differential Expression of miRNA in 24 hr Drug Treated vs. DMSO HCT-116 Cells (All Mean)



Michael Kahn – unpublished data

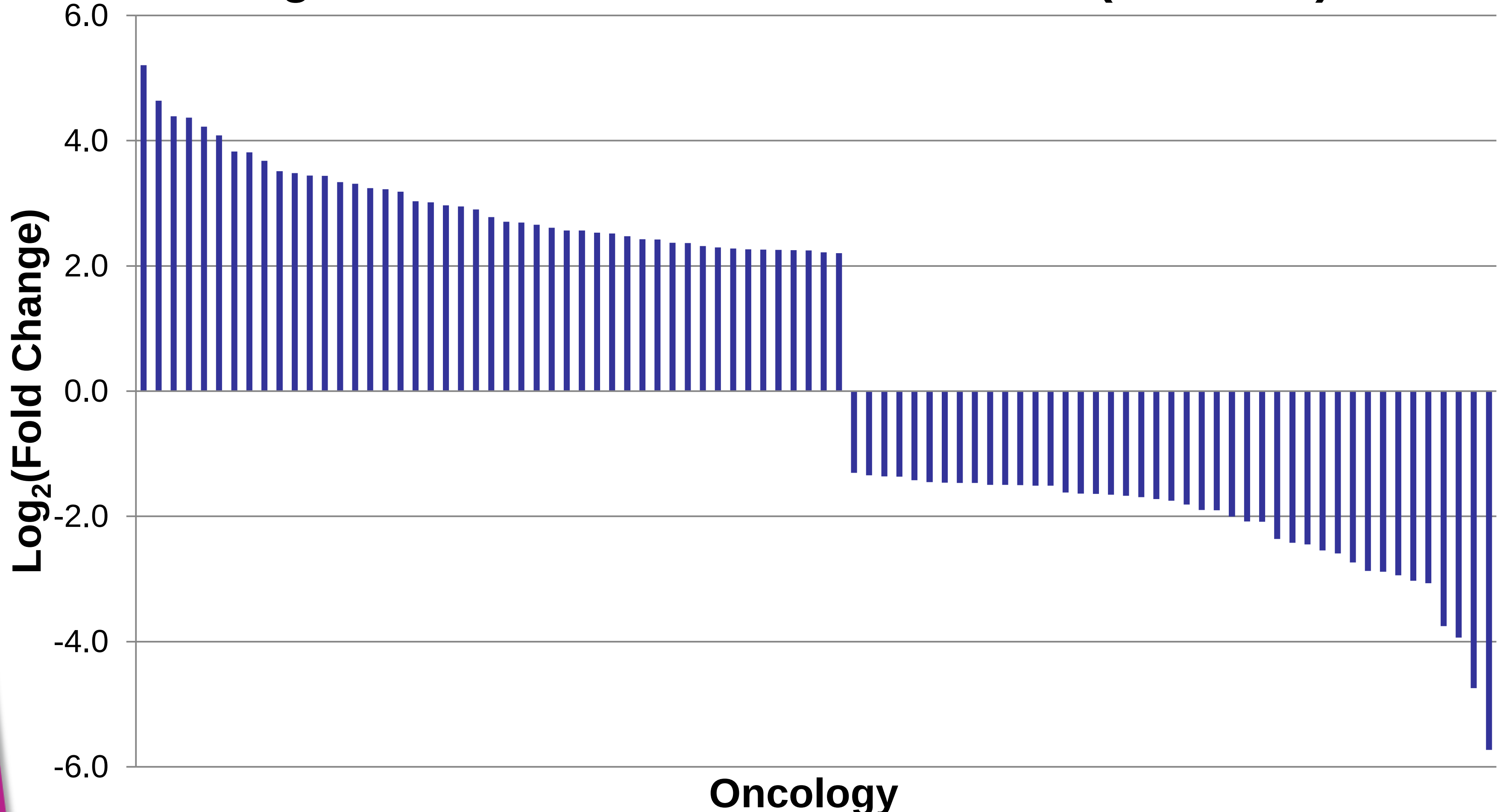
Advancing the science of tomorrow today.

miRNA DEG lists comparison (FC cutoff 1.5)



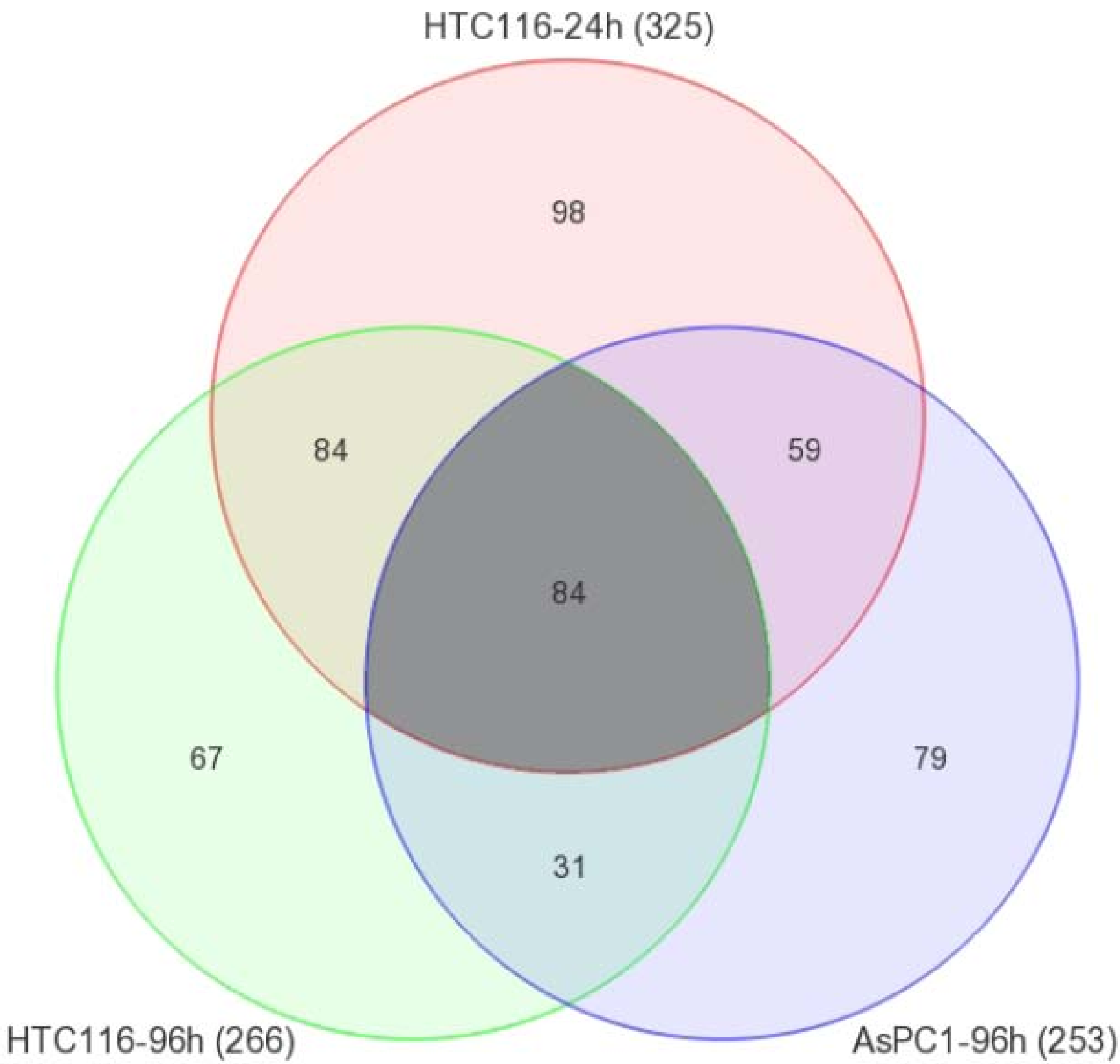
Michael Kahn – unpublished data

Differential Expression of Oncology panel in 24 hr Drug Treated vs. DMSO HCT-116 Cells (All Mean)



Michael Kahn – unpublished data

Oncogene mRNA DEG lists comparison-fold change cutoff=1.5



Michael Kahn – unpublished data

USC Study - Dr. Michael Kahn

- Differential expression levels were observed in the drug treated versus untreated
  - Human pancreatic adenocarcinoma cell line
  - Human colorectal carcinoma cell line
- Differential expression was detected with both the SmartChip miRNA and the SmartChip Oncology Panels
- These differences are being investigated further and have implications in multiple types of cancer

1. Fumi Takahashi-Yanaga and Michael Kahn. (*2010)* Targeting Wnt Signaling: Can We Safely Eradicate Caner Stem Cells? *AACR 16(12): 3153 - 3162.*

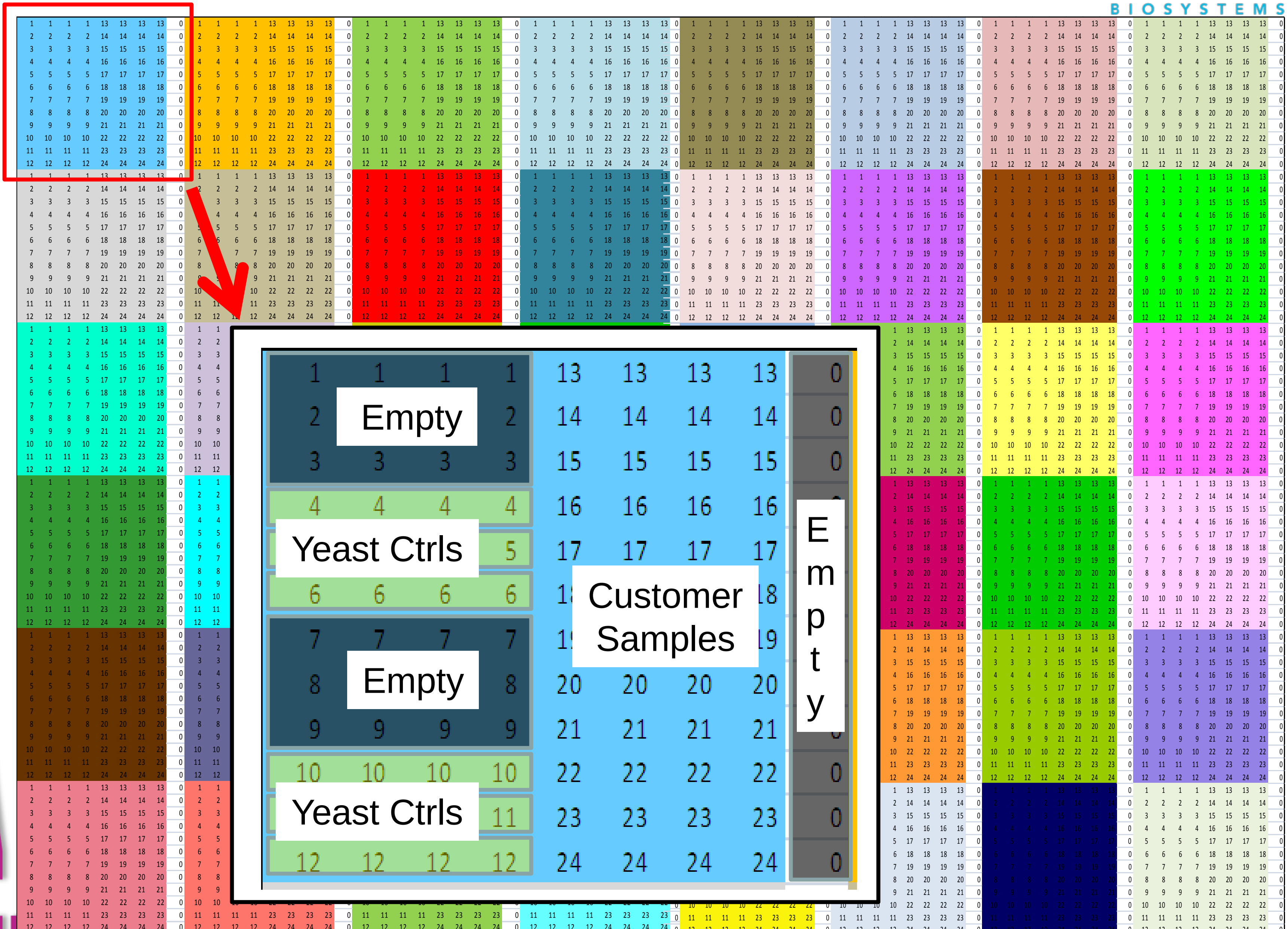
Collaborative Study: Using a Gene Signature to Stratify Patient Populations

Advancing the science of tomorrow today.

Multi-sample Capability

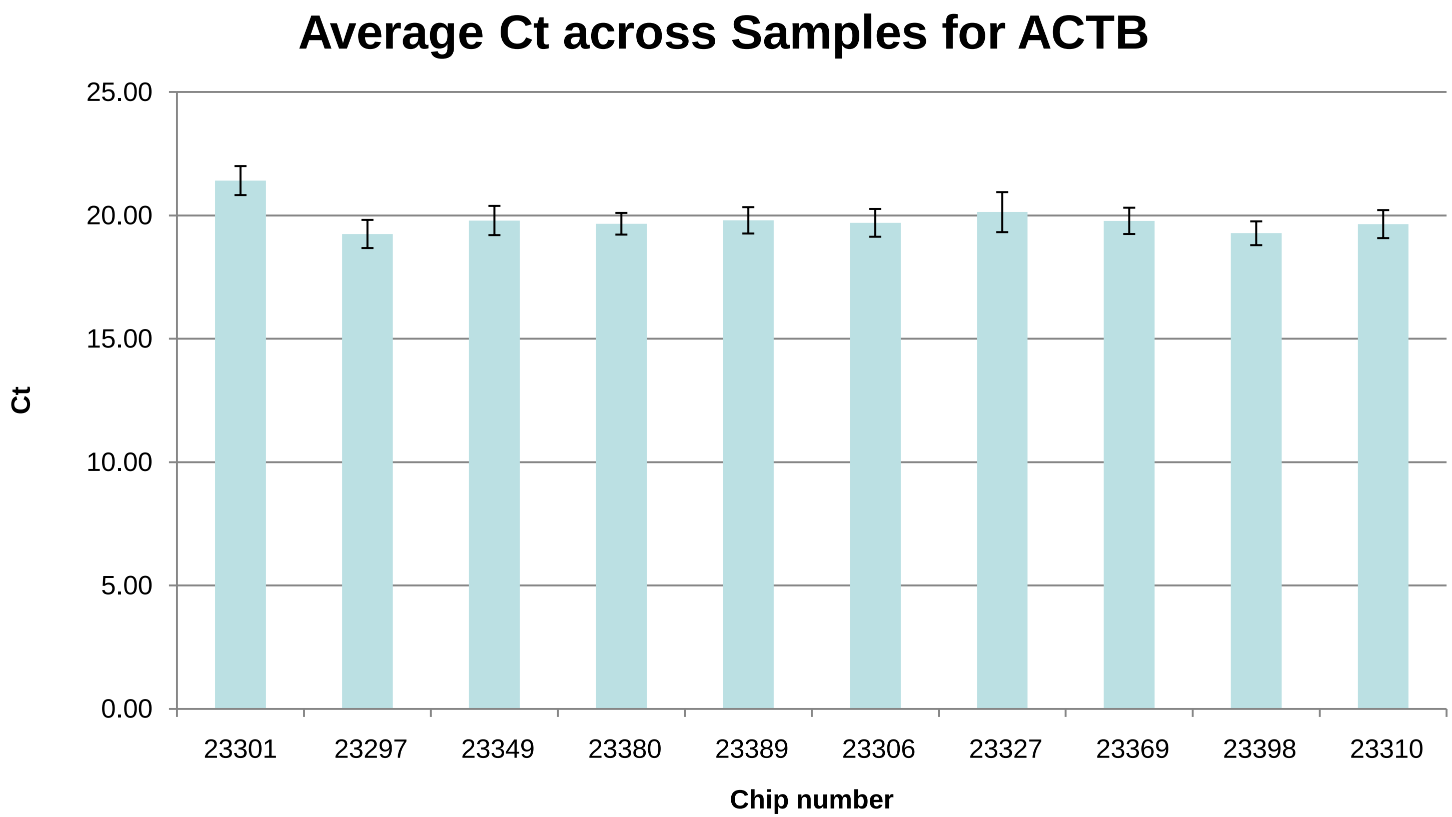
- A customer project involving validation of a 12 gene signature with >200 samples was contracted. The assays were designed and validated. A print pattern was designed to include 4 replicates of each of the 12 genes plus 4 replicates of the 6 spiked yeast control genes.
- Each chip was designed to be dispensed with 46 customer samples, one positive control (universal cDNA) and one negative control (NTC). A total of 5 chips were dispensed with the customer samples.
- Each primer pair was QC tested with universal cDNA and synthetic targets on a Roche LC480 and the amplicon was sequence confirmed.

Print pattern: Each color code represents a different cDNA sample and each number represents a different primer pair



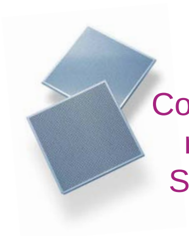
Advancing the science of tomorrow today.

Interchip variation of average Ct values for ACTB with universal cDNA



Advancing the science of tomorrow today.

The SmartChip System
Redefining Genomic Analysis



Content-ready nano-well SmartChips

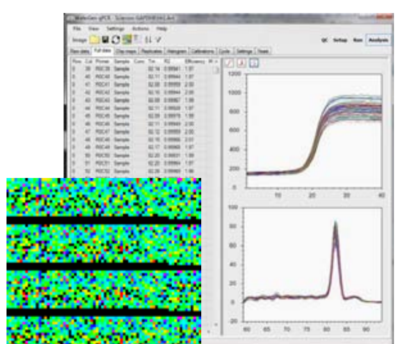
1,000's of miRNA and pathway-based PCR assays

Single-sample Nanodispenser

Multi-sample Nanodispenser

SmartChip Cycler

> 5,000 Real-Time PCR assays/chip



Benefits

- **Cost-effective**
  - High-throughput (5k-30k assays/chip)
  - Nano-scale assays
- **Faster results**
  - High-throughput (5k-30k assays/chip)
  - Simplified workflow
- **Superior Performance**
  - Real-Time PCR-like performance (sensitivity, dynamic range, quantification)
  - Pre-validated pathway based panels
- **Flexible content**
  - Off-the-shelf panels
    - Oncology pathways
    - miRNA
  - Custom panels

WAFERGEN BIOSYSTEMS

Thank You!

Advancing the science of tomorrow today.