



# A nanoliter-scale method for high throughput, real-time PCR gene expression profiling in oncology and miRNA gene sets using the SmartChip System

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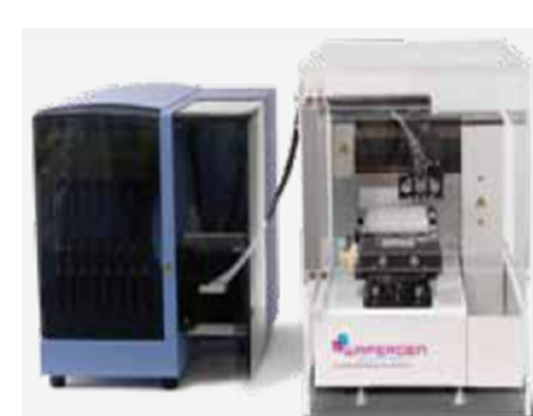
## Abstract

The SmartChip Real-Time PCR System from WaferGen Biosystems is a platform for performing high-throughput, real-time PCR assays for multiple applications, including gene expression studies. This system combines the benefits of real-time PCR, including superior sensitivity, precision and dynamic range, with the low cost-per-sample and high-throughput profiling power of hybridization arrays. The system consists of three components: a SmartChip Panel, comprising 5184 nanowells pre-loaded with gene-specific reaction content, a SmartChip Nanodispenser for automated delivery of sample and reaction mix to the SmartChip Panels, and a SmartChip Cycler for performing and collecting data from the real-time PCR assays. Using cDNA as an input for this system, one can generate thousands of data points comprising a thorough portrait of relative gene expression from a single sample.

The SmartChip System has been extensively tested with two expression profiling panels: The SmartChip Human Oncology Panel and the SmartChip Human microRNA Panel. These SmartChip Panels containing some 1250 cancer-related genes and 1200 miRNA's respectively, show strong intra-chip precision and inter-panel reproducibility. Additionally, various controls have been incorporated into the SmartChip qPCR Software to facilitate analysis. The SmartChip Panels were also used to test cDNA from a variety of samples, including commercially available RNA from tissue extracts, RNA from tissue culture cells and RNA from clinical samples for research.

The data generated on the SmartChip System demonstrates that you can achieve the simplicity and robustness of real-time PCR with the high throughput and cost effectiveness of microarrays.

## SmartChip Platform Overview



### SmartChip Multisample Nanodispenser

- Sample flexibility:** Enables automated loading of up to 384 samples on a single SmartChip Panel.
- Proven performance:** Demonstrated precision at nanoliter volumes.
- Reliable results:** Active evaporation control and chip cooling station.
- Quick loading:** Prepare an entire 5,184 nanowell SmartChip Panel in as little as 10 minutes.



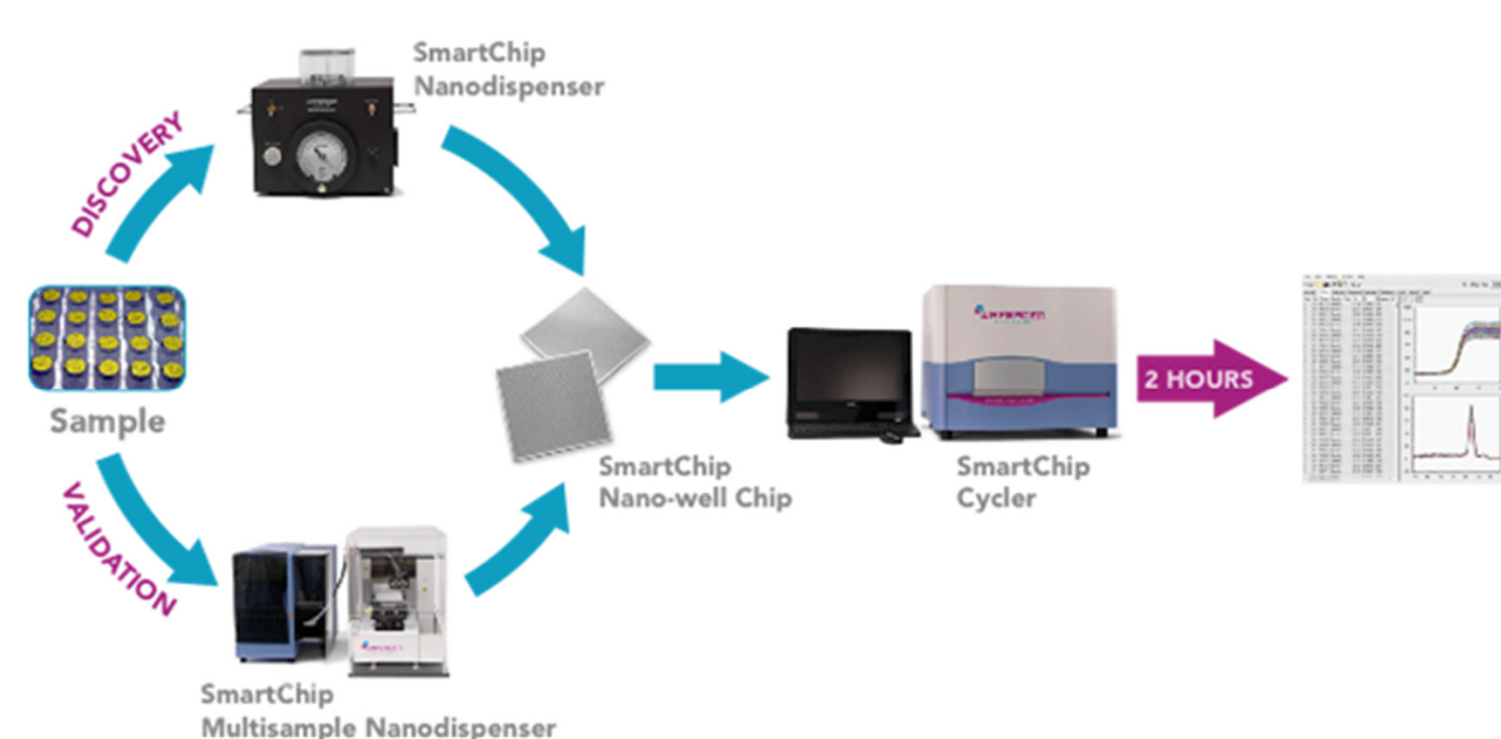
### SmartChip Nanodispenser

- Single-step loading:** Dispense a solution of cDNA and Real-Time PCR Master-Mix for an entire 5,184 well SmartChip in one easy step that takes less than 10 minutes.



### SmartChip Cycler

- Rapid results:** Thermal cycle and collect data on the 5,184 nanowell SmartChip Panel in ~ 2 hours.
- Temperature precision:** precise temperature control to produce intrachip Ct standard deviations < 0.1 Ct, demonstrated in control templates.



**Discovery:** Load a single cDNA sample and profile over 1000 genes in a simple workflow  
**Validation:** Profile 10's to 100's of genes on up to 384 samples in one experiment

## SmartChip Panel Overview

Every real-time SYBR® Green PCR assay contained in a SmartChip Panel undergoes many quality control steps to ensure the assay functions reproducibly at nanoliter scale (Table 1). The multiple levels of validation, including sequencing each amplicon, produce a basis for high quality quantitative PCR performance with these robust assays.

WaferGen Biosystems offers two focused panels for biomarker profiling in cancer with ~1200 targets and controls in quadruplicate. Additionally, WaferGen Biosystems has augmented this offering to include SmartChip Custom Panels which have flexible formats to address various throughput needs in Gene Expression profiling:

### The SmartChip Human Oncology Panel

1250 gene-specific assays covering 17 functional groups (Table 2). Includes 11 endogenous and 5 exogenous real-time PCR controls

### The SmartChip Human microRNA Panel

1200 microRNA specific assays, comprising all known human miRNAs, designed to the human miRBase version 16.0.

Includes 12 endogenous and 5 exogenous real-time PCR 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. A variety of formats are available, providing flexibility in the number of genes and samples per experiment (Table 3).

Table 1

Specifications	Criteria
Meets strict bioinformatics criteria	<ul style="list-style-type: none"> <li>Maximize primer specificity</li> <li>Minimize primer-dimers</li> <li>Targeted annealing Tm</li> <li>Short amplicon length</li> </ul>
Meets functional requirements	<ul style="list-style-type: none"> <li>Minimum 5-log dynamic range</li> <li>Specificity</li> <li>Melt analysis</li> <li>Get analysis</li> </ul>
Target verification	Assays sequenced to ensure specific targeted transcript is amplified

Table 2

Gene Family	Count
Endogenous Control	11
Exogenous Control	5
ADME (Toxicology, drug metabolism/transport)	173
Apoptosis	198
Cancer	325
Cell Cycle/Proliferation	52
Cardiovascular Disease	226
DNA damage repair	36
Drug Target	43
G-protein coupled receptor	31
Growth factor	12
Homeostasis/Metabolism	9
Inflammation	142
Kinase	194
Neuro Disease	5
Phosphatase	3
Proteinase	11
Signal Transduction	303
Transcription Factor	95
<b>Total</b>	<b>968</b>

Table 3

Number of Samples	Number of Genes	Number of Replicates
96	12	4
48	24	4
24	48	4
12	96	4
6	192	4
3	384	4

## Results

### Precision

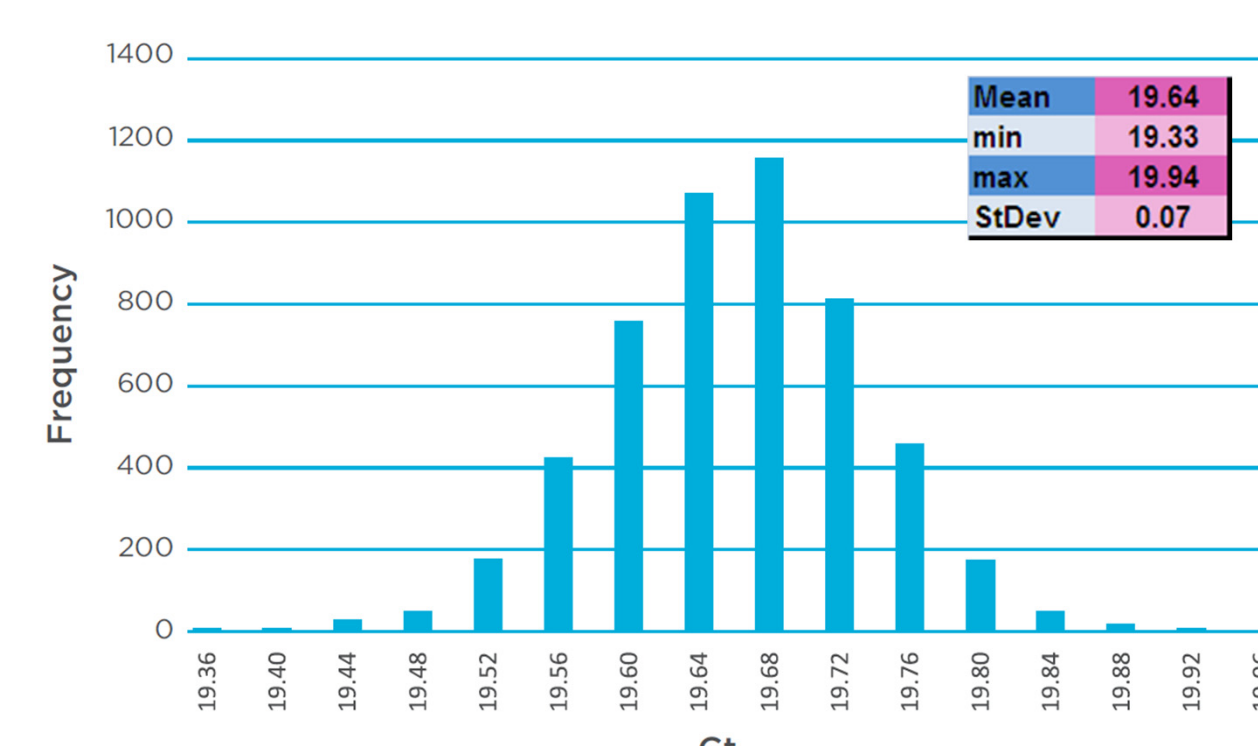
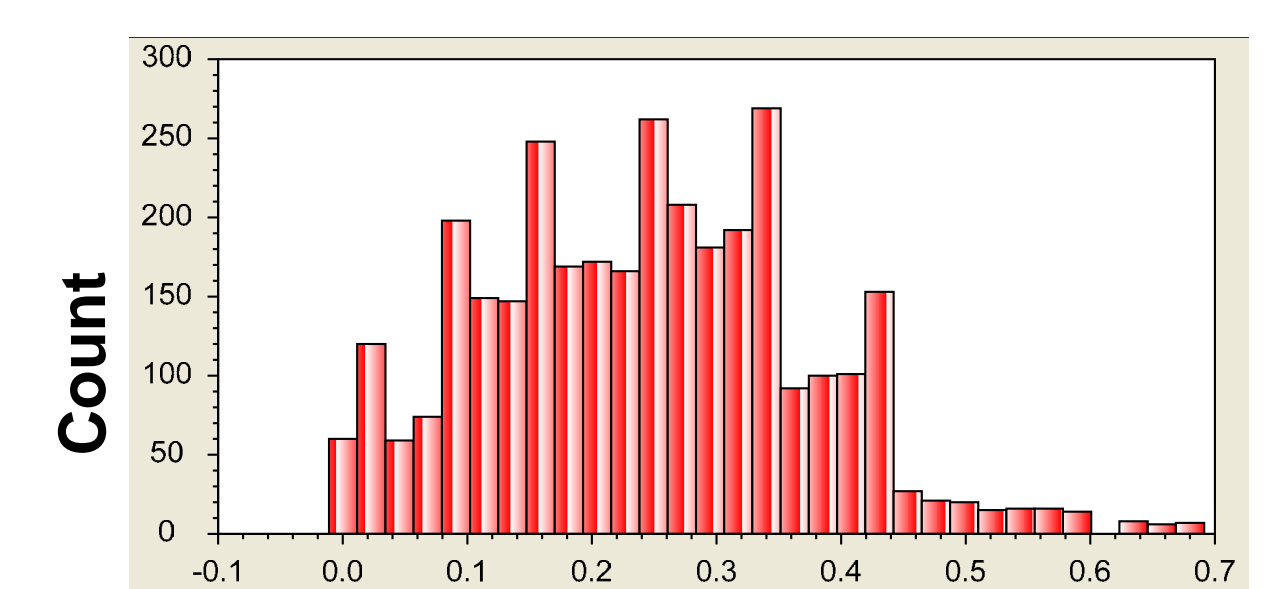


Figure 1

As a measure of system precision, the primers for a control target were loaded into the 5,184 SmartChip nanowell chip and control template was spiked into the PCR Master Mix and loaded before 40 cycles of real-time PCR. The resulting data contained 26 outliers, removed via Grubbs Method, and the resulting standard deviation of the Cycle Threshold (Ct) on the remaining wells is reported as 0.07 (Figure 1). Single target control runs are frequently run on the system and the average of standard deviation > 170 runs is < 0.1 SD of the Ct (data not shown).

Real-time PCR assay performance can also impact precision of the data. As a representative view of precision with contributions from both the system and assay, a representative SmartChip Human Oncology Panel is shown with standard deviations binned into 0.02 steps (Figure 2). Each of the genes in the panel are represented in quadruplicate, and the data is reflective of standard deviations of the replicate group after outlier removal by Grubbs Method.



Standard Deviation  
Figure 2

### Reproducibility

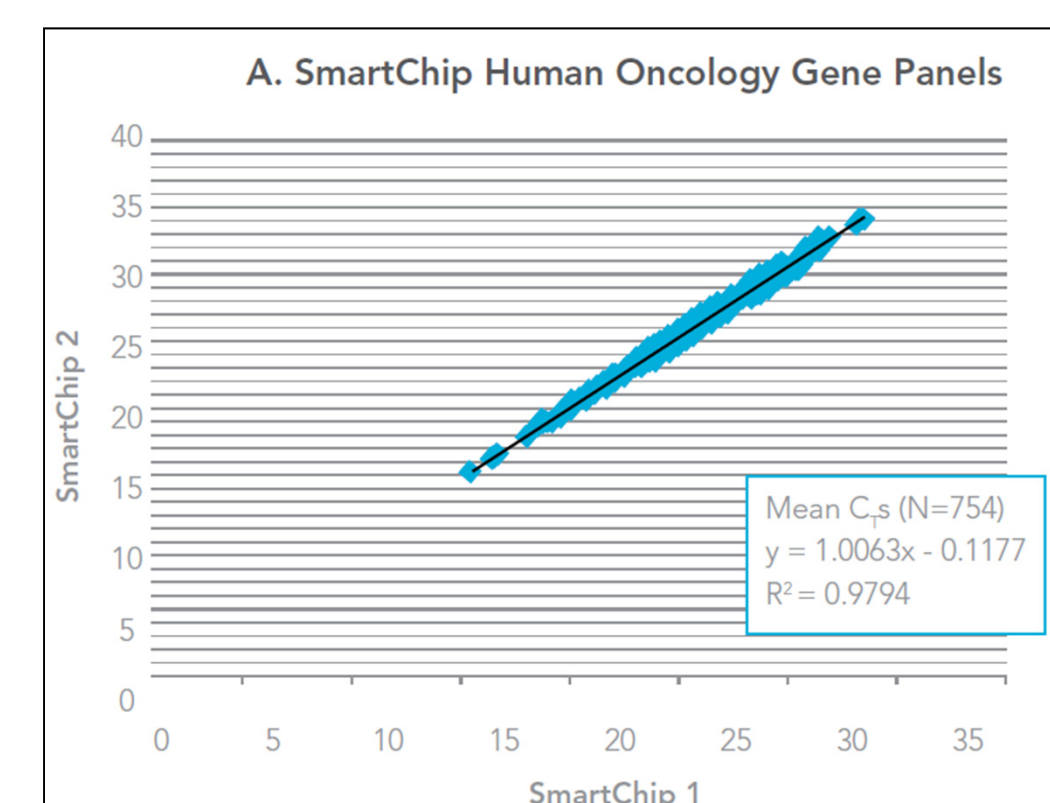


Figure 3

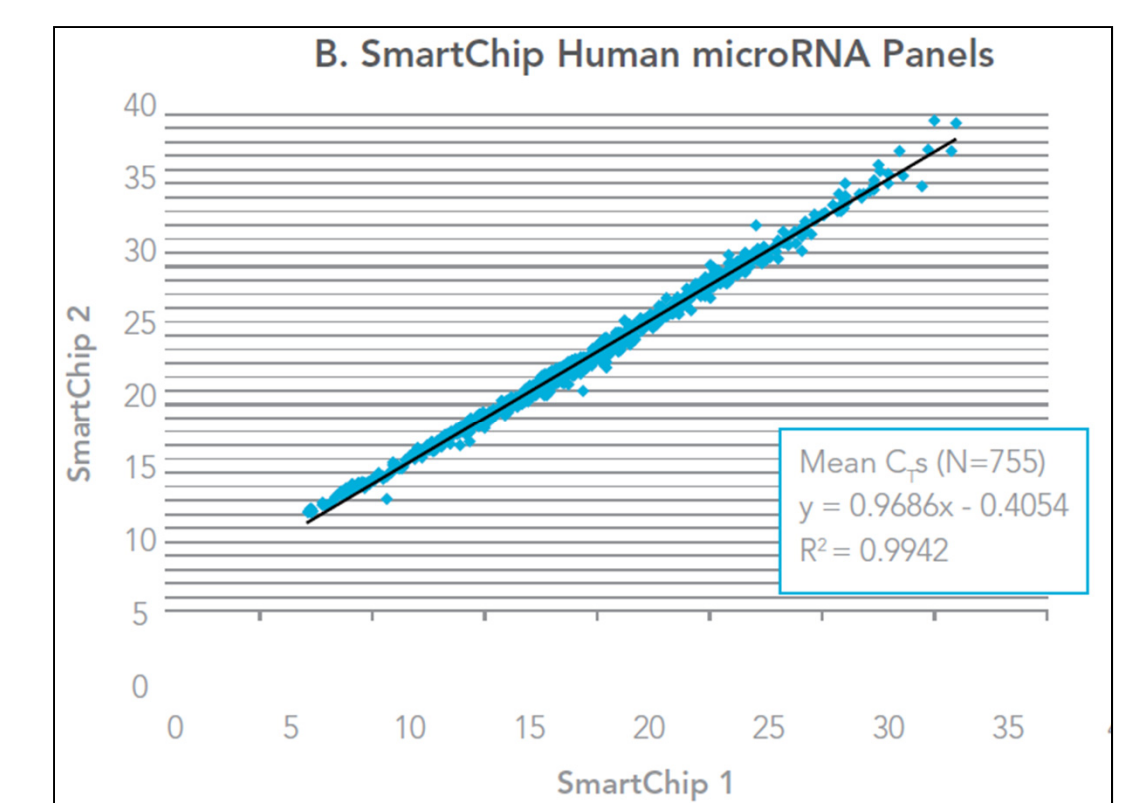


Figure 4

To demonstrate assay reproducibility, the same samples were repeated on two SmartChip Panels. Raw cycle thresholds (Ct) are plotted with associated curve fit and correlations. The SmartChip Human Oncology Panel data (Figure 3) was generated with 0.5µg of total RNA from fresh frozen lung tumor. The SmartChip Human microRNA Panel data (Figure 4) was generated with a panel of commercially available synthetic miRNA.

## SEQC Comparison

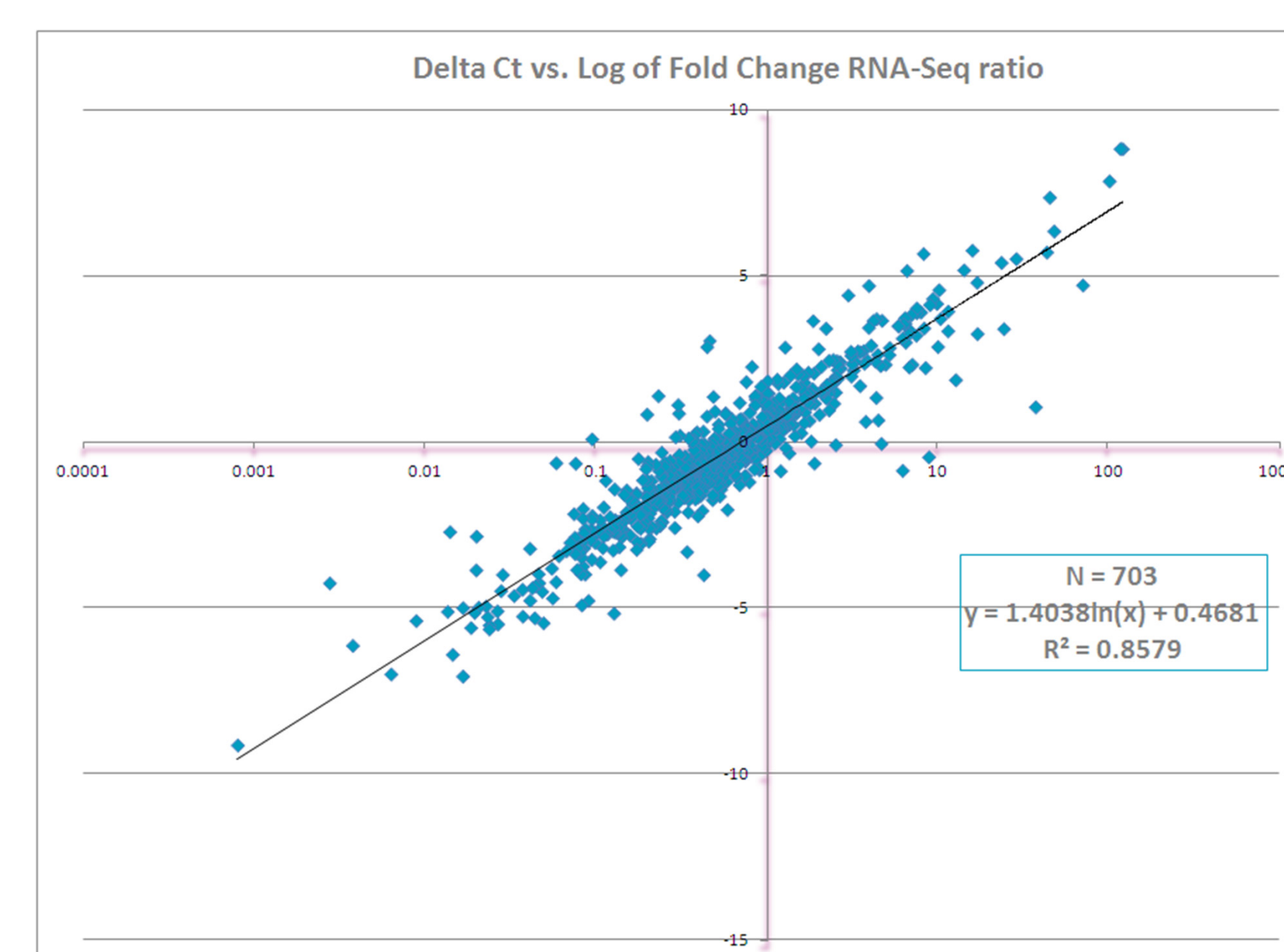


Figure 5

To compare the SmartChip System performance to an alternative technology, cycle thresholds (Ct) from both a human reference pool and brain RNA samples from the SEQC study were collected on the SmartChip Human Oncology Panel. Delta Ct's were determined for each gene that yielded significant Ct values collect for both samples (representing 703 genes on the panel). 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 (Figure 5).

## Conclusions

The SmartChip System is a high-density, high-throughput real-time PCR system that allows for a unique density of gene expression profiling with over 1,000 genes in a single experiment. The system also offers the flexibility to perform 10's to 100's of genes in custom gene panels to screen multiple samples on a single SmartChip Panel.

The SmartChip System has been designed to yield high quality data in a nanoliter format, demonstrating high precision with a standard deviation of < 0.1 on single control template across the 5,184 nanowells, and high precision of over 1,000 gene panels with control RNA from tissue.

Both the SmartChip Human Oncology and microRNA Panels have been designed to yield high reproducibility, demonstrated in a comparison of the same sample across independent runs, yielding correlation coefficients of > 0.97 for both panels.

The utility of the SmartChip System as a validation platform downstream of biomarker discovery systems has been demonstrated by a strong correlation to RNA-Seq methods. Using the SEQC data as a model, the SmartChip System yields a strong correlation of 0.86 compared to common genes detected in both data sets.