# A Novel Multiplexed Digital Gene Expression Technology

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TECHNOLOGY

One code = One mRNA

Biotin
Capture Probe Reporter Probe
mRNA
CodeSet/RNA Complex
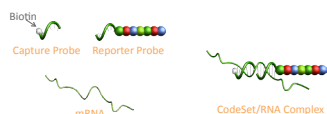
CodeSets are color coded "barcodes" that employ two 50-bp probes per mRNA which hybridize in solution. The Reporter Probe carries the signal, the Capture Probe allows the complex to be immobilized in the sample cartridge for data collection. Detection is direct and digital and the assay does not require cDNA synthesis or amplification.
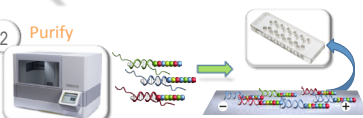
WORKFLOW

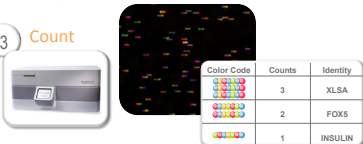**1 Hybridize**
1. Buffer
2. CodeSet
3. Sample
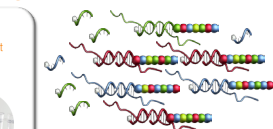
Sample material is mixed with excess CodeSet and hybridized overnight.

2 Purify

The Prep Station removes excess CodeSet and immobilizes CodeSet/RNA complexes in the nCounter Cartridge for data collection.

3 Count

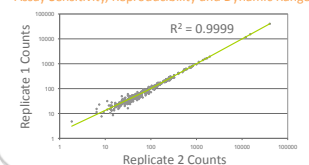| Color Code	Counts	Identity
	3	XLSA
	2	FOX5
	1	INSULIN

Sample cartridges are placed in the Digital Analyzer for data collection. Images of color codes on the surface of the cartridge are counted, and a running total of each target is tabulated.
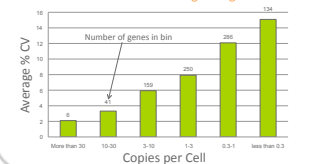
PERFORMANCE

Assay Sensitivity, Reproducibility and Dynamic Range

$R^2 = 0.9999$

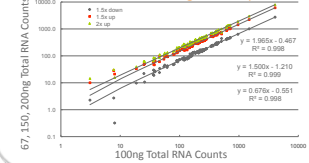Replicate 1 Counts vs. Replicate 2 Counts

Transcript counts for two technical replicates of a 509-plex CodeSet demonstrate the assay reproducibility over a wide dynamic range. 75 counts equals a [c] of ~1 copy/cell of a transcript, illustrating the high level of sensitivity and precision of the assay even at very low levels of expression.

Precision Across Large Ranges

Number of genes in bin

Average % CV vs. Copies per Cell (More than 30, 10-30, 3-10, 1-3, 0.3-1, less than 0.3) — 8, 45, 159, 250, 286, 134

Targets for the 509-plex assay were grouped by level of expression to evaluate the precision of the assay across the dynamic range. Percent CV was calculated for each group. The number of targets for each group are indicated by the number above each column. Targets expressed at below a single copy per cell demonstrated CVs less than 15%.
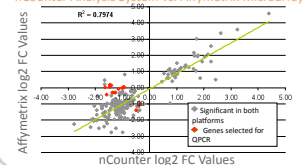
Fractional Fold Change Accuracy

67, 150, 200ng Total RNA Counts vs. 100ng Total RNA Counts

- 1.5x down
- 1.5x up
- 2x up

$y = 1.965x - 0.467$ $R^2 = 0.998$
$y = 1.500x - 1.210$ $R^2 = 0.999$
$y = 0.670x - 0.551$ $R^2 = 0.998$

The amount of input material was varied. The 100ng reaction was used as the baseline and 67, 150, and 200ng of input material was plotted against it. The slopes correlate closely with the expected values of 0.67, 1.5 and 2 which indicate the assay's ability to detect fractional fold changes.

CORRELATION

nCounter Analysis System vs. Affymetrix Microarray

$R^2 = 0.7974$

Affymetrix log2 FC Values vs. nCounter log2 FC Values

- Significant in both platforms
- Genes selected for QPCR

100ng of total RNA from Mock- and Polio Virus-infected cells were assayed for log2 fold change using both nCounter and Affymetrix's U133 Plus 2.0 arrays.

Correlation between nCounter, Affymetrix & TaqMan

- Microarray
- TaqMan
- NanoString

Gene Target vs. Reported log2 Fold Change
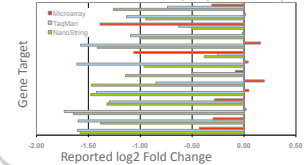
There was disagreement between nCounter and Affymetrix on 14 genes. TaqMan RT-PCR validated the nCounter results.

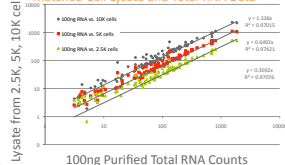### 20 Genes Analyzed by SYBR Green and nCounter

Signal Level vs. Timepoint
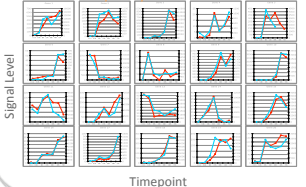
Expression levels for 20 sea urchin genes measured on the nCounter System at 7 development time points showed excellent correlation with SYBR Green Real Time PCR.

EFFICIENCY

Reaction Number Comparison Calculator		
Number of Samples	200	
Number of Genes	200	
Technical Replicates	3	
	nCounter	qPCR
Required #s of Reactions	600	120,000

SAMPLE TYPES

Matched Cell Lysate and Total RNA Data

2.5K, 5K, 10K cells

- 100ng RNA vs. 10K cells
- 100ng RNA vs. 5K cells
- 100ng RNA vs. 2.5K cells

$y = 1.336x$ $R^2 = 0.97015$
$y = 0.649x$ $R^2 = 0.97621$
$y = 0.3092x$ $R^2 = 0.97076$

Lysate from 2.5K, 5K, 10K cells vs. 100ng Purified Total RNA Counts
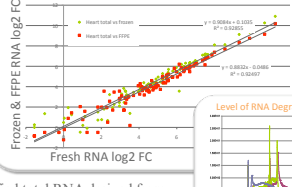
Raw cell lysate in varying amounts was input directly into the nCounter hybridization reaction and is compared here to a matched purified total RNA sample.

Matched Whole Blood Lysate and Total RNA Data

- Blood Lysate 1
- Blood Lysate 2

$y = 0.976x + 0.0272$ $R^2 = 0.98541$
$y = 0.9854x + 0.4538$ $R^2 = 0.9879$

Whole Blood Lysate log2 FC vs. 100ng Total RNA log2 Fold Change
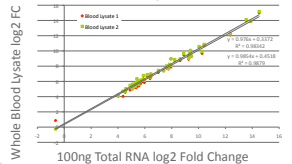
PaxGene lysed blood was input directly into the nCounter hybridization reaction and is compared here to a matched purified total RNA sample.

Matched Frozen and FFPE Derived Total RNA

- Heart total vs. frozen
- Heart total vs. FFPE

$y = 0.9884x + 0.5025$ $R^2 = 0.92851$
$y = 0.8832x + 0.0486$ $R^2 = 0.92497$

Frozen & FFPE RNA log2 FC vs. Fresh RNA log2 FC

Level of RNA Degradation

Purified total RNA derived from matched fresh, frozen and FFPE treated tissue were assayed with the nCounter System.

SUMMARY

The NanoString assay offers many advantages including:

- Direct digital detection
- Multiplex hundreds of genes
- High sensitivity & precision
- No enzymology
- Fully automated
- Cost competitive

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TECHNOLOGIES