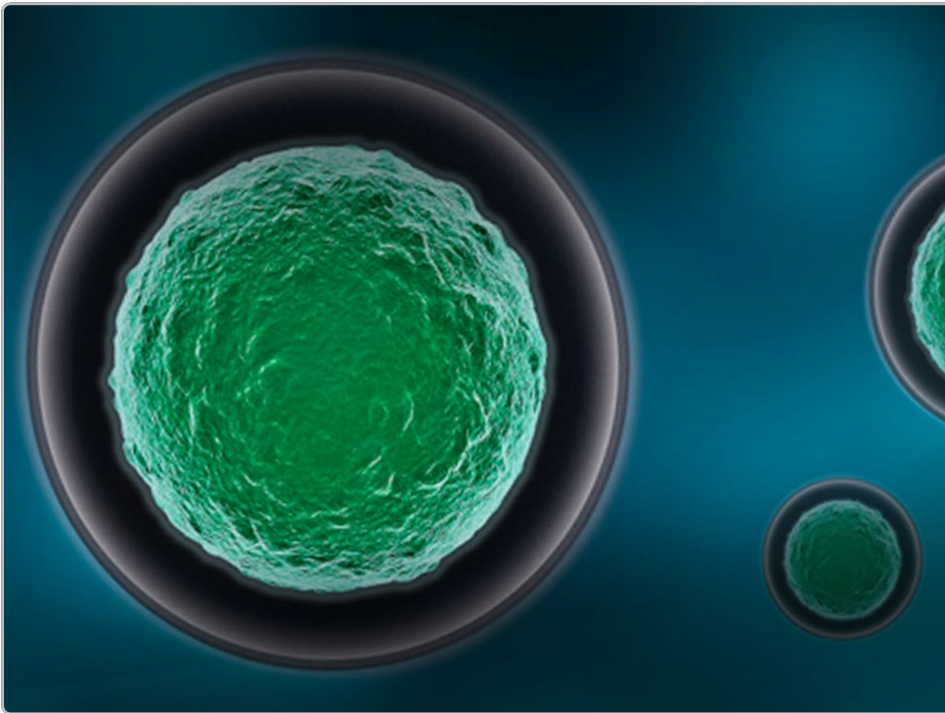




nCounter® Single Cell Gene Expression



Product Highlights

More Genes

- Analyze multiple pathways for up to 800 genes

High Sensitivity

- Eliminate sample splitting, minimize amplification - get better data from every cell

Digital Counting

- Determine fractional fold-changes - eliminate the variability of analog data

High Throughput

- Analyze hundreds of samples per day

nCounter® Single Cell Gene Expression

The nCounter® Analysis System offers an easy-to-use, cost-effective way to profile the expression levels of hundreds of genes simultaneously in a single tube, with high sensitivity and digital precision. Using molecular barcoding and single molecule imaging, the system detects and counts individual transcripts for up to 800 genes in a single reaction.

The introduction of the nCounter® Single Cell Gene Expression Assay greatly expands the types of studies that can be analyzed on the nCounter Analysis system, allowing researchers to address biological questions that have previously been impossible to answer. Entire biological pathways and custom gene signatures can now be studied for single cells without the necessity to match the gene number to the format of a microfluidic PCR consumable.

The nCounter Single Cell Gene Expression Assay provides an ultra-sensitive, reproducible, and highly multiplexed solution for profiling gene expression from single cells or as little as 10pg of total RNA. A **Multiplexed Target Enrichment (MTE)** step allows transcripts within individual cells to be linearly amplified after a reverse transcription step. As shown in **FIGURE 2**, MTE can linearly amplify up to 800 targets from a single cell in a single tube without bias. The resulting amplified material is then directly hybridized with an nCounter CodeSet targeting the genes of interest - no sample clean-up or sample splitting is required. After hybridization samples are processed on the nCounter Prep Station and Digital Analyzer using the standard gene expression protocols.

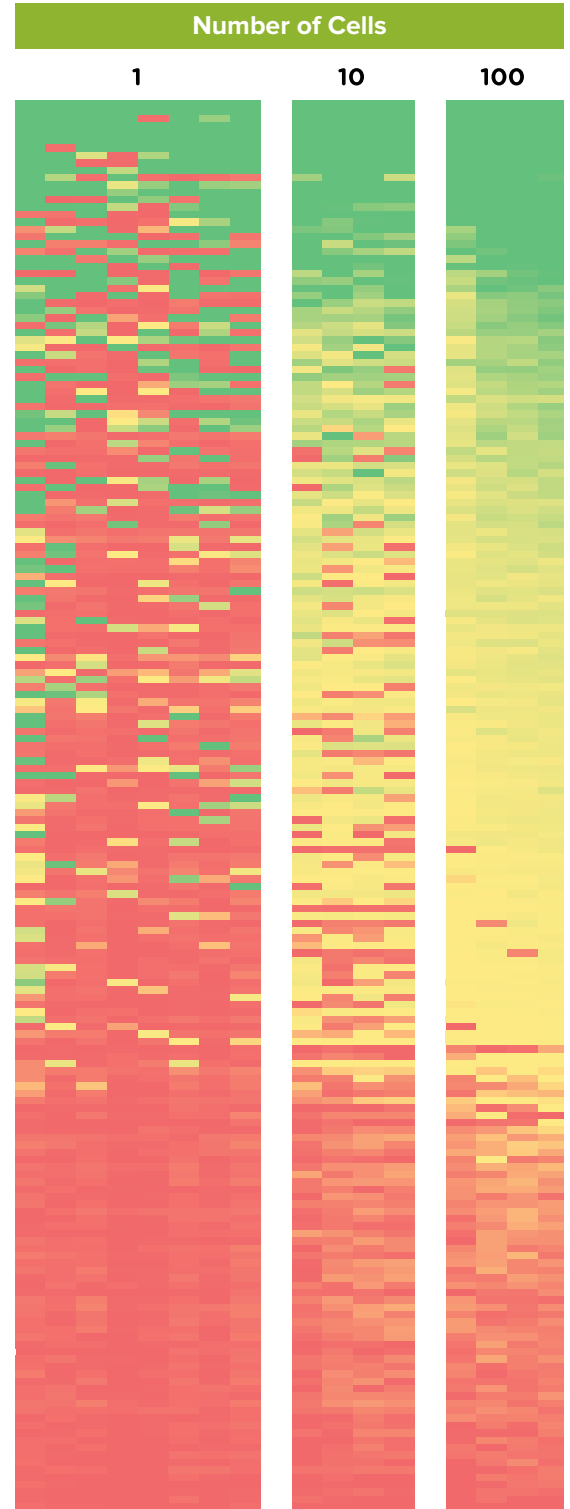
Molecules That Count®

The Power of Single Cell Analysis

Historically, analysis of gene expression data has relied upon the assumption that the expression state of any single cell of a given type, or within an otherwise seemingly homogenous population, is accurately reflected by an average expression profile. The unprecedented resolution afforded by single cell RNA analysis allows for relative gene expression level comparisons across cell types under different experimental conditions and disease states, providing an avenue to new mechanistic insights and a means of elucidating previously hidden relationships between individual cells within a population. As such, the ability to analyze single cell gene expression, rather than global and regional assessments of mRNA expression, has tremendous potential to unlock a wealth of biological information.

In order to demonstrate how analyzing single cells can uncover significant differences in gene expression profiles that are hidden when looking at larger cell populations, individual (and small populations) of CD8+ T-cells were flow-sorted. After reverse transcription and amplification using the nCounter Single Cell Gene Expression protocol, a gene expression profile for each sample was generated using a 191 gene custom CodeSet on the nCounter Analysis System (FIGURE 1). The data clearly demonstrates that individual single cells have very different expression profiles, however this diversity is masked in the samples containing 10 pooled cells, and almost completely hidden in the samples containing 100 pooled cells.

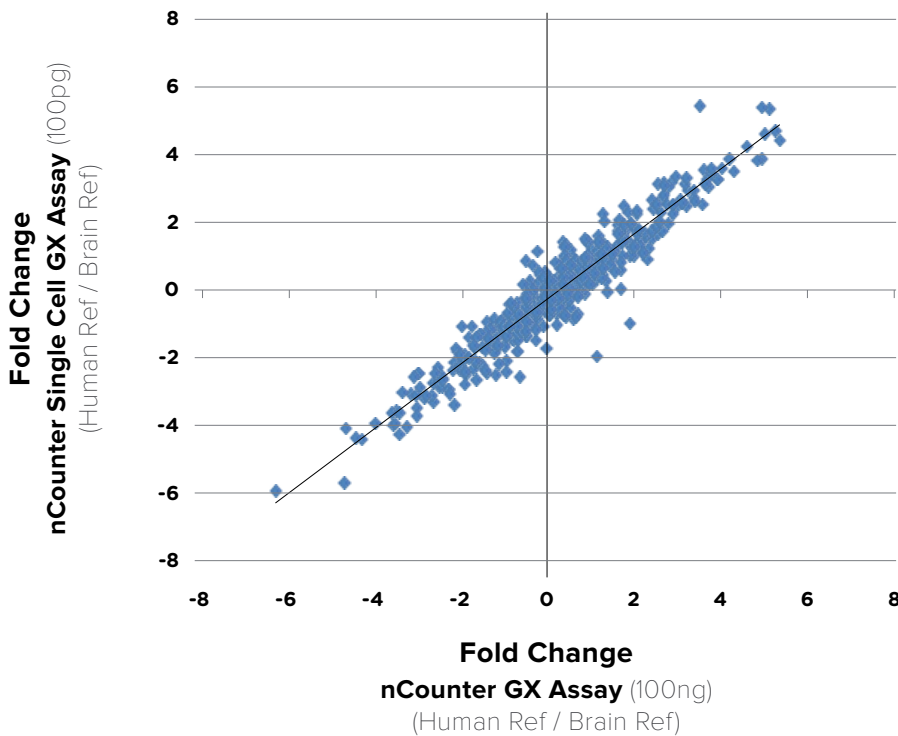
FIGURE 1: Gene expression profile



High Sensitivity with Linear Response

A **Multiplexed Target Enrichment (MTE)** was performed with 800 primer pairs using 100pg of either Human Reference or Brain Reference total RNA as sample input. Fold changes were calculated for all probes exhibiting significant detection and plotted against the fold changes observed in unamplified samples (100ng) for the same genes after hybridization with nCounter GX probes. These data show a 1:1 correlation of fold changes between assays, demonstrating the simultaneous, unbiased amplification of hundreds of target transcripts and preservation of fold change information provided by the nCounter Single Cell Gene Expression protocol (FIGURE 2).

FIGURE 2: Highly correlated fold change



Applications

Cancer

The study of rare cell populations such as drug resistant cell clones, tumor microenvironments and circulating tumor cells has been historically difficult. Single cell profiling technologies, especially those that allow for numerous pathways to be examined simultaneously, will greatly expand the ability to assess the diversity within these cell populations and pave the way for new clinical applications.

Stem Cells

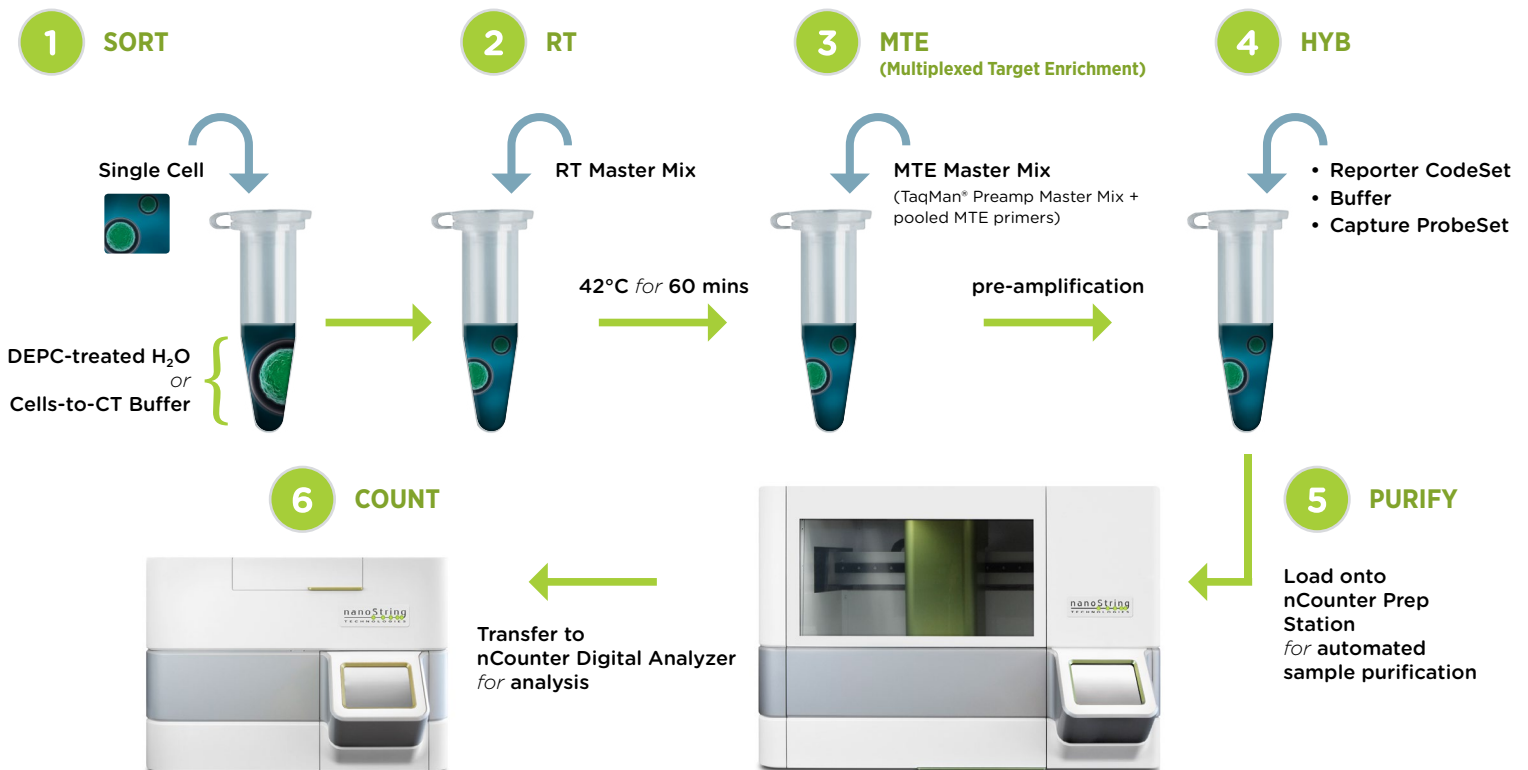
The nature of stem cells makes them well suited to single cell technology. To fully understand the function of each stem cell, scientists must be able to track an individual cell and monitor its differentiation potential. Because stem cells are often available in limited quantities, they are best queried with tools allowing for single cell sensitivity.

Immunology

The study of immune system cells is another research area for which single cell resolution is especially useful. Seemingly homogeneous populations of immune cells, such as T-cells and B-cells, have been found to contain heterogeneous mixtures of cells performing various functions, leading researchers to determine that there is far more diversity among these cells than previously thought.

Single Cell → Single Tube → 800 Genes

FIGURE 3: nCounter Single Cell workflow



Conclusion

The nCounter Single Cell Gene Expression application provides researchers with an exceptional approach to discovering differences in cell-to-cell gene expression profiles by offering high sensitivity and flexibility. Using this application, researchers can let the biology determine the number of genes they want to profile – anywhere from 20 to 800 genes. The ability to analyze up to 800 genes in a single tube without sample splitting frees researchers from the constraints of fixed-format consumables employed by existing technologies. The high correlation of results obtained with non-amplified material and amplified material (as shown in FIGURE 2) demonstrate that the nCounter Single Cell Gene Expression Assay does not introduce measurable bias.

nCounter® Analysis System

The nCounter Analysis System from NanoString offers a cost-effective way to easily profile hundreds of gene transcripts simultaneously with high sensitivity and precision. The digital detection of target molecules and high levels of multiplexing eliminate the compromise between data quality and data quantity, bringing better sensitivity, reproducibility, and linearity to your results. It is ideal for studying defined gene sets across a large sample set, e.g., microarray validation, pathway analysis, biomarker validation, and splice variation analysis.

The system utilizes a novel digital technology that is based on direct multiplexed measurement of gene expression and offers high levels of precision and sensitivity (<1 copy per cell). The technology uses molecular “barcodes” and single molecule imaging to detect and count hundreds of unique transcripts in a single reaction.

System Performance

Description	Specifications
Level of multiplexing	20 – 800 gene targets
Recommended amount of starting material	Single cell or down to 10 pg of purified total RNA
Sample types supported	Single cell or total RNA
Spike correlation	$R^2 \geq 0.95$
Linear dynamic range	7×10^6 total counts
nCounter Prep Station throughput	48 samples < 2.5 hours
nCounter Digital Analyzer throughput	48 samples / 4 hours
Controls	6 positive assay controls 8 negative assay controls

Ordering Information

Description	Quantity / Use	Part Number (P/N)
nCounter Single Cell Gene Expression Assay Kit		GXA-1CELL-###
nCounter Analysis System (includes the Prep Station and Digital Analyzer)	1	NCT-SYS-120
Additional nCounter Prep Station	1	NCT-PREP-120
Additional nCounter Digital Analyzer	1	NCT-DIGA-120

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