NanoString Technologies, Inc.

nCounter Elements™ Reagents

REV 4.0

OPEN ARCHITECTURE DIGITAL GENOMICS

Molecules That Count®

Gene Expression • Copy Number Variation • Single Cell Gene Expression

nanoString TECHNOLOGIES®

Open Architecture Digital Genomics

NanoString's **nCounter Elements™ reagents enable digital detection and counting of large sets of nucleic acids in a single reaction without amplification*.** They can be used for the development of highly multiplexed Gene Expression or Copy Number Variation assays.

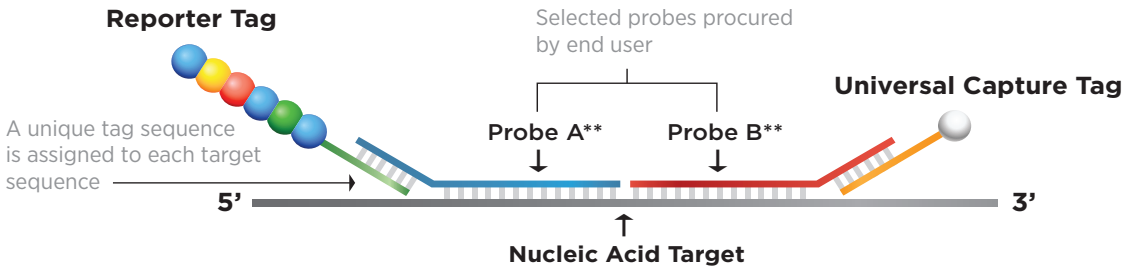
Design and Develop Your Own Assay

- **Digital** » nCounter Elements™ use molecular barcodes to enable **digital quantification of nucleic acids of interest**
- **Multiplexed** » Multiplex **up to 216 custom targets per sample in a single tube**
- **Simple Workflow** » 3 pipetting steps per sample; **no reverse transcription, no library prep, no amplification***
- **FFPE Compatible** » **Highly correlated results** from **Fresh Frozen and FFPE samples**
- **Reproducible** » Robust molecular barcoding chemistry and simple workflow **minimize variability**

Flexible Format Enables Wide Range of Study Types

- **Custom designs for as few as 12 samples**
- **Ideal for complex projects requiring iterative design**
- **Target-specific oligonucleotide probes can be re-used in multiple studies**

nCounter Elements™ Chemistry



** Single Cell assay requires reverse transcription and amplification prior to hybridization with nCounter Elements.*

*** Probes A and B are 65–85 bp oligonucleotide probes that may be purchased from an oligo supply company.*

nanoString TECHNOLOGIES®

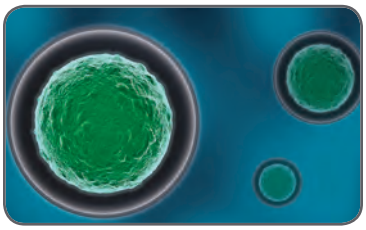
Innovation in Research *and* Translational Applications

nCounter Elements reagents enable analysis with color-coded molecular barcodes that facilitate detection and quantification of RNA and DNA targets by hybridizing to target-specific oligonucleotide probes that can be purchased from an oligo supplier. They are ideal for a range of applications requiring efficient, **high-precision quantitation of tens to hundreds of target molecules across a sample set.** This unique chemistry **generates high-quality results from challenging sample types, such as FFPE tissue or crude cell lysates.**

RNA Analysis

Gene Expression Analysis

- Analyze up to 216 genes simultaneously
- 3 pipetting steps per sample; no reverse transcription, no amplification, no enzymes*
- Directly assay tissue, cell and blood lysates, and FFPE extracts in a simple workflow
- Precise and reproducible results



Single Cell Gene Expression Analysis

- Analyze a single cell in a single tube for up to 216 genes of interest
- Reliable, digital results from as little as 100 pg of RNA
- Obtain single cell sensitivity while minimizing amplification cycles

Fusion Gene Analysis

Analysis of Expressed Fusion Genes

- Identify fusion events without knowledge of partner genes
- Characterize specific fusions by probing the junction sequence
- Characterize over 200 fusions in a single assay
- Study fusions and gene expression targets in the same assay

DNA Analysis

Copy Number Variation Analysis

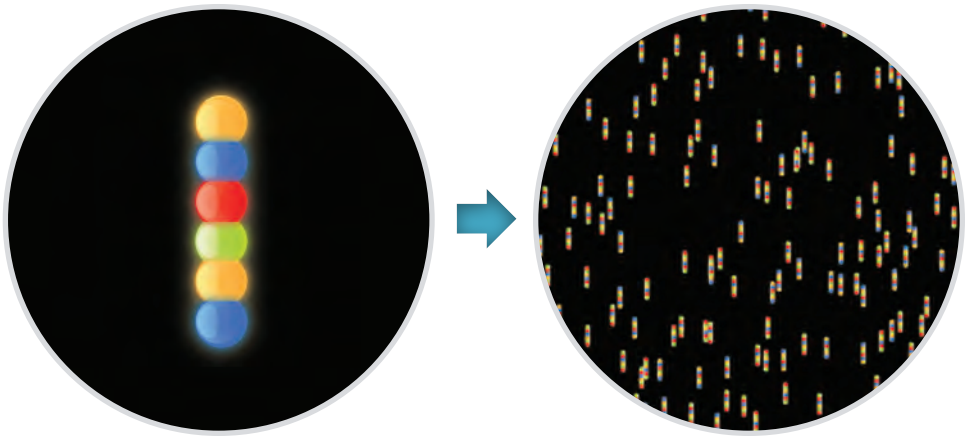
- Multiplex up to 216 target regions in a single reaction
- Just 25-minutes of hands-on time per 12 samples
- Linearity over wide dynamic range enables analysis of multiallelic CNVs
- Reliable data from FFPE samples

Innovation in Chemistry

Based on NanoString's **patented technology**, nCounter Elements is a digital, molecular barcoding chemistry that **allows users to assemble their own assays with oligonucleotide probes that target their genes of interest.** They enable **highly multiplexed, direct profiling of individual molecules in a single reaction without amplification*.**

Molecules That Count®

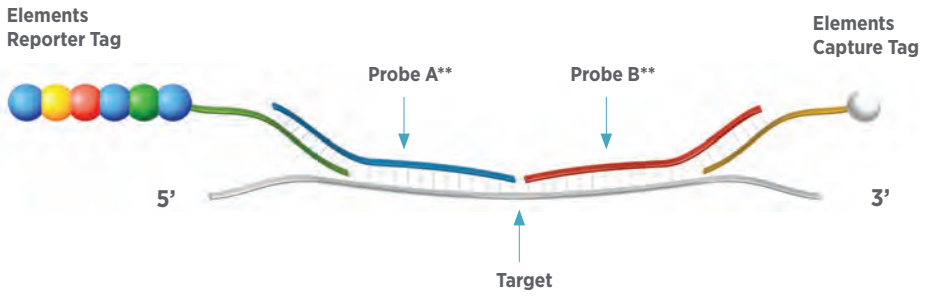
Each color-coded barcode represents a single target molecule. Barcodes hybridize to target-specific probes and can be individually counted without the need for amplification* – **providing high-quality digital data.**

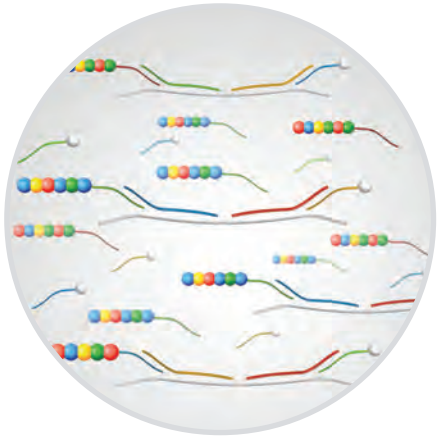


Single-molecule barcodes each hybridize to an individual target-specific probe via a unique tag sequence.

1

HYBRIDIZE





solution phase hybridization

Hybridization

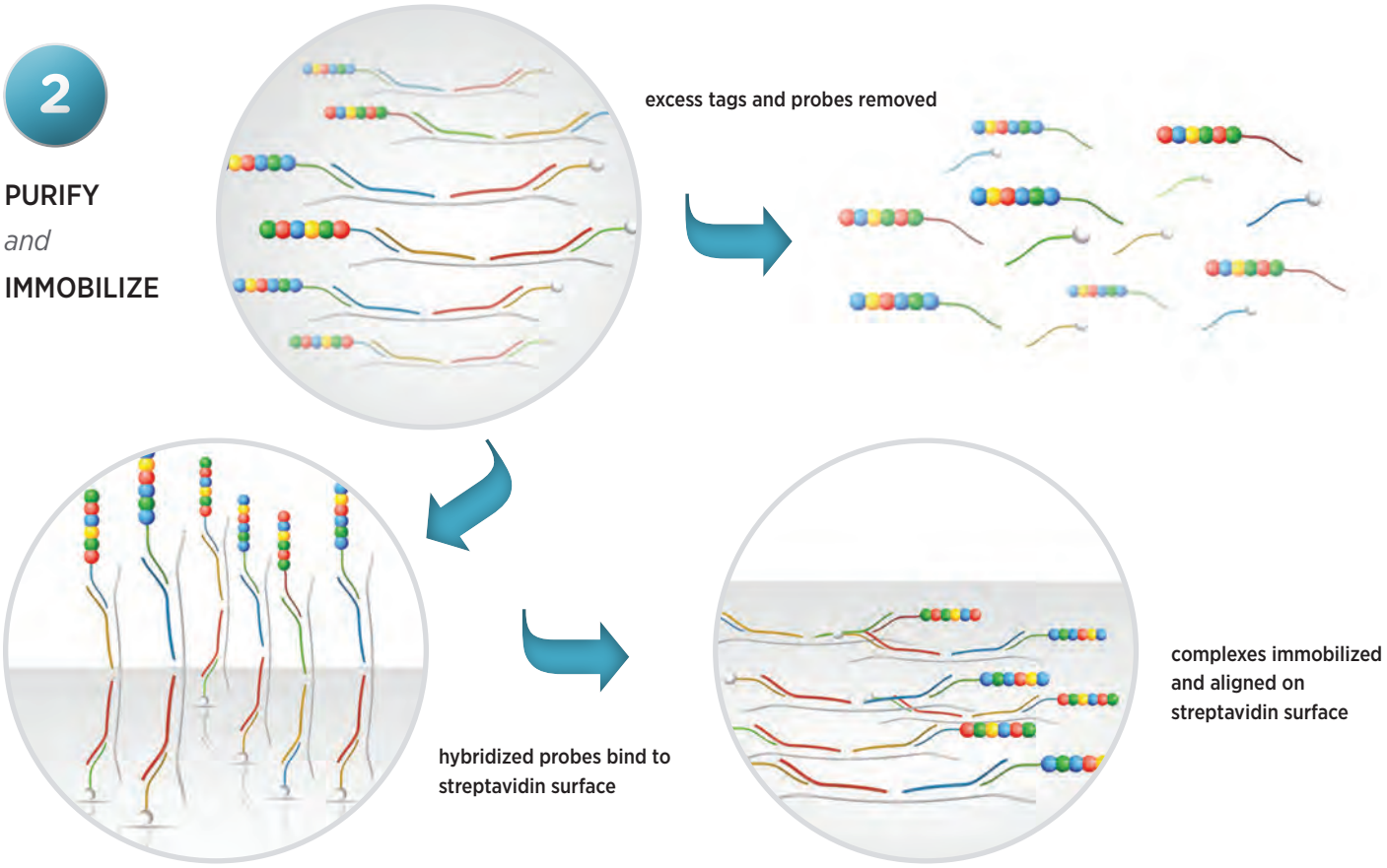
nCounter Elements **Reporter and Capture Tags**, target-specific probes (**Probes A and B****), and **Target** molecules hybridize to one another in solution. The **Reporter Tag** carries the signal and the **Capture Tag** allows the complex to be purified and immobilized for data collection.

** Single Cell assay requires reverse transcription and amplification prior to hybridization with nCounter Elements.*

*** Probes A and B are 65–85 bp oligonucleotide probes that may be purchased from an oligo supply company.*

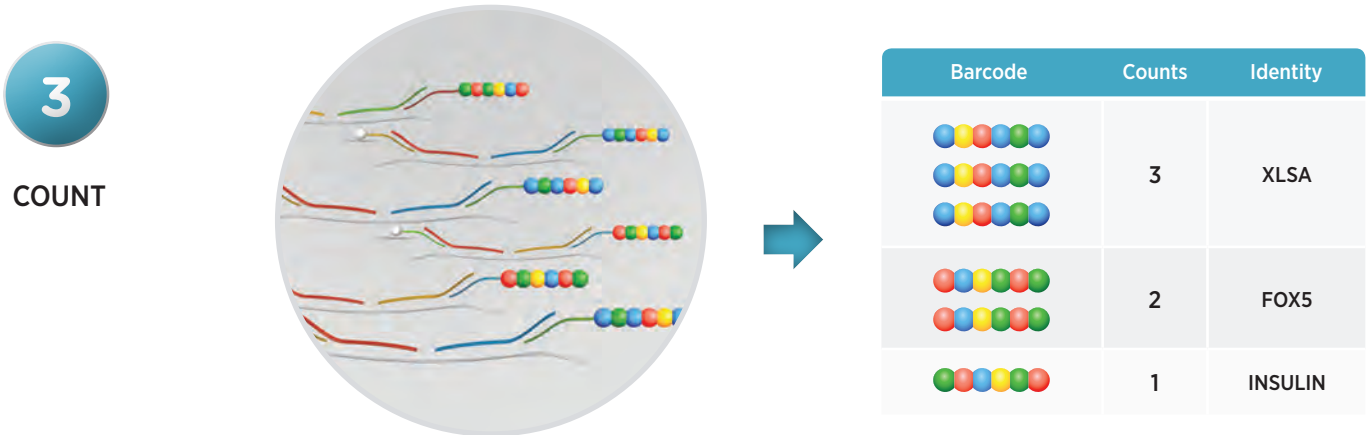
nanoString TECHNOLOGIES®

1 Molecule = 1 Count



Sample Processing

After hybridization, excess tags and oligonucleotide probes may be removed by magnetic bead purification while hybridized complexes are bound, immobilized, and aligned on a streptavidin surface.



Barcode	Counts	Identity
	3	XLSA
	2	FOX5
	1	INSULIN

Digital Data Acquisition

Barcodes can be counted and tabulated for each target molecule. Raw data are digital counts for each target nucleic acid which can be used to calculate fold-change (gene expression) or copy number calls.

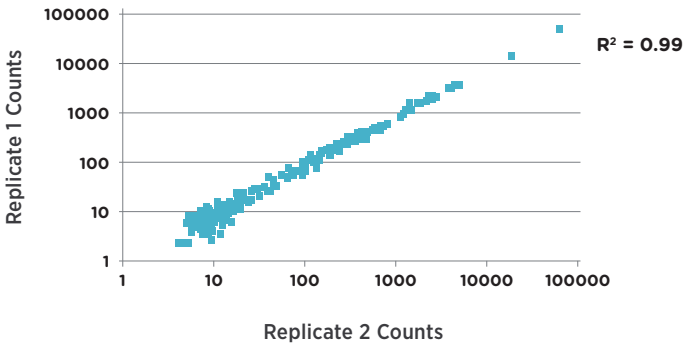
Robust Molecular Barcoding Chemistry

Generates Results You Can Rely On

The combination of digital counting with a simple, robust workflow minimizes variability. Up to 216 targets can be analyzed in a single reaction with a very high degree of reproducibility. **Digital counts increase linearly over a wide dynamic range facilitating accurate fold-change determination** of targets of varying expression levels.

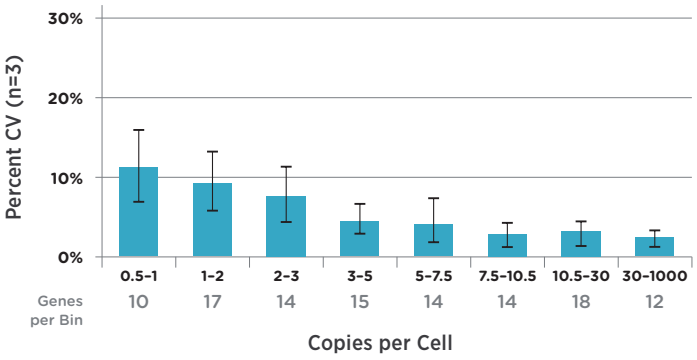
Precision Across a Large Range of Gene Expression

Reproducibility and Dynamic Range



Comparison of gene expression counts for 192 genes between 2 technical replicates. Replicates were hybridized with a 192-plex nCounter Elements TagSet. Data demonstrate very highly correlated counts over 4 logs of dynamic range.

Measurement Error *by* Expression Level (Counts)

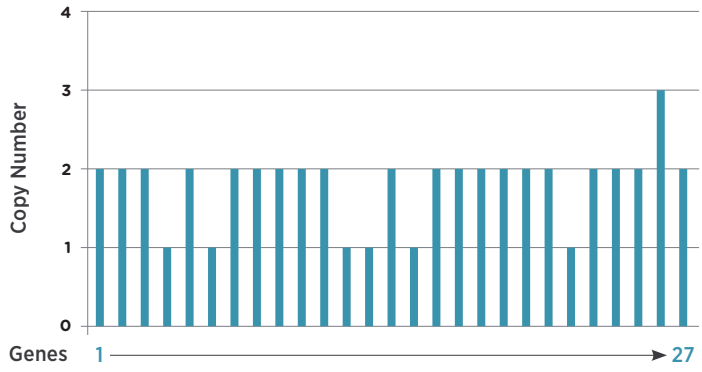


10,000 cells were hybridized to an 192-plex nCounter Elements TagSet. Genes were grouped by level of expression and percent CV was calculated for each group. Genes expressed at less than one transcript per cell can be measured with less than 15% CV, allowing for quantitation of 2-fold changes or less even at very low levels of transcript abundance. **Precision increases with expression levels, allowing for quantitation of less than 1.2-fold changes for more highly expressed genes.**

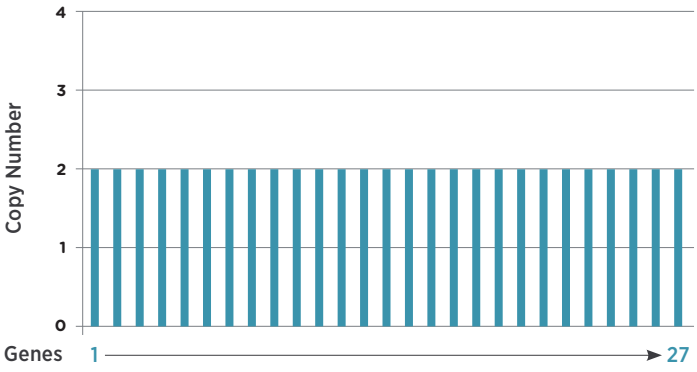
Reliable, Multiplexed CNV Analysis

Accurate Copy Number Calls from FFPE Samples

A. Tumor Tissue



B. Normal Tissue



Copy Number analysis was performed on an FFPE Tumor/Normal Pair from colon tissue (Panel A and B respectively). Standard Elements hybridizations were performed on 300ng of total DNA using a 96 gene Elements TagSet that included 27 cancer genes (3 probes per gene) and additional internal controls. Data was normalized to the internal controls and copy number determined relative to a set of normal FFPE controls. The copy number estimates from the 3 individual probes for each gene were averaged and rounded to the nearest integer.

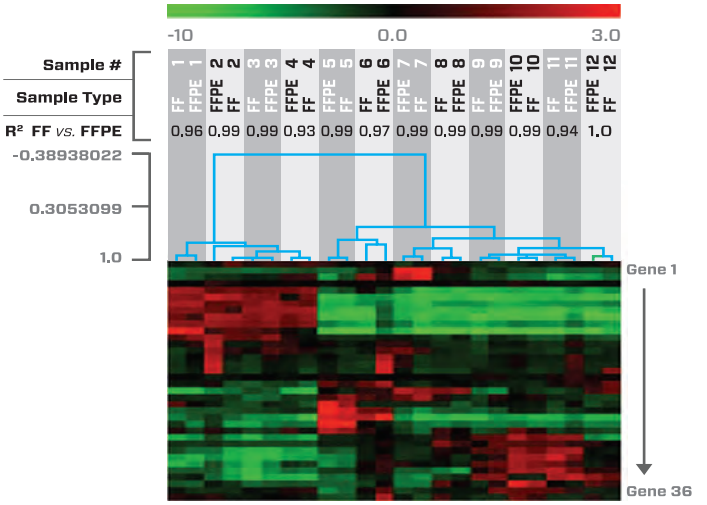
NOTE: Results may vary depending on assay design, sample input, or other factors.

Reliable Results *from* Challenging Sample Types

nCounter Elements reagents enable the generation of **reliable results even in challenging sample types such as Formalin-Fixed Paraffin-Embedded (FFPE) tissues or crude cell lysates.** The ability to efficiently perform large studies on archival tissues and cell lines are key advantages of nCounter Elements in translational research.

Reliable Results from FFPE Tissues

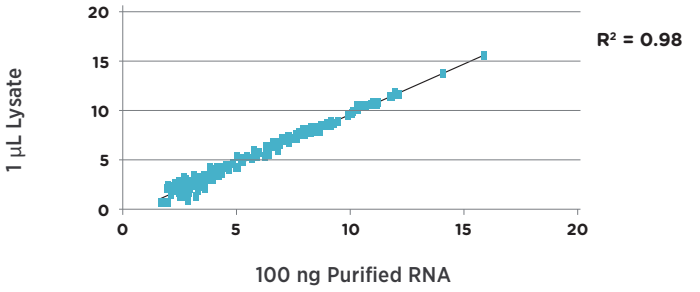
FFPE and Fresh Frozen (FF) Samples Cluster Together



FFPE and Fresh-Frozen Samples Cluster Together in Gene Expression Analysis. Twelve matched pairs of FFPE and Fresh Frozen tumor samples were analyzed with a 36-plex nCounter Elements TagSet. Each FFPE sample clusters most closely with its Fresh Frozen partner indicating that the FFPE fixation process is not confounding biological insights. Fresh Frozen datapoints were plotted against corresponding FFPE datapoints in linear regression generating an average R^2 of 0.98.

Results from Crude Cell Lysates

High Correlation - Purified RNA and Crude Cell Lysates



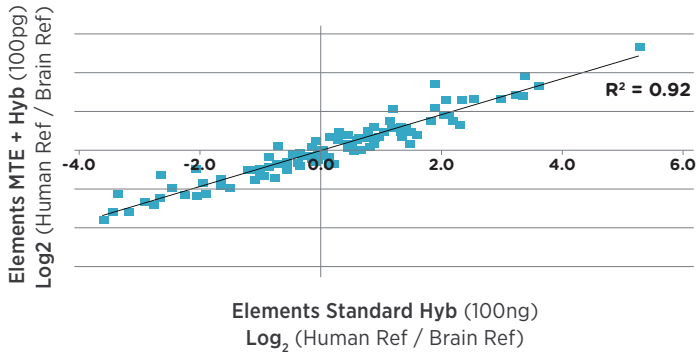
Log_2 gene expression counts from 1 µL crude cell lysate and 100 ng purified RNA from the same sample show high correlation. Each sample was analyzed with an 192-plex nCounter Elements TagSet.

Multiplexed Target Enrichment for Ultra-Low Sample Inputs

Achieve single cell sensitivity while minimizing amplification – single tube assay provides a simple workflow & utilizes the entire sample. **A Multiplexed Target Enrichment (MTE) step allows transcripts to be amplified** after a reverse transcription step. **MTE can amplify 216 targets from a sample in a single tube.** The resulting amplified material can then be directly hybridized with nCounter Elements and target-specific probes targeting the genes of interest - **no sample clean-up or sample partitioning is required.**

NOTE: Results may vary depending on assay design, sample input, or other factors.

Comparison of Analysis With and Without MTE



Multiplex target enrichment enables sensitive detection of fold changes from very low sample inputs. Fold changes observed with probes using a standard nCounter Elements hybridization and input of 100 ng RNA on the x-axis and 100 pg prepared with MTE prior to Elements hybridization on the y-axis.

Multiplexed Fusion Gene Analysis

nCounter Elements reagents can be used to create multiplexed assays capable of detecting and discriminating over 200 expressed gene fusions in a single reaction. Flexibility in design options and multiplexing capabilities enable development of assays for a variety of applications from discovery to diagnostics.

5’/3’ Imbalance Designs

Detect fusions without knowledge of the partner gene

Fusion events can be detected with a probe design that compares the ratio of gene expression upstream and downstream of the fusion junction. Each fusion partner may have promoters with different strength or activity, which will differentially affect expression of exons located 5’ or 3’ of this junction. A ratio of 5’/3’ expression that diverges from 1 is therefore indicative that a fusion event has occurred. The 5’/3’ design can be used to discover new fusions (Suehara et al., 2012) or to develop robust assays to support clinical research programs (Lira et al., 2013; Lira et al., 2014).

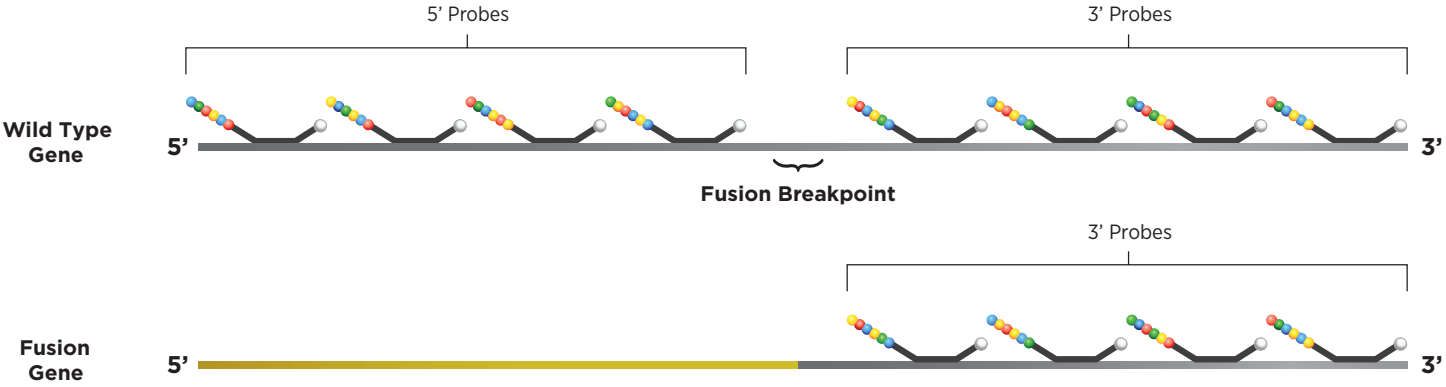
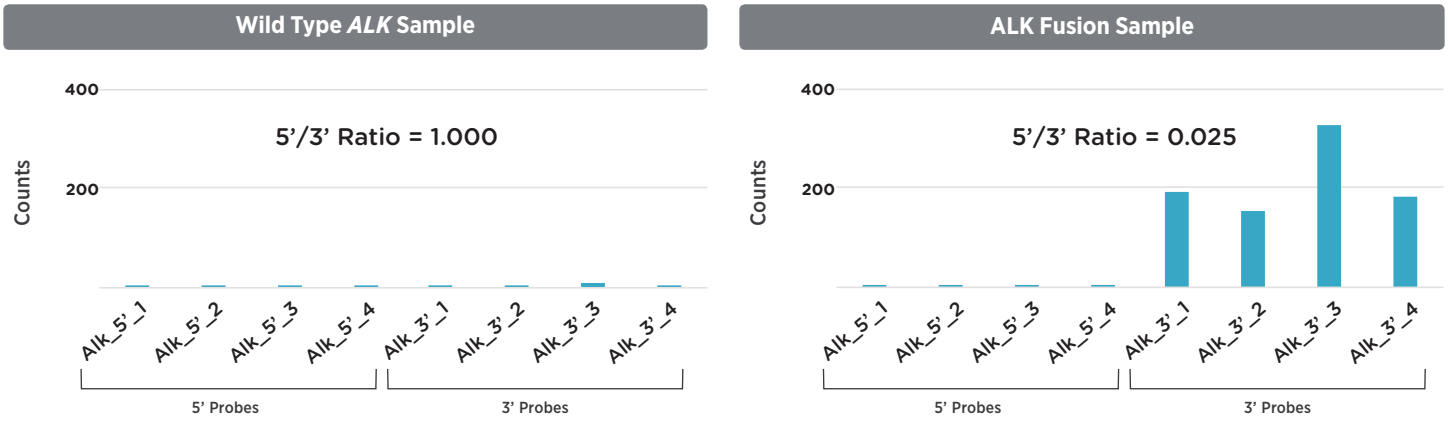


Diagram of a 5’/3’ imbalance design with 4 probes upstream and 4 probes downstream of the fusion junction.



A 5’/3’ imbalance design enables detection of fusion events involving the ALK gene without knowledge of the fusion partner. For this assay, 4 probes were placed upstream and 4 probes downstream of the fusion junction. The data show the counts generated by these probes for an ALK wild type and an ALK fusion sample. In the sample containing an ALK fusion, there is a clear imbalance in the expression levels of the 5’ probes compared to the 3’ probes indicating that a fusion event has occurred. Data kindly provided by Kindstar Global.

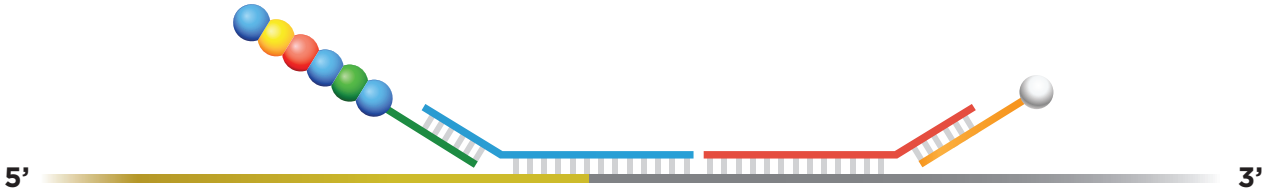
1. *Suehara et al. (2012) Identification of* KIF5B-RET *and* GOPC-ROS1 *fusions in lung adenocarcinomas through a comprehensive mRNA-based screen for tyrosine kinase fusions.* Clin Cancer Res *18(24):6599-6608.*
2. *Lira et al. (2013) Multiplexed gene expression and fusion transcript analysis to detect* ALK *fusions in lung cancer.* J Mol Diagn *15(1):51-61.*
3. *Lira et al. (2014) A single-tube multiplexed assay for detecting* ALK, ROS1, *and* RET *fusions in lung cancer.* J Mol Diagn *16(2):229-243.*

Characterize Specific Fusion Genes

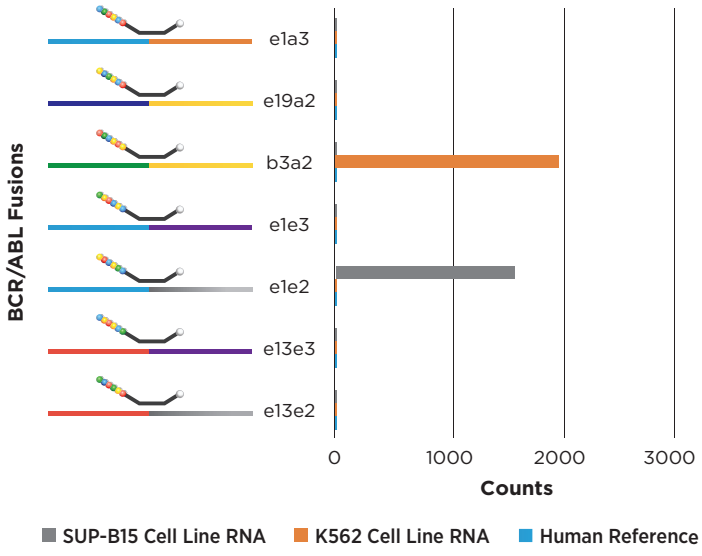
Universal Junction Sequence Designs

Targeting the Junction Sequence to Identify Specific Fusions

Highly specific probes for the junction sequence can be used to detect and discriminate unique fusion events. Junction probes can be used independently to develop robust assays for known fusions or combined with 5'/3' probes to create assays for orthogonal confirmation of known fusion events and detection of unknown fusion events.

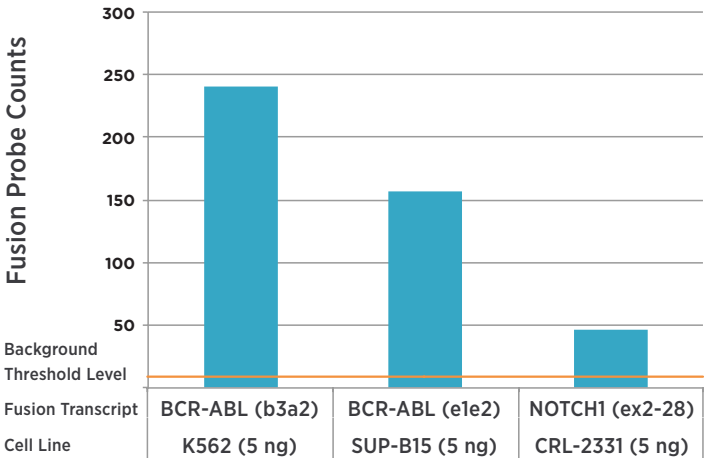


Multiplexed Analysis of *BCR/ABL* Fusions



A multiplexed nCounter Elements assay containing probes to seven distinct BCR-ABL fusions was run on total RNA from two cell lines, SUP-B15 and K562, each known to contain a specific fusion. Human Reference RNA was run as a negative control. Robust counts were obtained only for the correct fusion in each cell line; no cross-hybridization was detected for any of the incorrect probes. The difference in signal for the two fusions reflects the difference in the expression levels of the fusion genes in the two cell lines.

Detect Fusions in the Presence of 90% Normal RNA



Unique Fusion Transcripts are detected in 50 ng of total sample containing only 10% (5 ng) fusion sample. RNA extracted from fusion-containing cell lines was mixed with Human Reference RNA in a 1:10 ratio. 50ng of the combined RNA was run in a multiplexed nCounter Elements fusion assay. In each case the correct fusion was clearly detected. Cross-hybridization rates of all other fusion probes from the same fusion families were below background (data not shown). The differences in signal for the fusions reflect the differences in expression levels of the fusion genes in the cell lines.

Product and Ordering Information

nCounter Elements™ TagSets

A TagSet is a pool of nCounter Elements Reporter Tags and the Universal Capture Tag. Each Reporter Tag has a unique molecular barcode and tag that hybridizes to its complimentary sequence on target-specific oligonucleotide probes. The Universal Capture Tag enables the hybridized complex to be immobilized for counting. TagSets are available for analysis of 12 to 216 targets. Core TagSets are pre-mixed with ERCCs to enable analysis of 12 to 192 targets. Extension TagSets, without ERCCs, can be added to any core TagSet to expand the multiplexing capability by 12 or 24 targets.

nCounter Elements™ Master Kits

Master Kits contain consumables and reagents for post-hybridization processing of samples. They facilitate magnetic bead purification to remove un-hybridized capture and reporter tags and also enable capture and immobilization of barcodes.

Ordering Information

	Product Type	Product Number	Description
nCounter Elements™	**nCounter Elements TagSet** *Reagents for 12 reactions; includes ERCCs*	**ELE-P1TS-012**	12 Tags
		ELE-P1TS-024	24 Tags
		ELE-P1TS-036	36 Tags
		ELE-P1TS-048	48 Tags
		ELE-P1TS-060	60 Tags
		ELE-P1TS-072	72 Tags
		ELE-P1TS-084	84 Tags
		ELE-P1TS-096	96 Tags
		ELE-P1TS-108	108 Tags
		ELE-P1TS-120	120 Tags
		ELE-P1TS-132	132 Tags
		ELE-P1TS-144	144 Tags
		ELE-P1TS-156	156 Tags
		ELE-P1TS-168	168 Tags
		ELE-P1TS-180	180 Tags
		ELE-P1TS-192	192 Tags
	nCounter Elements Extension TagSet *Reagents for 12 reactions; no ERCCs*	**ELE-P1EX-012**	12 Tags
		ELE-P1EX-024	24 Tags
	nCounter Elements Master Kit	**ELE-AKIT-048**	48 Rxns
		ELE-AKIT-192	192 Rxns

CONTACT INFO

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