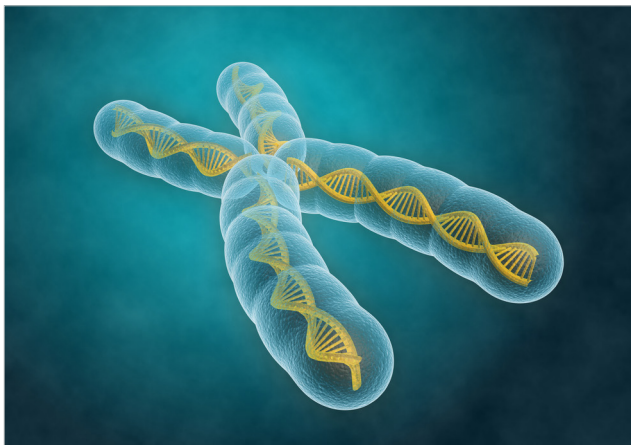


# nCounter® Vantage

## Gene Fusion Panel

Multiplexed Gene Fusion Detection Simplified

Increasing numbers of actionable fusions have made traditional technologies such as PCR and FISH inefficient and costly. NGS can profile multiple fusions in a single assay but is slow, complex, and costly. nCounter Gene Fusion Panels directly detect up to hundreds of actionable fusions from challenging samples such as FFPE in a single assay that is easy to implement, requires only 15 minutes of hands-on time, and produces results in less than 24 hours.



### Product Highlights

- Save time and sample material with multiplexed gene fusion detection in a single tube
- Detect specific fusion junctions or fusions with unknown partners in the same assay
- Ideal for use with challenging sample types including FFPE tissue
- Add-on probes for fusion targets of your choice or develop your own assay

### Assay Details

Feature	Specification
Input Material	50–100ng Purified Total RNA 150–300ng FFPE extracted RNA
Hands on Time	~15 minutes
Time to Results	<24 hours
Sample Type(s)	FFPE, fresh frozen tissue, cell extracts, cell lysates, blood lysates or blood fractions
Customizable Features	Design up to 228 probes for a de novo assay using nCounter Elements reagents or add on an additional 24 probes to off the shelf assays with the nCounter TagSet Extension product.
Data Analysis	nSolver™ Analysis software (For Research Use Only)
Required Reagents	Panel CodeSet and Master Kit (nCounter MAX or nCounter FLEX) Panel CodeSet and Cartridge and Reagent Pack (nCounter SPRINT™)

### Direct Detection and Counting of Fusion Events

No RT-PCR, No Library Prep, No more limitations with FISH assays

**STEP 1**  
Hybridization

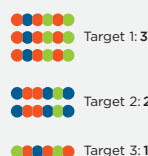


**STEP 2**  
Automated  
Sample  
Processing



**STEP 3**  
Data  
Analysis

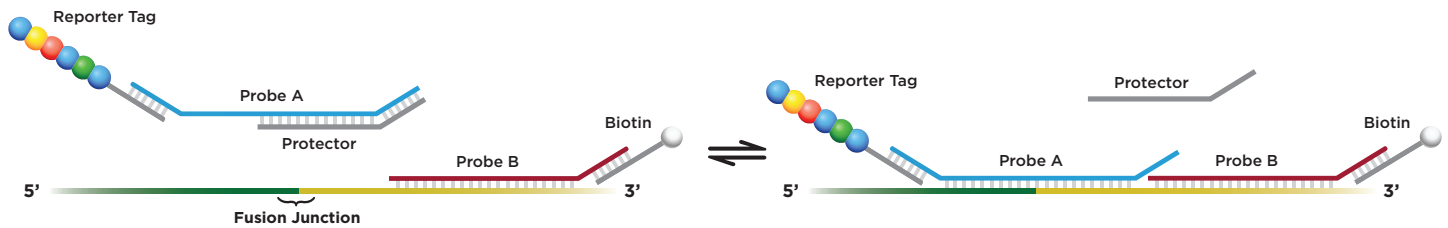
Target Count



## Highly Specific Detection of Fusion Events

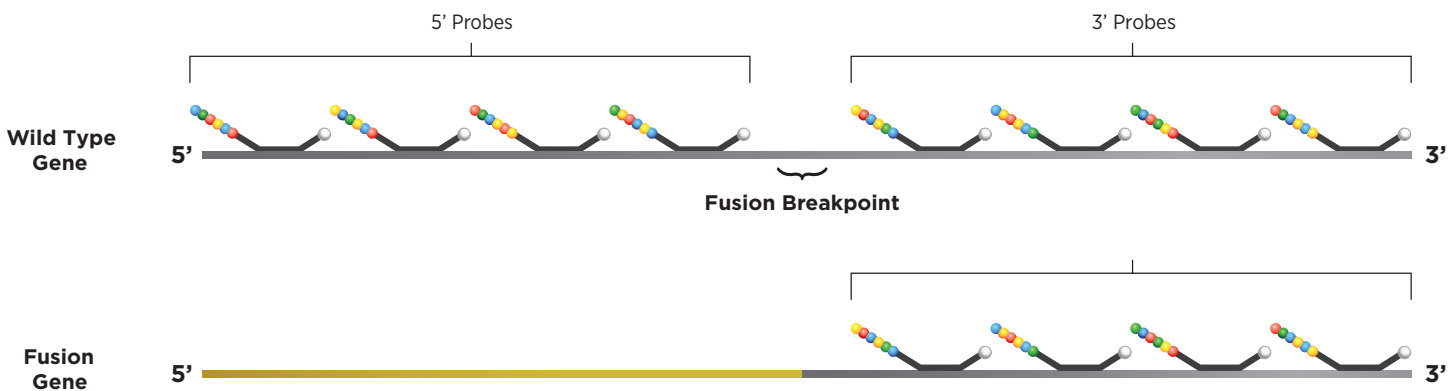
Gene Fusion panels are created using one or more patented probe design methods.

**Junction Probe Design:** NanoString's patented Junction Probe Design enables highly specific detection of fusion junction sequences. Specificity is conferred by toehold exchange technology that utilizes an additional probe, the Protector Probe, to create a thermodynamic balance that ensures signal is only generated in the presence of a perfect match of the target sequence.<sup>1,2</sup> This enables highly specific detection of fusions in a background of abundant normal tissue.



**FIGURE 1:** Illustration of the junction probe design methodology. Junction probes span a unique fusion junction, using toehold exchange technology for greater specificity. For a technical explanation of toehold exchange technology, see Zhang DY, Chen SX, Tin P. (2012) Optimizing the specificity of nucleic acid hybridization. *Nat Chem* 4(3):208-14.

**5'/3' Positional Imbalanced Probe Gene Expression Design:** Fusion events can also be detected with a probe design that compares the ratio of gene expression upstream and downstream of a fusion junction. Fusion partners with strong promoters result in increased expression of the 3' exons of the fusion gene. A ratio of 5'/3' expression that diverges from 1 is therefore indicative that a fusion event has occurred. The 5'/3' imbalance design can be used to confirm fusions detected with probes for specific junctions as well as to detect fusions with unknown partners or those not included in off-the-shelf assays.<sup>4,5</sup>



### REFERENCES:

1. Zhang et al. (2012) Optimizing the specificity of nucleic acid hybridization. *Nat Chem* 4(3):208-214.
2. Wu et al. (2015) Continuously tunable nucleic acid hybridization probes. *Nat Methods* 12, 1191-1196
3. 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.
4. 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.
5. 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.

## nCounter Lung Gene Fusion Panel

The nCounter Vantage Lung Fusion Panel includes **63 probes**: 35 for specific fusion detection, 24 for positional gene expression imbalance detection, and 4 internal reference genes.

**Specific Lung Gene Fusion Probes detect the following gene fusion families:**

ALK	RET	ROS	NTRK1
EML4-ALK	CCDC6-RET	CD74-ROS1	CD74-NTRK1
HIP1-ALK	KIF5B-RET	EZR-ROS1	MPRIIP-NTRK1
KIF5B-ALK		GOPC-ROS1	
TFG-ALK		LRIG3-ROS1	
TPR-ALK		SDC4-ROS1	
		SLC34A2-ROS1	
		TPM3-ROS1	

**Lung Imbalance probes detect gene expression imbalance in the following genes:**

ALK	RET	ROS1
8 probes	8 probes	8 probes

## nCounter Leukemia Gene Fusion Panel

The nCounter Leukemia Fusion Panel includes **42 probes in total**: 27 for specific fusion detection, differential expression of 12 leukemia genes, and 3 internal reference genes.

**Leukemia Gene Fusion Probes detect the following gene fusion families:**

CML	ALL	AML	AML/APL
BCR-ABL	E2A-PBX1	AML-ETO	PML-RARA
	MLL-AF4	CBFB-MYH11	
	TEL-AML1	DEK-NUP214	
		RPN1-EVI1	

**Leukemia Gene Expression Probes:**

Genes for AML, CML and ALL			
BAALC	ERG	FLT3	MECOM
MLLT11	MN1	NRAS	PRAME
RB1	SOCS2	TP53	WT1

**Customize any Fusion Panel:** nCounter Gene Fusion Panels can be customized with 24 additional probes using our TagSet Extension product. De novo assays using Elements Reagents or a Custom CodeSet can also be designed to include:

**Fusion Genes • Disease or Tissue-specific genes • Immune response genes**

## Detect unique and complex fusions in the presence of abundant normal tissue

### Fusion Data Analysis

Following hybridization with the nCounter Gene Fusion assay, samples are analyzed for fusion detection. Each target of interest is identified by the 'barcode' generated by six ordered fluorescent spots. The molecular barcode is then counted and tabulated for each target.

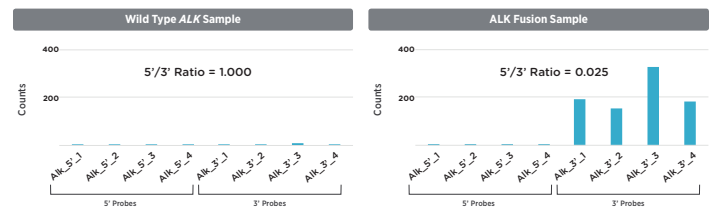
### Example Data:

#### Counts generated for Imbalanced Probe Gene Expression using the nCounter Lung Gene Fusion Panel

5'/3' Probes	ALK Fusion Positive Sample Counts	ALK Wildtype Sample Counts
ALK_5P-1	10	3
ALK_5P-2	4	1
ALK_5P-3	2	3
ALK_5P-4	4	1
ALK_3P-1	890	7
ALK_3P-2	841	3
ALK_3P-3	1166	5
ALK_3P-4	698	5

Detailed data analysis is performed using our nSolver data analysis software. NanoString's Field Application Scientists and Bioinformatics team can provide personalized training, data analysis and support. For more information or to speak with one of our support scientists please contact us at: [support@nanosttring.com](mailto:support@nanosttring.com)

### 5'/3' Imbalanced Gene Expression



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 were placed 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.

### Ordering Information:

Description	Format	Quantity	Catalog Number
nCounter Lung Fusion Panel	Code Set Only	12 reactions	VRXC-LGF-12
nCounter Leukemia Fusion Panel	Code Set Only	12 reactions	VRXC-LKF-12
nCounter Master Kit (Max or FLEX Systems)	Reagents and Cartridges	12 reactions	NAA-AKIT-012
nCounter SPRINT™ Cartridge	1 Cartridge, 12 lanes	12 reactions	SPRINT-CAR-1.0
nCounter SPRINT™ Reagent Pack	Reagents A,B,C & Hybridization Buffer	192 reactions	SPRINT-REAG-KIT

## About nCounter Elements™

nCounter Elements TagSets utilize digital, molecular barcoding chemistry based on NanoString's patented technology that allows users to assemble their own assays. They enable counting of individual molecules with color-coded molecular barcodes via fluorescent microscopy.

nCounter Elements TagSets are For Research Use Only. Assays can be developed using one of our pre-designed panels or in a customized assay. nCounter Elements enable highly multiplexed, direct profiling of individual molecules in a single reaction and are ideal for a range of applications requiring efficient, high-precision counting of tens to hundreds of target molecules across a sample set.

For more information, please visit [nanosttring.com](http://nanosttring.com)

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