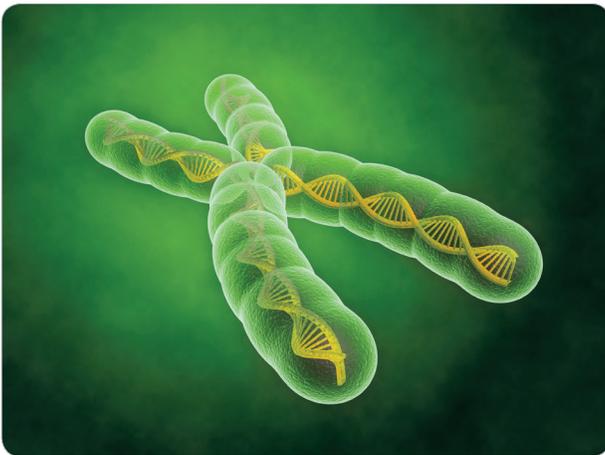


nCounter Vantage™ Gene Fusion Panels



Multiplexed Gene Fusion Detection Simplified

Lung Gene Fusion Panel

Leukemia Gene Fusion Panel

NanoString's Vantage panels for Lung and Leukemia gene fusion detection enable a highly multiplexed assay capable of simultaneously characterizing key fusion events and selected reference genes in a single tube. Combined with direct digital counting of the nCounter® system and our patented Junction Sequence probe design, the detection of fusion genes is highly sensitive, quantitative, and easy.

Product Highlights

- Save time and sample material with multiplexed gene fusion detection in a single tube.
- Detect rare fusions with highly specific probes designed using patented Junction Sequence design technology.
- Ideal for use with challenging sample types including FFPE tissue.
- Customizable and forward compatible with our growing line of 3D Biology™ products.

Assay Details

Feature	Specification
Input Material	50–100ng Purified Total RNA (~5000–10,000 cells) 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
Customizable Features	Add up to 24 additional genes or additional fusions with the nCounter® TagSet Extension product.
Data Analysis	nSolver™ Analysis software
Required Reagents	Panel CodeSet and Master Kit (<i>MAX</i> or <i>FLEX</i>) Panel CodeSet and SPRINT™ Cartridge and Reagent Pack

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



nSOLVER™
Analysis Software

The nCounter Analysis System utilizes a non-enzymatic protocol and novel digital color-coded barcode technology for the direct multiplexed measurement of gene expression.

For more information: www.nanostring.com/applications/technology

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

Highly Specific Detection of Fusion Events

Vantage 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.^{1,2} This enables highly specific detection of fusions in a background of abundant normal tissue.

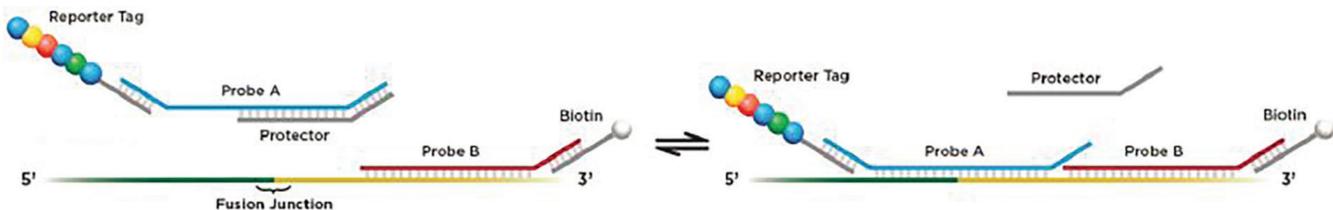
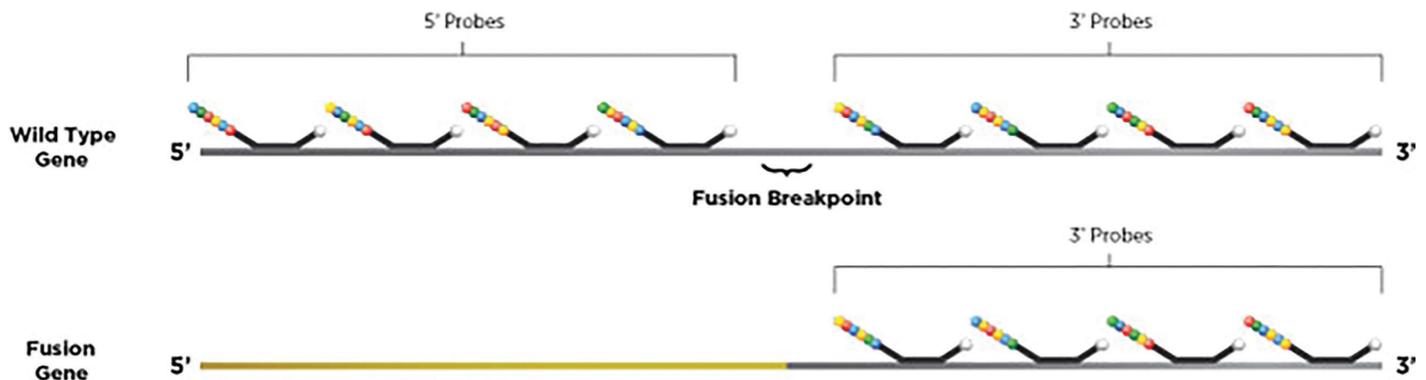


FIGURE 1 Illustration of the junction probe design methodology. Junction probes span a unique fusion junction, using toehold exchange technology for greater specificity. For a technical explanation of toehold exchange technology, see Zhang DY, Chen SX, Tin P. (2012) Optimizing the specificity of nucleic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

5'/3' Positional Imbalanced Probe Gene Expression Design: In some instances, fusion events can also be detected with a probe design that compares the ratio of gene expression upstream and downstream of a 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 fusions³ or to aid in the development of robust assays to support clinical research programs.^{4,5}



For more Information: nCounter Vantage Fusion Panels: www.nanostring.com/fusionpanels

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 Vantage™ Lung Fusion Panel

The nCounter® 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 Vantage™ Leukemia Fusion Panel

The nCounter Vantage Leukemia Fusion Panel includes **42 probes in total**: 27 for specific fusion detection, 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 Vantage Panels are available with the option to customize using our TagSet Extension product. With the additional purchase of the TagSet Extension product and custom designed probes, users can add up to 24-additional genes to any panel including:

Additional 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 Vantage 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.

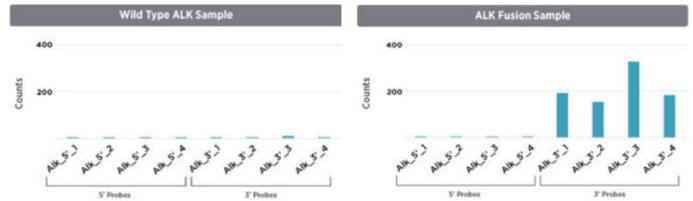
Example Data: Counts generated for Imbalanced Probe Gene Expression using the nCounter Lung Gene Fusion Panel

5'/3' Probes	Fusion + Sample Counts	Wild Type Sample Counts
ALK_5'_1	5	4
ALK_5'_2	7	7
ALK_5'_3	7	6
ALK_5'_4	12	6
ALK_3'_1	1366	550
ALK_3'_2	1488	623
ALK_3'_3	2208	909
ALK_3'_4	831	350

Detailed data analysis for Fusion panels 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 on data analysis or to speak with one of our support scientists please contact us at:

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 Vantage Lung Fusion Panel	Code Set Only	12 reactions	XT-CSO-LKFU1-12
nCounter Vantage Leukemia Fusion Panel	Code Set Only	12 reactions	XT-CSO-XLKFU1-12
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

Forward-compatible nCounter Vantage assays for DNA or Protein Analysis.

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Stop asking, "What can I measure?" Start asking, "What should I measure?" Thanks to nCounter Vantage™ assays, you get the full picture—targeted DNA, RNA, and Protein analysis in one process. Stop compromising. Stop performing sequential testing.

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