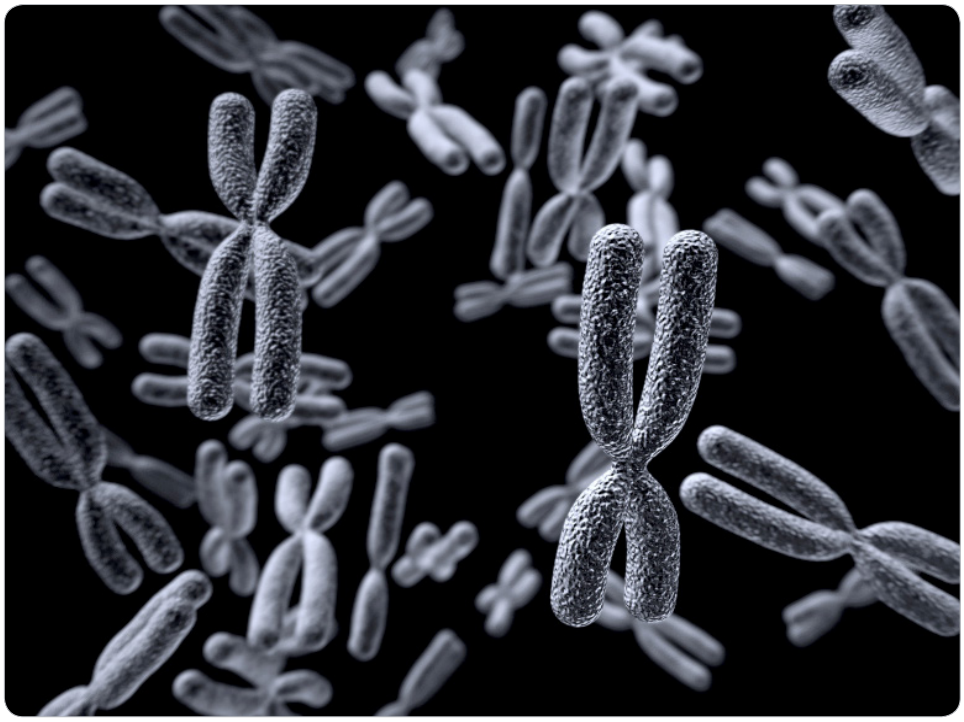
nCounter®
v2 Cancer CN Assay



Product Highlights

- **Copy number analysis of 87 genes commonly amplified or deleted in cancer**
- **Optimized for analysis of FFPE samples**
- **Unparalleled precision**
- **Accurate quantitation of highly amplified genes**
- **Simple, robust assay protocol**

nCounter® v2 Cancer CN Assay

The **nCounter v2 Cancer CN Assay Kit** is a highly multiplexed assay that enables copy number quantification for 87 genes commonly amplified or deleted in cancer including PIK3CA, AKT, PTEN, BRCA, ERBB2, and MYC (TABLE 1). Based on NanoString's proven digital, molecular counting chemistry, the assay delivers unparalleled precision and accurately quantifies genes that are amplified to 10s or even 100s of copies. The assay is optimized for analysis of FFPE samples and shows excellent correlation in comparisons of matched fresh frozen and FFPE samples.

For research use only, the nCounter v2 Cancer CN Assay Kit enables researchers to rapidly generate a copy number profile for hundreds of specimens per week. A run can be set up with only 45 minutes hands-on time and results are available the next day (FIGURE 1).

TABLE 1: v2 Cancer CN Assay Gene List.

nCounter® Cancer CN Assay Gene List												
AKT2	BCL2L2	CCNE1	CSMD1	ERBB2	GRB2	KIT	MDM2	MYCL1	PARK2	PTPRD	TERT	YAP1
AKT3	BIRC2	CDK4	DCC	FADD	HMGA2	KRAS	MDM4	MYCN	PAX9	RB1	TP53	YWHAZ
APC	BRCA1	CDK6	DCUN1D1	FGFR1	IGF1R	MAGI3	MELK	NCOA3	PDE4D	REG4	TP73	ZNF217
AR	BRCA2	CDKN1A	DYRK2	FHIT	IRS2	MAP2K4	MET	NF1	PDGFRA	REL	TRAF2	
AURKA	C8orf4	CDKN2A	E2F3	FOXO1	ITGB4	MAP3K5	MITF	NKX2-1	PIK3CA	RPS6KB1	VEGFA	
BBC3	CCND1	CDKN2C	EEF1A2	GAB2	JUN	MAPK7	MYB	NKX2-8	PRKCI	SHH	WHSC1L1	
BCL2L1	CCND2	CRKL	EGFR	GPC5	KDR	MCL1	MYC	ORAOV1	PTEN	SKP2	WT1	

The nCounter CN Assay protocol enables profiling in 4 easy steps:

45 Minutes Hands-on Time per Run

1 Fragment

Fragment DNA either via AluI digestion or Covaris AFA™.

30 minutes hands-on

Day 1

2 Hybridize

Hybridize fragmented DNA with the nCounter CodeSet.

5 minutes hands-on

Day 1

3 Purify

Place hybridized samples in the nCounter Prep Station for automated post-hyb processing.

5 minutes hands-on

Day 2 (automated)

4 Count

Transfer the nCounter cartridge from the Prep Station to the nCounter Digital Analyzer for image acquisition and analysis.

5 minutes hands-on

Day 2 (automated)

nCounter® Analysis System

The **nCounter Analysis System** from NanoString™ offers a simple, cost-effective way to profile hundreds of mRNAs, microRNAs, or CNVs 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.

The system utilizes a novel digital technology that is based on direct multiplexed quantification of nucleic acids and offers high levels of precision and sensitivity. The technology uses molecular "barcodes" and single molecule imaging to detect and count hundreds of unique targets in a single reaction. Unlike other methods, the protocol does not include any amplification steps that might introduce bias to the results.

nCounter® CNV Assays

The nCounter CNV Assays allow researchers to quantify copy number for up to 800 regions of the human genome in a single multiplexed reaction. The nCounter CNV Assays are based on the standard nCounter assay with two important additions: DNA fragmentation and denaturation. These two steps yield single-stranded targets for hybridization with nCounter probe pairs which are comprised of a Reporter Probe which carries the signal, and a Capture Probe which allows the complex to be immobilized for data collection. After hybridization, samples are transferred to the nCounter Prep Station where excess probes are removed and probe/target complexes are aligned and immobilized in the nCounter Cartridge. Cartridges are then placed in the nCounter Digital Analyzer for data collection. Each CNV probe pair is identified by the "color code" generated by six ordered fluorescent spots present on the Reporter Probe. The Reporter Probes on the surface of the cartridge are then counted and tabulated.

Cancer CN CodeSet Design

The nCounter v2 Cancer CN CodeSet is designed to genes that have shown copy number variation in many cancers. Several publicly available sources were used to generate the gene list (literature reviews, specific publications, public databases, feedback from experts). A preliminary list was generated combining genes from all public sources and then prioritizing and selecting genes based on the frequency of membership in the source lists. The preliminary list of genes was then refined further based on input from leading cancer researchers. The result is a list of 87 genes that are commonly amplified or deleted in cancer. The CodeSet includes multiple probes per gene to increase accuracy when analyzing FFPE samples. Also included are 54 probes targeting invariant regions, bringing the total number of probes in the CodeSet to 309.

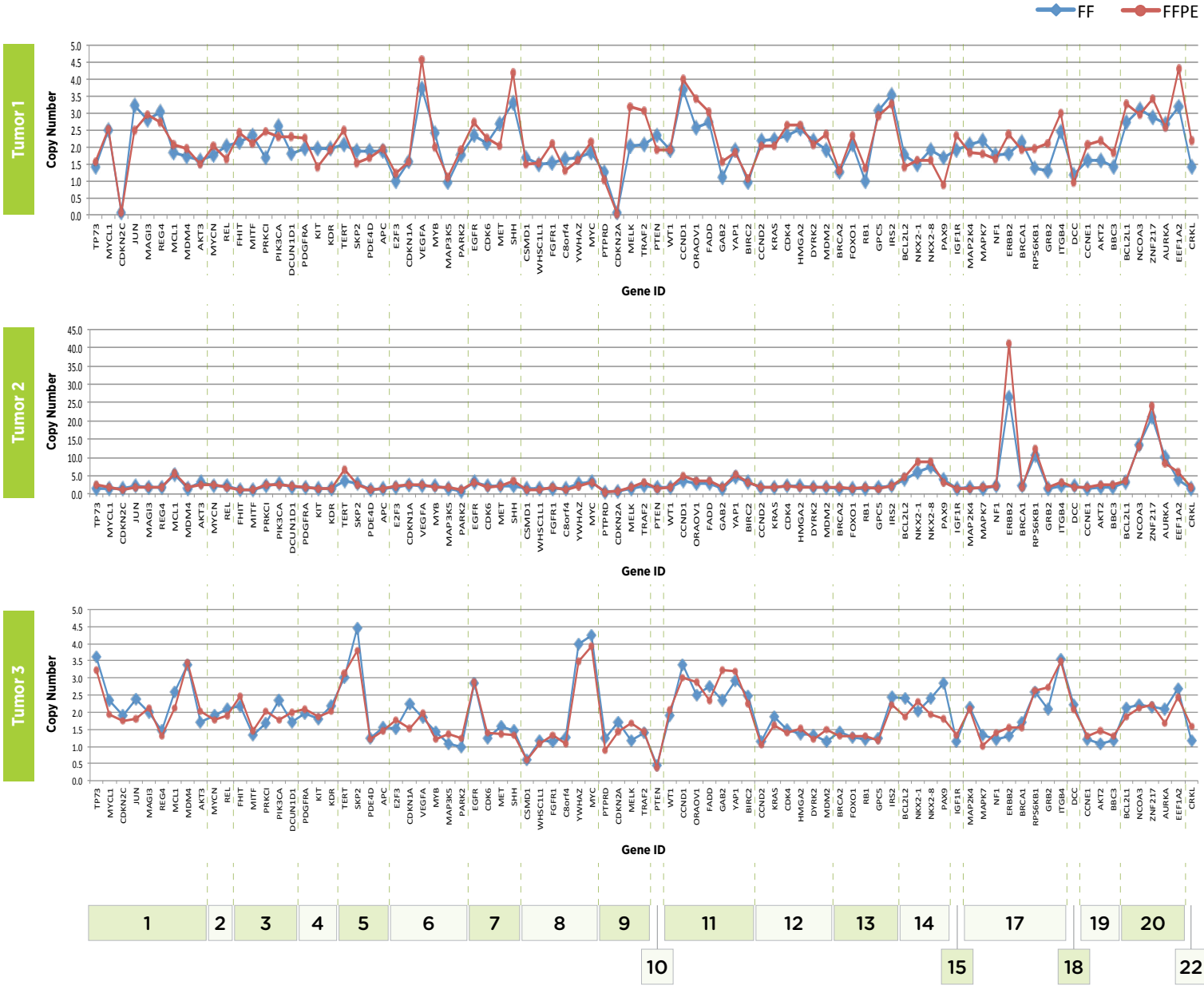
nanoString TECHNOLOGIES

Assay Performance

To evaluate the performance of the nCounter v2 Cancer CN Assay Kit in relevant sample types, we used the kit to characterize matching flash frozen and FFPE samples from tumors. Data from three representative matched pairs are shown in FIGURE 2. The copy number profiles were highly concordant between the matching FFPE and FF samples for both tumors. Several genes were clearly identified as deleted (see CDKN2C and CDKN2A for Tumor 1) or highly amplified (*e.g.*, ERBB2, NCOA3, ZNF217 for tumor 2). In addition, these data highlight the utility of the nCounter assay in identifying single copy deletions or amplifications, with several genes showing either 1 copy (E2F3, MAP3K5, and BIRC3) or 3 copies (GPC5, IRS2, and NCOA3) in both FF and FFPE.

Correlation of Copy Number Calls for Matching Flash Frozen and FFPE Samples

FIGURE 2: Copy number for genes included in the Cancer CN Assay CodeSet for 3 matched pairs of FF and FFPE tumor samples. The data was analyzed using diploid reference samples that matched the sample type as closely as possible. For the fresh frozen tissue, we used the cell line NA10851 as a control and for the FFPE samples we used the Cancer CN FFPE Reference data set (provided by NanoString with the Cancer CN Assay kit). DNA was fragmented by AluI digestion and the input amount was 300ng.



System Performance

Description	Specifications
Genomic regions analyzed in one reaction	87 genes commonly amplified or deleted in cancer (average 3 probes/region)
Recommended amount of starting material	300ng total genomic DNA
Sample types supported	Human genomic DNA from FFPE samples, fresh or frozen tissue, or cell lines
Reproducibility	> 99%
Number of copies detected	0 – 4, multiallelic
nCounter Prep Station throughput	12 samples < 2.5 hours
nCounter Digital Analyzer throughput	12 samples / 2.7 hours
Controls	54 invariant genomic regions, and spike-in process controls

Ordering Information

Description	Quantity / Use	Part Number (P/N)
nCounter v2 Cancer CN Assay Kit	12 assays 24 assays 48 assays 96 assays	CNV-CAN2-12 CNV-CAN2-24 CNV-CAN2-48 CNV-CAN2-96
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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