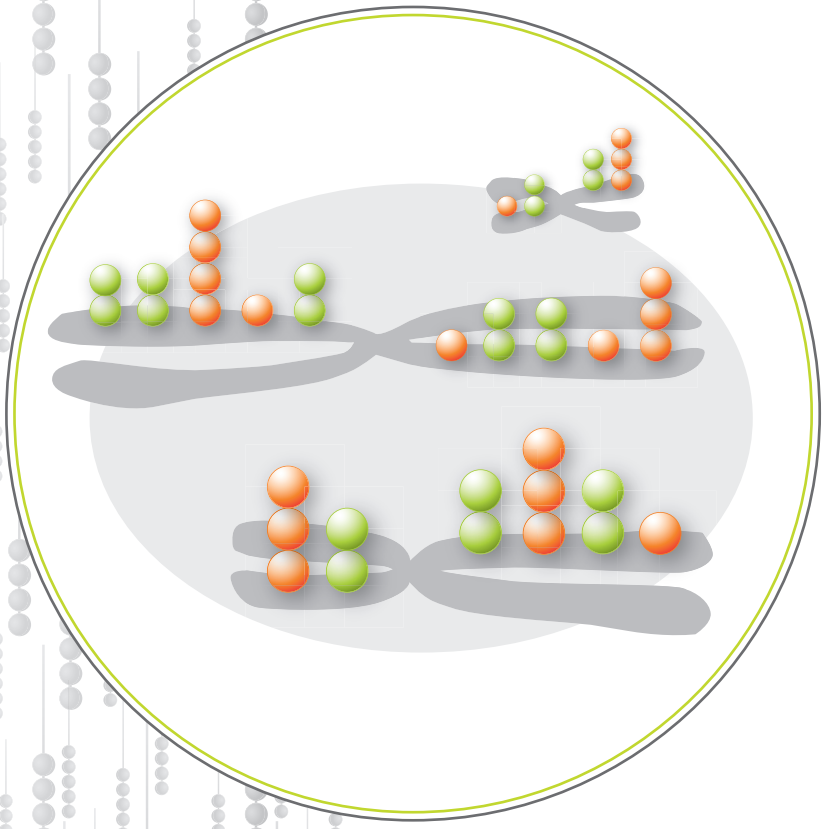
nanoString TECHNOLOGIES

nCounter® Custom Copy Number Variation Assay



The Early Access nCounter Custom Copy Number Variation Assay

Gold Standard Data Quality

- Provides 99% reproducibility
- Accurately quantify 0-4 copies
- Call rates of greater than 94%

Flexiblty

- Up to 200 regions in single tube
- Tolerates common DNA contaminants

Efficiency

- Multiplex 200 regions from as little as 200ng of DNA in a single tube
- 10 invariant region controls included in every CodeSet
- Complete studies in a fraction of the time it would take for qPCR

Ease of Use

- 35 minutes hands on time
- Fully automated target purification & data acquisition
- No amplification or technical replicates required

Copy Number Variation

Copy Number Variants (CNVs) are structural polymorphisms in the genome that include deletions, amplifications, and other complex rearrangements. CNVs have been associated with disease susceptibility, drug responses and cancer progression. They can be inherited, or they can arise spontaneously. Thousands of putative CNVs have been discovered in recent years using whole-genome technologies such as microarrays or sequencing. Existing technologies for CNV validation are time consuming and labor intensive, and are not readily scalable for analyzing many genomic regions at once, creating a bottleneck at the validation and screening stage of experiments.

The nCounter Custom CNV Assay delivers a highly multiplexed, accurate, precise and automated method to eliminate the validation bottleneck.

nCounter® CNV Assay

The nCounter Custom CNV Assay allows researchers to select up to 200 regions of the human genome for CNV analysis in a single multiplexed reaction, using the proven nCounter Analysis System in use today for mRNA and miRNA analysis.

The nCounter Custom CNV Assay is 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.

Optimized probe set design and process controls

The Custom CNV Assay incorporates a proprietary bioinformatics pipeline designed to optimize probe pair design taking into account CodeSet controls and genomic complexity. The system is based on locus-specific probe pairs that are hybridized to fragmented and denatured DNA samples in solution. The protocol eliminates any amplification steps that might introduce bias into the results.

The nCounter Custom CNV Assay includes multiple controls. A spike-in plasmid is provided in the CNV DNA Prep Kit and serves as a positive control for the entire process from fragmentation through digital read out. In addition, optimized probe pairs for 10 invariant regions of the genome are included in every CNV CodeSet to be used for data normalization thus eliminating the need to run the additional control reactions required by qPCR. Also included in every CodeSet are nCounter system controls for hybridization and purification efficiency.

Custom CNV Assay Performance

Reproducibility

To demonstrate the reproducibility of data generated via the nCounter Custom CNV Assay, we examined 20 genomic regions across 10 genomic DNA samples (purchased from Coriell Institute for Medical Research). For each region, 3 separate probe pairs were designed and mixed into a single multiplexed CodeSet. All samples were run in triplicate starting from 600ng of DNA; however, subsequent experiments have demonstrated that 200ng of DNA is sufficient to obtain similar results. Triplicates were run on separate slides on different days in order to mimic a typical laboratory workflow. Data was normalized to a set of 10 control probe pairs targeting invariant genomic regions, in order to account for slight differences in DNA input amounts and hybridization efficiency. Copy number calls were determined relative to a reference sample (NA10851) that was selected to have 2 copies of each region based on publicly available genotype data at the Database for Genetic Variants[1].

In the first analysis, we examined the technical reproducibility as well as the consistency of copy number calls for the 3 probe pairs for any given region. In Figure 1, the copy number data obtained from 10 samples across 2 genomic regions (Affy 6_12 and Affy6_31) is shown. For the majority of the samples tested, these two regions are present at 2 copies per cell; however, two of the samples (NA11930 and NA12716) have a single copy of region Affy6_12 and one sample (NA18608) had a deletion of region Affy6_31. These copy number variation results are consistent with the public data (Figure 2A and 2B, additional data not shown). The technical reproducibility of each probe pair is high, with an average standard deviation of only 8% for the samples shown. The copy number calls obtained from each of the 3 probe pairs within a region of interest are also consistent with one another.

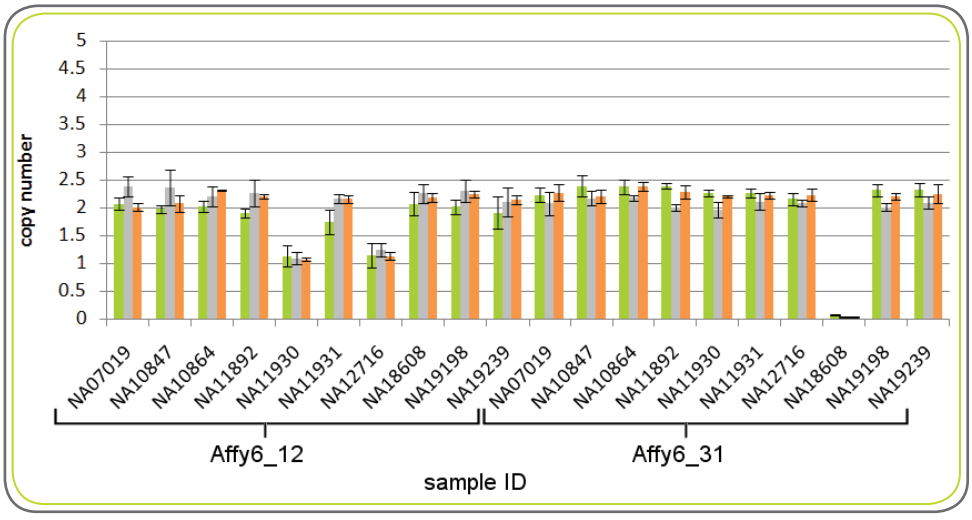


Figure 1: Copy number profiles for 10 samples across 2 genomic regions. *A single multiplexed nCounter Custom CNV CodeSet was used to determine copy number calls for 20 genomic regions in 10 samples. For each region, 3 independent probes were designed to different sequences within the regions. The copy number results for two of the ten regions (Affy6_12 and Affy6_31) are shown above. The color of the bars represents the individual probes. Error bars represent the standard deviation of the copy number call for individual probes run in triplicate. nCounter CNV calls were calculated relative to a reference sample NA10851 after normalization to a set of the 10 invariant control probes included in the CodeSet.*

Accuracy and Multiplexing

In order to illustrate the accuracy and the multiplexing capabilities of the nCounter Custom CNV Assay, we compared the results for 2 samples across all 20 copy number variant regions to the publically available data. The data for all 20 regions (60 probe pairs) was obtained in a single hybridization reaction per sample. Figure 2A shows a comparison of copy number values across 20 regions for sample NA11930. In all 20 regions the nCounter Custom CNV Assay calls (gray and orange bars) agree closely with the publically available CNV calls (green bars). Based on this data from a single multiplexed reaction we can determine that sample NA11930 has a single copy of region Affy6_12 and 3 copies of the region CNVR76.1. Figure 2B shows a similar analysis for sample NA12716 again, showing a single copy of Affy6_12 and 3 copies of CNVR76.1, but with an additional copy of region CNVR8221.1. The ability to measure many genomic regions in a single assay makes the nCounter Custom CNV Assay an ideal solution for validation of large sets of CNV candidates identified by Next Generation Sequencing or array-based platforms, or for screening many samples with known CNV regions.

In order to examine the need for replicate measurements, we compared the average CNV call for 3 probes per region performed in triplicate (gray bars) to the average CNV calls for 3 probes run in a singleton reaction (orange bars). In all cases, the average CNV calls were very close between the two analyses indicating that the replicate measurements are not required to obtain accurate CNV calls. This result should allow researchers with small amounts of material or large sample sets great flexibility when setting up experiments without suffering a loss of data quality.

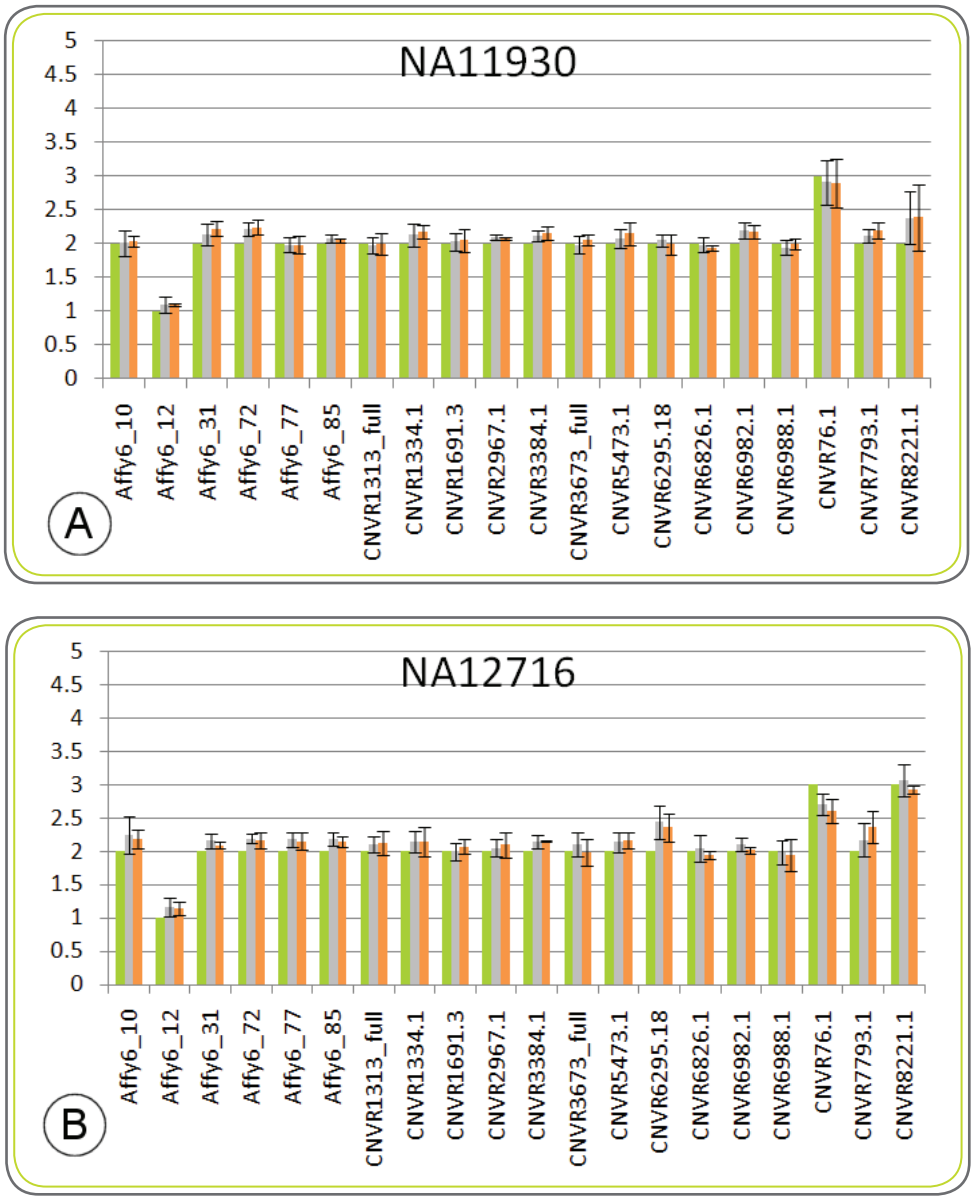


Figure 2: The nCounter Custom CNV Assay accurately measures multiple genomic regions in a single reaction without the need for technical replicates. *For each region, 3 independent probe pairs were designed to different sequences within the regions. Twenty genomic regions are shown for two DNA samples for which public data are available NA11930 (Panel A) and NA12716 (Panel B). Colored bars represent the copy number calls from public data (green), nCounter CNV calls run in triplicate on 3 probe pairs per region (gray) and nCounter CNV calls run in singleton on 3 probe pairs per region (orange). Error bars represent the standard deviations between probe pairs for the nCounter measurements (n=9 for triplicate reactions, n=3 for singleton reactions). nCounter CNV calls were calculated relative to reference sample NA10851 after normalization to a set of invariant control probe pairs that were included in the CodeSet.*

References:

[1]Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW, Lee C: Detection of large-scale variation in the human genome. Nat Genet. 2004 Sep;36(9):949-51.

System Performance

Critical Specifications	
Genomic regions analyzed in one reaction	200 regions of the human genome
CNV Size Detected	>2 kb
Minimum Sample Requirement	200ng genomic DNA
Sample Types Supported	Human genomic DNA from blood and saliva
Reproducibility	99%
Number of copied detected	0-4
Synthetic ssDNA Spike Titration Correlation	>0.95
nCounter Prep Station Throughput	12 samples < 2.5 hours
nCounter Digital Analyzer Throughput	12 samples / 4 hours (up to 72 samples per day unattended running in continuous mode)
Controls	10 invariant genomic regions, and spike in process controls

Ordering Information

Description	Quantity/Use	P/N
nCounter Custom CNV Assay	Increments of 192	CNV-P1CS -XXX
nCounter Master Kit	192 Assays	NAA-AKIT-192

NanoString Technologies, Inc.
530 Fairview Ave N, Suite 2000
Seattle, Washington 98109
(206) 378-NANO • (206) 378-6266
www.nanostring.com