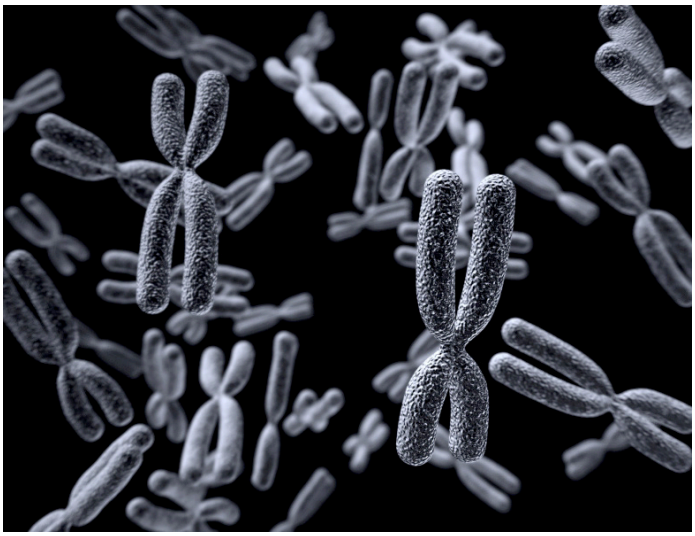
nanoString®

nCounter®
Copy Number Variation Assay



Product Highlights

Data Quality
- 99% reproducibility
- Superior accuracy for multiallelic CNVs
- Call rates of greater than 94%

Easy to Use
- Just 25 minutes of hands-on time
- Fully-automated target purification and data acquisition
- No amplification or technical replicates required

Efficiency
- Multiplex 800 regions from as little as 300ng 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 with qPCR

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 replication stage of studies.

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

nCounter® CNV Assay
The nCounter Custom CNV Assay allows researchers to select up to 800 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.

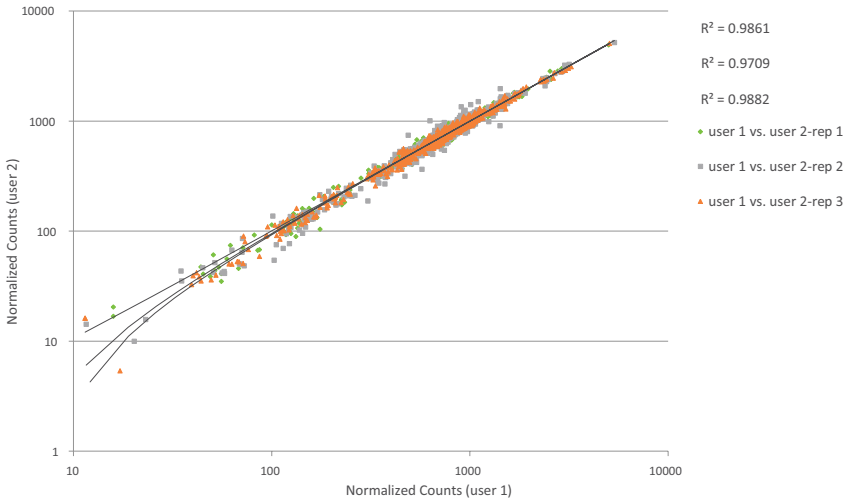
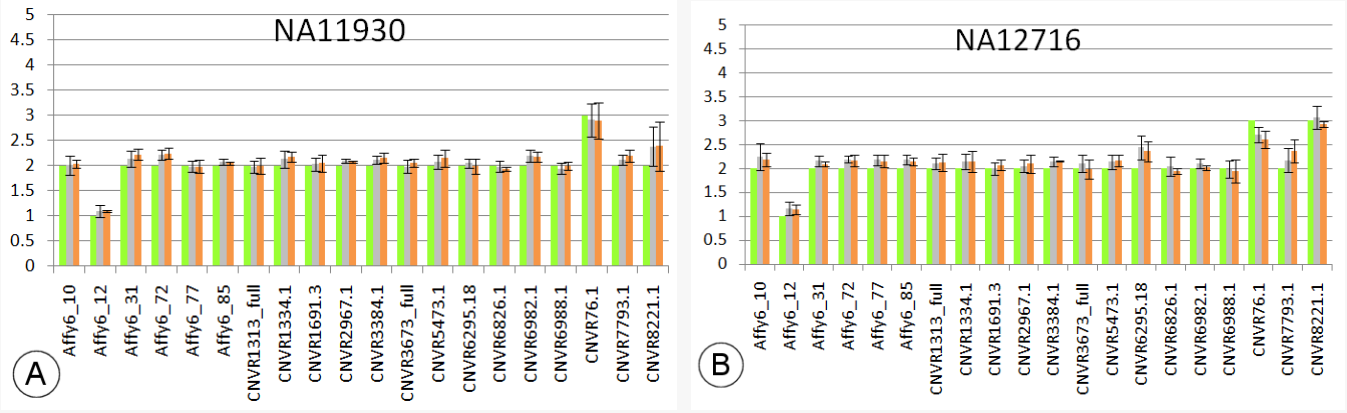


FIGURE 1: Precise copy number measurements across different users and cartridges. A series of 12 HapMap samples were run in triplicate on the nCounter CNV assay on different days by different users. Counts were normalized to the invariant controls. The scatter plot shows the normalized counts obtained by user #1 (x-axis) vs. the normalized counts obtained from user #2 (y-axis). The data from the 3 replicates, run on different cartridges over the course of 3 days, are shown in different colors. The R2 between counts run by different users was >0.97 for all 3 replicates. Over all precision for copy number calls for these experiments was 99.5% (data not shown).



FIGURES 2A and 2B: 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.

Custom CNV Assay Performance

Reproducibility

To demonstrate the reproducibility of data generated via the nCounter Custom CNV Assay, two different users performed assays on 12 genomic DNA samples (purchased from the Coriell Institute for Medical Research) on three different days, querying 20 distinct genomic loci. All assays were done using 300ng per hybridization reaction. Data was normalized to a set of 10 control probes representing 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 genomic locus based on data from the Database of Genomic Variants.

In Figure 1 we demonstrate the technical reproducibility of the nCounter Custom CNV Assay. Normalized counts for each probe and replicate are plotted for user 1 (x-axis) vs. user 2 (y-axis). A high correlation (R2= 0.9861) is observed for replicate 1 (day 1) between user 1 and user 2. Similar results are seen for replicates (days) 2 and 3, with correlations of 0.9709 and 0.9882, respectively. In this experiment over 2000 total copy number calls were generated, with an overall precision of 99.5% (data not shown).

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 publicly 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.

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.

Critical Specifications	
Multiplexing capability	Up to 800 regions of the human genome in each reaction.
Recommended amount of starting material	300ng purified genomic DNA
Sample Types Supported	Purified Genomic DNA from blood and saliva
Reproducibility	99%
Number of copied detected	0-4
Synthetic ssDNA Control 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

Description	Quantity / Use	Part Number (P/N)
nCounter Custom CNV Assay*	Increments of 192	CNV-P1CS-XXX
nCounter Master Kit	192 assays	NAA-AKIT-192

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