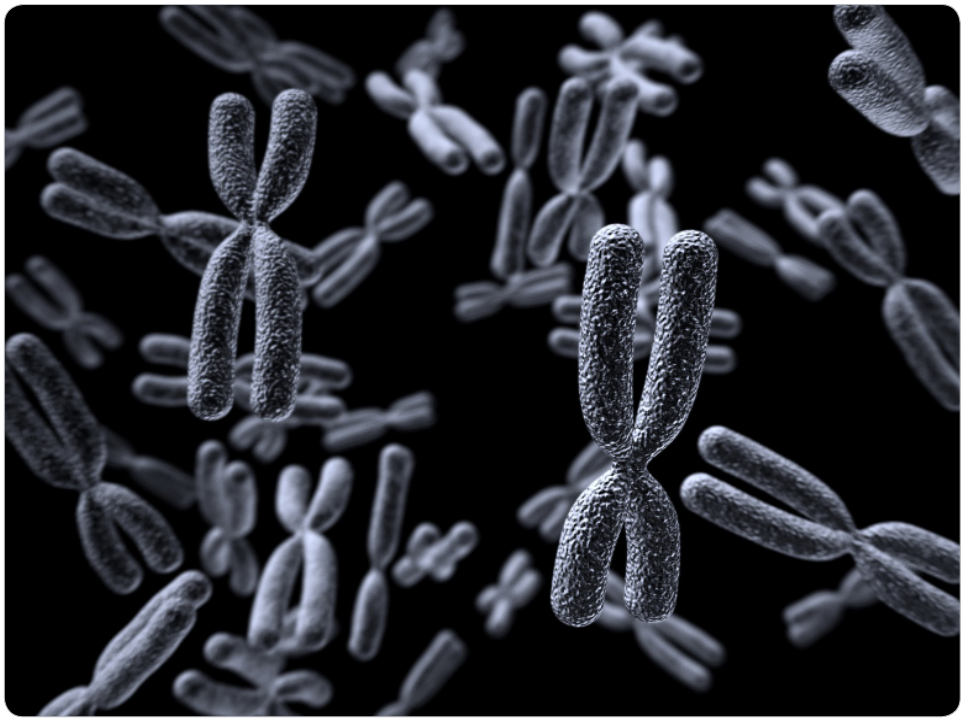
nCounter®
Human Karyotype Panel



Product Highlights

Comprehensive

- Monitor aneuploidy for all 24 human chromosomes in a single reaction
- Includes controls for normalization and sample digestion

Fast

- < 5 minutes hands-on time per sample
- Results next day

Reliable

- > 99% reproducibility
- > 99% accuracy

Cost-effective

- A fraction of the cost of traditional karyotyping
- No specialist skills required

Aneuploidy

When the karyotype of a human cell differs from 46 chromosomes (diploid), it is called aneuploid. Aneuploidies arising *in vivo* in early development (monosomy, trisomy, tetrasomy, etc.) can result in several developmental disorders. Furthermore, extended cell passage or altered growth conditions in vitro can also result in aneuploid cell lines with altered biological properties which can confound experimental results. Traditional methods of karyotype analysis (G-banding, FISH) are expensive, time consuming, labor intensive, and are not readily scalable for analyzing many samples simultaneously.

The NanoString nCounter® Human Karyotype Panel Assay delivers an accurate, precise and automated method of screening large numbers of samples for aneuploidies.

nCounter Human Karyotype Assay

The Human Karyotype Panel consists of 338 probes spanning all 24 chromosomes at a rate of approximately 8 probes per chromosome arm (Figure 1). This coverage enables highly accurate confirmation of diploidy and identification of aneuploidies for each chromosome.

The nCounter Human Karyotype Panel utilizes the same work flow and automation as the nCounter® Custom CNV Assay. Prior to hybridization, the genomic DNA is fragmented and denatured to yield single-stranded targets for hybridization with the Karyotype Panel CodeSet. Hybridization is then performed at 65°C for 12-30 hours. After hybridization samples are transferred to the nCounter® Prep Station where unhybridized 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 probe 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 digitally counted and tabulated. Analysis of Karyotype Panel data is automated by the nCounter CNV Collector Tool software provided free with the panel.

Karyotype Panel Assay Performance Data

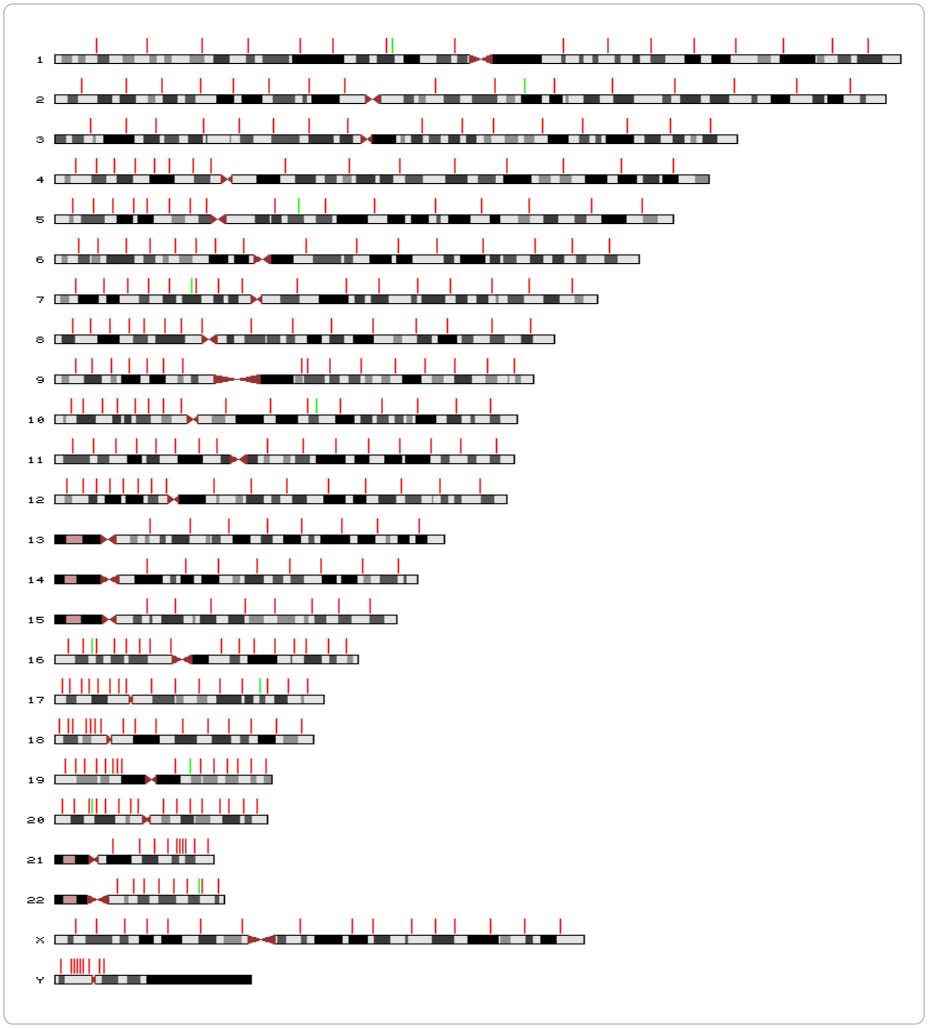
Accuracy

To demonstrate the accuracy of data generated via the nCounter Human Karyotype Panel, we assayed 100 genomic DNA samples purchased from Coriell Institute for Medical Research (Camden, NJ). All samples were run using 600ng of DNA input into the assay (300ng per hybridization reaction) as described in the nCounter® CNV Assay Manual. To account for slight differences in DNA input amounts and hybridization efficiency, data was normalized to the 10 invariant controls, across all samples. To calculate copy numbers for each probe, a reference value was created by averaging each probe across all 100 samples. A copy number estimate was then calculated for each probe by dividing the counts for each sample by the averaged counts of reference value. A single chromosomal copy number was calculated for each of the 24 chromosomes by averaging the values of all probes per chromosome. Since all samples are normal diploid individuals, accuracy is measured as a percentage of probes giving a copy number of 2 for autosomes and chromosome X within female samples, and copy number of 1 for chromosomes X and Y within male samples. The Human Karyotype Panel accurately quantifies of chromosomal copy number, with an extremely low false-positive rate for whole chromosomal aneuploidy (Table 1).

TABLE 1: Whole chromosome call accuracy and call rate for 100 normal diploid samples.

	Totals for 100 DNA Samples
Total calls	2400
Correct calls	2400
No calls	0
Accuracy	100%
Call rate	100%

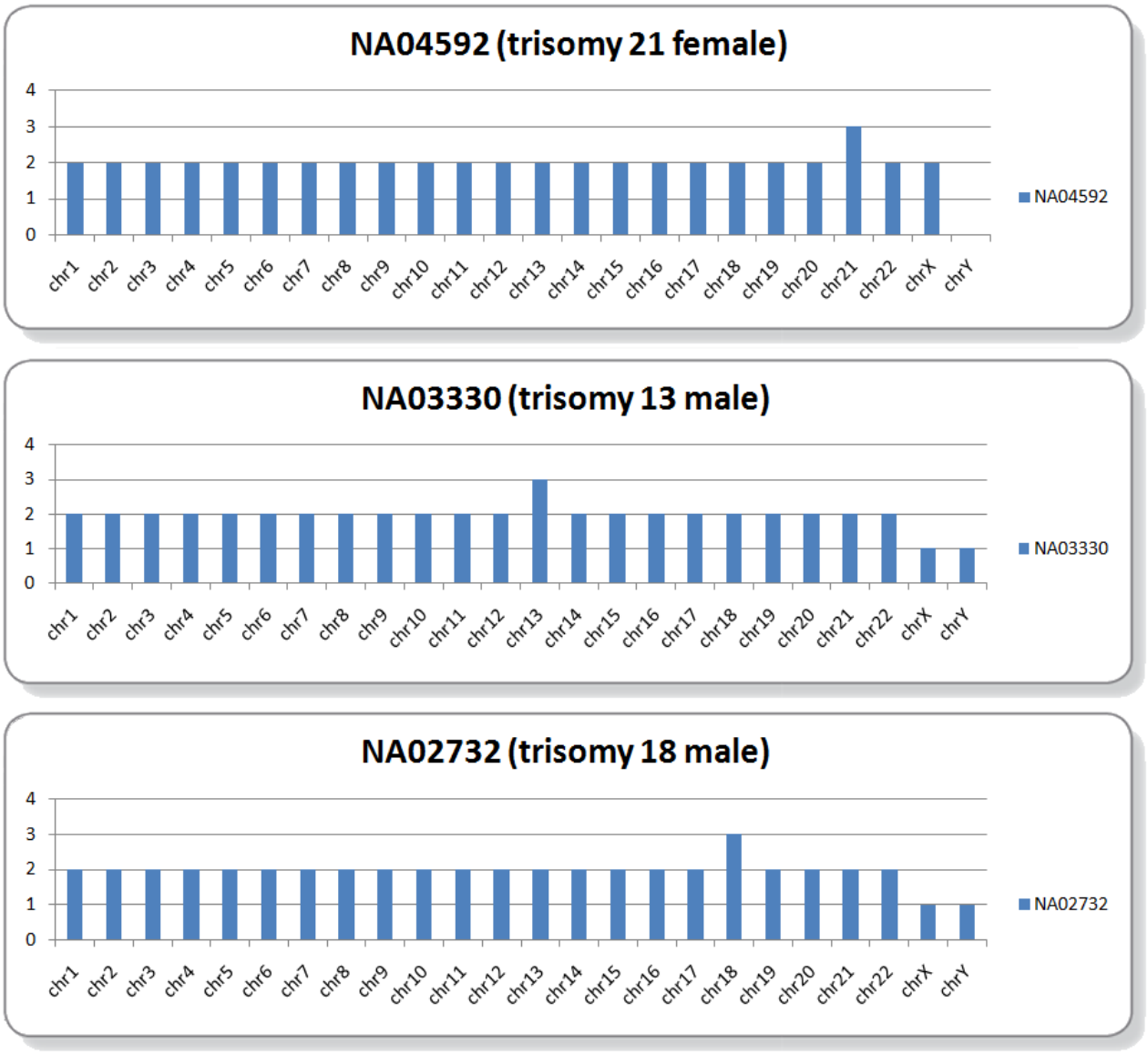
FIGURE 1: Graphical depiction of the location of the 338 karyotype probes on 24 human chromosomes (red). The locations of 2 probes within the Down Syndrome Critical Region (DSCR) of chromosome 21 are shown in brown, and locations of the additional 10 invariant control probes are shown in green. The Human Karyotype Panel has approximately 8 probes spaced to span the length of each chromosomal arm. Ideogram courtesy of the UCSC Genome Browser Website.



Detection of Aneuploidies

The Human Karyotype Panel also accurately measures known chromosomal aberrations in human samples. Three genomic DNA samples from Coriell known to contain aneuploidies by conventional G-band karyotype analysis were assayed as described above. Copy number calls for all probes were determined relative to a normal, diploid male sample, and a chromosomal copy number call was generated as described above. Aneuploid samples are clearly detected by the nCounter assay, with integer copy number calls of "3" for trisomy 21 (NA04592), trisomy 13 (NA03330), and trisomy 18 (NA02732) (Figure 2). All chromosomes expected to have a copy number of 2 gave integer copy number calls of "2". Copy number calls for X and Y gave the expected result for both male samples and the female sample (NA04592).

FIGURE 2: Accurate identification of known chromosomal abnormalities.



System Performance

Description	Specifications
Genomic regions analyzed in one reaction	24 chromosomes 338 individual loci/probes
Recommended amount of starting material	600ng total genomic DNA
Sample types supported	Human genomic DNA
Accuracy	> 99%
Reproducibility	> 99%
Call rate	> 99%
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	Part Number (P/N)
nCounter Human Karyotype Panel	12 assays	CNV-KAR1-12
	24 assays	CNV-KAR1-24
	48 assays	CNV-KAR1-48
	96 assays	CNV-KAR1-96

NanoString Technologies, Inc.

530 Fairview Ave N
Suite 2000
Seattle, Washington 98109

CONTACT US

info@nanostring.com
Tel: (888) 358-6266
Fax: (206) 378-6288
www.nanostring.com

SALES CONTACTS

United States: **us.sales@nanostring.com**
Europe: **europe.sales@nanostring.com**
Japan: **japan.sales@nanostring.com**
Other Regions: **info@nanostring.com**