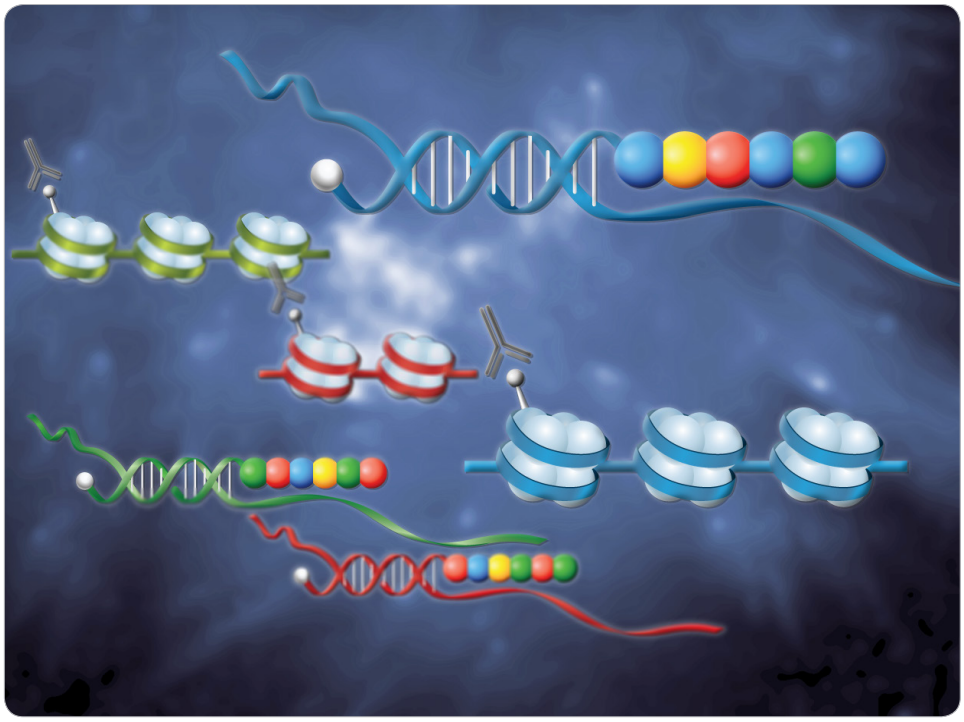
nCounter®
ChIP-String Assay



Product Highlights

Data Quality

- Accurate differentiation and quantification of enriched DNA
- Excellent correlation with ChIP-Seq results
- Valuable biological insights through focused analysis

Efficiency

- Analyze up to 800 loci with 15 minutes of hands-on time
- Results in 24 hours
- No library prep or amplification required

Ease of Use

- Just 15 minutes of hands-on time per run
- Fully-automated target purification and data acquisition
- No technical replicates required

nCounter Custom ChIP-String Assay Overview

The **nCounter Custom ChIP-String Assay** is a read-out method designed to measure dsDNA fragments that have been enriched via various user-defined chromatin immunoprecipitation protocols. Based on NanoString's molecular barcoding technology the assay can reliably quantify as few as 5,000 molecules of DNA without the need for amplification. The technology is ideal for validating ChIP-Seq results and screening large sample sets against focused sets of loci involved in chromatin remodeling and gene expression. It also provides a streamlined method for screening ChIP antibodies, optimizing IP protocols or performing library screening prior to sequencing runs. The nCounter Custom ChIP-String Assay delivers the same level of performance, flexibility, and low hands-on time as other nCounter applications.

Chromatin Modifications and Gene Regulation

The DNA of eukaryotic cells interacts with a variety of DNA binding proteins (histones, transcription factors and chromatin regulators) which serve to package the DNA in highly condensed chromatin structures and regulate transcription. Recent studies have shown that chromatin modifications (e.g., methylation and acetylation of key amino acids) are important in gene regulation and are thought to act in concert with chromatin binding proteins. An active area of epigenetic research is in characterizing patterns of chromatin modifications and associated binding proteins to define "chromatin states" which are associated with key transcriptional regulatory functions (initiation, active transcription, repression, etc.). Understanding the chromatin state associated with a gene makes it possible to predict whether a gene will be active or repressed. Studies to characterize chromatin state generally involve chromatin immunoprecipitation coupled with a method for sequence-specific quantification of DNA contained in the chromatin.

Chromatin Immunoprecipitation

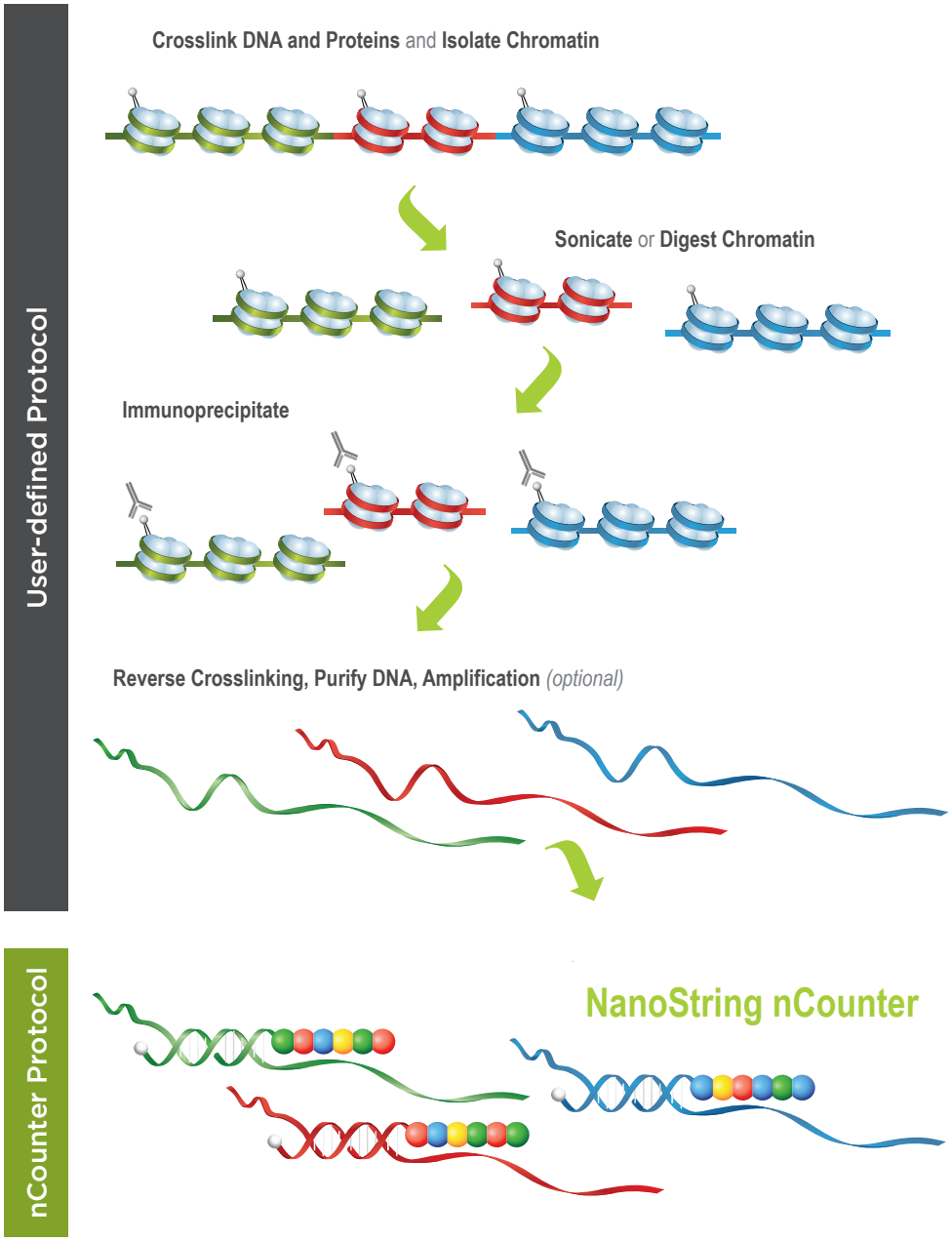
Chromatin immunoprecipitation (ChIP) is a process in which chromatin is precipitated with antibodies that are specific to histone modifications or chromatin-associated proteins. Briefly, DNA is crosslinked to proteins, fragmented, enriched via antibody selection, and purified away from unbound genomic DNA and proteins (FIGURE 1). The purified and highly enriched DNA is then interrogated on a sequence-specific basis to determine which genomic sequences are associated with specific histone modifications or bound by chromatin binding proteins. This has traditionally been done by PCR for low-plex studies and microarrays or sequencing for high-plex studies. nCounter ChIP-String Assays offer a solution for mid-plex ChIP validation studies that require sensitive, high-precision DNA quantification with minimal hands-on time.

nCounter ChIP-String Assay

The nCounter ChIP-String assay enables analysis of up to 800 target sequences in a single multiplexed reaction by using the proven nCounter Analysis System. nCounter's digital counting technology generates data with unparalleled precision and requires only 15 minutes of hands-on time to generate up to 9,600 data points. nCounter can quantify as little as 5 ng of enriched chromatin-immunoprecipitated DNA directly with no amplification. Prior to nCounter ChIP-String analysis, samples are prepared by an appropriate ChIP protocol as defined by the user. The only sample preparation required after ChIP is denaturation of the dsDNA. The nCounter Chip-String assay is ideal for studies of chromatin modifications and associated binding proteins for focused sets of genes. Potential applications include:

- **Assessing quality of ChIP-antibodies prior to sequencing**
- **Optimizing ChIP protocols or conditions prior to sequencing**
- **Analysis of focused sets of targets across many samples such as RNAi knockdowns, timecourse studies or large patient populations**
- **Orthogonal validation of ChIP-Seq or ChIP-Chip results**

FIGURE 1 Overview of ChIP-String Workflow



Custom ChIP-String Assay Performance

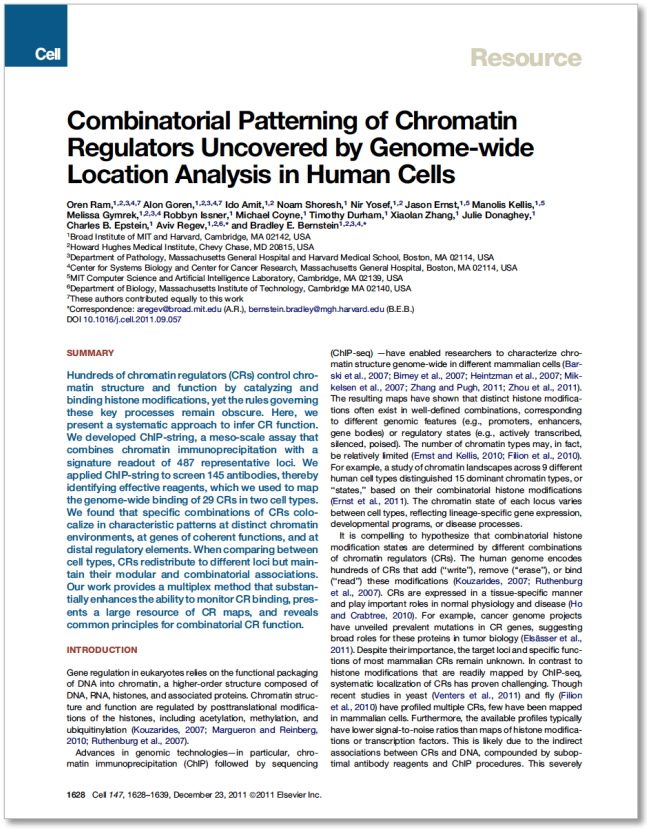
Measuring Enrichment of Genomic Sequences

A recent publication by researchers at the Broad Institute illustrates the utility of the nCounter ChIP-String Assay (FIGURE 2). The authors designed a comprehensive CodeSet containing sequences that are associated with a broad range of chromatin states that correlate to regulatory functions (transcription initiation, active transcription, distal elements polycomb repression, heterochromatin, etc). The CodeSet was used as a read-out method for samples that were enriched with a variety of antibodies via a ChIP protocol developed by the authors. The following advantages of nCounter ChIP String Assays were demonstrated in the publication:

- **ChIP-String is faster and lower cost than genome-wide profiling**
- **ChIP-String generates precise, digital results that correlate well with ChIP-Seq**
- **ChIP-String enables sampling of hundreds of regions in a single reaction, thus generating less biased results than ChIP-PCR (a method which would require a much smaller sampling)**

Data quality is highly dependent on the quality of enriched material and may vary across different protocols. NanoString does not support or endorse any immunoprecipitation protocol and relies on users to select an appropriate protocol for their experimental needs. FIGURE 3 provides an example of high quality data that can be generated when the nCounter ChIP-String Assay is utilized as a read-out method for a robust ChIP protocol.

FIGURE 2 Published work by the Broad Institute demonstrates the utility of the nCounter ChIP-String Assay



Cell

Resource

Combinatorial Patterning of Chromatin Regulators Uncovered by Genome-wide Location Analysis in Human Cells

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SUMMARY

Hundreds of chromatin regulators (CRs) control chromatin structure and function by catalyzing and binding histone modifications, yet the rules governing these key processes remain obscure. Here, we present a systematic approach to infer CR function. We developed ChIP-string, a meso-scale assay that combines chromatin immunoprecipitation with a signature readout of 487 representative loci. We applied ChIP-string to screen 145 antibodies, thereby identifying effective reagents, which we used to map the genome-wide binding of 29 CRs in two cell types. We found that specific combinations of CRs colocalize in characteristic patterns at distinct chromatin environments, at genes of coherent functions, and at distal regulatory elements. When comparing between cell types, CRs redistribute to different loci but maintain their modular and combinatorial associations. Our work provides a multiplex method that substantially enhances the ability to monitor CR binding, presents a large resource of CR maps, and reveals common principles for combinatorial CR function.

INTRODUCTION

Gene regulation in eukaryotes relies on the functional packaging of DNA into chromatin, a higher-order structure composed of DNA, RNA, histones, and associated proteins. Chromatin structure and function are regulated by posttranslational modifications of the histones, including acetylation, methylation, and ubiquitinylation (Kouzarides, 2007; Margueron and Reinberg, 2010; Ruthenburg et al., 2007).

Advances in genomic technologies—in particular, chromatin immunoprecipitation (ChIP) followed by sequencing (ChIP-seq)—have enabled researchers to characterize chromatin structure genome-wide in different mammalian cells (Barski et al., 2007; Birney et al., 2007; Heintzman et al., 2007; Mikkelsen et al., 2007; Zhang and Pugh, 2011; Zhou et al., 2011). The resulting maps have shown that distinct histone modifications often exist in well-defined combinations, corresponding to different genomic features (e.g., promoters, enhancers, gene bodies) or regulatory states (e.g., actively transcribed, silenced, poised). The number of chromatin types may, in fact, be relatively limited (Ernst and Kellis, 2010; Filion et al., 2010). For example, a study of chromatin landscapes across 9 different human cell types distinguished 15 dominant chromatin types, or "states," based on their combinatorial histone modifications (Ernst et al., 2011). The chromatin state of each locus varies between cell types, reflecting lineage-specific gene expression, developmental programs, or disease processes.

It is compelling to hypothesize that combinatorial histone modification states are determined by different combinations of chromatin regulators (CRs). The human genome encodes hundreds of CRs that add ("write"), remove ("erase"), or bind ("read") these modifications (Kouzarides, 2007; Ruthenburg et al., 2007). CRs are expressed in a tissue-specific manner and play important roles in normal physiology and disease (Ho and Crabtree, 2010). For example, cancer genome projects have unveiled prevalent mutations in CR genes, suggesting broad roles for these proteins in tumor biology (Ellässer et al., 2011). Despite their importance, the target loci and specific functions of most mammalian CRs remain unknown. In contrast to histone modifications that are readily mapped by ChIP-seq, systematic localization of CRs has proven challenging. Though recent studies in yeast (Venters et al., 2011) and fly (Filion et al., 2010) have profiled multiple CRs, few have been mapped in mammalian cells. Furthermore, the available profiles typically have lower signal-to-noise ratios than maps of histone modifications or transcription factors. This is likely due to the indirect associations between CRs and DNA, compounded by suboptimal antibody reagents and ChIP procedures. This severely

FIGURE 3 Demonstrated utility of nCounter ChIP-String Assay

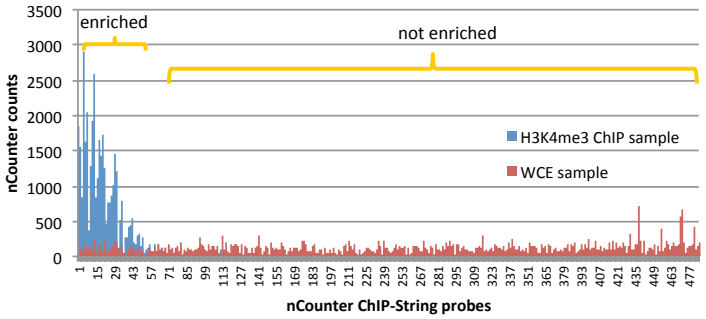
ChIP-String analysis provides a signature readout that is consistent with ChIP-Seq data and known chromatin states [data courtesy of Ram & Goren, *et al*., Broad Institute]:

(A) ChIP-String results for antibodies to H3K4me3 and un-enriched chromatin input (Whole Cell Extract, WCE). Approximately 50 ChIP-String probes were enriched (range 30-235 fold) relative to input WCE. Counts were normalized to DNA input (11ng and 143ng of unamplified DNA for H3K4me3 and WCE, respectively). X-axis represents 487 ChIP-String probes representing genomic loci associated with various chromatin states. Normalized counts are plotted on the y-axis.

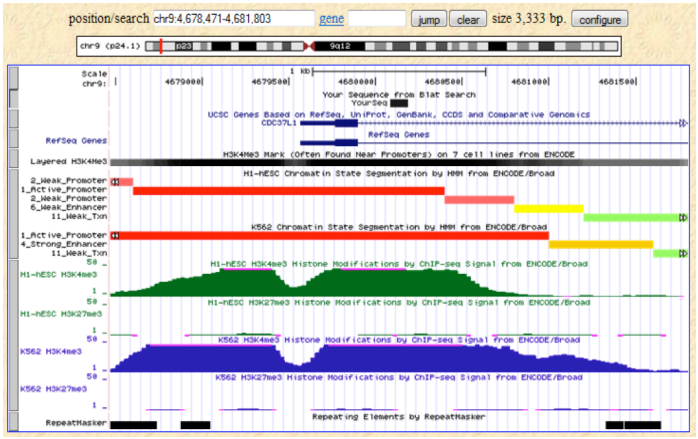
(B) UCSC genome browser view of genomic region for one ChIP-String probe. As expected, the location of this probe correlates with ChIP-seq data using antibodies to the same histone modification and is located within an active promoter for the CDC37L1 gene in K562 cells.

(C) Sequences enriched by H3K4me3 ChIP and measured by ChIP-String correspond primarily to active promoter and strong enhancer regions.

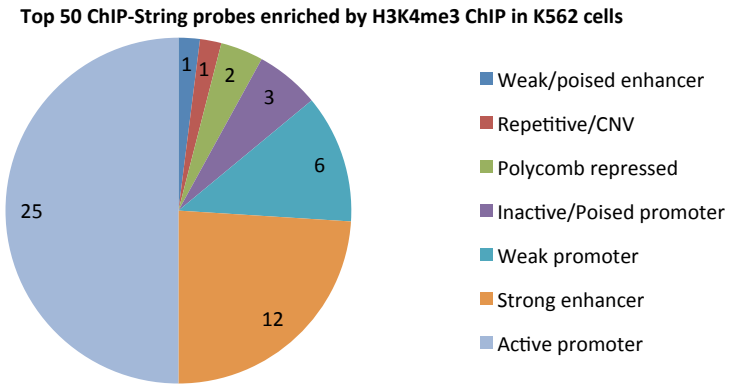
A Accurate differentiation and quantification of enriched DNA



B Excellent correlation with ChIP-Seq results



C Valuable biological insights through focused analysis



System Performance

Description	Specifications
Maximum number of probes per CodeSet	800
Recommended size of target region submitted	> 300 bases
Recommended amount of starting material	~10 ng unamplified ChIP DNA ~100 ng of WGA amplified ChIP DNA or NGS library prepared DNA
Sample types supported	Human genomic DNA prepared by ChIP
Synthetic spike titration correlation	> 0.95
Linear dynamic range	7 x 10^5 total counts
nCounter Prep Station throughput	12 samples / 2.5 hours
nCounter Digital Analyzer throughput	12 samples / 2.7 hours (up to 108 samples per day unattended running in continuous mode)

Ordering Information

Description	Quantity / Use	Part Number (P/N)
nCounter Custom ChIP Assay		XT-CIP-P1CS-XXX
nCounter Master Kit (all reagents, sample cartridges, and consumables necessary for processing 48 or 192 assays)	48 Assays 192 Assays	NAA-AKIT-048 NAA-AKIT-192
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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