Chromatrap

Chromatrap® 96: a new solid-state platform for highthroughput ChIP

Summary

Chromatrap® 96 (C96) is a high-throughput analysis platform that profiles up to 96 transcription factors and epigenetic modifications simultaneously in less than 1 d. It enables sensitive, selective and reproducible target amplification with excellent signal-to-noise ratios, even from samples as small as 0.1 µg. Compatible with automated handling, C96 allows simultaneous investigation of parallel epigenetic landscapes, offering unprecedented assay flexibility and speed.

Introduction

The field of epigenetics is rapidly expanding as researchers uncover the principles governing gene regulation through the dynamic binding of proteins to DNA. Profiling of heritable epigenetic modifications, a major driver of biological complexity, through target assays such as C96 is now beginning at the genomic level[1].

Understanding loci-specific, coordinated epigenetic mechanisms that affect gene expression and downstream cell phenotype is a focus for both the research and pharmaceutical communities. Such analysis – principally of chromatin remodeling through posttranslational modifications like histone acetylation, DNA methylation and the regulation of gene expression by noncoding RNAs – is crucial in biomarker identification, diagnostic development and therapeutic exploitation.

Chromatin immunoprecipitation (ChIP) enables the selective immunoprecipitation of a regulatory protein of interest or epigenetic mark to determine its associated DNA sequences[2,3]. ChIP has increased our understanding of the biological significance of DNA-protein associations and our ability to map the localization of modified histones, histone variants, transcription factors or other chromatin-modifying enzymes at given gene loci[4]. When paired with next-generation sequencing, ChIP can map epigenetic marks and transcription factor binding sites at the global genome level.

In cancer, both hypo- and hypermethylation of DNA have been linked to tumor growth. Aberrant patterns of histone marks also have a role in oncogenesis: hypoacetylation and hypermethylation of histones H3 and H4 can inhibit the expression of genes involved in tumor suppression independent of altered DNA methylation[5,6]. Beyond cancer, epigenetic factors have been implicated in inflammatory, autoimmune, metabolic, neurological and blood disorders[6].

Chromatrap® is a new technology that offers a quicker, easier and more efficient platform for ChIP[7]. Based on a patented solidstate platform, Chromatrap® enables fast, sensitive, selective immunoprecipitation of both high- and low-abundance targets from small cell numbers. It requires less manual handling than traditional ChIP methods and thus is less prone to operator error. The Chromatrap® protocol can be completed in as little as 5 h, offers excellent signal-to-noise ratios and has a straightforward process. It is compatible with single-loci and genome-wide profiling through ChIP-seq. It is available in a range of formats, including a unique multi-well plate format ('C96') that allows multiple proteins and/or samples of interest to be profiled simultaneously. Fully compatible with liquid handling, C96 lends itself to true high-throughput epigenetic screening in research, clinical profiling and epigenetic drug compound mechanism-of-action studies.

Chromatrap® technology—how it works

The solid-phase scaffold has a proprietary internal structure, composed of inert inner pore surfaces, functionalized with covalently bound Protein A or G. This microfluidic environment is specifically designed to maximize chromatin capture efficiency, reduce nonspecific binding and promote molecular mixing. It therefore allows more efficient immunoprecipitation than traditional bead-based methods (**Fig. 1**). All reactions take place inside the column, reducing handling time, processing complexity and error. The flow-through characteristics involved ensure excellent sample mixing and washing, further reducing protocol length. Because of the unique buffer chemistry, no DNA cleanup is necessary, streamlining time and cost to endpoint analysis. As a result Chromatrap offers unrivalled simplicity, efficiency and greater signal-to-noise results even from limited sample material.

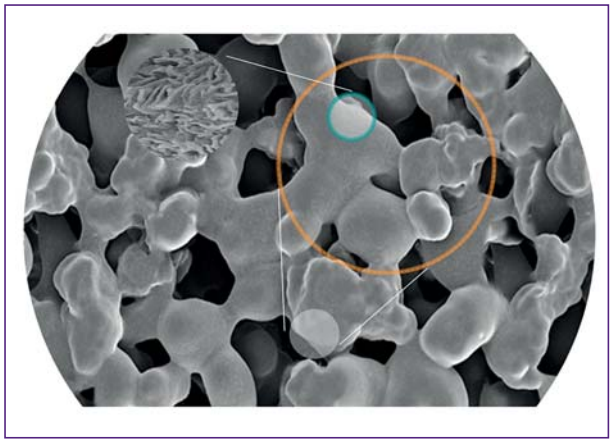

Figure 1 *Chromatrap® inert solid-phase scaffold for ChIP provides increased surface area for antibody binding, allowing more efficient immunoprecipitation whilst reducing nonspecific binding.*

Sensitivity from small samples. Excellent DNA enrichment is observed using C96. The signal is up to 25× higher than that for traditional bead-based assays from as little as 7,500 cells. The low background binding (typically 3× greater signal-to-noise ratio than competing procedures) enables protein immunoprecipitation from a wide, dynamic range of input chromatin concentrations (**Fig. 2**). Specifically developed for low chromatin concentrations, C96 performs equally efficiently with immunoprecipitations from 100 ng of input chromatin to 2 µg. This assay flexibility enables C96 to be tailored to ChIP from difficult clinical samples and to more everyday cell line–based assays, while a modified protocol enables higher loading (up to 50 µg) and is compatible with ChIP-seq. This dynamic range enables more assays per sample, thus allowing multiple pathways and transcription factors to be targeted from the same sample.

Simultaneous epigenetic modification and transcription factor targeting. The binding efficiency and occupancy of common epigenetic marks (H3, H4, H3K4me3 and RNA Pol II) on three gene targets of interest (GAPDH, b-globin and PABPC1) in three human chromatin samples (HepG2, HeLa and K562) was performed simultaneously. For every immunoprecipitation, 1 µg of chromatin from each cell type was loaded onto the C96 microplate with 2 µg of antibody for 1 h with gentle agitation. Following a series of quick and simple centrifugation washes, chromatin was released after 15 m of incubation in elution buffer. After reverse cross-linking and protein digestion, selectively enriched DNA was amplified by quantitative PCR and compared relative to the background and input signal. Immunoprecipitation can be carried out in less than 5 h, with no liquid handling. All assays were performed in triplicate; signal levels are shown as an average ± standard error of the mean (**Fig. 3**).

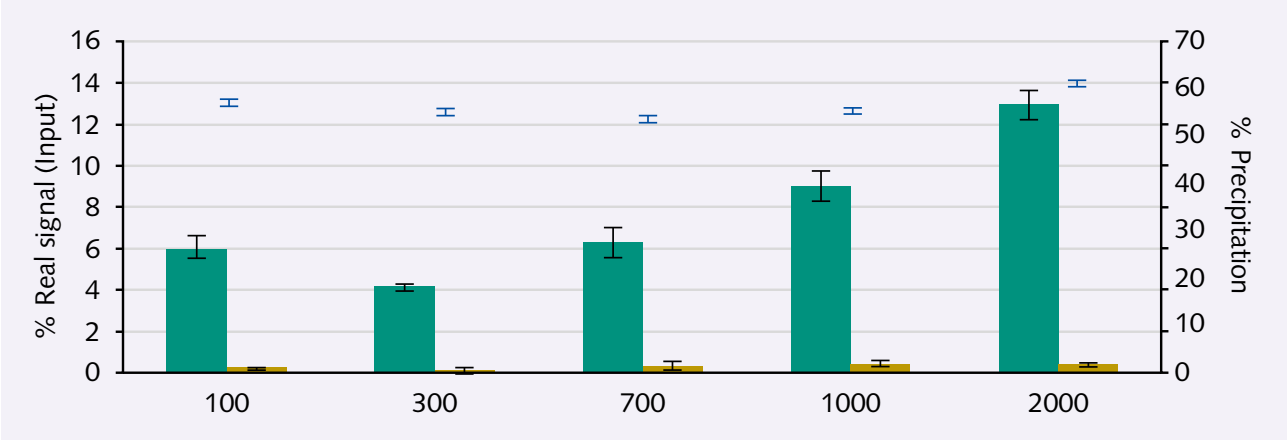

Figure 2 *Chromatrap® provides a wide dynamic range suitable for all sample types. Excellent signal-to-noise ratio can be achieved using as little as 100 ng per immunoprecipitation, demonstrated by sensitive enrichment of RNA Pol II onto the GAPDH locus. Chromatrap® has a full chromatin concentration range of 50-7,000 ng for qPCR and up to 50 µg for ChIP-seq.*

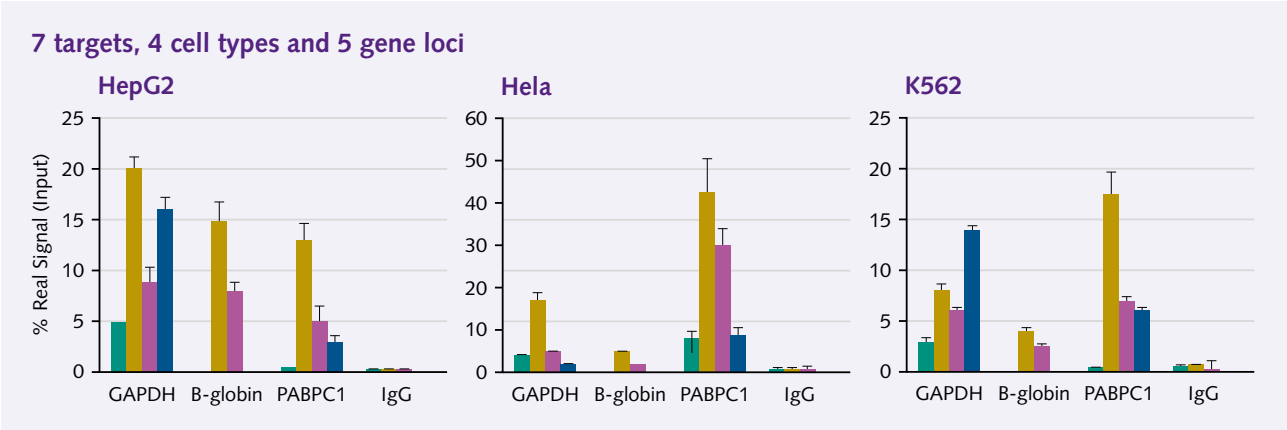

Figure 3 *C96: high throughput with speed and sensitivity. The ability of C96 to analyse multiple antibody target occupation of multiple gene loci in three different chromatin sample types is represented here by a subset of data on one C96 plate.*

C96 demonstrates strong signal strength with RNA Pol II presence mapped at key target gene loci, in the presence of associated H3 and H4 signatures. The sensitivity and selectivity of the assay is clearly shown, with all transcription factor targets tested against positive and negative control genes. C96 has been tested using a range of fully validated ChIP antibodies.

C96 offers a high-throughput platform to study epigenetic modifying enzymes, transcription factors and histones from small cell samples using low chromatin concentrations. The assay speed and reduced manual handling produces reproducible, sensitive and selective assay data (www.chromatrap.com).

Conclusion

Epigenetics is a fast-growing research area with wide applications, including disease mechanism profiling and personalized medicine strategies. ChIP is crucial to epigenetic research. Presented here is a new, enriched assay process for profiling simultaneous protein and DNA patterns that characterize disease mechanisms. The highthroughput, patented solid-state platform enables 96 simultaneous assays across a multitude of target proteins, cell-signaling mechanisms, cell types and/or patient samples in 1 d. Chromatrap® offers a step change in ChIP productivity that significantly improves the efficiency and scope of epigenetic research.

References

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