HaloCHIP™ System: Mapping Intracellular Protein:DNA Interactions using HaloTag® Technology

ABSTRACT The HaloCHIP™ System is a novel method that covalently captures intracellular protein:DNA complexes without using antibodies. DNA binding proteins of interest are expressed in cells as HaloTag® fusion proteins, crosslinked to DNA, and then captured on HaloLink™ Resin, which forms a highly specific, covalent interaction with the HaloTag® moiety on the fusion protein. Due to the complete covalent linkage established between the HaloLink™ Resin and the crosslinked protein:DNA complexes, the resin can be stringently washed to remove nonspecific DNA and protein more effectively than is possible by coimmunoprecipitation. The crosslinks are reversed to release purified DNA fragments from the resin. By improving specificity and reducing background interference during the isolation of protein:DNA complexes, this new method effectively increases the signal-to-noise ratio to permit detection of small changes in protein binding within a genome.

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The HaloCHIP™ System offers a new approach for the capture of crosslinked intracellular protein:DNA complexes and yields captured DNA fragments that are ready for further analysis.

INTRODUCTION

Regulation of chromatin structure and gene expression is essential for normal development and cellular growth. Transcriptional events are tightly controlled both spatially and temporally by specific protein:DNA interactions. Currently there is a rapidly growing trend towards genome-wide identification of protein binding sites on chromatin and characterization of the transcriptome using the chromatin immunoprecipitation (ChIP) method coupled with either DNA microarray or ultrahigh-throughput sequencing analysis (1,2). While significant advances and the development of new technology in the fields of DNA microarrays and sequencing have allowed genomic-level characterization of protein binding sites, the upstream ChIP (3,4) process for the capture of DNA fragments remains cumbersome and time consuming. The present challenges of the ChIP method include: the lack of highly specific antibodies certified to recognize crosslinked epitopes; the efficiency and specificity of capture during the coimmunoprecipitation step; and the multistep, time-consuming procedure required. The HaloCHIP™ System[a,b] was designed to address each of these challenges, providing a robust and efficient alternative to the standard ChIP method.

THE HALOCHIP™ SYSTEM

The HaloCHIP™ System uses a protein tag, the HaloTag® moiety, which can be fused to any protein and which mediates a covalent interaction with various interchangeable ligands. We have designed several fluorescent and solid-support ligands for the HaloTag® technology, allowing both in vivo and in vitro characterization of HaloTag® fusion proteins (5,6). In the HaloCHIP™ System, DNA binding proteins fused to the HaloTag® moiety can be transiently or stably expressed in cells. Figure 1 shows a schematic diagram of the HaloCHIP™ System. Similar to the ChIP protocol (3), cells are treated with formaldehyde, generating covalent protein:DNA crosslinks, lysed, and sonicated to shear chromatin into 500–1500 bp fragments. Unique to the HaloCHIP™ System, the crosslinked complexes are then directly and covalently captured from cell lysates on HaloLink™ Resin and washed stringently to remove nonspecific proteins and DNA. Captured DNA fragments are released from the HaloLink™ Resin[a,b] using heat, which reverses the formaldehyde crosslinks. The DNA can be purified using a PCR purification kit and analyzed by the user's preferred method. A control for the HaloCHIP™ experiment is established by splitting the cellular lysate prior to capture on HaloLink™ Resin and incubating the control sample with the HaloCHIP™ Blocking Ligand. The Blocking Ligand binds to the HaloTag® protein, preventing interaction with the HaloLink™ Resin. This control provides the best estimate of background DNA binding.

ADVANTAGES OF HALOTAG® PROTEIN IN THE HALOCHIP™ SYSTEM

The beneficial features of using HaloTag® protein and the HaloCHIP™ System are the rapid, covalent capture on the HaloLink™ Resin and the ability to retain activity after 0.75–1% formaldehyde treatment, lysis, and mild sonication. The fast on-rate or binding efficiency of the HaloTag® protein to its ligands is comparable to the biotin-streptavidin interaction, allowing greater than 75% binding of crosslinked HaloTag® protein:DNA complexes to

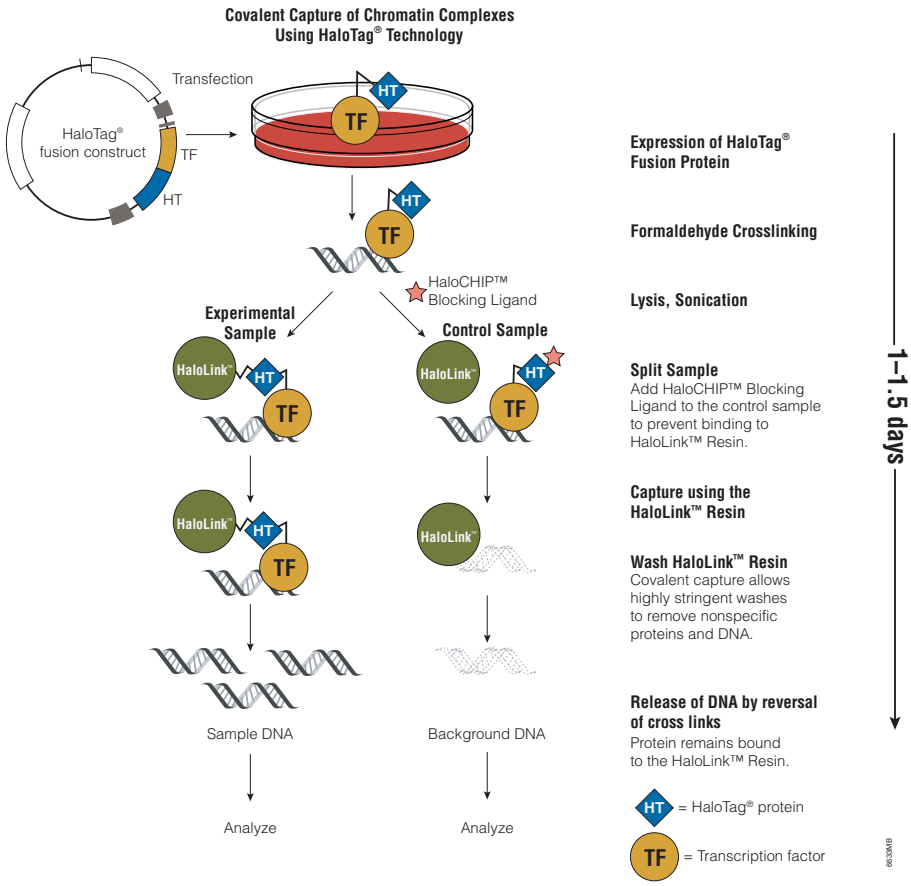

Figure 1. Schematic diagram of the HaloCHIP™ System.

HaloLink™ Resin within a short time frame of 2–3 hours (Figure 2). This is significantly faster than the time required for coimmunoprecipitation in the ChIP method (4).

The covalent interaction between the HaloTag® protein and the HaloLink™ Resin allows efficient capture of low concentrations of protein:DNA complexes within a complex mixture such as a cell lysate or from a small amount of cells (2–4 × 10^5 cells). The covalent linkage established between the HaloLink™ Resin and the crosslinked HaloTag® fusion protein:DNA complexes allows harsh and extensive washing after capture to remove nonspecific protein and DNA bound to the HaloLink™ Resin. Covalent linkage also eliminates the problem of losing protein:DNA complexes due to dissociation during the washing steps. These washing steps are not possible with noncovalent interactions. Additionally, with the HaloCHIP™ System, the washes can be performed with buffers containing high concentrations of detergents or salts without incurring sample loss. Water or TE can also be used.

The HaloCHIP™ System provides a 2- to 3-day time savings over ChIP methods.

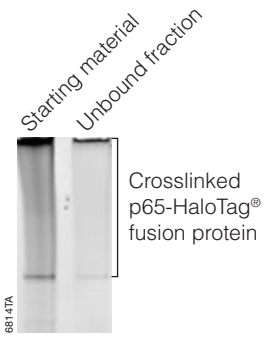

Figure 2. Capture of crosslinked p65-HaloTag® fusion protein on the HaloLink™ Resin. HeLa cells (2 × 10^5) were transfected with a p65-HaloTag® construct, crosslinked with 0.75% formaldehyde, lysed, and sonicated. A sample of the starting material was removed before a 2-hour incubation with the HaloLink™ Resin at room temperature. Following the incubation, the supernatant was collected, representing the unbound fraction of the crosslinked p65-HaloTag®:DNA complexes. Both the starting material and unbound fractions were labeled with the fluorescent HaloCHIP™ Blocking Ligand, subjected to SDS-PAGE, and visualized on a Typhoon® scanner to detect the fluorescence. Greater than 75% of the starting crosslinked p65-HaloTag®:DNA complexes were captured on the HaloLink™ Resin. In both lanes, the p65-HaloTag®:DNA complexes migrate as a smear due to the variable sizes of crosslinked complexes.

HALOCHIP™ EXPERIMENTS WITH VARIOUS TRANSCRIPTION FACTORS

To demonstrate the specificity and functionality of the HaloCHIP™ System, the model system of p65/NFκB was chosen (7). The p65 protein has been characterized using traditional ChIP, and promoters have been identified that are bound by p65, including IκB, IL-6, and IL-8 (8,9). For our experiments, the full-length human p65 protein was cloned into the pFC8A(HaloTag®) CMV Flexi® Vector and transiently expressed as a C-terminal p65-HaloTag® fusion protein in HeLa cells. The results show specific and enriched binding compared to the control for p65-specific promoters using both endpoint PCR and the Plexor® qPCR System (10) for DNA analysis (Figure 3). The background signal shown (Figure 3, Panel A) represents the control sample, which was incubated with the HaloCHIP™ Blocking Ligand prior to capture on the HaloLink™ Resin. The quantitative Plexor® data is plotted as the ratio of the signal from the sample to the background signal in the control sample.

The HaloCHIP™ System has also been used to study p53-HaloTag® and CREB-HaloTag® fusion proteins binding to their predicted respective promoters, p21/Waf and hCG (11,12). Both systems showed capture of specific promoters and amplification using standard endpoint PCR (Figure 4). Different cell lines, JEG-3 for CREB and U2OS cells for p53, were used for these experiments, demonstrating the versatility of the HaloCHIP™ System. While these examples show the use of standard PCR techniques for the analysis of isolated DNA fragments, other downstream applications such as microarray analysis and sequencing are possible with the HaloCHIP™ System, because typical yields of DNA from a single HaloCHIP™ experiment are greater than 10 ng.

The HaloTag® protein offers covalent binding, rapid capture, and resistance to mild formaldehyde treatment and sonication.

SUMMARY

The HaloCHIP™ System offers a new approach for the capture of crosslinked intracellular protein:DNA complexes and yields DNA fragments that are ready for further analysis. The significant benefits of using the HaloTag® protein include: covalent binding, rapid capture, and resistance to mild formaldehyde treatment and sonication. These benefits negate the need for transcription factor-specific antibodies and permit extensive washing of the complexes after capture. Ultimately, this results in reduced background and increased signal-to-noise ratios in the HaloCHIP™ System. The HaloCHIP™ System also provides a 2- to 3-day time savings compared to

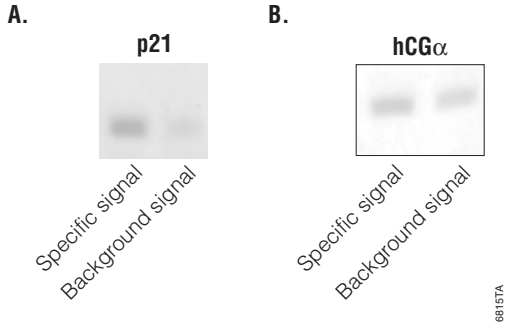

Figure 4. HaloCHIP™ capture of p53- and CREB-specific promoters. U20S cells were transfected with the p53-HaloTag® construct (Panel A), and JEG-3 cells were transfected with the CREB-HaloTag® construct (Panel B). The cells were processed using the HaloCHIP™ System protocol described in the *HaloCHIP™ System Technical Manual*, #TM075. **Panel A.** Ethidium bromide-stained 2% gels showing the PCR amplification products of the p53-specific p21/Waf promoter. **Panel B.** Ethidium bromide-stained 2% gels showing PCR amplification products of the CREB-specific hCG promoter. The PCR products for the signal and the control samples are indicated.

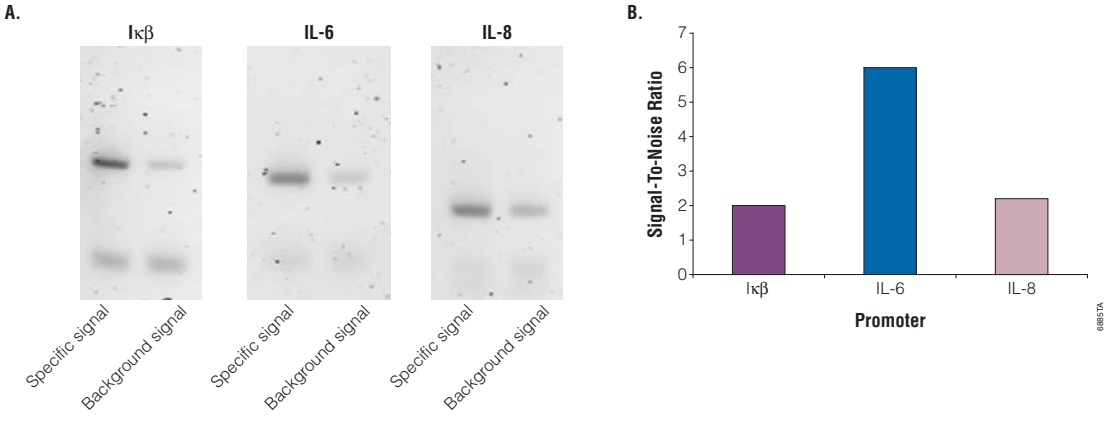

Figure 3. Standard and quantitative PCR analysis of HaloCHIP™ data with p65-HaloTag® protein. HeLa cells (4×10^5) were transfected with the p65-HaloTag® construct, crosslinked with formaldehyde and processed using the HaloCHIP™ protocol described in the *HaloCHIP™ System Technical Manual*, #TM075. DNA fragments obtained from the HaloCHIP™ procedure were further purified and the p65-specific promoters, IκB, IL-6, and IL-8, were amplified and analyzed using standard PCR (33 cycles) or quantitative PCR with the Plexor® System. **Panel A.** The PCR products for the specific signal and background/control signal are shown on ethidium bromide-stained 2% agarose gels. **Panel B.** The signal-to-noise ratio for each promoter was determined and plotted following quantitative Plexor® PCR analysis.

standard ChIP and has demonstrated success in a variety of cell lines with various DNA binding proteins. The HaloCHIP™ System complements all other HaloTag® applications, such as imaging (5) and protein:protein pull-downs (6), allowing complete characterization of a DNA-binding protein's life cycle with the use of a single HaloTag® fusion protein construct.

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PROTOCOL

- *HaloCHIP™ System Technical Manual*, #TM075, Promega Corporation
  www.promega.com/tbs/tm075/tm075.html

Want to learn more about the HaloCHIP™ System?
See an animation online at:
www.promega.com/halochipmovie/

ORDERING INFORMATION

Product	Size	Cat.#
HaloCHIP™ System	20 reactions	G9410
HaloLink™ Resin	2 ml	G1911
	5 ml	G1912
HaloLink™ Magnetic Beads	1 ml	G9311
HaloTag® pHT2 Vector	20 µg	G8241
pFC8A (HaloTag®) CMV Flexi® Vector	20 µg	C3631
pFC8K (HaloTag®) CMV Flexi® Vector	20 µg	C3641
HaloTag® Alexa Fluor® 488 Ligand	30 µl	G1001
HaloTag® Oregon Green® Ligand	30 µl	G2801
HaloTag® TMR Ligand	30 µl	G8251
HaloTag® diAcFAM Ligand	30 µl	G8272
HaloTag® Coumarin Ligand	30 µl	G8581
HaloTag® Biotin Ligand	30 µl	G8281
HaloTag® PEG-Biotin Ligand	30 µl	G8591
HaloTag® Amine (04) Ligand	5 mg	P6741
HaloTag® Succinimidyl Ester (04) Ligand	5 mg	P6751
HaloTag® Thiol (04) Ligand	5 mg	P6761
HaloTag® Iodoacetamide (04) Ligand	5 mg	P6771
HaloTag® Succinimidyl Ester (02) Ligand	5 mg	P1691
HaloTag® Amine (02) Ligand	5 mg	P6711
HaloTag® Iodoacetamide (02) Ligand	5 mg	P1681

[a] Patent Pending.

[b] For research use only. Researchers may use this product in their own research and may modify or link Promega HaloTag® ligands to Promega or customer-supplied moieties. No reach-through payments shall be owed to Promega relating to an organization's commercialization of products that are the discoveries resulting from research use of this product or its derivatives, provided that such products of the organization do not fall within the scope of the valid claims of any issued patents assigned or licensed to Promega, or that such commercialization would not be a violation of the terms of this label license. No other use or transfer of this product is authorized without the express, written consent of Promega. With respect to manufacture or sale of research products, or any diagnostic, therapeutic or prophylactic uses, please contact Promega for supply and licensing information. If the purchaser is not willing to accept the conditions of this limited use statement, Promega is willing to accept return of the unopened product and provide purchaser with a full refund. However, in the event that the product is opened, then purchaser agrees to be bound by the conditions of this limited use statement.

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