An Improved Method for Chromatin Immunoprecipitation Using Micro-Scale Affinity Columns

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Introduction
Covariant post-translational modifications of histone proteins are important factors in the regulation of gene expression. Chromatin immunoprecipitation (ChIP) coupled with PCR amplification is a powerful tool for studying histone modifications of specific loci. Traditional methods for ChIP utilize agarose beads to precipitate the antibody-chromatin-DNA immune complex by centrifugation and to isolate and wash the beads. We present an improved method for enrichment of the chromatin immune complex using micro-scale affinity columns. The columns provide improved performance and reduced background as well as reduced time and assay complexity.

Materials
MAGP2 antibody was obtained from Abcam, Ltd (Cambridge, UK). Histone H3 antibodies, SDS Lysis Buffer, ChIP Dilution Buffer, and salmon-sperm DNA/Protein A and Protein G agarose were obtained from Upstate Biotechnology (Lake Placid, NY). RKO and SW480 cell lines were obtained from the American Type Culture Collection. Protein G PhyTips® Columns were obtained from PhyNexus, Inc. (San Jose, CA).

Methods
Chromatin Preparation
Human colon carcinoma cell lines RKO and SW480 were grown to confluency, cross-linked with 1% formaldehyde, trypsinized, re-suspended in SDS/Lysis and sonicated to fragment the DNA. ChIP Dilution buffer was added to a final volume of 2 ml. 20 µl of each sample was retained for DNA input PCR analysis.

Pre-clearing and Antibody Incubation
The remaining samples were pre-cleaned with 80 µl salmon sperm DNA Protein A and Protein G agarose (3:1 ratio of Protein A to Protein G) and the supernatant from each cell line was divided equally into five fractions. 2.5 µl of each antibody was added to separate fractions and incubated overnight at 4°C. Control samples containing no antibody were also prepared for the two cell lines. Following the overnight incubation, each sample was split into two 200 µl fractions for analysis using conventional immunoprecipitation and PhyTip enrichment.

Conventional Precipitation
Ten microliters of the 3:1 Protein A and Protein G agarose were added to each sample for one hour at 4°C to capture the antibody-histone complex and collected by centrifugation. The beads were washed twice for 5 minutes each at 1000 rpm with 1 ml Low Salt, High Salt, and twice with LiCl. Following the final wash step, 120 µl elution buffer (1% SDS, 0.1M NaHCO3 added to the eluate to the elute the protein/DNA complex (Figure 1).

Enrichment with PhyTip™ Columns
PhyTip columns containing 5 µl of Protein G resin encapsulated at the end of a pipette tip (Figure 2) were pre-blocked with salmon sperm DNA. The samples, wash, and elution buffers were placed in individual columns of the Microtiter plate. Liquid handling steps were performed using the PhyNexus™ ME-200 computer-controlled multichannel pipettor (Figure 3). Capture of the immune complex was achieved by passing the 200 µl samples back and forth through the column for five cycles. The columns were washed with the same series of buffers as described in the preceding section by passing 200 µl of each wash solution through the columns for three cycles. The elution step was performed by passing 120 µl of elution buffer back and forth through the column for five cycles.

Results
Anti-acetyl-Histone H3

| Protein G beads | Protein G PhyTip | Input control |

| RKO | SW480 | SW480 | SW480 |

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Table 1. ChIP-PCR Results. The expected pattern of histone modification and DNA methylation as described previously are included along with the results obtained using the bead method and the PhyTip method for enrichment. Results that agree with the expected pattern are shown in green while the ambiguous or false results are shown in red.

Discussion
Acetylation and methylation patterns of Histone H3 surrounding the promoter region of the mismatch repair gene hMLH1 in colon cancer cell lines RKO (hMLH1 promoter silenced) and SW480 (hMLH1 promoter active) have been described previously [1]. In this study, we observe similar results from the PhyTip method using ten times less cellular material for each ChIP assay. The traditional bead method was not sensitive enough at this level to produce accurate results (Table 1). We also demonstrate detection of methylated CpG in the hMLH1 promoter using immunoprecipitation of the MeCP2 protein. Signals from the PhyTip enrichment method were consistent with expected results [2]. The traditional bead method for enrichment failed to generate sufficient sensitivity.

The results were consistent using PCR primers for two other regions of the hMLH1 promoter (data not shown).

Summary
PhyTip™ columns demonstrate improved performance for the enrichment of chromatin immunoprecipitation complexes. The design of the microscale affinity column format allows for decreased assay time and complexity as well and improved sensitivity and robustness. The PhyTip method is capable of processing multiple ChIP enrichment assays in parallel with reduced sample requirements, enabling increased resolution in multiplexed ChIP experiments.

References