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Chromatin immunoprecipitation for human monocyte derived macrophages

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ABSTRACT

The importance of Chromatin Immunoprecipitation (ChIP) technology has grown exponentially along with an increased interest in epigenetic regulation. The correlation of transcription factors with histone marks is now well established as the center of epigenetic studies; therefore, precise knowledge about histone marks is critical to unravel their molecular function and to understand their role in biological systems. This knowledge constantly accumulates and is provided openly in the expanding hubs of information such as the USCS Genome Browser. Nevertheless, as we gain more knowledge, we realize that the DNA–protein interactions are not driven by a "one size fits all" rule. Also, the diversity of interactions between DNA, histones, and transcriptional regulators is much bigger than previously considered. Besides a detailed protocol of sample preparation for the ChIP assay from primary human monocyte-derived macrophages (MDM) [an acceptable *in vitro* model for primary, human macrophage cells], we show that differences between various types of cells exist. Furthermore, we can postulate that such variations exist between transformed macrophage-like cell lines and primary macrophages obtained from healthy volunteers. We found that the most efficient fixation time for MDM is 10 min. Finally, to perform multiple analytical assays, we showed that even with thorough methodology, the yield of material obtained from primary cells is the major challenge.

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1. Introduction

Epigenetics is the examination of functionally relevant changes to the living organism that do not result from changes in the nucleotide sequence in the genome. Cell differentiation is an insightful example of the importance of epigenetic regulation [1]. An example of epigenetic regulation is a fertilized egg that gives rise to an organism that consists of hundreds of different cell types, morphologically and functionally, while preserving genetic information contributed by both parents [2,3]. The epigenetic regulation of gene expression is a highly complicated process; however, bringing to light the mechanisms that are responsible for the regulation of epigenetic modifications is imperative to advance personalized medicine and improve disease treatments and prevention.

Chromatin Immunoprecipitation (ChIP) is an epigenetic tool that compares transcription factors or modifications of DNA-bound proteins (*e.g.* histones) to specific genes across the whole genome [4]. An important feature of the ChIP assay is the fixation of protein–DNA interactions and histone post-translational modifications (PTM) in the cells, which reflect the influences on gene expression by capturing the state of regulation at a given point in time. Therefore, the importance of understanding epigenetic regulation extends beyond developmental biology into other biomedical fields, such as immune responses and toxicity. As such, in this study, we focus on the adoption and adaptation of methods to study epigenetic regulation of the primary, human monocyte-derived macrophages (MDM). This is a cell type that plays an integral role in the innate immunity system and also promotes crosstalk between innate immune cells and adaptive immune cells [5].

Macrophages originate from pro-monocytes and have high phenotypic plasticity to accommodate their need to rapidly respond to changes in the surrounding environment. For example, in response to inflammation, Ly-C6^{+} monocytes differentiate into inflammatory

Abbreviations: Center for Biomolecular Science and Engineering, CBSE; chemokine ligand 2, CCL-2; Chromatin Immunoprecipitation, ChIP; Chronic myelogenous leukemia, CML; Encyclopedia of DNA Coding Elements, ENCODE; human umbilical vein endothelial cells, HUVEC; immunoprecipitation, IP; Integrated DNA Technologies, IDT; interferon-gamma, IFN-γ; Interleukin, IL; lysis buffer, LBuf; Macrophage Serum Free Media, MSFM; macrophage colony stimulating factor, MCSF; monocyte-derived macrophages, MDM; nucleotide GC, Guanine-Cytosine; phenylmethanesulfonyl fluoride, PMSF; phosphate buffered saline, PBS; protease inhibitor cocktail, PIC; Qiagen Buffer EB, EB; Qiagen Buffer PB, PB; shearing buffer, SBuf; Sodium Dodecyl Sulfate, SDS; tumor necrosis factor-alpha, TNFα; base pairs, bp.

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macrophages that can further polarize into specific phenotypes, such as M1 or M2 macrophages [6,7]. A primary function of the macrophage is to survey the organism and respond quickly to pro-inflammatory signals, infection, or toxic insults to maintain homeostasis. Macrophages respond to such stimuli by secreting cytokines and producing reactive oxygen species, quinolinic acid, glutamate, arachidonic acid, and its metabolites, etc. contributing to the overall response of the innate immune system [8,9], all of which are regulated by epigenetic mechanisms. Therefore, abnormalities in epigenetic regulatory processes can be detrimental for their functions. Despite decades of research, we are still far from an in-depth understanding of the complex regulatory mechanisms by which pathogens and toxins contribute to a wide range of impairments of the innate immune system.

A more comprehensive understanding of the mechanisms controlling epigenetic regulation can be obtained by integrating multiple technologies, such as mass spectrometric analysis of histone PTM, profiling transcription regulatory expression and activity, investigating DNA methylation, and high-throughput sequencing of immunoprecipitated DNA fragments. Each of these technologies can provide unique information; however, the challenges that must be overcome can be daunting and not always obvious. In our research, we utilized both mass spectrometry-based quantification of histone PTMs as well as ChIP. We determined that the limited yield of DNA that can be efficiently isolated from primary cells is a severe impediment, but not impossible. Cell lines can provide abundant amounts of biological material as compared to primary cells obtained from human subjects.

Investigations into epigenetic regulation of macrophages have primarily used transformed macrophage-like cell lines, including THP-1, U937, and HL-60. Although beneficial, the findings of these studies have limited translatability to primary cells. While the UCSC Genome Browser provides the histone modifications for cells lines included in the Encyclopedia of DNA Coding Elements (ENCODE), it is also a great guideline for experimental design to determine the antibodies against PTMs for immunoprecipitation (IP). Nevertheless, we have identified differences between cell lines and primary MDM that need to be further characterized in future studies.

MDM are an excellent experimental model when looking at specific genes or globally to investigate specific pathways of regulation or to provide a much broader picture of intracellular mechanisms [10]. MDM from human donors reflect the *in vivo* situation more closely than transformed cell lines. Another important aspect of using primary cells from human donors is the ability to observe donor-to-donor variation and translate this information to broader conclusions about the function of specific mechanisms in diverse populations.

Despite numerous articles and book chapters describing methods used in biomedical research, ChIP techniques require optimization because of the experimental models and approaches, as well as the ever-increasing availability of new reagents and ready to use kits. Additionally, since most research employs cell lines, development of analytical methods to investigate primary cells is lagging. Therefore, we present detailed methodology to obtain DNA from MDM using ChIP techniques. Although this technique seems relatively straightforward and despite continuous progress [11], the limited amount of immunoprecipitated DNA is the biggest constraint. In this study, we describe our approach for modifying the protocol from Active Motif, one of the kits currently available. Because our study is focused on ChIP-PCR, only a select number of genes were investigated.

2. Methodology

ChIP is a multi-step procedure that requires optimization for each cell type or tissue. In this study, the ChIP-IT Express Enzymatic Kit (Active Motif) was used as the basis for development of

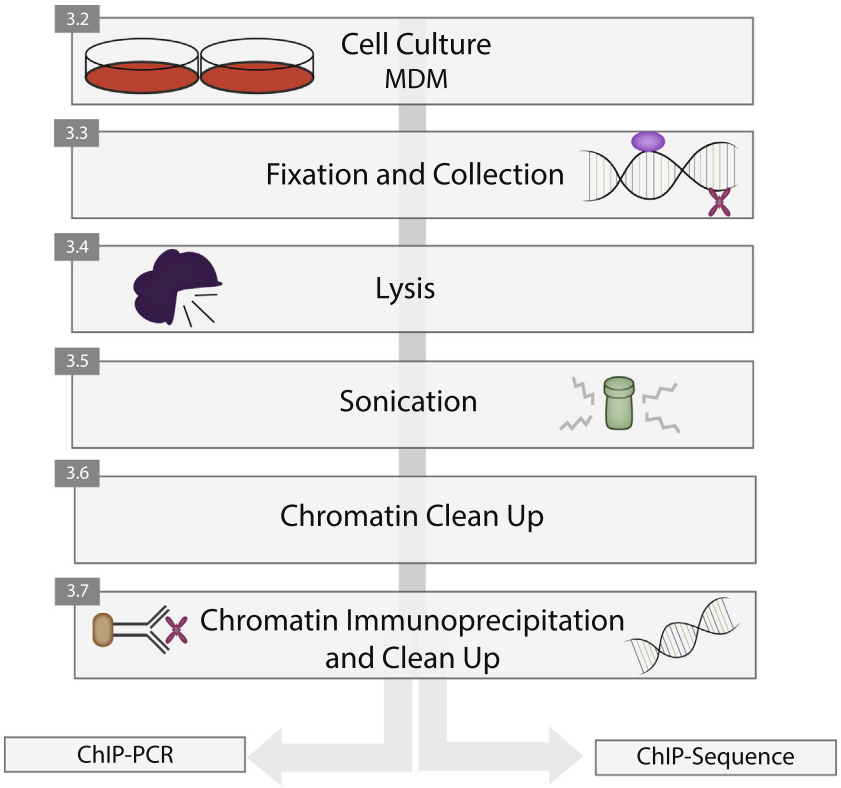

Fig. 1. Flow chart of methodology. General steps for ChIP that can be adapted for multiple experimental models. Numbers on the left side of the figure, correspond to the detailed description provided in Section 3.

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