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RESEARCH ARTICLE

CHROMATIN IMMUNOPRECIPITATION ASSAY AND ITS VARIANTS FOR THE ANALYSIS OF DNA-PROTEIN INTERACTIONS

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ABSTRACT

The chromatin immunoprecipitation (ChIP) assay is a powerful method for probing protein-DNA interactions within the natural chromatin context of the cell is versatile enough for adaptation for a variety of purposes. This assay can be used to identify multiple proteins associated with a specific region of the genome, or the opposite, to identify the many regions of the genome associated with a particular protein. It has been exploited for mapping the localization of post-translationally modified histones and histone variants in the genome, and for mapping DNA target sites for transcription factors and other chromosome-associated proteins. It has emerged as technique of choice to investigate protein–DNA interactions inside the cell. The amalgamation of ChIP with high-throughput technologies like microarray and high-throughput sequencing has enabled the identification of protein-DNA interactions on a global scale. Several novel biological insights have emerged from protein-DNA interactions that have been identified using ChIP. This review highlights the principle and methodology of ChIP assay along with variants on the theme of the ChIP assay, and examples of their applications.

INTRODUCTION

In the post-genome era, attention has focused on the expression of genome sequences and how they are regulated. Gene expression is regulated at many levels. Regulation of gene expression i.e. how genes are turned on and off, or up and down, provides the molecular basis that drives normal cellular function, differentiation and metabolism, mechanisms of disease, and response of cells to stimuli such as drug treatments. Gene regulation occurs primarily at the level of gene transcription, whereby the genetic information encoded by the DNA is copied into an RNA transcript that is subsequently translated into protein. Gene transcription is controlled by the interactions between transcription factor proteins and their binding sites. Transcription factors are proteins that bind to the promoter elements upstream of genes and either facilitate or inhibit transcription. These DNA-protein interactions are crucial for vital cellular functions including gene transcription, DNA replication and recombination, repair, segregation, chromosomal stability, cell cycle progression, and epigenetic silencing. Detection of the specific DNA binding sites of transcription factors in the genome is therefore fundamental to understanding the complex and varied regulatory pathways that direct gene expression in the cell and govern all biological processes, including disease development. Chromatin is composed of DNA, proteins and RNA (Bernstein and Allis, 2005; Schubeler and Elgin, 2005; Felsenfeld *et al*., 2003). Chromatin structure is dynamic, responds to extracellular signals, and controls gene expression. Chromatin immunoprecipitation (ChIP) assays are a powerful technique that allows detection of protein-DNA interactions in vivo (Orlando, 2000; Das *et al*., 2004). ChIP is a very versatile technique that enables investigators to identify regions of the genome associated with specific proteins within the context of the cell. The ChIP assay has been used to study both histones and non-histone proteins, such as transcription factors, within the context of the cell (Wells, 2002; Weinmann *et al.,* 2002, 2004). ChIP assay has become a very popular technique for fine-mapping the location of modified histones, transcription factors, and nonhistone chromosomal proteins. ChIP assays have proven to be a powerful means to investigate a host of DNA-dependent processes (Kuo and Allis, 1999; Impey *et al*., 2004). Along with other techniques (Dundr *et al*., 2002; Cheutin *et al*., 2003), ChIP reveals an extraordinarily rich and dynamic chromatin environment. Moreover, it is an in situ technique that offers a more physiological representation of nuclear events involved in the processing of DNA. Initially,

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the ChIP assay was used to study the association of hyperacetylated histones with specific DNA sequences to further understand the role of histone acetylation in transcription (Chen *et al*.1999). However, more recent studies have used the ChIP protocol to characterize DNA sequences associated with specific transcription factors (Shang *et al*., 2000; Sharma and Fondell, 2002).

DNA-protein interactions

DNA Protein interactions play very vital roles in any living cell. There are several types of proteins found in a cell. But only those proteins interact with DNA, which have the DNA binding domains. Each DNA binding domain has at least one motif, which is a conserved amino acid sequence of this protein, which can potentially recognize a double stranded or a single stranded DNA. These DNA binding domains possess an affinity to bind to either double stranded or single stranded DNA. Proteins can interact with DNA either specifically or non-specifically. In the case of non-specific interactions like Histone (protein) - DNA interactions, the sequence of nucleotides has no effect as far as the binding interactions are concerned. Histone-DNA interactions occur between functional groups on the protein and the sugar-phosphate backbone of DNA. Specific DNA - protein interactions, however, depend upon the sequence of bases in the DNA and on the orientation of the bases that can vary with DNA super coiling. Specific protein-DNA interaction is governed by sequence recognition (base readout) through direct contacts between protein's amino acid side chains and DNA bases (Koop *et al*., 1985, Rohs *et al*., 2010), as well as the spatial geometry of the two biomolecules (shape readout). Recognition of specific DNA sequences by specific DNA-binding proteins often leads to the formation of large protein complexes responsible for regulation of gene expression. These DNA-protein interactions are strong and are mediated by hydrogen bonding, Ionic interactions and other forces like van der Waals and hydrophobic (Luscombe *et al*., 2001, Rutledge *et al*., 2010). Sequence specific DNA protein interaction is found to occur in case of transcription. The transcription factors are a special kind of DNA binding proteins that recognize only a specific DNA sequence. The sequence non-specific DNA protein interaction occurs in replication where DNA double strand is melted by helicase enzyme and a replication fork is ready. A special kind of protein called single strand binding protein or SSB binds to the melted single strand of DNA and stabilizes the system by preventing them to be re-natured. There are several motifs present, which are involved in DNA binding, for example, helix-turn-helix, leucine zipper, zinc finger, helix-loop-helix etc. Understanding how proteins interact with DNA, determining what proteins are present in these protein-DNA complexes and identifying the nucleic acid sequence (and possible structure) required to assemble these complexes are vital to understanding the role these complexes play in regulating cellular processes. Analysis of DNA-protein interactions provides researchers with insight into regulation events that are critical for many biological processes and disease states. The physiological role of DNA-binding proteins is determined by the affinity and specificity of the DNA-protein interaction. These properties depend upon the precise interactions between amino acids in the DNA-binding protein and nucleotides in the DNA-binding site. Protein-protein interactions are required for efficient DNA-protein interactions as well. DNA-binding sites. Gene expression is often regulated by proteins that activate or repress transcription by binding to short, specific DNA sequences. Such cis-acting sites are usually located close to the promoter (RNA polymerase binding site) for the regulated gene. Sometimes DNA-binding proteins bring together two distant DNA-binding sites and loop out the intervening DNA. When positioned close enough to the promoter, almost any specific DNA binding protein may act as a repressor. Repression may be caused by competition with RNA polymerase binding to the promoter, blocking the isomerization of RNA polymerase required for initiation of transcription, bending the DNA, or inhibiting the elongation of RNA polymerase after transcription has initiated. Repression due to binding to a single site is usually strongest when the binding site is close to the promoter (usually within about 20 bp).

ChIP assay

ChIP assays are a powerful technique that allows detection of protein-DNA interactions in vivo. ChIP is a very versatile technique that enables investigators to identify regions of the genome associated with specific proteins within the context of the cell. ChIP reveals an extraordinarily rich and dynamic chromatin environment. Moreover, it is an in situ technique that offers a more physiological representation of nuclear events involved in the processing of DNA. It has been used to study both histones and non-histone proteins, such as transcription factors, within the context of the cell. Initially, the ChIP assay was used to study the association of hyper acetylated histones with specific DNA sequences to further understand the role of histone acetylation in transcription. However, more recent studies have used the ChIP protocol to characterize DNA sequences associated with specific transcription factors. There are two kinds of ChIP procedures including N-ChIP using 'native chromatin' input and X-ChIP using 'cross linked chromatin' input. N-ChIP is used to study proteins with strong and direct association with DNA, for example histone proteins. For cofactors which indirectly or transiently associate with DNA, a crosslink step to fix protein-DNA interactions is crucial for pulling down DNA elements with the protein of interest. Comparing to N-ChIP, X-ChIP is more frequently used for studying any protein-DNA interactions. Basic steps in ChIp procedure are as follows:

1. Formaldehyde-mediated DNA-protein crosslinking

The cells are incubated with the cross linker formaldehyde to prevent the separation of DNA-associated proteins from their target DNA sequence in subsequent steps. In a different set of approaches, DNA-bound proteins are first cross linked to DNA by treatments with UV light (Gilmour and Lis, 1984), dimethyl sulfate (Karpov *et al*., 1984), or a variety of other agents (Welsh and Cantor, 1984). Some of the above methods, in particular DNA modification by dimethyl sulfate (Church and Gilbert, 1984; Ephrussi *et al*.,1985) or UV light (Becker and Wang, 1984), have been used recently to footprint DNA-protein interactions in vivo. Since both treatments damage DNA extensively upon prolonged exposure, the use of the corresponding techniques is restricted. On the other hand, formaldehyde produces DNA-protein cross links both in vitro and in vivo (Ilyin and Georgiev, 1969) and at the same time

displays virtually no reactivity toward free double-stranded DNA (McGhee and Hippel, 1975; Trifonov *et al*., 1967). Since formaldehyde produces DNA-protein (Brutlag *et al*., 1969; Varshavsky and Ilyin,1974), RNA-protein (Moller, 1977), and protein-protein (Jackson,1978) crosslinks, its addition to living cells results within minutes in formation of crosslinked networks of biopolymers, thus preventing any large-scale redistribution of cellular components upon prolonged fixation. Formaldehyde-induced crosslinks, in particular DNA-protein crosslinks, can be reversed under relatively mild conditions (Jackson and Chalkley, 1981). Limit-digestion of formaldehyde-fixed eukaryotic chromosomes with relatively nonspecific proteinases, such as pronase or proteinase K, does not yield a completely peptide-free DNA (Doenecke, 1978). Moreover, DNA fragments containing Pronase resistant peptide-DNA adducts have reduced electrophoretic mobility. So they can be treated with specific antibody to remove the proteins.

2. Sonication

To fragment the DNA, the cells can be sonicated and then lysate is centrifuged to remove insoluble cellular debris. A whole-cell extract is prepared, and the cross-linked chromatin is sheared by sonication to reduce average DNA fragment size to 100-500 bp.

3. Immunoprecipitation

The resulting material is immunoprecipitated with an antibody against a desired protein, modi ed (e.g., acetylated, phosphorylated, methylated) peptide, or epitope (in situations where the protein of interest is epitope-tagged). DNA sequences that directly or indirectly cross-link with a given protein (or modi ed variant) are selectively enriched in the immunoprecipitated sample. The major limitation of the ChIP assay is the quality of the antibody, as some antibodies work poorly or not at all in ChIP. This limitation could be circumvented by using tagged protein constructs (hemagglutinin (HA), Flag or other) expressed from plasmids (Denisenko *et al*., 2002, Waugh, 2005).

4. Crosslinking Reversal and DNA Clean-up

Reversal of the formaldehyde cross-linking by heating permits the recovery and quantitative analysis of the immunoprecipitated DNA. The protein complement of the immune complexes can be digested away and the DNA can be isolated by ethanol precipitation.

5. DNA Quantitation

Standard PCR methods are often employed to identify the DNA sequences or regions of the genome associated with a particular protein or histone modification (1,2). PCR is used to measure the relative abundance of a particular DNA sequence enriched by a protein-specific immunoprecipitation versus an immunoprecipitation with a non-specific antibody control. PCR products are run on an agarose or acrylamide gel to facilitate quantification, and the level of enrichment of the DNA sequence is determined relative to the total amount of input DNA (percent of input). Appropriate numbers of PCR cycles are used to ensure that the specific and mock-precipitated DNAs are within the linear range of amplification. Enrichment of a specific DNA locus in immunoprecipitated material, as compared to mock precipitation, indicates that the factor of interest interacts with this genomic region, and the intensity of the PCR signal is proportional to occupancy at the binding site. Alternatively, an interaction of the factor with multiple genomic locations can be detected simultaneously by applying the isolated DNA to a DNA microarray (Liu *et al*., 2005, Pokholok *et al*., 2005).

Optimization of ChIP assay

The chief obstacles to successful ChIP assays are low sensitivity and reproducibility of the obtained results. Multiple factors influence the sensitivity and reproducibility of ChIP experiments during sample preparation. Major ones include variability in the formaldehyde crosslinking, chromatin shearing efficiency, the quality and specificity of the antibodies employed, and Protein-A/G beads. Only stringent control overall experimental conditions will reduce variability and assure the reproducibility of results, as well as the quality and sensitivity of the data (Haring *et al*., 2007). To optimize ChIP experiments, four critical steps have to be optimized: 1) sample fixation and preparation, 2) chromatin shearing, 3) the amount of input material and 4) the amount of antibody used. Each one of these is described as follows:

1. Sample fixation and preparation:

It is important to capture the DNA–protein interactions within a cellular context using a chemical cross-linking agent, such as formaldehyde. The aim of cross-linking is to fix the antigen of interest to its chromatin binding site. Histones themselves generally do not need to be cross-linked, as they are already tightly associated with the DNA. Other DNA binding proteins that have a weaker affinity for DNA or histones may need to be cross-linked. This holds them in place and avoids protein dissociating from the chromatin binding site. The crosslinking step should be optimized, since too little crosslinking will not sufficiently preserve the chromatin structure, and too much crosslinking will hamper the ChIP procedure (Das *et al*., 2004). Excessive cross-linking can lead to several issues including reduction in antigen availability and sonication efficiency. For example, epitopes may be masked or changed, affecting the ability of the antibody to bind the antigen, which in turn causes a reduction in the material in sample. The relative sensitivity of the antigen epitopes to formaldehyde also should be taken into consideration. Essentially, when the crosslinking is optimal for ChIP, decrosslinking is required to efficiently isolate DNA from the nuclei by phenol-chloroform extraction (Haring *et al*., 2007). The chromatin is over-crosslinked when it is impossible to recover a substantial amount of DNA from the nuclei by decrosslinking. A time-course experiment to optimize cross-linking conditions may be performed.

2. Fragmentation/ Shearing of chromatin

Sonication and Micrococcal nuclease (MNase) digestion are two commonly used methods for shearing of chromatin Both methods can show preferential fragmentation of certain chromosomal regions (Reneker *et al*., 1991). When using formaldehyde crosslinking, sonication is the preferred method,

as crosslinking restricts the access of MNase to chromatin (Bellard *et al*.,1989). Ideally, the bulk of the chromatin is sonicated to a length between 250 and 750 bp (Haring *et al*., 2007). For efficient fragmentation, sonication at low power, in combination with several pulses, is preferred over sonication at high power and few pulses, but conditions vary with the sonication device used. It is important to keep the chromatin sample cooled on ice during sonication, as heat released by the sonication probe can reverse the crosslinks. The presence of detergent (SDS) in the sonication buffer improves sonication efficiency considerably, but can induce foaming during sonication. Foam makes the chromatin sample unsuitable for ChIP, probably as a result of the surface tension imposed by the foam, which can disrupt protein conformation (Clarkson *et al*., 1999). Foaming can be prevented by decreasing the sonication power.

3. Amount of input material

As DNA-binding proteins are expressed over a wide range in various tissues, the amount of protein of interest available for IP varies widely. For example, for highly expressed proteins, the amount of total protein required for immunoprecipitation will be significantly lower than that of a low abundance protein. Therefore, ChIP must be optimized for the amount of input material (total protein) required for IP using each antibody against the protein of interest. In addition, before beginning to do any ChIP experiment, it is important to determine the expression level or pattern of the protein of interest using western blotting or in situ. Also in some cases less amount of protein may interact with DNA, although the protein may be widely expressing. In such cases, more ChIP-ed DNA may be required for the detection.

4. Amount of antibody used

The cross-linked DNA–protein complex is immunoprecipitated using a specific antibody against the protein of interest. The quality and specificity of the antibody contributes immensely to the outcome of the ChIP procedure Antibodies can be available as polyclonal or monoclonal preparations. Monoclonal antibodies have a high specificity compared to polyclonal sera, but the polyclonal sera may recognize several epitopes of the target, increasing signal levels of low-abundance templates (Haring *et al*., 2007). The successful use of a specific antibody in experiments other than ChIP (i.e. Western blotting, immunocytochemistry) does not automatically mean the antibody is suitable for ChIP; that has to be tested. Different antibody preparations have distinct properties, which can affect the ChIP results. The affinity for epitopes differs between antibodies, affecting the resulting signal levels. For example, antibodies can differ in their sensitivity towards crosslinks or adjacent modifications (Hanlon *et al*., 2004). In addition, the relation between the availability of epitopes and antibody binding may not be linear. Some antibodies are sensitive to inhibitory factors present in the input chromatin sample, resulting in a decrease in binding efficiency of the antibody when increasing the amount of input. To ensure the comparability of results obtained with different input samples, it is recommend determining the optimal antibody: chromatin ratio by titration of the amount of input chromatin, and the use of similar amounts of input chromatin when ChIP results are to be compared (Haring *et al*., 2007).

Variants of chip

Carrier ChIP (CChIP)

A major drawback of ChIP has for a long time been the requirement for large cell numbers. Conventional ChIP requires a large number of cells simply because: 1) the recovery rate of cross linked chromatin in ChIP varies from one to ten percent of the total cellular DNA content in the starting material; and 2) extensive wash steps during immunoprecipitation result in loss of specific interactions and therefore reduced signal to noise ratio (Hao *et al*., 2008). The underlying principle behind carrier ChIP, or CChIP, is that the immunoprecipitation of a small amount of chromatin prepared from few mammalian cells (100–1,000) is facilitated by the addition of carrier chromatin from Drosophila—or any species sufficiently evolutionarily distant from the species investigated (O'Neill *et al*., 2006). Application of a carrier in ChIP has been seen in a sequential ChIP method (Geisberg and Struhl, 2004) to make the second immunoprecipitation similar to the first immunoprecipitation and minimize background signal. With CChIP, O'Neill *et al* were able to immunoprecipitate modified histone bound chromatin from ~200 cells (O'Neill *et al*., 2006).

Quick and quantitative (Q^2) ChIP

A quick and quantitative (Q^2) ChIP protocol is suitable for up to 1,000 histone ChIPs or up to 100 transcription factor ChIPs from as few as 100,000 cells (Dahl and Collas, 2007). Increased specificity is obtained by moving the IP reaction to a fresh tube prior to reversing the protein-chromatin cross linking, leaving behind non-specific plastic bound chromatin. Q^2 ChIP involves a chromatin preparation from a larger number of cells than CChIP, but includes chromatin dilution and aliquoting steps which enable storage of many identical chromatin samples from a single preparation. Because Q^2 ChIP involves a cross-linking step, chromatin samples are also suitable for immunoprecipitation of transcription factors or other non-histone DNA-bound proteins. DNA protein cross-linking in suspension in the presence of a histone deacetylase inhibitor, elimination of essentially all non-specific background chromatin through a tube-shift after washes of the ChIP material, and combination of cross-linking reversal, protein digestion and DNA elution into a single 2-h step, shorten the procedure and enhance ChIP efficiency. Suitability of Q^2 ChIP to small amounts of chromatin has been attributed to the reduction of the number of steps in the procedure, increased ratio of antibody-to-target epitope, resulting in an enhanced signal-to-noise ratio. It has been used to illustrate changes in histone H3K4, K9 and K27 acetylation and methylation associated with differentiation of embryonal carcinoma cells on developmentally regulated promoters (Dahl and Collas, 2007).

Fast ChIP

Fast ChIP assay has introduced two modifications which dramatically shorten the procedure (Nelson *et al*., 2006). First, incubation of antibodies with chromatin in an ultrasonic bath substantially increases the rate of antibody-protein binding,

shortening incubation time to 15 min. Second, in a traditional ChIP assay, elution of the ChIP complex, reversal of cross-linking and proteinase K digestion of bound proteins require 9 h, and DNA isolation by phenol:chloroform isoamylalcohol extraction and ethanol precipitation takes almost 1 day. Instead, Fast ChIP uses a cation-chelating resin (Chelex 100)-based DNA isolation which reduces the total time for preparation of PCR-ready templates to 1 h. Under Fast ChIP, after cross-linking with formaldehyde, the cells are lysed, the fraction containing nuclear pellets is isolated and chromatin is sheared. Chromatin samples are incubated with the antibodies in an ultrasonic bath (15 min, 4 °C); after centrifugation, the precleared samples are mixed with protein A beads (45 min, 4 °C). After several washes, Chelex 100 suspension is added to the beads; the suspension is boiled (10 min), the tubes are allowed to cool and then proteinase K is added. The mix is incubated in a shaker (30 min, 55 °C at 1,400 rpm) and the beads are then boiled again (10 min). After centrifugation, the PCR-ready DNA is collected. Samples are stored at 20 °C and can be thawed and frozen repeatedly.

Fast carrier ChIP (Fast CChIP)

Fast CChIP method was adapted from a Fast ChIP protocol (Nelson *et al*., 2006) and a CChIP (O'Neill, 2006). The Fast CChIP procedure combines the Fast ChIP method with the CChIP method to benefit from their respective advantages, i.e., shortened time requirement of Fast ChIP and high sensitivity to detect protein and DNA interaction in a small number of cells of CChIP (Hao *et al*., 2008). It follows the basic procedure of Fast ChIP with the modification of using yeast Saccharomyces cerevisiae strain BJ5464 cells as a source of carrier chromatin. Hao *et al*. (2008) successfully demonstrated its application in analyzing against phosphorylated cyclic-AMP response element binding (CREB) transcription factor binding activity with c-fos gene promoter in an individual brain nucleus tractus solitarius (NTS) confirming CREB's role in mediating hypertension-induced c-fos expression. Fast CChIP is capable of determining changes in site-specific transcription factor binding from a small number of cells (~500) in a background of non-responding heterogeneous cell types

ChIP followed by microarray hybridization (ChIP-on-chip)

ChIp-on-chip is a high throughput identification and analysis of DNA fragments that are bound by specific proteins such as histones, transcriptional factors across the genome. Traditionally DNA-Protein interactions are studied on a gene-by-gene basis using ChIP followed by multiple PCR reactions. More recently ChIP has been further developed for compatibility with DNA microarray technologies. Combining ChIP with glass slide DNA microarrays has created the approach of "ChIP-on-chip" (also known as genome-wide location analysis), which enables the parallel mapping at the whole genome level. First described in 2000 (Ren *et al*., 2000), ChIP-chip couples chromatin IP to microarray analysis allowing genome-wide analysis of protein or modifications of interest distribution (Buck and Lieb, 2004). Similar to traditional ChIP method, a DNA binding protein is covalently attached to its target DNA using formaldehyde cross-linking. Then using an antibody specific for the protein in question, the protein-DNA adduct is immunopreciptated from nuclear extracts after shearing of the chromatin. After immunoprecipitation, the enriched DNA sample is commonly, but not always, amplified using a variety of methods. The last step is to fluorescently label the enriched DNA and the reference DNA. In mammalian ChIP, the reference is usually composed of the input sheared chromatin or mock immunoprecipitations using normal, nonspecific sera from mice or rabbits. Once the DNA samples have been amplified and labeled, the control and enriched samples are hybridized to various microarrays (Hudson and Snyder, 2006). The two most commonly used microarray formats used for the analysis of ChIP-chip samples are PCR product arrays and oligonucleotide arrays. In PCR arrays, each of the DNA features on the array are amplified using PCR and these products are mechanically spotted onto glass slides. Due to the limited packing density that can be achieved, this form of microarray is limited to 20,000 to 30,000 features. Subsequently, oligonucleotide arrays have been developed from controlled and combinatorial nucleotide sequences which facilitate screening of a much larger representation of a genome and, when arrayed in high-density on 'tiling' arrays, provide a higher resolution of e.g. histone modification patterns. The DNA from a single ChIP experiment can be examined at an unprecedented number of loci with microarrays, leading to high-confidence mapping of the chromatin landscape at a genome-wide level from a small biological sample amount. The loci covered by a ChIP-on-chip experiment depend on the array(s) being used; these loci could be from selected genomic regions, all promoters, or even the whole genome. ChIP-chip has been instrumental in understanding the structure and function of the human genome (Kim *et al*., 2005). Initial ChIP-chip studies were performed on Saccharomyces cerevisiae regulatory TFs (Iyer *et al*., 2001, Lieb *et al*., 2001), and replication origin recognition proteins (Wyrick *et al*., 2001), using microarrays spotted with PCR amplicons covering essentially all intergenic regions in yeast. Studies of yeast TFs in multiple cellular states (Simon *et al*., 2001) or environmental conditions (Harbison *et al*., 2004) highlighted that binding of TFs can be condition-dependent. Recent ChIP-chip studies have focused on two main areas: (1) histone modifications, histone-modifying proteins, and chromatin remodeling; and (2) combinatorial and condition-specific regulation by transcription factors (Bulyk *et al*., 2006). ChIP-on-chip has been utilized to map c-Myc binding sites in the genome (Dang *et al*., 2006, Lee *et al*., 2006), elaborate Oct4, Nanog, and Sox2 transcriptional networks in ES cells (Boyer *et al.,* 2005), identify polycomb target genes (Boyer *et al.,* 2006, Lee *et al*., 2006) or provide a histone modification landscape in T cells.

ChIP-Sequencing (ChIP-seq)

ChIP-seq combines ChIP with next-generation sequencing (Barski *et al*., 2007, Johnson 2007). ChIP-seq protocols have been adapted from ChIP-chip methods: proteins are cross-linked to their bound DNA by formaldehyde treatment, cells are homogenized, and chromatin is sheared and immunoprecipitated with antibody-bound magnetic beads. The mmunoprecipitated DNA is then used as the input for a next-generation sequencing library prep protocol, where it is sequenced and analysed for DNA binding sites. ChIP-seq first cross-links bound proteins to chromatin, fragments the

chromatin, captures the DNA fragments bound to one protein using an antibody specific to it, and sequences the ends of the captured fragments using next-generation sequencing (NGS). Computational mapping of the sequenced DNA identifies the genomic locations of bound DNA-binding enzymes, modified histones, chaperones, nucleosomes, and transcription factors, thereby revealing the role of these protein-DNA interactions in gene expression and other cellular processes. The use of NGS provides relatively high resolution, low noise, and high genomic coverage compared with ChIP-chip assays. ChIP-seq is now the most widely used procedure for genome-wide assays of protein-DNA interaction (Furey *et al*., 2012), and its use in mapping histone modifications has been seminal in epigenetics research (Ku *et al*., 2011). ChIP-seq has been used to generate 'chromatin-state maps' for ES and lineage-committed cells (Mikkelsen *et al*., 2007). Using the Illumina/ Solexa 1G platform, binding sites for the transcription factor STAT1 in HeLa cells (Robertson *et al*., 2007) and a profiling of histone methylation, histone variant H2A.Z binding, RNAPII targeting, and CTCF binding throughout the genome (Barski *et al*., 2007) have also been reported. All results claim robust overlap between ChIP-seq, ChIP-on-chip, and ChIPPCR data. Interestingly, the ChIP-seq data illustrate the potential for using ChIP for genome-wide annotation of novel promoters and primary transcripts, active transposable elements, imprinting control regions, and allele-specific transcription (Mikkelsen *et al*., 2007). As outlined above, ChIP-seq can be applied downstream of a sequential ChIP approach to identify genomic regions co-enriched in histone variants (Jin *et al.,* 2009).

Conclusion

ChIP aids in identification of the precise genomic DNA binding sites of specific transcription factors and co-regulatory factors with a high level of accuracy. Through direct measurement of the interactions between DNA and transcription factors that occur inside the nucleus of living cells and tissues, highly specific and quantitative information valuable for identifying and studying unique genetic regulatory events, genetic pathways, and novel biomarkers will be generated. Understanding gene transcriptional regulation offers opportunity to define molecular pathways that are associated with spatial and temporal pattern of biologic responses. ChIP-on-chip and ChIp-seq, the strategies of combining ChIP with microarray/ NGS techniques have been used to study global crosstalk between DNA and protein on a genome-wide scale. ChIP is extremely versatile; it may be used to compare the enrichment of a protein/protein modification at different loci, to map a protein/protein modification across a locus of interest or to quantify a protein/protein modification at an inducible gene over a time course. To observe the protein of interest and its interactions with the genome in its natural state ChIP is a great choice.

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