

Chip Assay for "Transcriptomics" - by Malvika Bhatt

MJS BioLynx Technical Support Specialist

Identification of the Pre-initiation Complex

With recent completion of the Human Genome project, more and more researchers are trying to identify the true coding regions of the Genome. Many computer based techniques have been developed to find the true coding sequences which have been estimated to be only 2% of the genome. The coding regions would also be the ones which would have the promoter sequences that could bind the transcription factors and initiate transcription. One could also identify the promoters of different cell lines under different conditions to see where the transcription factors/ cell's own promoter recognition proteins are binding and this could tell us which promoters are actively transcribing. The Chromatin Immunoprecipitation technique could be adapted to target the Pre-initiation complex that binds the active promoters, by using antibodies against both RNA Pol and a TFIID transcription factor subunit (TAF1) to pull down cross-linked DNA fragments. This way one could pull down all promoters that bind both the factors. These fragments could be then sequenced and profiled by a database scan to find the open reading frames of the genome. This methodology could be used to identify promoters for novel genes. Improvements to the technique could be made by using antibodies that recognize proteins which bind to different promoter recognition sequences. This can help to pull down the pre-initiation complex more accurately.

The ChIP Method

In the ChIP method, intact cells are fixed using formaldehyde, which cross-links and therefore preserves protein/DNA interactions. DNA is then sheared into small uniform fragments and the DNA/protein complexes are immunoprecipitated using an antibody directed against the DNA-binding protein of interest. Following immunoprecipitation, cross-linking is reversed, proteins are removed by Proteinase K treatment and the DNA is rapidly cleaned up using the included DNA purification columns. The DNA is then screened to determine which genes were bound by the protein of interest. The versatility of ChIP means that screening can be done using generalized hybridization or a more targeted PCR-based approach.

Classically, ChIP has been performed with antibodies directed against abundant chromatin components, such as acetylated histones. While this yields information about transcriptional activity of promoters, it does not reveal which transcription factor is bound to the promoter(s) of interest. In contrast, ChIP using transcription factor-specific antibodies enables direct monitoring of transcription factor/DNA interactions, but it is technically more challenging and requires the preparation of several complicated buffers, inhibitor cocktails and blocking reagents. In addition, result validation is difficult without antibodies, controls and a protocol proven to work in ChIP.

Alternative to Sonication

For successful ChIP, the chromatin must first be sheared to 200-1000 bp fragments. This has traditionally been performed by subjecting the isolated chromatin to different pulses of sonication. Although sonication can be an effective method for shearing DNA, it can also be time consuming and difficult to optimize due to complications arising from emulsification and overheating. And, because the quality of your sheared sample depends greatly upon the quality of your sonicator, it may be necessary to purchase an expensive, "high-end" sonicator to get reproducible shearing. To overcome these problems associated with the sonication, there's also available in the market a more robust and user-friendly method to shear chromatin for ChIP. **The Enzymatic Shearing Kit** uses a proprietary Enzymatic Shearing Cocktail that quickly and easily shears DNA into 200-1000 bp fragments. Because enzymatic shearing is solely time and temperature dependent, all of the problems associated with sonication are eliminated and ChIP results are improved. For more information on these techniques, please contact MJS BioLynx at 1-888-593-5969 or email tech@biolynx.ca.

Reference:

1. [Kim TH, Barrera LO, Qu C, Van Calcar S, Trinklein ND, Cooper SJ, Luna RM, Glass CK, Rosenfeld MG, Myers RM, Ren B.](#) 2005. Direct isolation and identification of promoters in the human genome. *Genome Res.* Jun;15(6):830-9.
2. Active Motif website www.activemotif.com