Assessment of sera for chromatin-immunoprecipitation

Juliette Nguyen¹, Daisuke Yamada¹, David C. Schultz², and Pierre-Antoine Defossez¹

¹Centre National de la Recherche Scientifique, Paris, France and ²The Wistar Institute, Philadelphia, PA 19104, USA

BioTechniques 44:66-68 (January 2008)
doi 10.2144/000112681

Chromatin-immunoprecipitation (ChIP) is a powerful technique for mapping the protein-DNA interactions that occur in living cells. The critical technical determinant for successful ChIP is the availability of an appropriate, "ChIP-grade" serum. Here we present a technique designed to assess whether sera are suitable for ChIP, and to quantify their efficiency relative to a positive internal reference. This approach is useful as a first step toward ChIP-on-chip or ChIP-sequencing, especially in the case of recently identified proteins for which no binding sites are yet known.

Chromatin-immunoprecipitation (ChIP) is a technique that allows the mapping of sites at which DNA binding proteins are present in vivo. The method, introduced nearly two decades ago (1), is especially powerful when combined with current genome-wide assays, such as microarray hybridization (ChIP-on-chip) (2), or high-throughput parallel sequencing (ChIP-sequence) (3). ChIP can be applied to any protein that associates with DNA, including histone proteins and their variants, chromatin remodeling factors, and transcription factors.

The critical technical parameter underlying the success of ChIP is the existence of a suitable serum or monoclonal antibody. There are no hard rules for raising "ChIP-grade" reagents, and antibodies that perform well in immunofluorescence, Western blotting, or even immunoprecipitation, are not necessarily suitable for ChIP, as the crosslinking treatment can mask epitopes and the presence of detergents in the buffers can disturb specific interactions. In fact, the only way to assess whether a given serum is "ChIP-grade" is experimentation, using as a positive control a site that has been previously shown to recruit the protein. This requirement presents a challenge in instances when an uncharacterized protein is being tested, as no positive control is then available.

As an example, our laboratory has recently cloned two uncharacterized transcription factors from the human genome: ZBTB4 and ZBTB38 (4). We now wish to identify their in vivo binding sites by ChIP-on-chip. To this end, we have raised different sera against various epitopes of ZBTB4, but were unable to test their usefulness in ChIP for lack of a known target.

To circumvent this problem, we have used a method that can be adapted to any other protein (Figure 1A). Our approach uses a human cell line with a Gal4-responsive luciferase gene integrated in the genome, HEK293 UAS-TK-Luc (5). Using cloning and transfection, the protein of interest is then expressed as a fusion to the Gal4 DNA binding domain in the reporter cell line. ChIP is then carried out with ChIP-grade antibodies against Gal4, and in parallel with sera directed against the protein of interest. The ChIP against Gal4 provides an internal positive control, as well as a standard against which the efficiency of the tested sera can be compared.

We carried out ChIP using a standard protocol (www.abcam.com), and protein A-agarose beads precoated with salmon-sperm DNA (Model no. 16-157; Upstate, Lake Placid, NY, USA). The DNA recovered after ChIP was assayed by quantitative PCR on a LightCycler apparatus (Roche, Meylan, France), using the Quantitect

SYBR Green kit (Qiagen, Courtaboeuf, France) at an annealing temperature of 55°C. The primers used are adjacent to the Gal4 binding sites; their sequence is 5'-CACACAGGAAACAGCTATGAC-3' (sense) and 5'-GAATTCGCCAATGACAAGAC-3' (antisense). The DNA binding domain of yeast Gal4 (amino-acids 1-147) was expressed from the SV40 promoter using the plasmid pGAP (6). The complete coding sequence of human ZBTB4 was PCR amplified and cloned in pGAP to generate the Gal4-ZBTB4 expression plasmid.

To verify the basic components of the system, we first expressed the Gal4 DNA binding domain in the HEK293 UAS-TK-Luc cell line. ChIP with antibodies (Model no. SC-577; Santa Cruz Technologies, Santa Cruz, CA, USA) directed against Gal4 worked efficiently, precipitating about 1% of the input material. As expected, little or no DNA was precipitated when the cells were not transfected, or when the ChIP was carried out with nonspecific rabbit serum (Figure 1B).

We then transfected Gal4-ZBTB4 in the reporter cell line. ChIP with the Gal4 serum recovered about 0.3% of the input material. This efficiency is lower than with the Gal4 protein alone. The proteins Gal4 and Gal4-ZBTB4 are expressed to similar levels as judged by Western blotting (not shown). A possible reason for the lower recovery is decreased antibody accessibility to the Gal4 protein due to the ZBTB4 moiety. Alternatively, a fraction of the Gal4-ZBTB4 protein may be titrated away from the Gal4 binding sites toward ZBTB4 binding sites, therefore decreasing the efficiency of ChIP at the site we assessed.

We then used this system to test two sera directed against different regions of human ZBTB4: sera no. 98 and no. 120. These sera perform equally well in Western blotting and in immunofluorescence (data not shown). As seen in Figure 1B, no.120 gives a positive result, and its recovery of GAL4-UAS sequence from the integrated luciferase transgene is similar to that obtained with the anti-Gal4 antibody. In contrast, serum no. 98 is markedly less effective. Therefore, we conclude that the serum no. 120 is highly efficient for ChIP, and

Figure 1. A system to test sera in chromatin-immunoprecipitation. (A) Experimental design. The human cell line HEK293 UAS-TK-Luc contains an integrated reporter in which the Luciferase gene is under the control of Gal4 binding sites (UAS) upstream of the Thymidilate Kinase promoter (TK). The cells can be transfected with constructs expressing the DNA binding domain of Gal4 fused to a protein of interest (Prot. X). ChIP can then be performed with antibodies against Gal4, or against the protein of interest. The primers used to measure DNA recovery after ChIP are depicted as a pair of inverted arrows. (B) Validation of the system with the human transcription factor ZBTB4. The reporter cells were transfected with expression vectors for the Gal4 DNA binding domain alone (Gal4), the Gal4-ZBTB4 fusion, or were left untransfected. ChIP was then carried out with the indicated antibodies. The result of two independent experiments is shown. Serum no.120 functions in ChIP as well as the anti-Gal4 antibodies, whereas serum no.98 is far less efficient.

we have made it our reagent of choice for downstream applications, including ChIP-on-chip.

We note that this approach may yield some falsely negative results: it could underrate some antibodies that are actually functional. For instance the Gal4 moiety could impede recognition of the attached protein by the antibodies. Also, the general conformation, and the accessibility of the epitopes, may be different when the protein of interest is linked to Gal4 than when the native protein is bound at its target sites. This last phenomenon could also potentially cause some falsely positive results, and it should be kept in mind that the crosslinking efficiency may be different for Gal4 and the protein under study. This too could cause sera to perform less well on their native target than might be expected from the results of this assay.

In spite of these potential limitations, we believe this method could prove generally useful. First, it can be adapted to any DNA-associated protein from any species. Second, the technique is particularly advantageous when

dealing with proteins for which no binding sites are yet identified, which is bound to become a problem of growing importance. As an example, there are an estimated 2600 transcription factors in the human genome, the vast majority of which have not yet been studied (7). The transcriptional networks of *Saccharomyces cerevisiae* have been entirely described using ChIP (8). If such an approach is to be eventually applied to mammalian cells, the adequate reagents will have to be generated and evaluated using methods such as the one we are describing.

ACKNOWLEDGMENTS

Work in the Defossez laboratory is supported by Centre National de la Recherche Scientifique (CNRS), Action Thématique et Incitative sur Programme, Association pour la Recherche contre le Cancer (grant no. 3727), and Ligue contre le Cancer. D.Y. is supported by a postdoctoral fellowship from Association pour la Recherche contre le Cancer. We thank

Guillaume Filion for his help with the ChIP experiments. We are also indebted to Manel Esteller and Esteban Ballestar with whom we have initiated a ChIP-on-chip project.

COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

REFERENCES

- Solomon, M.J., P.L. Larsen, and A. Varshavsky. 1988. Mapping protein-DNA interactions in vivo with formaldehyde: evidence that histone H4 is retained on a highly transcribed gene. *Cell* 53:937-947.
- Hudson, M.E. and M. Snyder. 2006. High-throughput methods of regulatory element discovery. *BioTechniques* 41:673, 675, 677 passim.
- Mardis, E.R. 2007. ChIP-seq: welcome to the new frontier. *Nat. Methods* 4:613-614.
- Filion, G.J., S. Zhenilo, S. Salozhin, D. Yamada, E. Prokhortchouk, and P.A. Defossez. 2006. A family of human zinc finger proteins that bind methylated DNA and repress transcription. *Mol. Cell. Biol.* 26:169-181.
- Sripathy, S.P., J. Stevens, and D.C. Schultz. 2006. The KAP1 corepressor functions to coordinate the assembly of de novo HP1-demarcated microenvironments of heterochromatin required for KRAB zinc finger protein-mediated transcriptional repression. *Mol. Cell. Biol.* 26:8623-8638.
- Defossez, P.A., J.L. Baert, M. Monnot, and Y. de Launoit. 1997. The ETS family member ERM contains an alpha-helical acidic activation domain that contacts TAFII60. *Nucleic Acids Res.* 25:4455-4463.
- Babu, M.M., N.M. Luscombe, L. Aravind, M. Gerstein, and S.A. Teichmann. 2004. Structure and evolution of transcriptional regulatory networks. *Curr. Opin. Struct. Biol.* 14:283-291.
- Lee, T.I., N.J. Rinaldi, F. Robert, D.T. Odom, Z. Bar-Joseph, G.K. Gerber, N.M. Hannett, C.T. Harbison, et al. 2002. Transcriptional regulatory networks in *Saccharomyces cerevisiae*. *Science* 298:799-804.

Received 28 September 2007; accepted 19 October 2007.

Address correspondence to Pierre-Antoine Defossez, Centre National de la Recherche Scientifique (CNRS), UMR 218, Institut Curie, 26 rue d'Ulm, 75005 Paris, France. e-mail: defossez@curie.fr

To purchase reprints of this article, contact: Reprints@BioTechniques.com