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Assessing protein-DNA interactions: Pros and cons of classic and emerging techniques

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Investigation of protein-DNA interactions provides important information for understanding gene function and regulation, but identification and validation of specific interactions remain major challenges in the post-genomics era. Therefore, effective and economical methods to assess protein-DNA interactions are highly sought-after by molecular biologists.

Choosing the appropriate method to examine a specific protein-DNA interaction also remains a crucial challenge for researchers. Since the 1970s, many methods and variants have been developed to study protein-DNA interactions based on different principles and incorporating different modifications. In this insight, we summarize the most commonly used methods for assessing protein-DNA interactions, so researchers can examine the strengths and weaknesses of each method at a glance and choose an appropriate technique to study the biological processes of interest (Table 1). The methods for protein-DNA interactions can be categorized into four types based on different principles:

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METHODS BASED ON THE ELECTROPHORETIC MOBILITY OF DNA

The electrophoretic mobility shift assay (EMSA) is a classic *in vitro* technique to investigate protein-nucleic acid associations based on differences in DNA mobility during electrophoresis. Free DNA generally moves faster than protein-bound DNA, resulting in a band shift in native polyacrylamide or agarose gels (Garner and Revzin, 1981). EMSA is a robust assay and has been modified into many variants using various DNA labeling methods (Hellman and Fried, 2007).

METHODS BASED ON DNA CLEAVAGE

Footprinting uses DNaseI to cut DNA randomly, and binding of a protein protects the bound DNA region from digestion. Therefore, comparison of the patterns of DNA fragments with and without protein on a sequencing gel reveals the footprint of the binding protein (Galas and Schmitz, 1978). DNase footprinting is the gold standard used to identify a protein's core binding sequence at single-nucleotide resolution. Another strategy, Restriction Endonuclease Protection Selection and Amplification (REPSA), can identify a protein binding site based on DNA cleavage and PCR am-

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Table 1 Comparison of current methods for assessing protein-DNA interactions

Type	Principle	Method & Reference	DNA labeling	Detection	Screening	Quantification	Sensitivity
I	DNA mobility	EMSA (Garner and Revzin, 1981)	Yes	Electrophoresis/ Autoradiography	No	Yes	High
II	DNA cleavage	Footprinting (Galas and Schmitz, 1978)	Yes	Electrophoresis/ Autoradiography	No	No	High
		REPSA (Hardenbol and Van Dyke, 1996)	No	PCR/Sequencing	Yes	No	Medium
		CBA (Xie et al., 2016)	No	Electrophoresis	No	Yes	Medium
III	Affinity chromatography	ChIP (Gilmour and Lis, 1984)	No	PCR/Sequencing	Yes	Yes	Medium
		DNA pull-down (Zhu et al., 2002)	Yes	Western blotting/MS	Yes	Yes	High
		Filter binding (Woodbury and von Hippel, 1983)	Yes	Autoradiography	No	Yes	High
IV	Transcriptional activation	Y1H (Wang and Reed, 1993)	No	Reporter gene	Yes	Yes	Low
		B1H (Meng et al., 2005)	No	Reporter gene	Yes	Yes	Low

plification (Hardenbol and Van Dyke, 1996).

METHODS BASED ON AFFINITY CHROMATOGRAPHY

To identify DNA sequences bound by a specific protein, chromatin immunoprecipitation analysis (ChIP) relies on the specific affinity between antigen and antibody to precipitate protein-bound DNA (Gilmour and Lis, 1984). The DNA sequences isolated by ChIP represent specific regions of the genome associated with the protein of interest *in vivo*. ChIP is also a powerful tool for determining the target sites of histone modifiers in epigenetics. ChIP, in combination with next-generation sequencing (ChIP-seq) or microarray technologies (ChIP-chip) can screen for the target sites of a specific protein at the whole-genome scale.

Conversely, to identify proteins bound by a specific DNA sequence, DNA pull-down, also called streptavidin-agarose pull-down, relies on the specific affinity between streptavidin and biotin. Proteins interacting with a biotinylated DNA probe are pulled down by streptavidin–agarose beads and then analyzed by western blotting or mass spectrometry (MS) (Zhu et al., 2002). Identification of novel, trace proteins depends heavily on the sensitivity of MS. If the DNA pull-down assay is conducted in combination with an enzyme-linked immunosorbent assay (DPI-ELISA), the detection sensitivity of the method increases significantly (Brand et al., 2010).

In contrast to the chemical affinity-based methods such as DNA pull-down and ChIP, filter-binding assays can measure the interactions between protein and DNA based on the charge-transfer affinity of the molecules. Most proteins have a net positive charge, and DNA and nitrocellulose have a negative charge; therefore, a nitrocellulose filter will immobilize proteins rather than DNAs. Thus, only protein-bound DNA will stay on the filter and the amount of DNA (labeled with a fluorescent or radioactive tag) on the nitrocellulose filter can be quantified by measuring the radioactivity or fluorescence remaining on the filter (Woodbury and von Hippel, 1983).

METHODS BASED ON ACTIVATION OF A TRANSCRIPTION FACTOR

One-hybrid assays are frequently used to screen for novel proteins that interact with a target DNA, based on the activation of a reporter gene by a reconstructed transcription factor; this reporter acts as a selectable marker and its activation allows the survival of the yeast or bacteria (Wang and Reed, 1993; Meng et al., 2005). However, self-activation sometimes causes artifacts in these assays.

Among these methods, EMSA and footprinting are very sensitive, especially when using radioisotope-labeled DNA. However, the manipulation of radioisotope labeling is complicated and requires a special license and training to avoid hazards to the environment and the users. For applications that do not require high sensitivity, we recommend non-isotopic methods to achieve the same goal.

DNA or proteins identified from one-hybrid, ChIP, DNA pull-down, or REPSA should be confirmed by another methods. The cleaved amplified polymorphic sequence (CAPS)-based binding assay (CBA) recently reported by Xie et al. is ideal for this purpose (Xie et al., 2016), as CBA provides a simple, low-cost, label-free strategy that does not require special training or equipment. In CBA, the interaction between a protein and DNA is revealed by the CAPS patterns derived from differences in the accessibility of a restriction endonuclease site (intrinsic or artificial) in am-

plified DNA in the presence and absence of the binding protein. In EMSA, the protein-DNA complex may disassemble due to the salt concentrations and pH of the electrophoretic buffer, thus producing a smeared pattern. By contrast, in CBA, cleavage of the DNA by restriction enzymes occurs prior to electrophoresis, producing simple and clear-cut CAPS patterns. Moreover, CBA provides a semi-quantitative readout of the interaction strength based on the dose of the binding protein or the efficiency of DNA cleavage. However, CBA is not ideal for high-throughput screening.

For evaluation of protein-DNA interactions, most current methods provide qualitative or semi-quantitative data. We lack truly quantitative techniques that can measure the binding strength of protein-DNA associations with parameters such as binding stoichiometries, affinity constants, and kinetics. Although several biophysical techniques such as Surface Plasmon Resonance (SPR), Bio-layer Interferometry (BLI), and Isothermal Titration Calorimetry (ITC) can quantitatively measure these parameters, they require expensive infrastructure that most researchers cannot afford. Therefore, development of a simple, low-cost, quantification strategy will be a promising future direction for the development of new technologies for assessing protein-nucleic acid interactions.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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