

An electrophoretic mobility shift assay using the protein isolated from host plants

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Abstract: **【Objective】** The electrophoretic mobility shift assay (EMSA) is a common technology to detect DNA-protein interactions. However, in most cases, the protein used in EMSA is obtained via prokaryotic expression, and rarely from plants. At the same time, the proteins expressed from prokaryotic systems usually cannot fold naturally and have no post translationally modification, which may affect the binding of proteins to DNA. Here, we develop a technique to quickly isolate proteins of interest from host plants and then analyze them using fluorescent EMSA. **【Method】** Special transient transformation was used to obtain transgenic plants encoded the target protein. DNA- protein interaction was determined through immunoprecipitation, protein prokaryotic expression and purification, chemiluminescent EMSA, and fluorescent EMSA. The binding between DNA and proteins with post-translational modifications was determined through western blotting and quantitative real-time reverse transcription PCR (qRT-PCR). **【Result】** This technology system is called: protein from plants fluorescent EMSA method (PPF-EMSA). In PPF-EMSA, a special transient transformation method is employed to transiently deliver genes into the plant, enabling efficient synthesis the encoded proteins. Then, the target protein is isolated using immunoprecipitation, and the DNA probes were labeled with cyanine 3 (Cy3). Both fluorescent EMSA and super-shift fluorescent EMSA can be performed using the proteins from plants. Three kinds of plants, *Betula platyphylla*, *Populus davidiana*×*P. bolleana* and *Arabidopsis thaliana*, are used in this study. The proteins isolated from plants are in a natural state, can fold naturally and are posttranslationally modified, enabling true binding to their cognate DNAs. **【Conclusion】** As transient transformation can be performed quickly and not depended on whether stable transformation is available or not, we believe this method will have a wide application, enabling isolation of proteins from host plant conveniently.