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Novel and Efficient Protocol for DNA Coating-Based Identification of DNA-Protein Interaction by Antibody-Mediated Immunodetection

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Abstract

Background: Studying protein-protein and protein-DNA interactions are prerequisites for the identification of function and mechanistic role of various proteins in the cell. Protocols for analyzing DNA-based Protein-Protein and Protein-DNA interactions are complicated and need to be simplified for efficient tracking of binding capabilities of various proteins to specific DNA molecules. Here, we demonstrated a simple yet efficient protocol for the identification of DNA coating-based Protein-DNA interaction using antibody-mediated immunodetection.

Methods: Briefly, we have coated specific DNA in the microtiter plate followed by incubating with protein lysate. Specific protein-DNA and/or protein-protein bind with DNA interactions are identified using specific fluorophore-conjugated antibodies. Antibodies are used to detect a protein that is bound to the DNA.

Results: Fluorescent-based detection identifies the specific interaction between Protein-DNA with respect to coated DNA fragments. The protocol uses indirect conjugated antibodies and hence the technique is sensitive for effective identification of Protein-DNA interactions.

Conclusions: Based on the results we conclude that the demonstrated protocol is simple, efficient and sensitive for identification of Protein-DNA interactions.

Keywords: DNA coating, Lamin A, Protein-DNA interaction.

Introduction

Identifying the sequence-specific DNA-protein interactions is a prerequisite to study the mechanism of various proteins at the molecular level. Many methods are available to find the DNA-protein interactions namely, DNA footprinting, filter binding and gel shift assays. The conventional and versatile method for identifying DNA-protein interactions is Electrophoretic Mobility Shift Assay (EMSA) (1). It is a quick and effective method to find out the interaction between proteins and nucleic acid (1–4). EMSA has less specificity and to improve the specificity, radio-labelled probes are introduced for effective identification of DNA-protein interactions (5, 6). However, radio-labeled probes provide high sensitivity. It has its own disadvantages, notably short half-life of the isotope, environmental safety issues and exposure requirements for detection of the signal. To overcome the issues, two-color EMSA is introduced to detect the nucleic acids and proteins in a gel (7). Antibody-based identification of proteins in the DNA-protein complex is interesting, which improves more sensitivity (6, 8). Hybrid systems such as eukaryotic hybrid systems are gold-standard tools for the detection of protein-protein as well as protein-DNA interaction studies. However, these methods are more complicated and obviously not simple to use. Hence, we designed

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DNA coating-based versatile technique for studying DNA-protein interaction.

In the present study, instead of separating DNA and protein in the gel for mobility shift assay, we directly coated the desired specific DNA fragments in the microtiter plate using the DNA coating solution. Briefly, we studied the interaction of lamin A and trf2 in connection with telomere sequences using this novel protocol approach. We demonstrated DNA-Protein binding assay for studying the interaction between telomeric DNA, trf2 and lamin A, making the method convenient and more suitable for any DNA-protein binding interaction studies. Genomic DNA is isolated from the Human Dermal Fibroblast (HDF) cells and the telomeric repeats $[TTAGGG]_n$ is separated from the genomic DNA with the help of restriction enzymes namely *Rsa* I and *Hinf* I. After purification, the telomere repeats are coated in the microtiter plate using DNA coating solution. The uncoated DNA fragments are washed with 1X TBST buffer. Protein lysate is prepared from the HDF cells and incubated with the telomeric DNA-coated microtiter plates. After incubation, the unbound proteins are washed and proceeded further for immunological reactions. Primary antibody, anti-lamin A and anti-trf2 are added and incubated overnight at 4 °C. The microtiter plates are washed three times and incubated with appropriate secondary antibodies. Fluorophore-conjugated secondary antibodies namely; anti-rabbit IgG Alexa Fluor 488 and anti-mouse IgG Alexa Fluor 647 are used for the studies. Excitation and Emission readings for the samples are taken using multi-plate reader according to the secondary antibody-conjugated fluorophores and documented.

Materials and methods

Reagents

RIPA Buffer: 50 mM Tris HCL, 150 mM NaCl, 1% NP-40, 0.5% Sodium Deoxycholate, 0.1% SDS.

PBS Buffer: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4.

DNA Coating solution (Thermo Scientific - Cat. No. 17250).

Restriction enzymes (*Rsa* I [NEB - Cat. No. R0167S] and *Hinf* I [NEB - Cat. No. R0155S]).

Primary Antibodies [anti-lamin A antibody (Sigma- Cat. No. L1293) and anti-trf2 antibody (Abcam – Cat. No. ab13579)].

Secondary Antibodies (anti-rabbit IgG Alexa Fluor 488 [Sigma - Cat. No. SAB4600036] and anti- mouse IgG Alexa Fluor 647 [Abcam - Cat. No. ab150119]).

TBST Buffer: 1X PBS with 0.1% Tween20.

4′,6-diamidino-2-phenylindole (DAPI): DNA staining solution.

Equipments

Cooling Centrifuge (Eppendorf)

Multi Plate Reader (Perkin Elmer Multimode Plate Reader)

Animal Cell culture chamber (Haier)

Minispin (D-LAB)

Preparation of Protein Lysate

1. Prepare protein lysate from Human Dermal Fibroblast cells using RIPA buffer.
2. Culture Human Dermal Fibroblast cells in T-25 flask up to 90% confluence. Then remove the medium and rinse the cells with ice cold 1X PBS.
3. Add 3 ml of Ice cold RIPA buffer along with 1 mM PMFS (protease inhibitor) and incubate at 4 °C for 10 minutes.
4. Collect the cells by vigorous pipetting, which helps to detach the cells from the flask.
5. Protein lysate is prepared by passing the cells through 18-gauge syringe needle.
6. Pellet the cells by centrifugation at 560 g at 4 °C for 10 minutes and collect the protein lysate.

DNA Isolation and Restriction Digestion

1. Culture Human dermal fibroblast up to 90% confluence, after that subject the cells to genomic DNA isolation using the HI media kit (Cat. No. MB506-50PR), as per the manufacturer protocol.
2. Check the purity of the DNA by Nano-drop at 260 and 280 nm.
3. Isolate the DNA and restrict it using two restriction enzymes namely *Rsa* I and *Hinf* I. (These two restriction enzymes cut the DNA randomly except telomeric repeats TTAGGG).

DNA Coating and Protein binding

1. Dilute 5 µg of restricted DNA in 1 ml of TE buffer and mix with 1 ml of DNA coating solution (1:1 ratio). After mixing, incubate the mixture for 10 minutes at room temperature (RT).
2. Add DNA-Coating solution mixture to the black well plate for coating.

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3. Incubate the microtiter plates at RT for 1–2 hours with gentle shaking.
4. Wash the microtiter plate three times with 1 X TBST to remove uncoated DNA fragments.
5. Add 10 µg of protein lysate to each well and incubate at 4 ℃ for 2 hours.
6. Wash the microtiter plate three times with 1 X TBST to remove unbound proteins.

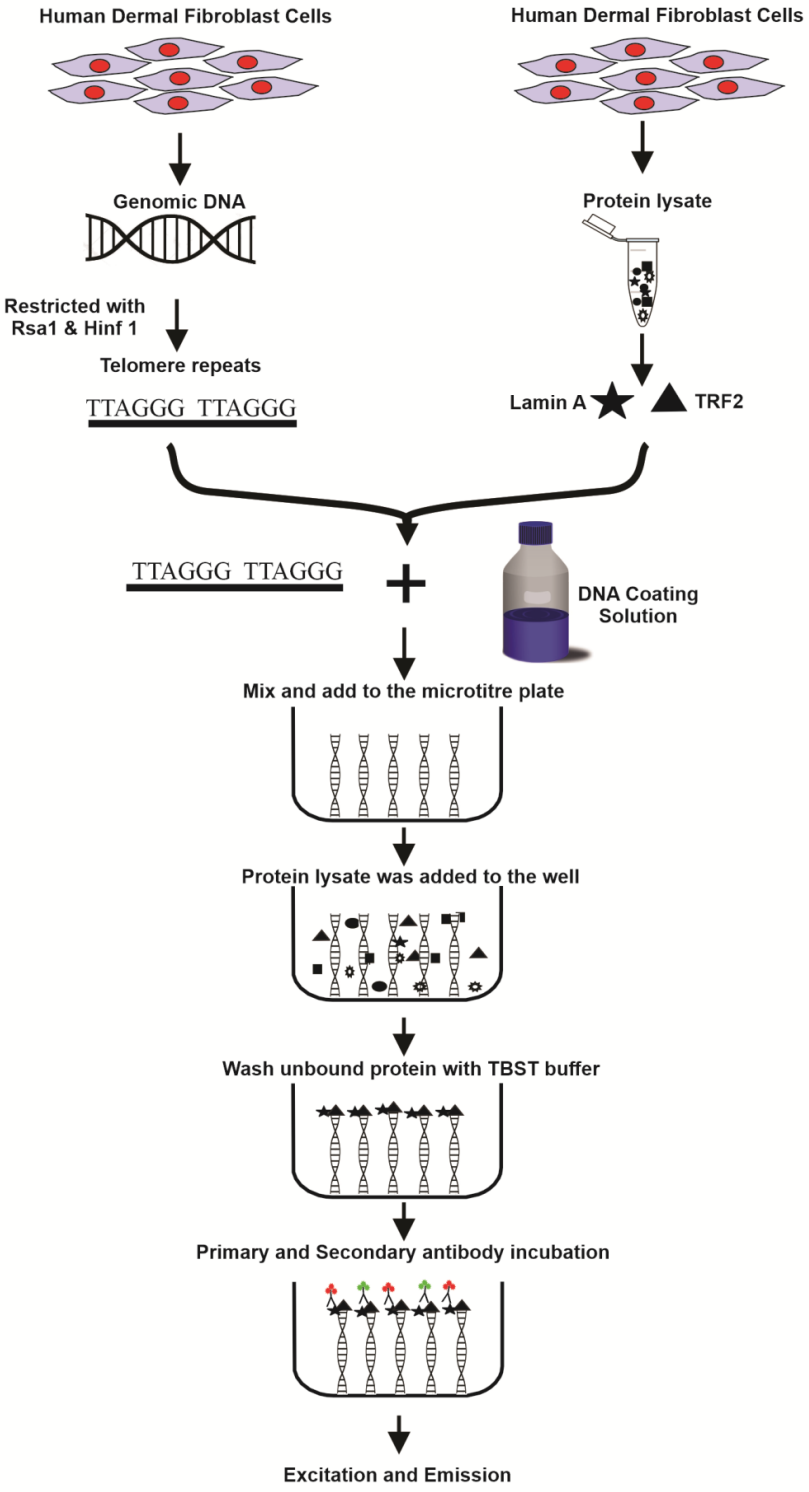

Fig. 1. Schematic representation of DNA coating-based Protein-DNA interaction.

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Antibody Incubation

1. Prepare primary antibody (lamin A [1:100] and trf2 [1:100]) in 1% BSA, and added to each well. Incubate the microtiter plates for overnight at 4 °C.
2. Wash the microtiter plates three times with 1X TBST buffer at RT.
3. After repetitive washing, incubate the plates with secondary antibody (anti-rabbit IgG Alexa Fluor 488 [1:1000] and anti-mouse IgG Alexa Fluor 647 [1:1000]) respectively, in a dark place for 2 hours at RT.
4. Repeat Step 1.
5. Excitation and Emission readings of the samples are taken using multiplate reader at 488 and 647 nm, respectively.
6. Schematic representation of the complete protocol was shown in the Fig. 1.

Results

Coating of specific DNA fragments

Rsa I and *Hinf* I restricted DNA fragments are coated in a microtiter plate using DNA coating solution as mentioned in the materials and methods. The coated DNA fragments are stained with DAPI and observed for excitation and emission at 358 and 461 nm, respectively. The graph shown in the Fig. 2 clearly confirms the presence of DNA in the coated microtiter plate. In addition, Fig. 2 illustrates that, no fluorescence was observed in the DNA molecules.

Antibody-based detection of DNA-Protein interaction

Protein lysate is obtained from HDF cells and incubated to the DNA-coated microtiter plates. Proteins specifically bound with DNA are identified using antibodies corresponding to the specific protein. In the present experiment, we used lamin A and trf2 antibodies to target the telomeric-specific DNA binding capability of lamin A and trf2. The data shown in the Fig. 2, confirm the DNA-binding capability of lamin A and trf2 with telomeric DNA fragments, respectively. Reports clearly suggest that the trf2 has a capability to interact with telomeric DNA sequences (9, 10). To validate the data, we perform immunoprecipitation with lamin A and trf2, vice versa to confirm the protein-protein interaction. Immunoprecipitation results clearly confirm the presence of lamin A and trf2 in trf2 as well as lamin A immunoprecipitated blotting, respectively. The data validate the DNA-binding capability of trf2 and lamin A. Thus, Fig. 2 results are validated.

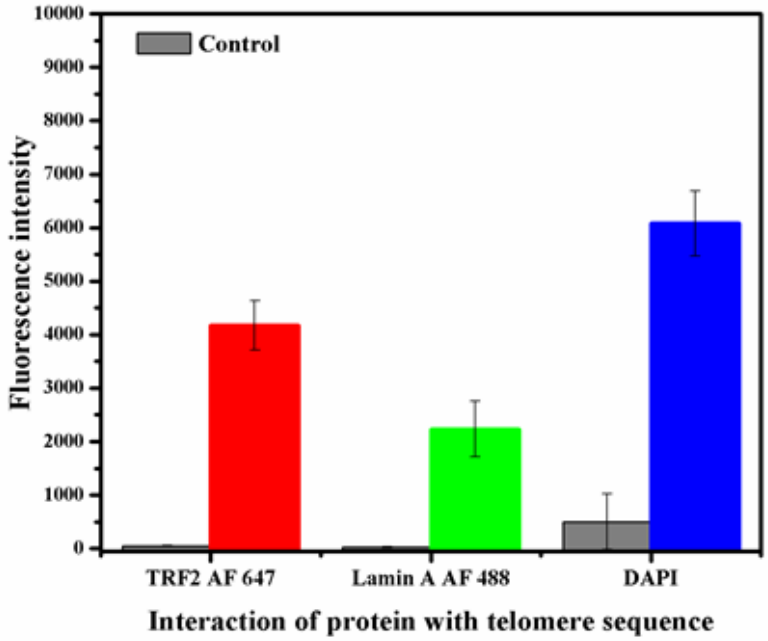

Fig. 2. Represents the fluorescence intensity of trf2 (red) and lamin A (green) interaction with telomeric DNA repeats. Coated telomeric DNA was stained with counterstain DAPI (blue).

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Discussion

Protein-DNA and Protein-protein interactions are the core components of all biological systems. These interactions are the basics found in all biological processes (11). Protein-protein interaction plays an extensive range of biological processes from cell-to-cell interactions to all metabolic and developmental process (12). DNA-Protein interaction plays a vital role in all regulatory protein functions (13). Overall, these protein-DNA/ protein-protein interactions are becoming one of the major objectives of system biology (14).

The main aim of our study is to develop a highly sensitive and stable DNA-Protein interaction for the determination of Lamin A and trf2 protein interaction with telomere. Finally, detection of this interaction is observed with the help of antibody-based fluorescent method. Previous findings use nitro cellulose filter binding assay to study protein DNA interactions (15–17). Similarly, EMSA is used to study protein interaction with nucleic acids (18). Chromatin immunoprecipitation method is also used to study the bio molecules interactions (19).

Moreover, sequence-specific coating of DNA molecules is possible in this technique and hence the interaction studies between the DNA and protein are more sensitive. In addition, using this technique, DNA bind protein-protein interaction is also possible. In general, DNA-coating based immunodetection is a novel approach to study the DNA-protein interaction.

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The authors declare no conflict of interest.

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