EMSA

Introduction

An **electrophoretic mobility shift assay** (EMSA) or **mobility shift electrophoresis**, also referred as a **gel shift assay**, **gel mobility shift assay**, **band shift assay**, or **gel retardation assay**, is a common affinity electrophoresis technique used to study protein–DNA or protein–RNA interactions. This procedure can determine if a protein or mixture of proteins is capable of binding to a given DNA or RNA sequence, and can sometimes indicate if more than one protein molecule is involved in the binding complex. Gel shift assays are often performed in vitro concurrently with DNase footprinting, primer extension, and promoter-probe experiments when studying transcription initiation, DNA replication, DNA repair or RNA processing and maturation, as well as pre-mRNA splicing.

Materials

Binding Buffer

- 10 mM Tris-Cl [pH 8.0],
- 200 mM NaCl,
- 5% glycerol

Dialysis buffer

- 200mM NaCl
- 200mM of KCl,
- LiCl,
- NH4Cl,
- MgCl2,
- Na2CO3,
- C2H3NaO2 or Na3C6H5O7

Procedure

1. Gel Shift Assay was carried out by standard procedure in which various concentrations of the purified transcription factor were incubated with 90ng of promoter DNA which harbors Operator region, in a binding buffer

2. The effect of the various cations and anions on interactions was investigated by replacing the 200mM NaCl in dialysis buffer with 200mM of KCl, LiCl, NH4Cl, MgCl2, Na2CO3, C2H3NaO2, or Na3C6H5O7. The pH values of all the buffers were checked and adjusted to pH 8.0, before use.

3. The purified transcription factor was thoroughly dialyzed against each of the above buffers and used for binding reactions and CD-spectra analysis.

4. To carry out a gel shift assay, a 6% non-denaturing PAGE was used. Individual reaction mixtures were loaded onto different lanes of the polymerized 6% non-denaturing PAGE. Electrophoresis was carried out in cold for 5hr at 80 V.

5. Finally, the gel was stained with SYBR Green II Stain-10,000X concentrate in DMSO (ThermoFisher Scientific) and the stained native gel pictures were captured by E-Gel® Imager System with UV Light Base used SYBR® Filter (Invitrogen, Life Technologies).

6. The gel pictures were analyzed by ImageJ version 1.51d (Schneider, C.A., et al. 2012). Plot profile function was used to determine the percentage of Promoter-TF bound complex and graphs were drawn using the sigmoidal fitting function of OriginPro 2016 Version b9.3.2.303 (Academic). The fitting curves had an R-square value above 0.9. The KD values were calculated from the graphs.

7. Each experiment has been repeated thrice