4. Electrophoretic mobility shift assay (EMSA)

I. Process workflow

a. **Preparation of samples**
b. **Incubation**
c. **Running native PAGE**
d. **Scanning**
e. **Data evaluation**

II. Motivation

In this experiment you will learn a method how to test the possible protein-DNA interaction using a native gel electrophoresis. This technique could be also used in other biochemical assays such as nuclease or helicase activity assays.

III. Theoretical background

An **electrophoretic mobility shift assay** (EMSA), also referred as a **gel shift assay**, or **mobility shift electrophoresis**, 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 have binding affinity to DNA or RNA sequence, and can sometimes indicate if more than one protein molecule is involved in the binding complex. EMSA could be performed *in vitro* concurrently with DNase footprinting, primer extension, and ChIP assays when studying transcription initiation, DNA replication, DNA repair or RNA processing and maturation (Ausubel 1994).

EMSA is based on the observation that complexes of protein and DNA/RNA migrate through a nondenaturing polyacrylamide/agarose gel more slowly than free DNA/RNA fragments or double-stranded oligonucleotides. In the classical assay, solutions of protein and nucleic acid are combined and the resulting mixtures are subjected to electrophoresis under native conditions. The speed at which different molecules move through the gel is determined by their size and charge, and to a lesser extent, their shape. After electrophoresis, the distribution of species containing nucleic acid is determined, usually by autoradiography of ^{32}P-labeled nucleic acid or by fluorescent scanner (Garner 1981, Fried 1981). Under the correct experimental conditions, the interaction between the DNA and protein is stabilized and the ratio of bound to unbound

nucleic acid on the gel reflects the fraction of free and bound probe molecules as the binding reaction enters the gel. If the starting concentrations of protein and probe are known, the binding affinity of the protein for the nucleic acid sequence may be determined (Ausubel, 1994).

In addition to a labeled DNA fragment, specific antibody that recognizes the protein can be added to the gel shift reaction to create an even larger complex. If the protein recognized by the antibody is not involved in complex formation, addition of the antibody should have no effect. If the protein that forms the complex is recognized by the antibody, the antibody can either block complex formation, or it can form an antibody-protein-DNA complex. This method is referred to as a *supershift assay*, and is used to unambiguously identify a protein present in the protein-nucleic acid complex.

In this assay we will test the binding affinity of Rad52 protein to fluorescently labelled single-stranded DNA.

IV. Design of the experiment

Material:

10xTBE (pH 8.3) buffer (0.89 M Tris, 0.89 M boric acid, 20 mM Na_2EDTA)

10% polyacrylamide gel in 0.5xTBE

6x loading buffer (10 mM Tris-HCl, 60% glycerol, 60 mM EDTA, Orange G)

5x reaction buffer B (150 mM Tris-HCl, 25 mM $MgCl_2$, 5 mM DTT)

Sterile H_2O

500 mM KCl

Protocol:

Important!!
Keep in ice all your solutions and your samples till the moment you incubate them.
Fluorescent dyes are sensitive to light, so maintain them in dark as much as you can.

a. Preparation of samples

1) Prepare the master mix:

12 µl 5xB buffer
1.8 µl ssDNA (49-mer, 0.2 nM)

Mix and spin shortly.

2) Prepare the samples according to the following table. Add the protein at the end to start the reaction, after you have pipetted all the other components.

	1	2	3	4	5
Master mix	2.3	2.3	2.3	2.3	2.3
H_20 (sterile)	7.7	6.4	6.3	6.1	5.7
500 mM KCl	-	1.1	0.9	0.6	-
Rad52	-	0.2	0.5	1.0	2.0

Total sample volume: 10 µl

b. Incubation

3) Incubate the samples for 10 min at 37°C.

4) Put the sample back in the ice and add 2 µl of 6x loading buffer.

c. Running native PAGE

5) Load the samples on the gel previously placed in the vertical electrophoresis unit filled with 0.5xTBE buffer.

6) Run the gel for 35 min at 80 V.

d. Scanning

7) After the run is over, remove the gel sandwich from the electrophoresis unit. Separate both glasses and place the gel on the glass plate of the scanner.

e. Data evaluation

Bands on the scanned the gel will be evaluated by using a special software.

V. References

1. Ausubel, Frederick M. (1994). *Current protocols in molecular biology*. Chichester: John Wiley & Sons. pp. 12.2.1–11.

2. Garner MM, Revzin A (1981). "A gel electrophoresis method for quantifying the binding of proteins to specific DNA regions: application to components of the Escherichia coli lactose operon regulatory system". *Nucleic Acids Res.* **9** (13): 3047–60

3. Fried M, Crothers DM (1981). "Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis". *Nucleic Acids Res.* **9** (23): 6505–25.

VI. Question

What concentration of Rad52 can bind about 50% of DNA?