5. Helicase activity assay

I. Process workflow

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

II. Motivation

The aim of this practice is to learn how to study the helicase activity of a protein, by means of native polyacrylamide electrophoresis and using fluorescently labeled synthetic oligonucleotides.

III. Theoretical Background

Helicases are enzymes that catalyze the unwinding of double-stranded DNA or RNA in a process coupled to the hydrolysis of nucleoside triphosphate. They are ubiquitous and diverse, and have important roles in almost all pathways of nucleic acid metabolism as DNA replication, DNA repair, recombination, transcription, RNA processing and translation. (Lohman et al, 2008; Patel & Picha, 2000)

Several human diseases are related to defects in helicases, therefore their importance. Research is focused in understanding their roles, activities and mechanisms of action, and discovering interacting protein partners.(Ellis, 1997)

One of the methods used to study the helicase activity of a protein combines the simplicity of native polyacrylamide electrophoresis with the sensitive fluorescent detection of labeled synthetic oligonucleotides. These are used to create a variety of nucleic acid structures that imitate structures appearing during DNA or RNA processing in the cell. One or more of the oligonucleotides constituting the structure of matter are labeled with fluorescent dyes. For example, once a DNA structure and a helicase are mixed together, the protein may bind to the DNA and unwind it producing intermediate structures and single-stranded DNA (ssDNA). The original structure, the intermediates and ssDNA will have different mobilities in a native polyacrylamide gel. They can be then distinguished and visualized in a fluorescent

scanner, and quantified by an image analysis software. This way, we can study and compare the helicase activity on different substrates, as a dependence on time or protein concentration. The method is relatively fast, of high reproducibility and requires low amount of material; all facts that are very valuable.

BLM, the protein we will use in our practice, belongs to the family of human RecQ helicases, a highly conserved family that is required for the maintenance of genome integrity. Mutations in the *BLM* gene lead to Bloom's syndrome, a disorder associated with a predisposition to cancer of all kinds. (Hickson, 2003)

IV. Design of the experiment

Material:

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

12% polyacrylamide gel in 1xTBE

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

5x reaction buffer H (150 mM Tris pH 7.5, 5 mM DTT, 0.5 mg/mL BSA, 100 mM creatine phosphate, 100 µg/mL creatine kinase)

30 mM $MgCl_2$ / 25 mM ATP solution

500 mM KCl

Sterile H_2O

BLM solution of concentration ….µM in Tris buffer with 150 mM KCl

3'flap DNA substrate solution of concentration 100 nM

5% SDS

10 mg/mL proteinase K

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 the dark as much as you can.

a. Preparation of samples

1) Prepare the master mix:

14 µL 5x buffer H
5.6 µL 30 mM $MgCl_2$ /25 mM ATP solution
2.1 µL 3' flap (100nM)

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	6
Master mix	3.1	3.1	3.1	3.1	3.1	3.1
H_2O (sterile)	4.9	4.72	4.55	4.2	3.5	4.9
500 mM KCl	2	1.93	1.85	1.7	1.4	2
BLM	-	0.25	0.5	1	2	-

Total sample volume = 10 µL

b. Incubation, stop of the reaction

3) Incubate the samples for 10 min at 30 °C.

4) Stop the reaction by adding 1 µL of **freshly** prepared stop solution. Prepare the stop solution by mixing 5 µL of 5% SDS and 5 µL of 10 mg/mL proteinase K .

5) Incubate for 3 min at 30 °C. Then remove the samples from the incubator.

6) In the meantime boil sample 6 for 1 min to denature the DNA substrate and then put it immediately in ice.

7) Add to each sample 2 µL of loading buffer and centrifuge shortly.

c. Running native PAGE

8) Load on the gel previously placed in the vertical electrophoresis unit filled with 1x TBE buffer.

9) Run the gel for 1 h at 100 V.

d. Scanning

10) 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. Ellis NA (1997) DNA helicases in inherited human disorders. *Curr Opin Genet Dev* **7**(3)**:** 354-363

2. Hickson ID (2003) RecQ helicases: caretakers of the genome. *Nat Rev Cancer* **3**(3)**:** 169-178

3. Lohman TM, Tomko EJ, Wu CG (2008) Non-hexameric DNA helicases and translocases: mechanisms and regulation. *Nat Rev Mol Cell Biol* **9**(5)**:** 391-401

4. Patel SS, Picha KM (2000) Structure and function of hexameric helicases. *Annu Rev Biochem* **69:** 651-697

VI. Question

What concentration of helicase unwinds the substrate so that we get 50% of single stranded DNA?