PS#2: We will begin at 10:40 am with Problem 1 in BH212

Assignments
Problem 1- group 4
Problem 2- group 3
Problem 3- group 2
Problem 4- group 1

Time limit: 25 min per group.

The beginning: Designate one person in each group to give a 5 min introduction: try to be succinct and remember to describe the biological significance of the question. You don’t need to describe the whole solution in these 5 min, but you can set the stage.

The discussion: Describe the solutions and the logic behind them.

The end: Designate one person in each group to give a short summary at the end of the problem discussion.
**Q1.** A new bacterium, *E. bioreg*, produces a key metabolite, called bioregia. A repressor, named Brr, controls the pathway producing bioregia. The operator sites were established by mutational analysis. The arrangement of regulatory sites is diagrammed below.

![Diagram of DNA regulatory sites](image)

Key:
- **O1** = operator 1 (Brr binding site)
- **O2** = operator 2 (Brr binding site)
- Upward arrow: start site of transcription

Both operator sites are required for repression:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Arbitrary units of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>1</td>
</tr>
<tr>
<td>brr-</td>
<td>100</td>
</tr>
<tr>
<td>O1-</td>
<td>100</td>
</tr>
<tr>
<td>O2-</td>
<td>100</td>
</tr>
<tr>
<td>O1-O2-</td>
<td>100</td>
</tr>
</tbody>
</table>

Propose several (2 or 3) hypotheses explaining why both operators are required. Describe experiments to test each hypothesis and indicate which experiments distinguish between the hypotheses.
**Q2.** The bacterium *E. bioreg* also produces a key metabolite, called hardwork. An activator, named Hda, controls the pathway producing hardwork; activation is observed when grit is present in the media. Grit is the ligand that binds to Hda, and is required for activation. The activator and its binding site were identified genetically. Hda is not an essential gene in *E. bioreg*. The arrangement of these regulatory sites is diagrammed below.

![Diagram of regulatory sites](image)

**Key:**
- Green: binding site of Hda
- Upward arrow: start site of transcription

The established genetic relationships are as follows:

<table>
<thead>
<tr>
<th>Strain</th>
<th>arbitrary units of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>10</td>
</tr>
<tr>
<td>WT + Grit</td>
<td>100</td>
</tr>
<tr>
<td>hda-</td>
<td>1</td>
</tr>
<tr>
<td>hda- + Grit</td>
<td>1</td>
</tr>
</tbody>
</table>

To determine whether binding of Hda is sufficient to activate transcription, you set up an *in vitro* transcription reaction. Hda is overexpressed in *E. coli* and then purified. The *in vitro* transcription reaction contains the following purified components: RNA polymerase holoenzyme, Hda, Grit, the double stranded DNA fragment encompassing the hardwork promoter and operator (shown below), and a standard transcription mix (salt, buffer, radioactively labeled ATP (ATP*), and the other 3 NTPs). RNA chains are precipitated with TCA and the radioactivity (cpm) determined. Although a template containing a control promoter transcribed well, Hda did not activate the hardwork promoter (results below). In the cell, the activated Hda promoter initiates as well as the control promoter.

<table>
<thead>
<tr>
<th>Template</th>
<th>radioactivity (cpm) in RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control promoter</td>
<td>10,000</td>
</tr>
<tr>
<td>Hardwork promoter</td>
<td>30</td>
</tr>
<tr>
<td>Hardwork promoter + Hda</td>
<td>30</td>
</tr>
<tr>
<td>Hardwork promoter + Hda +Grit</td>
<td>30</td>
</tr>
</tbody>
</table>

Propose 3 hypotheses for the inactivity of the *in vitro* system, and describe the experiments you would perform to test each hypothesis.
Q3. *E. bioreg* produces a 3rd metabolite, stress, controlled by the Sta activator. Activation occurs in the presence of the trx ligand. The arrangement of regulatory sites is diagrammed below.

![Diagram of regulatory sites](image)

Key:
- Green: binding site of Sta
- Upward arrow: start site of transcription.

You have performed a careful *in vitro* analysis of the mechanism of activation using the abortive initiation assay (2 NTPs, one of which is labeled), purified RNA polymerase holoenzyme and DNA, and appropriate buffer conditions for transcription. You determine the effect of the activator on $K_B$, the initial binding step, and $k_f$, the forward rate constant of the reaction leading to open complex formation. You find that the activator exclusively affects $k_f$, without affecting $K_B$.

Propose several models for how an activator could work exclusively at $k_f$, and describe how you would test these models. Indicate whether the experiments proposed distinguish between the models.
Q4. A newly discovered species of yeast makes a key biofuel precursor through the transcription of the gene Fire shown below. Transcription of Fire is controlled by the transcriptional activator, “Kindle”. High resolution nucleosome mapping indicates a well-positioned nucleosome just upstream of the transcription start site of Fire. ChIP data indicates that Kindle binds within the site of the positioned nucleosome. The DNA binding sequence of Kindle is known and is ~30 bp inwards from one end of the nucleosome binding site.

![Diagram showing nucleosome and Kindle binding site](image)

**Key**
- Green: footprint of positioned nucleosome
- Red: location of Kindle binding site
- Arrow: start site of transcription

Since ChIP is done on a population of cells it is possible that
(i) Kindle and the nucleosome do not co-occupy the same site or,
(ii) they do co-occupy the same site.

Propose a few different biochemical and *in vivo* experiments to discriminate between these two possibilities. Speculate on the regulatory value of each possibility.