PS#3: We will begin at 10 am with Problem 1 in BH212 on 2/19/2015

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

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.
Q1. A new species of scaly bird (G. westeros), unusually large and with fire-breathing ability, was recently discovered. RNAi revealed that G. westeros contains a pair of genes, Ned and Catelyn which are both essential for survival. Ned primary transcripts are processed into two products. The minor product, Ned2, exhibits features of a two exon spliced mRNA. It contains a 5’ splice site, a branch point, and weak 3’ splice site. The major product, Ned1, consists only of the sequences in exon 1. Ned1 assembles with the CATELYN protein into a Ribonucleoprotein particle (RNP).

Production of both the major and minor Ned RNAs depend on the G. westeros splicing machinery. Describe more than one model that can account for how the two products are generated. How would you test these models?
1. Describe more than one model that could explain how Walterwhite mRNA alternative splicing is regulated.

2. What kind of elements could be disrupted by the Gus mutation?

3. How would you test your hypotheses?

4. A protein, SAUL, is normally expressed in Jesse cells. Ectopic expression of SAUL in Skylar cells results in the Jesse splicing pattern of Walterwhite mRNA. What kinds of activities could SAUL have?

5. How would you test your hypotheses?

Q2: The Walterwhite gene encodes a transcription factor that is necessary to turn on many genes in the meth synthesis pathway. Walterwhite mRNA is subject to alternative splicing. In Skylar cells, exon 2, which encodes a stop codon, is included, yielding an mRNA that encodes a short and inactive WALTERWHITE protein. In Jesse cells, exon 2 is excluded and the resulting mRNA encodes a long WALTERWHITE protein that has full activity.

When a slow polymerase is introduced into Jesse cells, a Skylar splicing pattern of exon 2 inclusion occurs.

A Gus mutation of Walterwhite results in the Jesse splicing pattern (exon 2 exclusion) in Skylar cells.

Introduction of a slow polymerase into the Gus mutant rescues normal splicing in Skylar cells.
Q3. 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.

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.
Q4. A histone H3 variant, H3.Q has been identified in the newly sequenced yeast species, *S. chocolat*. Deleting H3.Q increases the levels of mRNAs coding for proteins involved in chocolate metabolism. H3.Q differs from H3 only in its N-terminal tail sequence. The N-terminal tail sequences of H3 and H3.Q are shown below.

**H3:** ARTKQTARKSTGGKAPRKLATKAARK  
**H3.Q:** ARTAQTTARASTGGAAPRAQLATKAARA

Propose two or three different hypotheses for how H3.Q might be exerting its effects. Describe how you would experimentally test these hypotheses.