Gene Expression II

Purpose:
To work jsGenex in order to help you to understand:

- rules for DNA & RNA structure
- base-pairing
- transcription including promoters & terminators
- translation including start & stop codons
- mRNA splicing

First: Notebook Review
To be sure that you have all the information you need for this lab, with your lab partners, go over the notes you took while doing the SPOC and from lecture and be sure you have the information listed below. You should fill in any gaps in your notes so everyone in your group has all they need. You can check these items off as you go.

- The rules for DNA and RNA from lecture
- How transcription is controlled and how to figure out which mRNA will be made from a given DNA molecule
- How mRNA splicing is controlled and the difference between introns and exons
- How translation is controlled and how to figure out which protein will be made from a given mRNA molecule

You will then discuss these with your TA as a class to clarify any issues that remain.

Part III: jsGenex

Up until now, you have worked with genes of increasing complexity on paper. This can be time-consuming and error-prone. We will now move on to examine the structure and function of the genes using a computer simulation that takes care of the tedious details.

This simulation allows you to explore gene expression in a hypothetical simplified eukaryote. Eukaryotic genes have promoters and terminators for controlling transcription as well as start and stop codons for controlling translation. Although promoter and terminator sequences are different in different organisms, the genetic code, including the start and stop codons, is **identical in all organisms**. The promoter and terminator sequences used in the hypothetical organism simulated by the gene explorer are:

a promoter:

```
5′–TATAA–3′
   |||||
3′–ATATT–5′
```

Transcription begins with the first base pair to the *right* of this sequence and continues to the right.

a terminator:

```
5′–GGGGG–3′
   |||||
3′–CCCCC–5′
```

Transcription ends with the first base pair to the *left* of this sequence.

Therefore, a gene would look like this:

```
5′–TATAAXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXGGGGG–3′
   |||||||||||||||||||||||||||||||||||||||
3′–ATATTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXCCCCC–5′
```

Where the region shown as X's would be transcribed into pre-mRNA.

In addition, eukaryotic genes have a few features that prokaryotic genes do not have. These are:

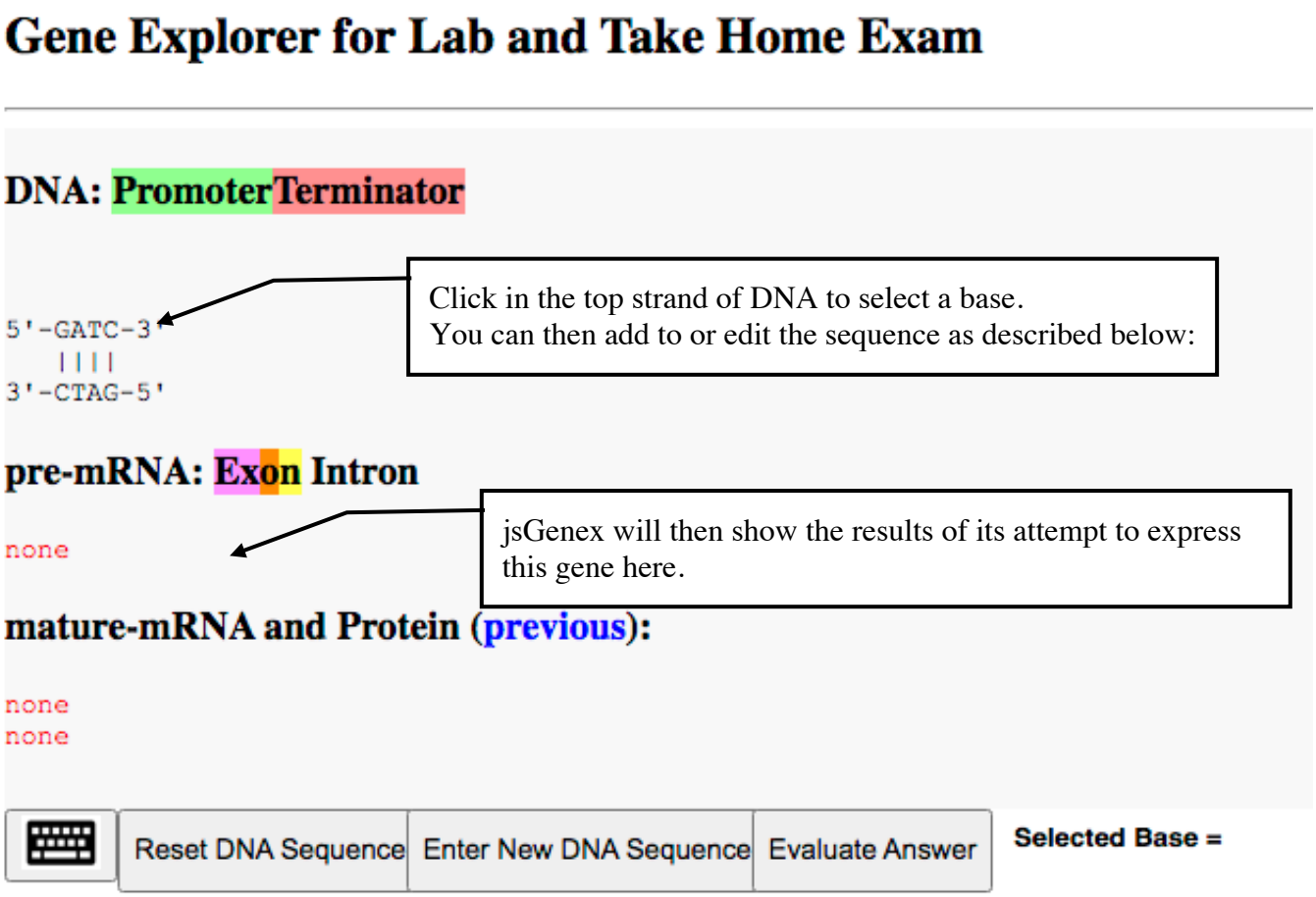
- Transcription produces an mRNA called a pre-mRNA that is not yet ready for translation.
- This pre-mRNA is then processed in several steps to produce the mature mRNA ready for translation:
    - The introns are removed and the exons are joined; this is called mRNA splicing. This is controlled by splice signal sequences. In real organisms, these sequences are not well known. In general, introns start with 5′-GU-3′ and end with 5′-AG-3′. In the hypothetical organism simulated by the Gene Explorer, introns start with 5′-GUGCG-3′ and end with 5′-CAAAG-3′.
    - A modified G nucleotide is added to the 5′ end of the mRNA; this is called the "cap." In the Gene Explorer, this is not shown.
    - Many A's are added to the 3′ end of the mRNA; this is called the poly(A) tail. In real organisms, as many as 400 A's can be added at a specific signal sequence; the Gene Explorer adds 13 A's as a tail to the 3′ end of any mRNA. Note that these A's do not correspond to T's in the DNA.

In previous problems, you did the work of expressing a gene by hand. Now that you are familiar with how these processes work, jsGenex will do all the tedious work of:

- Finding the promoter and terminator
- Reading the DNA sequence to produce the pre-mRNA
- Finding the splice sites
- Splicing and tailing the mRNA
- Finding the start codon
- Translating the mRNA

jsGenex will then allow you to make specific mutations in a gene sequence, and it will then calculate and display their effects on the mRNA and protein. You do not have to deal with all the details listed above; jsGenex will take care of it all. Researchers use tools like this to analyze the genes they are studying.

Launch jsGenex by clicking on the link in the OLLM. You should see something like this:



You can edit the DNA in several ways:

- To delete the selected base, use the "*delete*" or the "*backspace*" key.
- To replace the selected base with another base, type a *lowercase* letter (a, g, c, or t).
- To insert bases *to the left of the selected base*, type an *uppercase* letter (A, G, C, or T).

When you change the DNA sequence, the pre-mRNA, mature mRNA, and protein sequences are automatically updated. The *previous protein sequence*, the sequence of the protein before the latest change, is shown in blue for comparison purposes.

There are several useful buttons on jsGenex:

- (Button that looks like a keyboard) – Click this to bring up some buttons for entering and editing DNA – this works on tablets including the iPad.
- Reset DNA Sequence – Click this to reset the DNA to the starting gene sequence.
- Enter New DNA Sequence – Click this to enter a new DNA sequence.
- Evaluate Answer – this is not used

You should use what you know about gene expression to build a gene that expresses a small protein. You may find it useful to build your gene piece by piece like this:

1. Insert a promoter – it should show up in green and you should see a little mRNA being made.
2. Add a terminator – it should show up in orange.
3. Add a start codon – you should see a little protein being made
4. Add more codons to build a protein
5. Add a stop codon to control the C-terminus of the protein
6. Add an intron and see that it is spliced out properly and that you can change the sequence of the intron without changing the sequence of the protein

Preparing for the Take Home Exam

Take Home Exam 6 will be, in part, based on this lab. You should look at the exam on Blackboard before you leave lab today. You may want to use some of the remaining time in lab to prepare for the exam.

The Genetic Code

	U	C	A	G	
U	UUU **phe**	UCU **ser**	UAU **tyr**	UGU **cys**	U
	UUC **phe**	UCC **ser**	UAC **tyr**	UGC **cys**	C
	UUA **leu**	UCA **ser**	**UAA STOP**	**UGA STOP**	A
	UUG **leu**	UCG **ser**	**UAG STOP**	UGG **trp**	G
C	CUU **leu**	CCU **pro**	CAU **his**	CGU **arg**	U
	CUC **leu**	CCC **pro**	CAC **his**	CGC **arg**	C
	CUA **leu**	CCA **pro**	CAA **gln**	CGA **arg**	A
	CUG **leu**	CCG **pro**	CAG **gln**	CGG **arg**	G
A	AUU **ile**	ACU **thr**	AAU **asn**	AGU **ser**	U
	AUC **ile**	ACC **thr**	AAC **asn**	AGC **ser**	C
	AUA **ile**	ACA **thr**	AAA **lys**	AGA **arg**	A
	AUG met	ACG **thr**	AAG **lys**	AGG **arg**	G
G	GUU **val**	GCU **ala**	GAU **asp**	GGU **gly**	U
	GUC **val**	GCC **ala**	GAC **asp**	GGC **gly**	C
	GUA **val**	GCA **ala**	GAA **glu**	GGA **gly**	A
	GUG **val**	GCG **ala**	GAG **glu**	GGG **gly**	G