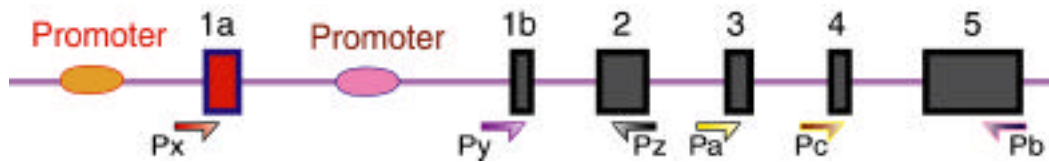


Bioc471a/571a Homework 6 - Due at the start of class October 16

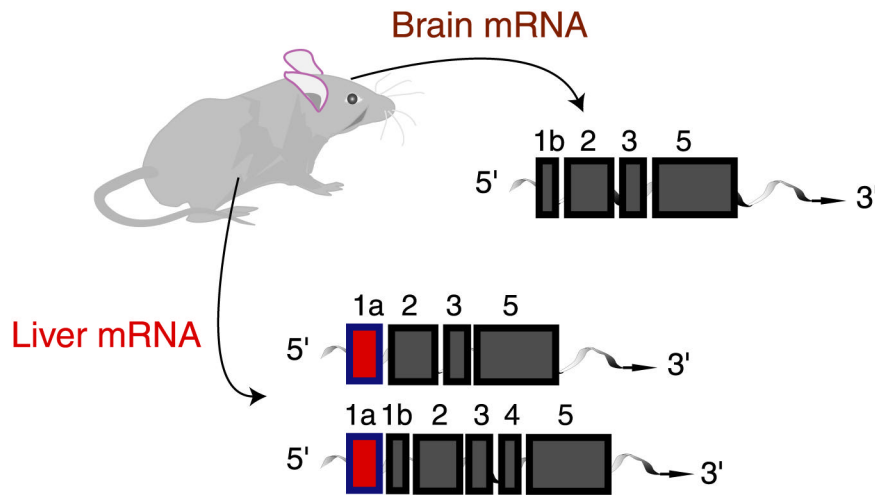
Lab Practicum Exercise - "Size Does Matter"

Background

A novel growth factor receptor (NGFR) gene had recently been identified in *C. elegans* using a genetic screen that targeted uncharacterized ORFs as a possible signal transducers. The orthologous NGFR mouse gene was subsequently isolated in the same lab by a technician. A graduate student then took on the task of studying the expression of NGFR in various mouse tissues to understand its role in mammalian physiology. Preliminary data obtained from genomic characterization of the NGFR gene revealed that *two promoters* were present, one of which is used exclusively in the liver. Moreover, Northern blots indicated that more than one transcript may be present in some tissues, presumably due to *alternative RNA splicing*. Based on sequence information derived from the NGFR genomic region, the student designed a number of PCR primers to use as diagnostic probes to characterize RT-PCR products produced from brain and liver RNA samples.



Six months later the student presented his work at the annual Research Symposium. The final slide shown below summarized the student's data based on his interpretations of his RT-PCR analysis.



Answer the following questions using the genomic map and the student's interpretation of his RT-PCR data.

1. (3 pts.) List all of the NGFR primer pairs that must have produced RT-PCR products using liver or brain RNA as the starting material. Which primer pairs would have generated multiple products when using liver or brain mRNA?

Primer pairs that will detect products: Px:Pz, Px:Pb, Py:Pz, Pa:Pb, Pc:Pb

Primer pairs that will detect multiple products in liver cells: Px:Pz, Px:Pb, Pa:Pb (brain NGFR is all one type of NGFR splice variant).

2. (2 pts.) Describe an additional primer pair that could be designed to determine the relative combined amounts of NGFR mRNA in brain and liver, i.e., a primer pair that would detect all known splice variants in these two tissues. What possible explanations would there be if this control primer pair generated two products using an RNA preparation prepared late on a Friday night, but not when a similar RNA sample was used from another preparation?

Any primer pair that spans exons 2 and 3 would work because all NGFR transcripts contain these two exons. A primer pair within a single exon, e.g., exon 5, would not be as good because the PCR product could originate from genomic DNA contamination.

If two products were observed with this new primer pair using one RNA prep, but not with a similar RNA prep made on another day, it would most likely be due to contamination of the sample, e.g., genomic DNA contamination that would show up as a larger PCR product containing intronic sequences. Primary transcripts that represent unspliced RNA (hnRNA) could presumably show the same result, nevertheless, it would represent an aberrant result since preps on different days gave different results).

Note that if the RT-PCR reaction used RTase in the first step, rather than a thermophilic polymerase (rTt) for both cDNA synthesis and amplification, it would be possible to distinguish between mRNA and DNA templates by showing that the larger PCR product was generated independent of RTase, i.e., not derived from cDNA.

3. (2 pts.) PCR assays can sometimes lead to artifacts due to its incredible sensitivity and specificity. Briefly describe two other RNA based assays that could be performed to corroborate the RT-PCR data.

RNA assays that are able to detect predictable splicing patterns and are RT-PCR independent:

***Northern blots* using specific oligonucleotide probes spanning unique exon junctions.**

***RNase protection assays (RPA)* that use specific cRNA probes to detect unique exon junctions.**

***In situ hybridization* using specific oligonucleotide probes spanning unique exon junctions.**

4. (3 pts.) After the seminar, the Department Head asked the student if he had any functional data that would address the bigger question of how do these three NGFR proteins differ in biological activity. The student answered, "Actually, my advisor doesn't want me talk about it until the patent is filed, but I can tell you confidentially that exon 4 encodes a portion of the receptor that distinguishes between two well characterized physiological ligands." Describe a set of experiments he most likely performed to make this discovery. (hint: he first had to get his hands on some NGFR cDNA)

The best way would have been to isolate cDNA clones all three of the spliced variants and to express them in a mouse cell line that lacked endogenous NGFR protein expression. The two liver NGFR cDNA clones would differ by both the absence/presence of exons 1b and 4, whereas, the brain transcript is identical to one of the liver transcripts with regard to exon 4 but they differ by the use of promoter 1a or 1b. By comparing the ligand binding activities of all three protein products, he could infer that exon 4 was the determining function for ligand discrimination. However, to test this more directly, he could create two NGFR proteins, one containing the 1a-2-3-4-5 combination, and the other 1b-2-3-4-5, both of which only differ by the use of exons 1a or 1b, and more importantly, differ from naturally occurring splice variants by the inclusion of exon 4. He must have had prior knowledge to predict which ligands to test!