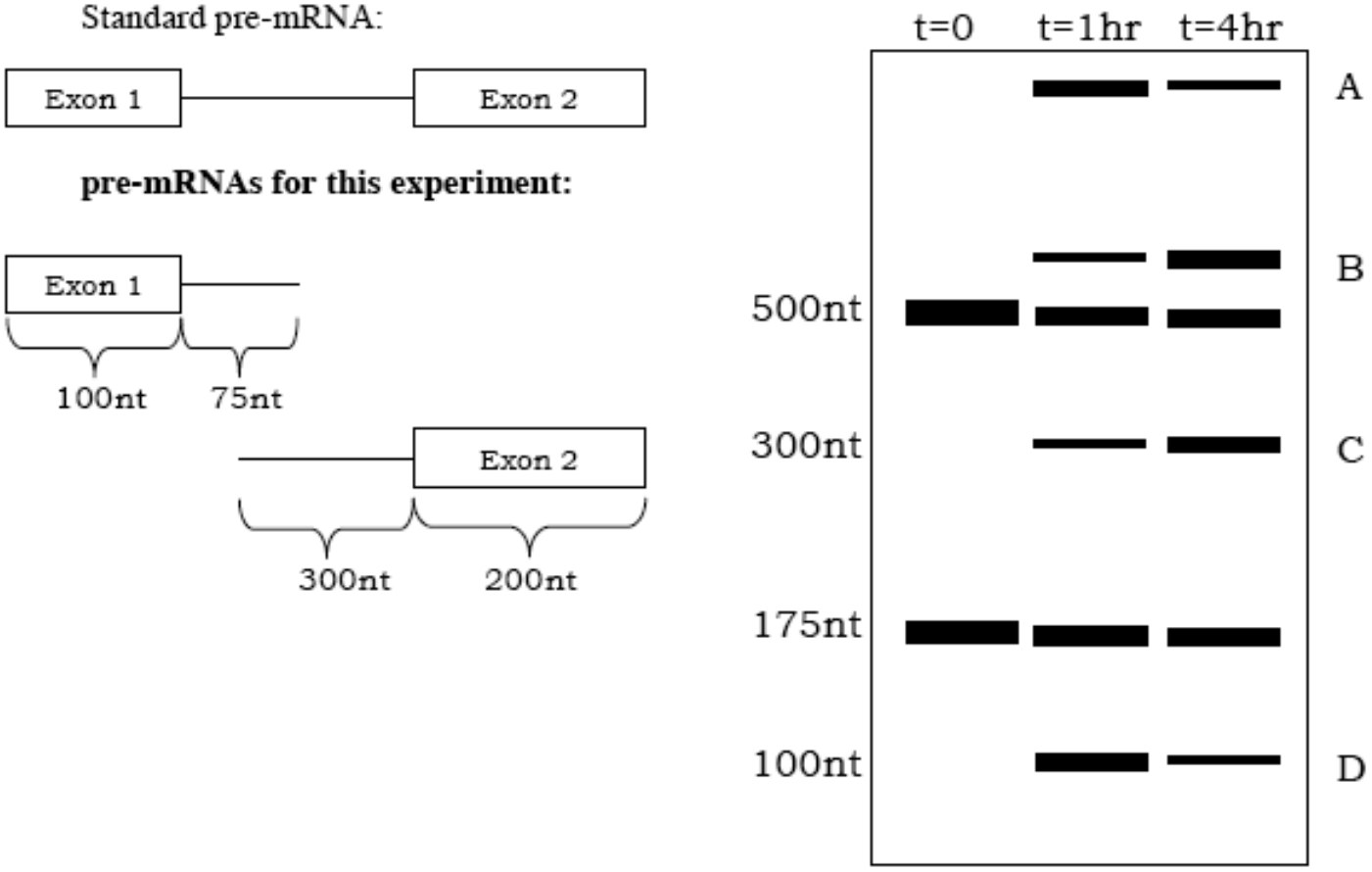
Name:_____
Biology 312 Problem Set 4

Pre-mRNA splicing is often studied in vitro by incubating a uniformly-32P labeled premRNA substrate with a nuclear extract that contains all of the components essential for splicingto occur. After an appropriate incubation time the RNA is recovered and analyzed on a denaturing polyacrylamide gel.

To study this process further, a researcher took the pre-mRNA substrate and split it into two uniformly-32P labeled pre-mRNA substrates show below. One of these pre-mRNAs contains Exon1, the 5' splice site, and some intron sequences. The other pre-mRNA contains the intron sequences that contain the branch site, the polypyrimidine tract, the 3'splice site and exon 2. In this researcher's experiment, excess amounts of these two pre-mRNA species were incubated with the nuclear extract for differing amounts of time after which the RNA species were isolatedfrom the extract and analyzed on a gel. The resulting autoradiograph of the gel is shown below.



1. Four new RNA species (A,B,C, and D) appear after the two pre-mRNAs are incubated in the nuclear extract. What do they represent? In the space below, draw the structures of the four new RNAs using boxes for exons and lines for introns. Make sure to label the exons in your drawing.

The two starting products have all the requirements for intron splicing to occur, the only difference between the two component system and splicing of the standard pre-mRNA is that the two component system will splice in trans since the intron is not continuous.

2. Why do bands A and D decrease in concentration from 1 hour to 4 hours, while bands B and C increase?