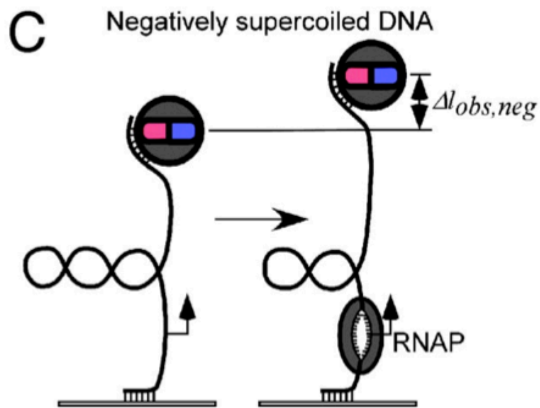
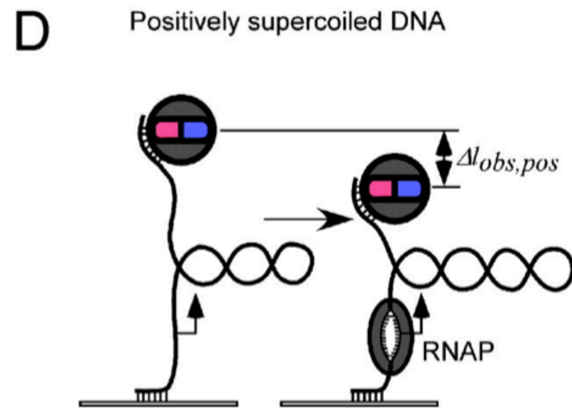
DNA replication
Transcription (magnetic bead paper)
Translation

(Lab Practicum)
PCR/DNA digest

5’-ATG AAA AAG ACC AAA ATT GTT TGC ACC ATC GG3’
5'- TTA CAG GAC GTG AAC AGA TGC GG -3'

Solution Calculation
429 uL BPB
2571 uL water 0.86 A_{595}





Name:_______________________________________ Lab Practicum (Exam 2)

Design primers that could be used to amplify the pyruvate kinase I gene from *E. coli str. K-12 substr. MG1655* during a PCR reaction. Below are the instructions provided on your homework. Note that you want to amplify the pyruvate kinase I gene not the alkaline phosphatase gene.

1. Find the sequence of the gene of interest. In this case, we are interested in the *phoA* gene from *E. coli* K-12 MG1655.
    a. Go to the Pubmed website.
    b. Search for your gene of interest in the "Gene" database. Choose the database from the pull-down tab next to the search box.
    c. Search through the results for the gene of interest from the organism we are using. You can limit the results by using the "Organisms Tree" found on the right panel.
    d. Select the "GeneBank" link under *Genomic regions, transcripts, and products*.
    e. Scroll through all the information available to you.
    f. At the bottom of the page, you'll find the DNA sequence of the phoA gene.
2. You will need both a forward and a reverse primer for your PCR reaction. Each primer should be 16 to 35 bases in length. It is best for your primer to end (3') with at least two "G" or "C" bases.
3. Go to Integrated DNA technologies' website. This company chemically synthesizes primers.
    g. Under *SciTool*s you'll find the *OlogoAnalyzer* application
    h. Analyze each primer sequence separately. Take note of the calculated melting temperature of each primer.
    i. Keep adding gene sequence to each primer until the melting temperature of the two primers are close to each other and around 60°C.

Primer 1: 5'____3'

Primer 2: 5'____3'

Use the NEBcutter V2.0 program to determine the number of times that each of the following DNA endonucleases will cleave the pyruvate dehydrogenase I DNA sequence. Determine the length of the resulting DNA fragments in basepairs.

EcoRI: _______(cut sites)_______________________________(fragment lengths)

XhoI: ________(cut sites)_______________________________(fragment lengths)

XbaI: ________(cut sites)________________________________(fragment lengths)

You will find a tube of stock 7x Bromophenol blue ($C_{19}H_{10}Br_4O_5S$; 669.98 g/mole).

Prepare 3 mL of a 1x Bromophenol blue (BPB) solution in a small glass test tube using dH_2O as a solvent.

____________________uL of 7x BPB
____________________uL of dH_2O
(total volume 3 mL)

Using dH_2O as a blank, determine the absorbance of your sample at 595 nm with an OceanOptic spectrophotometer.

____________________ AU_{595}

$$1\%\left(\frac{w}{v}\right)\ \text{BPB} = \frac{1\ g\ \text{BPB}}{100\ \text{mL solution}} = 970\ \text{AU}_{595}$$

What is the concentration of BPB in your 1x solution in units of grams per liter?

____________________ $\frac{\text{g BPB}}{\text{L solution}}$

What is the concentration of Bromophenol blue in the 7x solution in units of grams per liter?

____________________ $\frac{\text{g BPB}}{\text{L solution}}$

What is the concentration of Bromophenol blue in your 1x solution in units of Molar?

____________________M BPB

NAME:____

1. The following figures were presented in Revyakin *et al.* (2004). Promoter unwinding and promoter clearance by RNA polymerase: Detection by single-molecule DNA nanomanipulation.

Figure 1:

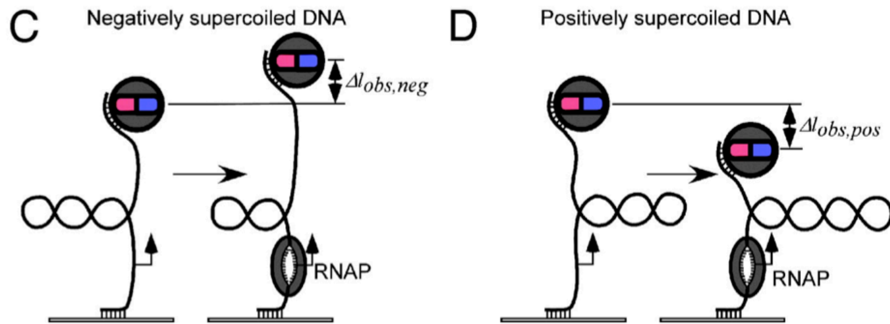
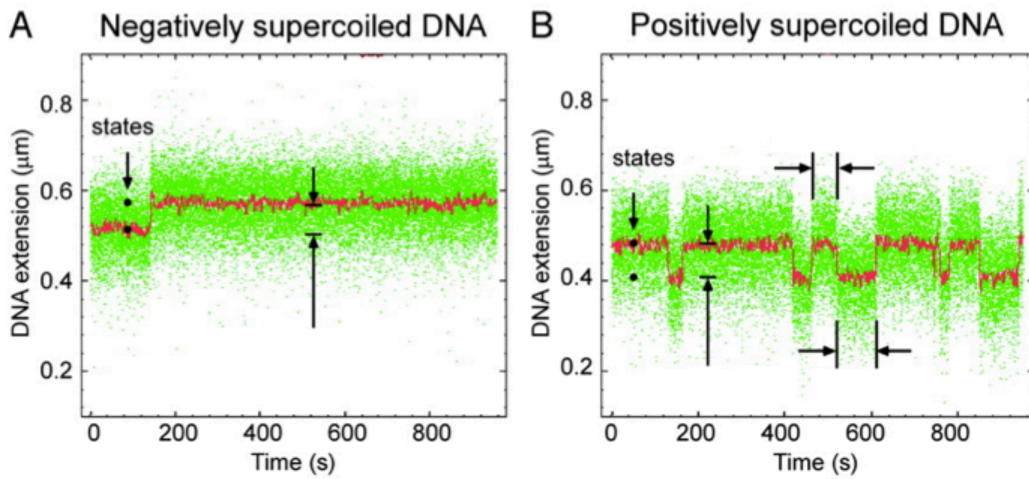
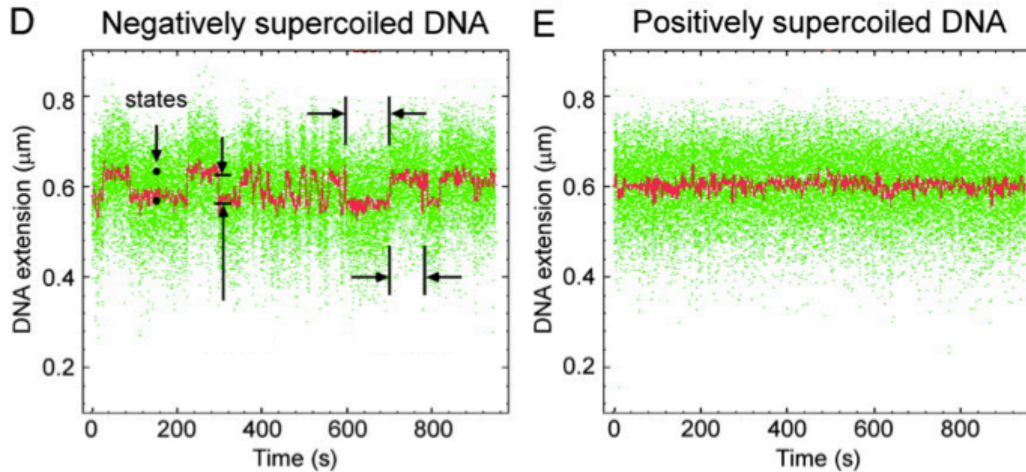


Figure 2:



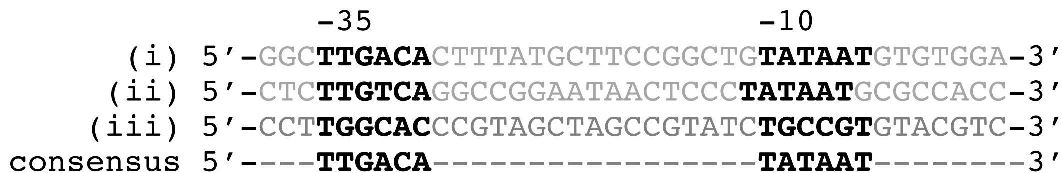


The data presented in Figures 2A and 2B were collected at Promoter Y, while the data presented in Figures 2D and 2E were collected at Promoter S.

A. **For the negatively supercoiled experiments (Figures 2A and 2D), at which promoter (Y or S) does the RNA polymerases spend more time in the open complex (RP_O)?**

B. **For the positively supercoiled experiments (Figures 2B and 2E), at which promoter (Y or S) does the RNA polymerases spend more time in the open complex (RP_O)?**

C. **Identify the sequence of Promoter Y (i, ii, or iii) and Promoter S (i, ii, or iii) that is consistent with your above observations.** The consensus sequence is provided for comparison.



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                  -35                            -10
      (i) 5'-GGCTTGACACTTTATGCTTCCGGCTGTATAATGTGTGGA-3'
     (ii) 5'-CTCTTGTCAGGCCGGAATAACTCCCTATAATGCGCCACC-3'
    (iii) 5'-CCTTGGCACCCGTAGCTAGCCGTATCTGCCGTGTACGTC-3'
consensus 5'---TTGACA-----------------TATAAT--------3'
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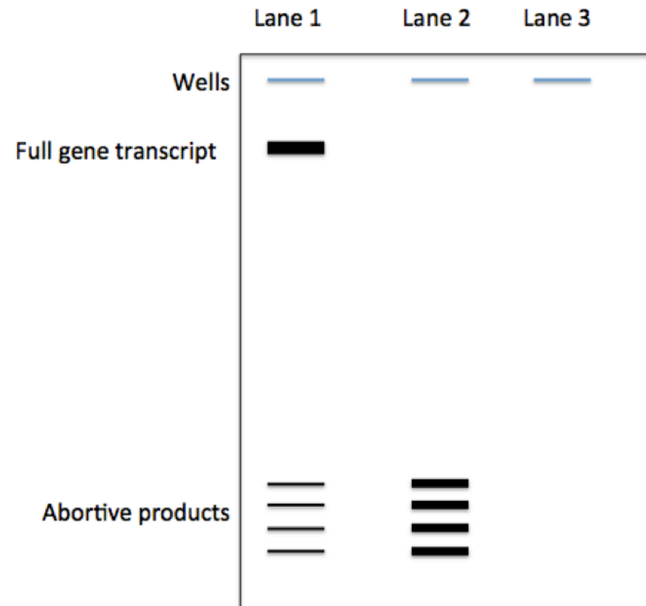
In the gel image below, increasing amounts of RNA product are indicated by wider/thicker bands.



D. Indicate the polarity of the applied electric field used to resolve the RNA products in the above gel.

E. Which lane on the above gel (1, 2, or 3) would result if nucleotide triphosphates were added to the initiation complexes (open complexes) formed at Promoter Y? Explain your answer.

F. Which lane on the above gel (1, 2, or 3) would result if nucleotide triphosphates were added to the initiation complexes (open complexes) formed at Promoter S? Explain your answer.

2. PCR is utilized by forensic chemists to compare small but unique regions of the human genome for identification purposes. Forensic samples submitted for this DNA typing analysis may originate as blood spatter at a crime scene. Human blood contains high amounts of the protein hemoglobin that binds four heme molecules (porphyrin rings). The heme molecules of hemoglobin bind Iron(II) and Iron(III) natively but are also capable of binding Magnesium ions. Forensic chemists have observed that hemoglobin/heme molecules from blood will inhibit (i.e. prevent) a PCR experiment. **Propose a mechanism for the inhibition of PCR reactions by hemoglobin/heme.** Provide mechanistic detail to backup your explanation. There is more than one correct answer; you need one.

3. The *phoA* gene is 1416 bp long. **How many ATP equivalents (i.e. ATP, GTP, CTP, and/or UTP) are hydrolyzed during the processes of transcription and translation to produce the alkaline phosphatase protein from the information stored in the gene?** Explicitly itemize the step of utilization of each equivalent in the table below.

Step	# of ATP Equivalents