Note: Do you own work.

Note: Have fun!

Question 0 (5 points). What is your name? ________________________________.

Question 1 (30 points). Synthesis Sam claims to have invented a new type of glass bead for use in de novo chemical synthesis of DNA oligonucleotides. In particular, he claims that the beads allow 10,000 copies of the same oligo to be synthesized on a single bead. Sam also claims that you can use these beads to synthesize oligos up to 1,000 nucleotides long, using standard phosphoramidite chemistry with a per base coupling efficiency of 99%. You have an exciting research idea that requires direct synthesis of a 1,000 bp DNA fragment. You don't care about overall synthesis yield, you only need to get a single copy of the full-length (i.e., 1000 bp) molecule.

Should you buy any of these fancy new beads from Sam? (Yes/No).

Please show your analysis or reasoning if you would like to be eligible for any partial credit.

Answer.

No.

As a rough calculation...

(0.99)^1,000 = 4.3E-5 <-- likelihood of any one oligo being full length

*4.3E-5 * 10,000 = 0.43 <-- approx. likelihood of one correct oligo per single bead*

Multiple beads might help, but would still be expensive and inefficient.

Likely a better decision to obtain smaller fragments from which to assemble a longer construct.

Question 2 (25 points). It's wartime, Coraline Coder has just sent you a message encoded via a double-stranded DNA sequence along with a partially completed sentence. What does she want you to do?

5'-cga gct gat gcg cgg-3'
3'-gct cga cta cgc gcc-5'

<Your Name Here>, Don't be a ___________! Send us the plans for the new ___________.

(Hint. Use the standard genetic code and single letter amino acid abbreviations.)

Answer.

Reading the top strand in the 5' -> 3' direction gives R-A-D-A-R.

Reading the bottom strand of the same sequence in the 5' -> 3' direction gives P-R-I-S-S.

Note that bottom strand is read CCG CGC CTA... (not GCC, CGC, CTA...)

		2nd base			
		U	C	A	G
1st base	U	UUU (Phe/F) Phenylalanine	UCU (Ser/S) Serine	UAU (Tyr/Y) Tyrosine	UGU (Cys/C) Cysteine
		UUC (Phe/F) Phenylalanine	UCC (Ser/S) Serine	UAC (Tyr/Y) Tyrosine	UGC (Cys/C) Cysteine
		UUA (Leu/L) Leucine	UCA (Ser/S) Serine	UAA Ochre (*Stop*)	UGA Opal (*Stop*)
		UUG (Leu/L) Leucine	UCG (Ser/S) Serine	UAG Amber (*Stop*)	UGG (Trp/W) Tryptophan
	C	CUU (Leu/L) Leucine	CCU (Pro/P) Proline	CAU (His/H) Histidine	CGU (Arg/R) Arginine
		CUC (Leu/L) Leucine	CCC (Pro/P) Proline	CAC (His/H) Histidine	CGC (Arg/R) Arginine
		CUA (Leu/L) Leucine	CCA (Pro/P) Proline	CAA (Gln/Q) Glutamine	CGA (Arg/R) Arginine
		CUG (Leu/L) Leucine	CCG (Pro/P) Proline	CAG (Gln/Q) Glutamine	CGG (Arg/R) Arginine
	A	AUU (Ile/I) Isoleucine	ACU (Thr/T) Threonine	AAU (Asn/N) Asparagine	AGU (Ser/S) Serine
		AUC (Ile/I) Isoleucine	ACC (Thr/T) Threonine	AAC (Asn/N) Asparagine	AGC (Ser/S) Serine
		AUA (Ile/I) Isoleucine	ACA (Thr/T) Threonine	AAA (Lys/K) Lysine	AGA (Arg/R) Arginine
		AUG (Met/M) Methionine, *Start* [A]	ACG (Thr/T) Threonine	AAG (Lys/K) Lysine	AGG (Arg/R) Arginine
	G	GUU (Val/V) Valine	GCU (Ala/A) Alanine	GAU (Asp/D) Aspartic acid	GGU (Gly/G) Glycine
		GUC (Val/V) Valine	GCC (Ala/A) Alanine	GAC (Asp/D) Aspartic acid	GGC (Gly/G) Glycine
		GUA (Val/V) Valine	GCA (Ala/A) Alanine	GAA (Glu/E) Glutamic acid	GGA (Gly/G) Glycine
		GUG (Val/V) Valine	GCG (Ala/A) Alanine	GAG (Glu/E) Glutamic acid	GGG (Gly/G) Glycine

Question 3 (40 points). Your local Laboratory for Total RNA Dominance has issued a challenge to all Stanford students!

"Sketch out the design of an engineered riboswitch that implements the following logical operation: So long as neither molecules D or E are present, and only if molecules A and B, or molecule C alone, is present then turn on the expression of green fluorescent protein."

Your friend, a computer science major, translated their challenge into a logical statement:

IF (((A and B) or C) not (D or E)) then GREEN

Sketch out the architecture of a riboswitch system that implements this 5-input logic gate. Be sure to clearly indicate the default state of any ribozyme actuators (i.e., activity in the absence of any input molecules) that you use.

(Hint: You are free to use any of the 3 integration schemes that we discussed in class, alone or in combination)

Answer.

Multiple solutions possible. One solution shown below. Red star indicates riboswitch with ribozyme core that is active in the absent of input ligands, and that mRNA will be cut and degraded by default. Green star indicates switch that is inactive, that mRNA will not be cut. Sensor A and B must both be bound by ligand to inactivate the first switch. Binding of A and B or C will inactivate the first switch. Either binding of D or E will activate their respective ribozyme cores, leading to cutting and degradation of mRNA.