

1. (4 pts) What is the main difference in the source of an siRNA versus a miRNA?

siRNAs come from an exogenous source, whereas miRNAs are endogenously encoded.

How do these different noncoding RNAs (ncRNAs) affect mRNAs?

siRNAs lead to mRNA cleavage, while miRNAs usually block translation (although they can also lead to cleavage or degradation).

2. (6 pts) During RNAi, explain how the double-stranded RNA is processed and how it leads to the silencing of a complementary mRNA. (Divide your answer into three basic steps).

Step 1: **The double-stranded RNA is processed by Dicer into small RNA fragments (siRNA).**

Step 2: **The siRNA associates with a complex called the RNA-induced silencing complex (RISC).**

Step 3: **The RISC complex binds to a complementary mRNA, leading to mRNA degradation.**

3. (4 pts) What do restriction enzymes recognize?

specific DNA sequences (often palindromic sequences)

What type of chemical bond does a restriction enzyme cleave?

covalent bond in each of the two DNA strands

4. (3 pts) Why is the use of a heat-stable DNA polymerase important to the success of PCR?

The first step of the reaction requires that the solution be heated to $> 90^{\circ}\text{C}$ to separate the two strands of DNA. Most enzymes are denatured at this temperature. With the use of a heat-stable polymerase, the enzyme can be added at the beginning of the reaction and will function throughout multiple cycles.

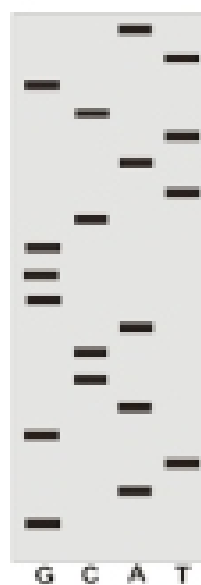
5. (3 pts) If you start with a single copy of DNA, how many PCR cycles would you need to run in order to have over 10,000 copies of the DNA? Assume 100% efficiency in your reaction.

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6. (2 pts) What happens when a dideoxynucleotide is incorporated into a growing DNA strand?

The strand can no longer grow (chain termination).

7. (5 pts) Write the FIRST FIVE NUCLEOTIDES (from 5' to 3') of the sequence of the DNA strand synthesized during the DNA sequencing experiment shown in the following gel. (Note: You will receive zero points for this question if you include more than five nucleotides in your answer.)



GATGA

7. (4 pts) List two methods that can be used to introduce a gene of interest into plant cells.

1) **agrobacterium infection (of Ti plasmid carrying the gene of interest)**

2) **biolistic transformation (gene gun) of the gene of interest**

8. (3 pts) Rank these three restriction endonucleases from best to worst with regard to the efficiency of binding their ends during a ligation experiment. List them in order, using ">" and "=" when appropriate.

Source	Restriction enzyme	Recognition site
<i>Escherichia coli</i>	EcoRI	5' GAATTC 3' 3' CTTAAG 5' ↓ ↑
<i>Bacillus amyloliquefaciens</i>	BamHI	5' GGATCC 3' 3' CCTAGG 5' ↓ ↑
<i>Haemophilus influenzae</i>	HindIII	5' AAGCTT 3' 3' TTCGAA 5' ↓ ↑

The symbols † and ‡ indicate where the DNA is cut.

BamHI = HindIII > EcoRI

9. (3 pts) Describe what it means for a crop to be "Roundup Ready."

It means that the plants have been genetically engineered to express an enzyme that allows them to break down the active ingredient (glyphosphate) in the herbicide called Roundup. This way, when farmers spray with Roundup, only the weeds will be killed, but the Roundup Ready crops will be resistant.