

Name: _____ Student ID: _____

1) Plasmids, viruses and transposons often capture (clone) genes & these genes benefit the host. Briefly, why do you think these genes are maintained on plasmids? ($\frac{1}{2}$ pt.)

For efficiency—bacteria with small genomes can grow faster than ones with very large genomes. See reader pg. 112

2) R-factor plasmids are conjugative plasmids that encode proteins that inactivate antibiotics. In less than 3 sentences, please list the three mechanisms of inactivation carried out by enzymes? ($\frac{1}{2}$ pt.)

- 1) Degrade the antibiotic
 - 2) Modify the antibiotic so its inactive
 - 3) Export the antibiotic
- See reader pg. 113

3) DNA restriction enzymes were discovered as a result of the study of bacteriophage lambda. Briefly explain 2 reasons why restriction nucleases do not cut up newly-made DNA? ($\frac{1}{2}$ pt.)

In order to be cut, DNA must usually be double stranded and completely unmethylated at the recognition sequence. Note that newly made DNA is hemi-methylated.

4a) Digesting DNA with restriction enzymes often yields multiple fragments of DNA. How can one visualize this DNA once it has been cut?

Stain with ethidium bromide (EtBr) which intercalates between the strands. Run it out on a gel and use UV light to detect fluorescence.

b) How can you know that you got the correct sized fragment? ($\frac{1}{2}$ pt.)

Run a ladder along side your sample on the gel --MW standards.

5) Recombinant DNA technology revolutionized the biotechnology industry. The first cloning experiment using *Xenopus* DNA was a giant breakthrough, why? ($\frac{1}{2}$ pt.)

See reader pg. 129

6) Please explain in less than 2 sentences, what reporter genes are? Name a commonly used reporter gene and why it is so useful. ($\frac{1}{2}$ pt.)

Reporter genes are fused to cloned DNA in order to monitor transcription and translation. LacZ is a commonly used reporter gene which encodes B-gal and when it hydrolyzes the dye-coupled B-gal, x-gal, it turns blue.

See reader pg. 139

7) The GFP gene was isolated from the jellyfish *Aequorea victoria*. Briefly explain one way in which this gene is used and how it is visualized? ($\frac{1}{2}$ pt.)

See reader pg. 140.

Turns bright green when it is irradiated with UV or blue light.

8) Briefly explain why most transposable elements encode transposase genes with promoters that deviate from the consensus sequence of all known promoters? ($\frac{1}{2}$ pt.)

Promoters that deviate from the consensus are poor promoters. Transposable elements create mutations every time they transpose so it is important for them to keep the rate of transposition low, so they don't kill their hosts.

9) Colony blotting is very similar to Southern blotting. Name two ways in which it is different? ($\frac{1}{2}$ pt.)

See reader pg. 147

10) Sanger DNA sequencing, site-directed mutagenesis and PCR are three important techniques based on the Klenow fragment. Name three things all of these techniques require. ($\frac{1}{2}$ pt.)

- 1) ss DNA template
- 2) DNA primer complementary to template
- 3) Annealing of the primer to the template

11a) PCR is a very powerful technique used in labs daily. In one sentence, what is the main thing that it accomplishes?

Allows DNA to be made at an exponential rate

b) List & briefly explain two problems with PCR ($\frac{1}{2}$ pt.)

- 1) error prone
- 2) contamination
- 3) use fee