

Exam 2 Spring 10 Key

Short Answer Questions (4 points each)

1. State 4 reasons why pigs are good candidates for producing organs for Xenotransplant into humans.
 - **Their organs are approximately the same size as humans**
 - **Compared to primates, they carry few diseases that afflict humans.**
 - **They are cheap to grow**
 - **They have large litters**
 - **They mature quickly**

2. Describe the initiation of translation in Eukaryotes.

The initiator tRNA (carrying methionine) binds to the small ribosomal subunit to form the initiation complex. This complex binds to the 5' cap of the mRNA and then scans 5' to 3' in search of the first AUG codon. The anticodon of the initiator tRNA basepairs with the AUG codon. The Large ribosomal subunit binds to form the whole, functional ribosome. A new aminoacyl tRNA enters and binds at the A site. A peptide bond is formed between the methionine and the A site amino acid. Initiation is now complete.

3. Transgenes can be introduced into animals by several methods. Describe the “DNA microinjection approach” to producing a transgenic mouse

A mouse egg is fertilized in vitro to produce a zygote. The transgene is injected into the male pronucleus of the zygote. The zygote is allowed to develop in vitro into a blastocyst, which is then inserted into the uterus of a surrogate mother mouse. The surrogate gives birth to transgenic mice.

4. Briefly describe the lytic cycle of bacteriophage that infect E. coli.

Phage bind to the cell wall of E coli bacteria and then inject their DNA into the cell. The viral DNA is replicated, transcribed and translated to produce new viral genome and new viral proteins, which self-assemble into new phage particles. Viral enzymes lyse the bacterial cell releasing new phage, which then infect neighboring cells where the cycle continues.

5. How does a nascent polypeptide get directed to the endoplasmic reticulum and then threaded into the lumen of the endoplasmic reticulum?

The signal recognition protein (SRP) binds to the N-terminal ER signal sequence on the nascent polypeptide. The SRP directs the nascent polypeptide (and bound ribosome/mRNA) to the ER where it binds to a receptor on the ER membrane. The polypeptide is threaded through a translocator protein and into the lumen of the ER. When translation is complete, the signal sequence is cleaved and the polypeptide is released into the ER.

6. What is the mechanism of polyadenylation? In other words, how is a polyA tail placed on to the 3' end of a eukaryotic mRNA

The cleavage and polyadenylation specificity factor (CPSF) binds to the polyadenylation signal sequence and cleaves off the 3' end of the mRNA. PolyA polymerase then adds a long chain of adenine nucleotides to the 3' end of the mRNA.

7. Transducing phage can be used to map the linear order of E. coli genes. Consider the following cross:

The genotype of the donor bacteria is gly+, trp+, phe+

The genotype of the recipient bacteria is gly-, trp-, phe-

Donor bacteria are infected with phage.

Progeny phage are purified and used to infect recipient bacteria.

Recipient bacteria are plated on media that lacks glycine (gly); 100 colonies grow.

The gly+ recombinant colonies are then replica plated on media that lacks both glycine and tryptophan (trp); 15 colonies grow

The ala+ recombinant colonies are also replica plated on media that lacks both glycine and phenylalanine (phe); 45 colonies grow.

- Which gene, trp or phe, is closer to the gly gene on the E. coli chromosome?

Phe is closer.

- These data support 2 alternative orders for the gly, trp, and phe genes on the E. coli chromosome. Draw the 2 alternative orders that these data support.

Try Phe Gly or Try Gly Phe

Next, the recipient bacteria are plated on media that lacks tryptophan; 100 colonies grow.

The trp+ recombinant colonies are then replica plated on to media that lacks both tryptophan and phenylalanine; 40 colonies grow

The trp+ recombinant colonies are also replica plated on to media that lacks both tryptophan and glycine; 15 colonies grow

Based on these data, what is the linear order of gly, trp, and phe genes on the E. coli chromosome?

Try Phe Gly

Essay Question

Describe how a gene might be inserted into a plasmid to yield a recombinant plasmid. Next, describe how you could transform *E. coli* bacteria with this plasmid and select for those cells that take up the plasmid (transformed cells).

A DNA sequence of interest (target DNA) can be inserted into a plasmid and propagated in a host cell. The procedures for doing so are as follows:

- a) Cut the source DNA (contains the target DNA) with the appropriate restriction enzyme. Linearize the plasmid with the same restriction enzyme.
- b) Mix the two DNAs in the presence of ligase. The sticky ends of the DNA molecules will anneal and ligase will form covalent bonds, thus linking the target DNA to the plasmid.
- c) Host cells are transformed with the recombinant plasmid. Transformation efficiency is very low, less than 0.01 % of cells will take up the DNA.
- d) In order to select those cells that contain the plasmid (transformed cells) from those cells that do not, the cells are grown in the presence of an antibiotic to which the host cell is sensitive. The plasmid contains a gene that confers resistance to the antibiotic. Only cells with plasmids survive.
- e) Surviving cells replicate to form colonies. Each colony is a clonal population of cells that possesses the target DNA.