Name\_\_\_\_\_\_\_\_\_KEY\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ UT EID\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

# BIO 330

**Exam 2**

**April 5, 2011**

Please read each question carefully before answering. Many questions have multiple parts. There are 9 questions on 4 pages.

1. Several virus families that we have studied encode proteins with signal peptides. Name two. (5 points)

Explain whether or not signal recognition particle (SRP) would participate in transport of a viral protein across the mitochondrial membrane (4 points)

1. Describe how rhabdoviruses generate a polyA tail on their mRNAs. (6 points)

Why doesn’t leader RNA need to be polyadenylated? (5 points)

1. What is pseudotyping and why is it useful? (5 points)

What is the maturational cleavage for togaviruses? (5 points)

1. Several patients with high fevers and neurological symptoms and are suspected to have a viral infection. You isolate this virus (called NEURO1). You decide to isolate temperature-sensitive (ts) mutants of this virus. Why do you avoid leaky mutants from your analysis? (5 points)

The resulting ts mutants were then used for recombination experiments in susceptible cells. You obtain the following results.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Yield at permissive temperature (PFU/ml)** | | | | |
| **Mutant** | **Ts1** | **Ts2** | **Ts3** | **Ts4** | **Ts5** |
| Ts1 | 5.0 X 106 | 3.5 X 107 | 2.3 X 107 | 3.9 X 107 | 1.9 X 107 |
| Ts2 | -- | 1.2 X 107 | 4.5 X 107 | 1.5 X 107 | 3.4 X 107 |
| Ts3 | -- | -- | 6.3 X106 | 2.9 X 107 | 1.8 X 107 |
| Ts4 | -- | -- | -- | 7.5 X 106 | 3.6 X 107 |
| Ts5 | -- | -- | -- | -- | 3.8 X 106 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Yield at non-permissive temperature (PFU/ml)** | | | | |
| **Mutant** | **Ts1** | **Ts2** | **Ts3** | **Ts4** | **Ts5** |
| Ts1 | 2.3 X 103 | 6.0 X 106 | 6.8 X104 | 6.6 X106 | 3.2 X 106 |
| Ts2 | -- | 4.5 X 103 | 7.7 X106 | 4.8 X104 | 1.0 X 105 |
| Ts3 | -- | -- | 7.8 X 103 | 4.9 X 106 | 3.1 X 106 |
| Ts4 | -- | -- | -- | 5.9 X 103 | 1.1 X 105 |
| Ts5 | -- | -- | -- | -- | 1.0 X 104 |

Draw a map of these mutants. Show your calculations and your rationale. (5 points) What is the likely number of gene represented by these mutants? (2 points)

Why do you need to multiply the results of recombination tests by 2? (3 points)

Why do you subtract results of single mutant infections at the non-permissive temperature? (5 points)

1. You label the cells infected by NEURO1 with 35S-methionine and analyze the size and number of virus-specific polypeptides by polyacrylamide gel electrophoresis. You decide to test TEX1 for its ability to be mapped using pactamycin. How does pactamycin work? (5 points)

You infect cells with NEURO1 and divide them into two tissue culture plates. You add pactamycin to one plate, and you use the other plate as an untreated culture. After an hour at 37oC, you add medium containing radioactive amino acids to each plate. After incubation for an additional hour, you lyse the pactamycin-treated and untreated cultures and subject them to polyacrylamide gel electrophoresis. Each gel is then analyzed, and you determine the percentage of radioactivity present in each viral polypeptide. You obtain the following results.

|  |  |  |
| --- | --- | --- |
|  | **Percentage of total radioactivity** | |
| **Polypeptide** | **Untreated cells** | **Pactamycin-treated** |
| A | 17 | 17 |
| B | 23 | 23 |
| C | 14 | 14 |
| D | 25 | 25 |
| E | 7 | 7 |
| F | 14 | 14 |

Assuming that you have verified that the pactamycin is working in a control experiment, what is your conclusion? Explain. (5 points)

1. What is the function of an IRES sequence? (5 points)

In the experiment to evaluate the effect of the IRES sequence on translation of chloramphenicol acetyl transferase (CAT) mRNA, what is the purpose of adding Actinomycin D after poliovirus infection? (3 points)

In this same experiment, why does the translation of a CAT mRNA lacking an IRES decline in a mammalian cell infected with poliovirus? (5 points)

1. One of the mRNAs from NEURO1 has the following structure:

m7G -5’ UTR----AUG—signal peptide----stop transfer signal---stop codon ---3’ UTR-polyA

What would this mRNA encode? Give your reasoning. (5 points)

What family of viruses have we studied that could encode this mRNA? Explain. (5 points)

1. Why would viruses transcribe separate mRNAs for structural and nonstructural proteins? (5 points)

Is the flavivirus genomic RNA translated on membrane-bound or free polyribosomes? Justify your answer. (4 points)

1. A togavirus ts mutant synthesizes minus strand RNA after 8, 10 or 12 hours at the non-permissive temperature, but it does not make positive stranded RNA. What could be the defect in this mutant? Give your rationale. (4 points)

Give an example of a *cis-*acting sequence and its function on the 26S togavirus RNA. (4 points)