Genome 466 Midterm Answer Key

1A. Infect adrenal cortical cells with virus from mosquito and with other viruses and see if it causes a tumor growth phenotype.
Second part: hybridization analysis between virus from the mosquito with other viruses to isolate the area that doesn't hybridize. That area is the one that most likely causes tumor growth.

1B. To know if it is through regulation use a northern blot to measure mRNA levels. To know if is a sequence mutation, there are three options:

-Southern: Use DNA from tumor cells and DNA from healthy cells or from healthy individuals as a comparison, cut both DNA samples with restriction enzymes and probe with the viral DNA. Compare band patterns between the two samples and identify differences. To know if it the mutation is causative or a polymorphism look if the pattern is seen only in tumor cells and absent in healthy cells is likely to be a causative mutation.

-RNAse protection assay or endonuclease assay: Use DNA (or RNA) from tumor and from healthy cells or individuals for comparison, and hybridize both with the viral DNA/RNA. Treat both samples with endonuclease/RNAse and probe with the viral DNA. Compare band patterns between the two samples and identify differences. To know if it the mutation is causative or a polymorphism look if the pattern see is seen only in tumor cells and absent in healthy cells is likely to be a causative mutation.

-Homologous recombination: Use DNA from tumor and from healthy cells or individuals for comparison. Use viral DNA to identify and isolate the fragment causing the tumor and build a restriction map. Run a homologous recombination test, by recombining different fragments and testing each fragment for a tumor phenotype. To identify polymorphisms vs. mutations, narrow down the region of causative mutation before sequencing it and look for differences between the normal sequence and the tumor causative sequence.

1C. The DNA repair protein encoded in the viral genome can't fix DNA mistakes well because although it can identify and bind to DNA mistakes, it can't interact with human DNA repair proteins. The protein encoded in the viral DNA does not cause tumor growth per se, but the fact that it can't fix DNA mistakes, increases the chances to accumulate mutations in important parts of the genome. Therefore the length of time and onset of tumors will depend on when an important region was affected. Dinucleotide repeat instability is the feature that characterizes mismatch repair problems.

2A. Look for reversion of his- mutants as a test of mutagenicity of extracts of the waste site (Ames test). Critical components include (any 2 were enough for full credit) : his- mutants with different types of mutations (point mutants, frame-shifts in different contexts) to assay different types of mutagens; liver extract to activate possible promutagens; bacteria with defective cell walls to allow better penetration of the chemicals; bacteria defective in DNA repair pathways to make them more sensitive to mutagens; his- plates to allow selection of revertants.

2B. If the waste extract reverts frame shift mutants, it is more likely to target tumor suppressor genes, because frame shifts are likely to inactivate a gene and tumor suppressors generally require inactivation. If, on the other hand, the waste extract preferentially reverts point mutants, its target is more likely to be oncogenes such as ras, which are activated by specific point mutations.

2C. Transfect NIH3T3 cells with DNA isolated from the tumor and normal samples. An activated oncogene of a ras type in the tumor should generate growth foci where the normal DNA will not. To detect myc like oncogenes, use rat fibroblasts already transfected with ras.

2D. If the carcinogen alone is generally only capable of inducing benign tumors, the presence of more aggressive tumors in this pedigree suggests that these family members carry an inherited mutation in gene in a second pathway that interacts with the gene(s) mutated by the carcinogen to produce more aggressive tumors. Most likely, the inherited mutation is in a tumor suppressor gene and thus predisposes the carriers to develop more aggressive tumors when the normal copy is knocked out.

3A. Bilateral tumors. Early age of onset.

3B. Allele G is most likely associated with the causative gene because it is present in all affecteds across all three generations. I would combine family 1, which shows inheritance of the C allele in all affecteds through three generations. Family 3 looks to be sporadic with no common allele for affecteds, and should be excluded.

3C. Deletions are indicative of a tumor suppressor gene. Southern blotting can be used to determine which genes are deleted by using the cloned genes as probes and hybridizing each gene individually to the samples. To narrow to a single, causative gene, I would need to find an internal deletion for a single gene, or see that the only commonly deleted region across all samples is within a single gene.

3D. Using Southern blotting with tumor DNA and blood cell DNA from the patient, as well as blood cell DNA from each parent, inheritance can be determined. The gene is used as the probe. A somatic mutation, in this case the deletion, would only be seen in the tumor sample. An inherited mutation would be seen in the tumor and

patient's blood cell DNA. If neither parent is a carrier (the deletion is absent in their blood DNA samples), then it is a germline mutation. If mutation is found in the sample from either parent they were carriers and passed on the mutation.