Format of Practical Exam 2

1. In the laboratory, follow the procedure below to isolate plasmid DNA and load it on a gel.

Miniprep Isolation of DNA

1. Obtain a tube of E. coli cells (vol = 100µl)
2. Add 200µl of NaOH/SDS solution. Mix contents by inverting 5X vigorously, store cells on ice.
3. Add 150µl 5M KOAc. Mix contents by inverting 5X vigorously, incubate cells on ice five minutes.
4. Microcentrifuge sample on highest speed for 5 minutes.
5. Transfer 400µl of supernatant to new tube, store at room temperature. (Discard tube with pellet.)
6. Add 800µl ethanol to supernatant. Mix vigorously and incubate at room temperature for 2 minutes.
7. Microcentrifuge sample on highest speed for 5 minutes.
8. Observe pelleted nucleic acids at bottom of tube.
9. Carefully pour off supernatant
10. Gently add 1ml 70% ethanol to tube with pellet.
11. Observe pelleted nucleic acids at bottom of tube.
12. Carefully pour off supernatant
13. Use twisted kimwipe to remove remaining droplets of supernatant.
14. Air dry pellet 10min
15. Add 200µl of TE buffer to the dried pellet. Resuspend by vortexing vigorously.

Gel electrophoresis

1. Transfer 15µl of plasmid DNA to new microfuge tube.
2. Add 15 µl of 2XLoading dye
3. Mix by vortexing and microcentrifuge briefly to bring sample to bottom of tube.
4. Load 20 µl of sample in a lane on the agarose gel indicated by instructor.

2. In the computer lab your instructor will give you a CD containing the Virtual Genetics Lab computer program and a fly population. You will be given a single data collection sheet for the populations. Use the program to investigate the basic genetics of your trait and the genotypes of the population. Complete the data collection sheet.

3. The CD will also contain a fragment of a DNA sequence. You will use this to search the DNA databases and identify the gene to which it corresponds, identify what protein it encodes, what species where it came and the sequence of the first leucine codon.