Read the entire question carefully before answering. Please provide complete answers limiting yourself to the space provided.

Scenario 1 (50 points):
1.1 The vulva is a structure on the ventral surface of the adult nematode, *Caenorhabditis elegans*, which connects the gonad to the outside environment. It has been determined that six cells, known as Pnp cells, give rise to the vulva.

1.1a (5 points) Describe an experiment that might have been performed to show that the Pnp cells give rise to the vulva.

*Inject early developing worms with a vital dye and watch which cells give rise to the vulva.*

1.1b (1 point) What is the term that you would use to describe this type of experiment?

**Fate mapping**

1.2 Studies using lasers to ablate (i.e. kill) individual cells in the developing worm indicate that a single cell, known as the anchor cell, is required for the formation of the vulva. The anchor cell does not contribute to the final vulva (i.e. it is not a Pnp cell).

1.2a (5 points) Formulate a hypothesis suggesting a role for the anchor cell in vulval development.

*The anchor cell secretes some factor that causes the Pnp cells to form the vulva.*

1.2b (1 point) What is the developmental term that describes the action of the anchor cell?

**Induction.**

1.3 You have identified a gene named *lin-3* that you believe to be important for vulval development. *lin-3* encodes a growth factor that acts through a receptor tyrosine kinase known as *let-23*.

1.3a (5 points) Describe an experiment that would ask whether expression of *lin-3* correlates with vulval development. You may use any reagents you wish to.

*Raise an antibody to *lin-3*. Use this antibody to do immunocytochemistry on developing worms, looking specifically at the vulva.*

1.3b (4 points) Describe a result that would **support** (be consistent with) this correlation.
lin-3 is expressed in the anchor cell (and not in other cells) of developing worms.

1.3c (4 points) Describe a result that would not support (be inconsistent with) this correlation.

lin-3 is not expressed in either the anchor cell or the Pnp cells of developing worms.

1.4 You hypothesize that lin-3 is necessary for vulval development.

1.4a (4 points) Describe an experiment to test the necessity of lin-3 in vulval development. You may use any reagents you wish to.

Find a worm with a mutation in the lin-3 gene by mutational screen that completely blocks lin-3 function (i.e. knock it out) and look at vulval development.

1.4b (4 points) Describe a result consistent with this hypothesis.

The mutant worm does not form a normal vulva at all.

1.4c (4 points) Describe a result inconsistent with this hypothesis.

The mutant worm forms a vulva normally (at the right time and place).

1.5 You hypothesize that lin-3 is sufficient to direct vulval development.

1.5a (5 points) Describe an experiment to test the sufficiency of lin-3 to direct vulval development. You may use any reagents you wish to.

Ablate the anchor cell with a laser. Place a bead coated with purified lin-3 protein in the ablated worm (in the location of the anchor cell) and look at vulval formation.

1.5b (4 points) Describe a result consistent with this hypothesis.

Placing the purified lin-3 protein in the ablated worm leads to normal vulval formation.

1.5c (4 points) Describe a result inconsistent with this hypothesis.

Placing the purified lin-3 protein in the ablated worm leads to worms developing with no vulvas.

Scenario 2 (50 points):

2.1 You discover a mouse mutant called “gutless”, where the gut fails to form and embryos die early due to multiple problems. Initial analyses indicate that the transcription factor otx2 is expressed in all cells of the mutant, whereas in wild type embryos, otx2 is expressed only in the nervous system.

2.1a (2 points) The gut comes from one of the three earliest cell types in the embryo. What are these called collectively?
Germ layers

2.1b (2 points) The gut is one fate arising from the endodermal lineage. Define cell fate.
   The ultimate differentiated state to which a cell has become committed.

2.1c (4 points) Does the "gutless" mutant necessarily correspond to a mutation in the otx2 gene? Explain your answer.
   No! The mutation could lie in a different gene that acts to activate the expression of otx2. A mutation that leads to overexpression of the upstream gene would lead to overexpression of otx2.

2.1d (4 points) Assuming that the gutless mutation does lie in the otx2 gene, describe two regions in the otx2 gene that, when mutated, could lead to its expression throughout the embryo (ubiquitous expression)? For each region, list a mechanism by which gene expression could be altered.

<table>
<thead>
<tr>
<th>Gene region mutated</th>
<th>Mechanism leading to ubiquitous otx2 expression</th>
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<tbody>
<tr>
<td><strong>Promoter Region</strong></td>
<td>Increases the binding of RNA polymerase leading to increased otx2 transcription</td>
</tr>
<tr>
<td><strong>Coding Region</strong></td>
<td>Increases the stability of the otx2 mRNA leading to more translation (i.e. more protein).</td>
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</tbody>
</table>

2.2 You wonder about the normal function of otx2.

2.2a (5 points) Formulate a hypothesis regarding a role for otx2 in normal development.
   otx2 is required in the nervous system to repress expression of gut genes.

2.2b (5 points) Describe a loss of function experiment that would test this hypothesis. You may use any reagents you wish to.
   Produce a mouse knockout of the otx2 gene by homologous recombination. Look at nervous system development in the knockout.

2.2c (4 points) Describe a result from this experiment that would be inconsistent with your hypothesis.
   The nervous system of the knockout mouse develops completely normally.
2.2d (4 points) Describe a result from this experiment would be consistent with your hypothesis.

The nervous system of the knockout mouse expresses genes normally only seen in the gut.

2.3 You wonder also about how ubiquitous $otx2$ expression in “gutless” caused the mutant phenotype.

2.3a (5 points) Formulate a hypothesis to explain why gut formation did not occur in this mutant.

Expression of $otx2$ in a cell leads that cell to adopt a nervous system fate.

2.3b (5 points) Describe a correlation experiment to test this hypothesis. You may use any reagents you wish to.

Look at the expression of nervous system genes in the ubiquitous $otx2$ mutant.

2.3c (5 points) Describe a result consistent with your hypothesis.

Nervous system genes are expressed throughout the embryo in the ubiquitous $otx2$ mutant.

2.3d (5 points) Describe a result inconsistent with your hypothesis.

Nervous system genes are expressed only in the nervous system in the ubiquitous $otx2$ mutant.