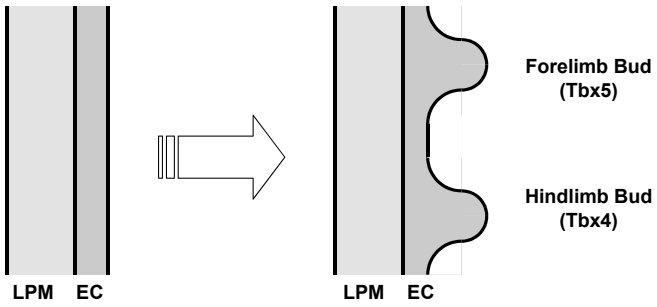
Name: _____

2007 7.013 Problem Set 5

Due before 5 PM on WEDNESDAY, April 18, 2007.
Turn answers in to the box outside of 68-120.
PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

Question 1.

The limb (arm or leg) forms when a group of cells called "lateral plate mesoderm" (LPM) induces overlying ectoderm (EC) to form an outgrowth called the "limb bud".



The limb bud forms an outgrowth because the number of cells it contains increases relative to surrounding ectoderm.

1a. One possibility is that cells in the bud undergo increased cell division relative to surrounding ectoderm. What technique could you use to ask whether this possibility is correct? (5 words or fewer)

1b. Give another possible mechanism that would increase cell number. (5 words or fewer)

1c. Interestingly, part of the brain (the "hindbrain") can substitute for the LPM and induce limb bud outgrowth. Discuss the molecular biology that is likely to explain this result (15 words or fewer).

1d. Young lateral ectoderm can give rise to many fates, including the limbs, brain and spinal cord, the epidermis of the skin, pigment cells. However, in older embryos, the lateral ectoderm can form only a limb bud. What is the term for the possible fates that a cell can form?

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The front and back legs of the chicken are very different, where the front limb is covered with feathers, and the hindlimb is covered with scales. In order to figure out why these limbs are different from one another, the following transplant experiment was performed. LPM from the hindlimb area was placed under future forelimb ectoderm, and LPM from the forelimb region was placed under future hindlimb ectoderm. The resulting chick embryos were assayed after the limbs had differentiated, and the following results were obtained.

future forelimb LPM	+	future forelimb ectoderm	→→	feathers
future hindlimb LPM	+	future forelimb ectoderm	→→	scales
future hindlimb LPM	+	future hindlimb ectoderm	→→	scales
future forelimb LPM	+	future hindlimb ectoderm	→→	feathers

1e. What do these data tell you regarding the mechanism by which fore- and hindlimbs make feathers or scales? (15 words or fewer)

Chickens have white feathers, quail have black feathers. You perform a transplant experiment like the one above, as detailed below, with the following results:

Chick forelimb LPM	+	chick ecto	→ → →	white feathers
Quail forelimb LPM	+	chick ecto	→ → →	white feathers
Chick forelimb LPM	+	quail ecto	→ → →	black feathers
Quail forelimb LPM	+	quail ecto	→ → →	black feathers

1f. What do these data tell you regarding the mechanism by which chick and quail make different color feathers? (15 words or fewer)

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The Tbx4 transcription factor is expressed exclusively in the developing hindlimb, and is necessary for development of hindlimb characteristics, including whether it has scales or feathers. A mutant has been isolated where the hindlimb now looks like a forelimb.

Linkage analysis indicates that this mutant lies in the Tbx4 gene. However, in order to confirm mutant gene identity, you sequence the Tbx4 genomic DNA from this mutant, and find that there are no changes in any exon. On the other hand, you find two nucleotides in the mutant DNA, which differ from wild type. The first is at the exon1/intron1 splice acceptor sequence. The second is 25 bp 5' to the transcriptional start site, where the wild type sequence 5' TATAAAAT is instead 5'TATAGGGC.

1g. In the splice acceptor mutant, what changes would you observe in the RNA made from this gene, relative to wild type? (15 words or fewer)

1h. Could this change account for the Tbx4 mutant phenotype? Explain in 15 words or fewer.

1i. If the mutant that maps to the non-transcribed part of the gene (TATAAAAT), what changes would you observe in the RNA made from this gene, relative to wild type? (15 words or fewer).

1j. Could this change account for the mutant phenotype? Explain in 15 words or fewer.

1k. Tbx5 is required for forelimb development, and normally expressed only in the forelimb. In the Tbx4 mutant, you find that Tbx5 is now expressed in both sets of limbs. Explain (use a diagram if you like).

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Question 2.

2a. Tube formation is often accompanied by lengthening or extension of an initially short tube. What two processes lengthen a cell sheet? Explain what each entails (15 words or fewer).

(i)

(ii)

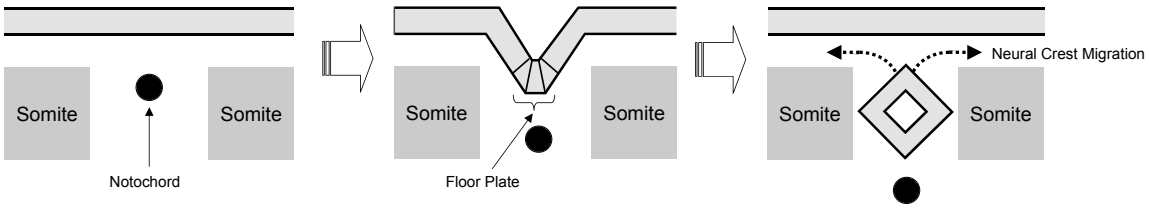
The neural tube forms the brain and spinal cord. Wnt signaling is required for lengthening the tube. The future neural tube lies next to the notochord and the somites.

2b. Give two ways you could determine where the Wnt signal comes from? Your answers should be independent, and include…

(i) use of an explant assay

(ii) use of gene expression assays

Formation of the neural tube involves a sharp ventral bend as diagrammed. This bend is associated with formation of wedge-shaped cells to form the "floorplate".



2c. One gene which is expressed in the floorplate is "sonic hedgehog" (shh). How could you determine whether shh is necessary for floorplate formation? (15 words or fewer)

2d. What structural cytoskeletal protein would you expect to change in the floorplate cells as they become wedge-shaped?

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2e. How would this protein change?

2f. The compound “blebbistatin” inhibits myosin function. If you applied blebbistatin to the developing floorplate, what effect would you expect to observe? Explain in 15 words or fewer.

2g. As shown in the above diagram, the floorplate lies above a structure called the “notochord”. shh is also expressed in the notochord, and is responsible for inducing expression of shh in the floorplate. How does shh expression in the notochord activate shh expression in the floorplate? (15 words or fewer)

As diagrammed above, from the dorsal neural tube, an important group of cells called the “neural crest” begins to migrate to many parts of the embryo. When they reach their final destinations, they differentiate into pigment cells, the bones and muscles of the face, the peripheral nerves and part of the adrenal gland.

2h. Are the migratory neural crest cells likely to be single cells or a sheet of cells?

2i. What cell type transition do the neural crest cells undergo as they leave the neural tube to become migratory?

The protein collagen is the most abundant in the human body. It is crucial part of the extracellular matrix. Mutants with no collagen die very soon after fertilization, but those with decreased collagen function survive longer, but show no neural crest migration.

2j. Name one function of the extracellular matrix is important for cell migration? (15 words or fewer).

Neural crest cells that arrive at similar destinations stick to one another and go on to differentiate. Future adrenal medulla cells, and future jaw cells, can be dissociated into single cells by calcium removal, after they reach their destinations, but prior to differentiation. Addition of calcium will cause reassociation.

2k. After mixing the two dissociated populations together in the presence of calcium, what would you predict would happen? Include the term for this process. Explain in 15 words or fewer.

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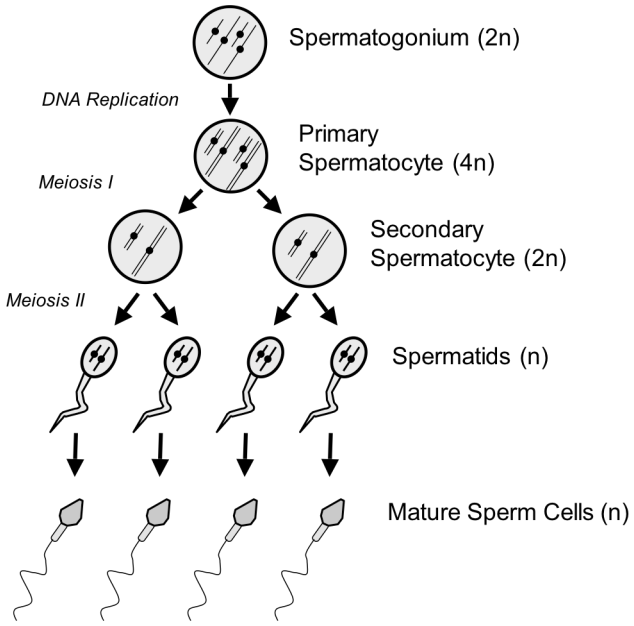
Question 3.

The search for pluripotent stem cells is intense. Spermatogonial cells are the diploid precursors of haploid sperm according to the following scheme.



3a. In order to ask whether mouse spermatogonia are stem cells, they were isolated from a donor and introduced into the testis of a host male of the sterile1 strain, where the testis looks normal, but lacks germ cells. The mouse recovered fertility. If the donor cells were from a mouse with black fur (B/B), and the host had white fur (b/b), devise a genetic cross to confirm that restoration of fertility was due to the donor B/B spermatogonia, and not to some other recovery of fertility by the b/b mouse. Assume b is recessive to B.

3b. You wonder whether other stages of sperm have stem cell capacity, and find that secondary spermatocytes do, but only for a few weeks after injection into the sterile1 strain. In contrast, the spermatogonia restore fertility for the rest of the sterile1 mouse' life. What property of a stem cell is missing in the secondary spermatocytes? (5 words or fewer)

The Sertoli cells normally surround the spermatogonial cells. In the sterile2 strain, there are no Sertoli cells, nor any germ cells.

3c. If Sertoli cells normally comprise the "niche" of spermatogonial cells, would you predict that wild type donor spermatogonia introduced into the testes of the sterile2 strain would differentiate into sperm? Explain (15 words or fewer).

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3d. What is the relationship between cell-cell signaling, induction and the niche? (15 words or fewer).

You also wonder whether what the potency the spermatogonial cells possess. You therefore make a transgenic animal, ROSA52, which specifically expresses the lacz gene in the spermatogonia, which makes all cells in the animal blue, after appropriate staining. You then perform various repopulation experiments to test spermatogonial potency.

3e. What is the point of using blue cells?

You purify spermatogonial cells from a ROSA52 mouse and introduce them into an unlabelled lethally irradiated mouse.

3f. What is the point of the lethal irradiation?

3g. You find that all blood and immune cells are blue in the repopulated mouse. What would you conclude regarding spermatogonial potency?

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Question 4.

4a. What are the two meanings of “cloning” to which you have been exposed in this course?

(i)

(ii)

The challenge of neuropsychiatry is to understand whether a disorder is caused by genetic mutation, or by other means. Identical twin studies are useful in this regard.

4b. What is the definition of “concordance” in this context? (5 words or fewer)

4c. Studies on autistic disorder show a concordance of 0-23% for siblings, and 90% for monozygotic twins. What does this data indicate? (15 words or fewer)

4d. In contrast, most forms of schizophrenia show a concordance of less than 50% in monozygotic male twins. What does this data indicate? (15 words or fewer)

4e. A mouse model for schizophrenia has been developed, by disruption of a gene called DISC1, implicated in the human disorder. As in humans, only about 50% of the mice (which are a genetically identical homogenous strain) develop the disorder. You treat the mice with 5-azacytidine, a cytosine analog that cannot be methylated. You now find that 100% of the mice develop the disorder. Explain this result (15 words or fewer).

Embryos derived from fathers with loss of function in the (Insulin-like growth factor) IGF1 gene are very small. Embryos derived from mothers with loss of IGF1 function are of normal size.

4f. Of what is this phenomenon an example? (1 word)

Mice derived by somatic cell nuclear transfer usually suffer from “large offspring syndrome”, where the neonates are several times larger than normal neonates.

4g. What prediction would you make regarding IGF1 protein expression in these embryos derived from SCNT? (5 words or fewer)