Name……

Question	**Points**	**Score**
Total for Final: 200		
1	6	
2	6	
3	6	
4	6	
5	6	
6	6	
7A	8	
7B	15	
8	12	
9	12	
10	12	
11	14	
12a	10	
12bc	12	
12d	8	
13	8	
14	6	
15	20	
16	15	
17	12	
	200	

Name……

Question 1 (6 points) Predict both the molecular and morphological consequences of misexpressing the Abd-B Hox gene throughout otherwise normal Drosophila embryos.

Question 2. (6 points) Why do Drosophila embryos that completely lack *eve*$^{+}$ gene activity have no engrailed expression and no segment boundaries?

Name……

Question 3. (6 points) What is the consequence of expanding the Snail expression pattern on heart development in the Drosophila embryo? Be sure to provide both molecular and morphological details.

Question 4. (6 points) In Drosophila, why does the misexpression of Oskar at the anterior pole cause the formation of ectopic pole cells AND the development of a second abdomen?

Name……

Question 5 (6 points)
Dorsal and Twist work synergistically to activate the transcription of a variety of target genes. Propose a general mechanism for this synergy.

Question 6 (6 points)
Give examples of a maternally localized mRNA, and its role in early pattern formation
a) From Dorsal ventral patterning of Drosophila

b) From Xenopus

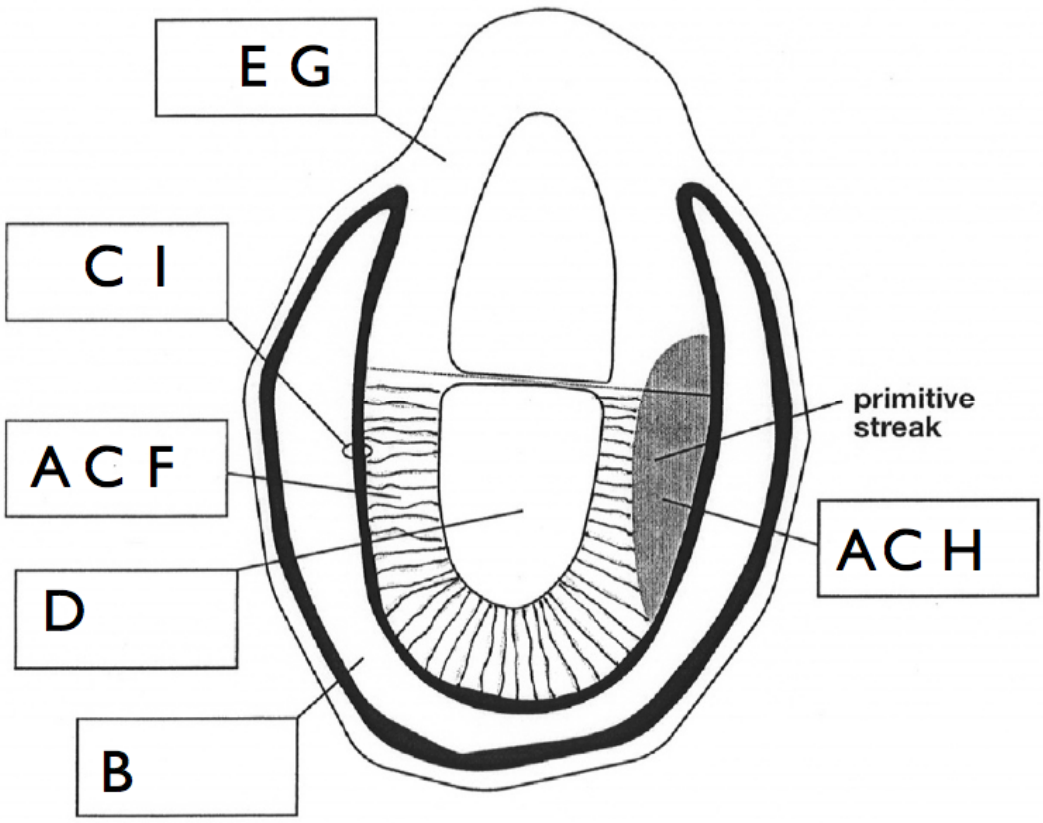
C) From Ciona, chicken or mouse

Name……

Question 7. Part A (8 points)

Below is shown a schematic cross section of a 6.5 day mouse embryo, implanted in the uterine wall. Epiblast cells are outlined, and the primitive streak (darkened area) has begun to form on one side.
Put letters from the list into the boxes to identify the designated parts of the embryo.

A. provides cells of the embryo and of some extraembryonic tissue.
B. was previously the blastocyst cavity.
C. descended from inner cells of the 64-cell compacted embryo
D. pro-amniotic or amniotic cavity
E. descended from outer cells of the 64-cell compacted embryo
F. will become anterior neural plate of the embryo
G. trophoblast or trophectoderm
H. site of endo-mesoderm induction
I. formed from inner cell mass cells closest to the blastocyst cavity



Name……

Question 7b (12 Points)

Explain

i) Why the primitive Streak of the 6.5 day mouse embryo forms on the side of the epiblast away from the Anterior Visceral Endoderm (AVE) and

ii) Why the anterior neural plate later forms on the side of the epiblast towards the AVE

For full credit, specify the signaling molecules, tissue layers and signaling processes.

i)

Nodal and nodal signaling is turned on **throughout the proximal epiblast,** but it is inhibited by **nodal antagonists such as Cerberus and lefty1** secreted by the **anterior visceral endoderm** which **migrated from the distal tip of the epiblast to lie next to the proximal epiblast.** These antagonists **prevent nodal signaling** thus **preventing mesoderm induction** outside **the primitive streak** and allowing the epiblast to form alternative fates such as **epidermis/surface ectoderm**, and **neural plate.** This inhibition of mesoderm induction therefore **specifies that the primitive streak only forms** on the side away from the AVE

ii) The anterior visceral endoderm secretes **nodal antagonists** which prevent **mesoderm induction.** It secretes **BMP antagonists,** such as **cerberus** which prevent **BMP-mediated epidermis induction**. It also secretes **Wnt antagonists**, such as the multifunctional **Cerberus, dkk1,** these **protect the anterior neural plate** from the **posteriorizing influencec of Wnts**, resulting in anterior inductions. Since the AVE has also constrained the streak to form on the opposite side, then the node will form there, and produce **axial mesoderm,** which migrates between the Primitive endoderm and epiblast, and also secretes **BMP antagonists,,** such as **noggin and chordin,** to help prevent epidermal induction (and Wnt antagonistsfrom the anterior mesoderm/prechordal plate).

Name……

Question 8 (15 points)

The sclerotome of the vertebrate embryo develops into the vertebral skeleton. In the case of the frog embryo, trace the development in three steps backwards to the precursor cells in the animal hemisphere of the mid-blastula stage, indicating the signals that the tissue must receive to continue development to the sclerotome fate.

a) back one step: From the somite of the neurula to the sclerotome. What signals reached the cells and from where?

The somite receives signals from the **notochord**/floor plate in the form of both Sonic **Hedgehog** (SHH) and **BMP antagonists, noggin, chordin**. The antagonists block **BMP signaling** (which can affect the somite from structures such as the **lateral plate** and roof plate of the neural tube), and in this environment high doses of **SHH induce** the sclerotome fate.

b) back two steps: from lateral-ventral mesoderm of the gastrula to the somite of the neurula: What signals reached the cells and from where?

The mesoderm is already specified, but **BMP signals** are synthesized and have the ability to affect the entire embryo, but particularly **outside the organizer**, where they **specify the ventral types of mesoderm**. During gastrulation, morphogenetic movements bring the mesoderm close to the organizer, where **BMP antagonists** such as **noggin, chordin, follistatin** are expressed and block the BMP, **permitting the default fate** of becoming **somite**.

c) back three steps: From animal hemisphere cells of the mid-blastula to lateral- ventral mesoderm of the gastrula: What signals reached the cells and from where?

The maternally localized VegT is active in turning on **nodal signals** in the **marginal zone**, where they **specify mesoderm**, in the absence of high beta catenin signaling on the dorsal side, this **dose of nodal is sufficient to specify ventrolateral mesoderm**, but not dorsal mesoderm/organizer.

Name……

Question 9

a) (6 points) Predict the phenotype of the *Xenopus* embryo that would develop from an oocyte that had been injected with an antisense oligonucleotide directed against maternal beta-catenin mRNA (after which the oocyte was matured into an egg and fertilized). Briefly explain why you predicted this outcome.

The embryo undergoes **ventral/posterior development**, to form a **radially/cylindrically symmetrical** embryo with **no dorsal structures**, but with an **excess** of **ventral mesoderm, epidermis,** and posterior endoderm. Normally, maternal beta catenin mRNA is needed to provide **elevated beta catenin** levels on the prospective dorsal side, where it accumulates in the nucleus as a transcriptional activator **cooperates/synergizes** with **VegT and nodal to specify dorsal structures**. In the absence of the ability to produce enough beta catenin to act in this dorsalizing pathway, the entire embryo is subject to the **basal levels of nodal signaling** activated by **VegT alone,** that specify **ventrolateral mesoderm**, and then in the **absence of an organizer**, there is **no axial mesoderm formation**, nor **dorsalization** of mesoderm to make somite, nor **induction of neural plate**.

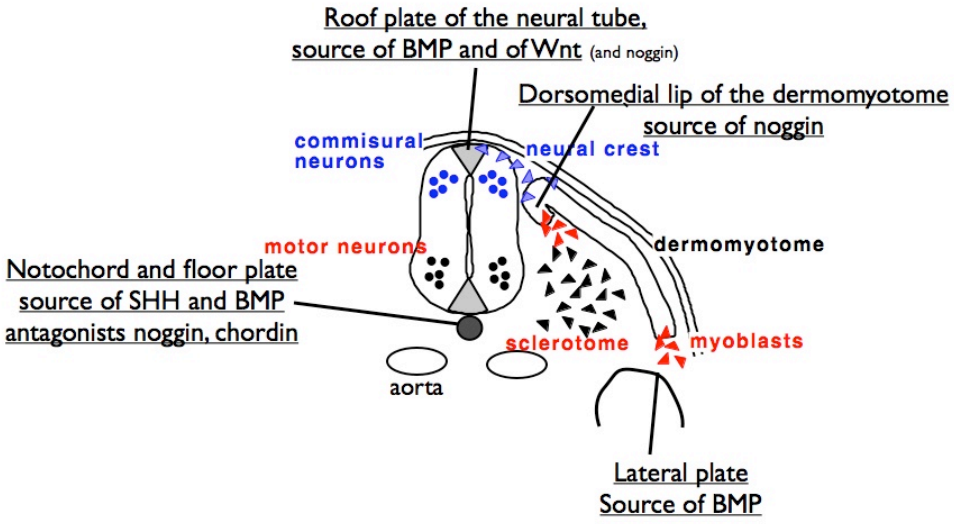
b) (6 points)

Predict the phenotype of the *Xenopus* embryo that would develop from one of the manipulated eggs of Part A that was injected shortly after fertilization with antisense morpholino oligonucleotides that would block translation of Bmp mRNAs (Bmp 2,4 and 7). Briefly explain why you would predict this outcome.

The embryo would develop as a **cylindrically/radially symmetrical**, **dorsalized** and **neuralized** embryo, and would die due to lack of enclosing epidermis. Although the embryo would **start out** as uniformly **ventralized** (see above), the blastula/**gastrula-stage activity of BMP**, that would normally **induce epidermis**, and **ventralize the mesoderm** would be **absent**, and so the **epidermis** would be directed to the **neural fate**, and the **mesoderm** to a **dorsalized mesodermal fate**.

Name……

Question 10 (12 Points) Draw a picture of a cross section of an early chick embryo at the stage of somite formation where the dermomyotome has segregated from the sclerotome (trunk level).
Indicate on the diagram the various molecular signals and name the structures that are sources of signals that act in muscle induction. Label the parts of the somite that differentiate into muscle as a result of signals. The roof plate of the neural tube is a source of BMP signals, which act to suppress muscle formation. Indicate on the diagram and explain below why these BMPs do not suppress muscle determination during normal development.



Roofplate Wnt signals induce noggin in the dorso medial lip of the dermomyotome, which protects this region from BMP signals which would also come from the roofplate of the neural tube. This allows muscle formation.

Name……

Question 11 (12 points)

Discuss a case where an enhancer mediates gene activation and executes a positive feedback loop in the mouse gastrula; Explain the mechanism that prevents this positive feedback loop from dominating the entire embryo.
For full credit, describe the extracellular and intracellular events, proteins and DNAs that mediate these feedback loops.

The **nodal enhancer mediates positive feedback**, while the **lefty2 enhancer mediates negative feedback**.
Nodal protein that is expressed in the **proximal epiblast** activates the **type I and II receptors** by bringing them together in the **membrane** as a **dimer** (along with the co-receptor cripto/oep). The type II receptor has serine threonine **kinase activity** that phosphorylates a juxtamembrane **domain** on the **typel receptor**, thus **activating** it. **Smad2** is prebound to the type I receptor and is **phosphorylated** by the **kinase** of the **type I receptor**, which kicks it off into the **cytoplasm**. There is gets together with the **co-smad/smad4**, and the **complex moves into the nucleus**. In the nucleus, the **weak** DNA binding activity of the Smad complex is augmented by making a complex with a **specific DNA-binding protein** (Fast1, foxH1, fox protein, forkhead protein) which by itself has **no activating activity**, but with the smad complex has strong **transcriptional activation activity**. This binds to a **specific DNA binding region/enhancer** in the first **intron of the nodal gene**, **promoting more nodal expression** of mRNA and therefore of protein. Thus the positive feedback loop is activated.
The complex in the nucleus also activates a **similar enhancer in the lefty2 gene**, thus promoting lefty2 expression. **Lefty2 is a nodal antagonist** (thought to act by binding up the cripto/oep). This **inhibits nodal activation of the smad2** pathway.
The extra wrinkle is that **lefty2 is thought to diffuse faster and further than nodal**, so in the epiblast, there is a **region just ahead of the nodal activity** where **lefty2 is in excess**, and prevents small amounts of nodal activity, and therefore **prevents initiation of the nodal positive feedback loop**,and therefore restricts the spread of nodal

Name……

Question 12:
An embryologist, Hilde Weissenegger, wishes to understand developmental mechanisms in a new marine organism that is named the Schmoo. The organisms live in the mud, but can be induced to ovulate and be fertilized at any time of year by exposure to light. The large egg and embryo is easy to inject with a variety of materials. As with Xenopus embryos, injected mRNAs are slow to diffuse after injection, while fluorescent dextrans diffuse fast, and lipophilic dyes permanently mark the membranes of the cells into which they are injected.
The early embryo develops three head segments very quickly, and then adds on a segment every day from a mass of undifferentiated tissue at the posterior end, until it has ten segments. The embryologist carries out an experiment from which she concludes that the ten segments were already specified at an early time, when just the three head segments were morphologically distinct.
a) (10 points) What experiment would you do to test whether this model might be correct? Provide the alternative outcomes and interpretation in the cases that your experiment either supports or refutes the model.

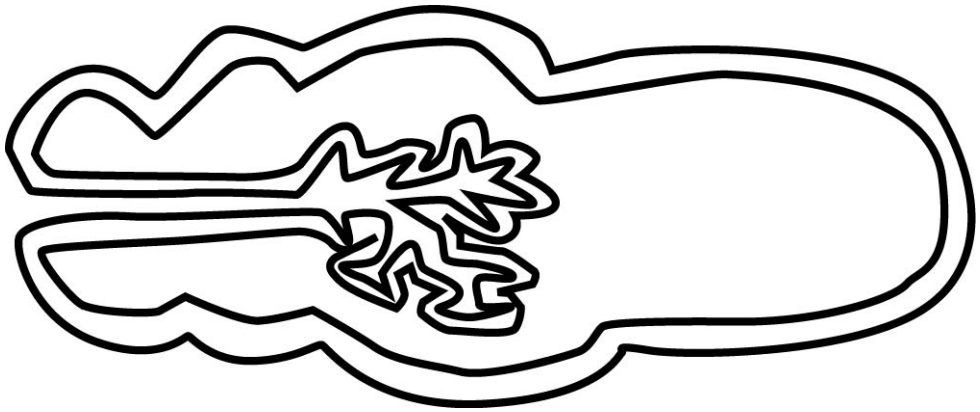
This is analogous to the early specification model of limb development, where a **lineage tracing** experiment was used to argue that all of the structures were **already specified in the early bud**.
So we can use a lineage tracer experiment, where a **small dot** of **DiI (lipophilic dye)** is injected into **different embryos** at **different positions** in the **tailbud/posterior zone**. Some embryos are **examined immediately to verify that the dot is local** and not spread. **Other embryos are allowed to develop** their segments. There are two extreme outcomes:
if the tailbud is a **stem cell zone/analogous to the progress zone** that **lays down segments as it grows** out, then one would **predict** that a locally applied dot of DiI in this zone would **spread to label a long stripe that covers more than one segment**, and in the extreme version, would label most of the length of the additional segments. This is analogous with labeling the node of a chick embryo which labels the entire axial mesoderm. This **result would refute** the early specification idea.

The other extreme, which would **support the early specification model**, is that each embryo developing after local application of the DiI dot, would **bear label in just one** (or maybe a couple) **of segments**, since the local application would label **only a prespecified segment** (or two), which would then **grow and differentiate** to form the segment.

Name……

Question 12:b (6 points) The schmoo develops a branched gut with a single opening to the mouth. In a screen for gene expression patterns, the embryologist finds a tantalizing pattern of expression of FGF and its receptor. Provide an analogy with branching morphogenesis in a vertebrate organ, and draw how these mRNAs might be expressed in the embryo.

By analogy with the expression of **GDNF** and its **ret receptor** in branching morphogenesis of the **ureteric bud** of the vertebrate **metanephros/kidney**, **FGF** would be expressed in a **cloud around the branching structure** (promoting growth) and the **FGF receptor** would be expressed in the **branching epithelium** (extra credit for anyone who notes that ret is actually expressed at higher levels in the growing tips of the branching structure, though I didn't mention this in lecture)



c (6 points) Provide a plausible mechanism whereby branching might be initiated by deployment of an antagonist. Base this on molecules that were discussed in the lectures.

By analogy with the model suggested for **feedback inhibition** of tip growth by an FGF antagonist, **sprouty**. The tip would grow into a region of high FGF, and **turn on sprouty** as a **target of the FGF** signaling, this would **prevent further growth** of the tip, but **FGF acting around the sides** of the tip would **promote growth** there, which would result in **lateral growth/branching** and renewal of the cycle. A **diagram** or series of diagrams would help here.

Question 12 d) (8 points) The schmoo develops neurons that are scattered throughout the three head segments, and Hilde decides to test whether Notch signaling might regulate neurogenesis, by analogy with its role in other animals. She finds there is a single, zygotically expressed Notch gene. How would you increase or decrease Notch signaling to see whether the mechanism of neurogenesis is similar between other animals and the schmoo, and if the mechanism is similar what outcomes would you predict?

Increase Notch signaling:
Inject the fertilized egg with **mRNA** encoding the **intracellular domain of Notch/ inject excess delta mRNA** which would **activate Notch signaling**. To **trace** the domain that inherits Notch (**only those that inherit mRNA** in the **ectoderm of the three head segments are informative**), one can co-**inject a tracer**, such as **lacZ** or GFP mRNA, which is expected to diffuse at the same rate as the Notch reagent, and thus mark where it is active.
If this **is like neurogenesis in flies or frogs**, then excess Notch would be expected to **inhibit neurogenesis**. So one would take the injected embryos, **visualize the region with the injected mRNA** (fix and stain, or fix and look at GFP), then use some specific **stain** to **examine the neurons/histological examination**, and see whether the numbers are **increased or decreased relative to control** embryos that received **just the tracer**, or tracer plus something inert (globin).
Decrease Notch signaling:
One can reduce Notch with a **dominant negative delta** (as with the Xenopius experiments) or use a **morpholino oligonucleotide directed against Notch mRNA** to **reduce** Notch expression (there is no maternal pool so this should be effective). In the case of the latter, one would use an appropriate tracer such as **fluorescent dextran**, or **labeled MO** to trace the inheritance of the injected material. **Fix, stain for tracer** and also for **neurons** and **compare** to controls injected with tracer plus irrelevant oligonucleotide. Predict there should be an **increase in neurons** in the head segments, when the **tracer** overlap with these tissues.

Name……

Question 13 (8 points) Name six derivatives of the neural crest. Among these, one fate is dictated by BMP signaling. What is the source of signal and what does it do?

Head: intramembranous bone producing cells/osteoblasts
Dorsal root Ganglia
Neurons of trunk ganglia (sympathetic/parasympathetic)
Endocrine cells of the adrenal medulla/chromaffin cells
Enteric nervous system
Melanocytes
Schwann cells/glial cells of the peripheral nervous system
Muscle cells of the cardiac outflow tract
etc.

Neurons are formed from cells that migrate past the BMP-producing aorta. This signal instructively induces these cells to become neurons.

Could also name osteoblasts, but then one would have to figure out where it is made.

Question 14 (6 points)
Provide examples of three roles of BMP signaling in the development of the limb, with the consequence of preventing BMP signaling in each case.

1. suppresses the apical ectodermal ridge. Inhibition of BMP leads to a more robust and longer lasting AER, which in turn can lead to ectopic outgrowth of the limb

2. Induces condensation of mesenchyme into cartilage
Prevents condensation, so no cartilage is formed

3 Induces cell death in the interdigital mesenchyme .prevents cell death, so the fingers are webbed (soft tissue syndactyly)

Name……

Question 15

From a paper describing hairy expression in the chick: "hairy1 mRNA Expression in the Presomitic Mesoderm Defines a Highly Dynamic Caudal-to-Rostral Expression Sequence Reiterated during Formation of Each Somite"

a) (5 points) From the fixed embryos that were used in the initial experiments, it was not immediately clear what underlay the expression pattern. What experiment ruled out that the patterns observed reflected static patterns that simply varied from embryo to embryo?

To visualize dynamic patterns, the presomitic region was explanted, and cut in half longitudinally. One half was fixed immediately,a nd the other was left for some time period (less than one somite's worth). Both tissues were stained for hairy, and the pattern was not the same, indicating that the pattern was not a static one, but was dynamic.

b) (5 points) What experiment addressed whether there was an oscillator in the tailbud that drove a propagating wave of gene expression? What did the experiment show?

Similar to above, the presomitic mesoderm and tailbud was explanted, but the tailbud was cut off. Again the explant was split longitudinally, and processed at different times (immediate and 30 min e.g.) the two halves still showed different dynamic pattens, indicating that they do not need a signal from the tailbud to drive the dynamic expression

Question 15 c) (5 points) What would be the effect of suddenly eliminating Notch function on the pattern of hairy gene expression in the presomitic mesoderm?

The pattern would progress, using its cell-autonomous oscillator normally for a short time, but the cells would no longer be coordinated locally, since they have lost the important Notch component of the intercellular coordination of hairy cycling. Therefore the pattern gradually assumes a more salt and pepper pattern of expression of hairy.

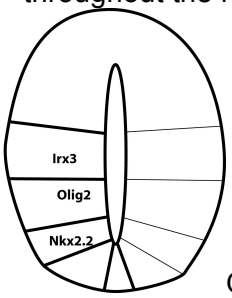
d) (5 points) What would be the effect of introducing a mouse hairy gene transcript into all the cells on one side of the midline?

This side would be affected, but not the unmanipulated side. The mouse hairy transcript would be translated, and produce excess hairy repressor, which would inhibit transcription of the endogenous chick hairy gene. However, as the mouse hairy mRNA and protein declined (since both are unstable), this would allow the ubiquitous activators of hairy to turn chick hairy back on. SO there would be a region of uniform inhibition of hairy, followed by uniform activation of hairy, which would result in a totally messed up somitogenesis, possibly no somite divisions at all.

Name……

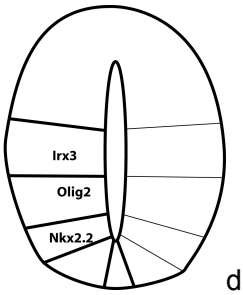
Question 16 In this question we are going to manipulate neural tube patterning; the initial pattern of gene expression is indicated on the left side of the neural tube:

a) **(3 points)** what do you predict is the consequence of ectopically expressing Olig2 throughout the right side on expression of the indicated transcripts



Olig2 would inhibit irx3 and Nkx2.2

b) **(6 points)** Draw in a smoothened mutant clone of cells that spans the neural epithelium on the right side and overlaps the olig2 and Nkx2.2 boundary. Draw in the consequences of this clone of cells on expression of the indicated genes, and also of pax3.



dorsal expansion of nkx/olig (irx), with pax 3 dorsally and in the clone

c) **(6 points)** Label the source of Shh in the picture above. All of the transcripts indicated are activated by Shh. What would you predict is the enhancer structure of the Nkx2.2 gene, and how does it differ from that of Olig2?

Floorplate
Nkx2.2 should have binding sites for the olig2 repressor, and activation sites for the gli activator which would be predicted to be low affinity- only activated by high levels of HH signaling
In contrast, olig2 qould have higher affinity sites for gli activators, and nkx2.2 and irx3 repressor binding sites

Name……

Question 17 **4 points**
Introduction of the "stripe assay" to examine the migration of growth cones or neural crest cells was an extremely important step in understanding attractive and repulsive cues. What is it about the assay that makes it so powerful?

Instead of simply providing a substrate for cells to crawl on (and given enough time they will crawl on many things), the stripe assay provides a choice, so that small differences in either attractive cues or repulsive cues are visualized by the choice of where to crawl

b) (10 points) Describe the mechanism by which neural crest cells aggregate locally into ganglia along the sides of the spinal cord. What are the molecular similarities with the projection of retinal axons to the optic tectum? Draw a diagram illustrating the retina and tectum and explain how high and low signaling strength affects the destination of the axons (no need to worry about which is nasal and temporal- just provide the logic of the system)

The somites express ephrin on the posterior, which repels Eph receptor epressing neural crest cells. Given the choice, these cells crawl on and therefore aggregate on the anterior somite.

Retinal ganglion cells express Eph Receptor, either A high, or B low
Tectum expresses ephrin either C high or D low.

When A cells get to even low levels of ligand (D), they receive a signal strong enough to make their growth cones collapse, so they stop
In contrast, B cells keep going until they find a higher dose of ephrin, enough to make their growth cones collapse at the distal end of the tectum