Spring 2005
7.02/10.702 Development Exam
Study Questions

Please note:

1. **While the exam given on May 19th will not be "cumulative," you should note that many of your interpretation questions and a number of these study questions (taken from old DEV exams) ask you to make connections between the DEV and other modules in the course. You should think about these connections as you prepare for the exam!**

Question 1 (x points)

Your UROP project focuses on two genes, Par1 and Mst5, which you suspect are involved in the embryonic development of the mouse. To study these genes, you isolate total DNA, total RNA, and total protein samples from mice at the following developmental stages:

1) early embryos (8 days post fertilization, or 8dpf)
2) late embryos (15 days post fertilization, or 15dpf)
3) newborn pups (NB)
4) 3 month old adults (A)

The expression of Par1 and Mst5 during development may be controlled at the RNA level (by regulating either transcription or mRNA stability) or at the protein level (by regulating translation or protein stability). To begin to understand Par1's regulation, you decide to perform Northern and Western blots on your samples.

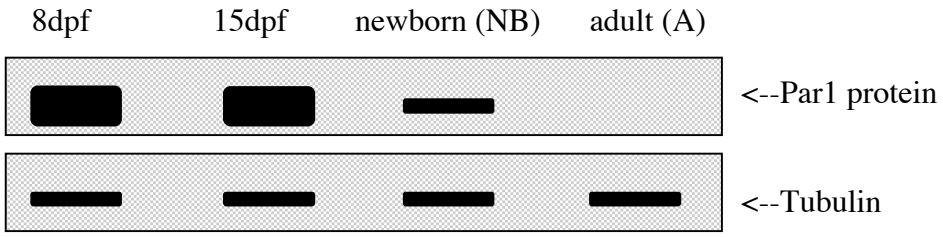
b) Compare and contrast the Northern and Western blot procedures by completing the following table:

	Northern blot	Western blot
What method(s) will you use to denature your samples?		
What type of gel will you run?		
In what direction will your samples migrate in the gel? What ensures this?		
What specific type of "probe" will you use to detect Par1 mRNA or protein?**		

**you may assume you use the same general type of "probe"—and the same reagents to "visualize" the probe—as you used in 7.02 lab.

Question 1 (continued)

You perform the Western blot first, and probe the membrane for Par1 and another protein, tubulin. (Tubulin is a cytoskeletal protein expressed at constant levels throughout development and in the fully developed mouse.) You observe the following on your blot:

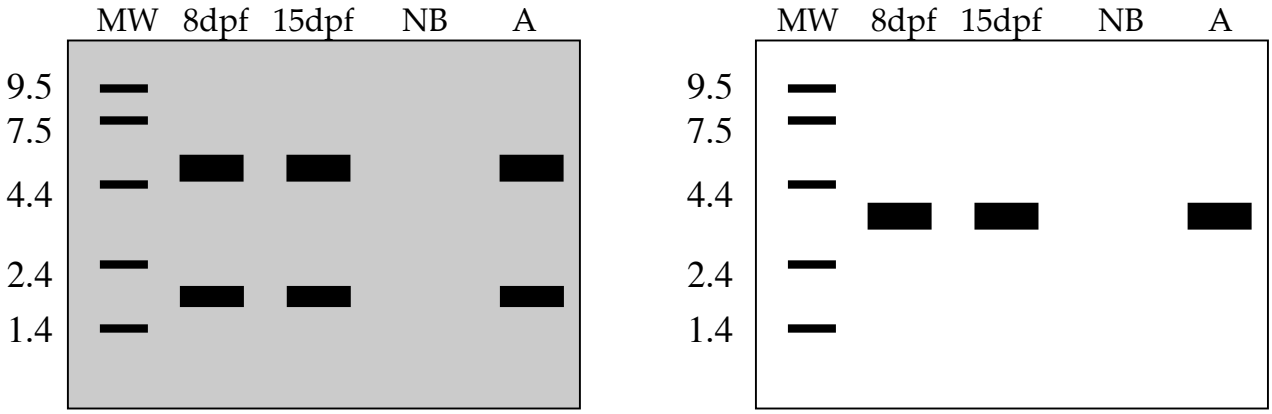


b) Why did you probe your membrane for tubulin as well as for Par1?

c) Is Par1 protein expressed during embryonic development? (circle one) YES NO

d) Is Par1 protein required in the adult mouse? (circle one) YES NO

You next perform a Northern blot, probing for Par1. You develop the blot, expose it to film, and obtain the result shown below:



e) Your labmate concludes that Par1 is **not transcribed** in newborn mice. Do the gel and Northern blot data definitively support his conclusion? Why or why not?

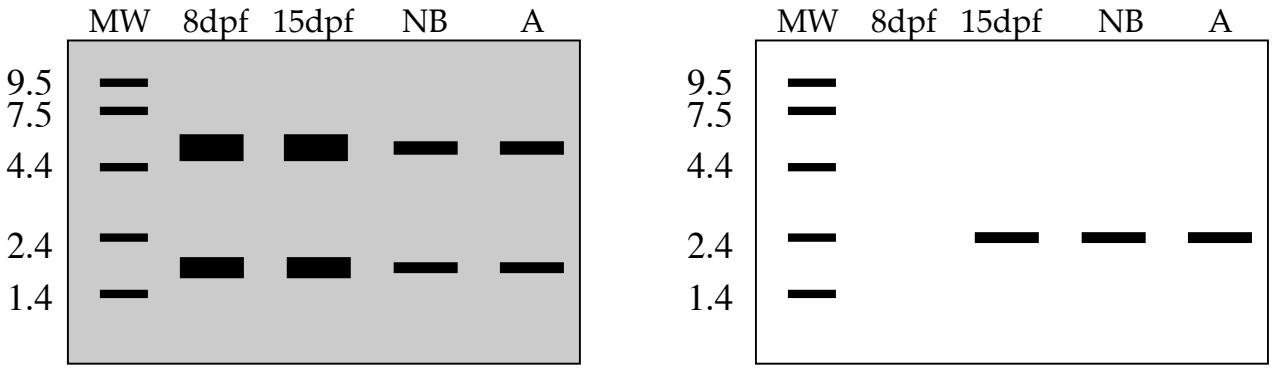
Question 1 (continued)

f) Based on the data from the Par1 Western and Northern blots, how is Par1 expression regulated in mice? (circle one)

RNA level protein level

g) Justify your answer to part f), referring to **specific lanes** of the blot/film that support your conclusion.

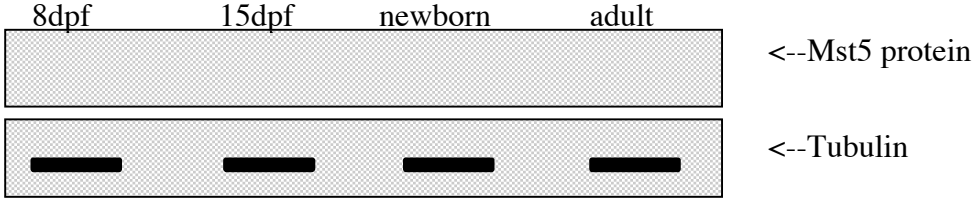
To study Mst5 regulation, you first decide to perform a Northern blot. You re-extract total RNA from mice at the four stages, run a gel, transfer the RNA to the membrane, and probe for Mst5. You observe the following:



EtBr stained gel Film

h) Interpret the results of the Mst5 Northern blot.

i) You hypothesize that Mst5 expression is regulated solely at the level of **transcription**. Draw what you will see on a Western blot probed for Mst5 protein if your hypothesis is correct.



Question 1 (continued)

Now that you know something about how Mst5 is regulated in mice, you'd like to determine what tissues in the mouse express this gene.

j) Identify two different experiments you could use to answer this question.

Experiment 1: _____

Experiment 2: _____

You know that the zebrafish genome contains an Mst5 homolog, which is 80% similar to the mouse gene at the nucleotide level.

k) Name 3 specific changes that you could make to your mouse Mst5 Northern blot protocol that may allow you to detect the zebrafish Mst5 transcript.

1.

2.

3.

A Southern blot allows a researcher to detect the presence of a particular DNA molecule in a mixture of other DNA molecules. You perform a Southern blot using the following protocol:

1. Isolate total DNA;
2. Cut DNA into pieces of various sizes using an "8-cutter" restriction enzyme;
3. Denature DNA and separate the pieces on an agarose gel;
4. Transfer the DNA to a membrane and prehybridize;
5. Hybridize with a labeled probe specific to Mst5;
6. Detect the probe.

l) Assuming that you loaded total DNA from an equivalent number of cells in each lane—and that transfer was 100% efficient—draw what you'd expect to see on the Southern "film" below. Justify your answer **in one sentence.**

Film

Question 2 (x points)

In 7.02 lab, you observed that LiCl disrupts the development of zebrafish embryos. Design a microarray experiment to determine the effects of LiCl treatment on gene expression in zebrafish by filling in the blanks with the appropriate word or phrase.

a) Each spot on the DNA chip contains millions of copies of the cDNA of _____ gene.

b) In my experiment, I will use two populations of zebrafish embryos. The _____ population will be treated with LiCl, and the _____ population will be untreated.

c) To observe an effect of LiCl on embryonic development, I will treat the embryos at the following developmental stage (circle one):

between 2-6 hpf between 10-24 hpf between 24-48 hpf

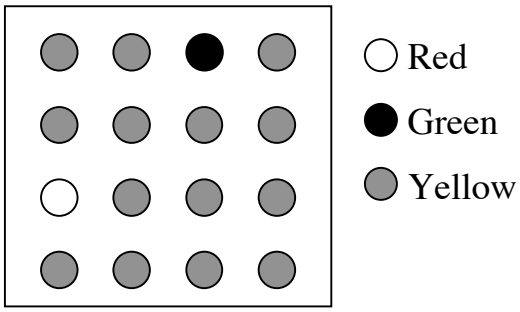
d) I will isolate _____ from each population and convert it to cDNA using the enzyme _____.

e) The cDNA from the LiCl-treated population will be labeled "red" and the cDNA from the untreated population will be labeled "green" using _____.

f) The labeled cDNA from both populations will bind to the chip via _____.

g) To visualize the expression pattern, I will expose the "chip" to _____.

Here is what your DNA "chip" looks like after visualization:



h) On the diagram above, **<u>circle</u>** a "spot" that represents a gene whose transcription is **<u>decreased</u>** in the presence of LiCl.

i) On the diagram above, **<u>draw an "X" through</u>** a "spot" that could represent a gene that is a **<u>negative regulator</u>** of zebrafish eye development.

j) On the diagram above, **<u>draw a square</u>** around a "spot" that represents a gene whose transcription is **<u>unaffected</u>** by LiCl.

Question 3 (x points)

You are studying a new gene, *knighted*, which you suspect is involved in the formation of the zebrafish embryonic shield. Previous work has demonstrated that *knighted* is transcribed in the **gastrula** stage (stage 2) of development, and that the transcript size is **1.5 kb**. To confirm this result, you decide to perform Northern blots using RNA from the four stages of development used in lab (1, 2, 3, 4) and the detection reagents used in 7.02.

Listed below are the partial sequence of the *knighted* cDNA and the sequences of three potential probes (A, B, C) that you could use to detect *knighted* in your Northern blot:

```
5'.....GATCGGGAATGATAGCCTAGGG....3'<-same sequence as knighted mRNA
3'.....CTAGCCCTAACTATCGGATCCC....5'
```

Probe A: 5'—GATCGGGAAUGAUAGCCUAGGG—3'
* * *

Probe B: 5'—CCCUAGGCUATCAAUCCCGATC—3'
* * *

Probe C: 5'—CCCTAGGCTATCAATCCCGATC—3'

Probe D: 5'—UAUCAU—3'
* * *

Note: the (*) indicates the presence of DIG-dUTP

You decide to use each of the four potential probes (A, B, C, D) to the knighted mRNA to probe a different Northern blot membrane. For each probe, answer the following questions:

1) How many band(s) do you expect to see on your Northern film?
2) In what stage(s) of development do you expect to see band(s)?
3) What is the size or size(s) of the band(s)?
4) A brief explanation of your answers to questions 1-3.

Question 4 (x points)

Many of the techniques, reagents, or enzymes used or encountered in the laboratory in the four 7.02 modules (GEN, PBC, RDM, and DEV) can be grouped together based on a common "activity" or "outcome," as shown in the example below:

Category: Techniques used for introducing DNA into bacterial cells.

GEN: ___**transduction**_____

RDM: ___**transformation**_____

For each of the categories listed below, identify an enzyme, reagent or technique from each of the modules listed that fit into this category.

a) Enzymes that **create** the phosphodiester bond between nucleotides.

RDM: _____
DEV: _____

b) Enzymes that **break** the phosphodiester bond between nucleotides

DEV: _____
RDM: _____

c) Enzymes that **remove** phosphate groups from other molecules

DEV and PBC (same enzyme):_____
RDM: _____

d) Reagent that you used in 7.02 lab to change the **phenotype** of an organism

DEV: _____
GEN: _____

e) Techniques for the separation of macromolecules based on their mobility in/through a porous matrix.

PBC (2 different techniques): _____ and _____
RDM and DEV (same technique): _____

e) Techniques whose success depends on the creation of **specific** DNA-DNA (or RNA-DNA) duplexes.

RDM: _____
DEV: _____

Question 5 (x points)

On the next page, you will find drawings or photographs of zebrafish embryos in various stages of development (labeled A-H). An adult zebrafish is also pictured (labeled I).

Answer questions a) through k) by placing the appropriate CAPITAL letter or letter(s) in the blank. Some letters may be used more than once.

a) Which one(s) contain totipotent cells? _____

b) Which one(s) contain an immune system? _____

c) Which one(s) are at the mid-blastula transition (MBT)? _____

d) Which one(s) are undergoing epiboly? _____

e) Which one(s) represent the four stages of zebrafish from which you isolated RNA for your Northern blot in 7.02 lab:
stage 1_____ stage 2 _____ stage 3 _____ stage 4 _____

f) In which one(s) are somites are forming? _____

g) Which one(s) have a clearly visible embryonic shield? _____

h) In which embryo are the epiblast and hypoblast forming? _____

i) If you performed fate mapping in these stages, tissues from all three germ layers would be labeled. _____

j) At which stage(s) would you be able to visually see a difference between wild type and albino zebrafish? _____

k) Which is the earliest stage embryo in which you could see the heart beating?

You have a dish of zebrafish embryos that were fertilized between 10-24 hours ago, but you are not sure of the exact age of these fish.

l) What could you "count" in order to accurately tell the time of fertilization? _____

m) In your own words, define the following:

a. anterior:

b. posterior:

c. dorsal:

d. ventral:

n) Label the axes defined above on the embryo labeled "F."

Figures removed due to copyright reasons.

Question 6

a) What is a teratogen?

b) How does a teratogen differ from a mutagen?

c) What do you think would happen if you added 0.3M LiCl to an adult zebrafish? Explain your answer briefly.

Question 7

What was the purpose of the following reagents in the Development module? A complete answer will **BRIEFLY** describe when it was used in the module (what experiment) and what it is/how it works/what it does.

a) diethylpyrocarbonate (DEPC)

b) 10X SSC

c) guanidine isothiocyanate

d) NaOAc, pH 4.0

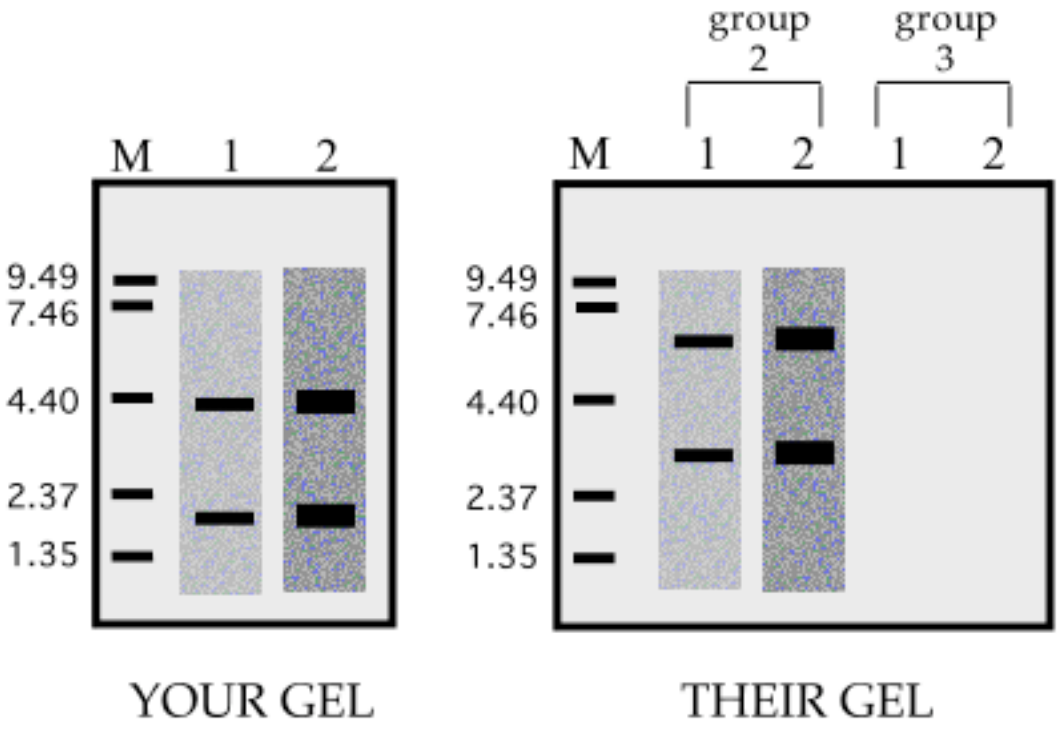
e) 1 kb *zcyt1* fragment

f) pBSK

Question 8

After a very successful (or so you hope!) RNA purification on Development Day 1, you are ready to run your analytical gel to check your RNA purity and quality. You pour a gel and run two of your samples (sample 1 and sample 2) on it. Two other groups in the class (group 2 and group 3) also check their RNA samples by running another gel.

As an extra precaution, all three groups confirm that they **DO** have RNA present in their samples using UV spectrophotometry. YOUR GEL and THEIR GEL (groups 2 and 3) are shown below:



Answer the following questions about YOUR GEL:

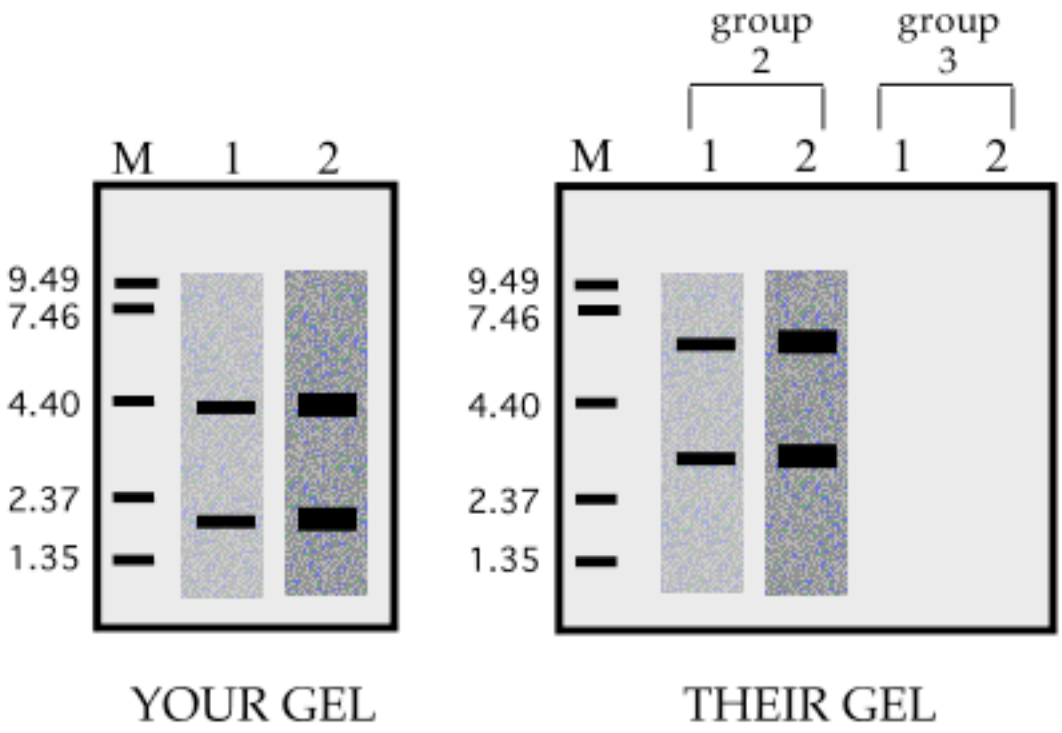
a) What do the two thick bands in each of samples 1 and 2 represent?

b) What makes up the background "smear" that you observe?

c) Which of your samples (1 or 2) has more total RNA in it? How do you know?

Question 8 (continued)

Your colleagues in groups 2 and 3 are confused by their gel results, and ask you to help them figure out what why their results are different than yours. Here are the two gels again:



d) What two reagents or steps might group 2 have omitted when preparing their samples? Explain your reasoning.

e) What reagent did group 3 omit? Explain your answer.

Question 9

Describe the two types of approaches (classical and molecular) used in the 7.02 laboratory to study zebrafish development. What problem/question were you trying to address with each approach?

Question 10

Name the three germ layers that develop during gastrulation and give an example of a tissue or organ that develops from each.

1.

2.

3.

Question 11

Name three advantages of using zebrafish as a model organism:

1. _____

2. _____

3. _____

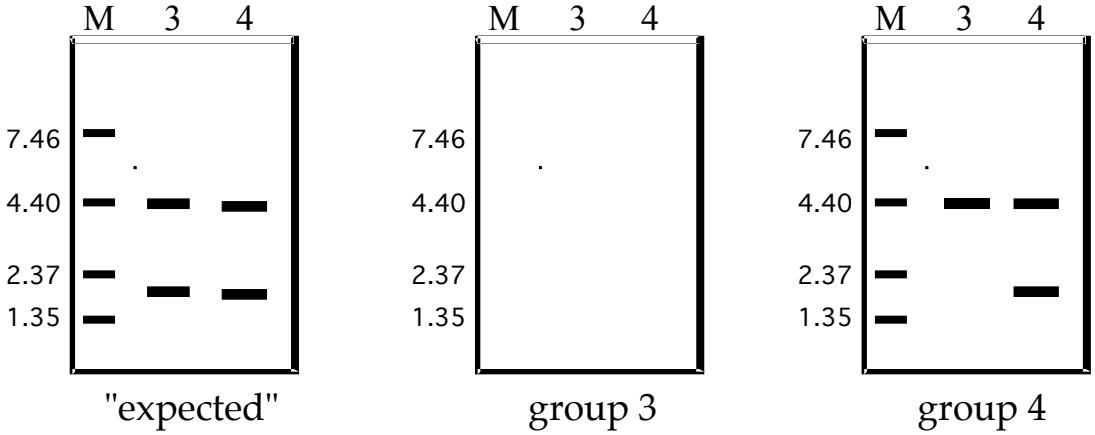
Question 12

In the Development module, you ran a Northern blot to determine the expression pattern of the *Danio rerio* (zebrafish) *z-cyt1* gene. The following are the "expected results" for two different steps in the process, and examples of "bad results" obtained by different student groups for the same step. (Note: for simplicity, only 2 stages of RNA—3 and 4—are shown.)

In analyzing your results, you should consider each "step" to be an independent experiment. That is, the results of the first step do not impact the results of later steps.

For each "bad" result, BRIEFLY identify what went wrong, how you know, and how you'd prevent the problem from happening again.

Northern Blot transfer (Day 3/3.5)
(pictures show RNA on blot visualized on UV box; assume that prior to transfer, denaturing gel looked "as expected")

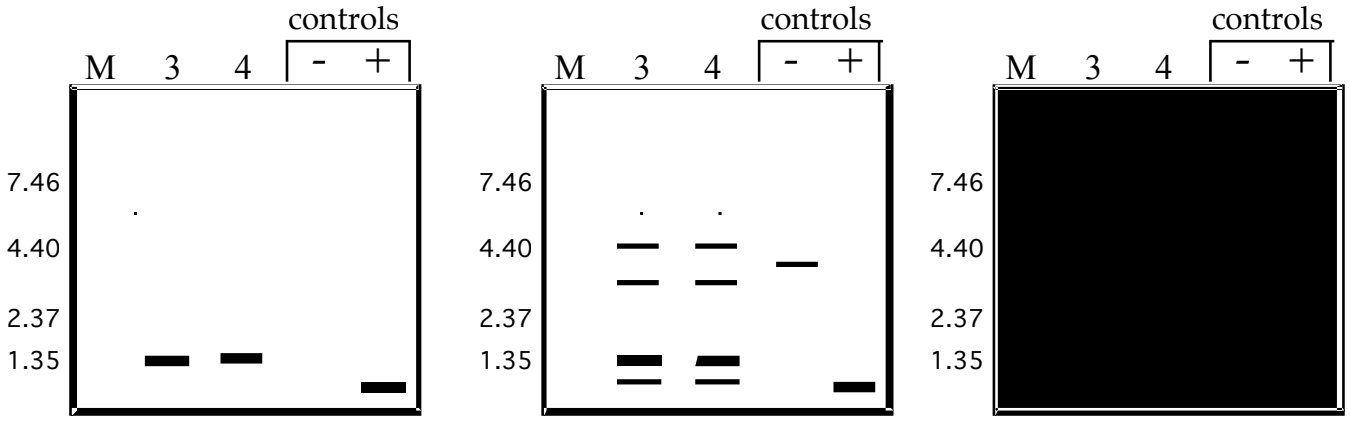


Group 3:

Group 4:

Question 12 (continued)

Northern Blot Film: Day 5
(assume that all transfer occurred "as expected" and that the same labeled *zcyt1* probe was used by all groups)



Group 5:

Group 6:

Question 13

You have two *E. coli* strains that are identical in every way except that one has an intact copy of the *lacZ* gene (LacZ+) and one has a deleted *lacZ* gene (LacZ-). The strains are unlabeled, and you need to figure out which strain is which!

In the chart below, describe five **different** types of experiments/techniques that you could use to help you distinguish the two strains. Tell me in what module you learned about the experiment/technique, and what you'd expect to see if your strain is LacZ+, and if it is LacZ-!!

(Remember: Many experiments learned in 7.02 can be adapted to study any gene or protein of interest!)

Experimental description	what module?	if the strain is LacZ+, I'd observe...	if the strain is LacZ-, I'd observe...

Question 14

After completing a strenuous but satisfying year of 7.02, you can't stay away—and find yourself back in 7.02 as an undergraduate TA (UTA). On DEV Day 3, you spot your students constructing their Northern apparatuses in the following combinations:

Apparatus A

TOP

Lid with heat block
Stack of Wet Paper towels
Whatman
Nylon Membrane
Inverted Agarose Gel
Wet Wick
Inverted Gel Tray

BOTTOM

Apparatus B

TOP

Lid with heat block
Stack of Dry Paper towels
Whatman
Nylon Membrane
Wet Wick
Inverted Agarose Gel
Inverted Gel Tray

BOTTOM

Apparatus C

TOP

Lid with heat block
Stack of Dry Paper towels
Whatman
Nylon Membrane
Inverted Agarose Gel
Wet Wick
Inverted Gel Tray

BOTTOM

Apparatus D

TOP

Lid with heat block
Stack of Dry Paper towels
Whatman
Upright Agarose gel
Nylon Membrane
Wet Wick
Inverted Gel Tray

BOTTOM

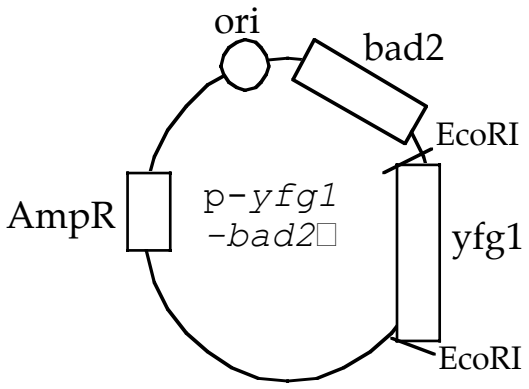
All of the gel chambers were correctly filled with 10XSSC, covered with Saran Wrap as described in the 7.02 manual, and the transfer proceeded overnight.

a) Which apparatus(es) were set up correctly for optimal Northern Transfer? _____

b) Choose **ONE** suboptimal apparatus, and briefly explain why its setup will result in a failed Northern transfer.

Question 14 (continued)

During your semester as a 7.02 UTA, we changed the Northern blot part of the DEV module to look at the developmental regulation of another zebrafish gene, *yfg1*. The instructors ask you to create a labeled probe specific for *z-yfg1*. Your starting template DNA is the plasmid p-*yfg1-bad2* (shown below). This plasmid contains the cDNA sequences of the *yfg1* gene and another zebrafish gene, *bad2*.



c) You want to create a labeled probe that is **specific for *yfg1***, and plan to follow the probe labeling protocol used in 7.02 lab. What is problematic about using the p-*yfg1-bad2* plasmid as the template DNA for creating/labeling your probe?

d) Describe **two different methods** you could use to create an "improved" template DNA for probe creation/labeling. You have access to p-*yfg1-bad2* plasmid DNA, any reagents used in the 7.02 lab this semester, and the ability to synthesize any DNA molecule you choose.

(Note: This question can be answered in one or two sentences—BE BRIEF!!)

Question 14 (continued)

Below are the sequences of portions of the *yfg1* mRNA and three non-*yfg1* mRNAs found in zebrafish embryos (A, B, and C). All four mRNAs are of different lengths, and thus can be separated from one another on a denaturing agarose gel.

5′--...GAUGAAAGAUCAGGUCUGAAUGUAU...–3′	*yfg1* mRNA
5′--...UUUGAAAGAUCAGGUCUGAAUGUAU...–3′	A
5′--...CUACUUUCUAGUGGUCUGAAUGUAU...–3′	B
5′–-...CUACUUUCUAGUCCUCUGAAUGUAU...–3′	C

The probe you've created is **100% complementary** to the sequence of the *yfg1* mRNA. given above.

You hybridize the probe to your blot at room temperature (20˚C), then want to adjust the stringency of your washes by increasing **temperature** of the buffer (but leaving salt constant).

e) You perform your highest stringency wash at a temperature of **50˚C.** Which of the four mRNA(s) will you "see" as bands on film when you develop your Northern blot? Circle one, and explain your reasoning.

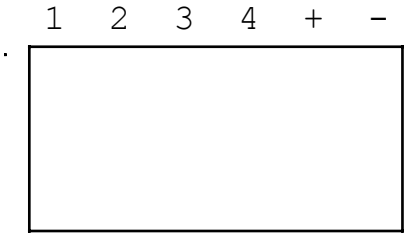
i *yfg1* alone
ii *yfg1* and A
iii *yfg1* and B
iv *yfg1* and C
v all of the above
iv none of the above

Question 15

After 7.02, you decide to come back as an undergraduate TA. Your student groups in the DEV module can't seem to follow the protocol in lab; instead, they insist on doing it "their way." Help them understand why following the protocol is a good idea by deciphering the following scenarios:

a) One group decides to use the lysis protocol from the PBC module (25 mM Tris, 10 mM EDTA, pH 8.2 + lysozyme + detergent) instead of the lysis buffer from DEV to lyse their zebrafish embryos. They are surprised when they don't get any RNA from their samples. Are you surprised? Why or why not?

b) A second group decides to help Debbie make the *zcyt-1* specific probe. They set up the labeling reaction with the following reagents: 1kb *zcyt1* cDNA, hexanucleotide mixture (primers, buffer, salts, etc.), dNTPs (1mM dATP, 1 mM dGTP, 1 mM dCTP, 0.65 mM dTTP, 0.35mM dUTP) and Klenow. They perform the Northern blot as in lab, and get a blank film (no bands, as in the picture below). Assuming that transfer was successful, why is their film blank?



c) Your final group of students (C4) decides to change the prehyb/hyb conditions. They prehybridize their membrane at 0.5X SSC and 68˚C, then hybridize at 5X SSC and 50˚C. They share RNA with group C2, who does the experiment exactly as done in 7.02 lab. Assuming both groups have the same amount of RNA on their blots, what do you expect to see when you compare their blots? Circle one and explain your reasoning.

i. C2 has a stronger *zcyt1* signal (darker bands) than C4
ii. C4 has a stronger *zcyt1* signal than C2
iii. The two groups have the same intensity *zcyt1* signal.

Question 16

Many of the techniques or reagents used in the 7.02 DEV module can be compared and contrasted with other techniques/reagents used in the 7.02 Genetics, Protein Biochemistry, and Recombinant DNA Methods modules.

For each pair of techniques/reagents listed below, please do the following:

1. Describe how the members of the pair are **similar** (in origin, function, and/or outcome);
2. Describe how the members of the pair are **different**.

For example:

Mg+2 and Ca+2 (GEN)

Similar: both are divalent cations important for phage binding to cells
Different: Mg+2 is required for lambda binding, whereas Ca+2 is required for P1 binding

a) agarose gel electrophoresis (DEV) and agarose gel electrophoresis (RDM)

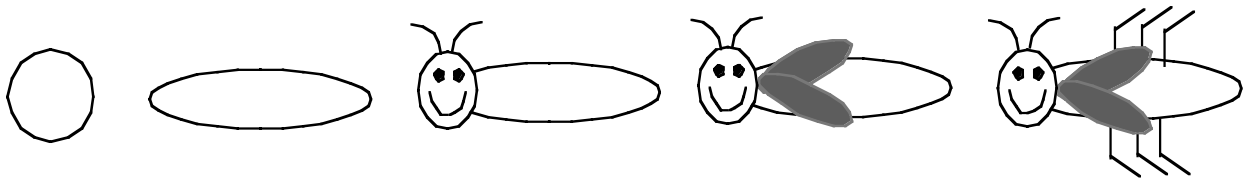
b) Klenow (DEV) and Taq (RDM)

c) Northern blot **transfer step** (DEV) and Western blot **transfer step** (PBC)

d) precipitation of RNA (DEV) and precipitation of proteins (PBC)

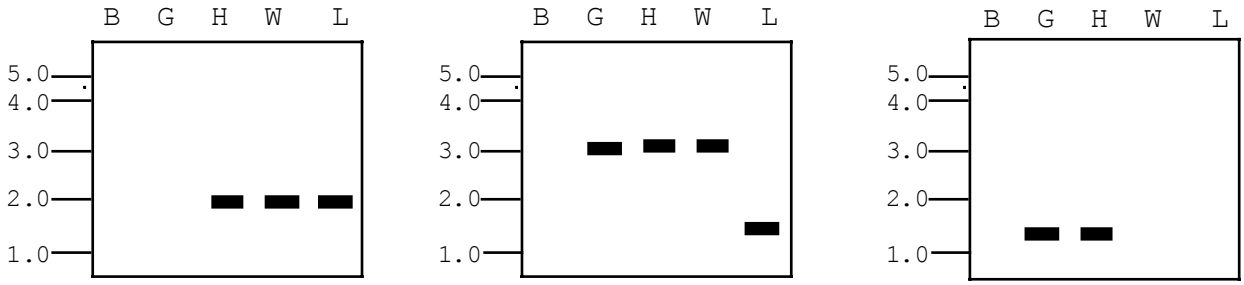
Question 17

You are interested in the early development of the Happy fly, a relative of the fruit fly, *Drosophila melanogaster*. You suspect that Happy fly development requires careful temporal ("timed") expression of genes required for the creation of different body parts, and set out to investigate this process. The development of the Happy fly proceeds as diagrammed below:



Blastula (B) Gastrula (G) Head (H) Winged (W) Legged (L)

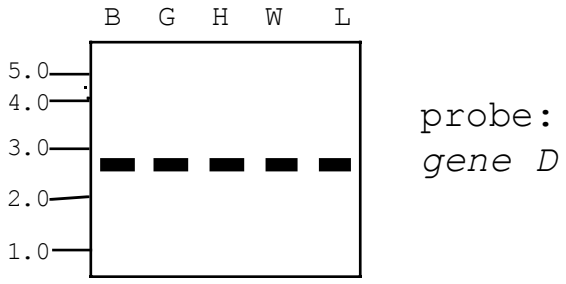
You have cloned three genes (*A*, *B*, and *C*) that appear to be required for Happy fly development. To study their expression patterns, you create DIG-labeled probes for each gene using the appropriate **cDNA** as a template for probe labeling. You isolate RNA from wild type embryos at the stages of development pictured above, load a gel with equal volumes of RNA from each stage, and perform Northern blots like in 7.02 lab. Below are the developed blots (film):



a) What body part(s) do genes *A*, *B*, and *C* likely affect the development of? Do these genes likely act as positive or negative regulators of development? Explain your reasoning.

Question 17 (continued)

You next strip the blots from part a) (that is, remove the bound probe), and reprobe the membrane with a probe specific for another Happy fly mRNA, *gene D*. You observe the following pattern on each blot:



b) Is *gene D* likely to be important for the <u>development</u> of the Happy fly? Why or why not?

Question 18

You have discovered that your quest in life is to study heart development in *Danio rerio* (zebrafish). You perform transposon mutagenesis on zebrafish, and screen for mutants with altered heart development. One of your mutants, *romeo*, has a heart that is twice as big as that of a wild type zebrafish! You clone the *romeo* gene, and decide to study its expression by performing a Northern blot on wild type embryos at different stages of development.

a) What **three** pieces of information can your Northern blot provide about the expression of *romeo*?

b) Name one additional piece of information you could gain about *romeo* if you did an *in situ* hybridization instead of a Northern blot. _____

Question 18 (continued)

In order to perform your Northern, you need to create a *romeo*-specific, labeled probe. Your labmate offers to help you, and sets up the probe labeling reaction with the following components: buffer, template (zebrafish chromosomal DNA), Klenow polymerase, random hexamers, DIG-dUTP, and dNTPs. He then programs the thermocycler to carry out the following program, and heads to lunch:

95˚C 5 minutes
95˚C 1 minute◊ 52˚C 2 minutes◊ 72˚C (repeat 29x)
72˚C 5 minutes

c) Explain why your labmate's protocol will **NOT** create a *romeo*-specific, labeled probe. (Hint: three reasons)

To your dismay, you cannot reprogram the thermocycler—it only runs the program listed above.

d) What **two** changes can you make to the probe labeling reaction (i.e. components) to allow you to create the probe you need?

You have also been studying the effects of different chemicals on heart development. To your surprise, zebrafish treated with ethanol also have enlarged hearts! You hypothesize that ethanol may cause enlarged hearts by affecting the transcription of *romeo*.

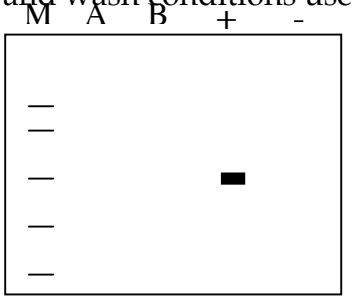
e) In list form (step 1, 2, etc.), describe the experiment you would perform to test this hypothesis. Be **brief** but **specific**.

e) What results would you expect to see if your hypothesis is correct? Explain your reasoning (HINT: how was *romeo* identified?).

Question 18 (continued)

While fishing in the Charles River, you stumble upon a new species of fish which you name *Almstdon 702us* . While observing the transparent embryos of *A. 702us*, you notice that their hearts have a similar structure to those of the zebrafish. Suspecting that these fish might express *romeo* as well, you decide to perform a Northern blot.

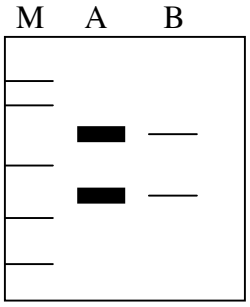
To do this, you first isolate *Almstdon 702us* embryos at two developmental stages (A and B); these stages correspond to the zebrafish stages where *romeo* is expressed. After isolating total RNA, you perform a Northern blot using your zebrafish *romeo* probe and the same hybridization and wash conditions used for zebrafish. You observe the following:



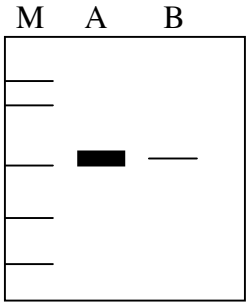
M = MW markers
+ = zebrafish *romeo* cDNA
- = pBSK

f) Assuming that *romeo* plays a similar role in *Almstdon 702us* as it does in zebrafish, why might you have gotten these results? What conditions could you change (and how would you change them) to increase the success of this Northern?

You finally get the conditions worked out, and observe the following result on your Northern blot probed for *romeo*:



EtBr stained gel



Northern film

g) What do you conclude about the pattern of *romeo* expression in *Almstdon 702us*? Explain your reasoning.

Question 19

The following statements are related to the laboratory experiments performed in the 7.02 DEV module. Please mark whether each statement is **true** or **false**. **If a statement is false, correct it by crossing out and/or substituting words or phrases.**

(For example: __False__ The winter sky over Boston is usually gray blue).

_____ a) GITC is an alkylating agent that inactivates RNAses by modifying active site histidine residues.

_____ b) *zcyt1* encodes cytokeratin, a protein that gives strength to the zebrafish skin.

_____ c) The shield forms on the ventral side of the embryo and is involved in determining the embryo's anterior/posterior axis.

_____ d) Formaldehyde and formamide denature RNA by disrupting the hydrogen bonds between bases.

_____ e) Three examples of mutagens are LiCl, alcohol, and transposons.

_____ f) The *zcyt1* probe used in the Northern blot was synthesized using specific primers and DIG-labeled dCTP.

_____ h) SDS and BSA are used to prevent nonspecific binding of the probe and anti-DIG antibody to the Northern membrane.

_____ i) CSPD is cleaved by β-galactosidase, which is conjugated (attached) to the anti-DIG antibody.

_____ j) RNA degradation occurs when the 2' OH attacks the RNA backbone, breaking the phosphodiester bond.