

**Spring 2005  
7.02/10.702 Development Exam  
Study Questions  
ANSWER KEY**

**Note: To save paper, only the answers are provided here. The questions can be downloaded as a separate file.**

## Question 1

a)

	Northern blot	Western blot
What method(s) will you use to denature your samples?	<b>Formaldehyde Formamide Heat</b>	<b>SDS BME Heat</b>
What type of gel will you run?	<b>Denaturing agarose gel</b>	<b>Polyacrylamide</b>
In what direction will your samples migrate in the gel? What ensures this?	<b>Toward the positive pole  The phosphates of the RNA (sugar-phosphate) backbone make it negatively charged.</b>	<b>Toward the positive pole  SDS coats the proteins, giving all proteins a net negative charge (constant charge/mass ratio)</b>
What specific type of "probe" will you use to detect Par1 mRNA or protein?*	<b>A DIG labeled probe that is complementary to (or specific to) the Par1 mRNA</b>	<b>A rabbit anti-Par1 antibody</b>

b) Since tubulin is expressed at constant levels at all stages, probing for this protein tells you if you have loaded constant amounts of total protein from each stage of development.

c) YES

d) NO

e) No. The EtBr gel does not show any staining for the rRNA bands—indicating that there is no RNA present at all in that lane of the Northern blot. Thus, you cannot conclude anything about the expression of Par1.

f) protein level

g) On the Northern blot film, we see that the Par1 mRNA is present in equal amounts at 8 dpf, 15 dpf, and in adults. Yet on the Western film, we see that there is more protein in early stages (8. 15) than in the adult (absent). Thus, though the mRNA is always there, there are different amounts of protein made-->regulation at the protein level.

## Question 1 (continued)

h)

- The Mst5 gene is transcribed in the following stages: 15 dpf, NB, and A.
- The Mst5 mRNA levels are equal in the newborn and adult mice, and there is 2X as much Mst5 mRNA in these stages as in the 15 dpf mouse. (You know this because while the Northern film signal is equal, there is 2X as much total RNA in the 15 dpf lane as in the NB and A lanes, which each have 1X total RNA. Therefore, the actual transcript levels are 1X, 2X, 2X.)
- The Mst5 transcript is 2.4 kb in length.

i) There should be 2X as much protein in NB and A lanes as in the 15 dpf lane, and no protein in the 8 dpf lane.

j) Possible answers include:

- Northern blot, where each lane is from different tissues
- In situ hybridization
- GFP fusion to Mst5

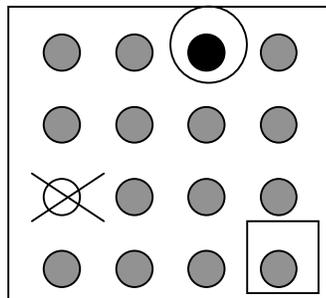
k) Possible answers include:

- increase the salt concentration
- decrease the temperature
- use a more neutral pH
- reduced concentration of denaturants (e.g. formamide)
- use a probe specific for the zebrafish Mst5 gene

l) The bands in the Southern film should be of equal intensity. This is because every cell in the mouse (at every stage of development) has two copies of the Mst5 gene; therefore, the signal will be the same at every stage if an equivalent number of cells are loaded.

## Question 2

- one
- experimental; control
- between 2-6 hpf
- total mRNA; reverse transcriptase
- fluorescent dyes
- basepairing
- UV light
- h-j) see figure



### Question 3

Probe A:

- 1) no bands
- 2) and 3) N/A
- 4) Probe A has the same sequence as the *knighted* mRNA, and therefore cannot basepair to *knighted* mRNA on the blot.

Probe B:

- 1) one band
- 2) stage 2 (gastrula)
- 3) 1.5 kb in length
- 4) Probe B has a sequence that is complementary to the *knighted* mRNA, and will therefore hybridize to the *knighted* mRNA on the blot. It is also labeled with DIG, which is required for detection by anti-DIG Ab with AP and CSPD. There will only be one band because the probe is long enough to be specific; the band will be 1.5 kb and in stage 2 because we know that is where *knighted* is transcribed (see intro to problem).

Probe C:

- 1) no bands
- 2) and 3) N/A
- 4) Though the probe is complementary to the *knighted* mRNA, it does not contain DIG for visualization.

Probe D:

- 1) many bands
- 2) all stages of development
- 3) all sizes
- 4) Probe D is complementary to the *knighted* mRNA, and will therefore hybridize to the *knighted* mRNA on the blot. However, since the probe is only 6 basepairs long, it will also likely bind to other mRNAs in every stage of development (anything that has a complementary sequence!). As in probe B, you see bands because the probe is labeled with DIG, which is required for detection by anti-DIG Ab with AP and CSPD.

### Question 4

- a) Taq or ligase (RDM); Klenow (DEV)
- b) restriction enzymes (RDM); RNAses (DEV)
- c) CIP (RDM); alkaline phosphatase
- d) lambda 1205 or miniTn10 transposon (GEN); lithium chloride (DEV)
- e) SDS-PAGE and gel filtration (PBC); agarose gel electrophoresis (RDM/DEV)
- f) PCR (RDM); Northern blot (DEV).

**Question 5**

- a) A
- b) I
- c) H
- d) B (F)
- e) stage 1 = H; stage 2 = B; stage 3 = D; stage 4 = G
- f) C
- g) B
- h) B
- i) A, E, H
- j) G, I
- k) D
- l) somites
- m)
  - a) anterior = "head end"
  - b) posterior = "tail end"
  - c) dorsal = "back"
  - d) ventral = "belly"
- n)

Figure removed due to copyright reasons.

## Question 6

- a) A teratogen is a chemical/substance that causes a phenotypic change (defect) in the organism that is exposed to it—without affecting that organism's genetic material (DNA).
- b) A mutagen, unlike a teratogen, causes a change in the organism's DNA. As a result, the effects of mutagens MAY be heritable, but only if the DNA change occurs in the germ cells.
- c) Probably nothing. LiCl acts as a teratogen during embryonic development in zebrafish. Since adult fish have already completed development, there is likely to be no effect of LiCl exposure. However, if the fish was pregnant, her offspring may be affected.

## Question 7

- a) DEPC was used to treat the water used to make all the solutions in the DEV module. DEPC is a chemical that inactivates RNAses (by esterifying the histidines in the RNase active site). Using DEPC-treated water prevents RNase degradation of our RNA samples.
- b) 10X SSC was the liquid phase used in the transfer step of the Northern blot on Day 3. The movement of 10X SSC by capillary action allowed the transfer of RNA onto the nylon membrane.
- c) Guanidine isothiocyanate is a strong detergent. It was used during the lysis of zebrafish embryos to denature proteins (including RNAses).
- d) NaOAc, pH 4.0 was used during RNA isolation on Day 1. At pH= 4.0, the DNA and RNA backbones are protonated. When protonated, DNA becomes nonpolar, and partitions to the organic phase; RNA, with its 2' OH, remains in the aqueous phase. The Na<sup>+</sup> ions also are required for precipitation of RNA at the end of Day 1.
- e) The 1 kb *zcyt1* fragment was used in two experiments in the Development module. It was the template DNA for probe labeling (Day 3), as was also the positive control on the Northern blot. This control helps ensure that our probe is specific for the *zcyt1* mRNA (if so, the 1 kb *zcyt1* fragment should give a band on our blot).
- f) The pBSK plasmid DNA was run as a negative control on the Northern blot. This ensures the specificity of the *zcyt-1* probe we made on Day 3.

## Question 8

a) 28S and 18S rRNA bands

b) mRNA

c) Sample 2 has more RNA. Both the rRNA bands and the background “smear” are more intense. Since EtBr binds proportionally to size and RNA concentration, a more intense band means more RNA. If rRNA bands are darker, we can assume that total RNA is greater as well.

d) The ribosomal RNA bands in group 2's lanes are not running “true to size.” This suggests that their RNA is not fully denatured. They may have forgotten to add formaldehyde and formamide, which form hydrogen bonds with RNA and thus prevent the formation of RNA secondary structures. They also may have forgotten to heat their samples to 65°C, which also helps denature the RNA.

e) Group 3 is likely to have forgotten the 2X denaturing mix, which contains ethidium bromide. They have RNA present (based on UV spectrophotometry, see previous page!), but without EtBr, there is no way to visualize their RNA bands.

## Question 9

The classical approach makes use of the phase contrast microscope and the researcher's observational and recording skills. In this module, you were interested in recording the progression of wild type zebrafish embryonic development, and then comparing that development to that of embryos treated with the teratogen LiCl. This approach allows you to look at phenotypic changes, but does not allow you to correlate changes in phenotype with changes at the molecular level.

The molecular approach made use of the Northern blotting technique to study the expression of a particular mRNA, *zcyt-1*, at four stages of embryonic development (blastula, gastrula, straightening, and hatching.) *z-cyt1* encodes cytokeratin, a structural protein of the epidermis. Thus, studying the expression of *zcyt-1* gives the researcher a molecular marker with which to follow the development of the epidermis.

## Question 10

1. endoderm: stomach and intestine (“gut”), lungs
2. mesoderm: muscles, bone, heart
3. ectoderm: skin, brain, nervous system

## Question 11

Here are four possible answers:

1. transparent embryos
2. short generation time, ability to get large numbers of embryos
3. external fertilization (easy to obtain embryos)
4. they are vertebrates, so they are a good model system for vertebrate (human) development

## Question 12

### Group 3:

Group 3 got no bands on their Northern blot after transfer (not even their molecular weight standards). Since the previous denaturing gel was “as expected,” this must have been a problem with the transfer of RNA from the gel to the blot. Perhaps they forgot to add the 10X SSC to the wells of their gel apparatus--such that there was no mobile phase to allow capillary action—or forgot the wick to move the buffer from the chamber and through the gel. Alternatively, if their paper towels fell into the buffer, the SSC would bypass the gel/blot, and transfer would not occur. To avoid these problems, be sure to add the 10XSSC to the buffer chamber, add the wick, and cover the chambers with Saran Wrap during Northern blot setup.

### Group 4:

Group 4’s blot shows a “spot” where no transfer of RNA has occurred in sample 3. This suggests that there was an air bubble between the gel and the membrane when the Northern blot was assembled. Since the RNA travels from the gel to the blot in the 10X SSC buffer (and cannot travel through air), air bubbles will eliminate transfer of RNA in the spot where the bubble occurred. To avoid this in the future, be sure to run a pipette over the membrane/Whatman paper to eliminate air bubbles.

### Group 5:

Group 5’s film shows hybridization of the probe to mRNAs other than the *zcyt1* mRNA (as indicated by the “expected” result), including a band in the negative control lane. This results suggests that the stringency of the washes was not high enough to eliminate non-specific probe binding; perhaps they forgot to do one or more of the wash steps. In the future, be sure to do both low and high stringency washes to ensure specific binding of the probe to the *zcyt1* mRNA.

### Group 6:

Group 6’s film indicates binding of the probe and antibody all over the nylon membrane. The subsequent enzymatic reaction (CSPD + alkaline phosphatase--> CSD + light) leads to the results observed on the film. This suggests that the students in Group 6 forgot to add the blocking reagents (casein) to their prehybridization/ hybridization solution. To avoid this, remake the prehyb/hyb solution with a blocking reagent.

### Question 13

Here are a number of experiments learned about in 7.02 that would work. For experiments other than PCR, cells would need to be grown in lactose or IPTG-containing media to induce the production of *lacZ* mRNA/protein in order to “see” a signal.

Growth on plates containing IPTG (or lactose) and Xgal	GEN	blue colonies	white colonies
$\beta$ -galactosidase assay with ONPG on crude lysate from each strain	PBC	yellow color produced	no yellow color produced
Western blot with anti- $\beta$ -galactosidase antibody, AP conjugated secondary antibody, and developing reagent (NBT/BCIP)	PBC	band on Western blot at the expected size of $\beta$ -galactosidase	no band on Western blot at the expected size of $\beta$ -galactosidase
PCR with primers specific to the <i>lacZ</i> gene	RDM	PCR product on agarose gel that is the size of the <i>lacZ</i> gene	no PCR product of the appropriate size
Northern blot with labeled probe specific to the <i>lacZ</i> gene	DEV	band on film of the size of the <i>lacZ</i> mRNA	NO band on film of the size of the <i>lacZ</i> mRNA
In situ hybridization with <i>lacZ</i> -specific probe	DEV	signal in cells where <i>lacZ</i> mRNA is present	NO signal in cells where <i>lacZ</i> mRNA is NOT present

## Question 14

a) Apparatus C was set up correctly for the Northern Blot transfer.

b) Apparatus A:  
The problem with this apparatus is that the stack of paper towels is WET (not DRY). Northern transfer relies on capillary action, and without dry paper towels to “pull” the SSC up, no RNA will be transferred.

Apparatus B:  
The problem with this apparatus is that the wick is placed above the gel. Thus, the liquid moving by capillary action will never pass through the gel, and the RNA will not be transferred to the nylon membrane.

Apparatus D:  
The problem with this apparatus is that the nylon membrane is between the wick and the agarose gel. Thus, the RNA will not be transferred to the nylon membrane by capillary action (in fact, it will transfer to--and likely through--the Whatman paper).

Note: Many people said that the problem was that the gel was not “inverted.” Remember that the purpose of “inverting” the gel in the correct Northern setup was to place the RNA in close proximity to the nylon membrane to ensure transfer. In Apparatus D, the RNA is closest to the membrane already, so “inverting” the gel is not correct.

c) The probe labeling protocol used in 7.02 lab relies on random hexamers as primers. Thus, using this template, you will create labeled primers that are specific for BOTH *yfg1* (the mRNA you want) and *bad2* (the mRNA you don't want), as well as the AmpR gene. Since both *yfg1* and *bad2* are zebrafish genes, your developed Northern blot will show bands for both, and thus will be difficult to interpret.

d)

Experiment 1:  
Use EcoRI to digest p-*yfg1-bad2*, and separate the fragments on an agarose gel. Purify the *yfg1* fragment, and use this your template DNA in the probe creation/labeling reaction.

Experiment 2:  
Design and synthesize PCR primers specific for the *yfg1* gene. Perform PCR to amplify *yfg1*, and use the PCR product as template in the probe labeling/creation reaction.

## Question 14 (continued)

### e) ii yfg1 and A

Reasoning:

The probe is 100% complementary to the portion of the *yfg1* gene shown here. The  $T_m$  of the probe/*yfg1* mRNA duplex is  $68^\circ\text{C}$  (9 GC basepairs =  $36^\circ\text{C}$  + 16AT basepairs =  $32^\circ\text{C}$   $\rightarrow$   $68^\circ\text{C}$ ), so the probe will remain bound to *yfg1* after the  $50^\circ\text{C}$  wash.

Similar calculations can be performed for the other probe/mRNA combos, noting that only nucleotides that are the same as *yfg1* are included in the  $T_m$  calculation:

A:  $62^\circ\text{C}$  (8 GC basepairs, 15 AT basepairs are identical)

B:  $36^\circ\text{C}$  (5 GC basepairs, 8 AT basepairs are identical)

C:  $28^\circ\text{C}$  (3GC basepairs, 8 AT basepairs are identical)

Thus, at  $50^\circ\text{C}$ , the probe will also bind to mRNA A. Since the temperature is way above the  $T_m$ s of the probe/mRNA B and probe/mRNA C duplexes, these duplexes will not survive this high stringency wash, and you will not see bands B and C on your blot.

## Question 15

a) No! The lysis buffer from PBC does not contain any strong denaturants (like guanidinium isocyanate) that will denature RNAses. Thus, RNAses will be active in the embryo lysates and will destroy the RNA.

b) They never created a labeled probe! The labeling reaction mixture failed to include the DIG-dUTP (used dUTP instead!). The anti-DIG antibody is conjugated to alkaline phosphatase; it is the reaction of AP with CSPD that produces light (and therefore bands on film). Without DIG, there is nowhere for the antibody to bind.

c) The two groups have the same intensity *zcyt1* signal (answer iii). Prehybridization does not include the probe, so stringency is irrelevant here (stringency has to do with probe binding to the mRNA on the blot). Since they hybridized their probe under the same temperature/salt conditions as C2, they should get the same amount of probe bound, and thus the same signal on the film.

### Question 16

- a) agarose gel electrophoresis (DEV) and agarose gel electrophoresis (RDM)  
 Similar: both separate nucleic acids based on size and shape  
 Different: agarose gels in DEV are denaturing and are used for separating RNA molecules; the agarose gels run in RDM are nondenaturing and were used to separate DNA molecules
- b) Klenow (DEV) and Taq (RDM)  
 Similar: both are DNA polymerases  
 Different: Klenow was isolated from *E. coli*, works at 37°C, and was used in the probe labeling experiment; Taq was isolated from *Thermus aquaticus*, works at 72°C, and was used in PCR.
- c) Northern blot **transfer step** (DEV) and Western blot **transfer step** (PBC)  
 Similar: both involved the transfer of a macromolecule from a gel to a membrane  
 Different: Northern blot involves the transfer of RNA from an agarose gel to a nylon membrane via capillary action; Western blot involves the transfer of protein from a polyacrylamide gel to a nitrocellulose membrane by electroblotting.
- d) precipitation of RNA (DEV) and precipitation of proteins (PBC)  
 Similar: both precipitation methods involve disrupting "solvation cages"  
 Different: precipitation of RNA is performed using isopropanol (and Na<sup>+</sup> to shield the RNA backbone); precipitation of proteins was performed using ammonium sulfate.

### Question 17

- a) *gene A* is likely to regulate the development of the head. It acts as a positive regulator of development, as its mRNA first appears when the head appears and persists. *gene B* is likely to regulate the development of the legs. It acts as a positive regulator of development, as the spliced form appears when the legs first appear in the Happy fly. *gene C* is likely to regulate the development of the wings. It acts as a negative regulator of wing development, as its mRNA disappears when the wings form.
- b) Probably not. as it is present throughout development at constant levels. mRNA from developmentally important genes usually change in abundance, splicing, or localization (though this is not tested here) during the process of development.

## Question 18

- a) The three pieces of information you can obtain are:
1. When *romeo* is expressed (what stage)
  2. relative abundance of *romeo* mRNA at different stages
  3. size of the *romeo* transcript
- b) An *in situ* hybridization experiment can also tell you where *romeo* is expressed (location in the embryo).
- c) The three reasons are:
1. Your template is zebrafish DNA, and you are using random primers. Some fraction of your probe will hybridize to every gene in the zebrafish genome (and not just to *romeo*).
  2. Klenow is an *E. coli* enzyme; it will not function at the high temperatures used in this protocol (95°C, 72°C) like Taq does.
  3. Primers are only 6 nucleotides long; 52°C is too high for primer annealing (greater than the T<sub>m</sub> of the primer).
- d) Here are three possible answers:
1. generate *romeo* specific primers to use in the PCR reaction.
  2. Use Taq polymerase instead of Klenow
  3. use *romeo* cDNA as template instead of chromosomal DNA
- e) Here is the experimental protocol you should follow:
1. Fertilize embryos and separate into two groups (A and B).
  2. Treat “A” with ethanol and allow them to develop; Use group “B” as untreated control group.
  3. Harvest embryos from A (treated) and B (untreated) at different stages during development and isolate total RNA.
  4. Perform a northern blot—probing for *romeo*—and compare gene expression in treated and untreated embryos at each stage.
- f) You would expect that treated embryos would have lower levels of *romeo* expression than untreated. *romeo* was isolated as a loss of function mutant (transposon insertion) that had a “large heart” phenotype. Therefore, reducing *romeo* levels must give rise to large hearts. Thus, if ethanol treated embryos have large hearts, it is likely that *romeo* expression will be lower.
- g) Your hybridization and/or wash conditions, while suitable for zebrafish, may have been too stringent for detecting *romeo* in *Almstdon 702us*. You can lower the stringency by increasing the amount of salt or decreasing the temperature of your hybridization or washes.
- h) *romeo* is expressed at equal levels in stages A and B. Though the northern blot shows greater *romeo* signal in A, this is due to greater total RNA levels—as shown by the darker rRNA bands on the EtBr stained gel.

### 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 <sup>gray</sup>blue).

Corrections are noted in **BOLD**.

- false a) **DEPC** is an alkylating agent that inactivates RNAses by modifying active site histidine residues.
- true b) *zcyt1* encodes cytokeratin, a protein that gives strength to the zebrafish skin.
- false c) The shield forms on the **DORSAL** side of the embryo and is involved in determining the embryo's **DORSAL/VENTRAL** axis.
- true d) Formaldehyde and formamide denature RNA by disrupting the hydrogen bonds between bases.
- false e) Three examples of **TERATOGENS** are LiCl, alcohol, and **COCAINE** (or other teratogen of choice).
- false f) The *zcyt1* probe used in the Northern blot was synthesized using **RANDOM** primers and DIG-labeled **dUTP**.
- false g) SDS and **CASEIN** are used to prevent nonspecific binding of the probe (and anti-DIG antibody) to the Northern membrane.
- false h) CSPD is cleaved by **ALKALINE PHOSPHATASE**, which is conjugated (attached) to the anti-DIG antibody.
- true i) RNA degradation occurs when the 2' OH attacks the RNA backbone, breaking the phosphodiester bond.