

Advantages of zebrafish over bacteria in biological studies

- **Eukaryotic**
- **Multicellular**
- **Develops!!**

**Development: from a single-cell zygote
to a multi-organ organism**

ONLINE APPENDIX

Advantages of zebrafish as a MODEL ORGANISM in developmental biology

- _ It is a **VERTEBRATE**, and thus can provide clues to human biology.
- _ It **BREEDS** quickly and often (daily).
- _ Its embryos develop **OUTSIDE** the body where they can be easily observed.
- _ Its embryos are **TRANSPARENT** so defects in development can be seen easily.
- _ Embryonic development is **QUICK**.
- _ They can **ABSORB** small molecules, such as mutagen/teratogen, from the aquarium water.

Module overview

Goal	Technique	
<ul style="list-style-type: none">• Zebrafish development observation	<ul style="list-style-type: none">• Phase contrast microscopy• Teratogenesis	} DAY 2 ON
<ul style="list-style-type: none">• Gene expression analysis	<ul style="list-style-type: none">• RNA isolation• Northern blot	} TODAY

**GOAL I: study development
at morphological level**

GOAL II: study development at **molecular level**

- Gene expression pattern

How would you analyze gene expression?

1. PROTEIN!

-Western blot

or

-GFP

How would you analyze gene expression?

2. RNA!

-PCR (reverse transcriptase)

or

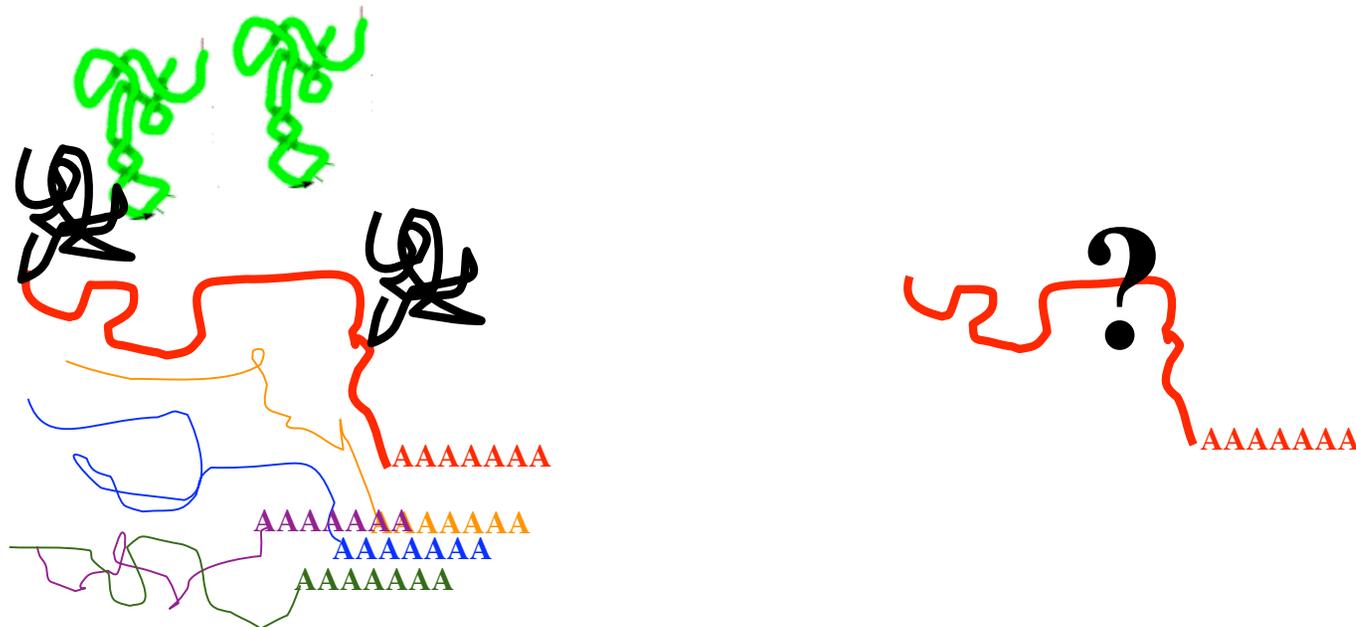
-Northern blot

Northern blot: molecularly monitor development progression

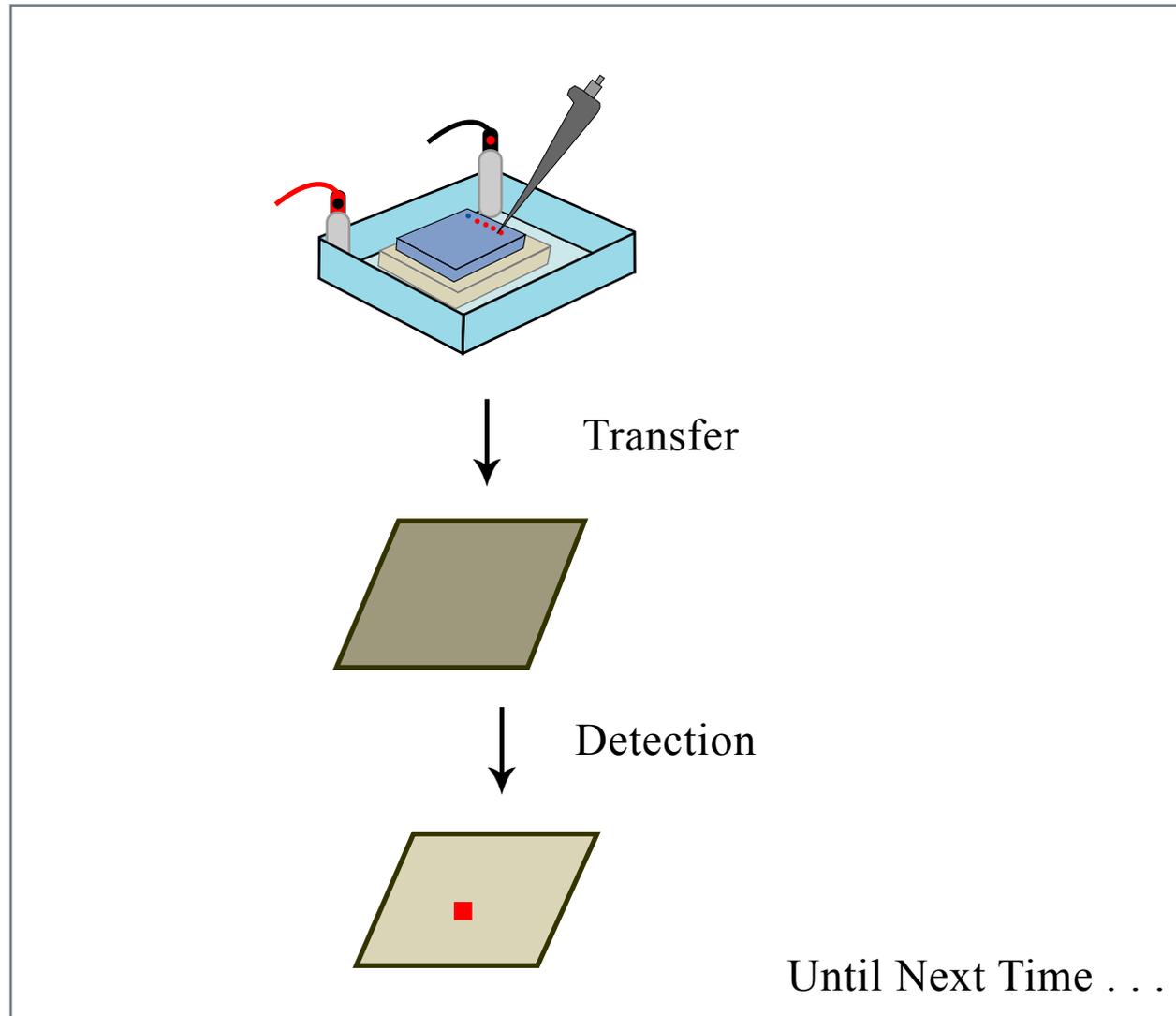
Total RNA from different developmental stages



Amount of a specific mRNA?

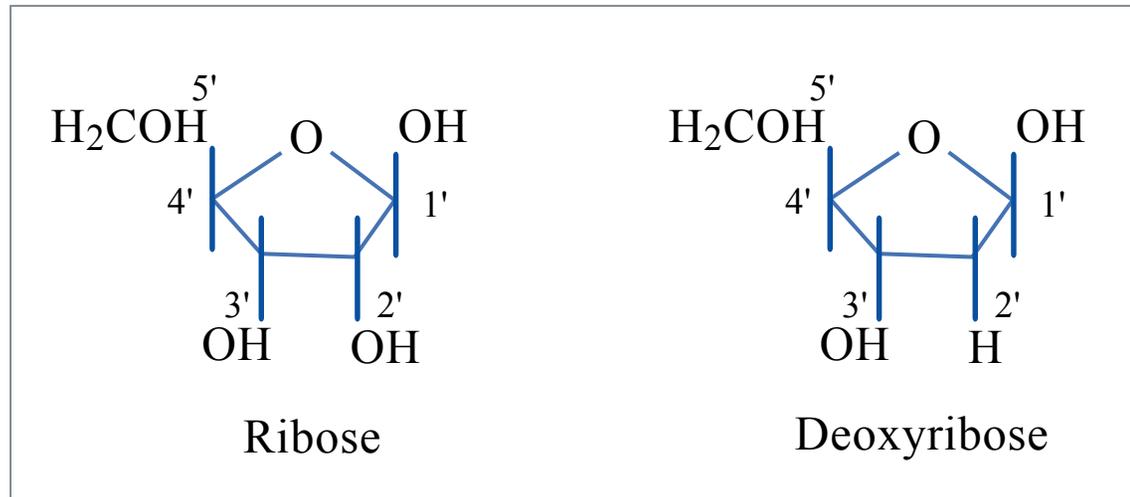


Northern is very similar to Western blot.



RNA vs DNA

sugar



Figures by MIT OCW.

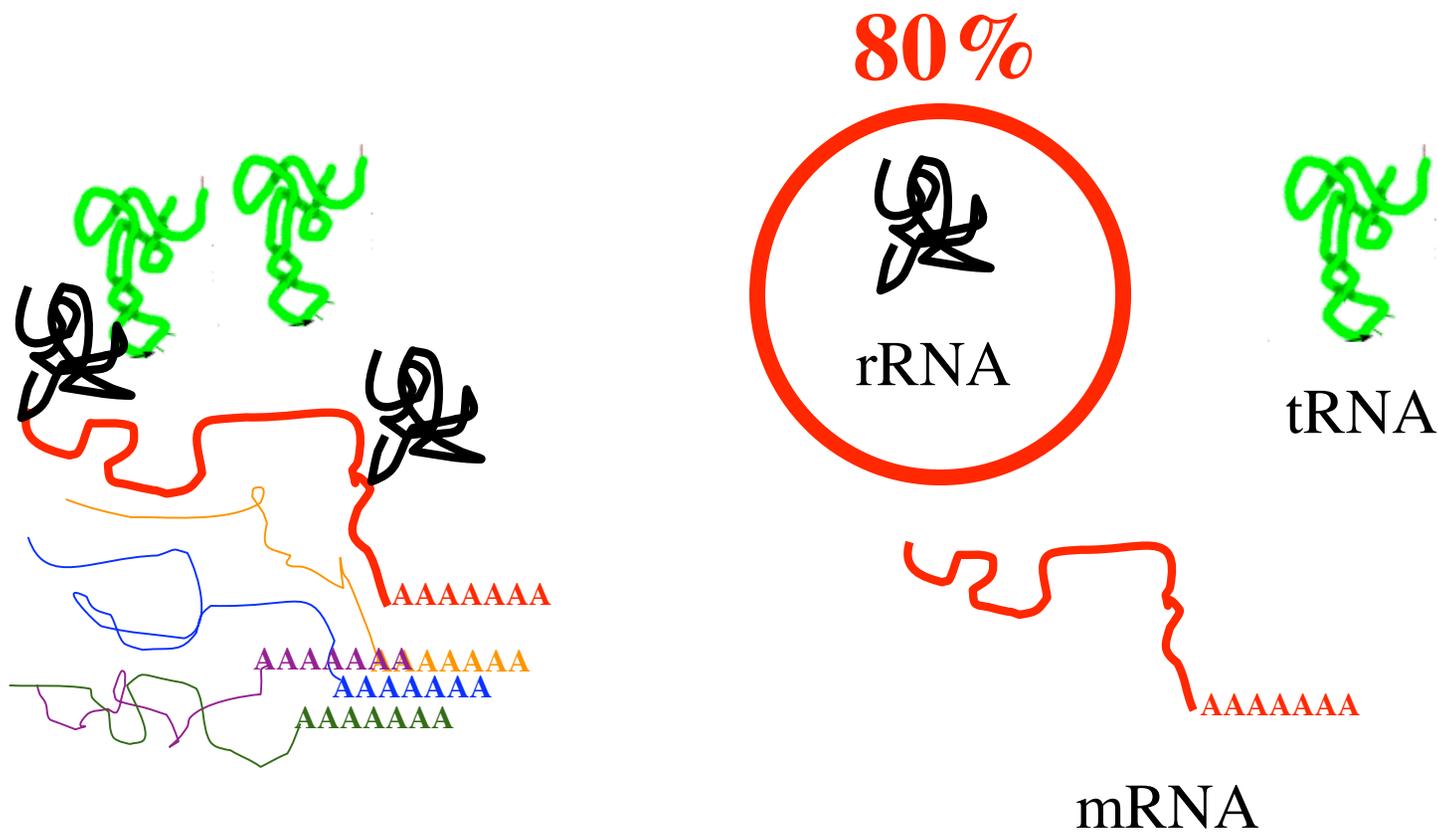
bases

A C U G

A C T G

structure Single stranded double stranded

Total RNA



RNA isolation: RNase ALERT!

- Wear gloves and lab coats
- Use sterile tips and tubes
- Don't speak directly to your sample
- Keep samples on ice
- Use DEPC water

DEPC: disrupts RNases' active site by chemically altering His and Tyr.

RNA isolation step 1: lysis of embryos

- Lysis buffer contains:
 - buffer NaCitrates
 - detergent sarkosyl
 - protein denaturant GITC
 - reducing agent β ME

DONE by the teaching staff.

RNA isolation step 2: separate RNA from the others

- Phenol/chloroform: Extract hydrophobic protein
- NaOAc (PH 4.0): Low PH allows RNA but not DNA to stay in the aqueous phase

Phenol/chloroform: very nasty!!

- **Eats through gloves in 7 min!!**
- Wear gloves, lab coats and goggles.
- Work with and dispose in the hoods.

RNA isolation step 3: precipitate, wash and dissolve RNA

- isopropanol
- 80% ethanol
- DEPC-treated ddH₂O