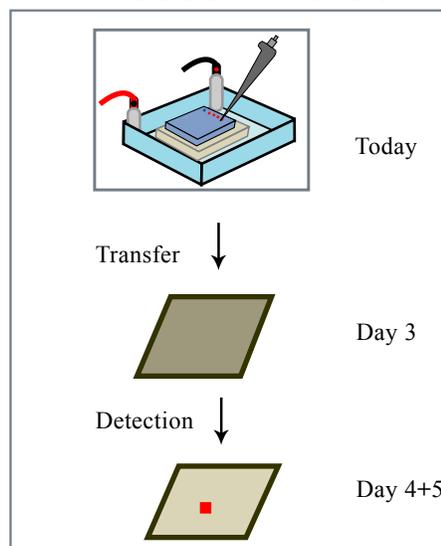


Module overview

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

Northern is very similar to Western blot.



Figures by MIT OCW.

To separate RNA: denaturing agarose gel

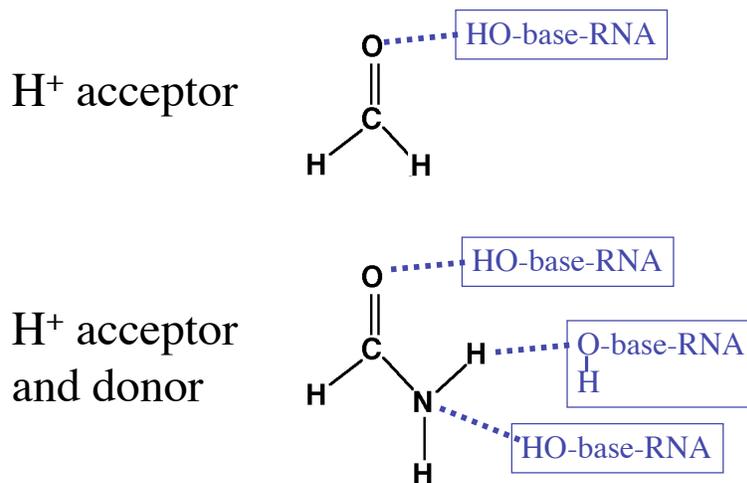
- **Why?** -- single-stranded RNA tends to form secondary structures.
- **Denaturant?** -- formaldehyde and formamide
- **Where?** -- denaturing mix and gel

Figure removed due to copyright reasons.



How do denaturants work?

Disrupt base-pairing.



Total RNA



tRNA



rRNA

**Control for both RNA
quality and quantity**



mRNA

**Target of Northern blot
(*z-cyt1* in our experiment)**

Total RNA on a gel

Figure removed due to copyright reasons.

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Module overview

Goal

- Zebrafish development observation
- Gene expression analysis

Technique

- Phase contrast microscopy
- Teratogenesis
- RNA isolation
- RNA gel
- Northern blot

Figure removed due to copyright reasons.

Embryonic staging by morphological criteria (28°C)

Cleavage period (0.7-2.2h)

Figure removed due to copyright reasons.

Blastula period (2 1/4 - 5 1/4h)

Figure removed due to copyright reasons.

Gastrula period (5 1/4-10h)

Figure removed due to copyright reasons.

Segmentation period (10-24h)

Figure removed due to copyright reasons.

Pharyngula Period (24-48 h)

Figure removed due to copyright reasons.

Hatching period (48-72h)

Figure removed due to copyright reasons.

Today....

- Embryos laid ~9AM.
- Therefore, you'll see **mostly gastrula and possibly blastula.**
- On day 1, you were given lysates from: blastula, gastrula, segmentation+pharyngula, and hatching.

Module overview

Goal

- Zebrafish development observation
- Gene expression analysis

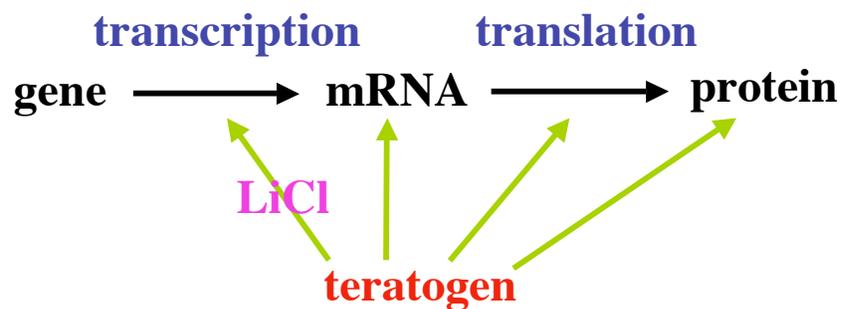
Technique

- Phase contrast microscopy
- **Teratogenesis**
- RNA isolation
- RNA gel
- Northern blot

Teratogen and teratogenesis

- A teratogen is an agent that can cause malformations of an embryo or fetus.
- It can be a chemical substance, a virus or ionizing radiation.
- Teratogenesis helps developmental biologists understand developmental patterning and the mechanisms behind that.

How does teratogen work?



Teratogen vs mutagen

Example	<ul style="list-style-type: none">• LiCl	<ul style="list-style-type: none">• Transposon
Target	<ul style="list-style-type: none">• mRNA/protein NOT DNA	<ul style="list-style-type: none">• DNA sequence
Consequence	<ul style="list-style-type: none">• Abnormal development	<ul style="list-style-type: none">• Varies
Heritability	<ul style="list-style-type: none">• NO	<ul style="list-style-type: none">• YES