

# Zebrafish Development Lab

---

**Instructor:** Kelsey Temprine

## Introduction

Zebrafish (*Danio rerio*) are freshwater fish found in the Ganges River in India as well as in many aquariums across the world. They are also a great model organism for the study of development as well as diseases, such as cancer. Zebrafish have many advantages related to developmental biology, including:

1. Like humans, they are vertebrates, meaning they have a spinal cord. In addition, humans share 70% of their genome (and 84% of genes linked with human disease) with zebrafish.
2. Unlike human embryos, zebrafish embryos develop externally, which means that you can watch a zebrafish egg be fertilized and then grow into a fish under the microscope!
3. Zebrafish embryos are transparent, making them great for both brightfield and fluorescent imaging.
4. Zebrafish embryos develop really quickly. During the first 5 days of development, all major organ systems develop and begin functioning, pigmented cells arise, and the larvae begin moving.
5. In addition to modeling normal development, zebrafish can be used to model various developmental diseases, including anemia, Duchenne muscular dystrophy, and diabetes.

Today you will observe zebrafish embryos at different stages of development.

## Learning Objectives

1. Practice identifying zebrafish embryos at different stages of development
2. Understand why zebrafish are a valuable model organism for studying both normal and abnormal development

## Activity: Stages of Development

After fertilization of the egg by the sperm, zebrafish embryos begin in the 1-cell stage, which consists of a single cell on top of a giant yolk. This yolk will provide the embryos with all the nutrients needed during their first week of life (similar to a human placenta). After ~30 minutes, the first cell division occurs, and the embryo is now at the 2-cell stage. These cell divisions continue until there are over a thousand cells at ~3 hours post fertilization (hpf).

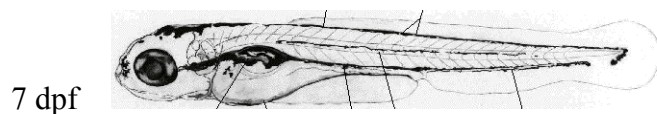
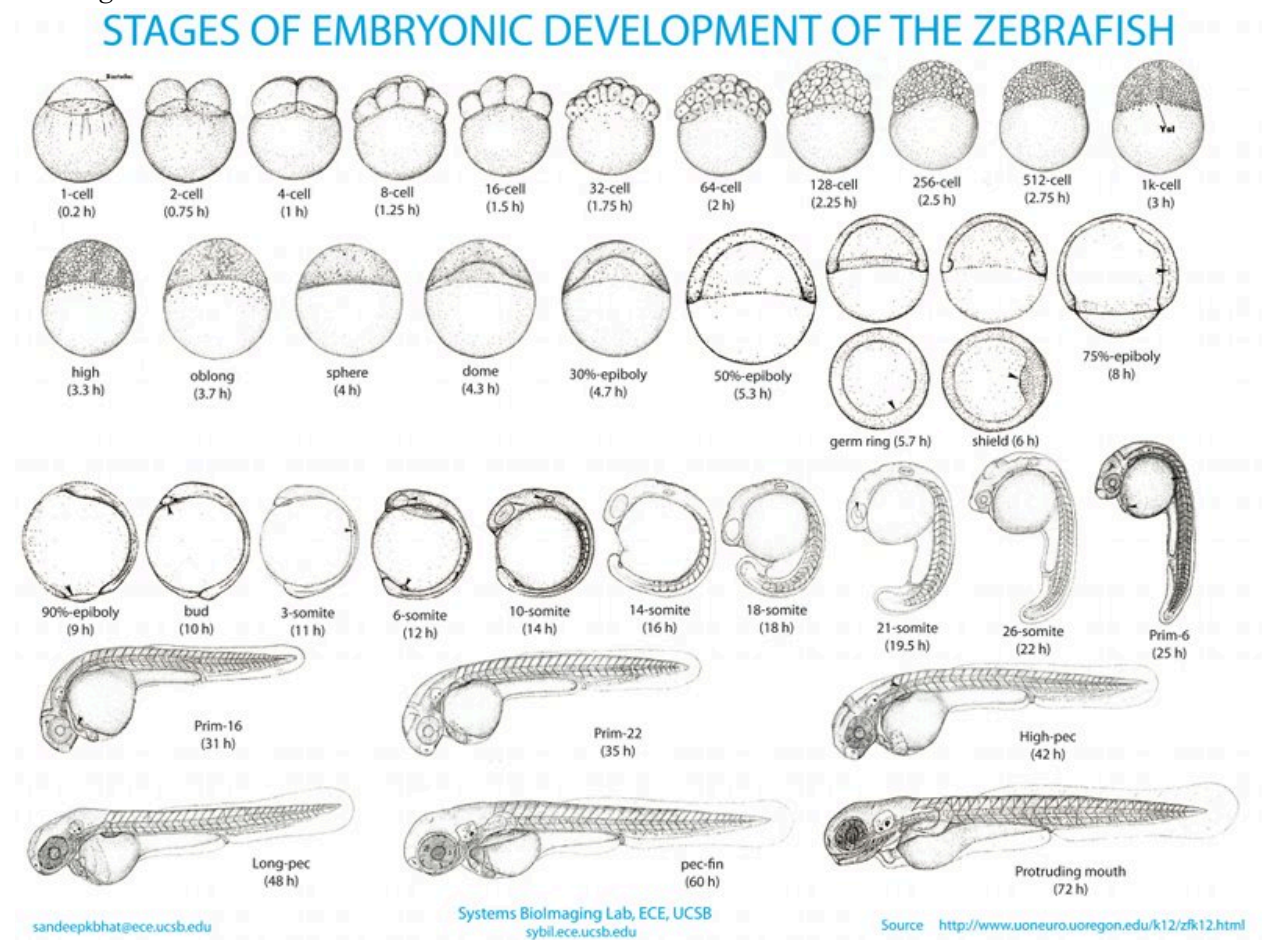
The next major step is gastrulation, during which the three germ layers (endoderm, mesoderm, and ectoderm) are formed, and then the cells migrate to cover the yolk via a process called epiboly. This

massive cell migration ensures that each germ layer is in the right place so that the organs and tissues will form in the correct locations. The percentage of the yolk that is covered is called %-epiboly.

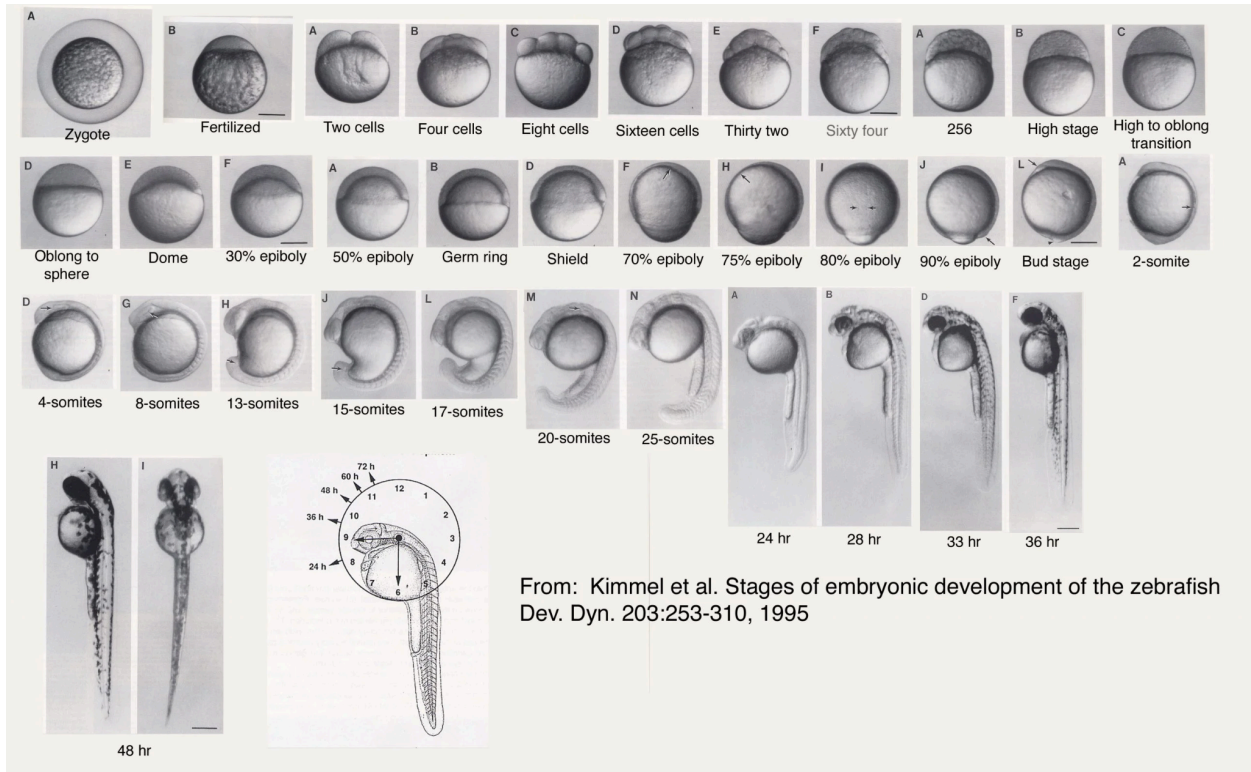
Starting at ~10 hpf, the somites (precursors to vertebrae) begin to form. This is followed by primary organogenesis, including eye formation. The zebrafish embryo grows a tail and begins to move. The heart develops, and blood is pumped throughout the body. Around 48 hpf, pigmented cells form in a characteristic pattern. Eventually, the zebrafish embryo hatches from its chorion (the transparent shell in which it initially develops) and begins swimming around. The jaw fully develops, and the embryo begins seeking out food as the yolk is depleted. All of this occurs in less than a week! And within just a few months, the zebrafish larvae will reach the adult stage and become sexually mature.

Check out the images (and links) below to observe what these stages look like!

*Drawings*



*Images (including pigmentation)*



You can watch an embryo develop over the first 24 hpf here:

<https://www.youtube.com/watch?v=ahJLZyioWM>.

And you can find additional images/drawings of the various stages of development here:

- [https://zfin.org/zf\\_info/zfbook/stages/](https://zfin.org/zf_info/zfbook/stages/)
- <http://www.uoneuro.uoregon.edu/k12/Development%20Stages.html>

In this activity, you will be given embryos and asked to determine each embryo's stage of development.

**Materials:**

- Vial with zebrafish embryos
- Plastic pipettes
- Concave slides
- Coverslips
- Foldscope
- Petri dish

**Procedure:**

*What Kelsey did prior to shipping out the embryos to you:*

Day 1 (afternoon):

1. Place one male zebrafish and one female zebrafish into a breeding cage with a divider. This divider keeps the fish separate until the next morning.

Day 2 (morning):

2. Transfer breeding cage to fresh water and remove divider. Fertilized eggs will fall through the holes in the bottom of the breeding cage and be collected below.
3. Transfer fertilized eggs to a petri dish containing embryo water.
4. Transfer some ~0-1.5 hpf embryos to a 6 well plate and fix them using a 4% PFA solution at 4°C overnight. Let the rest of the embryos continue developing.

Days 3+:

5. At various timepoint, transfer some embryos to a 6 well plate and fix them using a 4% PFA solution at 4°C overnight. Let the rest of the embryos continue developing.
6. After overnight incubation, transfer embryos at various stages into vials containing glycerol for shipping.

***What you will do for the lab:***

7. Use the plastic pipette to transfer the contents of the vial to the petri dish.
8. Using the plastic pipette, transfer one embryo to the concave slide.
9. Place coverslip on top and tape in place.
10. Place slide in foldscope and view embryo. Move the slide around to see all the different parts. You can use your phone camera to take pictures if you would like.
11. Try to determine which stage of development it is.
12. Repeat for the other embryos.
13. When you are done, transfer the embryos back to the vial for long-term storage.