CELLMORPHODYNAMICS
LATINAMERICA

Course "Optics, forces & development"

Basic Module 4

Handling and Mounting Zebrafish Embryos

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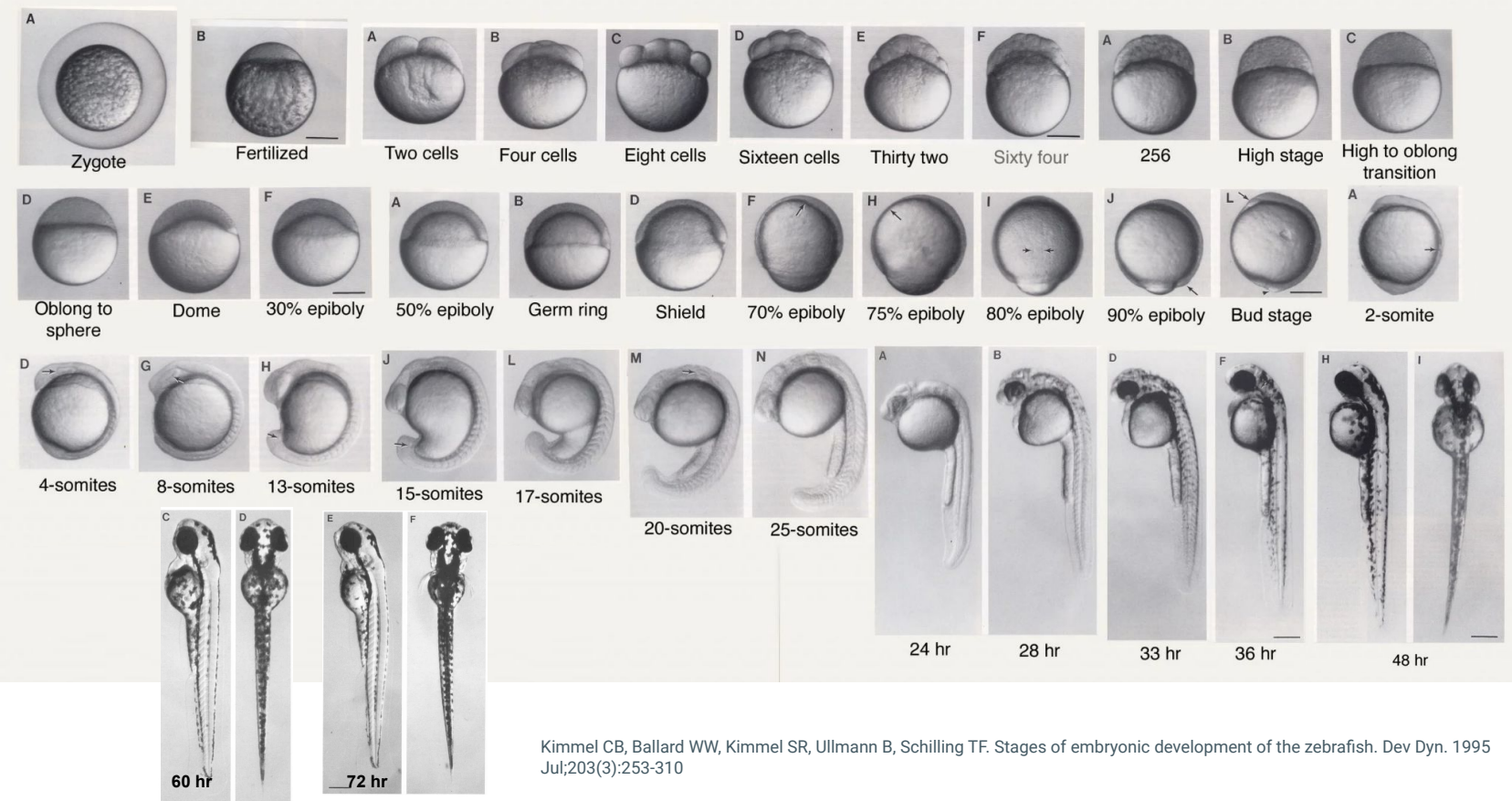
Objectives

- Familiarise with the distinct stages of development in zebrafish.
- Acquire theoretical knowledge and practical skills for handling and mounting zebrafish embryos in an agarose chamber to visualise them using a confocal microscope.
    - Dechorionate embryos using forceps.
    - Low melting agarose handling.
    - Orient and position the embryos using hair loops.

Why Zebrafish?

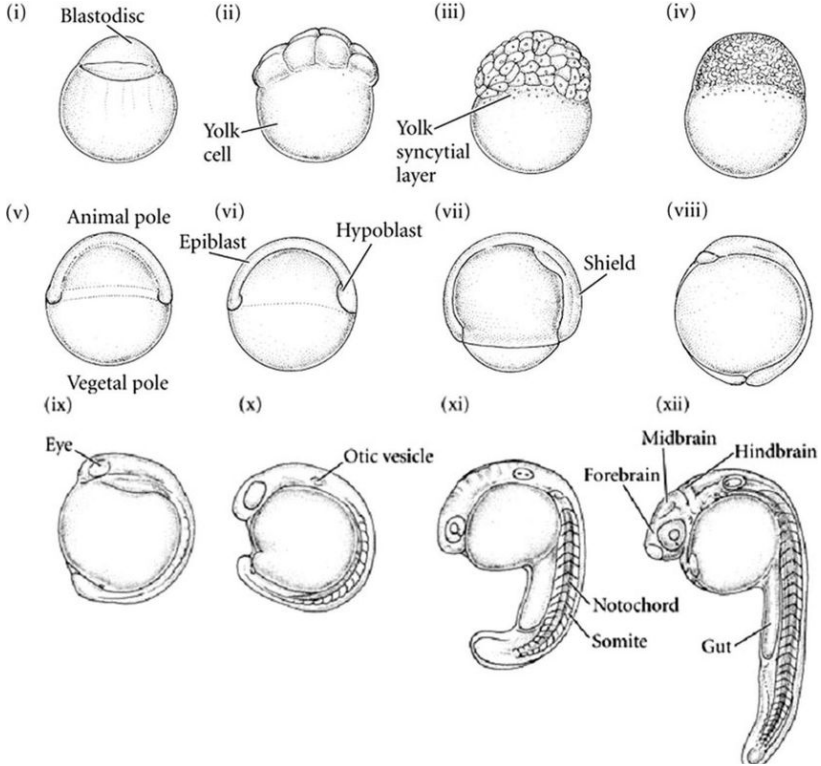
- Numerous offspring
- Transparent embryos
- Rapid development
- Exo-uterus development
- Availability of transgenics and mutants
- High genetic homology with humans

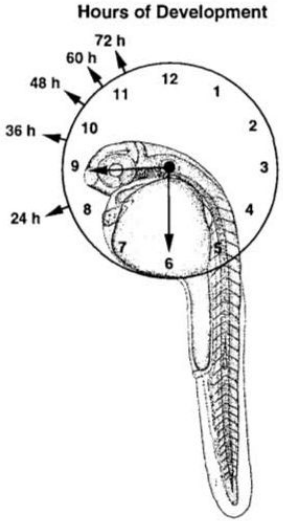
Stages of embryonic development of zebrafish

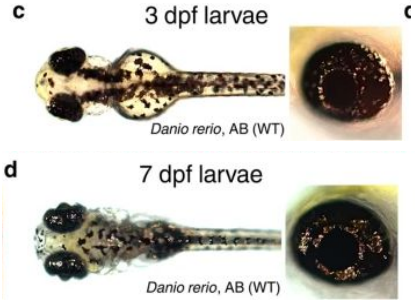


Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. Dev Dyn. 1995 Jul;203(3):253-310

Stages of embryonic development of zebrafish







Pigmentation phenotypes of wild type zebrafish embryos (3 dpf, (c)) and larval (7 dpf, (d)) stages. Antinucci, P., Hindges, R. A *crystal*-clear zebrafish for *in vivo* imaging. *Sci Rep* 6, 29490 (2016).

Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. Dev Dyn. 1995 Jul;203(3):253-310

Dechorionation

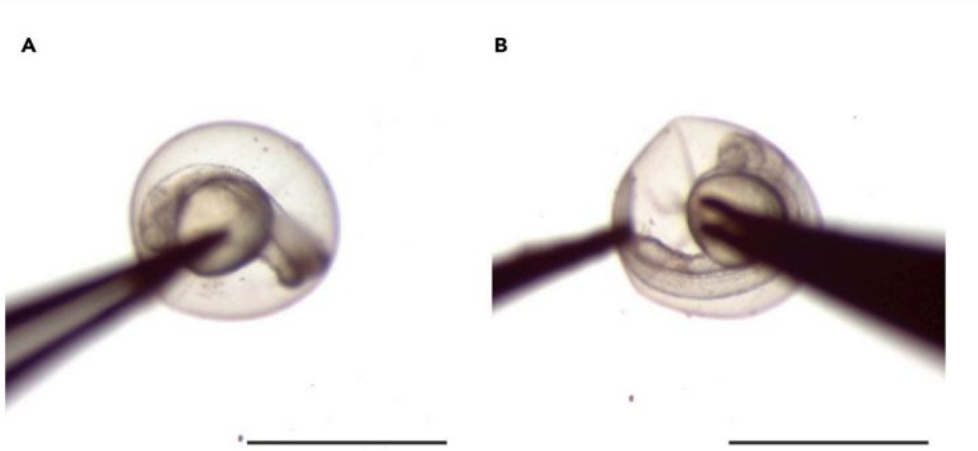
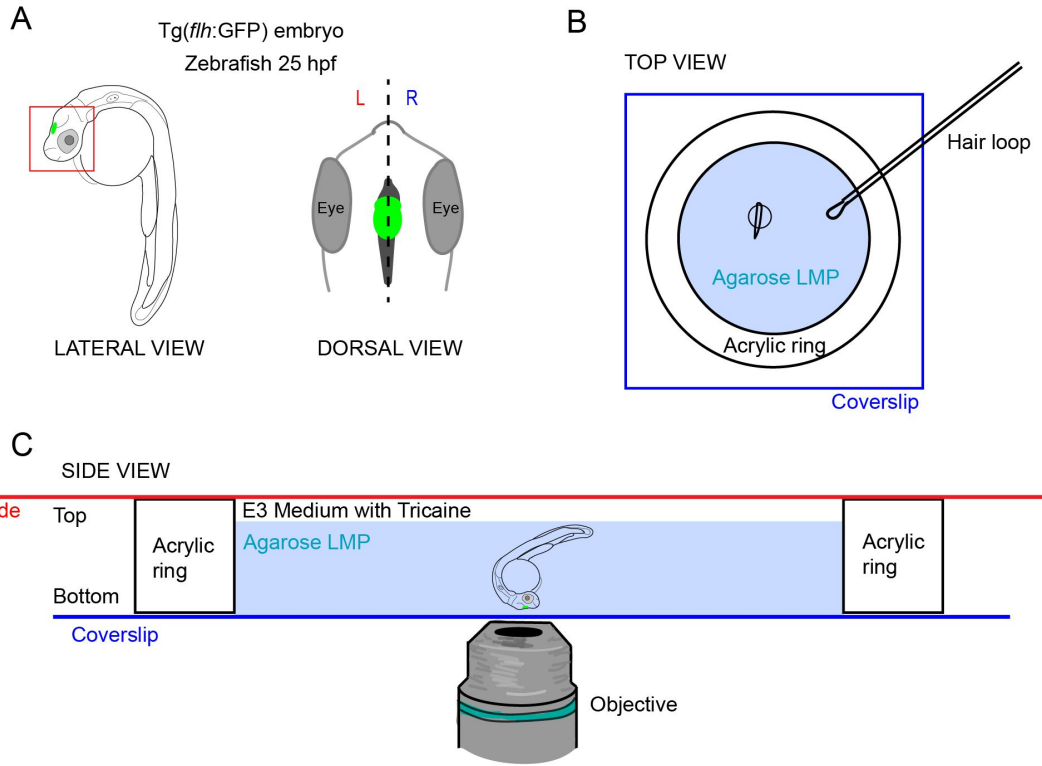


Figure 2. Zebrafish mounting in agarose for imaging (A) Embryos are dissected using fine forceps by first pinching the chorion with forceps. (B) A second pair of forceps is then used to peel away the chorion. Embryos are then anesthetized in 0.003% tricaine in E3 and oriented with a hair loop in 1% Low Melting Point agarose. Scale bar 1 cm.

Harris et al. Optogenetic axon guidance in embryonic zebrafish, STAR Protocols, Volume 2, Issue 4, 2021.

Mounting



Dadda M, Early differences in epithalamic left–right asymmetry influence lateralization and personality of adult zebrafish. Behavioural Brain Research, Vol 206, Issue 2, 2010. 208-215

Figure BM4.1: Mounting zebrafish embryos/larvae. (A) Transgenic embryos *flh*:GFP for fluorescence visualisation of the pineal complex (green). (B) Top view and (C) side view of an acrylic ring over a coverslip. Inside the ring, the embryo within LMP agarose. A drop of 1% (wt/vol) LMP agarose is added to cover the embryos. Tricaine containing fish water is added to keep the preparation from drying out.


Questions?