Guillemin Lab Protocol for the Derivation of Germ-free Zebrafish

Transcribed by Keaton Stagaman with addition of new antibiotic embryonic medium on 19 Apr 2017

Protocol

1. Soak fertilized embryos in sterile antibiotic EM until ~6 hours post fertilization (antibiotic EM can also be used when fertilizing squeezed eggs)
2. Pour embryos into sterile 50 mL beaker
3. Wash embryos in 3× sterile EM
4. Immerse embryos in 0.1% PVP-I solution for *exactly* 2 minutes. At two minutes, immediately add sterile EM to dilute the PVP-I solution. Any longer could result in embryo lethality
5. Pour off PVP-I and rinse embryos 3× in sterile EM. Let embryos sit in sterile EM for 3-5 minutes (recovery time)
6. Transfer embryos to new sterile 50 mL beaker. *(If using a gnotobiotic isolator, transfer embryos to 15 mL Falcon tubes).* Pour off sterile EM
7. Immerse embryos in 0.003% bleach solution
8. Soak embryos in bleach solution for 10 minutes. Do not bleach for longer than 30 minutes as this could result in embryo mortality. *(If using a gnotobiotic isolator, Clidox tube into isolator during this step for at least 20 minutes)*
9. While embryos are in bleach, fill flasks or beakers with sterile EM
10. Pour off bleach solution and rinse embryos 3× in sterile EM
11. Transfer embryos to flasks or beakers containing sterile EM

(see next page for solution recipes)

Solutions (make fresh each time)

Antibiotic EM – 500 mL

- 500 µL Ampicillin (100 mg/mL) [100 µg/mL final]
- 15.6 µL Amphotericin B (8 mg/mL) [250 ng/mL final]
- 500 µL Gentamycin (10 mg/mL) [10 µg/mL final]
- 50 µL Tetracycline (10 mg/mL) [1 µg/mL final]
- 25 µL Chloramphenicol (20 mg/mL) [1 µg/mL final]
- 500 mL embryonic medium (EM)
- Filter Sterilize

0.003% Bleach solution – 250 mL

- 125 µL 6.0% bleach solution
- 250 mL EM
- Filter sterilize

0.1% PVP-I solution (Polyvinylpyrrolidone-iodine [0.01% free iodine] Sigma #PVPI-100G) – 250 mL

- 2.5 mL 10% PVP-I stock (5 g PVP-I in 50 mL nanopure water)
- 247.4 mL EM
- Filter sterilize