

## Dechorionating embryos

Prior to completion of epiboly (~ 10-12 hpf at 27°), the embryos are very fragile. Exposed yolks will dissolve into plastic surfaces, or rupture if exposed to air-water interface. We prepare dechorionated eggs before this transition on agarose gel-covered plates to keep the yolks intact. After 10-12 hours, we perform dechoronation in regular petri dishes.

1. Add one thawed 1 mL tube of frozen pronase solution (20 mg/mL) to 50 mls egg water in 50 ml conical tube.
2. Transfer 250 fertilized eggs to agarose petri dishes. Eggs should be just barely submerged in water (dimpling water surface).
3. Add 5 - 15 mL of pronase solution to eggs. After ~ 30 seconds, pour off excess liquid. Again, eggs should dimple surface.
4. Keep eggs in pronase solution for:
  - a. 5 minutes for early embryos (3 – 5 h)
  - b. 6 minutes for late embryos (24h) (note, 24 hr embryos dont need agarose plates)
5. Stop digestion by adding egg water, then rinse at least three times:
  - a. Gently squirt in fresh egg water on top of eggs to remove shells.
  - b. Pour out liquid into a deep petri dish to catch floating eggs.
  - c. Continue until all embryos are dechorionated
6. For metabolomic extractions, count 200 undamaged embryos into ice-cold water.