Appendix 6-2. Conventional method for preparation of hatching solution

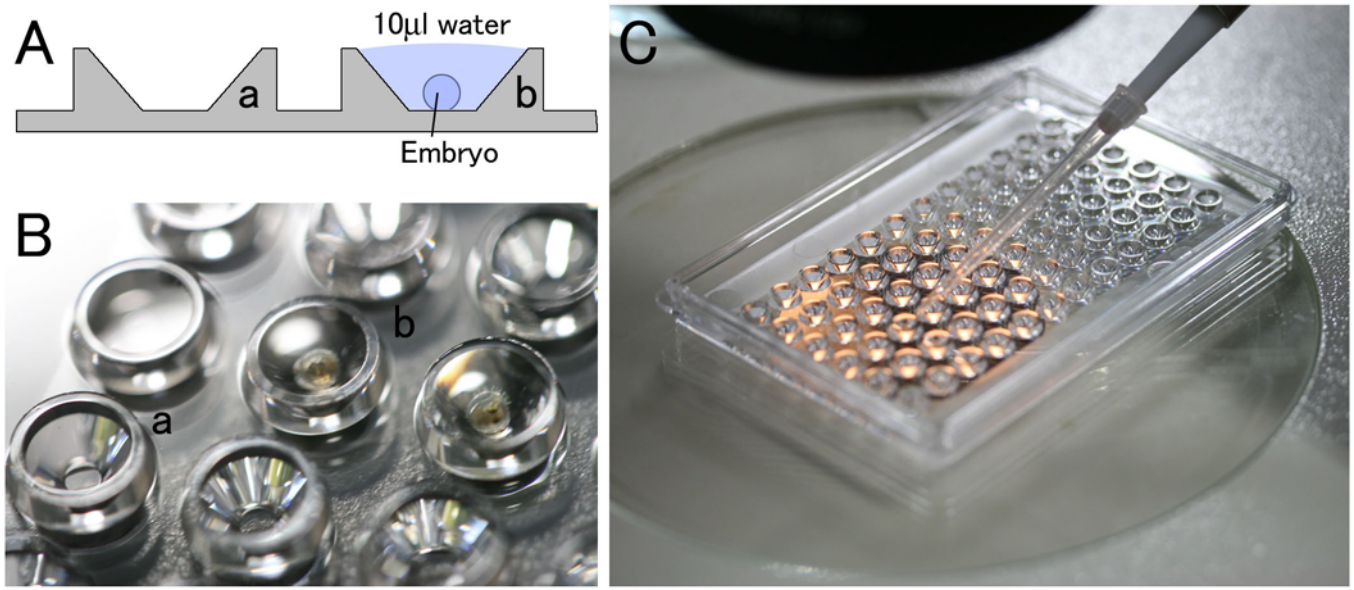

Figure A6-2.
Terasaki plate for collection of hatching solution. The shape (A) and a photo (B) of the plate. (a) indicates an empty well and (b) indicates a well filled with about 10 μm of water and an embryo (before hatching). At 1–3 days before hatching, embryos should be set using a syringe into each well with about 10 μm of water. Just after hatching, the solution should be collected from each well by micro pipette (C).

Procedure:

1. Collect fertilized eggs.
2. Sort eggs and incubate for 5–7 days in the Petri dish.
   At 1–3 days before hatching, prepare the Terasaki plate.
3. Rinse eggs at 5–7 days after fertilization with the medium (i.e., 0.03% seawater).
4. Embryos should be set using a syringe into each well (1 embryo/1 well) with about 10 μL of medium.
5. Incubate at about 27 ℃ while maintaining wet conditions, and check every day.
6. When the egg hatches, collect the medium by micro pipette.
7. The medium collected each day should be centrifuged (i.e., 10,000g) to separate the egg envelope debris.
8. The supernatant can be used for dechorionation, and the solution should be stored at –20 ℃.

Note:
Check the plate every day, and collect hatching medium within 24 hours. Leaving the medium for more than 24 hours may reduce the dechorionation activity.