

Natural and Artificial Fertilization of Frog Eggs - Hiroki Kuroda

A) Natural Fertilization of Frog Eggs

(One day Before)

- Inject 600 U of Chorionic Gonadotropin (Sigma) into female frog and 200 U into male frogs 18 h before eggs are to lay.
- Keep injected frogs together in 0.1 x Steinberg's solution in the dark (covered with dark plastic bag is fine)..

(The day you use)

- Frogs will lay their eggs in 0.1 x Steinberg's solution. Frogs will not lay below 19°C. A good idea is to start mating at 25°C.
- Remove eggs from the bottom by using a photo X-ray film.
- Move eggs from the cage to a 500 ml beaker using a 25ml plastic pipet that has been cut at the tip (to make a larger diameter mouth).
- Add enough Cysteine solution to cover the eggs (4.5% cysteine in 0.1 x Steinberg's solution, pH 7.8).
- Wait until jelly is completely removed. (Note. Until eggs touch with no space between them).
- Decant the Cysteine solution and wash 3 times with 0.1x Steinberg's solution.
- Incubate eggs in 0.1x Steinberg's solution.
Note. In an ideal day, you can get many stage 2-6 embryos, throughout the day.

10 x Steinberg's solution

NaCl 34g (580 mM), KCl 0.5g (6.7 mM), CaNO₃-4H₂O 0.8g (3.4 mM), MgSO₄-7H₂O 2g (8.3 mM), Kanamycin 0.1 g, Tris (MW=121) 6.0g (50 mM), adjust pH between 7.35-7.45 by HCl, adjust volume to 1L with H₂O and autoclave.

1 x Steinberg's solution

Kanamycin 0.1g, 10x Steinberg's solution 100 ml, adjust volume to 1L with H₂O and autoclave.

B) Artificial Fertilization of Frog Eggs

(One day Before)

- Inject 600 U of Chorionic Gonadotropin (Sigma) into the dorsal lymph sac female frog 12 h before eggs are to be laid. Go deep into the subcutaneous space.
- Keep injected frogs in the dark (covered with a black plastic bag is fine).
- Dissect male frog for testis.
- Keep testis into 1x Steinberg's solution with 0.1% BSA. (You can keep testis up to two weeks in this condition). We usually keep testis dry in a glass Petri dish.

(The day of use)

- Dice half of testis in 1x Steinberg's solution (Sperm solution).
- Squeeze female frogs and lay their eggs dry in a Petri dish.
- Add 3-5 drops of Sperm solution and spread so that they form only one layer.
- Wait for 2 minutes.
- Add enough volume of 0.1 x Steinberg's solution to cover the eggs. This is the beginning of the fertilization.
- After 20 min add the cysteine solution (3% cysteine in 0.1x Steinberg's solution, pH 7.8) for about 7 min or until eggs can touch each other with no jelly separating them.
- Decant the Cysteine solution and wash 6 times with 0.1x Steinberg's solution.
- Incubate eggs in 0.1x Steinberg's solution.

Note. The embryos should be at 2-cell stage one hour and twenty minutes after fertilization.

10 x Steinberg's solution

NaCl 34g (580 mM), KCl 0.5g (6.7 mM), CaNO₃-4H₂O 0.8g (3.4 mM), MgSO₄-7H₂O 2g (8.3 mM), Kanamycin 0.1 g, Tris (MW=121) 6.0g (50 mM), adjust pH between 7.35-7.45 by HCl, and adjust volume to 1L with H₂O and autoclave.

1 x Steinberg's solution

Kanamycin 0.1g, 10x Steinberg's solution 100 ml,. Adjust volume to 1L with H₂O and autoclave.