

May 1, 2018

## Centaurian gonadotropin frog mating protocol

### Preparation of hormones:

Gonadotropin, pregnant mare serum (PMSG) from Prospec, 1000IU – Add 1mL 0.9% saline  
Gonadotropin, human chorionic (HCG): from Sigma, 10000 IU – Add 10 mL 0.9% saline, final concentration = 1000 IU. Will need to transfer into a saline vial because original vial is small.  
Store hormones at 4°C for up to three months.  
Always write date, volume and initials when preparing new hormone solution.

### Coupling:

**Day 1:** Inject 50µl (50UI) PMSG into a female frog (this is only 0.05ml which is *less than* 0.1ml) and place in priming tank. The PMSG can be injected intraperitoneally into the ventral side of the frog or into the lymph sac on the dorsal side (see pictures below). Squeeze the frogs firmly in the net to avoid their moving. Make sure to hold the syringe at an angle nearly parallel to the skin. If you see blood after removing the syringe you went too deep!!!!

Repeat for second female frog, and you can place both primed female frogs into the same priming tank on the rack (rack 2).

**Always change the number written in whiteboard marker on the laminated tag on the tank (indicating the number of frogs) when you remove or add a frog. Tell Phil what you did for him to record by Wednesday of the following week.**

If you get your eggs, return animals from the couples back to their original tanks. This is usually “Last Used” but *may* be different for the animals you are returning so double check! **Always change the number written in whiteboard marker on the laminated tag on the tank (indicating the number of frogs) when you remove or add a frog.**

**Day 4:** Inject 150 UI (0.15ml) of hCG into the dorsal lymph sac of the male and place in a prepared breeding tank. (This step is recommended but optional.) Repeat for second male, and place into a second breeding tank.

Inject 150 UI (0.15ml) hCG into the male and 400 UI HCG (0.4ml) into the female, and place couple together in breeding tank.

Repeat for second couple.

If the two animals are not in amplexus one day after injection, wait another day or repeat the Day 4 injections.

**Note:** It is ok to push the Day 3/4 injections to Days 4/5.

### Moving labels:

When all frogs of the current group (tank labeled “current males”/ “current females”) have been used change the labels as follows:

The now empty tank that is labeled “Current” becomes “Last Used”. The tank “Last Used” becomes the last tank for that sex, and the one that was previously the last tank moves up the queue. So this is how the rotation would work:

- Current → Last Used, Last Used → Females 11, Females 11 → Females 10 ... Females 2 → Females 1, Females 1 → Current.
- Current → Last Used, Last Used → Males 7, Males 7 → Males 6 ... Males 2 → Males 1, Males 1 → Current.

Remember to keep count of frogs for each tank. That means change the count on each label to match the number of frogs that this tank contains.

Let Phil know what you did so he can update the database.

Don't be scared. You can do it!

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### Frog Breeding Schedule

	Day 1 Mon	Day 2 Tues	Day 3 Wed	Day 4 Thurs	Day 5 Fri	Day 6 Sat	Day 7 Sun
<b>C o u p l i n g</b>	Return females and males from last week's coupling into appropriate tanks.  Prime your females for the 1 <sup>st</sup> coupling.			AM: Prime males for the 1 <sup>st</sup> coupling. PM: Inject and do your 1 <sup>st</sup> couplings.  Prime your females for the 2 <sup>nd</sup> coupling.			AM: Prime males for the 2 <sup>nd</sup> coupling. PM: Inject and do your 2 <sup>nd</sup> couplings  Tell next week's frog whisperer AND Phil what you did.
<b>E g g s</b>	Collect eggs from last week.  CLEAN EGGS!!!	CLEAN EGGS!!!	CLEAN EGGS.	CLEAN EGGS	Collect eggs.  CLEAN EGGS!!!	CLEAN EGGS!!!	Collect more eggs.  CLEAN EGGS!!!

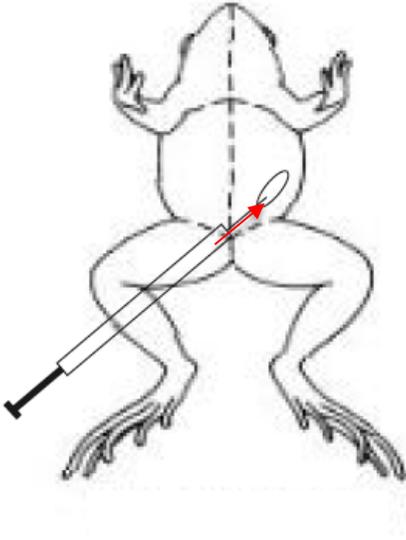
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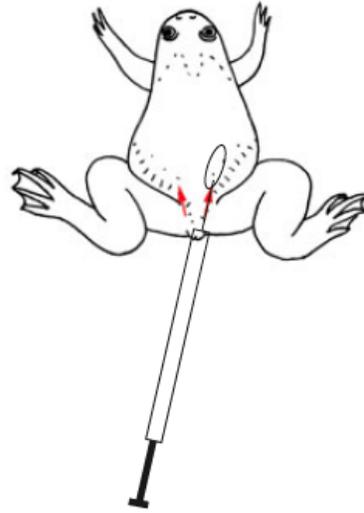
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The sites at which frogs may be injected to administer the priming dose of pregnant mare serum gonadotropin PMSG into either the peritoneum or the dorsal lymph sac are indicated (red arrows and circles):

Ventral:



Dorsal:



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