

Mice Super-Ovulation Protocol  
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### Materials

Materials	Size	Details	Manufacturer
PMSG (Pregnant Mare Serum Gonadotropin)	5IU (100ul)	Cat#: HOR-272, 1000IU	Prospec
hCG (Chorionic Gonadotropin)	5IU (100ul)	Cat#: 230734, 1mg	Sigma Aldrich (Millipore Sigma)
IP Needle and Syringe	1ml or 0.5ml insulin syringes	Cat#: 329461	BD
Ice			

### Preparation of materials

1. Purchase the two hormones and create stock
  - a. PMSG (Similar to FSH) - Cat#: HOR-272
  - b. hCG (similar to LH) - Cat#: 230734
  - c. Create 1000IU/ml stock and dilute in 19ml of PBS
  - d. Maintain hormones on ice when in use and aliquot stock in 500ul or 1ml. Store aliquots and stocks at -20C.

### Injection

1. Obtain female mice into new cages (separate from male mice)
2. Inject PMSG on Day 1 in the evening\*
  - a. Take any syringe that allows for IP injection for 5IU of hormone (100ul increments). We use BD 0.5ml insulin syringes
  - b. Prepare the syringes with appropriate total volume of hormone for the number of mice you wish to inject. (For example, if you have a total of 5 female mice, then load 500uL of hormone)
  - c. Perform IP injection on the mice with 100ul PMSG by holding the mouse in non-dominant hand and putting them in Trendelenburg position (head lower than the body). At a 45-degree angle, perform IP injection to the lower left or right quadrant of abdomen to avoid puncturing any internal organs.
  - d. Use a card to note the both date and time of injection and put all female mice back into cage.
3. In 48 hours, or Day 3, perform the second IP injection of LH hormone and make new breeding cages.
  - a. Prepare syringe once again with 100ul of hCG per mouse.
  - b. IP inject 100 uL of hCG into mice in Trendelenburg position
  - c. Immediately after, begin breeding cages. Place injected female mouse with male mouse (if necessary, you can add two super-ovulated female mice with one male mouse)

- d. Use a card to note both the date and time of injection and put all female mice back into cage.
4. On Day 4, or the next morning after hCG injection, check female mice for vaginal plug (sign that the mice have mated)
  - a. If necessary, add male mouse into new breeding cage with another round of super-ovulated female mice
5. Separate female mice after 2-3 weeks before they give birth

\*We recommend starting the first IP injection of PMSG at 5 pm so that on Day 4, you can check for the plug in the morning. We begin injections on Monday or Tuesday because this process is a 4 day process (Monday PMSG injection means checking for plug on Thursday).