

SOP Setting up Mouse Matings Procedures

How to set up female mice to produce embryos (natural mating):

Males: should be placed in separate cages 1-2 weeks before being presented with a female (because the dominant male in a litter may suppress testosterone synthesis in the others). Males reach sexual maturity between 6 and 8 weeks of age and can be used for breeding up to 1 year (we use them for 8-10 month). The female should be presented to the male for 4 days. If a male fails to plug several times in a row, it should be replaced.

Females: should be used from 6 weeks to 4 months of age we usually present two females to one male. The copulation plug should be checked every morning (for 4 days). A plug consists of coagulated proteins from the male seminal fluid and in most strains can easily be seen (as a white spot covering the vaginal entrance of the female)

Usually 50-70% of the females will mate, most of them on day 3 (40-50%). Depending on the strain, one expects to find 8-10 (NMRI) or 4-6 (C57Bl/6) embryos per female. That means if you need 5 pregnant females per week, you should breed 10-12 females (with 5-6 males).

Some check the stage of estrous cycle, but that is sometimes not easy and we have good results without taking "in estrus" females.

Females are examined in the afternoon and those in estrus are placed with males.

Usually 50% of the females will mate within 12-14 hrs.

The following table, from Champlin et al. (1973), describes the appearance of the vagina at different phases of the estrous cycle.

Stage of estrous cycle	Appearance of the vagina
Diestrus	Vagina has a small opening; tissues are blue and very moist
Proestrus	Vagina is gaping; tissues are reddish-pink and moist; numerous longitudinal folds or striations are visible on both the dorsal and ventral lips
Estrus	Vagina signs are similar to proestrus, but the tissues are lighter pink and less moist, and the striations are more pronounced
Metestrus 1	Vaginal tissues are pale and dry; dorsal lip is not as edematous as in the estrus
Metestrus 2	Similar to metestrus 1, but the lip is less edematous and has receded; whitish cellular debris may line the inner walls or partially fill the vagina

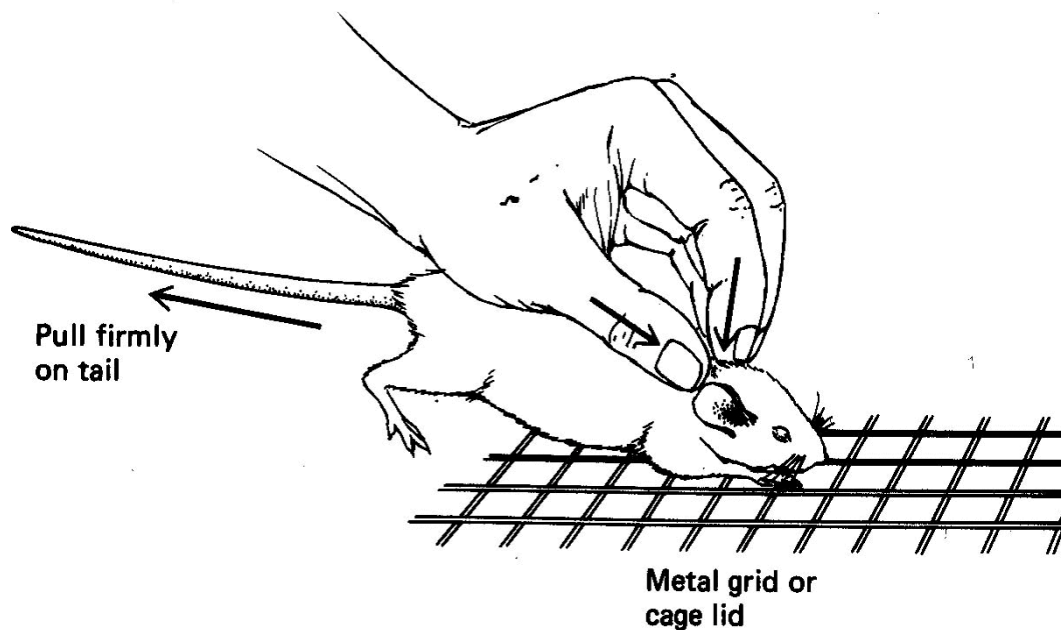
How to sacrifice mice:

The most recommended method is the cervical dislocation:

- Quickly place the mouse on top of the cage, so that it grips the bars firmly with its front paws.
- Then break its neck by applying firm pressure to the base of the skull and sharply pinching and twisting between thumb and forefinger while at the same time pulling backward on the tail.
- The action is one of stretching rather than snapping and only little force is needed.
- Alternatively, a spatula or pencil can be used to apply pressure to the base of the skull.

Cervical dislocation will result in a gap in the neck region of the spine. This gap can be felt when touching the neck with the thumb and forefinger and is proof for successful cervical dislocation.

You can also anaesthetize the mouse with isoflurane before the cervical dislocation, but with a little practice it is quicker and less stressful without anaesthesia (the cervical dislocation constitutes anaesthesia and sacrificing in one action).

**Signs of death:**

Complete relaxation of muscles (cave: rigor mortis!)

plus no breathing

plus no heart beat (cave: automatism of the heart!)

