

Breeding recommendations from the NIDA IRP Transgenic Rat Project (formerly the Optogenetic and Transgenic Technology Core)

RAT: Oprm1-iCre

- 1) We are breeding as heterozygote (or “het” or “carrier”) x CD (SD) from Charles River Laboratories (catalog # 001). It is important to use the same background strain if you want to be able to compare your results with what has already been published or observed. Please be aware that the CD (SD) from CRL may be different from other sources as the colonies have been separated and evolved differently over the years (potentially many generations). We have been crossing our animals with CD (SD) for at least 6 generations in our facility.
- 2) We prefer to use a male heterozygote x female CD (SD) but female heterozygote x male CD (SD) works as well.
- 3) In our facility we obtain on average one litter of 9-10 pups every 2 months per breeding pair. Half of the pups are heterozygotes (and half WT). Therefore if, for example, you set up 10 pairs, you can expect a production of 25 experimental animals (heterozygotes, males and females) per month in average.
- 4) We wean the litters ideally at 21 days but can do it between 19 and 24 days of age, to take in account weekends and holidays.
- 5) We set up the breeders when they are between 2 and 4 months of age to keep the breeding colony at its optimum production (younger than 2 months old, the rats are not mature enough to breed, older animals (past 4-5 months old) can be delayed in starting breeding and are slower breeders in general. The first litter appears usually 30-45 days after the setting up of the pair. One extra trick is to make sure that the female is not too much older than the male: an older and bigger female can get intimidating to the male and he will then not breed. Having a female way younger than the male is usually not an issue.
- 6) Breeders are retired (stop breeding and euthanized or used for experiments) generally following the general rules below:
 - Breeders become older than 10 months old
 - The pair had not had a litter in 3 months
 - The most recent litters have been getting smaller and smaller (for example if you see a pair having normally 10 pups per litter getting only 7 then only 5 then 3, it is good to be retired)
 - The female had 7 or 8 litters
- 7) We generally refresh the breeders every 5-6 months or so (unless the breeders had to be retired earlier as indicated above, in which case we replace them immediately with a new pair): after 5-6 months of breeding a particular pair, we set up a new one (with young new breeders) that will eventually replace the old one. We keep both pairs breeding in parallel until the younger pair gets a successful litter (surviving litter presenting at least one confirmed heterozygote) and only then it is safe to retire and euthanize the old pair. With that schedule, you can use the first 2 or 3 litters from a pair for experimental use and keep some animals for breeding from the 3rd or 4th litter and by the time you get these breeding, the parents would have had 2 or 3 more litters before they retire.
- 8) You can set up males in harems with 2 females each: you will need to separate the first pregnant female to avoid to have 2 litters in the same cage. From that point on, the male will be switched back and forth between the 2 cages (each one having a female):
 - On a regular basis, you can switch that male every 2-3 weeks for example with the exception below

- Do not re-introduce the male with a female who is pregnant or who has a litter: the male might not “recognize” his pups and kill them.
- 9) Depending on which size of cages you are using, you might need to separate the male at birth, leaving only the female + pups in the cage (to avoid overcrowding the cage). If that is the case, re-introduce the male as soon as the litter is weaned. Females with pups can become very protective and aggressive and if it happens, the male will have to be separated as well (if there are signs of biting for example).
 - 10) Always keep a few young heterozygotes at all times (preferably males) for backup, to use as breeders if you need to (breeder that dies or stopped producing unexpectedly). For that, set aside a few (a number equals to $\frac{1}{4}$ of your total number of breeding pairs is a good number) heterozygotes from the first litters you get. As you get new litters, keep some of the younger pups at which point you can use the previous backups for experiments (that way, your backups are always of the youngest age possible).
 - 11) Always try to use breeders coming from different pairs (do not pick all your breeders from the same pair, we use at least 4 origins at each generation): that way if a “harmful” mutation occurs, it will not affect all your colony but only a fraction of it, which can be easily reverse. Mutations are rare but can happen naturally: they can involve the transgene (loss of copies, loss or change in expression), they can also involve the general genome and if affecting an essential gene, could be a problem (physical abnormality, breeding difficulties). Using that same idea, never use for breeding an animal presenting a gross abnormality (i.e. missing part, abnormal behavior). On the other hand, do not go into the inverse excess like trying to select breeders from your best breeding parents (the ones that have the most pups for example): some people try to do so and it is not a good idea as it introduces a selection which can make variations in the experiments (bigger litters tend to have smaller pups for example).
 - 12) Keep in mind: 1 pair will give you an average of 2-3 heterozygote (males + females) per month.
 - 13) NOTE: the statistical information provided are based on our facility and should be used as reference but breeding in your facility may be different.