MOUSE BREEDING 101
Guidelines, Tips, and Suggestions

4 Rs
- Replacement
- Reduction
- Refinement
- Responsibility

Before You Started
- Think clearly what you want to do, and ... how many you need!
- Know the basic information of the background strain
  - Gestational range
  - Average litter size
  - Maternity
  - Genetic predisposition to disease
- Determine the breeding strategy
**Reproduction performance of common strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Litter size</th>
<th>Gestation</th>
<th>Maternity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD-1</td>
<td>8 +</td>
<td>19</td>
<td>Good</td>
</tr>
<tr>
<td>Hybrid</td>
<td>8 +</td>
<td>19</td>
<td>Good</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>6 +</td>
<td>19-20</td>
<td>Poor</td>
</tr>
<tr>
<td>FVB/N</td>
<td>7 +</td>
<td>18-20</td>
<td>Good</td>
</tr>
<tr>
<td>BALB/c</td>
<td>4 +</td>
<td>20-21</td>
<td>Good</td>
</tr>
</tbody>
</table>

**Basic knowledge of breeder selection**

- **Sexual maturation**
  - Male: 6 weeks; Female: 4-5 weeks
  - Best breeding start: 7-8 weeks
    - Somewhat variable by age and size
  - Post-partum estrus (use or not use?)
  - Gestation: 17 to 21 days
  - Wean @ 17-28 days
    - Duke standard is 21 days
    - May need later if problem animals
    - IACUC approval for late weaning age: > 21 days
  - Productive breeding life: 8 months

- **Housing males**
  - Fighting:
    - Do not pool more than 2 if they did not live together before weaning
  - Territory:
    - Always put females into male cage
  - Dominant status:
    - Do not put more than 3 females with 1 male
**Influence of Genetic Background on Breeding Performance**

- Behavior
- Hybrid vigor
- Birth defects

**Physiological Effects**

- Bruce Effect:
  - A form of pregnancy disruption in which exposure of a female to an unknown male results in pre- or post-implantation failure. Pregnancy disruption may occur at any time from conception to 17 days postmating, depending on the species and experimental conditions.

- Whitten Effect:
  - Mouse pheromone-laden urine synchronizes the estrus cycle among unisexualy grouped females.

- Vendenbergh Effect:
  - Early induction of the first estrous cycle in prepubertal female mice due to exposure to the pheromone-laden urine of a sexually mature (dominant) male mouse.

**Breeding Strategy**

- Most strains (NIH recommendation):
  - 1:1 breeding scheme
    - Pros: 
      - Prevent overcrowding
      - Easy to identify problematic breeders
    - Cons: 
      - May cannibalize pups

  - 1:2 (poor breeders such as B6, 129)
    - Pros: 
      - Increase litter size
      - Less cannibalization
    - Cons: 
      - Miss post-partum estrus
      - Trampling of newborn pups
Retirement
- **Male**: around 1 year
- **Female**: after 6 litters or around 1 year

Cannibalization
- **Reasons**:
  - First litter
  - Stillbirth:
    - phenotypes of transgenic mice
    - too young
    - undersized mom
  - Poor maternity: B6, 129, and some of the transgenic lines (non-healthy mom)
  - Environmental change
- **Prevention**
  - Nesting material
  - High-fat diet for B6 (peanut butter? dog food?)
  - Do not disturb 2 days prior to and 3 days after delivery
  - C-section: do not perform earlier than 24 hrs before the birth date

Non-genetic Factors That Influence Breeding Performance
- **Environment**
  - Temperature
  - Light intensity and light cycle
  - Noise and vibrations (construction, equipment)
  - Air pressure
  - Odors (toxic fumes, perfumes)
- **Handling consistency**
  - Over handling-leave pregnant mothers alone
- **Nutrition**
- **Health status**
**Timed-mating**

- Estrus cycle: 4-5 days
  - Identify female mice in estrus by:
    - Gross inspection
    - Vaginal smear
  - At any given time, about 20% of the females in the mouse overall population are in estrus
  - Grouped females will usually synchronize by the 3rd cycle (15 days)
  - Not every female will show a plug (60+ %)
  - ‘Plug’ ≠ Pregnant
  - The best choice is use ‘proven studs’
  - Remove both plugged and unplugged females
    - Unplugged: May go to 1:1 mating

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**Sexing / Genotyping Animals**

- Day 0:
  - Sexing:
    - black/agouti-black dot of scrotum
    - albino: anal-genital distance, nipple
- Day 7:
  - Sexing: Nipple
- Day < 12: Genotyping if toe clipping
- Day < 21: Genotyping for tail snips
- Day 17 – 28:
  - Wean
  - Separate sexes
  - Euthanize undesirable genotypes

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**Maintain a line**

- Requires (min) 4 males and 10 females
- Replacement
  - When last generation reaches 6 month old, set up at least 2 males and 4 females (1:2) for breeding
  - Keep only 2 males and 5 females from each cage. Terminate the rest.
- Cryopreservation is an option ....
  - Minimized the maintenance cages
Cryopreservation

- Protects against:
  - Contamination
  - Disease
  - Genetic drift
  - Disaster
  - Breeding cessation
  - Loss of copy number
  - Lower cost (than keeping breeding animals over a long time)

Cryopreservation

- Embryos, Sperm
- Superovulate ten 3-week-old females
  - Outbred/ FVB: ~100-150 morula, ~85-95% recovery (live pups)
  - Inbred: ~80-100 morula
    ~70-80% recovery (live pups)
- Minimize live animal maintenance cages
- Synchronize embryo stages

Special notices for breeding a transgenic line

- Make sure you have more than 1 line
- Transgenic founder breeding
  - Mosaic (positive F1 <30%)
  - Multiple integration sites (positive F1>80%)
    - Might diverge into more than one distinct line
- Genetic background interference
**Inbreeding Stabilization**

- Three family rotations (within 1 line)
  - Group your breeding cages into three families
  - AF female-BM male, BF female-CM male, CF female-AM male
  - Round 1 Selections: AF-BM, BF-CM, CF-AM
  - Round 2 Selections: AF-CM, BF-AM, CF-BM
  - Round 3 Selections: AF-AM, BF-BM, CF-CM

- Keeping the same pair together and always breeding from those offspring will encourage genetic drift and a new subline after 40 generations!

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**Data Collection and Record keeping**

- Critical for successful colony management!
- Collect breeding statistics
  - Birth dates for every litter
  - Interval between litters
  - Litter size
  - Number of mice that wean (born:wean ratio)
  - Genotype shift
  - Gender frequencies
- Pedigree records
  - Who is related to whom?

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**Non-Productive Breeder Criteria**

- No litter produced
  - 40 days from date of first mating
- No new litters
  - 40 days from last born date
- No weaned pups
  - 2-3 litters with none weaned
The ‘cage density’ policy reflects federal regulations, not just Duke’s requirement! These are not new regulations, just ones our community has had a difficult time with.

Requirements:

- 5 adults / cage (if all are ~ 25 grams)
- 4 adults / cage (if any > 45 grams)
- 3 adults + 5 pups / cage
- 2 adults + 10 pups / cage
- 1 adult + >10 pups / cage
- No litters >14 days apart in age
- Wean pups at 21 days unless IACUC approval for delayed weaning has been received
- Use “special husbandry” DLAR card if special husbandry is required.

OVERCROWDING IS AN ANIMAL WELFARE CONCERN!

ON-GOING OVERCROWDING MUST BE REPORTED TO THE NIH.

- Duke uses ‘5 incidents in 3 month / running average’ to define overcrowding

DLAR IS REQUIRED TO REPORT TO OAWA FIVE OR MORE OVERCROWDING INCIDENTS IN A 3 MONTH PERIOD.

Most repeat overcrowded notices are generated from:

- **PROBLEM:** Harem breeding resulting in 3 adults with > 5 pups or pups >14 days apart in age.
  **SOLUTION:** Remove dams once pregnant and single house or house with 1 male. Do not take advantage of post-partum estrus if approved for delayed weaning.

- **PROBLEM:** Pups not weaned at 21 days = too many adults in cage on day 22
  **SOLUTION:** Track breeding records accurately and wean at 21 days.

- **PROBLEM:** Two dams give birth and there are more than 10 pups (over 7 days of age) between both litters
  **SOLUTION:** Single house dams once pregnant to avoid confusion when separating litters.
**Tips for Colony Management**

- Mate mice early, between 7-12 weeks of age
- Establish/collection breeding statistics
- Replace breeders on a rotation (weekly, monthly)
- Have young breeders available (plan ahead)
- Keep good records, evaluate data regularly

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**Tips for Colony Management**

- Observe your colony AT LEAST once a week (good records are necessary if you choose this option).
  - If overcrowding notice is placed on cage …
  - … you have 24 hours to reply … then it will be separated and you will be charged for the service.
- Daily observation is necessary for husbandry / medical
- Record and investigate deviations immediately
- Keep older generations available (until the youngsters are proven breeders!)
- Choose breeders carefully

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**Tips for Colony Management**

- When genetic shifting genetic occurs, fertility changes may occur.
- Move a single female to a cage by herself within 7 days of parturition, especially if approved for delayed weaning so that the post-partum estrus IS NOT used!
- Harem breeding is ok, BUT avoid overcrowded cages!
- Strains that have >5 pups/litter should have dams singly housed (or housed with a male) to prevent 2 adults + >10 pups.
• You can use any system of numbers you choose
• Below are two different numbering systems:
Breeding Facts and Needs:
- You need 15 male newborns every 2 weeks for at least 3 months
- Mice are in CD-1 background, synchronized
- CD1 will have at least 8 pups/litter, so you’ll probably get 4 males/litter
- You need at least 4 pregnant moms every 2 weeks ... so ......

Example 1
- Week 1, set up 3 breeding cage (1:2)
- Week 3, set up 3 breeding cage (1:2)
- Week 4, first 6 females due - pups for exp.
  - identify non-preg. females, change to 1:1
- Week 6, second 6 females due - pups for exp.
  - identify non-preg. females, change to 1:1
- Week 7, wean pups for exp.
  - Set-up new breeders for next round of pups
- Week 9, wean pups for exp.
  - Set-up new breeders for next round of pups

Example 1
- Replace the females that didn’t get pregnant after week 4 and week 6
- Replace the male if the replaced females didn’t get pregnant
- Record breeding data
- Stop breeding by pooling females together if only temporarily (such as holiday season)
- Do not euthanize good breeding pairs without thinking!!! Once they are gone, that is it ...