



Reducing breeding overproduction: aiming for a realistic goal?

7th ANNUAL SYMPOSIUM IC-3Rs
REDUCTION AND REFINEMENT CHALLENGES IN 3Rs RESEARCH

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In house breeding

- In most facilities
- Wild type and genetically altered
- Not all animals can be used in experiments
- Mice, rats, zebrafish, other
- Overproduction



In house breeding

- Overproduction
 - Used as breeding animal – used in experiment – not used
 - Not all genotypes are needed (distribution over genotypes is not always the same)
 - Experiments are postponed/cancelled

Numbers – EU (Alures)

SECTION 1: NUMBERS OF ANIMALS USED FOR RESEARCH, TESTING, ROUTINE PRODUCTION AND EDUCATION AND TRAINING PURPOSES IN THE EU

Source: [ALURES statistics - Section 1 : Numbers of animals used for research, testing, routine production and education and training purposes - European Commission \(europa.eu\)](#) 20222, EU27 and Norway

Species	Number of uses	Percentage
Mice	4010766	47.86%
Rats	625777	7.47%
Guinea-Pigs	85167	1.02%
Rabbits	372239	4.44%
Pigs	86953	1.04%
Domestic fowl	417903	4.99%
Zebra fish	362449	4.33%
Sea bass	116706	1.39%
Salmon, trout, chars and graylings	1289139	15.38%
Other fish	691587	8.25%
Other	326711	3.90%
Total	8385397	100,00%



Numbers – EU (Alures)

SECTION 3: NUMBERS AND USES OF ANIMALS FOR THE **CREATION AND MAINTENANCE** OF GENETICALLY ALTERED ANIMALS (GAA) IN THE EU

Source: [ALURES statistics - Section 3 : Numbers and uses of animals for the creation and maintenance of genetically altered animals \(GAA\) - European Commission \(europa.eu\)](#) 20222, EU27 and Norway

Species	Number of uses	Percentage
Other fish	7012	0.59%
Zebra fish	75035	0.66%
Rats	1910	2.70%
Mice	250509	96.02%
Other	2607	0,76%
Total	341233	100,00%

Numbers – The Netherlands

Animals that died or were killed after use in breeding and/or before use in experiments

Source: NVWA

	Animals killed after use in breeding	Animals killed before use in breeding or experiments
Wild type animals	29.723	59.040
Genetically modified animals	69.436	183.688
- Of which are mice	33.137	163.416
- Of which are rats	882	1.109
- Of which are fish	35.417	19.163
Total	99.159	242.728

2022

Numbers - Flanders

Animals that died or were killed after use in breeding and/or before use in experiments in 2022.

Source: [Dierproeven in Vlaanderen 2022 cetwuv.pdf](#) (Dierenwelzijn | Vlaanderen.be)

	Animals killed after use in breeding (GAA lines)	Animals killed before use in breeding or experiments *
Mice	6.027	206.578
Rats	148	<5.000
Zebrafish	48	21.700
Total	6.663	316.937

* animals that were bred to create a new genetically modified line (0,62%); animals bred to maintain a genetically modified line (75,24%); animals that were not bred for any of the above reasons (19,84%).

The total number of animals used for testing vs breeding

	2018	2019	2020	2021	2022
Total	10,572,305	10,260,822	7,938,064	9,406,233	8,385,397

Table 1: Total numbers of animals used for the first time for research, testing, routine production and education purposes in the Union

	2018	2019	2020	2021	2022
GA creation	588062	508076	388729	356706	341233
GA maintenance	932729	699580	297899	308259	510912

Table 2: Total numbers of animals used for the creation and maintenance of genetically altered animal lines

The accurate reporting of animals under the maintenance of existing GA lines is particularly challenging, which may partly explain the continued fluctuation of numbers.

<https://circabc.europa.eu/ui/group/8ee3c69a-bccb-4f22-89ca-277e35de7c63/library/10ad28d6-e17e-4367-b459-20883402cfcc/details?download=true>

Numbers – NL Groningen

Mice

Killed before use in breeding or experiment (overproduction)

16364

Used in experiments

10089

Conclusion

Not all data available

Not the same manner of presenting the data (especially the numbers about breeding)

Doubts about the number in Allures, adjusted (years) later

E.g. only numbers are presented as used in breeding (overproduction is not used in experiment and also not used in breeding)

Reducing breeding overproduction

- Mice are the most used species
- High numbers of animals are being used for creation and maintenance of genetically altered lines
- Numbers for breeding overproduction should be reduced

Reducing breeding overproduction

- Background (things to consider)



Breeding – Types of genetic modification

- Gene knockout (remove DNA or such a portion that no protein can be made)
- Gene overexpression (extra proteins)
- Gene knock-in (gene is replaced; e.g. to make a specific mutation)
- Transgenic (Extra gene; containing an exogenous DNA cassette; e.g. gene reporters)
- conditional knockout mouse (Cre-loxP; under a ubiquitous, inducible, or tissue-specific promoter and tetracycline trans-activator (tTA))

Breeding – Use of genetic modification

- Gene knockout/overexpression - Study the role of a gene in biology process (e.g. disease development)
- Gene knock-in (mimic a specific mutation found in patients)
- Transgenic (usually reporting expression of a gene of interest)
- Conditional knockout mouse (limit expression of gene to cells of interest)



Making new lines

- Pronuclear injection (PNI) in ES cells
- Targeted nucleases (ZFNs, TALENs, and CRISPR-Cas systems)
- Genome editing via Oviductal Nucleic Acids Delivery (i-GONAD) that delivers CRISPR ribonucleoproteins to E0.7 embryos via in situ electroporation

No (new) genetic modification needed

- Crossing lines with the desired mutations
- Exchange of GA lines (databases; EMMA; MMRRC, MGI (Jax))



Making new lines

Background strains (FVB, C57Bl/6)

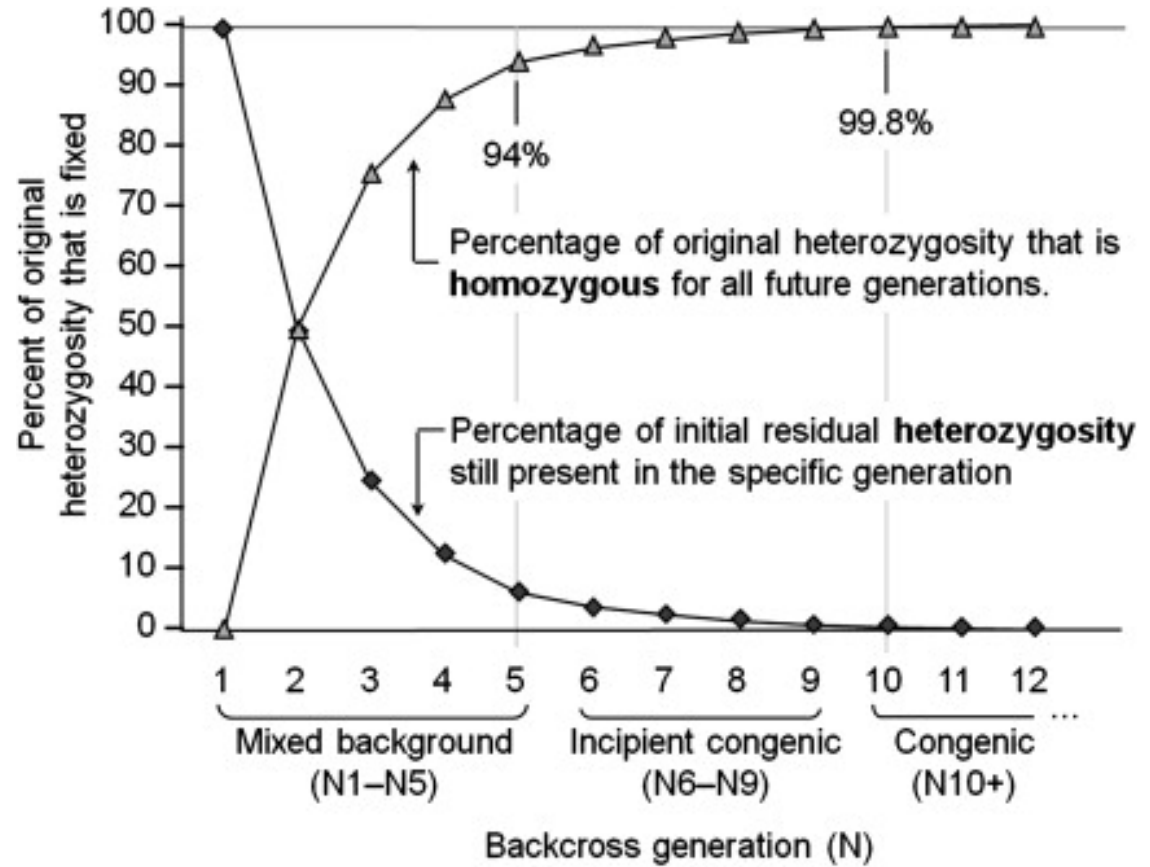
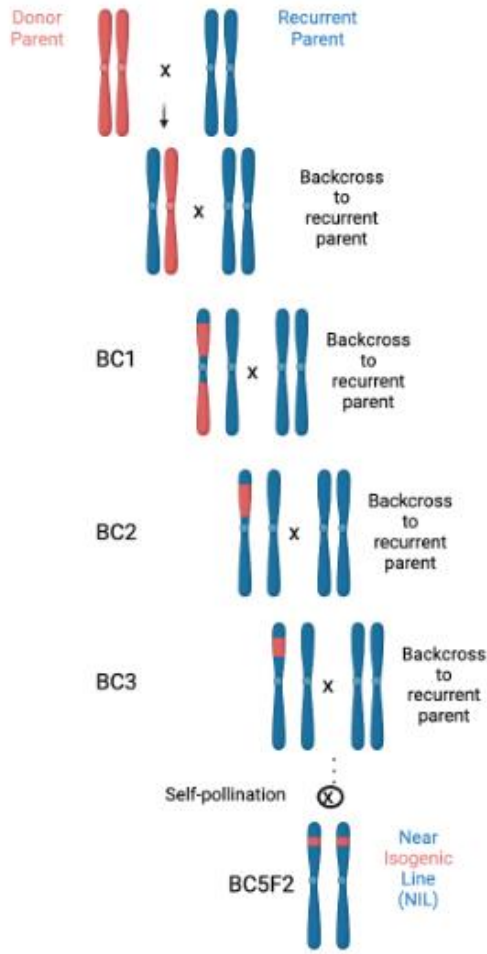
1. Pronuclear injection: GM C57Bl/6 ES cells into FVB foster mothers
 - Requires backcrossing for 8+ generations
2. Targeted nucleases: GM C57Bl/6 oocytes into foster mothers
 - Requires high numbers of young oocyte donors and many foster mothers (and infertile males)
3. Genome editing (iGONAD): GM C57Bl/6 oocytes in pregnant mothers
 - Requires less pregnant females and fertile males

Making new lines

Depending on the method, this may also require animals for generation of a new line and breeding to obtain the desired genotype (e.g. backcrossing)

Breeding towards the optimal breeding strategy

Making new lines - Backcrossing



Source: <https://www.sciencedirect.com/science/article/abs/pii/B9780123820082000039?via%3Dihub>

Breeding – Policies

Breeding:

- Continuous (postpartum conception); one litter every 3 weeks
- Separate male and female when pregnancy is visible (~2 weeks); one litter every 6-7 weeks

To maintain a solid background (e.g. C57Bl/6) regular backcrossing is required (2-3 generations) allowing better reproducibility with other labs and avoids genetic drift.

Making new lines – Avoiding genetic drift

Large Colony



Small Colony

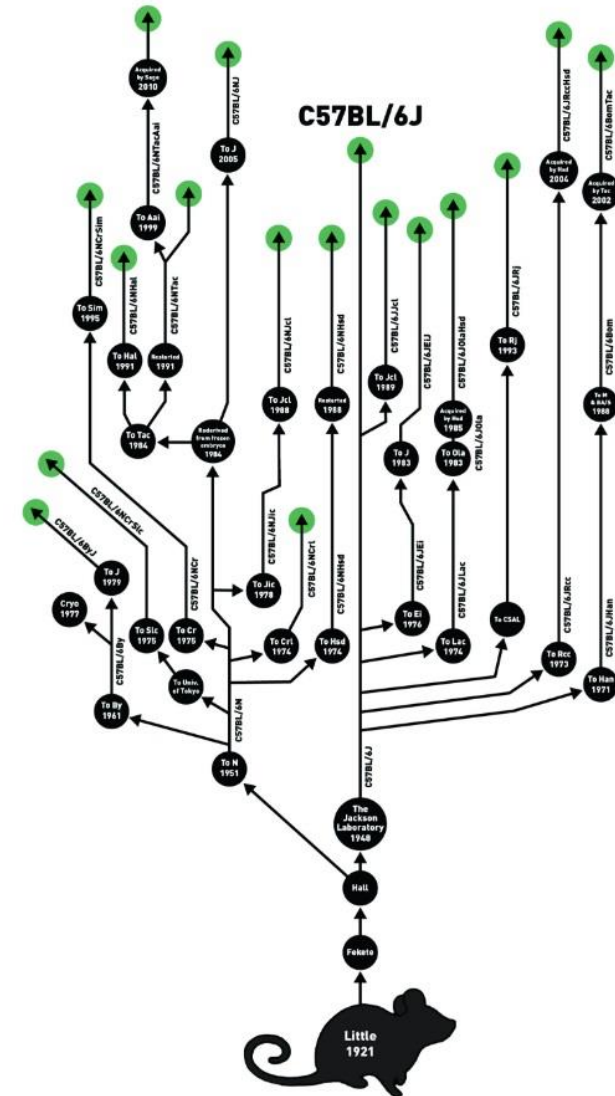


Spontaneous mutations can carry over to offspring

Sources for figures:

[Left: Strategies to Minimize Genetic Drift \(genengnews.com\)](#)

[Right: Here's What You Need to Know about the C57BL/6 Substrains \(genengnews.com\)](#)



Breeding – Policies

When to stop breeding (No immediate need for animals of a certain strain (unique genetic alteration)).

- Stop breeding without actions
- Cryopreservation (sperm or embryo's)
- Exchange of GA lines (databases; EMMA; MMRRC, MGI (Jax))

Breeding – Avoiding overproduction

Wild type breeding

- All offspring (100%) have the desired genotype
- In theory, all offspring can be used
 - To maintain the line
 - To perform experiments
- Restrictions in usefulness of offspring
 - Desired age
 - Desired sex



Breeding – Avoiding overproduction

GAA breeding

- Not all offspring have the desired genotype for expts (max 50%)
- One mutation: KO (Hom-Het-WT) or Tg (Tg (Het)- WT)
- In theory, all offspring can be used
 - To maintain the line (mainly Het)
 - To perform experiments (either HOM/WT or Het/WT)
- Restrictions in usefulness of offspring
 - Desired age
 - Desired sex
 - Desired numbers (for breeding)

Breeding – Avoiding overproduction

GAA breeding

- Not all offspring have the desired genotype (max 50%)
- Two or more mutation
- Complicated breeding
 - Genotype for breeding may vary
 - To maintain the line (all mutations should be carried over)
 - To perform experiments multiple combinations of genotypes may be needed
- Restrictions in usefulness of offspring
 - Desired age
 - Desired sex
 - Desired numbers (for breeding)
 - Desired genotypes

Wrap up – Avoiding overproduction

Overproduction is unavoidable, but we should limit this as much as possible:

- choosing the correct genetic alteration
- choosing the optimal method to create the line (or obtain an already existing line)
- breeding efficiently (genetic drift and planning!!)
- stop breeding when possible (after cryopreservation)