University of Basel

Center for Transgenic Models

Euthanasia of laboratory animals.
(from transgenic laboratory perspective)

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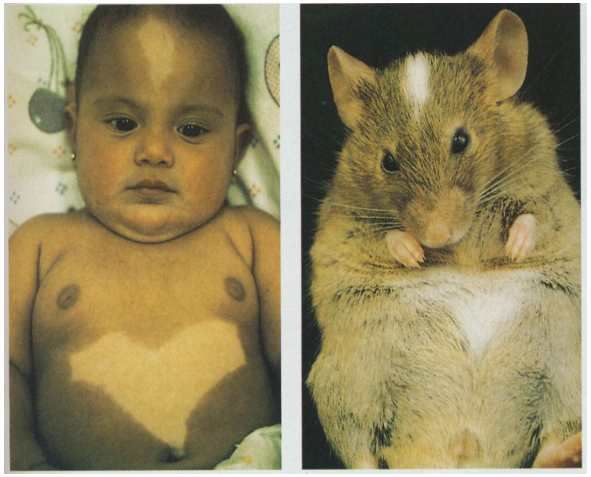
What types of animals are we euthanizing?

How can we use and euthanize less animals?

How do we euthanize lab animals?

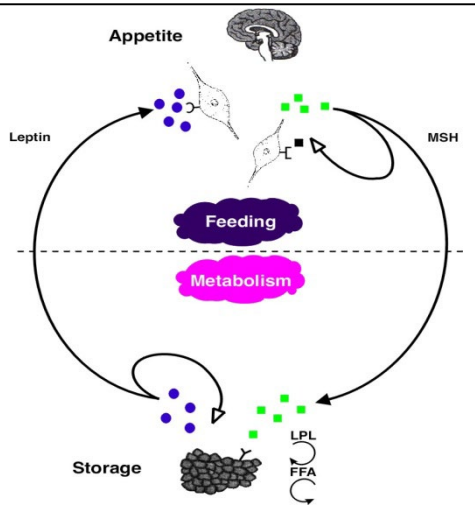
Why use an animal (mouse) as a research model?

- **Very close to humans**



Piebaldism
Mutation in the KIT gene that affects melanocyte development

- **Sufficiently complex**



Integrated Control of Appetite and Fat Metabolism by the **Leptin-POMC Pathway**

- **Extensive transgenic methods**

Including classical (DNA microinjection, ES cells) and new transgenic methods
ZFNs, TALENs, CRISPR/Cas9, Base editors, Prime editors

Most laboratory animals are mice and most mice are GMOs

Species

Genetically Modified Species

Over 97% of all genetically modified (GM) animals were **mice,** with relatively small numbers of fish, rats, rabbits and pigs.

Further more, in the mouse transgenic population, over 91% were used with the purpose of **biological basic research.**

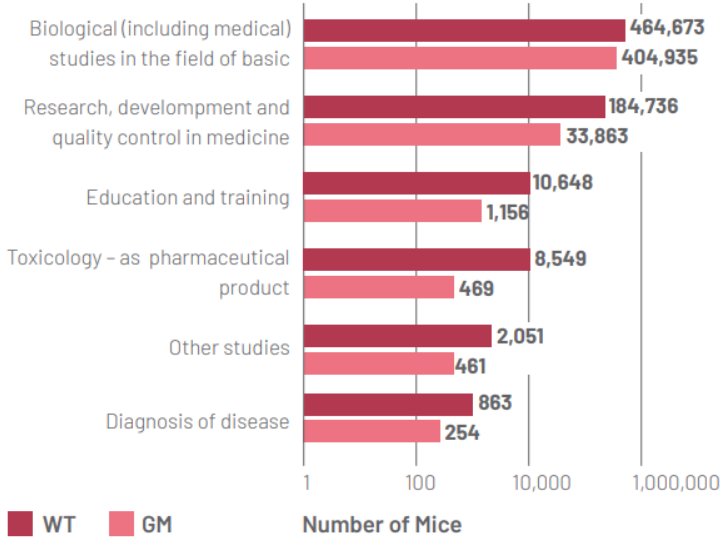
Studies of the **immune system** used the most GM mice (117'919), accounting for~40% of the total mice used. The nervous system (96'257) and oncology (63'223) followed.

Note that "wild-type mice" (WT) simply indicates that no specific modifications were made to the genome of the animal

Animal Numbers of Genetically Modified Animals for each Species

Species	Wild-Type	Genetically Modified
Mice	671,531	441,138
Fish	138,853	7,003
Rats	222,390	3491
Rabbits	3,113	8
Pigs	11,512	2
Grand Total	**1,047,399**	**451,642**

Number of Genetically Modified Mice split by Study Purpose



Two main classes of euthanized laboratory animals (mice)

1. **Laboratory animals in experiment**

Predetermined number of animals used (and euthanized) under an animal experiment license

License application granted only if:

- No viable alternative exists to animal experiments
- Balance of interest evaluation shows scientific gains outweigh the burden placed on animals
- Experimental design is sound - statistically meaningful data obtained with lowest number of animals
- Effort is made to alleviate pain and stress (anesthesia/analgesia, social groups, humane end points)
- Annual and final reports account for all animal used and euthanized during the experiment

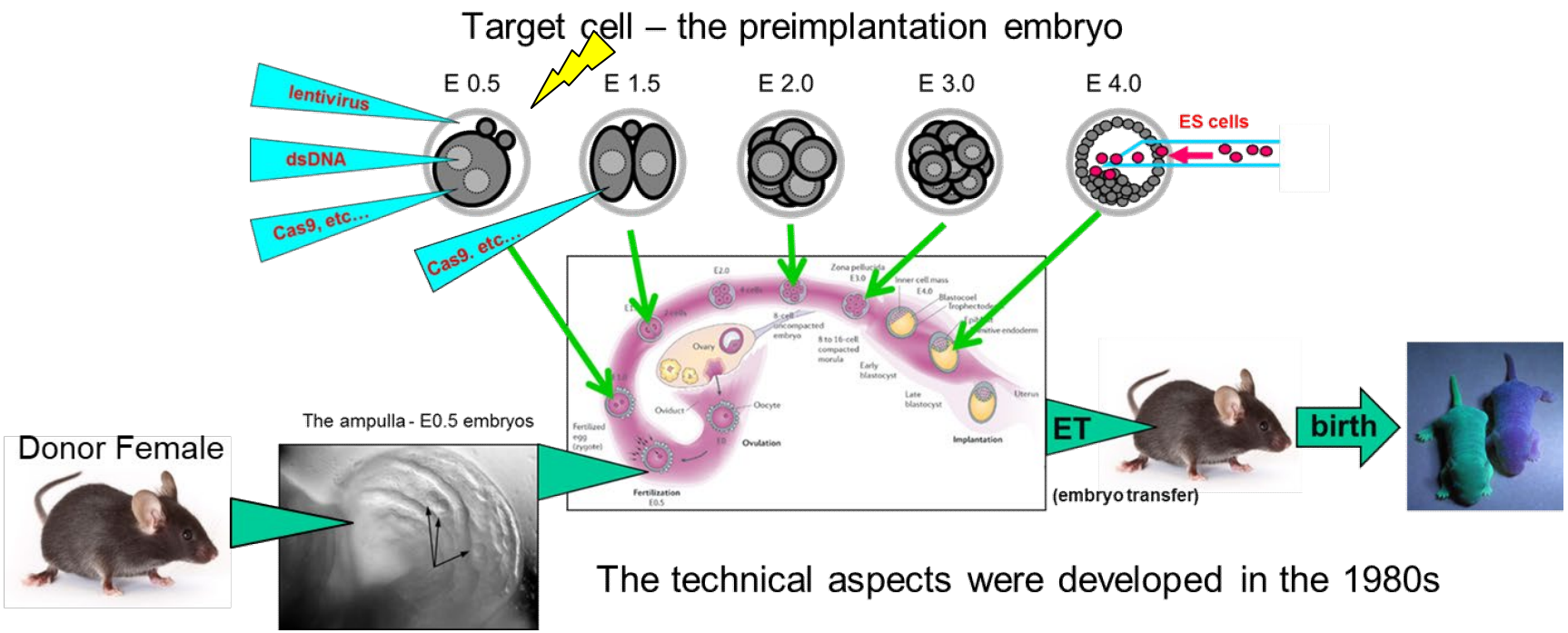
2. **Other laboratory animals** covered by breeding license that are **not in experiment**.

Majority of these are:

- animals bred and euthanized to generate meaningful experimental groups
- animals used in strain rederivation, expansion and maintenance
- **animals used in the course of transgenic techniques (embryo donors, unwanted offspring)**

Its estimated that such mice outweigh the experimental mice by ~3-4:1

How to better make a transgenic mouse ?

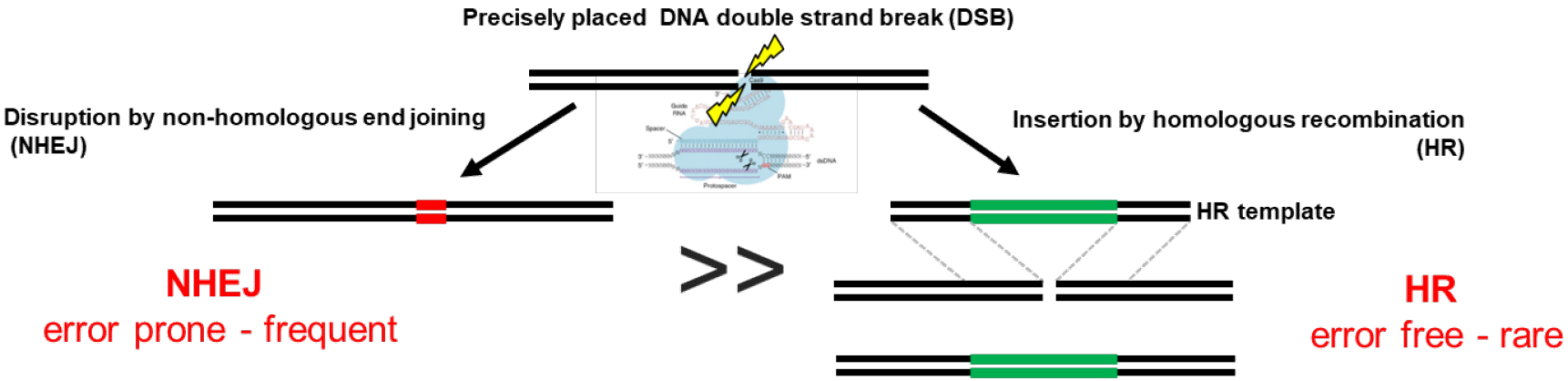


Every aspect of the process is covered by the breeding license.

This includes the use of CRISPR/Cas9 as well as all the euthanasia procedures.

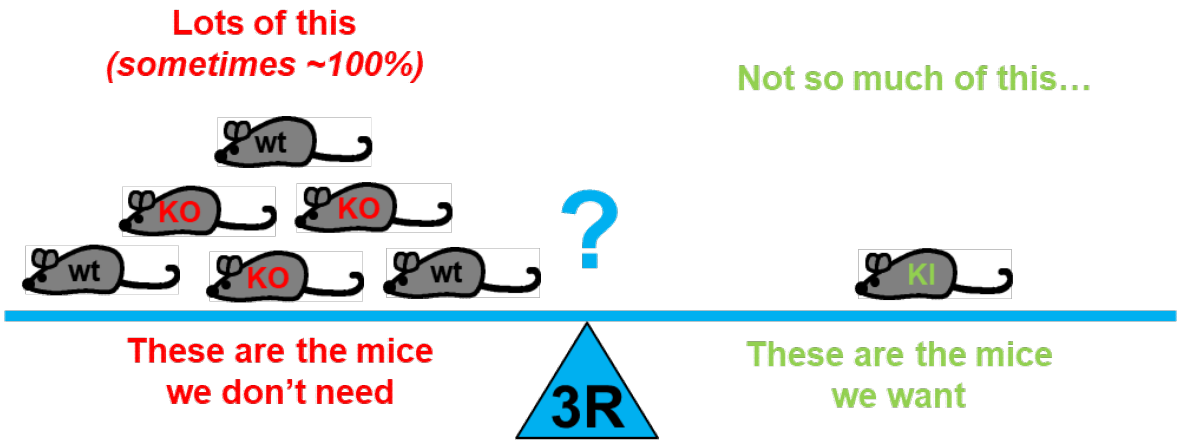
Euthanasia is part of the embryo collection and selection of transgenic offspring.

CRISPS/Ca9 systems for genome engineering



Cas9 creates the DSB – host repair does “genome engineering”

This has an impact on the resulting animals



How to justify making a transgenic mouse ?

Is the scientific reason sound? This is not part of an experimental license.
Does the animal (line) exist somewhere else? Redundancy not a factor.

How to better make a transgenic mouse ?

Incremental technical improvements that make genome engineering more efficient

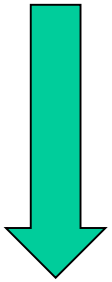
CRISPR/Cas9 – zygote electroporation
Most efficient way to generate all knock-out alleles (1bp to 100+kb)

CRISPR/Cas9 + ssDNA oligonucleotide – zygote electroporation
Most efficient way to generate small knock-in alleles (1bp - 50bp)

CRISPR/Cas9 + AAV – zygote electroporation
Most efficient way to generate medium size knock-in alleles (0.1kb – 4.8kb)

CRISPR/Cas9 + dsDNA – 2-cell embryos microinjection
Most efficient way to generate medium size knock-in alleles (5kb – 10kb)

Easy



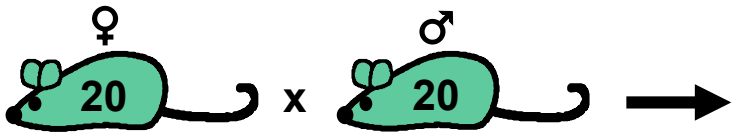
Difficult

Increasing the efficiency of genome engineering (less mice)

Generation of large knock-in (5kb+) mice with CRISPR/Cas9

CTM - **2020 vs. 2023**

2020

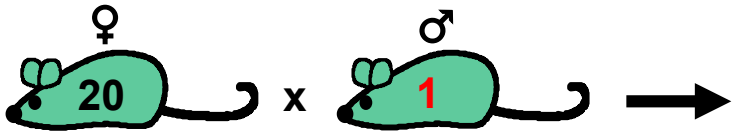


superovulation
mating
1-cell injection

Recover and inject ~200 1-cell embryos (Cas9RNP + dsDNA)
ET ~160 surviving embryos
Obtain and genotype ~25 offspring – hope for founders
Repeat if necessary

At least 20 females/project

2023



hyperovulation
IVF
2-cell injection

Recover ~1500 1-cell viable oocytes
Perform the IVF and culture overnight to 2-cell stage
Recover ~800 viable, synchronized 2-cell embryos
Inject ~200 2-cell embryos – freeze the remaining 600 for later
ET ~190 surviving embryos (better survival with ePore device)
Obtain and genotype ~30 offspring – identify the founders
Thaw additional 200 2-cell embryos for repeats or new projects

20 females and 1 male for 3-4 projects

3 - 4 fold reduction in animal numbers per project - 2020 vs. 2023

We use fewer animals, save time and money…

Continuous refinements of the humane euthanasia process

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Eidgenössisches Departement des Innern EDI

Externe Benutzer ME

Fachinformation Tierversuche

Fachgerechte und tierschutzkonforme Euthanasie von Versuchstieren 3.01

Maus, Ratte, Hamster, Meerschweinchen, Kaninchen, Zebrafisch, Krallenfrosch

Published
8.09.2020

Allowed methods

Maus	Pentobarbital
Ratte	
Hamster	
Meerschweinchen	
Kaninchen	
Krallenfrosch	

Maus	Euthanasie unter Inhalations- oder Injektionsanästhesie
Ratte	
Hamster	
Meerschweinchen	
Kaninchen	

Conditionally allowed methods

Maus ab 2 Wochen	CO_2 (Kohlendioxid)
Ratte ab 2 Wochen	

Maus bis 2 Wochen	Dekapitation ohne Anästhesie
Ratte bis 2 Wochen	

Maus bis 2 Wochen	Zervikale Dislokation ohne Anästhesie mit Entbluten
Ratte bis 2 Wochen	