

Knowing what you don't know: Phenotypic assessments and more....

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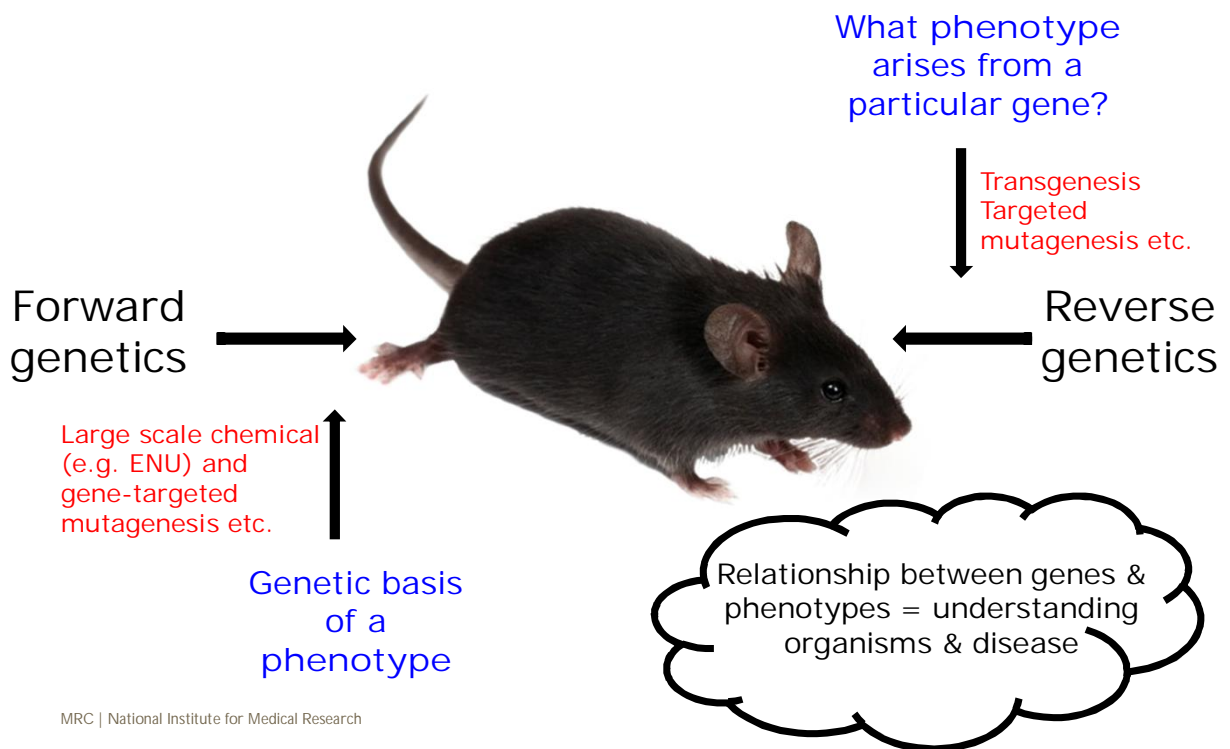
Knowing what you don't know....

- Generating GA mice
- Mice and the 5W's
- Phenotyping
 - ✧ Definitions
 - ✧ How, what, then what?
- Welfare considerations
- Humane endpoints
- Sharing mice
 - ✧ Information storage
 - ✧ Dissemination of information
 - ✧ Passports & transport of GAs

} James



Generating GA mice



Genetically altered mice: before you start!!

- **5Ws**

- Why? (*check all resources for alternatives*)
- Which mice?
- Where from?
- Who from? (*MTA's, frozen stock, least distance*)
- What **information is available?**



MRC | National Institute for Medical Research

Which mice & where from?

- “Ready made” mice – already available, published etc.
- ES cell, gene trap resources
- Make your own



Which mice?
Where from?

Check on the International Mouse strain resource, that your mouse is not already available.

Federation of International Mouse Resources (FIMRe)

International Mouse Strain Resource (IMSR)



Search Repositories Participate Glossary Contact Us About Us

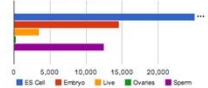
Welcome to the IMSR

The IMSR is a searchable online database of mouse strains, stocks, and mutant ES cell lines available worldwide, including inbred, mutant, and genetically engineered strains. The goal of the IMSR is to assist the international scientific community in locating and obtaining mouse resources for research. Note that the data content found in the IMSR is as supplied by strain repository holders.

For each strain or cell line listed in the IMSR, users can obtain information about:

- Where that resource is available (Repository Site)
- What state(s) the resource is available as (e.g. live, cryopreserved embryo or germplasm, ES cells)
- Links to descriptive information about a strain or ES cell line
- Links to mutant alleles carried by a strain or ES cell line
- Links for ordering a strain or ES cell line from a Repository
- Links for contacting the Repository to send a query

Available Strains by state



Search for: Search Reset Hide Options

Strain State: Strain Type:

Any
ES Cell
embryo
live
ovaries

Any
closed colony
coisogenic strain
congenic strain
consomic or chromosome substitution strain

Repository:

Any
APB (Australian Phenome Bank) Australia
CARD (Kumamoto University) Japan
CMMR (Canadian Mouse Mutant Repository) Canada
EM (European Mouse Mutant Archive) Italy
EMS (Dr. Elizabeth M. Simpson, Ph.D.) Canada
HAR (Hannam Genetics Laboratory) UK
HLB (JAX-PGA at The Jackson Laboratory) U.S.A.
JAX (The Jackson Laboratory) U.S.A.

View Repository Reports



All regions and repositories are selected by default; to limit your search to a specific region, click on the map, or select one or more specific repositories from the select list.

Mutations: chemically induced mutation chromosomal aberration deletion duplication gene trap insertion inversion other radiation induced mutation

reciprocal translocation recombinase(cre/flip) robertsonian translocation spontaneous mutation targeted mutation transgenic transposition

www.findmice.org

Mouse Locator UK

Strain Information Request

Mouse Locator (Transgenic Res. 2003 Oct;12(5):637.) provides a means to quickly search across UK research sites to source a colony or stored embryos from specific lines of mice. Permissions and the originators stipulations should then be addressed via the respondents.

Full Name	<input type="text"/>
Type	<input type="text" value="Transgenic"/>
Jax Number (if applicable)	<input type="text"/>
Common or Short Name	<input type="text"/>
Genetic Background	<input type="text" value="Any"/> If other please specify <input type="text"/>
Contact Email Address	<input type="text"/>
Additional Information	<input type="text"/>

If you know a strain of mouse exists and you want to find a UK source – send out a search on mouse locator

Only the fields that best identify the strain need completion.

Submit

For further information or enquiry please contact locator@cancer.org.uk



International Knockout Mouse Consortium

[Home](#) [About IKMC](#) [MartSearch](#) [Download](#) [Nominate gene](#) [FAQ](#) [Order Products](#) [Contact IKMC](#)

Welcome to the IKMC



The International Knockout Mouse Consortium (IKMC) aims to mutate all protein-coding genes in the mouse using gene trapping and gene targeting in C57BL/6 ES cells. [Read more...](#)

[Download the IKMC Gene List](#)
[View targeting strategies](#)
[View all allele types](#)

Search or Browse

Search IKMC database

Enter gene symbols, gene IDs or genome location

e.g., *Adam19*, *Pax*, *ENSMUSG00000020681*, *Chr13:22210730-22311689*
(coordinates from NCBI mouse genome assembly 37)

[Advanced Search](#)

Browse IKMC database

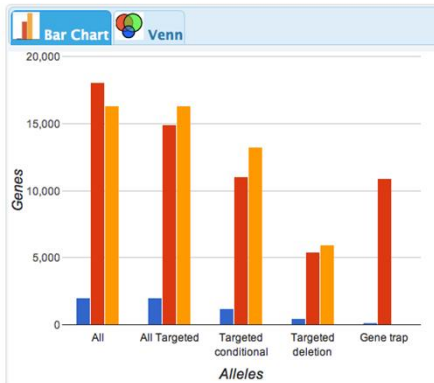
Use the following links to browse genes

- [Browse by Gene Symbol](#)
- [Browse by Chromosome](#)

If no mice,
check cells and
reagents: ES
cell KO
Resource

Status

IKMC Gene Progress Summary



Targeted alleles

Total Genes	KOMP		EUCCOMM/ EUCCOMMTools	NorCOMM	mirKO
	CSD	Regeneron			
Vectors available	6599	4731	9821	839	
ES cells available	5441	4011	7724	569	224
Mutant mice available	686	412	836	4	

[View details and project goals](#) [View details about the acronyms used](#)

Gene trap alleles

Total Genes	TIGM	EUCCOMM	NorCOMM
ES cells available	9411	4419	4593
Mutant mice available	151	10	

NOTE: Not all gene traps are in C57BL/6 ES cells. [View strain information](#)

www.knockoutmouse.org

ES cells: Practical concerns & potential pitfalls

- Keep considering 3Rs!
- Ordering EUCOMM clones
 - ✧ Order at least 3 ES cell clones (hugely variable GLT)
 - ✧ Expand on feeders (at least initially, reduces trisomies)
 - ✧ Karyotype/chromosome-count the clones
 - ✧ Confirm the genotype (this can be done for you!)
 - ✧ Choose the right host embryo (agouti & black JM8 cells)
 - ✧ Remove the floxed neo region prior to phenotypic analysis (reduce unpredictable effects)

Host blastocyst strain (genotype)	Chimera with C57BL/6N ESC line (ESC genotype)	Test-cross strain (genotype)	G ₁ Host-derived coat color (genotype)	G ₁ ESC-derived coat color (genotype)	Genetic background of ESC-derived G ₁
C57BL/6- <i>Tyr^{oBrd}</i> (a/a; <i>Tyr^{+/+}</i>)	● JM8 (a/a; <i>Tyr^{+/+}</i>) × C57BL/6- <i>Tyr^{oBrd}</i> (a/a; <i>Tyr^{o/c}</i>)	C57BL/6- <i>Tyr^{o/c}</i> (a/a; <i>Tyr^{o/c}</i>)	(a/a; <i>Tyr^{o/c}</i>)	(a/a; <i>Tyr^{+/c}</i>)	Mixed C57BL/6N × C57BL/6 <i>Tyr^{oBrd}</i>
	● JM8A3 (A/a; <i>Tyr^{+/+}</i>) × C57BL/6N (a/a; <i>Tyr^{+/+}</i>)	C57BL/6N (a/a; <i>Tyr^{+/+}</i>)	(a/a; <i>Tyr^{+/c}</i>)	(A/a or a/a; <i>Tyr^{+/+}</i>)	Pure C57BL/6N
BALB/c (A/A; <i>Tyr^{o/c}</i>)	● JM8 (a/a; <i>Tyr^{+/+}</i>) × C57BL/6N (a/a; <i>Tyr^{+/+}</i>)	C57BL/6N (a/a; <i>Tyr^{+/+}</i>)	(A/a; <i>Tyr^{+/c}</i>)	(a/a; <i>Tyr^{+/+}</i>)	Pure C57BL/6N
	● JM8A3 (A/a; <i>Tyr^{+/+}</i>) × C57BL/6N (a/a; <i>Tyr^{+/+}</i>)	C57BL/6N (a/a; <i>Tyr^{+/+}</i>)	(A/a; <i>Tyr^{+/c}</i>)	(A/a or a/a; <i>Tyr^{+/+}</i>)	Pure C57BL/6N
C57BL/6J- <i>A^{W/J}</i> (A ^{W/J} W/J; <i>Tyr^{+/+}</i>)	● JM8 (a/a; <i>Tyr^{+/+}</i>) × C57BL/6N (a/a; <i>Tyr^{+/+}</i>)	C57BL/6N (a/a; <i>Tyr^{+/+}</i>)	(A ^{W/J} W/J; <i>Tyr^{+/c}</i>)	(a/a; <i>Tyr^{+/+}</i>)	Pure C57BL/6N
C57BL/6J (a/a; <i>Tyr^{+/+}</i>)	● JM8A3 (A/a; <i>Tyr^{+/+}</i>) × C57BL/6N (a/a; <i>Tyr^{+/+}</i>)	C57BL/6N (a/a; <i>Tyr^{+/+}</i>)	(a/a; <i>Tyr^{+/c}</i>)	(A/a or a/a; <i>Tyr^{+/+}</i>)	Pure C57BL/6N

Making your own mice

- Lots of help is available!

www.biotec.tu-dresden.de/research/stewart/group-page/genetargeting

The screenshot shows the Biotec website's 'Gene Targeting Guide' page. The page has a header with the Biotec logo and navigation links. A right-hand navigation menu lists various categories like Home, About, Research Faculty, and specific researchers. Below the menu is a search bar and an 'Upcoming Events' section. The main content area is titled 'Gene Targeting Guide' and includes an introduction: 'Here we describe a way to design conditional alleles for mouse as well as practical hints for creating them. However, the strategies are transferable to other applications.' It also mentions that the creation of constructs is based on recombining and refers to a 'Recombining Guide' for practical hints. The page is dated 01.11.2011. At the bottom, there is a table of contents with 10 numbered items, each with a dropdown arrow:

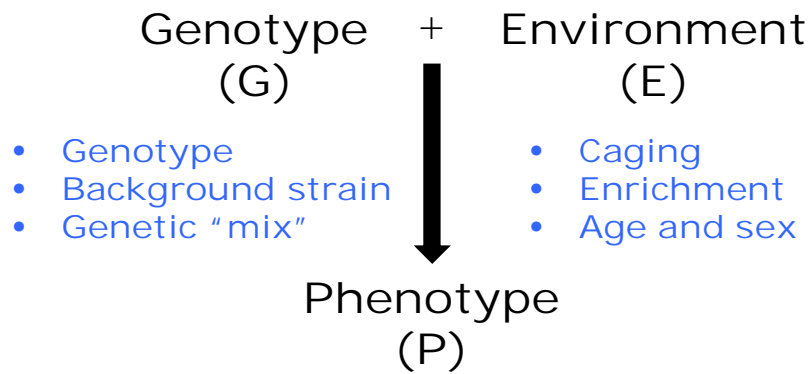
- 1) Knock-out First Allele Constructs
- 2) Gene of Interest
- 3) Expression
- 4) Exon Selection
- 5) Regulatory Elements and Repeats
- 6) Oligo design
- 7) Annotation in a Vector Program
- 8) In-Silico Creation of the Targeted Allele
- 9) Southern Strategy Design
- 10) Targeting Vector

Maintaining GA lines & phenotyping

- A day's worth of talks!!!
- Mendelian genetics
 - Prenatal lethality, homozygous lethal
- Age-dependent phenotypes
- Conditional/inducible models
 - Refine & modify phenotype
 - Reduce severity



Phenotype: An observed or measured quality such as morphology, physiology, development or behaviour

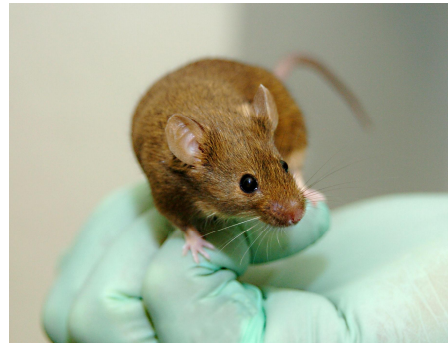


[G + E + genotype & environmental interaction = P]



Looking for phenotypes

- What's wrong with my mouse?
- What's right with my mouse?
- Expected vs unexpected.....



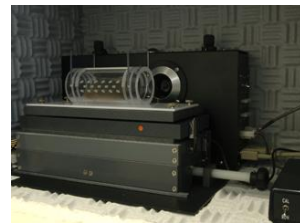
**Simple laboratory
equipment**



**Simple specialist
equipment**



**Advanced electronic
equipment**



The Best Phenotyping Equipment

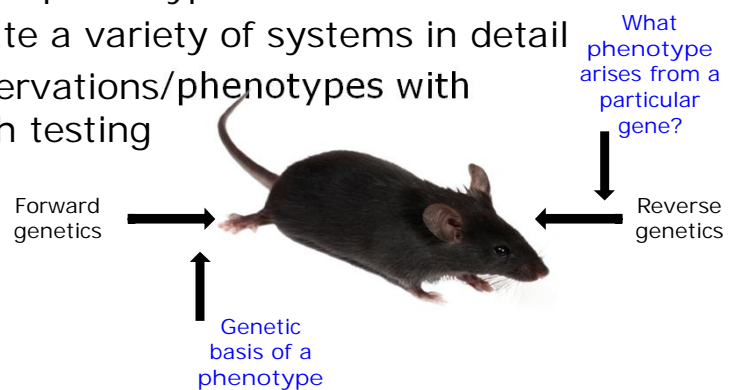


Key observation skills

Knowledge of normal behaviour

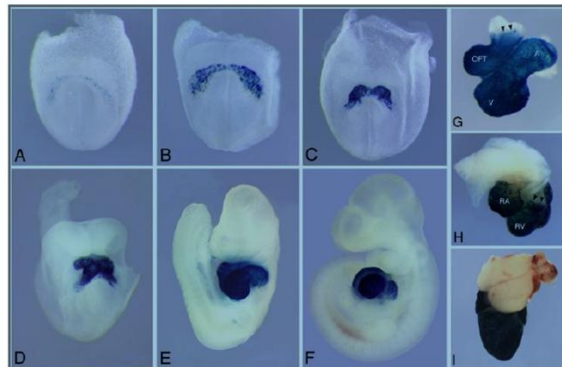
Different phenotyping strategies

- Direct approach
 - Know what systems the genetic alteration may affect
 - Want a profile of how the genetic alteration may affect a particular system
- Systematic comprehensive phenotyping
 - No preconception of phenotype
 - Primarily investigate a variety of systems in detail
 - Follow up any observations/phenotypes with secondary in-depth testing



Direct approach

- From phenotypic point of view most GA lines have been generated to analyse specific pathways or biological approaches
- Phenotyping is therefore conducted at the level of interest and expertise
- Pleiotropy!
- Miss other interesting phenotypes, reducing potential
- Should go hand in hand with welfare assessments
- Good observation
- Consider controls carefully (genetic drift, homozygous GA & inbred lines)





Welcome to the International Mouse Phenotyping Consortium

SEARCH OUR GENE LIST

SEARCH

Find the latest status of your favorite gene, register for updates or just [browse](#) / [download](#) the complete IMPC gene list.



BROWSE GENE LIST

Find out the latest status of your gene of interest



REGISTER FOR GENE STATUS UPDATES

Register to be kept up to date in the latest progress of your gene of interest



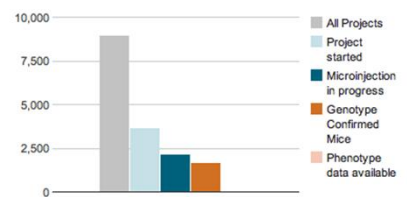
PHENOTYPE PROTOCOLS (IMPRESS)

View Phenotype Procedures and Parameters held in the IMPReSS Database



The International Mouse Phenotyping Consortium (IMPC)
Comprises a group of major mouse genetics research institutions along with national funding organisations formed to address the challenge of developing an encyclopedia of mammalian gene function.

Project Status



Validated protocols - IMPC

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Heart Weight [MPC_HWT_001]

[View as PDF](#) / [Display Ontologies](#)

[Purpose](#) [Experimental Design](#) [Equipment](#) [Procedure](#) [Notes](#) [Parameters](#) [Metadata](#)

Purpose

To evaluate cardiac size using heart weight and body weight.

Experimental Design

Minimum number of mutant animals required: 7 males + 7 females

Minimum age of animals: 16 weeks

Equipment

- fine forceps
- surgical scissors
- fine surgical scissor
- kim wipes (tissues) or surgical compress
- laboratory balance
- labelled jar with fixative
- corkplate or wax board
- pins
- jar containing tap water to rinse the tools

Procedure

Methods and procedures used not including center-specific data entry methods.

1. Sacrifice the mouse

Systematic comprehensive phenotyping

- Primary tests/pipelines
 - Comprehensive, standardised characterisation
(dysmorphology, cardiovascular, energy metabolism, clinical chemistry, lung function, expression profiling)
 - Basic parameters reveal traits of interest
 - Non-invasive
 - Efficient
 - Statistics, power analysis
- Secondary & tertiary tests/pipelines
 - Initial phenotypes are further investigated
(Behaviour tests, circadian rhythm, quantitative imaging, telemetry)
 - Validation and more delayed analysis
 - May be invasive



[International Mouse Phenotyping Consortium](#)

Describing phenotypes

www.informatics.jax.org/searches/MP_form.shtml

Use standard,
defined
terminology



[About](#) [Help](#) [FAQ](#)

[Search](#) [Download](#) [More Resources](#) [Submit Data](#) [Find Mice \(IMSR\)](#) [Analysis Tools](#) [Contact Us](#)



Mammalian P
Te

MP term: **abnormal adipose tissue morphology**
Synonym: **adipose tissue abnormalities**
Synonym: **adipose tissue dysplasia**
MP id: **MP:0000003**
Alternate id: **MP:0000011**
Definition: **any structural anomaly of the connective tissue composed of fat cells enmeshed in areolar tissue**
Number of paths to term: 1

denotes an 'is-a' relationship
 denotes a 'part-of' relationship

mammalian phenotype

- [adipose tissue phenotype](#)
 - [abnormal adipose tissue morphology \[MP:0000003\] \(1173 qenotypes, 2151 annotations\)](#)
 - [abnormal adipose tissue amount +](#)
 - [abnormal adipose tissue development](#)
 - [abnormal adipose tissue distribution](#)
 - [abnormal brown adipose tissue morphology +](#)
 - [abnormal fat cell morphology +](#)
 - [abnormal fat pad morphology +](#)
 - [abnormal white adipose tissue morphology +](#)
 - [abnormal adipose tissue physiology +](#)

Dysmorphology

- Full visual inspection of mice at appropriate time points

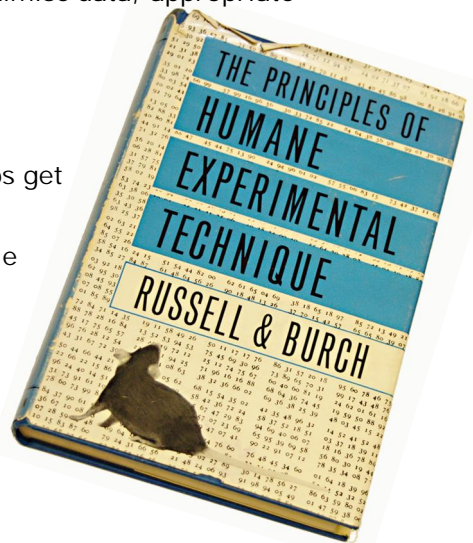


- Coat
- Skin
- Head Shape
- Ears
- Eyes
- Teeth
- Vibrissae
- Limbs
- Paws
- Digits
- Nails
- Teeth

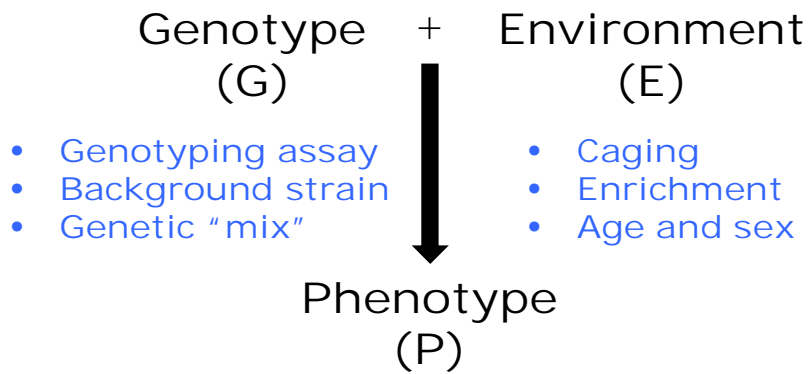


Characteristics of good phenotyping tests

- 3Rs
 - **Replacement** – consult the experts, keep up to date with tests
 - **Refinement** – know baseline data, analyse and compare data frequently
 - **Reduction** – select controls carefully, maximise data, appropriate experimental design & statistical analysis
- More 3Rs
 - **Reliable** – equipment, consistent data
 - **Robust** – measure differences, different labs get similar results
 - **Repeatable** – consistent results every time



Phenotype: An observed or measured quality such as morphology, physiology, development or behaviour



[G + E + genotype & environmental interaction = P]



Genetic Background: PVR mice

Tg21 – BALB/c



Tg66 – C57BL/6J



Tg66 – CBA/CaJ



Same transgene, same site of insertion, same copy number

Different susceptibility to infection with poliovirus.
Different routes of inoculation cause different phenotypes and disease/paralysis progression.

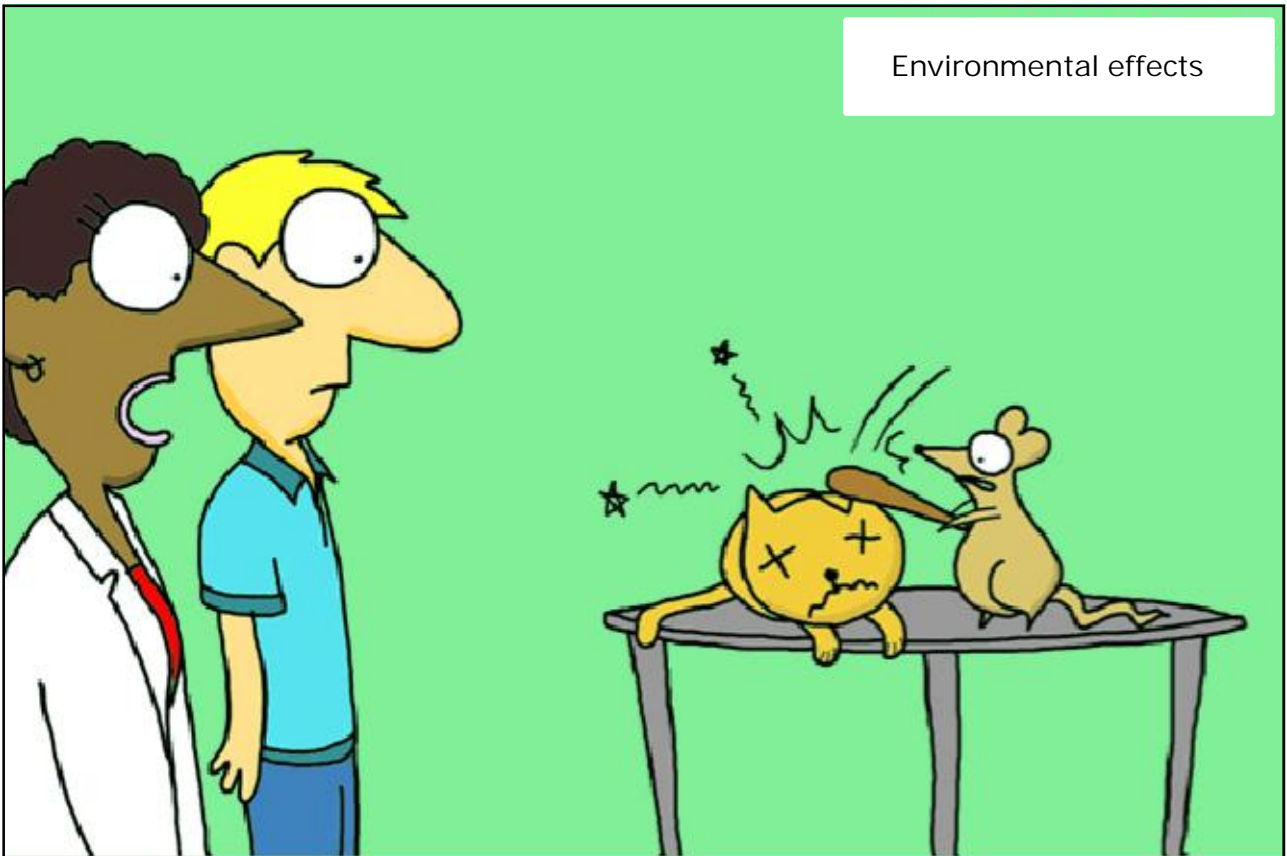
Environmental factors

- Noise
- Light
- Enrichment
- Handling
- Diet
- Microbiological status (IL-10^{-/-}) – watch this space!
- Order of tests!

- Treatment regimes (at cage or colony level)
- Health observations (changes of frequency may affect behaviour)
- Transport to equipment.....

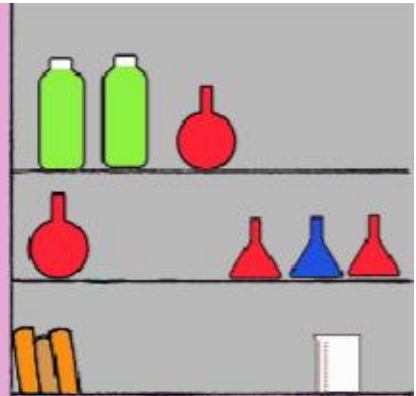
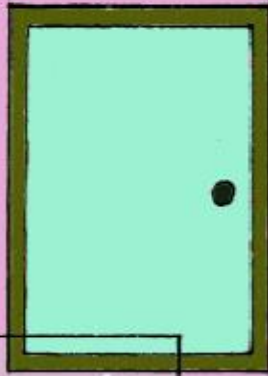


Environmental effects



**“Mouse had no discernible phenotype..
till the cat jumped on him!”**

www.VADLO.com

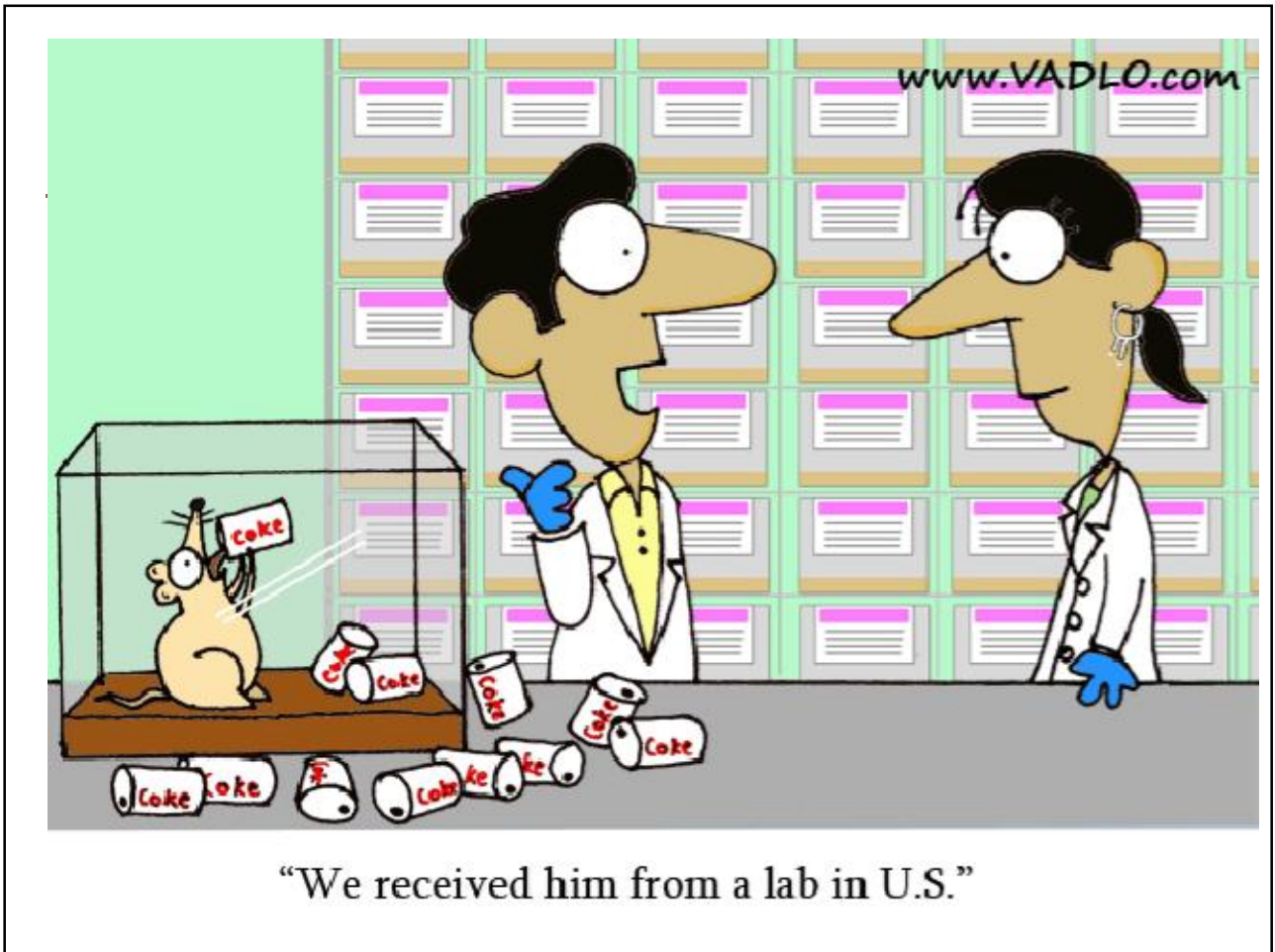


“Hey Ted, I would rather work with Tiffany.”

Sharing & archiving

- Information databases
 - MGI
- Importance of archiving
(Cryopreservation and appropriate cryopreservation strategy)
- Mouse passports





“We received him from a lab in U.S.”

Summary & Conclusion

“ There are known knowns; there are things we know that we know. There are known unknowns; that is to say there are things that, we now know we don't know. But there are also unknown unknowns – there are things we do not know we don't know. ”

Donald Rumsfeld, US Secretary of Defense



Acknowledgements



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MRC Harwell



The Mice