

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

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MRC National Institute for Medical Research

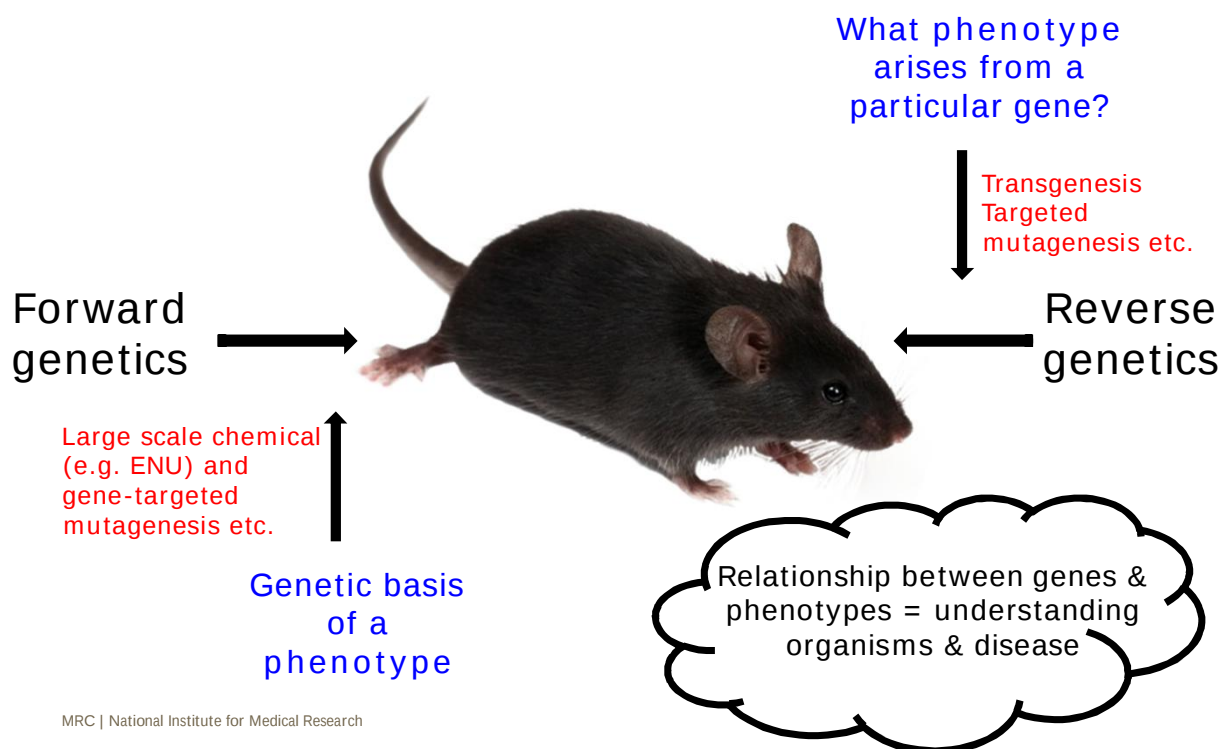


Knowing what you don't know....

- 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



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?**



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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

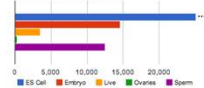
Welcome to the IMSR

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 gamplasm, 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
cologenic 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 (Mammalian 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(cro/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.

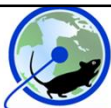
Full Name	
Type	Transgenic
Jax Number (if applicable)	
Common or Short Name	
Genetic Background	Any If other please specify
Contact Email Address	
Additional Information	

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

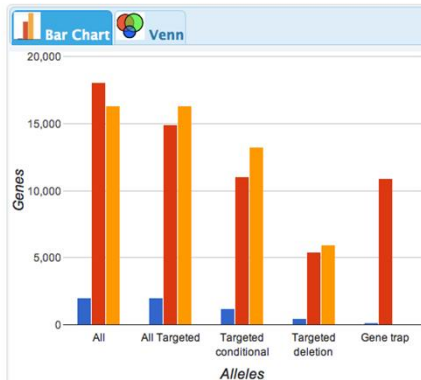
Use the following links to browse genes

- [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		EUCOMM/ EUCommTools	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	EUCOMM	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

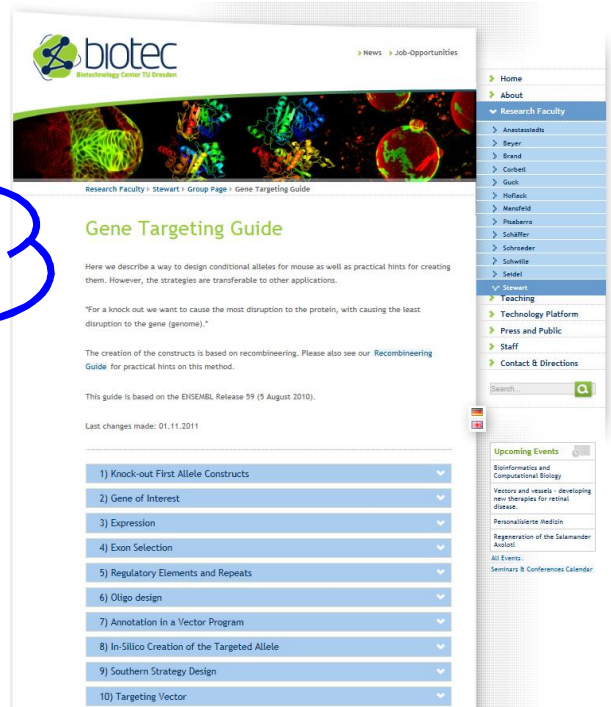
- Keep considering 3Rs!
- Ordering EUComm clones
 - ✧ Order at least 3 ES cell clones (hugely variable GLT)
 - ✧ Expand on feeders (at least initially, reduces trisomies)
 - ✧ Karyotype/chromosome-count the clones
 - ✧ Confirm the genotype (this can be done for you!)
 - ✧ Choose the right host embryo (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^{oBrd}</i>)	 JM8 (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^{oBrd}</i>)  (a/a; <i>Tyr^{+/+}</i>)	 (a/a; <i>Tyr^{oBrd}</i>)  (A/a or a/a; <i>Tyr^{+/+}</i>)	Mixed C57BL/6N × C57BL/6 <i>Tyr^{oBrd}</i> Pure C57BL/6N
BALB/c (A/A; <i>Tyr^{oBrd}</i>)	 JM8 (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^{oBrd}</i>)  (A/a; <i>Tyr^{+/+}</i>)	 (A/A; <i>Tyr^{oBrd}</i>)  (A/a or a/a; <i>Tyr^{+/+}</i>)	Pure C57BL/6N Pure C57BL/6N
C57BL/6J- <i>A^{W/J}</i> (A ^{W/J} ; <i>Tyr^{+/+}</i>)	 JM8 (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 ^{W/J} ; <i>Tyr^{+/+}</i>)  (a/a; <i>Tyr^{+/+}</i>)	 (A ^{W/J} ; <i>Tyr^{+/+}</i>)  (a/a; <i>Tyr^{+/+}</i>)	Pure C57BL/6N
C57BL/6J (a/a; <i>Tyr^{+/+}</i>)	 JM8 (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^{+/+}</i>)  (a/a; <i>Tyr^{+/+}</i>)	 (A/a or a/a; <i>Tyr^{+/+}</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



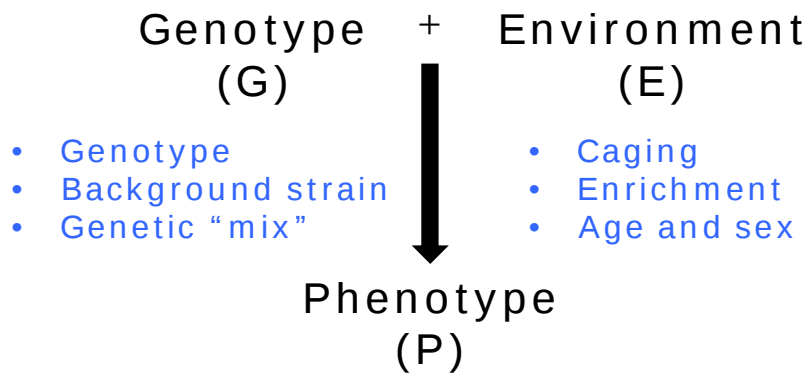
Maintaining GA lines & phenotyping

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



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

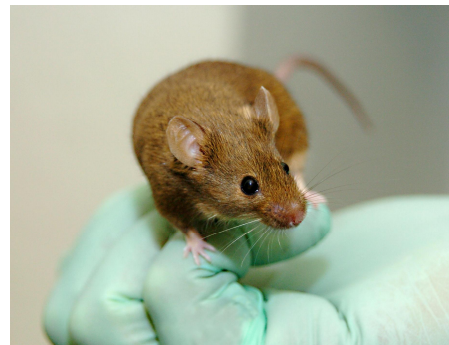


[G + E + genotype & environmental interaction = P]



Looking for phenotypes

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



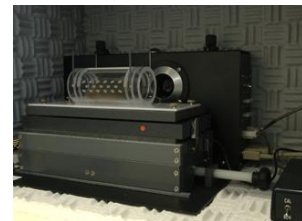
**Simple laboratory
equipment**



**Simple specialist
equipment**



**Advanced electronic
equipment**



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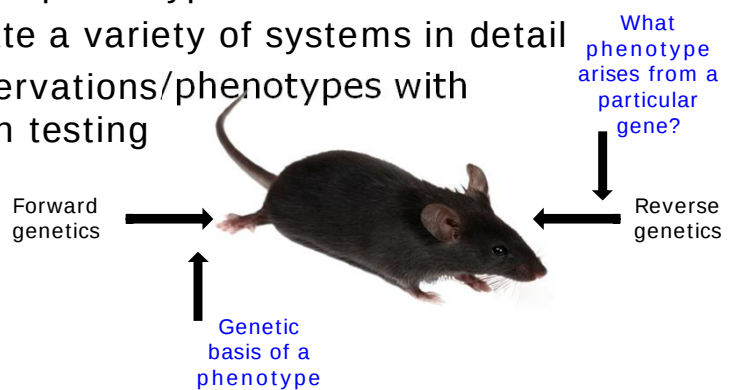
The Best Phenotyping Equipment



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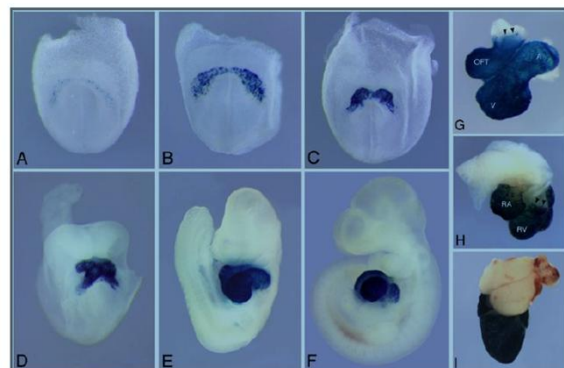
Different phenotyping strategies

- 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)



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Welcome to the International Mouse Phenotyping Consortium

SEARCH OUR GENE LIST

Use a gene symbol e.g. sox

[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 \(IMPRESS\)](#)

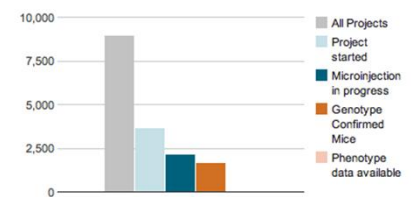
View Phenotype Procedures and Parameters held in the IMPReSS Database



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

[Home](#) [Pipelines](#) [Ontologies](#) [Glossary](#) [Help](#) [Contact Us](#)

Heart Weight [IMPC_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

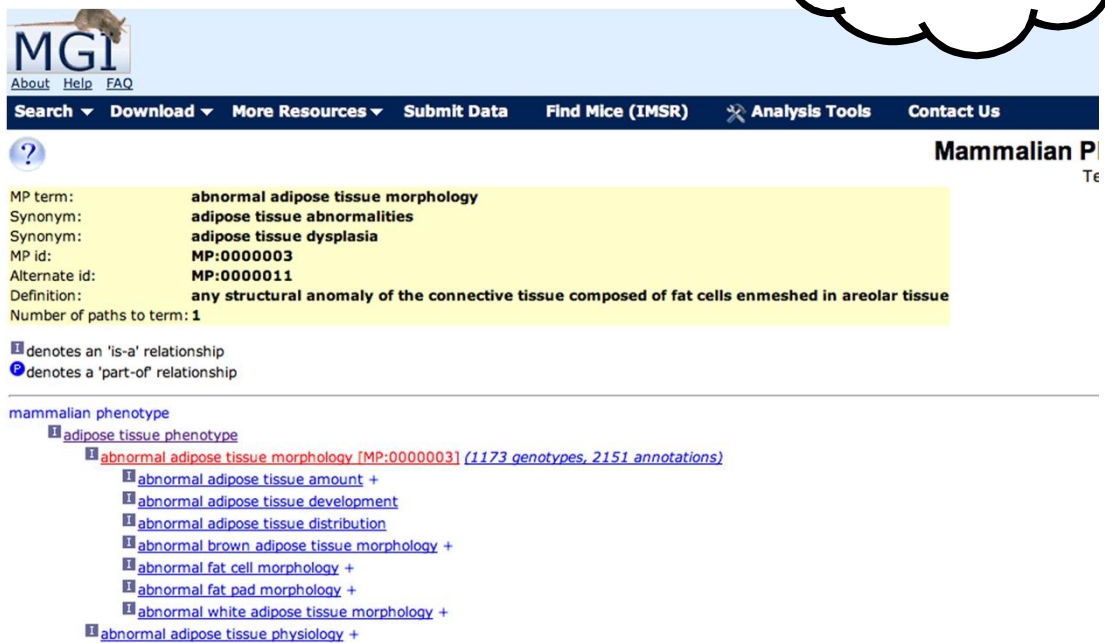


[International Mouse Phenotyping Consortium](#)

Describing phenotypes

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

Use standard,
defined
terminology



The screenshot shows the MGI website interface. At the top, there's a navigation bar with links: Search, Download, More Resources, Submit Data, Find Mice (IMSR), Analysis Tools, and Contact Us. Below this, a search bar contains a question mark icon. The main content area displays the phenotype details for 'abnormal adipose tissue morphology' (MP:0000003). The details include synonyms like 'adipose tissue abnormalities' and 'adipose tissue dysplasia', and a definition: 'any structural anomaly of the connective tissue composed of fat cells enmeshed in areolar tissue'. Below the details, there's a section for 'mammalian phenotype' which lists related terms like 'abnormal adipose tissue amount', 'abnormal adipose tissue development', etc., each preceded by an 'is-a' relationship icon.

MGI
About Help FAQ

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?

Mammalian Phenotype

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 \[MP:0000003\] \(1173 genotypes, 2151 annotations\)](#)
 - [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

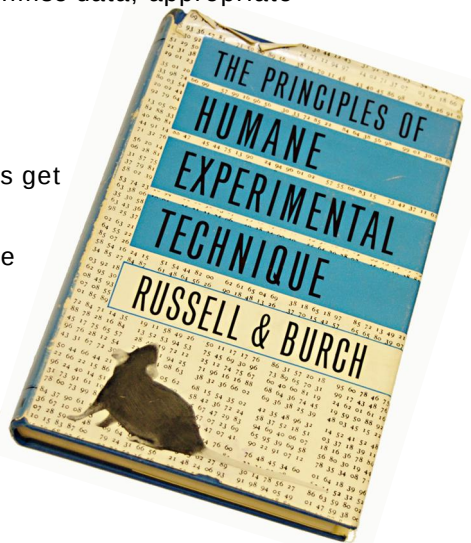


- Coat
- Skin
- Head Shape
- Ears
- Eyes
- Teeth
- Vibrissae
- Limbs
- Paws
- Digits
- Nails
- Teeth

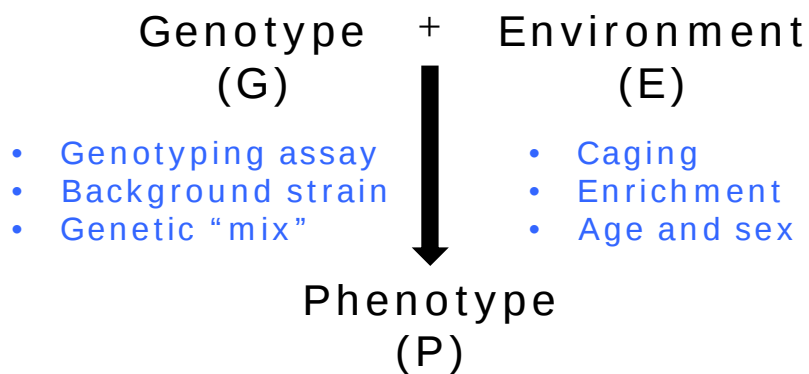


Characteristics of good phenotyping tests

- **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

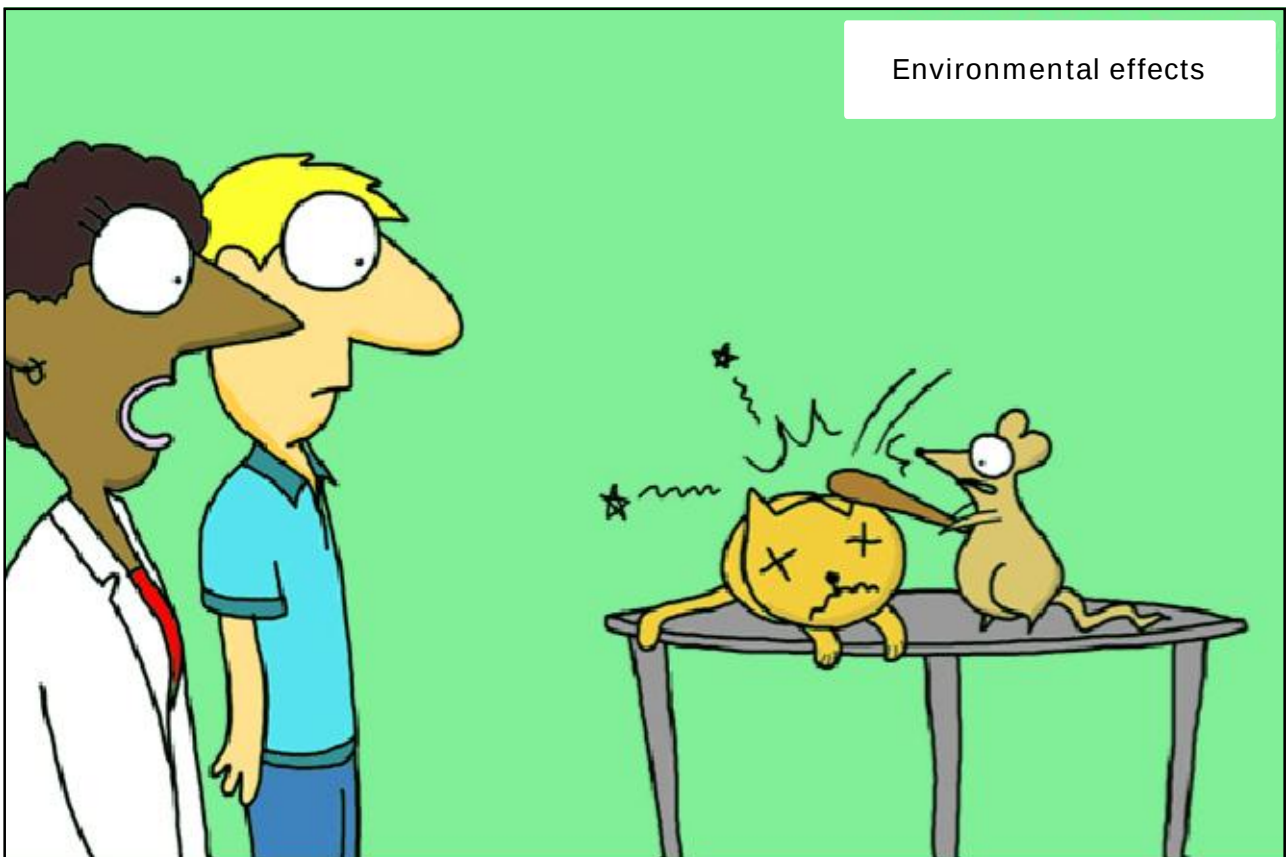
Different susceptibility to infection with poliovirus.
Different routes of inoculation cause different
phenotypes and disease/paralysis progression.

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.....

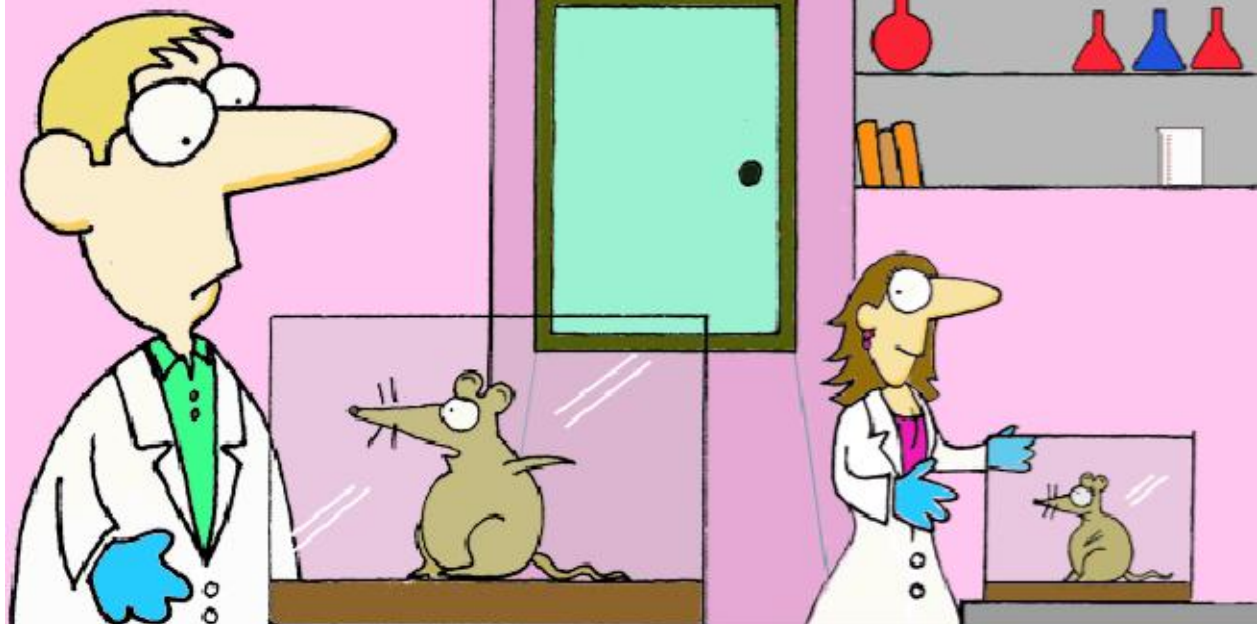


Environmental effects



“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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Sara Wells
MRC Harwell



The Mice