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

James

- Sharing mice
  - ✧ Information storage
  - ✧ Dissemination of information
  - ✧ Passports & transport of GAs

Generating GA mice

What phenotype arises from a particular gene?

Transgenesis
Targeted mutagenesis etc.

Forward genetics

Reverse genetics

Large scale chemical (e.g. ENU) and gene-targeted mutagenesis etc.

Genetic basis of a phenotype

Relationship between genes & phenotypes = understanding organisms & disease

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Genetically altered mice: before you start!!

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

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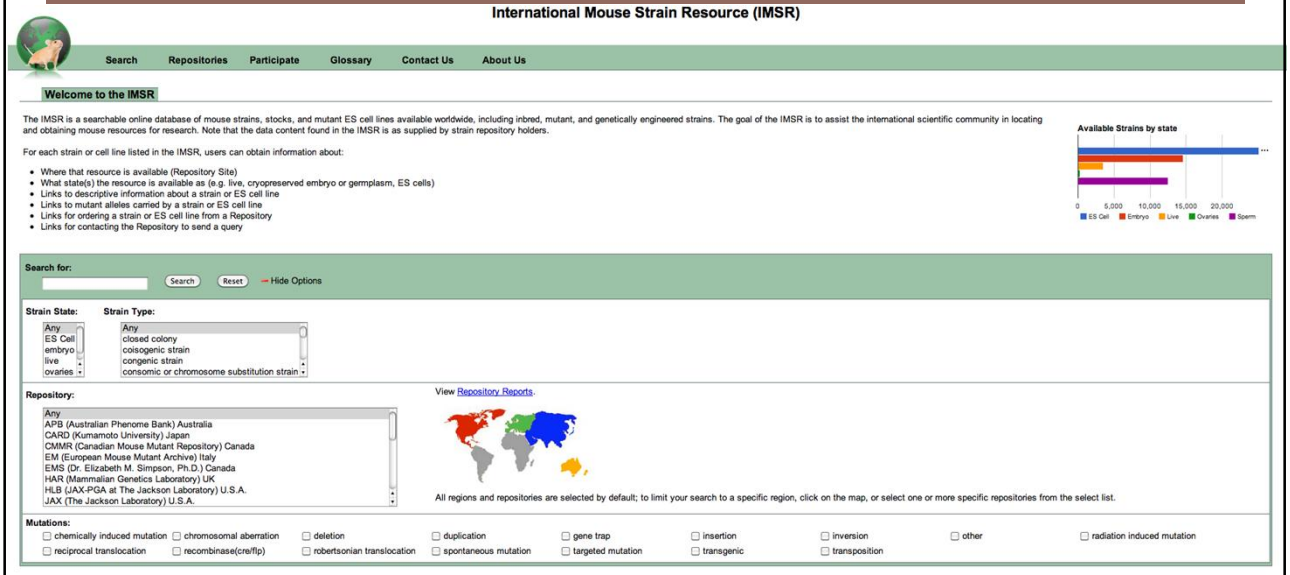
Which mice & where from?

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

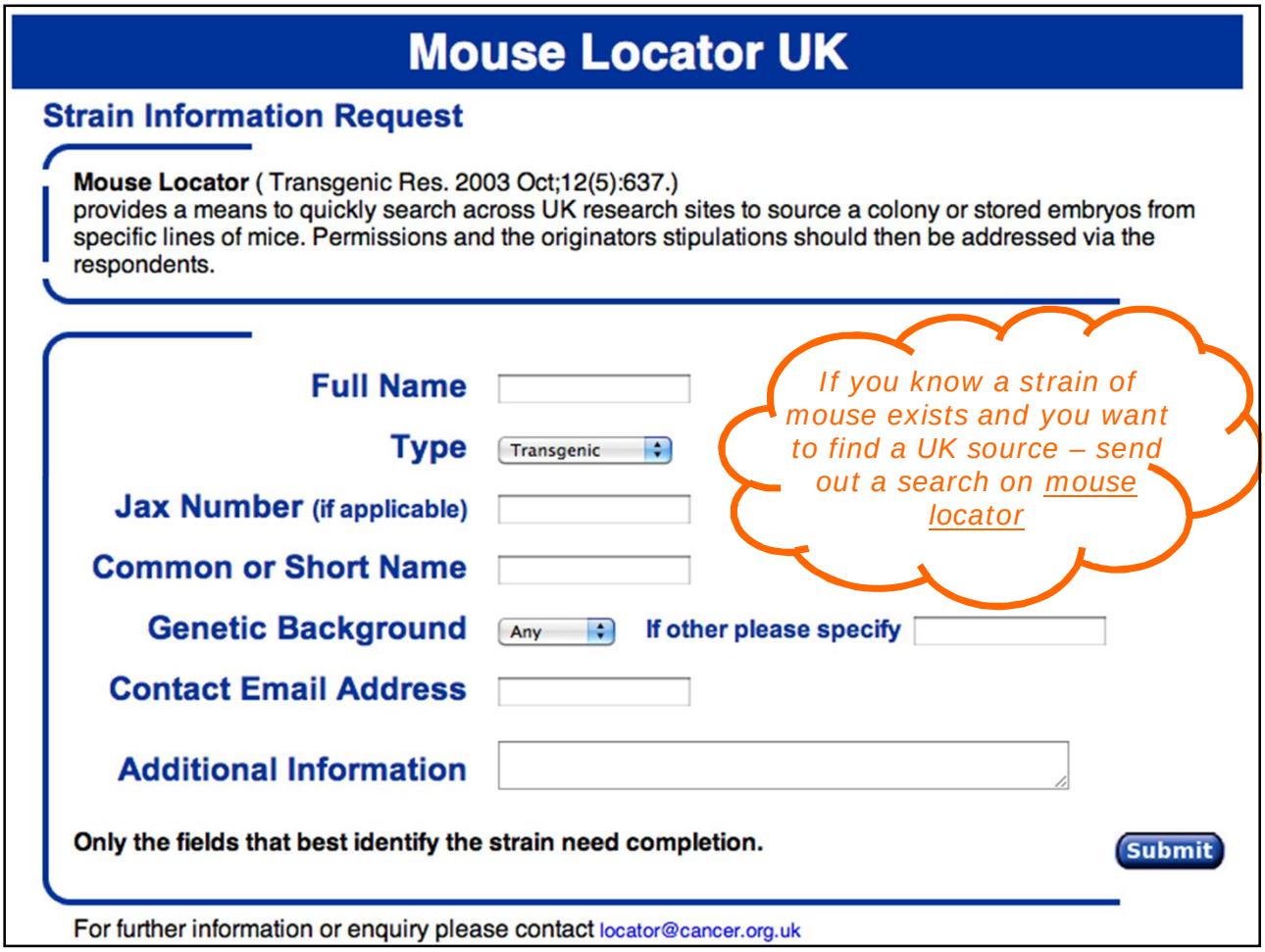
Which mice?
Where from?

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

Federation of International Mouse Resources (FIMRe)



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
Only the fields that best identify the strain need completion.
Submit
For further information or enquiry please contact locator@cancer.org.uk
If you know a strain of mouse exists and you want to find a UK source – send out a search on mouse locator

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

Search

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

- Advanced Search

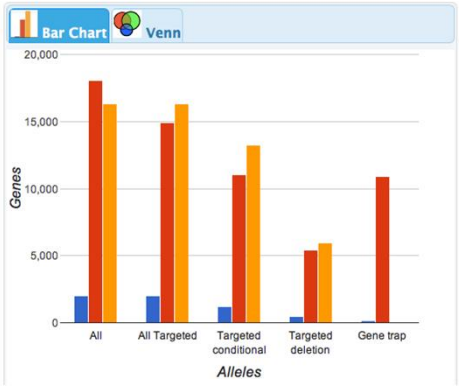
Browse IKMC database
Use the following links to browse genes

- 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

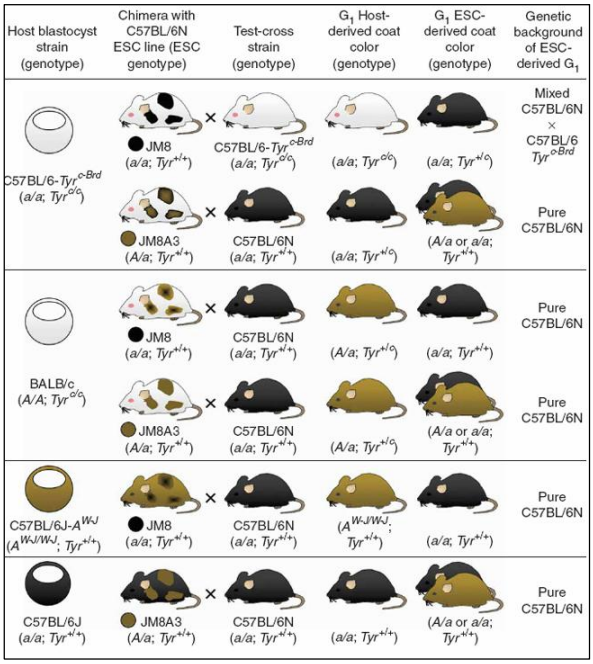
Total Genes	TIGM	EUCOMM	NorCOMM
ES cells available	9411	4419	4593
Mutant mice available	151	10	

NOTE: Not all gene traps are in C57BL/6 ES cells. View strain information

www.knockoutmouse.org

ES cells: Practical concerns & potential pitfalls

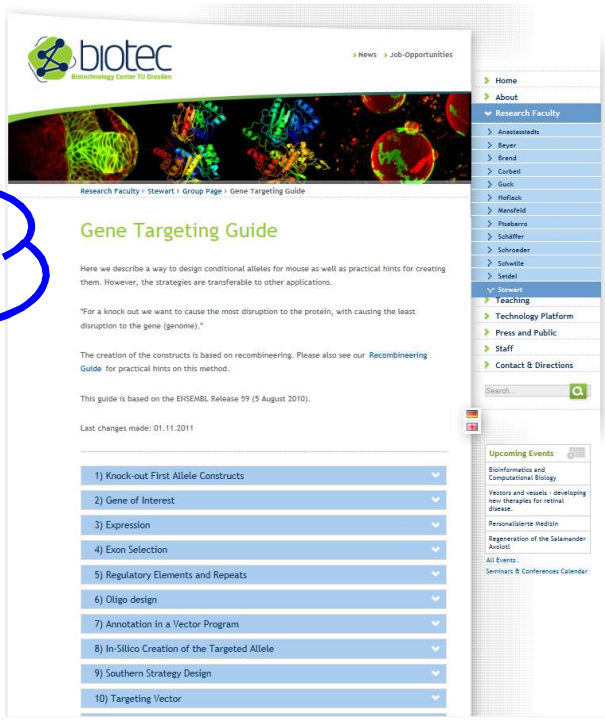
- Keep considering 3Rs!
- Ordering EUCOMM clones
  - ✧ Order at least 3 ES cell clones
    (hugely variable GLT)
  - ✧ Expand on feeders
    (at least initially, reduces trisomies)
  - ✧ Karyotype/chromosome-count the clones
  - ✧ Confirm the genotype
    (this can be done for you!)
  - ✧ Choose the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



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

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

Genotype (G) + Environment (E)

- Genotype
- Background strain
- Genetic "mix"

- Caging
- Enrichment
- Age and sex

Phenotype (P)

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

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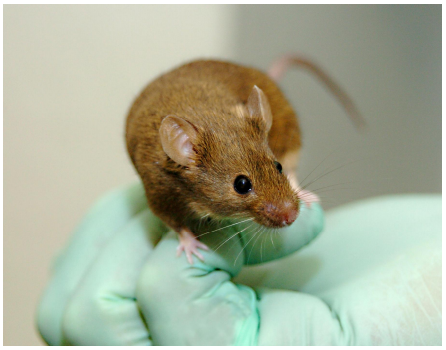
Looking for phenotypes

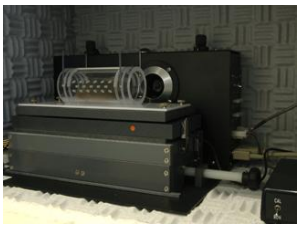
- What's wrong with my mouse?
- What's right with my mouse?
- Expected vs unexpected……



Simple laboratory equipment

Simple specialist equipment

Advanced electronic equipment

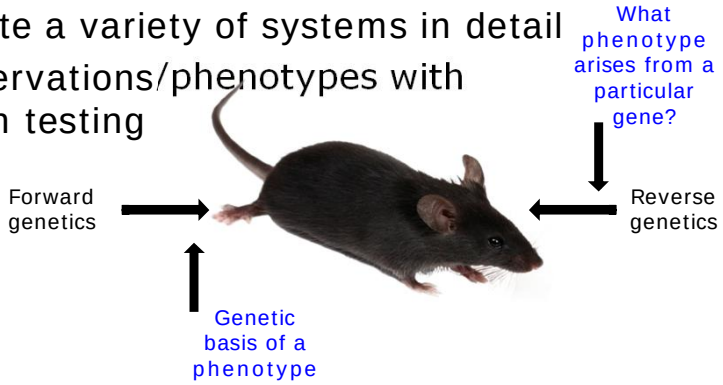


The Best Phenotyping Equipment

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

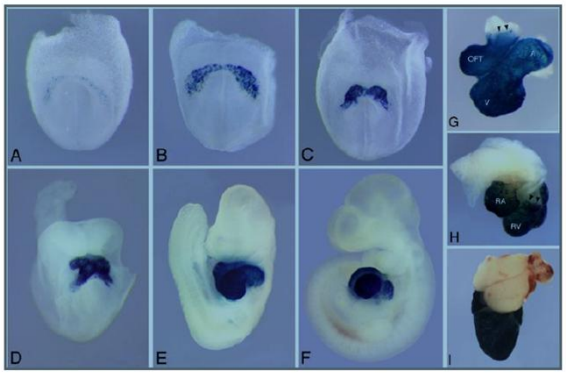
- Systematic comprehensive phenotyping
  - No preconception of phenotype
  - Primarily investigate a variety of systems in detail
  - Follow up any observations/phenotypes with secondary in-depth testing

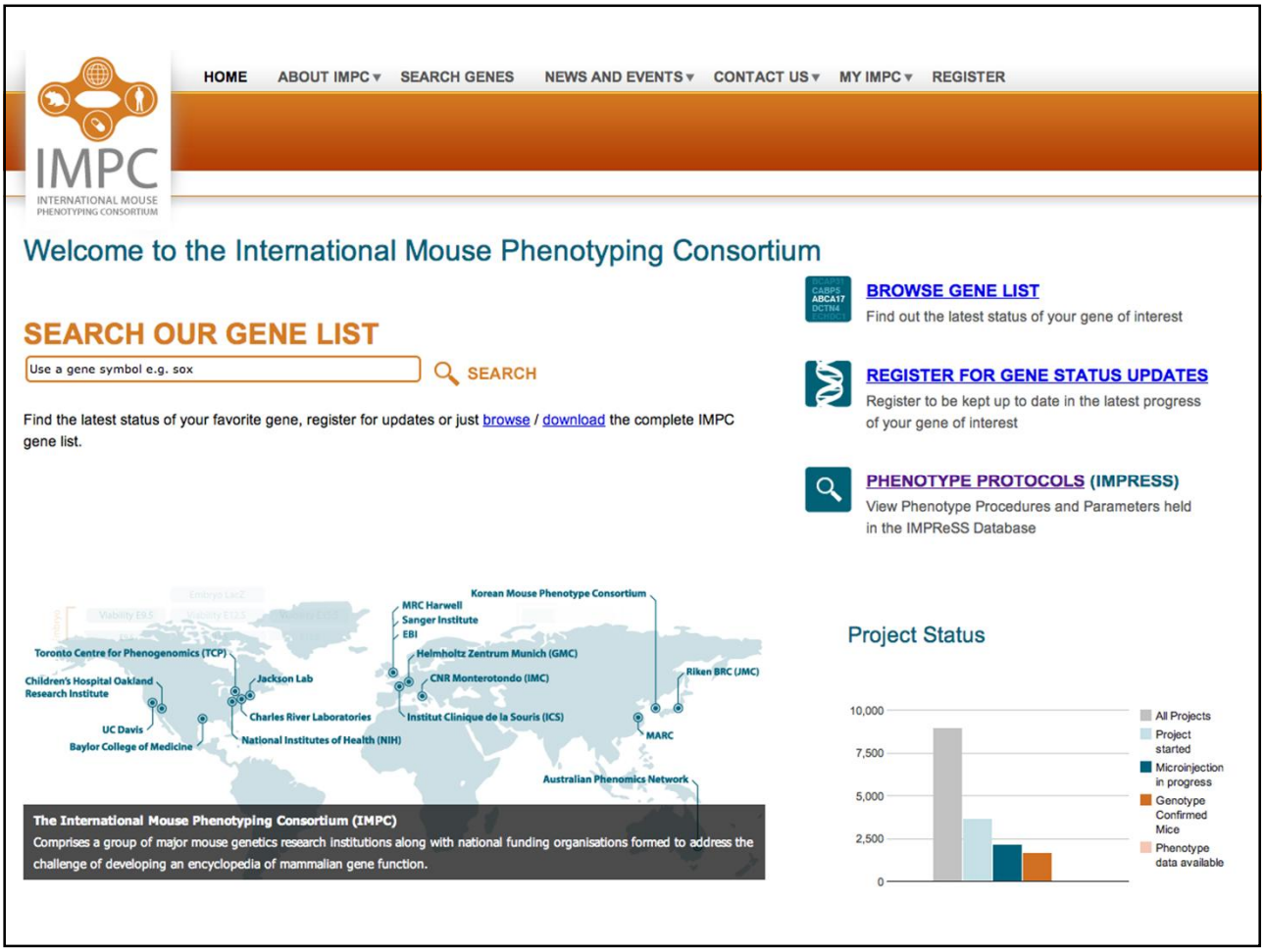


Direct approach

- 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)




IMPC
INTERNATIONAL MOUSE PHENOTYPING CONSORTIUM
HOME
ABOUT IMPC
SEARCH GENES
NEWS AND EVENTS
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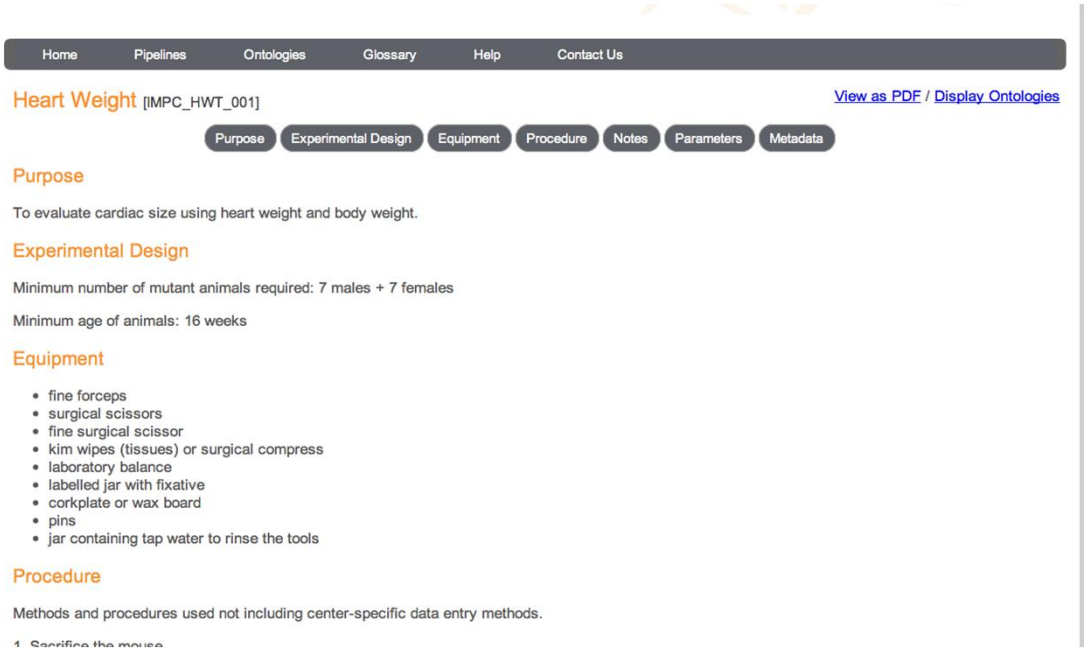
MY IMPC
REGISTER
Welcome to the International Mouse Phenotyping Consortium
SEARCH OUR GENE LIST
Use a gene symbol e.g. sox
SEARCH
Find the latest status of your favorite gene, register for updates or just browse / download the complete IMPC gene list.
BROWSE GENE LIST
Find out the latest status of your 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
Korean Mouse Phenotype Consortium
MRC Harwell
Sanger Institute
EBI
Toronto Centre for Phenogenomics (TCP)
Helmholtz Zentrum Munich (GMC)
Children's Hospital Oakland Research Institute
Jackson Lab
CNR Monterotondo (IMC)
Riken BRC (JMC)
Charles River Laboratories
Institut Clinique de la Souris (ICS)
UC Davis
MARC
Baylor College of Medicine
National Institutes of Health (NIH)
Australian Phenomics Network
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
10,000
7,500
5,000
2,500
0
All Projects
Project started
Microinjection in progress
Genotype Confirmed Mice
Phenotype data available

Validated protocols - IMPC



Systematic comprehensive phenotyping

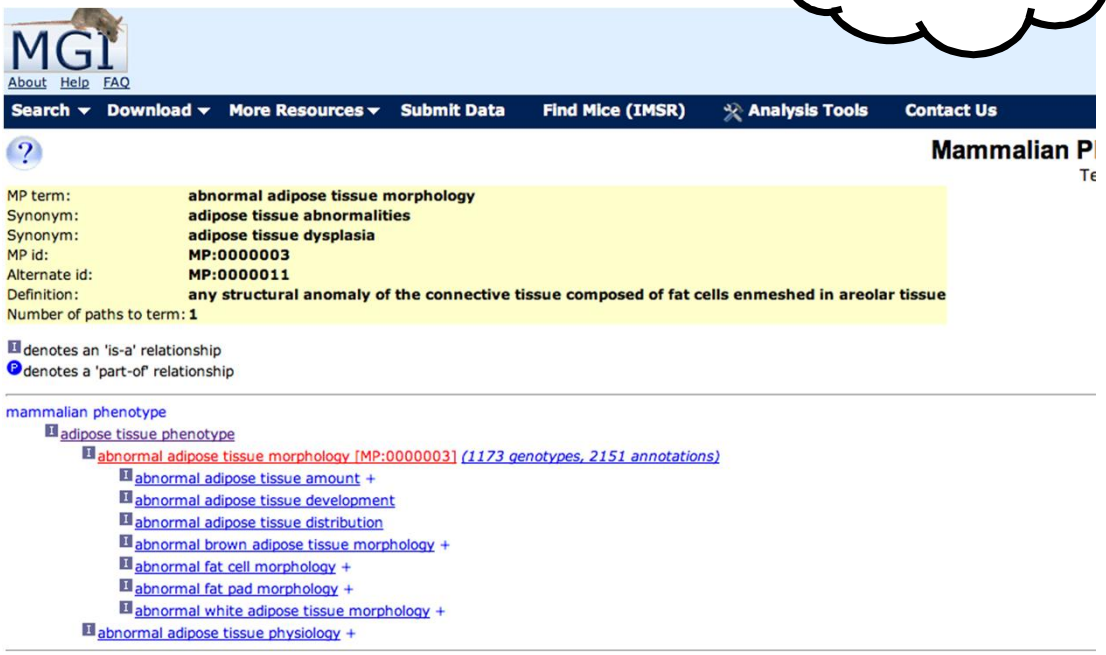
- Primary tests/pipelines
  - Comprehensive, standardised characterisation
    (dysmorphology, cardiovascular, energy metabolism, clinical chemistry, lung function, expression profiling)
  - Basic parameters reveal traits of interest
  - Non-invasive
  - Efficient
  - Statistics, power analysis
- Secondary & 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

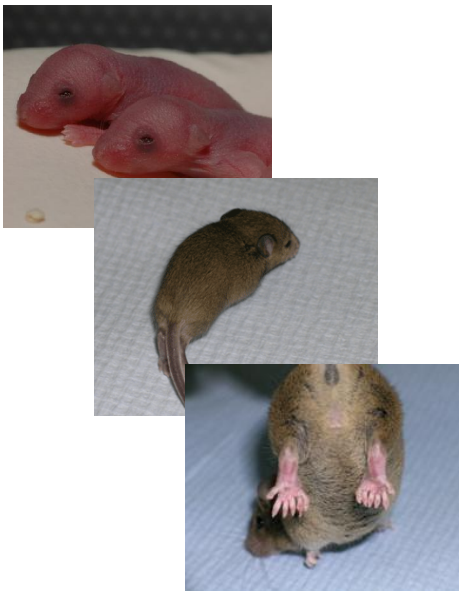
Use standard, defined terminology



Dysmorphology

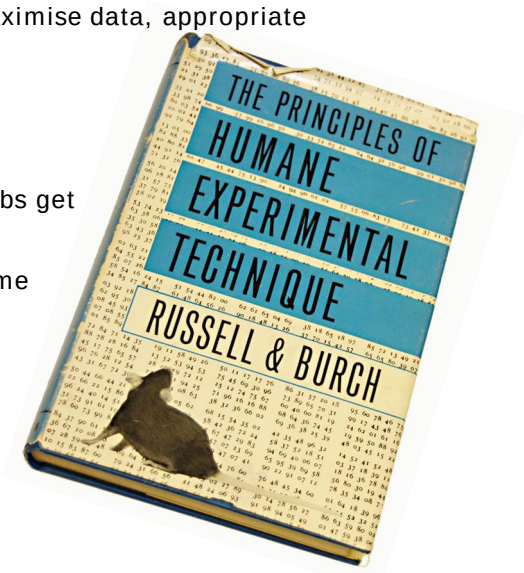
- Full visual inspection of mice at appropriate time points

- 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

Genotype (G) + Environment (E)

- Genotyping assay
- Background strain
- Genetic "mix"

- Caging
- Enrichment
- Age and sex

Phenotype (P)

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

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Genetic Background: PVR mice

Tg21 – BALB/c

Tg66 – C57BL/6J

Tg66 – CBA/CaJ

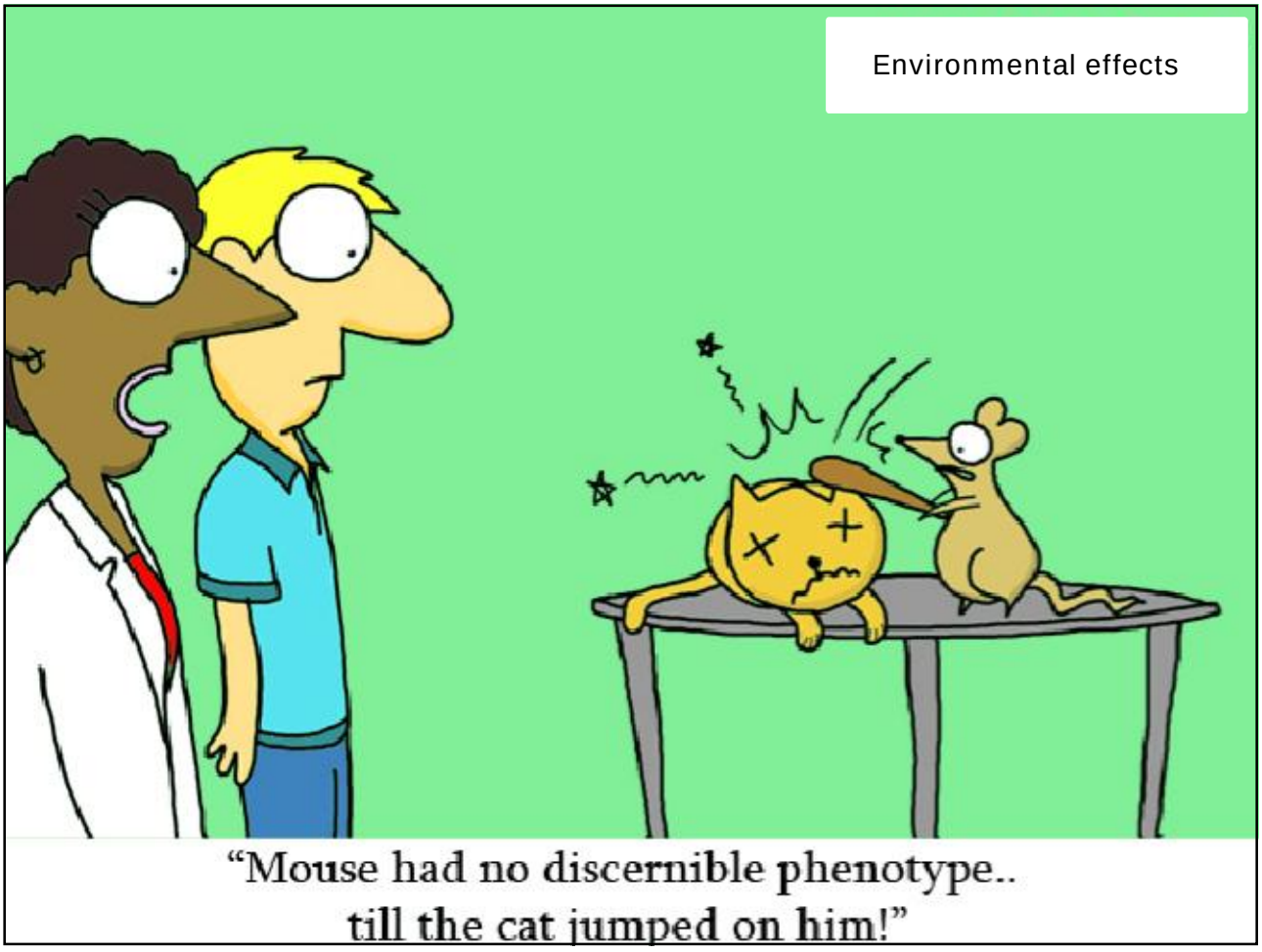
Same transgene, same site of insertion, same copy number

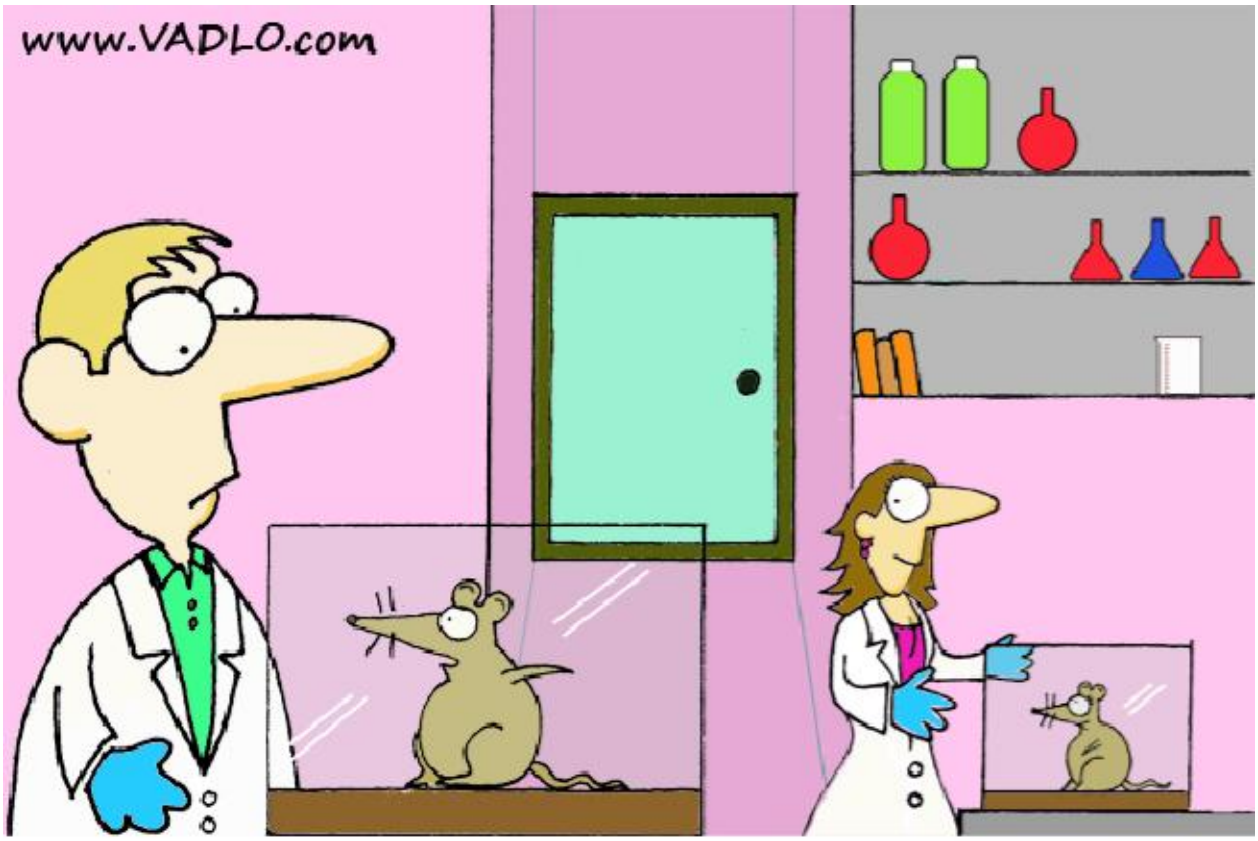
Different susceptibility to infection with poliovirus. Different routes of inoculation cause different phenotypes and disease/paralysis progression.

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!"



"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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The Mice