MRC | Harwell

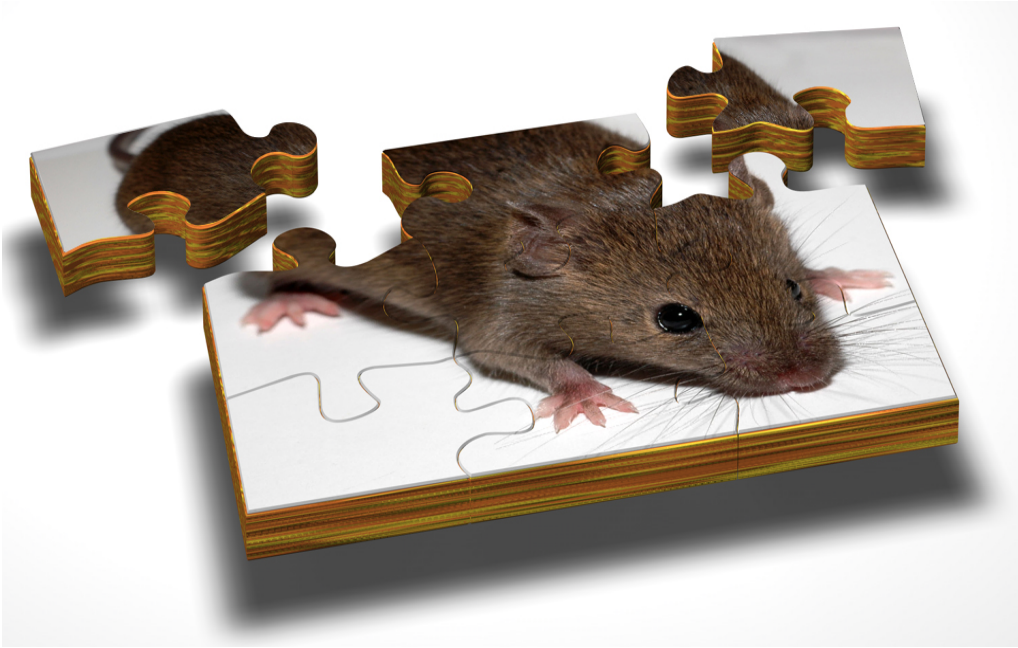
Mary Lyon Centre

Genetically engineered animals and their use in understanding disease

Lydia Teboul

Head of Transgenics

The Mary Lyon Centre



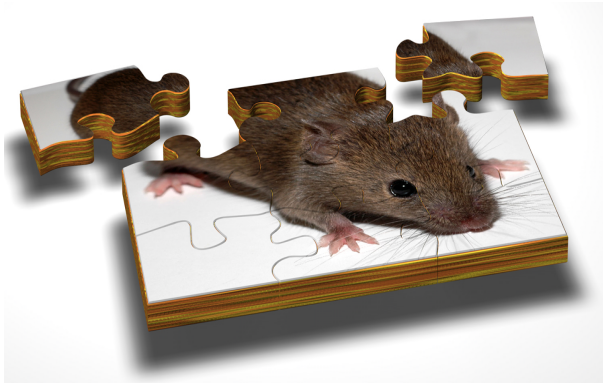
Genetically engineered animals and their use in understanding disease

MRC Harwell
Mary Lyon Centre

Targeting the genome
- Genome sequence + Gene targeting technology
- Publicly available allele collections
- Quality control of the EUCOMM library

Large scale phenotyping
- EUMODIC: Large scale phenotyping proof of principle
- IMPC

Aging screen
- ENU mutagenesis
- Screens



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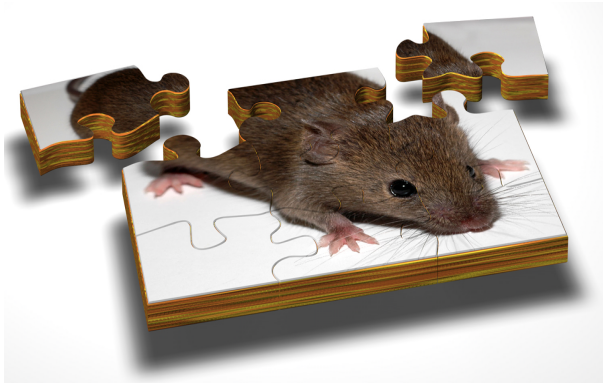

The mouse genome

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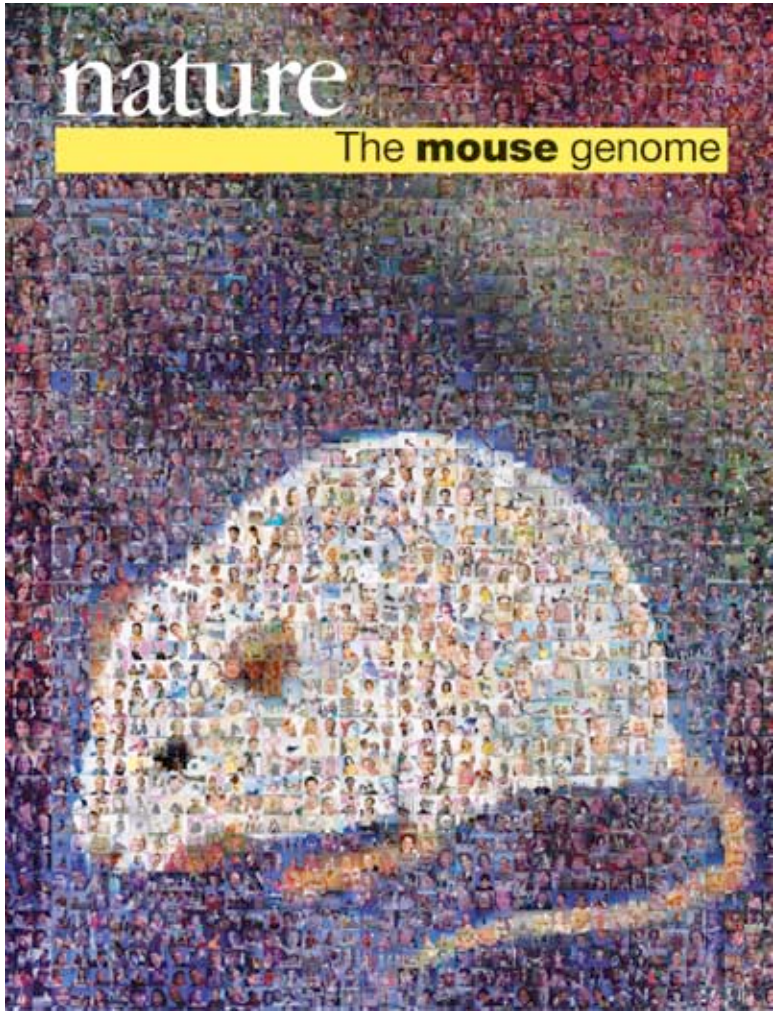
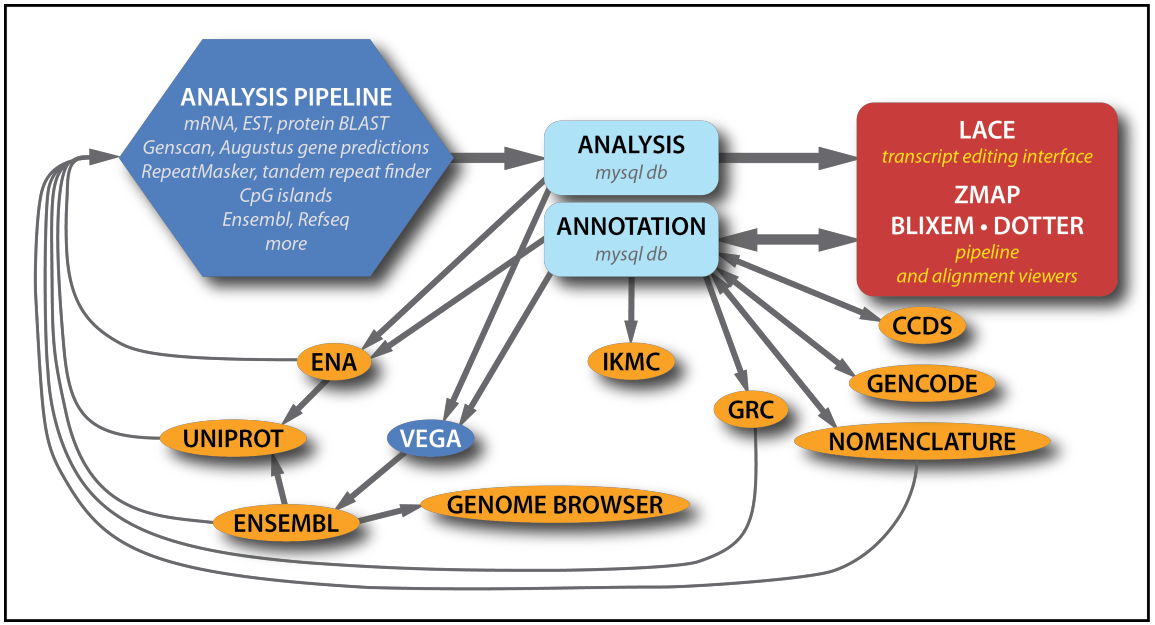


Sequence data



VEGA annotation by HAVANA group (WTSI)
Manual functional annotation of the genome: where are the protein coding genes?

2007 Nobel prize in Physiology and Medicine

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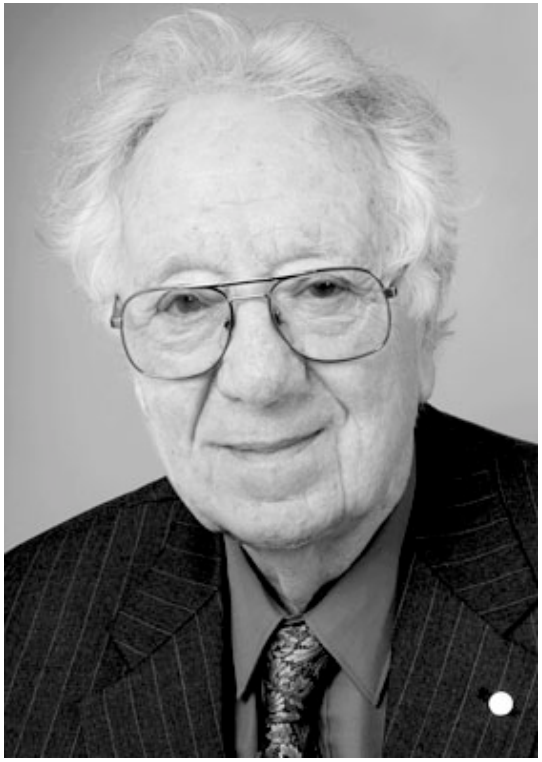

Oliver Smithies

Martin J. Evans

Mario R. Capecchi

"principles for introducing specific gene modifications in mice by the use of embryonic stem cells"

Principles of gene targeting

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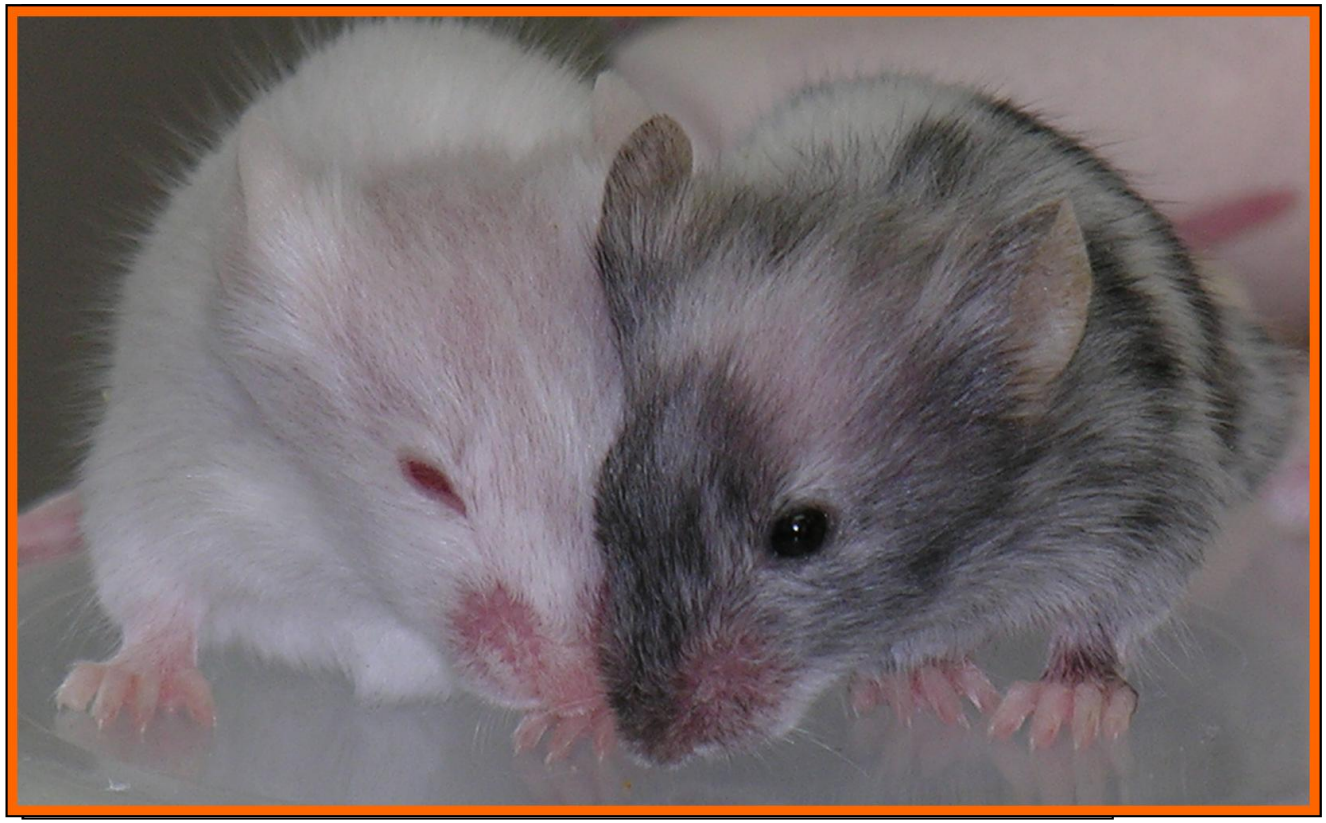
Mary Lyon Centre

Principles of gene targeting

MRC Harwell
Mary Lyon Centre



Principles of gene targeting

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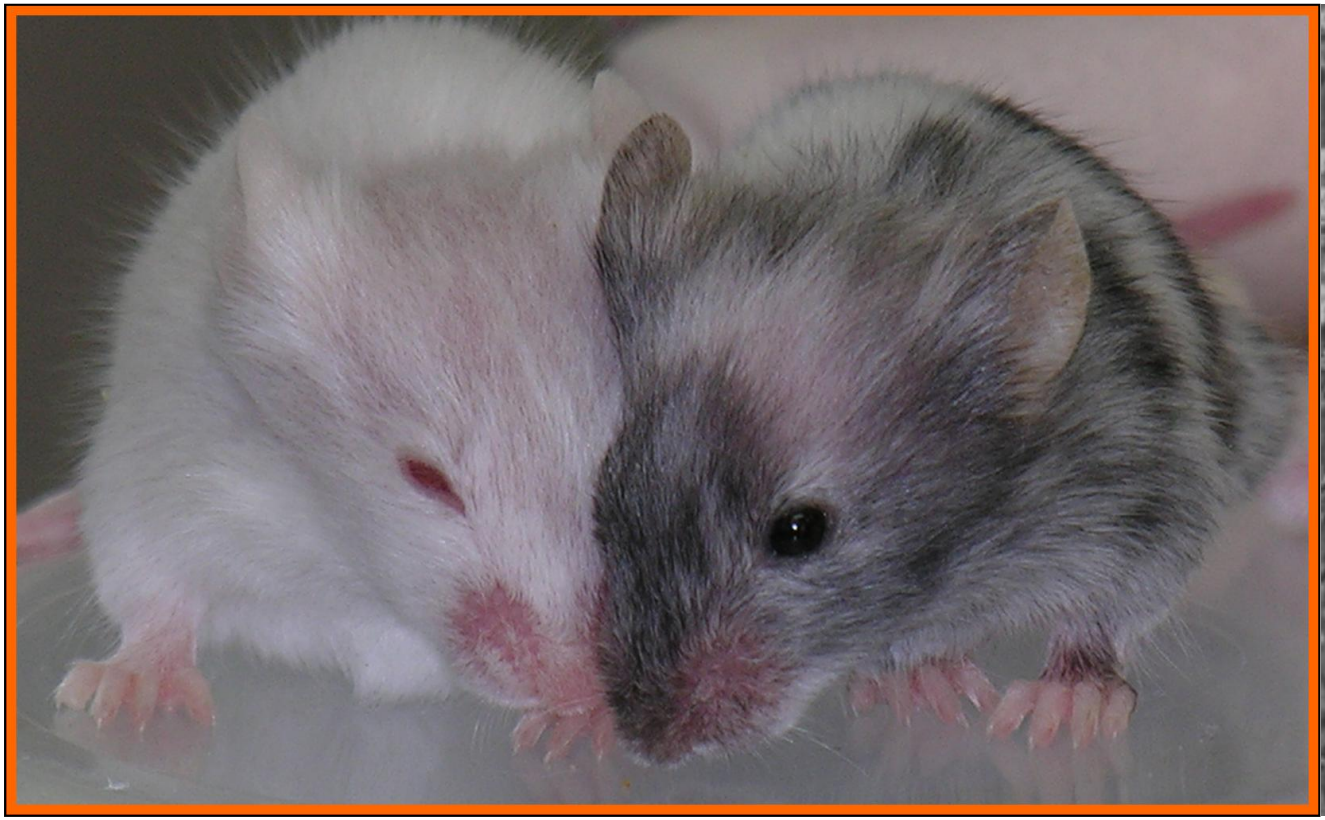
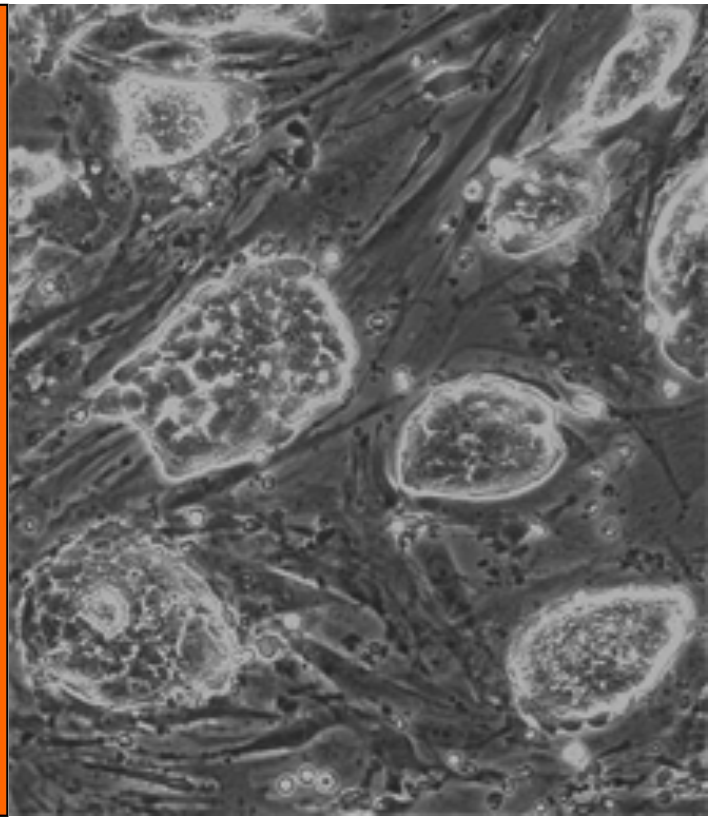
Mary Lyon Centre





Principles of gene targeting

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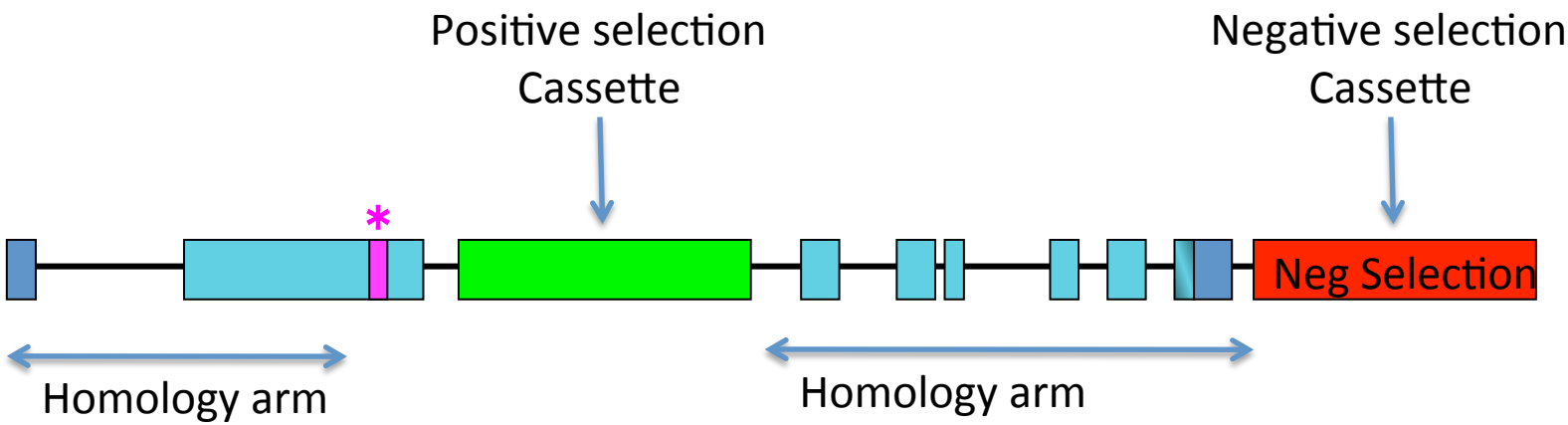
- Homologous recombination in ES cells
- Blastocyst injection
- Germ line transmission

Principles of gene targeting

Mary Lyon Centre

Targeting construct



Principles of gene targeting

MRC Harwell
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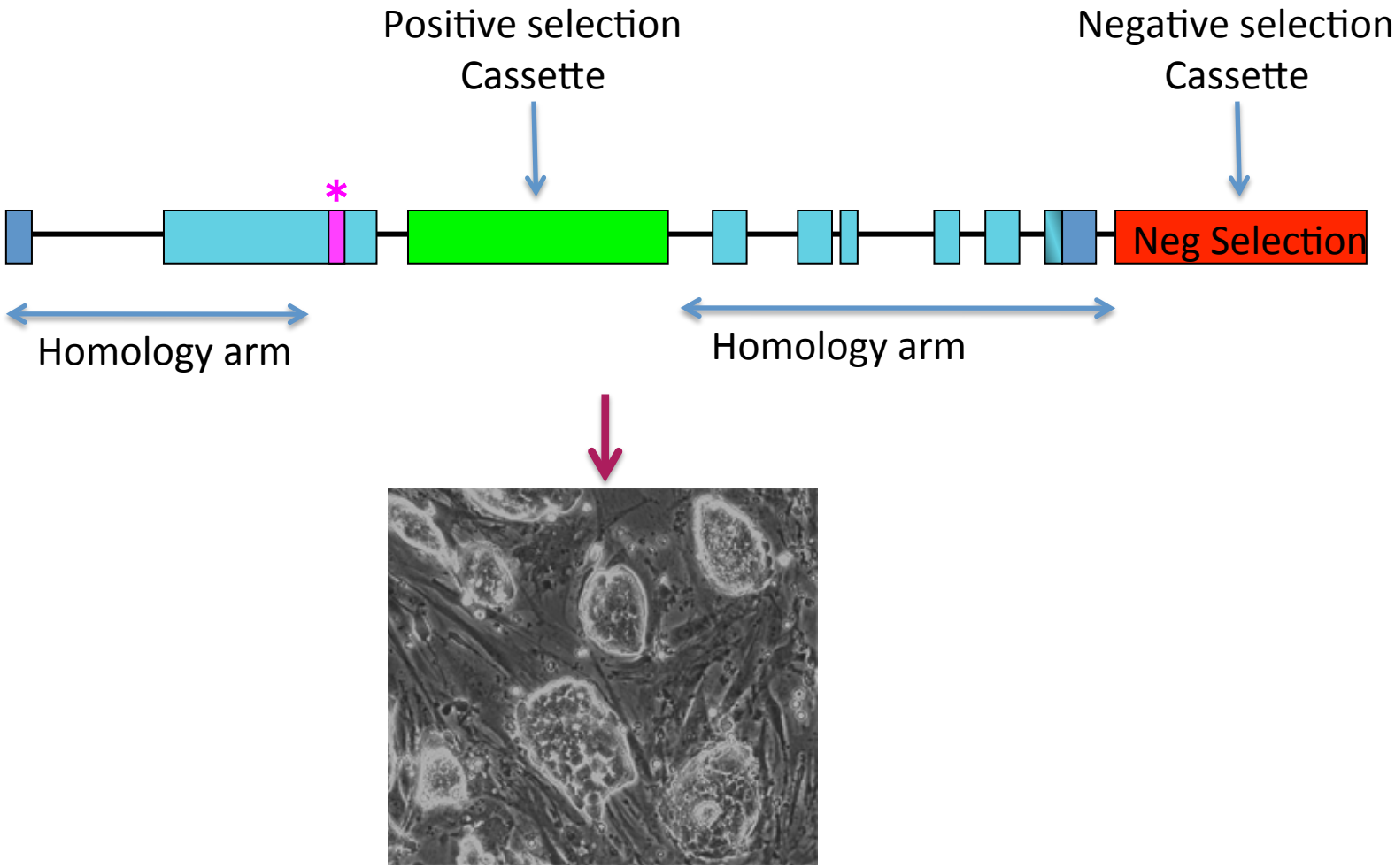
Targeting construct



Principles of gene targeting

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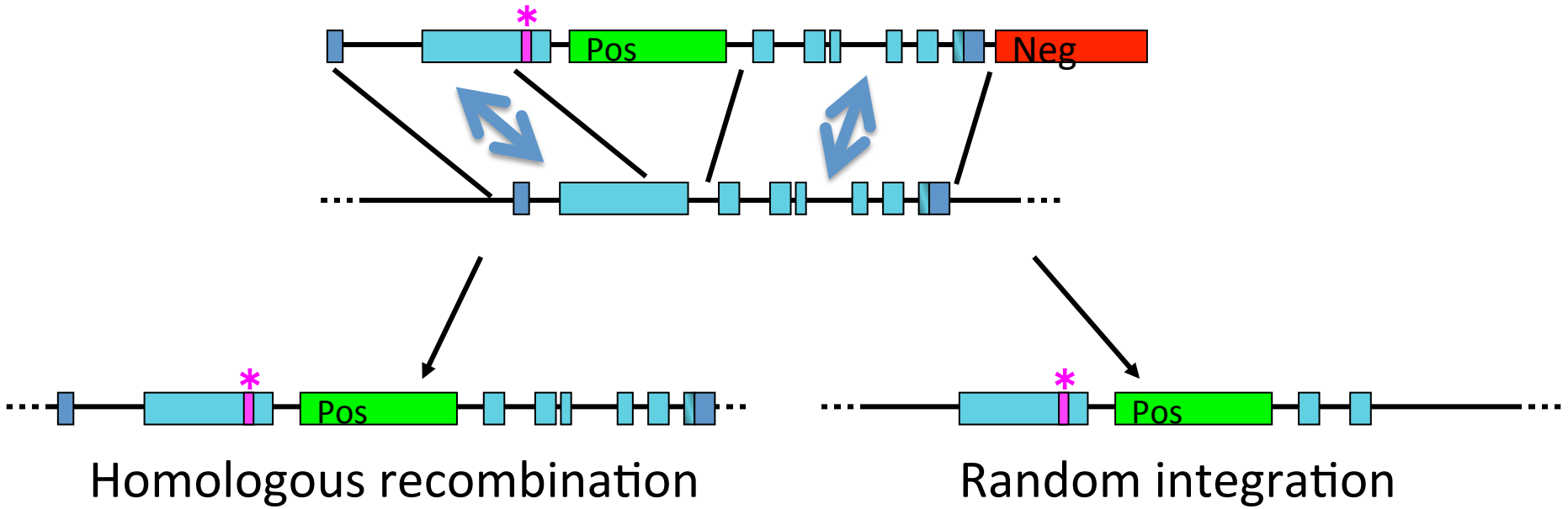

ES cells screen: finding homologous integration event

- Southern blotting
- Long Range PCR
- Loss of allele copy counting

Principles of gene targeting

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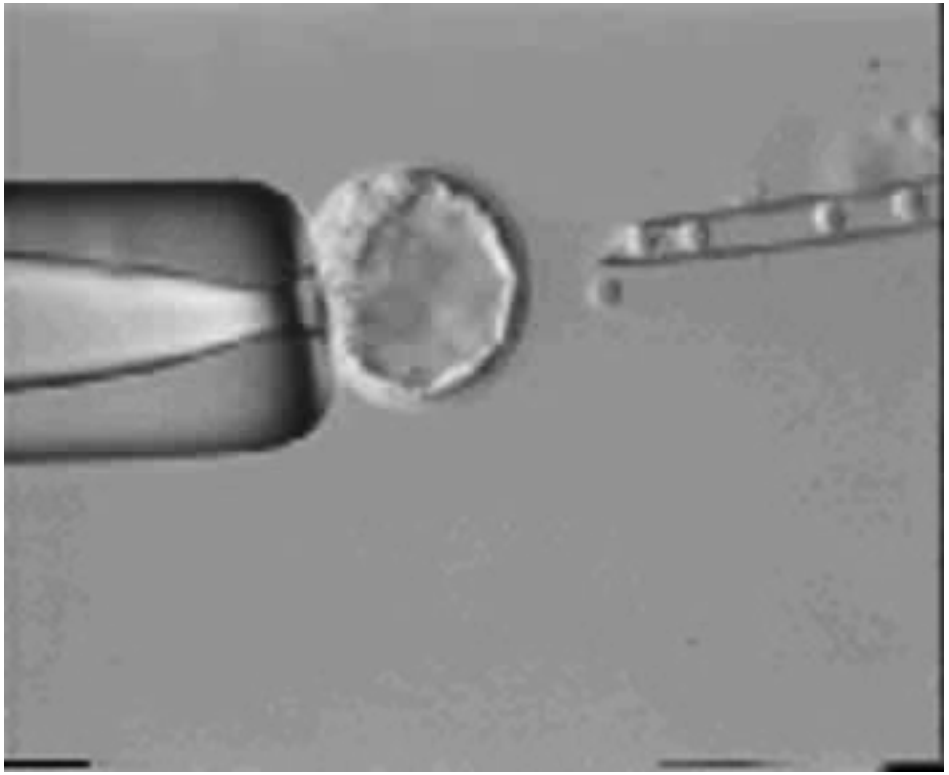
Blastocyst injection



Principles of gene targeting

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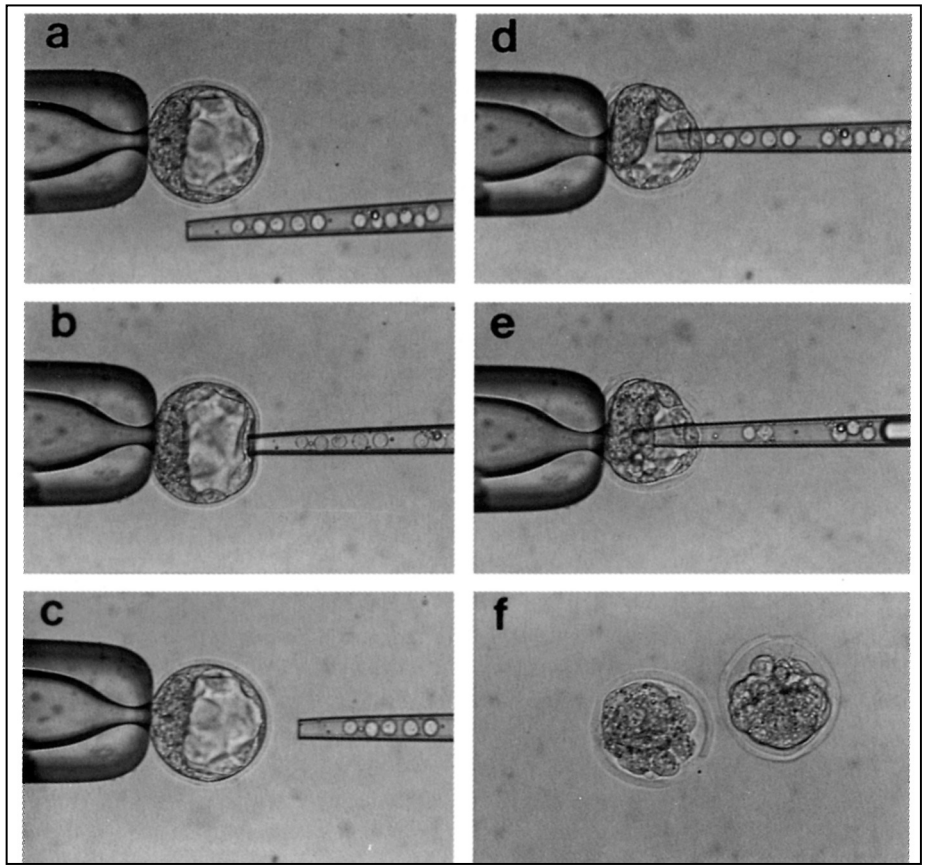
Blastocyst injection



Principles of gene targeting

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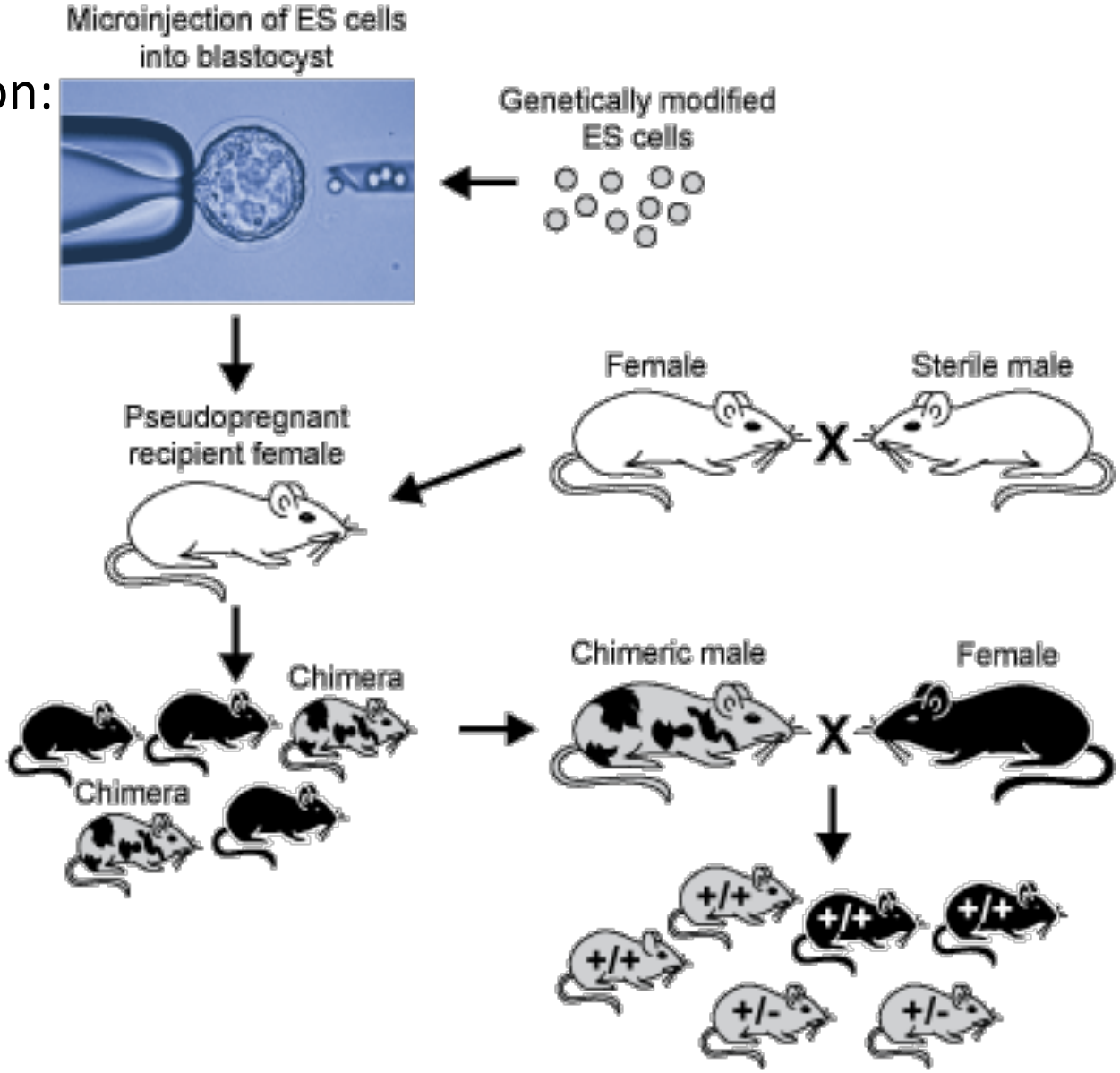
Germ Line Transmission:



Targeting the genome

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Bill Skarnes

Francis Stewart

Wolfgang Wurst

-> EUCOMM and KOMP programmes

EUCOMM and KOMP Gene targeting: KO first

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FLPe: allele is expressed normally, selection cassette deleted

Cre: frameshift allele is expressed

loxP

FRT

Gene targeting collections: IKMC

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IKMC: International Knockout Consortium : **Libraries of targeted ES cells**

- Targeted deletions: Velocigene
- Conditional targeting: EUCOMM, KOMP, NorCOMM

Number of protein coding genes with mutant ES cell lines in the IKMC resource:

Total Genes	KOMP CSD	KOMP Regeneron	EUCOMM	NorCOMM
Vectors available	6590	4733	8837	839
ES cells available	5252	3959	7165	569
Mutant mice available	450	382	687	4

Figures on 2nd May 2012

Quality control of the EUCOMM library

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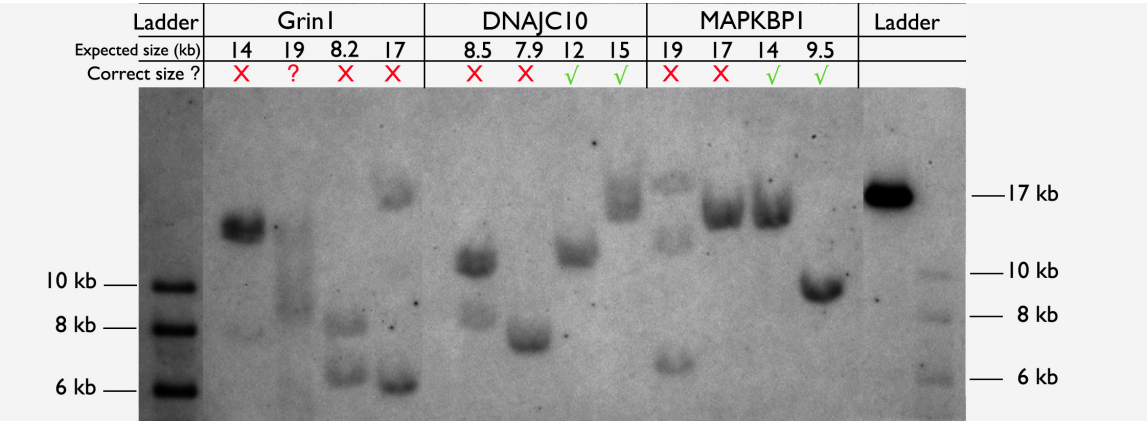
ES to mouse conversion:

- 568 conditional mouse lines (536 genes) produced for distribution through EMMA

- Germ Line Transmission rate (generally 1 injection session/clone): about 50%

Allele integrity:

- Early data suggested 1/5 clone was incorrect
- Clone screening strategy revised, extra check are now performed before cell distribution
- Advise to users/mouse producers: Check clones by Southern blot before injection



Distribution of targeting vectors, ES cells and mouse mutants

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2 gene targeting constructs and ES cell distribution centre:

EuMMCR: in Munich, distributes European collections

MMRRC: in Davies, CA, distributes US collections

European Mouse Mutant Archive:

Node In Harwell

- Archiving of GA mice lines
- ~1500 mouse lines archived
- Rederivation
- Assisted reproduction

Genetically engineered animals and their use in understanding disease

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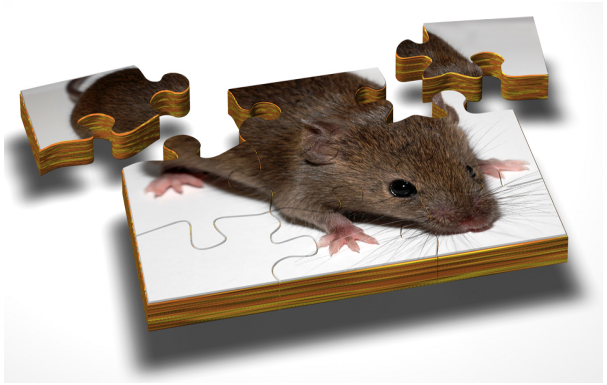

European Mouse Disease Clinic
Multi-system phenotyping

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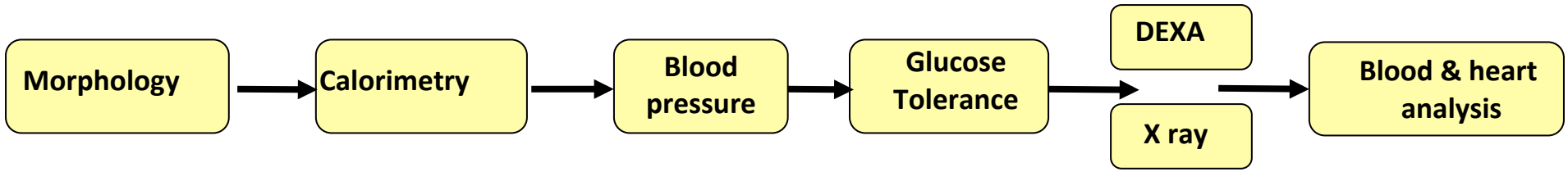
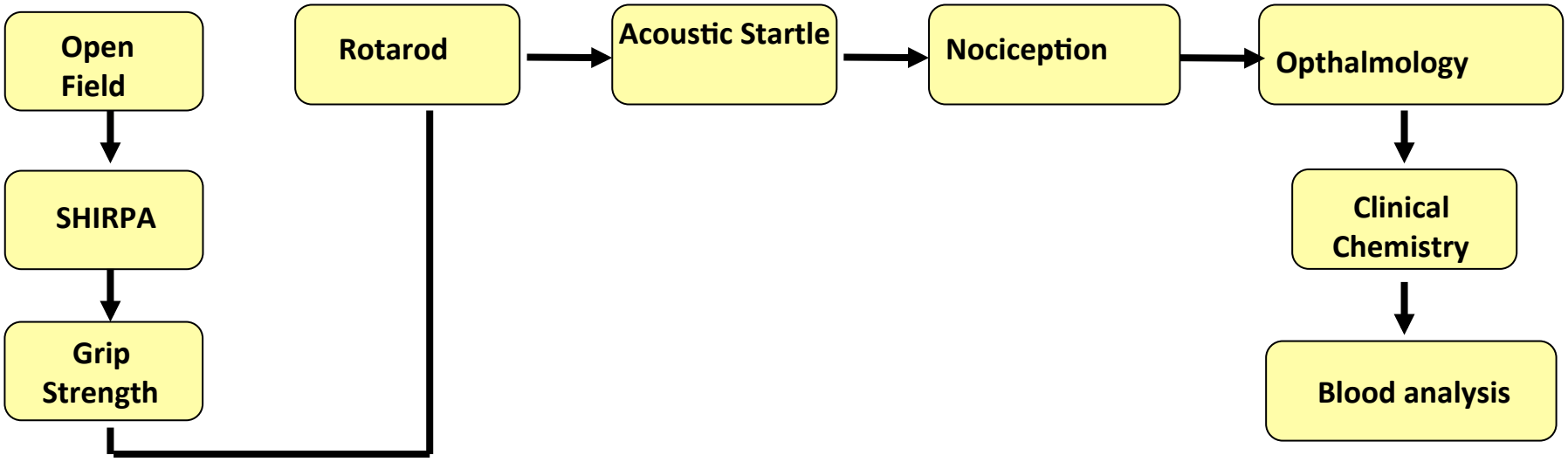
Pipeline 1- Metabolic Profiling

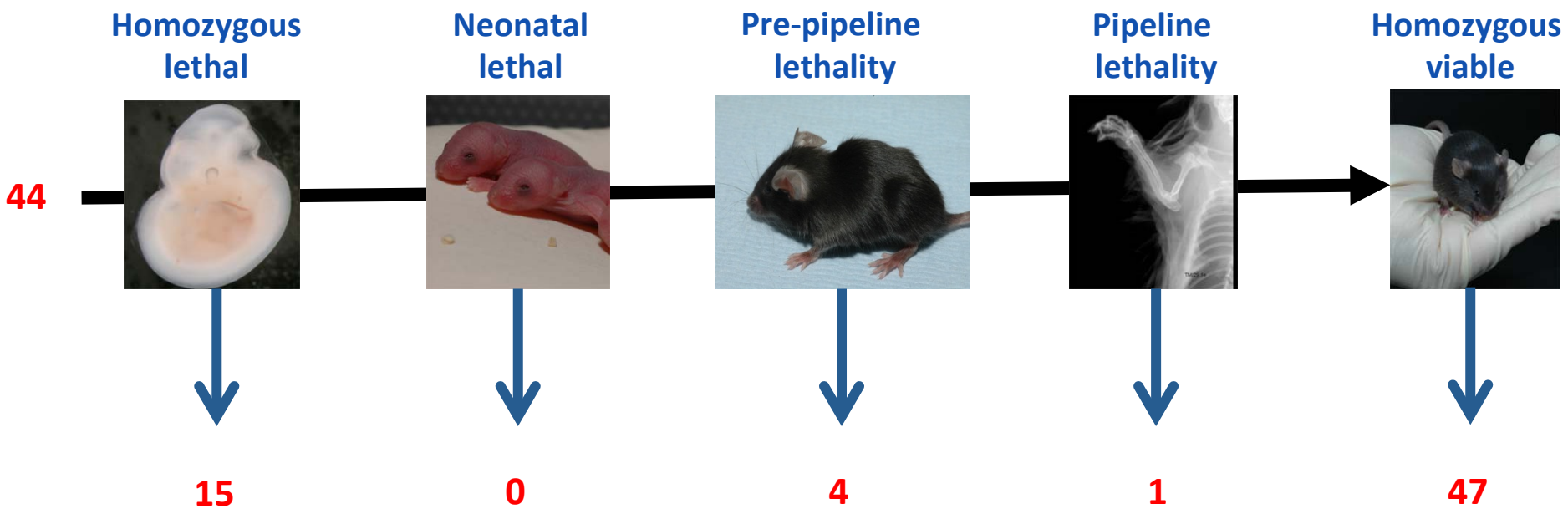


Pipeline 2- Neurosensory Profiling



Viability

Mary Lyon Centre



Viability data on first 67 lines

Homozygous Lethal

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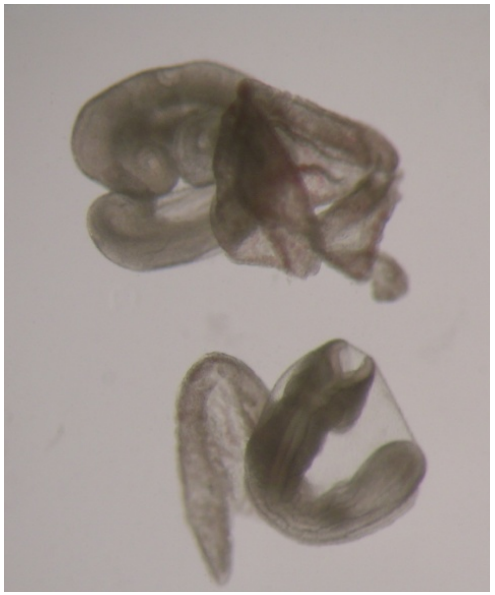
Homozygous lethal

15

Secisbp2
Selenocysteine insertion sequence–binding protein 2 (dies before dpc9.5)



Lack of neural tube closure and turning defect

Pre-pipeline Lethality

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Pre-pipeline lethality

2

Bag3
Is involved in anti-stress and anti-apoptotic pathways.

Back limb paralysis (d22-30)

Npc1
Niemann Pick type C1
.

Tremors, uncoordinated gait (d19-28)

Pipeline Lethality

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Mary Lyon Centre

Pipeline lethality

1

Cisd2

Previously implicated in human longevity, mouse knockouts of this gene show premature-ageing phenotypes.

Piloerect coat
Hunched
Rapid Breathing
Tremors
Abnormal gait

Onset of disease
(average 132 days, earliest 96 days)

Disease Progression
(2-32 days after onset)

No histological abnormalities

Phenotypes

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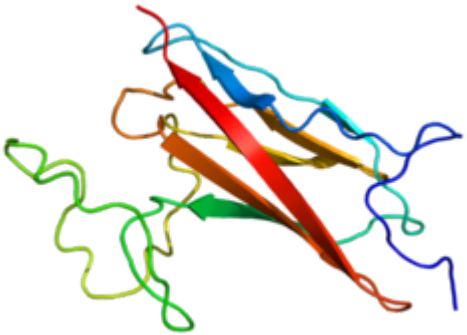
The message is:

That 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
And each year we discover a few more
Of those unknown unknowns.

—Feb. 12, 2002, Department of Defense news briefing

- **Known Knowns-** Confirming phenotypes in existing mutants
- **Unknown Knowns-** New phenotypes in existing mutants
- **Known Unknowns-** Confirming phenotypes seen in other mutations of the same gene
- **Unknown Unknowns-** New phenotypes in new mutants

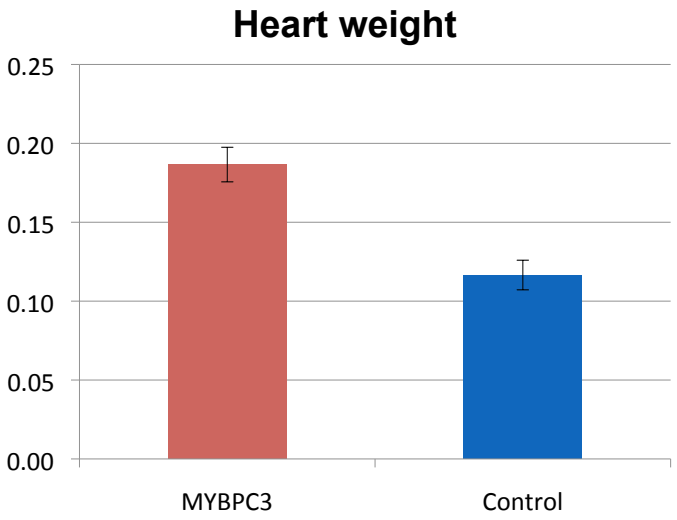
EUCOMM Lines

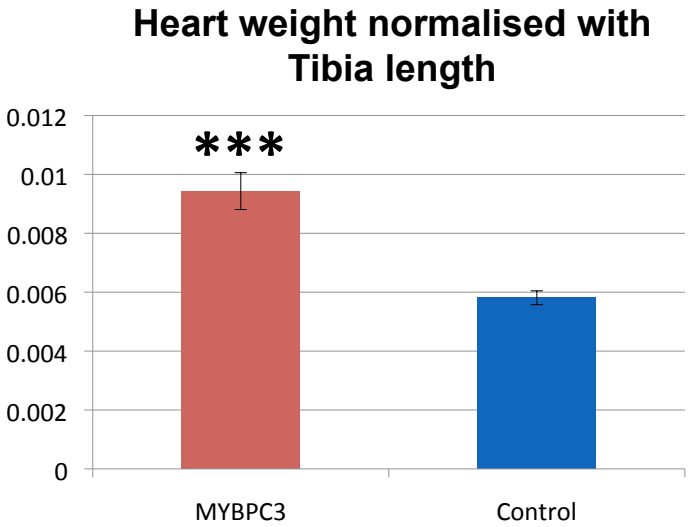


MYBPC3

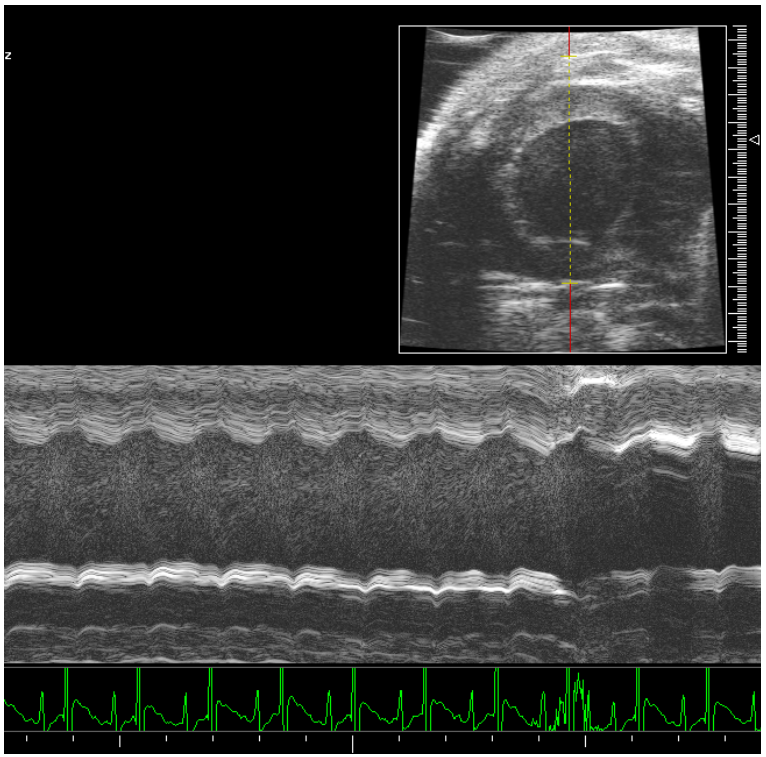
Myosin binding protein C.

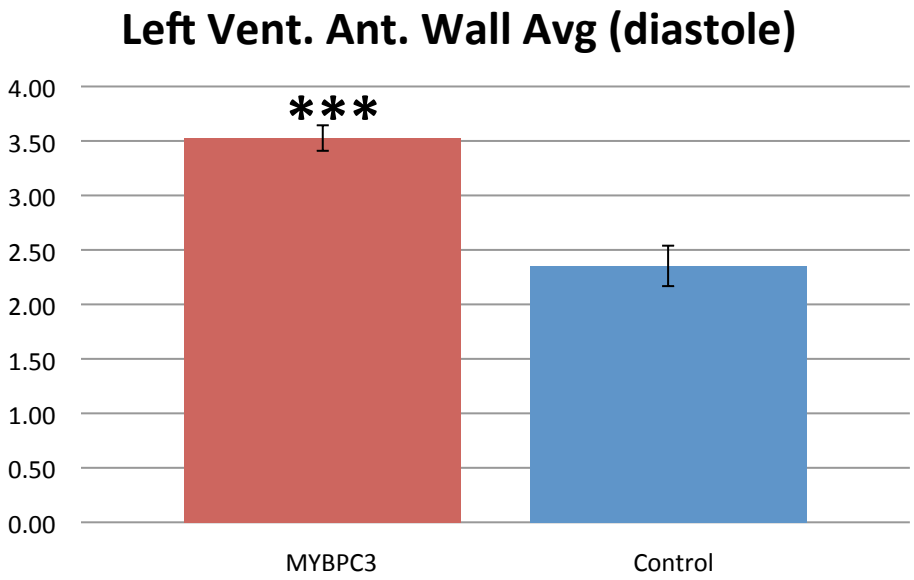
Hypertrophic cardiomyopathy





MYBPC3

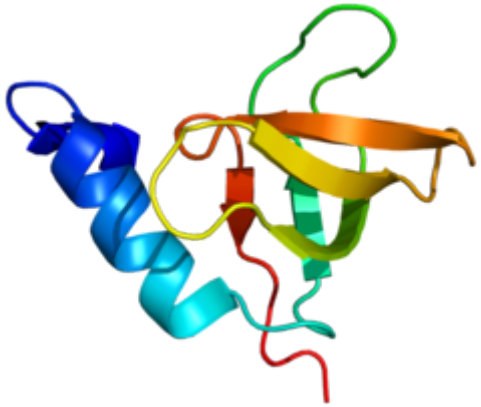




Left Ventricle Hypertrophy

Echocardiogramme

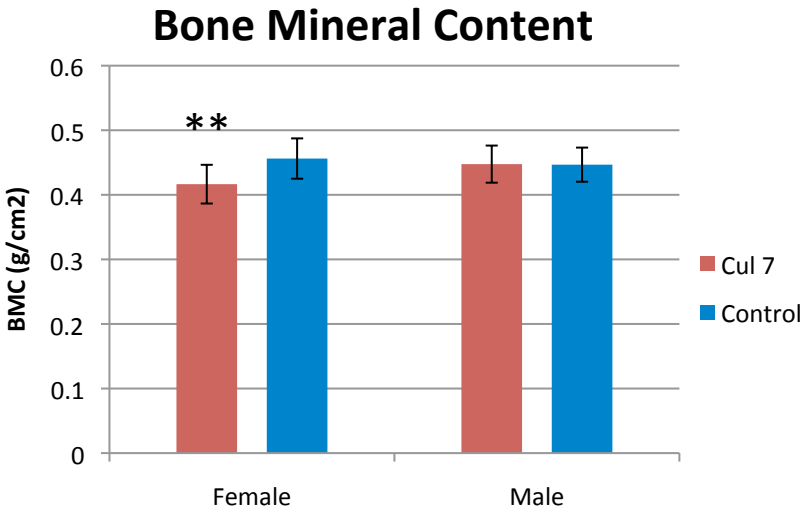
Cul-7

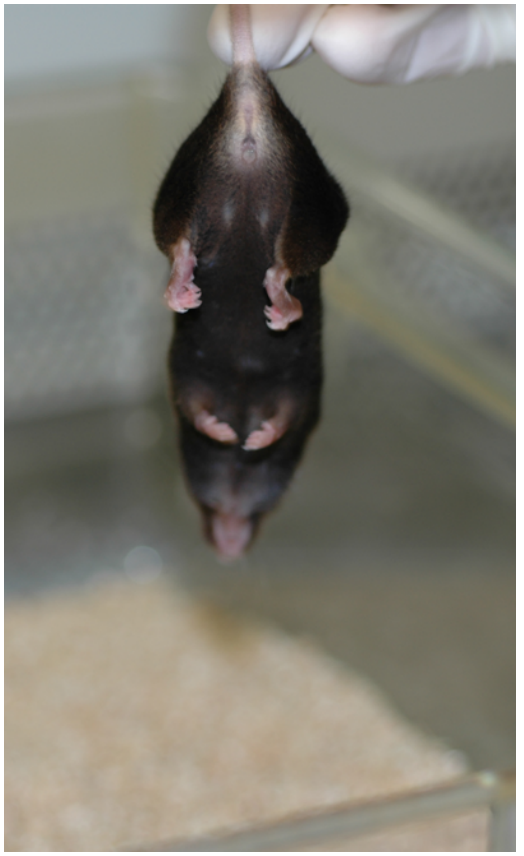


Cul-7

Component of an E3 ubiquitin-protein ligase complex.

Defects have been associated with 3M syndrome type 1.





Elmod-1

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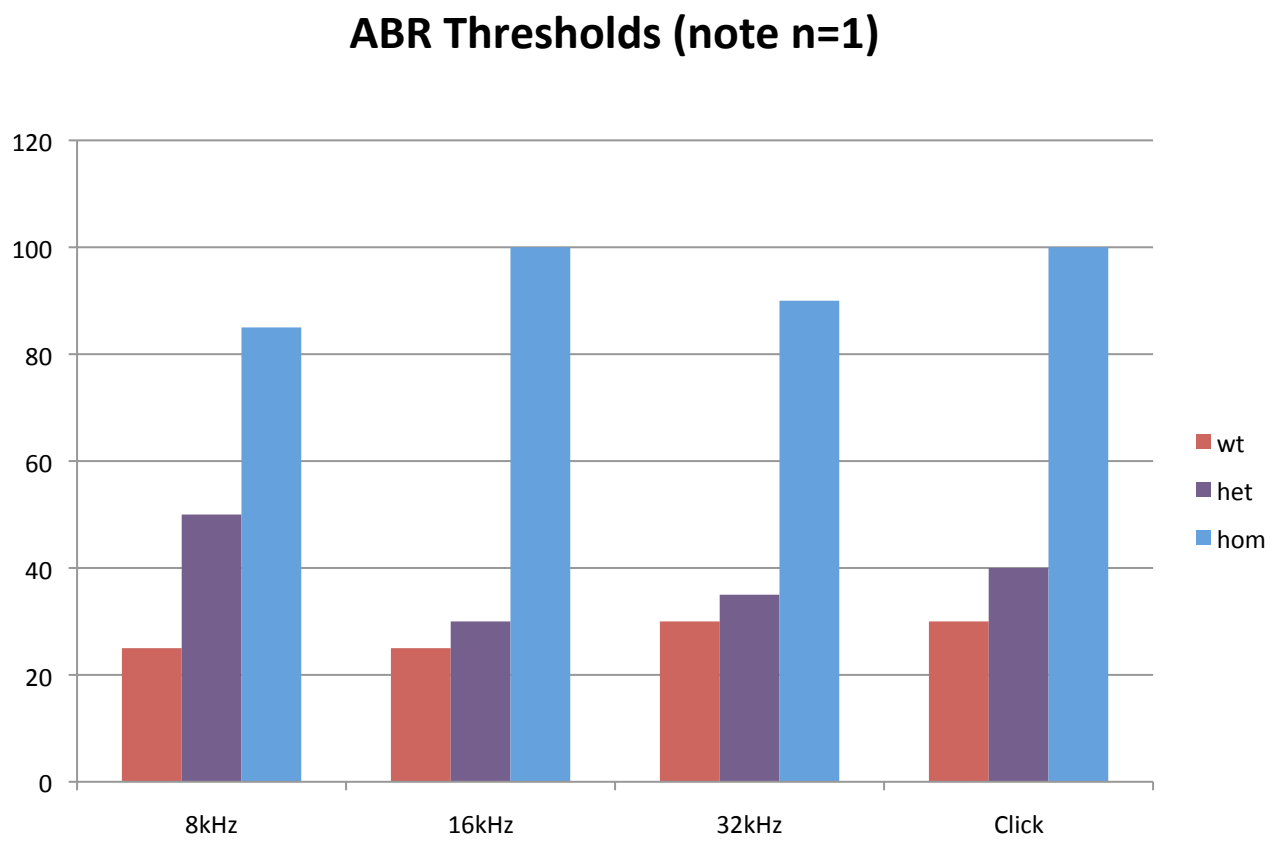

Data presented in EuroPhenome

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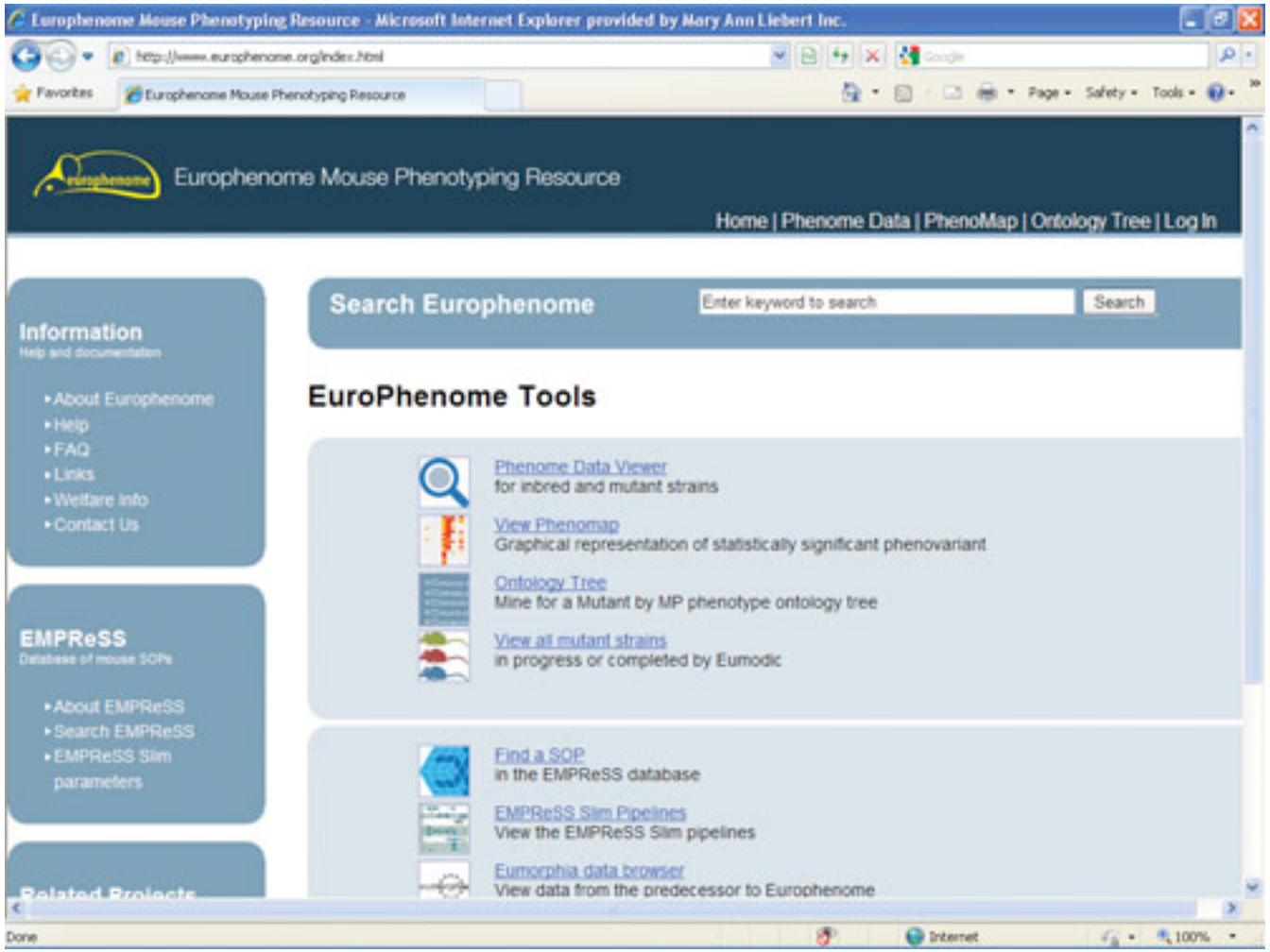
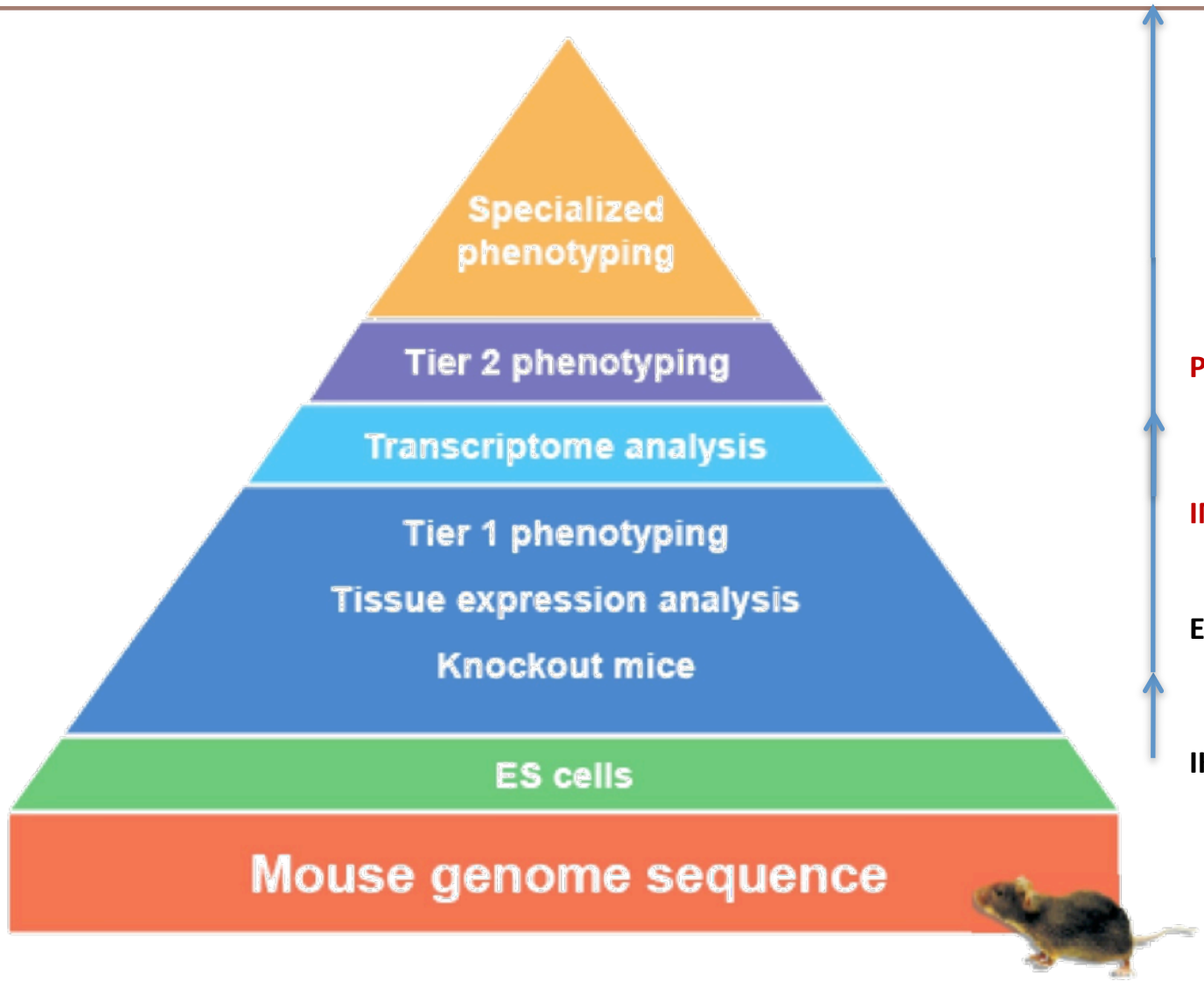
Mary Lyon Centre



IMPC: International Mouse Phenotyping Consortium

MRC Harwell
Mary Lyon Centre



Vision For Next 10 Years...

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- An Encyclopaedia of Mammalian Gene Function
  - Create KO mice for ALL GENES
  - **Database with associated primary phenotype info**
  - Discover unforeseen gene function
  - **Free access to MICE**
  - Free thousands of researchers from tool generation;
  - A rich seam for future hypothesis driven research, with the potential for breakthrough discoveries

- A transformative project that will underpin the future of biomedical science and the biology of disease systems.

IMPC

International Mouse Phenotyping Consortium

MRC | Harwell

Mary Lyon Centre

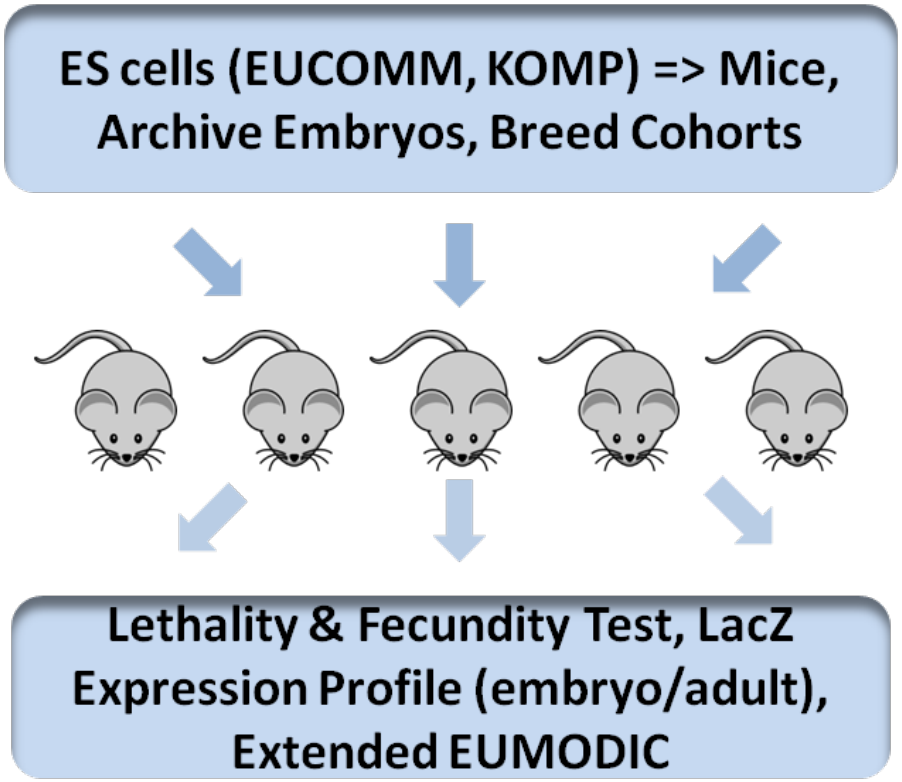
22 Partners, 13 Production Centres, 9 Countries

www.mousephenotype.org

MRC | Medical Research Council

Harwell part in IMPC

MRC Harwell
Mary Lyon Centre



- 100 Lines per year (funding secured)
- Node for mouse distribution

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Mary Lyon Centre

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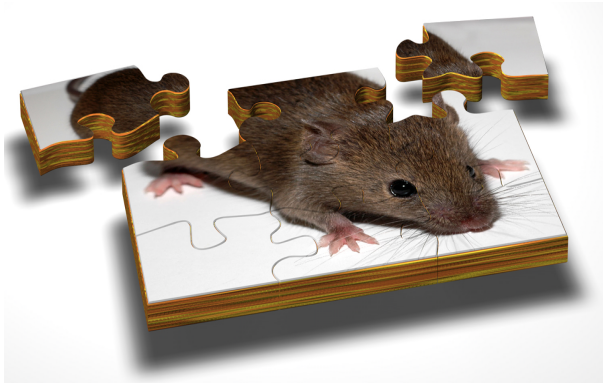

Chemical mutagenesis

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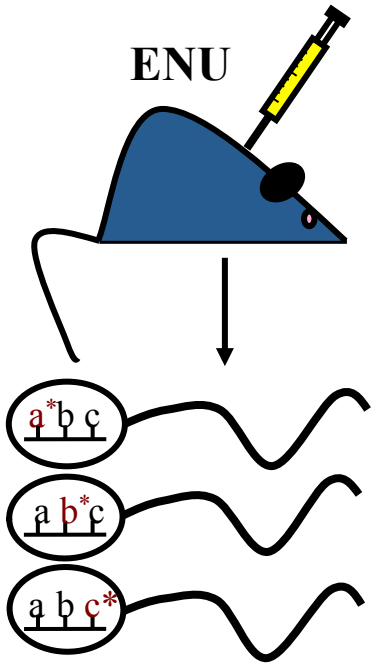
Large scale ENU mutagenesis programmes:



Gene driven screens: ENU DNA/Sperm archive

- >10000 DNA and sperm samples, allowing the identification of point mutations for all coding and non-coding sequences in mouse genome

Phenotype driven screens

- generating mouse models screening for a phenotype of interest

Building on Success

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Mary Lyon Centre

Recessive Screens	Pedigrees screened	Mice screened	Pedigrees containing Phenodeviants	Confirmed phenotypes
ADULT				
Dysmorphology	223	7520	39	25
Imprinting/Fertility	26	648	7	7
Circadian Rhythm	67	1296	24	12
Memory/Learning	45	967	13	3
Deafness	154	4485	26	8
Bone and Mineral Disorders	35	1163	4	4
Innate Immunity	146	954	23	4
Vision	137	3965	12	7
Brain Histology	19	331	1	1
Totals	**395**	**9122**	**150 (39% of pedigrees)**	**72**
DEVELOPMENT				
Development	187	1287	79	8
L-R development	135	615	39	13
Cardiac Development	76	2626	44	22
Totals	**398**	**2626**	**162 (41% of pedigrees)**	**43**

Phenotype Driven Screens

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Mary Lyon Centre

- **Makes no assumptions about genetic basis of disease**
  - Potential to identify novel genes or pathways involved in disease
  - Bias screens to search models of human disease
  - Employ challenges or genetic modifications for a sensitised screen
  - Screens need to be large (1000s of mice)

- **Point mutations**
  - Easier to map than QTLs
  - Hyper-, hypo- and neomorphic mutations

- ***Generate novel models of human disease***

Late Onset/Age-Related Disease

MRC Harwell
Mary Lyon Centre

- **Concept**
  - Let the mice grow old: look at apparition of phenotype
  - Would need to be a consortium of researchers
  - Makes use of existing expertise and technologies
  - Harwell well positioned to carry out such a screen
- **Importance**
  - Ageing society - Increasing pressure on society from age related diseases
  - Some models are acute/early onset which does not truly reflect human disease
  - Focus of funding bodies

Screening Pipeline

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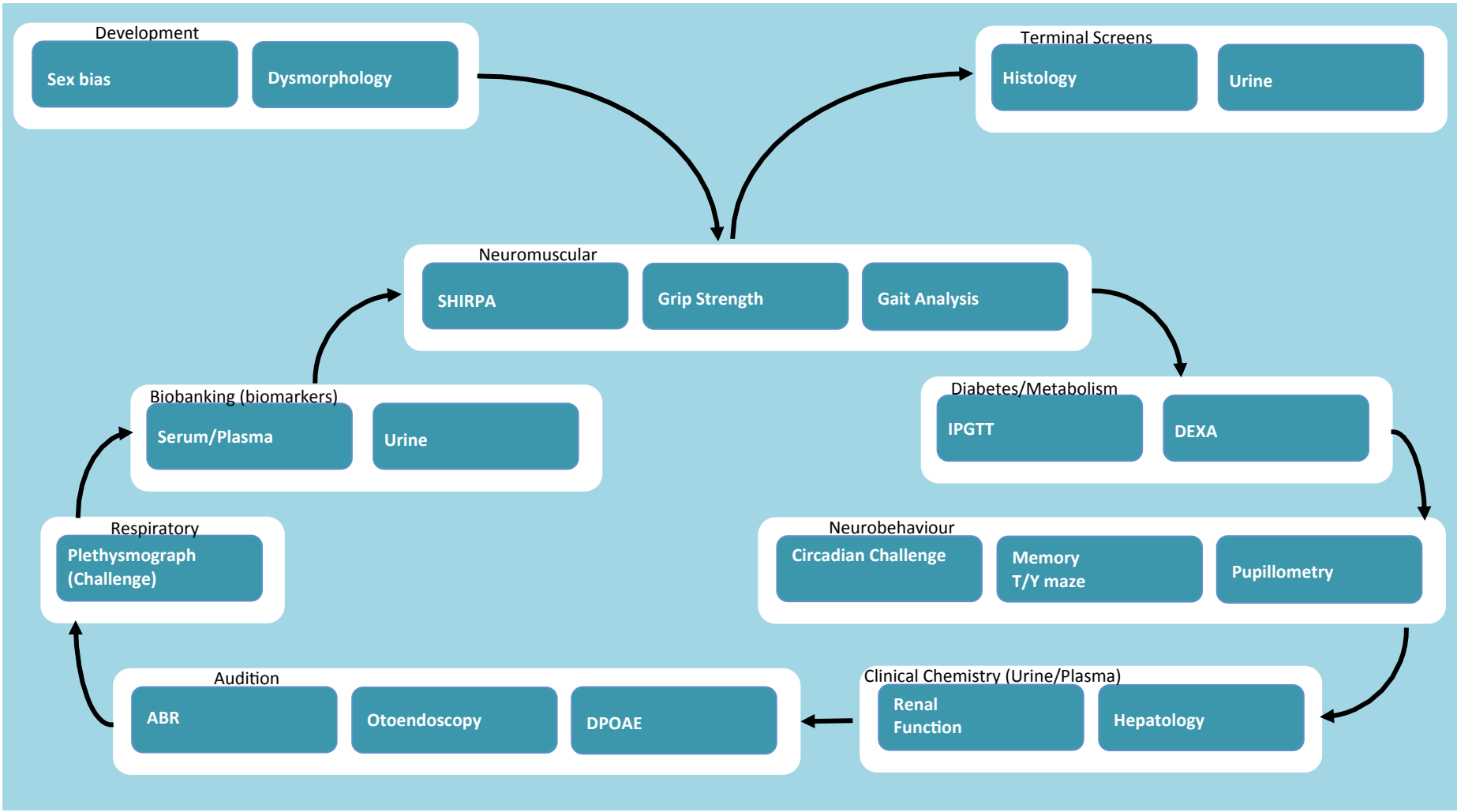
Mary Lyon Centre



Ageing Mutants MP-86 Hypercholesterolaemia

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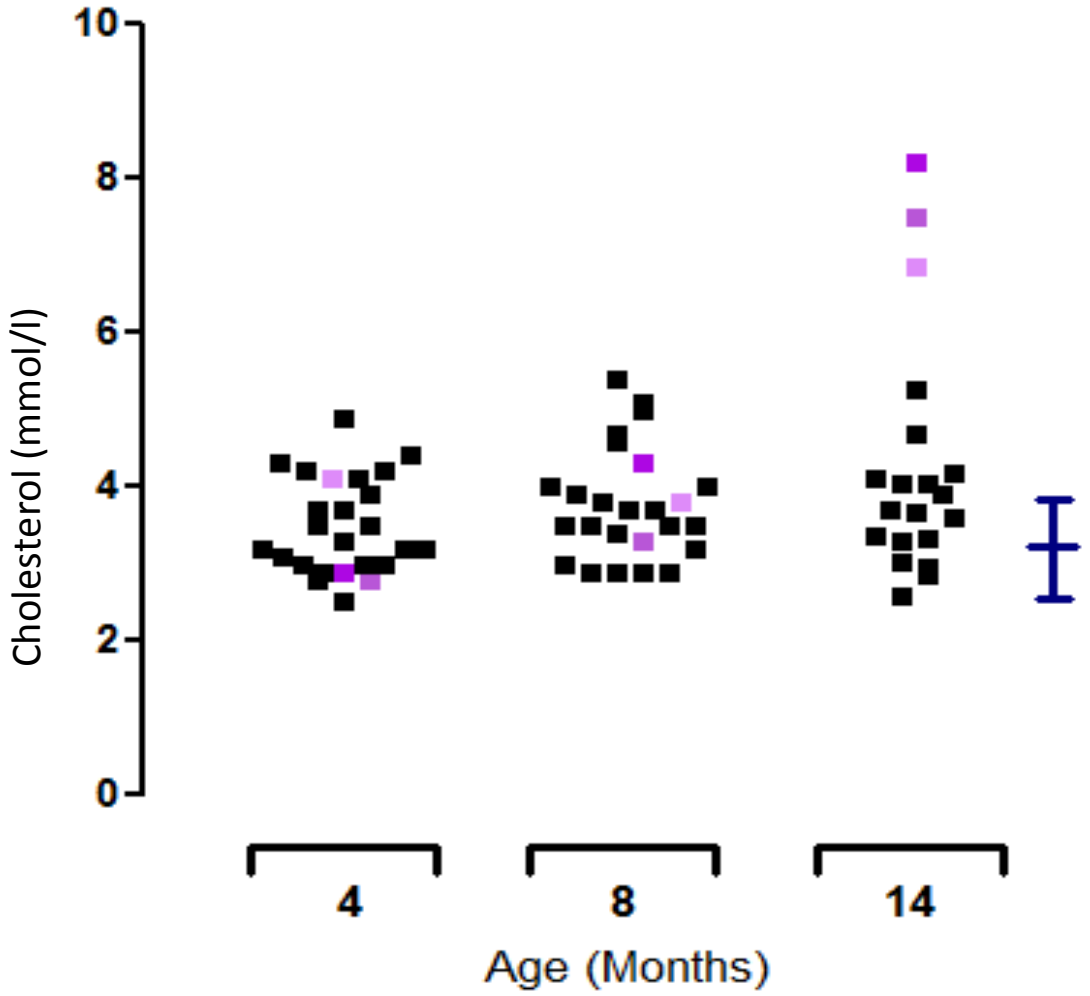
Mary Lyon Centre



Ageing Mutants MPC-46
Reduced Renal Function?

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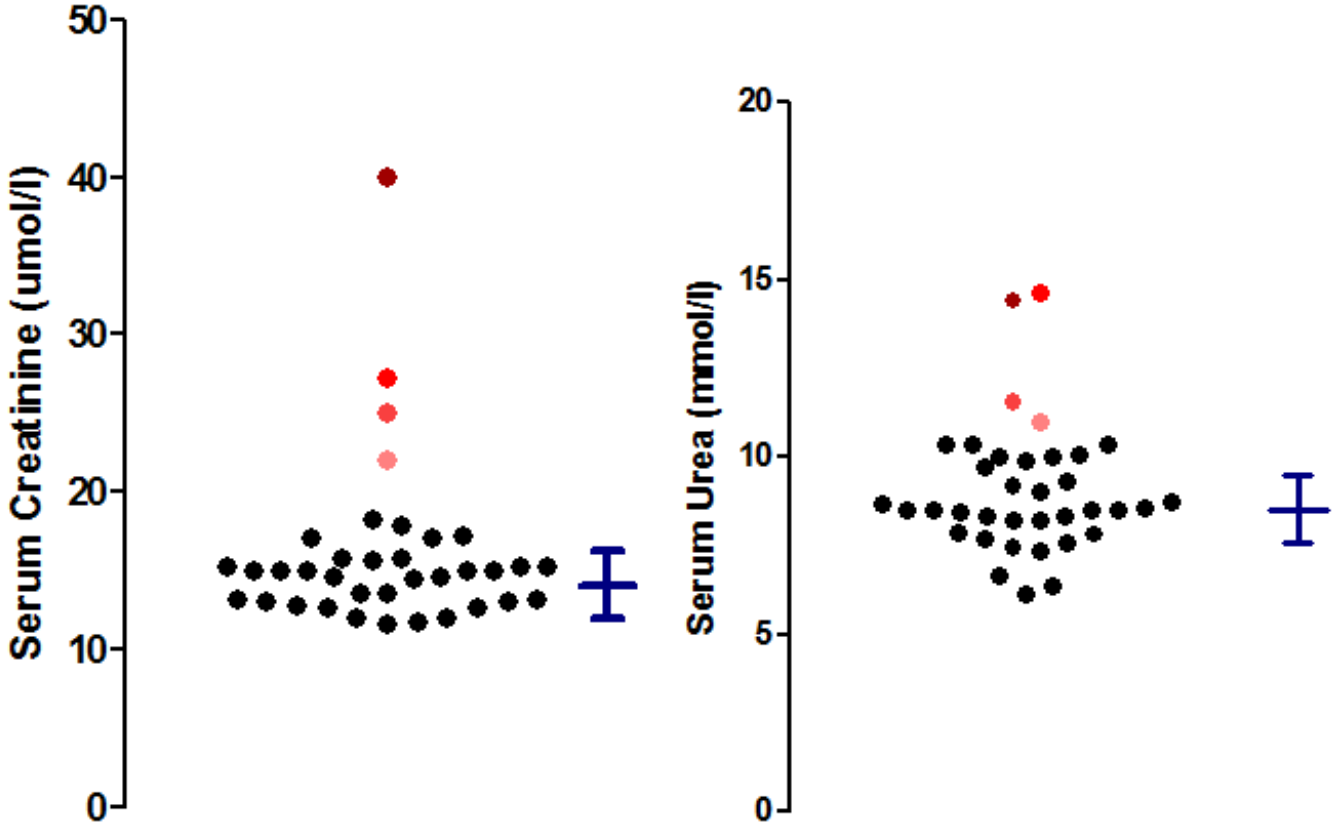

Increased serum urea and creatinine at 12 months

MPC-81 Lean mice

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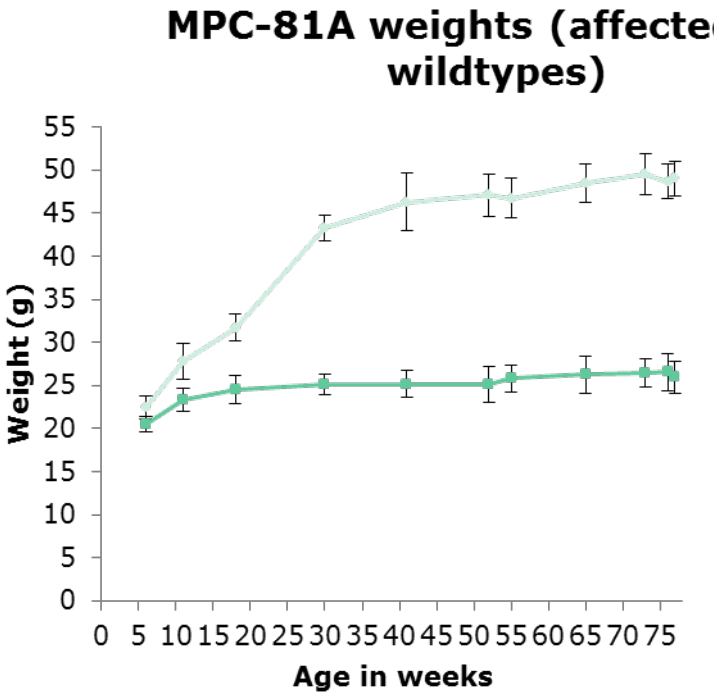

MPC-81 females 18 months old

MPC-81/2.2l

MPC-81/2.2d

Currently undergoing detailed metabolic phenotyping – activity, metabolism, food intake..

Mapping

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Mary Lyon Centre

- **Conventional Mapping**
  - Large pedigrees/multiple rounds of screening
    - Confidence in phenotype
    - Enough affected individuals for mapping
    - NGS to identify associated mutation

Acknowledgements

MRC Harwell
Mary Lyon Centre

Mary Lyon Centre, MRC Harwell

- Director: T Weaver
- FESA: M Fray
- Phenotyping: S Wells

Mammalian Genetics Unit, MRC Harwell

- Director: S Brown
- Aging programme: P Potter

EUCOMM (WP7) & EUMODIC consortia

- CNR (Monterotondo)
- Helmhotz Zentrum (Munich)
- ICS (Strasbourg)
- WTSI (Hinxton)

IMPC
International Mouse Phenotyping Consortium

EUMODIC Phenotyping

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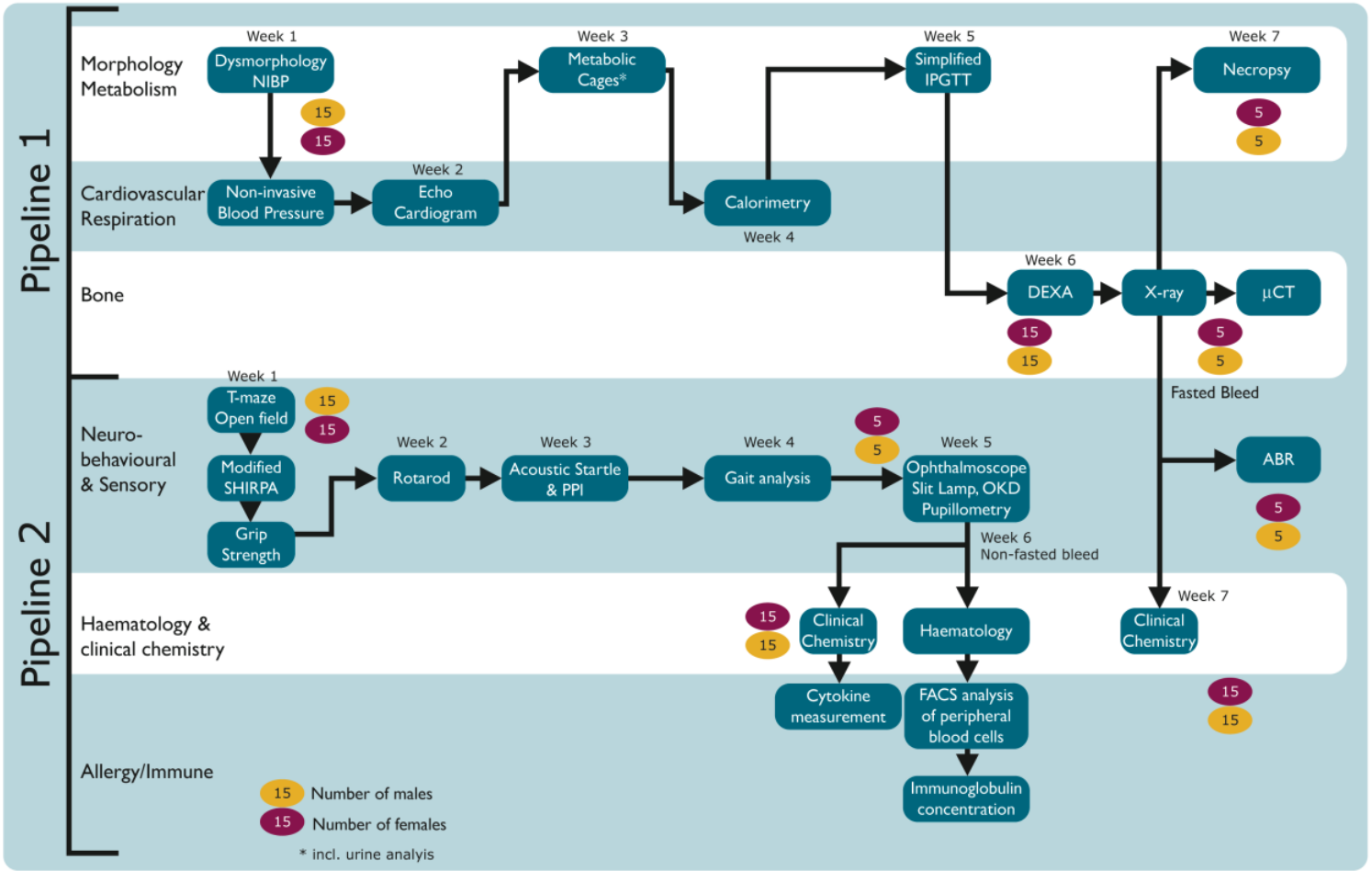

Principles of gene targeting

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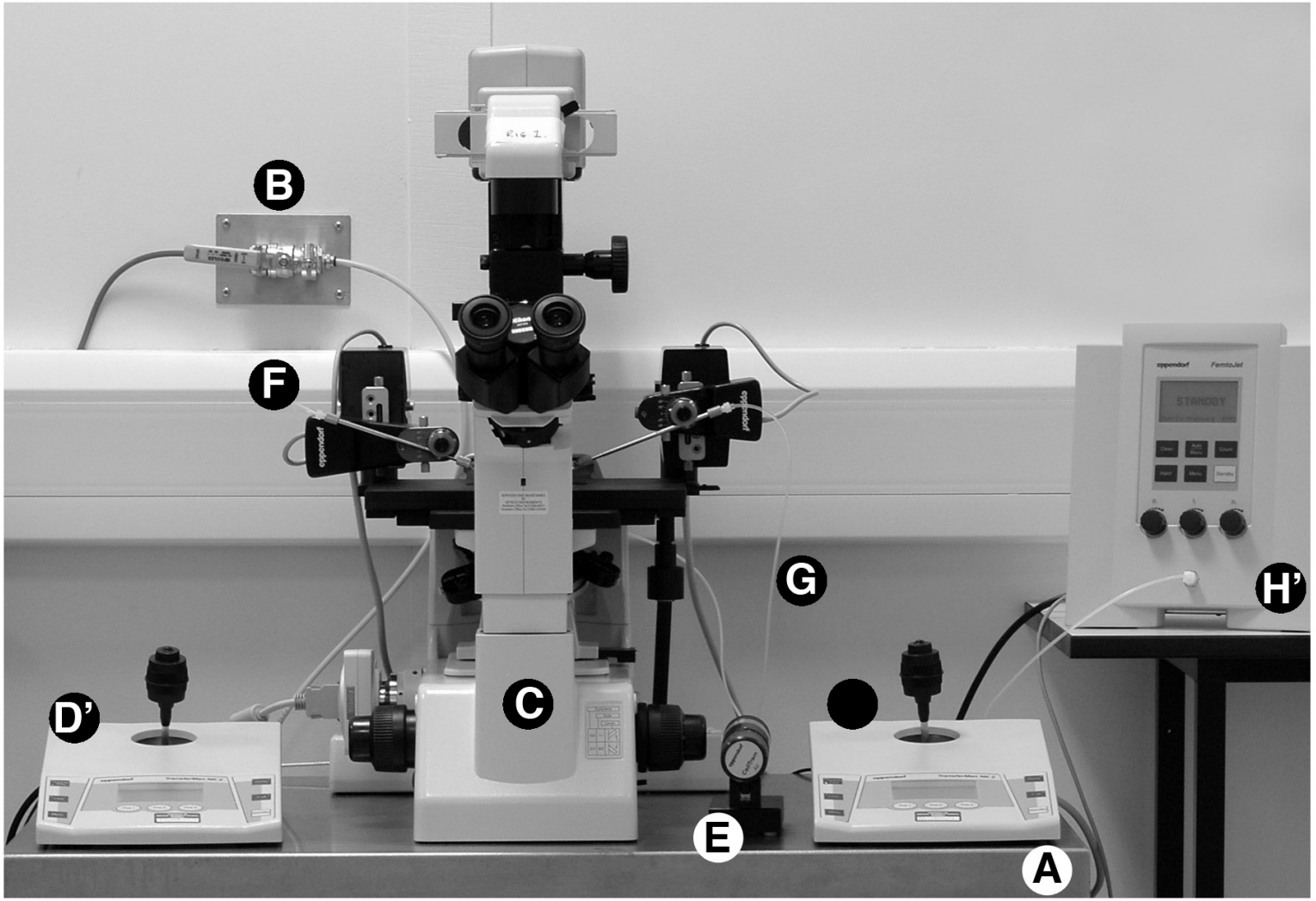

Dominant and Recessive phenotype driven screens

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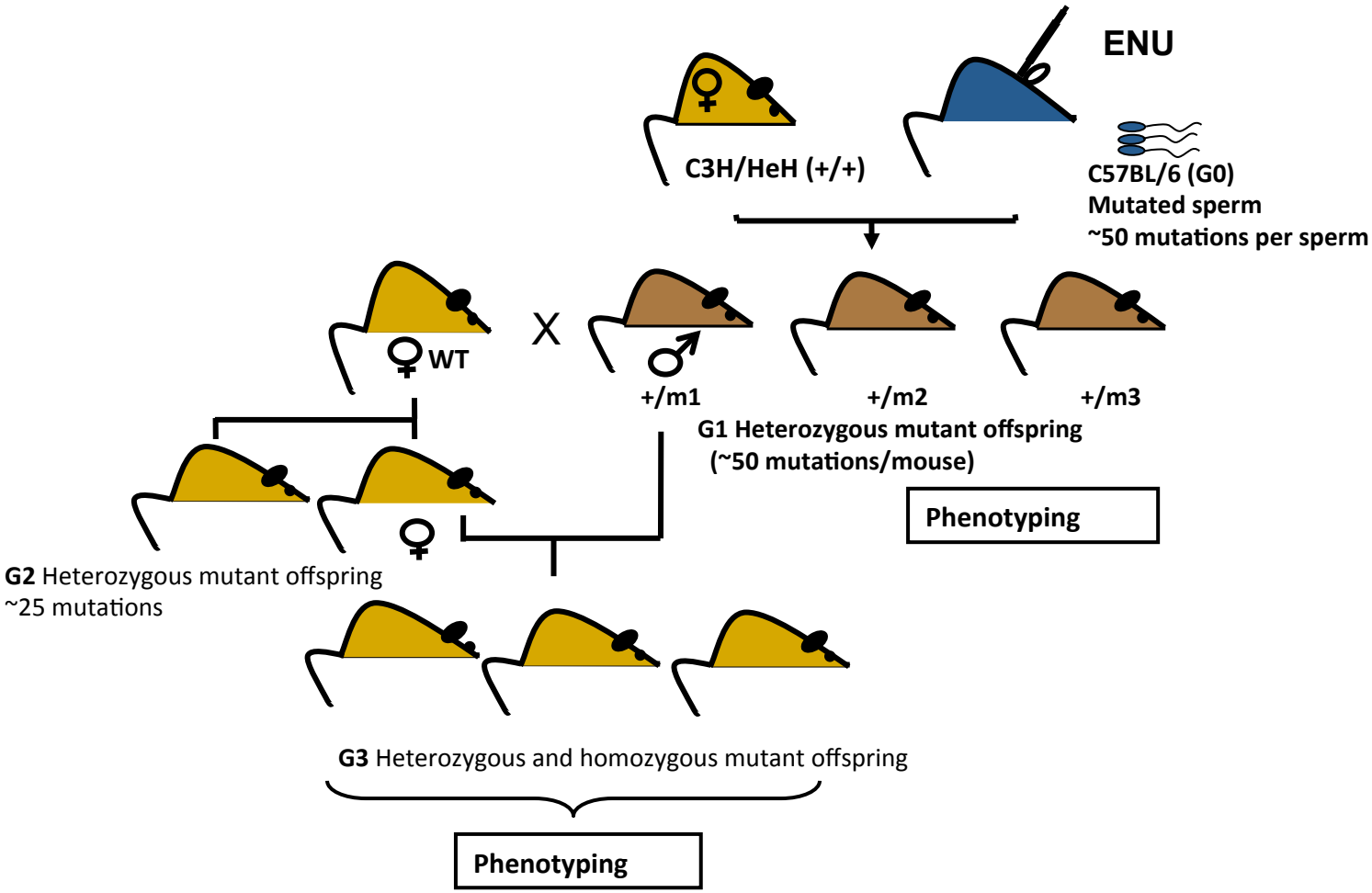

Mapping

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- **Conventional Mapping**
  - Large pedigrees/multiple rounds of screening
    - Confidence in phenotype
    - Enough affected individuals for mapping
    - NGS to identify associated mutation

- **Early (mild) disease/biomarkers**
  - Early indicators of late onset disease
  - Map on mild disease/biomarker