

Animal models to investigate diseases



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Animal models to investigate diseases

1. Possibilities to study humans and avoid animal experiments
2. Animal models in general
3. Why use mammal models, laboratory animals
4. Genetics of laboratory animals
5. Gnotobiology of laboratory animals
Germfree and gnotobiotic mice
Why interesting ?
6. Reverse genetic or gene driven approaches
Knockout, knockin, transgenic mice, techniques and examples
7. Forward genetic or phenotypic driven approaches
Screening approaches (ENU)
8. Alternative models

The ideal model : humans

Not ethical, risk for life to high

Exceptions:

1. Highly controled clinical trials and studies

2. Study of inborn errors

Rare genetic disorders of humans, best evidence to understand gene functions

3. Association studies

Determination of SNPs single nucleotide polymorphisms which are leading to increased disease risk. Animal models helpful to further investigate candidate genes

e.G. IBD NOD2

Rare genetic disorders of humans, best evidence to understand gene functions

Example: Toll-IL1Rs domain (TIR) signaling

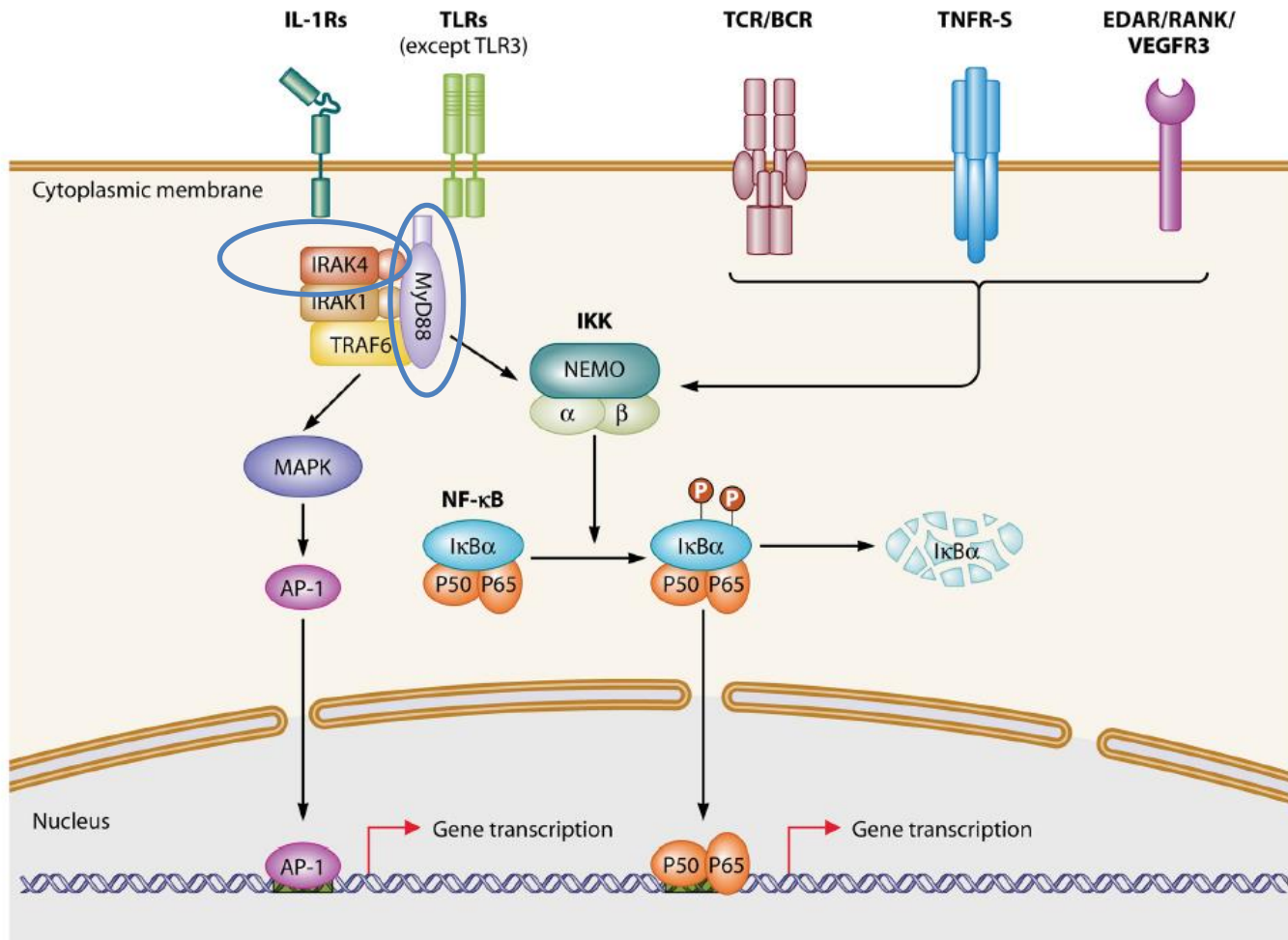


FIG. 1. TIR and NF- κ B signaling pathways. Immune receptor signaling pathways leading to NF- κ B activation can be grouped into four categories on the basis of the surface receptors involved: members of the TIR superfamily (IL-1Rs/TLRs), antigen receptors (TCR and BCR), members of the TNF-R superfamily (TNF-Rs), and RANK, VEGFR3, and EDAR. The two proteins of the TIR signaling pathway (MyD88 and IRAK-4) and the two proteins of the NF- κ B signaling pathway (NEMO and I κ B α) responsible for primary immunodeficiencies are shown.

Inborn errors of the TIR pathway: IRAK-4 and MyD88-/-

Autosomal recessive IRAK-deficiency >50 patients known

Autosomal recessive MyD88 deficiency more than 20 patients known

TABLE 1. Percentages of IRAK-4- and MyD88-deficient patients with bacterial infections at various sites^a

Infection	% of patients with infection		
	All patients (n = 71)	IRAK-4-deficient patients (n = 49)	MyD88-deficient patients (n = 22)
Meningitis	58	63	45
Sepsis	41	37	50
Arthritis	24	29	14
Osteomyelitis	13	14	9
Abscess	24	29	14
Lymphadenitis	27	29	23
Skin infection	35	45	14
Pneumonia	17	18	14
ENT infection	28	35	14

^a Based on data from references 11 and 58 and unpublished data.

TABLE 2. Documented bacterial infections in IRAK-4- and MyD88-deficient patients^a

Infection organism	% of patients with invasive infection (n = 71 patients)		% of patients with noninvasive infection (n = 71 patients)	
	IRAK-4-deficient patients (105 infections)	MyD88-deficient patients (44 infections)	IRAK-4-deficient patients (63 infections)	MyD88-deficient patients (15 infections)
<i>S. aureus</i>	14	20	43	53
<i>S. pneumoniae</i>	54	41	16	20
Other <i>Streptococcus</i> spp. (A and B groups)	6	11	8	
<i>P. aeruginosa</i>	19	16	22	13
Other Gram-negative bacteria	7	11	10	13
<i>Shigella sonnei</i>	2			
<i>Neisseria meningitidis</i>	2			
<i>Haemophilus influenzae</i>	2	2		
<i>Salmonella enterica</i> serovar Enteritidis		7		
<i>Klebsiella pneumoniae</i>				7
<i>Escherichia coli</i>			5	7
<i>Serratia marcescens</i>			2	
<i>Moraxella catarrhalis</i>		2	2	
<i>Clostridium septicum</i>	1			
<i>Citrobacter freundii</i>			2	
<i>Mycobacterium avium</i>			1	

^a Based on data from references 11 and 58 and unpublished data.

Requirement of animal models to understand diseases

- Today, scientists are creating models of human genetic disease using mice, flies, worms, and other animals. But what do these models reveal about us?
- Animal models are just models and give only a crude approximation to the human system

Why use mammal models to study human disease ?

- Models selected because of similarity to genetics, anatomy, and physiology
- Unlimited supply and ease of manipulation
- Defined nutrition, defined environment, defined genetic background
- Important manipulate only one variable and observe in consequence change
- Perform statistics

The most important species of laboratory animals

- **Mouse** – most frequently used. Pharmacology, genetics of mammals, virology, models of human diseases (mutant strains, transgenic and knock-out mice) Rodents most common models for experimental studies
Genomes similar to humans (80% 1:1 orthologus relationship with mouse genes)
Availability
 - Ease to handle
 - Unique technologies to manipulate mouse genomeHigh reproductive rate
Low costs
- **Rat** – physiology of cognitive processes, behaviour, models of diabetes
- **Rabbit** – serology, insulin quantification, pyrogens quantification, tests of irritable effect of chemical substances on the cornea
- **Cat** – study of CNS and respiratory system
- **Dog** – e.g. beagle, use in electrophysiology, neurophysiology
- **Guinea-pig** – in microbiology and serology, physiology of the auditory system
- **Hamster** - genetics
- **Pig** – training of surgical techniques, temporary covering of burns with porcine skin
- **Primates** – rhesus monkey, baboon, chimpanzee – use in neurology, virology, behaviour
- **Frog** – physiology of blood circulation, electrophysiology
- **Fish, molluscs, insects** – development, infections

GENETICS OF LABORATORY ANIMALS

- 1. Isogenic = genetically defined strains
(isogenicity= genetic uniformity of all individuals)**
- 2. Non-isogenic = genetically undefined strains**
- 3. Genetically semi-defined strains**

Isogenic strains - 1

Inbred strains

- obtained by close breeding for more than 20 generations (brother + sister or offspring + one of the parents)
- homozygosity higher than 98 % (Degree of homozygosity is expressed as a coefficient of inbreeding.)
- features: isogenicity, phenotype uniformity (low variability of reactivity), usually low fertility, disposition to diseases
- advantages of use in experiment: homogenous statistical set, lower number of individuals is sufficient
- disadvantages of use in experiment: a risk, that the findings are strain-specific and are not valid for other strains, problematic generalization of the results
eg. Different susceptibility against infection diseases, or disease models
DSS colitis C3HeJ > C57BL/6 mice
Leishmania C57BL/6 > Balb/c mice
Yersinia, Listeria Balb/c > C57BL/6 mice

Isogenic strains - 2

Ways to understand a distinct susceptibility for a certain disease

Coisogenic strains (mutant strains)

- differ from the original strain only in one gene, in which a mutation occurred

Congenic strains

- strains originated by cross-breeding of two strains and following back-cross-breeding with one of the original strains (at least 10 times, selection of some specific feature)
- presence of specific genes of one strain on genetical background of the second strain

Recombinant-inbred strains

- crossing of 2 strains, the hybrids give origin of new lines which are then crossed brother x sister, what leads to establishing of a new strain

Consomic strains

- a complete chromosome of one strain is transferred on the background of the second strain with back-crosses (similarly as for individual genes in congenic strains, but the process is more complicated)

Non-isogenic strains

Outbred lines

- genetically heterogeneous population without crossing with individuals coming from different, in the frame of the population close crossbreeding is avoided so that the coefficient of inbreeding remains as low as possible
- features: some level of phenotype variability (higher variability of reactivity), higher fertility and resistance to diseases
- advantages of use in experiment: cheaper and easier production, the findings have more general validity
- disadvantages of use in experiment: less homogeneous set, higher number of animals is necessary

SYSTEMS OF BREEDING OF LABORATORY ANINMALS

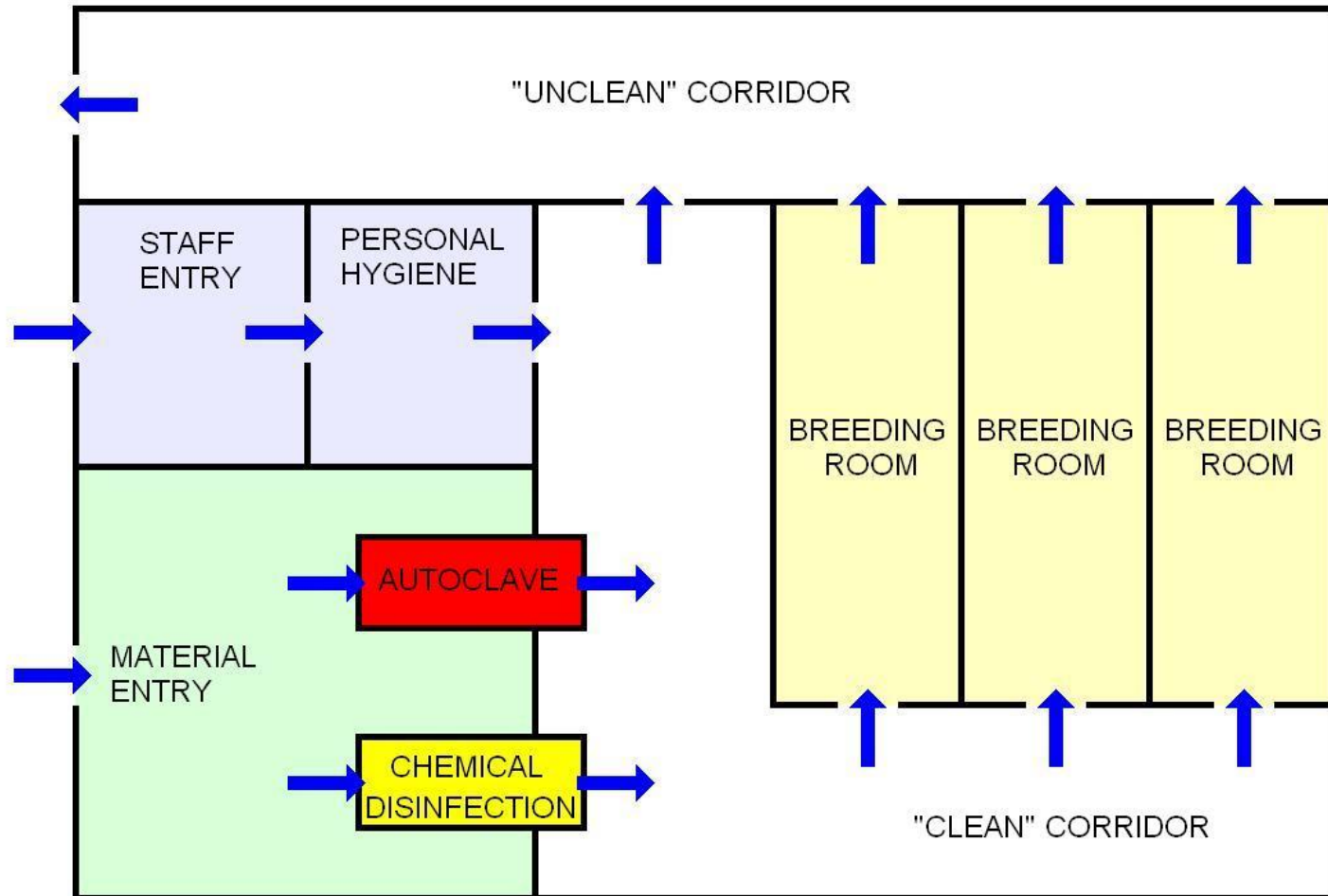
Open – communication without the barrier

Barrier – the space with the animals separated from external environment and movements of animals, people and material are controlled to eliminate possible introduction of alien factors from the external environment (infection) – sterilization of coming water, food, sawdust used for bedding, perfect personal hygiene of the personal.

Isolator – the space for the animals is permanently separated by a barrier from external environment as well as from people who manipulate with the animals.

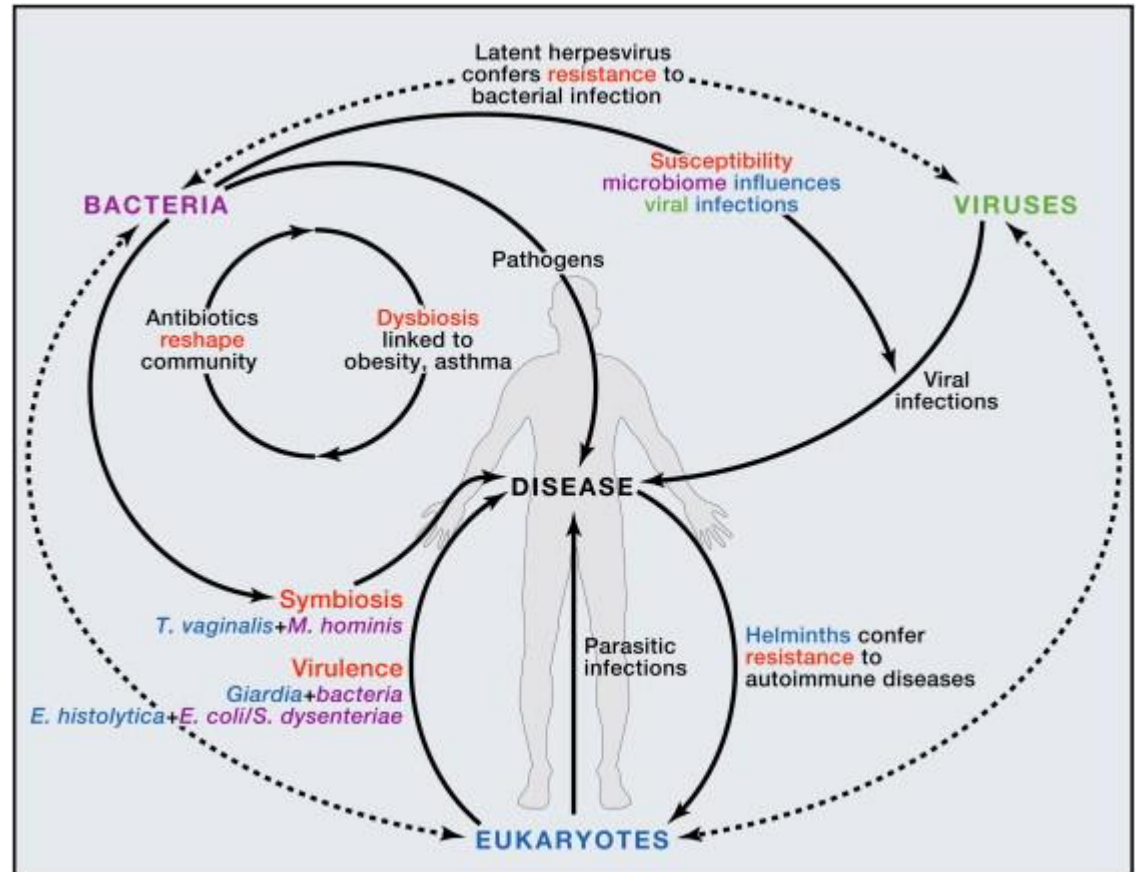
Question of costs

Scheme of barrier facility



Impact of microbiota

- Obesity
- Inflammatory bowel disease
- Diabetes
- CNS disorders
- Asthma
- Infectious diseases
- And so on



GNOTOBIOLOGY OF LABORATORY ANIMALS

Conventional animals

- undefined microbiota
- open breeding facility complying basic hygienic conditions

SPF animals = specified pathogen free

- microbiota of the animals certainly does not contain specified pathogens.
- barrier breeding facility
- everybody defines it different
- variations of microbiota can be different from one room to the next

Gnotobiotic animals

- breeding isolators

1. Axenic animals = germ free

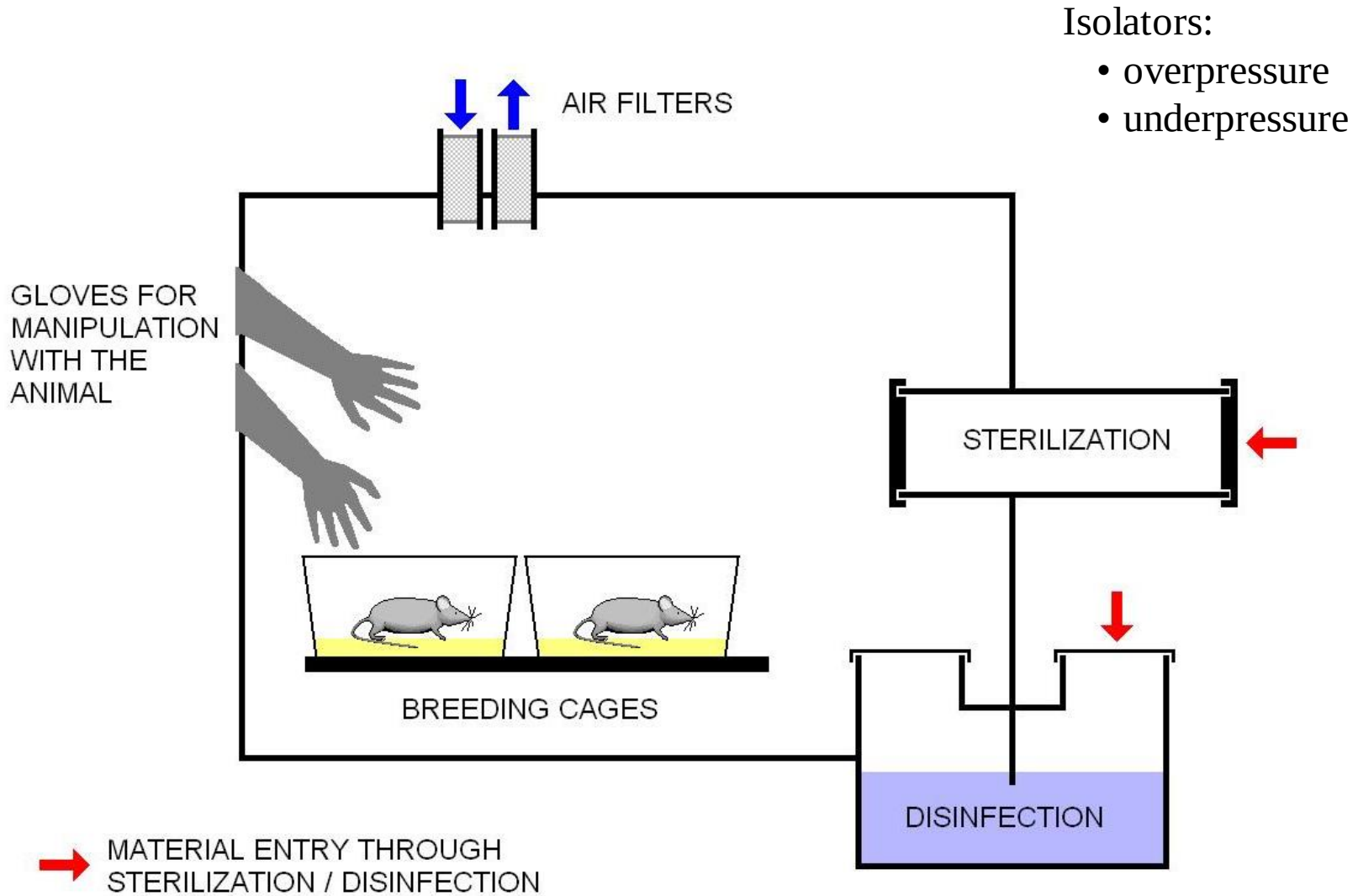
- without any microbes
- pups obtained with sterile hysterectomy or hysterotomy into sterile atmosphere of the isolator

2. Associated animals

- derived from axenic animals colonising them artificially with one or more species of microorganisms
- monoxenic, dixenic, polyxenic



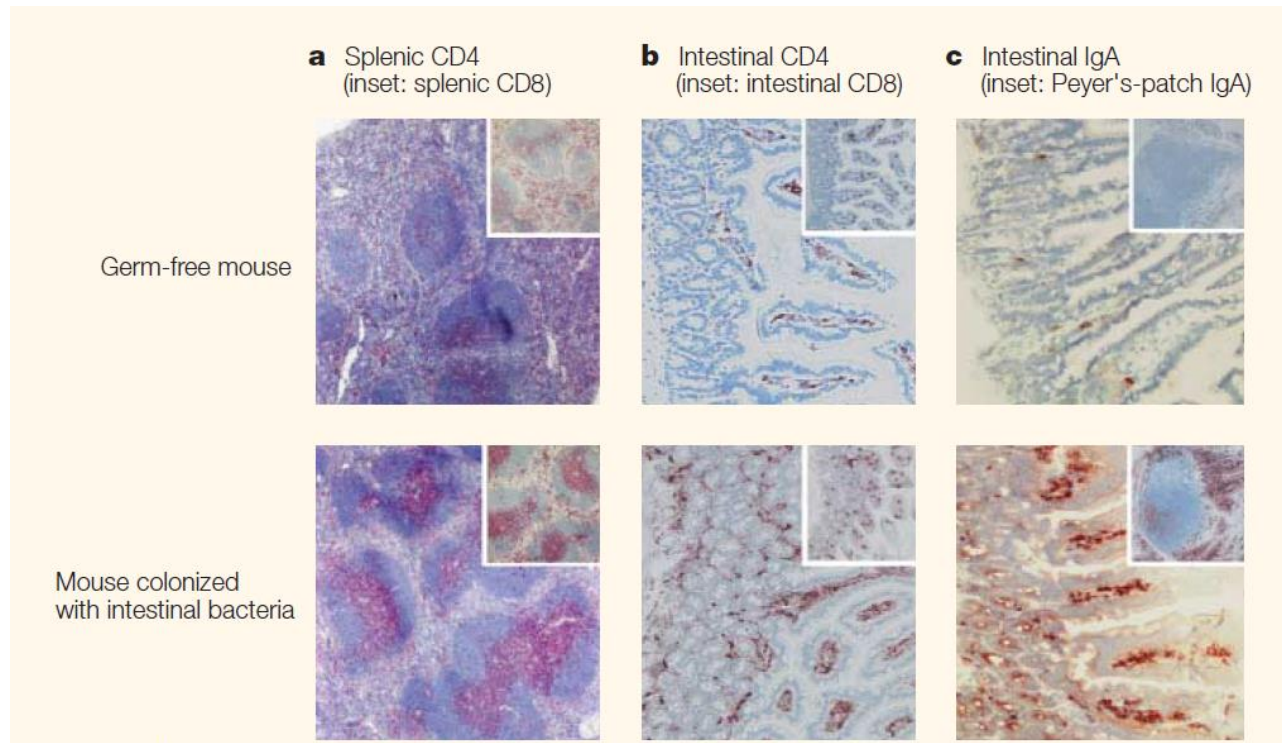
Scheme of isolator



Germ-free mice

Microbiota strongly affects immune system

- altered splenic architecture (less germinal centers)
- Less intestinal CD4 cells
- less IgA producing cells
- Addition of microbiota leads to restoration of immune system



Germ-free mice

To understand the impact of the microbiota on immune system, disease development (microbiota 100 fold more genes)

GF mice develop less inflammatory bowel disease, thus microbiota associated with IBD

GF mice more susceptible to gastrointestinal infections (colonization resistance, altered Immune system)

Basis to get gnotobiotic animals

Gnotobiotic mice with a defined microbiota

Colonization with microbiota (defined) to understand role of microbiota in disease development (cancer treatment, pharmacology, infections, autoimmunity, IBD)

e.G. SPF Interleukin-2-deficient mice: develop inflammatory bowel disease

GF IL-2-deficient mice: no IBD

Bacteroides vulgatus, no IBD

E. coli mpk IBD

Bacteroides prevents *E.coli* mpk induced IBD

Understanding interaction of bacteria in gnotobiotic mice may allow to develop strategies
To prevent complex disease (like IBD, but also diabetes, multiple sclerosis) etc.

Use of gnotobiotic mice to develop strategies to cure IBD

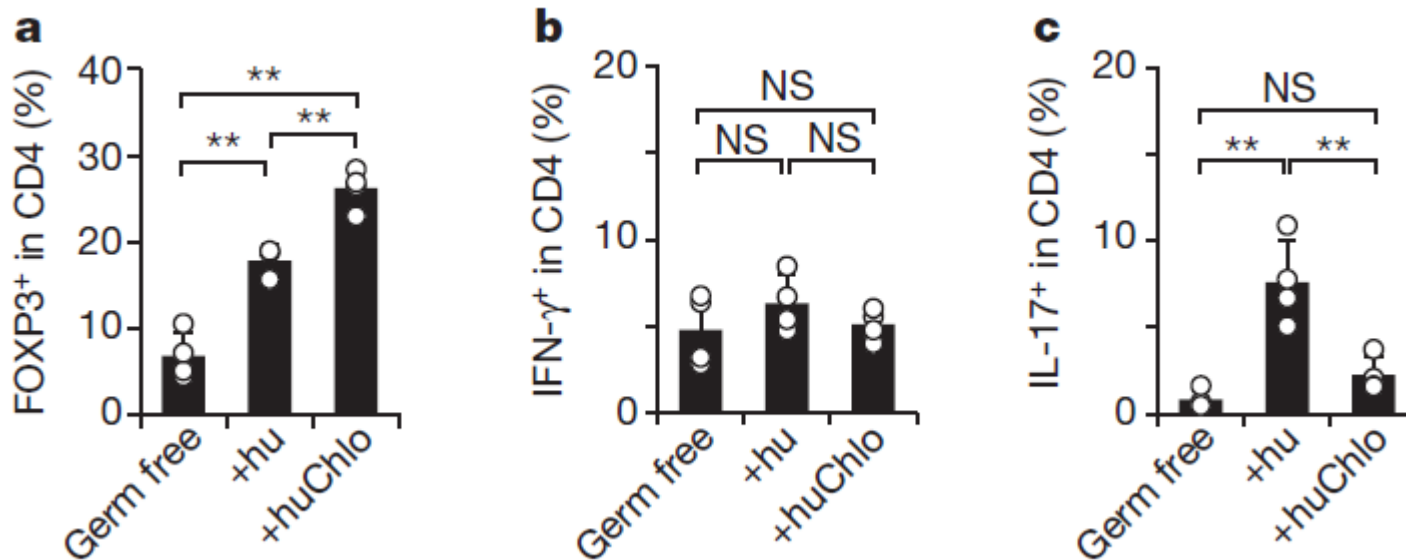
Atarashi et al 2013 Nature

Manipulation of gut microbiota to cure inflammatory bowel disease or allergic reactions

CD4+FoxP3 T regs in the lamina propria of the gut

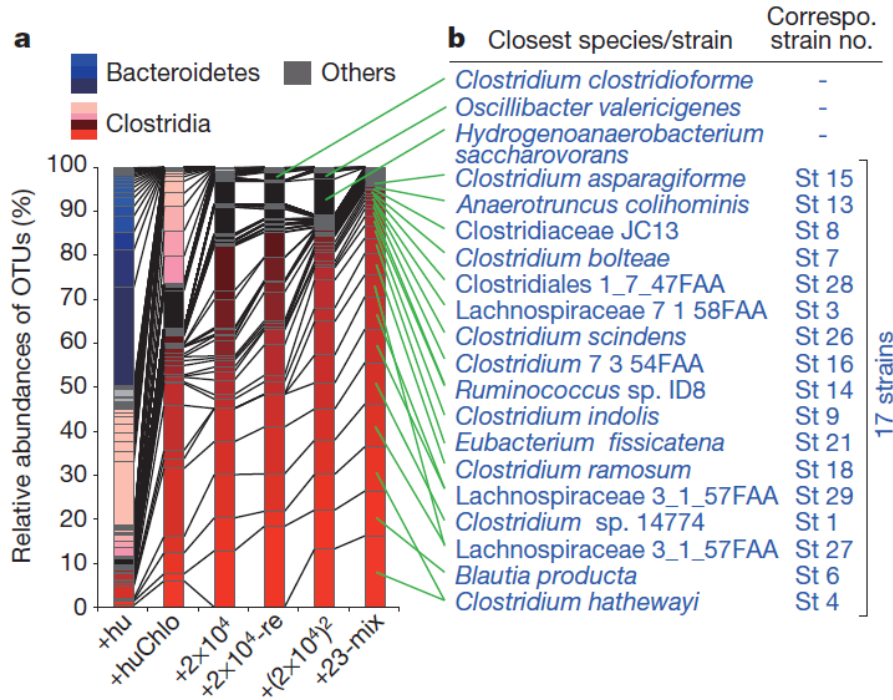
contribute to immune homeostasis and are involved in preventing IBD

Introduction of human feces isolates increases Treg percentage

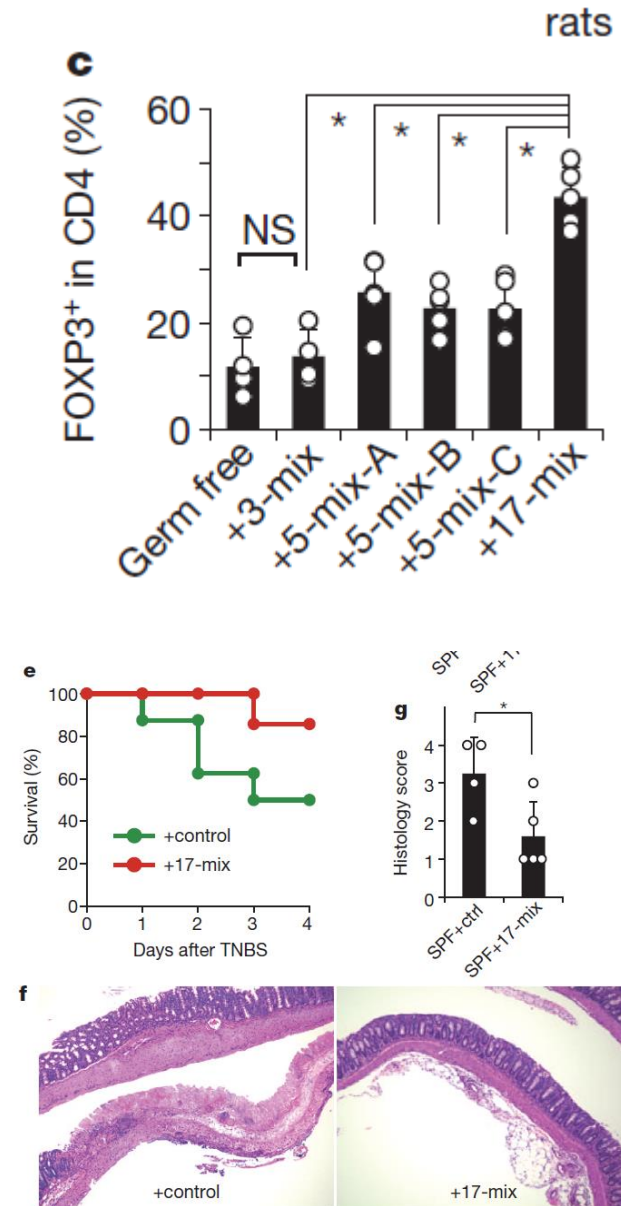


Hu: human feces HuChlo: Chloroform extracted/resistant bacteria

Distinct pattern of bacteria increases Treg number and prevents colitis



Current clinical trials using a mixture of bacteria to cure *C. Difficile* colitis IBD



SYSTEMS OF BREEDING OF LABORATORY ANINMALS

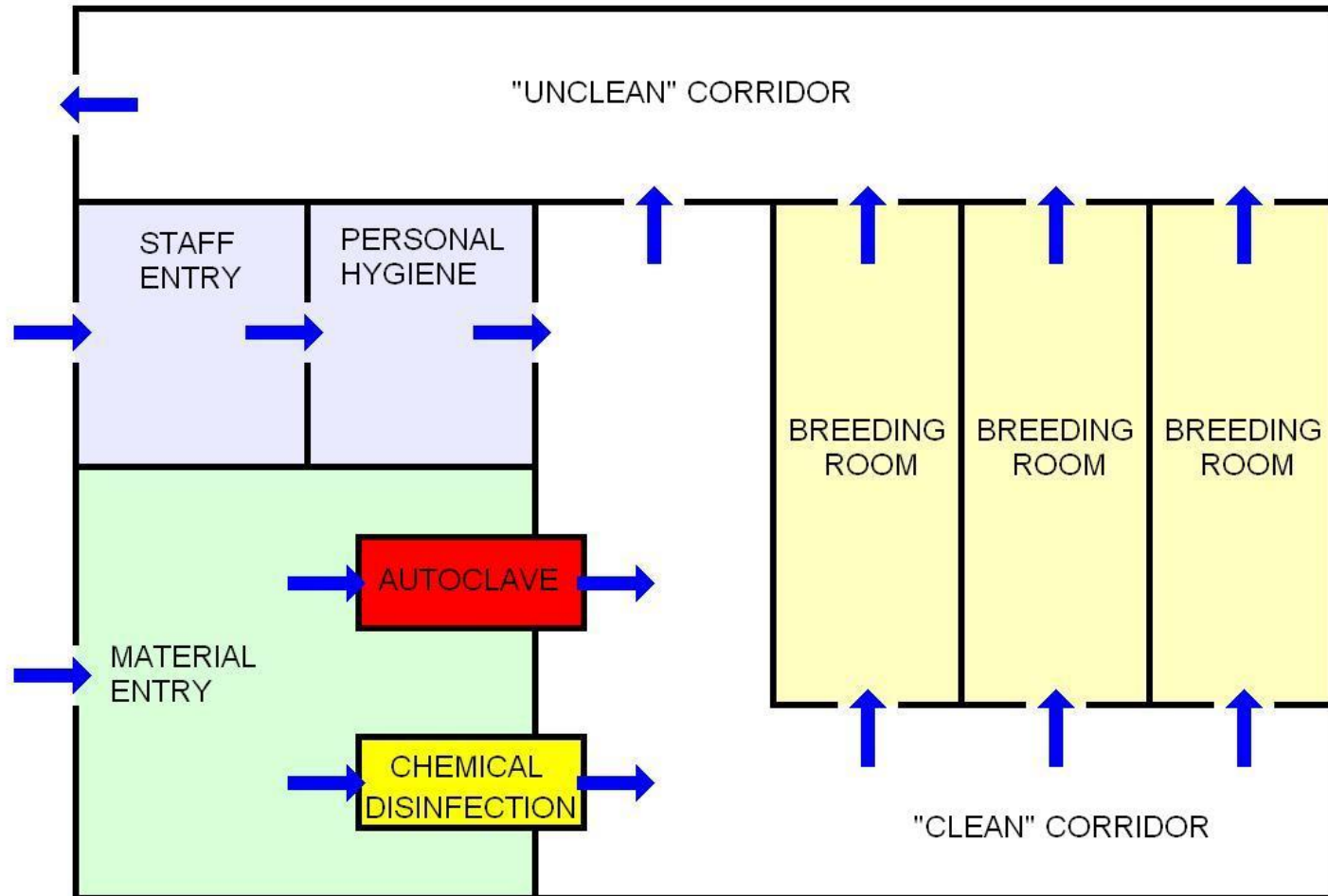
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Scheme of barrier facility



Animal models of diseases – Genetics

1. Reverse genetic or gene-driven approach

Targeted mutation of genes to determine the function of a gene in the context of a whole organism

Initial hypothesis required

approximately 50% of about 34000 known mouse have some some

Functional annotations

Sometimes inconclusive (embryonic lethality, undistinguishable because of gene redundancy)

Single gene knock-outs

Knock-Ins

Constitutive or conditional gene modifications

Chromosomal rearrangements

Animal models of diseases

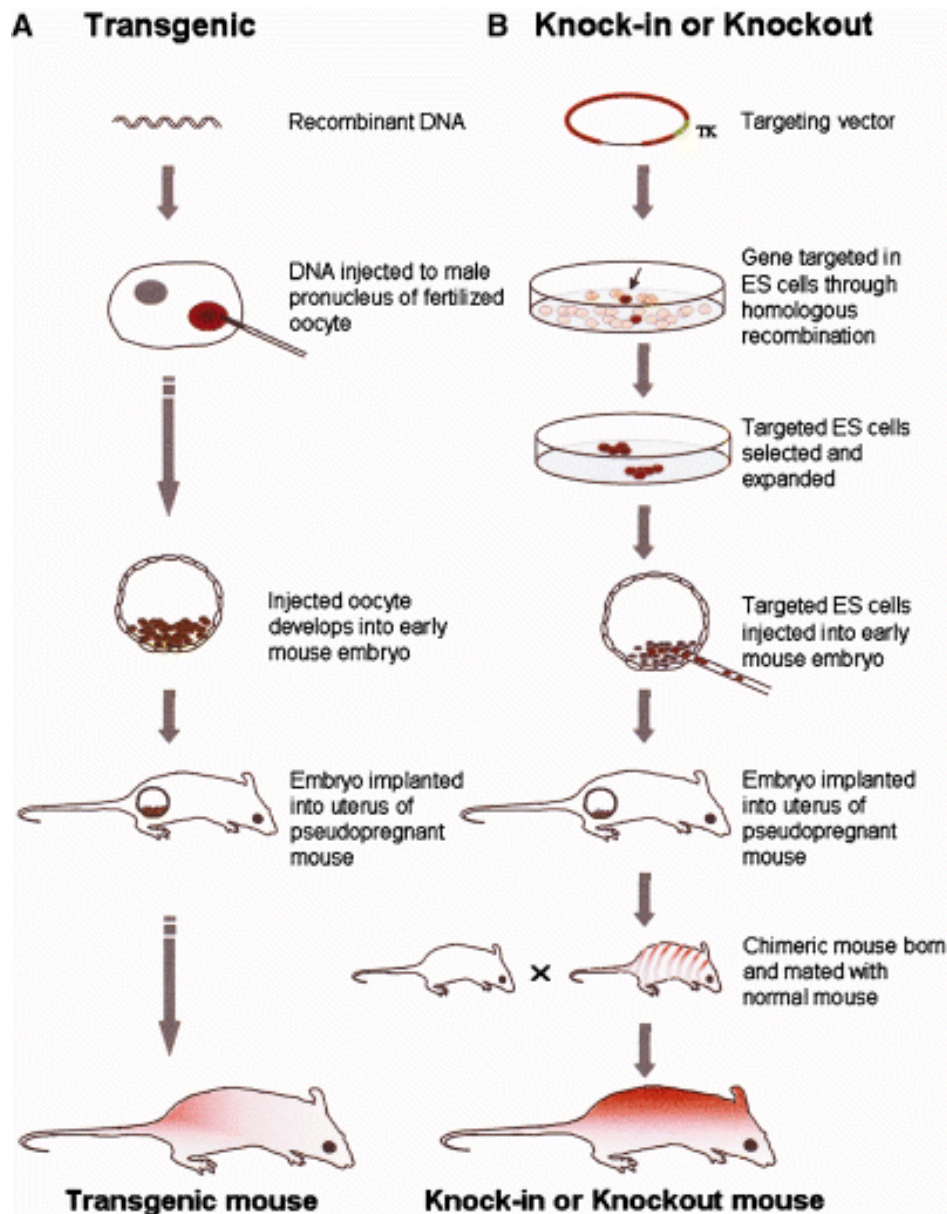
2. Forward genetic or phenotypic-driven approach

Definition of susceptibility loci by backcrossing approaches

- Generation of congenic/consomic strains and testing phenotypes

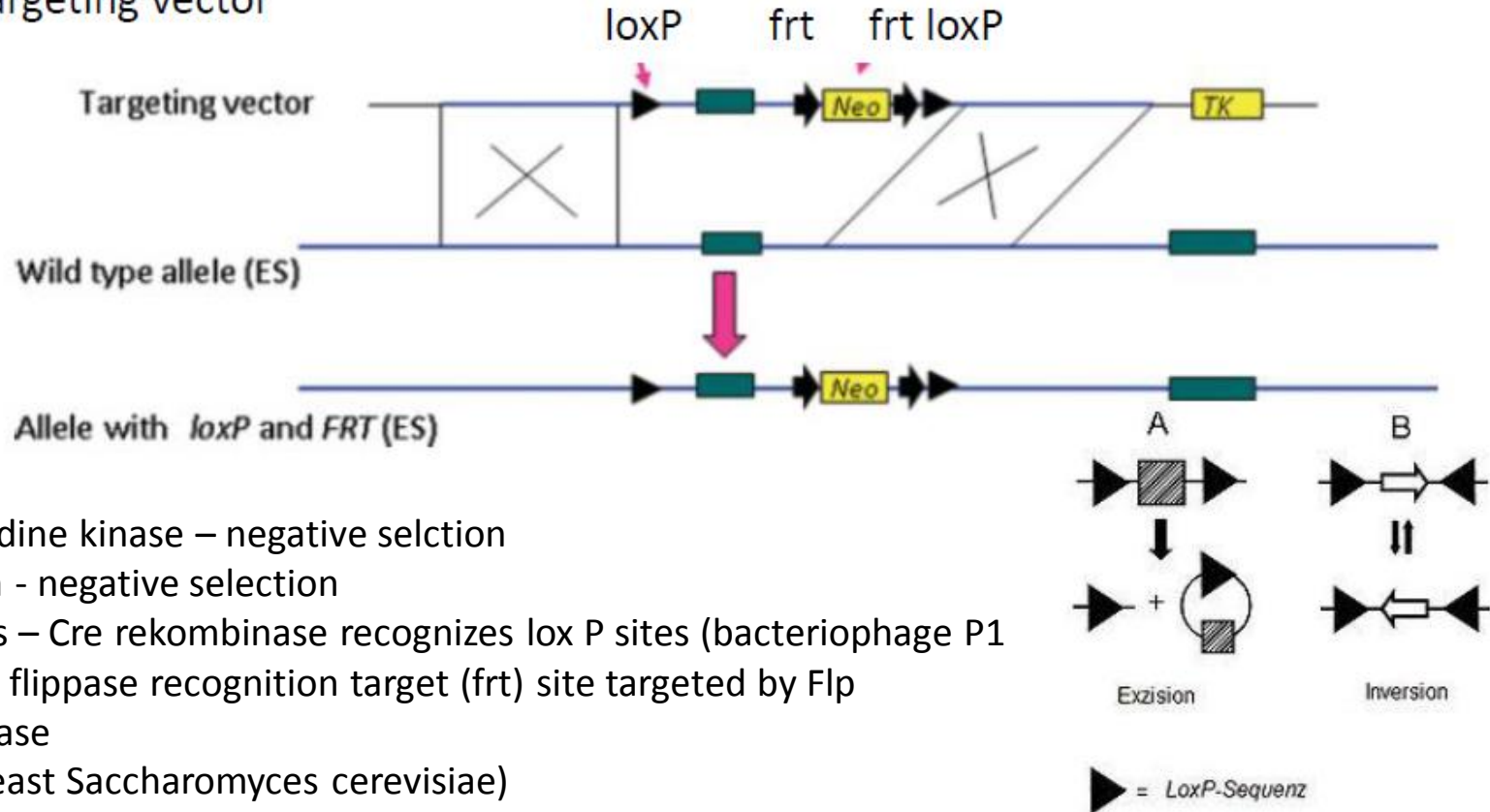
Mutation-driven approach uses radiation and chemicals to cause mutations. Large scale mutation screens to test phenotypes

Reverse genetic approach- Generation of transgenic, knockin or knockout



Generation of knockout mice

Targeting vector



Tk Thymidine kinase – negative selection

Neomycin - negative selection

Lpx P sites – Cre rekombinase recognizes lox P sites (bacteriophage P1

Ftr sites – flippase recognition target (frt) site targeted by Flp

recombinase

(bakers yeast *Saccharomyces cerevisiae*)

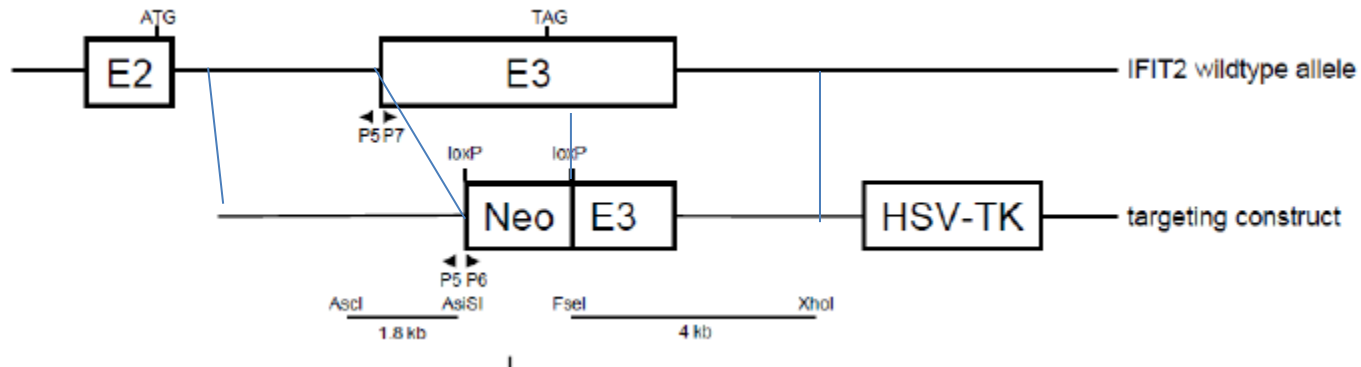
Transfection of embryonal stem cells (129Sv agouti coloured) with plasmid

Selection with Neomycin and gancyclovir

Neomycin-positive and Tk-negative cells survive

Finally deletion of selection marker, e.g with Flp rekombinase

1. Generation of targeting vector

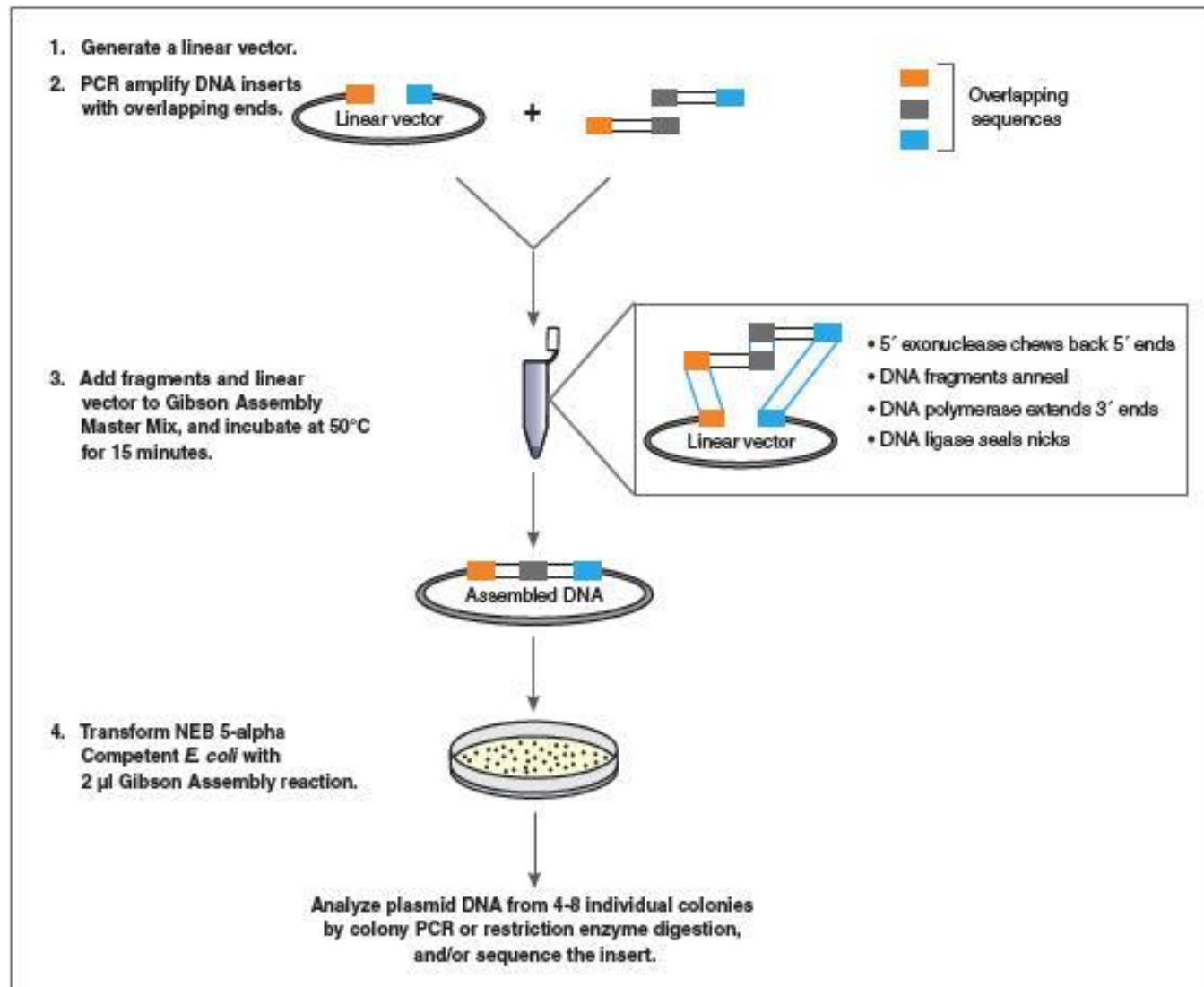


Generation of appropriate 5' and 3' flanking regions of 3 exons and cloning

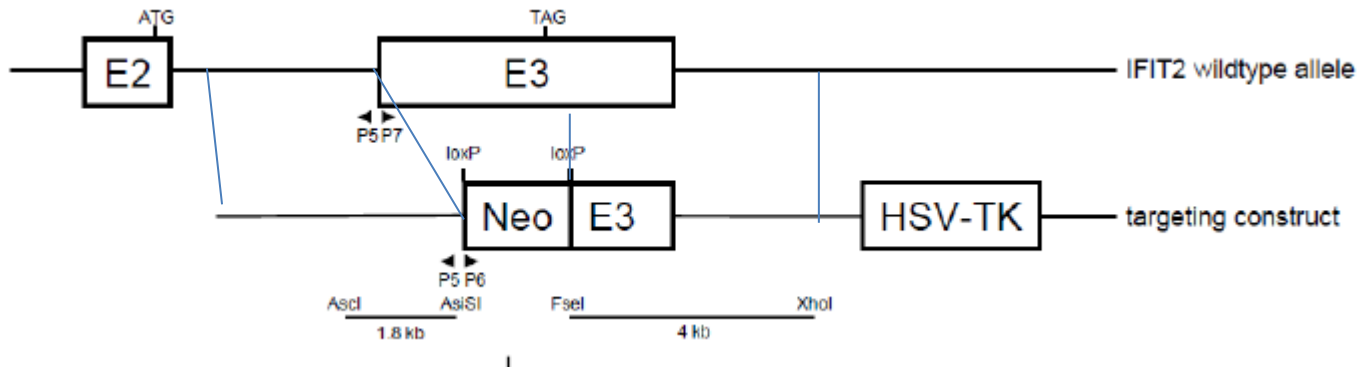
Generation of PCR fragments with restrictions site (Restriction endonuclease)

Restriction digest and ligation in expression vector.

Vector cloning with Gibson assembly



2. Transfection of targeting vector in embryonal stem (ES) cells



Liposome-mediated transfection

Liposomes are synthetic analogues of the phospholipid bilayer.

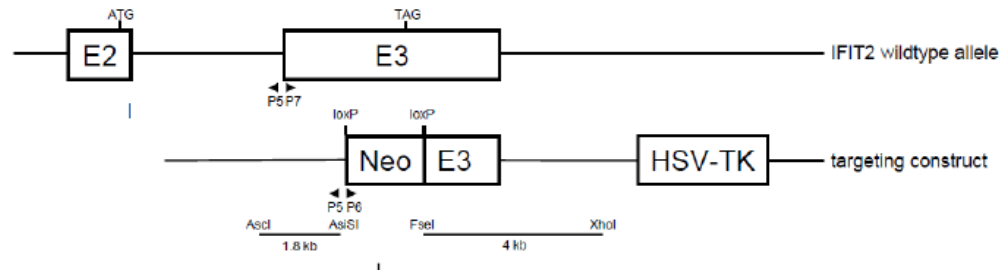
Non-liposomal transfection agents (lipids and polymers)

non-liposomal lipids and polymers capable of forming complexes with nucleic acid to form micelles, or tiny encapsulating droplets.

Electroporation

Electroporation is a highly efficient technique for delivering exogenous nucleic acids to suspension cells and non-adherent primary cells (like lymphocytes).
electricity to create transient pores (electropores) in the cellular membrane
to enable the uptake of charged nucleic acid molecules (RNA or DNA) into the target cells.

3. Selection of ES cell with homologous recombination – Stable ES cell line



Positive Selection: Resistance cassette

Neomycin, Puromycin oder Hygromycin - antibiotics

After successful recombination or resistance cassette – cells survive

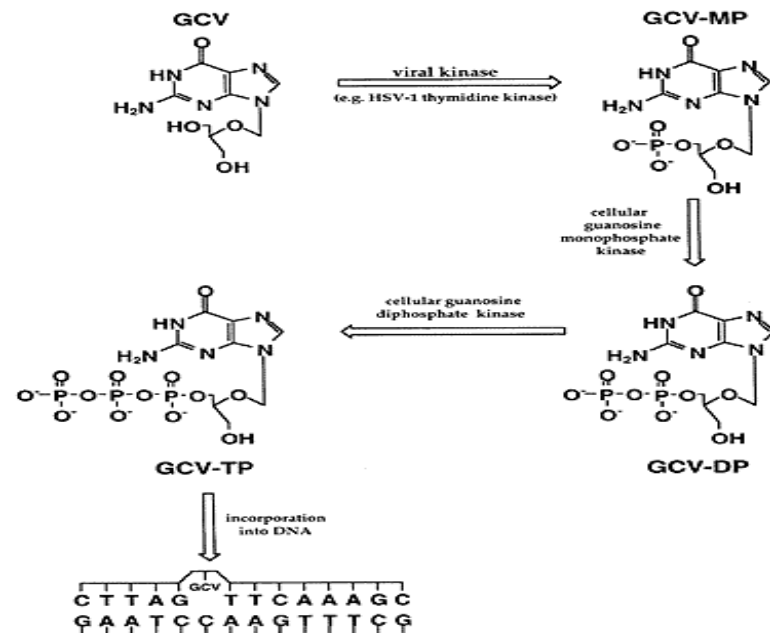
Negative Selection: only cells survive which lack TK (Thymidinkinase)

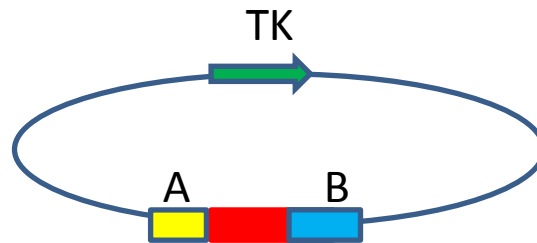
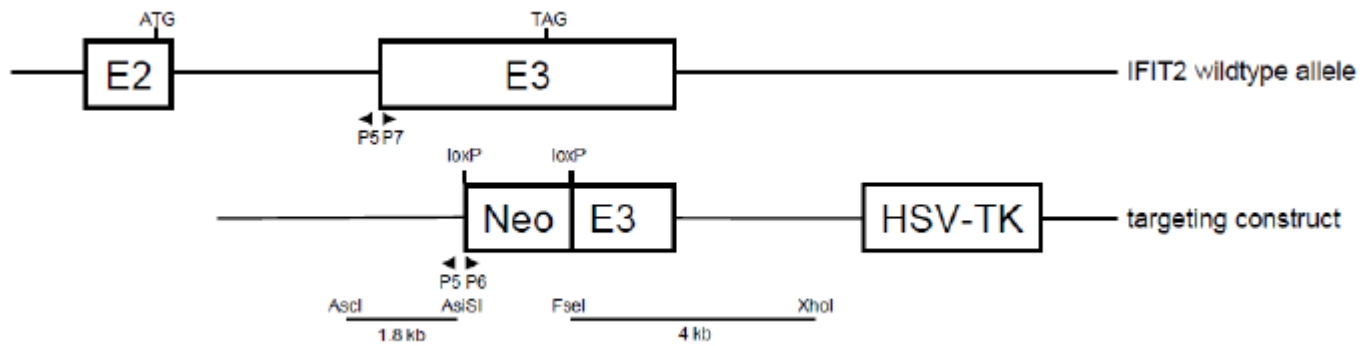
Treatment with Ganciclovir

Ganciclovir (GCV): Nucleosidanalogon

HSV-Tk produces GCV-MP

Cellular enzyme GCV-TP cell death



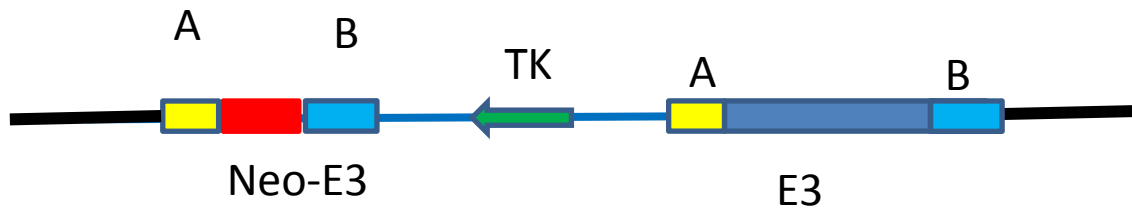


Neo-E3

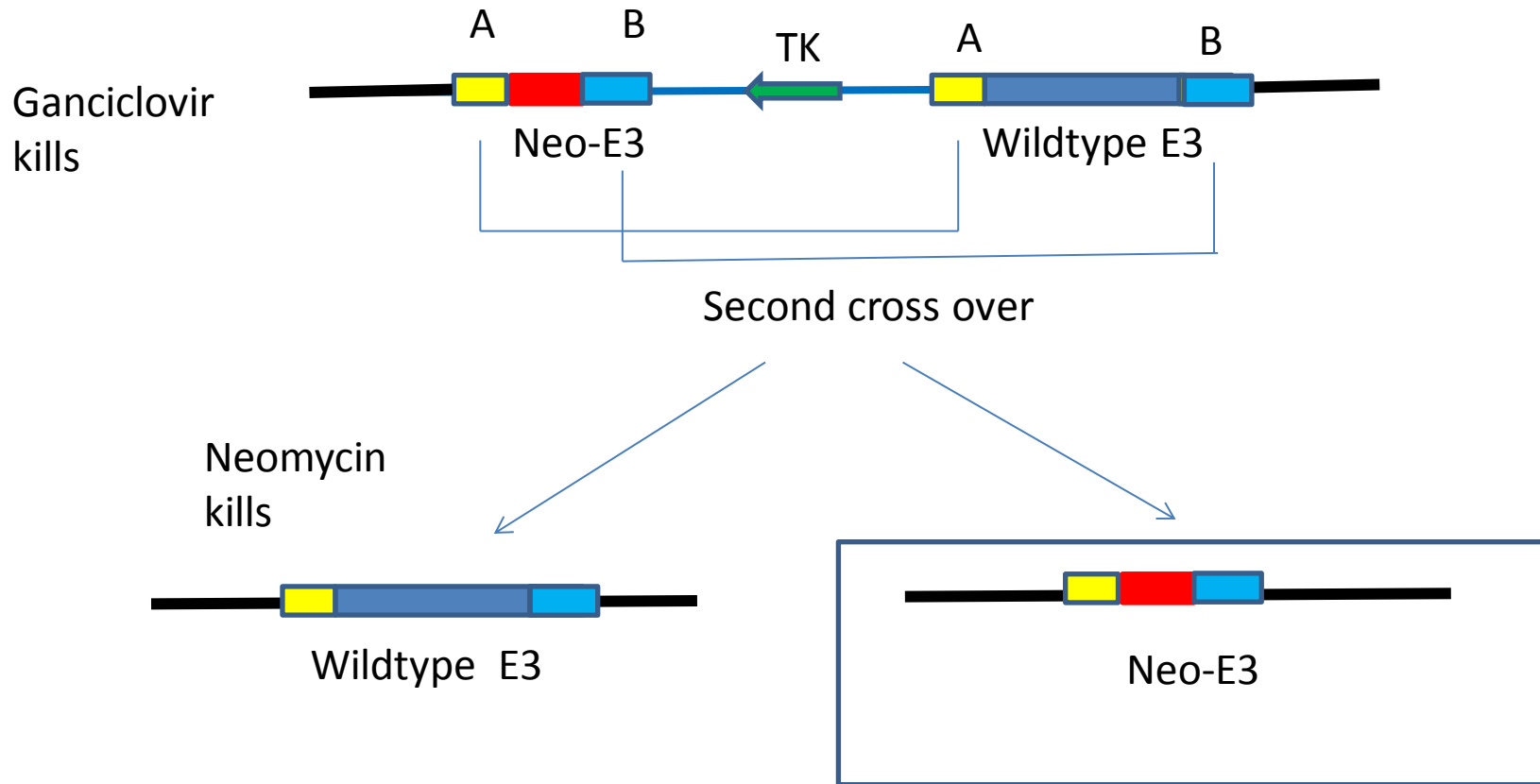
Single cross over



Neomycin selection
(Positive selection)



Presence of neomycin and gancyclovir drives second crossover



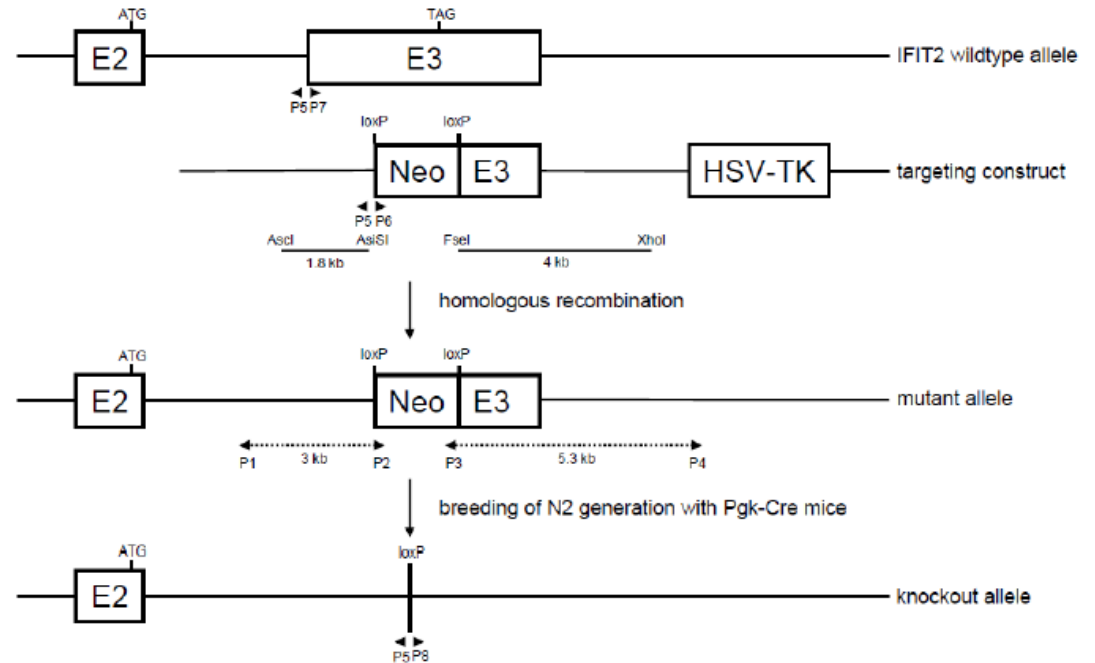
4. Selection of ES cells with homologous recombination – ES cell clones

Generation of ES single clones

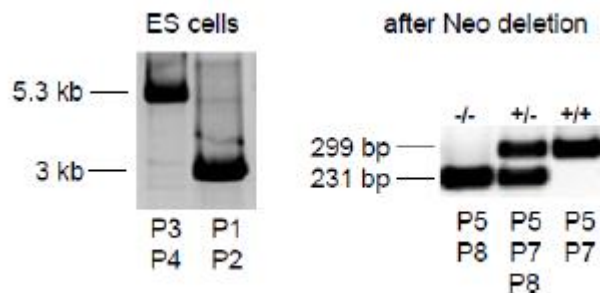
Testing for recombination

PCR

Southernblot



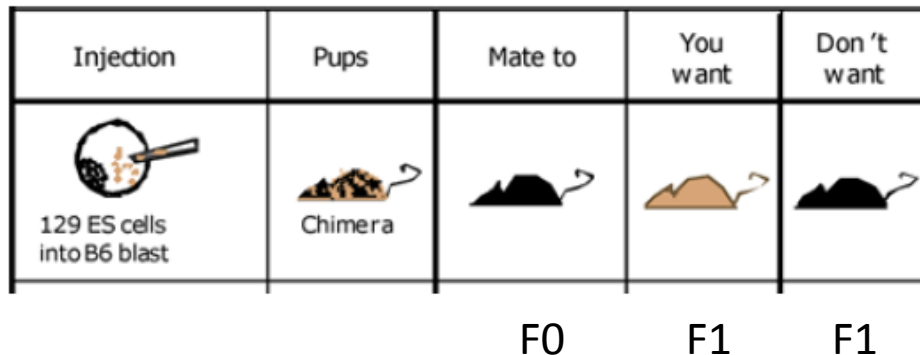
Mating with mice
expressing Cre Recombinase



IFIT2 deficient mice

Generation of knockout mice

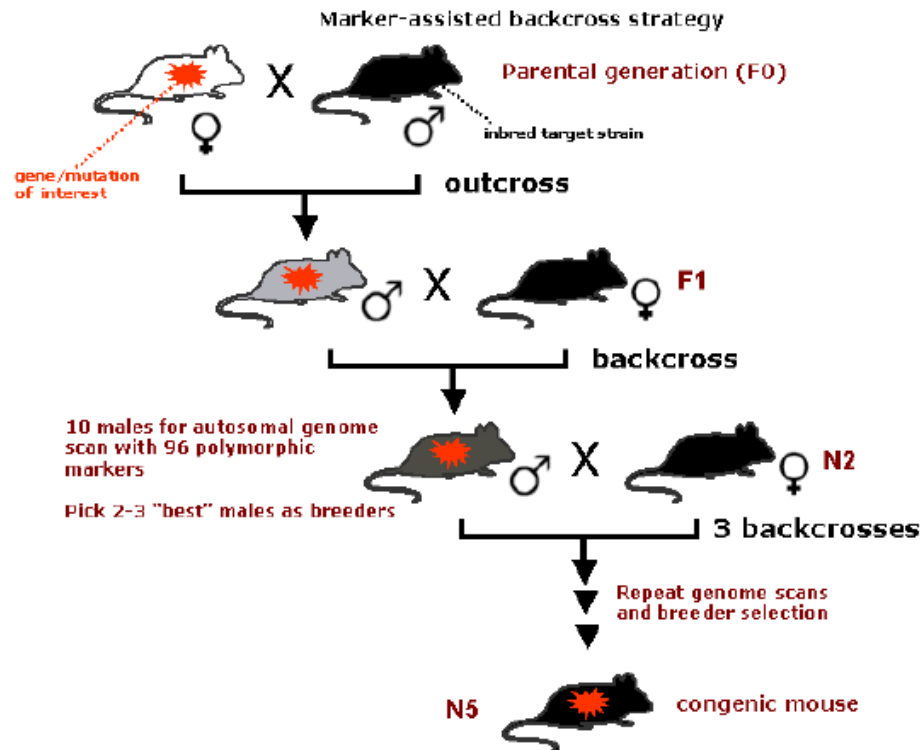
- Injection of ES cells into early embryo
- Implantation of embryo into foster mother
- Offspring: chimera
- Mating and screening for mice with germline transmission



- Example: 129Sv ES cells in C57BL/6 blastocyst
F1 generation 50% 129Sv/C57BL/6 mixed background
Back crossing to C57BL/6 > 10-20 generations to receive inbred strain

Faster: speed congenics

Generation of knockout mice – Speed congenics

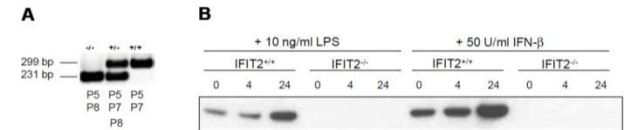


Even faster: Use of ES cell of C57BL/6 albino mice (white), no backcrossing required

IFIT-2 deficient mice

Interferon induced tetratricopeptide repeat protein 2

- Induced by type I (IFN alpha, beta) and type III (IFN-lambda) interferons
- Antiviral effects (VSV, Coronavirus)
- Working hypothesis: involved in inflammatory response



The Primary Workflow

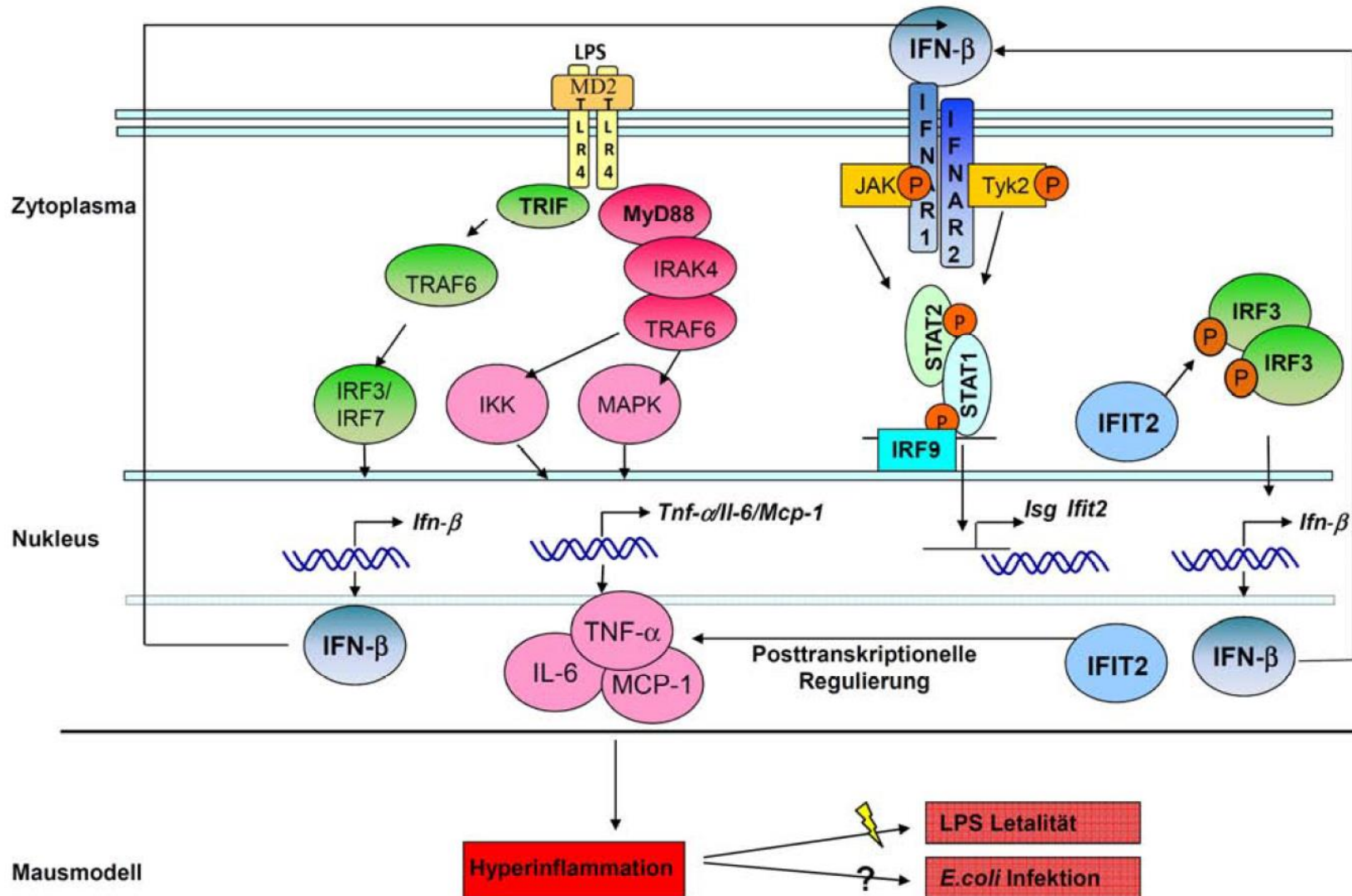


	Age [weeks]	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Screens	Methods															
Behaviour	Open field															
	Acoustic startle response & PPI															
Neurology	Modified SHIRPA, grip strength															
	Rotarod															
Clinical Chemistry	Clinical Chemistry after fasting															
Nociception	Hot plate															
Dysmorphology	Anatomical observation															
Allergy	Transepidermal water loss (TEWL)															
Energy Metabolism	Indirect calorimetry, NMR															
Clinical Chemistry	IpGTT															
Cardiovascular	Awake ECG / Echocardiography															
Eye	Scheimpflug imaging, OCT, LIB, drum															
Clinical Chemistry	Clinical Chemical analysis, hematology															
Immunology	FACS analysis of PBCs															
Allergy	BIOPLEX ELISA (Ig concentration)															
Steroid Metabolism (optional)	Corticost., Androst., Testosterone															
Neurology	ABR (Auditory brain stem response)															
Dysmorphology	X-ray, DEXA															
Energy Metabolism	NMR															
Clinical Chemistry	Clinical Chemical analysis, hematology															
Lung Function (optional)	Lung challenge															
Molecular Phenotyping	Expression profiling															
Pathology	Macro & microscopic analysis															

No severe phenotypes

1. First screen
German mouse clinics

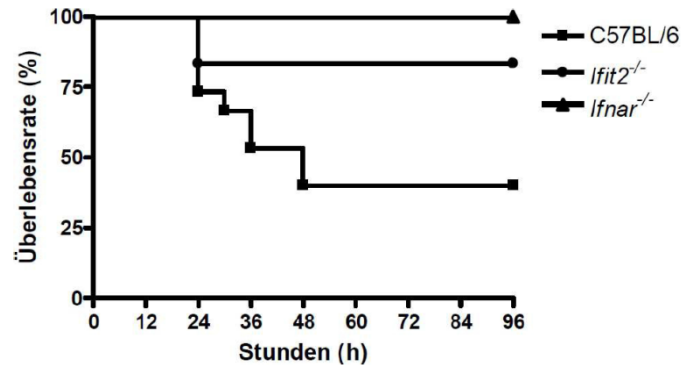
IFIT2 amplifys inflammatory response



IFIT2 promotes immunopathology in inflammation

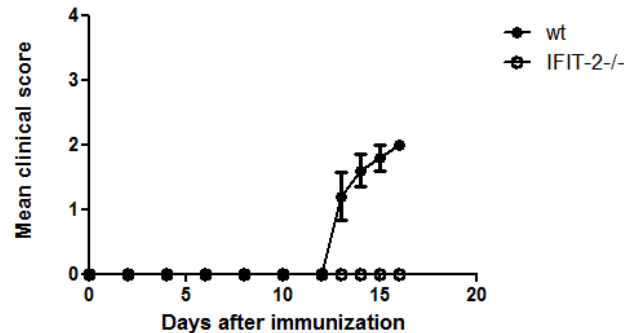
Systemic inflammation

Less LPS lethality (Sepsis model)
(iv injection of lipopolysaccharide (LPS))



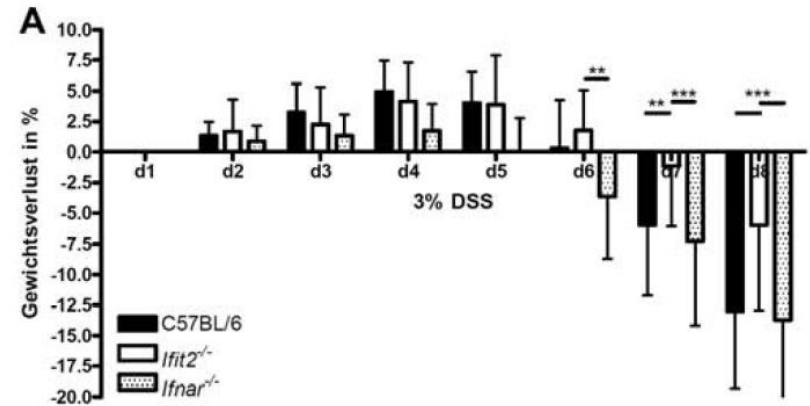
Brain inflammation

Experimental Autoimmune Encephalitis (EAE) – Multiple sclerosis model
Immunization with myelin basic protein fragment (MOG peptide) induces EAE.
Paralysis of limbs



Gut inflammation

DSS colitis (IBD model)
Oral application of Dextran sodium Sulfate) weight loss, colitis

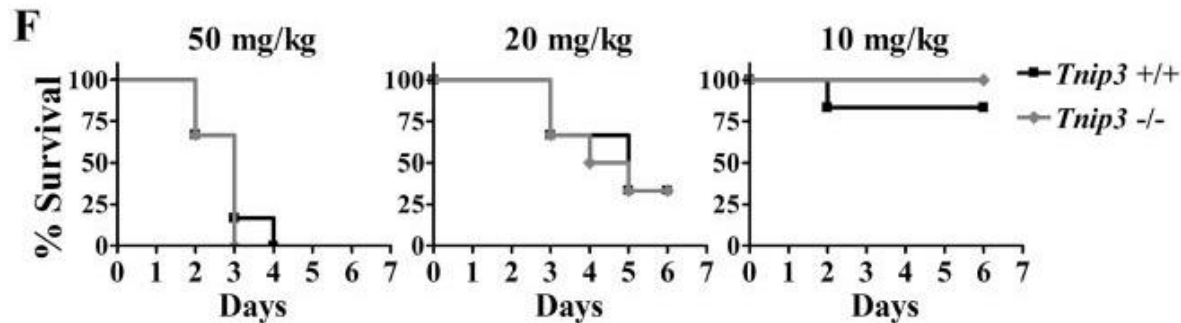


Outlook: therapy of MS or IBD by reduction of IFIT2 expression

ABIN-3: a molecular basis for species divergence in interleukin-10-induced anti-inflammatory actions.

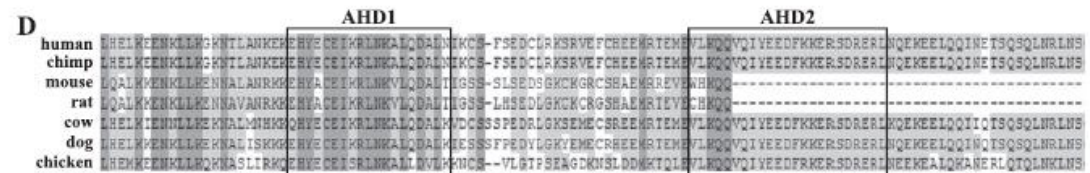
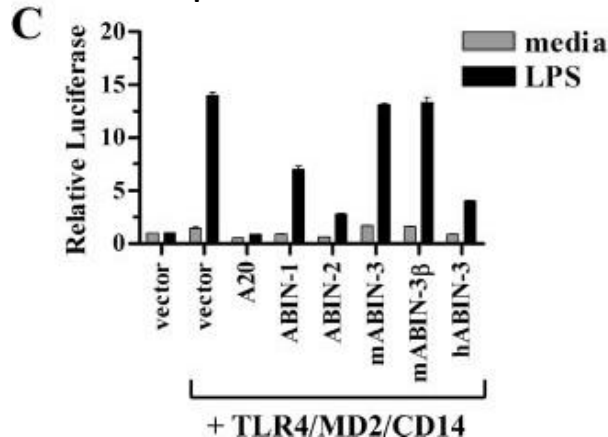
Weaver et al 2007 MCB

Hypothesis: LPS induces IL-10; IL-10 induces ABIN3 and promotes inhibition of TNF- α secretion (anti-inflammatory action)



ABIN-3

NF-Kb promoter luciferase reporter



AHD2 domain critical for ABIN3 function, lacks in mice

Conclusion: mouse results cannot always be transferred to human system

Simulation of humans- humanized mice

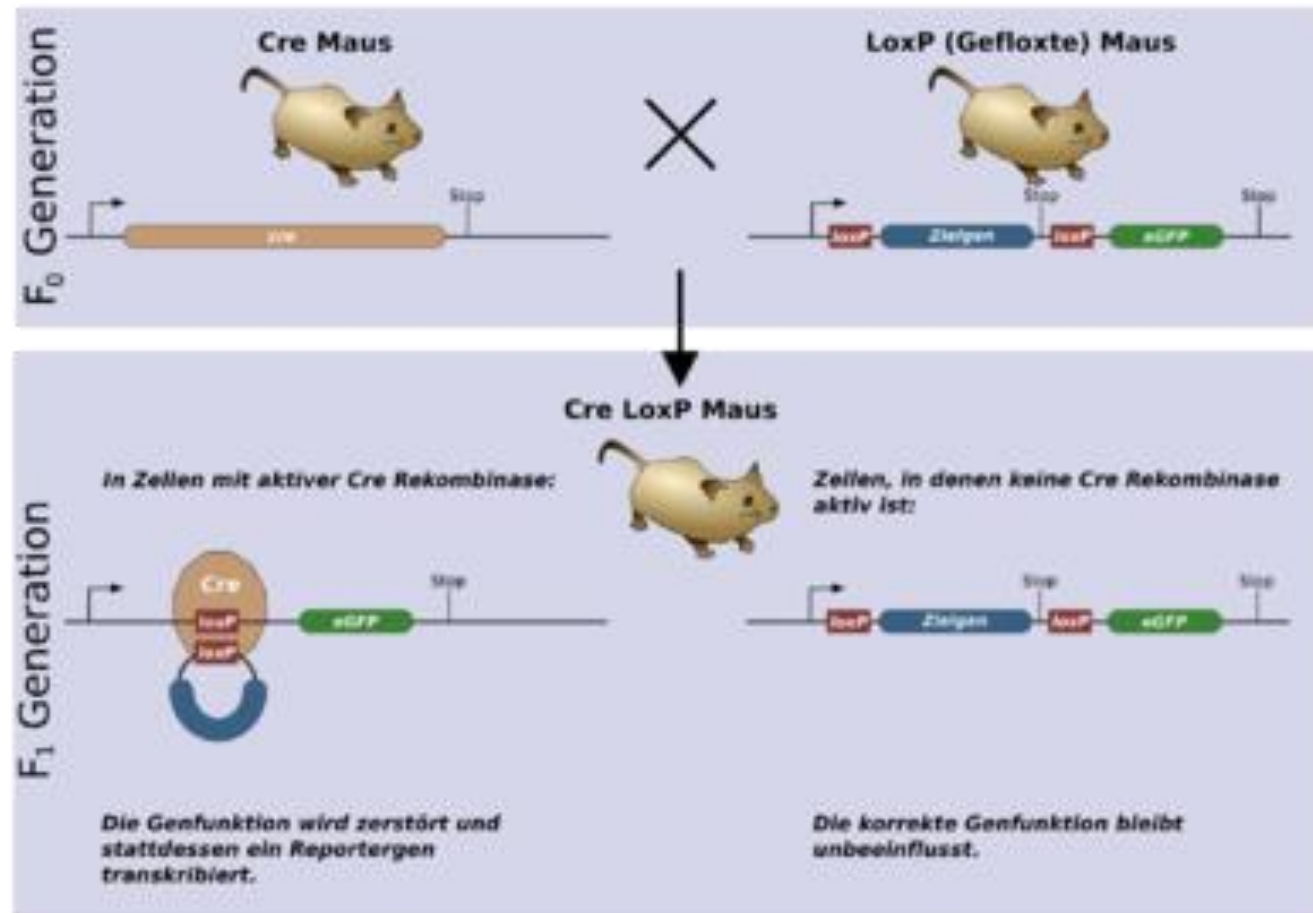
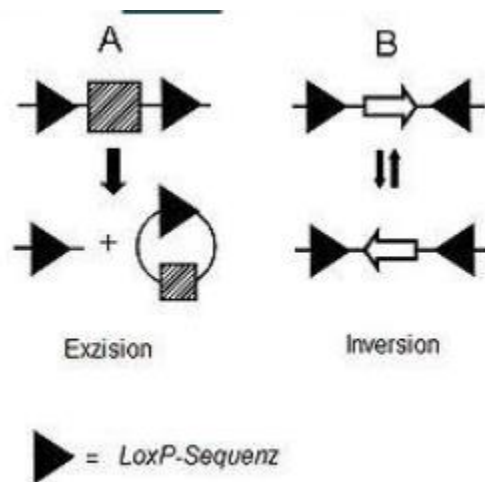
Mice engrafted with human cells or transgenic expression of human gene

Model	Approach	Characteristics	Use
Hu-PBL-SCID (Human Peripheral Blood Lymphocyte)	Engraftment of mature PBMC obtained from blood, spleen or lymph nodes	Predominately engrafts human CD3 T cells. Study of mature T cell function	Study of mature immune cell function
Hu-SRC-SCID (Human Scid Repopulating Cell)	Engraftment of hematopoietic stem cells	Develops human hematopoietic and naïve immune systems	Study of complete hematopoietic system and naïve immune system
SCID-Hu	Engraftment of human fetal liver and thymus	Develops human hematopoietic and immune systems	Study of complete hematopoietic system and naïve immune system
Hu-Tg Mice	Transgenic expression of human genes	Expresses specific human genes <i>in vivo</i>	<i>In vivo</i> study of human gene function

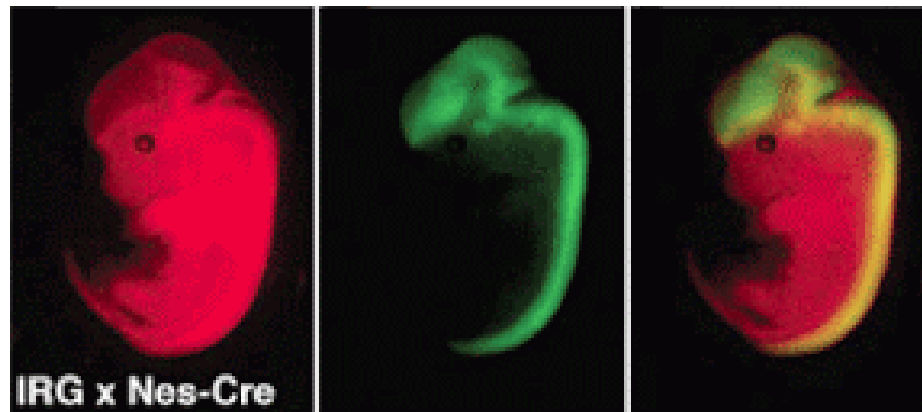
Cre-Mice und floxed mice – conditional knockouts

Aim: in case that null mice are embryonic lethal
or tissue specific expression of mice

Expression of Cre under control of inducible or tissue specific promoter



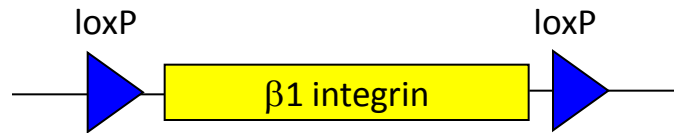
Cre-Mice



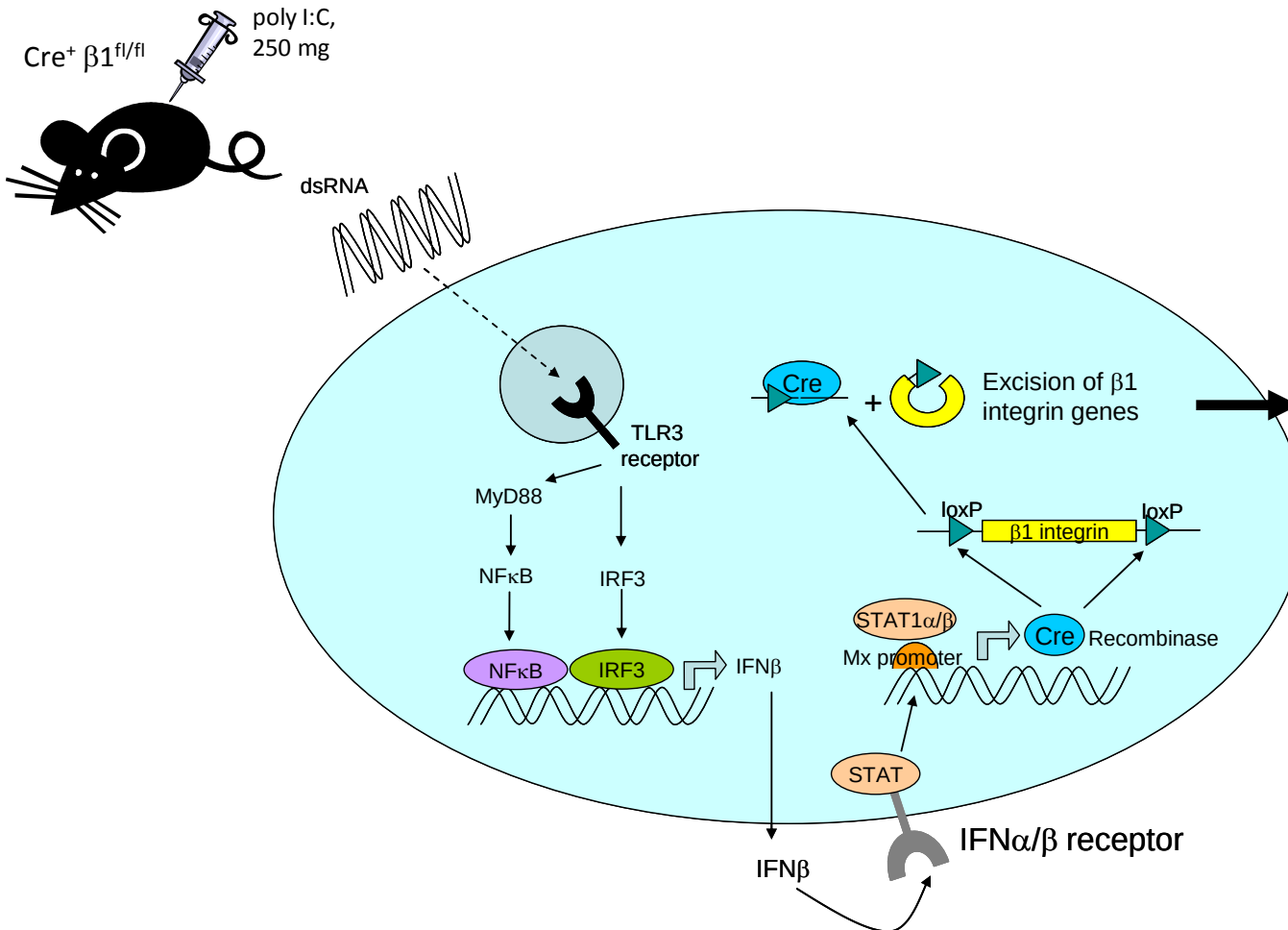
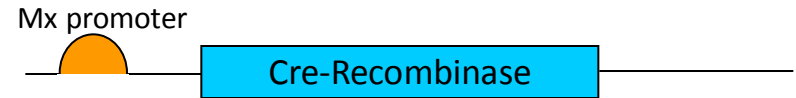
Nestin-Cre: Nerve cell specific expression

Inducible Cre-Promotor

$\beta 1$ integrin floxed mice:



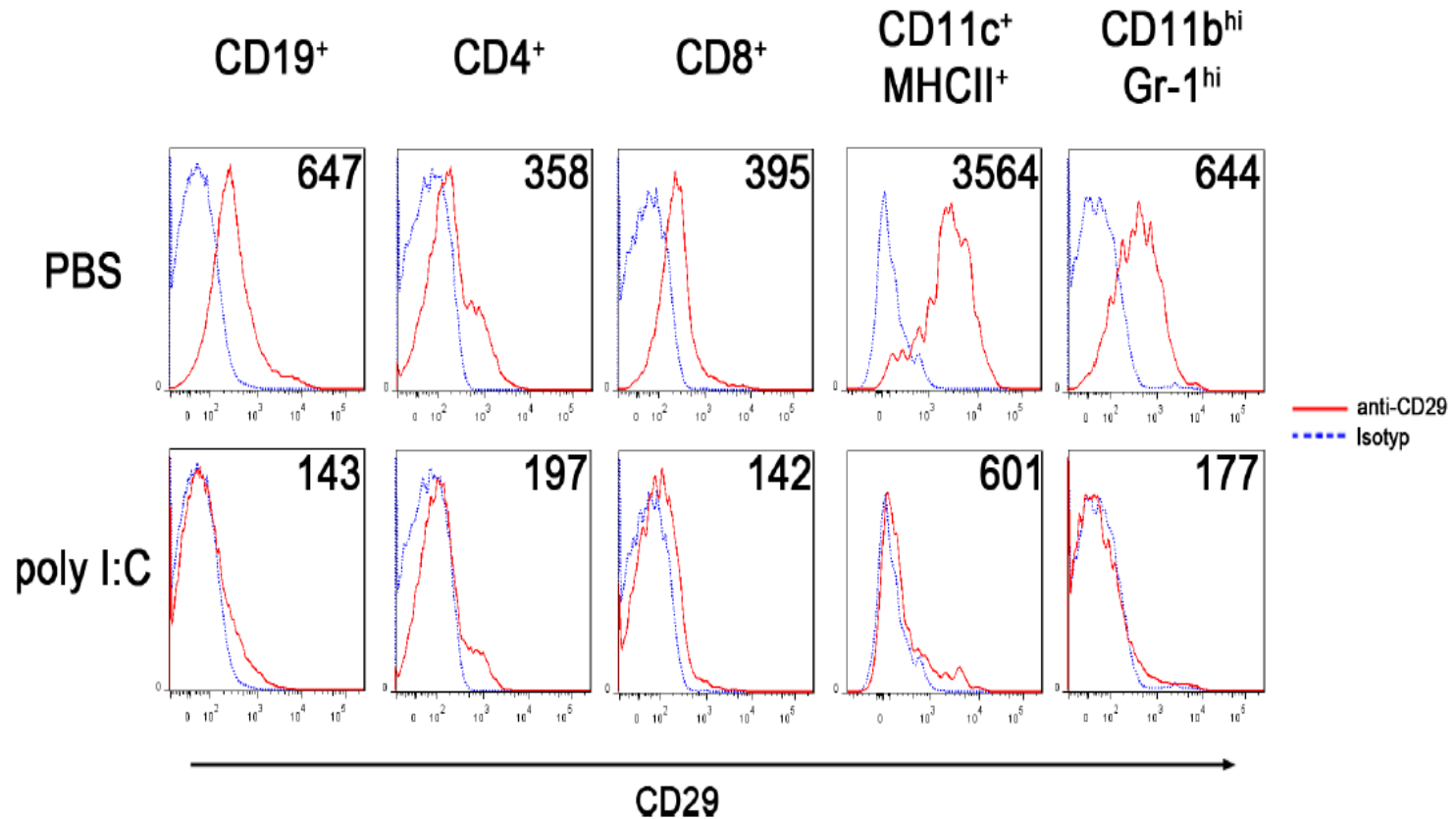
Cre⁺ mice:



No $\beta 1$ integrins on newly derived cells

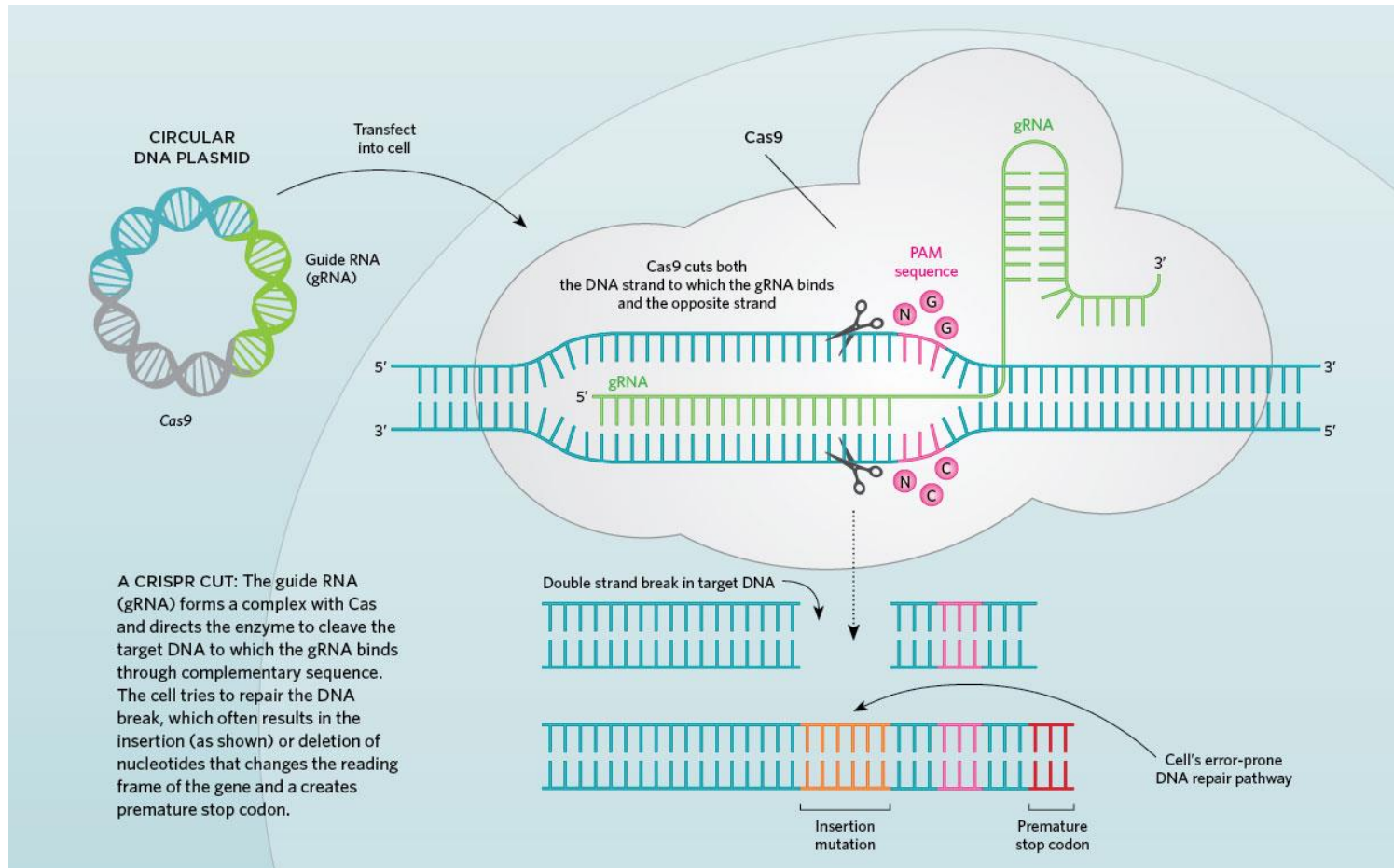
Kühn et. al., 2005

Depletion of $\beta 1$ integrins (CD29) after poly IC treatment



Fast generation of knockout mice using the CAS/CRISPR system

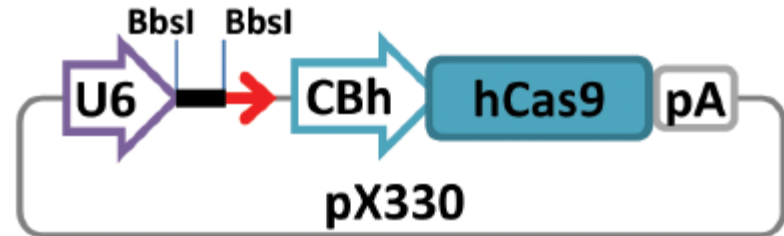
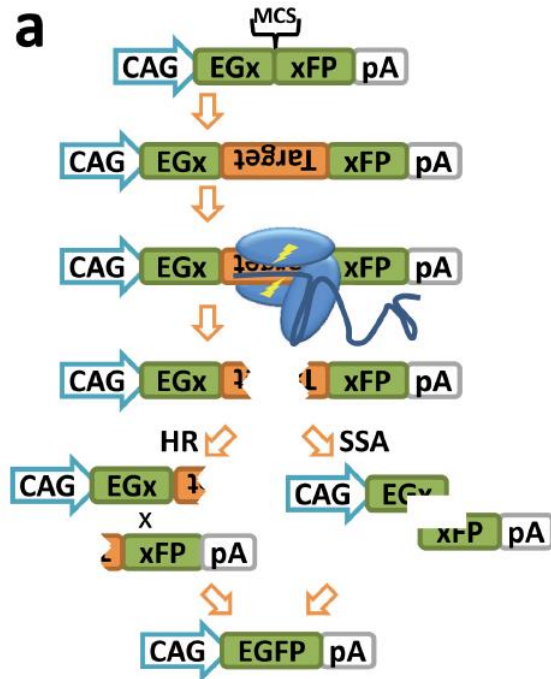
Type II Crispr (clustered regulatory interspaced short palindromic repeat/Cas (Crispr associated system) forms double strand breaks in mammalian cells error-prone NHEJ (non homologous end-joining) leads to targeted gene mutation



Protospacer adjacent motif (PAM)

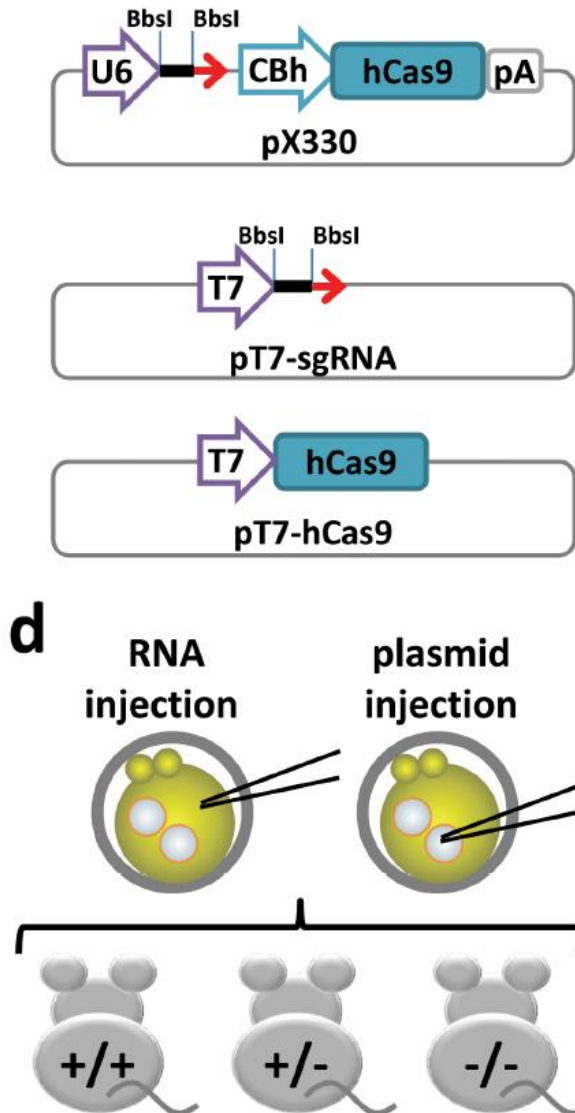
Selection of good target usable for generation of knockout mouse

Transfect vector expressing interrupted gfp including target sequence and vector expressing gRNA and Cas9 into HEK293 cells



Homologous recombination or single strand annealing, reconstruction of gfp

Generation of gene-targeted mice using CRISPR



a

wt: ATGGCGTCCA **CCTTCA** / GGAAGTCAAACGTTGCC TCTACCAGCT

em1: ATGGCGTCCA **CCTTCA** GGAAGTCAAACGTTGCC TCTACCAGCT (0, 1)

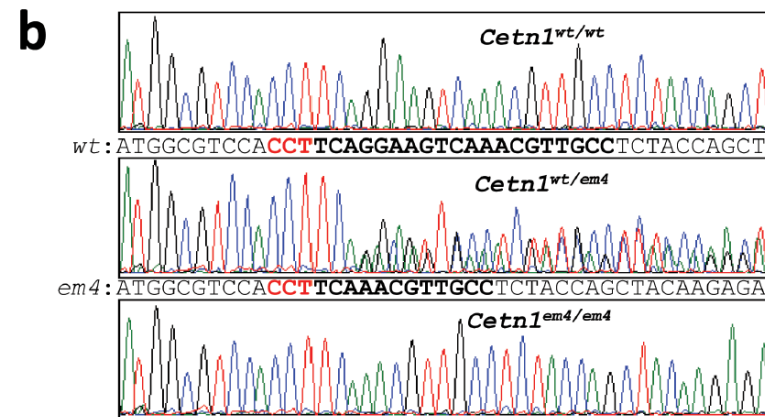
em2: ATGGCGTCCA **CCTTCC** / -GGAAGTCAAACGTTGCC TCTACCAGCT (1, 2)

em3: ATGGCGTCCA **CCTTC** - / GGAAGTCAAACGTTGCC TCTACCAGCT (1, 0)

em4: ATGGCGTCCA **CCTTCA** / ----- AACGTTGCC TCTACCAGCT (6, 0)

em5: ATGGCG ----- / ----- TCAAACGTTGCC TCTACCAGCT (2, 1)

RNA plasmid



Results

Table 1 | Generation of mutant mice via *hCas9* mRNA and sgRNA injection

target	<i>hCas9</i> :sgRNA (ng/uL)	injected	two-cell/survived	GMO/pups	hetero:homo
<i>Cetn1</i>	10:1	67	52/54 (96.3%)	5/20 (25.0%)	5:0
	10:1	72	45/45 (100%)	0/5 (0%)	-
<i>Prm1</i>	10:10	51	33/35 (94.3%)	7/15 (46.7%)	7:0
	40:10	23	15/16 (93.8%)	4/4 (100%)	4:0

hCas9 mRNA and sgRNA were mixed and injected into mouse zygotes. The injected eggs were transferred into pseudopregnant females. The mutations were identified by sequencing PCR amplified 0.5 kbp genomic fragment containing target in the center. GMO; gene modified organism.

Table 2 | Generation of mutant mice via pX330 plasmid injection

target	pX330 (ng/uL)	injected	two-cell/survived	GMO/pups	hetero:homo
<i>Cetn1</i>	1	91	63/73 (86.3%)	4/10 (40.0%)	4:0
	5	111	61/67 (91.0%)	10/17 (58.8%)	4:6
<i>Prm1</i>	1	31	10/14 (71.4%)	0/3 (0%)	-
	5	26	9/18 (50.0%)	2/3 (66.6%)	2:0

pX330 plasmids containing different sgRNA were injected into mouse zygotes. The injected eggs were transferred into pseudopregnant females. The mutations were identified by sequencing PCR amplified 0.5 kbp genomic fragment containing target in the center. GMO; gene modified organism.

Fast and efficient method

Disadvantage: possible off target effects (mutation of other genes)

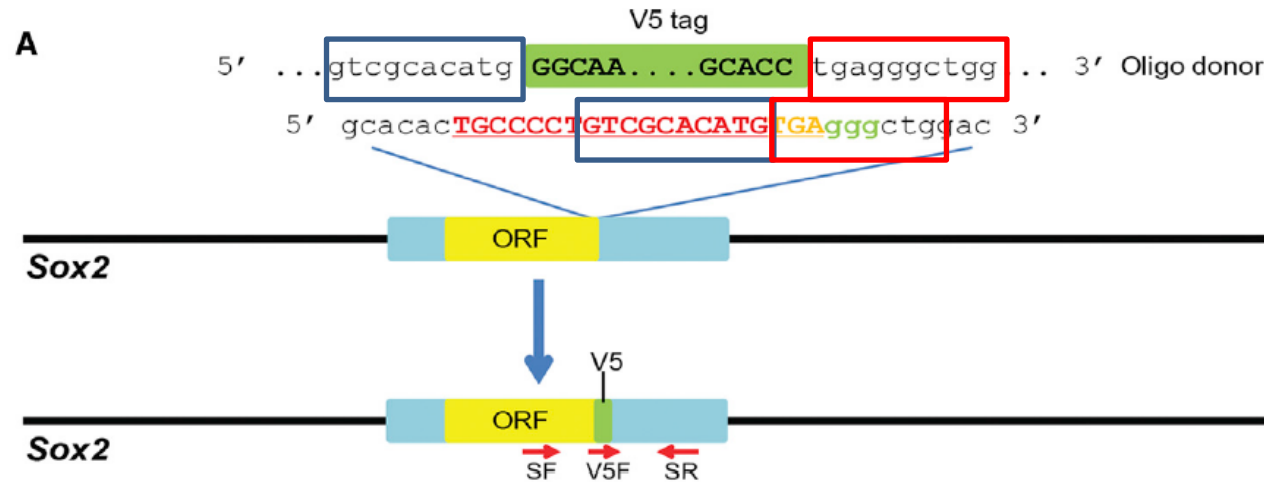
Insertion of a tag into a gene using a single step method- CRISPR

Yang et al Cell 2013

Oligo DNA with 60 bp flanking V5 tag homologous to sequence flanking DSBs
gRNA

Cas9 mRNA

Inject into zygotes



414/498 injected blastocytes

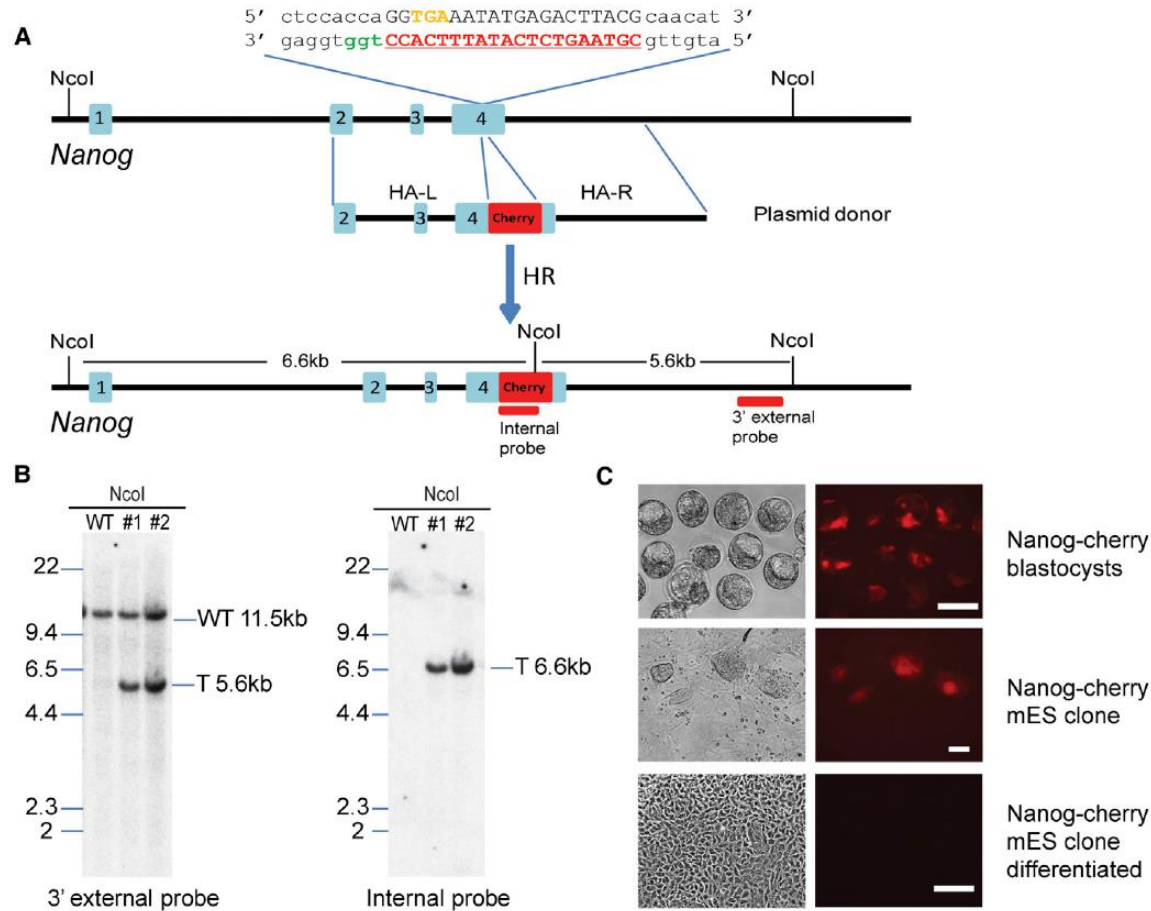
Targeted ES cells: 7/16

Knockin: 12/35

Insertion of a marker gene in an endogenous locus - CRISPR

Insertion of longer gene fragments like a cherry reporter

Injection of CAS9, guide RNA and Plasmid donor in blastocysts



50% targeted ES cells
10% knockin mice

Helpful tools

[www.mgi](http://www.mgi.org) mouse genomic information

KOMP is a trans-NIH initiative to generate, together with the other members of the [International Knockout Mouse Consortium \(IKMC\)](#), a public resource of mouse embryonic stem (ES) cells containing a null mutation in every gene in the mouse genome. By using [two complementary targeting strategies](#), KOMP produces both conditional and null mutants.

EUCOMM

Forward genetic or phenotypic-driven approaches -Large Scale Mutation Screens

X-ray or ENU screen

ENU: N-ethyl-N-nitrosourea

- Supermutagen“

- Irreversible translocation of ethyl group to nuclein base:

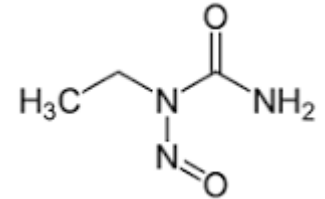
- Alkylation leads to mismatch during replication

- Premeiotic male germ line (extremely potent).

- Average point mutation rate of 1.5 per Mb of genomic DNA

- 1900 genetic variants per genome and about 30 mutations which are coding

- 45% AT to GC transitions, 28% AT to TA transitions



ENU mutagenesis

Makes all classes of mutations:

Null (lack of function)

Hypomorphs (reduced expression)

Antimorphs (a gain of function [mutation](#) that acts in opposition to the normal gene's function).

Neomorphs (a gain of function, novel function)

Hypermorphs (overexpression)

Point mutations often mimic human disease states, yielding highly relevant animal models.

Definition of unsuspected genes

the process of screening requires organization and tenacity.

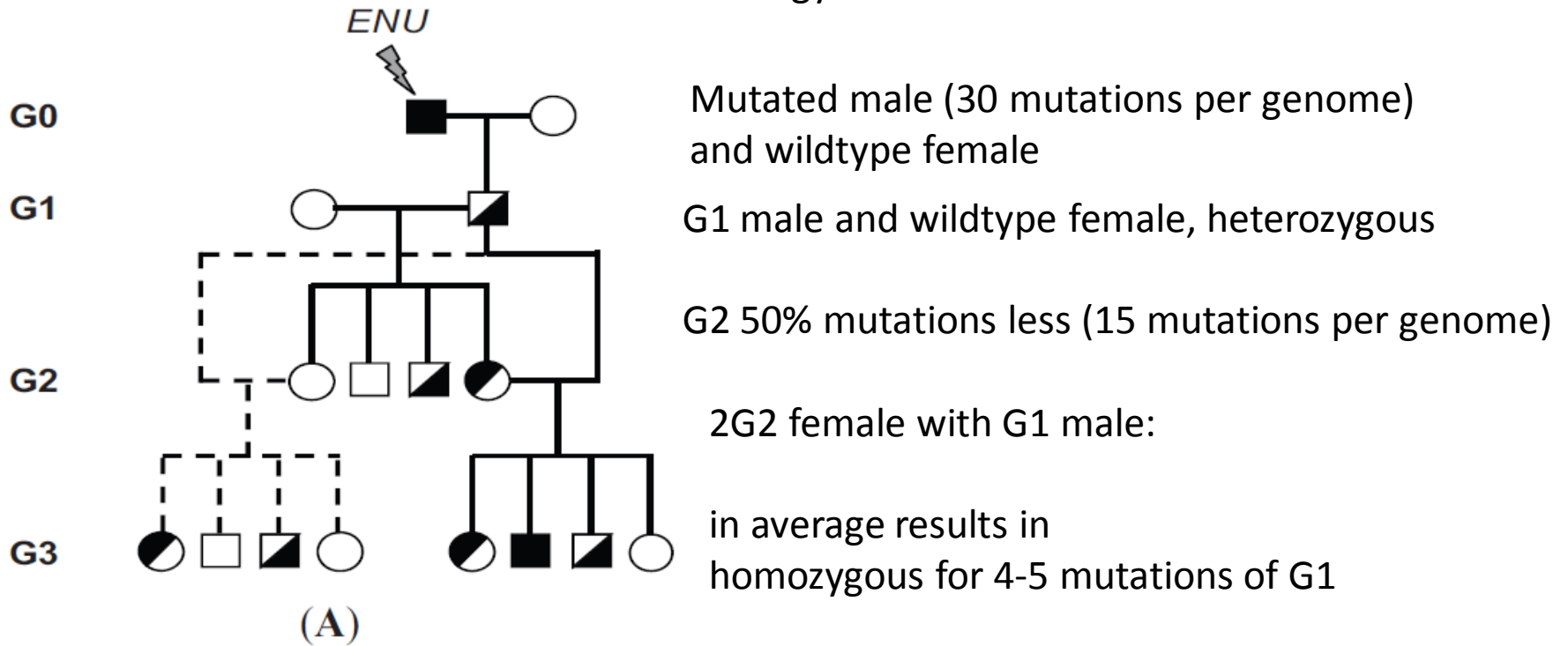
Autosomal recessive 68%

Dominant segregation 23%

X-linked recessive 1%

X-linked dominant 4%

ENU strategy



Mutated male (30 mutations per genome) and wildtype female

G1 male and wildtype female, heterozygous

G2 50% mutations less (15 mutations per genome)

2G2 female with G1 male:

in average results in homozygous for 4-5 mutations of G1

G3 screening for phenotype

If eight to ten G3 animal display a new immune recessive trait:

Perform genome wide scan by next generation sequencing

Examples for ENU screens and identified traits important for immune defense against pathogen

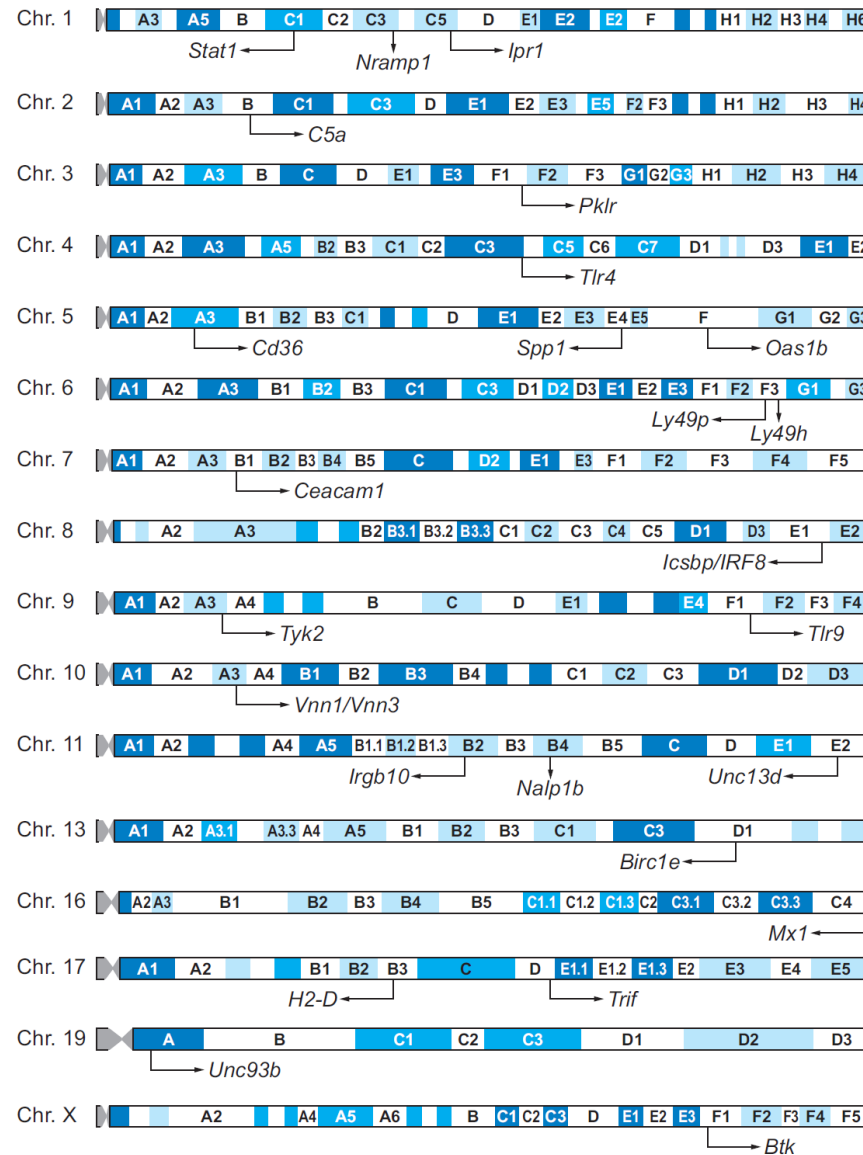
Caignard et al 2014 Genes

Pathway	Gene	Screen	Phenotype	Reference
TLR signaling	<i>Cd36</i>	Immunity→ <i>S. aureus</i>	Susceptible	[165]
	<i>Map3k8</i>	Immunity→Group B streptococcus	Susceptible	[166]
	<i>Ptpn6</i>	Autoimmunity→ <i>L. monocytogenes</i>	Susceptible	[172]
	<i>Tlr9</i>	Immunity→MCMV	Susceptible	[179]
	<i>Trif</i>	Immunity→MCMV	Susceptible	[180]
	<i>Unc93b1</i>	Immunity→MCMV	Susceptible	[181]
	<i>Ikbkg</i>	Immunity→MCMV	Susceptible	[183]
Type I IFN signal	<i>Usp18</i>	<i>S. Typhimurium</i>	Susceptible	[156,159]
	<i>Stat1</i>	MCMV	Susceptible	[182]
Effector	<i>Eif2ak4</i>	MCMV	Susceptible	[184]
	<i>Jak3</i>	<i>P. Berghei</i>	Resistant	[119]
Cellular immunity	<i>Foxn1</i>	<i>P. Berghei</i>	Resistant	[125]
	<i>Stat4</i>	<i>S. Typhimurium</i>	Susceptible	[155,156]
	<i>Tnf</i>	Immunity→ <i>L. monocytogenes</i>	Resistant	[167]
	<i>Gfi1</i>	Immunity→ <i>S. Typhimurium</i>	Susceptible	[170]
	<i>Gimap5</i>	Immunity→MCMV	Susceptible	[194]
	<i>Unc13d</i>	MCMV	Susceptible	[195]
	<i>Flt3</i>	MCMV	Susceptible	[197]
	<i>Slfn2</i>	MCMV	Susceptible	[169]
	<i>Ptprc</i>	HSV-1	Susceptible	[209]
	<i>Ank1</i>	<i>S. Typhimurium</i>	Susceptible	[154]
Red cell cytoskeleton	<i>Ank1</i>	<i>P. Chabaudi</i>	Resistant	[127,128]
Homeostasis	<i>Kcnj8</i>	MCMV	Susceptible	[198]
Lipid metabolism	<i>Scd1</i>	Immunity→ <i>S. Pyogenes</i>	Susceptible	[171]

Table summarizing experiment with 30000 mice

Genetic traits associated with infectious diseases

Many many studies



Alternative models

Galleria melonella (Wax moth)



Galleria melonella as a infection model and for drug screening

Cheap

Larvae can be easily purchased (used for feeding of reptils or fishing)

Larvae: 150-200 mg and 2-3 cm long

High temperature tolerance, tolerate 37°C (temperature of infection)

Easy and rapid injection of bacteria or substances possible

Innate immunity can be studied

Hemocytes function similar to mammalian macrophages and neutralization

Comparison mouse - Galleria

	Mouse	Galleria
Application	Yes	No
Costs	expensive	cheap
Immune system	innate and adaptive	innate
Intestinal tract	similarities	
Cell adhesion	similarities	
Antimicrobial peptides	similarities	
Adaption to oxidative stress	similarities	
High throughput screening e.g. new antibiotics	difficult	easier

Infection of Galleria

A



B



C



D



Figure 1. (A) Automated syringe pump injector. (B) Dead (top, black) and alive (bottom, white) *G. mellonella* larvae. (C) Bacteria are injected in the haemocoel of *G. mellonella* larvae. (D) *G. mellonella* larvae are force-fed with bacterial preparation: the needle enters the mouth of the insect larvae.

A

Time post injection

PBS

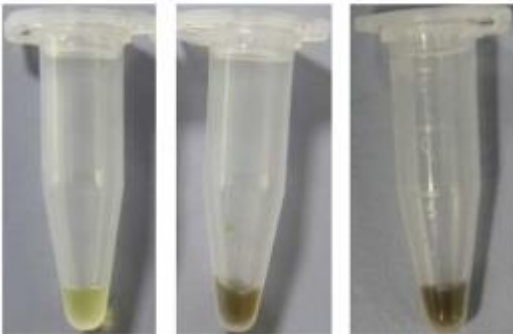
5 h

10 h

Larvae



Haemolymph



E

Melanin production as indication for immune Response

Dead animals are black

Pseudomonas aeruginosa infection - Good correlation between virulence in mice and *Galleria*

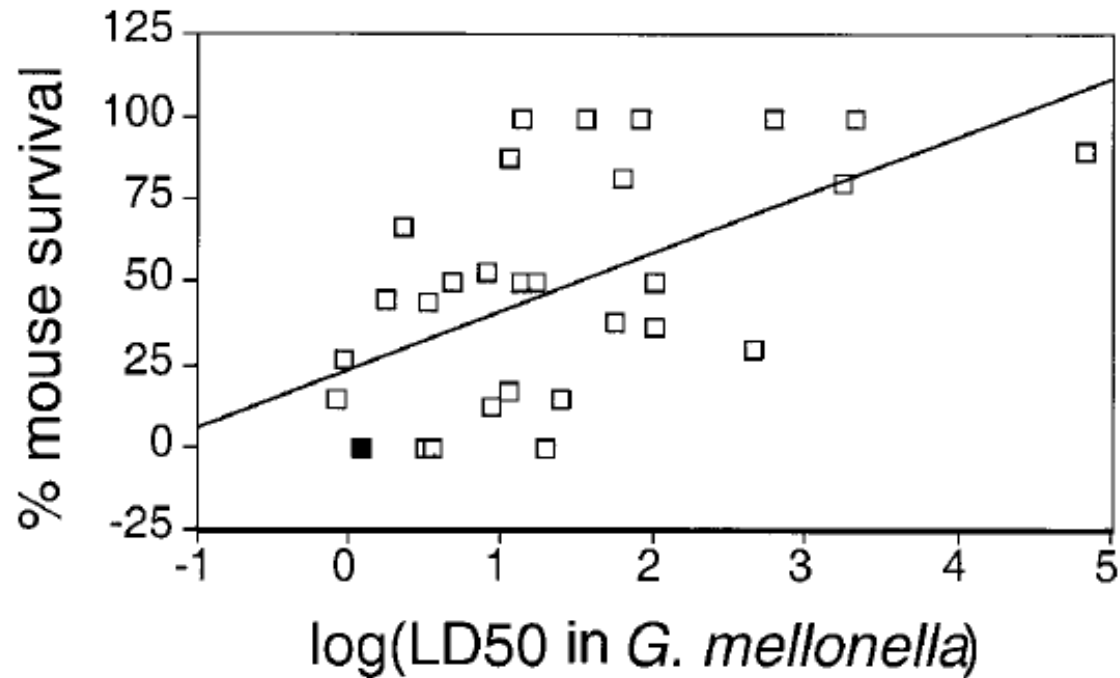


FIG. 3. Comparison of LD₅₀ in *G. mellonella* and percent survival of burned mice after infection with 10⁵ *P. aeruginosa* bacteria. Solid square, PA14; open squares, individual mutants derived from PA14. $y = 17.7 \cdot x + 23.7$, $P < 0.001$, $R = 0.565$.

Conclusion

Cell culture experiments are often limited to simulate complexity of diseases

Therefore: Animal models important to prove initial hypothesis in disease models

But still a mouse and not a human.

Disease models are only models but often helpful

Nevertheless

Three R rule (replacement reduction refinement) should be always in mind

Educational content

1. Animal models – overview
2. Importance of animal models and limitations
3. Basics of genetics of lab animals
4. Basics of breeding conditions
 - 4.1. Gnotobiology of Lab animals
5. Animal models of disease:
 - 5.1. Reverse genetics or gene driven approach
 - 5.1.1 Generation of transgenic and knockout mice and examples
 - 5.1.2 Overcoming limitations: Humainzation of Lab animals
 - 5.1.3 Conditional knockout mice and examples
 - 5.1.4 Novel approaches to manipulate genome of mice
 - 5.2 Forward genetics or phenotypic-driven approaches
 - 5.2.1 Examples of forward genetics
6. Alternative models for mammals, advantages and disadvantages