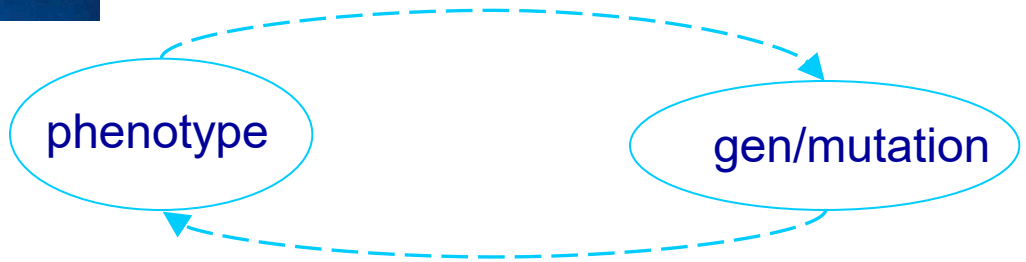
Functional genetics

Functional genetics

Link particular mutations/genes with phenotype

„Forward“ genetics

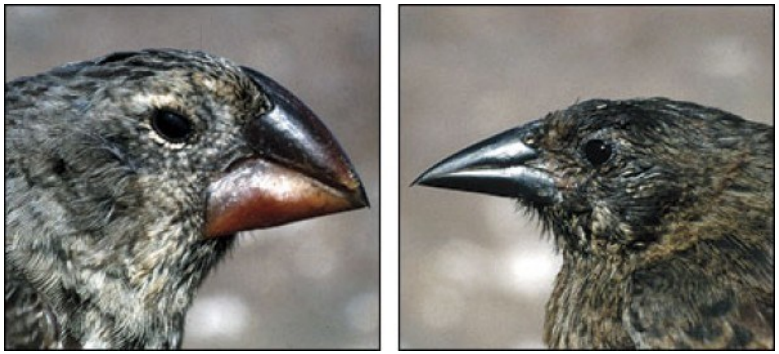


„Reverse“ genetics

Phenotypes

Natural variation in populations

- Adaptations, diseases

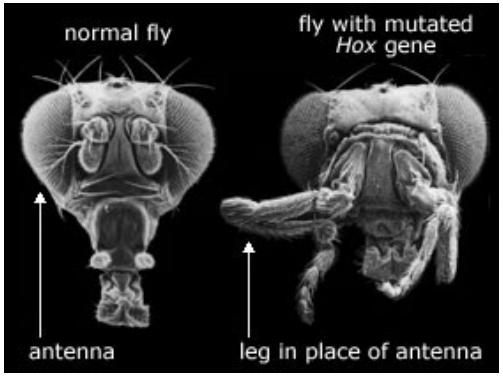


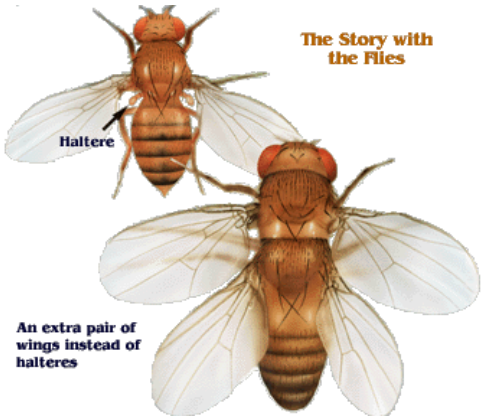
Phenotypes

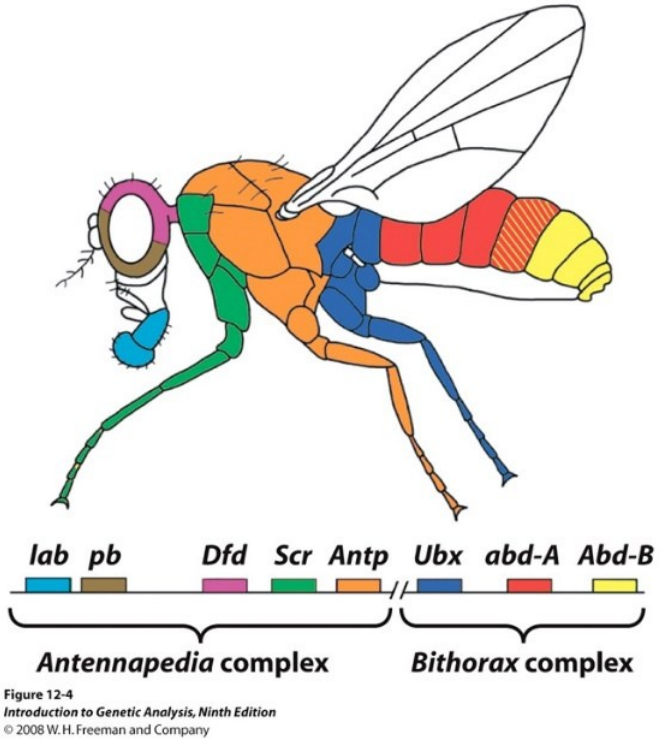
Artificial variation originated by mutagenesis

- random mutagenesis
  - physical mutagens: UV light, X-ray, γ-ray
  - chemical mutagens: ethylnitrosourea (ENU)
  - insertional mutagenesis: transpozons, retroviruses, vectors

Mutations in Hox genes







Forward genetics

How to find particular mutations/genes responsible for the phenotype?

Method of candidate genes

- Analysis of candidate genes possibly associated with the phenotype.
- This method was used in the past to find genes for some diseases (e.g. sickle cell disease) as well as adaptive traits (e.g. coat color in rodents).

Rock pocket mouse, light color in desert, black color on lava flows. Black color due to mutation in melanocortin-1-receptor gene (*Mc1r)* (Nachman et al. PNAS 2003).

Rock pocket mouse (*Chaetodipus intermedius)*

Genetic mapping

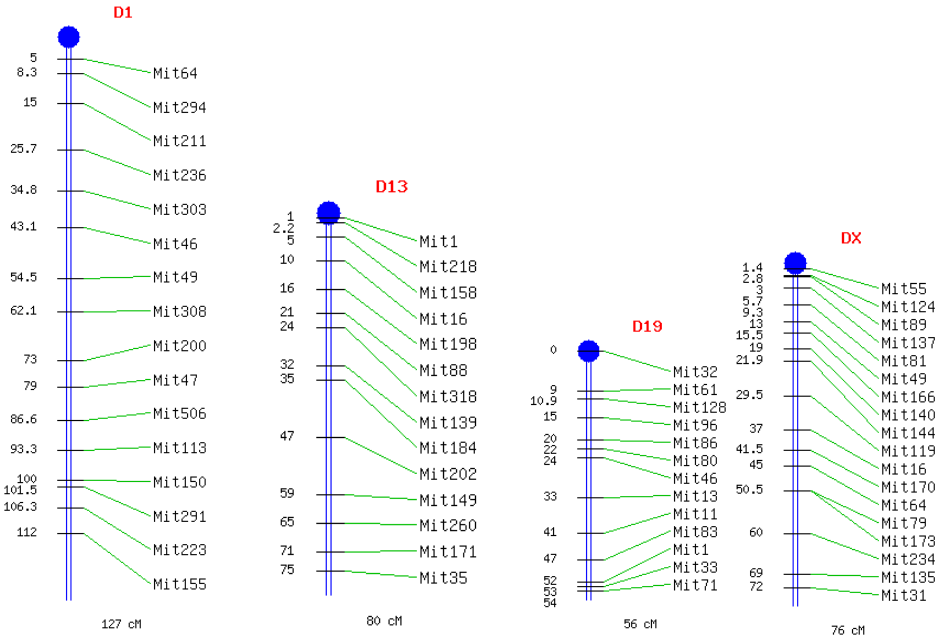
- Aims to find linkage of the phenotype to molecular markers for which we know the possition in the genome (genetic map).

1. Experimental crosses
2. Pedigree analysis
3. Association mapping in natural populations
4. Admixture mapping (in natural hybrid populations)

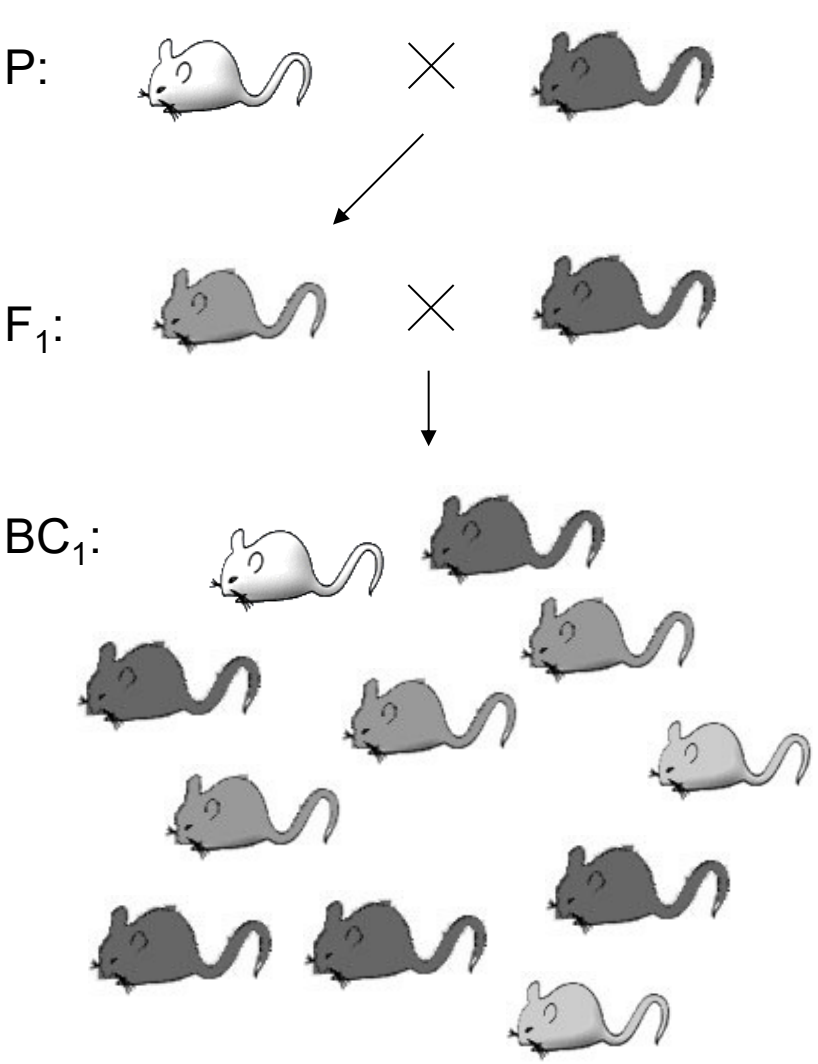
Genetic mapping using experimental crosses

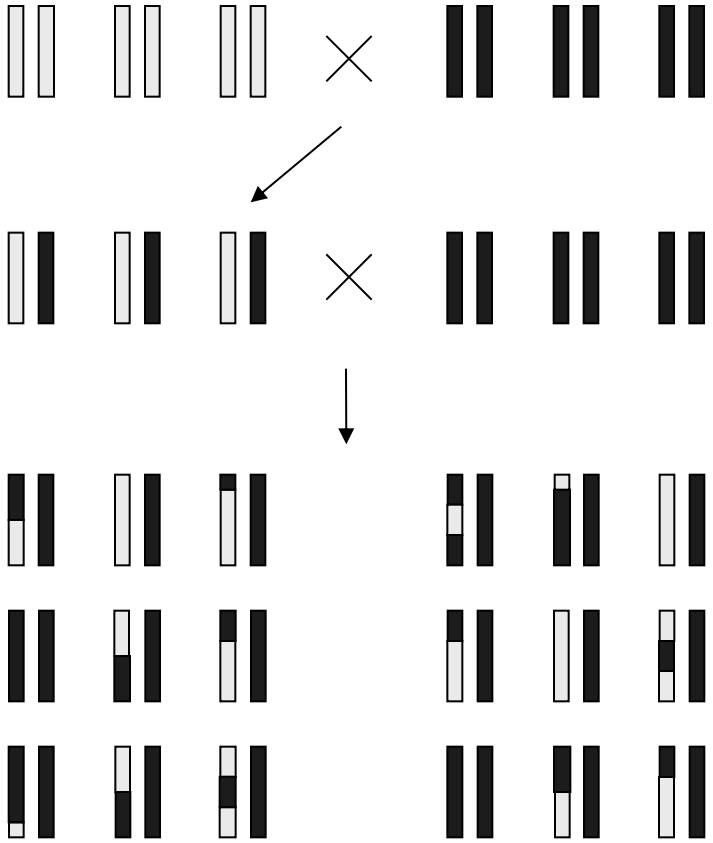
(QTL mapping)

- By crossing two strains/species differing in the studied trait, we will prepare segregating population (např. backcross population or F_2 population).
- Searching for association between the phenotype and molecular markers (polymorphic between the strains/species – SNPs, microsatellites).

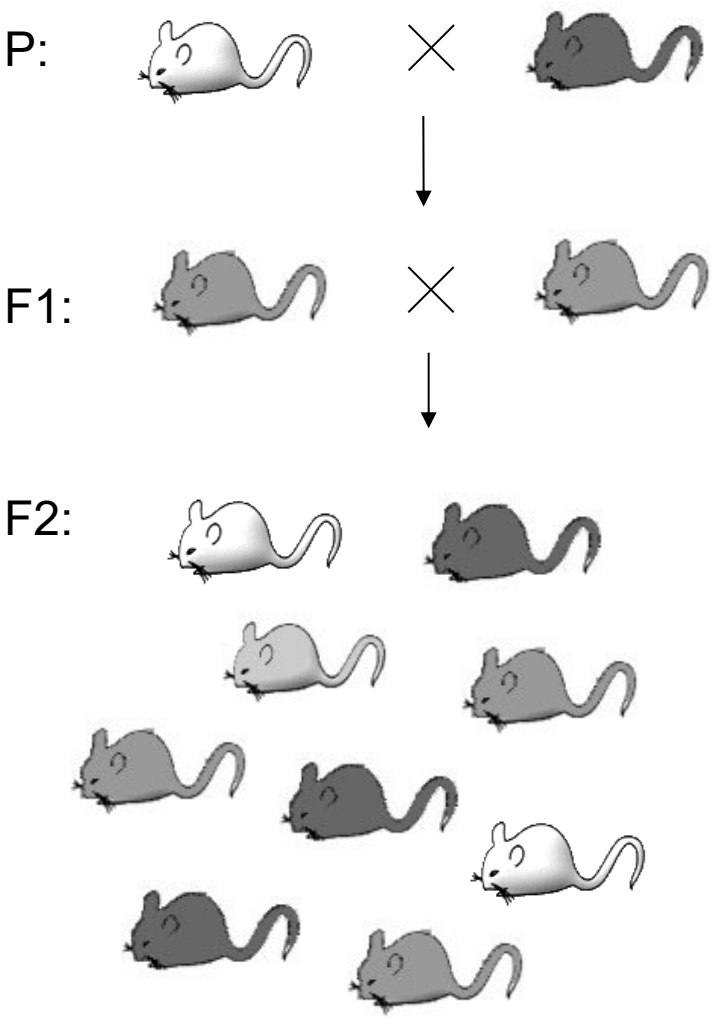


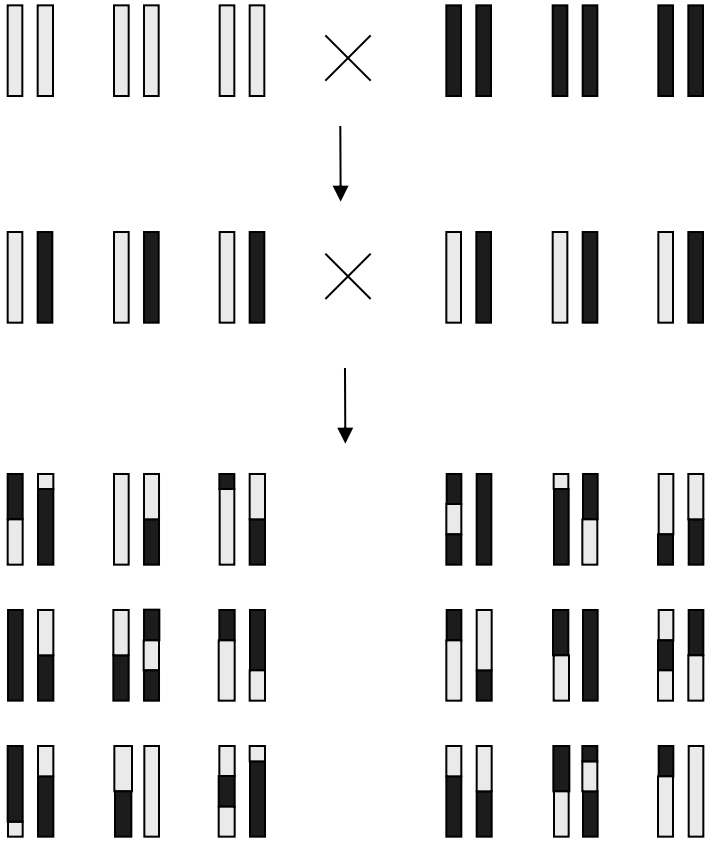
Backcross





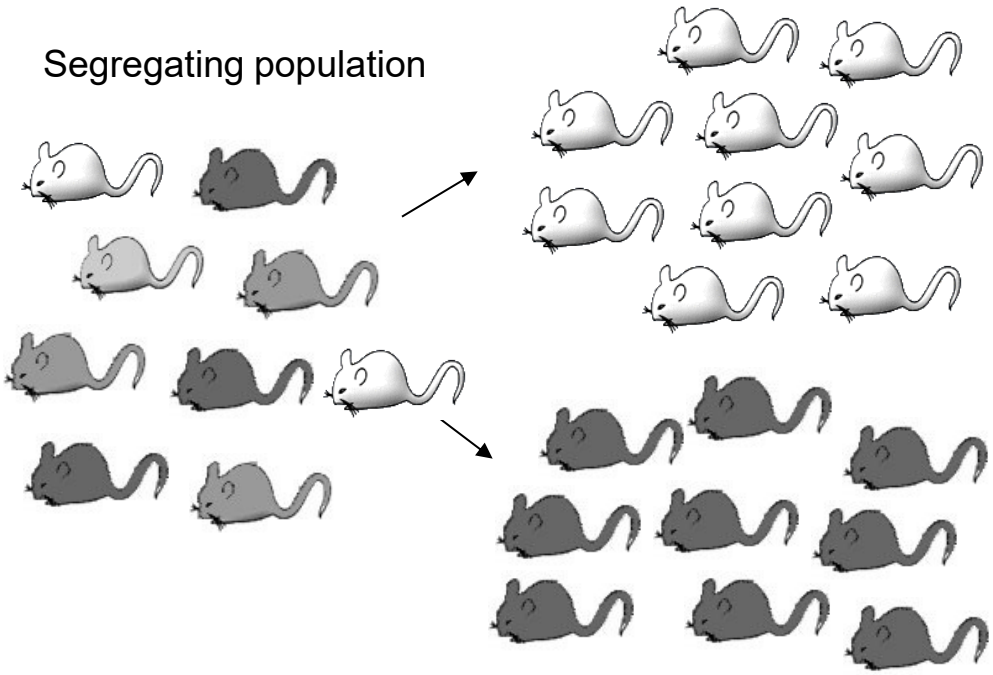
F_2 intercross

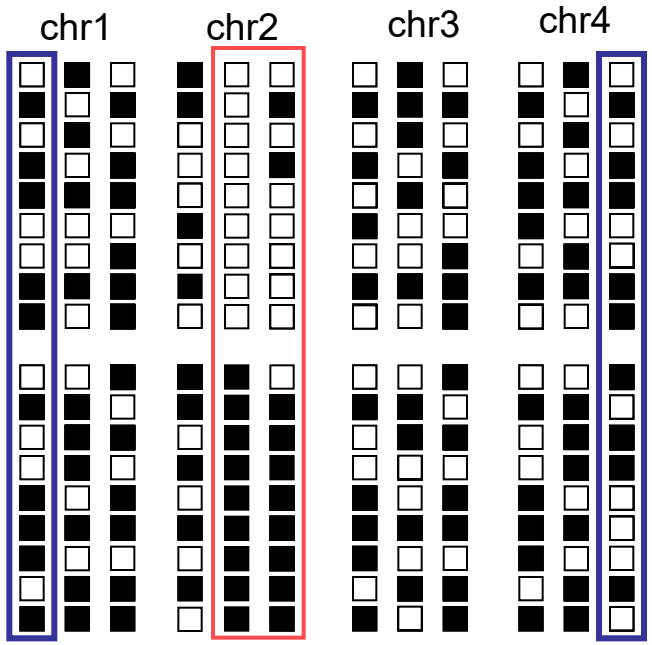




Genetic mapping using experimental crosses

Segregating population



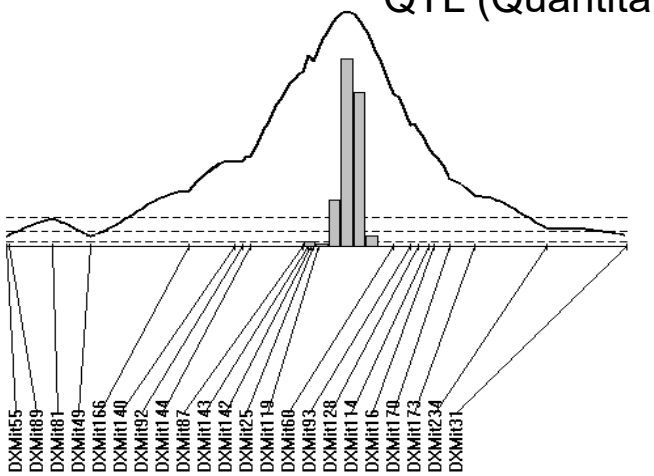


LOD score

$LOD = LOD_a + LOD_i$

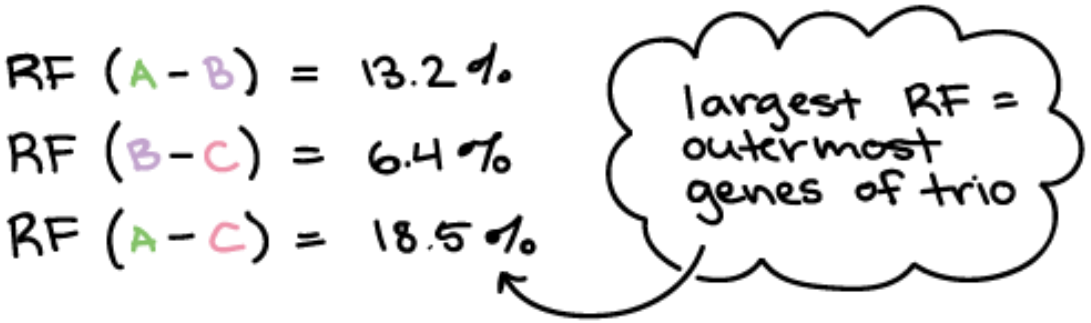
LOD_a additive effects
LOD_i gene interactions

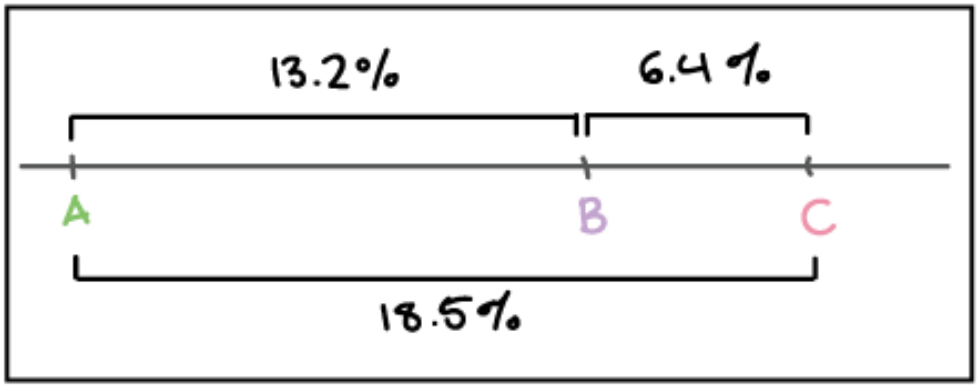
QTL (Quantitative Trait Locus)



How to create genetic map?

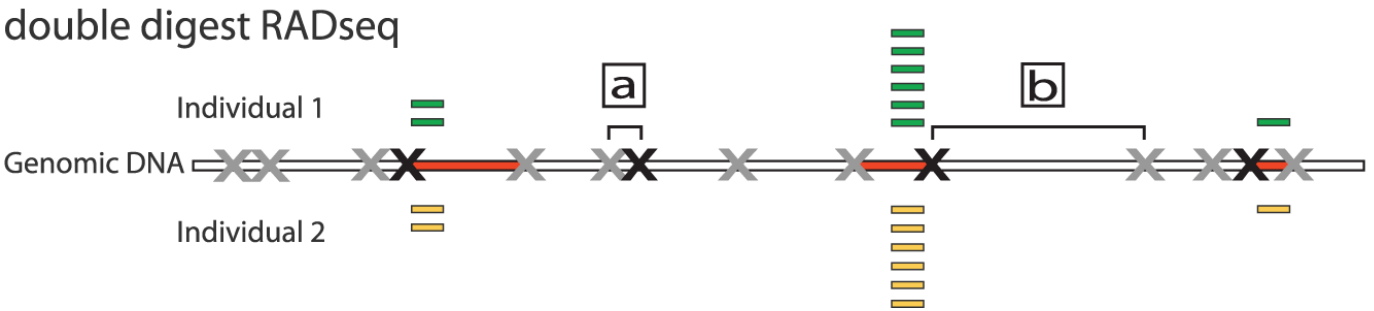
Recombination frequency (1% = 1cM)

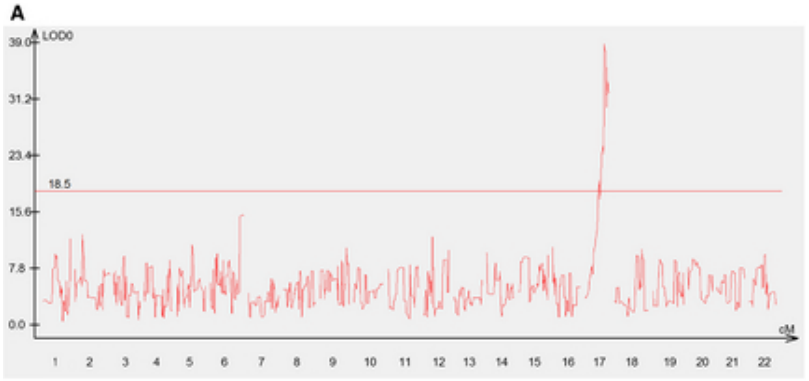


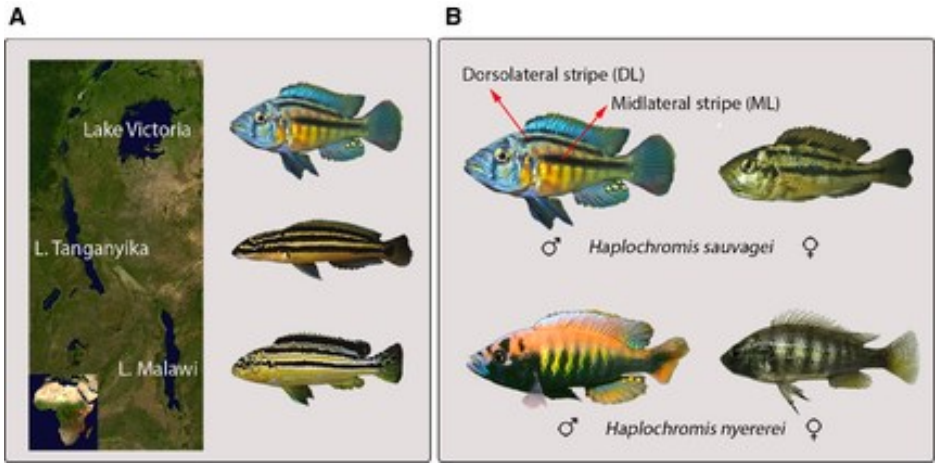


The same cross can be used to create the genetic map and to perform genetic mapping

- Segregating population can be sequenced using for example ddRAD sequencing.





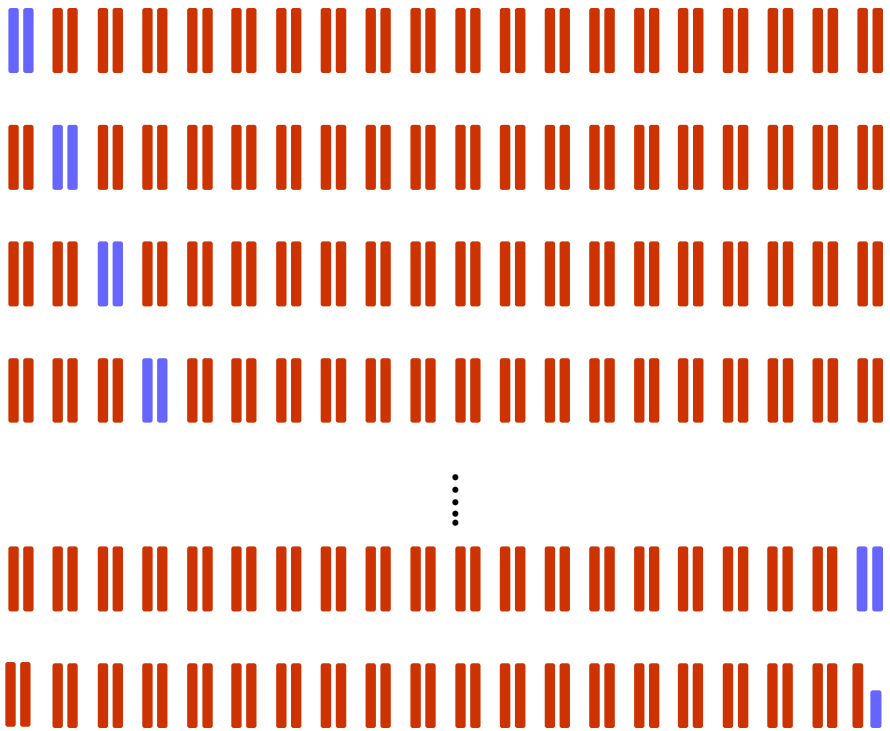


Henning et al. 2014

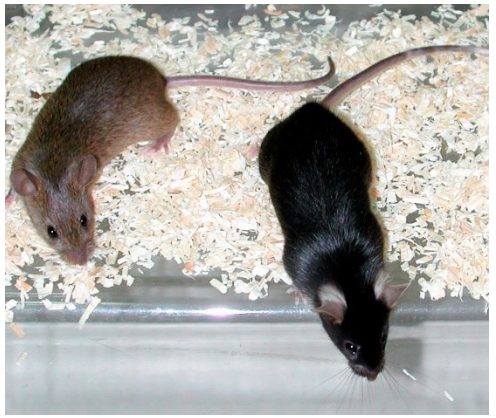
Genetic mapping using experimental crosses

- Preparation of segregating population can take a lot of time in some species.
- Coarse-scale mapping (cca 10 cM).
- We will determine number of loci responsible for the phenotype, their position in the genome and interactions between them. But not the particular genes.

Consomic strains
(Chromosomal substitution strains)



PWD/Ph
(*M.m.musculus*)



C57BL/6J
(M.m.domesticus)

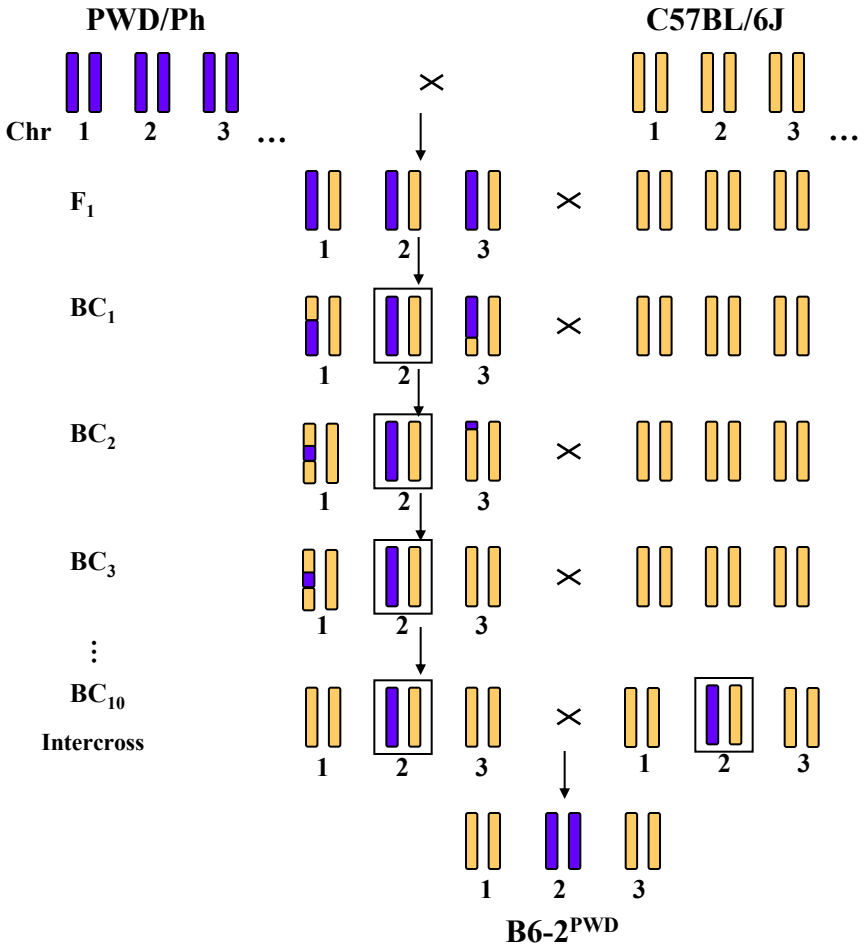
Resource

Mouse consomic strains: Exploiting genetic divergence between *Mus m. musculus* and *Mus m. domesticus* subspecies

Sona Gregorová,[1] Petr Divina,[1] Radka Storchova,[1,3] Zdenek Trachtulec,[1] Vladana Fotopulosova,[1] Karen L. Svenson,[2] Leah Rae Donahue,[2] Beverly Paigen,[2] and Jiri Forejt[1,4]

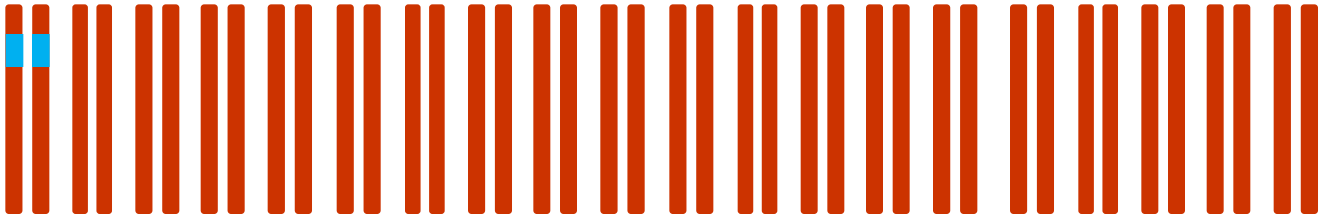
[1]*Institute of Molecular Genetics, Department of Mouse Molecular Genetics, Academy of Sciences of the Czech Republic, Prague 142 20, Czech Republic;* [2]*The Jackson Laboratory, Bar Harbor, Maine 04609, USA*

How to create the chromosome substitution strain



Genetic mapping using consomic strains

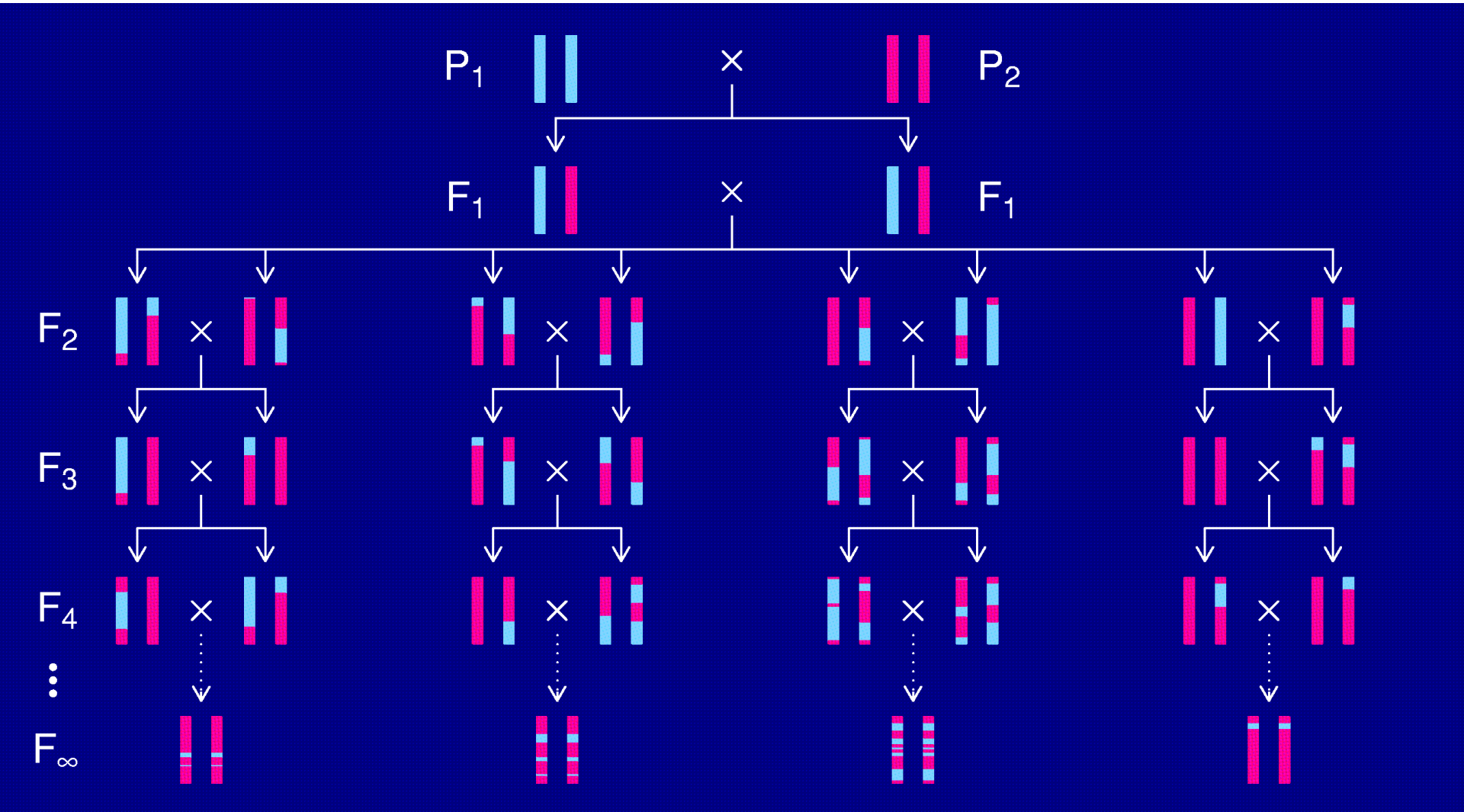
- Construction of strains is time consuming, but once they are available mapping to individual chromosomes is very fast and simple.
- For finner-scalle mapping further crossing is needed.
- From the consomic strain, **congenic strain** can be relatively quickly prepared.



- Similar crossings are used in improvement breeding in agriculture – transfer of a selected train from one species to another

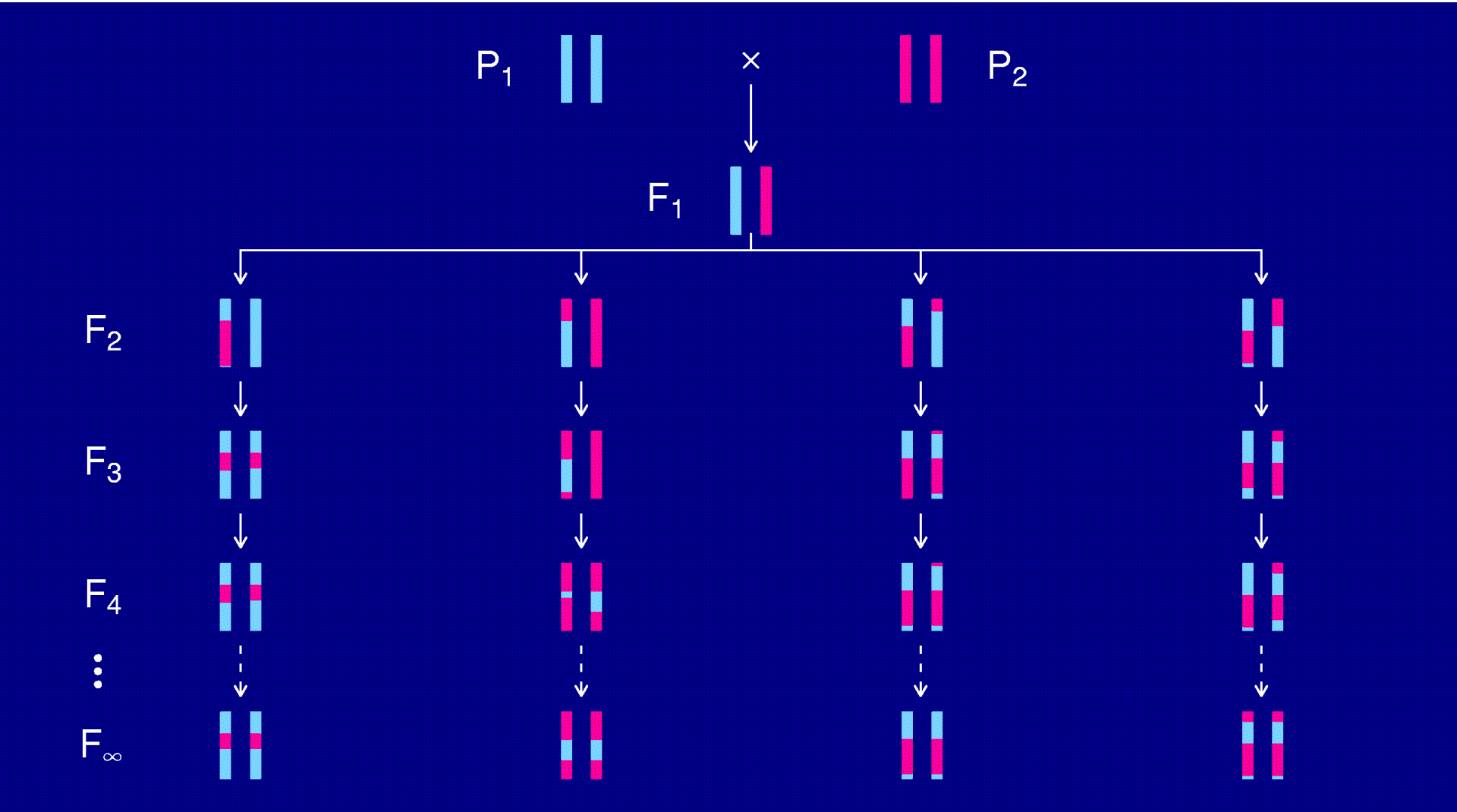
Recombinant inbred lines

- 20 generations of sibling crossing



Rekombinantně inbrední linie

- 10 generation of selfing in plants

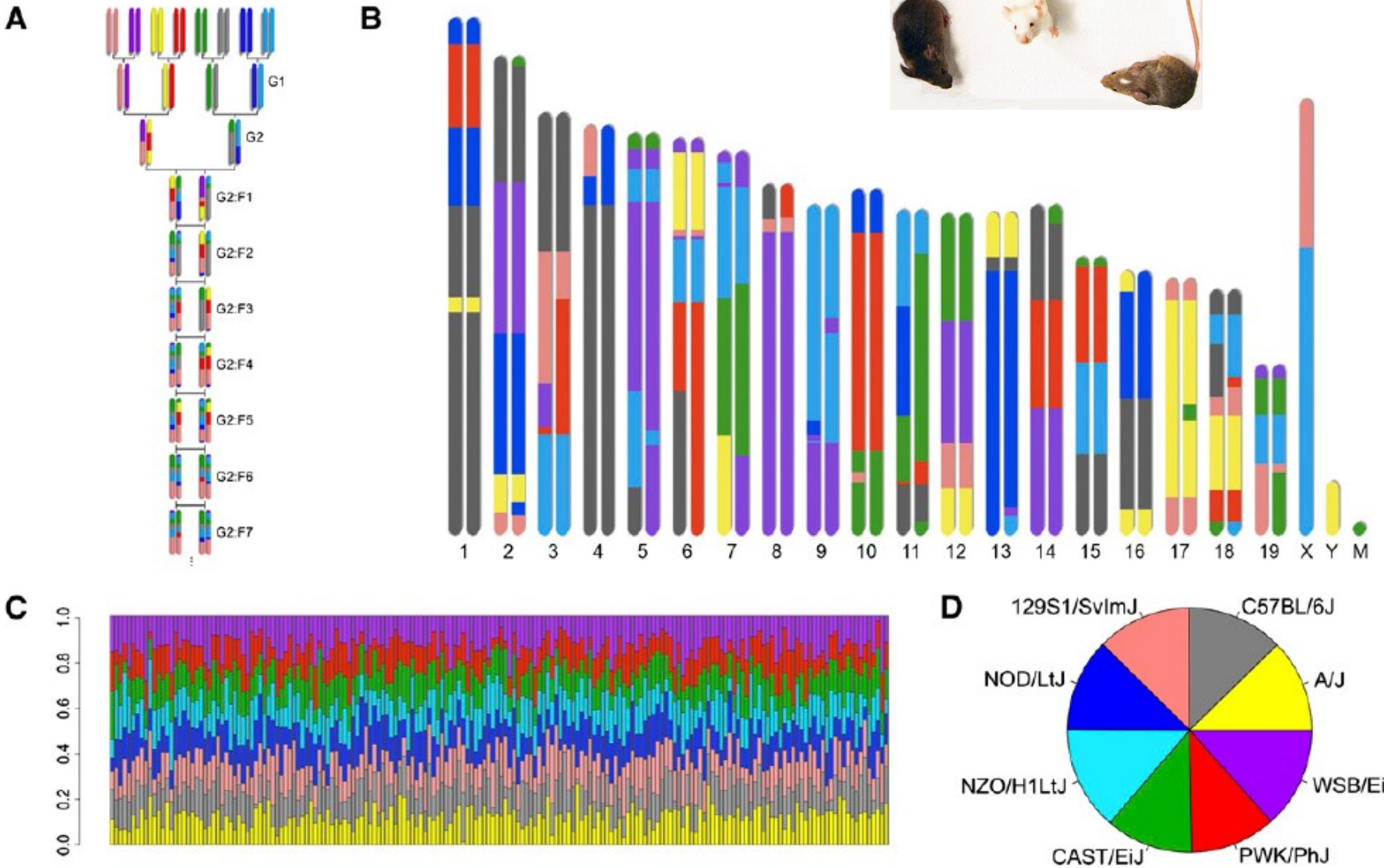


Genetic mapping using recombinant inbred lines

- Construction of strains is time consuming (20 generací křížení), genotyping of the resulting lines, but not during the crossing.
- Once they are constructed mapping is fast and relatively fine-scalle (~1cM or less depending on the number of strains).

Collaborative cross in mice

More strains, more phenotypes



Genetic mapping of white head-spot using collaborative cross

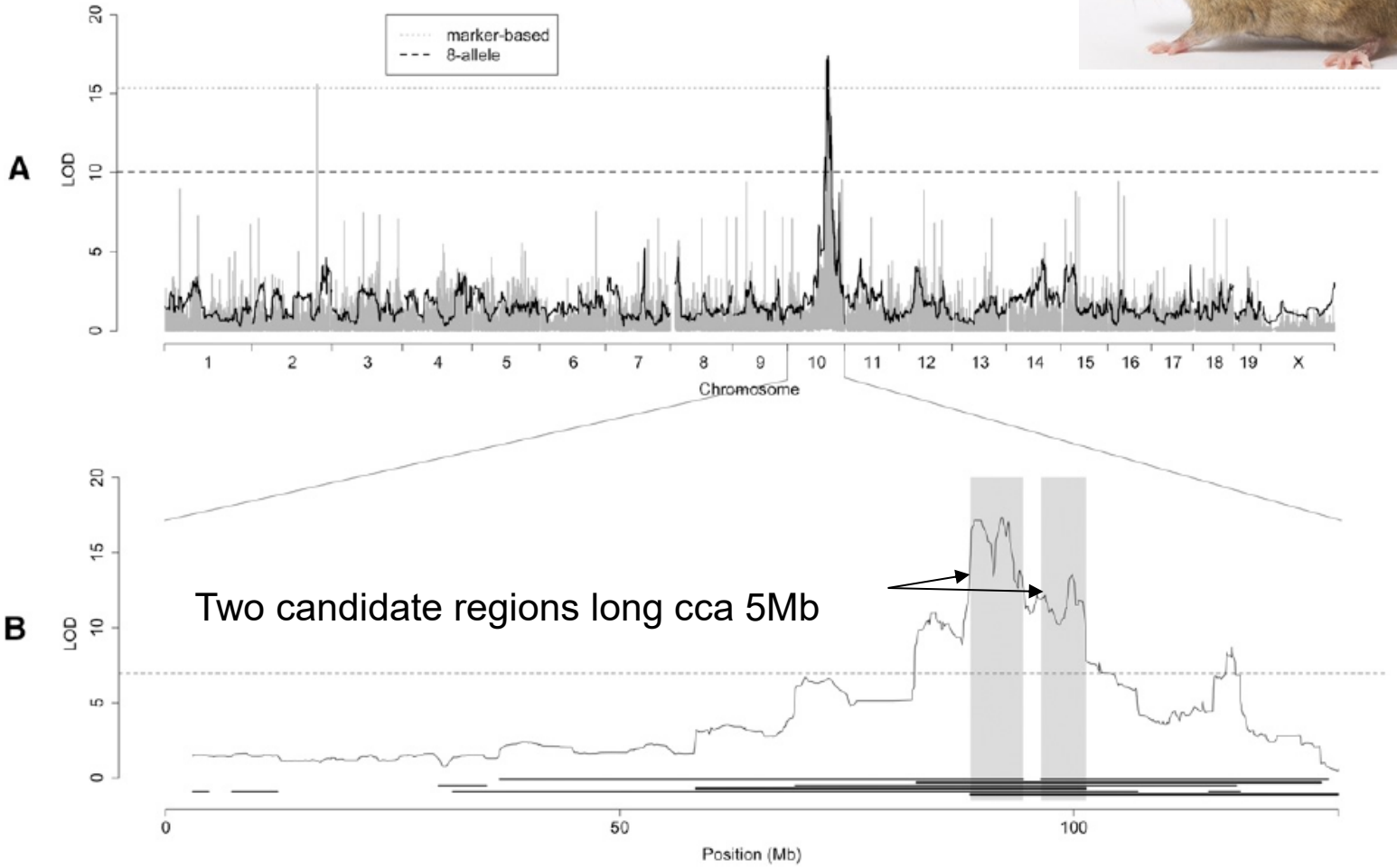
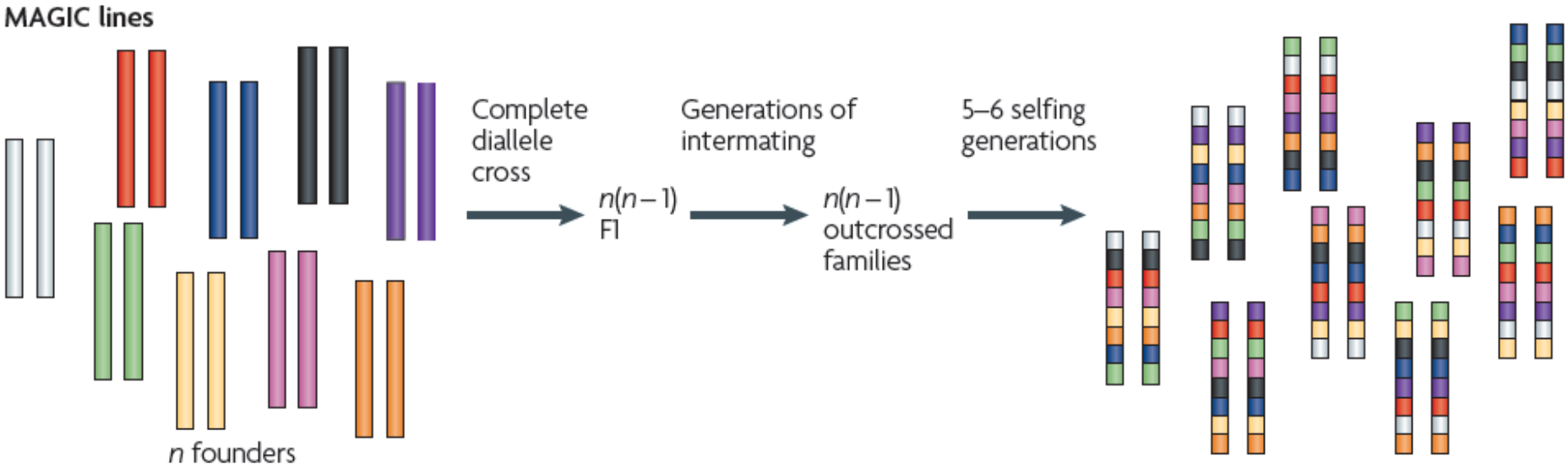


Figure 3. White head-spot genome scan. (*A*) Marker-based (light gray) and eight-allele (black) models implicate an allele on Chr 10. (*B*) Superimposing WSB/EiJ homozygous regions from white head-spotted samples reveals two candidate regions from 88.6 to 94.3 Mb and from 96.4 to 101.3 Mb.

MAGIC lines in *Arabidopsis thaliana*



Animal QTL database

AnimalQTLdb

Release 15
(Sep 22, 2011)

This Animal Quantitative Trait Locus (QTL) database (**AnimalQTLdb**) is designed to house all publicly available QTL data on livestock animal species for easily locating and making comparisons within and between species. The database tools are also added to link the QTL data to other types of genome information, such as RH maps, physical maps, and human genome maps. Besides the QTL data from species listed below, we also plan to apply the tool to other animal species where feasible. JAS, among other journals requires that new QTL data be entered into a QTL database as part of the publication requirements.

Pig QTL
There are **6,347** QTLs from **281** publications curated into the database. Those QTLs represent **593** different traits (see **data summary** for details).

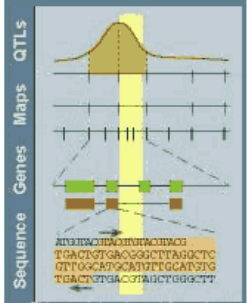
Cattle QTL
There are **4,802** QTLs from **285** publications curated into the database. Those QTLs represent **382** different traits (see **data summary** for details).

Chicken QTL
There are **2,451** QTLs from **125** publications curated into the database. Those QTLs represent **248** different traits (see **data summary** for details).

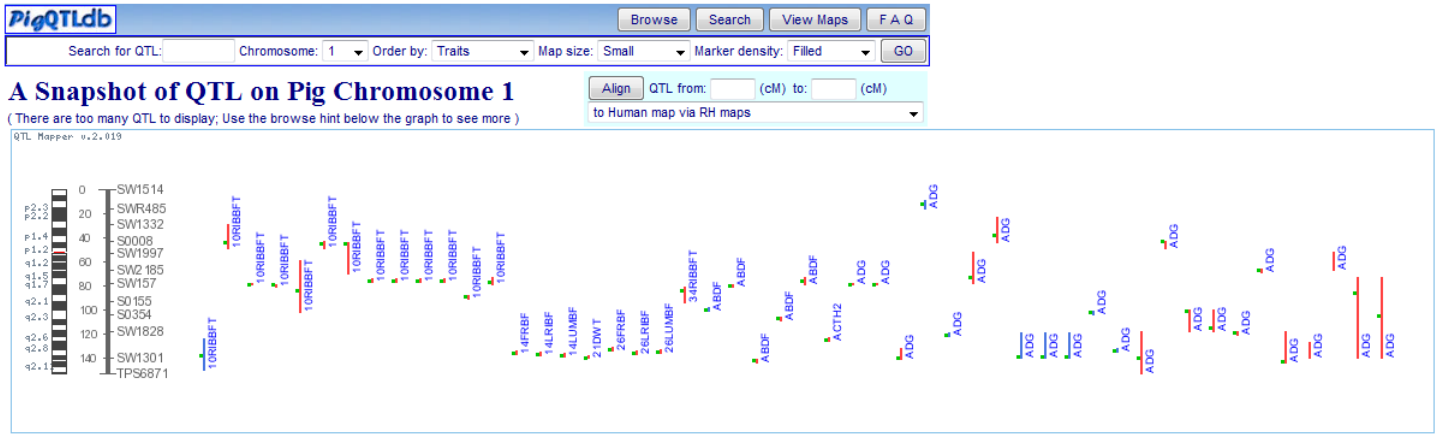
Sheep QTL
There are **639** QTLs from **74** publications curated into the database. Those QTLs represent **184** different traits (see **data summary** for most recent updates).

Database summary:

Publications:	765
Species:	4
Traits:	1,407
QTL:	14,239



This graph is adopted from the Rat GDB with kind permission



Physical mapping and analysis of candidate genes

- Delimitation of the critical region in the DNA sequence
- Annotation of genes in the critical region
- Searching for polymorphism in candidate genes (non-synonymous substitutions, changes in gene expression)
- Experimental confirmation. Transgenes and knock-outs.

např. in mouse 1cM ~ 2 Mb

Genetic mapping and identification of genes for F1 hybrid sterility

PWD/Ph
(*M.m.musculus*)

C57BL/6J
(M.m.domesticus)

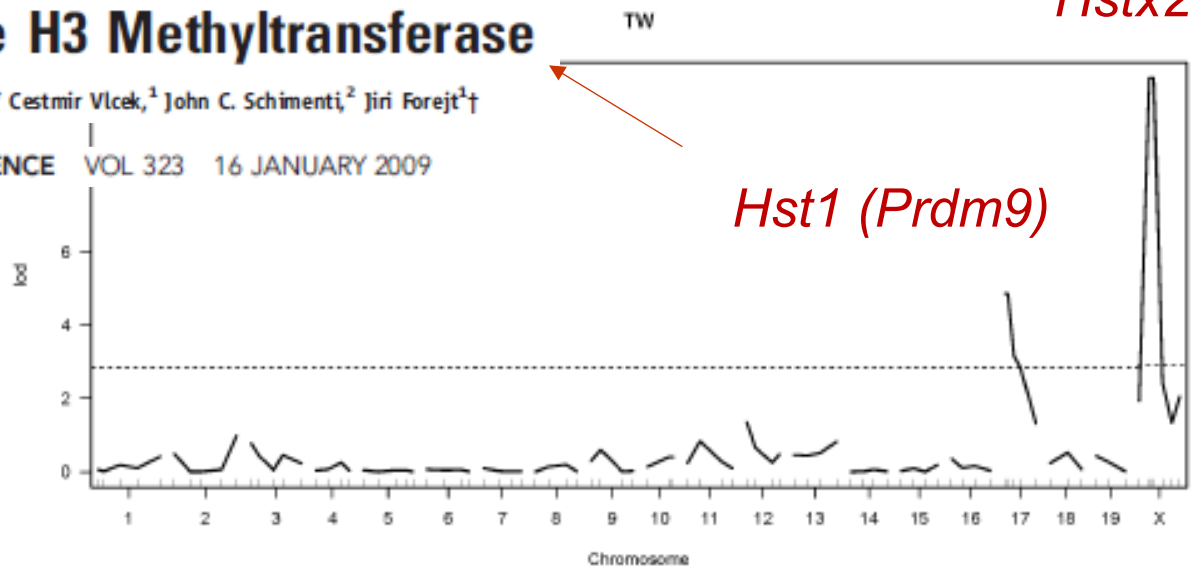
A Mouse Speciation Gene Encodes a Meiotic Histone H3 Methyltransferase

Ondrej Mihola,[1*] Zdenek Trachtulec,[1*] Cestmir Vlcek,[1] John C. Schimenti,[2] Jiri Forejt[1†]

www.sciencemag.org SCIENCE VOL 323 16 JANUARY 2009

Hstx2

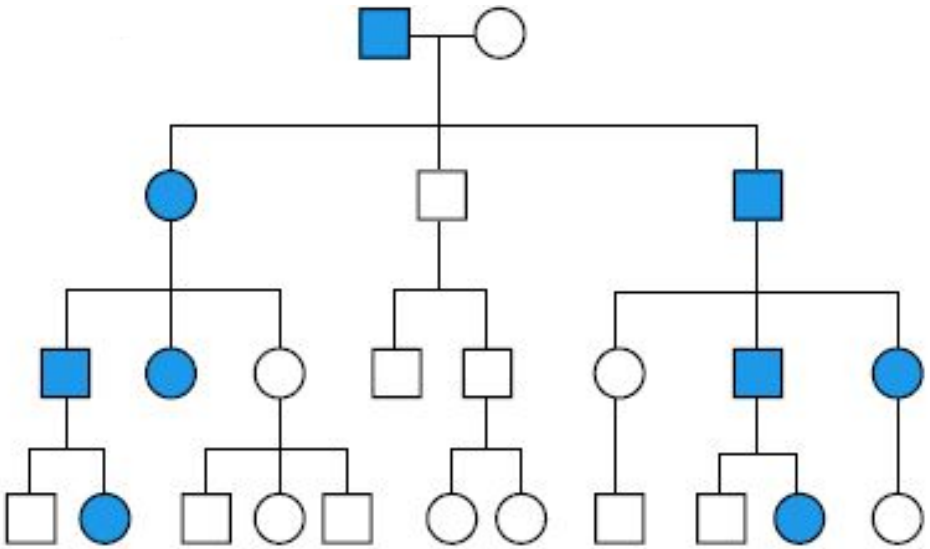
Hst1 (Prdm9)



Dzur-Gejdošová et al. (2012) Evolution

Pedigree analysis

- Pedigrees with a segregating trait (e.g. disease).
- Coase scale mapping (~1-10cM).
- Useful for mapping traits with simple Mendelian inheritance.
- Useful in organisms which cannot be crossed in the laboratory.



Important method from the historical perspective.

Identification of some human disease genes.

Identification of the Cystic Fibrosis Gene: Genetic Analysis

BAT-SHEVA KEREM, JOHANNA M. ROMMENS, JANET A. BUCHANAN, DANUTA MARKIEWICZ, TARA K. COX, ARAVINDA CHAKRAVARTI, MANUEL BUCHWALD, LAP-CHEE TSUI

Approximately 70 percent of the mutations in cystic fibrosis patients correspond to a specific deletion of three base pairs, which results in the loss of a phenylalanine residue at amino acid position 508 of the putative product of the cystic fibrosis gene. Extended haplotype data based on DNA markers closely linked to the putative disease gene locus suggest that the remainder of the cystic fibrosis mutant gene pool consists of multiple, different mutations. A small set of these latter mutant alleles (about 8 percent) may confer residual pancreatic exocrine function in a subgroup of patients who are pancreatic sufficient. The ability to detect mutations in the cystic fibrosis gene at the DNA level has important implications for genetic diagnosis.

Science (1985)

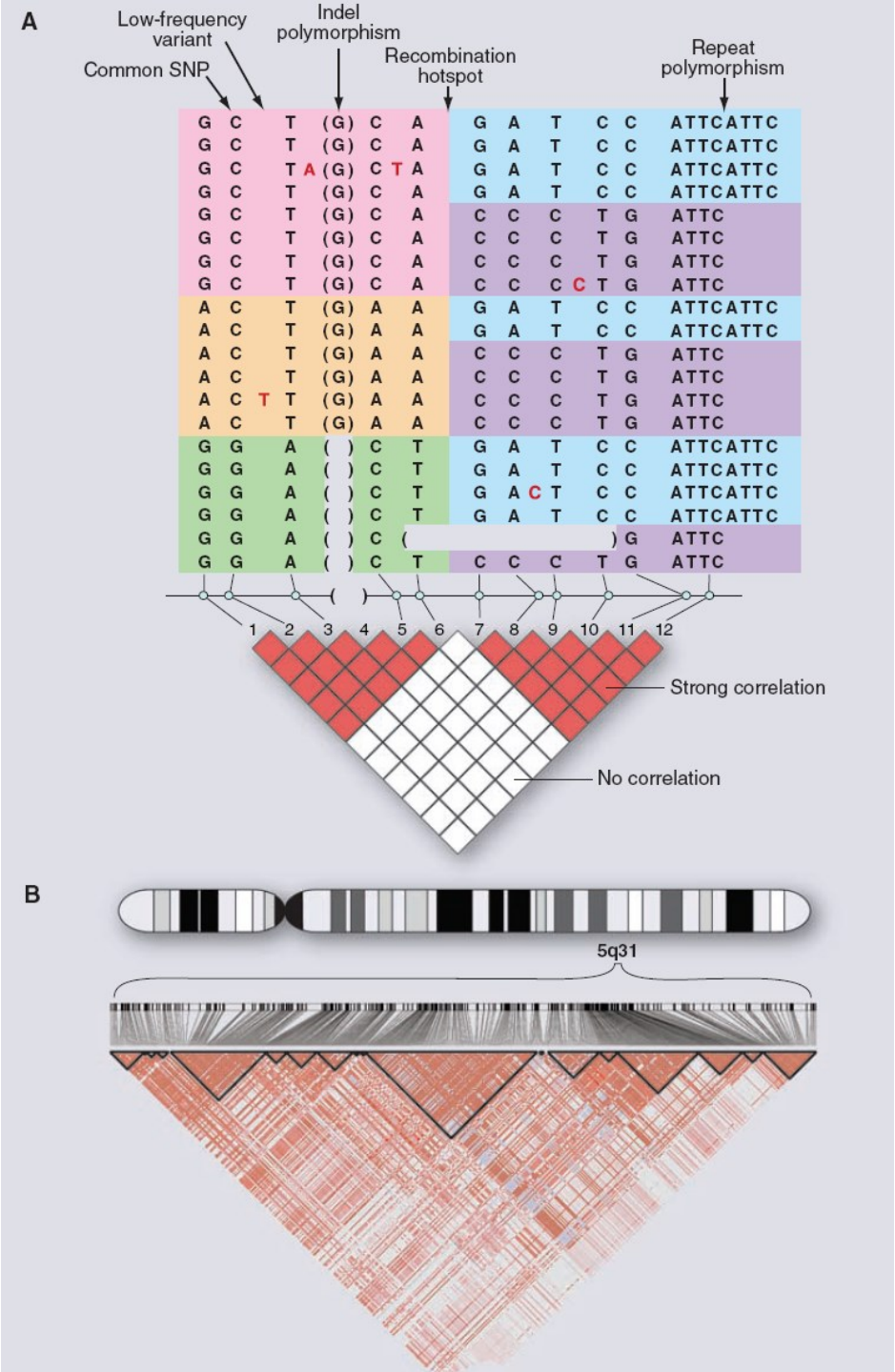
Association mapping

- Mapping in natural populations based on linkage disequilibrium (LD).
- To find association between the train and genetic marker, we need very high density of markers (~ 1 mil markers, depends on the level of LD).

Haplotype map of human genome (HapMap projekt)

- Genotypes of 1 mil SNPs located each 5 kb in the genome in more than 250 individuals.
- Genome consists of blocks (haplotypes) with high LD. Borders between these blocks correspond to recombination hotspots.
- To find association between a trait and the marker we need ~ 0.5 mil SNPs in European population and 1 mil SNPs in African population
  (i.e. capacity of one SNP genotyping chip)

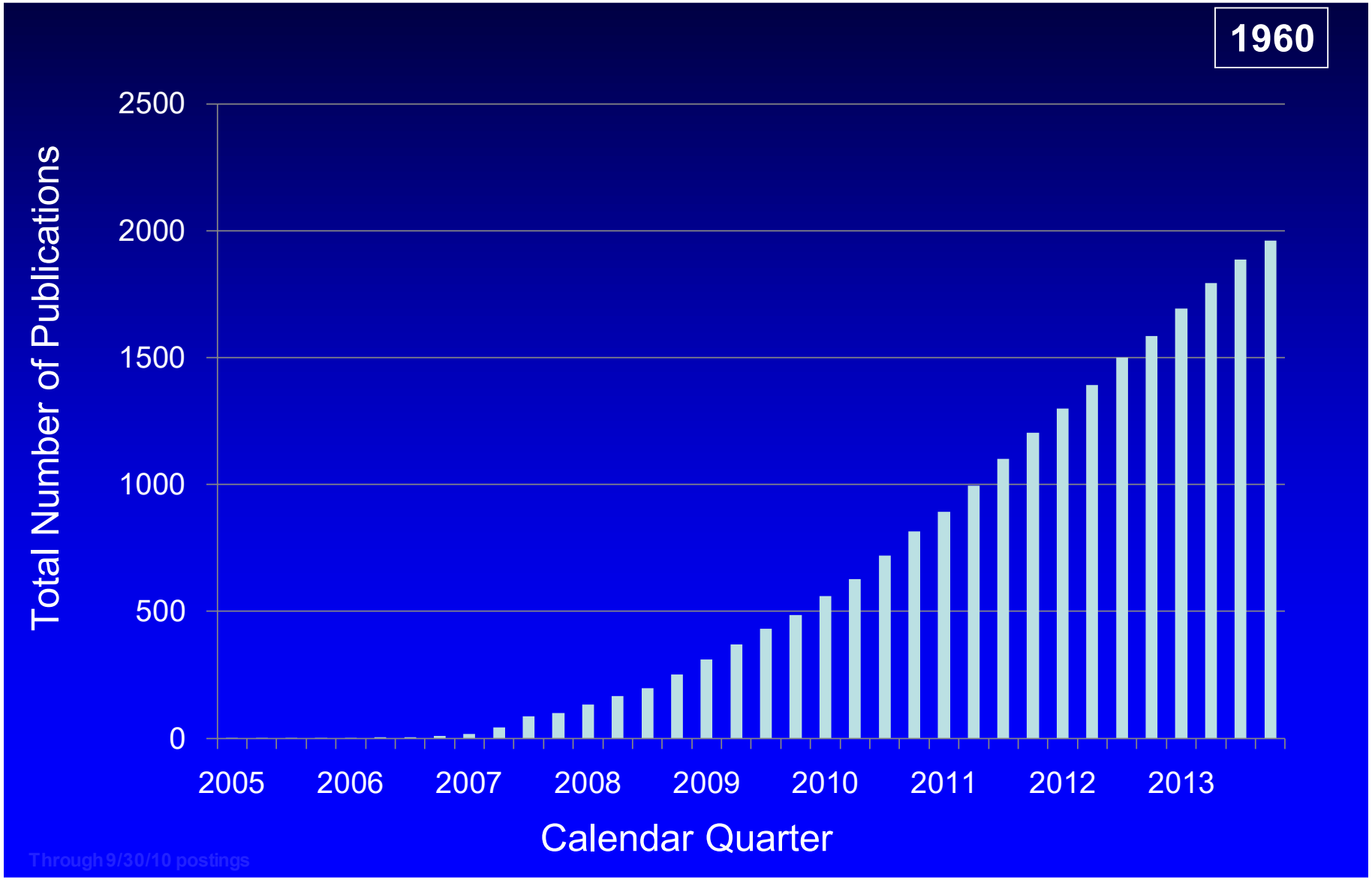
Nature 437, 1299-1320. 2005

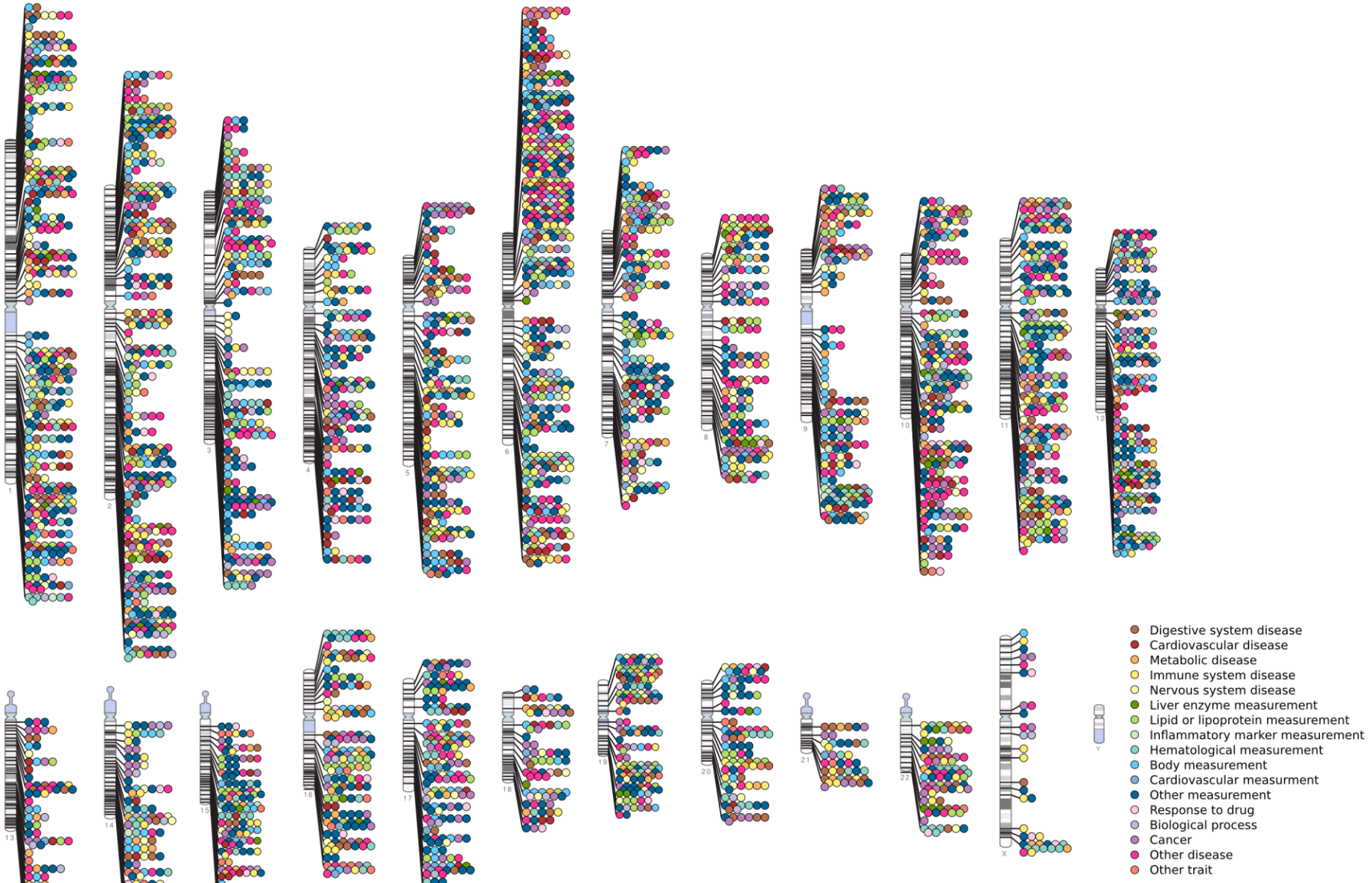


Association mapping

- Very fine scale mapping (~10kb).
- Mapping qualitative as well as quantitative traits.
- High number of individuals is needed (~ 1000).
- One should be careful about the population structure.

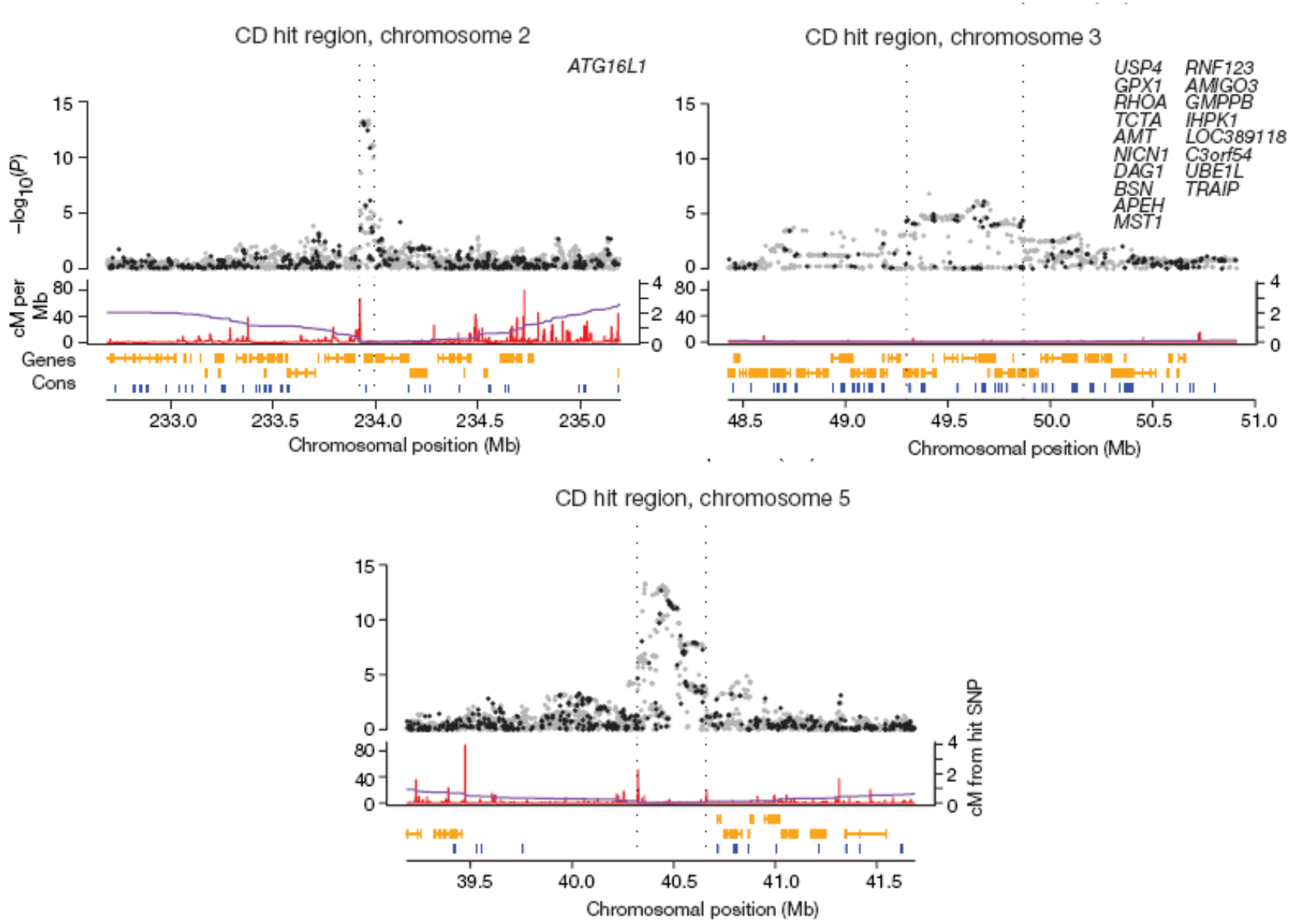
Published genome-wide association studies (GWA)





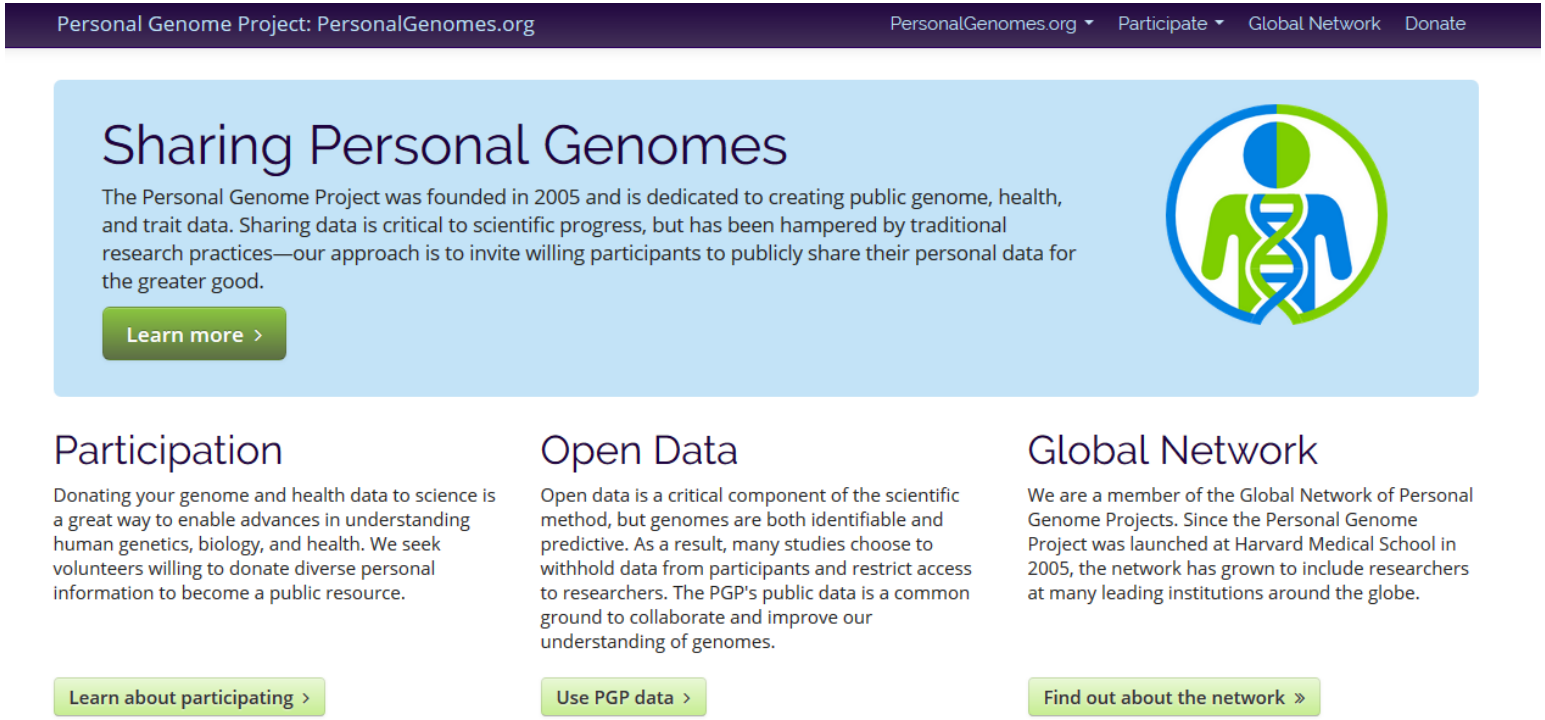
NHGRI GWA Catalog
www.genome.gov/GWAStudies
www.ebi.ac.uk/fgpt/gwas/

Association study of 7 diseases in British population



The Wellcome Trust Case Control Consortium, Nature 2007

Personal Genome Project

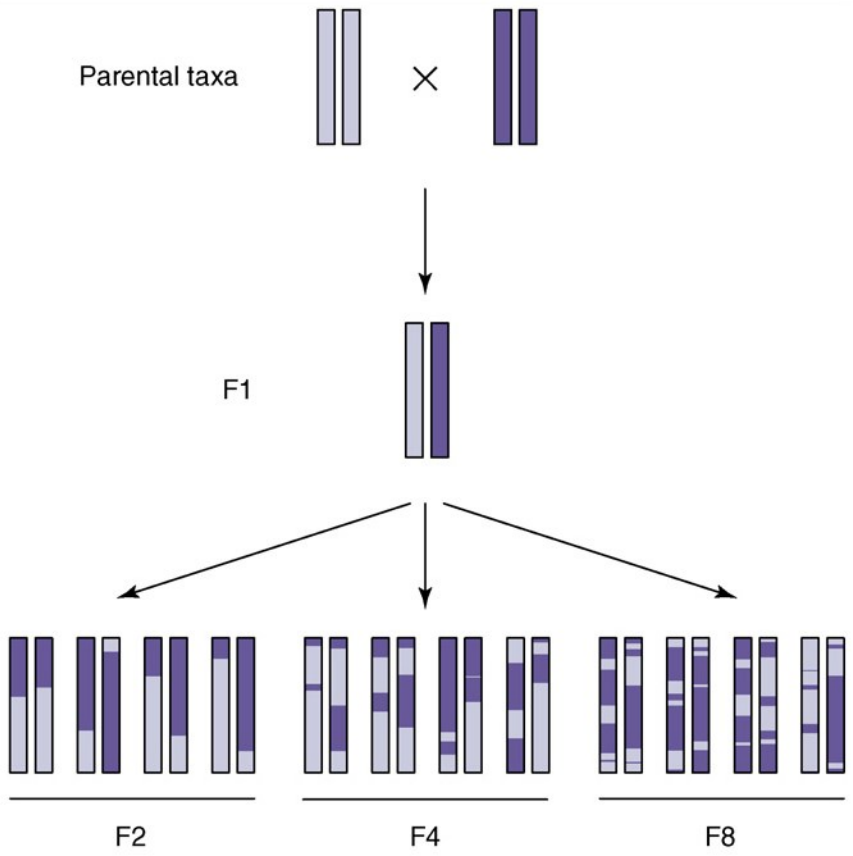


Risk of participation

- infer paternity or other features of the participant's genealogy
- claim statistical evidence that could affect employment or insurance or the ability to obtain financial services for the participant
- claim relatedness to criminals or incriminate relatives
- make synthetic DNA corresponding to the participant and plant it at a crime scene
- reveal propensity for a disease currently lacking effective treatment options

Admixture mapping

- Genetic mapping in natural hybrid populations.
- Finer resolution compared to classical mapping by experimental crossing, but coarser compard to association mapping.
- ~ 1000 - 5000 markers.



	Experimental cross	Admixture mapping	Association mapping
Number of markers	~100	~1 000	~ 1 000 000
Resolution	~10 cM	~1 cM, ~1 Mb	~10 kb

Combination of more approaches

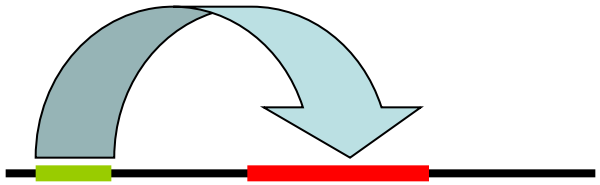
1. Experimental cross → 2. association mapping

Genome-wide coarse-scalle mapping

Local fine-scalle mapping

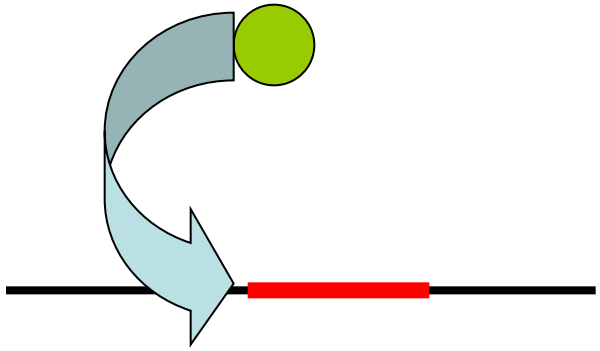
Expression (e) QTL mapping

- Difference in gene expression as quantitative trait.
- eQTL maps regulatory regions (cis or trans) responsible for gene expression change.



cis regulation

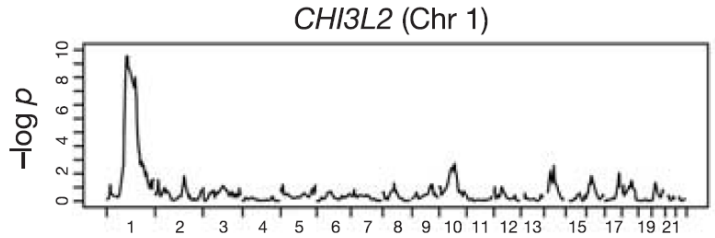
promotors,
nearby enhancers,



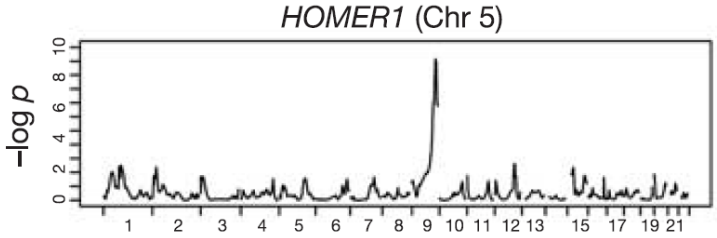
trans regulation

transkription factors,
miRNAs

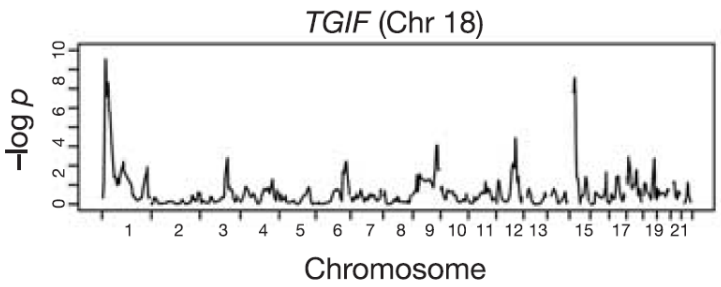
cis e-QTL



trans e-QTL



multiple e-QTL

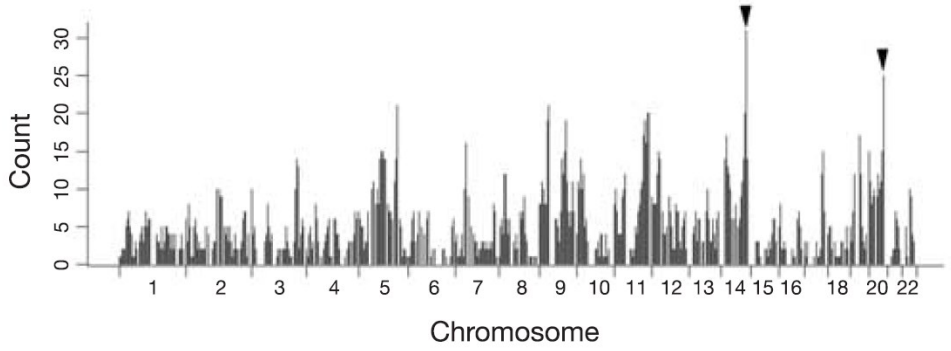


Morley et al. 2004, Nature

In one experiment, we can study expression of all genes, and create regulation networks.

Some trans eQTLs expression of many genes

„Master transkriptional regulators“



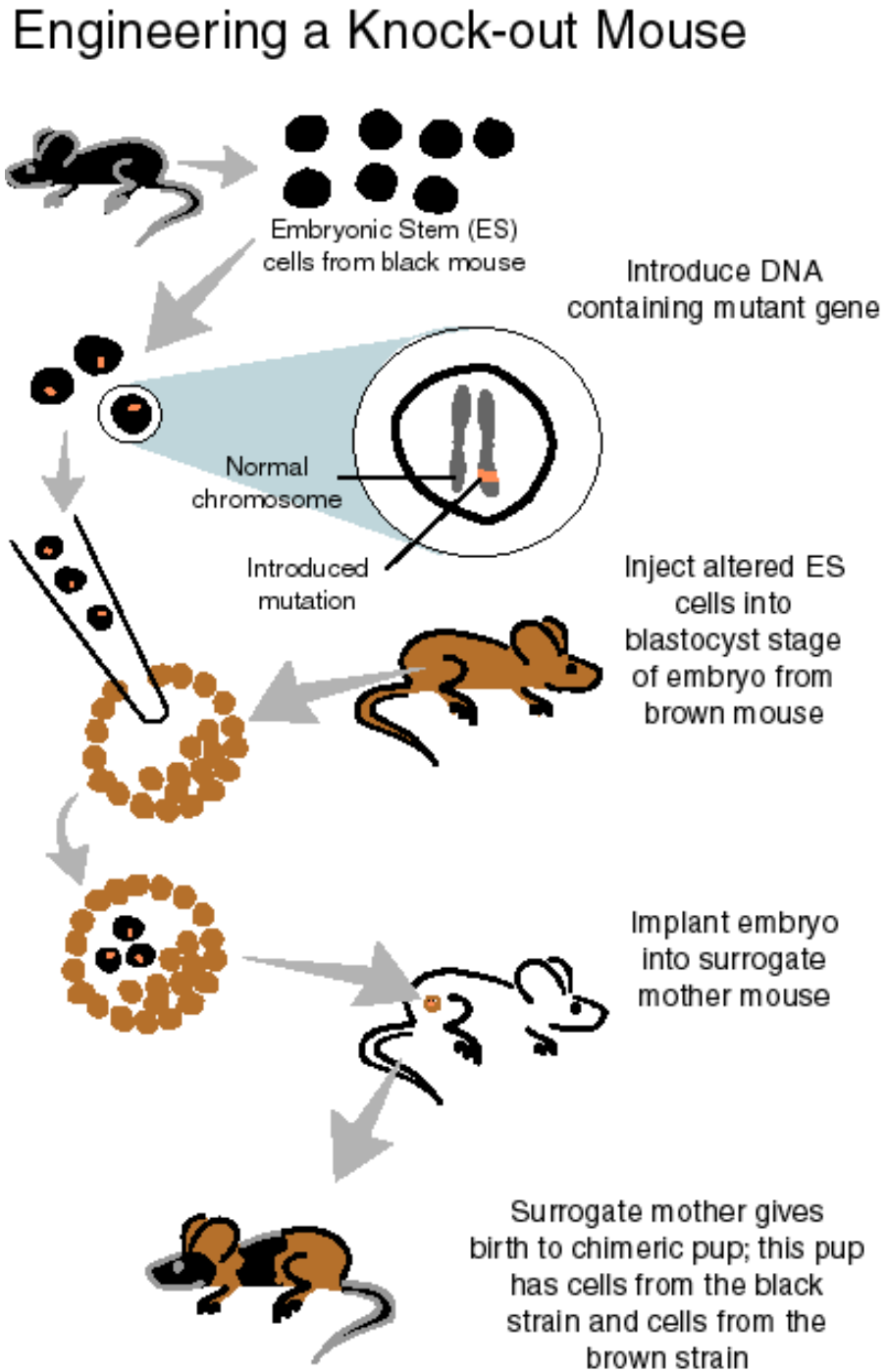
Morley et al. 2007, Nature

Reverse genetics

Methods of targeted mutagenesis

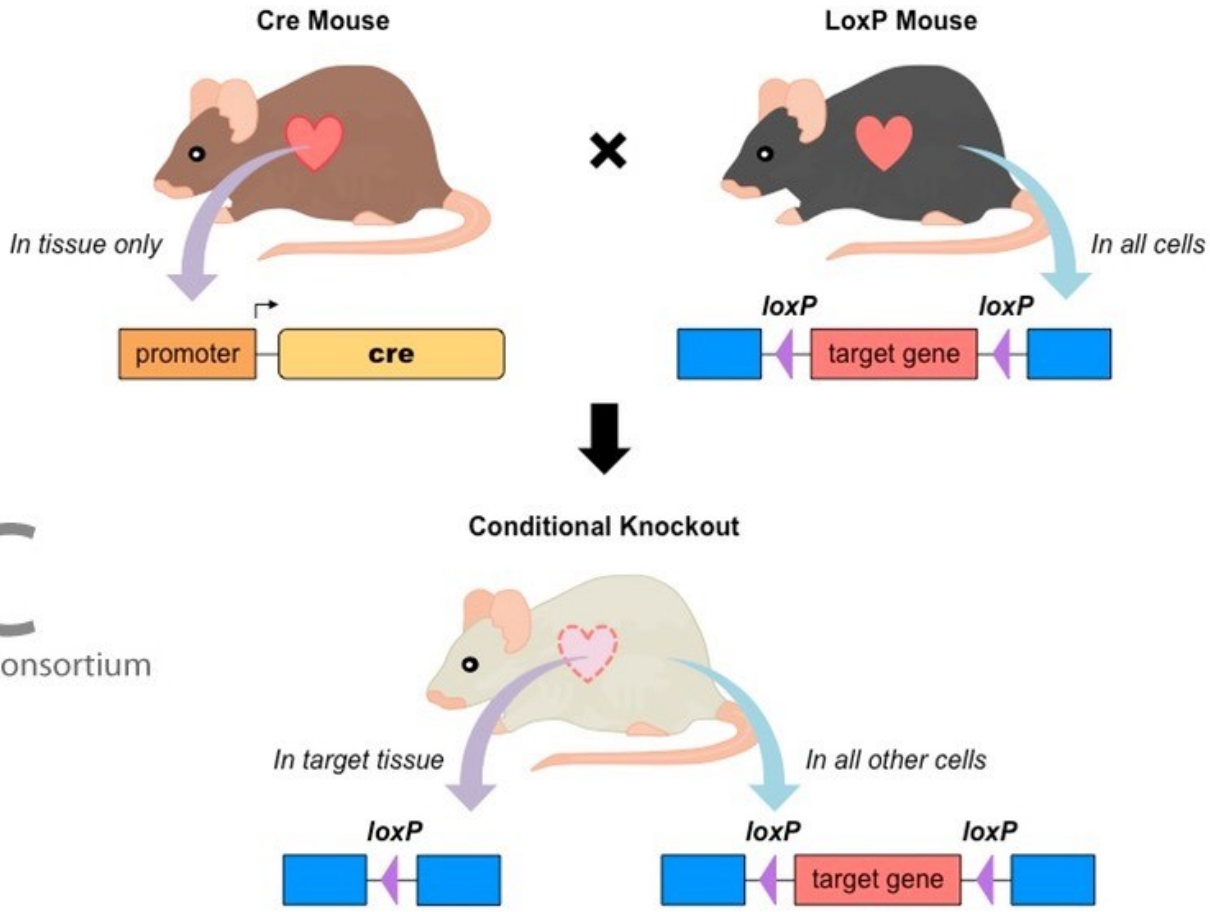
Gene knockout

- Inactivation or deletion of a particular gene.
- Homologous recombination in embryonic stem cells.



Conditional gene knockout

- Allows gene deletion in a tissue or time specific manner.
- Cre-lox system.



IMPC
International Mouse Phenotyping Consortium

Gene knockin

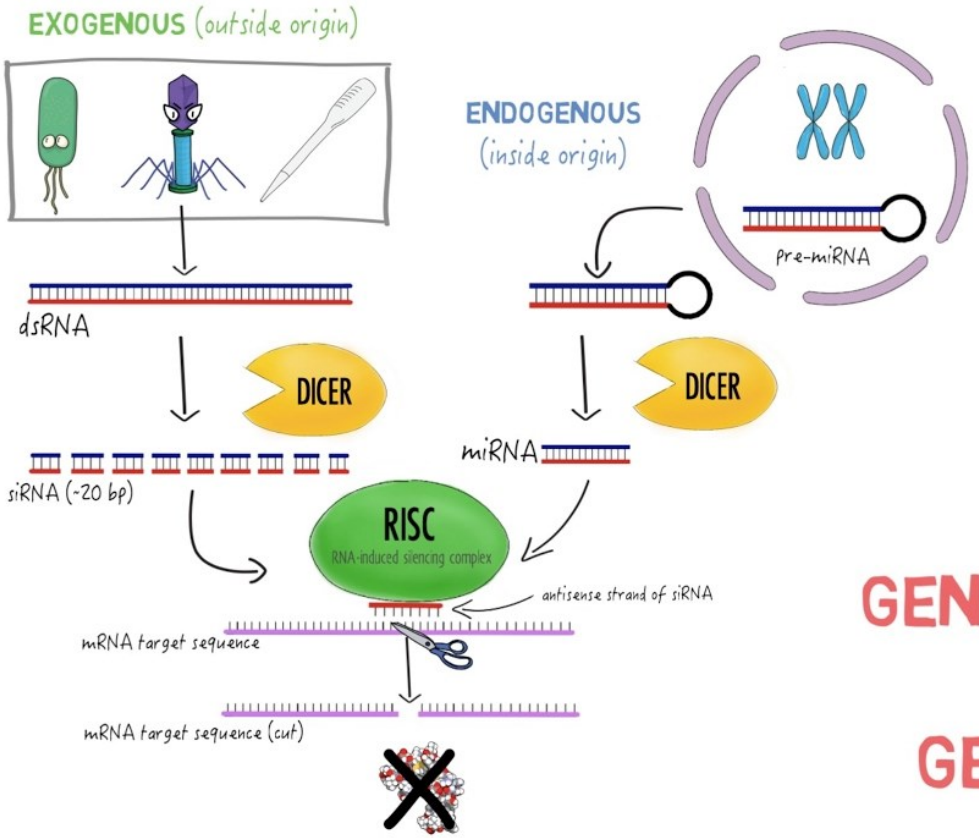
- Modification (rather than deletion) of the gene sequence.

Transgen

- Insertion of one or more copies of the gene to the genome. Insertion occurs randomly in the genome.

Gene knockdown

- Reduction of gene expression.
- Based on RNA interference.



GENE KNOCK-DOWN
OR
GENE SILENCING

Appl Microbiol Biotechnol (2017) 101:7091–7111
DOI 10.1007/s00253-017-8433-z

MINI-REVIEW

Therapeutic potentials of short interfering RNAs

Chit Tam[1] · Jack Ho Wong[1] · Randy Chi Fai Cheung[1] · Tao Zuo[2] · Tzi Bun Ng[1]

Critical Reviews in Oncology/Hematology 98 (2016) 159–169

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Nanoparticle-siRNA: A potential cancer therapy?

Samuel Wang Sherng Young[a], Martina Stenzel[b], Jia-Lin Yang[a,*]

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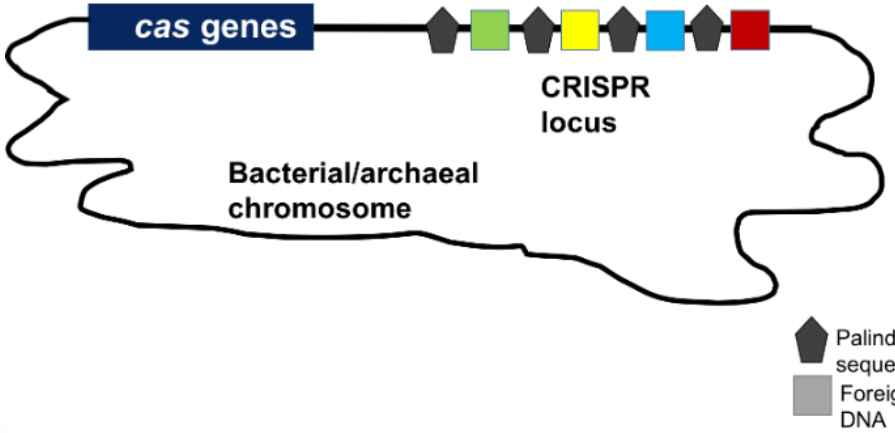
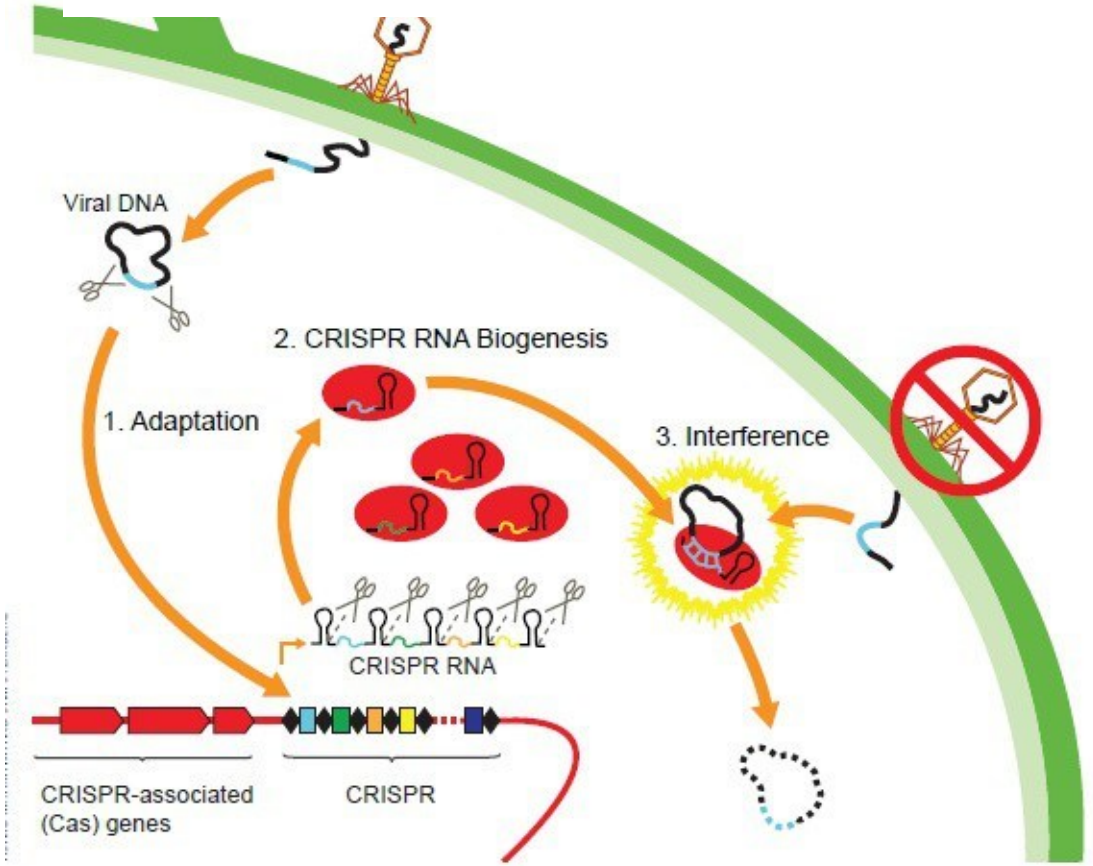
[b] Centre for Advanced Macromolecular Design, Faculty of Science, University of New South Wales, Sydney, NSW, Australia

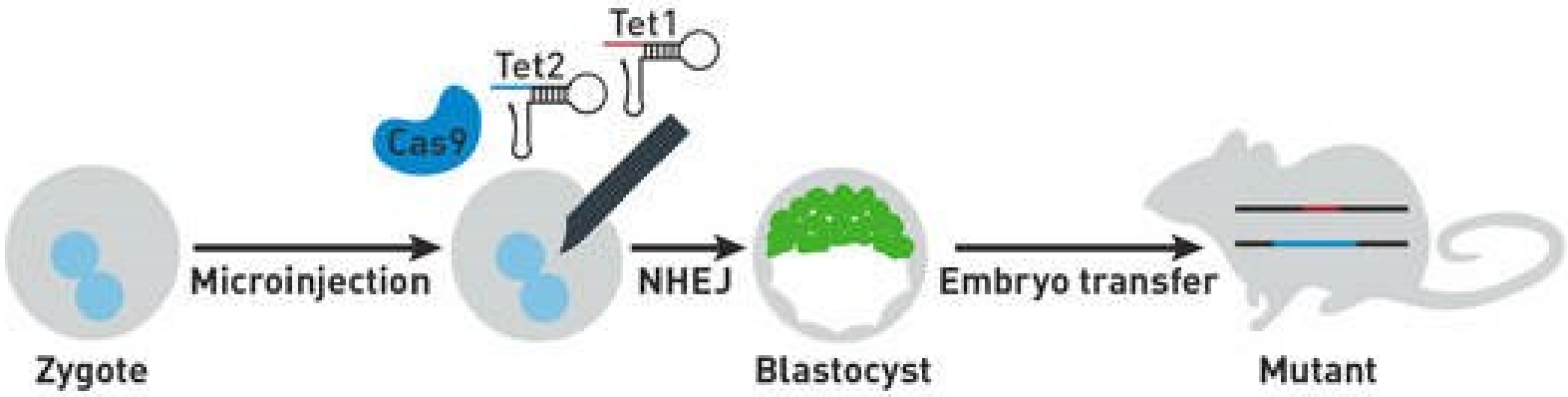
CRISPR-Cas

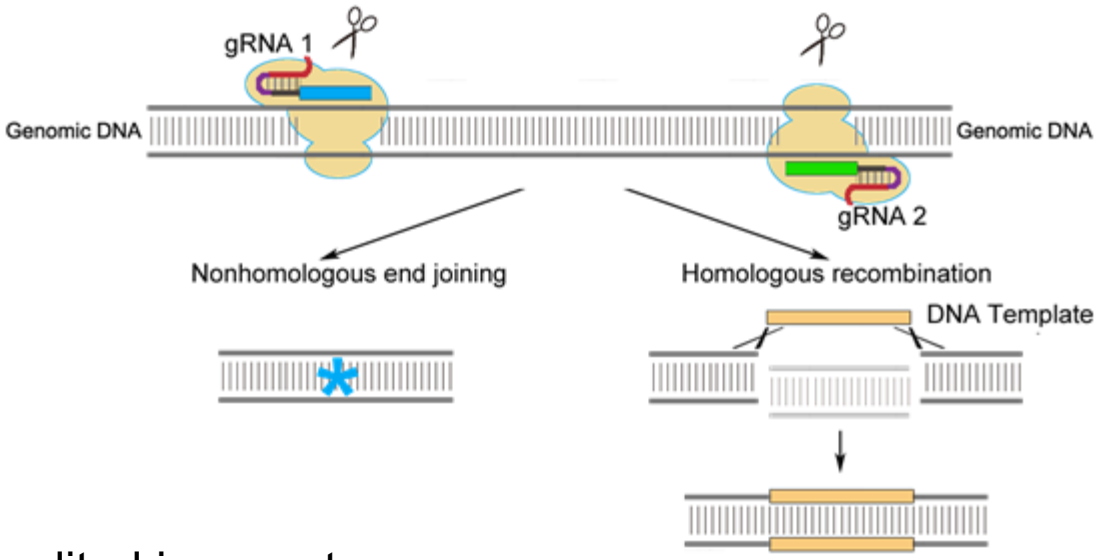
- CRISPER (Clusters of Regularly Interspaced Short Palindromic Repeats.
- Cas (CRISPER-associated) genes. Nucleases.
- Bacterial imune system





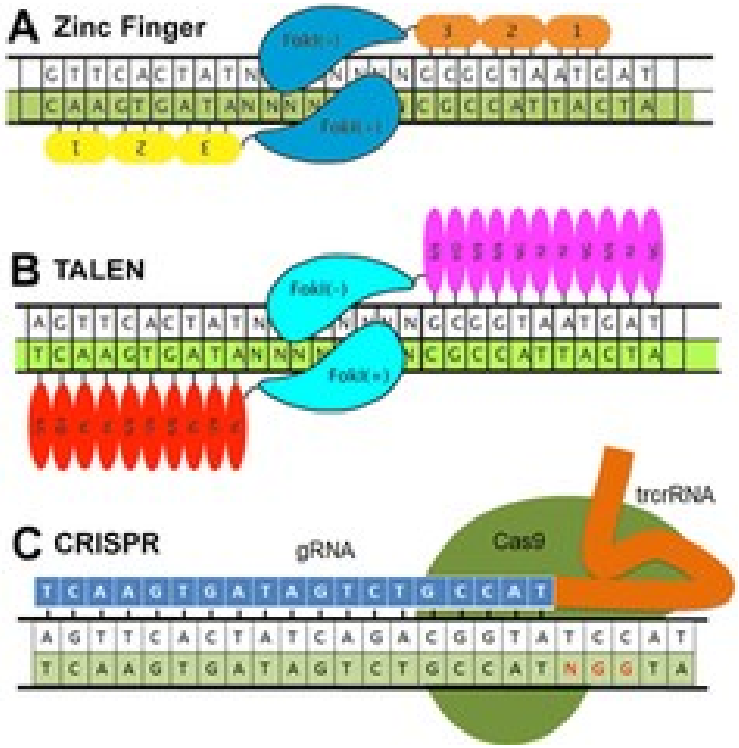
Genome editing by Crisper-Cas9

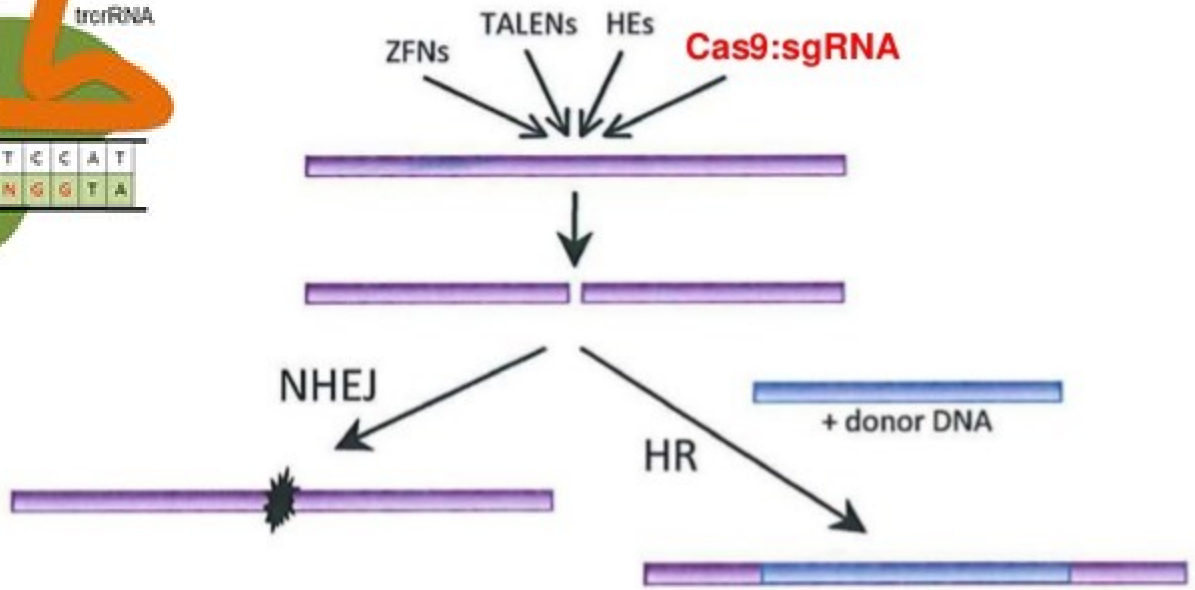




- Multiple genes can be edited in one step.

Other technologies for genome editing.





CRISPR'd babies: human germline genome editing in the 'He Jiankui affair'*

Henry T. Greely †

Corresponding author. E-mail: hgreely@stanford.edu

ABSTRACT

The world was shocked in Nov. 25, 2018 by the revelation that He Jiankui had used clustered regularly interspaced short palindromic repeats ('CRISPR') to edit embryos—two of which had, sometime in October, become living babies. This article is an effort to provide some deep context for the He Jiankui affair and to begin analyzing it. It focuses on He's experiment, without delving into the broader ethical issues around 'human germline genome editing' in the abstract. It begins by carefully defining 'human germline genome editing'. It then describes the little we know about the experiment before providing background on CRISPR, the pre-He ethical and legal status of human germline genome editing, and on He himself. The fourth, and longest, section provides a detailed narrative of the revelation of the He experiment and its fallout. The fifth section critiques the experiment, which I believe merits unequivocal condemnation on several grounds. The last section suggests some important immediate reactions, by 'Science' and by China.

AP

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AP Exclusive: US scientists try 1st gene editing in the body

MARILYNN MARCHIONE November 15, 2017

Treatment of Hunter syndrom by gene editing using zinc-finger protein

UK IS FIRST NATION TO APPROVE CRISPR THERAPY FOR DISEASES

Landmark decision could transform the treatment of sickle-cell disease and β-thalassaemia.

By Carissa Wong

In a world first, the UK medicines regulator has approved a therapy that uses the CRISPR–Cas9 gene-editing tool to treat disease. The decision marks another high point for a biotechnology that has been lauded as revolutionary in the decade since its discovery.

The therapy, called Casgevy, will treat the blood conditions sickle-cell disease and β-thalassaemia. Sickle-cell disease, also known as sickle-cell anaemia, can cause debilitating pain, and people with β-thalassaemia often require regular blood transfusions.

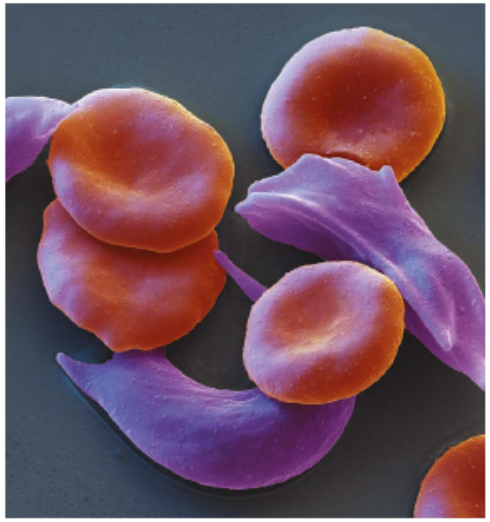
"This is a landmark approval which opens the door for further applications of CRISPR therapies in the future for the potential cure of many genetic diseases," said Kay Davies, a geneticist at the University of Oxford, UK, in comments to the UK Science Media Centre (SMC).

Nature explains the research behind the treatment and explores what's next.

What research led to the approval?

The approval by the Medicines and Healthcare products Regulatory Agency (MHRA) follows promising results from clinical trials that tested a one-time treatment, which is administered by intravenous infusion. The therapy was developed by the company Vertex Pharmaceuticals in Boston, Massachusetts, and biotechnology company CRISPR Therapeutics in Zug, Switzerland.

The trial for sickle-cell disease has followed 29 of 45 participants for long enough to draw interim results. In 28 of those people, Casgevy completely relieved debilitating episodes of pain for at least one year after treatment.



Sickled red blood cells affected by disease.

EYE OF SCIENCE/SPL

blood vessels. These blockages reduce the oxygen supply to tissues, which can cause periods of severe pain, known as pain crises.

β-thalassaemia occurs when mutations lead to low haemoglobin levels in the blood, low numbers of red blood cells and symptoms such as fatigue, shortness of breath and irregular heartbeat.

Clinicians administer Casgevy by taking blood-producing stem cells out of the bone marrow of people with either disease, then using CRISPR–Cas9 to edit the genes encoding haemoglobin in those cells. The gene-editing tool relies on an RNA molecule that guides the Cas9 enzyme to the correct region of DNA, which the enzyme cuts.

Once Cas9 reaches the gene targeted by Casgevy, called *BCL11A*, it cuts both DNA strands. *BCL11A* usually prevents the production of a form of haemoglobin that is made only in fetuses. By disrupting this gene, Casgevy unleashes the production of fetal haemoglobin, which does not carry the same abnormalities as adult haemoglobin in people with sickle-cell disease or β-thalassaemia.

Before the gene-edited cells are infused back into the body, people must undergo a treatment that prepares the bone marrow to receive the modified cells. Once administered, the stem cells give rise to red blood cells containing fetal haemoglobin. This relieves symptoms by boosting the oxygen supply to tissues. "Patients may need to spend at least a month in a hospital facility while the treated cells take up residence in the bone marrow and start to make red blood cells with the stable form of haemoglobin," the MHRA stated in a press release.

How safe is Casgevy?

Participants involved in the ongoing trials experienced side effects including nausea, fatigue, fever and an increased risk of

Genome editing in agriculture

A personal take on science and society

World view

EU proposal on gene-edited crops doesn't go far enough

The door should be opened further: CRISPR has huge potential to boost food security in the face of pathogens and climate change.

Speeding up plant