Functional genetics

Functional genetics

Link particular mutations/genes with phenotype



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



Phenotype has to be genetically determined



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 F₂ 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



 $LOD = LOD_a + LOD_i$

LOD_a additive effects LOD_i gene interactions



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.



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

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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 finescalle (~1cM or less depending on the number of strains).

Collaborative cross in mice

More strains, more phenotypes



PWK/PhJ

CAST/EiJ'

0.0

Genetic mapping of white head-spot using collaborative cross WSB/EiJ 20 marker-based 8-allele 5 o] ₽ Α 4 0 2 10 11 12 13 15 16 17 18 19 х 3 9 14 Chromosome 20 2 Two candidate regions long cca 5Mb в 0⁰ ю 0 50 100 Position (Mb)

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

Animal QTLdb

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).





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)



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 train 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)





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

Sharing Personal Genomes

The Personal Genome Project was founded in 2005 and is dedicated to creating public genome, health, and trait data. Sharing data is critical to scientific progress, but has been hampered by traditional research practices—our approach is to invite willing participants to publicly share their personal data for the greater good.



Participation

Donating your genome and health data to science is a great way to enable advances in understanding human genetics, biology, and health. We seek volunteers willing to donate diverse personal information to become a public resource.

Open Data

Open data is a critical component of the scientific method, but genomes are both identifiable and predictive. As a result, many studies choose to withhold data from participants and restrict access to researchers. The PGP's public data is a common ground to collaborate and improve our understanding of genomes.

Global Network

We are a member of the Global Network of Personal Genome Projects. Since the Personal Genome Project was launched at Harvard Medical School in 2005, the network has grown to include researchers at many leading institutions around the globe.

Learn about participating >

Use PGP data >

Find out about the network »

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



Genome-wide coarsescalle mapping 2. association 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

Engineering a Knock-out Mouse

brown strain



Conditional gene knockout

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



Gene knockdown

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



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MINI-REVIEW

Therapeutic potentials of short interfering RNAs

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



Nanoparticle-siRNA: A potential cancer therapy?



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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.

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.

CRISPR'd babies: human germline genome editing in the 'He Jiankui affair'* Henry T. Greely 10

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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.

Other technologies for genome editing.





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

MARILYNN MARCHIONE November 15, 2017

Treatment of Hunter syndrom by gene editing



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.