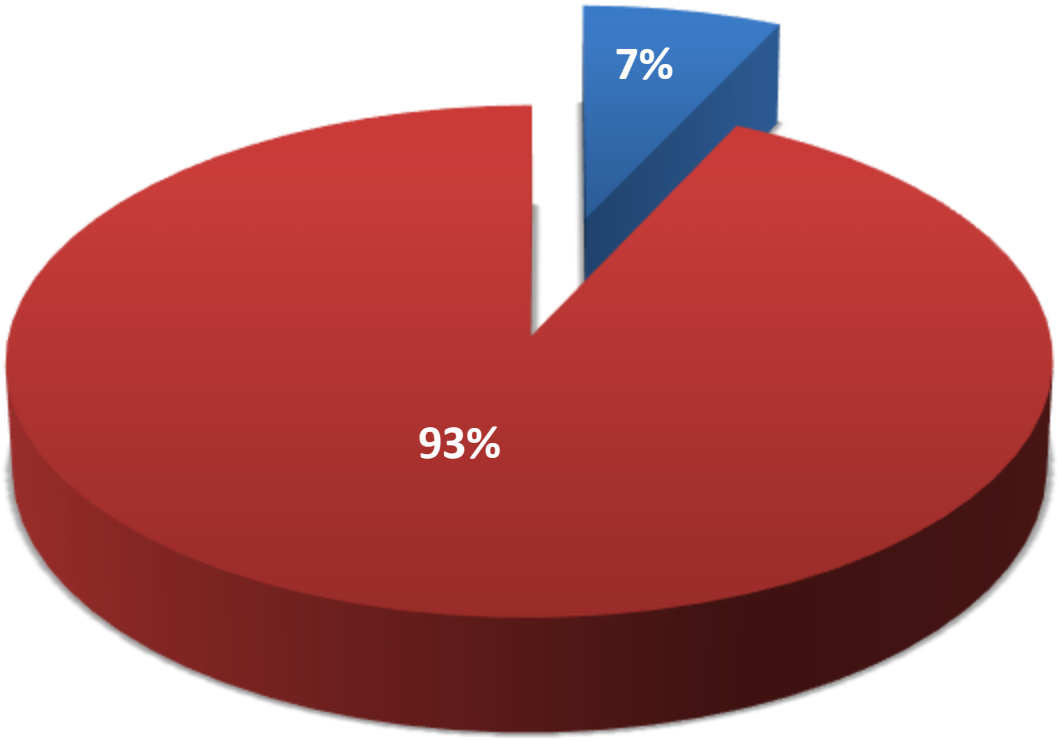
Lecture 4: Regulatory variation & the dawn of precision medicine

All SNPs linked to quantitative traits / diseases so far

The majority of trait-associated variants (SNPs) map to non-coding regions

>1,200 GWAS → 6,500 disease- or trait-predisposing SNPs



Easy to infer function

↑

Coding

Non-coding

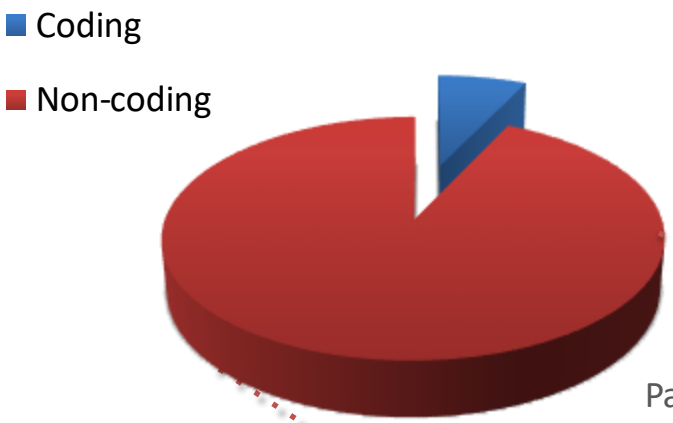
↓

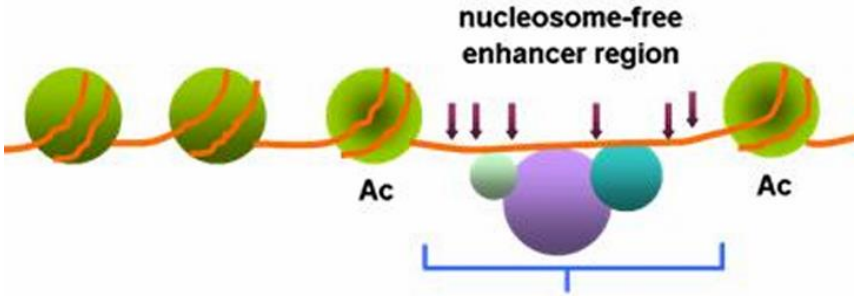
Difficult to infer function → *Still mostly a black box*

Q1

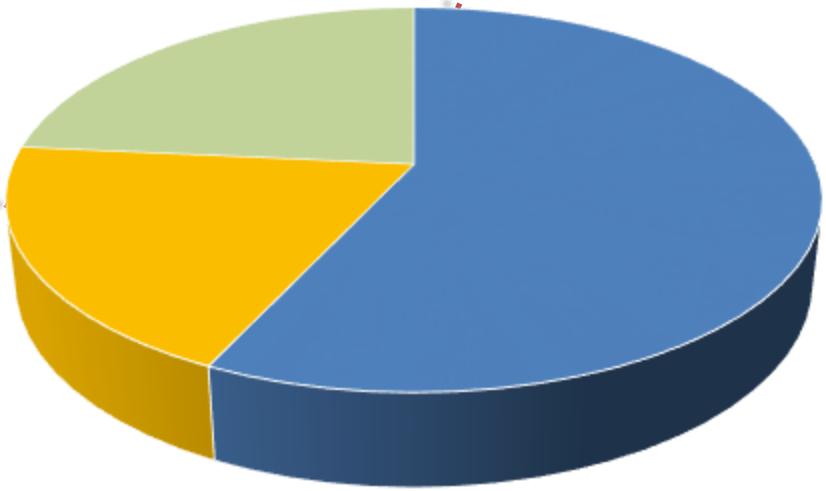
Hindorff et al., PNAS, 2010

The majority of trait-associated variants (SNPs) map to Q1 *functional* non-coding regions





Partition of non-coding GWAS SNPs according to their location

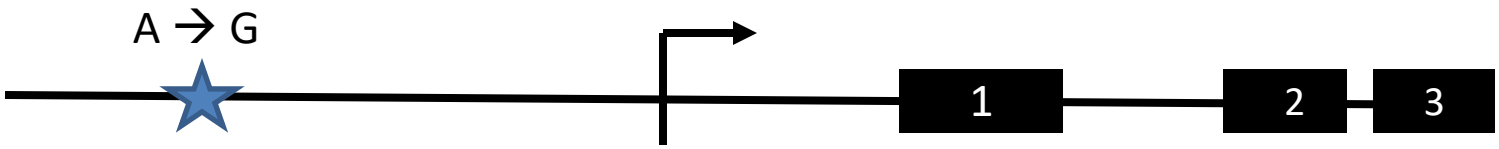


GWAS SNPs in nucleosome free regions (NFR)

GWAS SNPs in perfect LD with those NFR SNPs

Maurano et al., Science, 2012

Regulatory polymorphisms



• humans are heterozygous at more functional *cis*-regulatory sites than at amino acid positions (Rockman and Wray. Mol. Biol. Evol., 2002: 19, 1991).

• Case study with the CC chemokine receptor 5, a major chemokine co-receptor of HIV-1 necessary for viral entry into cells

- G to A SNP of *CCR5* at –2459 nt
- CCR5 density – low (homozygous GG), intermediate (GA), and highest (homozygous –AA) (correlates with disease progression, i.e. fastest in AA individuals)

Q2

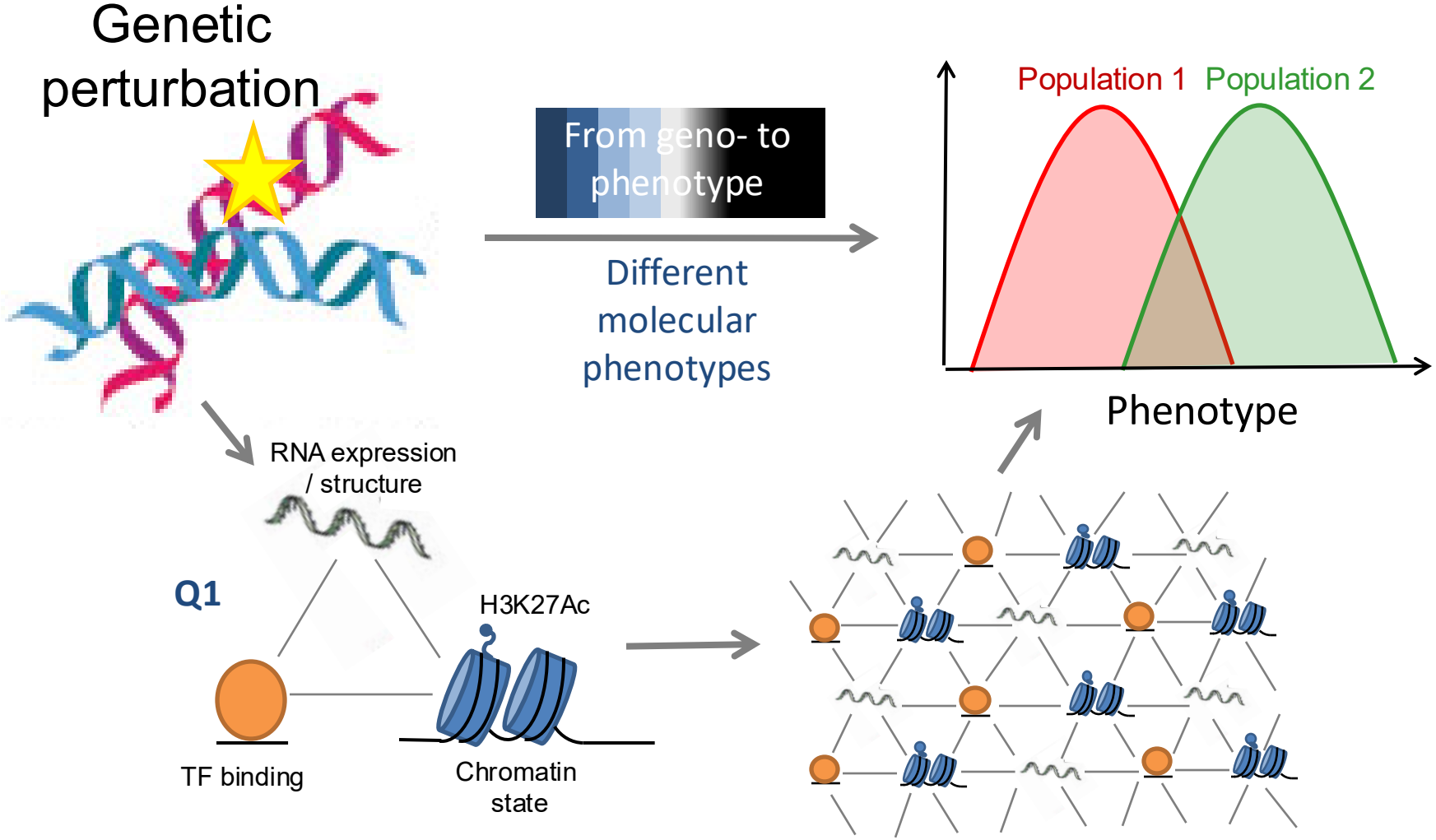
(Salkowitz et al., Clin. Immunol., 2003: 108, 234)

- Potential site for binding of the TF CREB1?

(Gornalusse et al., PNAS, 2015)

Whole genome association studies

From association to molecular mechanism

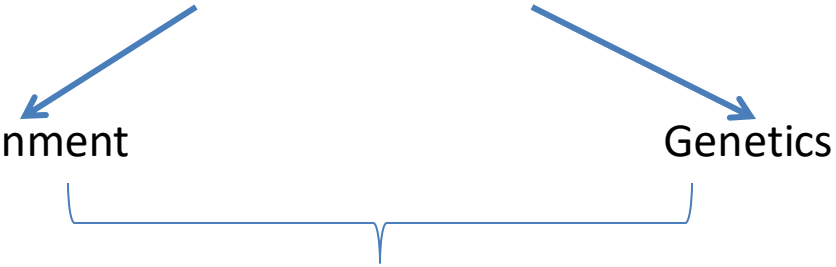


How genomic variation and especially regulatory variation results into phenotypic variation is still a big black box. That is why we need to bridge this gap by looking at how genomic variation causes molecular variation which then leads to organismal phenotypic variation.

Whole genome association studies

Gene expression variation: mapping eQTLs

Transcript abundance = a quantitative trait that can be mapped with considerable power

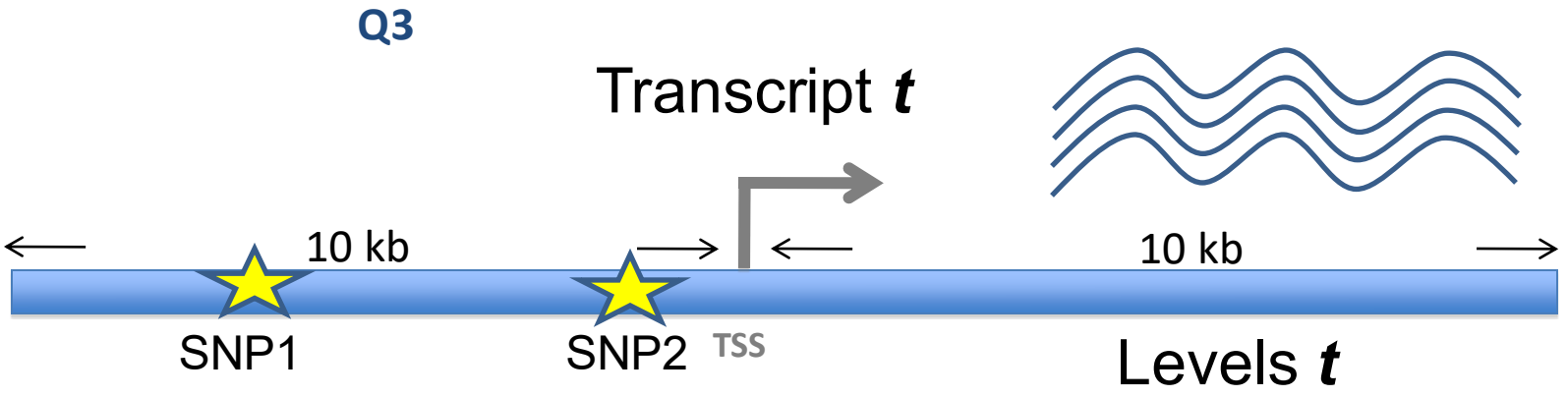


Heritability (H^2) = genetic variance over total trait variance with 0 = no genetic effects and 1 = all variance is under genetic control

Q3 **eQTL is a locus that induces a heritable change in gene expression**

Whole genome association studies

Gene expression variation: mapping cis-eQTLs



Individual 1	A	C	100
Individual 2	T	C	100
Individual 3	A	C	100
Individual 4	T	C	100
Individual 5	A	G	500
Individual 6	T	G	500
Individual 7	A	G	500
Individual 8	T	G	500
	Not associated	**Highly associated**	

Whole genome association studies

Gene expression variation: mapping cis-eQTLs

Q4

cis-eQTLs are highly abundant in the human genome!

→ GTEx (Genotype Tissue Expression Consortium): **cis-eQTLs for about 18k genes with a total of 4,278,636 genetic variants**
(~50 tissues from up to ~1000 postmortem donors)

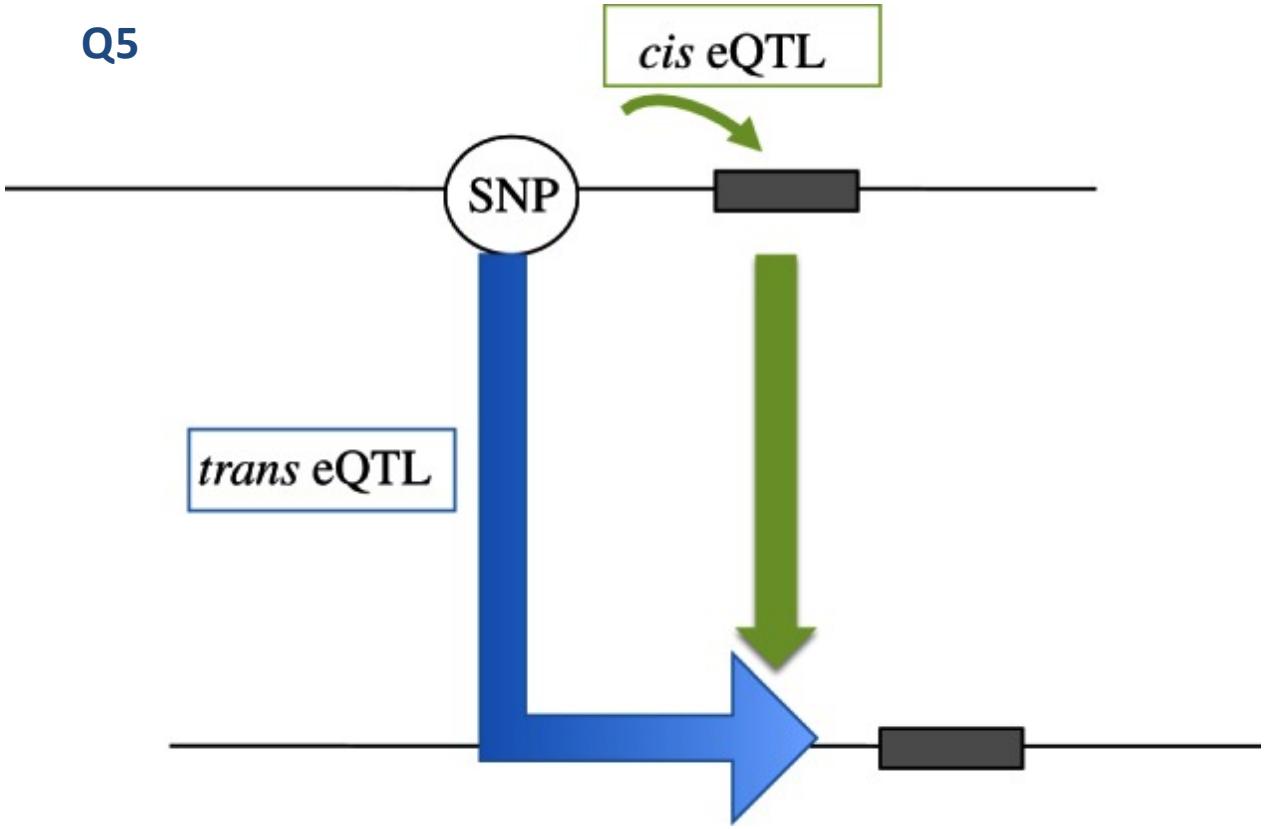
→ cis-eQTLs are enriched about 1.5-fold among GWAS QTLs
(compared to all variants tested in GWAS)

GTEx, Science, 2020

Many eQTLs are not *cis* but *trans*

(*trans*: they operate at a distance)

Q5



Nica and Dermitzakis, Philos Trans R Soc Lond B Biol Sci, 2013

Need many more samples / individuals to map *trans* eQTLs, why?

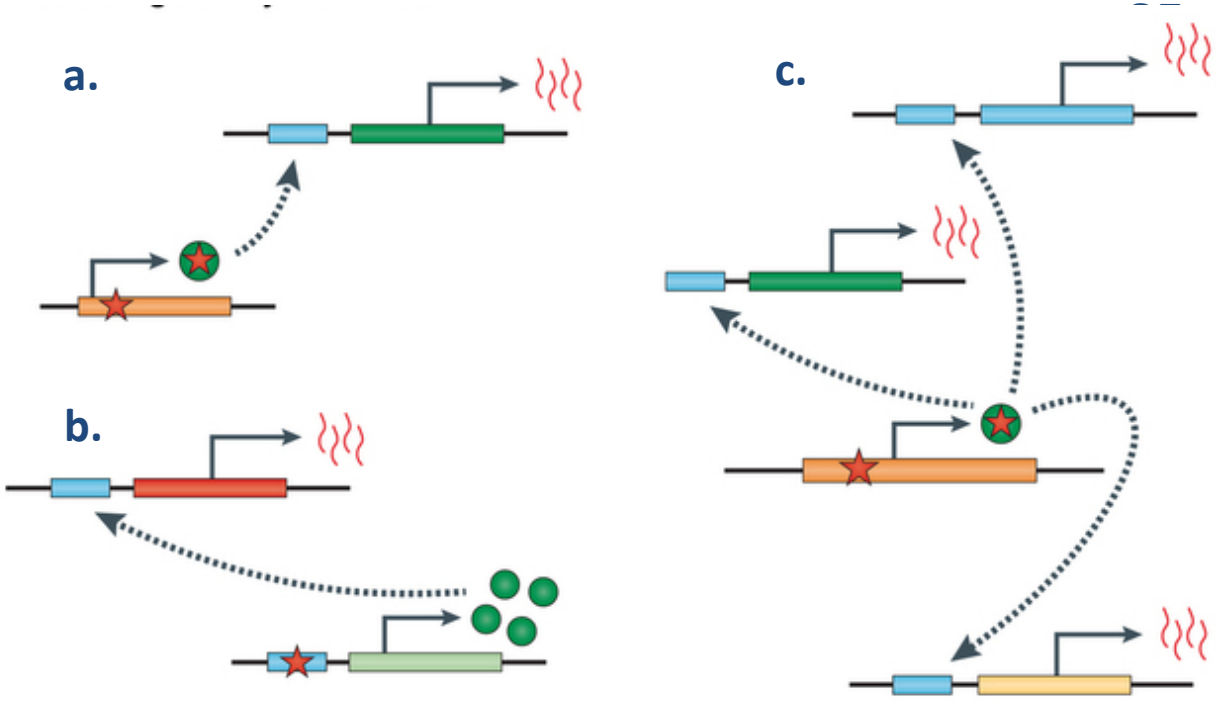
Q5

A. Because of LD between the eQTLs
B. Because of the multiple testing problem
C. Because most trans eQTLs involve SNPs with a low MAF
D. Because the signal is weaker because these variants modulate gene expression at a far distance

80

Whole genome association studies

Q6 Gene expression: mapping *trans* eQTLs



Albert and Kruglyak, Nature Reviews genetics, 2015

Trans-eQTLs are due to polymorphisms that alter the function **(a)** or expression **(b)** of a diffusible factor. **(c)** shows that the effect can act on many genes at once in *trans* (e.g. changes in actin levels)

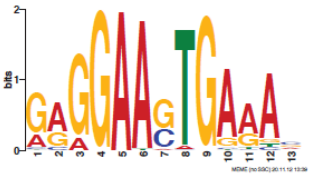
Whole genome association studies

Mapping tfQTLs: bridging genome variation with gene regulation

ChIP-seq of PU.1 in lymphoblastoid cell lines (LCLs) of several unrelated individuals

PU.1 *de novo* motif

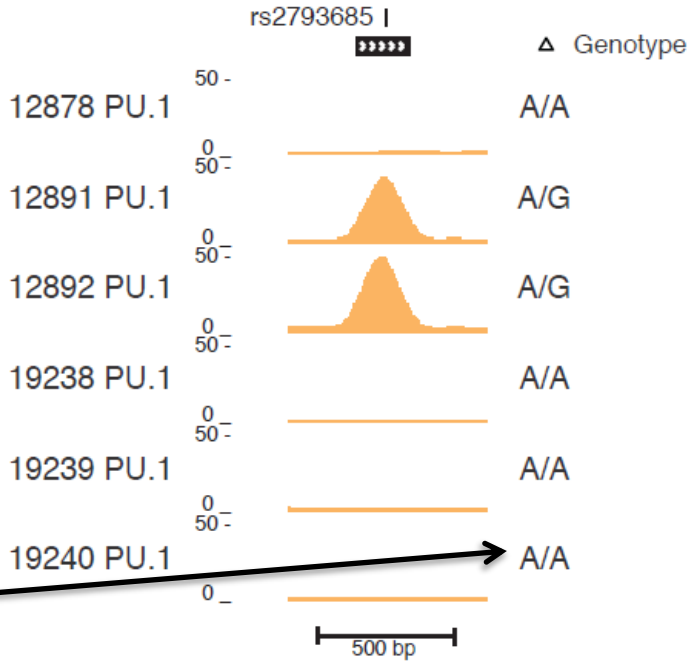
P=4.7e-2240
(1000/1000)



Q7

tfQTLs: Polymorphisms that induce heritable variation in TF DNA binding

Example of a PU.1 motif-disrupting SNP



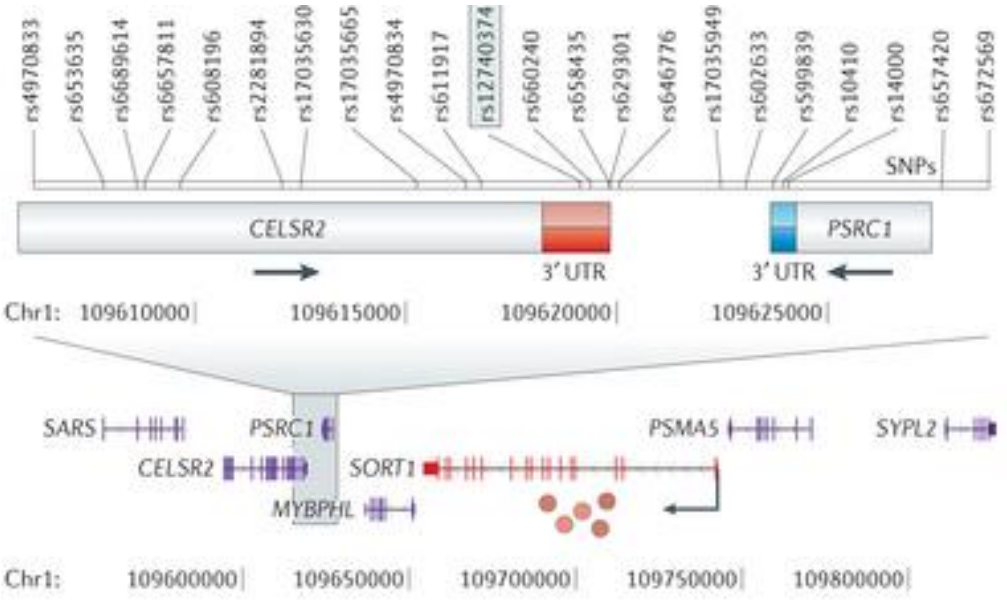
PU.1 (EICE) motif

Kiplinen et al., Science, 2013

Whole genome association studies

Solving the molecular mechanism underlying GWAS variants using "xQTLs"

The variant rs12740374 is a GWAS QTL linked to variation in myocardial infarction susceptibility



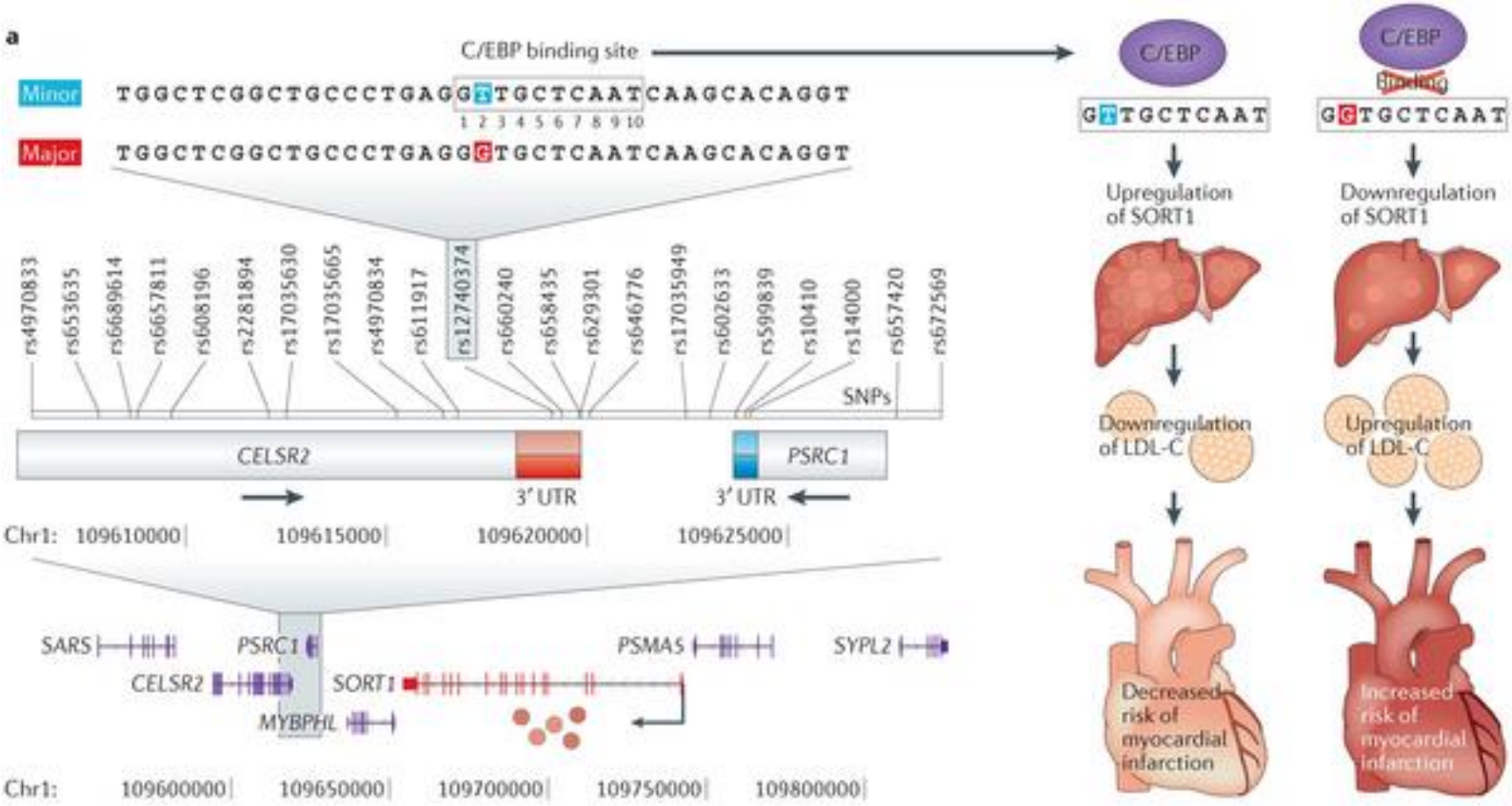
The variant is located in the 3'UTR of the *CELSR2* gene, so should we investigate *CELSR2* for its impact on myocardial infarction, or how do we dissect the underlying molecular mechanism?

Albert and Kruglyak, Nature Reviews Genetics, 2015

Q8

Whole genome association studies

Solving the molecular mechanism underlying GWAS variants using "xQTLs"



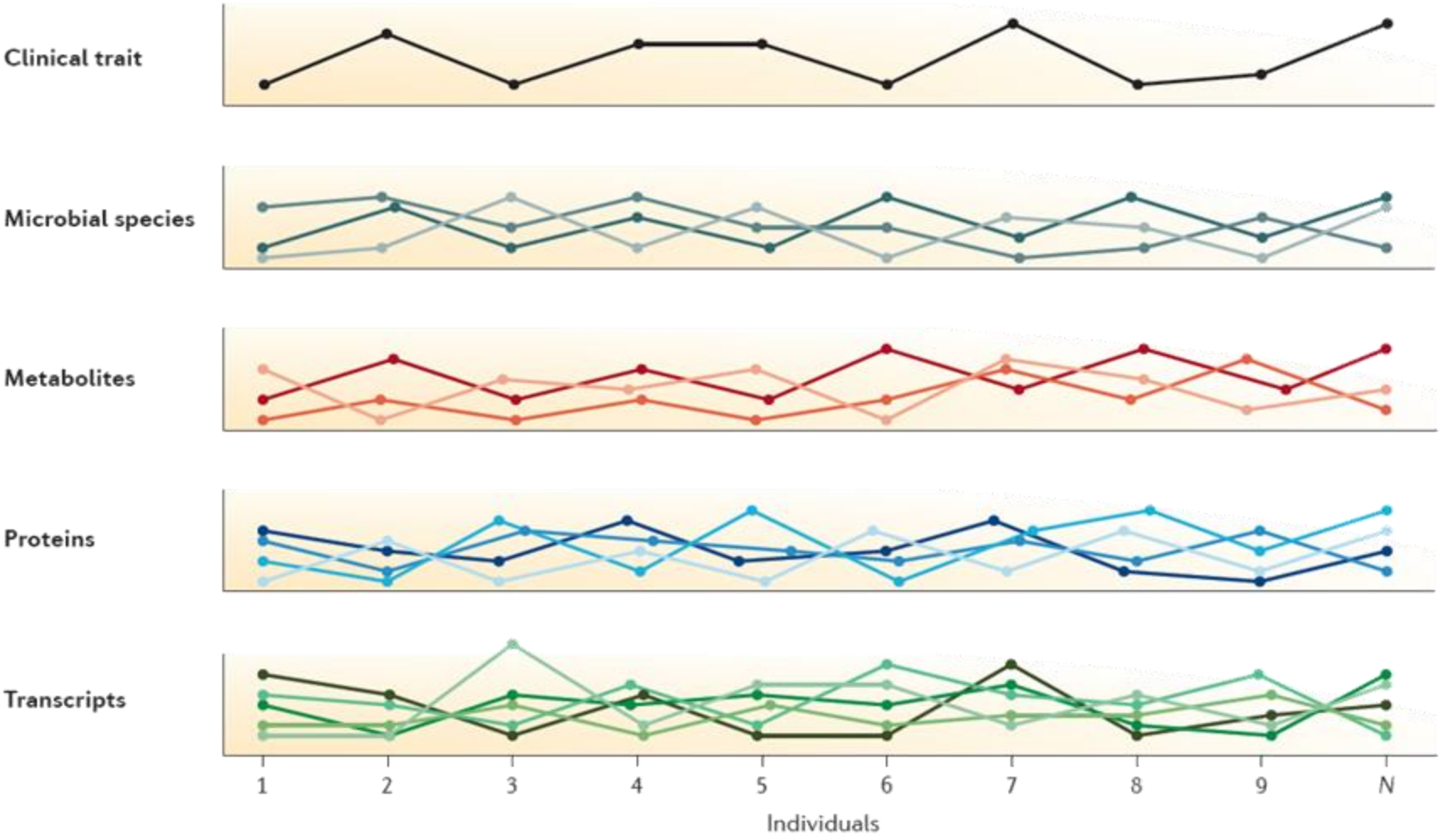
Albert and Kruglyak, Nature Reviews Genetics, 2015

- Minor allele of rs12740374 creates a TF binding site for CCAAT/enhancer-binding protein (C/EBP) → tfQTL (C/EBP)
- Binding of C/EBP at this site leads to increased expression of the sortilin 1 (*SORT1*) gene (40 kb downstream) in liver cells → eQTL (*SORT1*) (chromatin conformation!)

Q8 **→ overlapping these "x"QTLs uncovers the molecular mechanism**

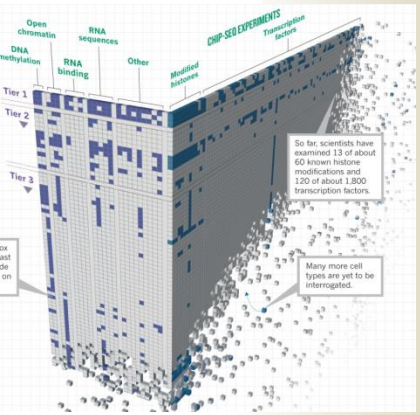
Whole genome association studies

The future: Mapping xQTLs: bridging genome variation with phenotypic variation at all levels



Civelek and Lusis_Nat Rev Genet_2013

From 1 to…


ChIP-SEQ EXPERIMENTS
Open chromatin
DNA methylation
RNA binding
RNA sequences
Other
Modified histones
Transcription factors
Tier 1
Tier 2
Tier 3
So far, scientists have examined 13 of about 60 known histone modifications and 120 of about 1,800 transcription factors.
Many more cell types are yet to be interrogated.

How can genomic analyses improve the practice of personalized medicine?

Q9 **What is most obvious to you?**

80

Entering the age of personalized medicine

Toward the elucidation of each person's genetic make-up

Necessary for:

Q9 **1) DNA-based risk assessment for common complex disease**

Breast Cancer → One of the better established disease prediction models

→ Women with ***BRCA1* mutation** ~65% chance of developing breast or ovarian cancer before the age of 70. Why not all?

→ Genotype-dependent (e.g. certain alleles of the *TNRC9* gene increase the effect of having the mutant *BRCA1* allele)


The New York Times

The Opinion Pages

WORLD U.S. N.Y. / REGION BUSINESS TEC

OP-ED CONTRIBUTOR

My Medical Choice

By ANGELINA JOLIE

Published: May 14, 2013 | 1712 Comments

LOS ANGELES

- Has Mother who died at 57 of breast cancer
- Had double Mastectomy
- Carries BRCA1 allele

Entering the age of personalized medicine

Toward the elucidation of each person's genetic make-up

Necessary for:

1) DNA-based risk assessment for common complex disease

Breast Cancer → One of the better established disease prediction models

→ Women with ***BRCA1* mutation** ~65% chance of developing breast or ovarian cancer before the age of 70. Why not all?

→ Genotype-dependent (e.g. certain alleles of the *TNRC9* gene increase the effect of having the mutant *BRCA1* allele)

→ *How to calculate the risk?*

→ Several different types of prediction models exist:

- Incorporate breast and ovarian cancer in 1st- and 2nd degree relatives
- Age of cancer onset
- Incorporate racial / ethnic backgrounds (e.g. Ashkenazi Jews)
- Other variables defining the person's personal and family history
- Tools: Logistic regression, Bayesian etc.

Entering the age of personalized medicine

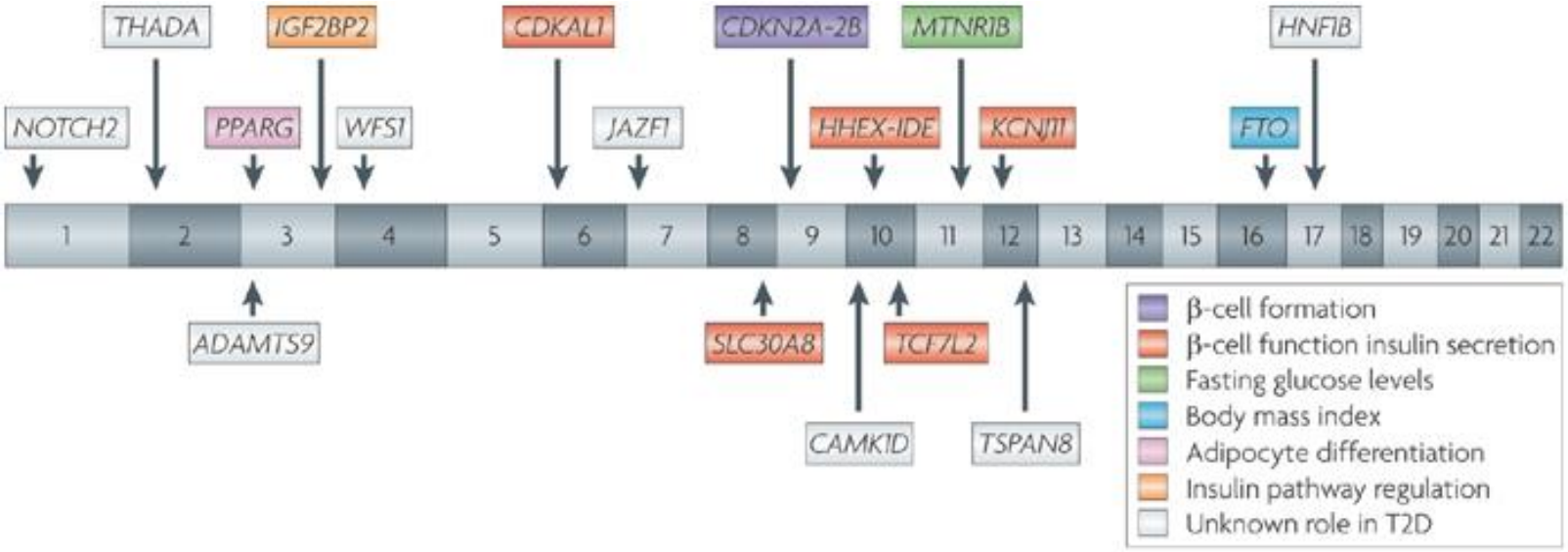
Toward the elucidation of each person's genetic make-up

Q9

Necessary for:

2) Identification of novel molecular signatures for disease diagnosis, prognosis, or drug design

e.g. Type II Diabetes → many possible disease predisposition markers
→ how to calculate probability?



Each person may have a different genetic pre-disposition, resulting in a different prognosis or drug treatment

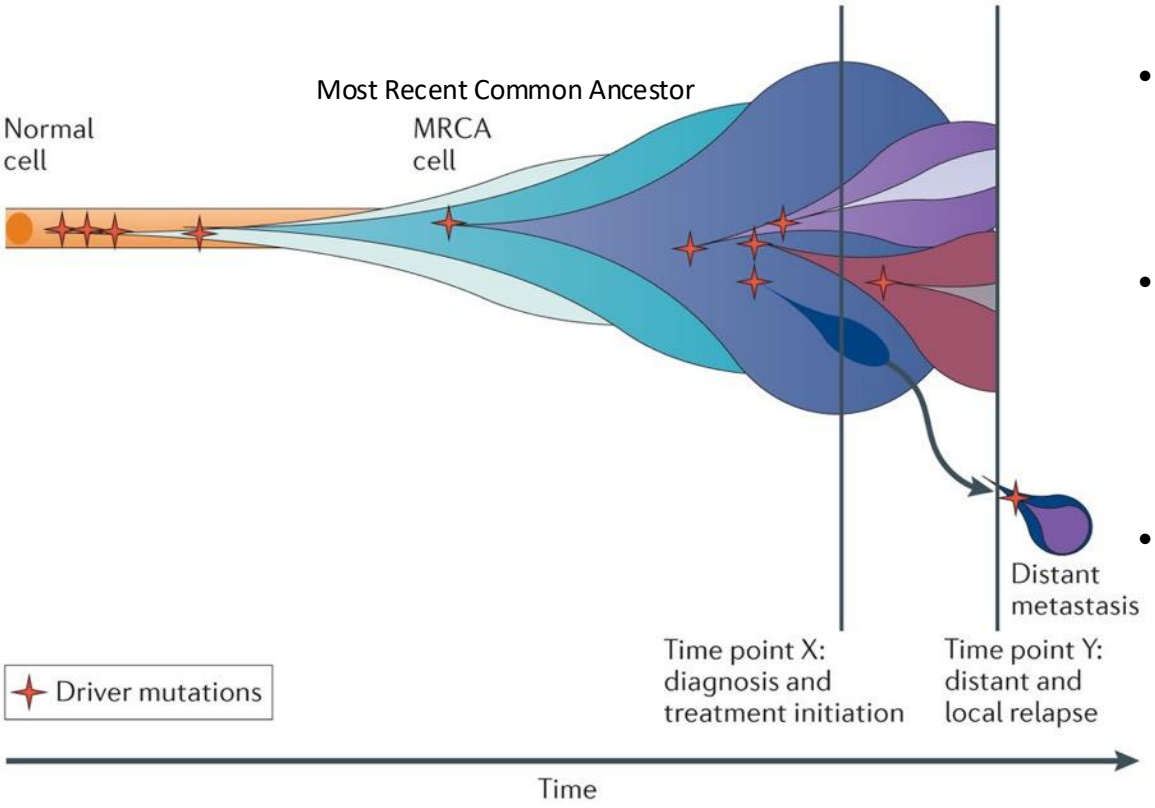
Entering the age of personalized medicine

Toward the elucidation of each person's genetic make-up

Necessary for:

2) Identification of novel molecular signatures for disease diagnosis, prognosis, or drug design

Another striking example: cancer



Q10 Cancers are genomically diverse and dynamic entities:

- Unique clones (colored bubbles) arise because of accumulating driver mutations in MRCA cell progeny
- Ongoing linear and branching evolution results in multiple subclones which drive disease relapse and metastasis.
- The dynamic clonal architecture is shaped by mutation and competition between subclones given specific environmental selection pressures, including cancer treatments.

Yates and Campbell, Nat Rev Genet, 2012

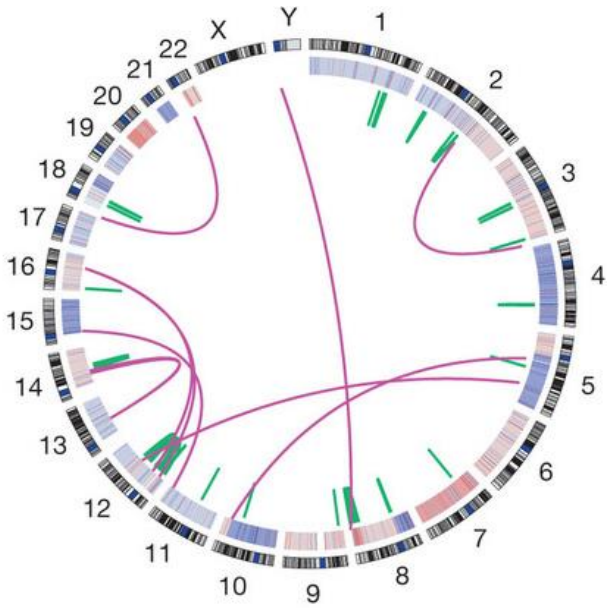
Entering the age of personalized medicine

Toward the elucidation of each person's genetic make-up

Necessary for:

2) Identification of novel molecular signatures for disease diagnosis, prognosis, or drug design

Another striking example: colorectal cancer genome of one patient (wildtype versus cancer cells)



Interchromosomal translocations
Intrachromosomal translocations
Amplifications and deletions
Individual nucleotide mutations not shown

The molecular diversity of human cancer is staggering (scary!)
(Patients with = disease) ≠ (Patients with = underlying biological disorder)

Lander, Nature, 2011

What is chromotrypsis (cancer context)?

A. A mutation that leads to “trypsis”, i.e. fragmentation of a cell?
B. Fragmentation and re-ligation of chromosomes
C. Fragmentation of a tumor into individual cells resulting in cell spread and metastasis
D. Chromatin state fragmentation such that all genes on a particular chromosome become activated

70

Entering the age of personalized medicine

Toward the elucidation of each person's genetic make-up

Necessary for:

3) A DNA-guided therapy and dose selection

A person's genetic make-up significantly affects the efficacy of a drug

- Polymorphisms in the *VKORC1* and *CYP2C9* genes dictate the effective dose levels of the anti-coagulant **Warfarin**

- Polymorphisms in the *UGT1A1* gene correlate with increased toxicity of the anti-colon cancer drug **Irinotecan**

- Polymorphisms in the *MTHFR* gene are associated with increased toxicity of **Methotrexate** used to treat Crohn's disease

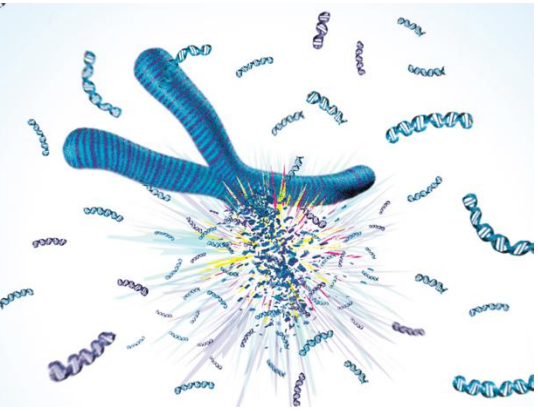
- Polymorphisms in the *CYP2D6* gene dictates the probability of relapse in women with breast cancer treated with **Tamoxifen**

Entering the age of personalized medicine

Toward the elucidation of each person's genetic make-up

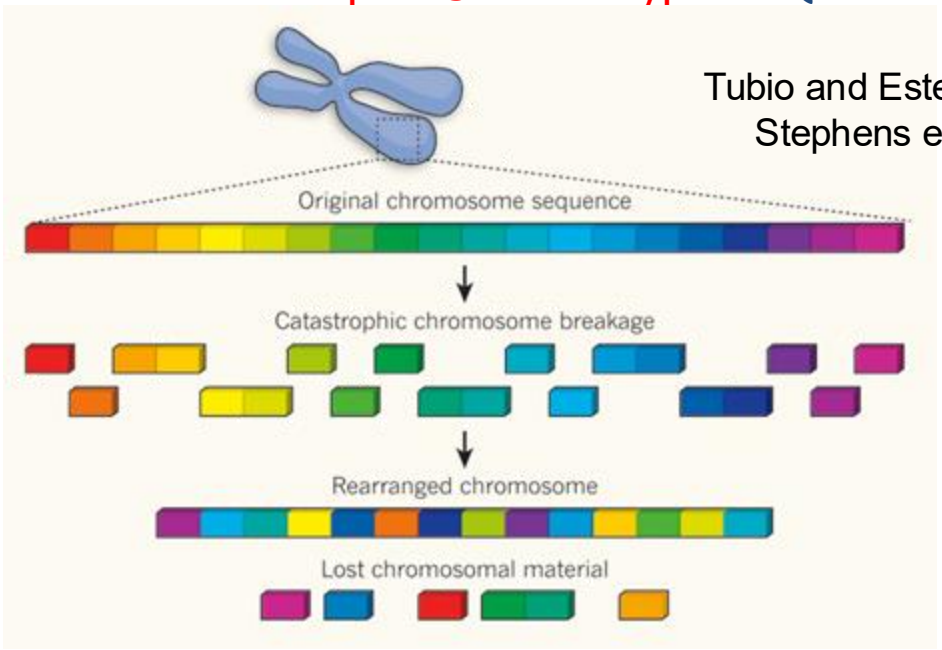
Necessary for:

2) Identification of novel molecular signatures for disease diagnosis, prognosis, or drug design



EMBL/P. Riedinger

Extreme example: Chromotrypsis **Q10**



Tubio and Estevill, Nature, 2011;
Stephens et al., Cell, 2011

- 1000's of clustered chromosomal rearrangements in a single catastrophic event in confined genomic regions (both cancer (2-3%) and congenital diseases)
- This phenomenon opposes the conventional theory that cancer is the gradual acquisition of genomic rearrangements and somatic mutations over time

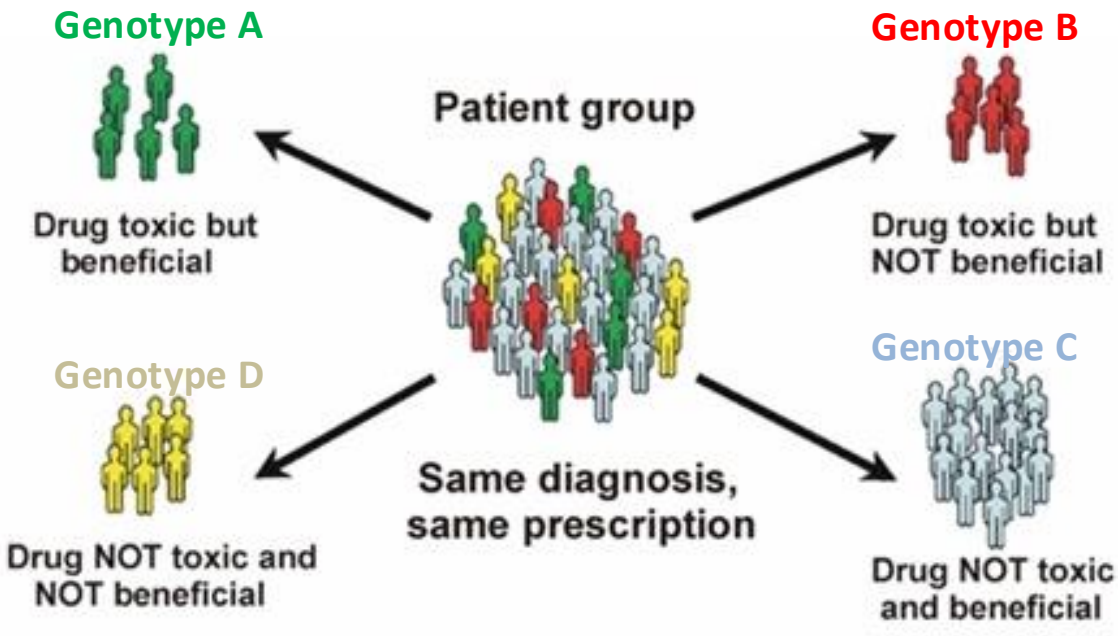
Entering the age of personalized medicine

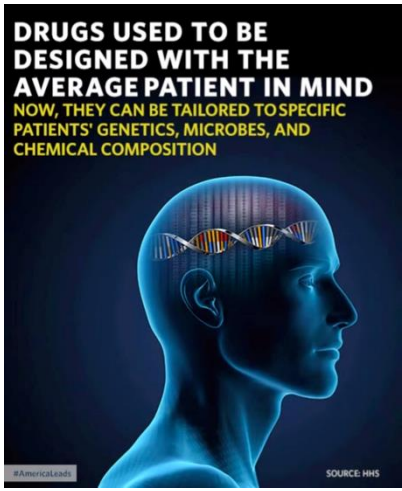
Toward the elucidation of each person's genetic make-up

Necessary for:

3) A DNA-guided therapy and dose selection

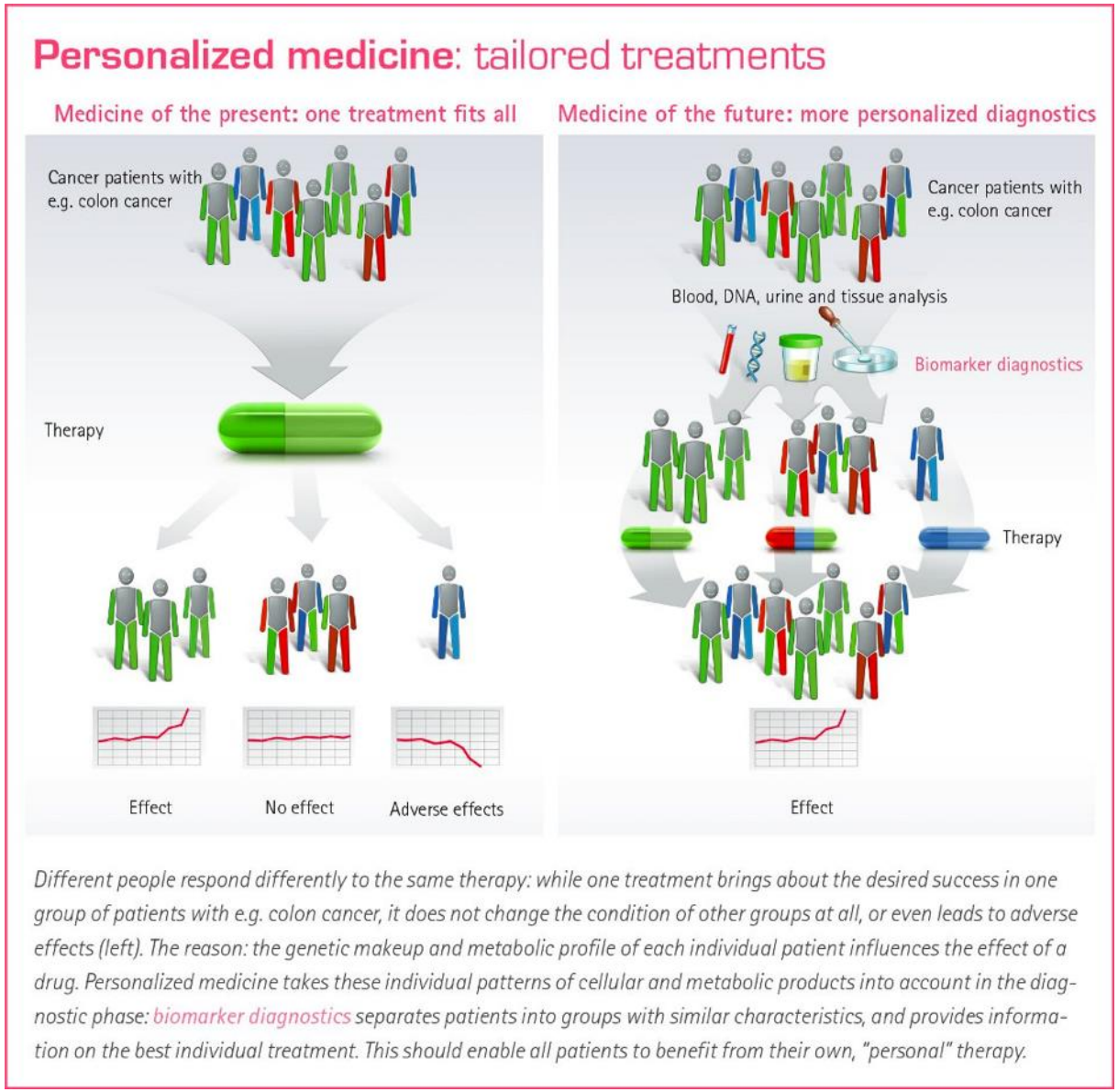
New strategy in clinical trials to test the efficacy of a new drug:





Q9

Drug may be as efficient as existing drug on a whole, but optimal for a specific subpopulation: *Genotyping is now essential in trials*



http://pharma.bayer.com/en/innovation-partnering/research-and-development-areas/oncology/personalized-medicine/#&gid=1&pid=1

Q9

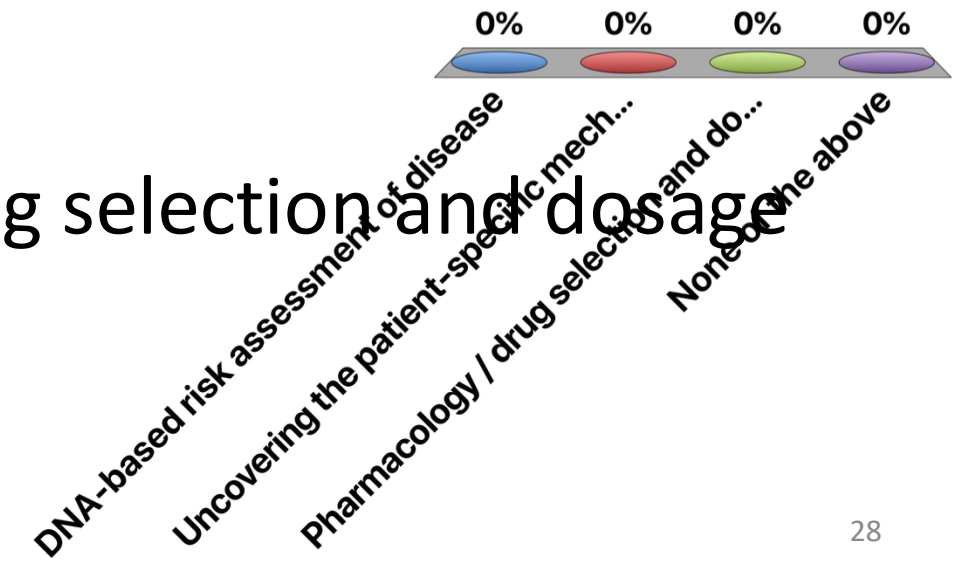
How can genomic analyses improve the practice of personalized medicine?

Summary

A. DNA-based risk assessment of disease

B. Uncovering the patient-specific mechanism underlying disease

C. Pharmacology / drug selection and dosage



From precision medicine to genetic engineering

Entering the age of personalized medicine

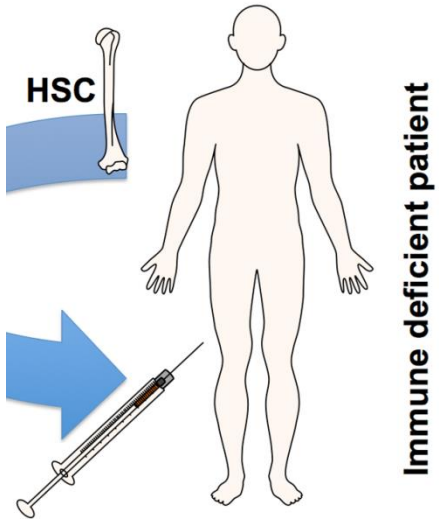
From a person's genetic make-up to gene therapy

RIP: «Bubble boy» David

Entering the age of personalized medicine

From a person's genetic make-up to gene therapy

RIP: «Bubble boy» David



- Received unmatched bone marrow transplant
- Died of lymphoma (12 yrs old)

SCID: severe combined immunodeficiency

γ chain mutation of cytokine receptor in T, B and NK cells (X-linked)

Entering the age of personalized medicine

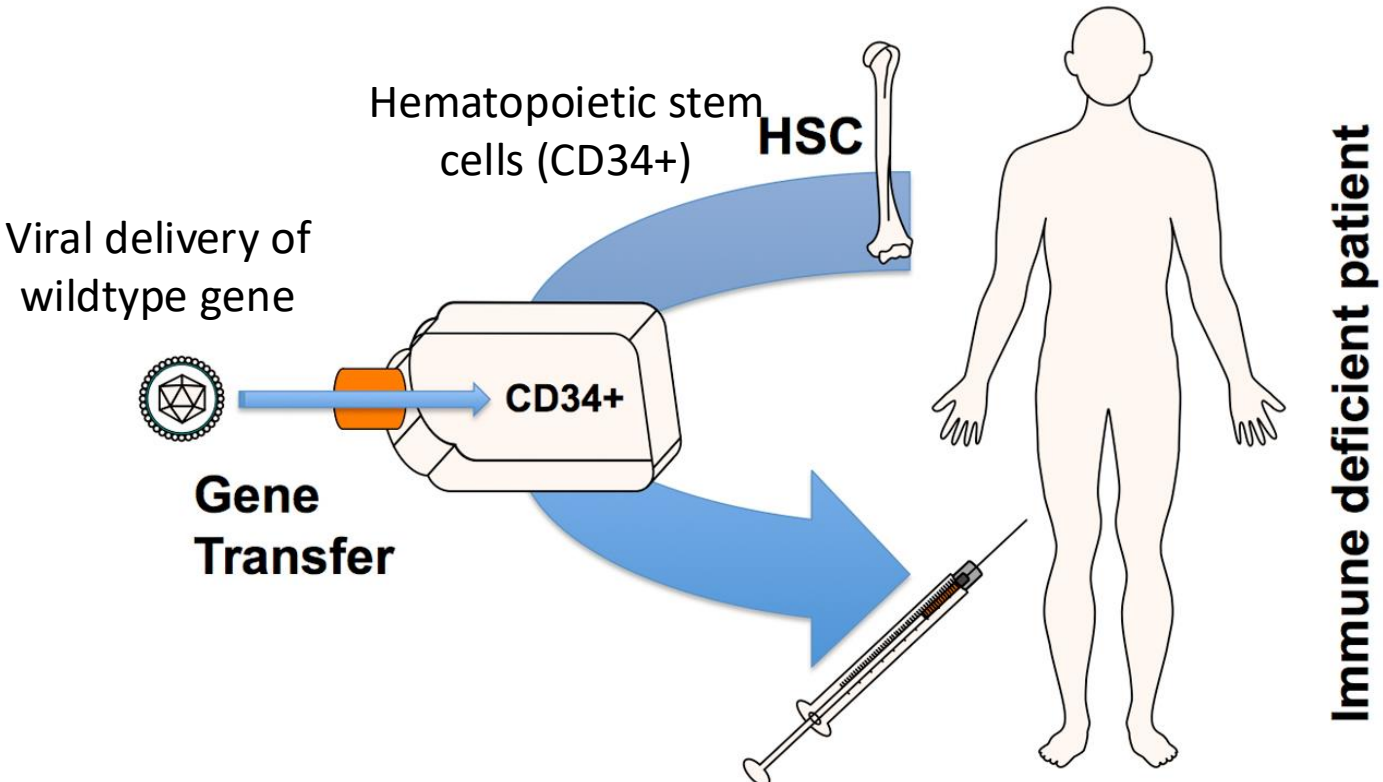
From a person's genetic make-up to gene therapy

RESEARCH ARTICLE

IMMUNODEFICIENCY Gaspar et al., Science Translational Medicine, 2011

Q11

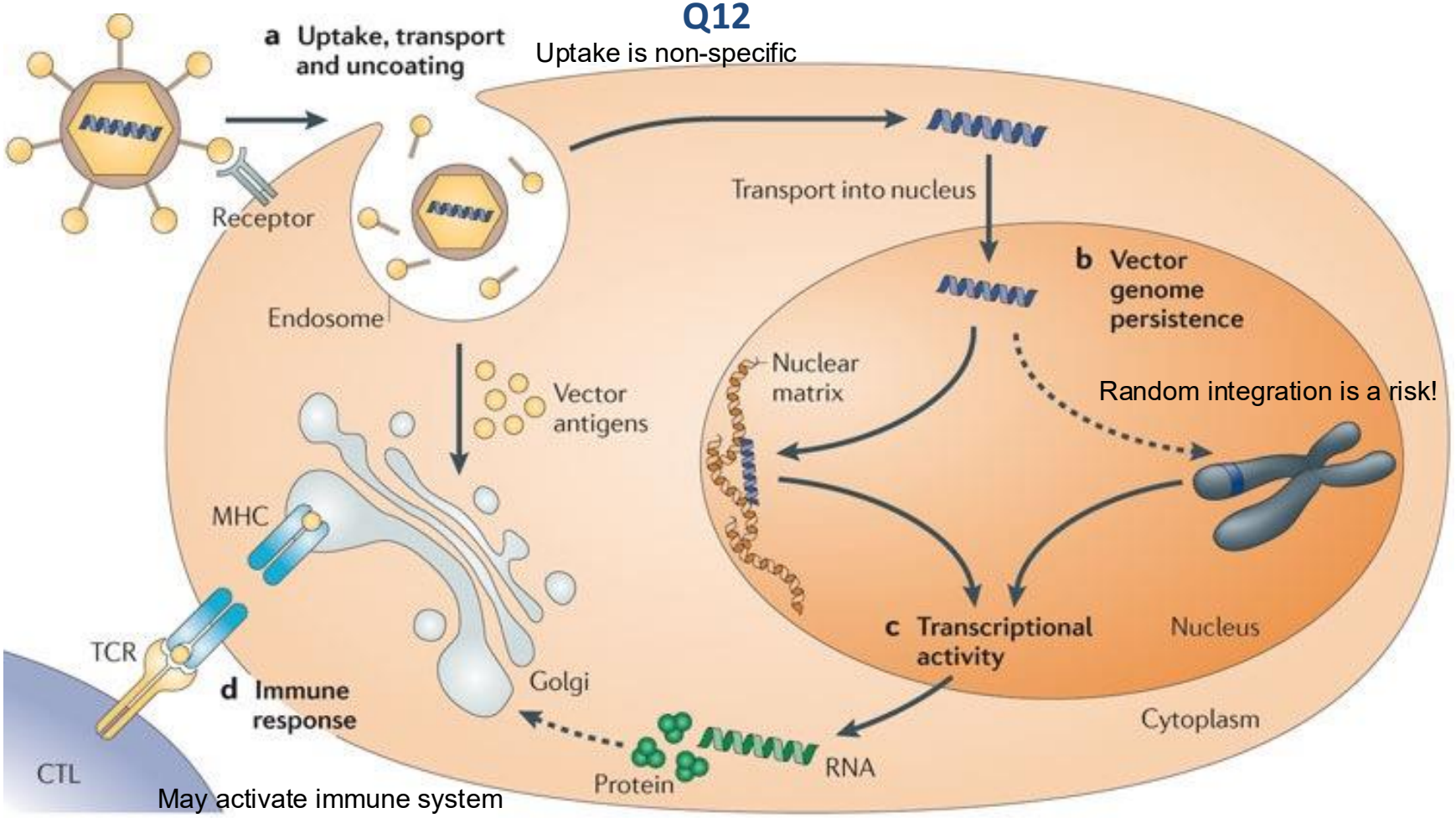
Long-Term Persistence of a Polyclonal T Cell Repertoire After Gene Therapy for X-Linked Severe Combined Immunodeficiency



Entering the age of personalized medicine

From a person's genetic make-up to gene therapy

But a long road ahead….: challenges



Kay, Nat Rev Genet, 2011

Entering the age of personalized medicine

From a person's genetic make-up to gene therapy

Genome engineering: **CRISPR**, a new kid on the block

Entering the age of personalized medicine

From a person's genetic make-up to gene therapy

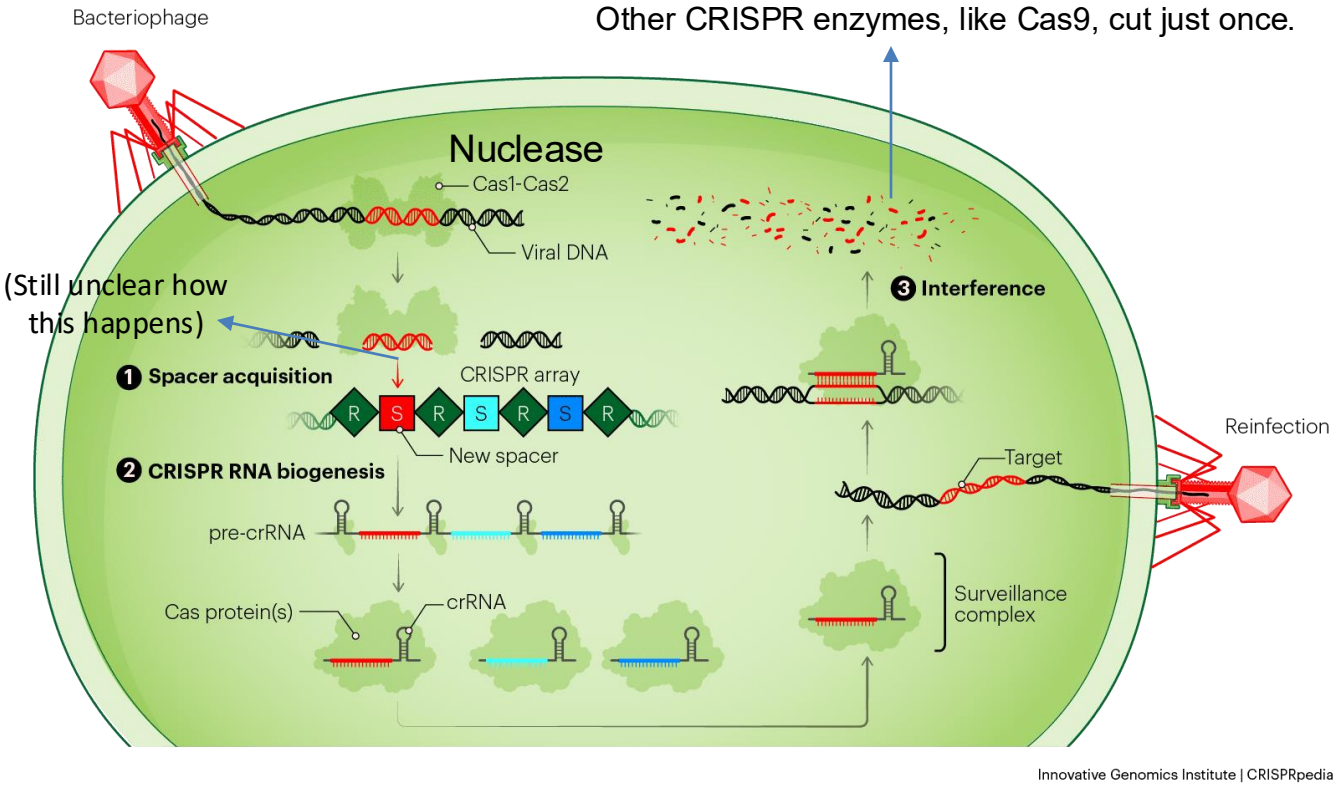
Genome engineering: **CRISPR**, a new kid on the block

Q13

CRISPRs (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats) → DNA loci that contain multiple, short, direct repetitions of base sequences (based on *Streptococcus thermophilus*, but in many bacteria)
CAS= CRISPR-associated proteins

Each repeat = series of bases followed by the same series in reverse and then by 30 or so base pairs known as "spacer DNA" → short segments of DNA from a virus and serve as a 'memory' of past exposures.

The Cas3 enzyme, found in type I systems, moves along target DNA, chopping it up like a lawn mower. Other CRISPR enzymes, like Cas9, cut just once.



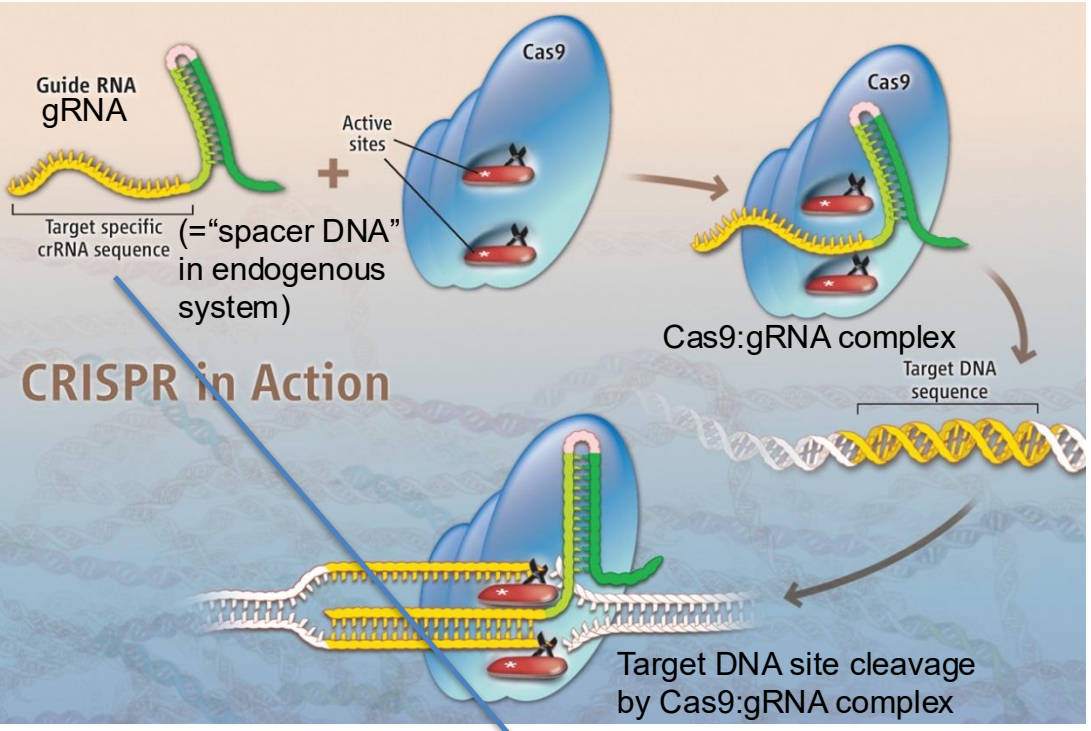
https://www.wur.nl/en/show/CRISPR-Cas-small-RNA-based-Adaptive-Immunity-in-Prokaryotes.htm

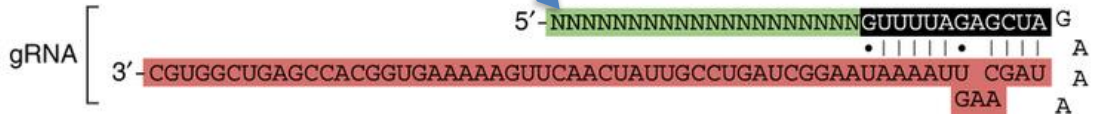
Entering the age of personalized medicine

From a person's genetic make-up to gene therapy

Genome engineering: **CRISPR**, biotech application

Q13





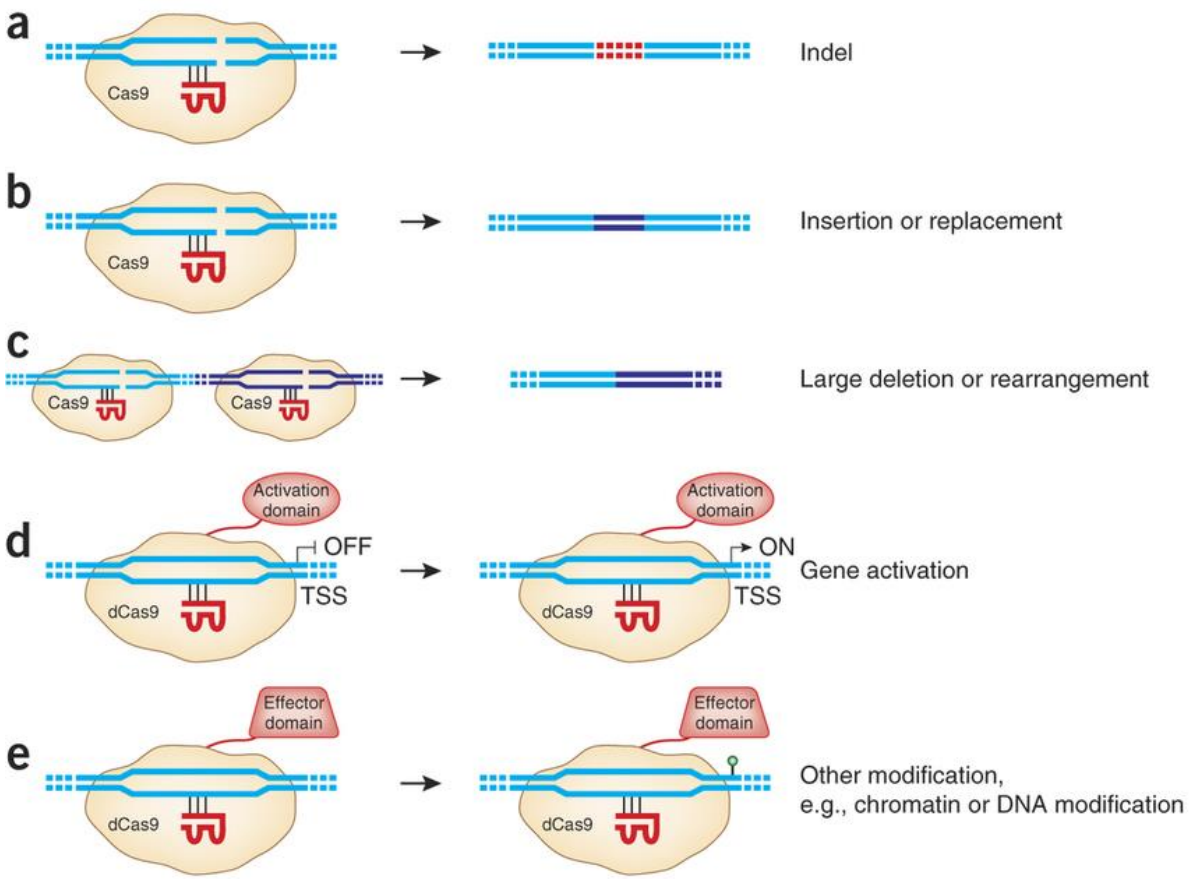
With just a guide RNA and a protein called Cas9, researchers first showed that the CRISPR system can home in on and cut specific DNA, knocking out a gene or enabling part of it to be replaced by substitute DNA.

Pennisi, Science, 2013; Sander and Joung, Nature Biotech, 2014

Entering the age of personalized medicine

From a person's genetic make-up to gene therapy

Q13 Genome engineering: **CRISPR**, biotech application



(**a**,**b**) gRNA-directed Cas9 nuclease can induce indel mutations (**a**) or specific sequence replacement or insertion (**b**).

(**c**) Pairs of gRNA-directed Cas9 nucleases can stimulate large deletions or genomic rearrangements (e.g., inversions or translocations).

(**d**–**f**) gRNA-directed dCas9 can be fused to activation domains (**d**) to mediate upregulation of specific endogenous genes, heterologous effector domains (**e**) to alter histone modifications or DNA methylation, or fluorescent proteins (**f**) to enable imaging of specific genomic loci. (TSS, transcription start site)

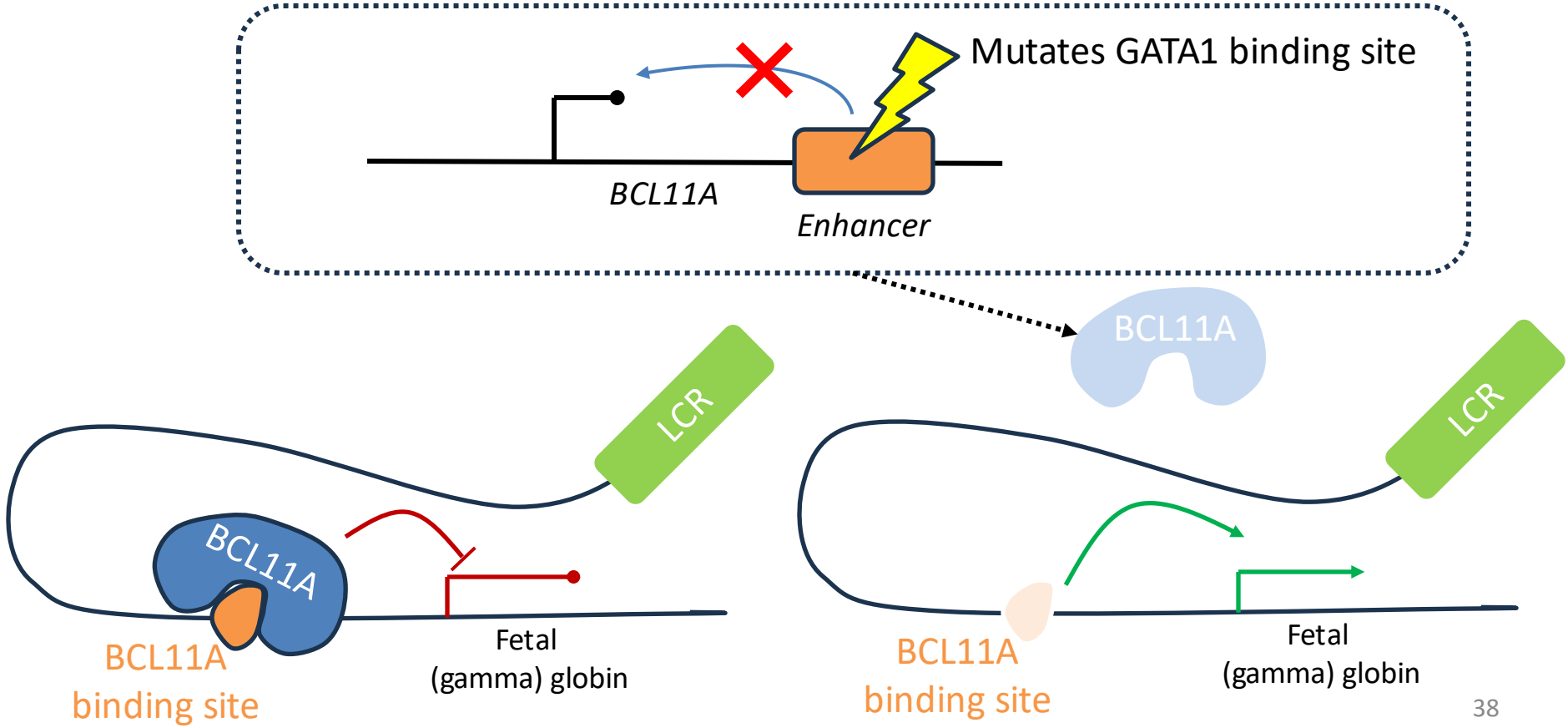
Sander and Joung, Nature Biotech, 2014

CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia

Haydar Frangoul, M.D., David Altshuler, M.D., Ph.D., M. Domenica Cappellini, M.D., Yi-Shan Chen, Ph.D., Jennifer Domm, M.D., Brenda K. Eustace, Ph.D., Juergen Foell, M.D., Josu de la Fuente, M.D., Ph.D., Stephan Grupp, M.D., Ph.D., Rupert Handgretinger, M.D., Tony W. Ho, M.D., Antonis Kattamis, M.D., et al.

New England J Medicine, 2011

- Does NOT target genetic mutations responsible for this deficiency
- Mutates a binding site for GATA1 in a key *BCL11A* enhancer that results in *BCL11A* downregulation and derepression of fetal hemoglobin



CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia

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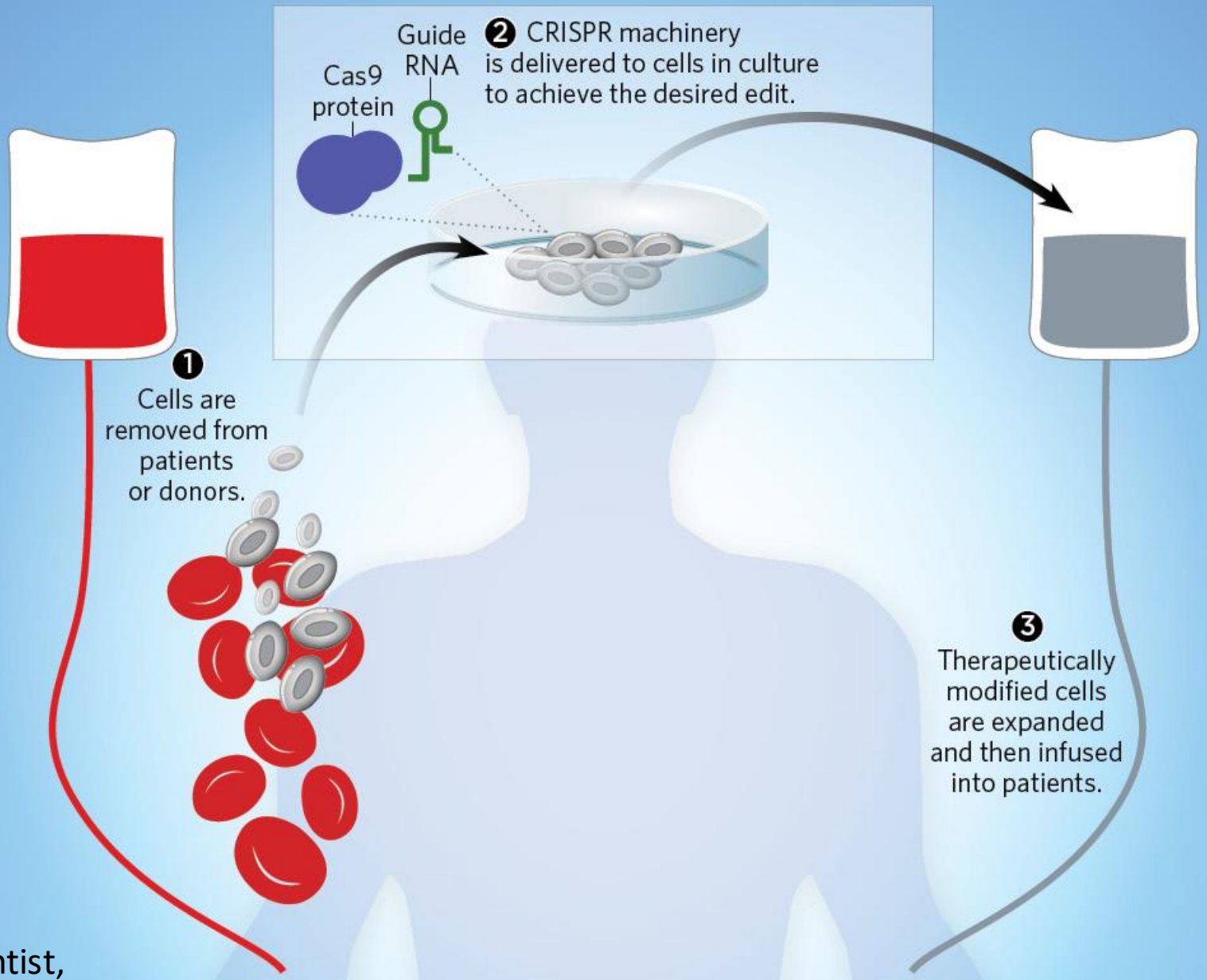
January 21, 2021
N Engl J Med 2021; 384:252-260
DOI: 10.1056/NEJMoa2031054
Chinese Translation 中文翻译

The UK's regulator has approved the world's first CRISPR–Cas9 gene editing therapy, which aims to cure sickle cell disease and transfusion-dependent β-thalassemia. Casgevy (exagamglogene autotemcel) is a first-of-its-kind treatment made by Vertex Pharmaceuticals and CRISPR Therapeutics in Zug, Switzerland. It comes just 11 years after Jennifer Doudna and Emmanuelle Charpentier invented the technology. The green light from the Medicines and Healthcare Products Agency represents a major scientific achievement for Vertex and CRISPR and a landmark for the biotech industry.

Approved: Jan 2024!

Q14

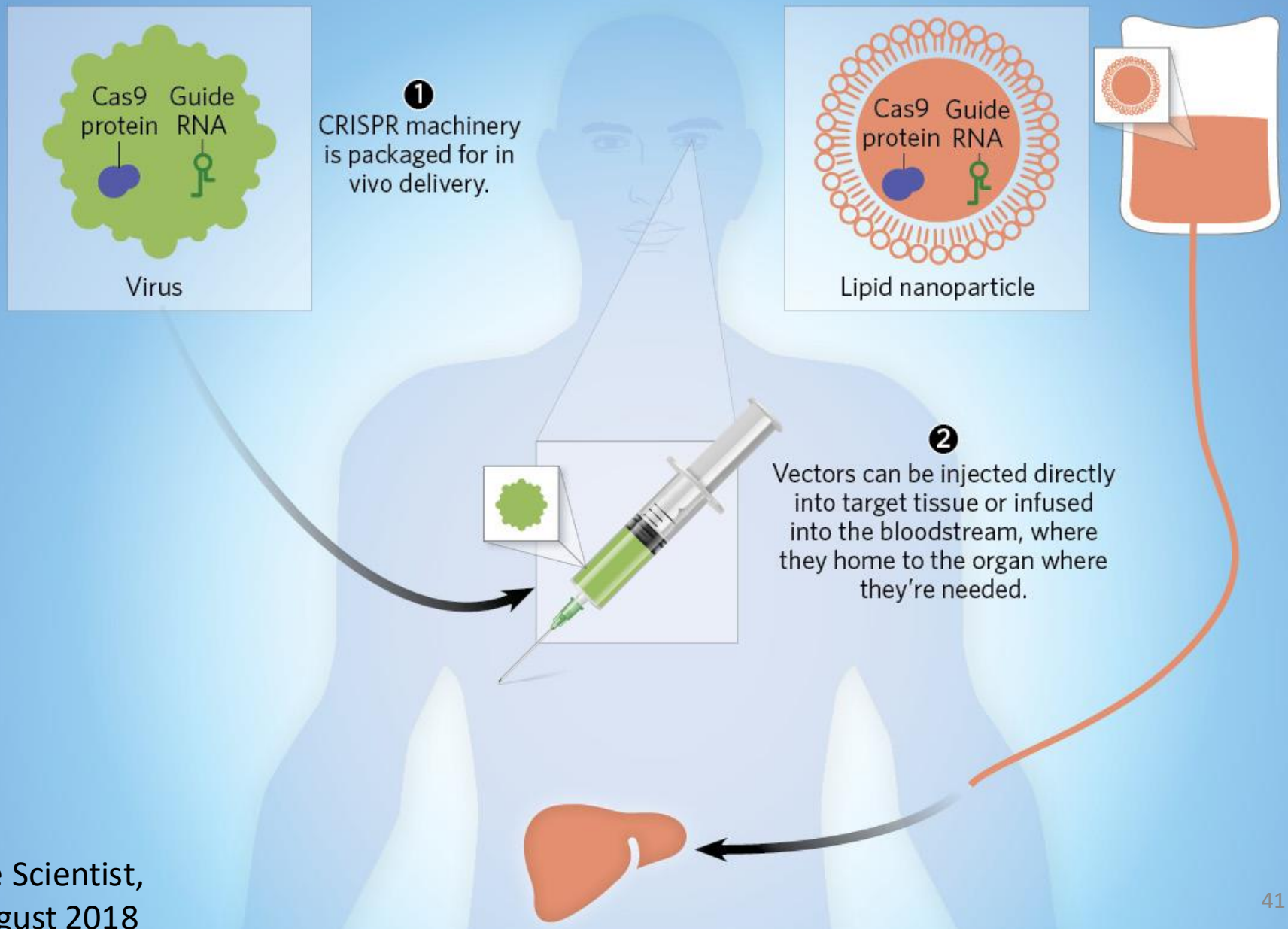
Ex vivo CRISPR therapy strategy



The Scientist, August 2018

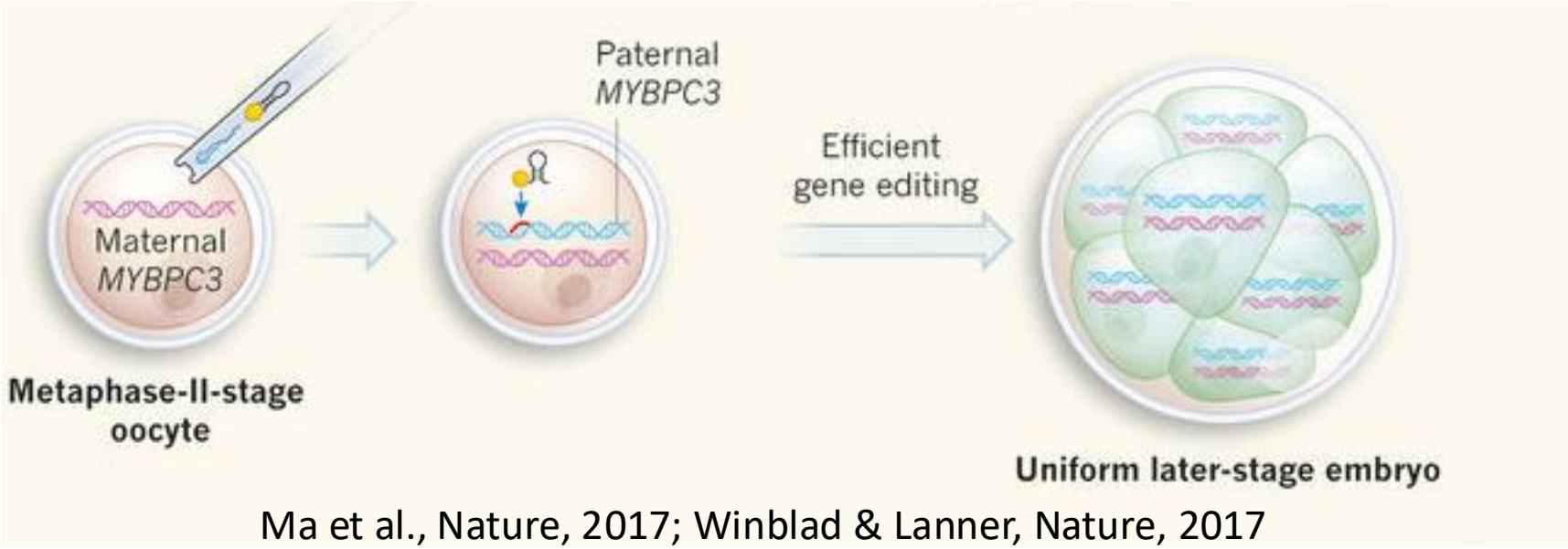
Q14

In vivo CRISPR therapy strategy



The Scientist, August 2018

Correction of a pathogenic gene mutation in human embryos



Ma et al., Nature, 2017; Winblad & Lanner, Nature, 2017

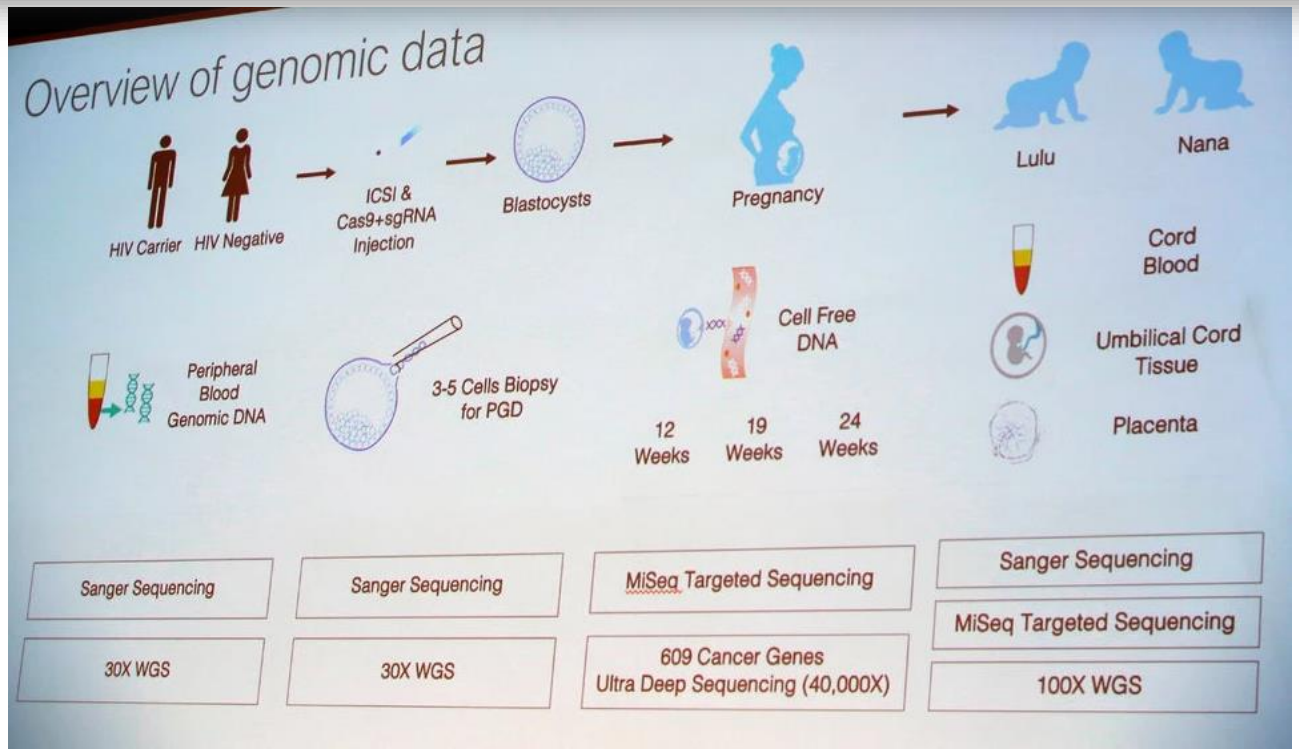
Aim: correct a mutation in the *MYBPC3* gene (which is associated with heart disease) in human embryos:

- Gene-editing components and sperm were injected into oocytes that contained non-mutated versions of *MYBPC3*
- Half the sperm used had a *MYBPC3* mutation
- 42 of 58 embryos tested (72.4%) did not have the *MYBPC3* mutation, indicating that at least “half” (around 25%) of the embryos were rescued

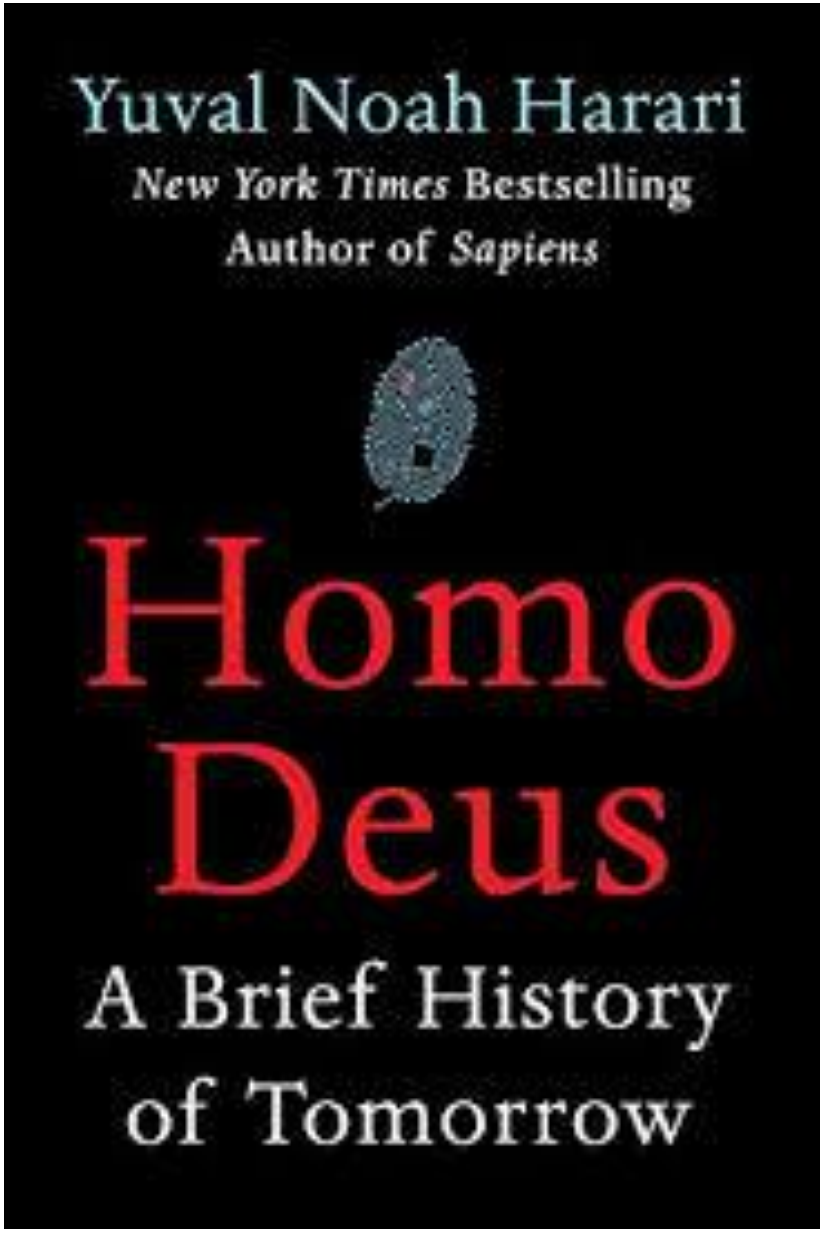
The New York Times

Why Are Scientists So Upset About the First Crispr Babies?

Only because a rogue researcher defied myriad scientific and ethical norms and guidelines. We break it down.



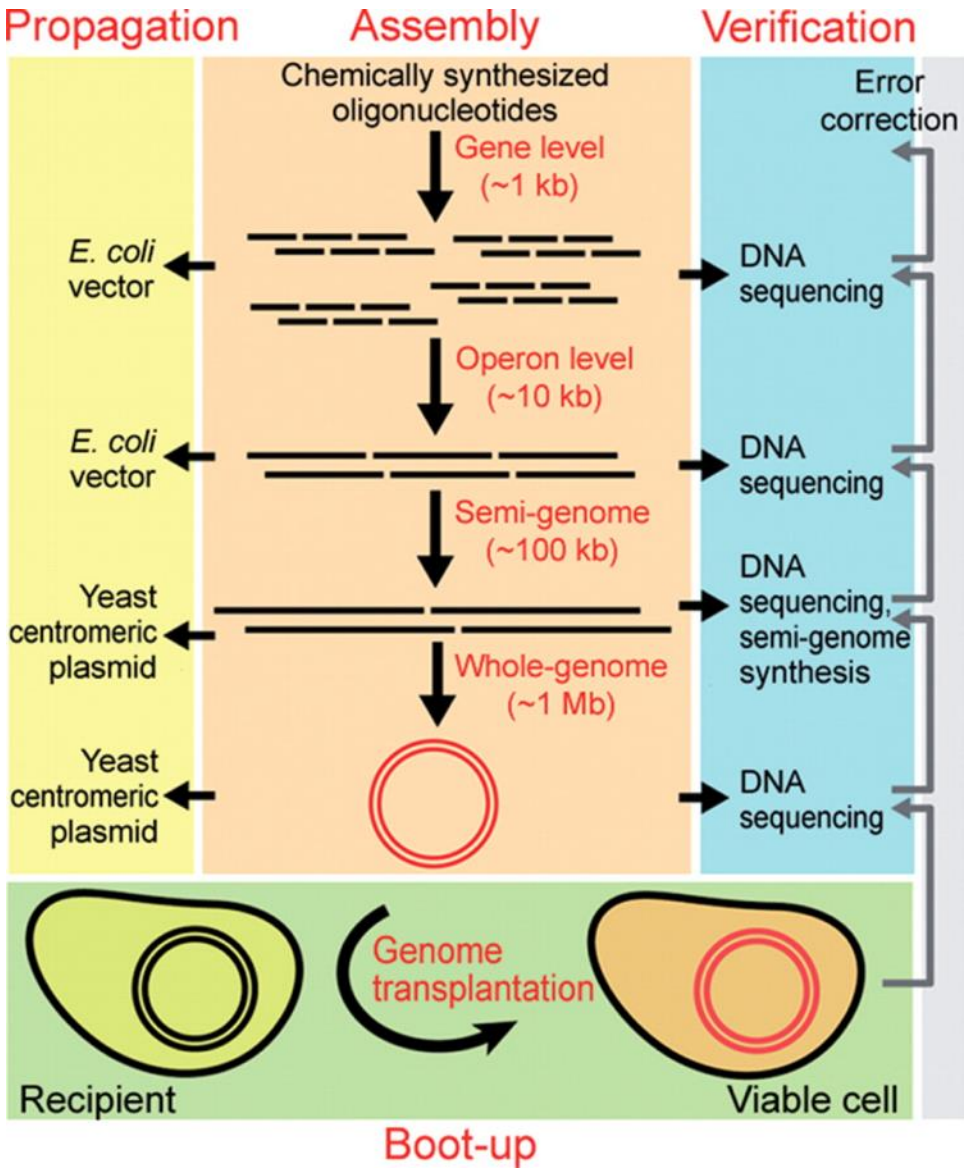
https://www.nytimes.com/2018/12/05/health/crispr-gene-editing-embryos.html

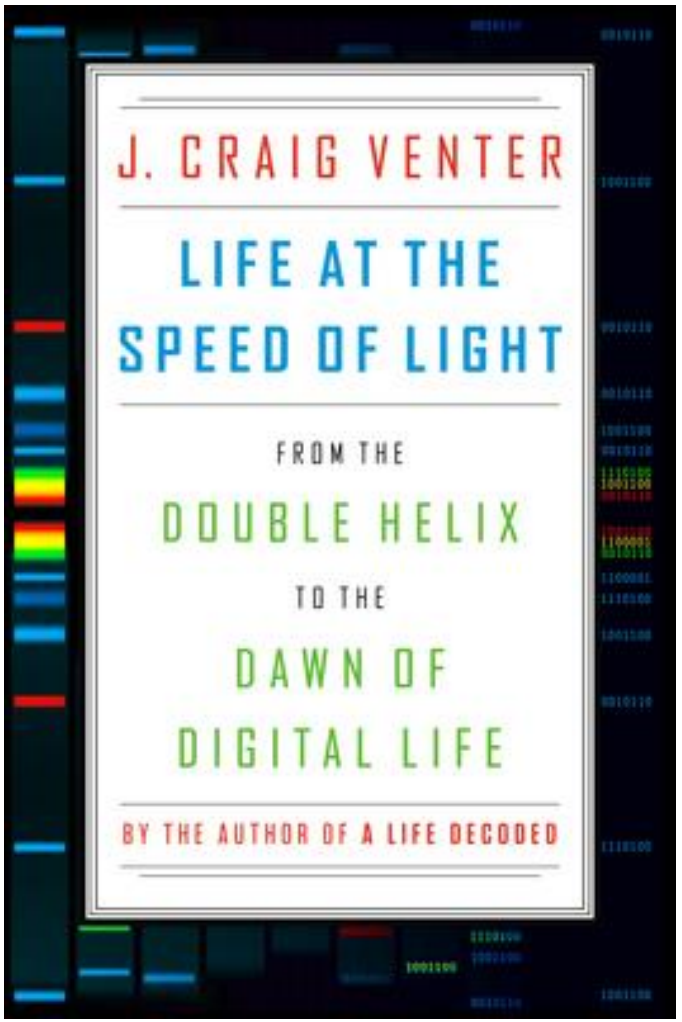

Yuval Noah Harari
New York Times Bestselling
Author of Sapiens
Homo
Deus
A Brief History
of Tomorrow

The Synthetic Genome

Goal: Build a bug

17 Oct 2013





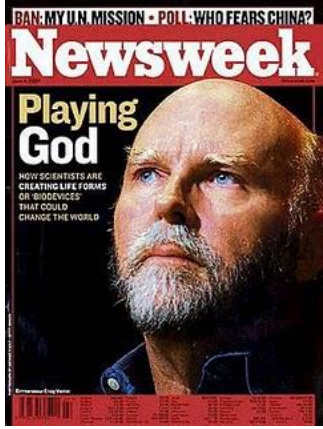
Gibson et al., Science, 2010

The Synthetic Genome

Synthetic biology and ethical implications

Synthetic "Life," Ethics, National Security, and Public Discourse

Cho and Relman, Science, 2010



"Synthetic genomics and synthetic biology may necessitate a new model for addressing ethical and policy issues because of the complexity of the biological systems being mimicked and manipulated. The complex interactions of biological parts and their evolution will likely lead to unpredictable, emergent behavior in engineered organisms and ecosystems."

"The greatest challenge in addressing biosecurity and ethical concerns has been, and will be, to design effective oversight mechanisms that avoid undue harm to the overwhelmingly beneficial life sciences enterprise. "

"A realistic assessment of likely benefits is important because it highlights potential issues of distributive justice and fairness, especially with growing skepticism about the practical application of genomics to date, and the tendency toward hype."

"Further discourse in this area should be informed by perspectives from theology, philosophy, the social sciences, and the general public."

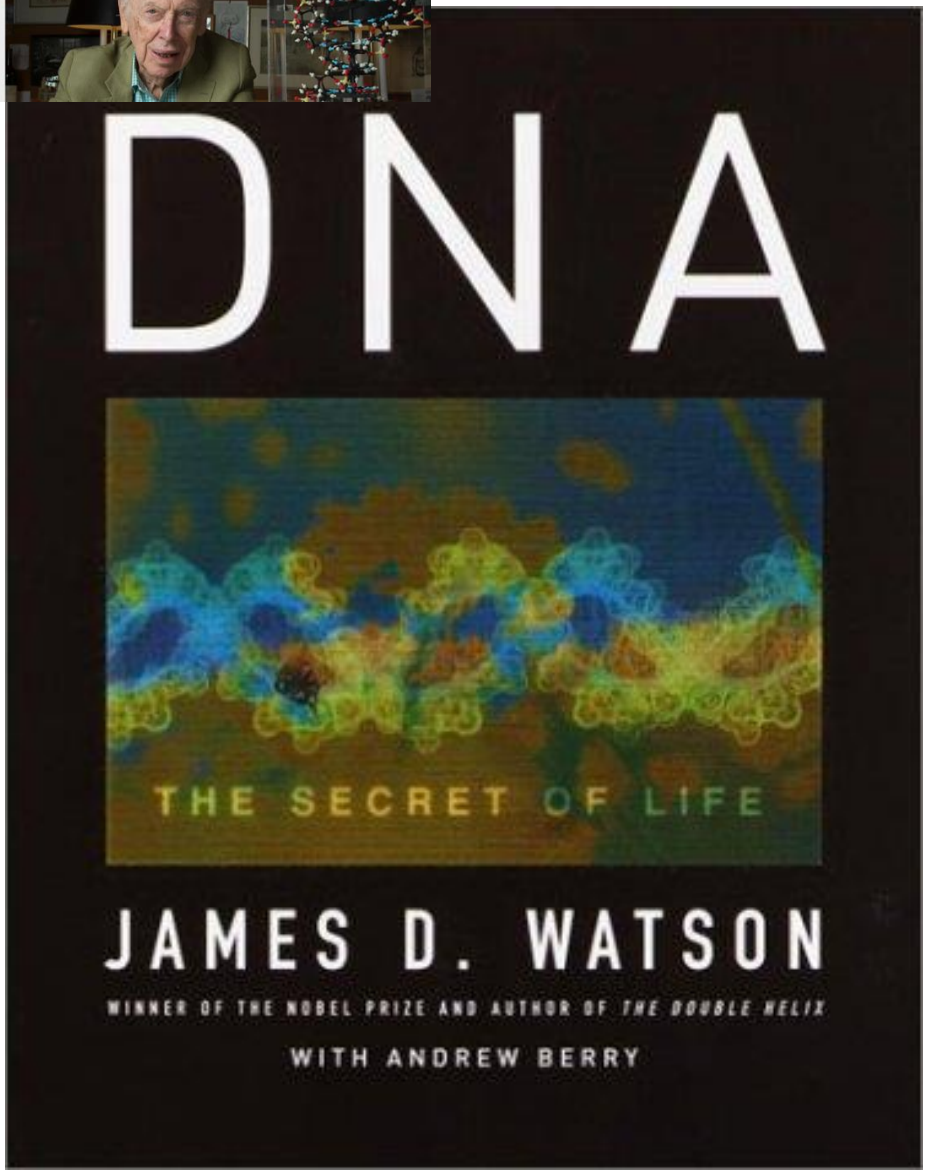
James Watson, Who Helped Discover the Structure of DNA, Is Dead at Age 97

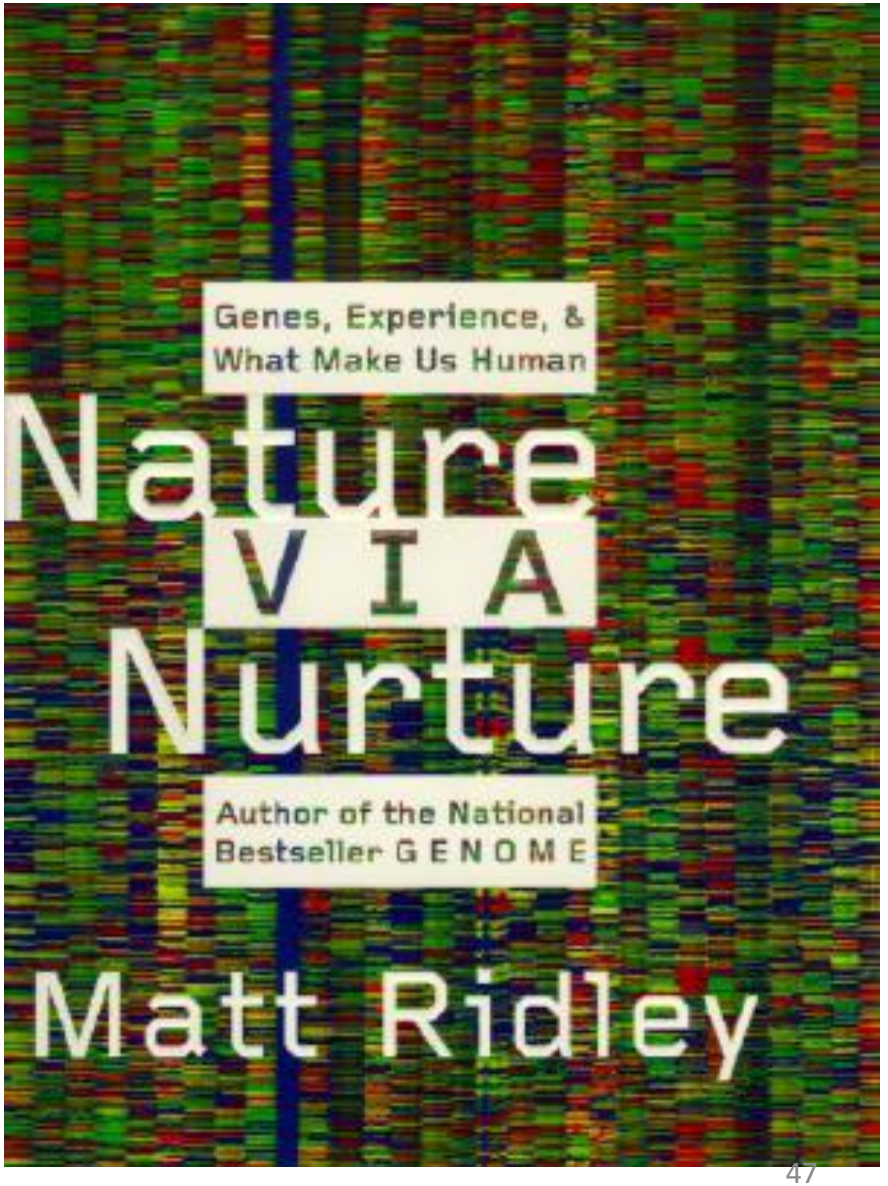
James Watson's work on the discovery of the double-helix structure of DNA led to a revolution in biology and genetics

BY DAVID M. EWALT

Nov 7th 2025

Other Christmas reading





Follow-up courses

BIOENG-420 Single Cell Genomics (MA2)

BIO-411: Life Sciences Engineering: genome to function (MA2)

BIO-463 Genomics and Bioinformatics (MA1 or 3)

BIO-468 Scientific literature analysis in Computational molecular biology (MA1)

BIOENG-455 Computational cell biology (MA1)

CS-433 Pattern classification and machine learning (MA1 or 3)

CS-502 Deep learning in Biomedicine (MA1)

BIO-491 New tools and research strategies in personalized health (MA2)

ChE-411 Principles and Applications of Systems Biology (MA1 or 3)

MATH-493 Applied biostatistics (MA2) (further expansion of R)

BIO-455 Introduction au droit et à l'éthique en STV (MA1 or 3)

Did this class change your opinion about sequencing your genome?

A. Yes and now I want to know
B. Yes and I no longer want to know
C. No, I always wanted to know
D. No, I never wanted to know

60

What do you think are opportunities? (sequencing your genome)

70

What are your principal concerns? (sequencing your genome)

70