INFRAFRONTIER/ IMPC
Stakeholder Meeting

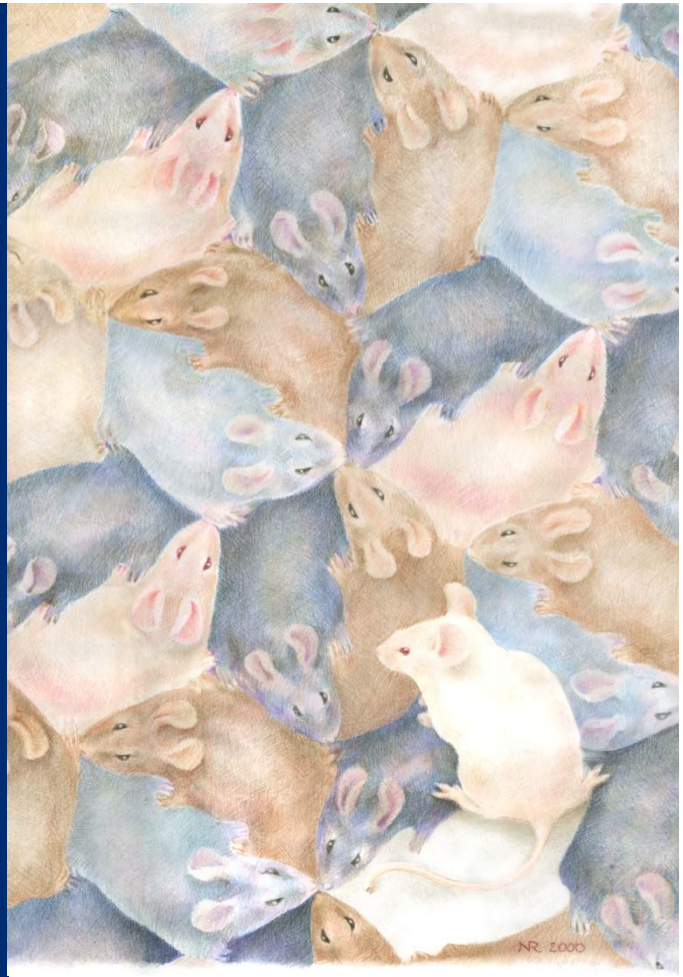
Advancing Personalised Medicine with Animal Models

Athens November 2017

Of mice and CRISPR

Nadia Rosenthal

FMedSci FAAHMS

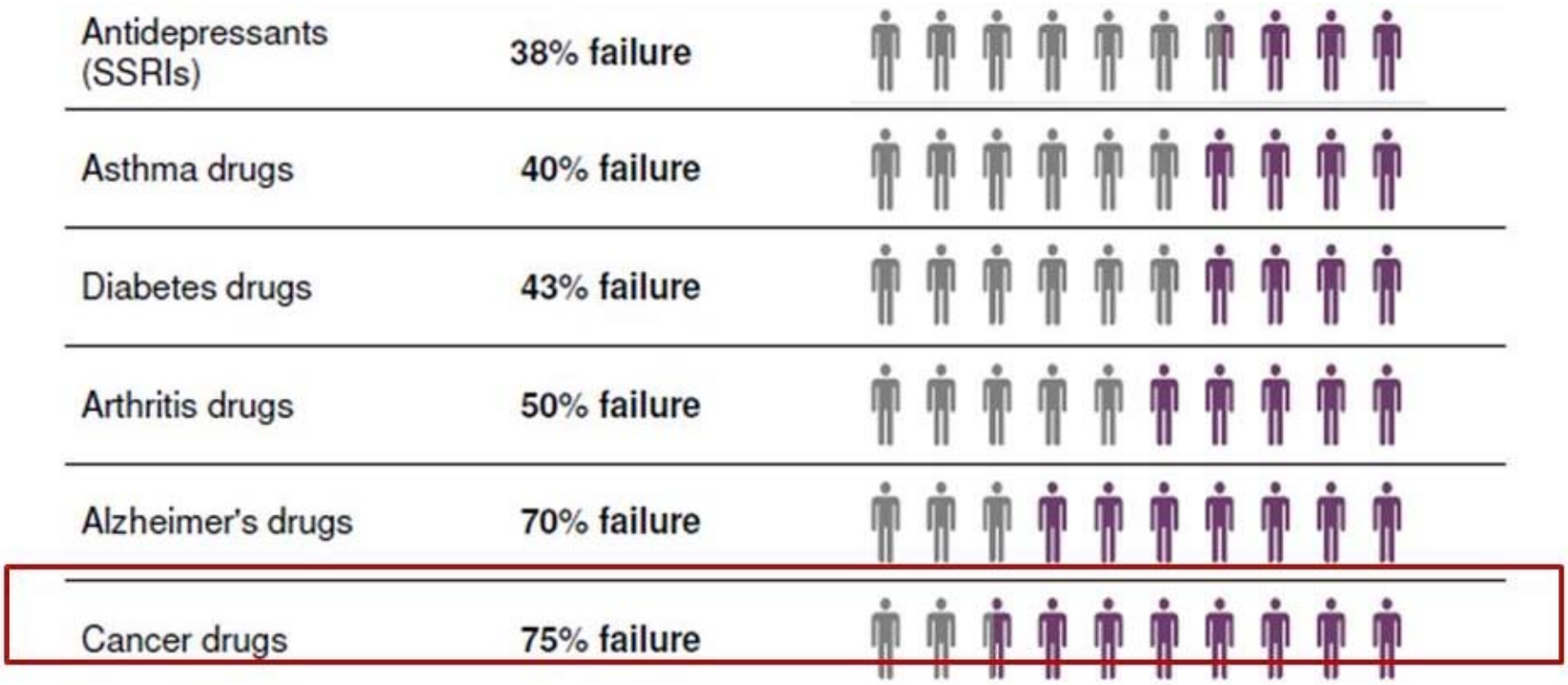


The Jackson Laboratory

Imperial College London

Why precision medicine?

Patients respond differently to the same medicine:



Achieving precision medicine

"..a revolution in the way we cure and prevent disease, using cutting edge technology to match patients with treatment."

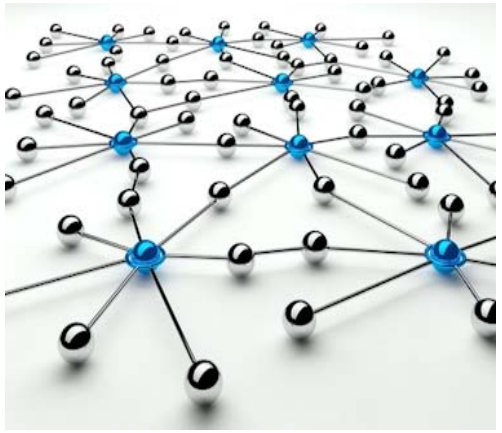
Medical research then: *Defined molecular pathways* *govern health and disease*

Medical research now: *Individual's genetic makeup* *governs the unique set of features and functions underlying health status and disease susceptibility*

Challenges of precision medicine

Different diseases disrupt **distinct sets of biological networks**:

- genetic networks
- molecular networks
- cellular networks
- organ networks

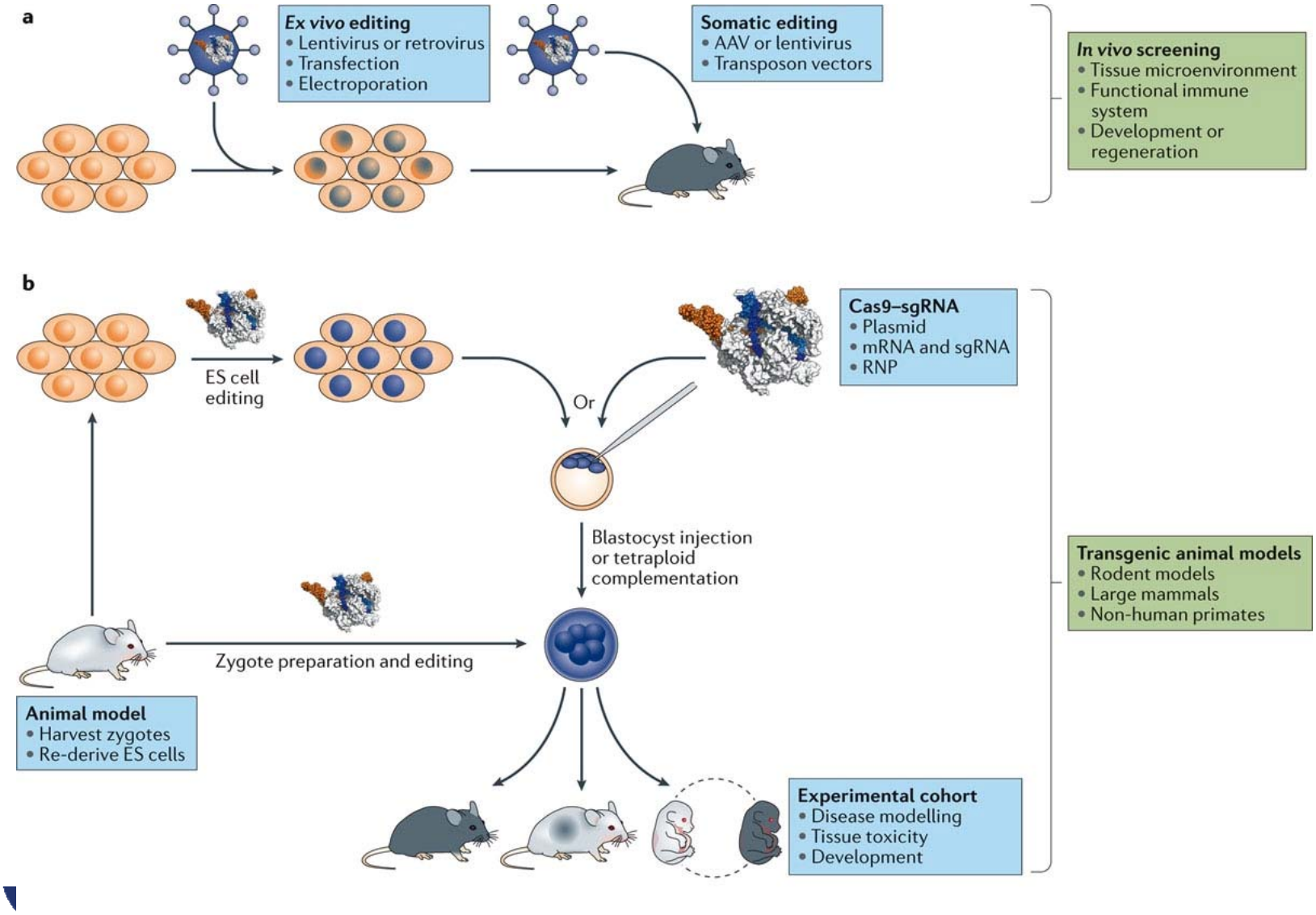


Variation in disease susceptibility and outcome between individuals reflects distinct **disturbances in an assembly of networks**.

What scientific vistas will CRISPR open for precision medicine?

- Creation of **complete allelic series** of a genetic disease
- **Experimental validation** of the phenotypic output of combinatorial gene variations
- **Humanization**: the progressive reshaping of the whole mouse genome
- Comprehensive understanding of **genetic background** and its effect on Mendelian disease mutation

CRISPR-Cas in mouse modelling



? How to model complex traits with CRISPR?

- **Multiplex mutations** in a single mouse
- **Stack variations** by crossing multiple mutant mice for combinatorial power
- **Reengineer functional portions** of the mouse genome (eg immunoglobulins)
- **Expand the genetic diversity** of mouse backgrounds on which to test mutations

Inbred mice: pros and cons

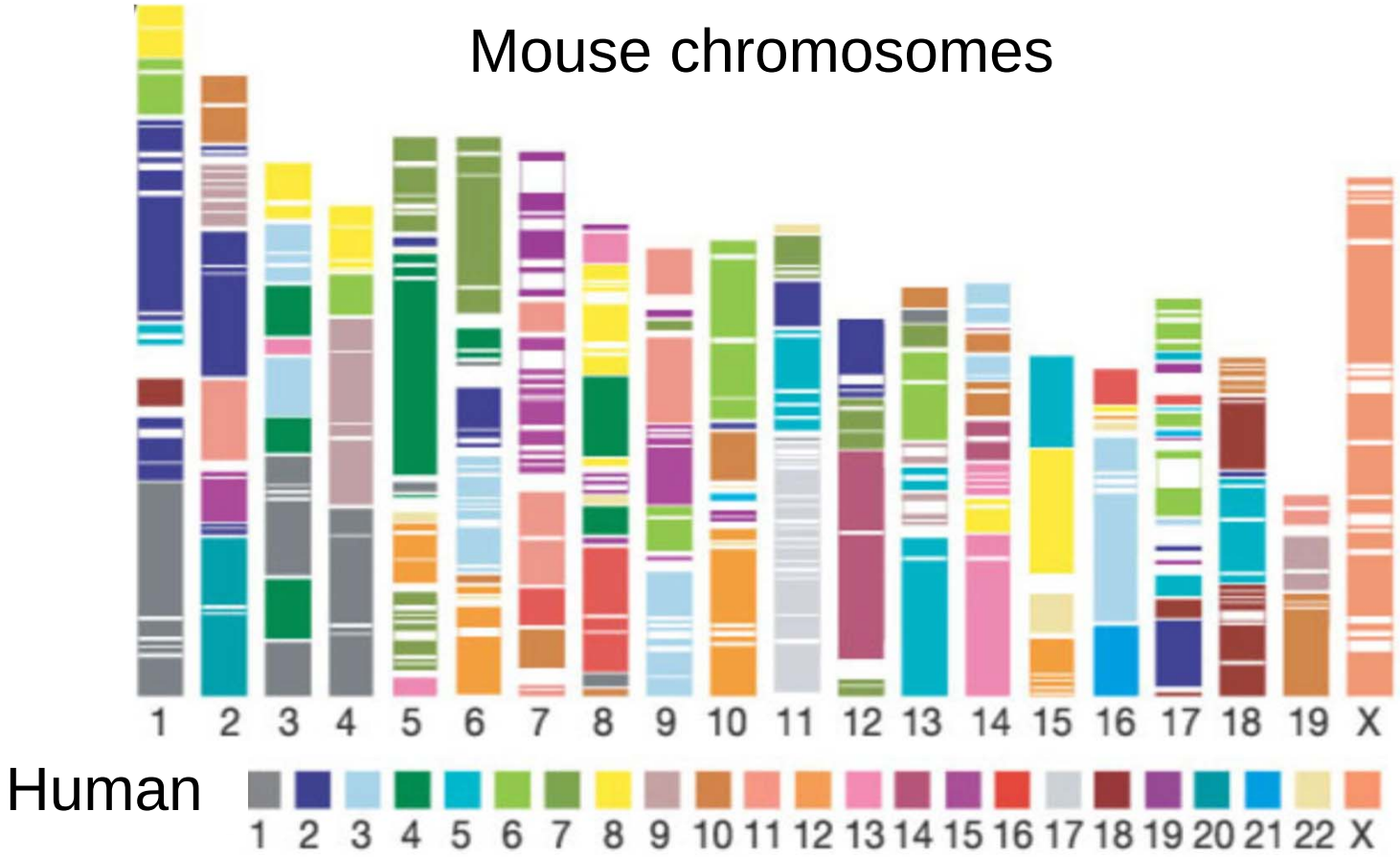
- Homozygous across their entire genome
- All mice within a strain are genetically identical
- Reproducible models of disease

A/J: high incidence of spontaneous lung adenomas

C3H/HeJ: high incidence of spontaneous hepatomas

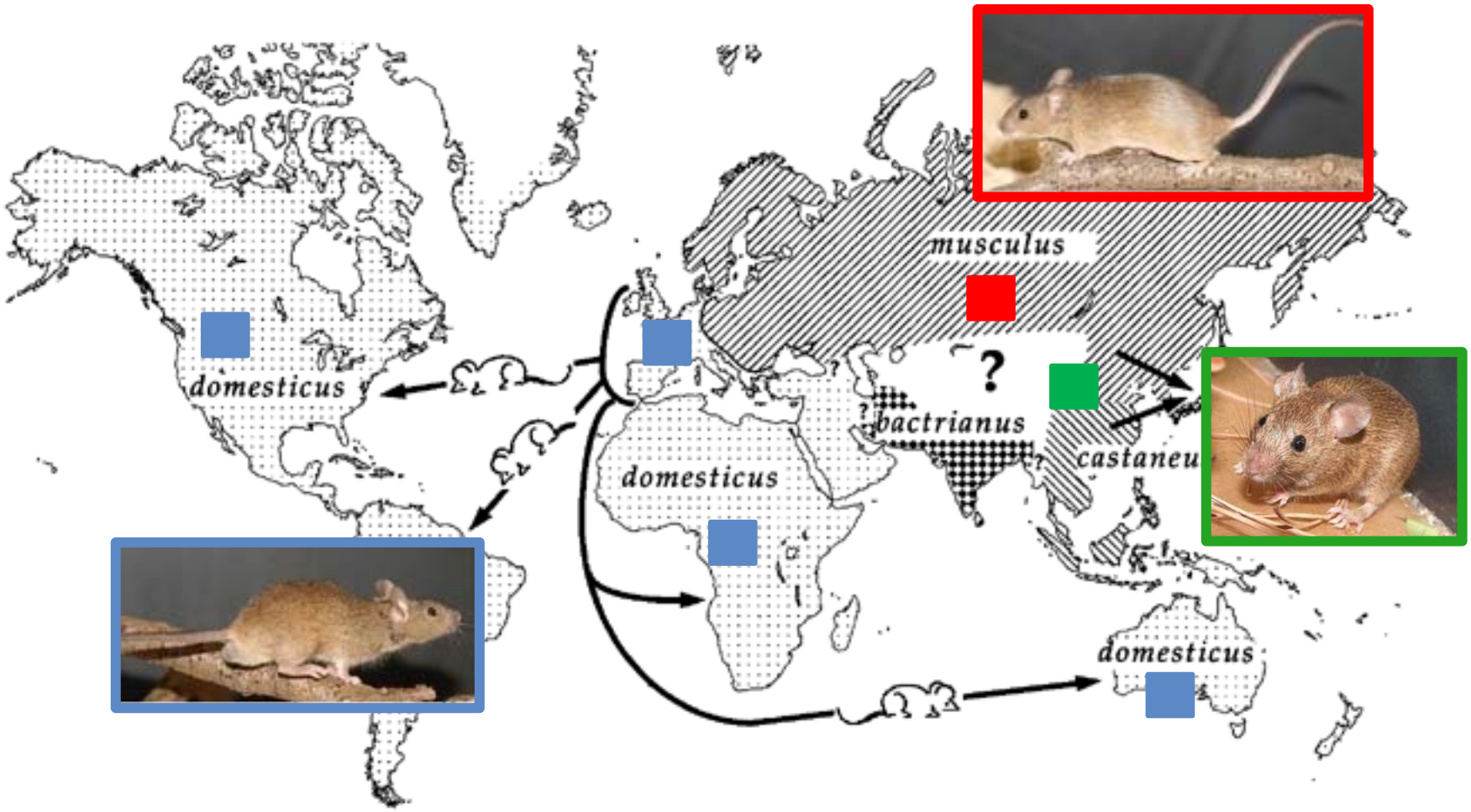
C57BL/6J or N:
a.k.a. “The Mouse”

Much of our genetic information can be mapped onto the mouse genome (synteny)

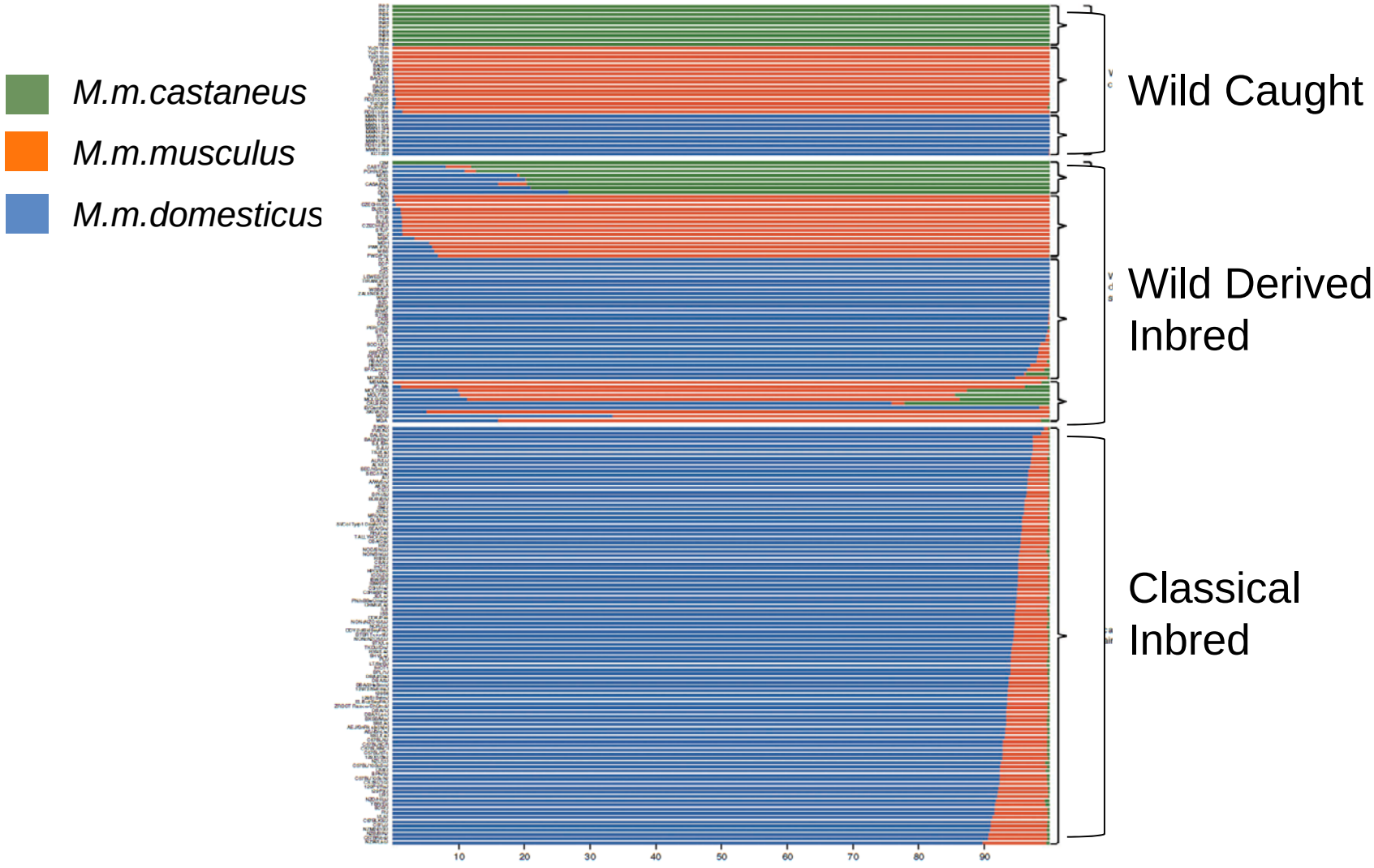


Ali et al., *BMC Bioinf.*, 2013

Laboratory mice are derived from three major sub-species

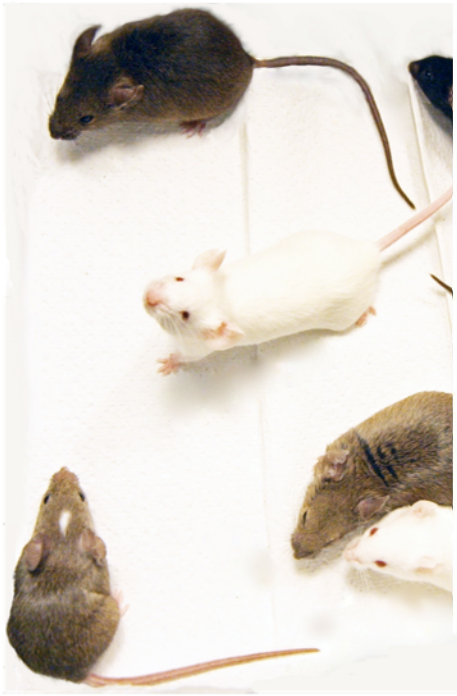


Limited genetic diversity of inbred mouse lines



Yang et.al., *Nat.Genetics.,* 2011

Is an inbred mouse a good model for the genetically diverse human population?

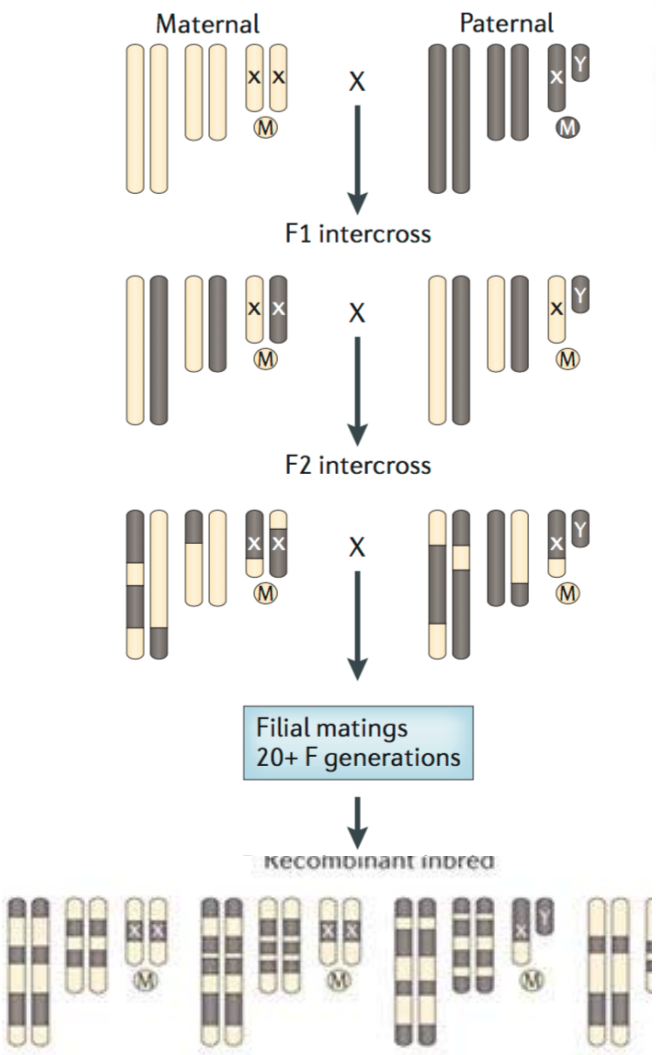


Genetically diverse mice

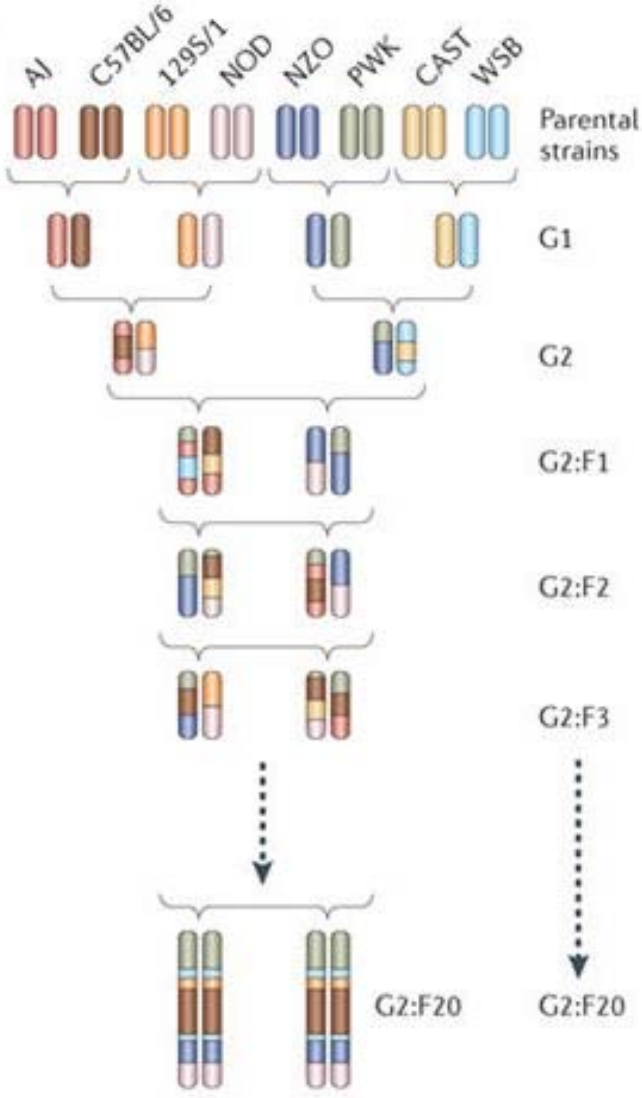
Genetically diverse human popoulation

Recombinant inbred resources

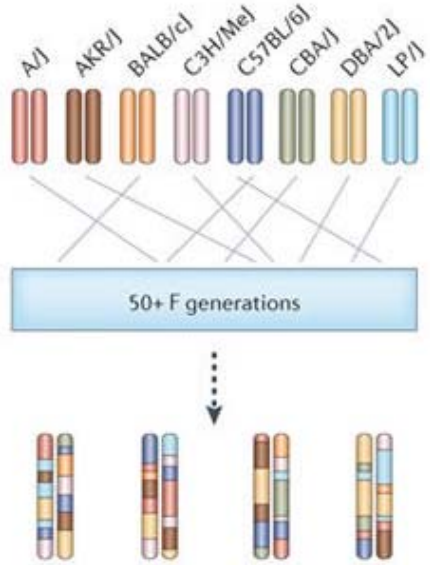
F2 intercross (BxD)



Collaborative Cross



Diversity Outcross

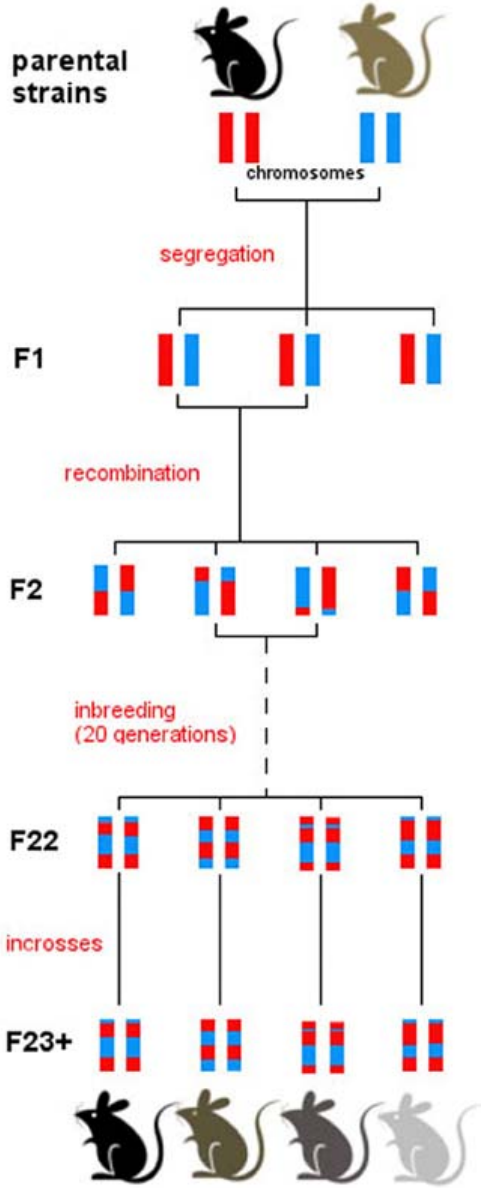


BxD Recombinant Inbred strains

The BXD family of strains were derived by crossing C57BL/6J (B6) and DBA/2J (D2) and inbreeding progeny for over 20 generations.

Data for thousands of phenotypes and nearly 100 gene, protein, and metabolite expression data sets have been acquired over a nearly a 40-year period.

Two strains differ at approximately 4.8 million SNPs. Variants are mostly single nucleotide polymorphisms and about 500,000 insertion-deletions)



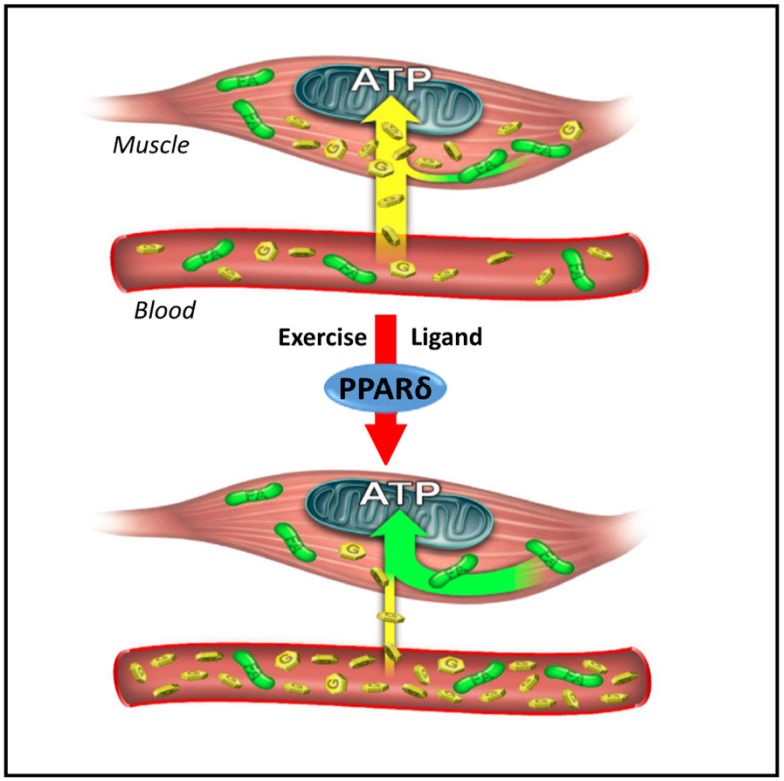
Mapping endurance parameters with BxD

Fan et al, 2017

Cell Metabolism

PPARδ Promotes Running Endurance by Preserving Glucose

Graphical Abstract



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Weiwei Fan, Wanda Waizenegger, Chun Shi Lin, ..., Johan Auwerx, Michael Downes, Ronald M. Evans

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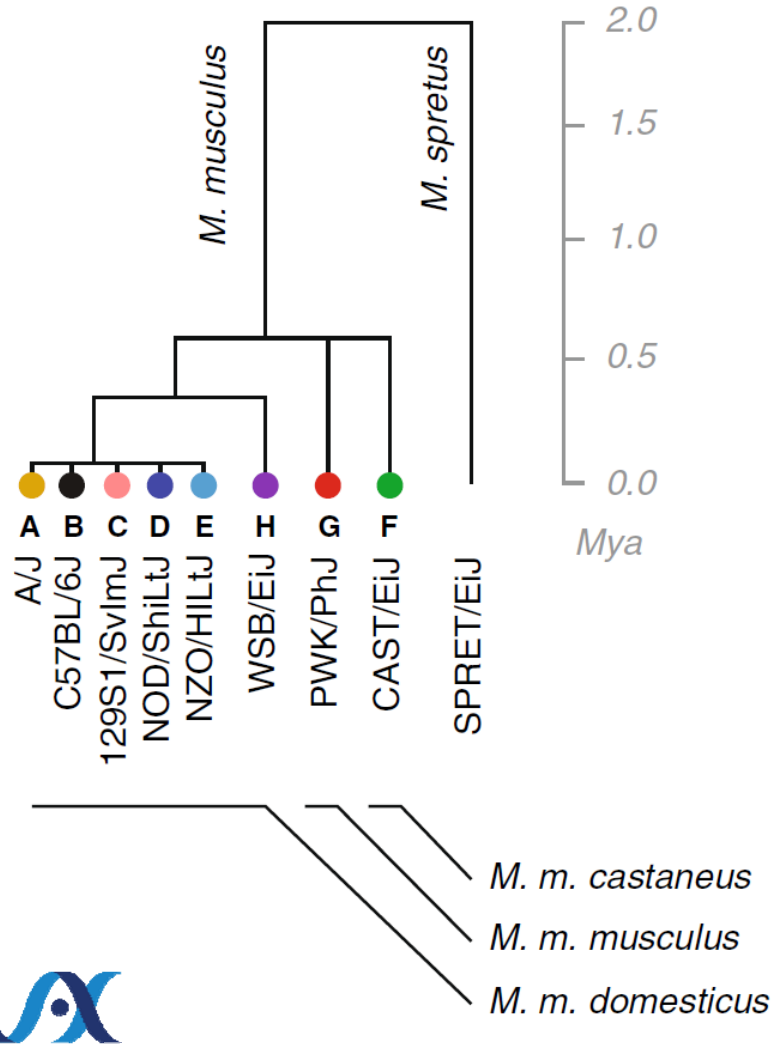
downes@salk.edu (M.D.), evans@salk.edu (R.M.E.)

In Brief

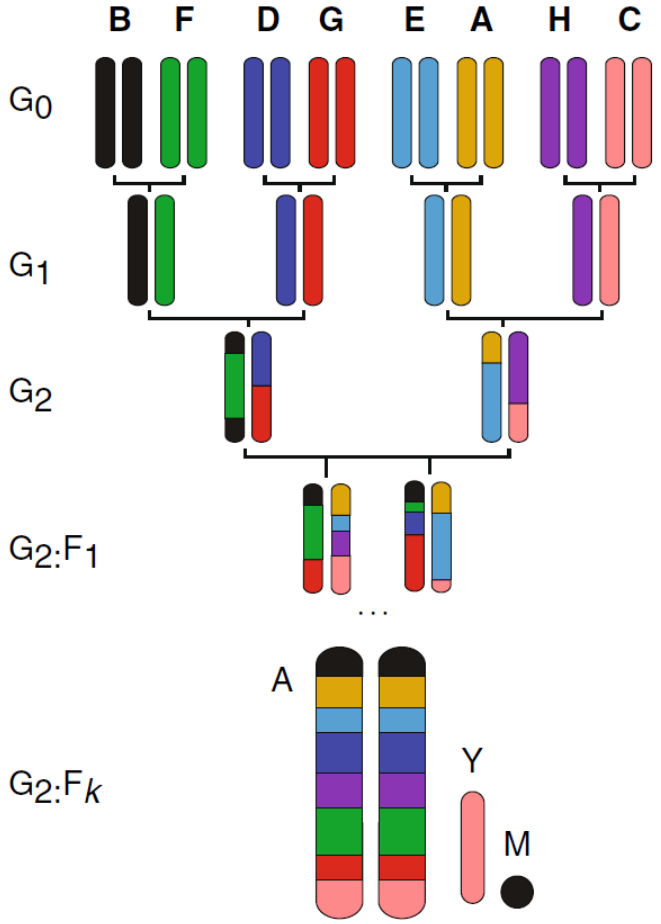
Carbohydrate depletion in endurance sports leads to the "hitting the wall" phenomenon, which is mitigated through sports training. Fan et al. show that muscle PPARδ actively suppresses glucose catabolism. Glucose sparing by PPARδ delays the onset of hypoglycemia and extends running time by ~100 min in agonist-treated mice.

Collaborative Cross (CC) structure

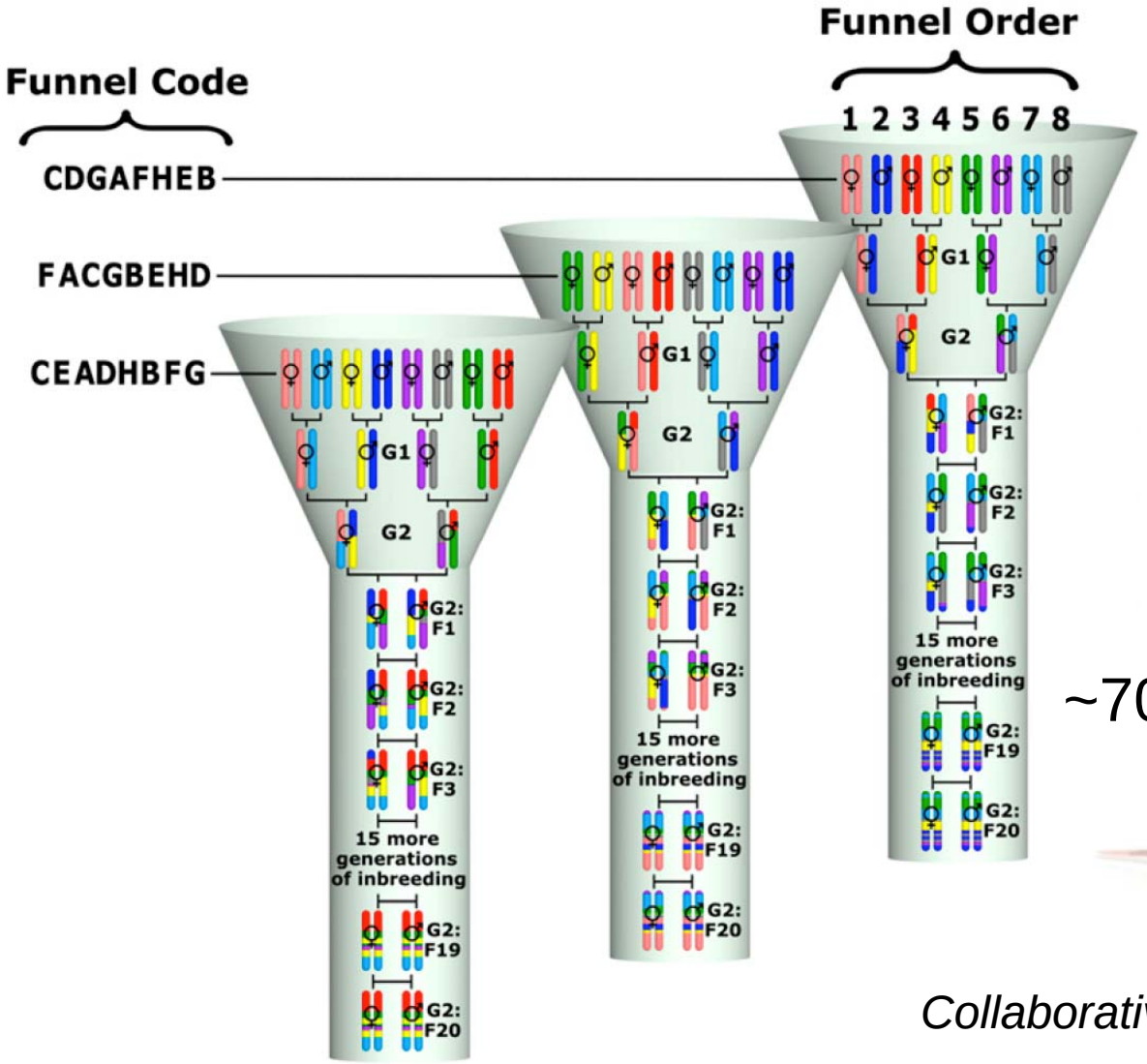
Select 8 inbred founder lines



Multiparent crosses



CC mice: RI strains with high diversity



~70 strains available

Collaborative Cross Consortium, Genetics 2012

CC mice recapitulate variation in human infectious disease outcomes

Modeling Host Genetic Regulation of Influenza Pathogenesis in the Collaborative Cross

Martin T. Ferris[1,2,3*], David L. Aylor[2], Daniel Bottomly[3,4], Alan C. Whitmore[1], Lauri D. Aicher[3,5], Timothy A. Bell[2], Birgit Bradel-Tretheway[3,5], Janine T. Bryan[3,5], Ryan J. Buus[2], Lisa E. Gralinski[1,6], Bart L. Haagmans[7], Leonard McMillan[8], Darla R. Miller[2], Elizabeth Rosenzweig[3,5], William Valdar[2], Jeremy Wang[8], Gary A. Churchill[9], David W. Threadgill[10], Shannon K. McWeeney[3,4], Michael G. Katze[3,5], Fernando Pardo-Manuel de Villena[2,3,11], Ralph S. Baric[1,4,6], Mark T. Heise[1,2,3,11]

PLoS Pathogens (2012)

Genetic Diversity in the Collaborative Cross Model Recapitulates Human West Nile Virus Disease Outcomes

Jessica B. Graham,[a] Sunil Thomas,[b] Jessica Swarts,[a] Aimee A. McMillan,[b] Martin T. Ferris,[c] Mehul S. Suthar,[d] Piper M. Treuting,[e] Renee Ireton,[b] Michael Gale, Jr.,[b] Jennifer M. Lund[a,f]

mBio mbio.asm.org May/June 2015 Volume 6 Issue 3

Expression Quantitative Trait Loci for Extreme Host Response to Influenza A in Pre-Collaborative Cross Mice

Daniel Bottomly,*,† Martin T. Ferris,*,‡ Lauri D. Aicher,*,§ Elizabeth Rosenzweig,*,§ Alan Whitmore,*,‡ David L. Aylor,‡,** Bart L. Haagmans,†† Lisa E. Gralinski,*,‡‡ Birgit G. Bradel-Tretheway,*,§ Janine T. Bryan,*,§ David W. Threadgill,§§ Fernando Pardo-Manuel de Villena,*,‡,** Ralph S. Baric,*,‡‡,*** Michael G. Katze,*,§ Mark Heise,*,‡,***,1,2 and Shannon K. McWeeney*,†,†††,‡‡‡,§§§,1,2

G3 (2012)

Genetically diverse mice are novel and valuable models of age-associated susceptibility to *Mycobacterium tuberculosis*

David E Harrison[1†], Clinton M Astle[1], M Khalid Khan Niazi[2], Samuel Major[3] and Gillian L Beamer[3*†]

Immunity & Ageing (2014) 11:24

Host genetic diversity enables Ebola hemorrhagic fever pathogenesis and resistance

Angela L. Rasmussen,*[1] Atsushi Okumura,*[1,4] Martin T. Ferris,[2] Richard Green,[1] Friederike Feldmann,[3] Sara M. Kelly,[1] Dana P. Scott,[3] David Safronetz,[4] Elaine Haddock,[4] Rachel LaCasse,[3] Matthew J. Thomas,[1] Pavel Sova,[1] Victoria S. Carter,[1] Jeffrey M. Weiss,[1] Darla R. Miller,[2] Ginger D. Shaw,[2] Marcus J. Korth,[1] Mark T. Heise,[2,5] Ralph S. Baric,[5] Fernando Pardo-Manuel de Villena,[2] Heinz Feldmann,[4] Michael G. Katze[1]†

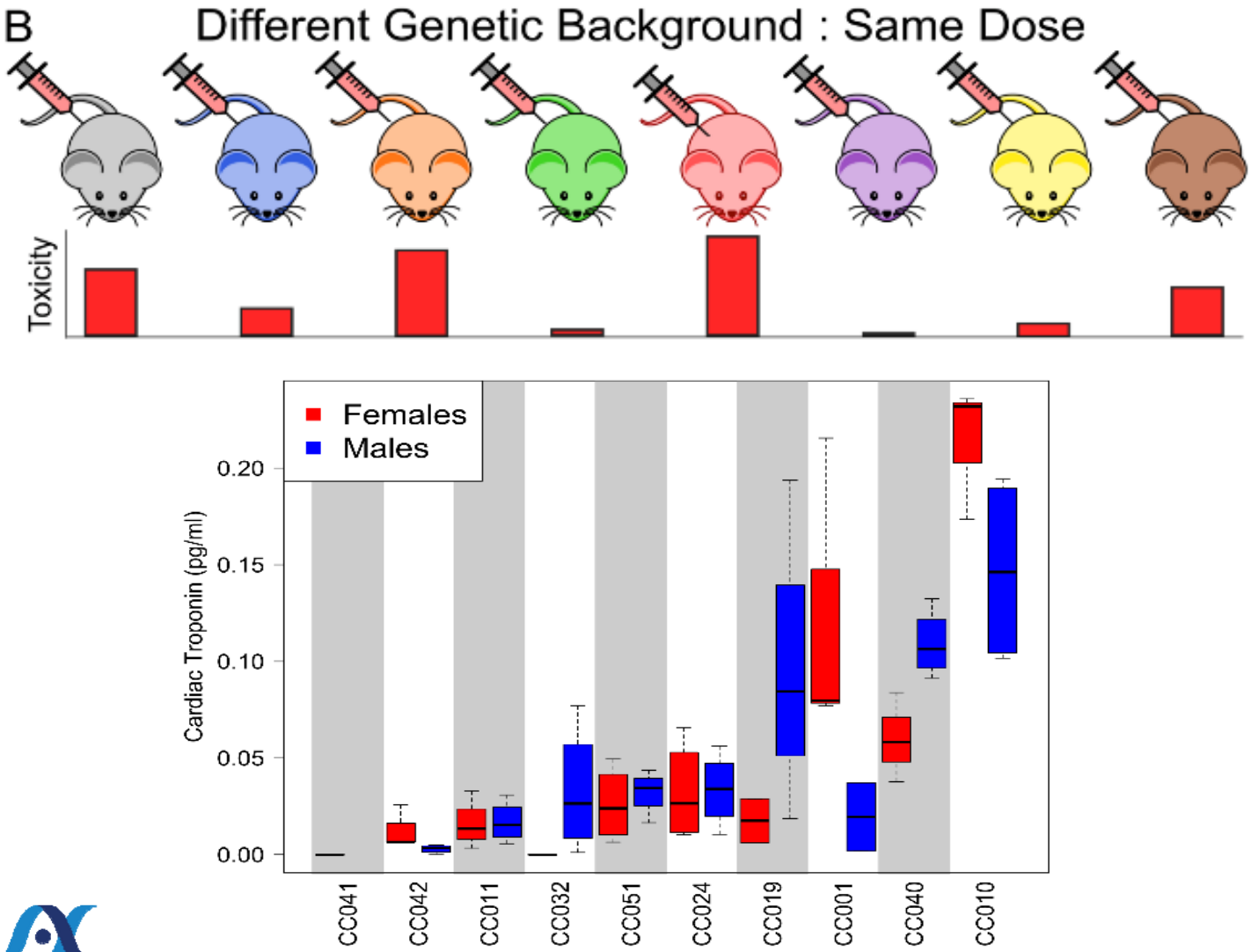
Science (2014) 346:987-991

Genome Wide Identification of SARS-CoV Susceptibility Loci Using the Collaborative Cross

Lisa E. Gralinski[1], Martin T. Ferris[2], David L. Aylor[2¤a], Alan C. Whitmore[2], Richard Green[3], Matthew B. Frieman[1¤b], Damon Deming[1¤c], Vineet D. Menachery[1], Darla R. Miller[2,4], Ryan J. Buus[2,4], Timothy A. Bell[2,4], Gary A. Churchill[5], David W. Threadgill[6], Michael G. Katze[3], Leonard McMillan[7], William Valdar[2], Mark T. Heise[2], Fernando Pardo-Manuel de Villena[2,4], Ralph S. Baric[1]*

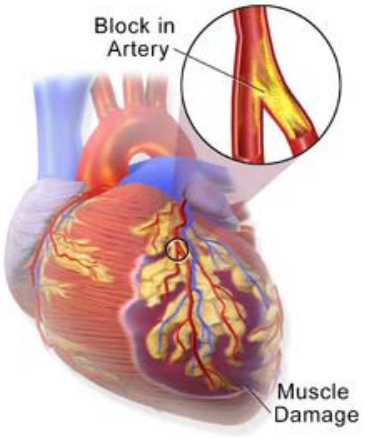
PLOS | GENETICS October 9, 2015

CC inbred strains recapitulate cancer drug-induced cardiotoxicity outcomes



D. Gatti, unpublished

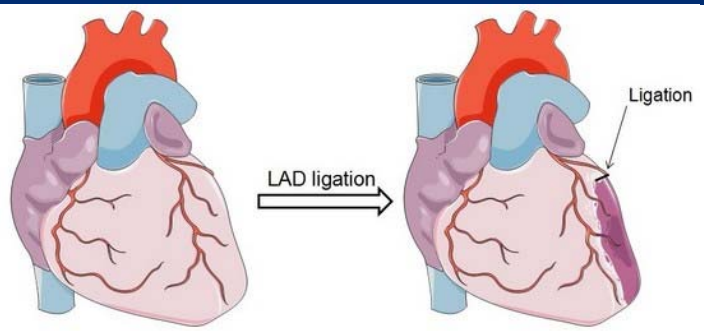
How to predict the clinical variation in response to heart attack?



Genetics?

Stress Gender Lifestyle

Varied response to heart attack in CC mice



BOM-GB

Survival: 95%
Best performer: no dilation, robust and opaque scars

New model of dilated cardiomopathy

TOFU-FB

Survival: 60%
Dilated hearts with huge scars, cachexia and heart failure

C. Salimova and M. Furtado, unpublished

Mapping modifiers of heart failure: Myo18b

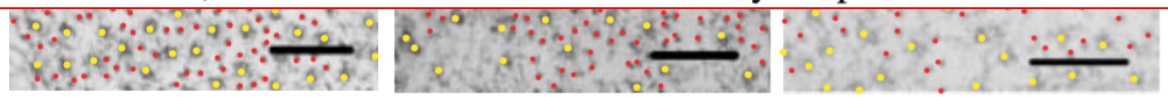
Myo18b knockout in mice:
Disordered myofibrillar structures in skeletal muscles

Journal of Neuromuscular Diseases 2 (2015) 219–227
DOI 10.3233/JND-150085
IOS Press

Research Report

A Premature Stop Codon in *MYO18B* is Associated with Severe Nemaline Myopathy with Cardiomyopathy

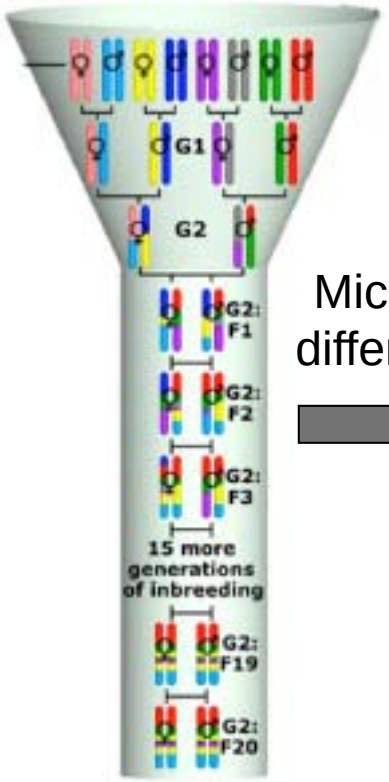
Edoardo Malfatti[a,b,1], Johann Böhm[c,d,e,f,1], Emmanuelle Lacène[a,b,g], Maud Beuvin[a,b], Guy Brochier[a,b,g], Norma B. Romero[a,b,g,2,*] and Jocelyn Laporte[c,d,e,f,2,*]



Ajima et al, Genes to Cells 2008

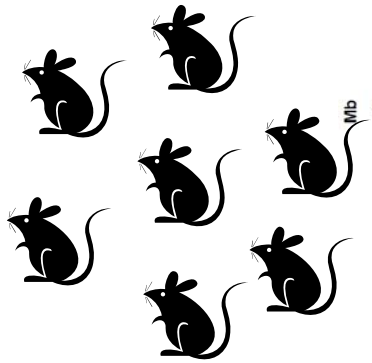
Diversity outcross (DO) mice: reaching individual human variation levels

Collaborative Cross Funnel

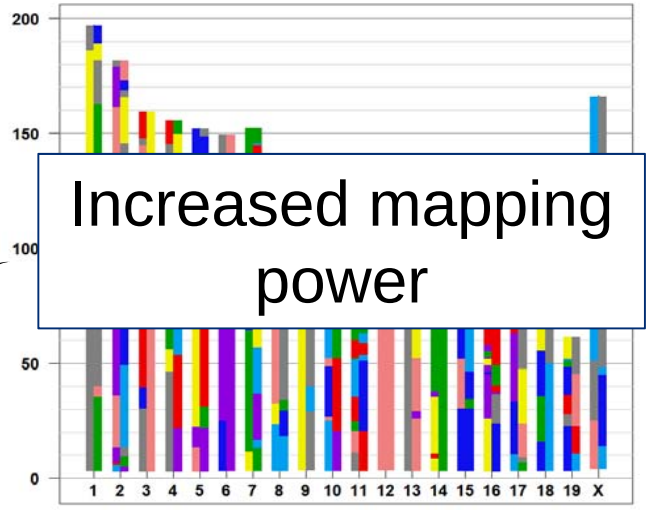


Mice from 144 different funnels

Random breeding



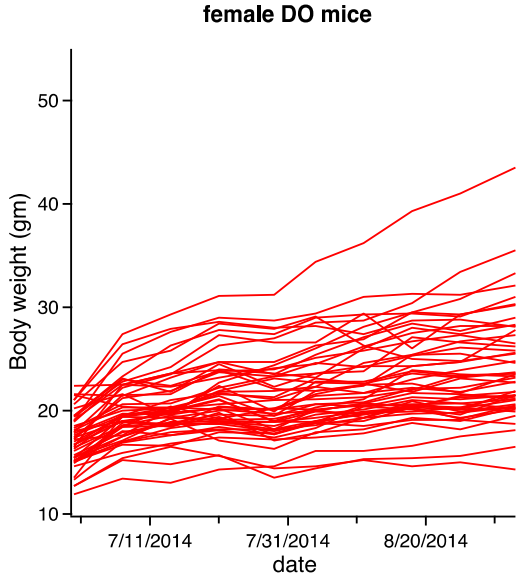
Diversity Outcross chromosomes

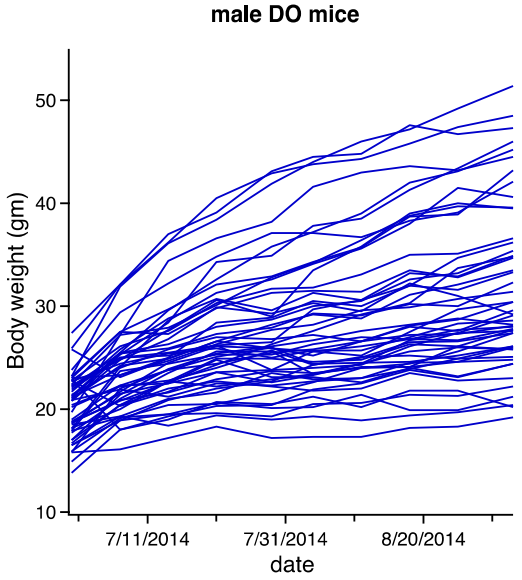


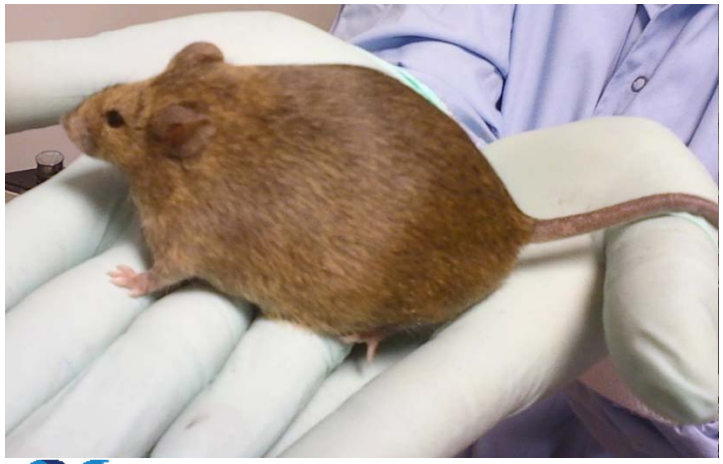
Increased mapping power

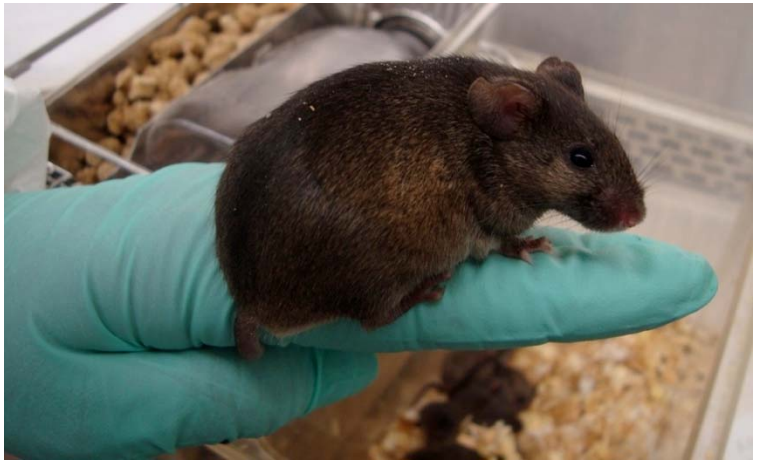
- DO mice contain 40 million SNPs
- Humans have ~15 mil. common SNPs
- Each mouse is genetically unique

Diverse characteristics of individual DO mice

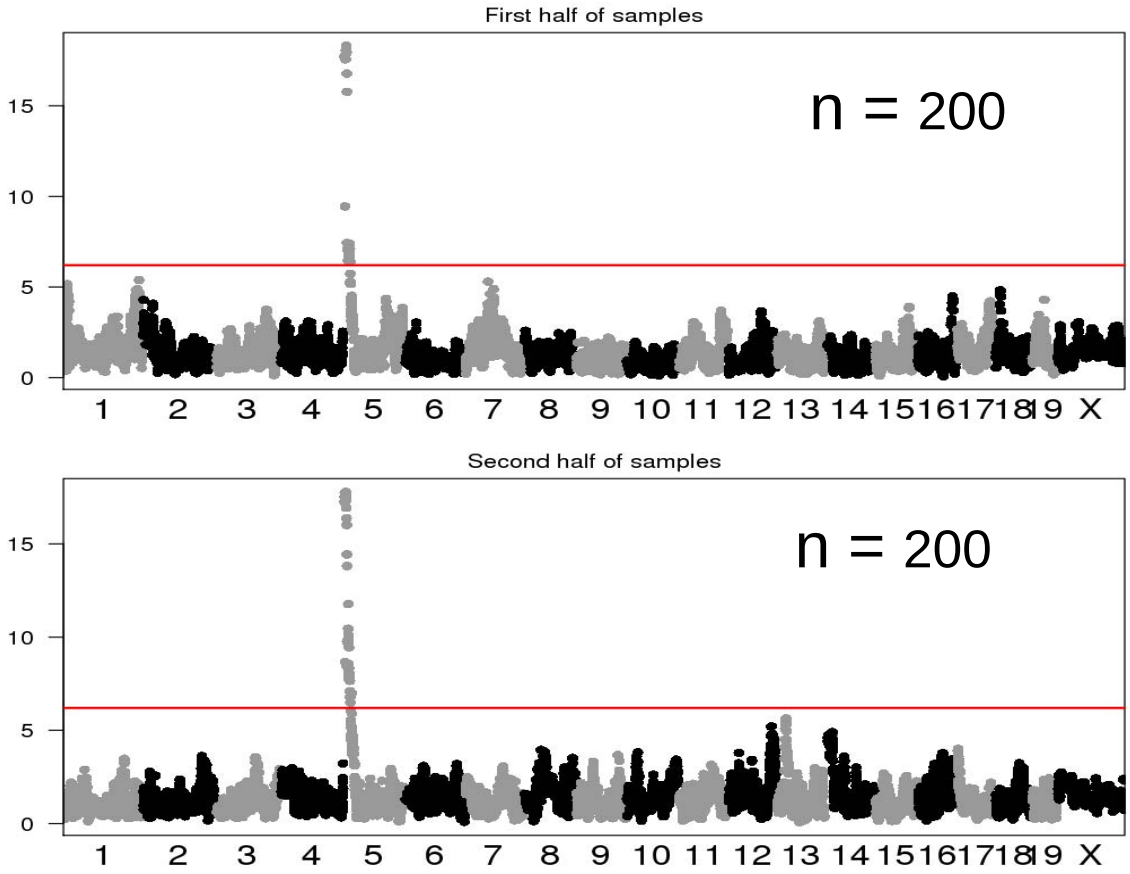








Are DO studies reproducible?



- Change in neutrophils in response to DOX
- Split by cohorts run several months apart

D. Gatti, unpublished

Using genetic diversity for network analysis

Defining the consequences of genetic variation on a proteome-wide scale

Joel M. Chick[1]*, Steven C. Munger[2]*, Petr Simecek[2], Edward L. Huttlin[1], Kwangbom Choi[2], Daniel M. Gatti[2], Narayanan Raghupathy[2], Karen L. Svenson[2], Gary A. Churchill[2]§ & Steven P. Gygi[1]§

NATURE | VOL 534 | 23 JUNE 2016

By measuring genome-wide transcript and protein expression in livers from **192 Diversity Outbred mice**, 2,866 protein quantitative trait loci (pQTL) were identified with twice as many local as distant genetic variants:

1. support **distinct transcriptional and post-transcriptional models**
2. reveal an **extensive network** of direct protein-protein interactions
3. local genotype can provide **accurate predictions of protein abundance**

Harness mouse diversity resources and CRISPR to functionalize the human genome

Human genomics: where are we headed?

Reading the genome

Understanding genetic & genomic complexity

Molecular mechanisms of disease

Writing the genome

Complex disease models: mice and cells

Genetic solutions to treating human disease

Goal 1: “reading” the human genome

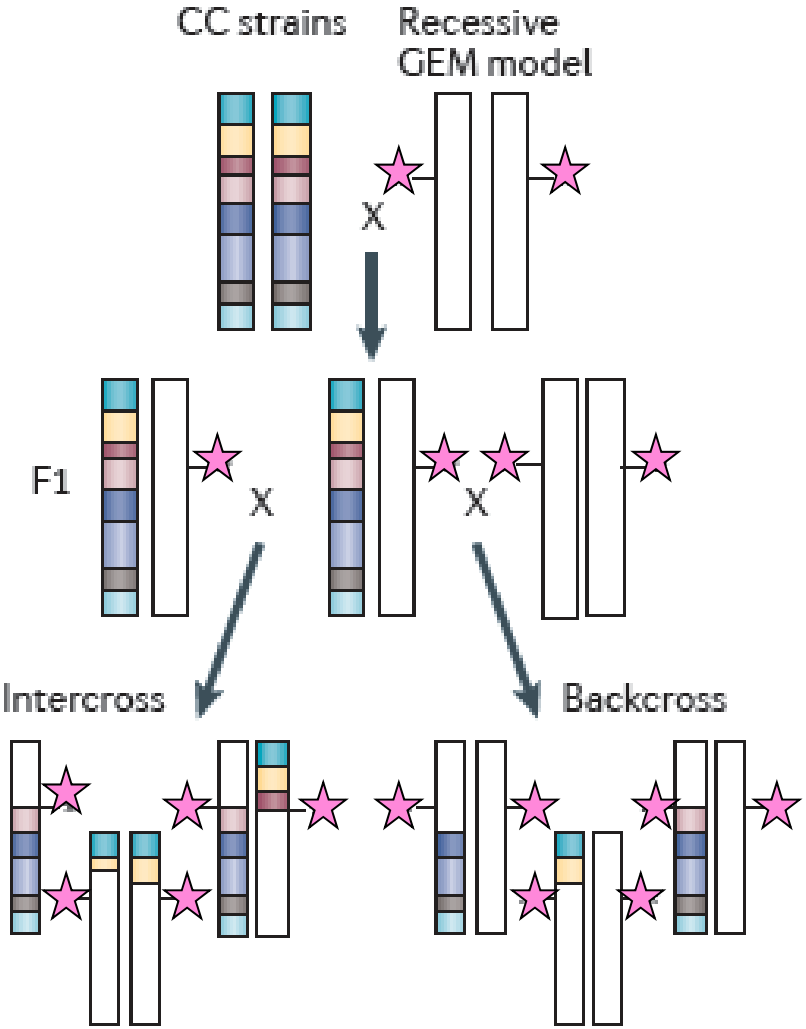
Decoding human variation with mouse diversity pipelines:

- Genetic mapping of variable disease phenotypes
- Validation of candidate modifiers with mutation
- Mapping gene networks onto human genome data

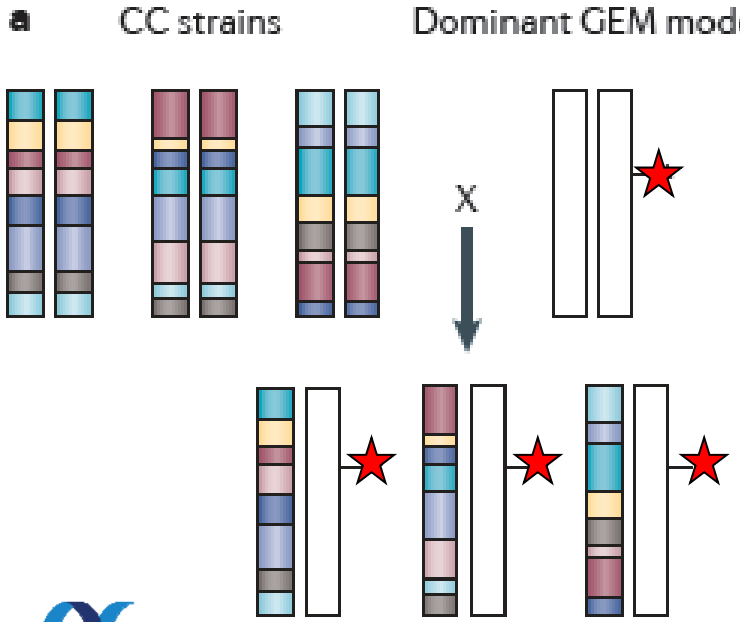
Validation of candidate modifiers with mutation

Crossing CRISPR mutants into CC inbred panels

Recessive gene



Dominant gene



Map networks onto human genome data

Genomics England: sequencing 100,000 human genomes with associated medical records (NHS): 39,540 completed

Genomics england

It is estimated half of all Britons will get some form of cancer at some point in their lives.

A rare diseases is one that affects 1 in 2,000 or less of the UK population. There are up to 8,000 rare diseases – affecting a total of 3 million people in the UK.

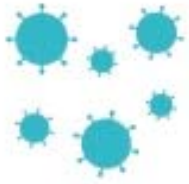
8,000
rare diseases affecting

3,000,000
people in the UK

There are over 100 rare diseases included in the Project and 7 common cancers.

7 common cancers

100+ rare diseases



Map networks onto human genome data

All of Us Research Program (NIH) seeks to extend the Precision Medicine Initiative to all diseases by building a national research cohort of one million or more U.S. participants, with the statistical power to detect associations between environmental and/or biological exposures and a wide variety of health outcomes.

Enrollment opens soon (eg US way behind)

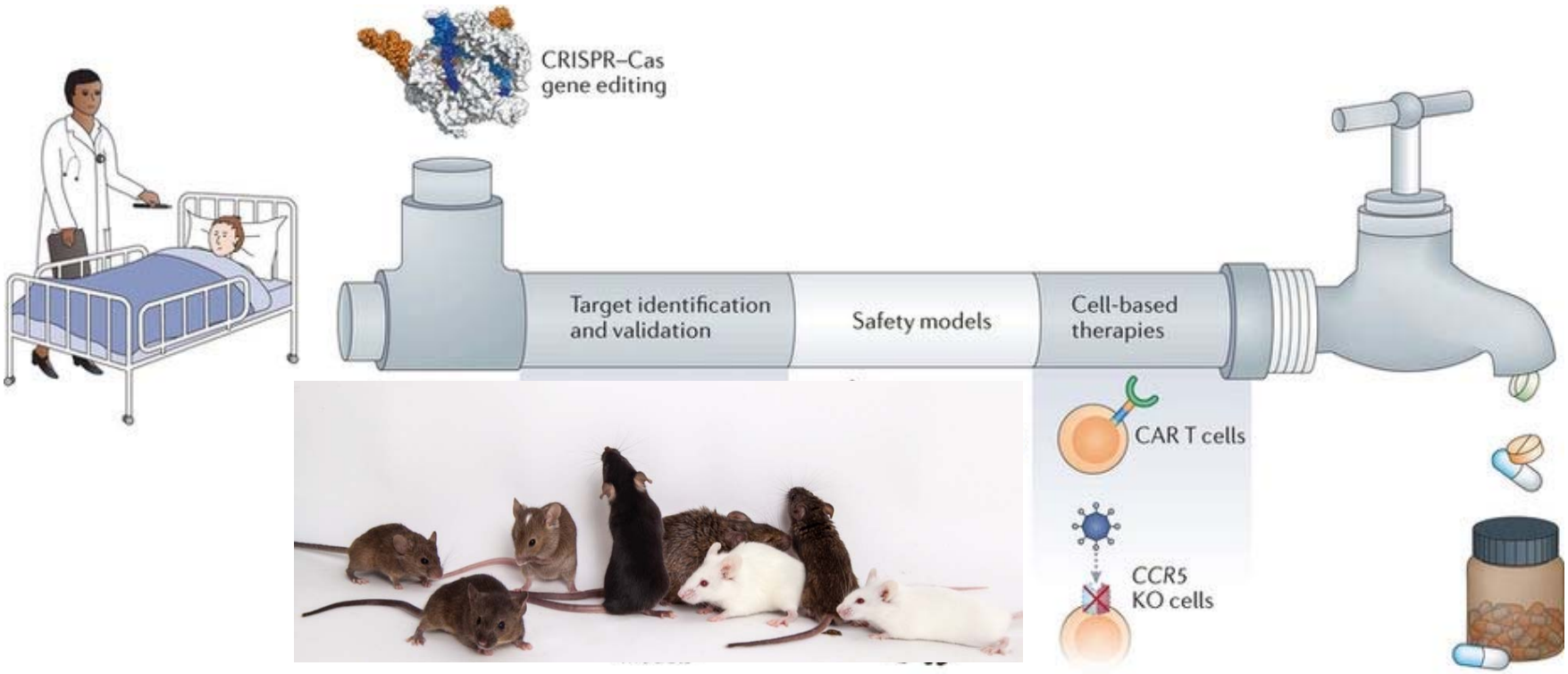
All of Us RESEARCH PROGRAM | The Future of Health Begins With You

Goal 2: “writing” the human genome

Curing complex diseases in mouse diversity panels:

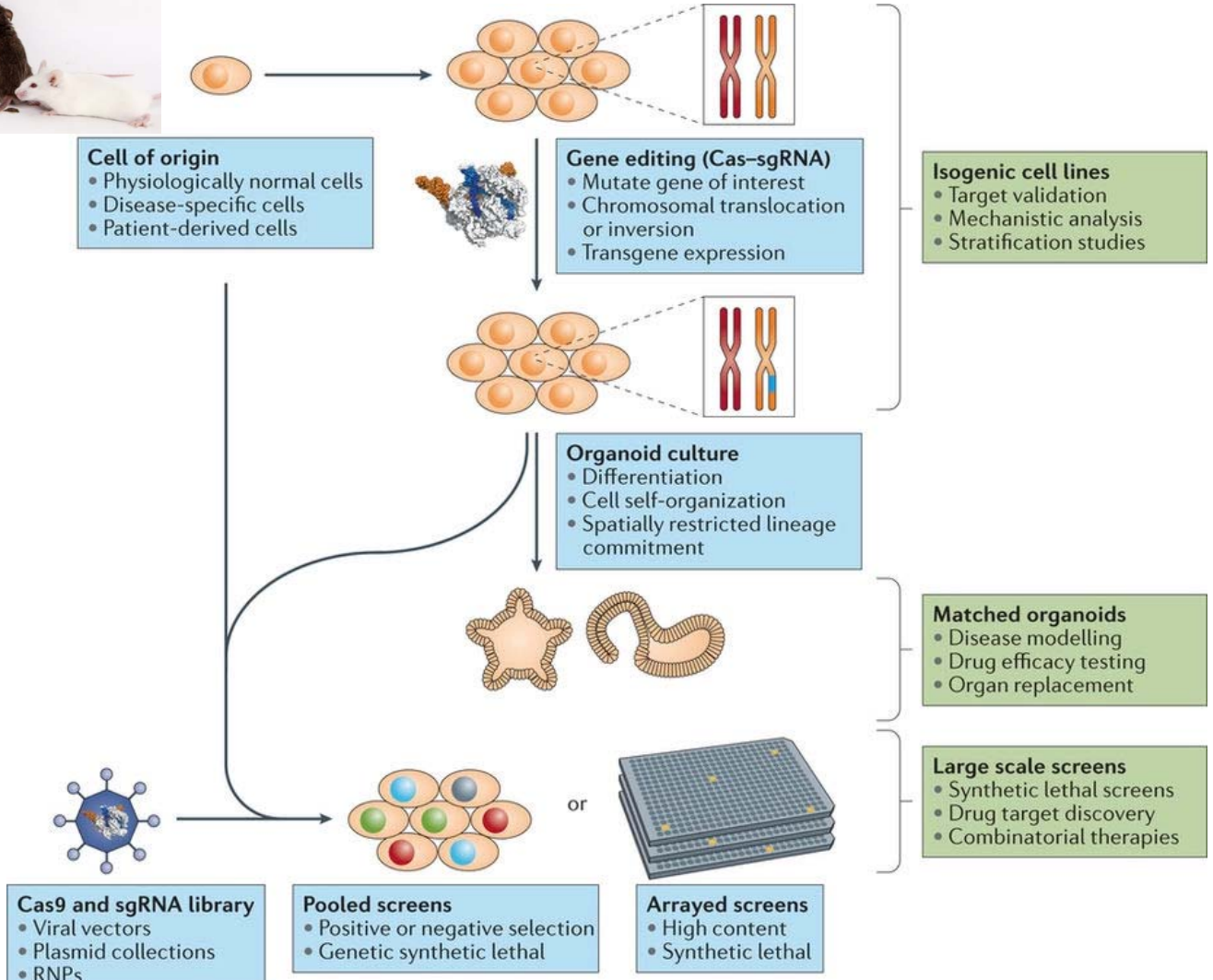
- Testing therapeutics targeted to complex disease traits
- “Repairing” these traits with CRISPR editing

Drug discovery with mouse diversity pipelines



Fellmann et al, Nat Drug Disc 2017

Mouse diversity panels as cell resources for CRISPR editing



Fellmann et al, Nat Drug Disc 2017

Somatic gene correction with CRISPR

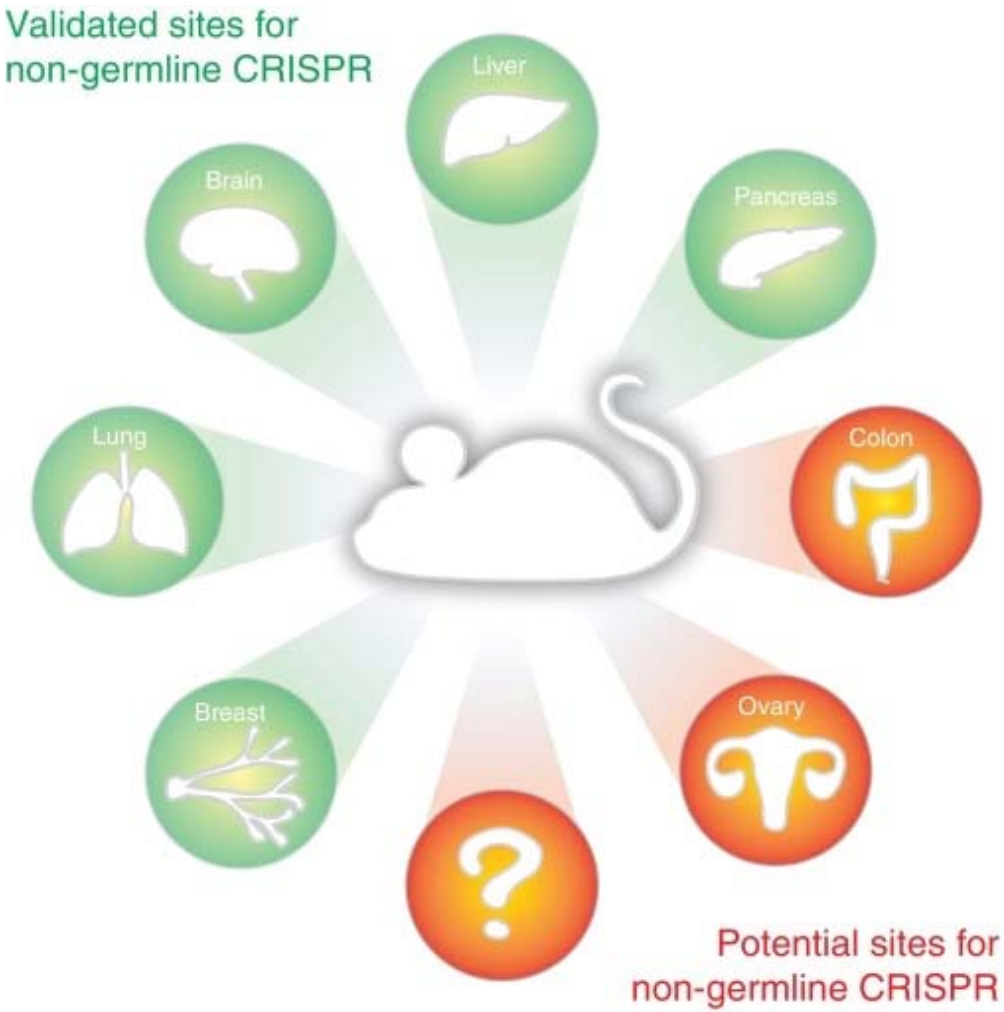
Viral or plasmid-based gene editing:

Pros:

- Avoids germline modification
- Tissue-targeted gene editing
- Tested in human (CarT)

Cons:

- Cas immunogenicity
- Developmental defects?
- Co-morbidities missed?



Somatic gene editing in humans

Functional CAR T cells evade host immunity in unmatched recipients

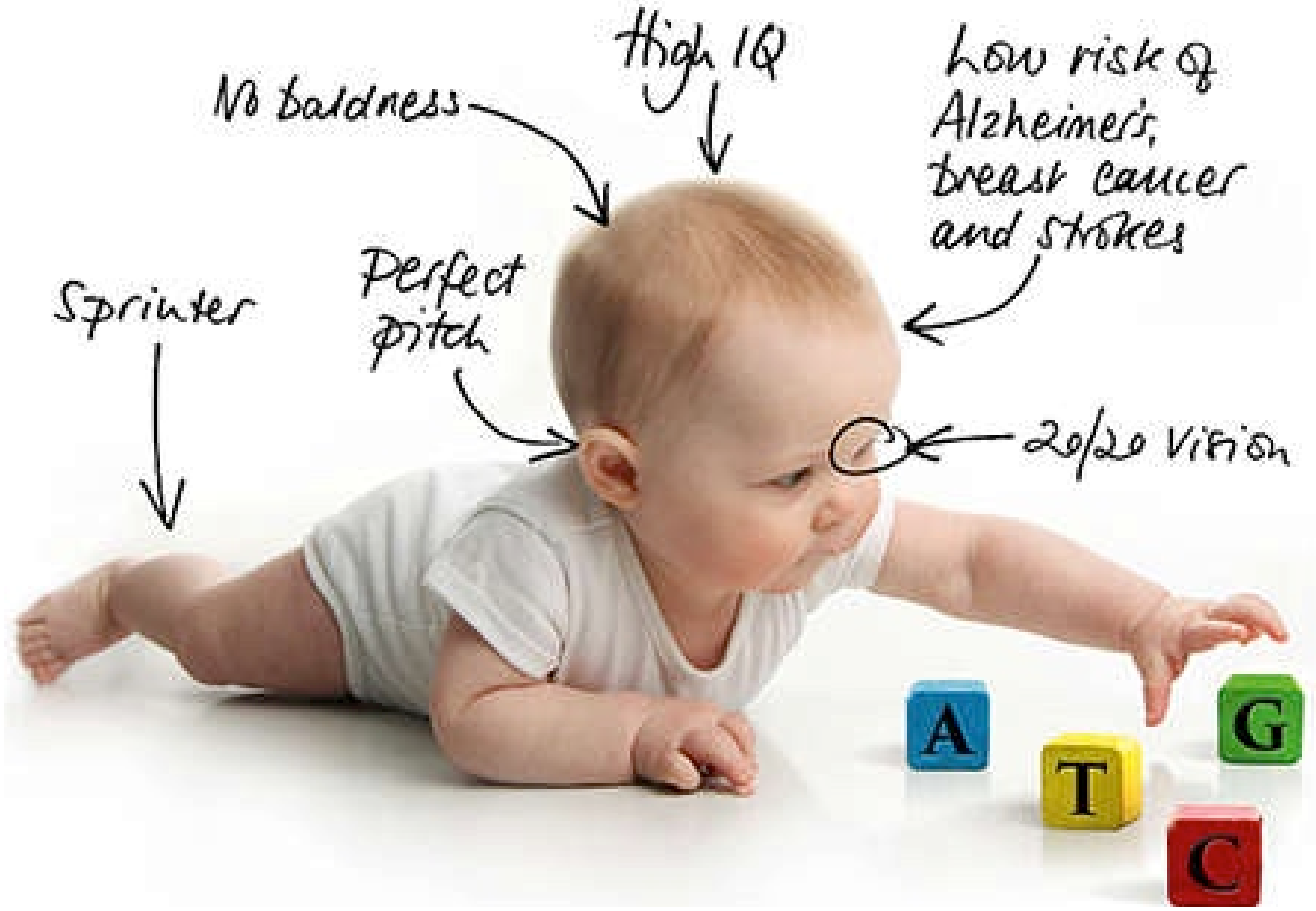
Produced by TALEN editing to simultaneously introduce CAR and disrupt TCR and CD52 in T cells

“Off-the-shelf” CAR T cells used to treat two infants with relapsed refractory acute lymphocytic leukemia and bridge them to allogeneic stem cell transplantation.

Layla Richards

Qasim et al, Science Trans Med 2017

Writing our genomes? Don't know enough yet



Thanks to:

Rosenthal lab:

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Dan Gatti
Steve Murray
Laura Reinholdt
Cat Lutz
Ed Liu