

perspectives and challenges for quantitative trait editing

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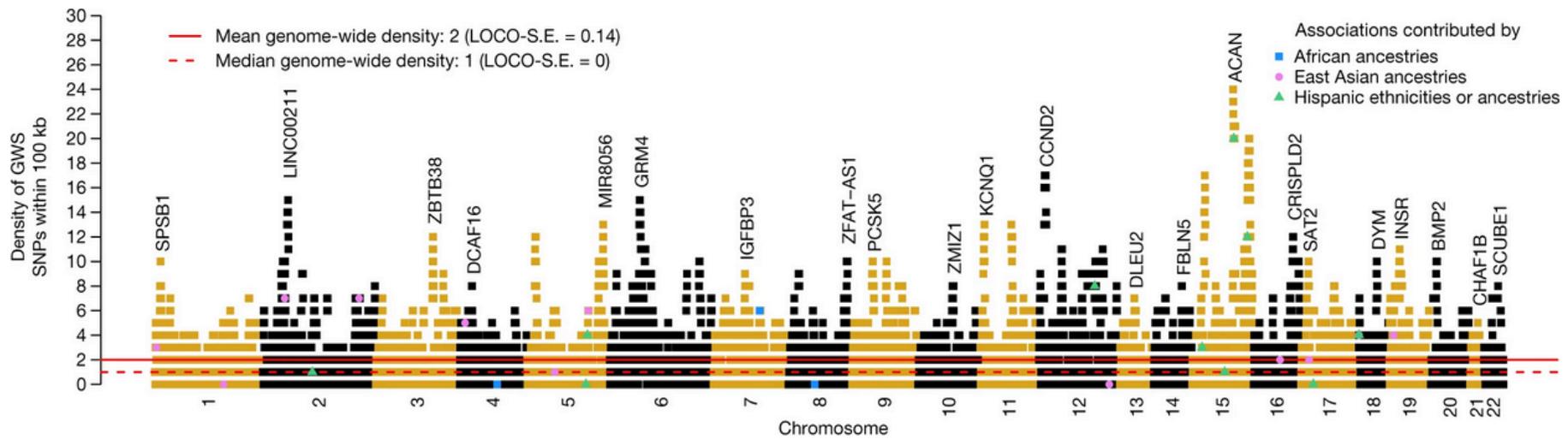
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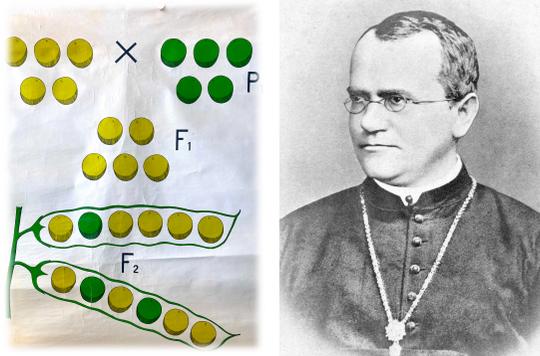
<https://www.unil.ch/cig/soyk>

What is a quantitative trait ?



What is a quantitative trait ?

seed coat color in pea



body height in human

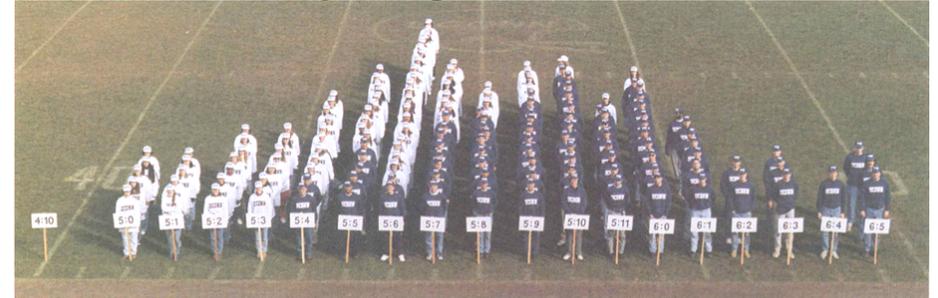
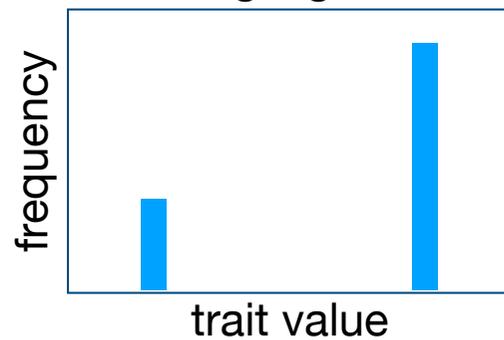


FIGURE 3.—A modern version of Figure 2, from Connecticut State University in 1996. The means and standard deviations in inches are as follows: males, 70.1 ± 3.0 ; females, 64.8 ± 2.7 ; combined, 67.6 ± 4.0 . Photo from LINDA STRAUSBAUGH.

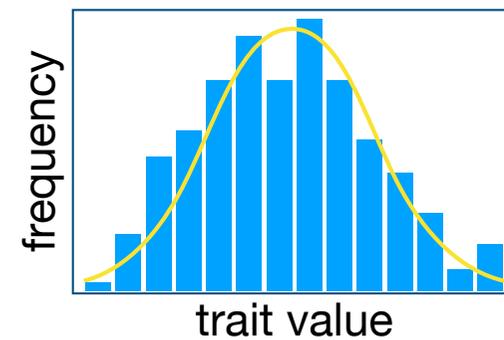
qualitative trait

discrete
single gene

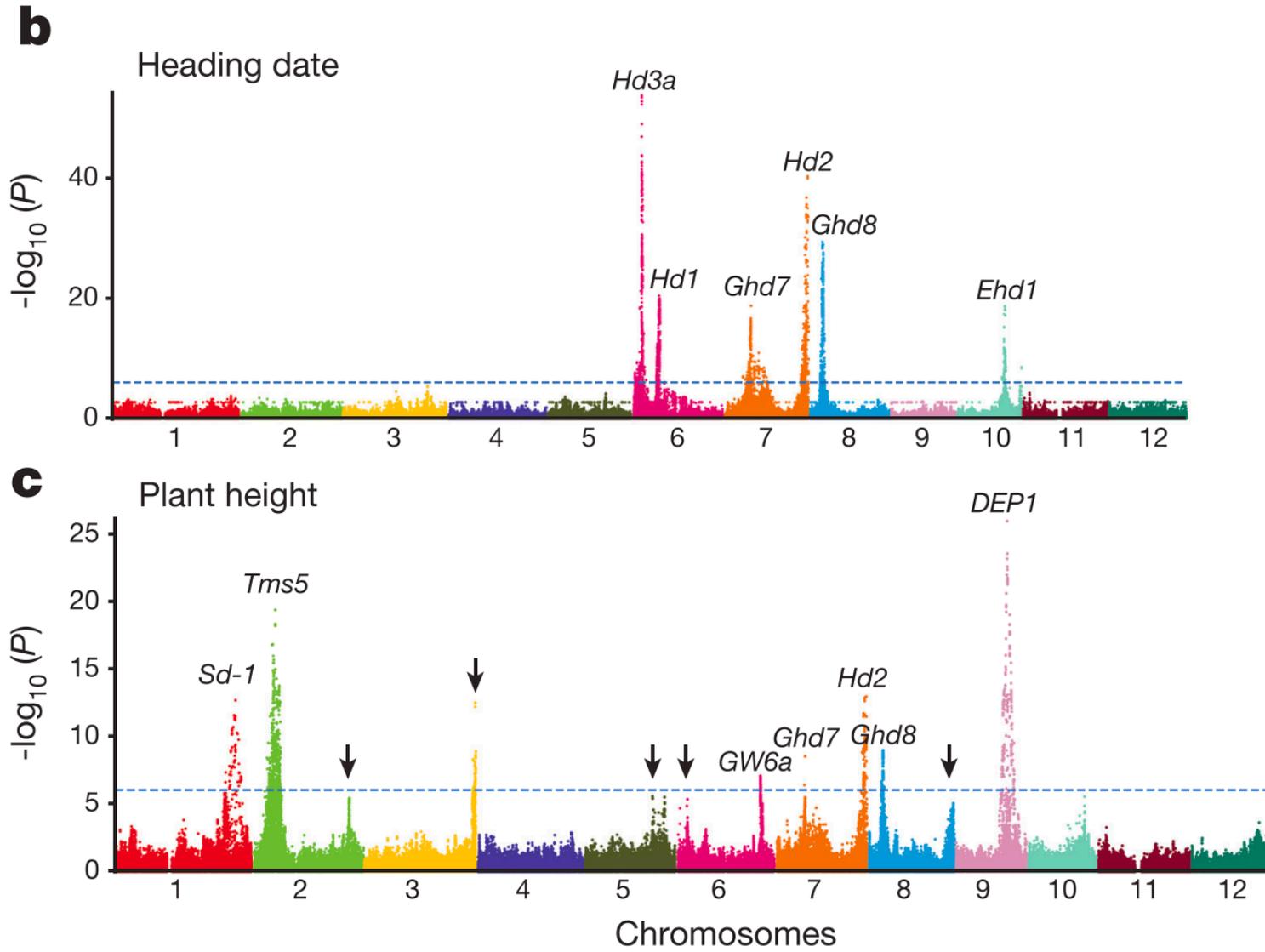


quantitative trait

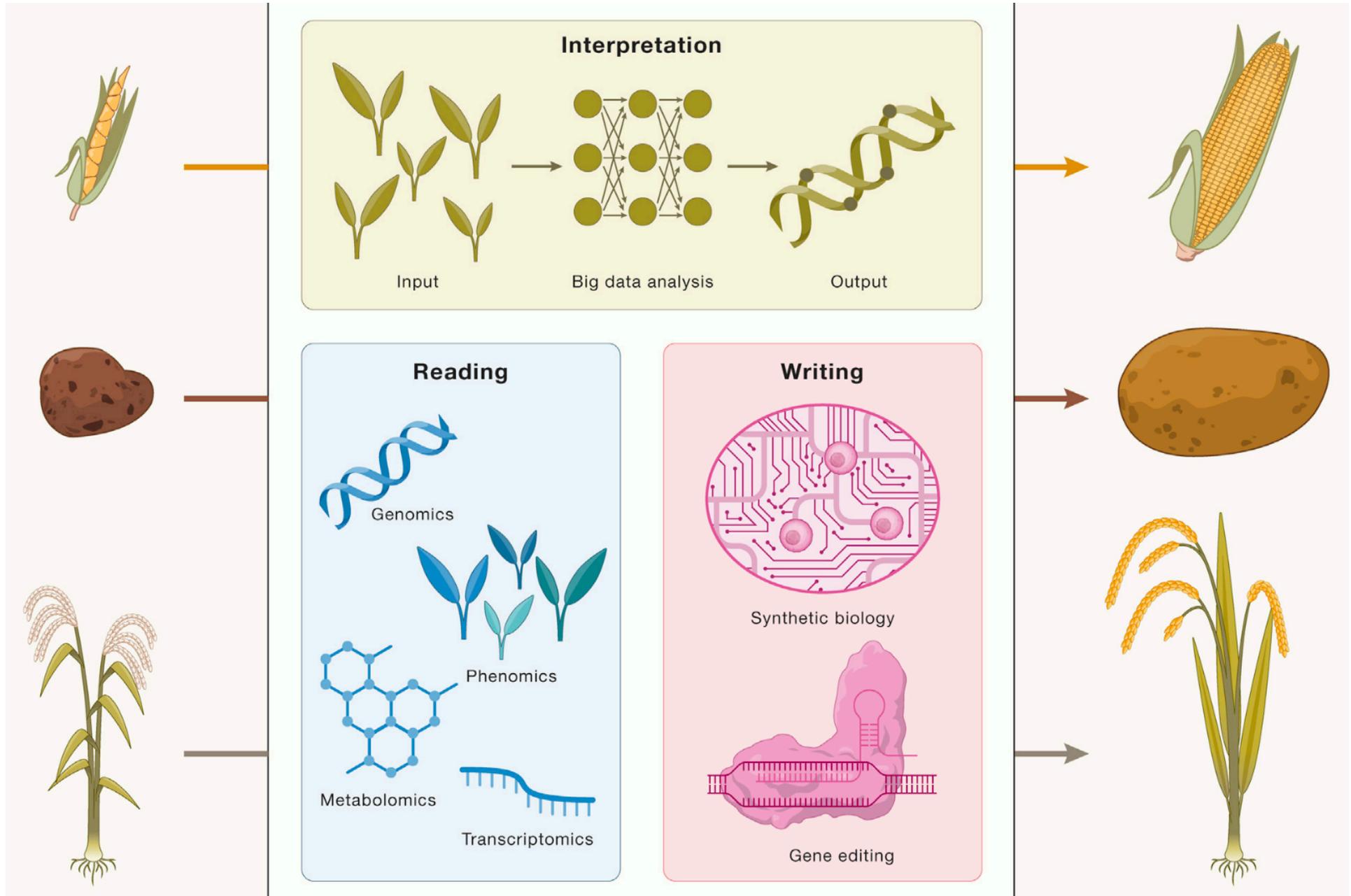
continuous
multiple genes (polygenic)



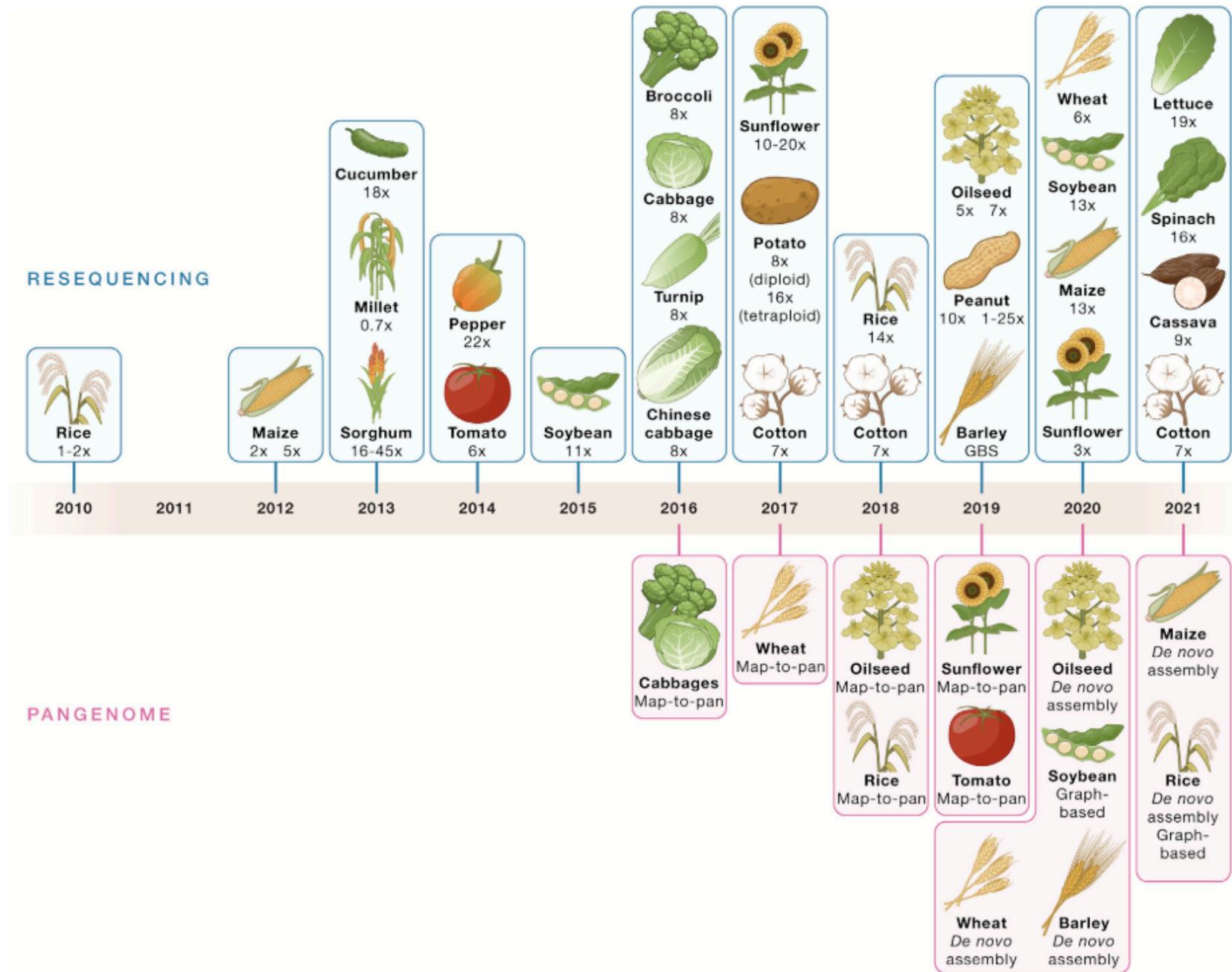
What is a quantitative trait ?



New genetic approaches for dissecting quantitative traits



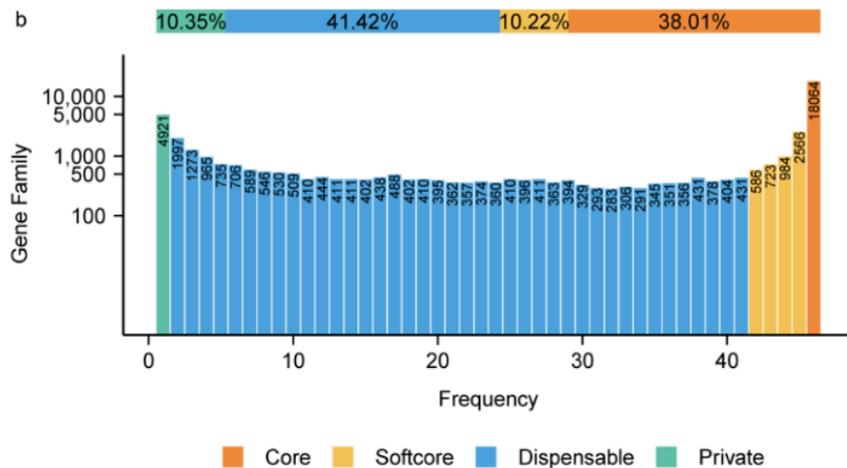
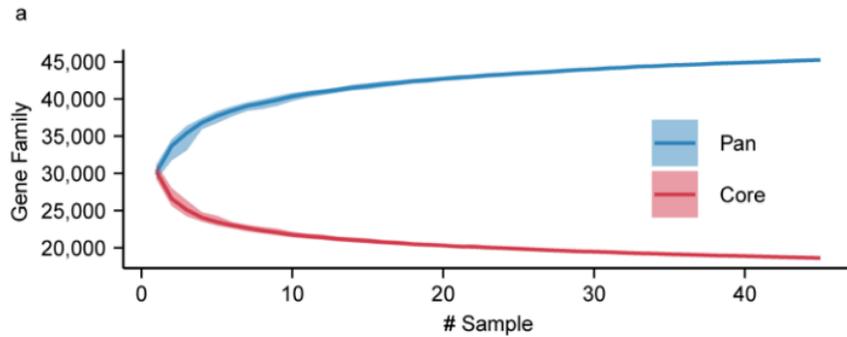
Pangenomes are available for many crop species



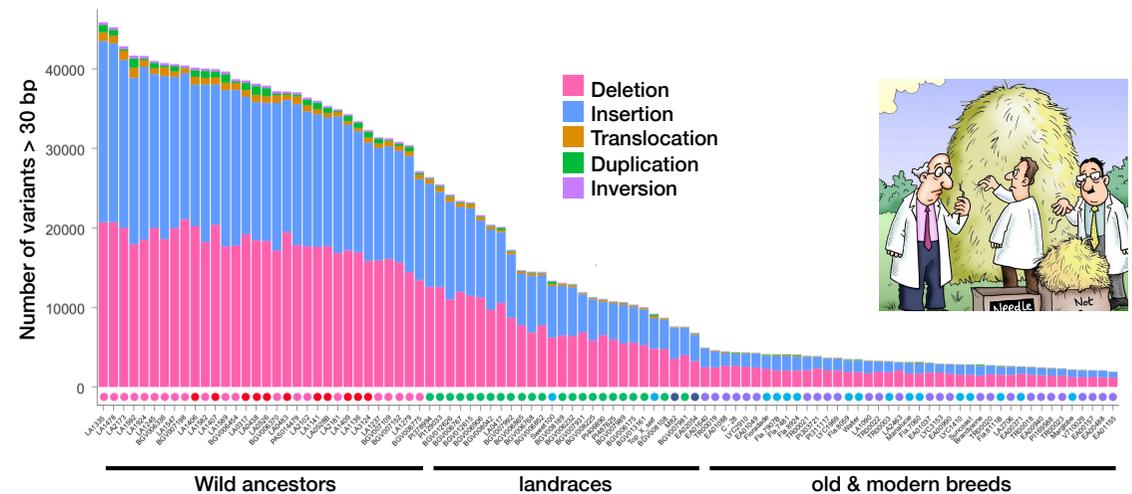
Pangenomes are available for many crop species



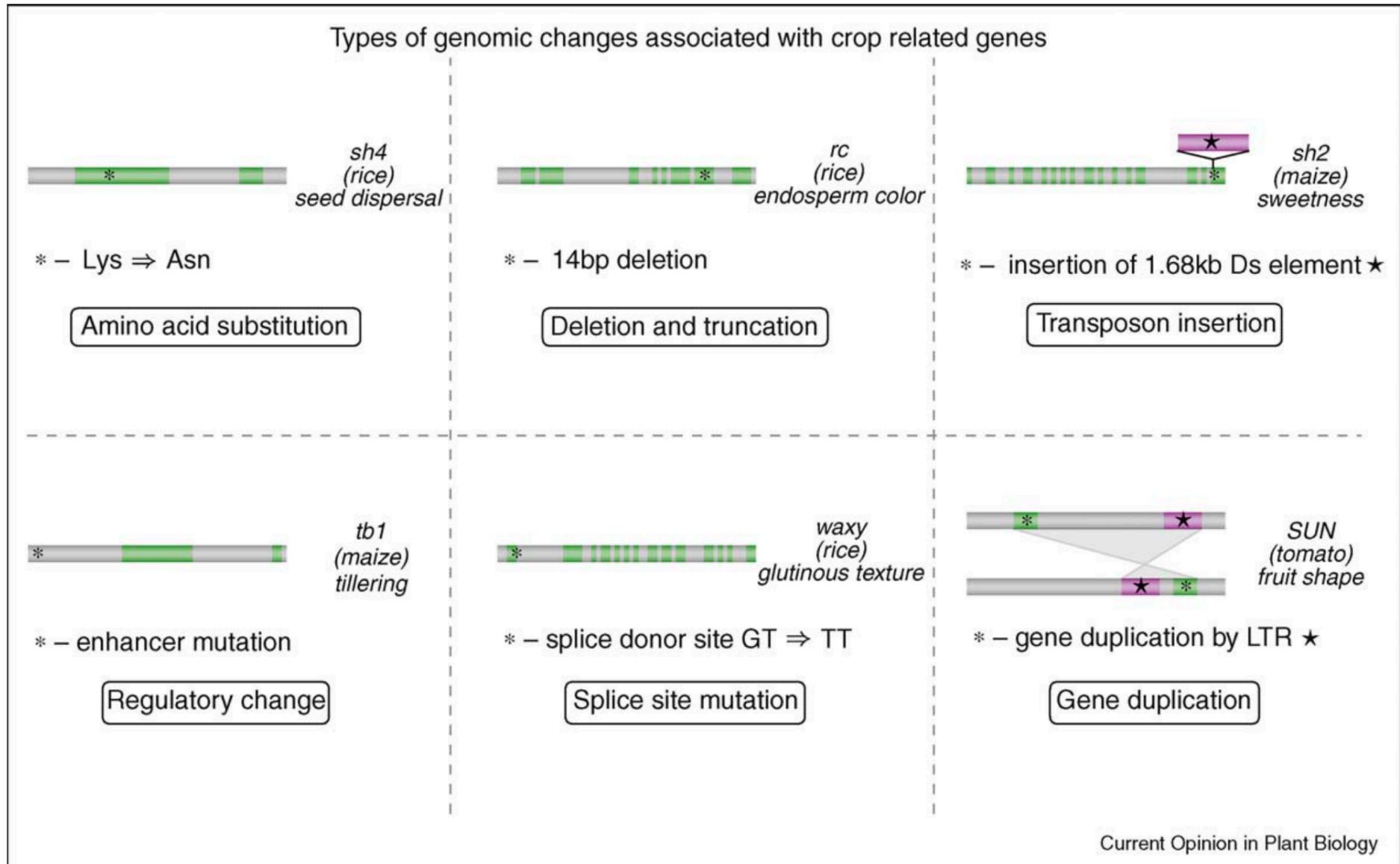
Presence-absence variants (PAVs) in 46 distinct genomes



Structural variants (SVs) in 100 distinct genomes



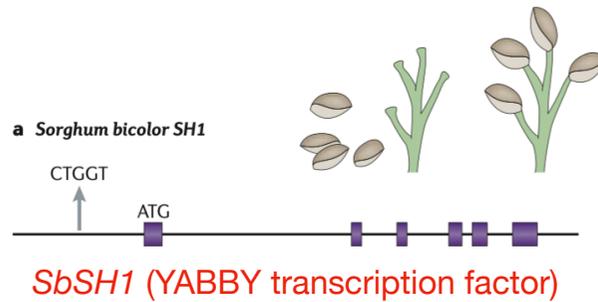
Genes and mutations that explain QTLs in crops



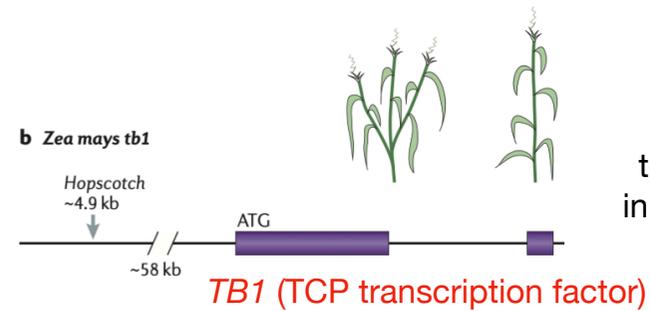
diverse genomic changes underly QTLs

Genes and mutations that explain QTLs in crops

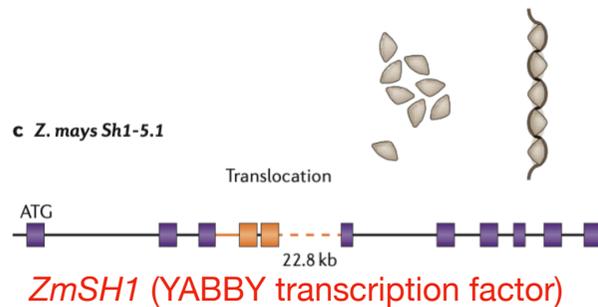
deletion
in **regulatory element**



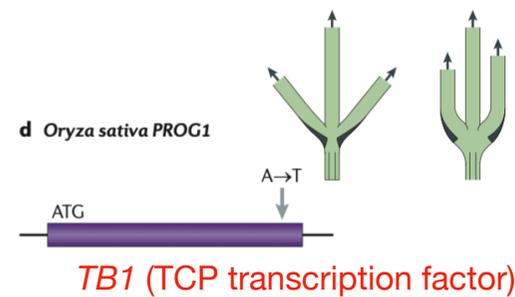
transposon insertion
in **regulatory element**



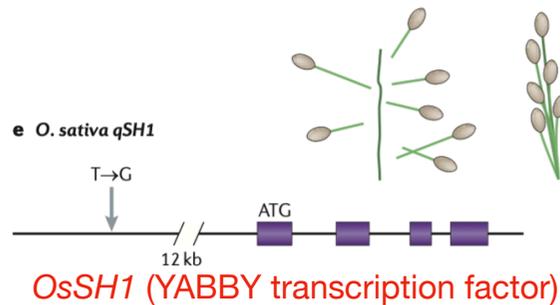
Translocation
affects **coding sequence**



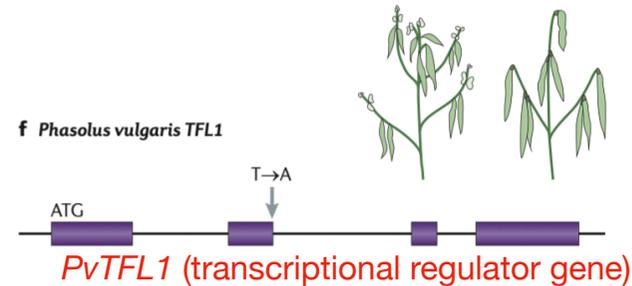
missense mutation
in **coding sequence**



Single Nucleotide
Polymorphism (SNP)
in **regulatory element**

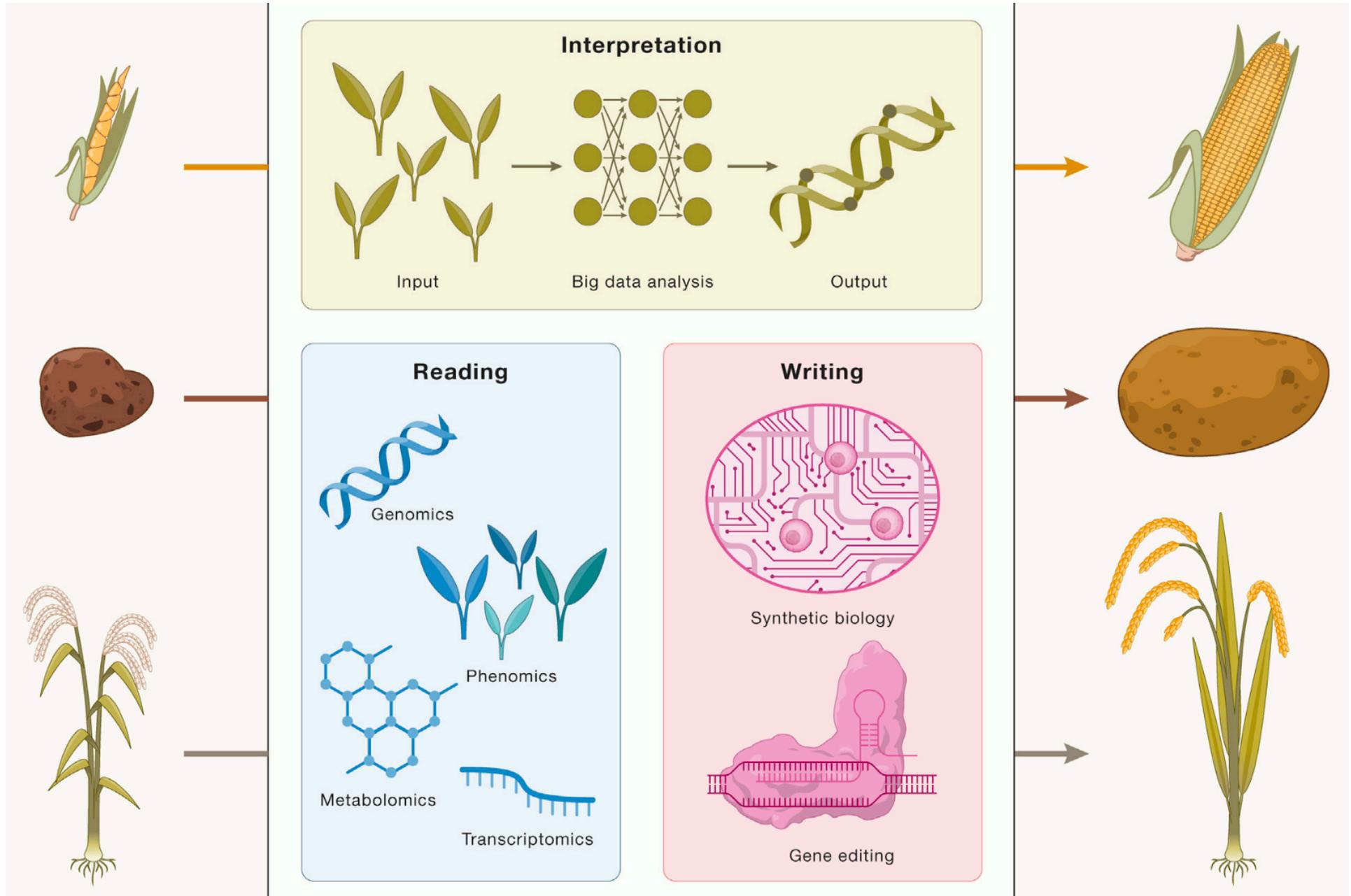


Mis-splicing mutation
affects **coding sequence**

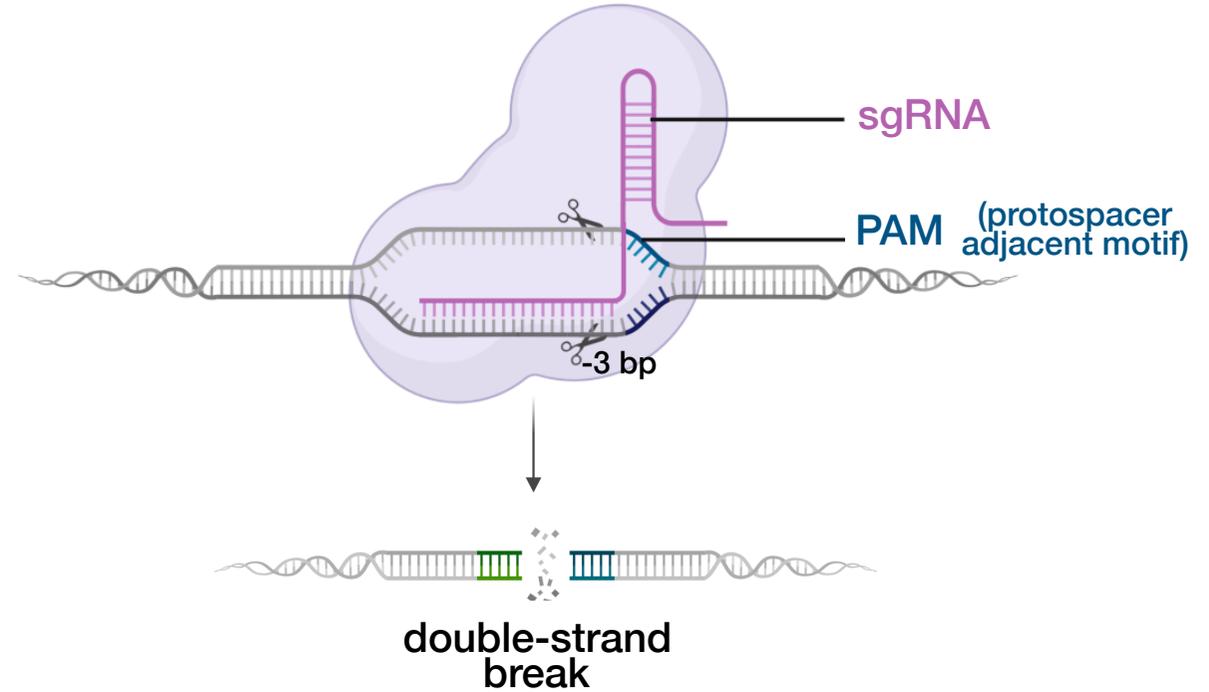
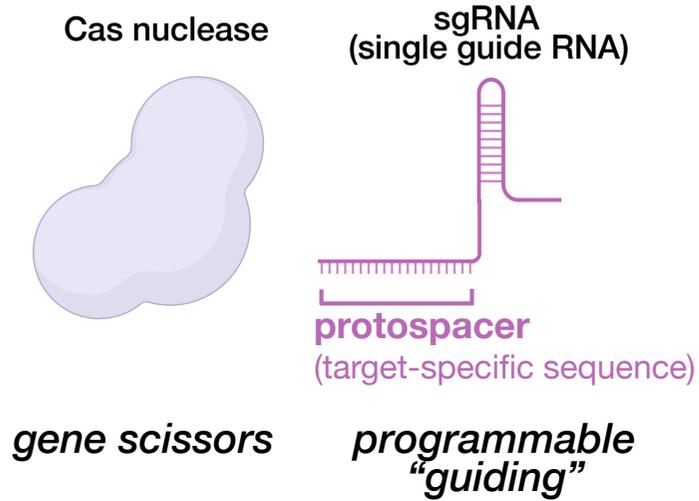


- Natural variants often affect **regulatory genes** and **cis-regulatory elements**
- Natural variants have often only **weak effects** on gene activity

New genetic approaches for dissecting quantitative traits



Genome editing using CRISPR-Cas9



Emmanuelle Charpentier

Nobel prize
2020

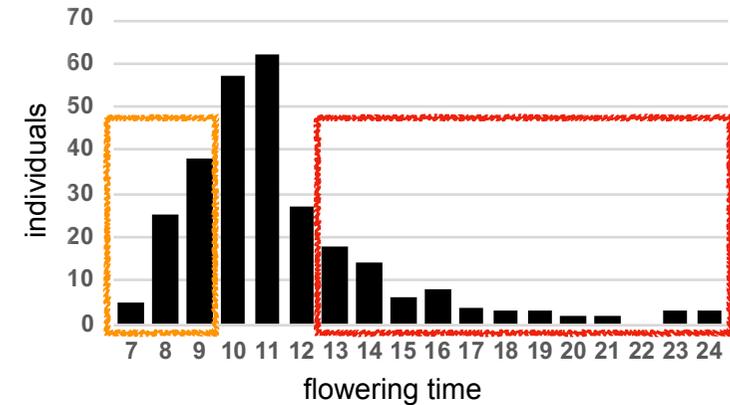
Jennifer Doudna

Mapping photoperiodic flowering in tomato

genetic cross

wild tomato (*S. galapagense*) × cultivated tomato (*S. lycopersicum*)

F2 phenotyping



early Progeny

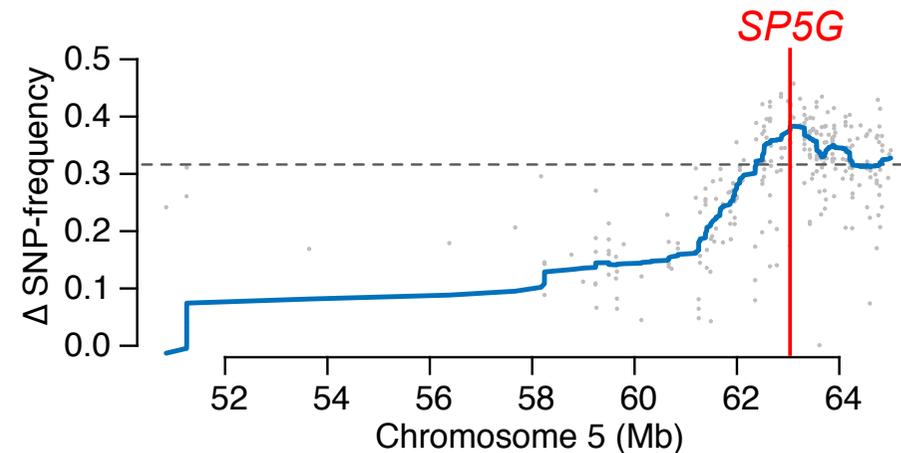
late Progeny

genome sequencing

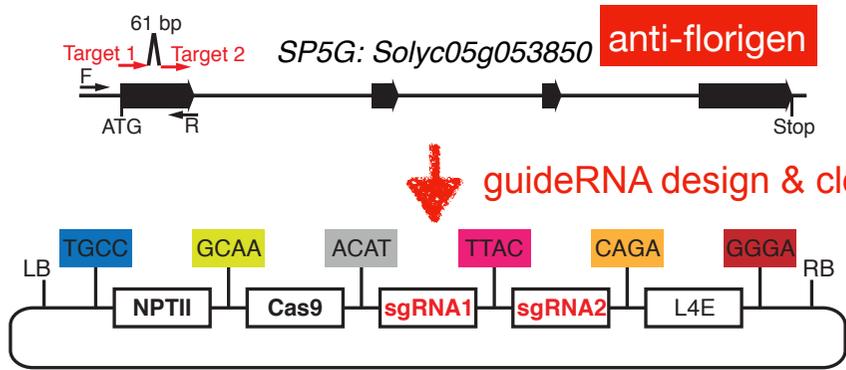


allele frequency analysis

an "anti-florigen" gene



Editing photoperiodic flowering in tomato

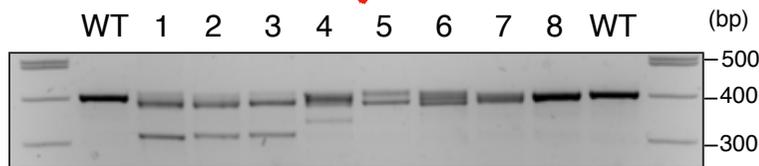


guideRNA design & cloning

plant transformation



genotyping

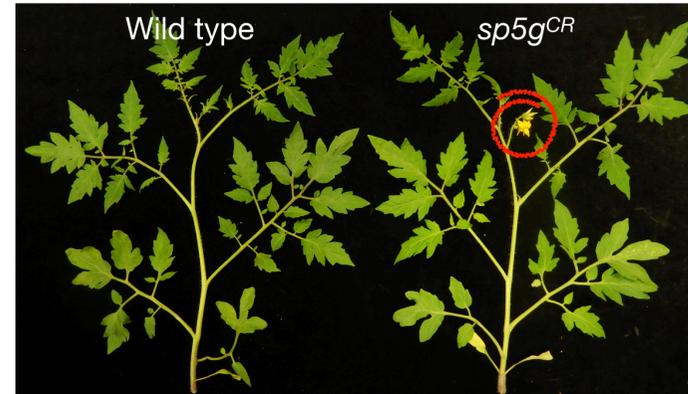


sequencing

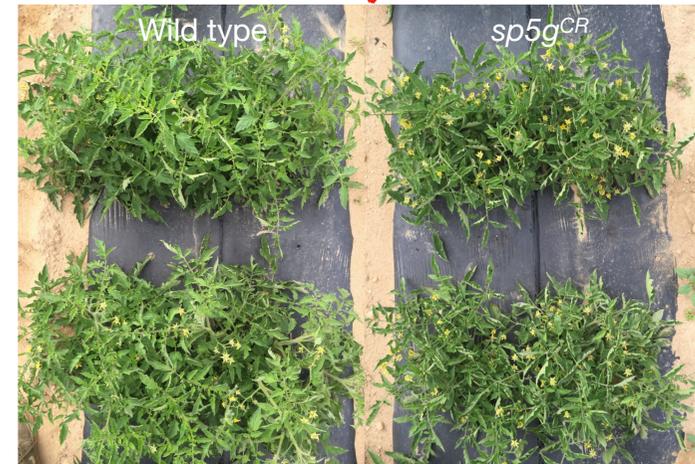
WT CCTAGAGATCCTTTAATAGTTTCGGAGTTG (47bp) GTTTACAACAATAGGGTGGTCTATAATGGA

a1 CCTAGAGATCCTTTAAT----- (47bp) -----GGA

a2 CCTAGAGATCCTTTAATAGTTTCGGAGTTG (47bp) GTTTACAACAATA-----TAATGGA



earlier
flowering



earlier
flowering



earlier
fruit set

Editing photoperiodic flowering in tomato

cherry tomato
(Sweet-100)



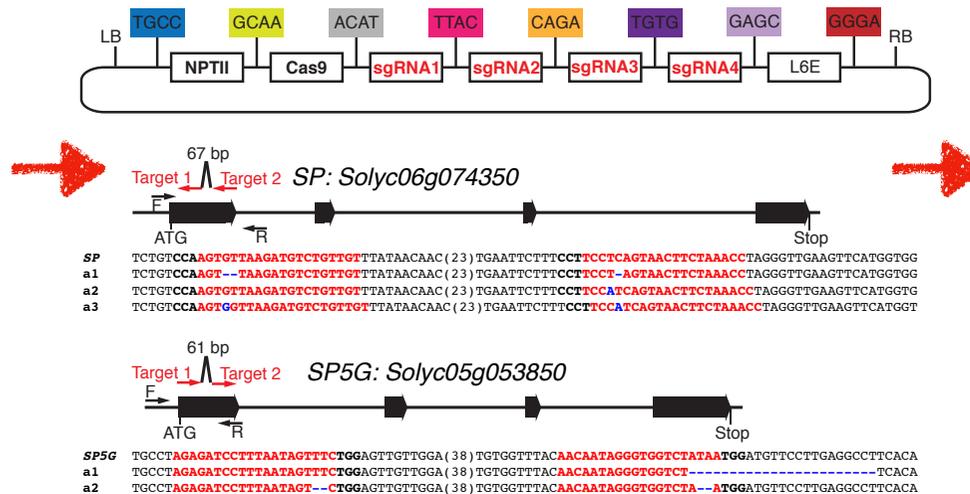
tasty fruits
late flowering & fruit set
large plants (indeterminate)

genome-edited
cherry tomato
(Sweet-100)



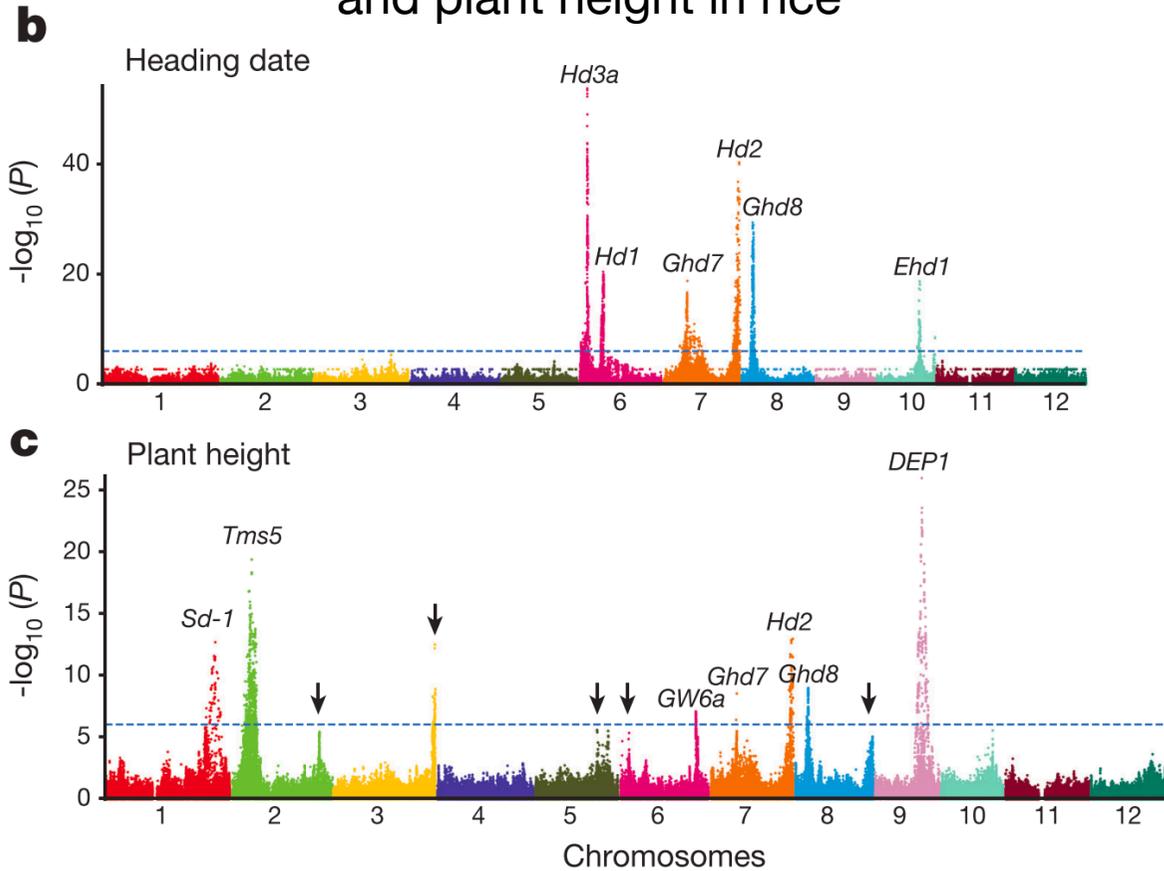
tasty fruits
early flowering & fruit set
compact plants (determinate)

multiplexed genome editing

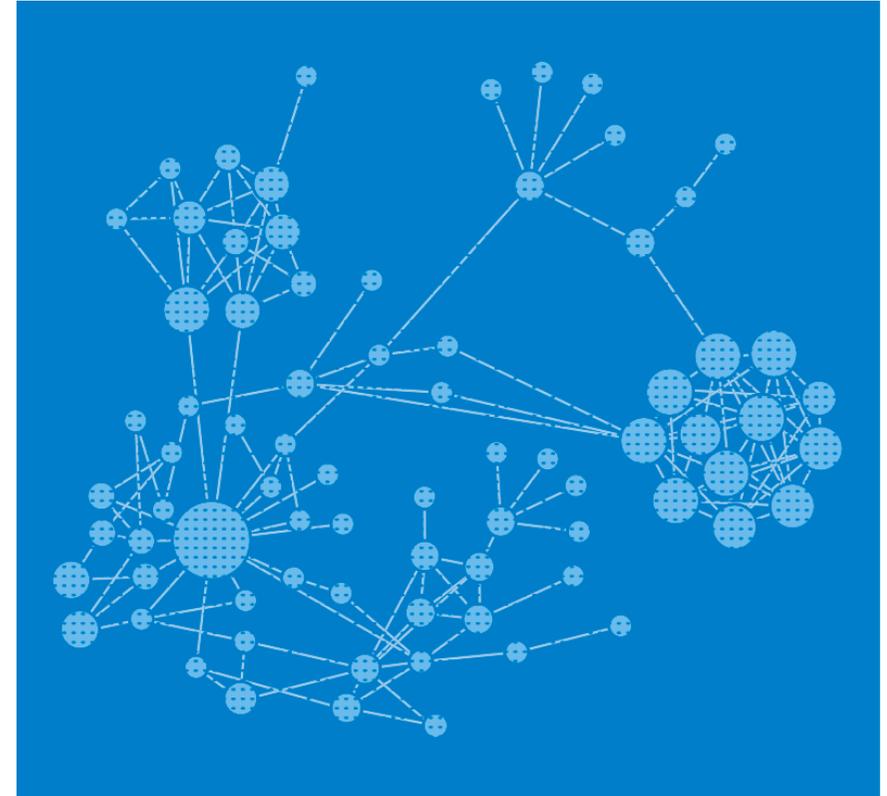


Engineering quantitative traits by **editing gene networks**

Genetic architecture for flowering time and plant height in rice



How can gene networks be rewired ?

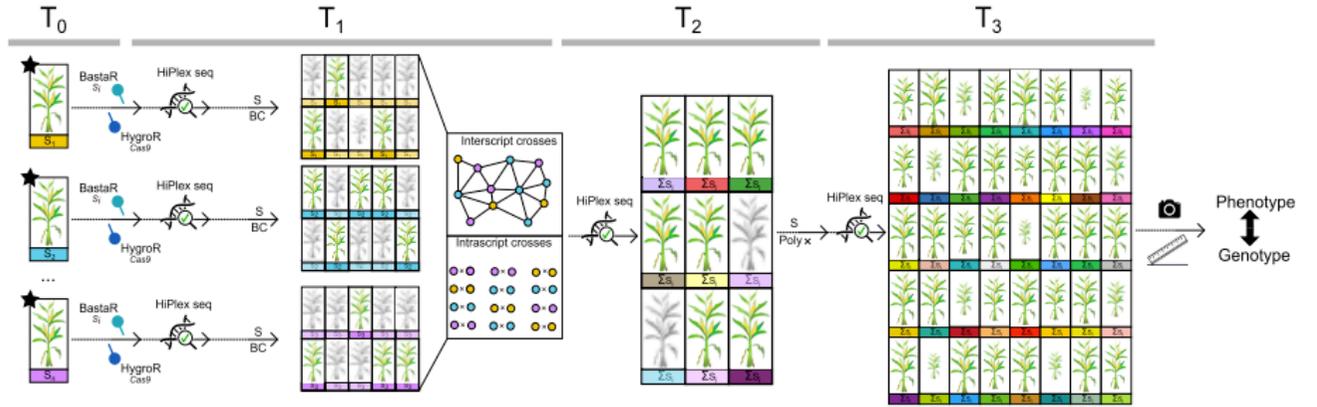
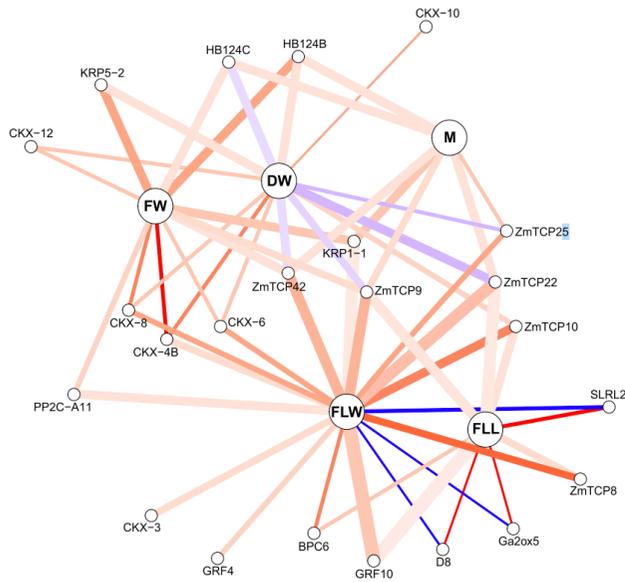


- **Agronomic traits are often polygenic (controlled by many genes)**
- **Engineering single mutations fails to reconstitute full phenotypic effects**

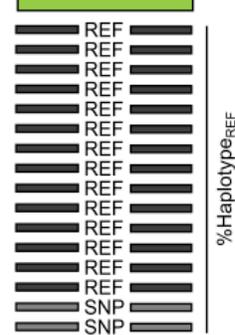
Engineering quantitative traits by editing gene networks

Multiplexed targeting of 48 genes

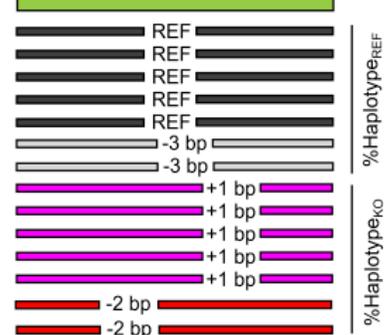
Network of growth-related genes in maize



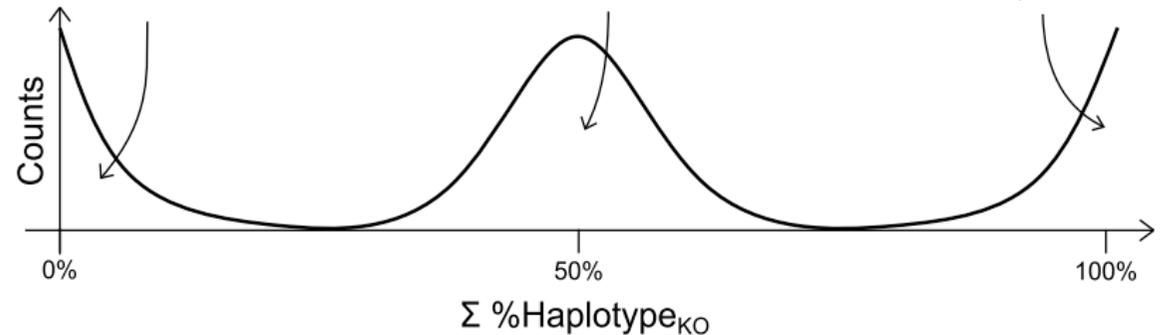
Gene A



Gene B



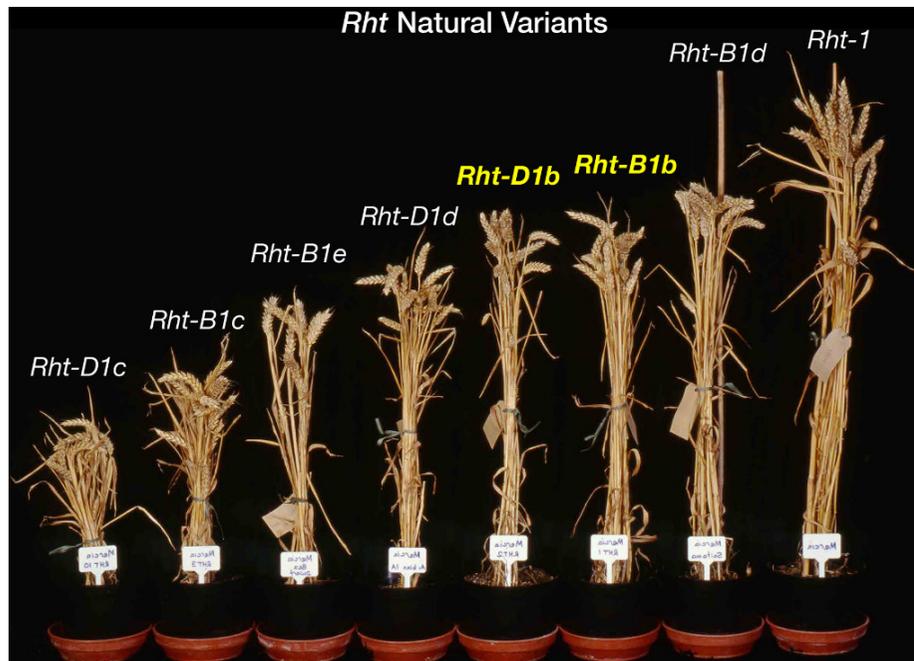
Gene C



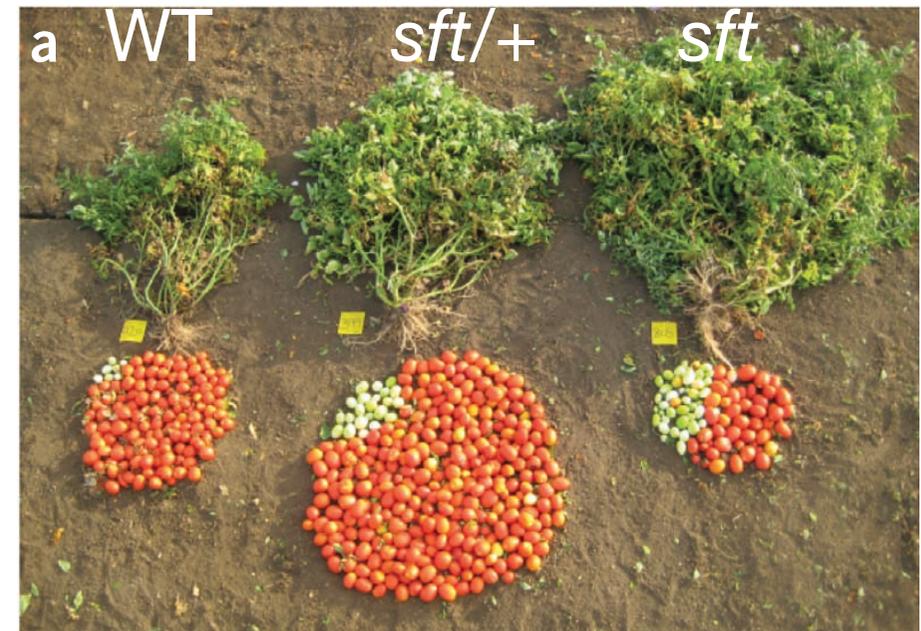
Engineering quantitative traits by **tuning gene activity**

Known single mutations with agronomic value have weak or moderate molecular effects

Hormone (GA) mutations

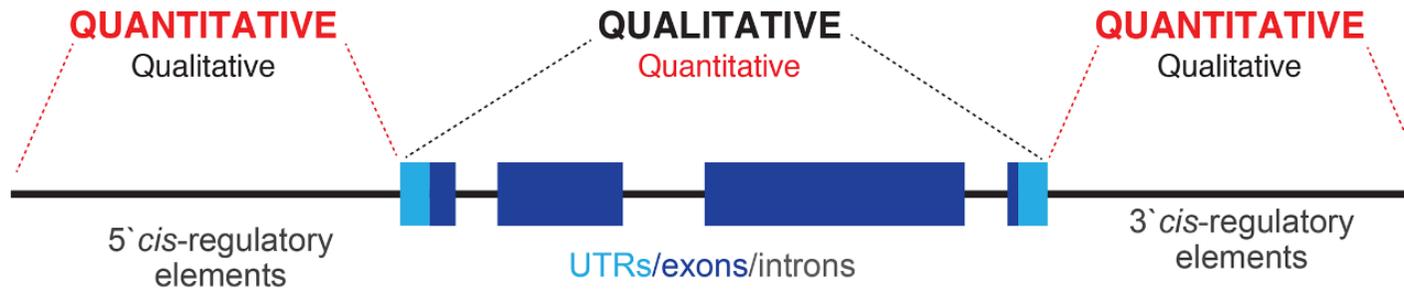


Hormone (florigen) mutations



How can we engineer such mutations?

Engineering quantitative traits by **tuning gene activity**



Qualitative Changes

- New loss-of-function alleles into old and new crops
- Generating identical alleles in elite backgrounds
- Introduction of species-specific gene modifications
e.g. Male sterility for hybrid seed production, Disease resistance, Allergen or toxin removal, etc.



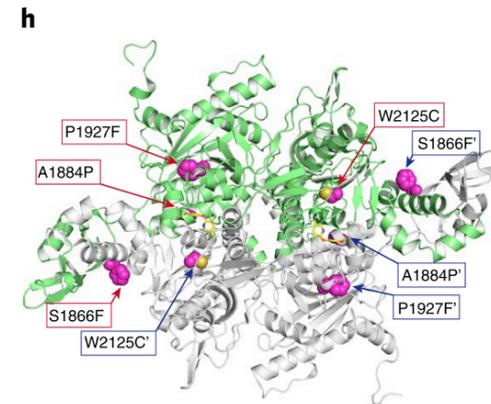
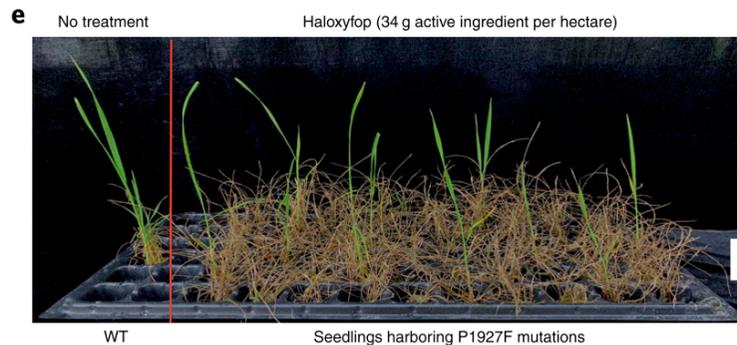
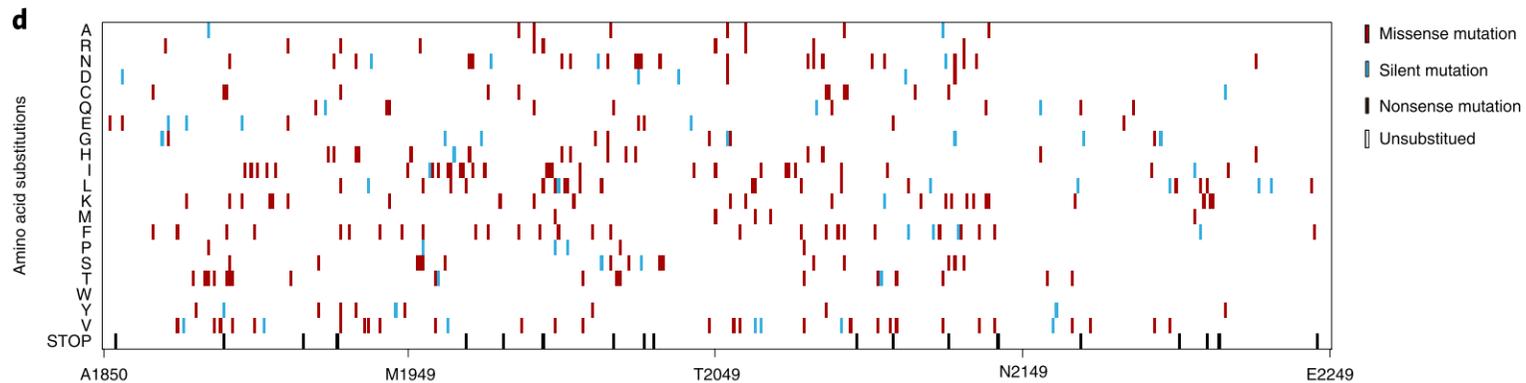
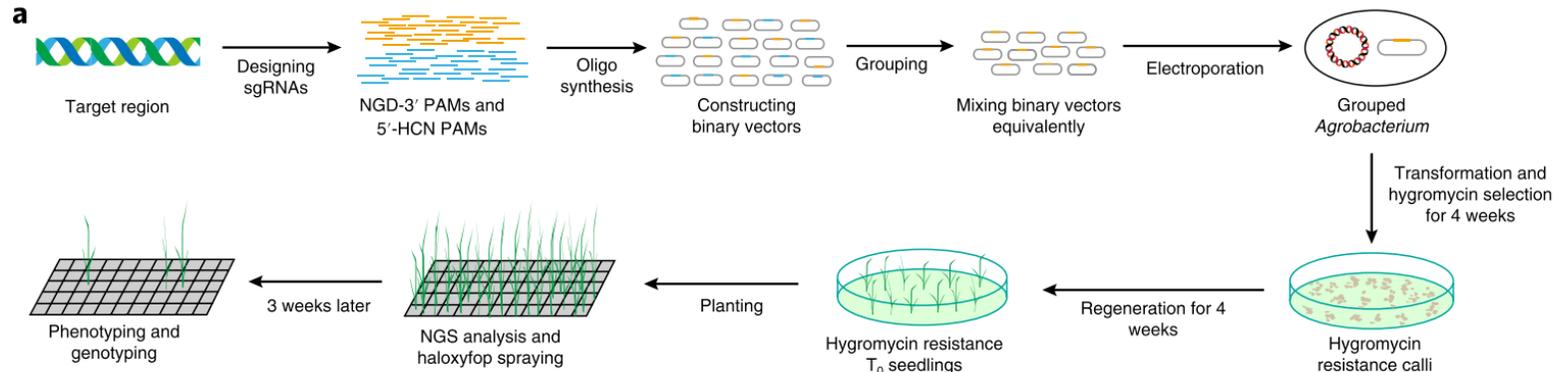
Quantitative Changes

- Generating allelic series for phenotypic selection
- Base edits or in-frame deletions in coding regions
- Interfering with RNA or protein stability
- Modifying *cis*-regulatory elements (activators/repressors)



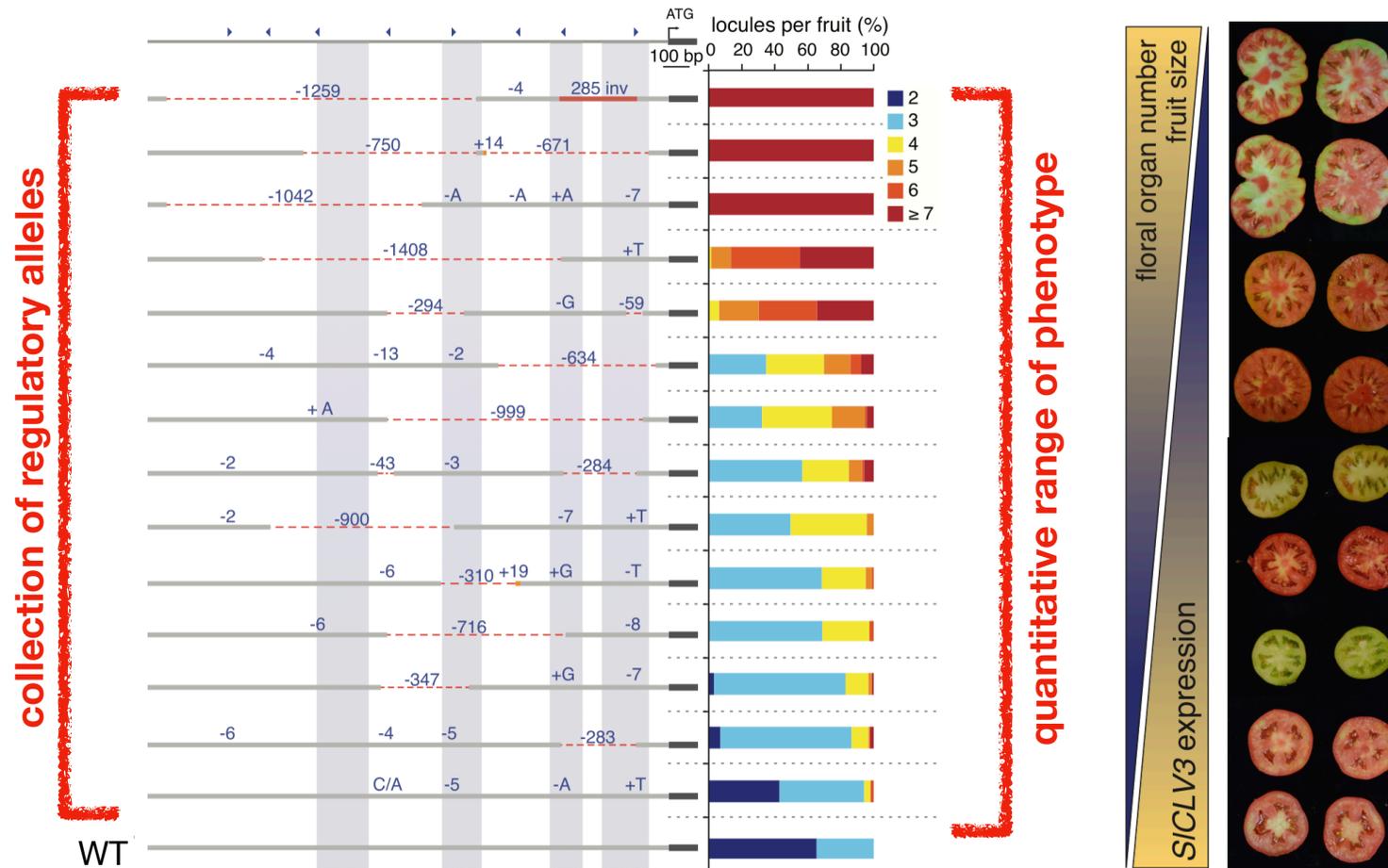
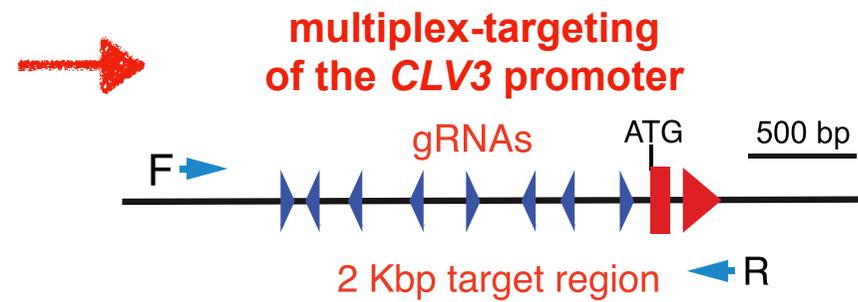
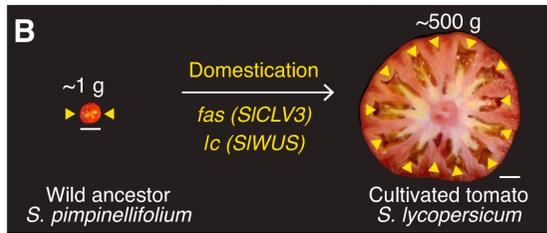
Engineering quantitative traits by **base-editing protein sequences**

Proof-of concept: directed evolution of herbicide resistance gene using base editing



Engineering quantitative traits by editing *cis*-regulatory regions

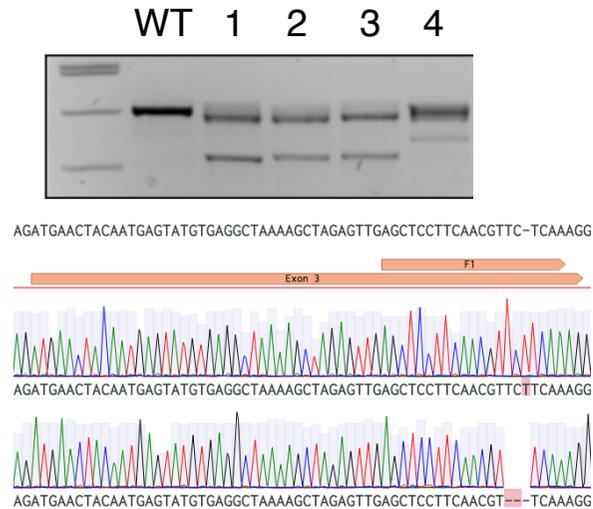
fruit size regulator gene *CLV3*



Engineering mutations in regulatory regions allows quantitative changes in gene activity

Genome edits can be challenging to detect

PCR + Gel + Sanger



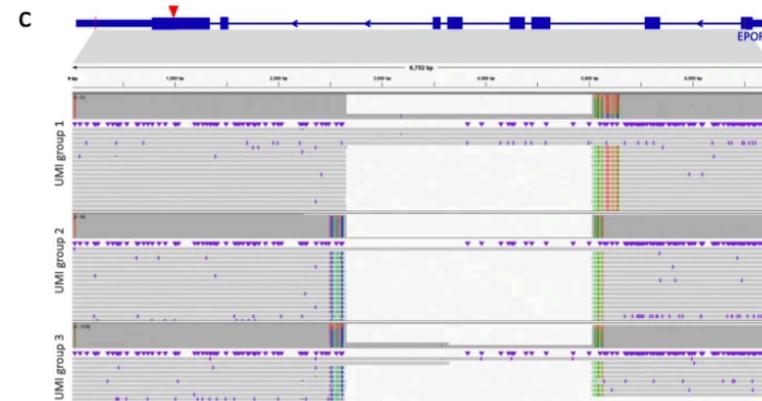
Advantages

- Low cost (at low sample sizes)
- High accuracy

Disadvantages

- Low throughput
- Mainly for homozygous edits

NGS



Advantages

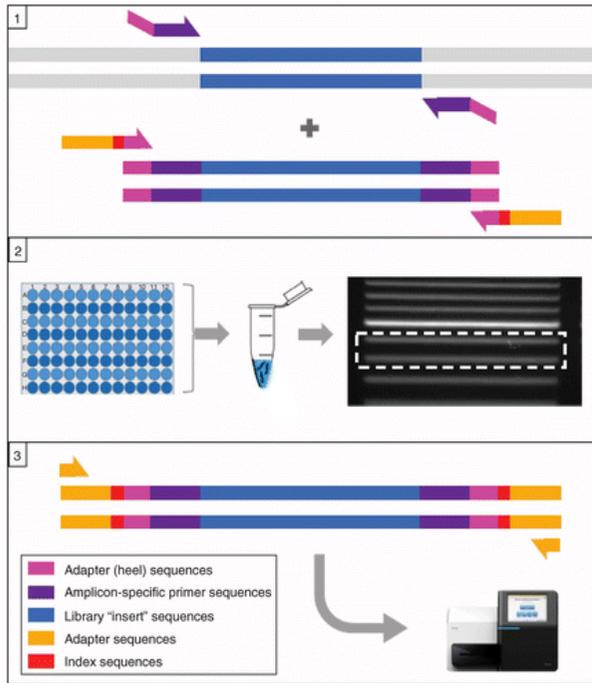
- High throughput
- High accuracy
- Low frequency edits

Disadvantages

- High cost (at low multiplexing level)
- Computational expertise

NGS approaches for analysing genome editing

Short-read (Illumina)

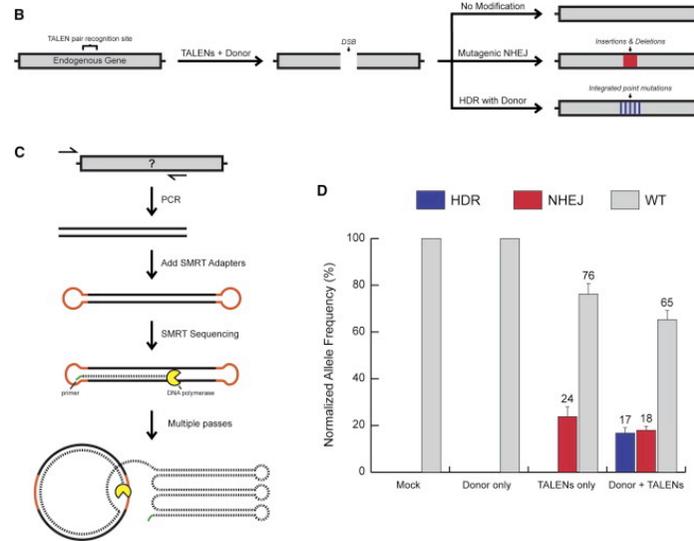


HiPlex2

Hammet et al., 2019, Biotech

...

Long-read (PacBio)

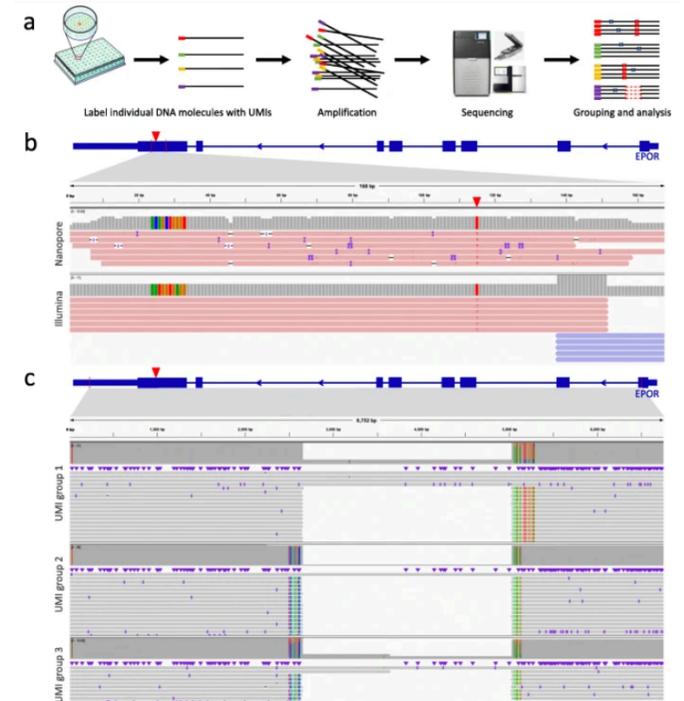


SMRT-Seq

Hendel et al., 2014, Cell Rep
Karst et al., 2021, Nat Method

...

Long-read (Nanopore)



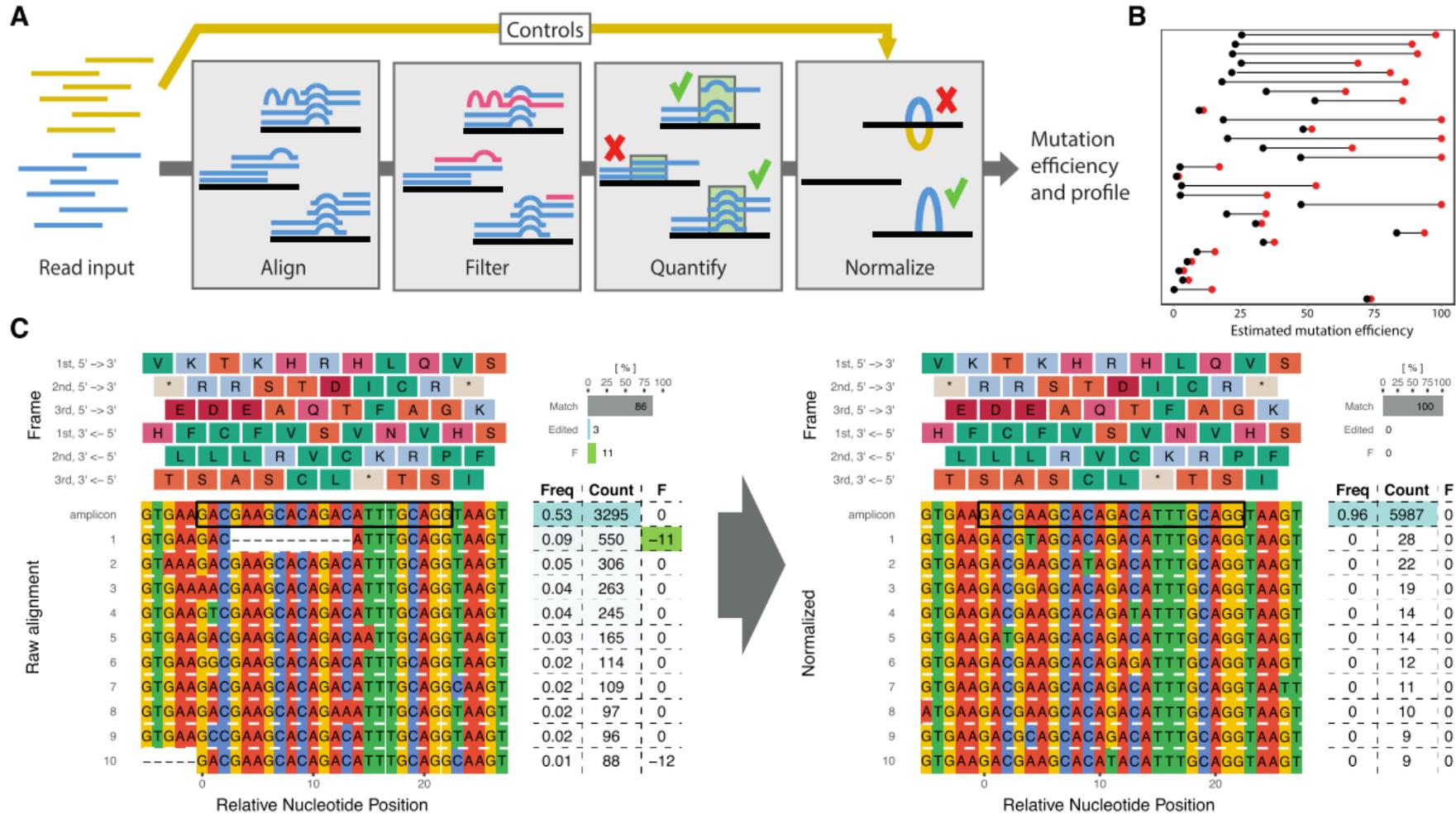
IDM-Seq

Bi et al., 2020, Genome Biol

...

Computational tools to analysing genome editing events

AmpliCan (Labun et al., 2019, Genome Research)



Review on methodologies for editing quantitative traits

What approaches are used for editing quantitative traits ?

- Rewiring gene regulatory networks
- Editing of cis-regulatory sequences
- Editing of protein coding sequences

What quantitative approaches allow the detection of genome edits ?

- short read / long read
- amplicon / whole genome
- multiplexing
- detection tools

What are the advantages and disadvantages per methodology ?

- accuracy
- scalability and costs
- limitations (complex mutations, rare edits, etc.)

Where is the field at ?