

# Engineering Trait Variation

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# Quantitative Traits in Crops

- Examples?
- Limited genetic variation
- Evaluating phenotypes is hard

# Quantitative Traits in Crops

- How to make gains with QTs
  - Forward genetic screens
  - Gene introgression
  - Genetic engineering

# Cis-regulatory elements

- Promoters ideal for studying trait variation
- Less pleiotropic
- Often contain significant SNPs markers found in GWAS/QTL analysis
- But things like epistasis and gene regulation make variants in these regions hard to directly study
- Not “saturated” for variation
  - Adding more variation can help engineer trait variation, but can also help expand our knowledge of the role of cis-regulatory elements

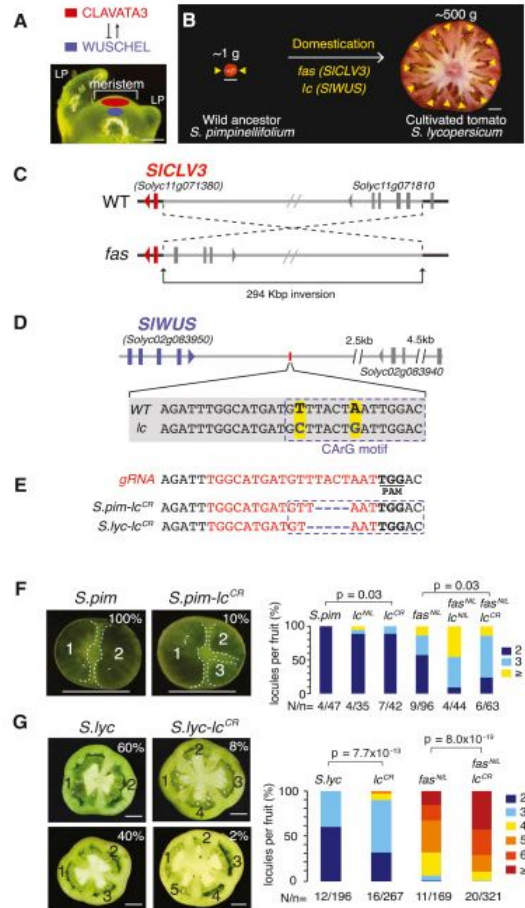
# CRISPR-Cas9 Allele Engineering

- Mostly used in coding sequence
- Used to create null alleles (complete loss of function) for functional analysis

# Tomato Fruit Size

- Increase in floral organs -> increase in fruit locules -> larger fruit
- Obviously a large increase due to domestication

# Recreating a Fruit Size QTL

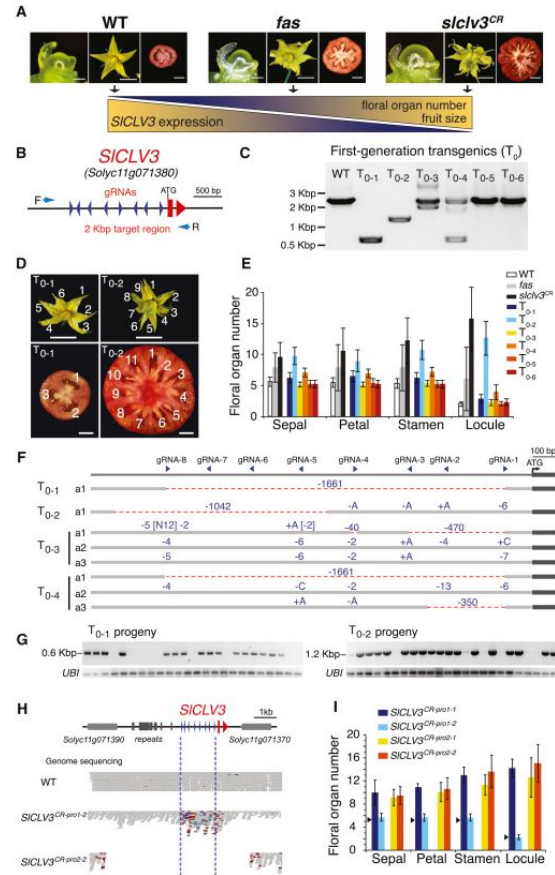


# Takeaways

- CRISPR/Cas9 can be used to recreate known cis-regulatory QTLs
- But, what about traits with unknown causative variants/QTLs?
- What CRE should be targeted?
  - Multiple CREs?



# Novel Alleles of the *SICLV3* Promoter



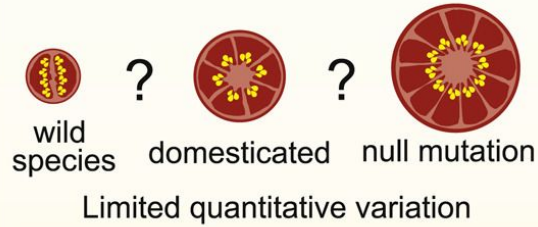
# Takeaways

- Novel alleles were created, with various effects, in the T0 plants
- The gRNAs were restricted to the region of interest
- Null and hypomorph alleles in box H are super important for the next result!

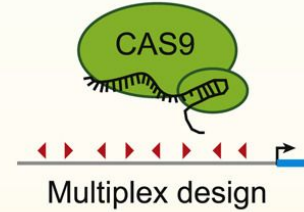
# trans-Acting CRISPR/Cas9 mutagenesis

- Introduce transgene to express the Cas9 protein along with the various gRNAs used in the previous study.
- How to find new alleles that may be complemented and masked?
  - Panel H alleles from previous figure. Top had no effect, bottom null, top complemented bottom
- Answer: Give the plant one null allele at beginning. If the second allele is loss of function, you will observe that phenotype.

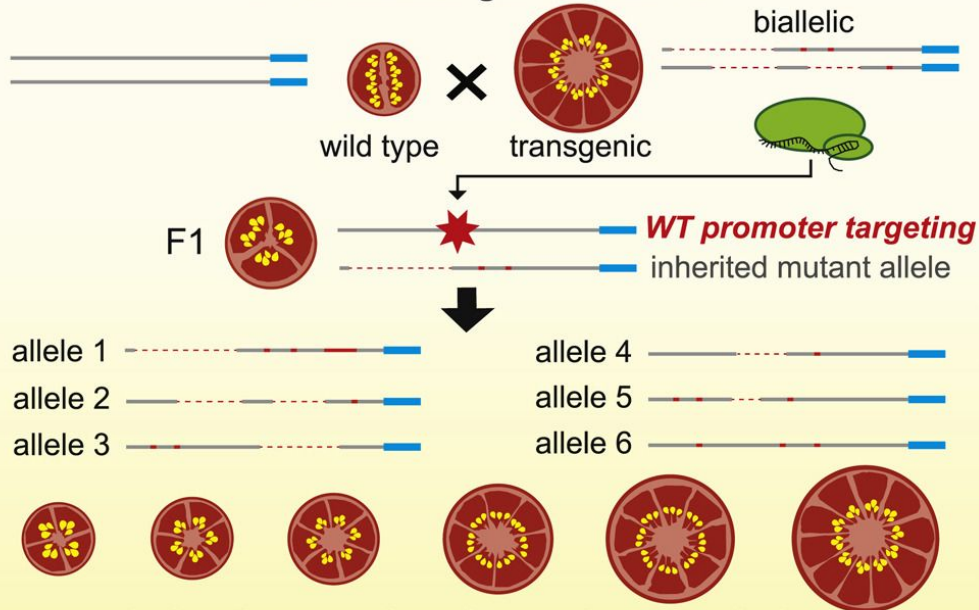
### 1. Yield traits (tomato locule number/fruit size)



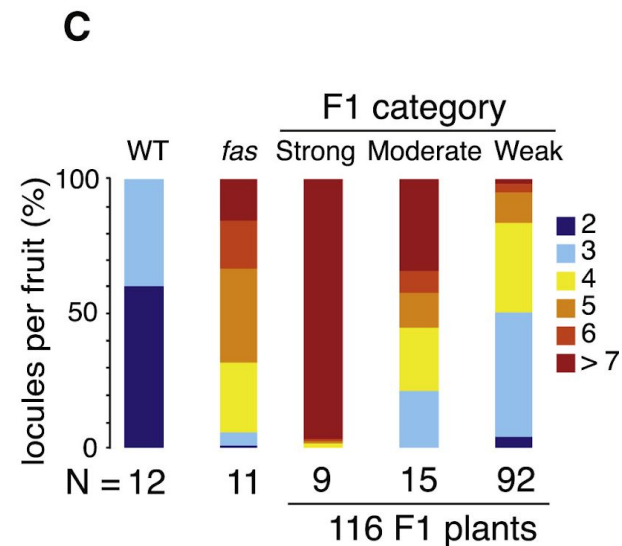
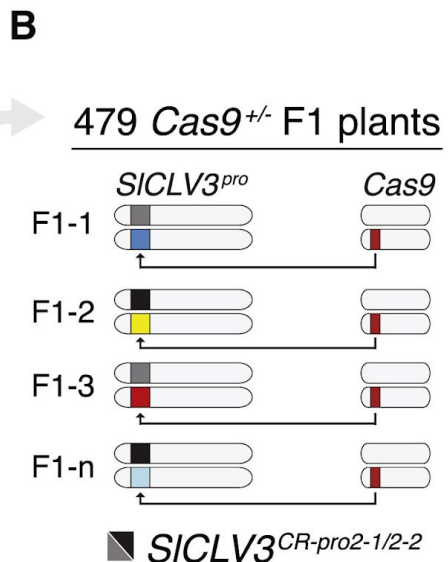
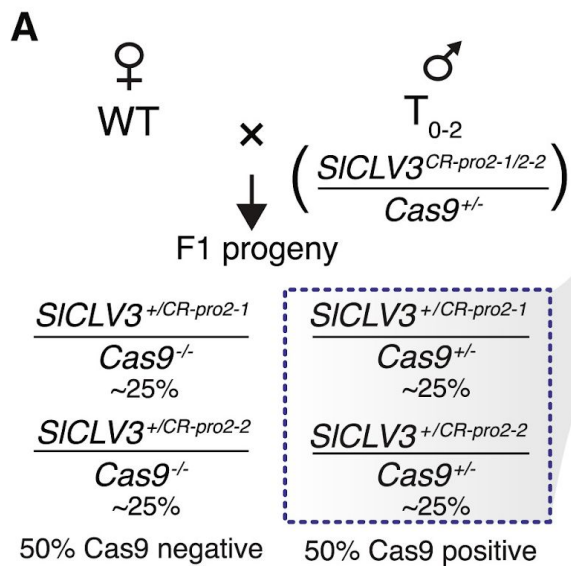
### 2. CRISPR/Cas9 *cis*-regulatory mutagenesis

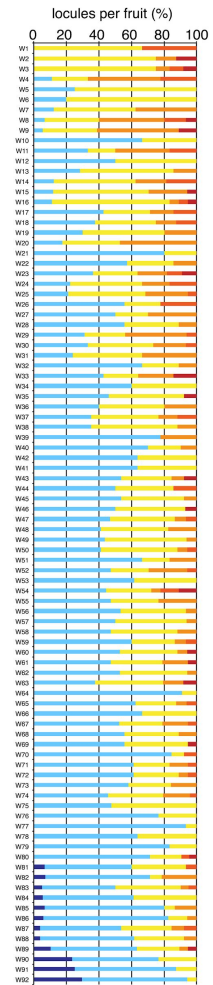
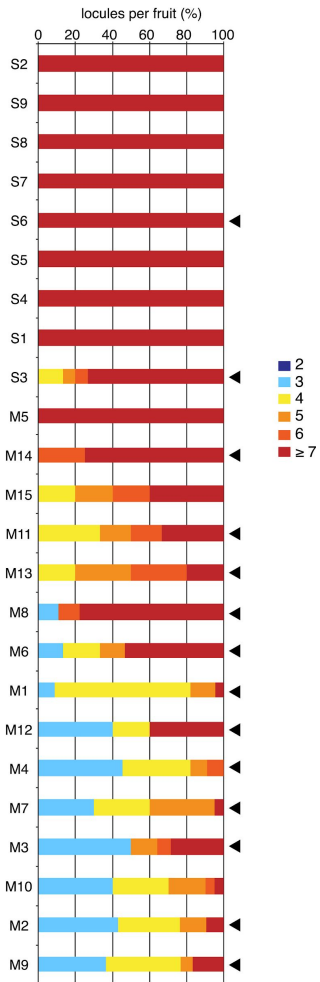


### 3. Sensitized genetic screen



### 4. Continuum of engineered trait variation



**A****B**

# Fixing Novel Alleles in Transgene Free Plants

- Ok we have novel alleles, but now what?
- But we know these genotypes are all homozygous for a strong loss of function allele, so what is there effect without that allele?
  - Homozygous new allele,
  - Hets new/+
- **Solution? Self them!**
  - Dihybrid cross with 9-3-3-1 ratio between the promoter allele and the Cas9 transgene.
  - 1/16 will be homozygous for new allele and no Cas9 transgene.

