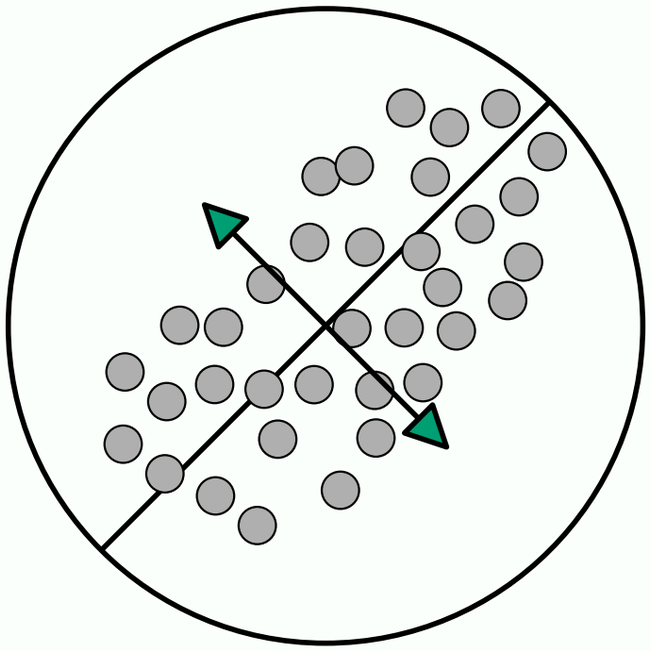
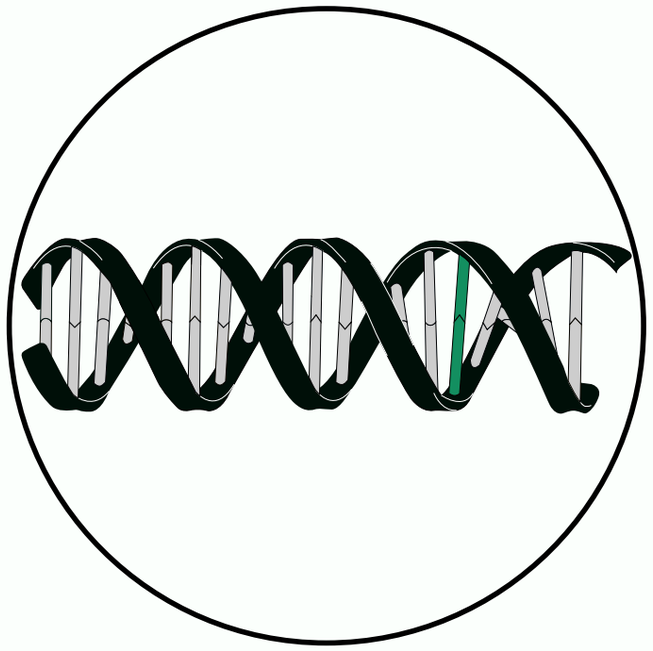
Distinct molecular mechanisms underlie natural variation in *C. elegans* toxin responses

Stefan Zdraljevic
Andersen Lab

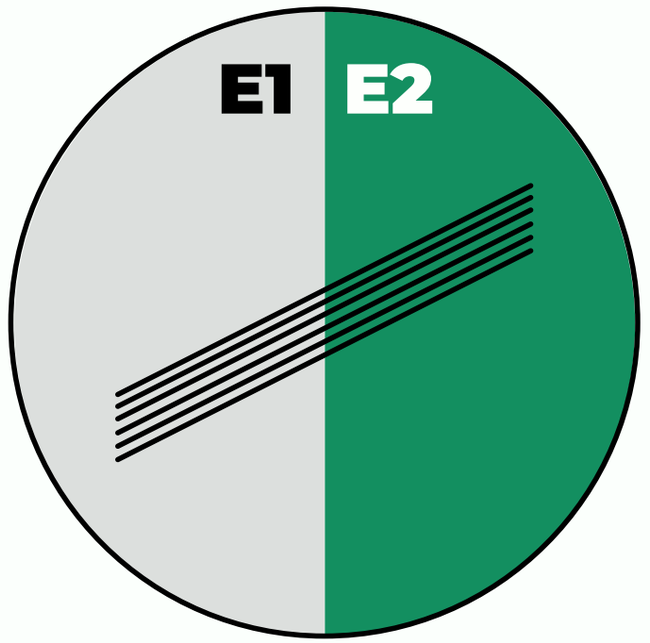
Sources of phenotypic variation



Stochastic

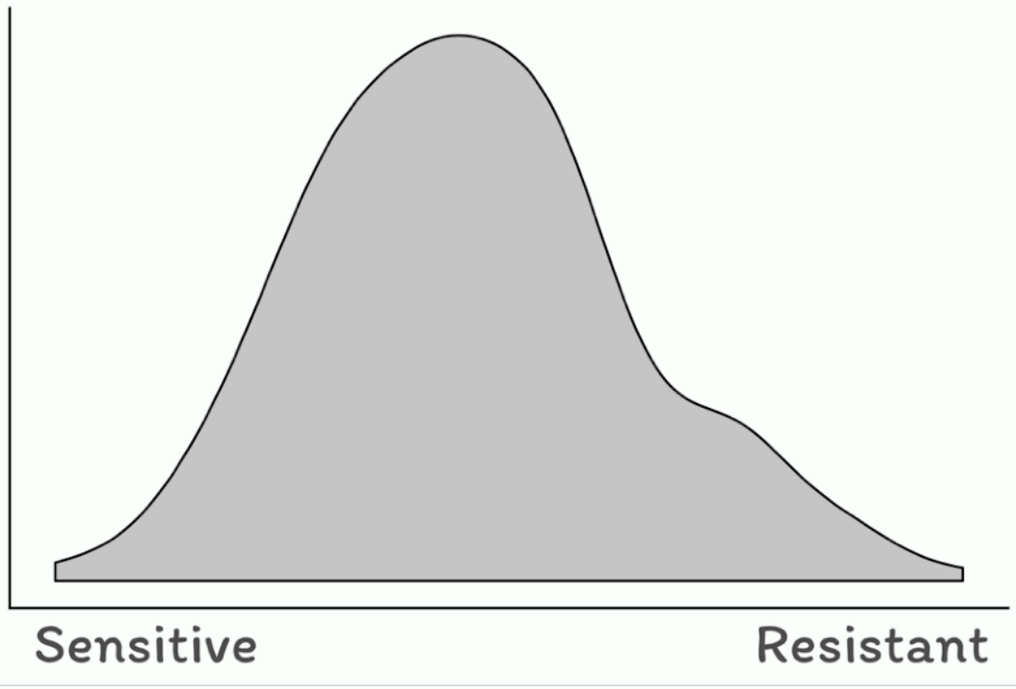


Genetic



Environmental

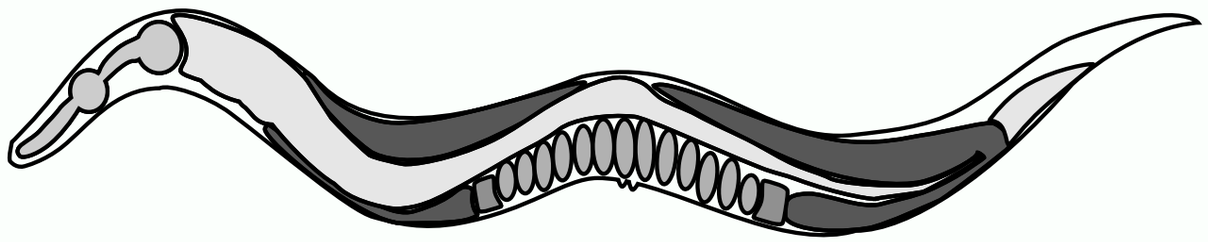
Leveraging natural genetic variation to identify sources of phenotypic variability



Genetic variation

Phenotypic variation

C. elegans is an excellent metazoan model to study mechanisms of phenotypic variation



Short life-cycle

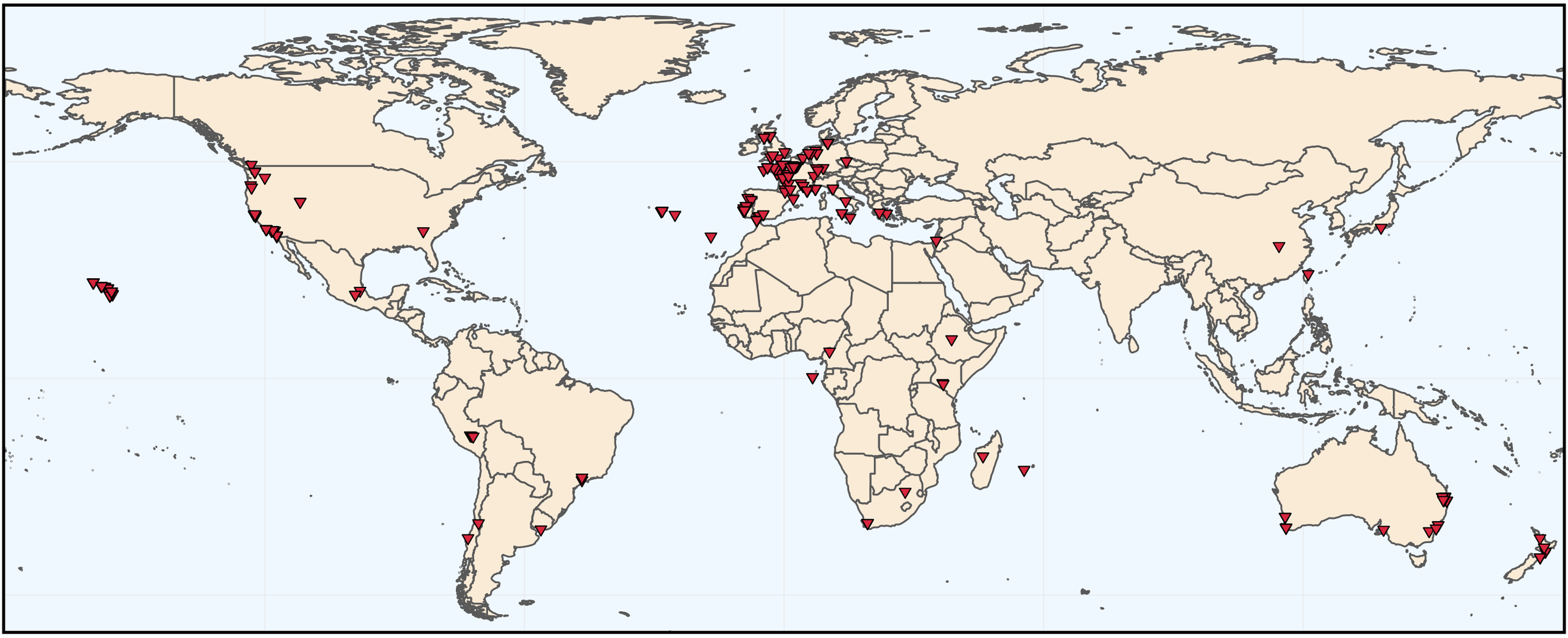
Hermaphroditic reproduction

Cheap and easy to handle

Well annotated genome

Cryopreservation

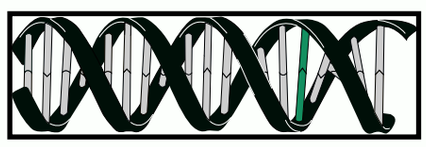
A global collection of 330 C. *elegans* strains



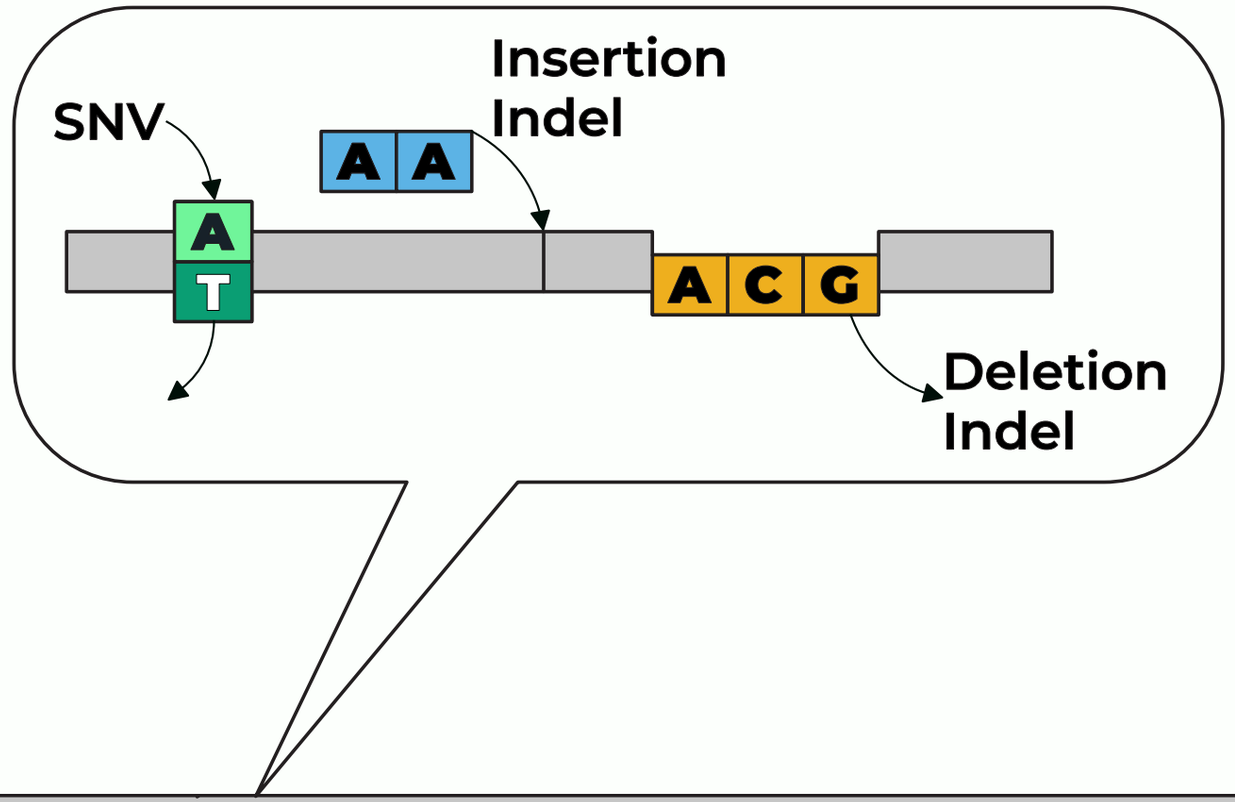
www.elegansvariation.org

Characterization of genomic variation

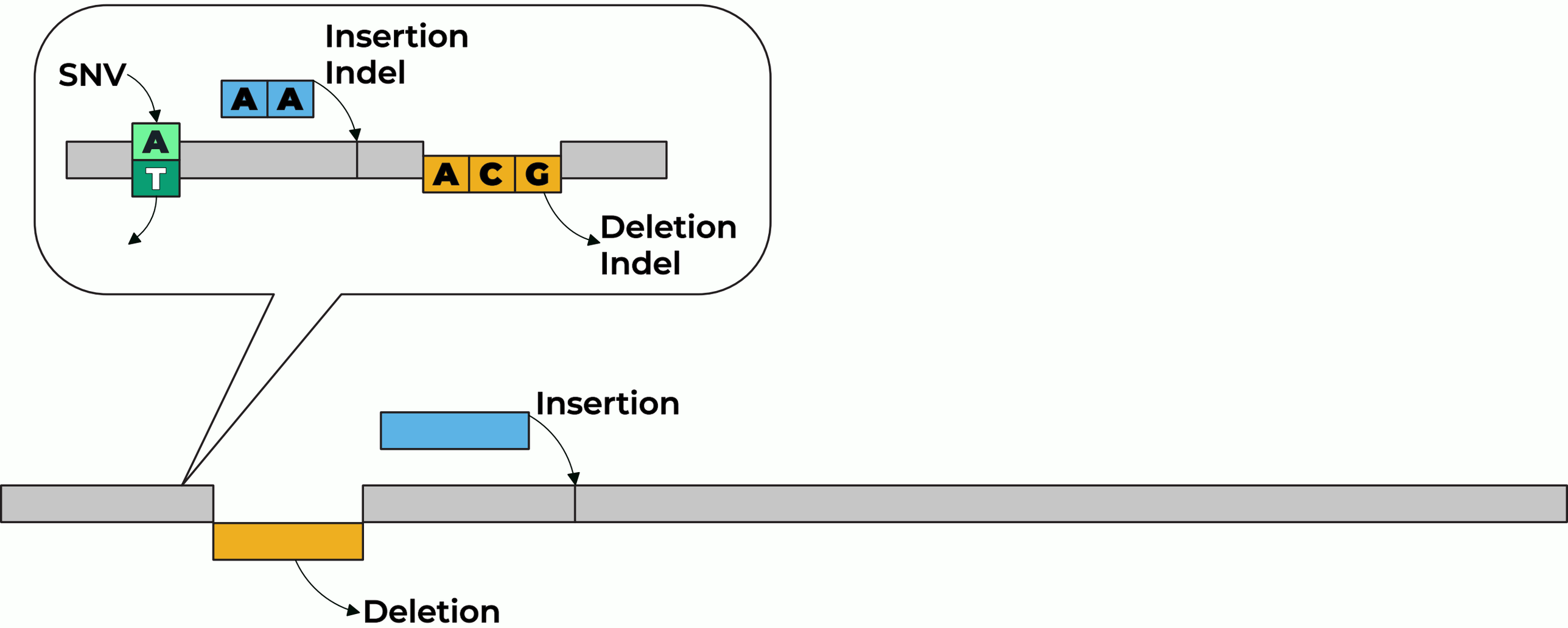
N2 reference genome



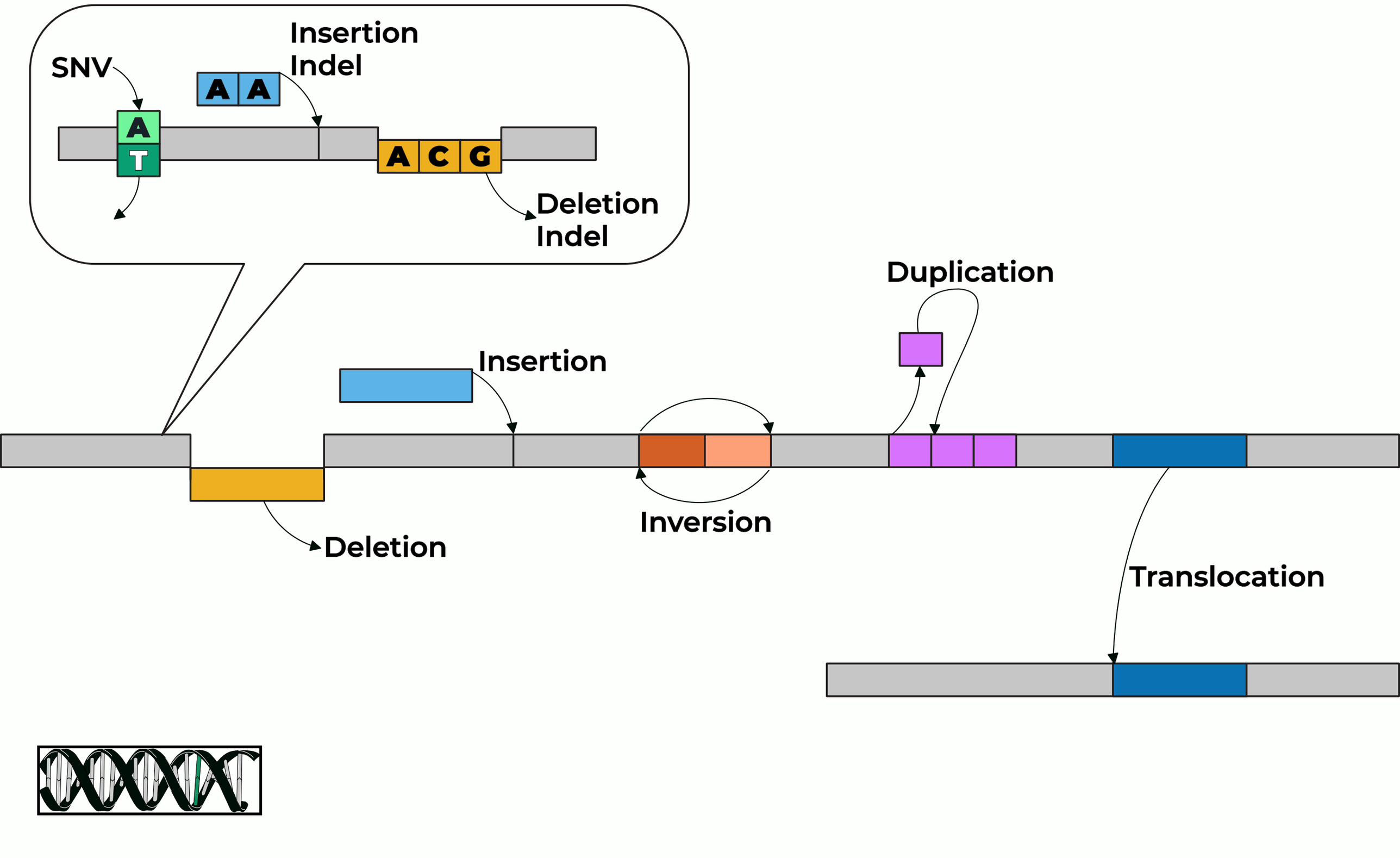
Characterization of genomic variation



Characterization of genomic variation



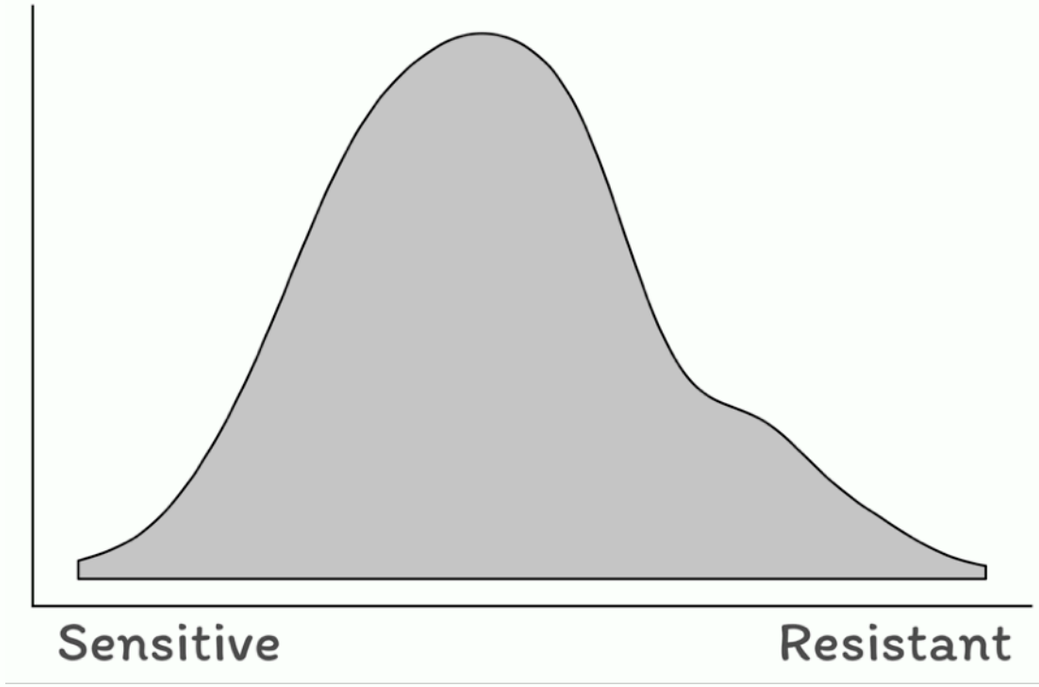
Characterization of genomic variation



We have identified 4.3 million variants in the *C. elegans* population

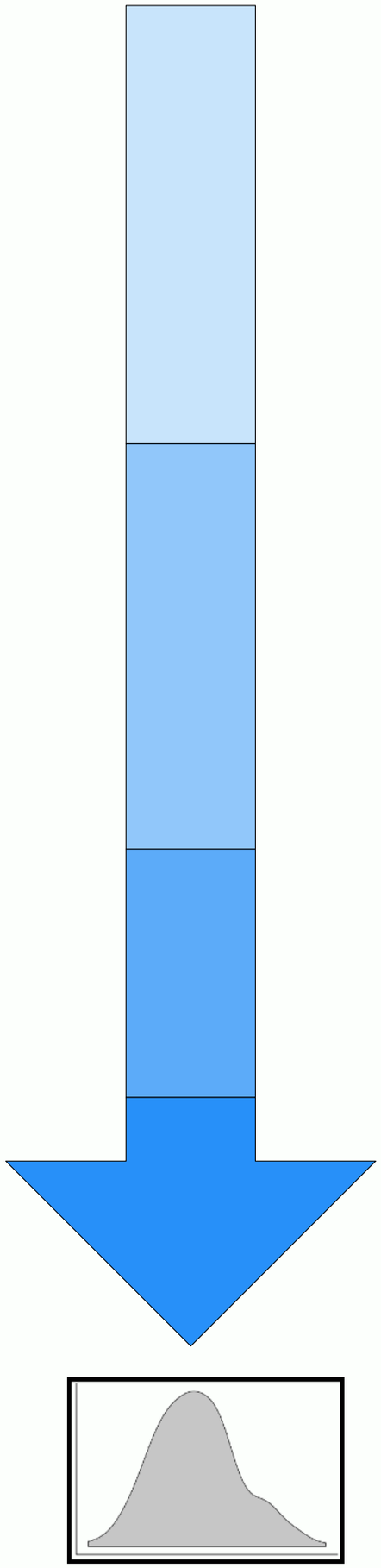
	Type	Count
< 50 bp	SNV	3144490
	Deletion-Indel	498292
	Insertion-Indel	447306
Structural variants	Deletion	82877
	Insertion	14217
	Inversion	35229
	Duplication	34684
	Translocation	87229
	Total	4,344,324

Genetic variation

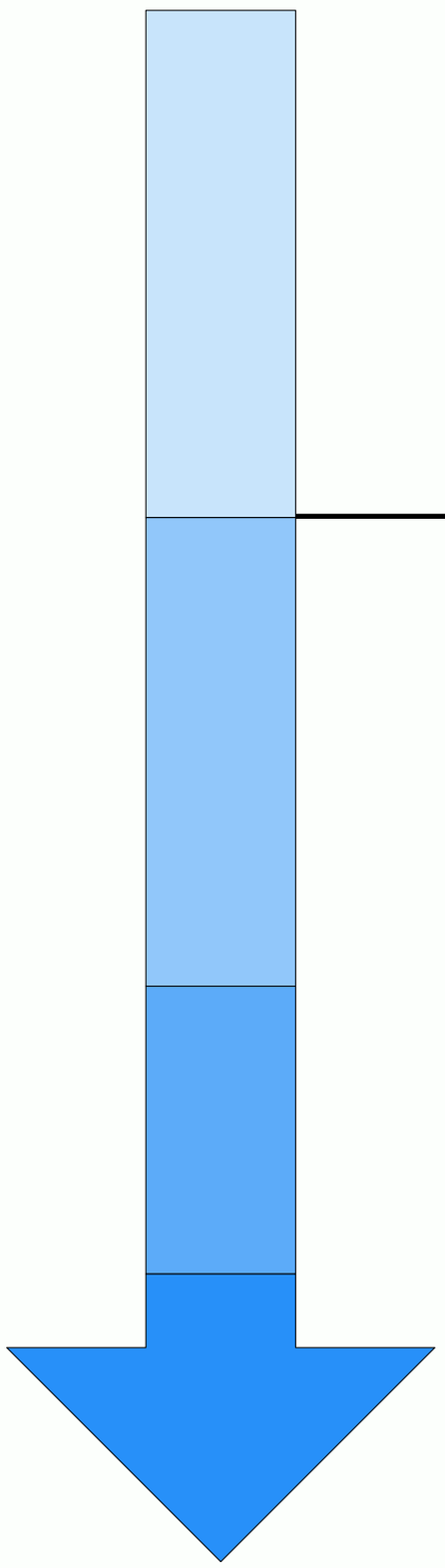


Phenotypic variation

High-throughput toxin response assay



High-throughput toxin response assay



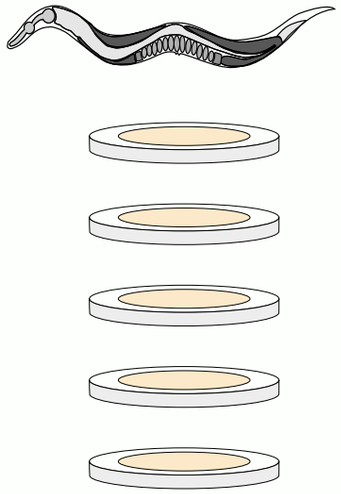
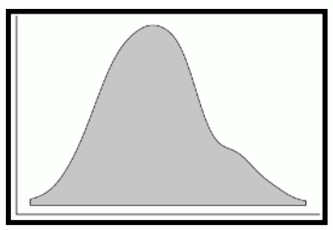


Plate Growth



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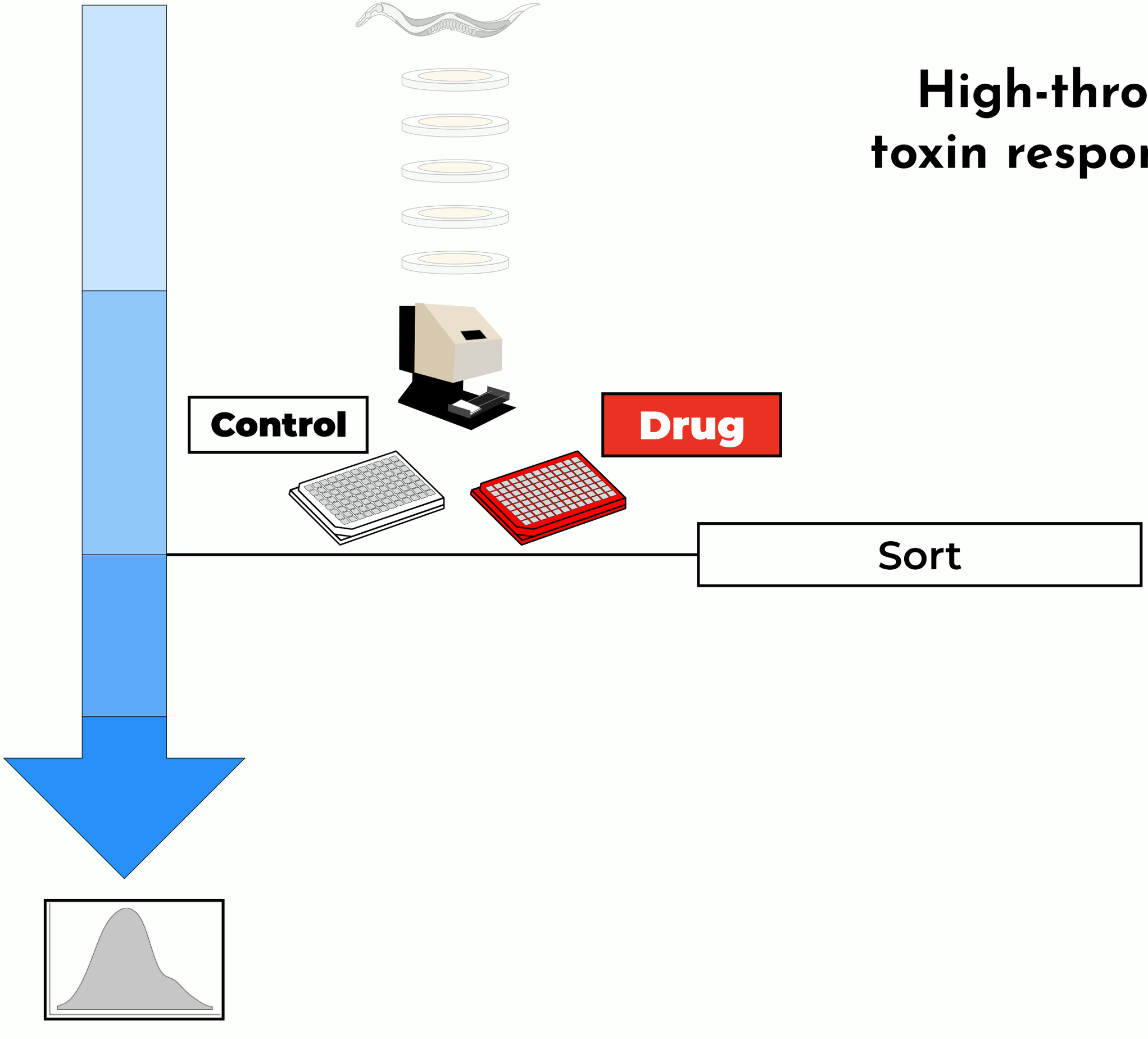

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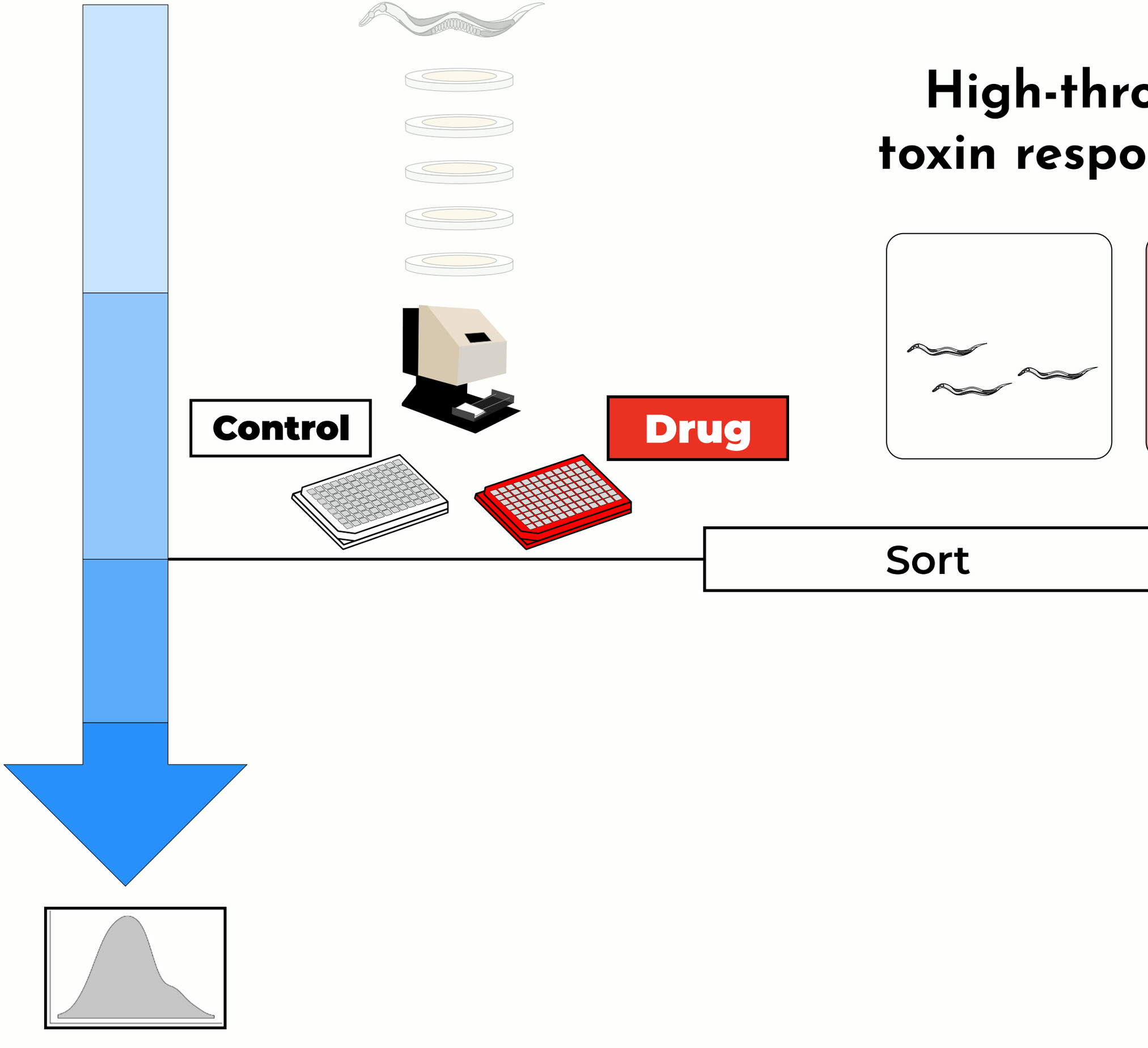

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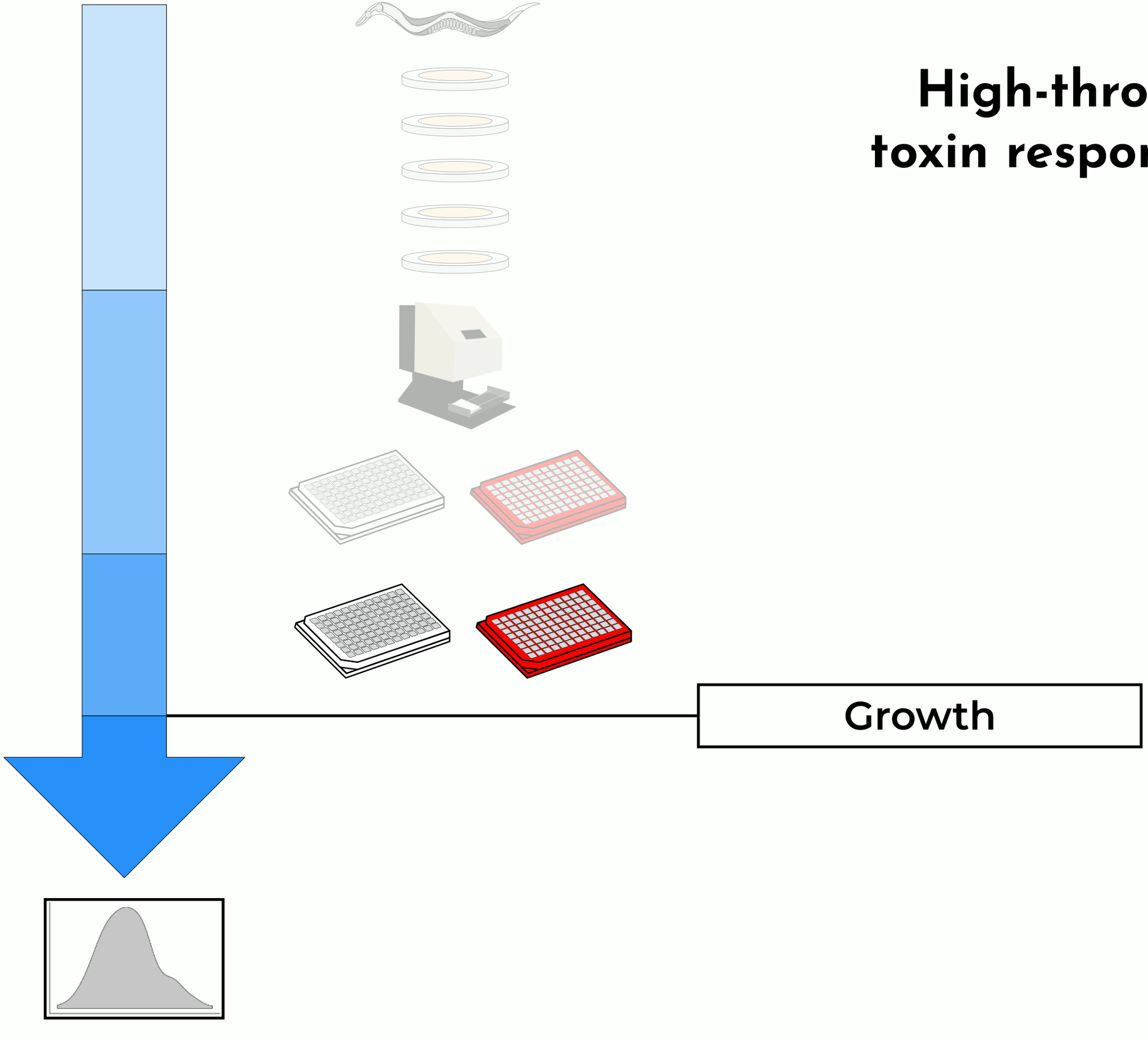
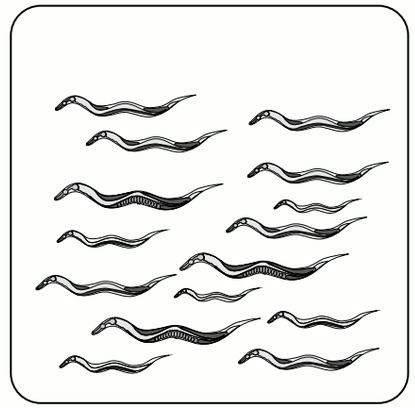
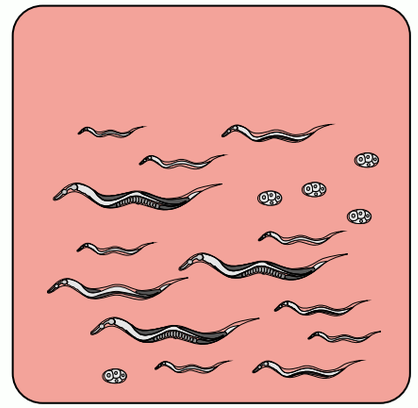


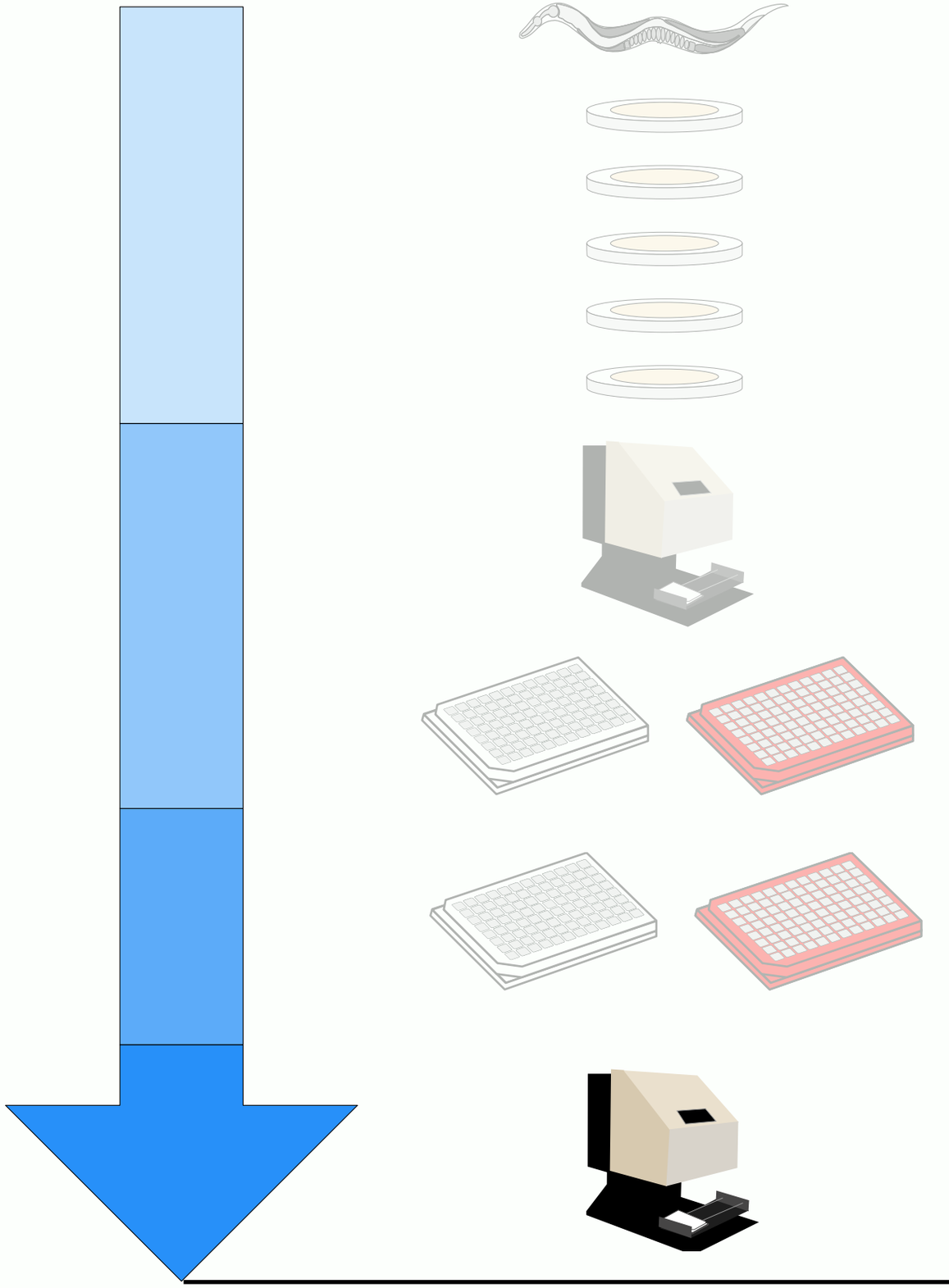
Growth

High-throughput toxin response assay

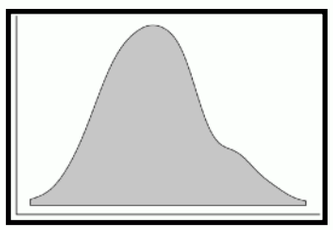
Strain A







Score



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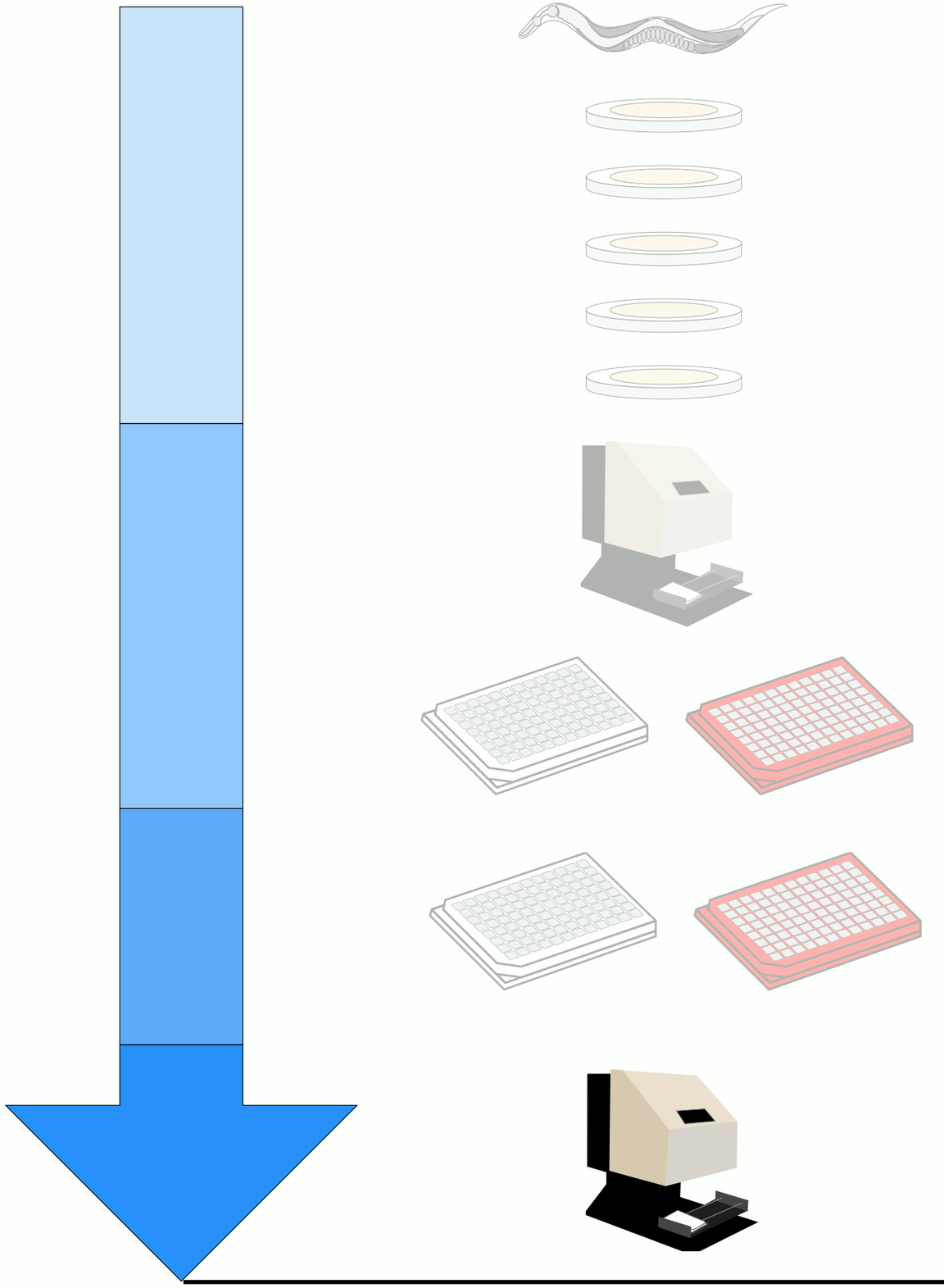
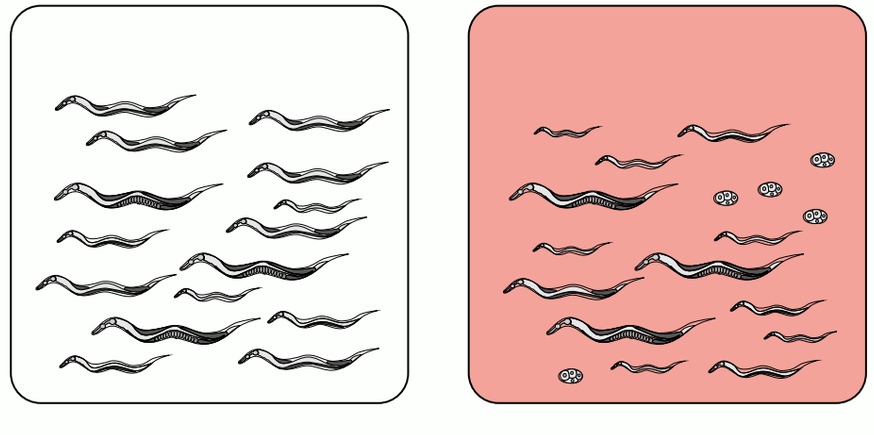
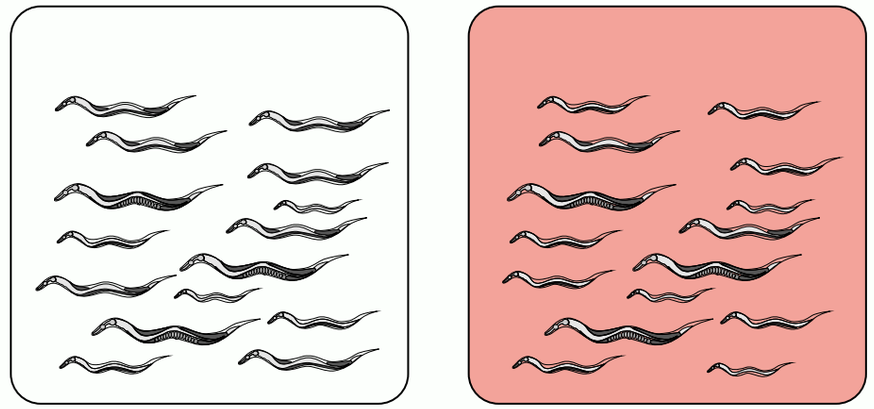


Strain A



Strain B



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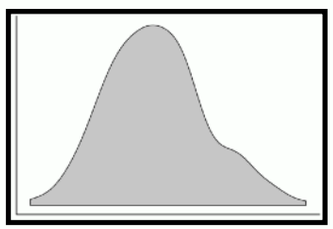

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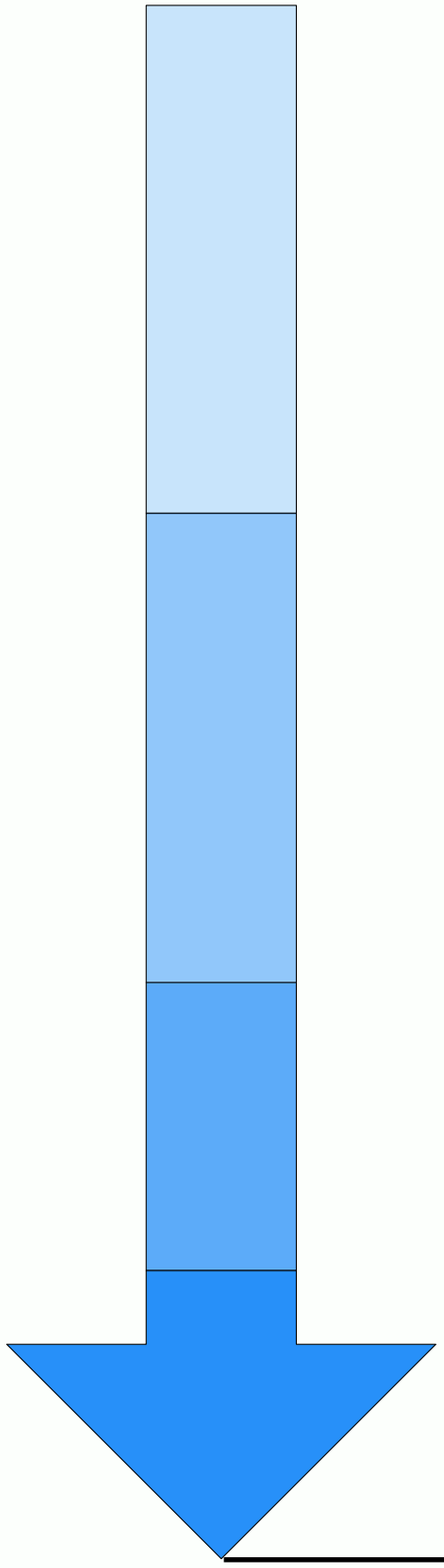

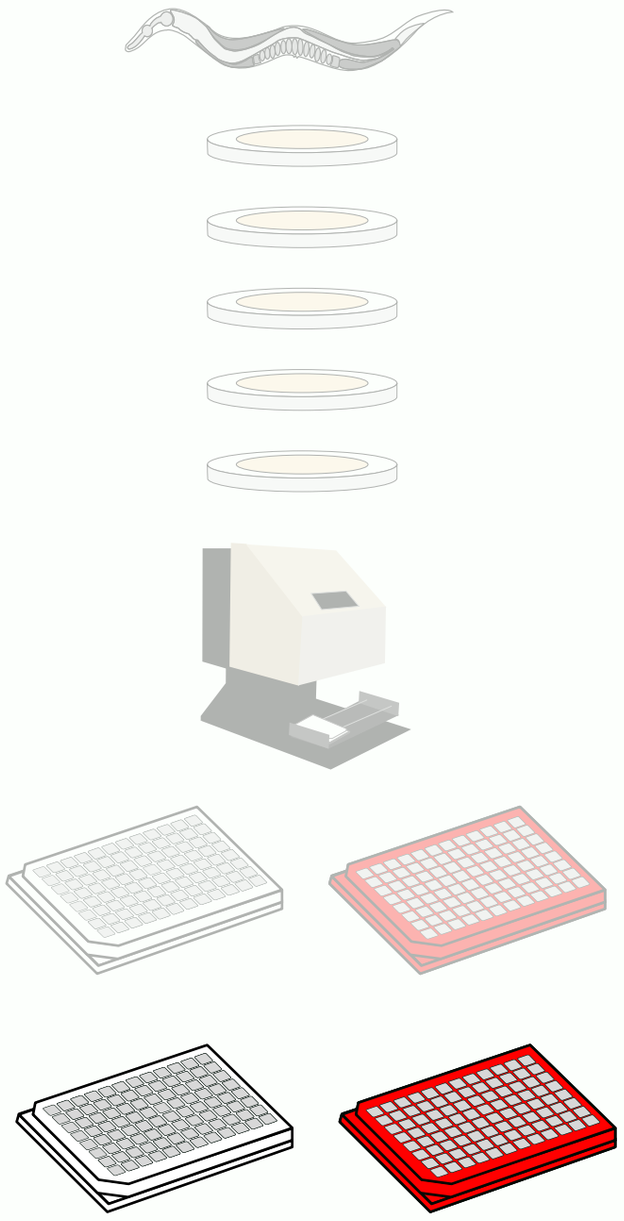

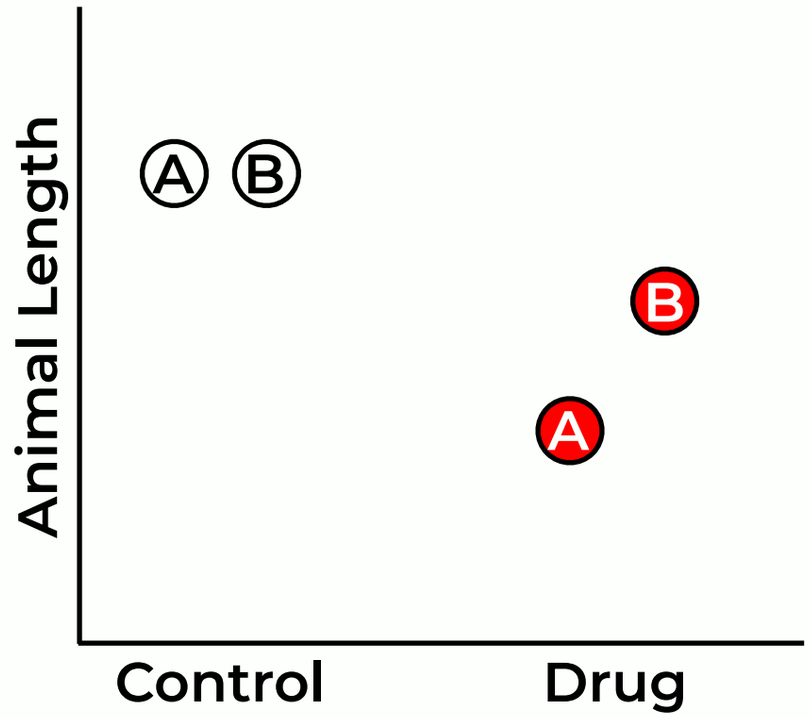

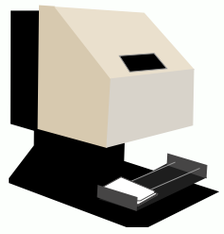

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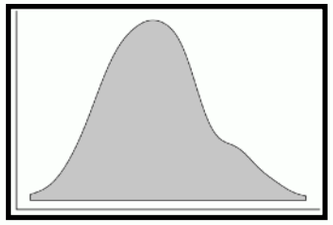

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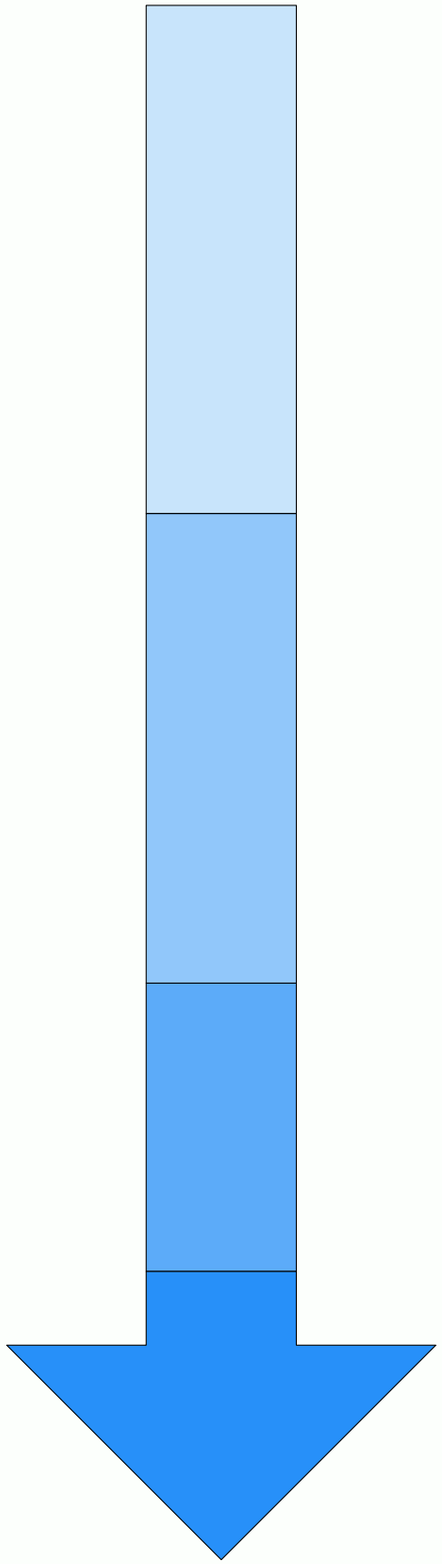
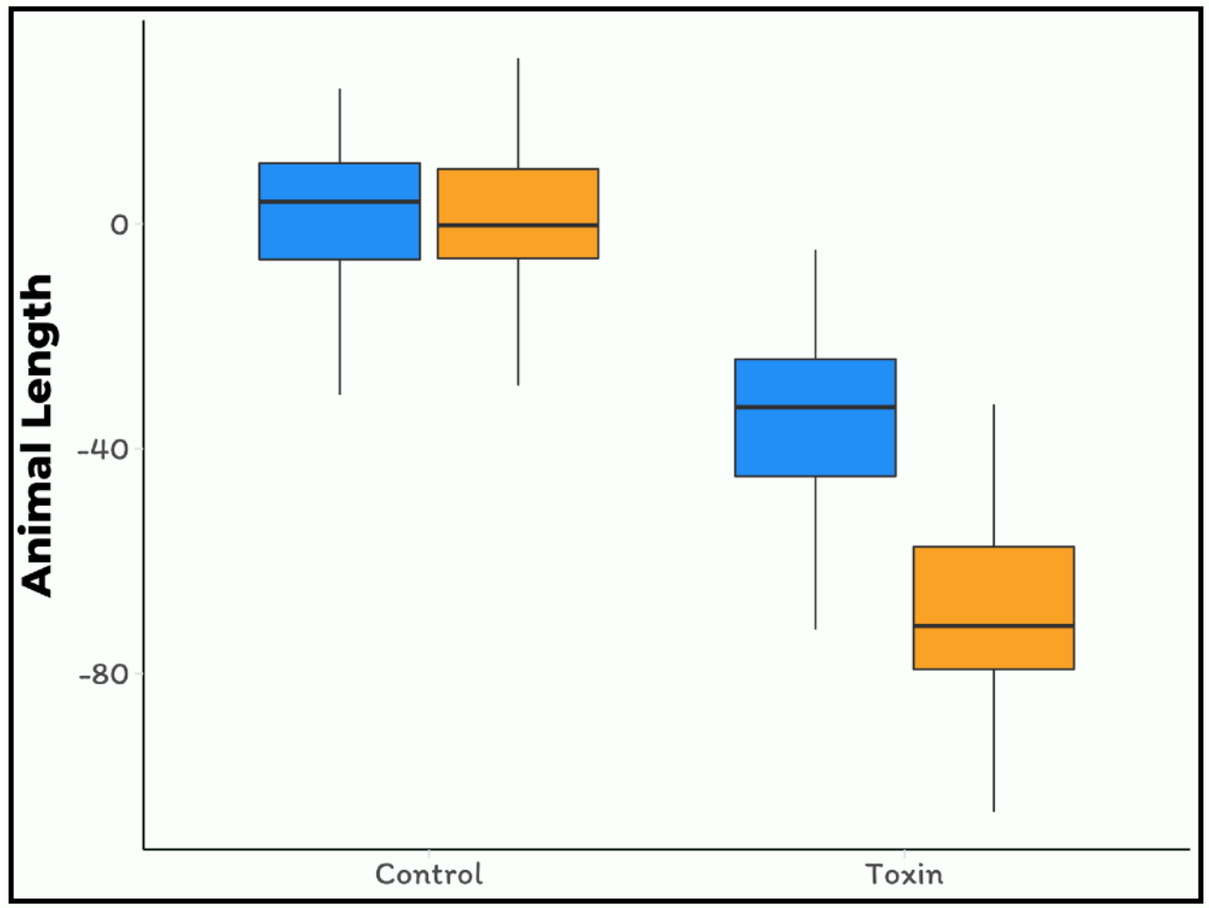
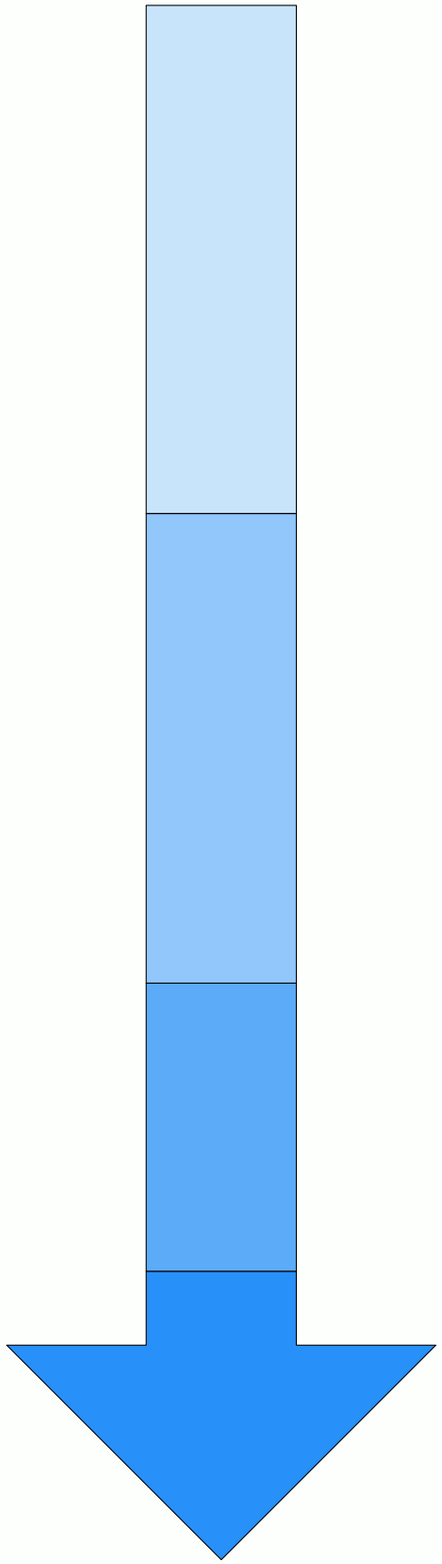
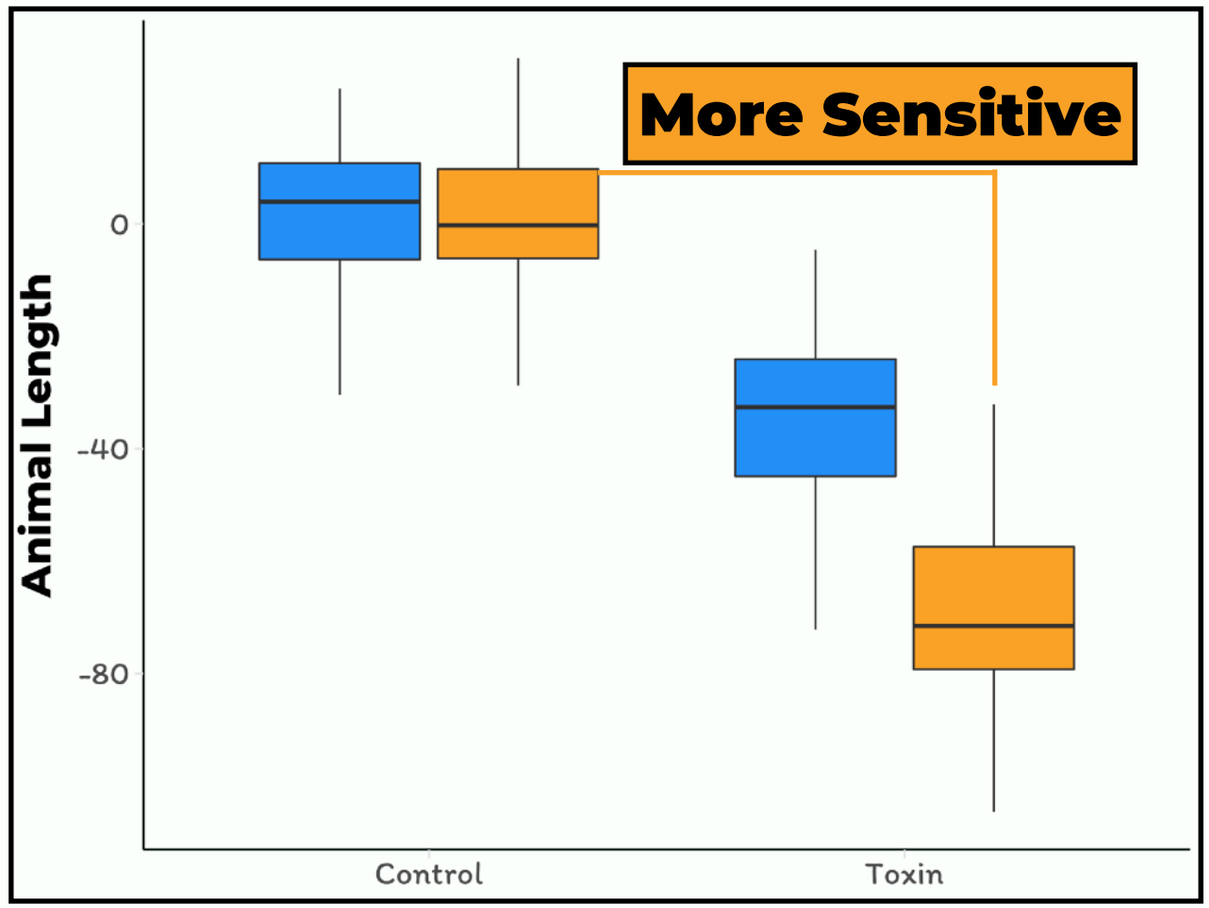


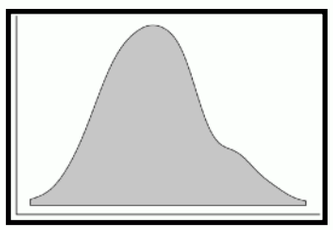


N2 CB4856

High-throughput toxin response assay

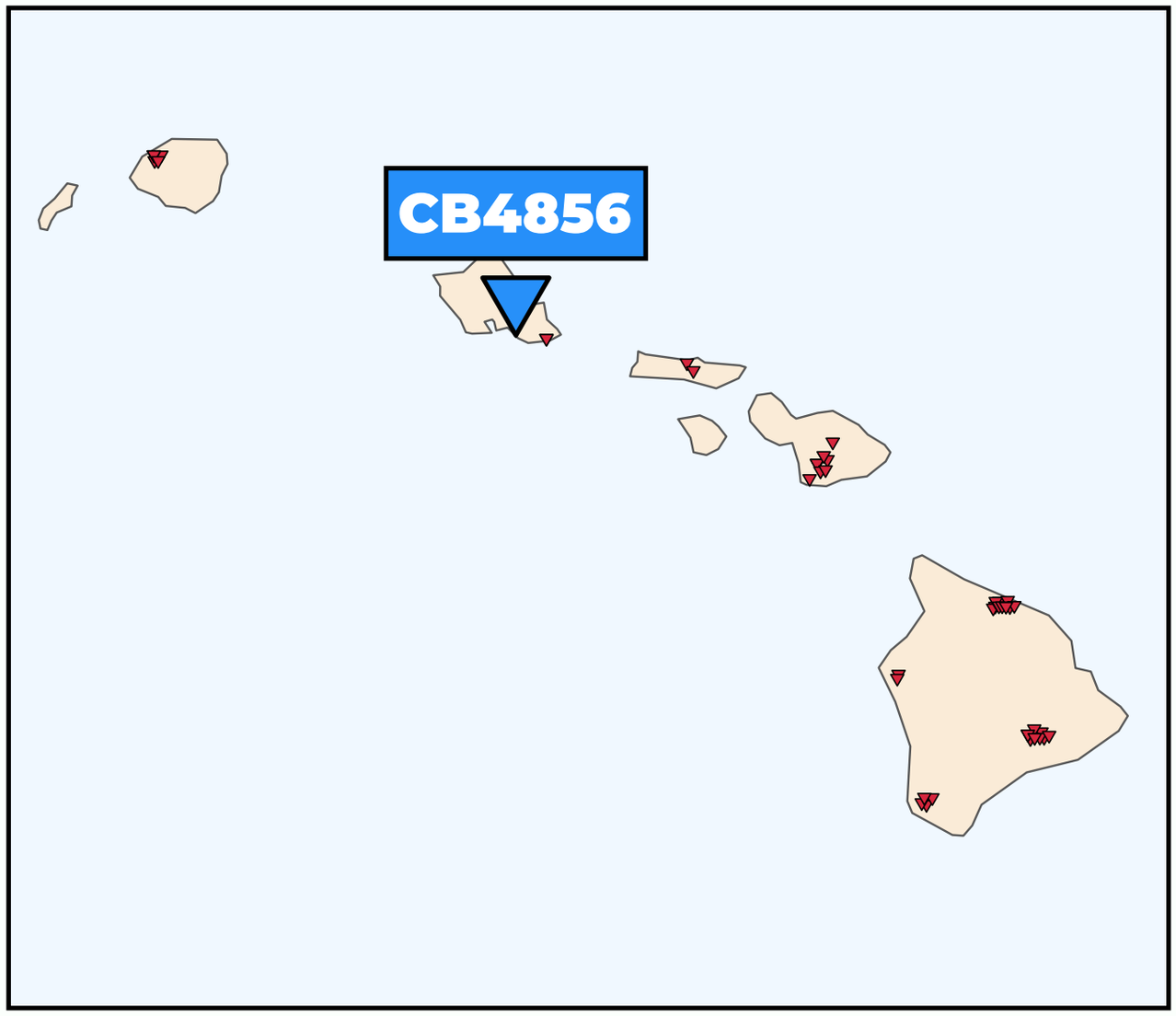


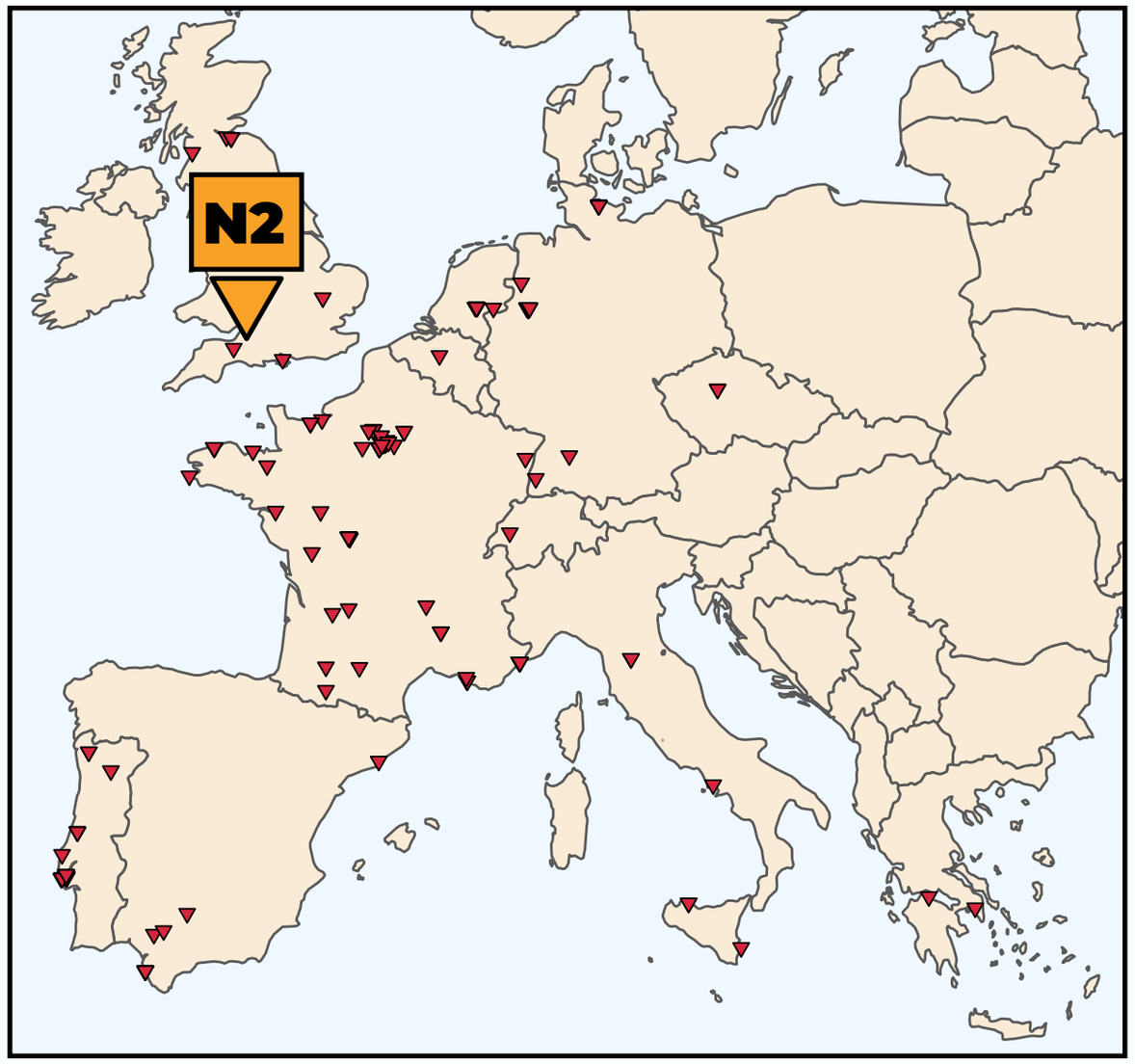




N2 CB4856

Two diverged strains were used to generate a panel of recombinant lines





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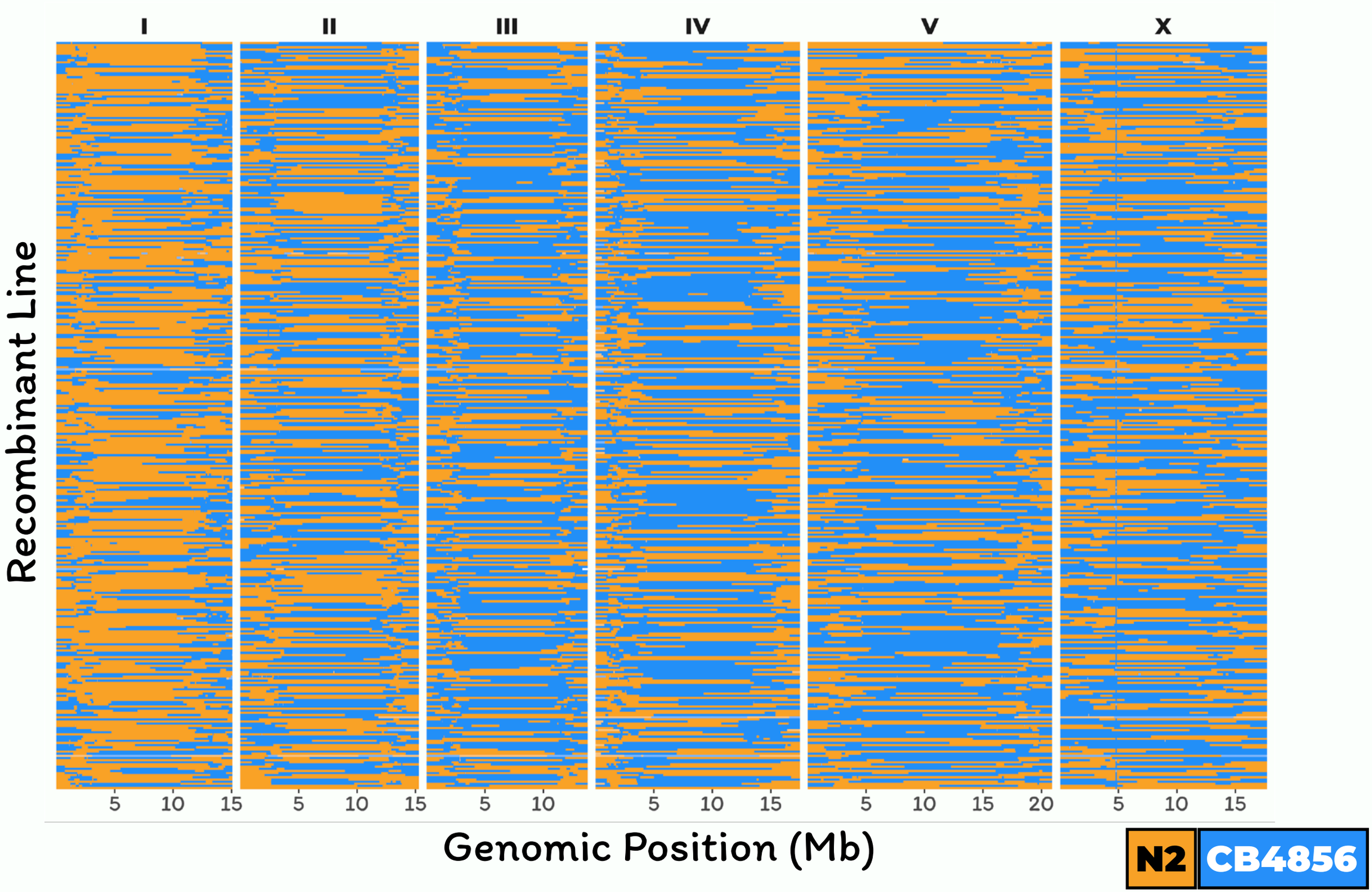
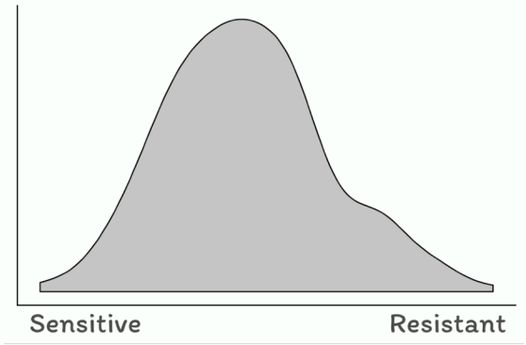
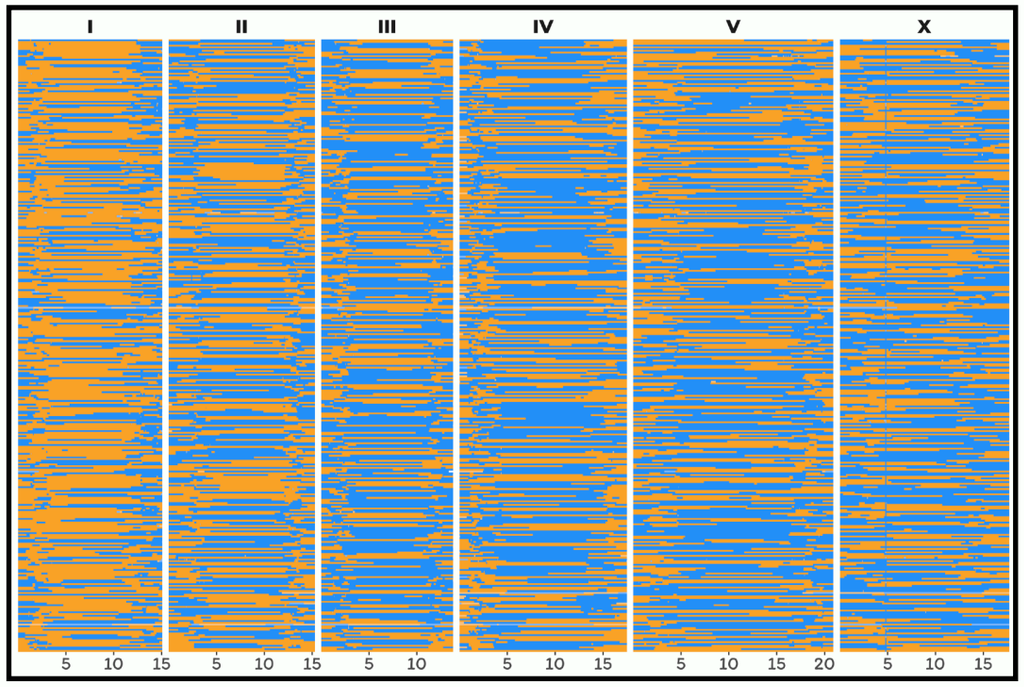
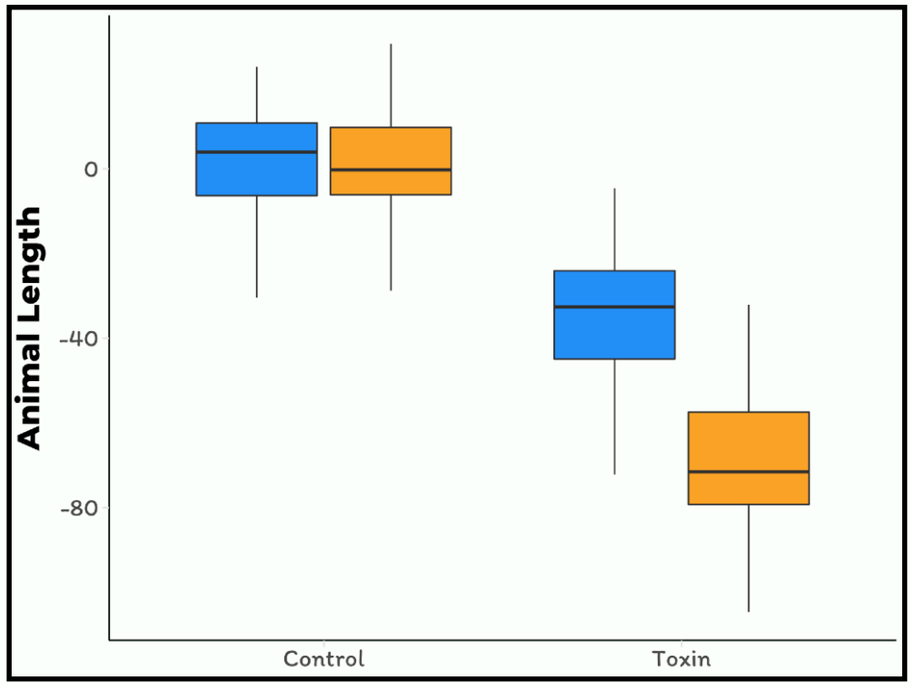


Genotype - Phenotype association



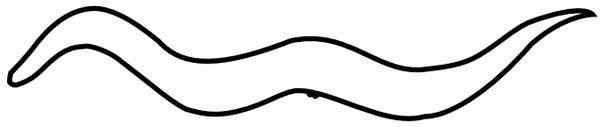
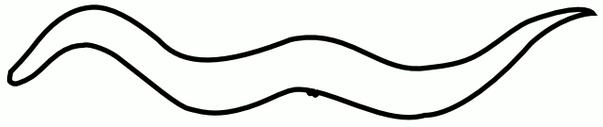
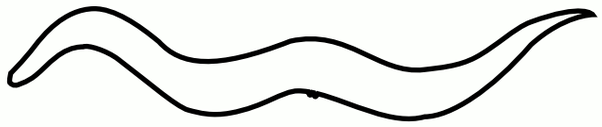




N2 CB4856

Genotype	Phenotype
ATGCAAGCCCATTATGAA	
ATGCTAGCCGATTATGGA	
ATGCAAGCCGATTATGGA	
ATGCTAGCCCATTATGGA	
ATGCTAGCCCATTATGAA	
ATGCAAGCCGATTATGAA	

N2 CB4856

Genotype	Phenotype
ATGCAAGCCCATTATGAA	
ATGCTAGCCGATTATGGA	
ATGCAAGCCGATTATGGA	
ATGCTAGCCCATTATGGA	
ATGCTAGCCCATTATGAA	
ATGCAAGCCGATTATGAA	

Genotype

ATGCAAGCCCATTATGAA

ATGCTAGCCGATTATGGA

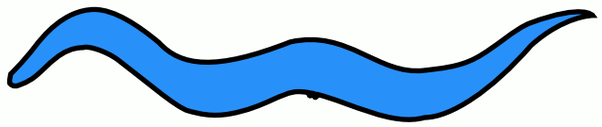
ATGCAAGCCGATTATGGA

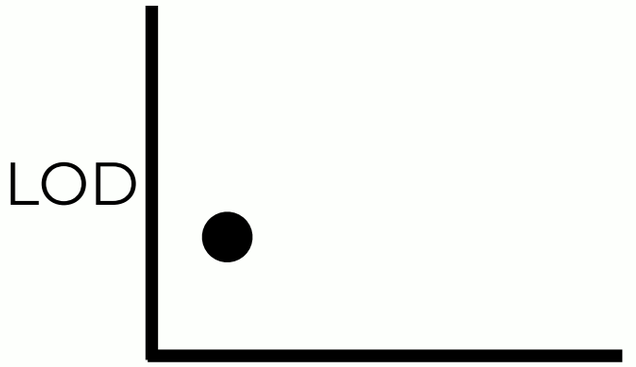
ATGCTAGCCCATTATGGA

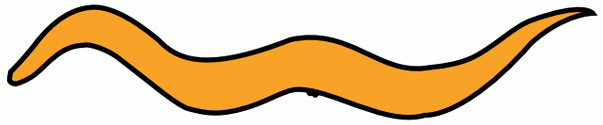
ATGCTAGCCCATTATGAA

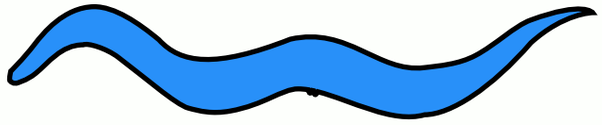
ATGCAAGCCGATTATGAA

Phenotype









N2 CB4856

Genotype

ATGCAAGCCCATTATGAA

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ATGCAAGCCGATTATGGA

ATGCTAGCCCATTATGGA

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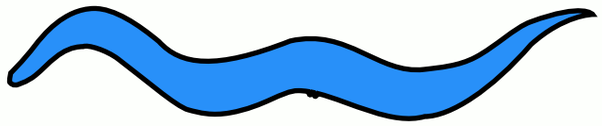
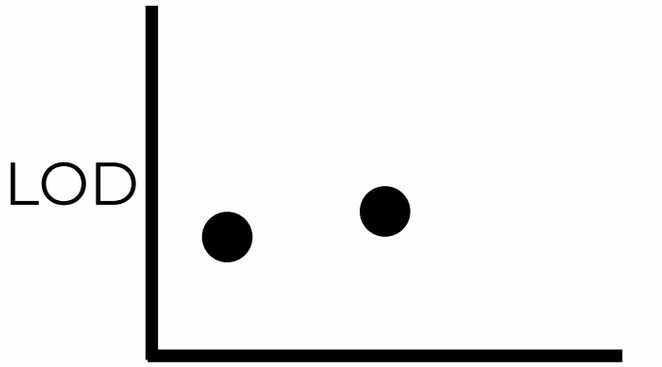
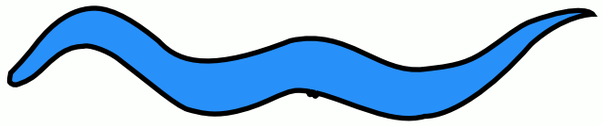
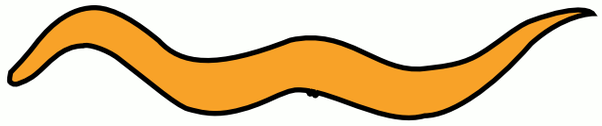
Phenotype









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ATGCAAGCCGATTATGGA

ATGCTAGCCCATTATGGA

ATGCTAGCCCATTATGAA

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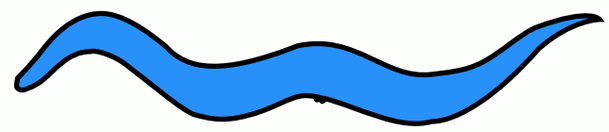
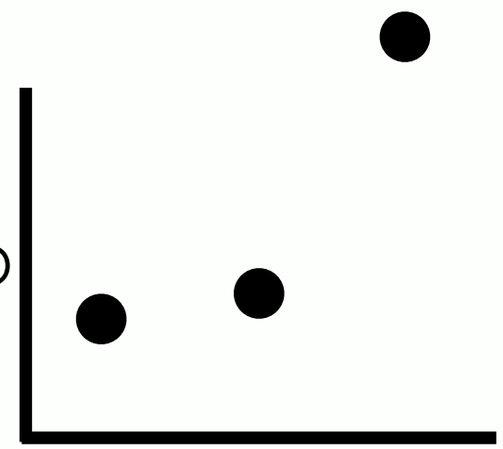
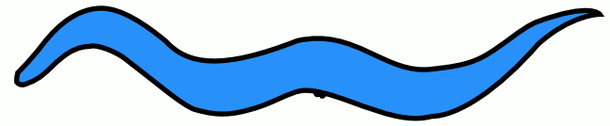
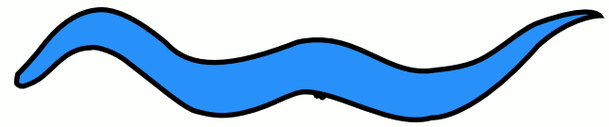








N2 CB4856

Genotype

ATGCAAGCCCATTATGAA

ATGCTAGCCGATTATGGA

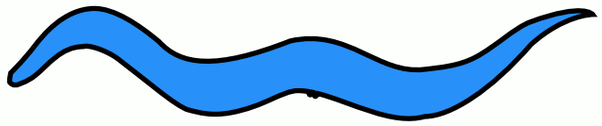
ATGCAAGCCGATTATGGA

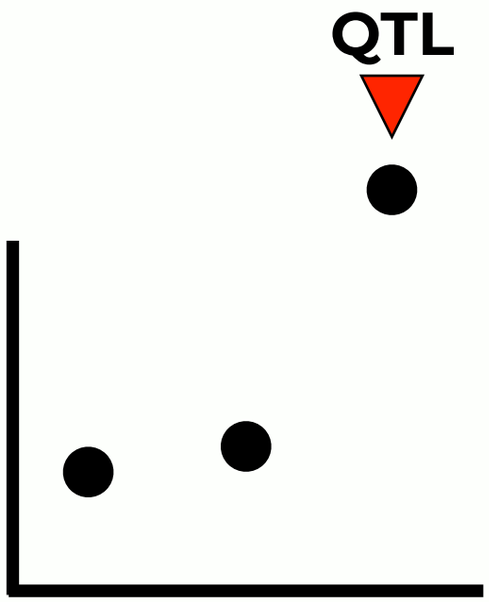
ATGCTAGCCCATTATGGA

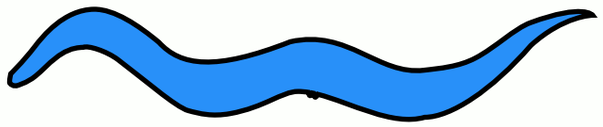
ATGCTAGCCCATTATGAA

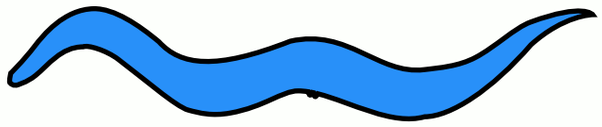
ATGCAAGCCGATTATGAA

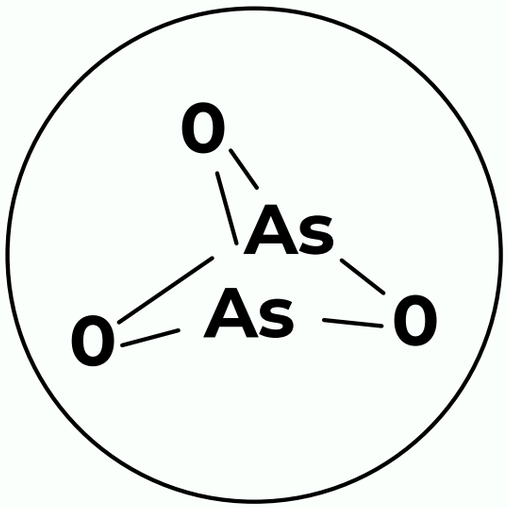
Phenotype



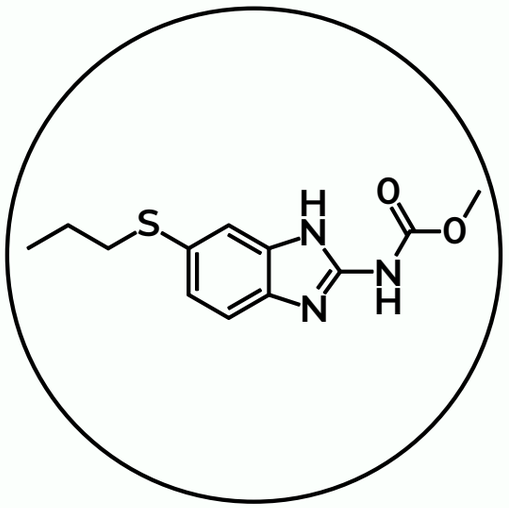






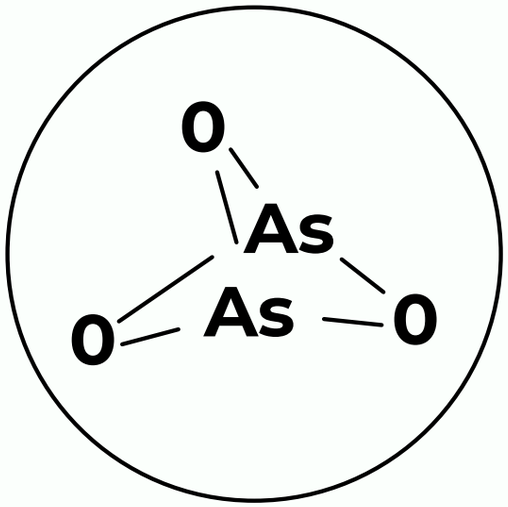


Arsenic



Albendazole

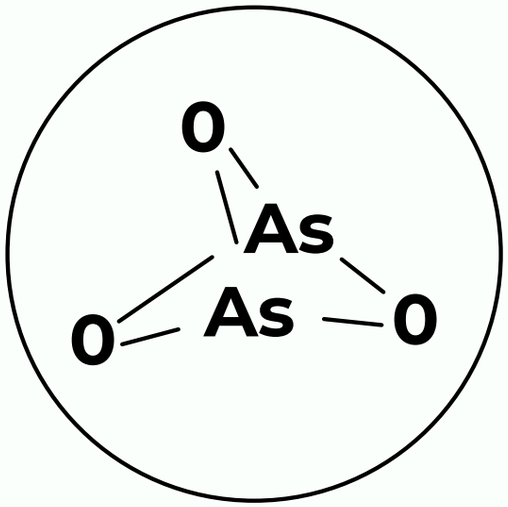
Arsenic is a pervasive toxin with pleiotropic cellular effects



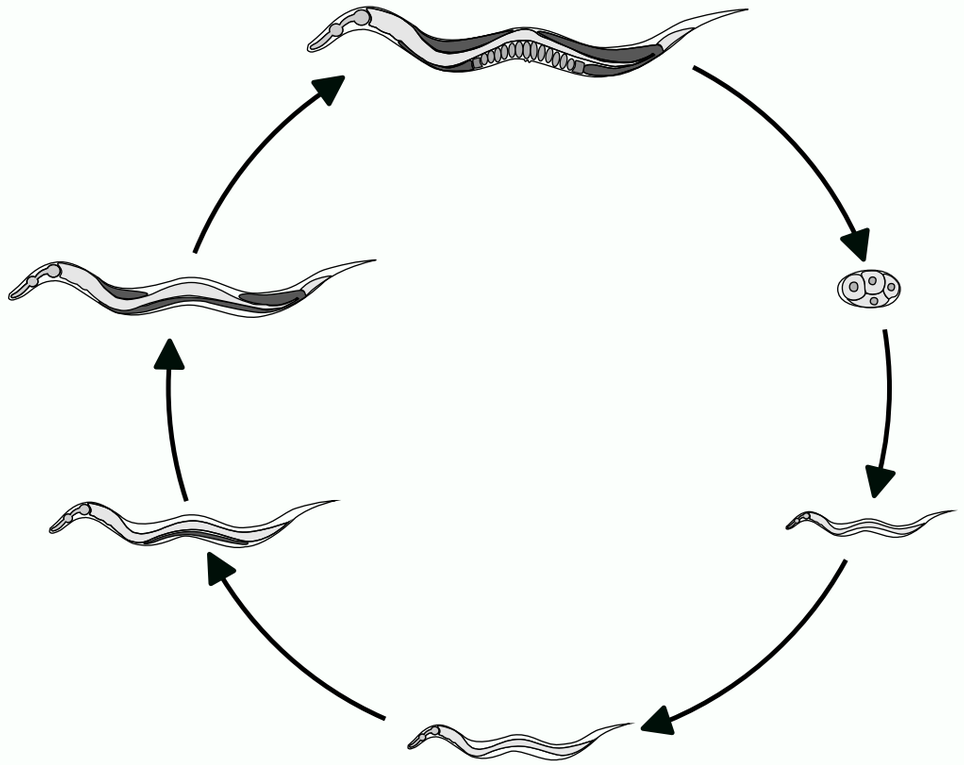
Arsenic

- ~100 million people are at risk of exposure
- ~200 enzymes are inhibited
- 66 clinical completed or active clinical trials

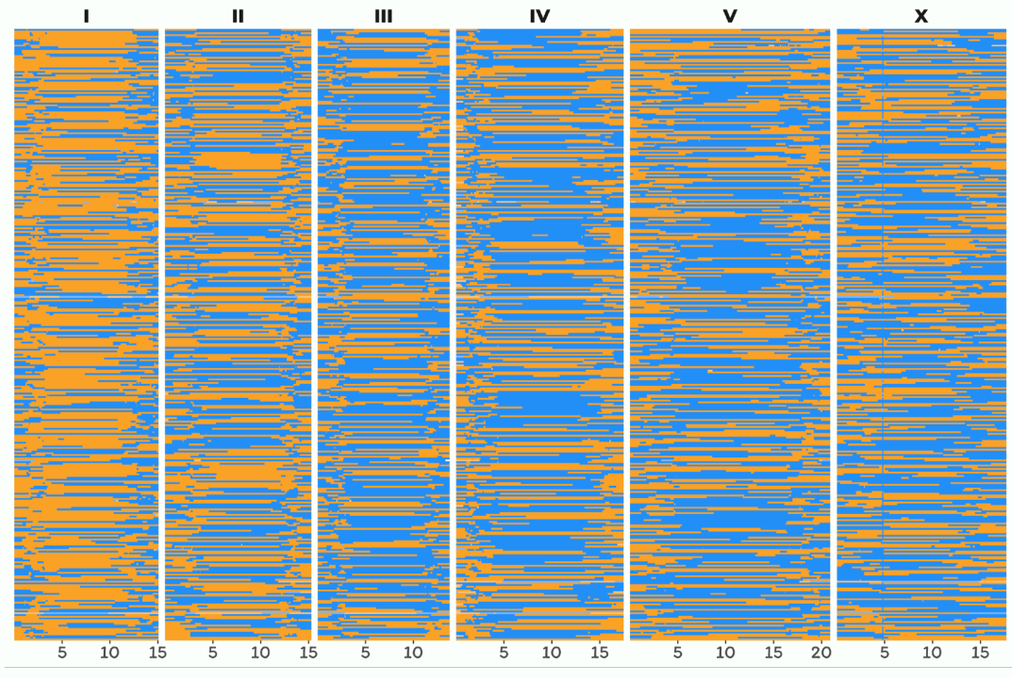
Developmental delay in *C. elegans* is a proxy for arsenic toxicity in humans

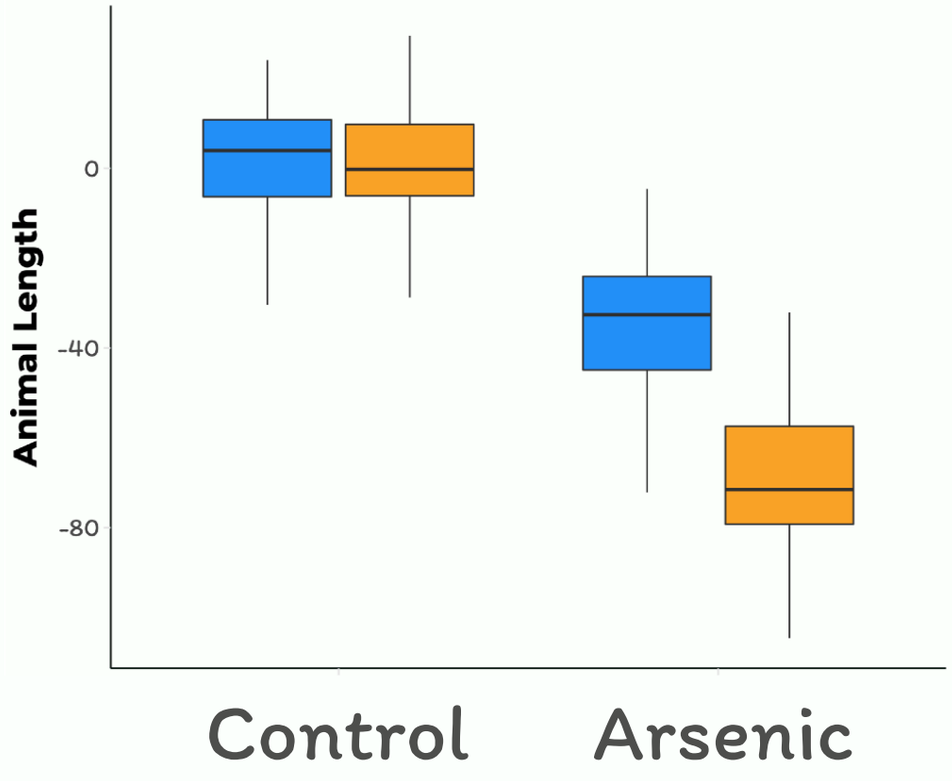


Arsenic



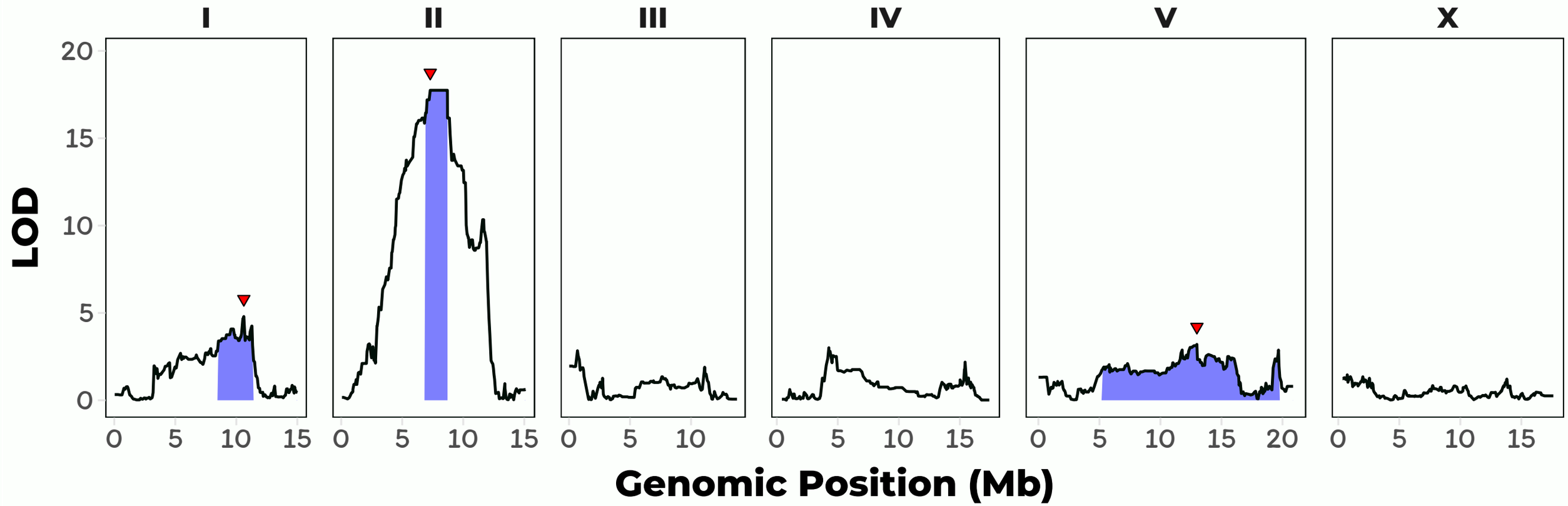
N2 and CB4856 are differentially affected by arsenic

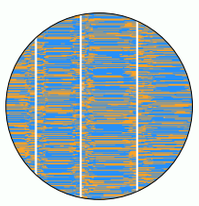




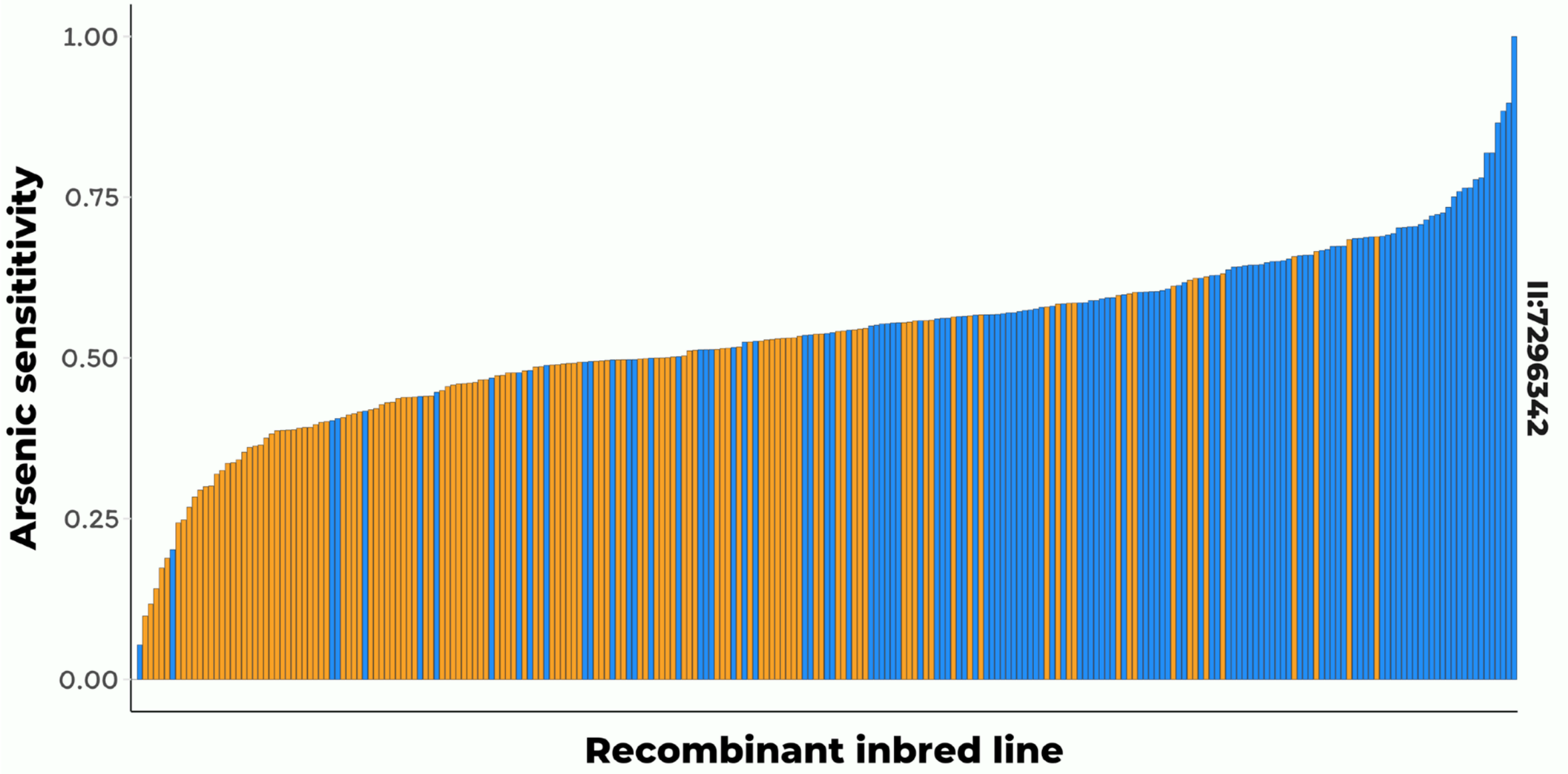
N2 CB4856

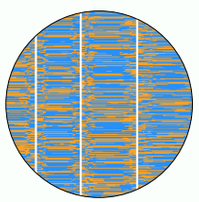
A large-effect QTL explains variable arsenic responses among recombinant lines





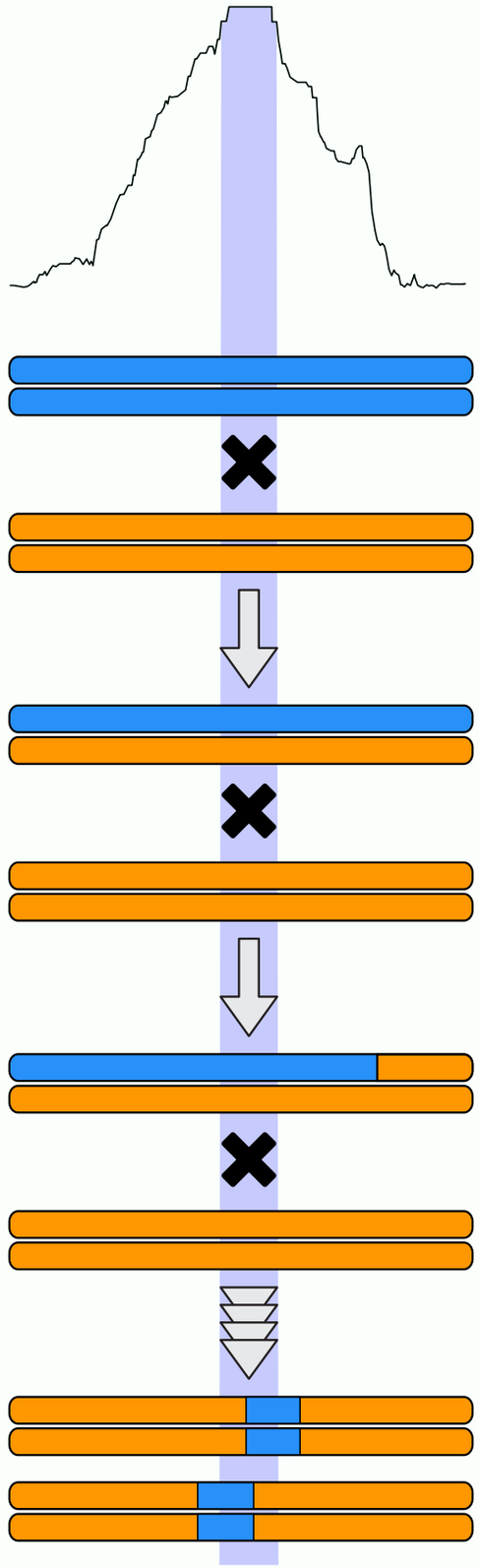
The CB4856 genotype confers arsenic resistance





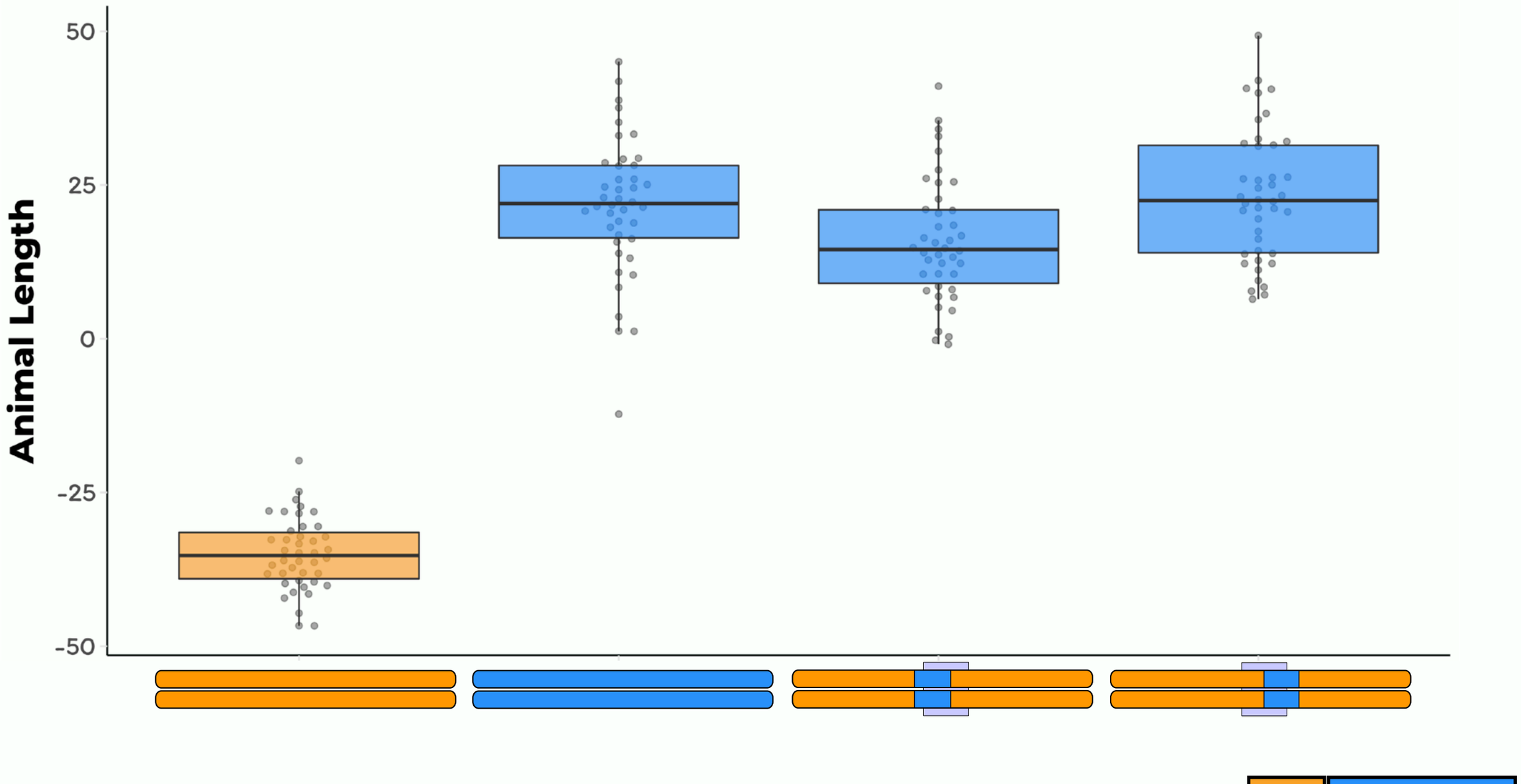
N2 CB4856

Construction of near-isogenic lines

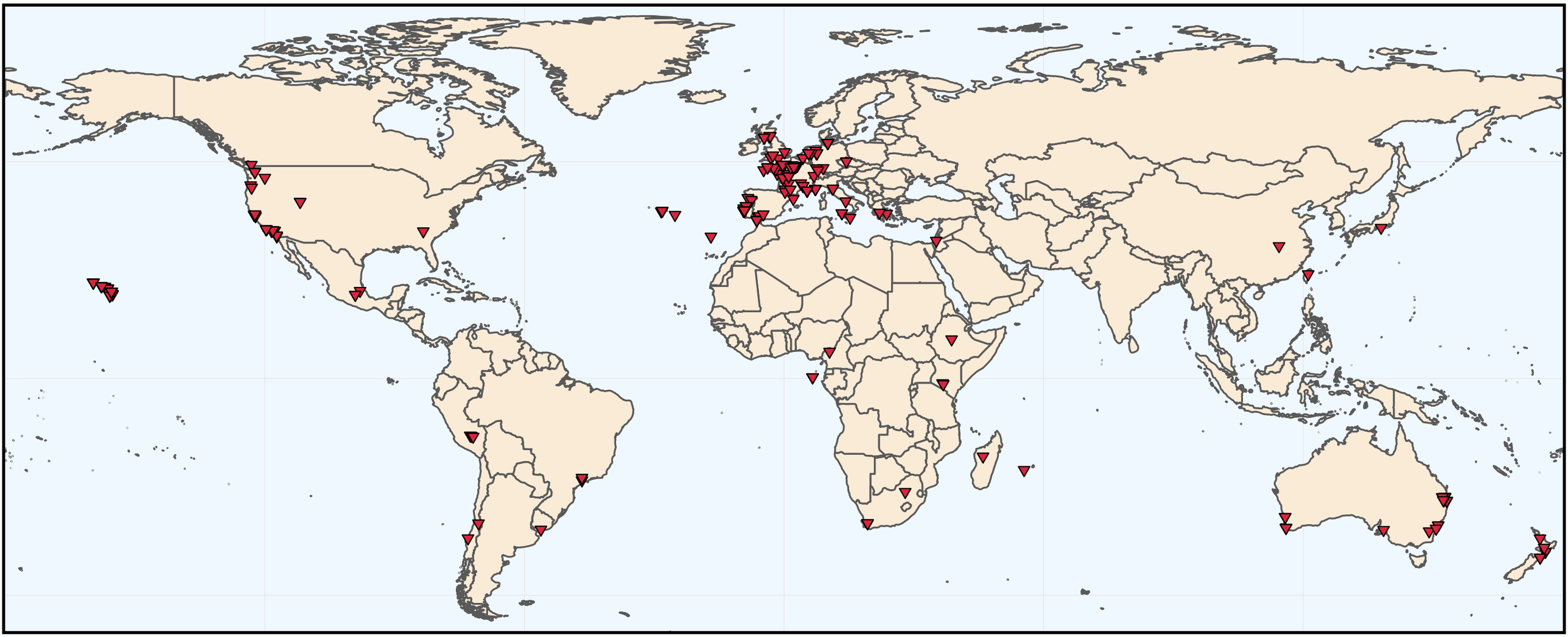


N2 CB4856

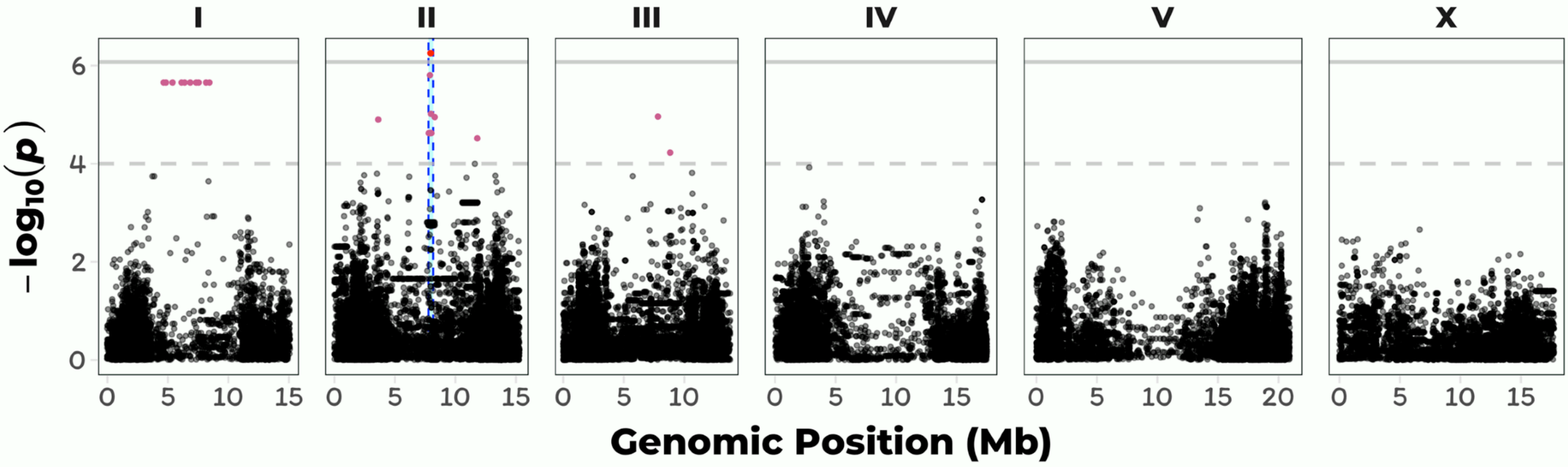
Two near-isogenic lines isolate the arsenic QTL confidence interval



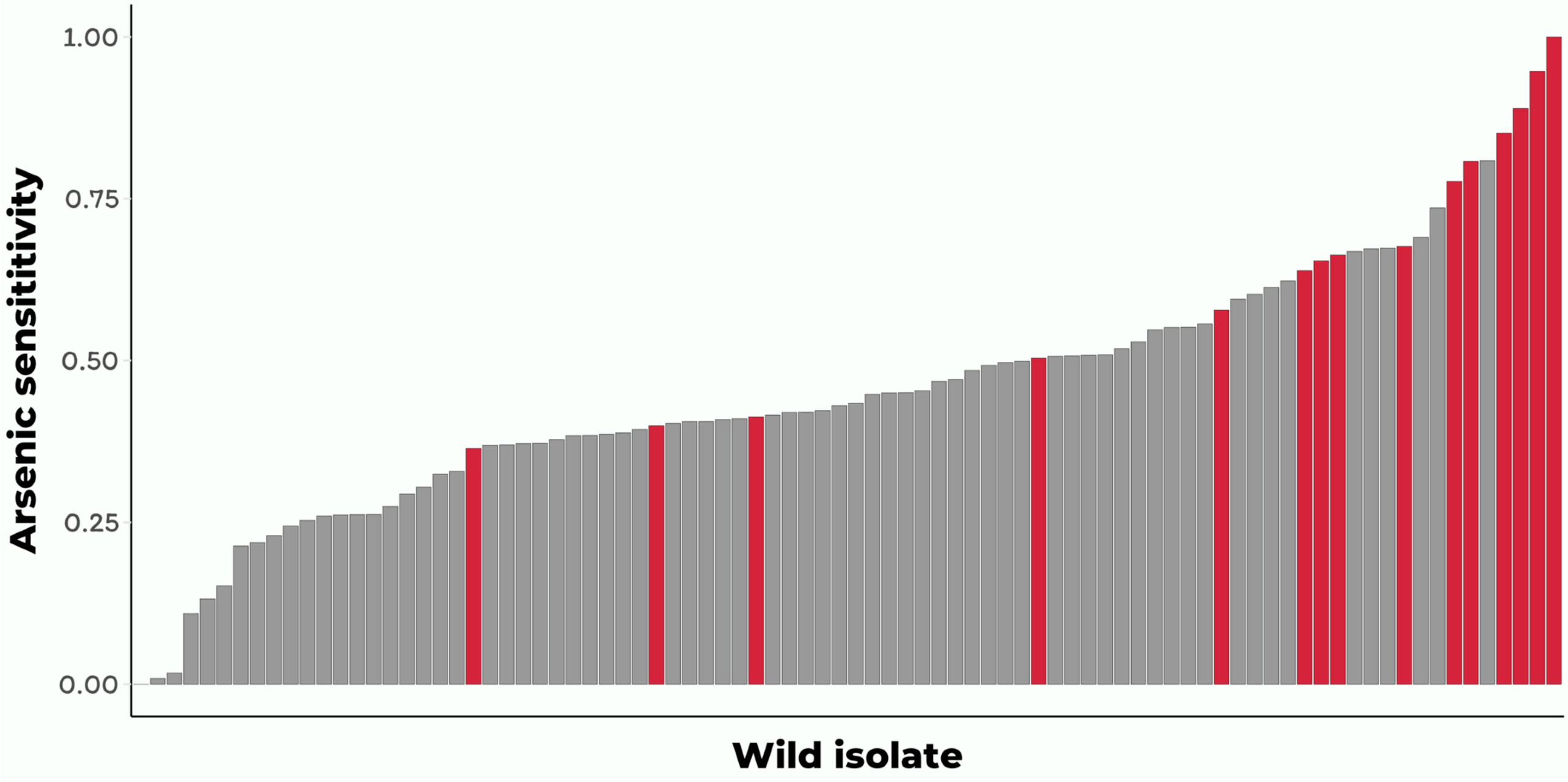
We simultaneously exposed a panel of wild *C. elegans* strains to arsenic



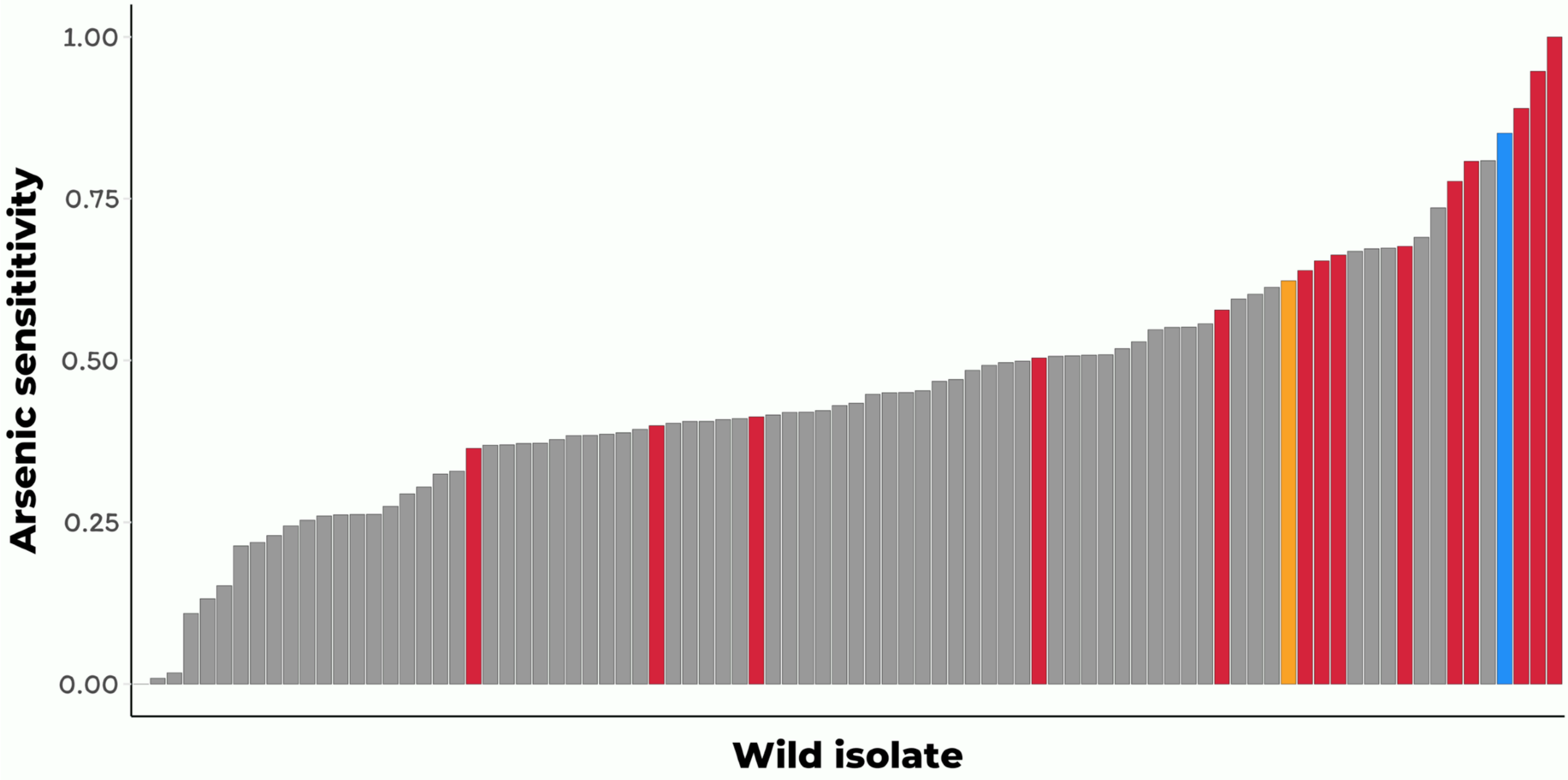
An overlapping QTL explains variable arsenic responses among 86 wild isolates



Strains with the alternate allele at the peak marker are resistant to arsenic

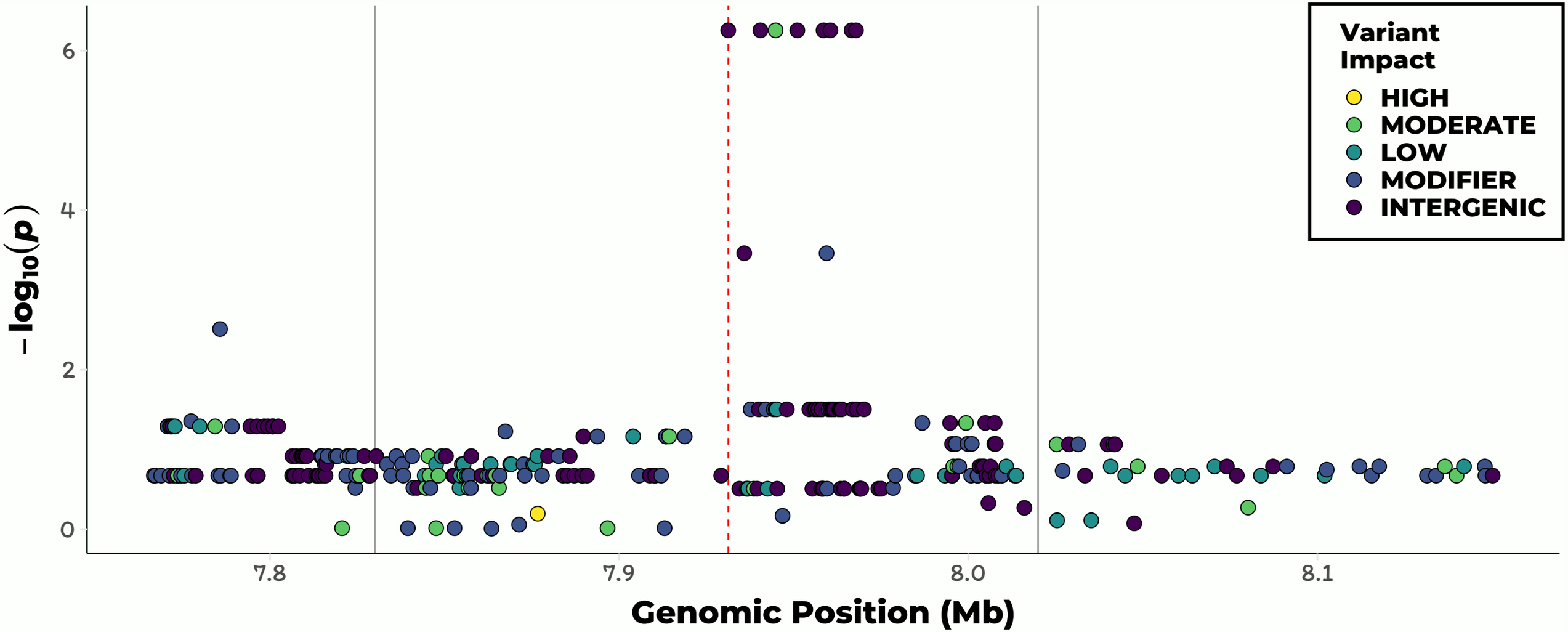


CB4856 has the alternate genotype at the QTL peak marker

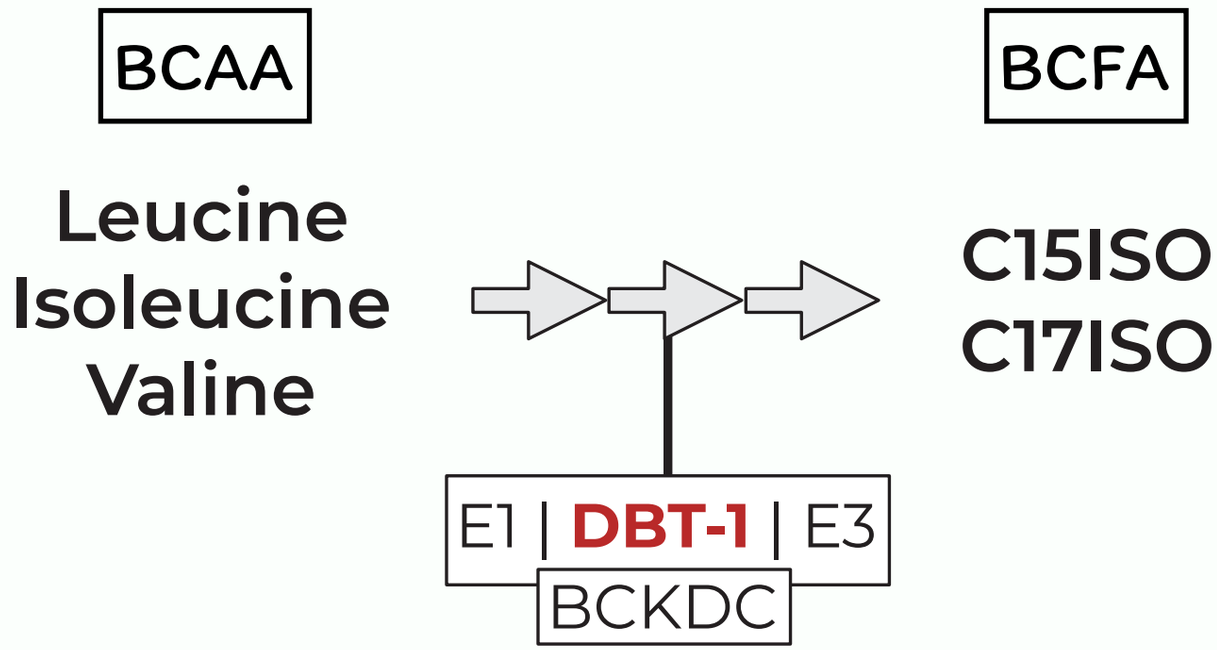


N2 CB4856

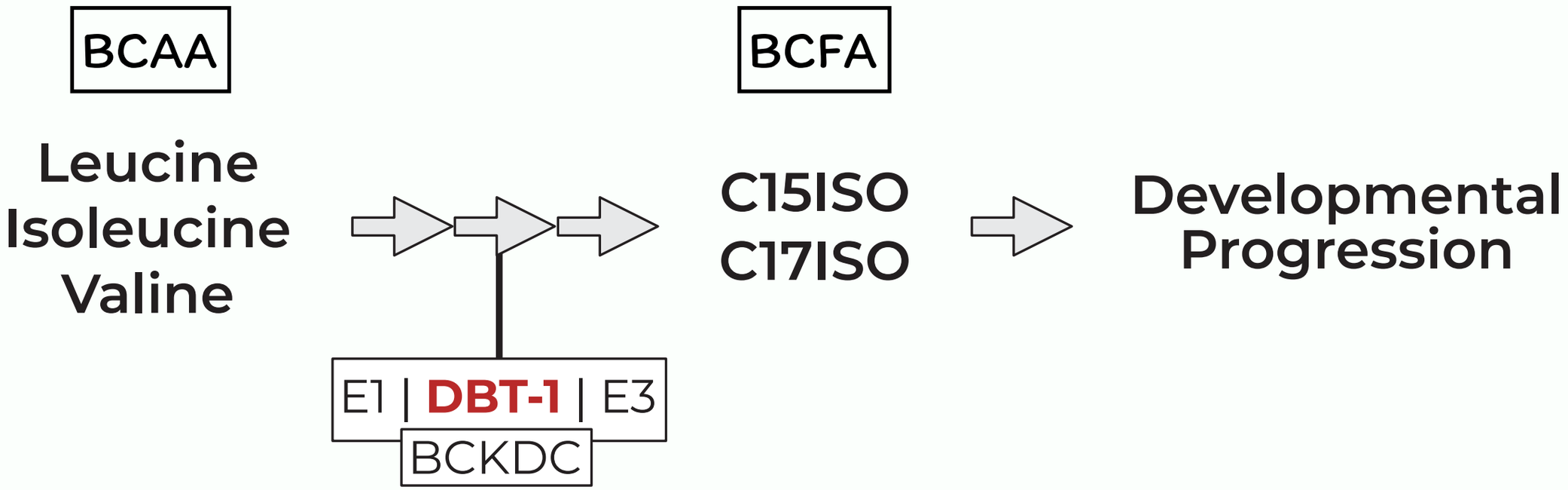
Fine mapping of GWA QTL identifies a candidate variant in the *dbt-1* gene



dbt-1 encodes the E2 domain of the branched-chain α-ketoacid dehydrogenase complex

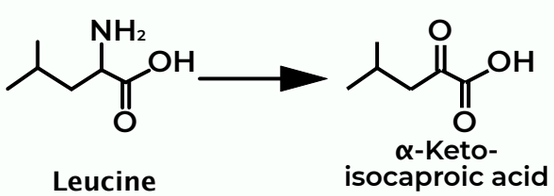


Branched-chain fatty acids are required for developmental progression



Jia et. al. 2015, JBC

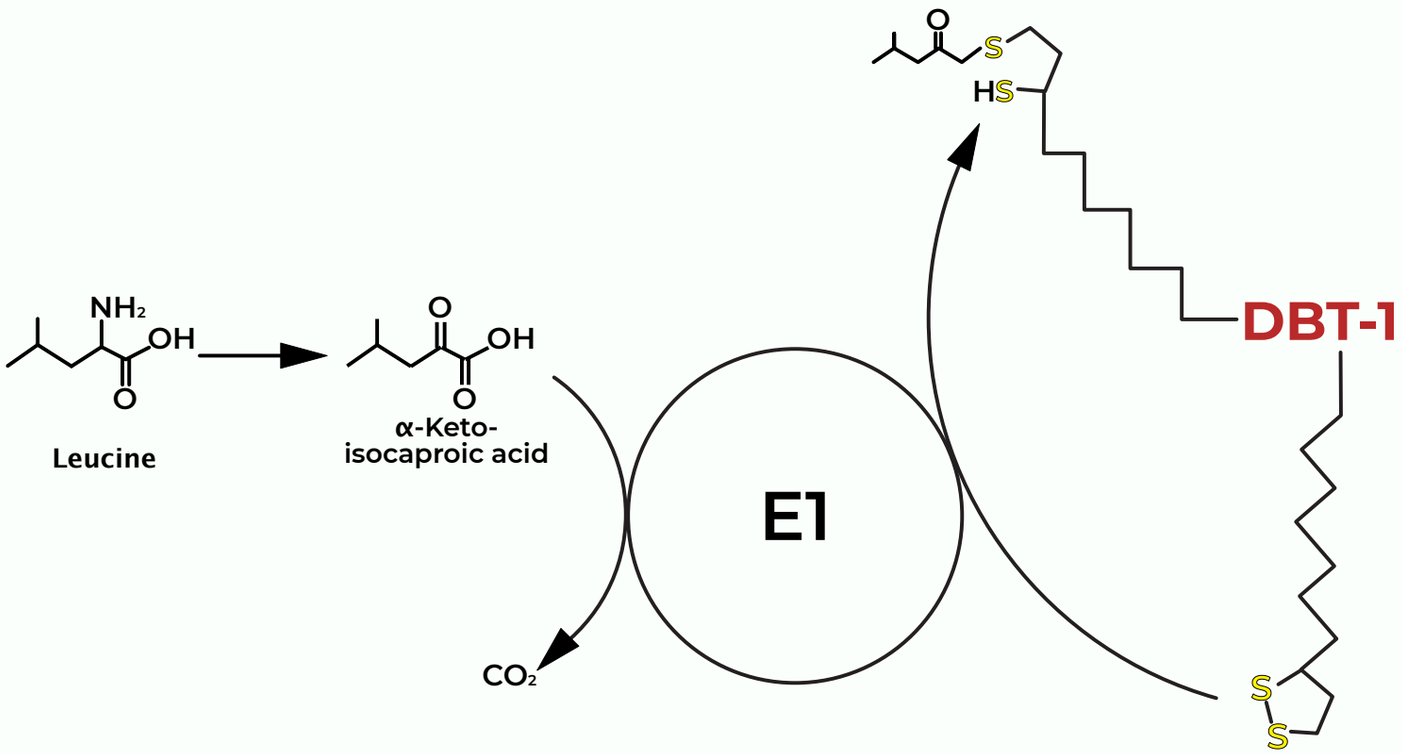
DBT-1 catalyzes the oxidative decarboxylation of amino-acid derived branched-chain α-ketoacids



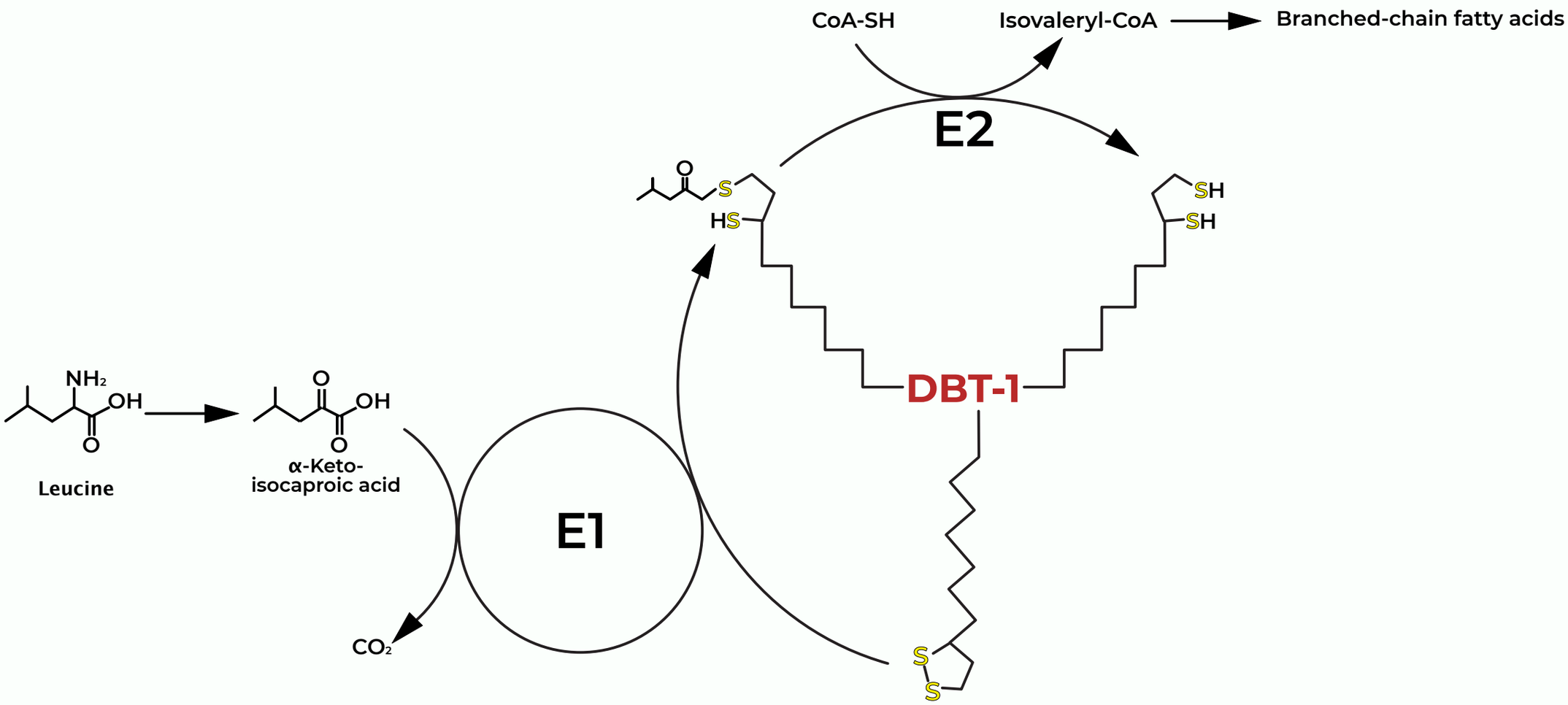
Leucine

α-Keto-
isocaproic acid

DBT-1 catalyzes the oxidative decarboxylation of amino-acid derived branched-chain α-ketoacids



DBT-1 catalyzes the oxidative decarboxylation of amino-acid derived branched-chain α-ketoacids



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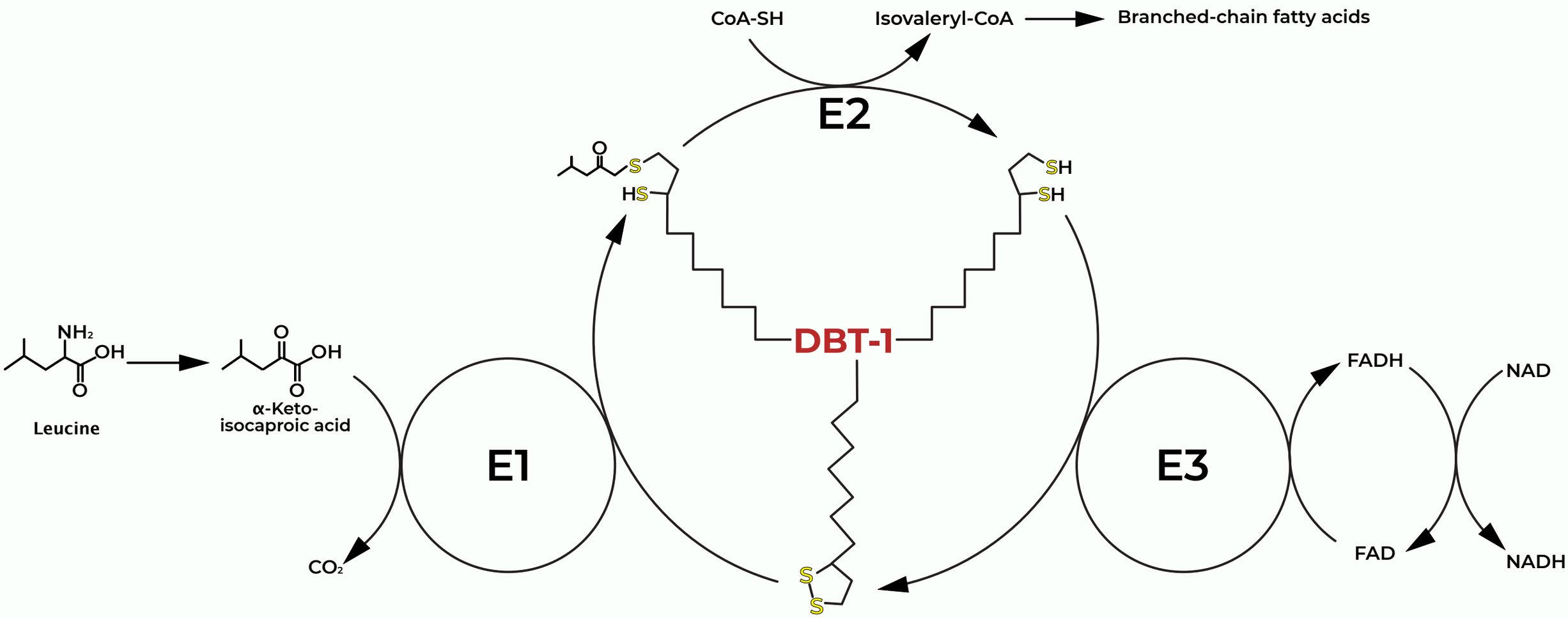
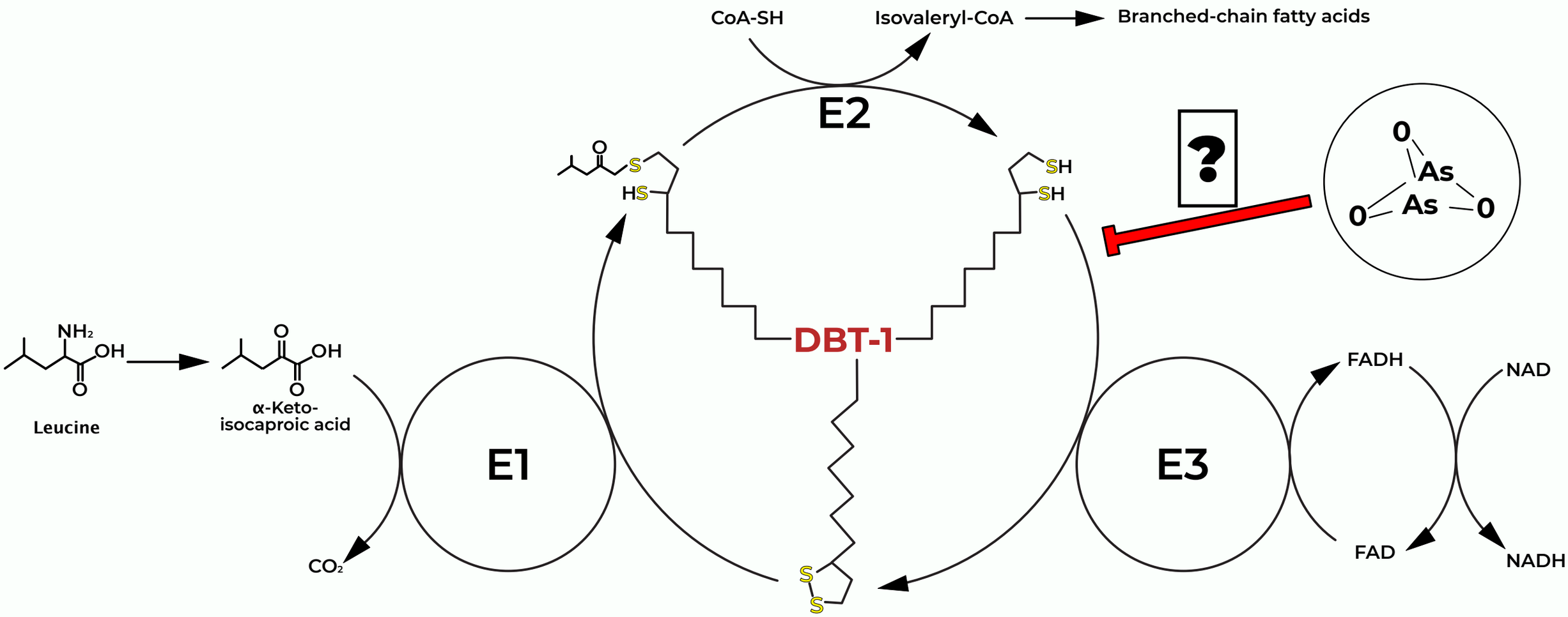
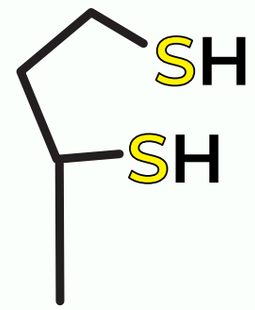


Arsenic has been shown to inhibit other α-ketoacid dehydrogenase complex

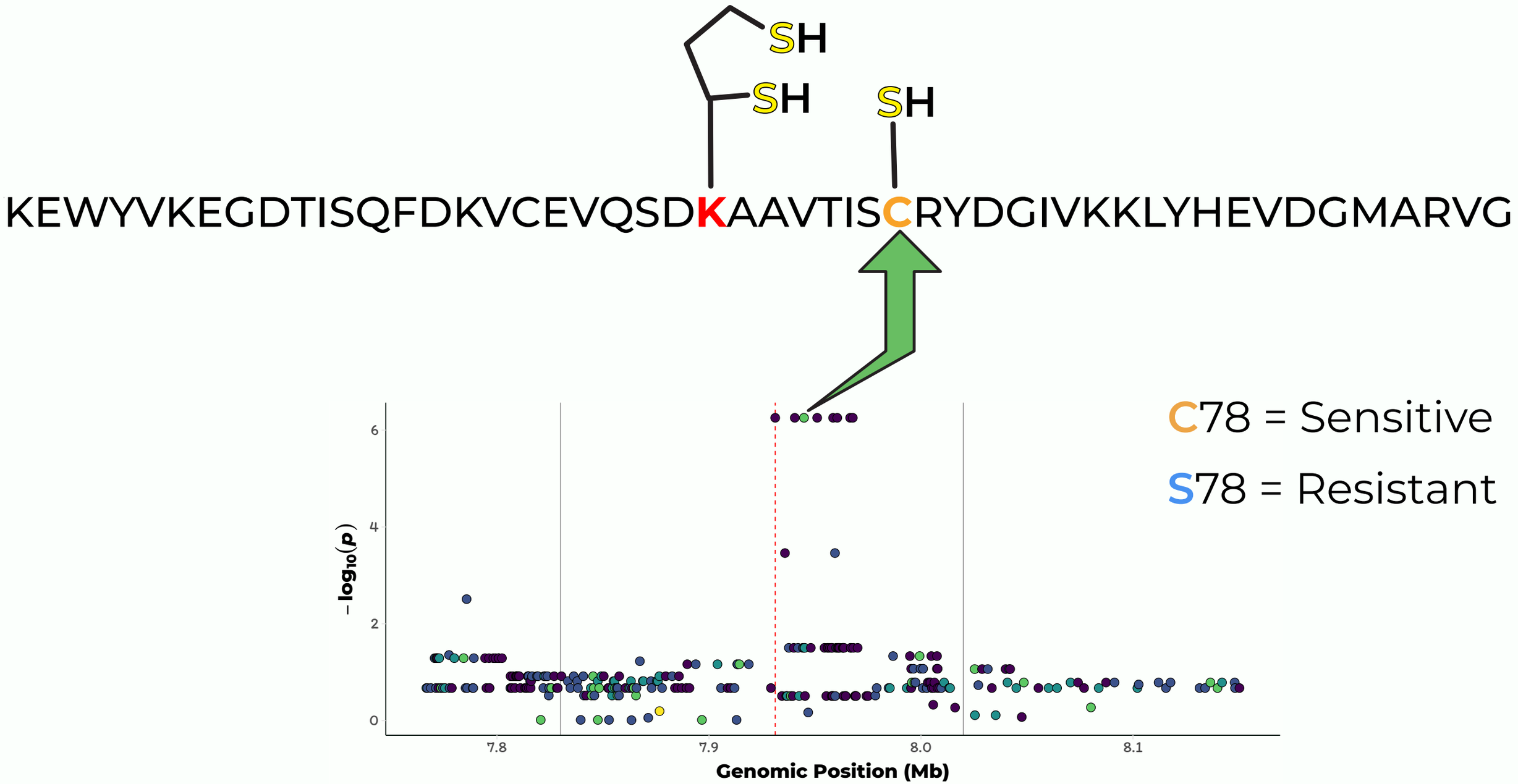


The C78S variant is in lipoyl domain of DBT-1



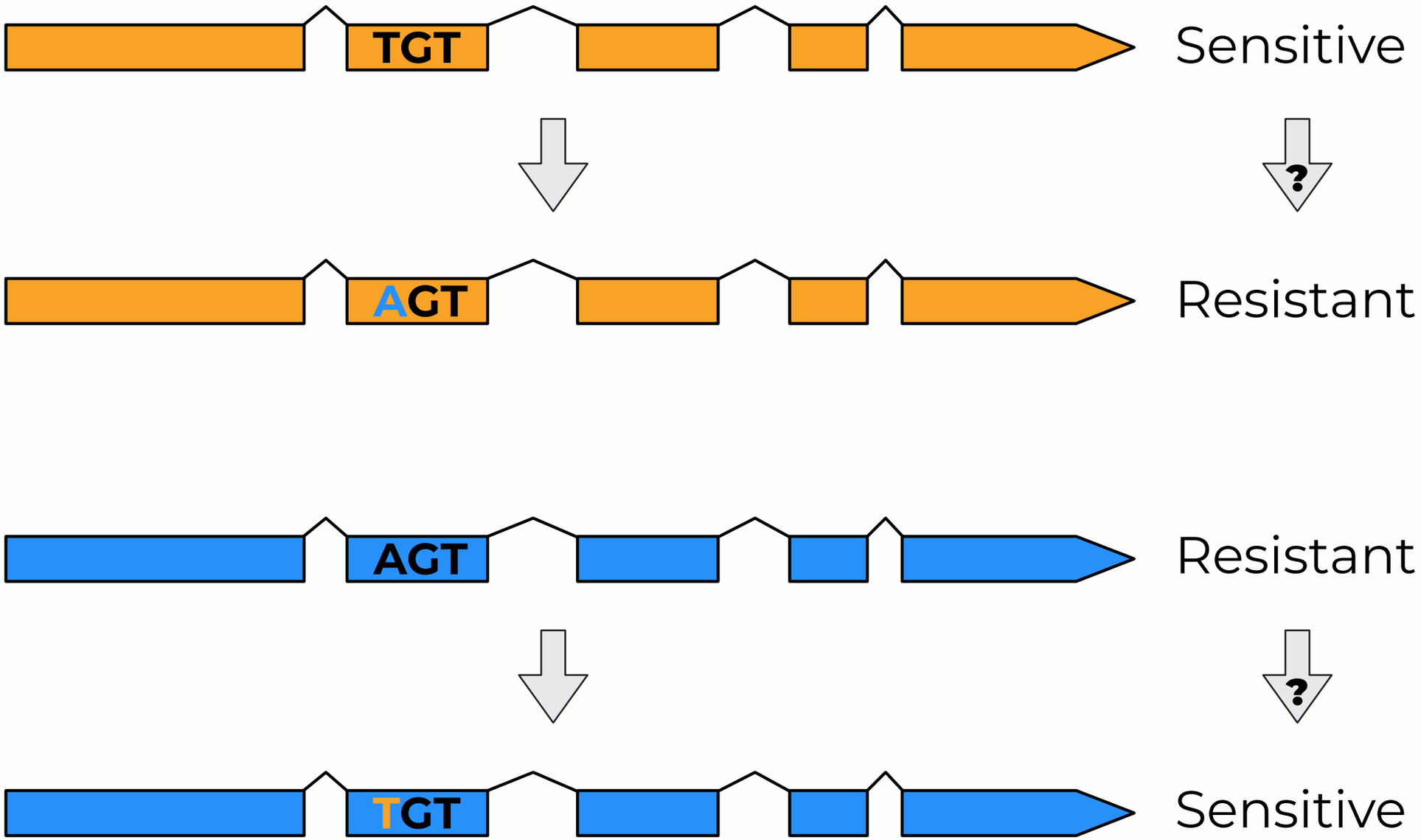
KEWYVKEGDTISQFDKVCEVQSDKAAVTISCRYDGIVKKLYHEVDGMARVG

Three thiol groups coordinate arsenic binding

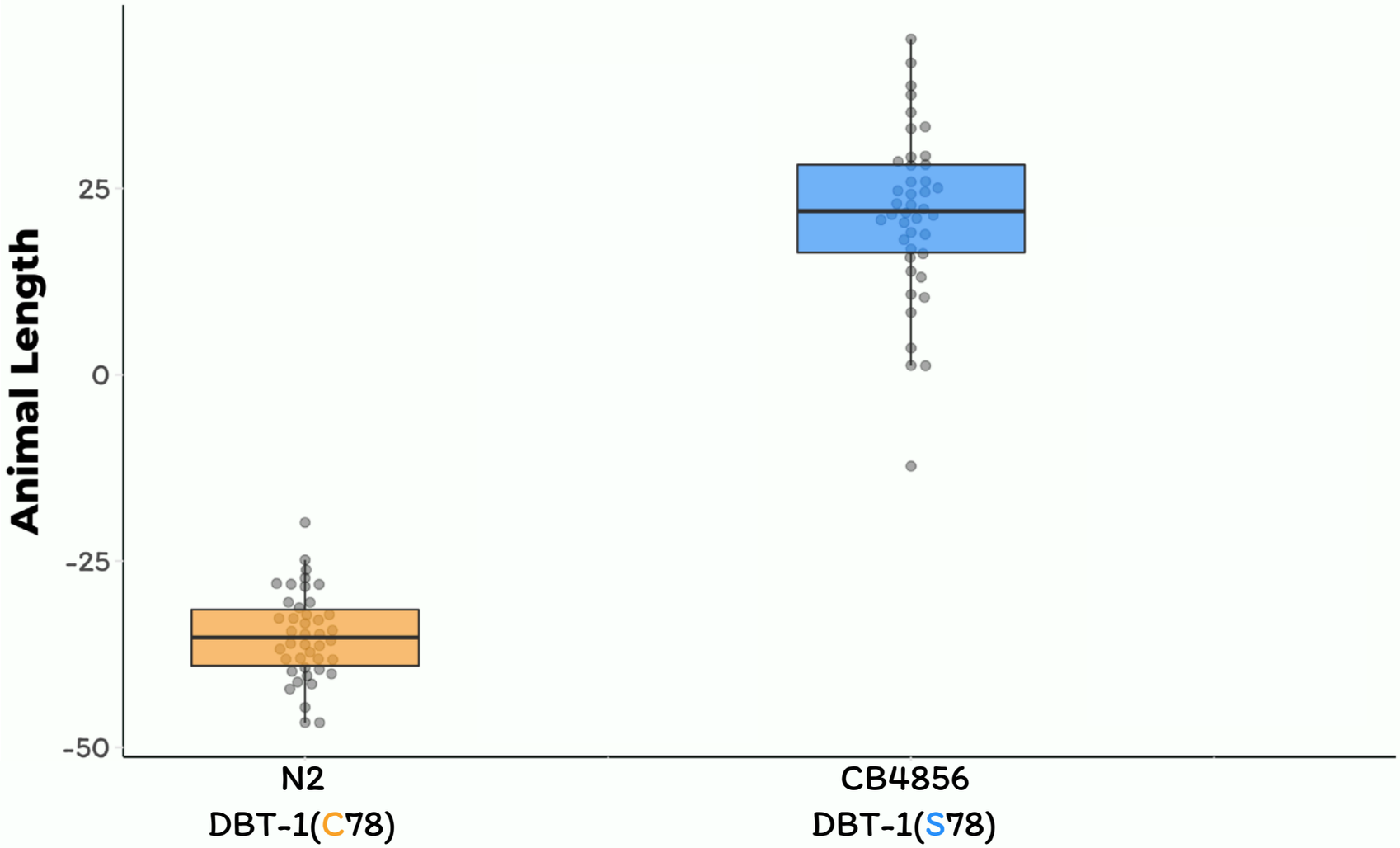


Shen et. al. 2013, Chem. Rev.

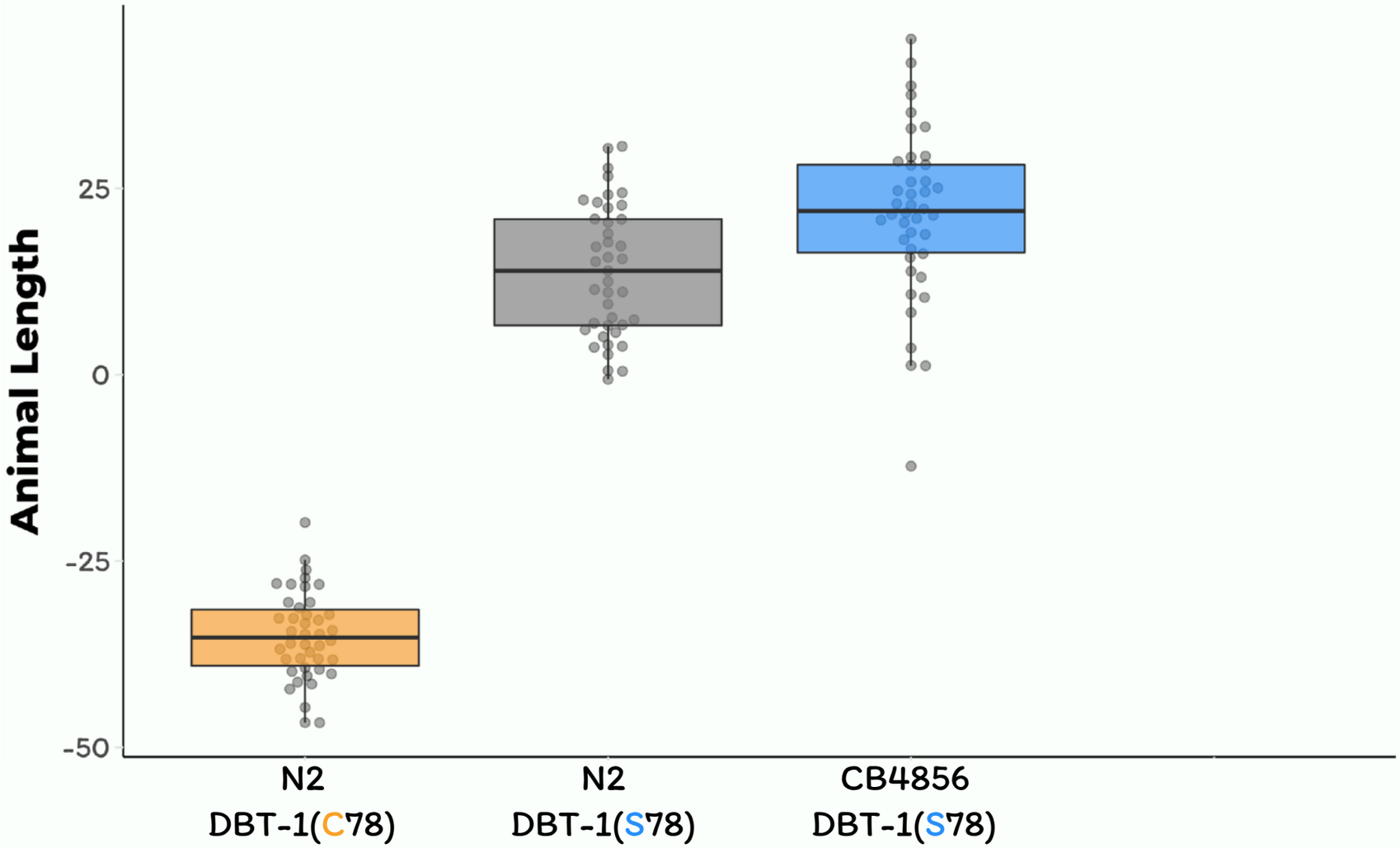
Testing the effect of the C78S variant with CRISPR/Cas9 allele-replacements



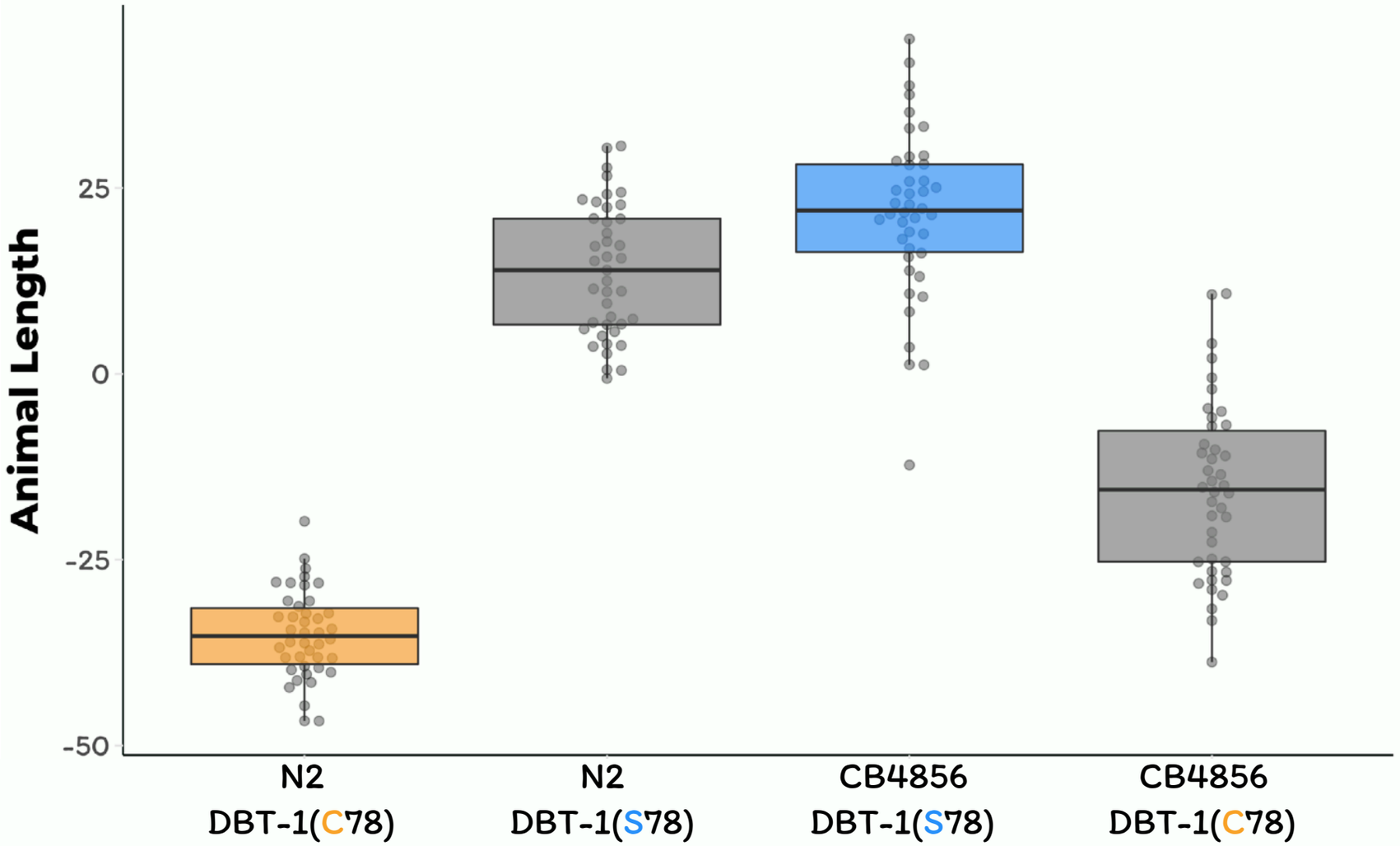
The DBT-1(C78) allele confers arsenic sensitivity



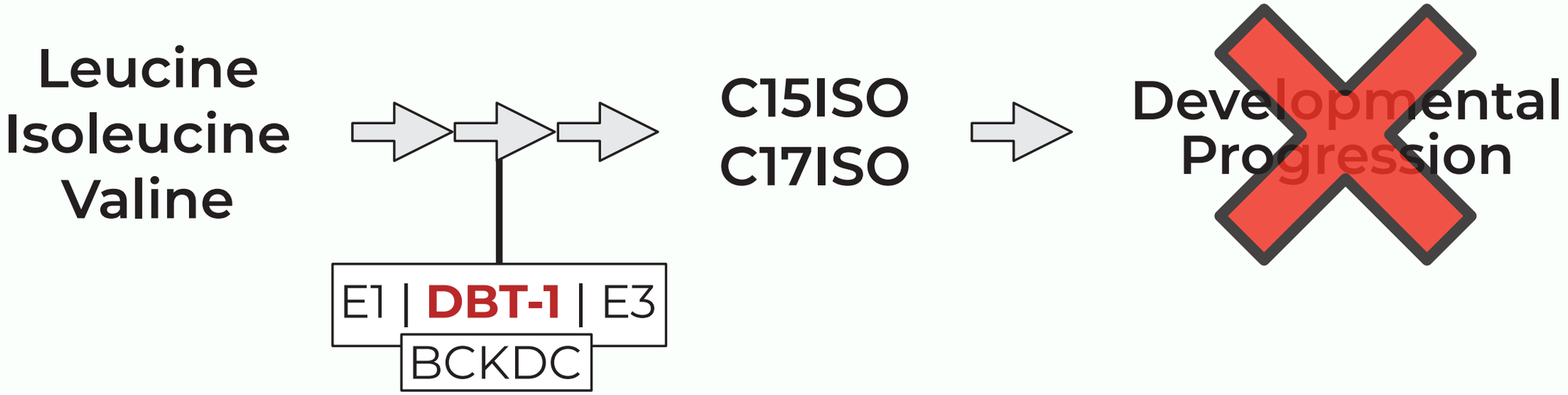
The DBT-1(C78) allele confers arsenic sensitivity



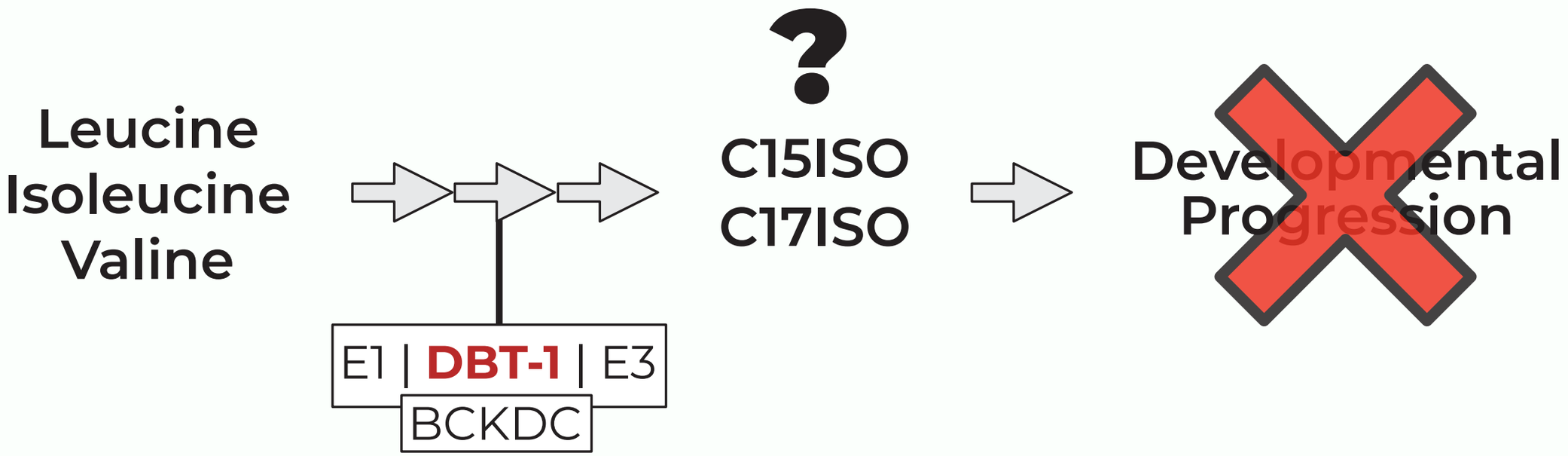
The DBT-1(C78) allele confers arsenic sensitivity



DBT-1 allele-specific developmental delay in the presence of arsenic

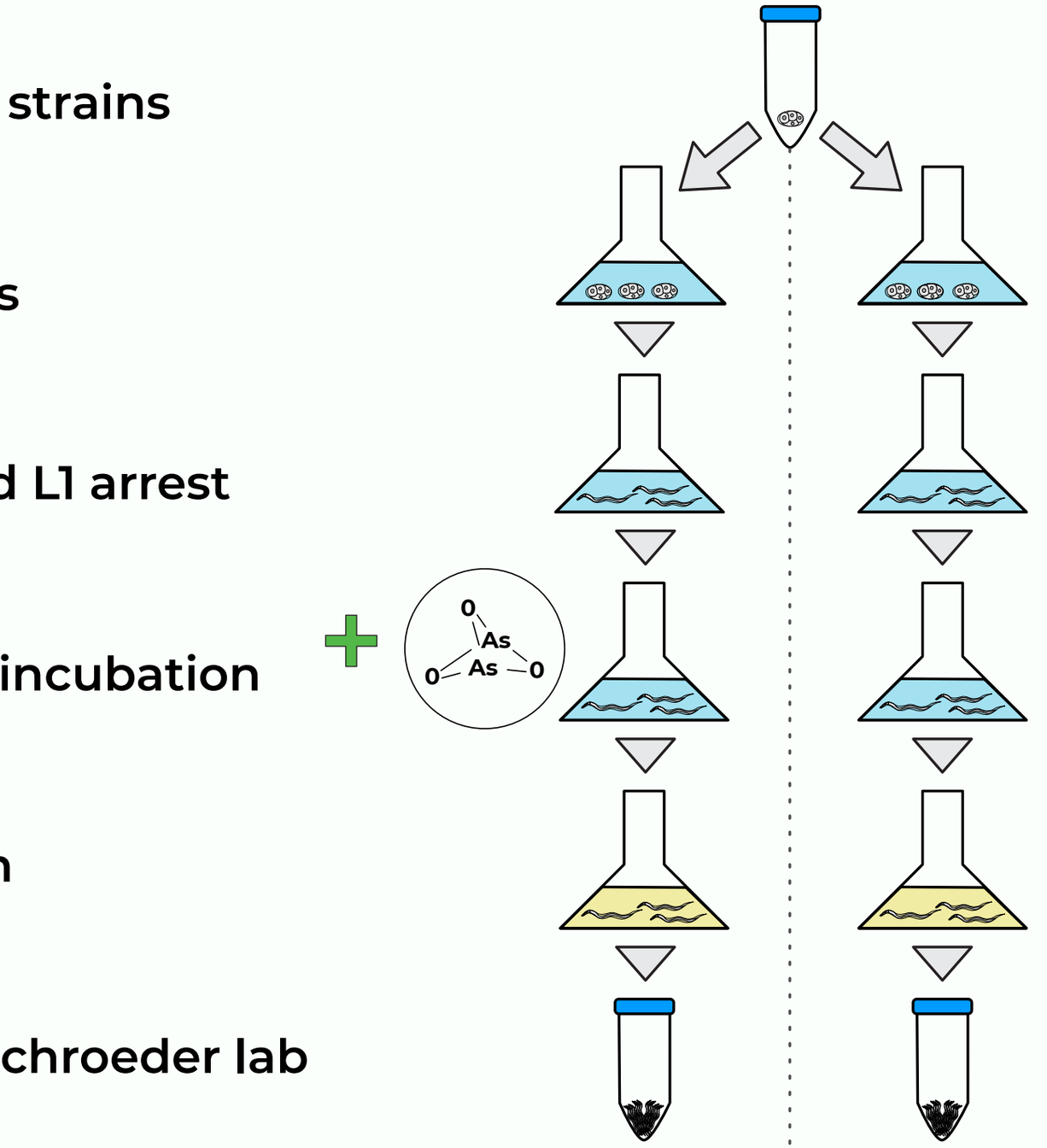


Are down-stream metabolites affected in an allele specific manner?

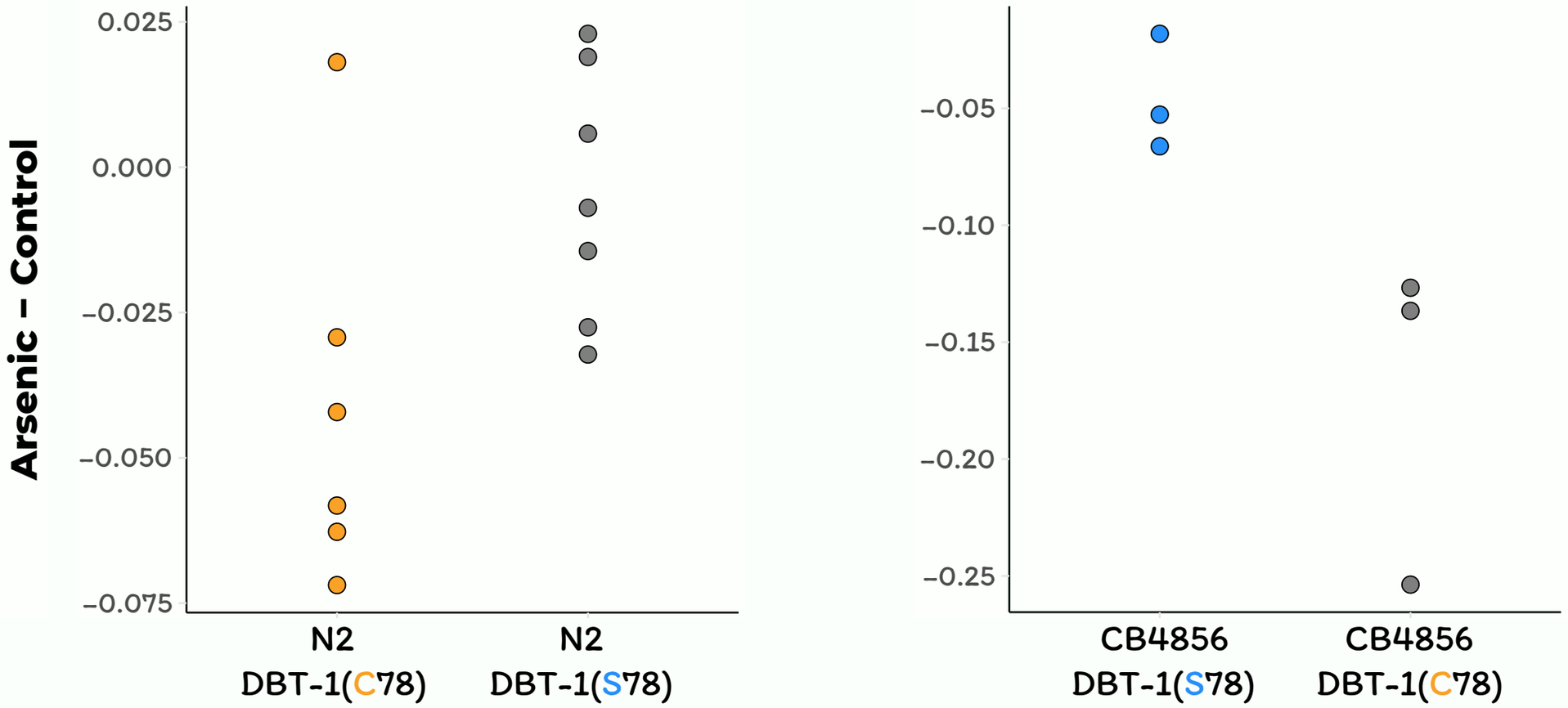


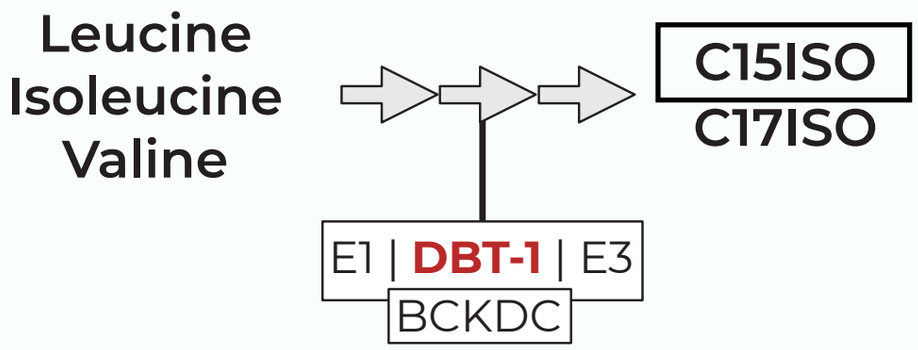
Are down-stream metabolites affected in an allele specific manner?

- Bleach-synchronize strains
- Paired 100 k aliquots
- Hatch overnight and L1 arrest
- 24 hr arsenic/water incubation
- 4 hr food incubation
- Pellet and send to Schroeder lab
- Quantify BCFA/SCFA ratios

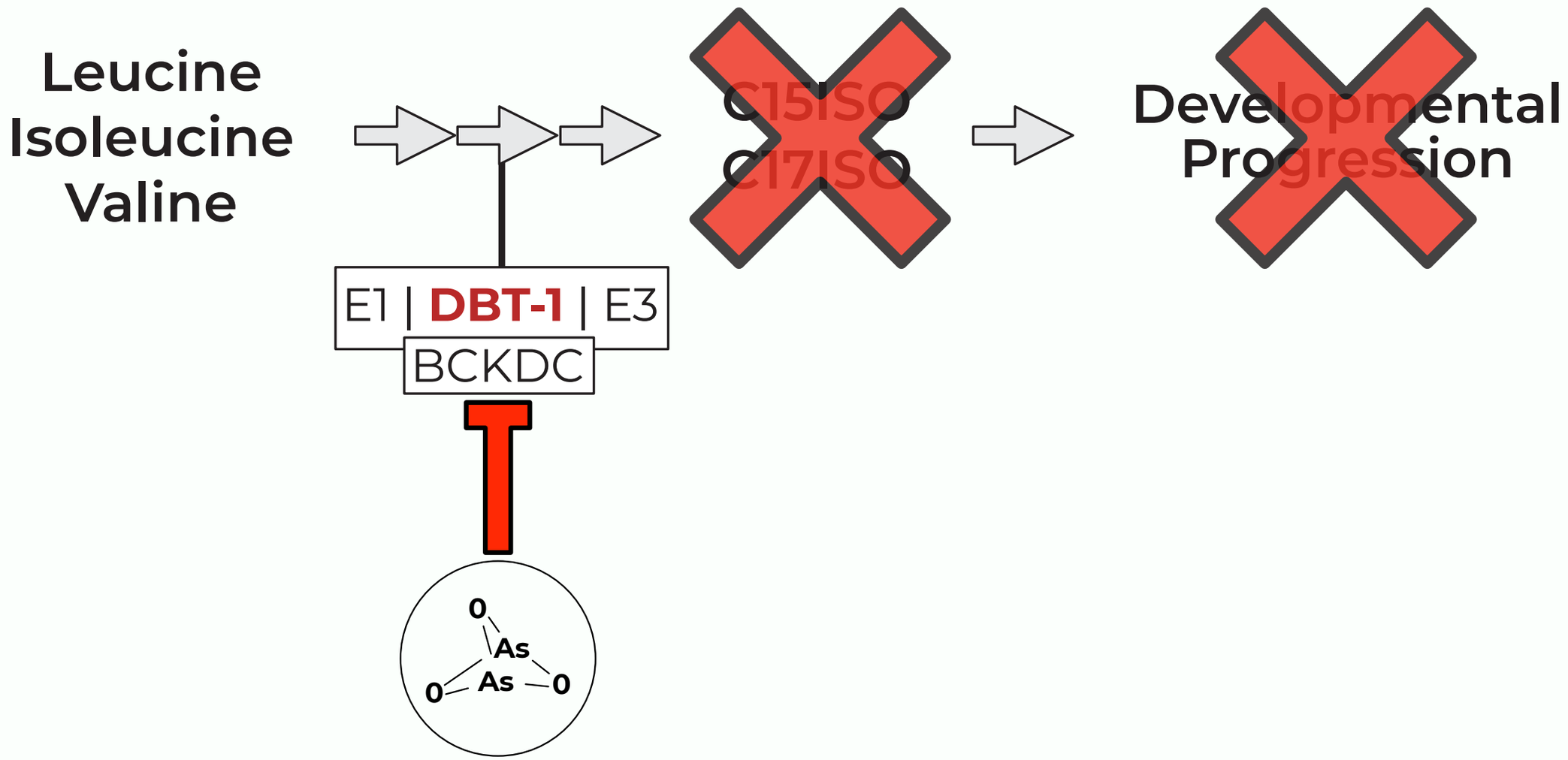


Strains with DBT-1(C78) produce fewer BCFAs in the presence of arsenic

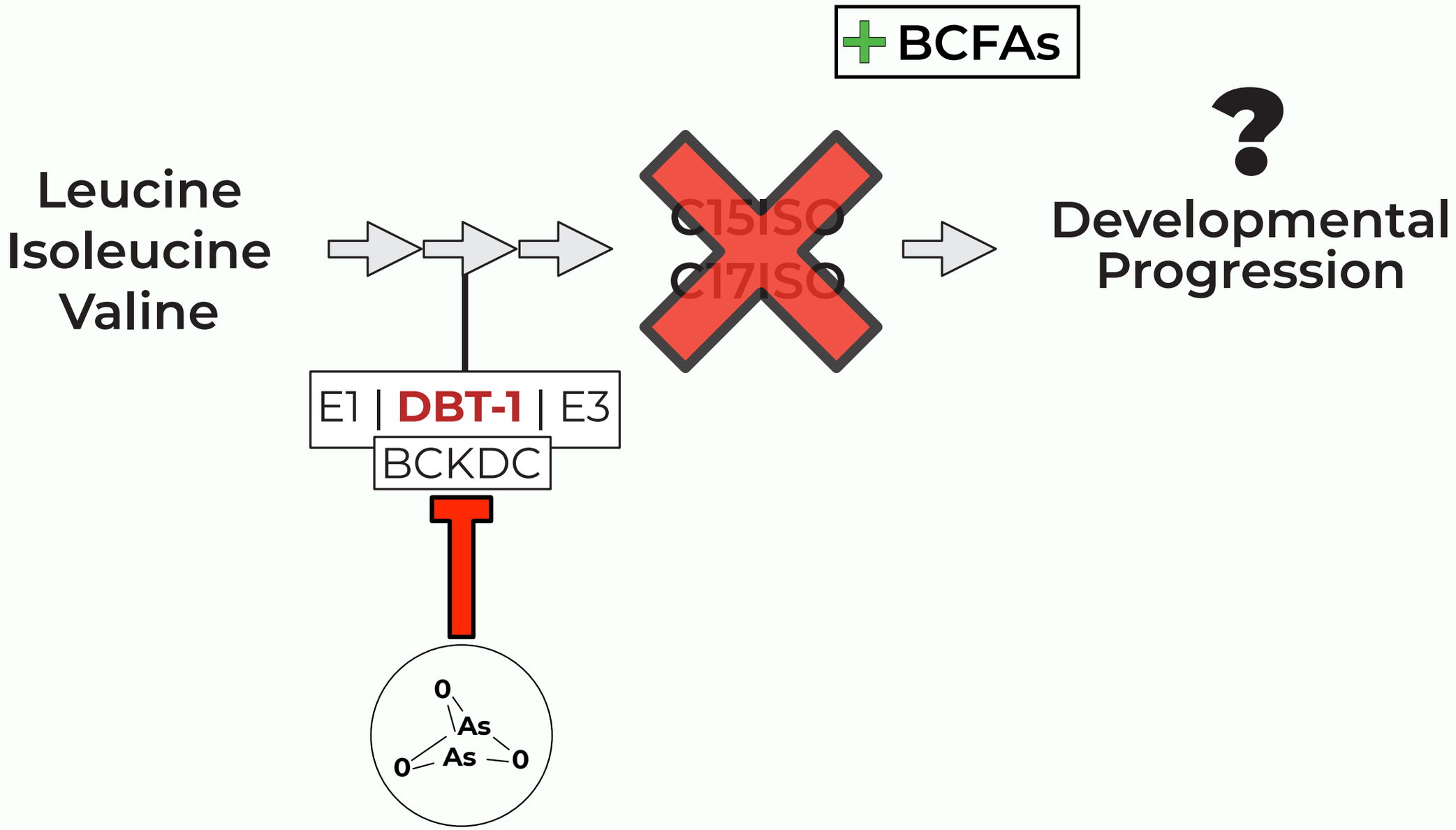




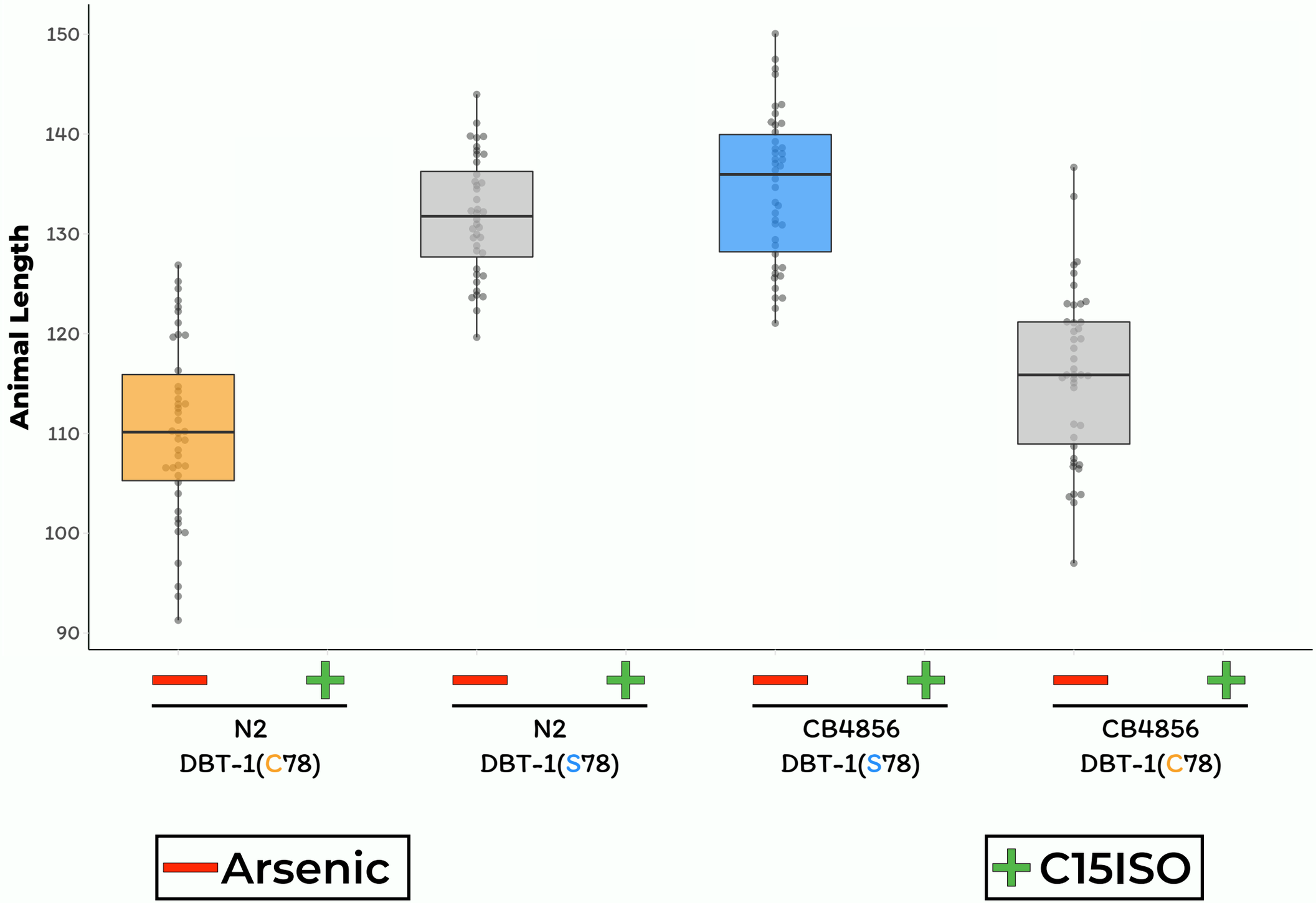
Strains with DBT-1(C78) produce fewer BCFAs in the presence of arsenic



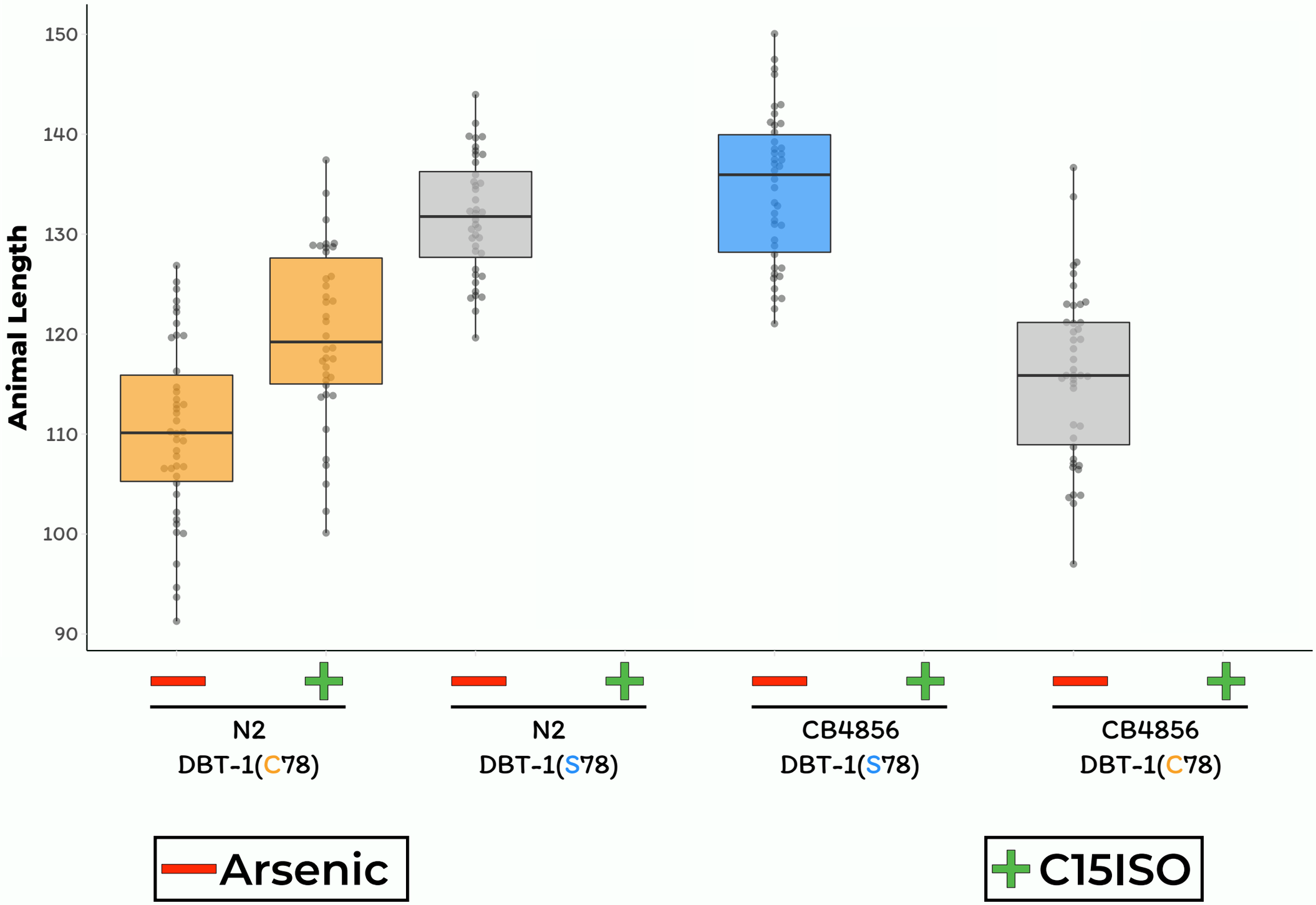
Arsenic-induced developmental delay might be caused by depletion of BCFAs



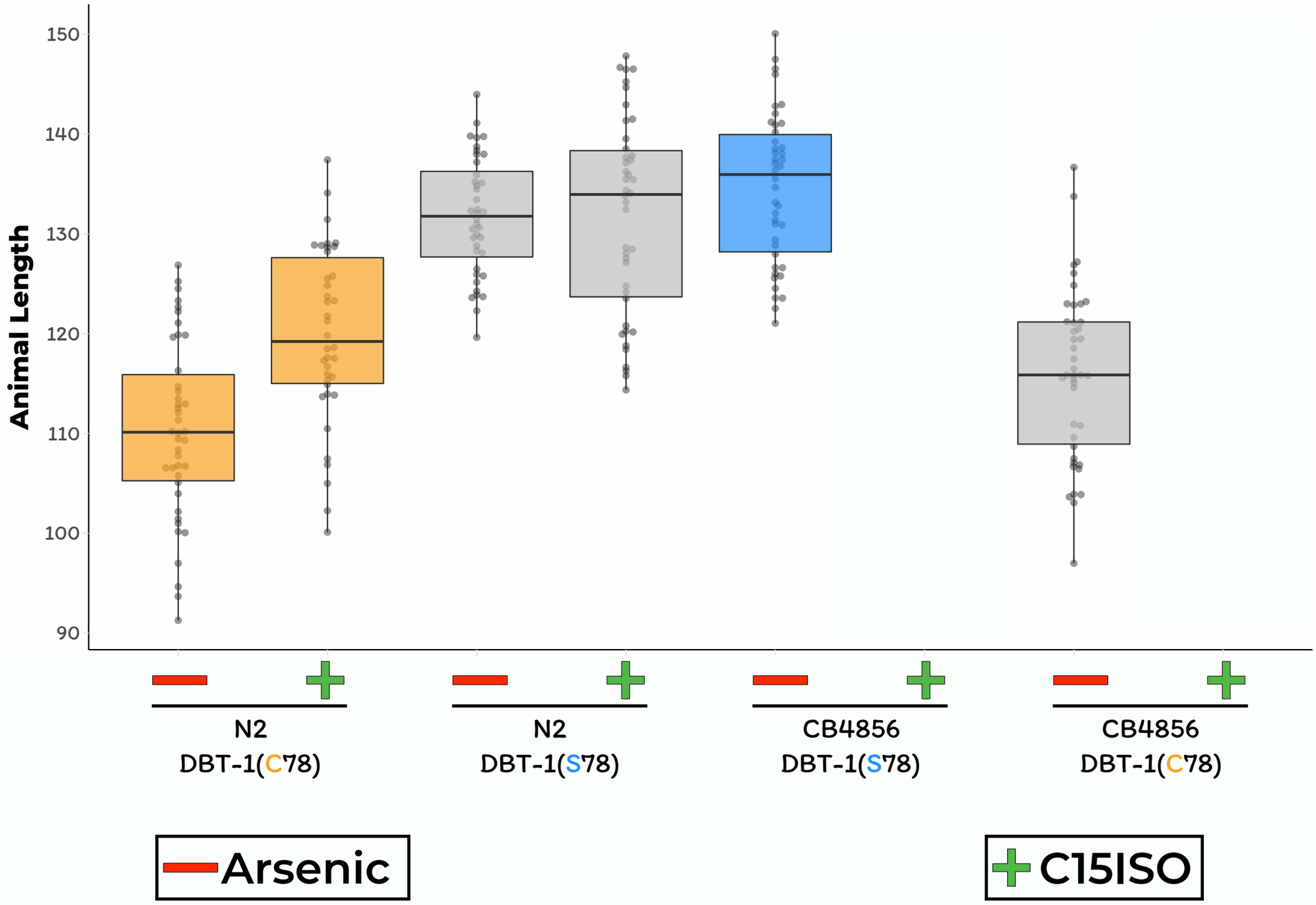
C15ISO supplementation of arsenic media partially rescues allele-specific arsenic toxicity



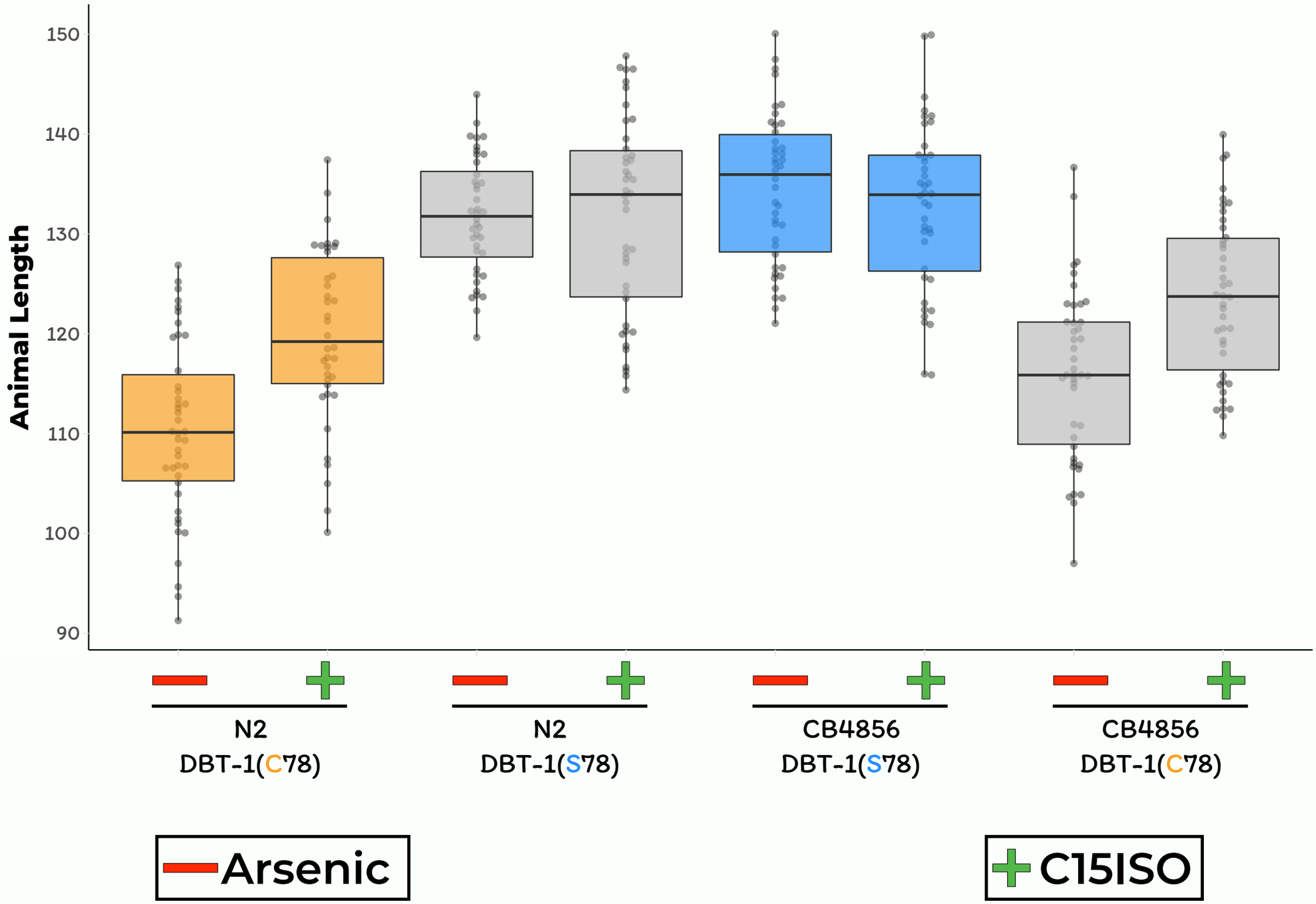
C15ISO supplementation of arsenic media partially rescues allele-specific arsenic toxicity



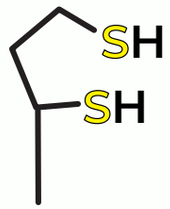
C15ISO supplementation of arsenic media partially rescues allele-specific arsenic toxicity



C15ISO supplementation of arsenic media partially rescues allele-specific arsenic toxicity



The lipoyl domain of DBT-1 is highly conserved



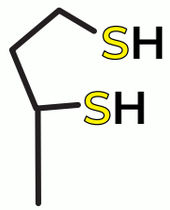
C. elegans

VVQFKLSDIGEGIAEVQVKEWYVKEGDTISQFDKVCEVQSDKAAVTISCRYDGIVKKLYHEVDGMARVGQALIDVEIEG

VVQFKLSDIGEGIREVTVKEWYVKEGDTVSQFDSICEVQSDKASVTITSRYDGVIKKLYYNLDDIAYVGKPLVDIETEA

H. sapiens

Human cell-line experiment to test if the mechanism of arsenic toxicity is conserved



C. elegans

VVQFKLSDIGEGIAEVQVKEWYVKEGDTISQFDKVCEVQSDKAAVTISCRYDGIVKKLYHEVDGMARVGQALIDVEIEG

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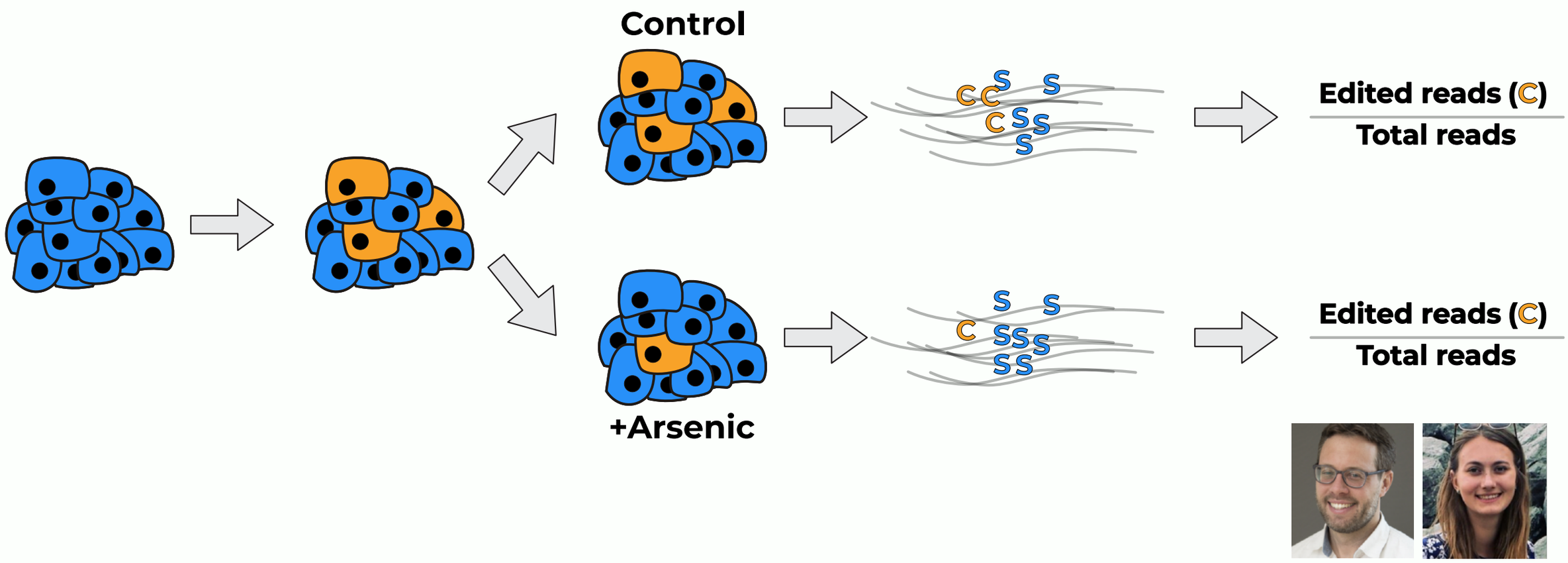
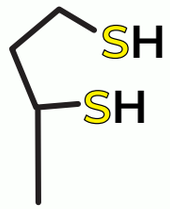
H. sapiens



The cysteine allele provides slight protection to arsenic treatment

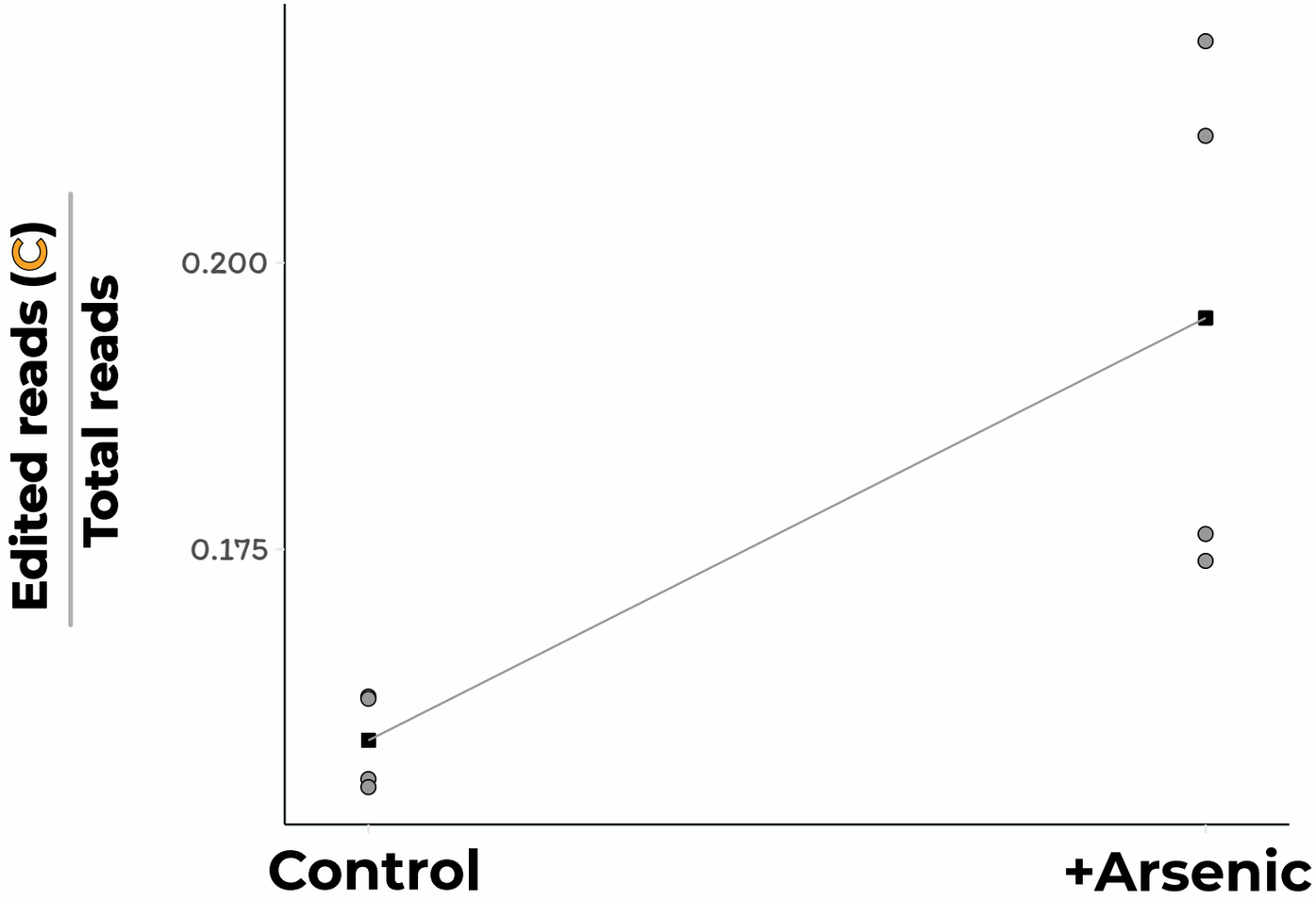


C. elegans

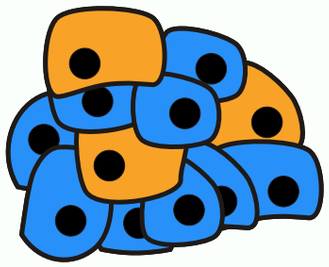
VVQFKLSDIGEGIAEVQVKEWYVKEGDTISQFDKVCEVQSD**K**AAVTIS**C**RYDGIVKKLYHEVDGMARVGQALIDVEIEG

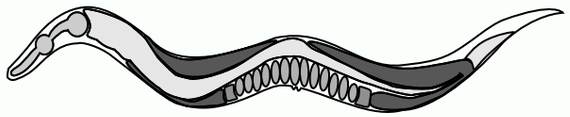
VVQFKLSDIGEGIREVTVKEWYVKEGDTVSQFDSICEVQSD**K**ASVTIT**S**RYDGVIKKLYYNLDDIAYVGKPLVDIETEA

H. sapiens

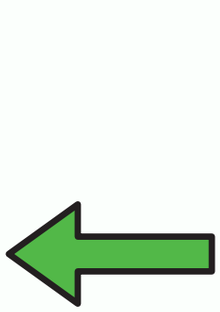


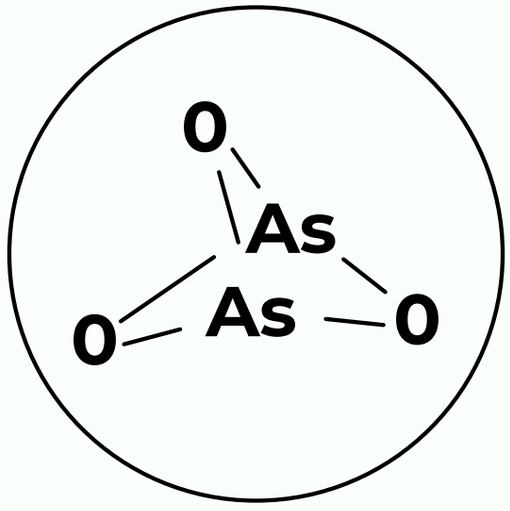
Differential effects of arsenic in *C. elegans* and human cell lines

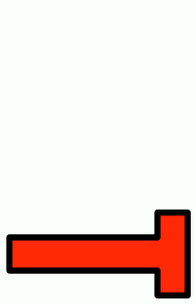




DBT-1(C78)

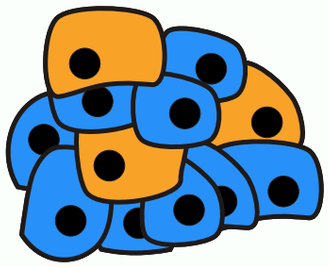




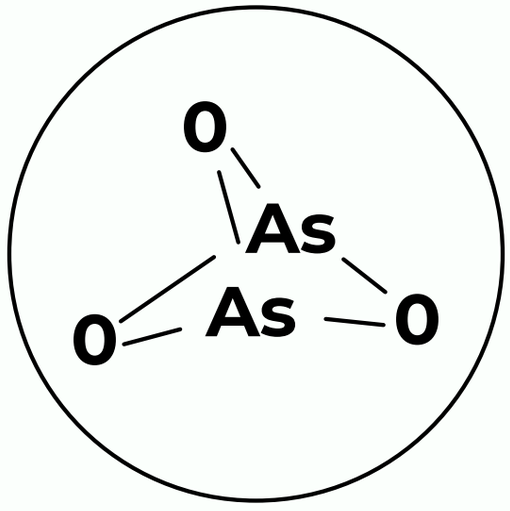


DBT-1(C78)

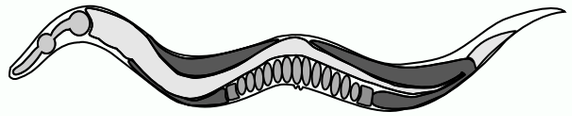
Neuronal expression of *dbt-1* in *C. elegans* rescues the developmental effects of *dbt-1(o)*



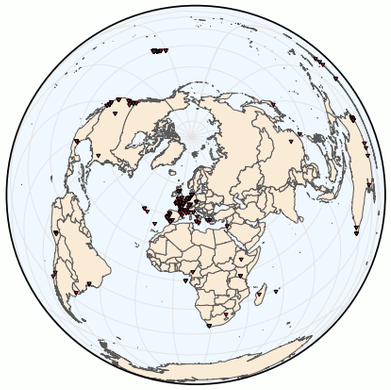
DBT-1(C78)

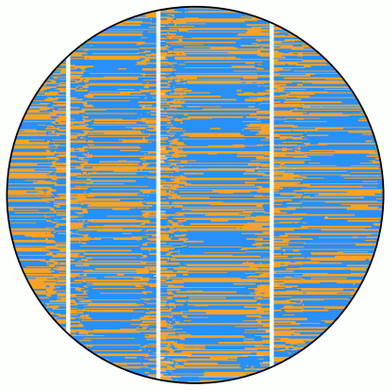


DBT-1(C78)

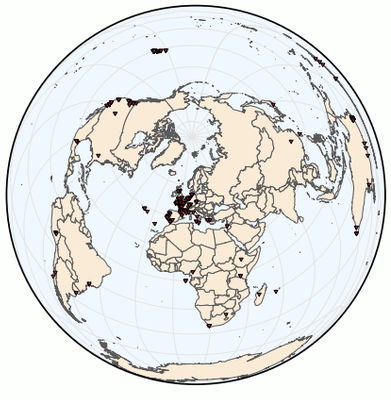


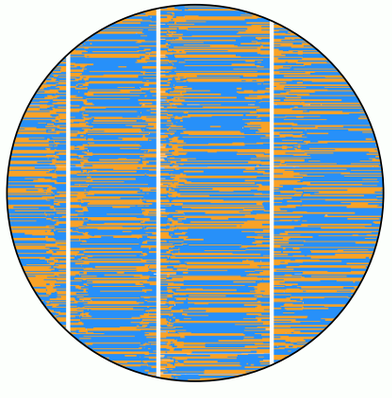
Jia et. al. 2015, JBC





DBT-1(C78S)





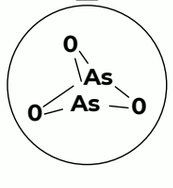
DBT-1(C78S)

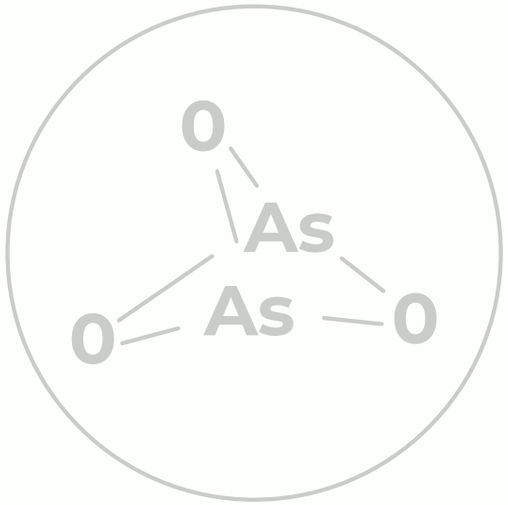
Leucine
Isoleucine
Valine

E1 | **DBT-1** | E3
BCKDC

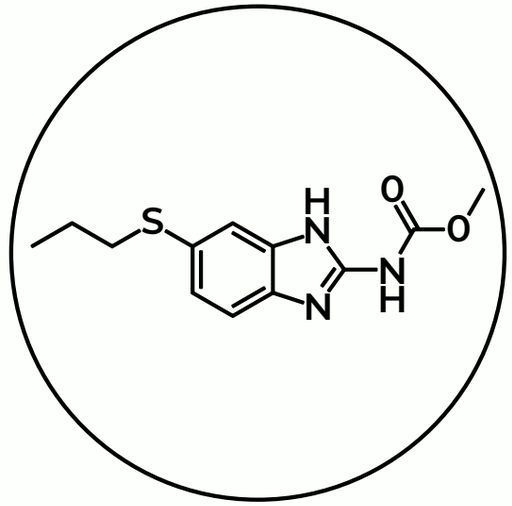
C151SO
C171SO

Developmental
Progression



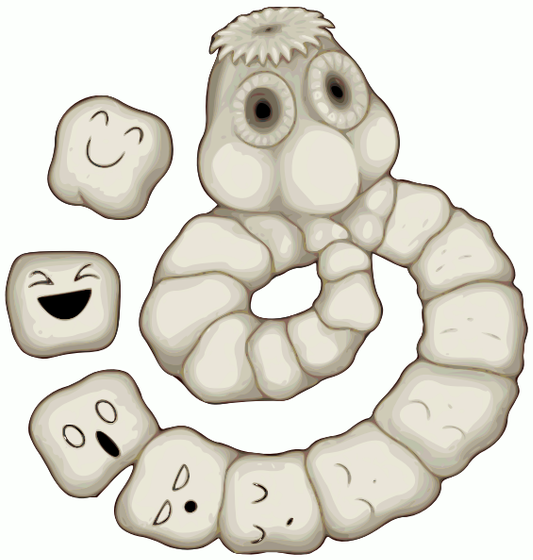


Arsenic

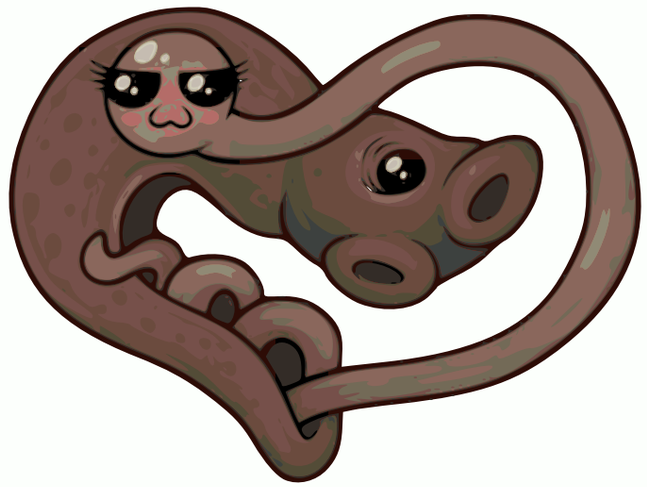


Albendazole

A LOT of parasitic worms want to infect you, your pets, and your food!



Cestoda

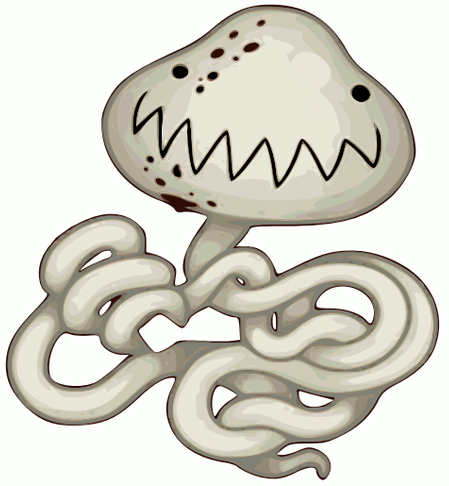


Schistosoma

Necator

Enterobius

Ascaris

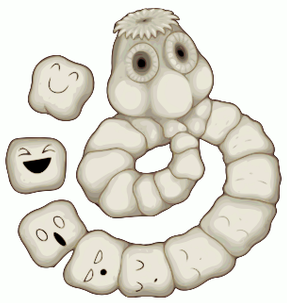


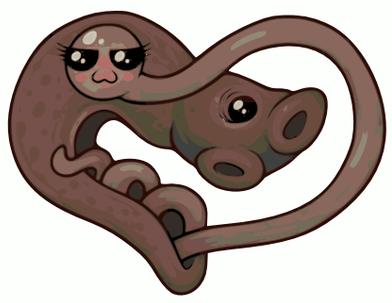
Dracunculus

A LOT of parasitic worms want to infect you, your pets, and your food!

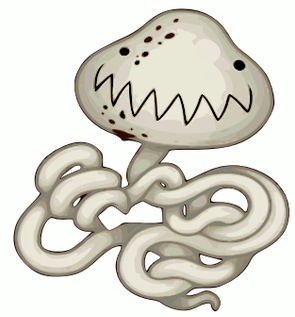
Helminths

Flatworms

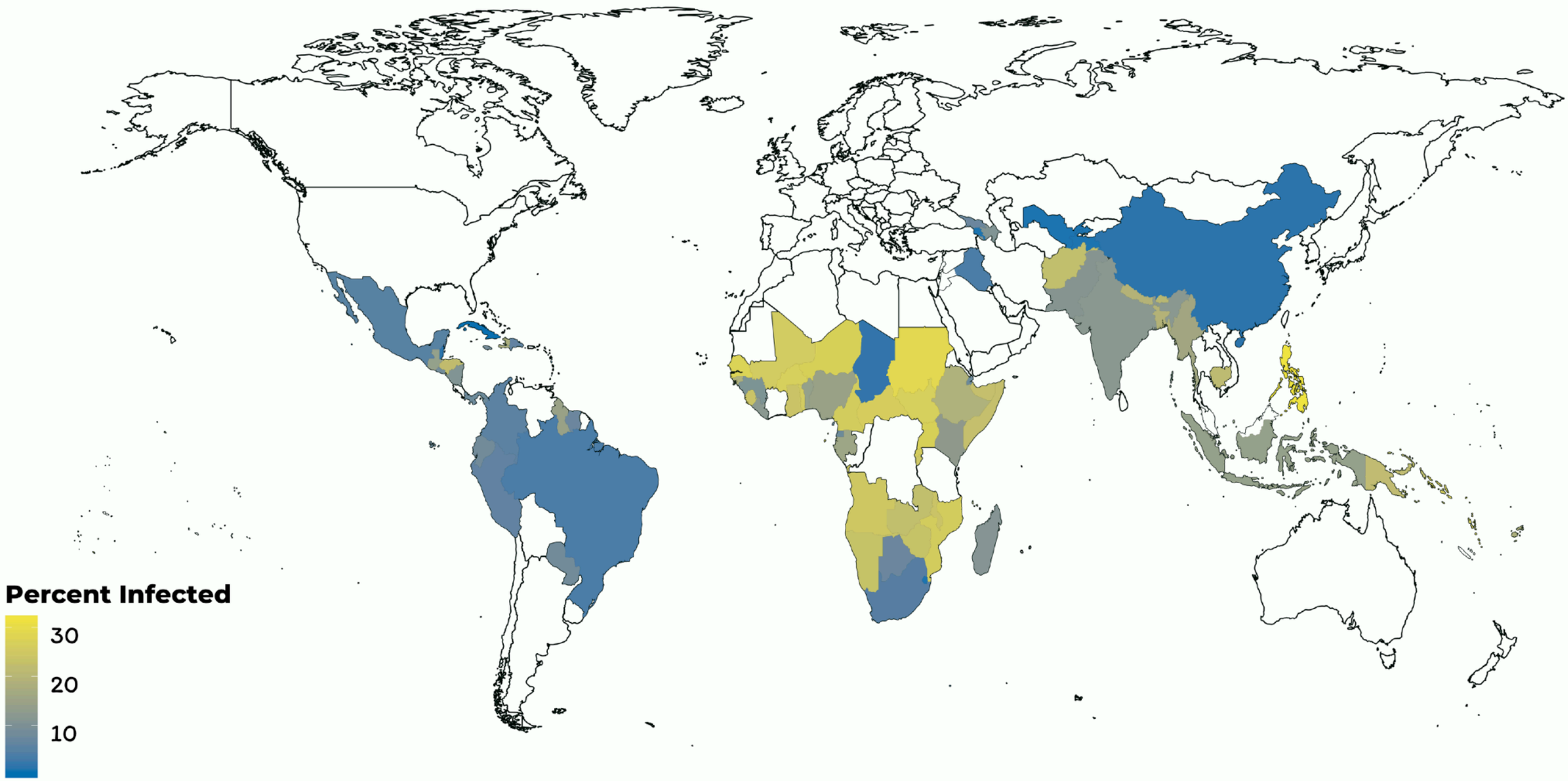




Roundworms



Approximately 1.2 billion people are infected by soil-transmitted helminths



World Health Organization: 2016

The human burden of parasitic nematodes

DALY =

Disability-Adjusted Life Year

YLD

Years Lost to Disability

YLL

Years of Life Lost

The human burden of parasitic nematodes

DALY =

Disability-Adjusted Life Year

YLD

Years Lost to Disability

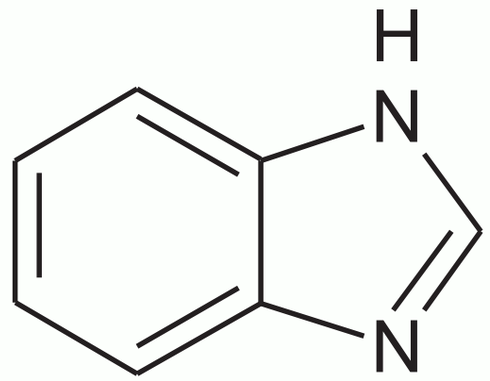
> 8 million

YLL

Years of Life Lost

< 0.5 million

Benzimidazoles (BZs) are among the most used anti-parasitic nematode compounds



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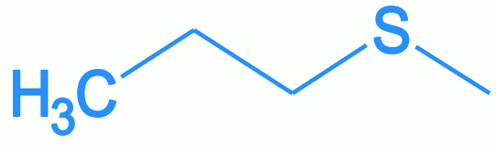
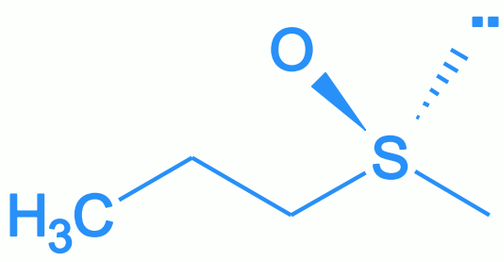
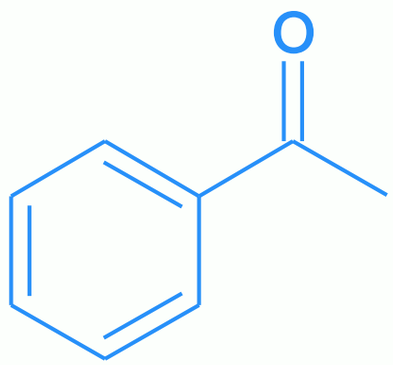
Albendazole

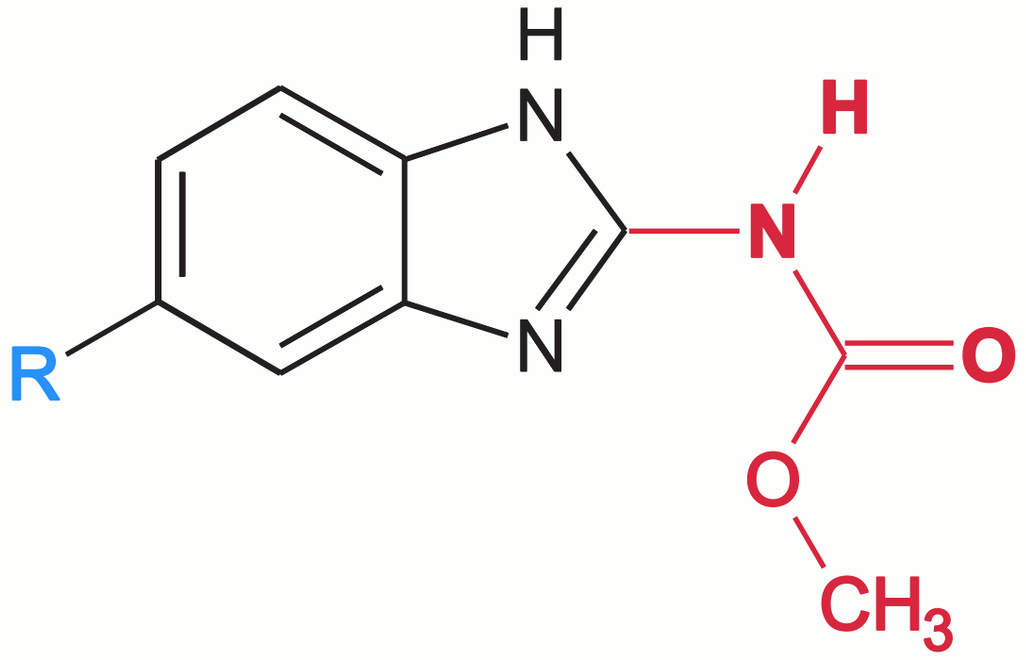


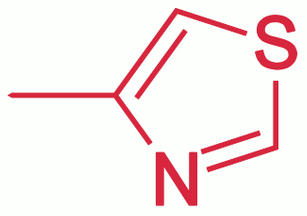
Albendazole sulfoxide



Mebendazole

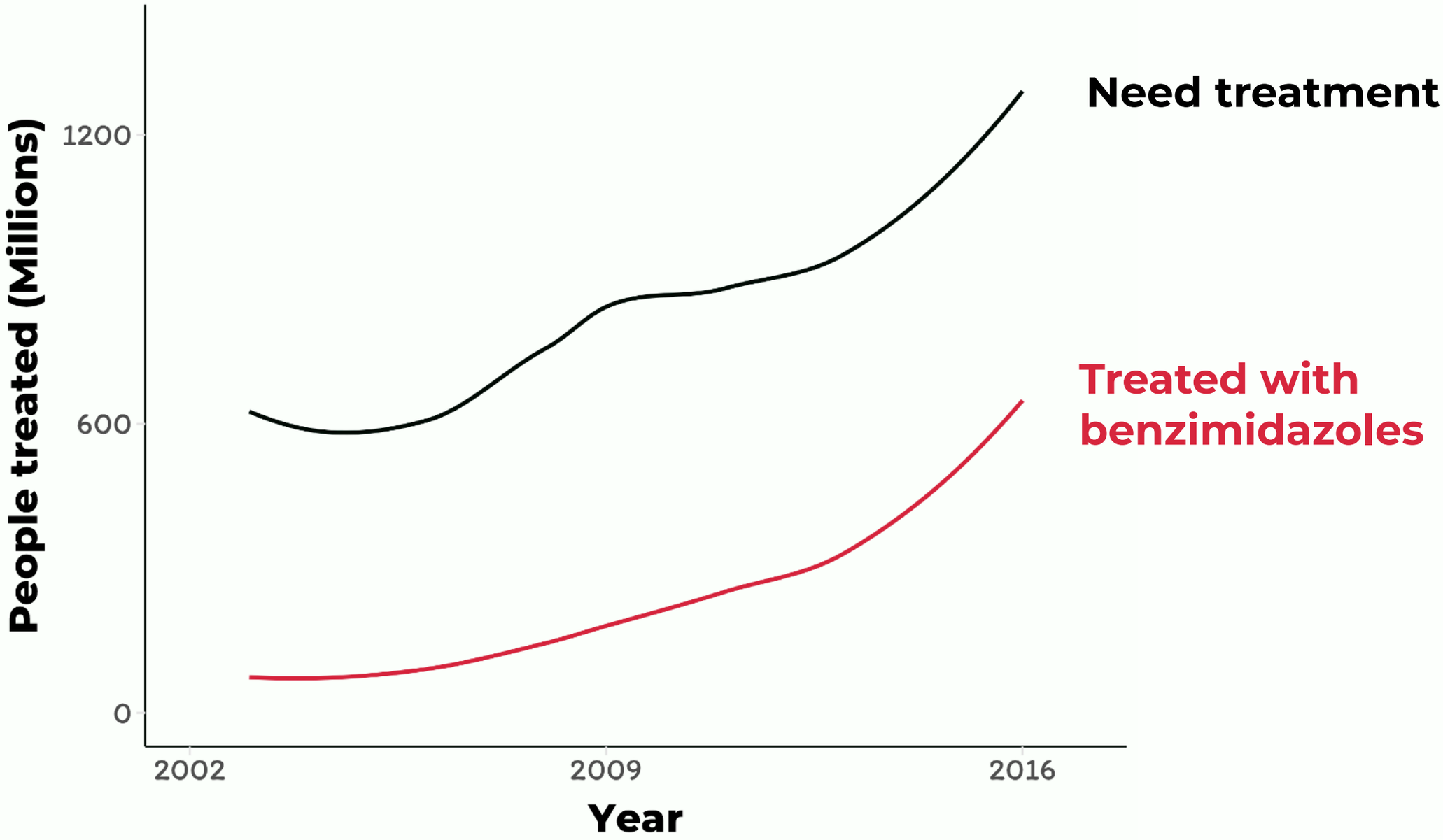






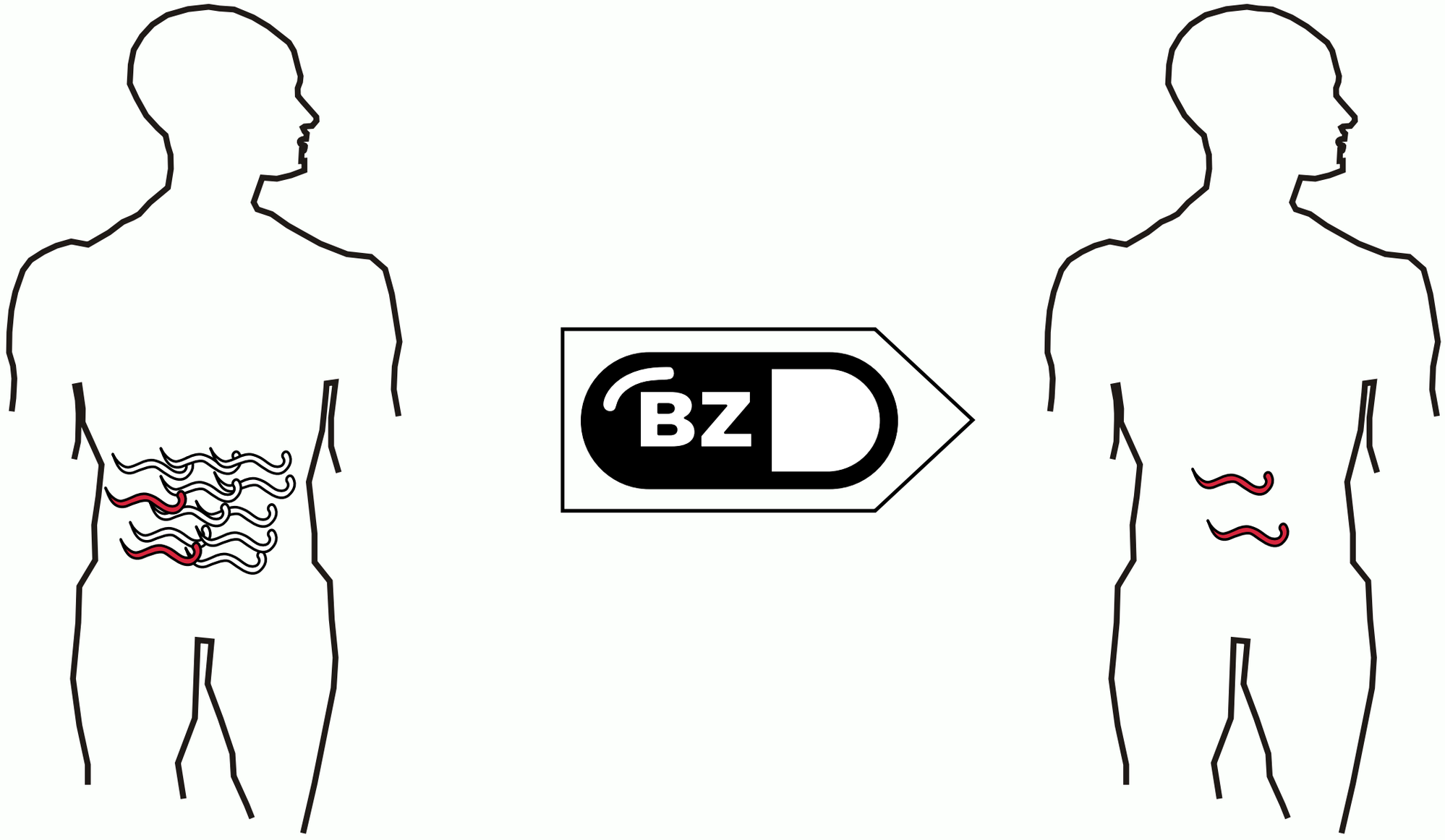
Thiabendazole

Benzimidazoles (BZs) are among the most used anti-parasitic nematode compounds

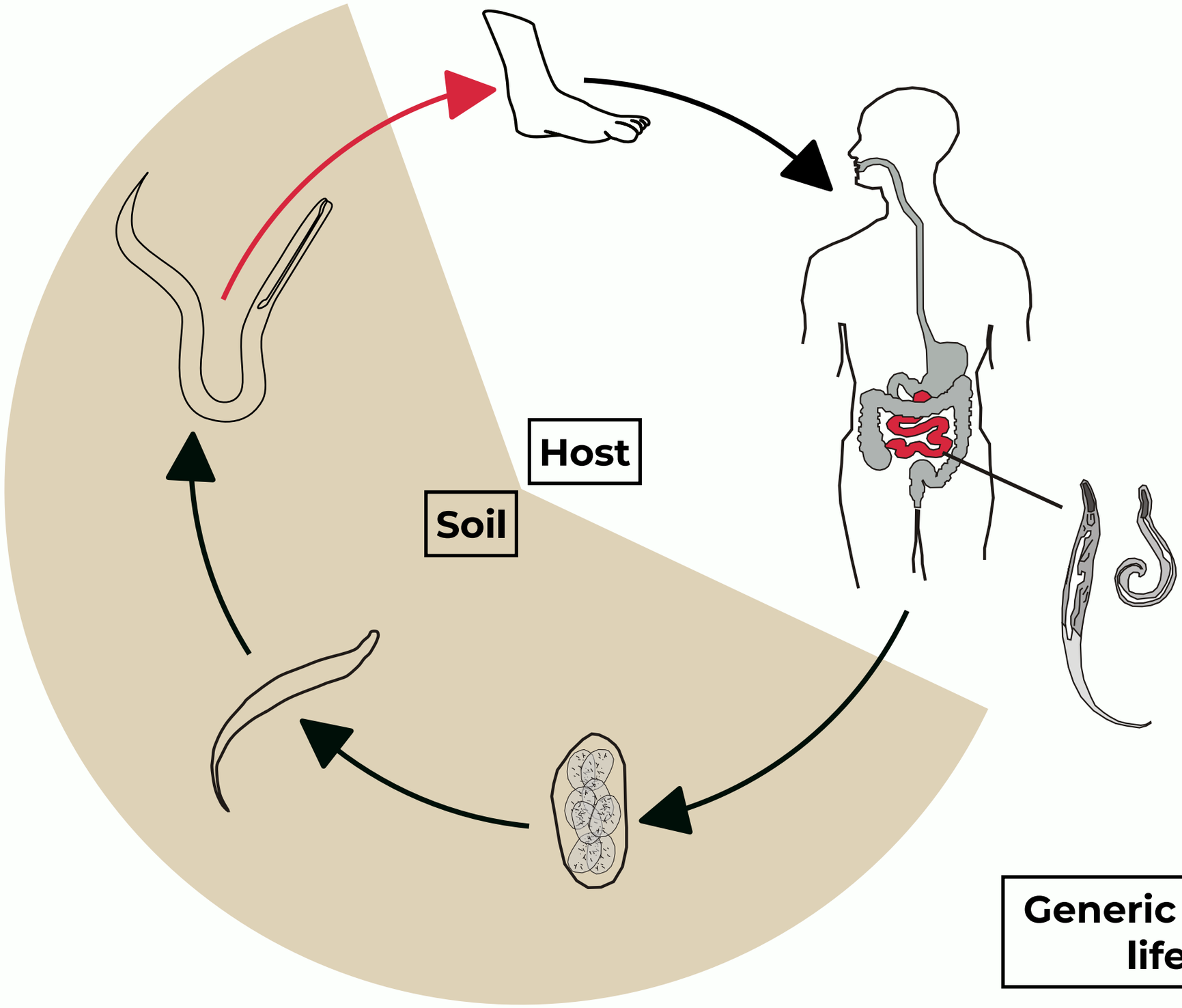


World Health Organization

A limited therapeutic repertoire promotes the selection of resistant alleles



A cycle of reinfection establishes resistant alleles



Generic hookworm life cycle

β-tubulin alleles predicted to confer BZ resistance

Redman et al. (2015) PLoS NTD

1. **Parasites with β-tubulin resistance alleles exhibit >10,000 fold variation in BZ response**

2. **Predicted β-tubulin resistance alleles have never been experimentally validated**

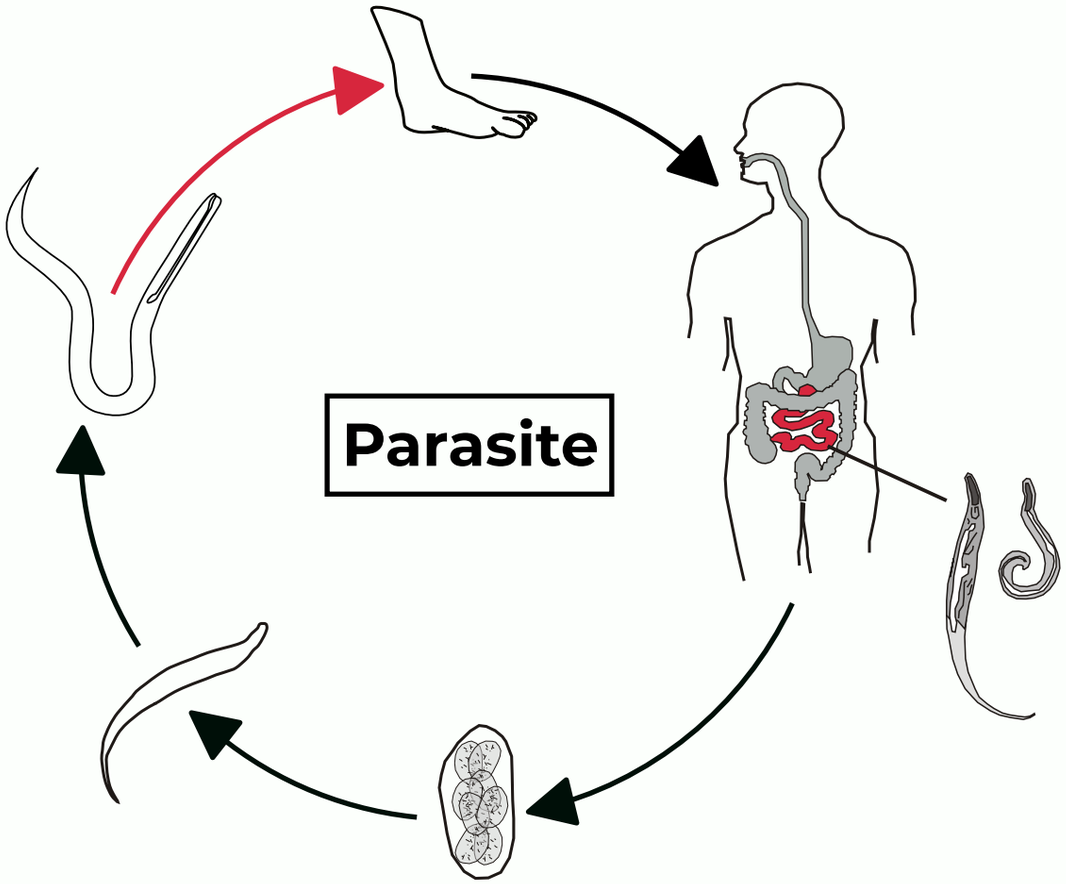
1. **Parasites with β-tubulin resistance alleles exhibit >10,000 fold variation in BZ response**

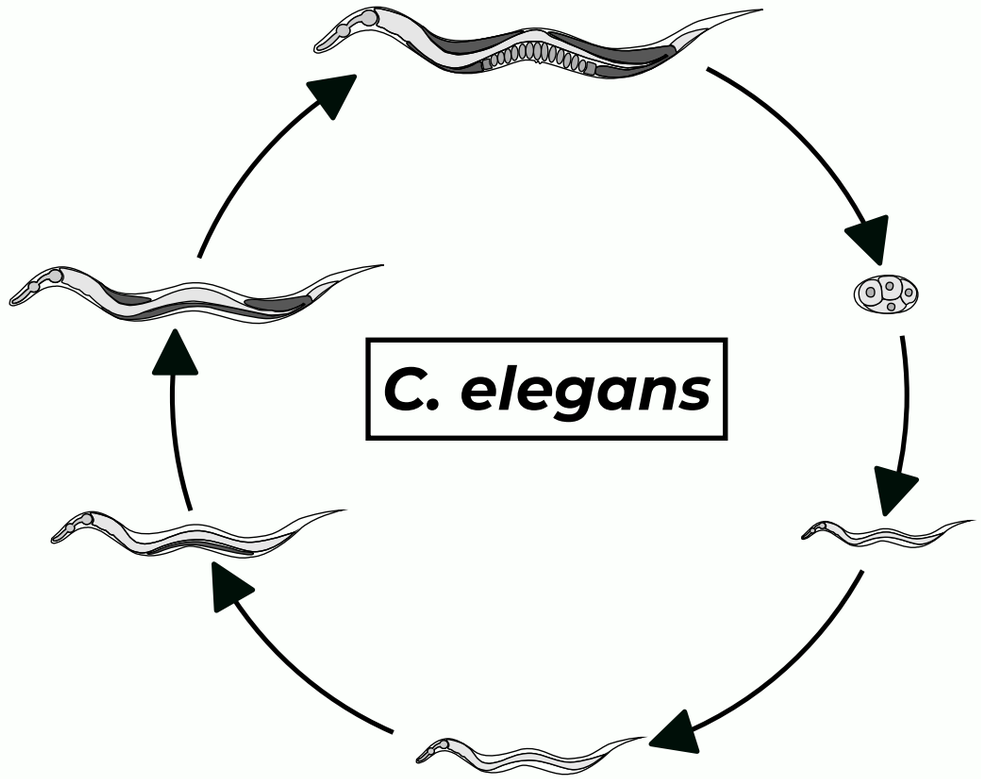
   - **Can we identify additional resistance alleles?**

2. **Predicted β-tubulin resistance alleles have never been experimentally validated**

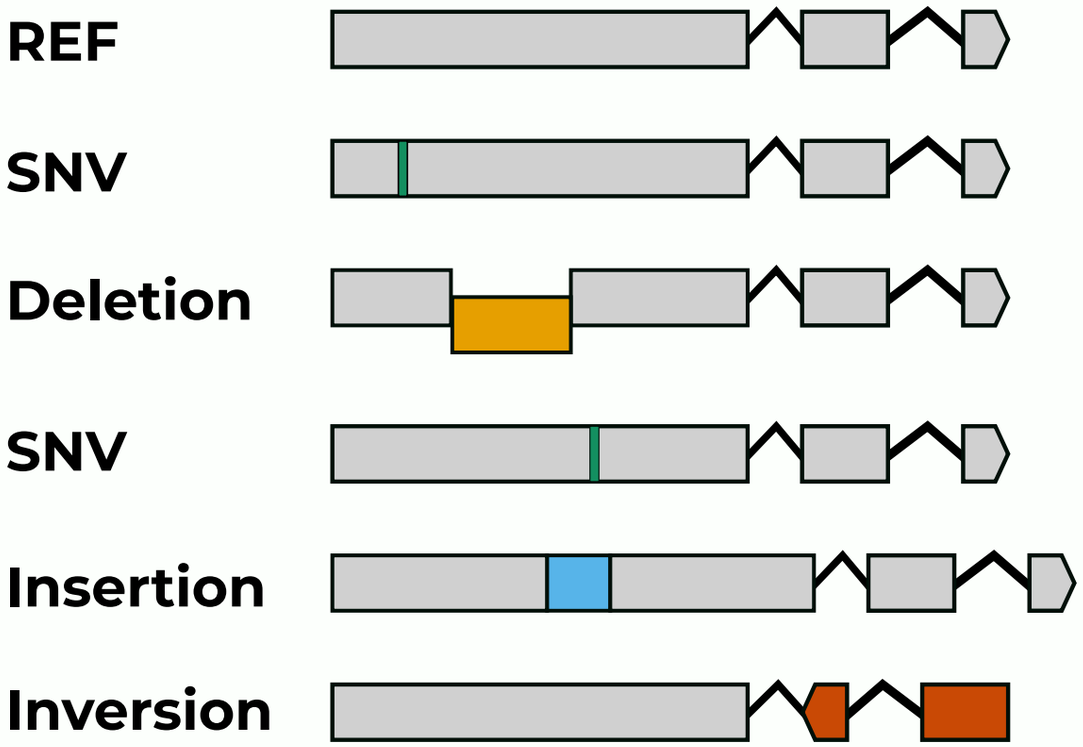
   - **Can we use *C. elegans* to experimentally validate predicted resistance alleles?**

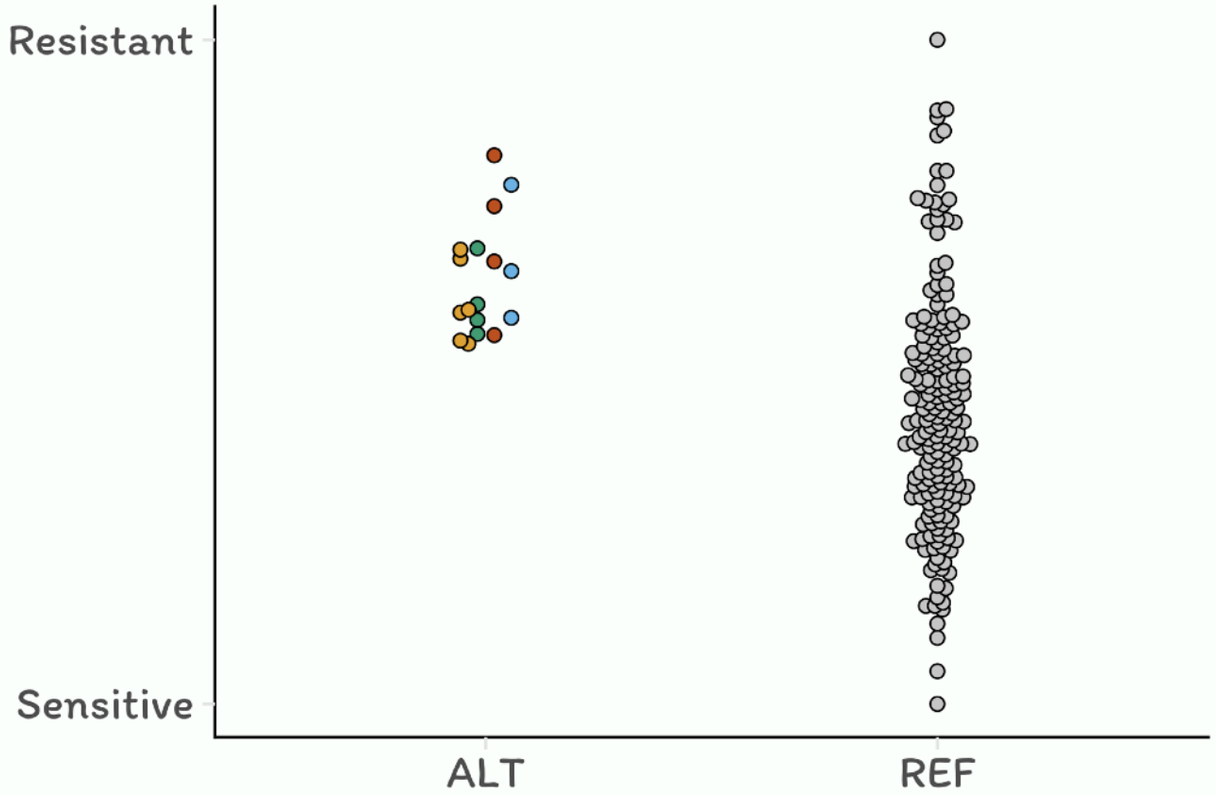
C. elegans as model system to study mechanisms of BZ resistance





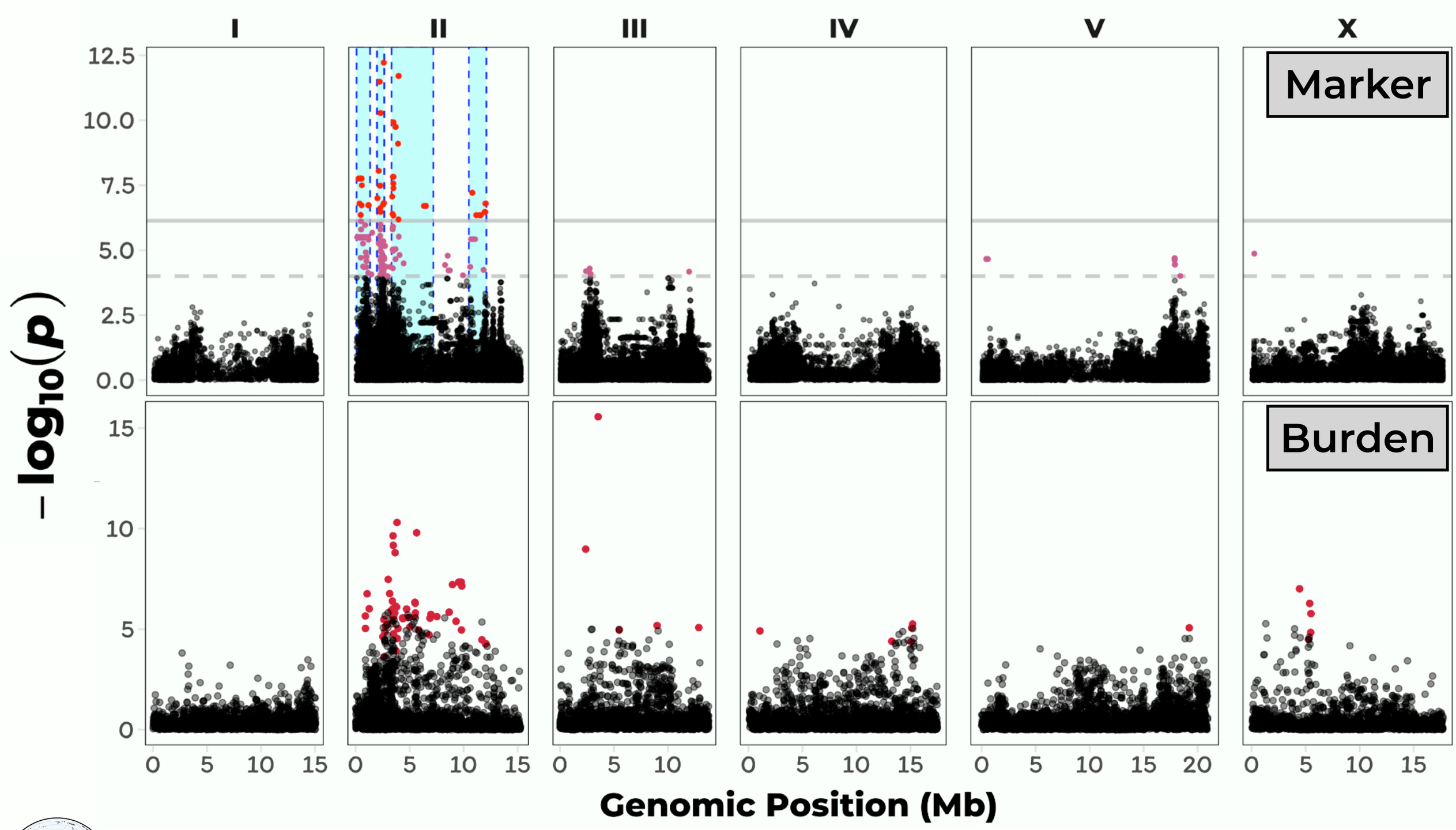
Burden-based GWA mapping



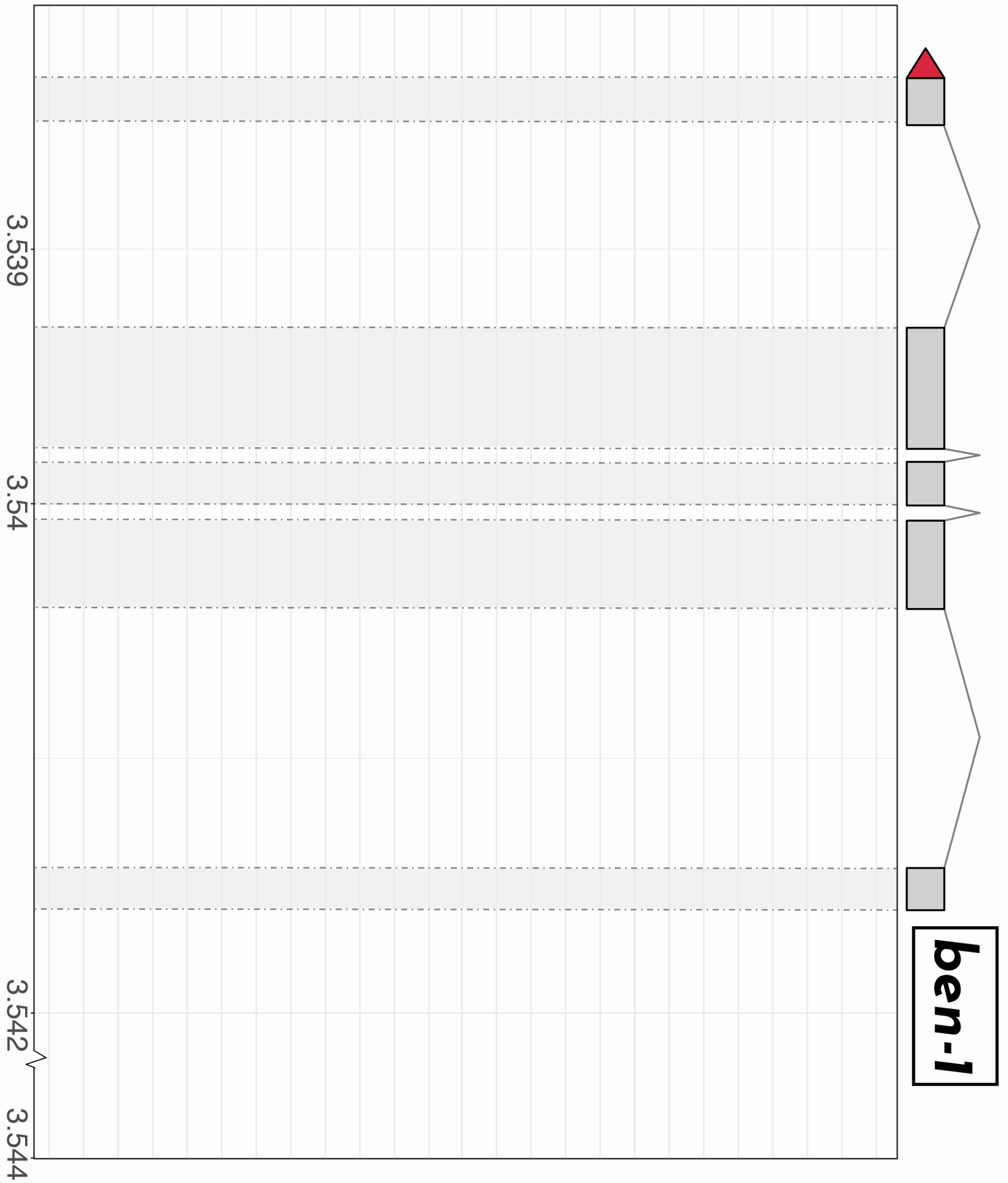


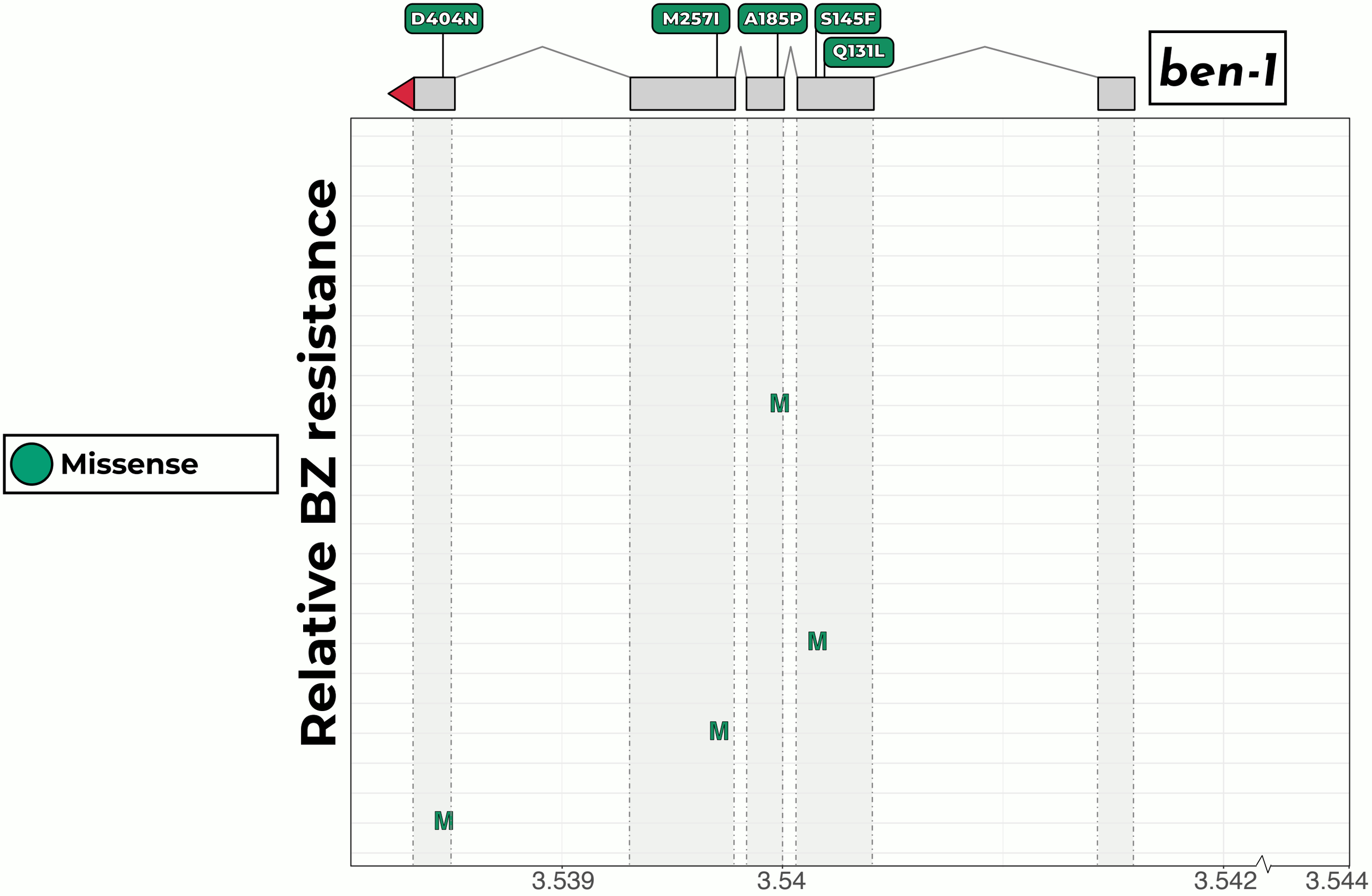
Burden

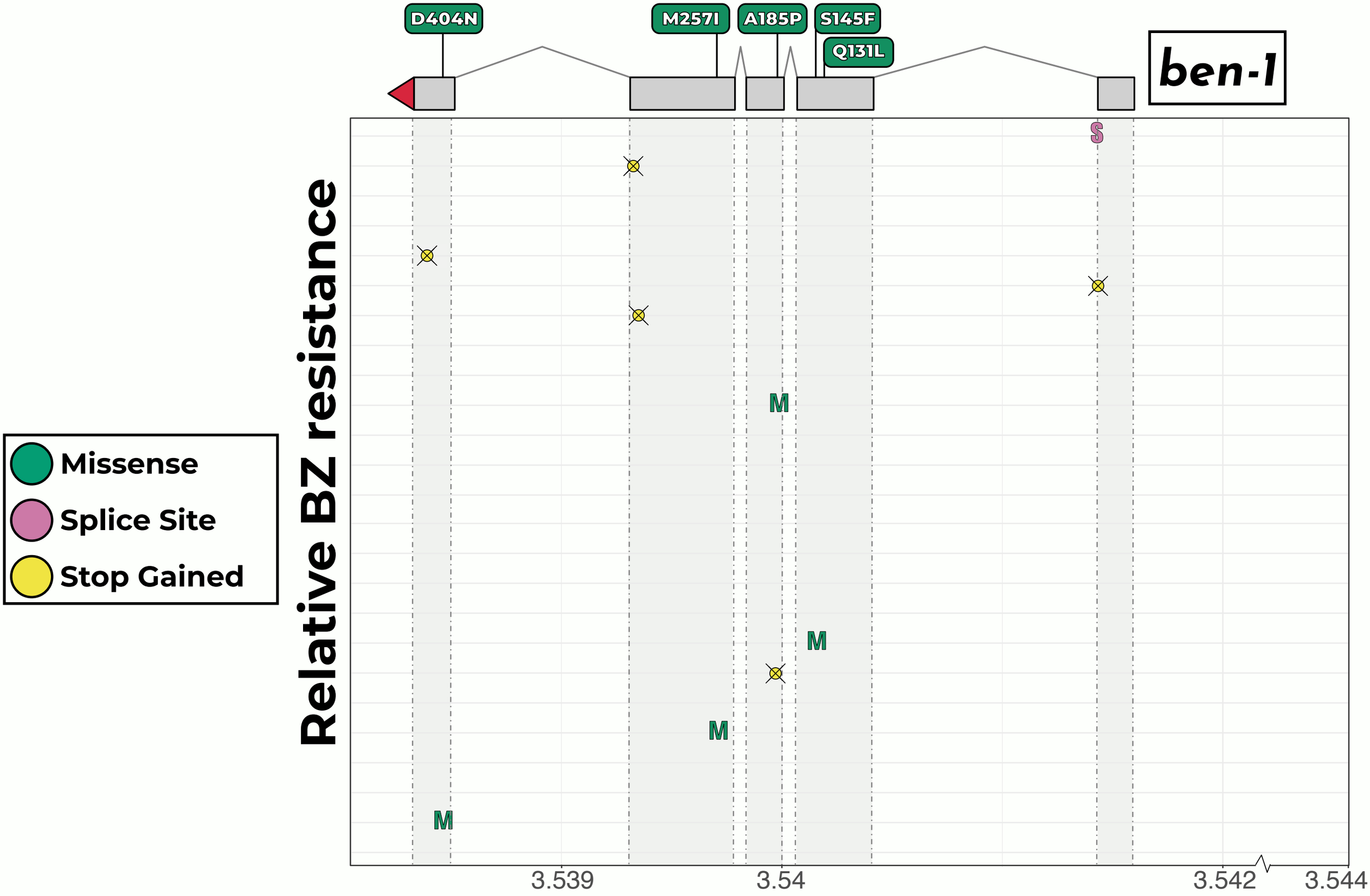
Multiple QTL are correlated with BZ response

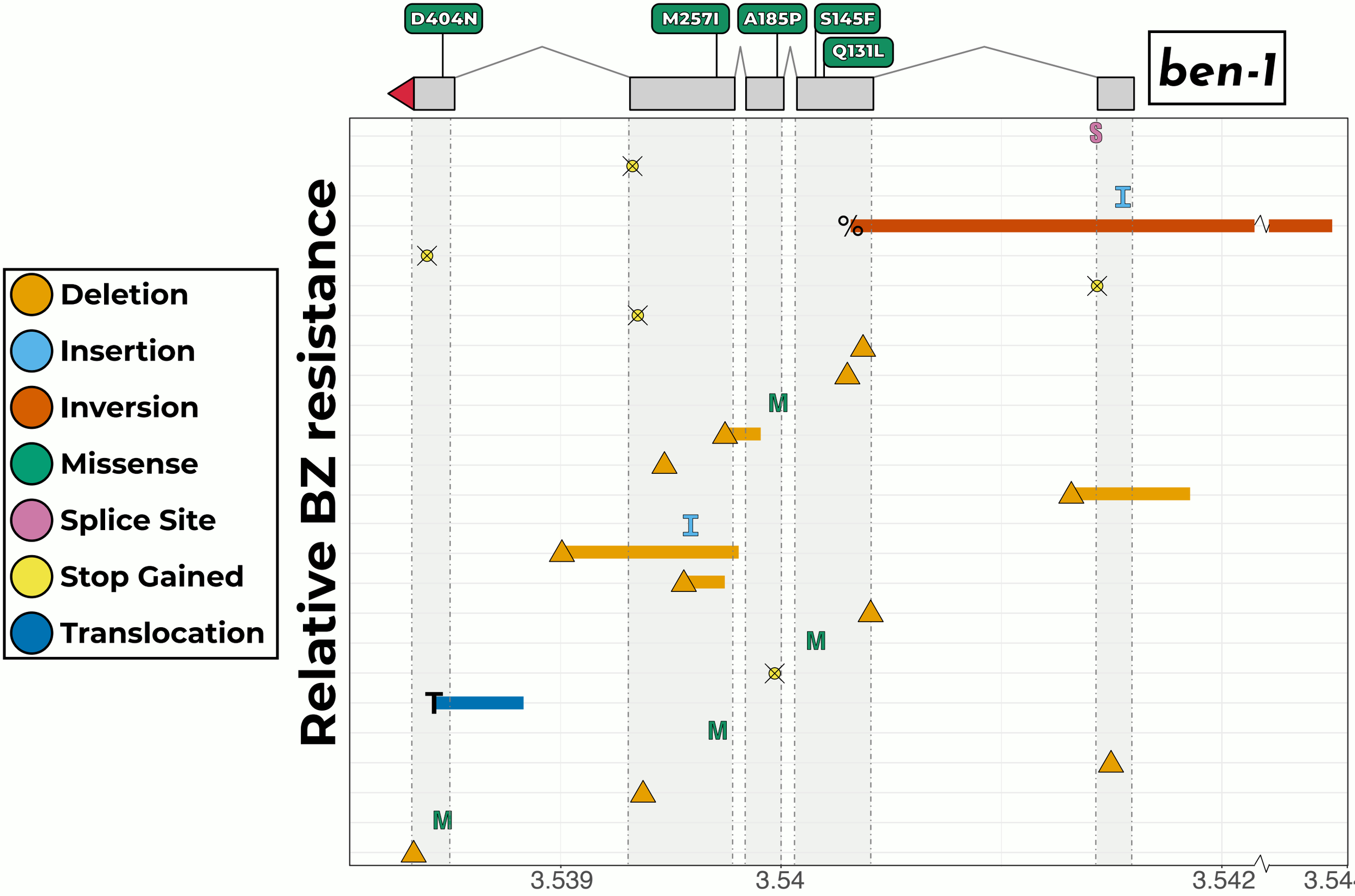


Relative BZ resistance

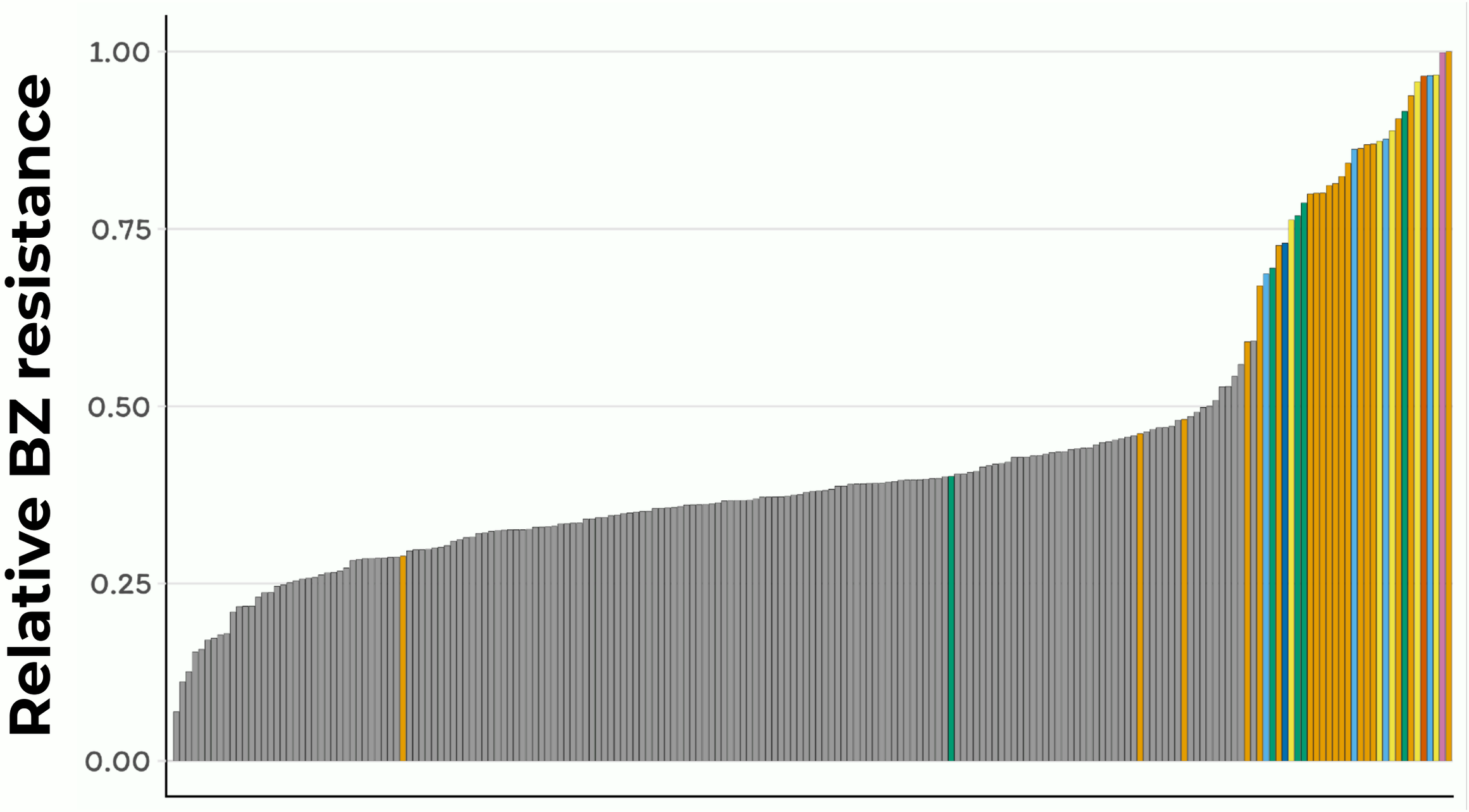



D404N
M257I
A185P
S145F
Q131L
ben-1
Missense
Relative BZ resistance
M
M
M
M
3.539
3.54
3.542
3.544

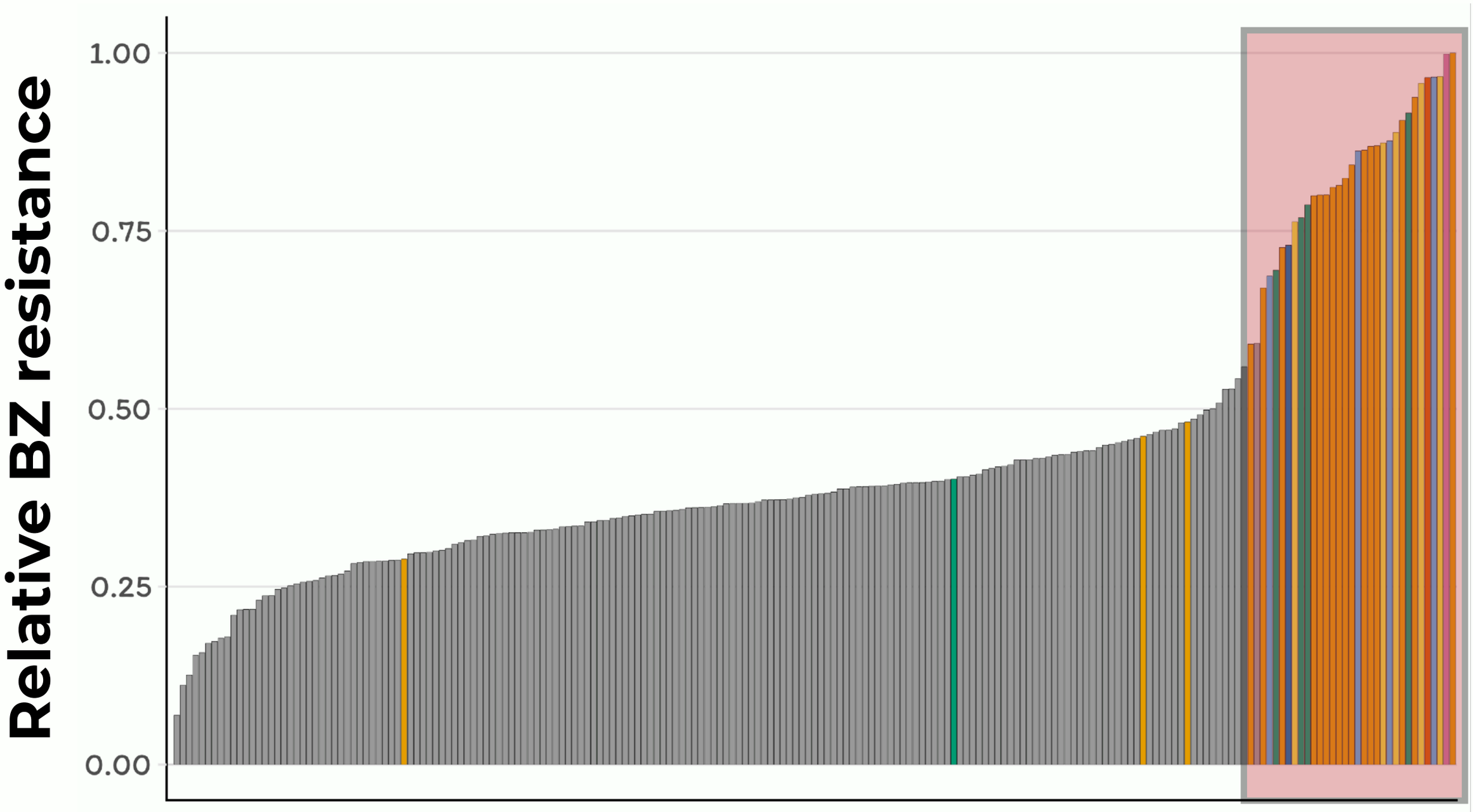

D404N
M257I
A185P
S145F
Q131L
ben-1
S
M
M
M
M
M
Relative BZ resistance
Missense
Splice Site
Stop Gained
3.539
3.54
3.542
3.544


D404N
M257I
A185P
S145F
Q131L
ben-1
Deletion
Insertion
Inversion
Missense
Splice Site
Stop Gained
Translocation
Relative BZ resistance
S
I
%
M
I
M
T
M
M
3.539
3.54
3.542
3.544

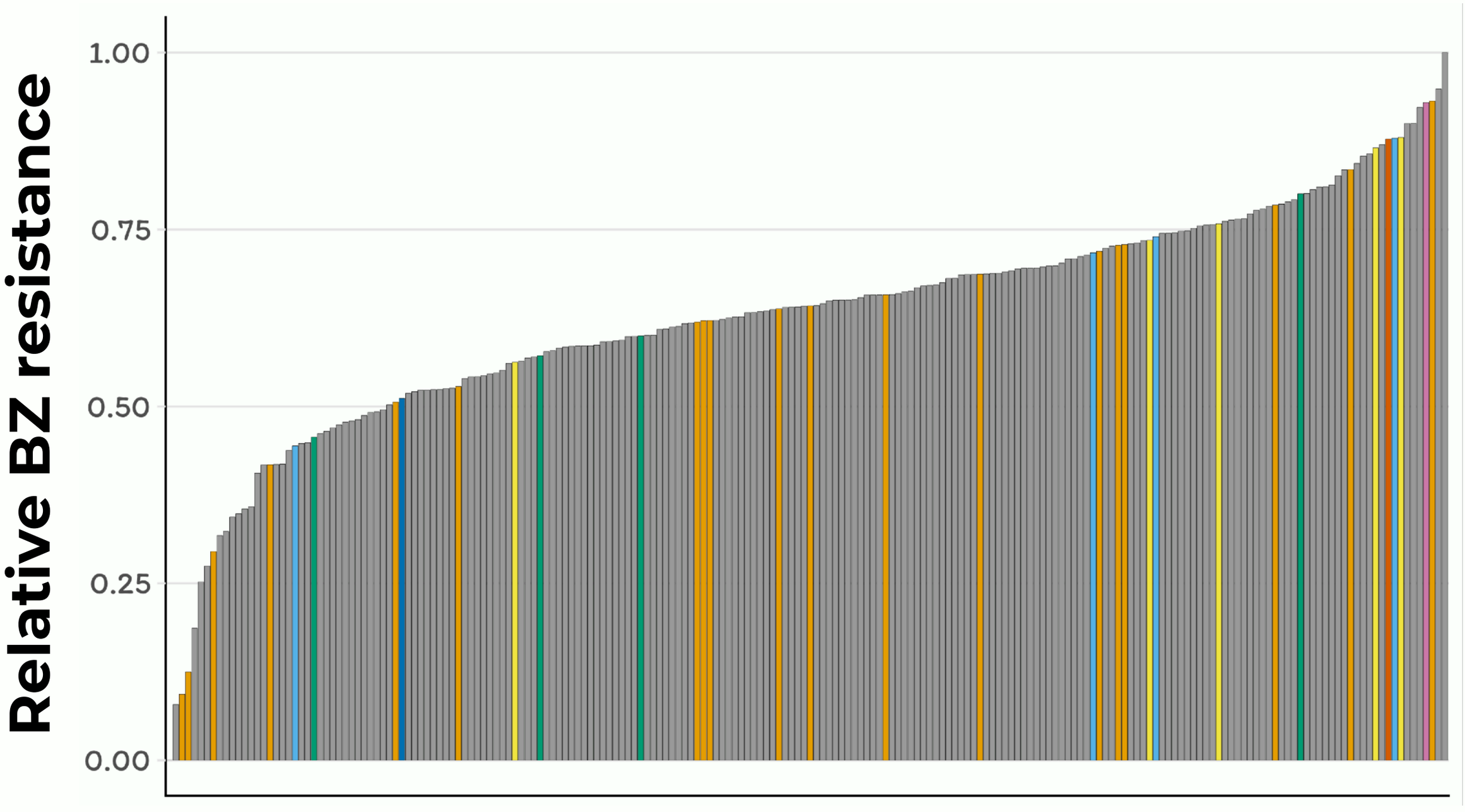
Strains with *ben-1* variants are resistant to BZs



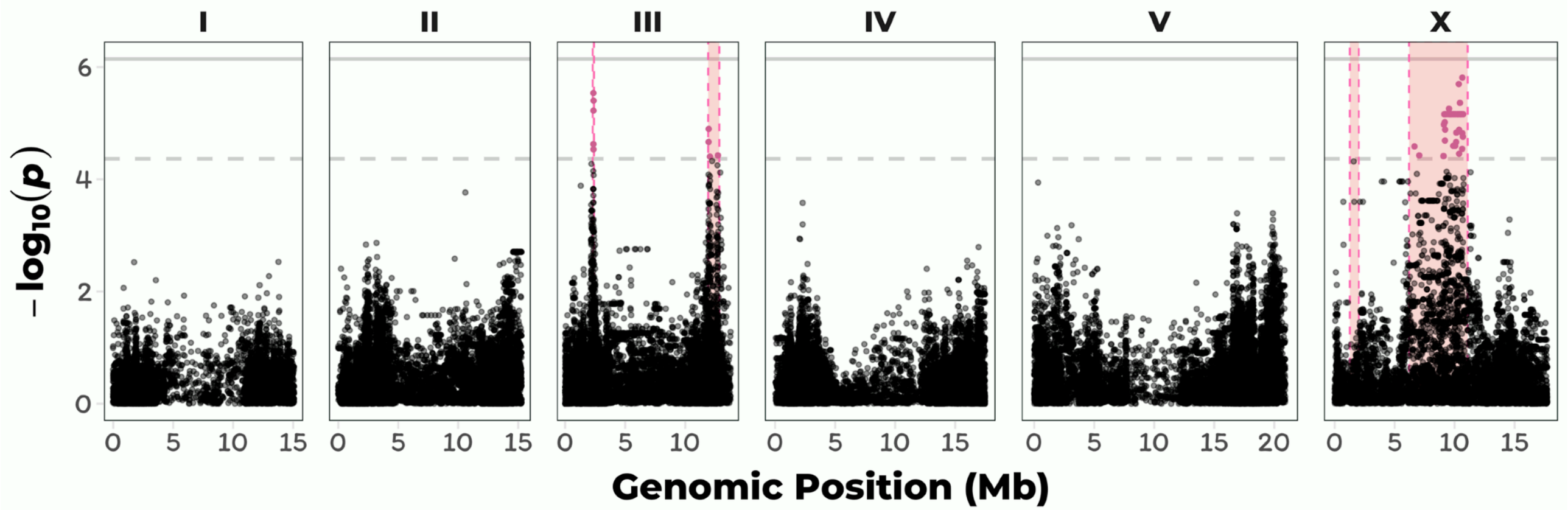
Correcting for the presence of *ben-1* variants



Correcting for the presence of *ben-1* variants

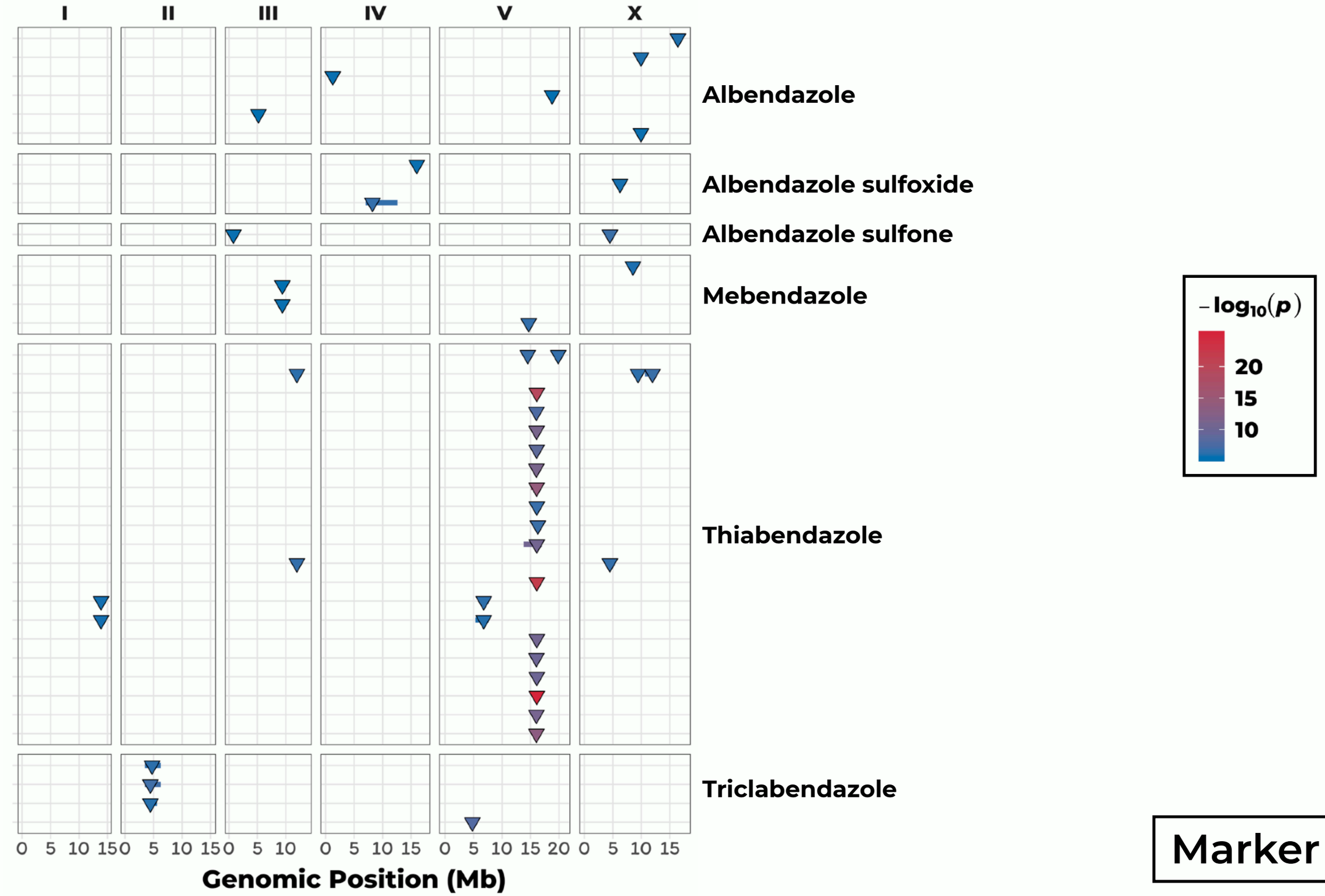


Common variation on chromosomes III and X is correlated with BZ resistance



Marker

We still have a lot to learn from *C. elegans*



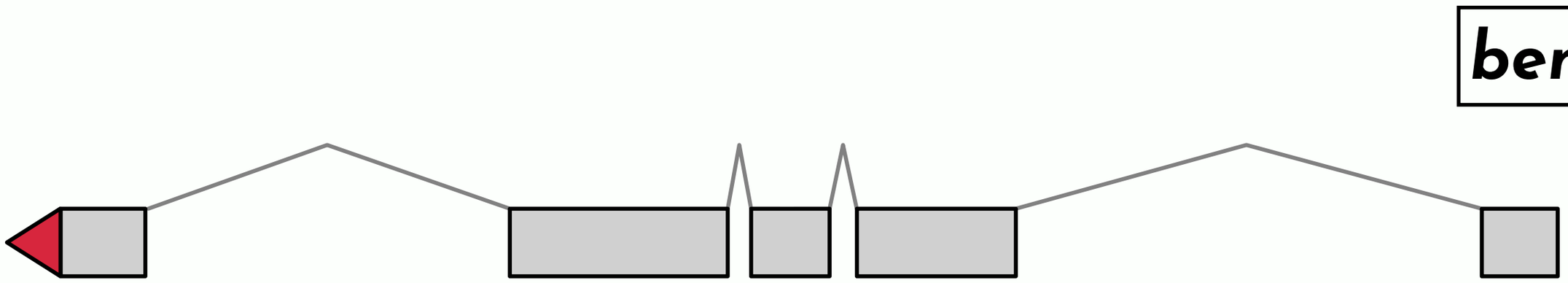
1. Parasites with β-tubulin resistance alleles exhibit >10,000 fold variation in BZ response

- Can we identify additional resistance alleles?

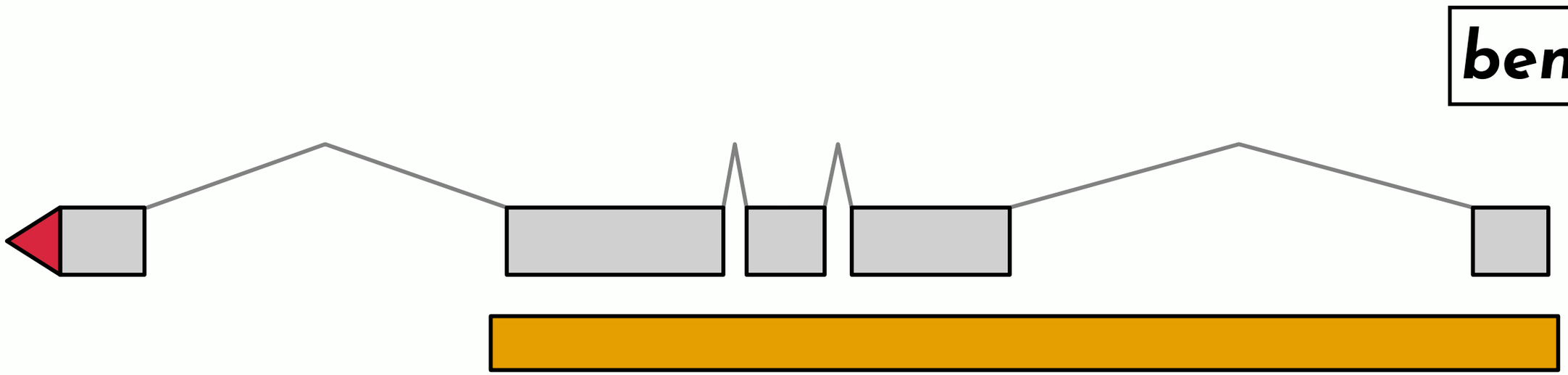
2. Predicted β-tubulin resistance alleles have never been experimentally validated

- Can we use *C. elegans* to experimentally validate predicted resistance alleles?

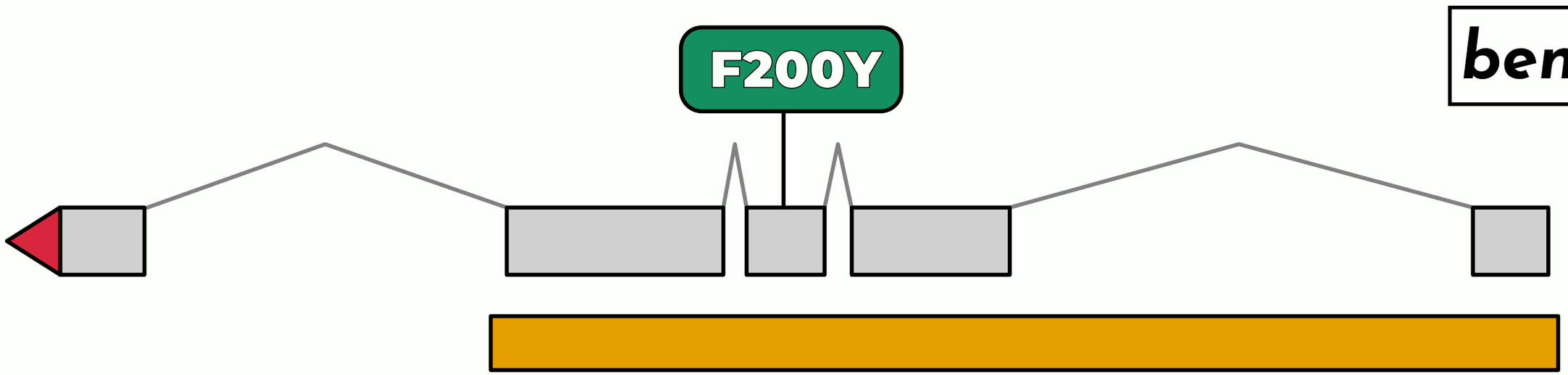
Establishing a causal connection between predicted BZ resistance alleles



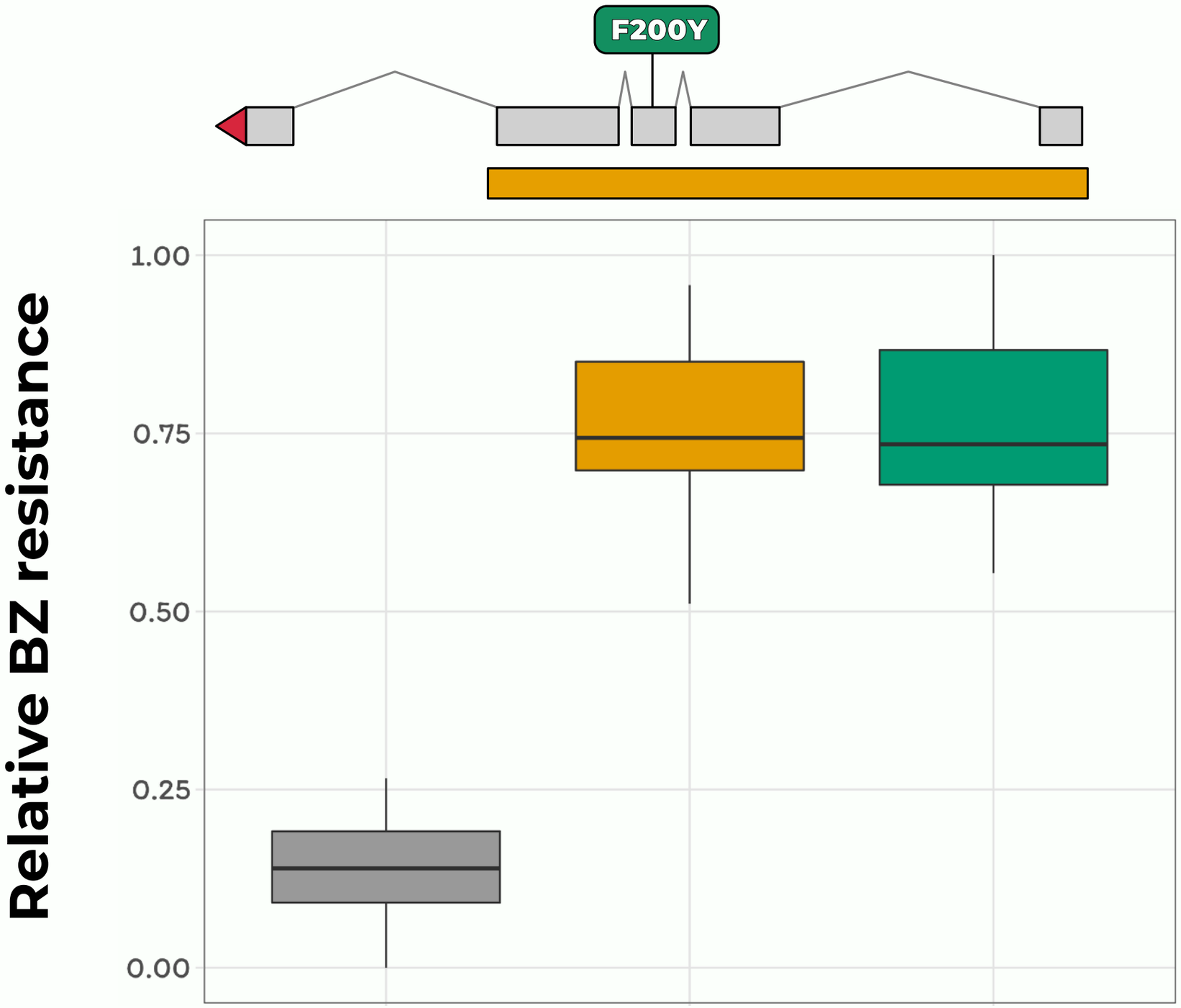
Establishing a causal connection between predicted BZ resistance alleles



Establishing a causal connection between predicted BZ resistance alleles

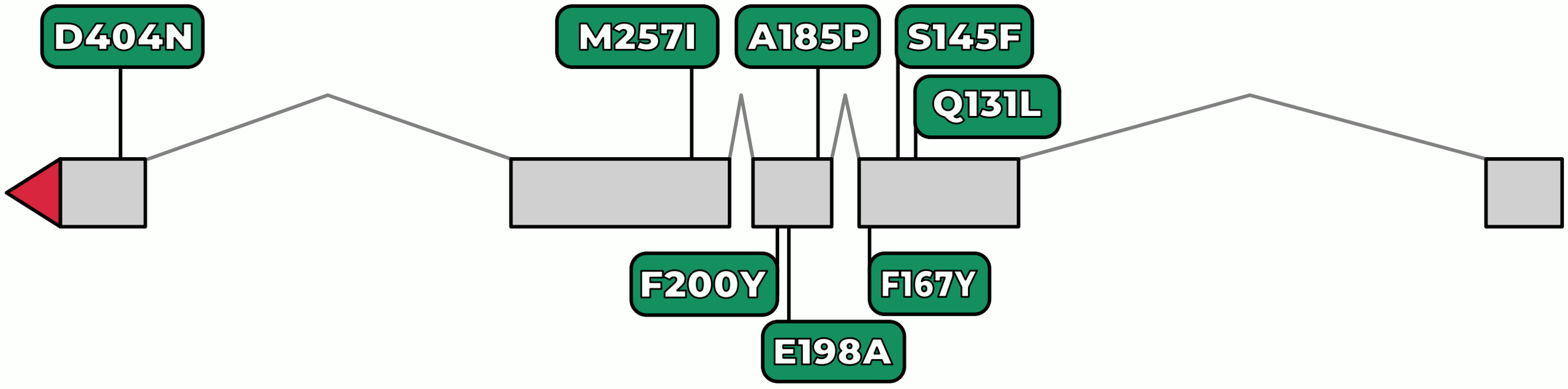


ben-1 alleles confer similar BZ resistance



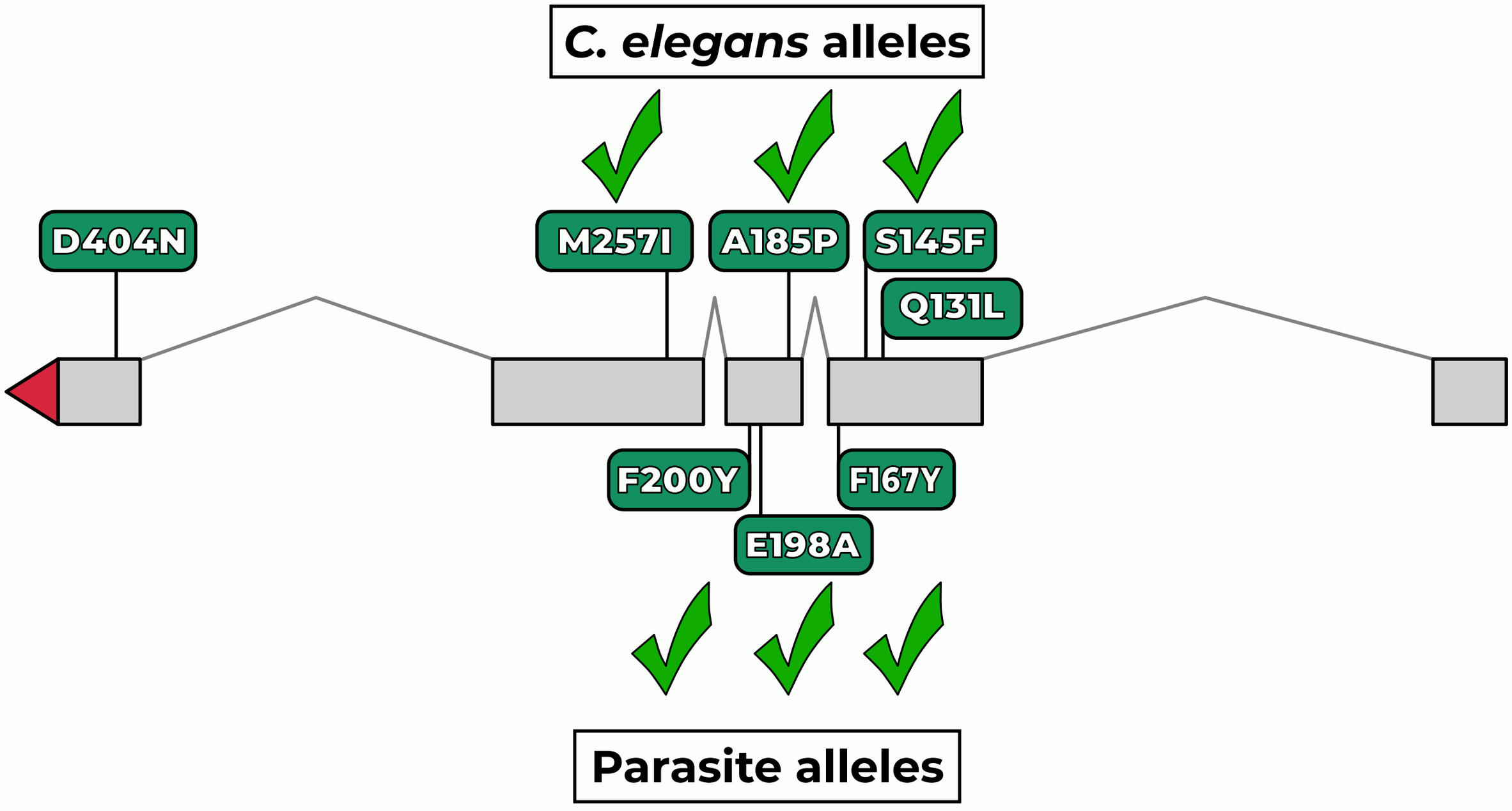
We are constructing additional *ben-1* missense alleles

C. *elegans* alleles



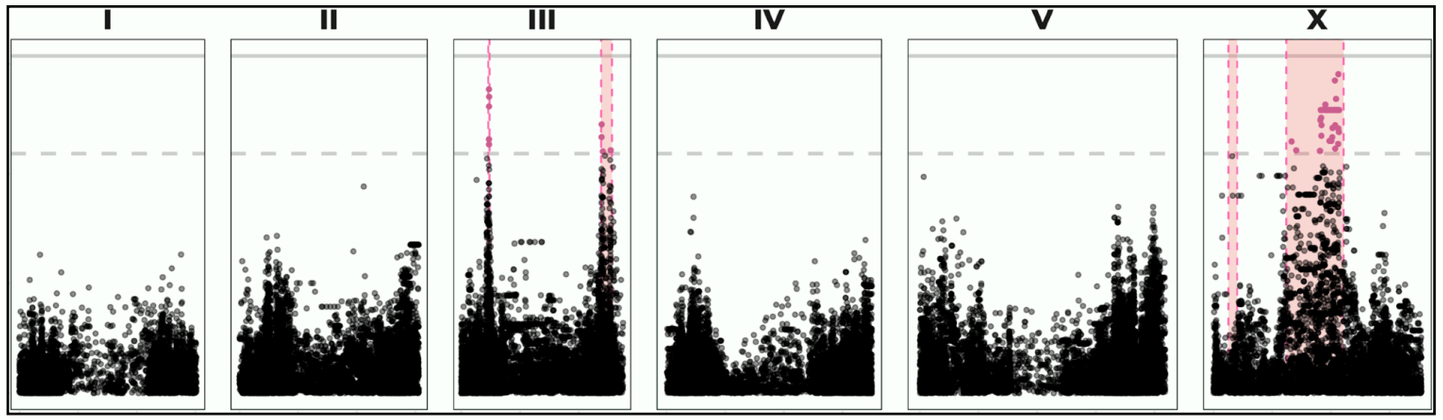
Parasite alleles

We are constructing additional *ben-1* missense alleles



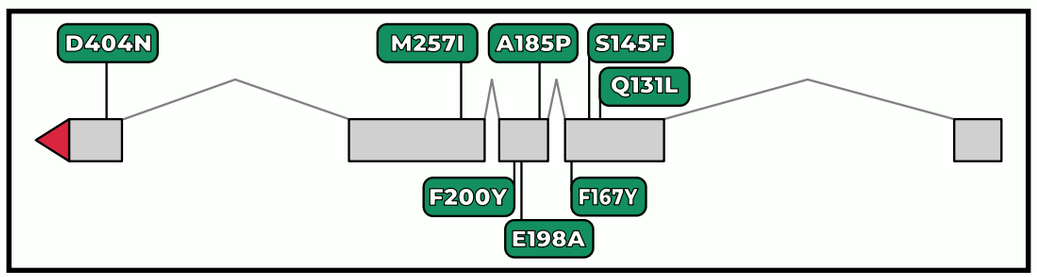
1. **Parasites with β-tubulin resistance alleles exhibit >10,000 fold variation in BZ response**

- **Can we identify additional resistance alleles?**



2. **Predicted β-tubulin resistance alleles have never been experimentally validated**

- **Can we use *C. elegans* to experimentally validate predicted resistance alleles?**



Independent occurrence of putative *ben-1* loss-of-function alleles

Quantifying differences among orthologs

$$\frac{\text{Non-synonymous changes}}{\text{Possible number of non-synonymous changes}}$$

K_A

$$\frac{\text{Synonymous changes}}{\text{Possible number of synonymous changes}}$$

K_S

Quantifying differences among orthologs

Neutral

$$1 = \frac{K_A}{K_S}$$

Quantifying differences among orthologs

Constrained

0

$$\frac{K_A}{K_S}$$

Neutral

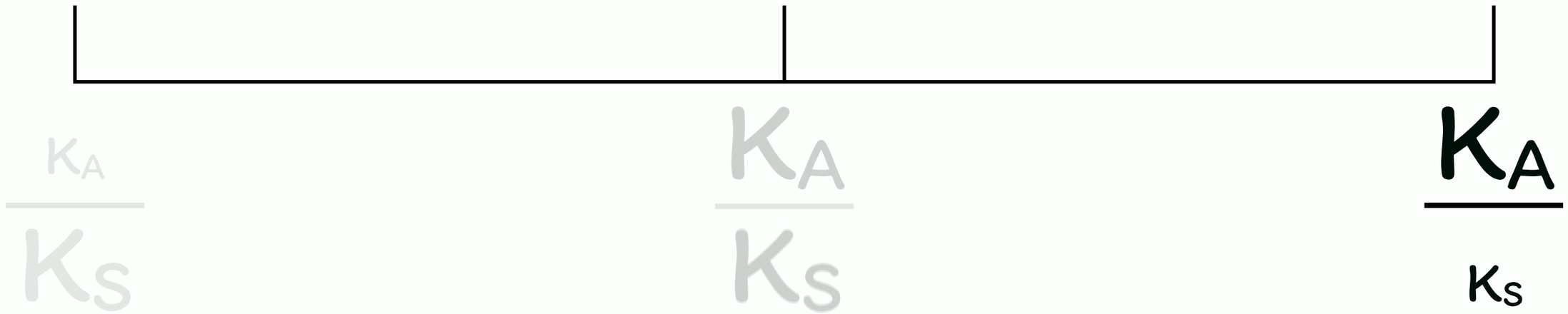
1

$$\frac{K_A}{K_S}$$

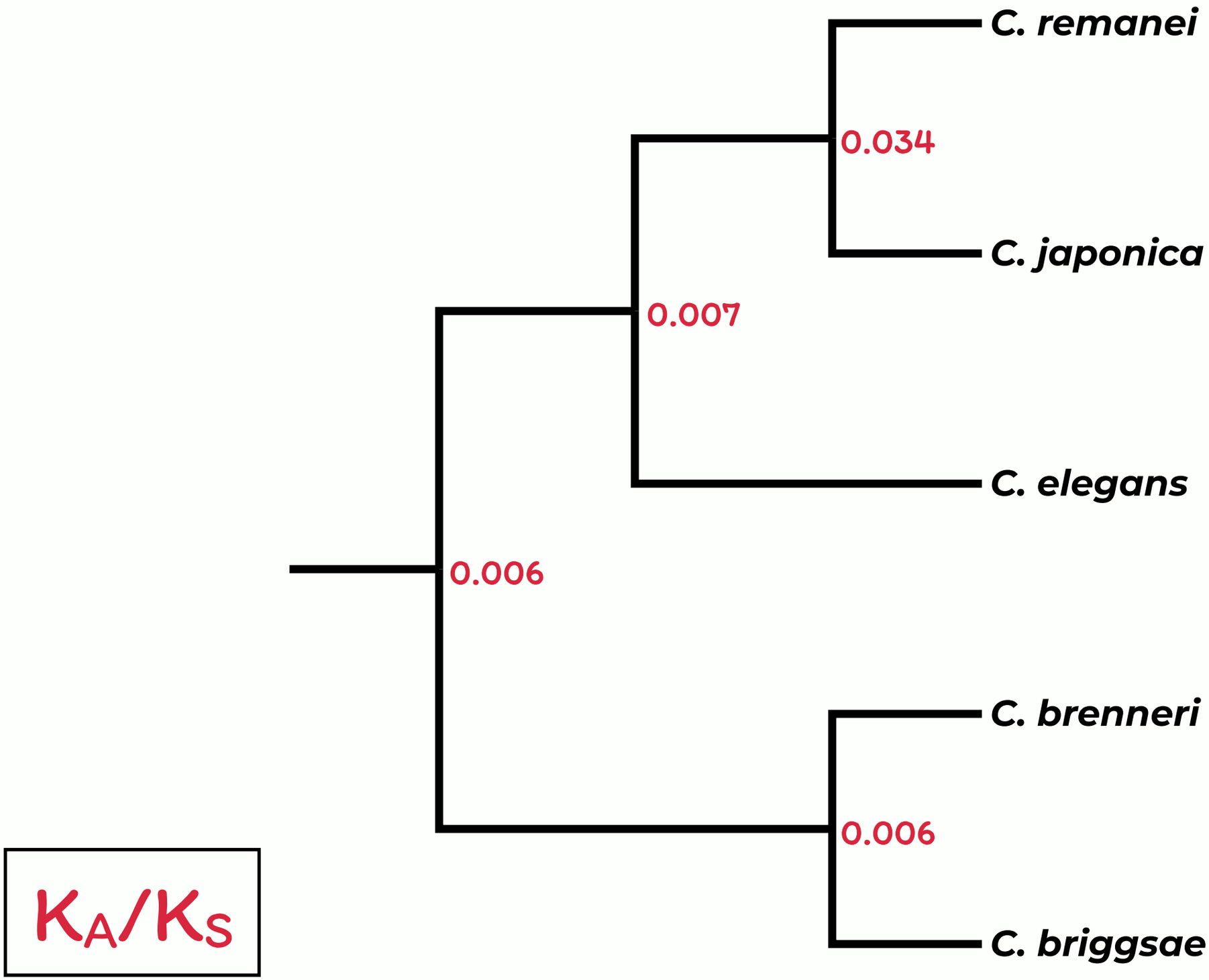
Quantifying differences among orthologs

Constrained **Neutral** **Diversifying**

0 1 2

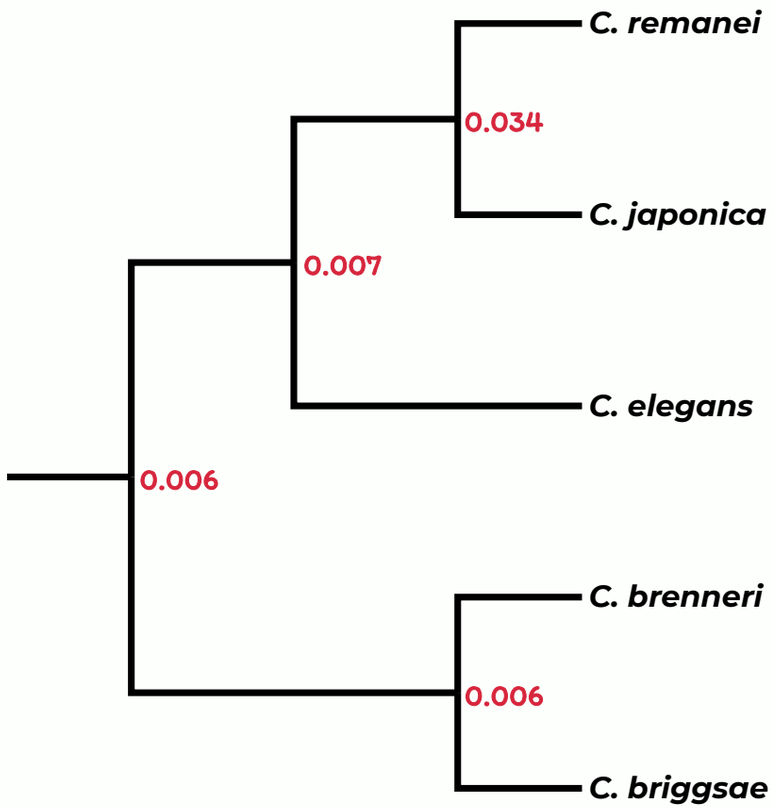


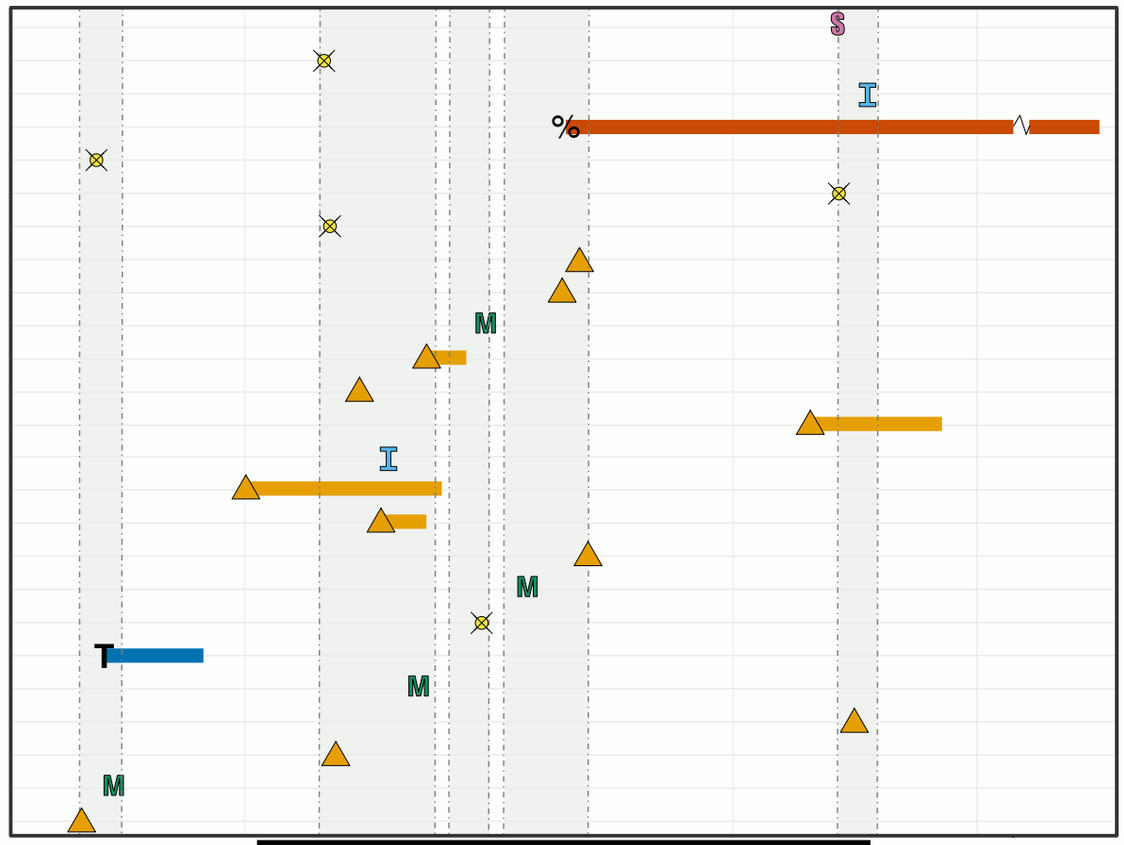
ben-1 is evolutionarily constrained



K_A/K_S

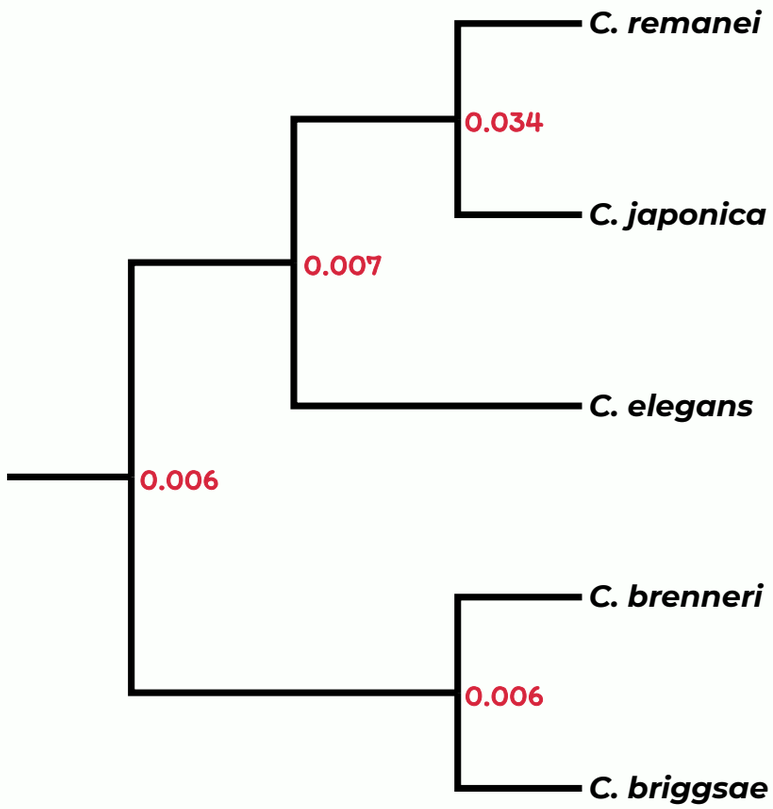
...and yet is highly variable within *C. elegans*

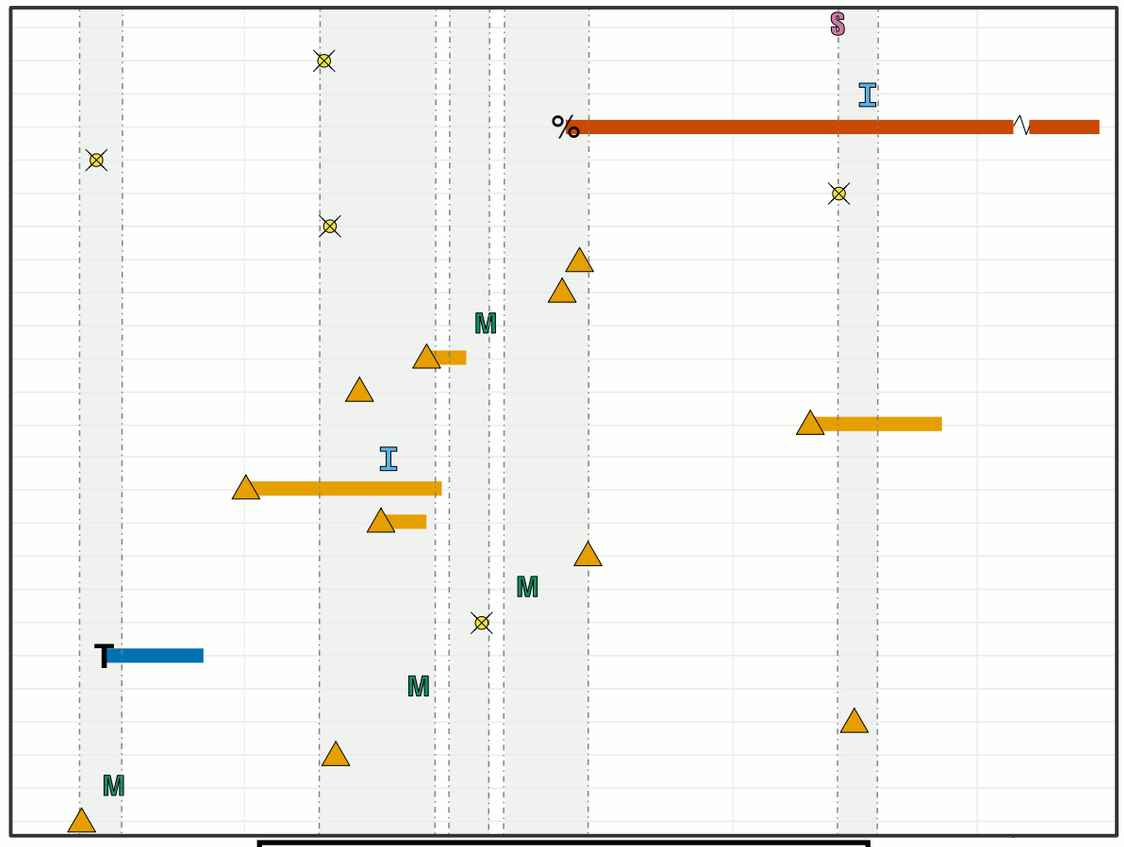




"K_A/K_S" > 2

...and yet is highly variable within *C. elegans*

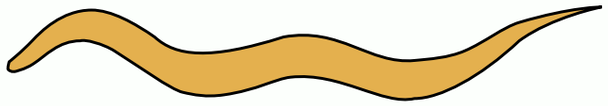




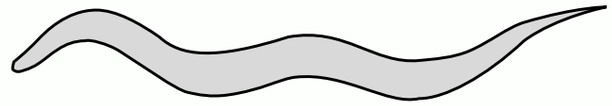
"K_A/K_S" > 2

Does loss of *ben-1* result in a reduction in fitness?

Competition assay to detect fitness effects of *ben-1* alleles

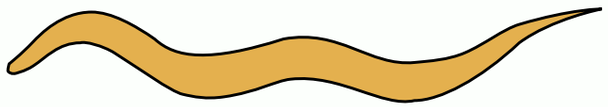


***ben-1* deletion strain**

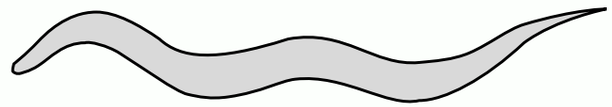


Barcoded wild-type *ben-1*

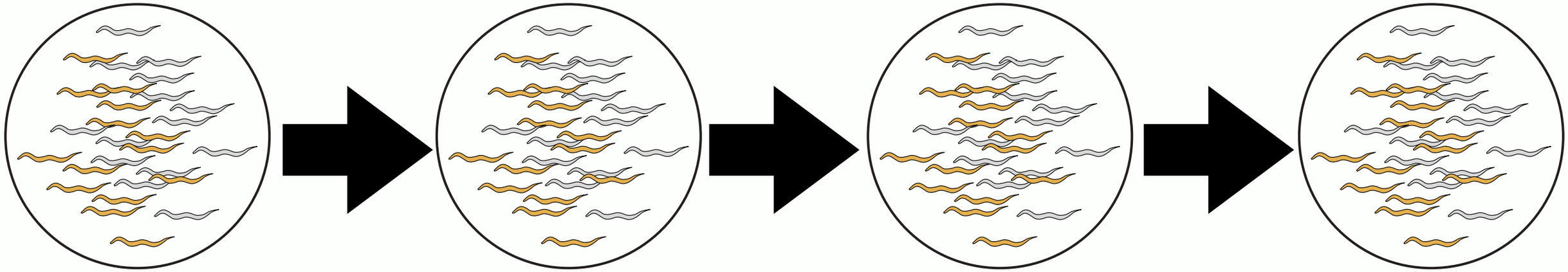
Competition assay to detect fitness effects of *ben-1* alleles



***ben-1* deletion strain**

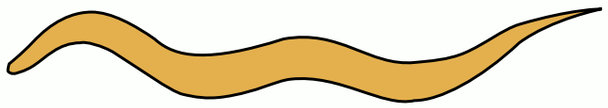


Barcoded wild-type *ben-1*

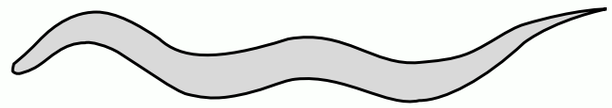


Generation

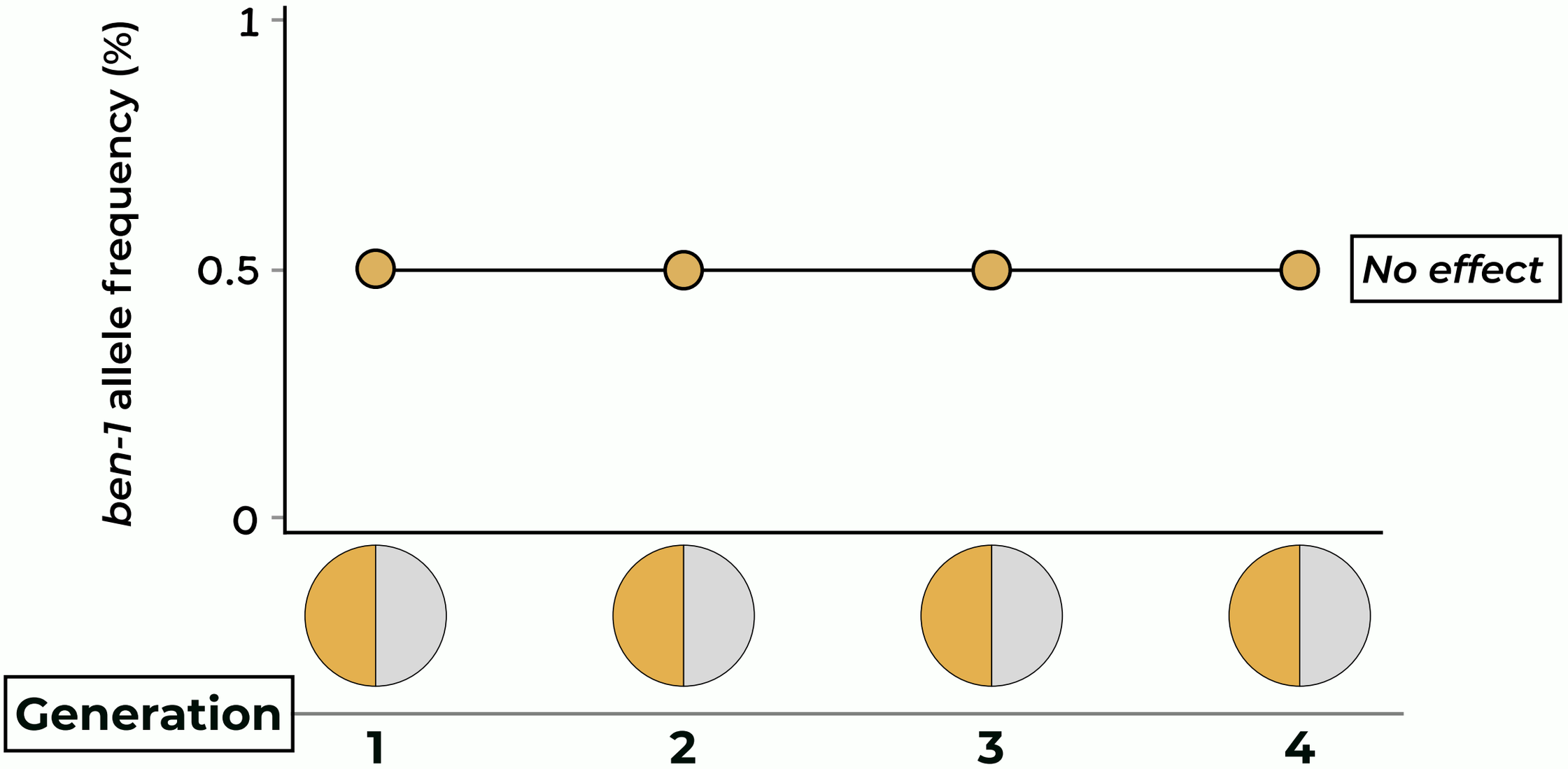
Competition assay to detect fitness effects of *ben-1* alleles



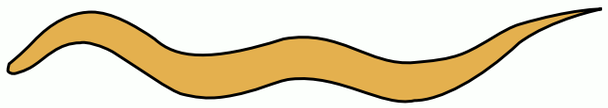
***ben-1* deletion strain**



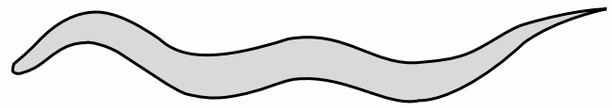
Barcoded wild-type *ben-1*



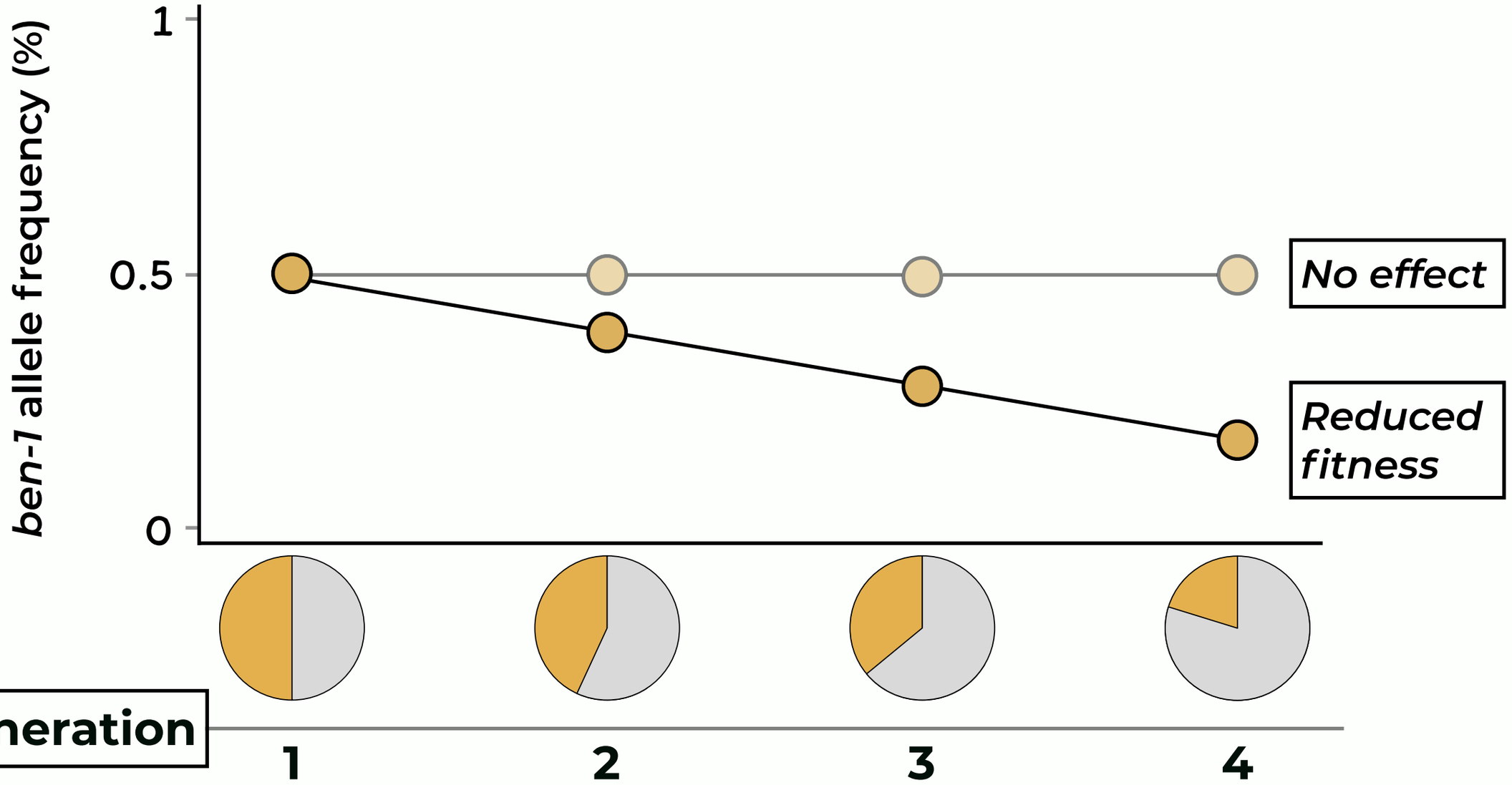
Competition assay to detect fitness effects of *ben-1* alleles



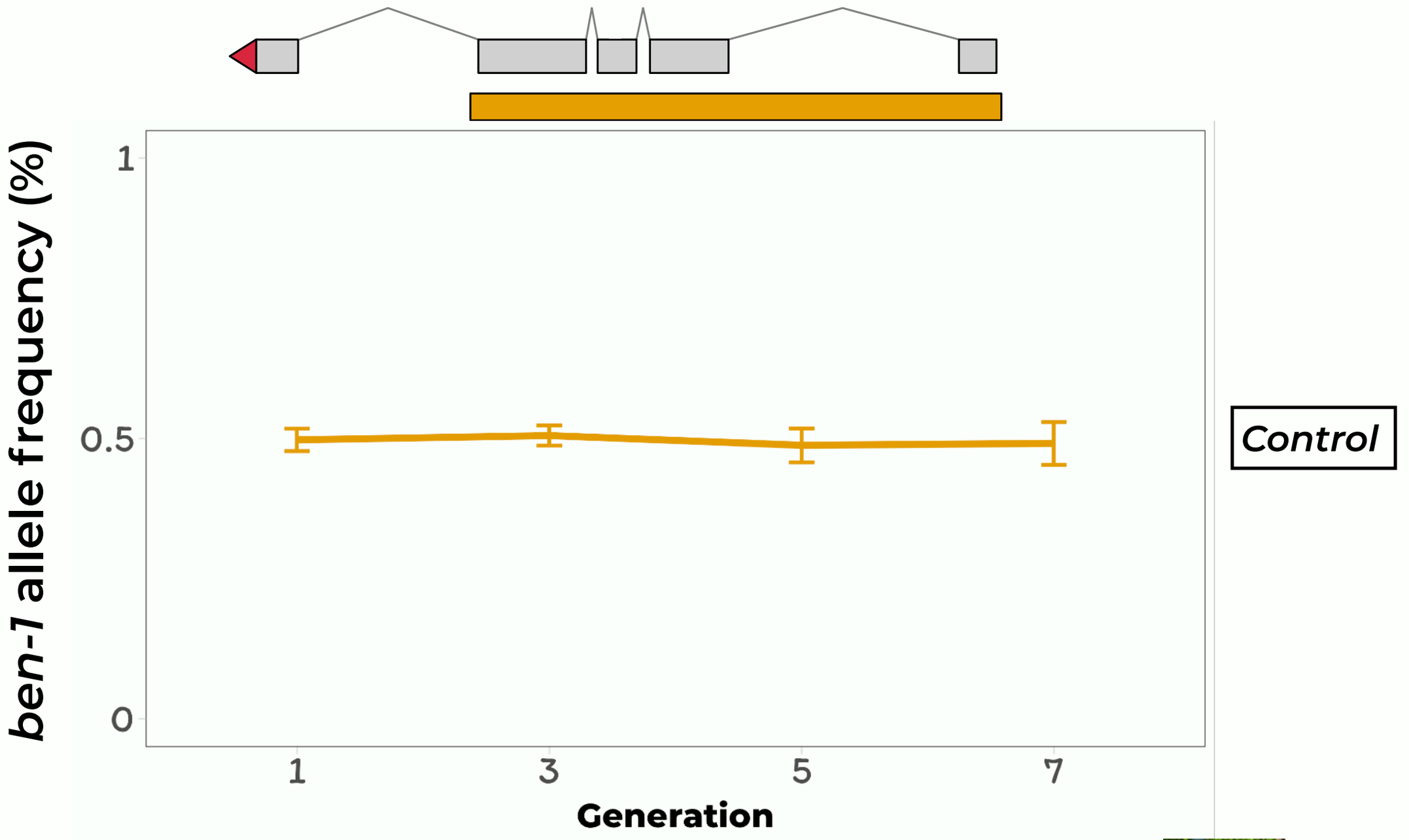
***ben-1* deletion strain**



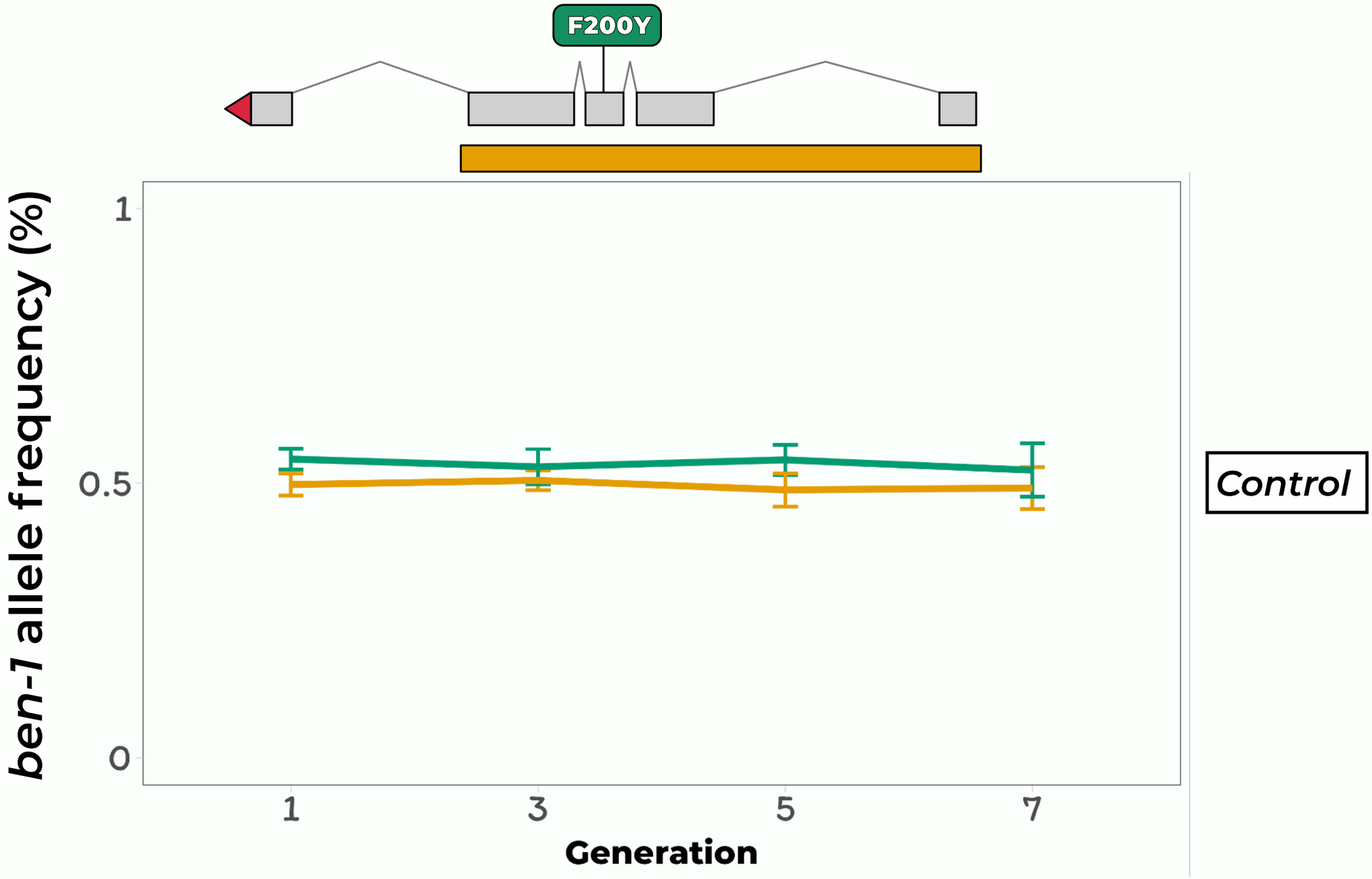
Barcoded wild-type *ben-1*



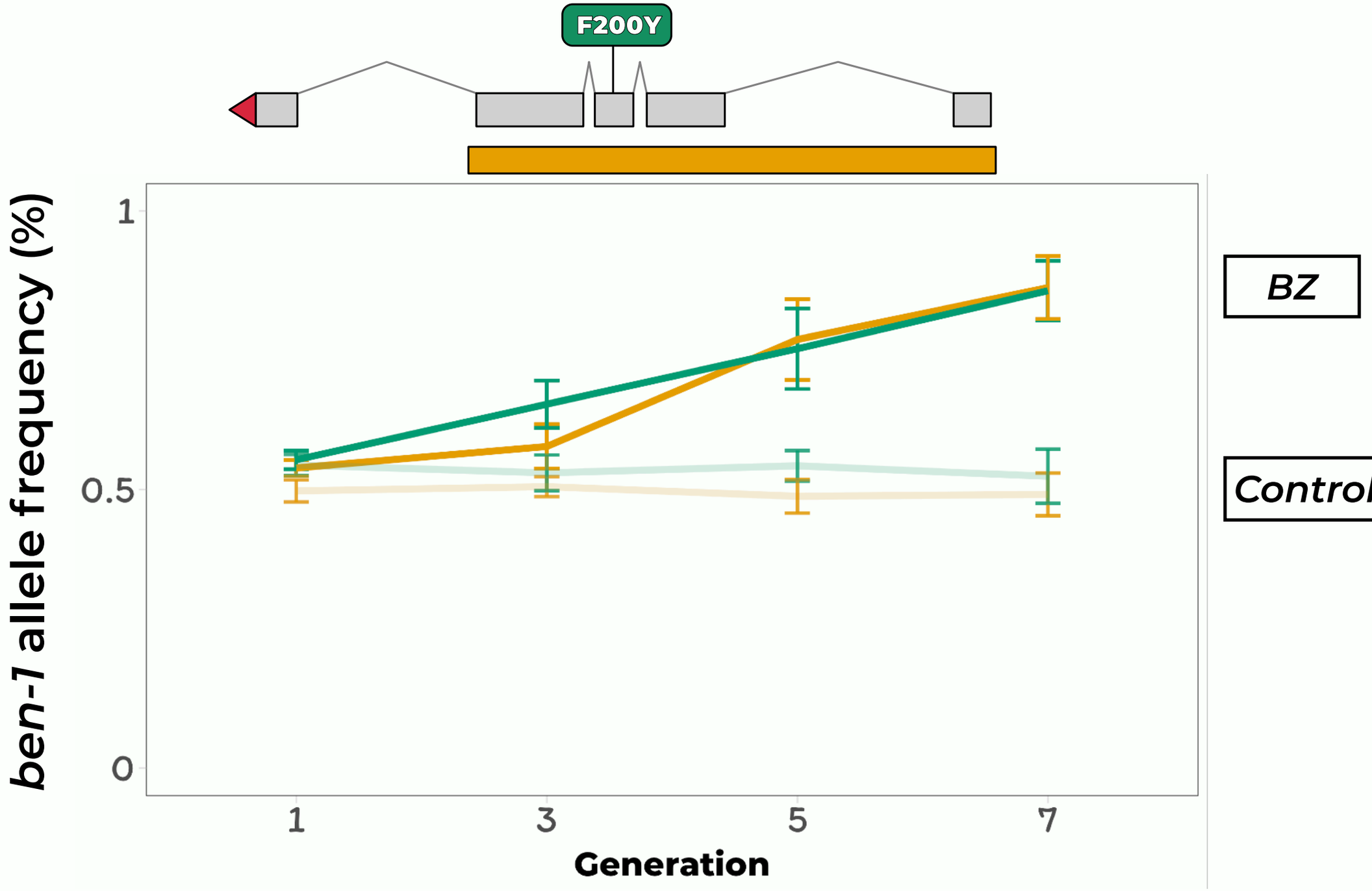
No observable fitness consequences of the *ben-1* deletion



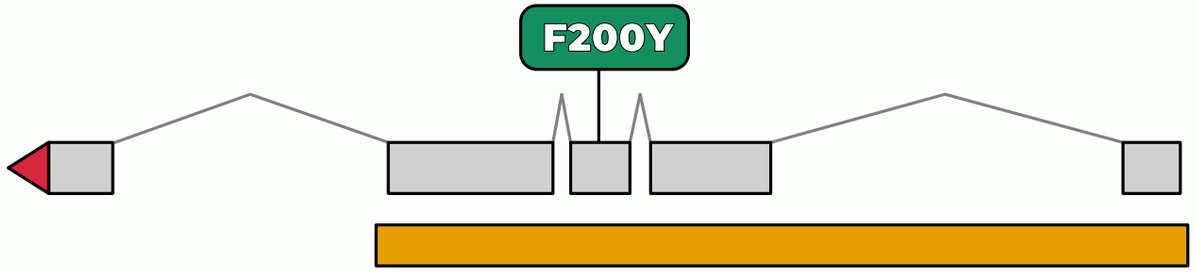
No observable fitness consequences of the *ben-1* F200Y allele

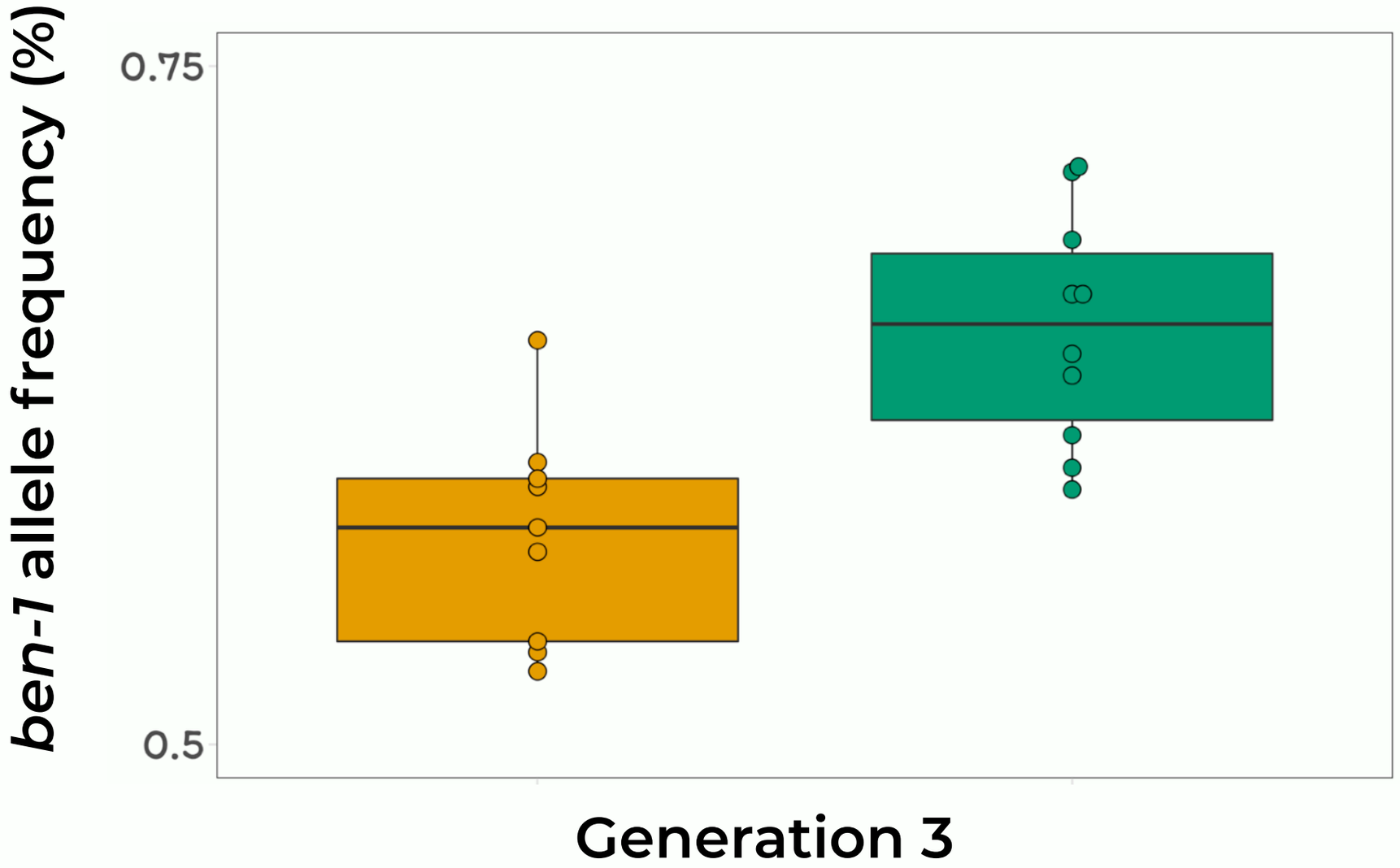


ben-1 alleles are enriched after BZ exposure



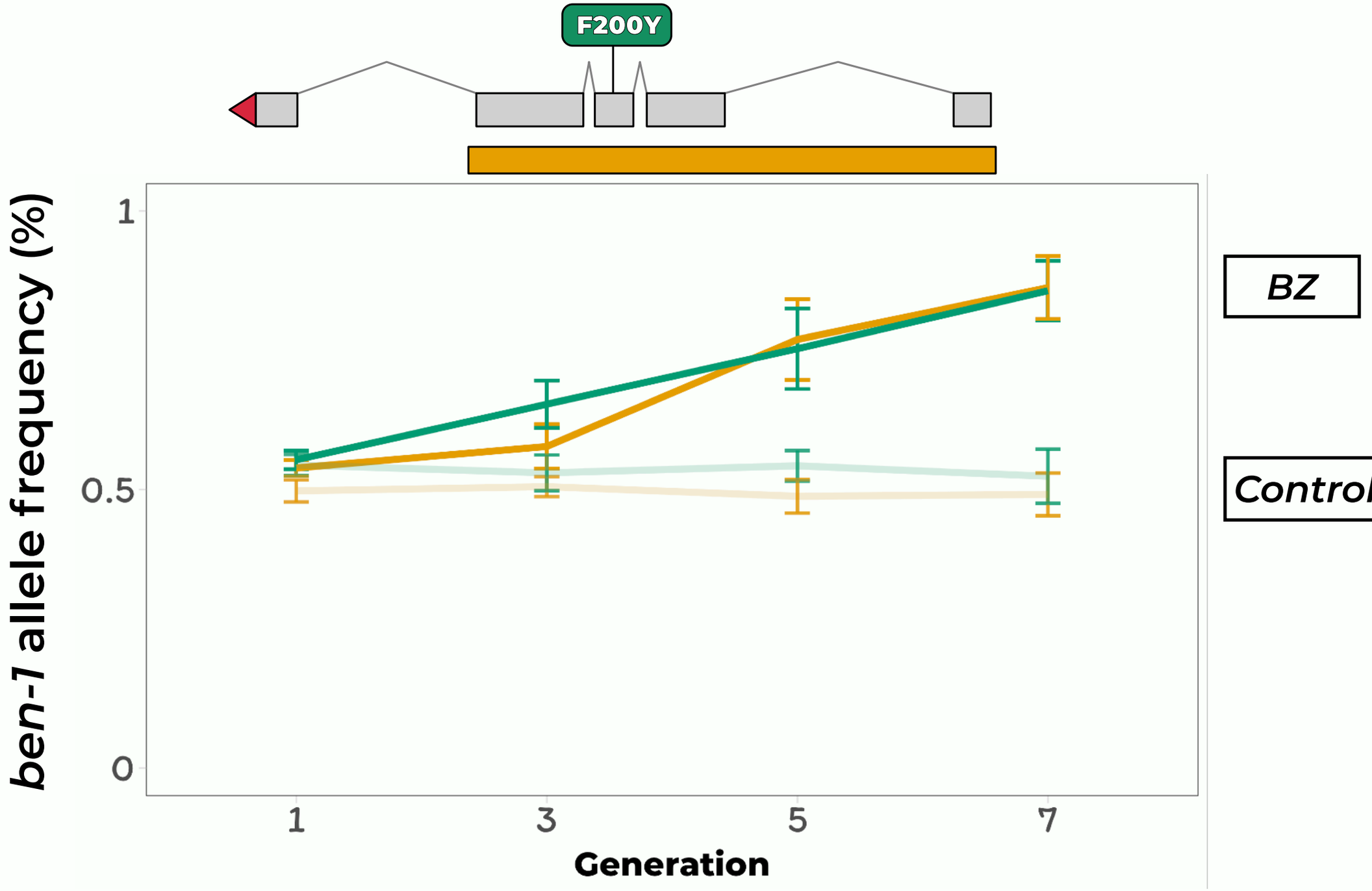
F200Y exhibits greater BZ resistance at the third generation

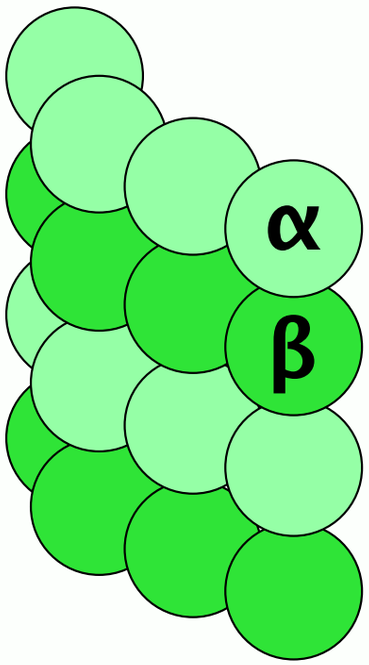




$p = 0.001$

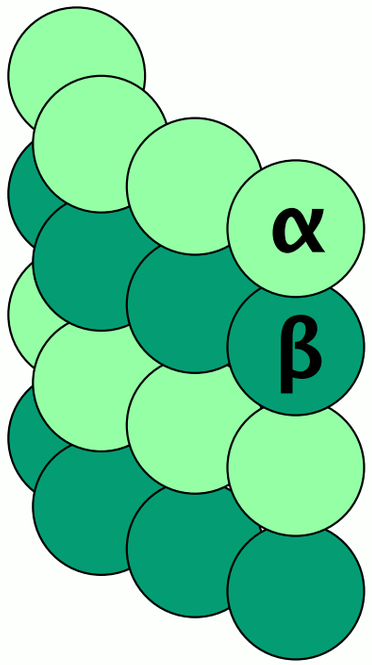
ben-1 alleles are enriched after BZ exposure





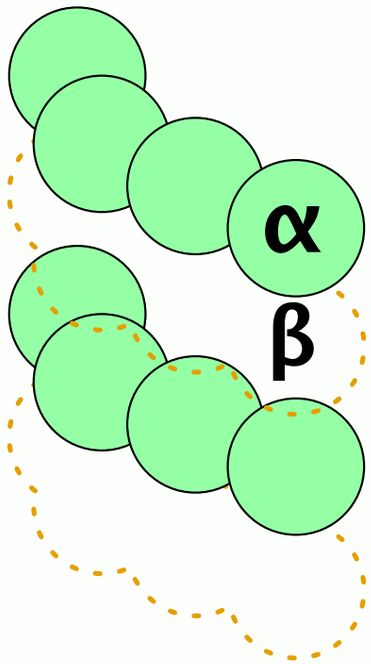
wt

Sensitive



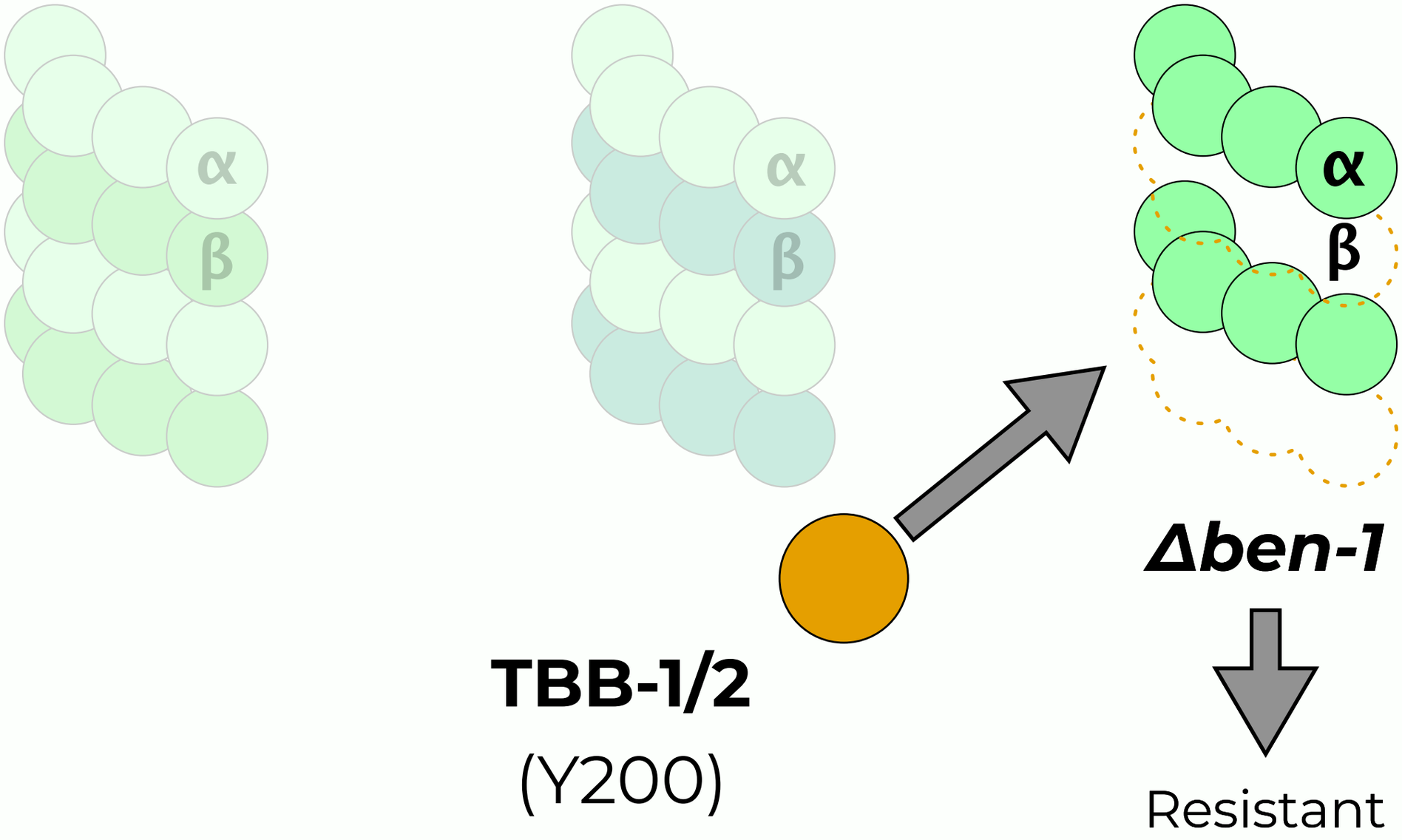
F200Y

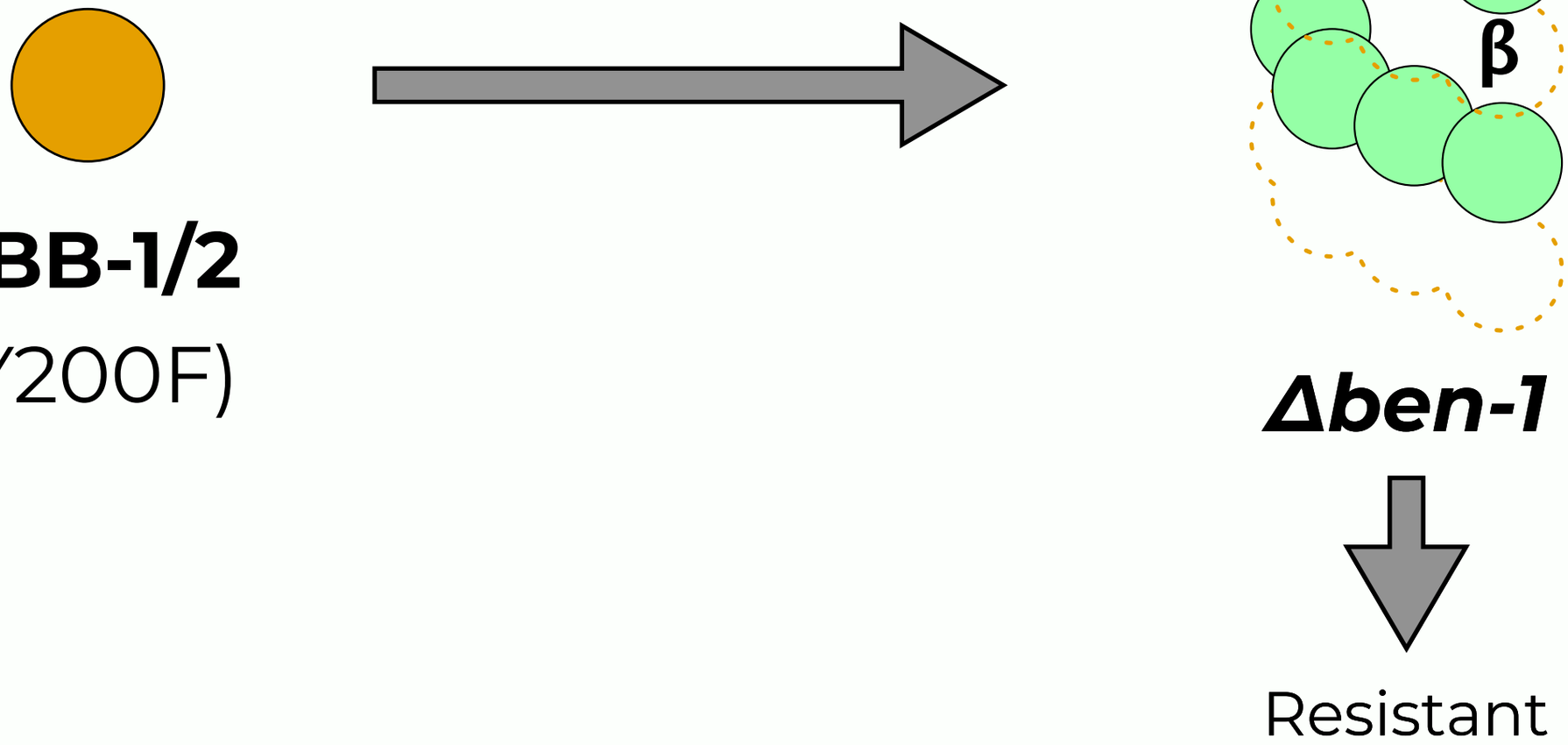
Resistant



Δben-1

Resistant


α
β
α
β
α
β
TBB-1/2
(Y200)
Δben-1
Resistant

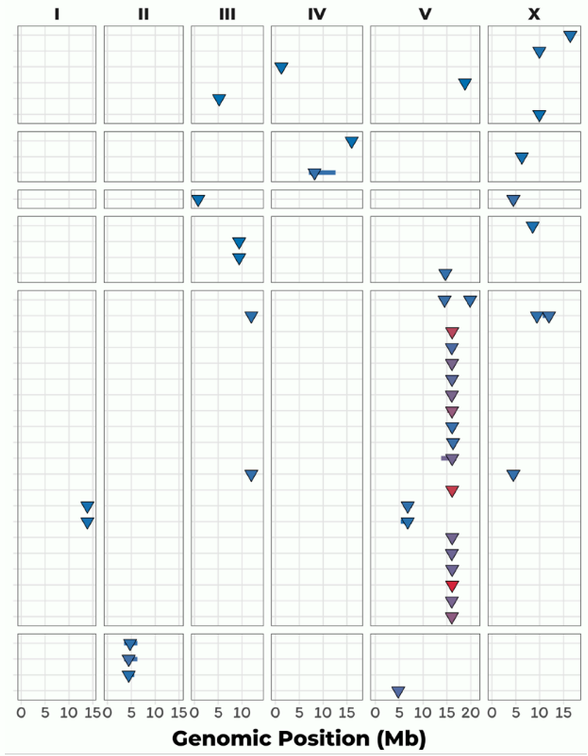


TBB-1/2
(Y200F)

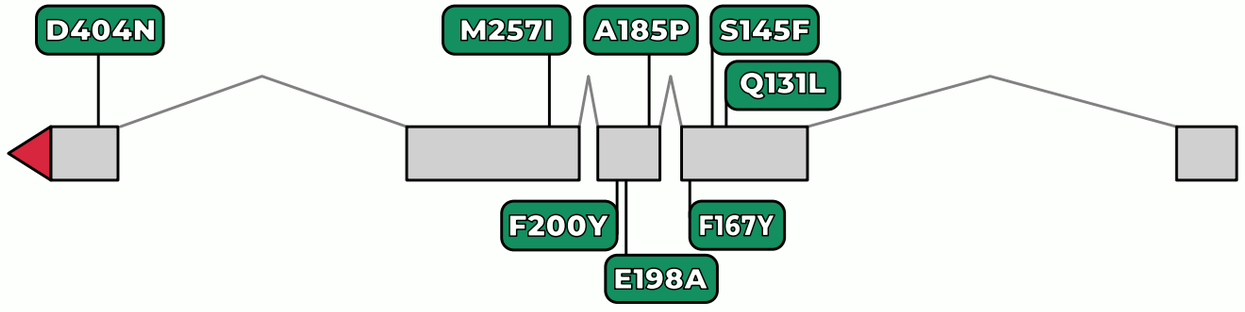
Δben-1

Resistant

Additional BZ resistance alleles



C. elegans is a powerful model for testing BZ resistance alleles



We still have a lot to learn about the evolutionary history and function of β-tubulins