



DETERMINING THE MECHANISMS BY WHICH NATURAL GENETIC  
VARIATION IN *CAENORHABDITIS ELEGANS* CONTRIBUTE TO  
PHENOTYPIC VARIABILITY IN RESPONSE TO TOPOISOMERASE II  
POISONS

Stefan Zdraljevic

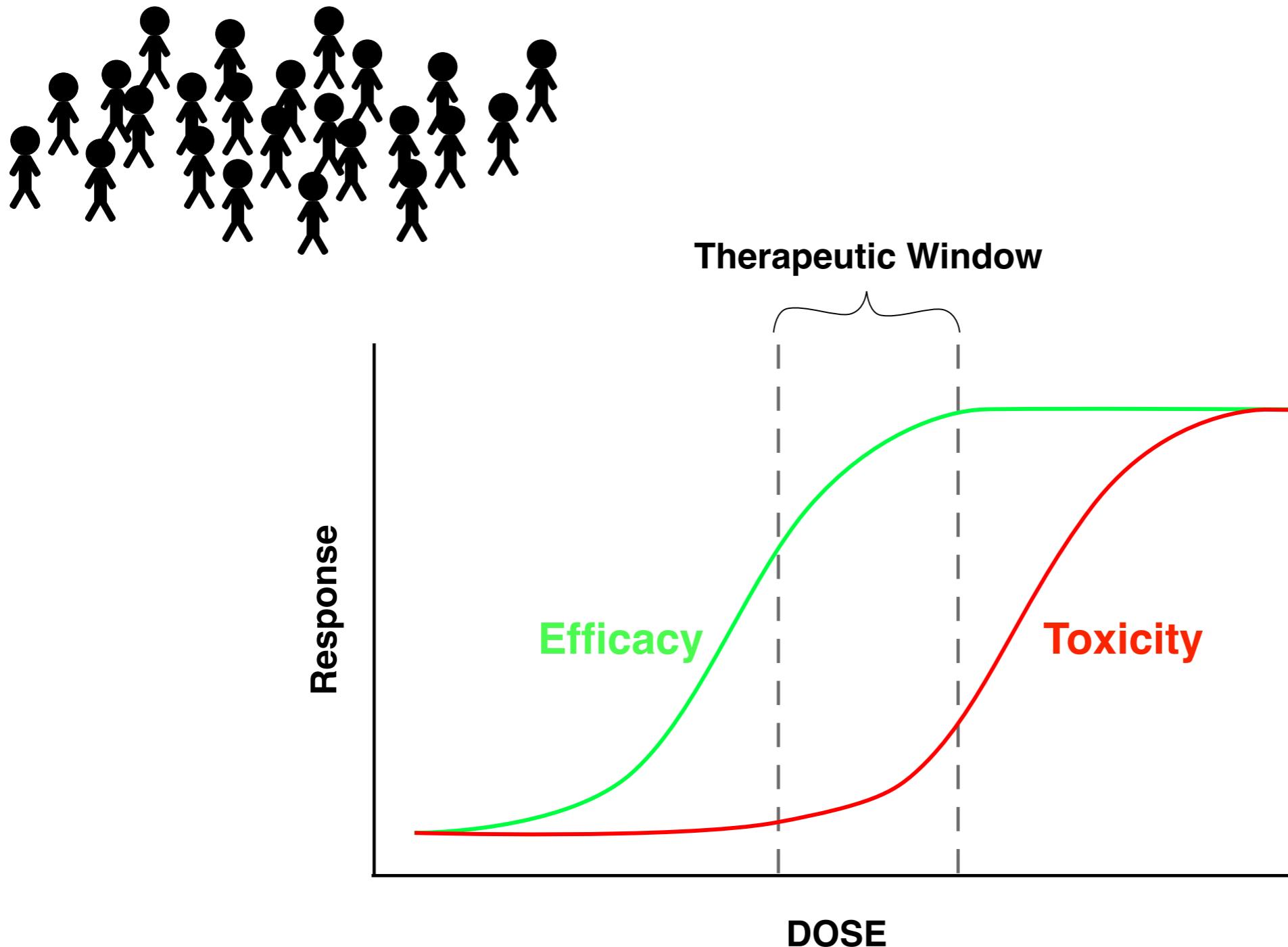
Advisor – Erik C. Andersen, Ph.D.

June 2nd, 2015

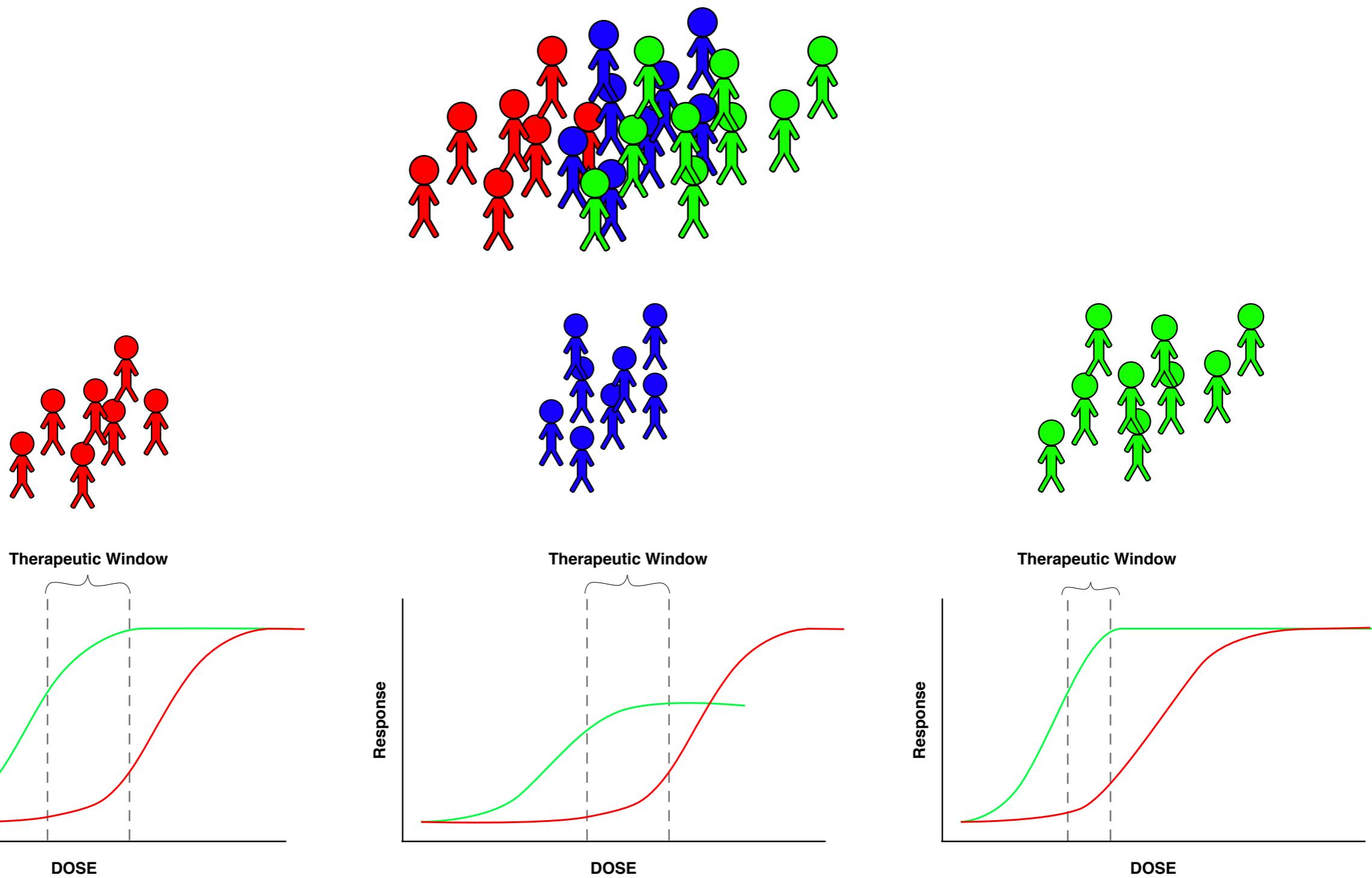
# Outline

- Motivation
- Preliminary Results
- Specific Aims

# Therapeutic Window



# Identifying an Ideal Therapeutic Window



# Sources of Variation in Response to Therapeutics

Adherence to treatment (both patients and doctors)

Medication access

Drug-drug interactions

Drug transport/metabolism

Genetic variation in drug targets

Environment

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Environment

**Difficult to control**

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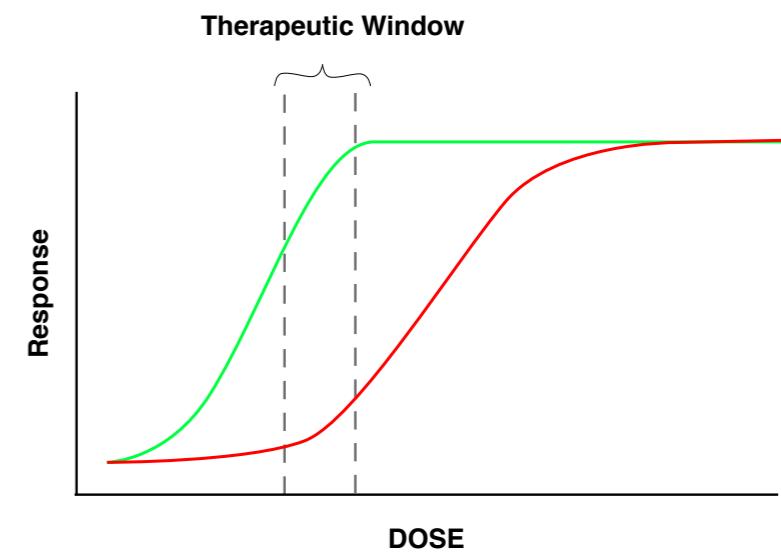
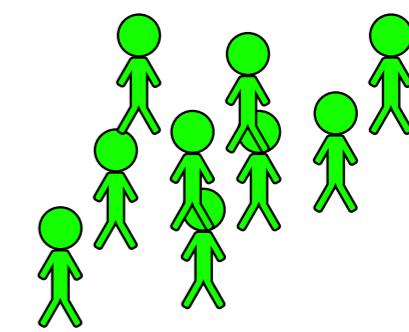
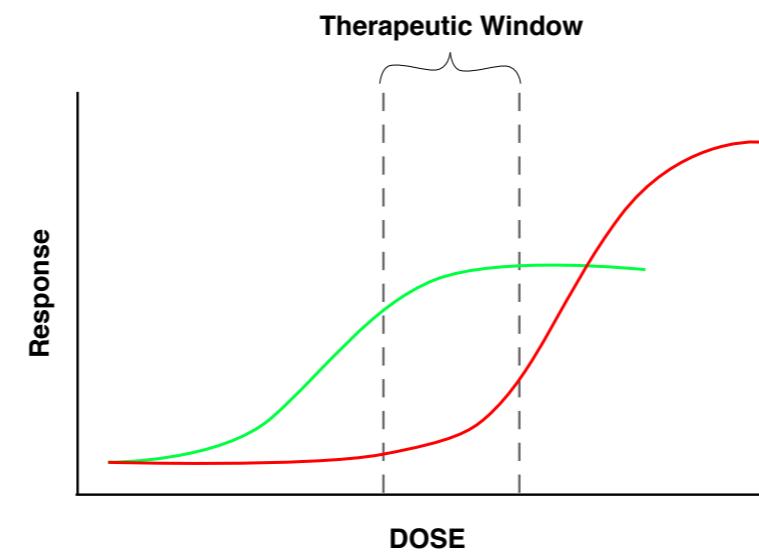
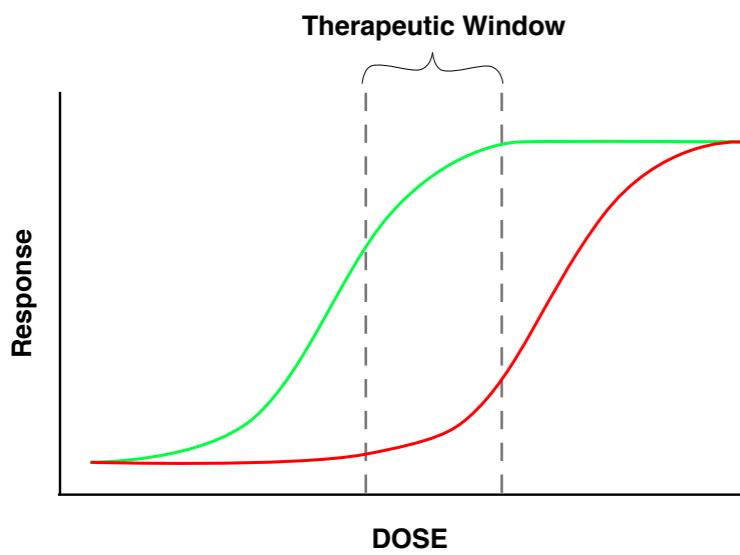
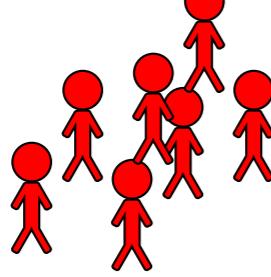
Genetic variation in drug targets

Environment



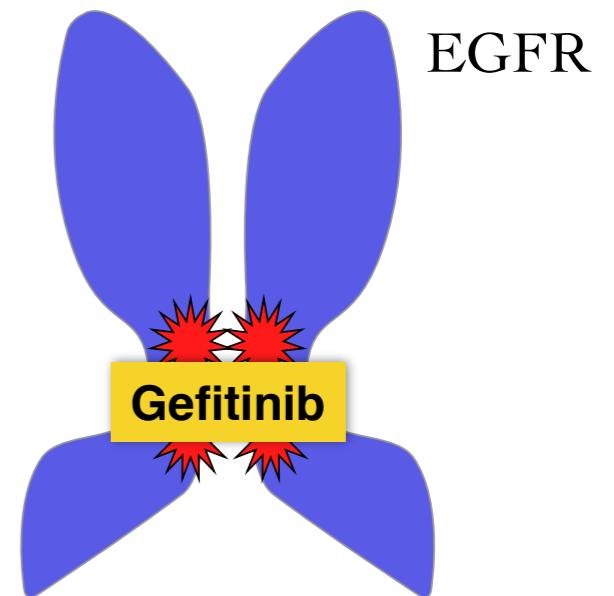
**Influenced by genetic factors**

# Gene and Environmental Factors Contribute to Differences in Therapeutic Windows

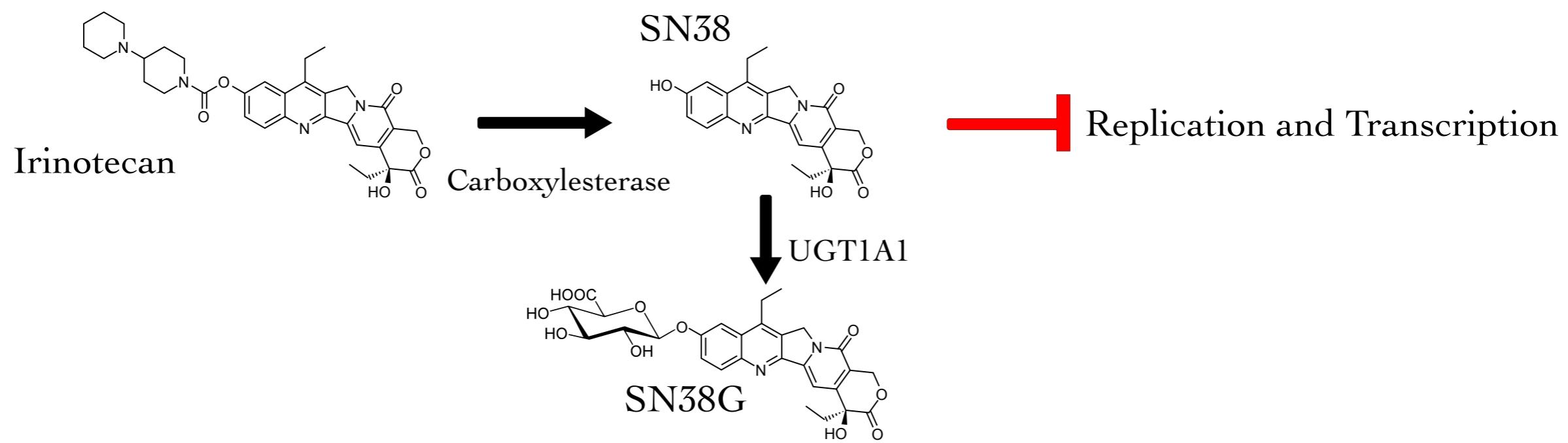


# Examples of Clinically Relevant Variants

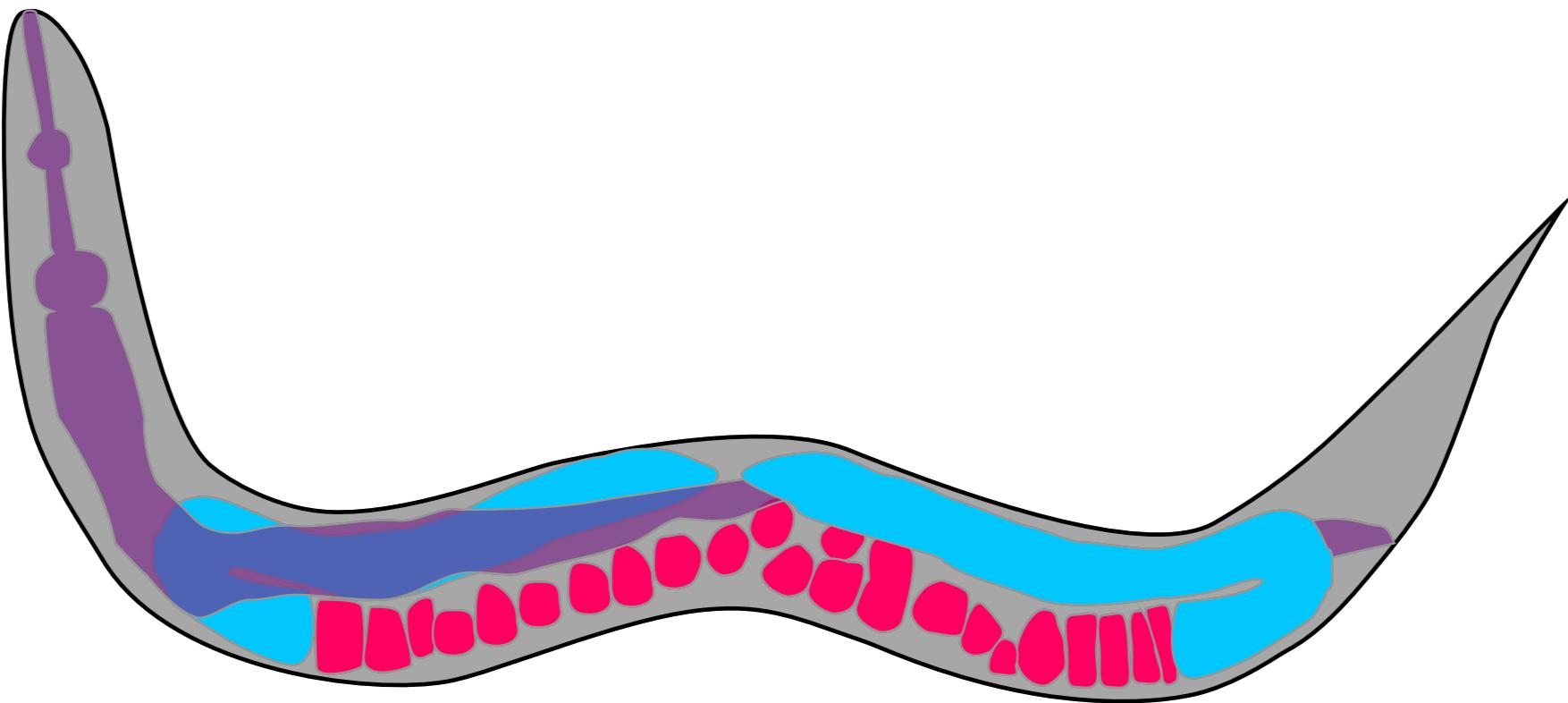
Genetic variation in drug targets



Genetic variation in drug metabolism



“An ideal experiment would involve carefully observing thousands of patients that undergo treatment from start to finish with only one chemotherapeutic medication without switching the dose, even if side effects become severe.”



Easy to handle

Low cost

High replication of experiments

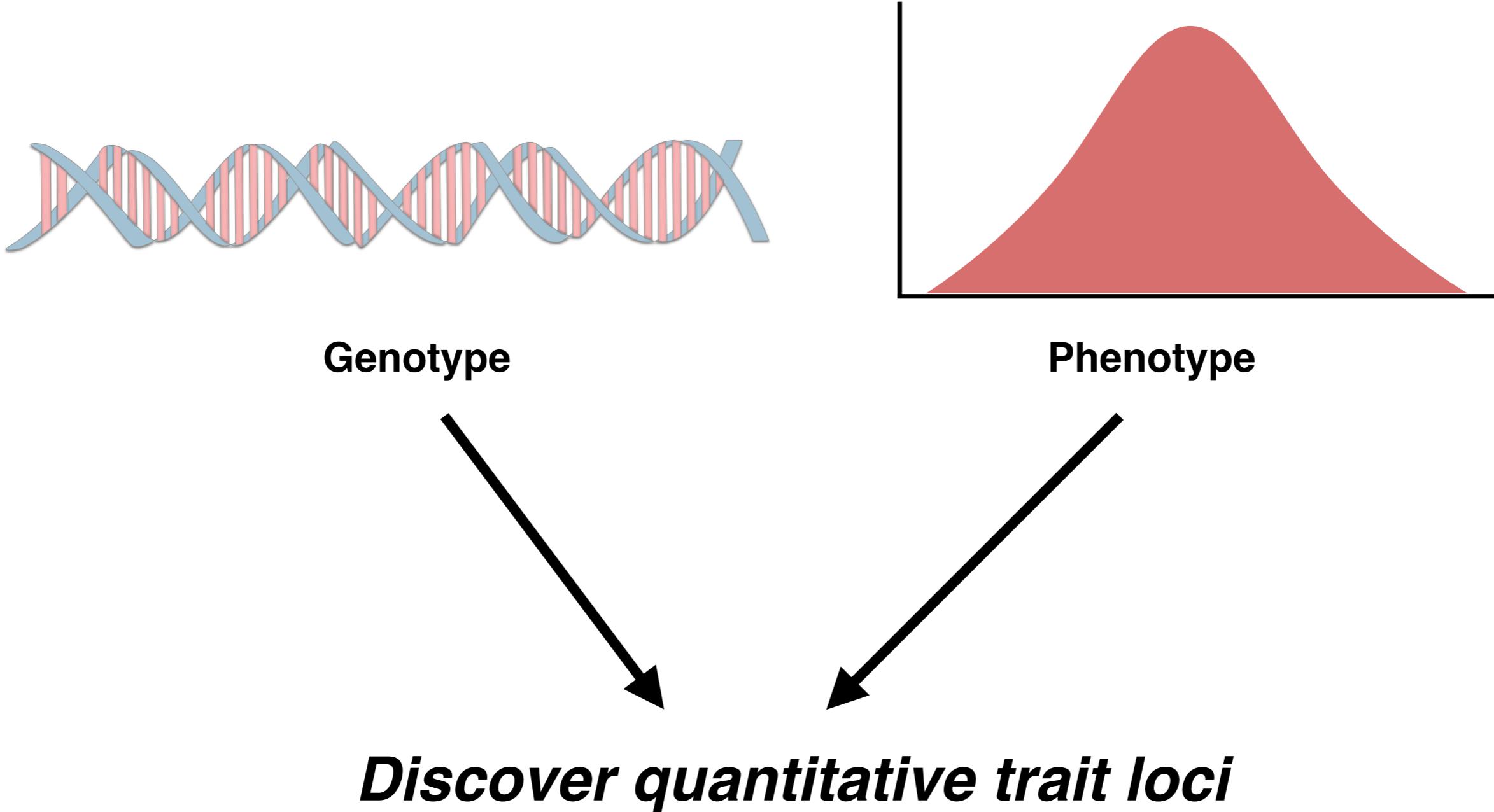
Short lifespan

Numerous genetic tools

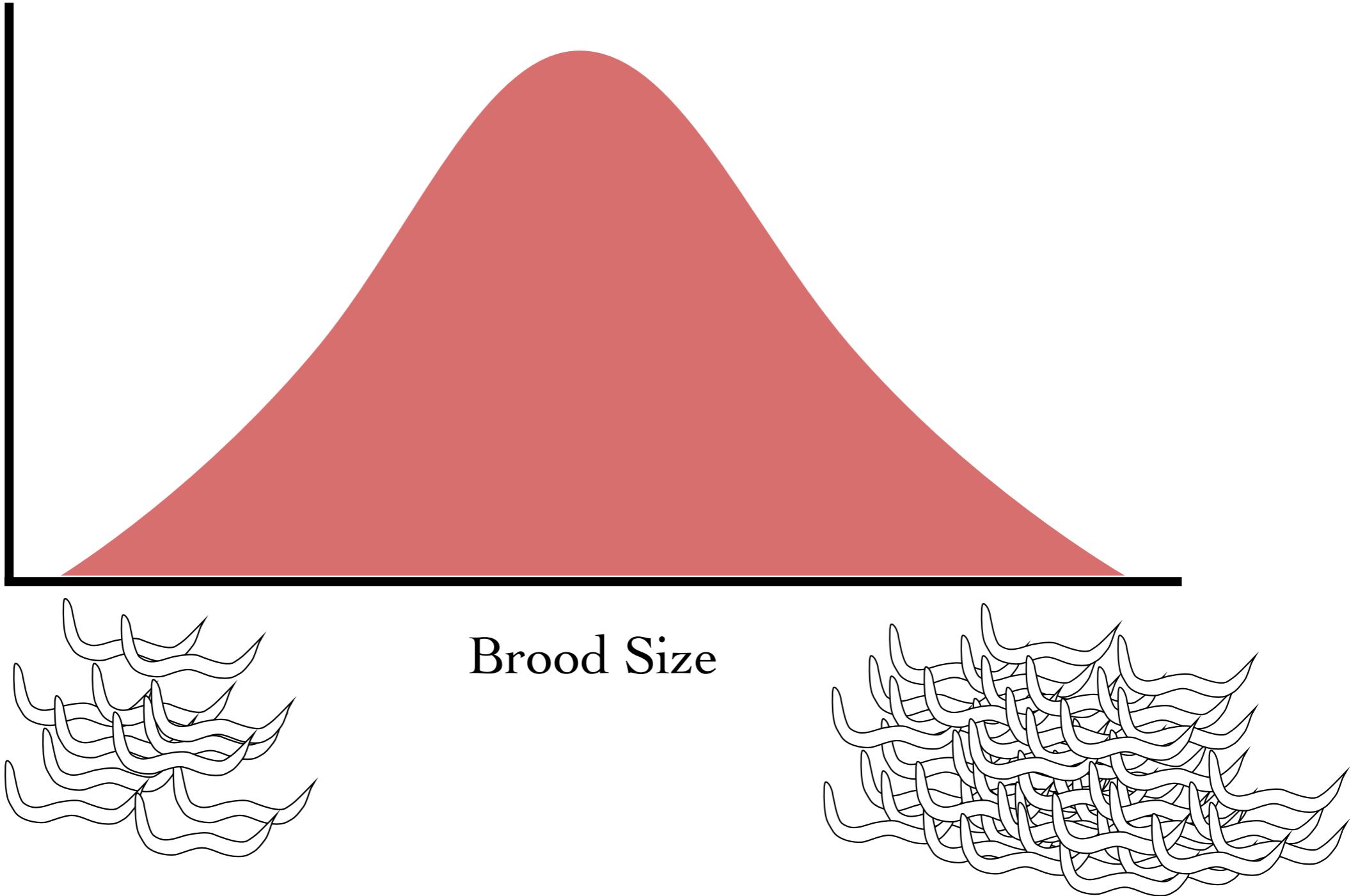
Ability to freeze strains

Excellent genomic annotation

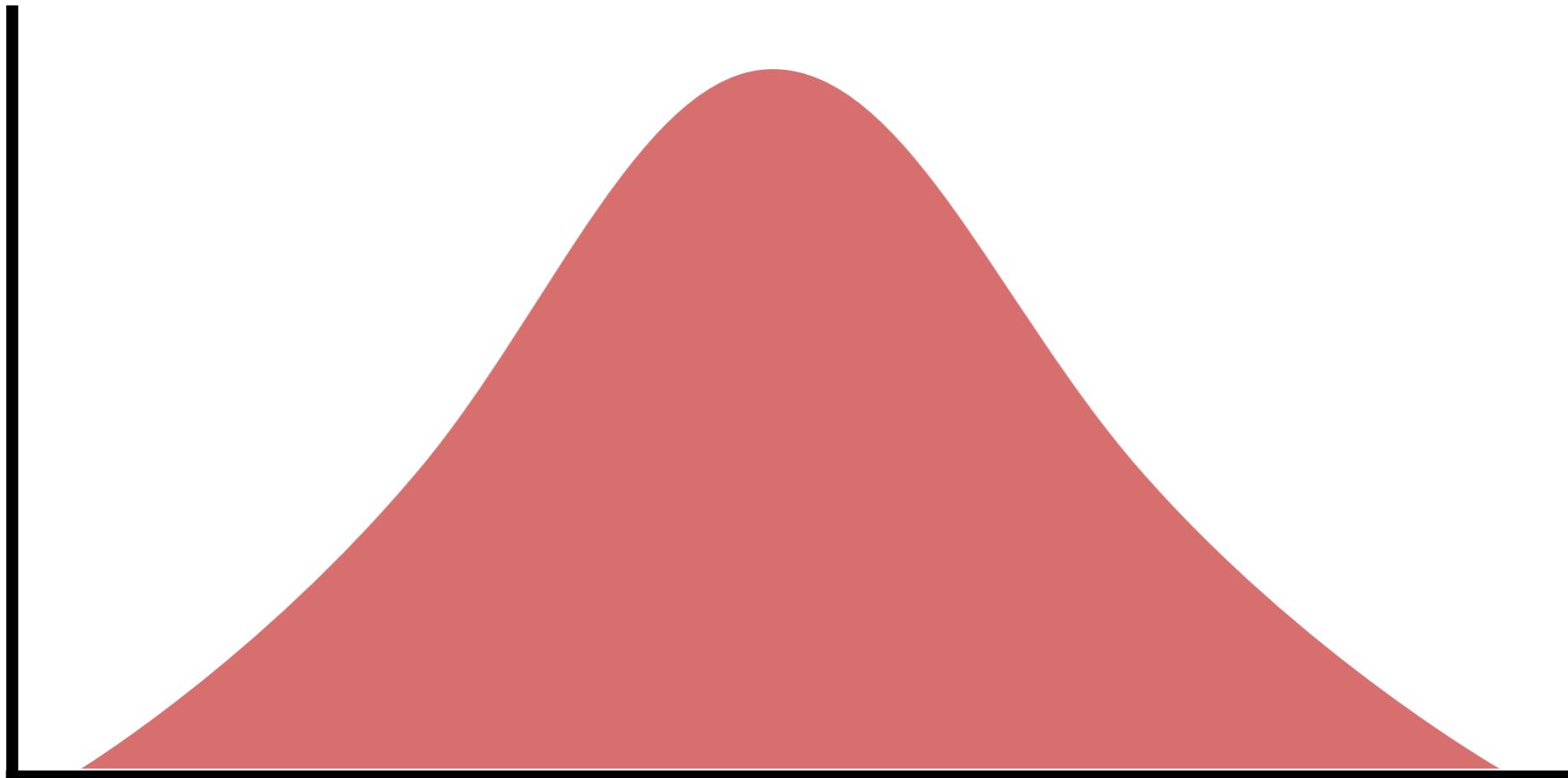
# Genotype – Phenotype Associations



# Quantifying Relative Fitness

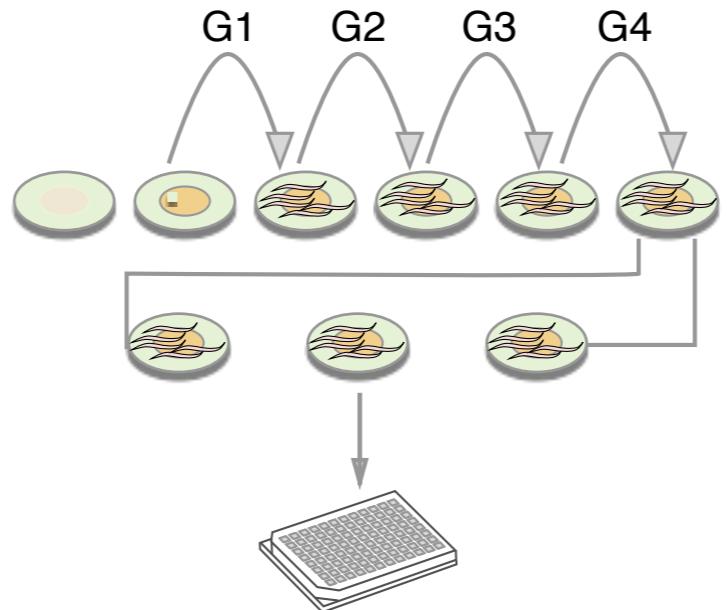


# Quantifying Relative Fitness



Animal Length





Bleach Synchronize  
G5

Aliquot to Growth Plates

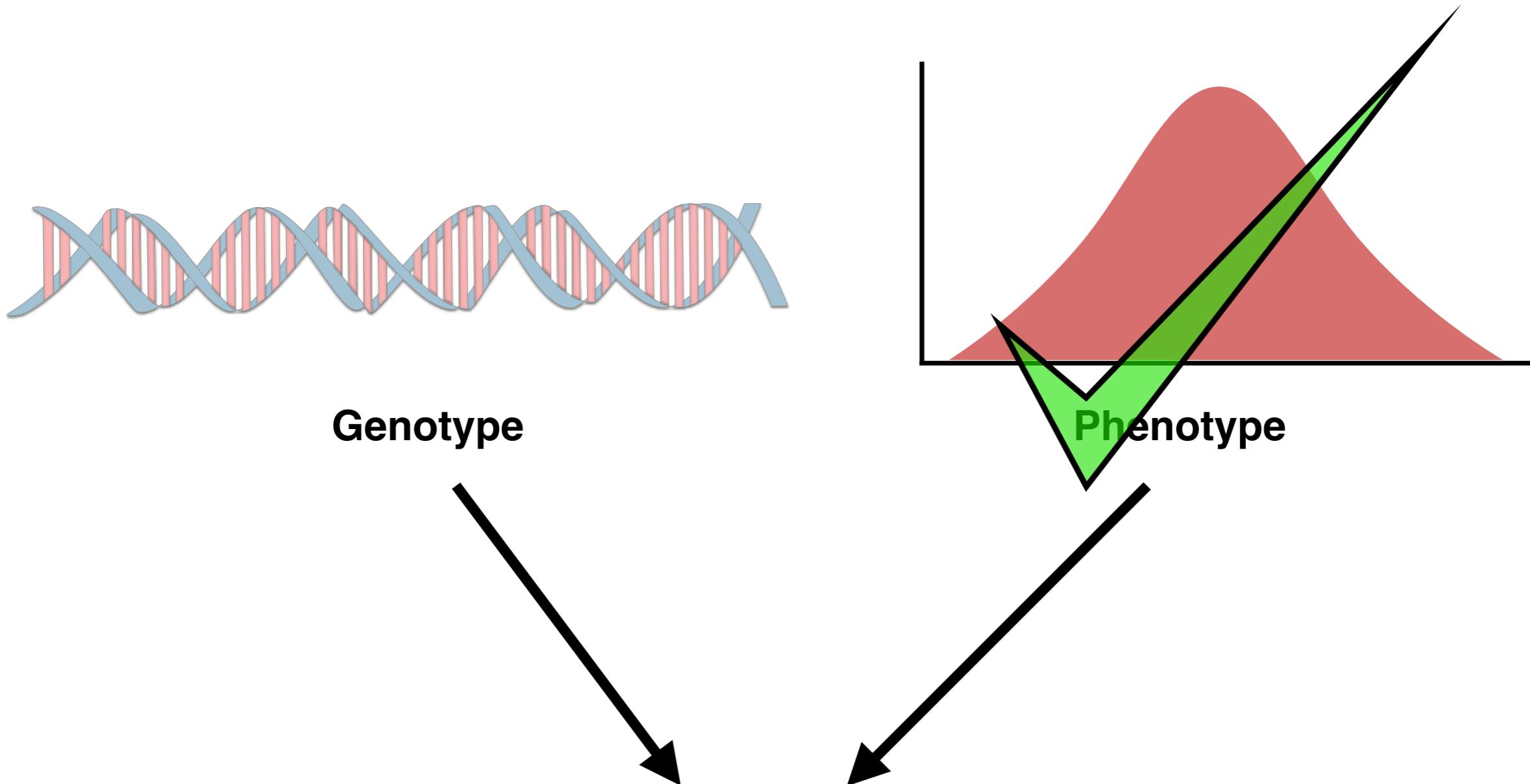


Sort 3 Animals into Assay Plate



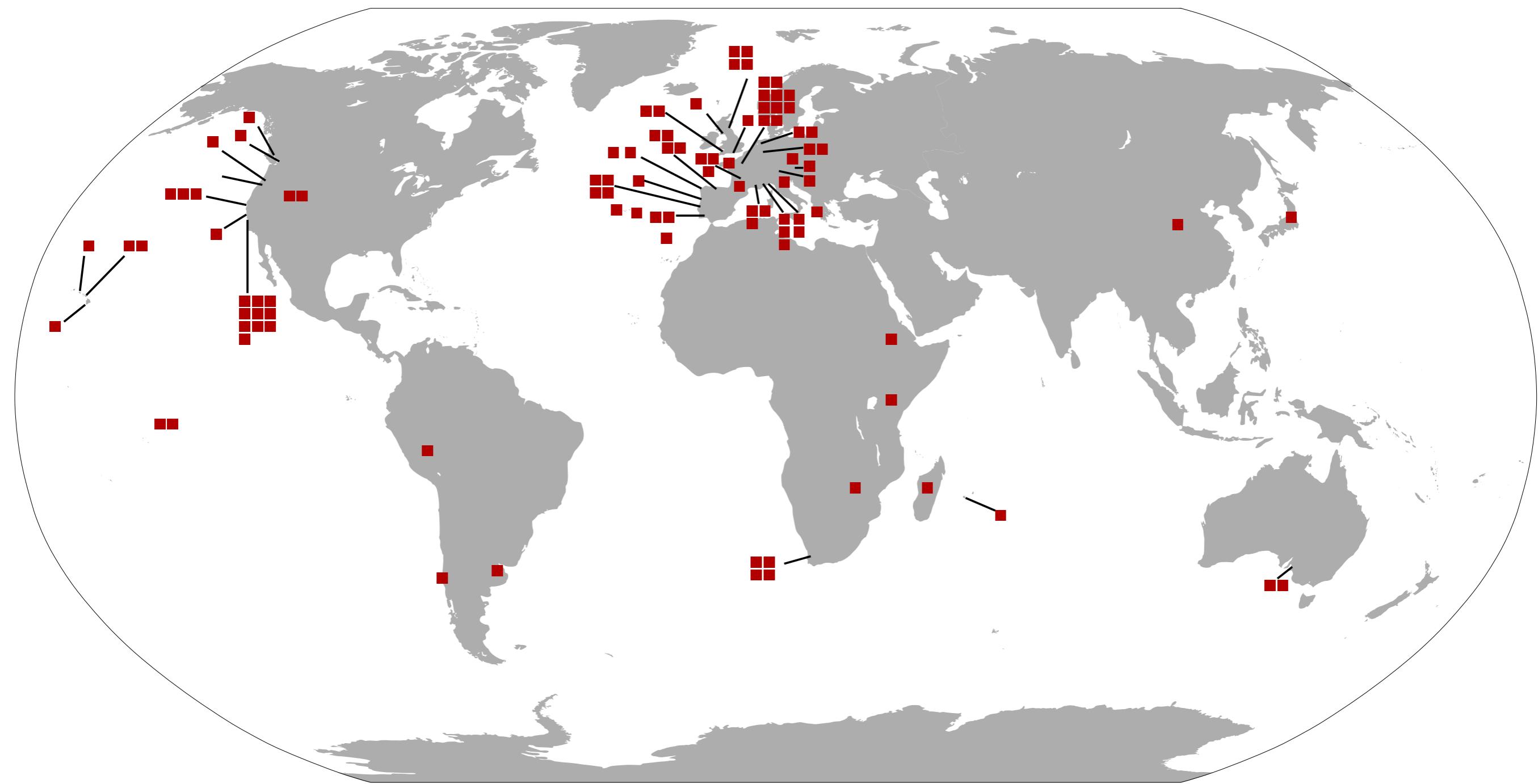
**Score**

# Genotype – Phenotype Associations

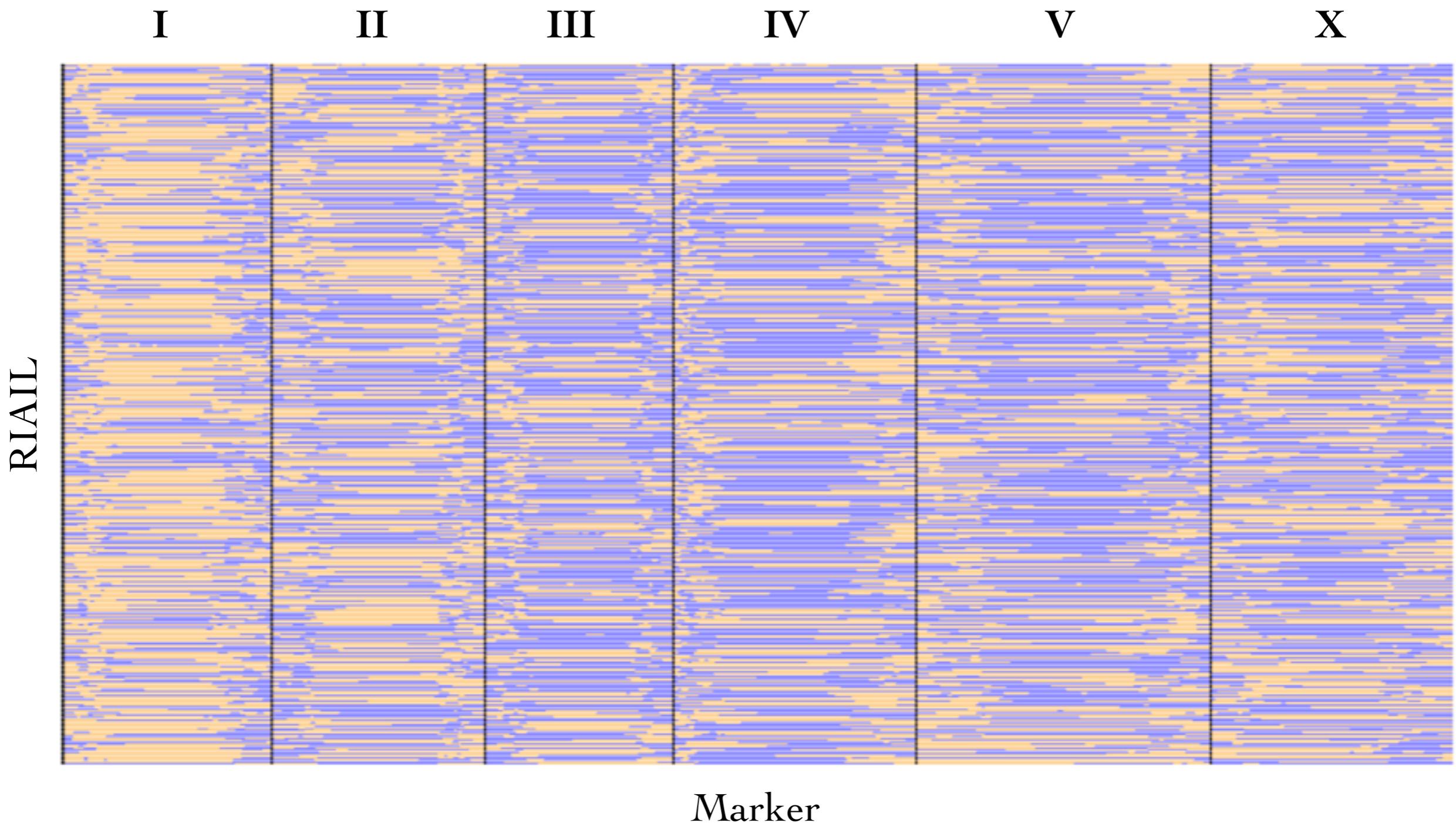


*Discover quantitative trait loci*

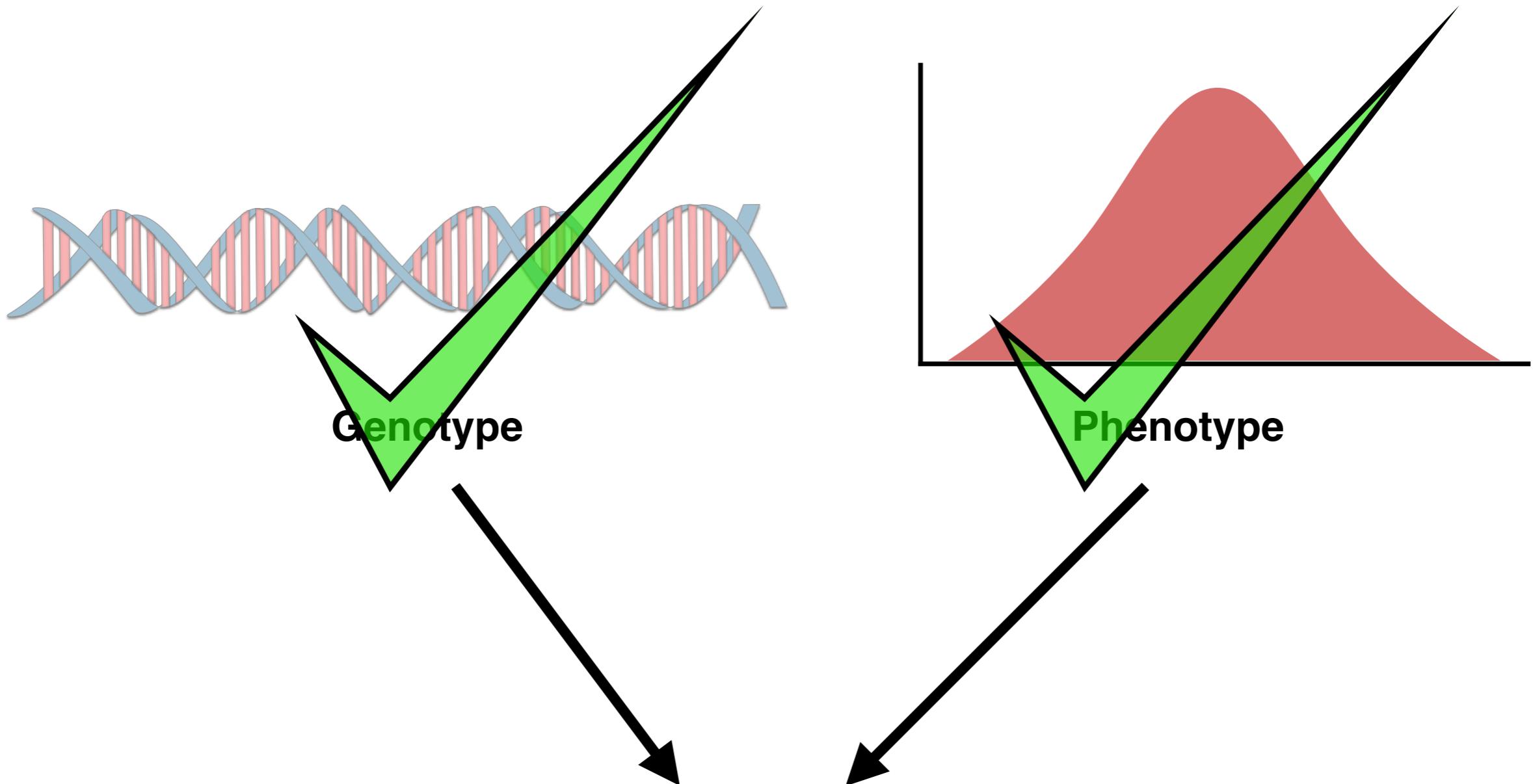
# Global Distribution of *C. elegans*



# Recombinant Inbred Lines

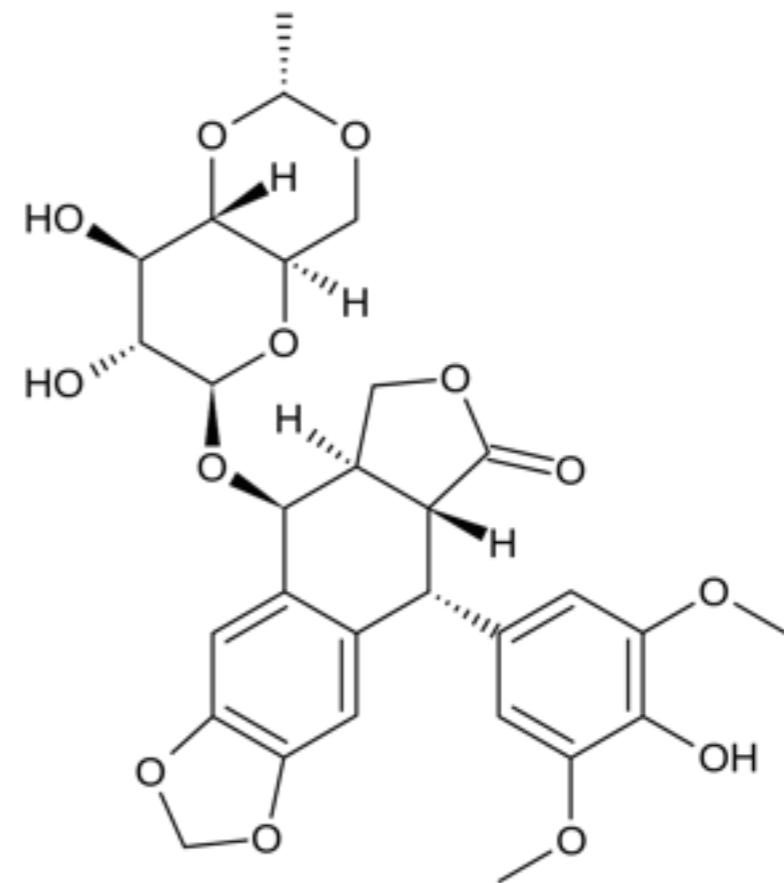
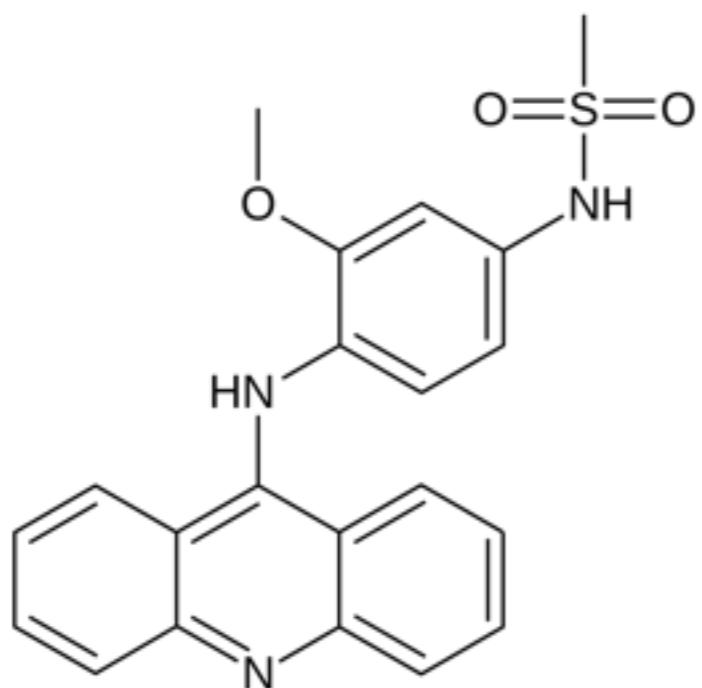


# Genotype – Phenotype Associations



*Discover quantitative trait loci*

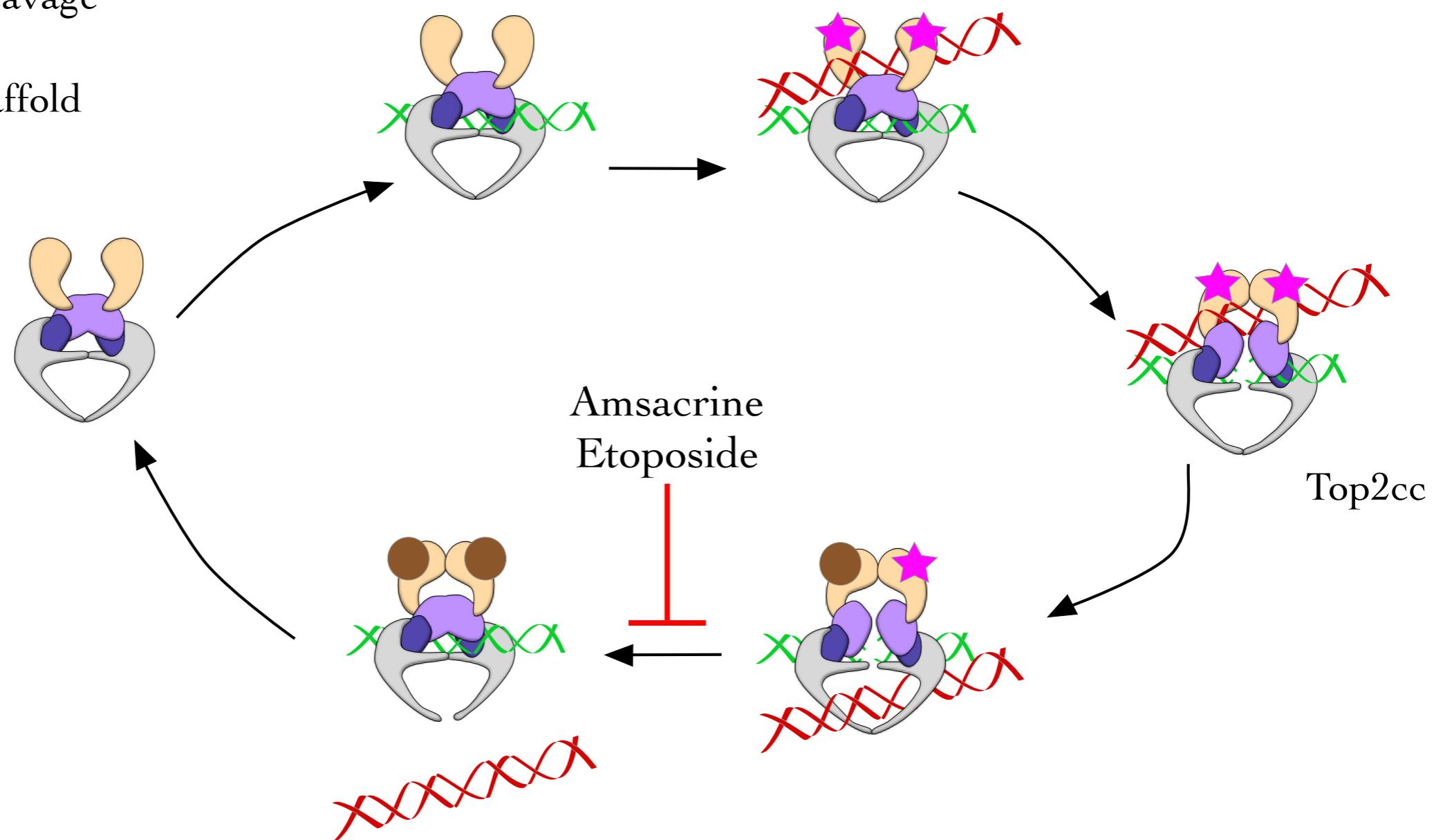
# Amsacrine and Etoposide



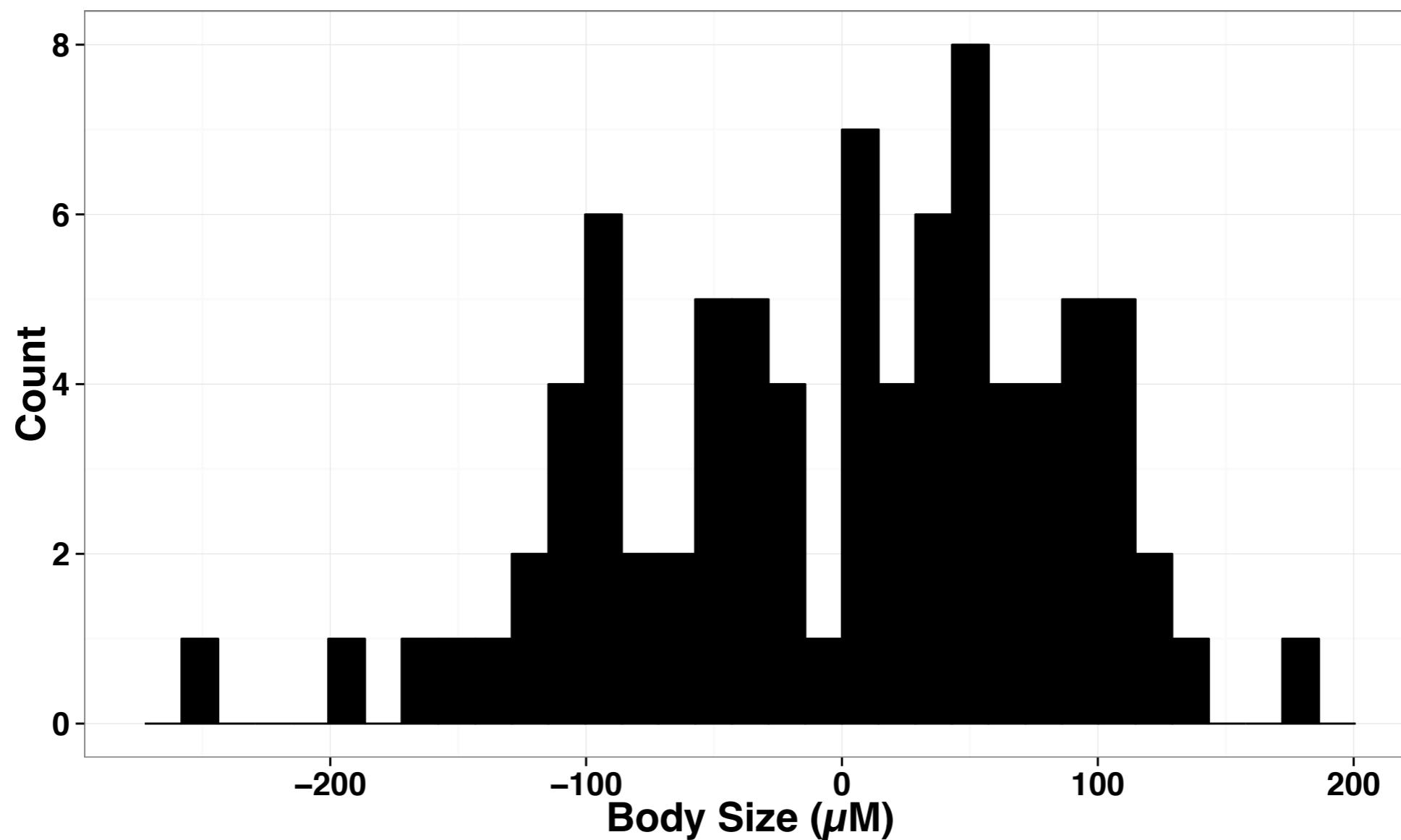
# Topoisomerase II Catalytic Cycle

- ★ ATP
- ADP
- ATPase
- TOPRIM
- WHD
- C-gate/Tower

Cleavage  
Scaffold

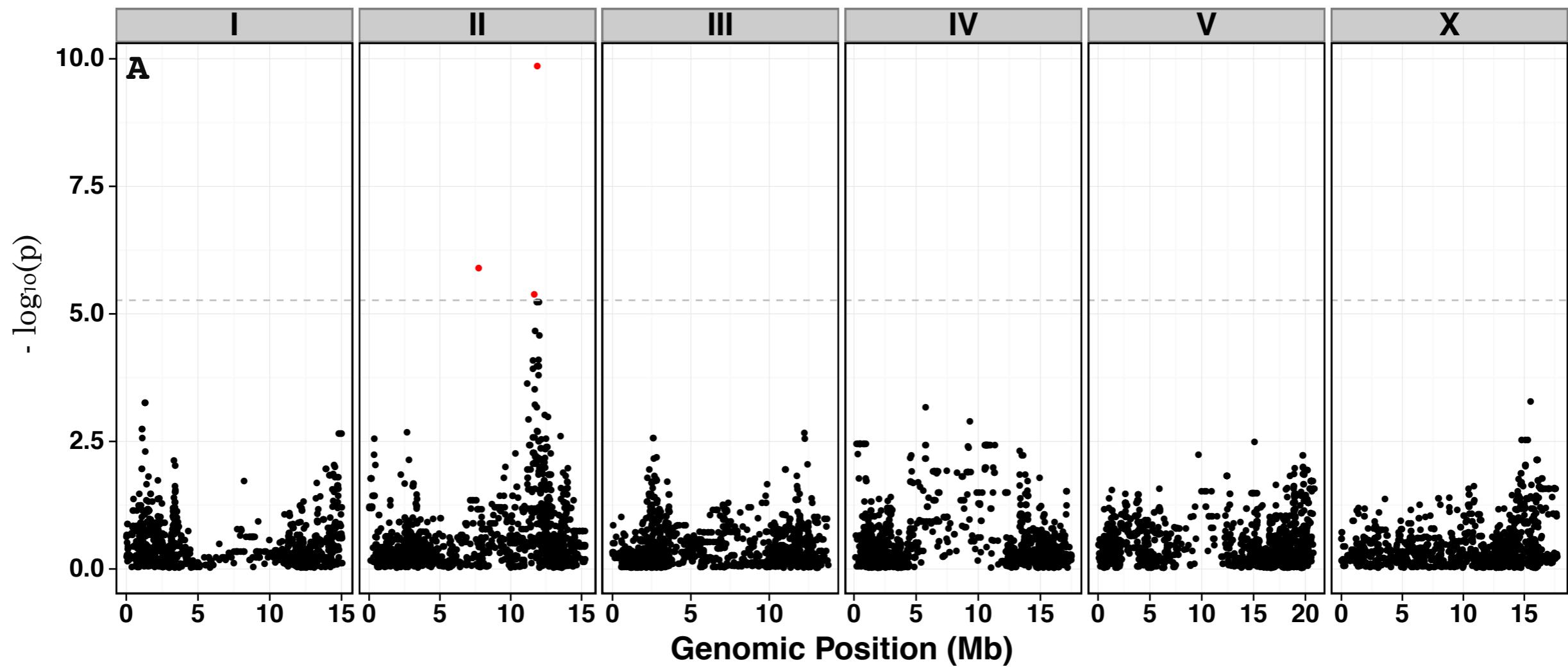


# Variation in Response to Etoposide

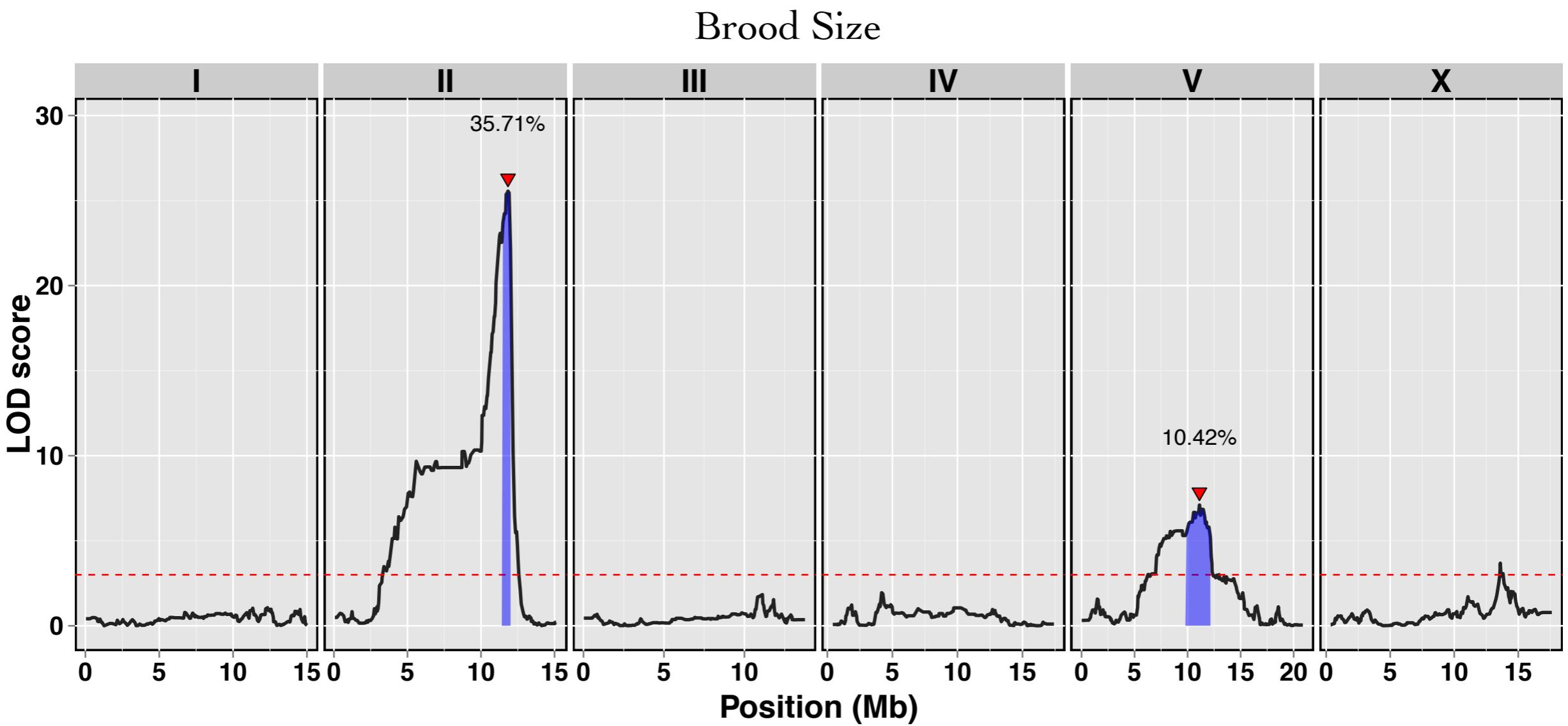


# Variation in Response to Etoposide

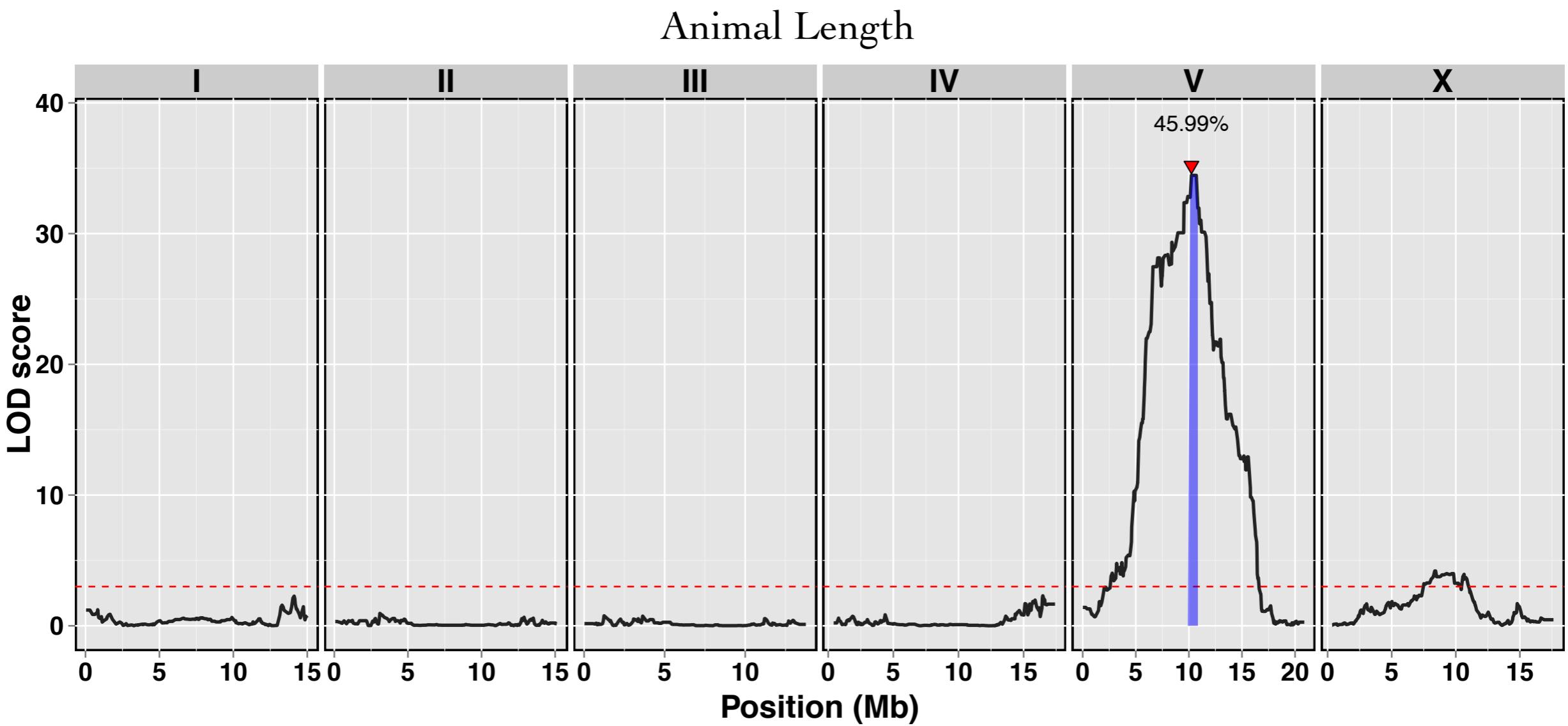
Animal Length



# Variation in Response to Etoposide



# Variation in Response to Amsacrine

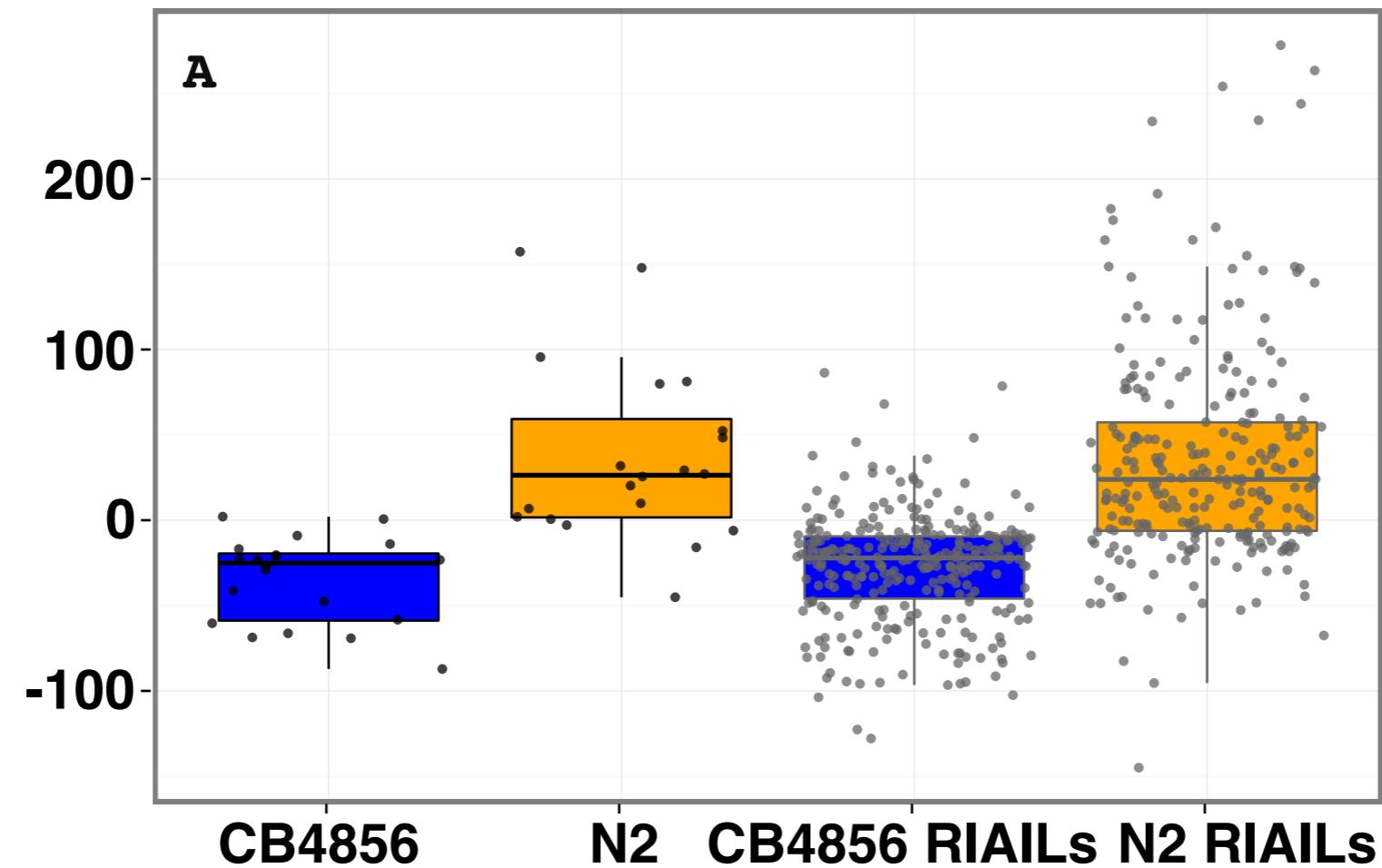


# Specific Aims

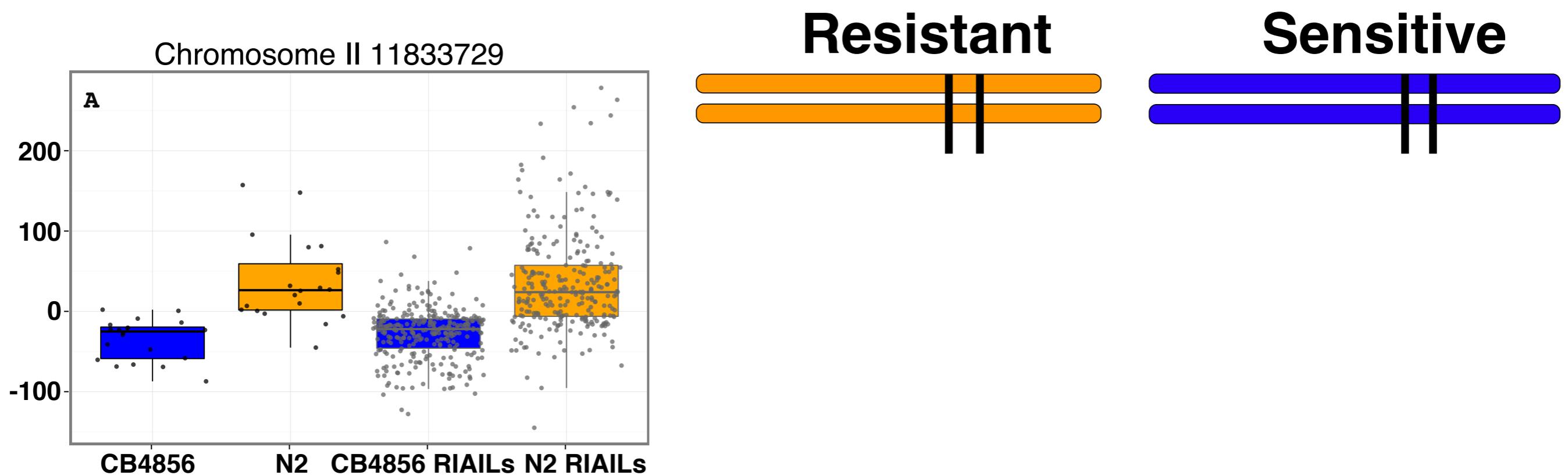
- **Specific Aim 1:** Narrow genomic regions underlying QTL
- **Specific Aim 2:** Identify specific genes and variants contributing to phenotypic variability in response to etoposide and amsacrine
- **Specific Aim 3:** Determine the altered molecular mechanisms contributing to phenotypic variation in response to topoisomerase II poisons

# Nearly Isogenic Lines

Chromosome II 11833729

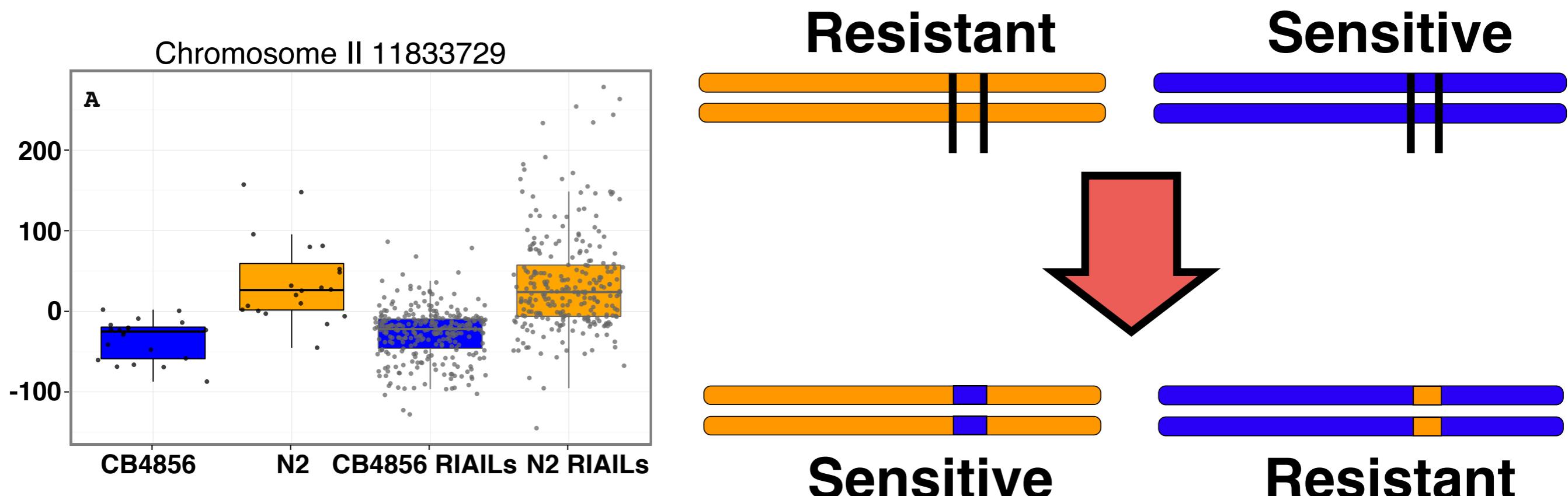


# Nearly Isogenic Lines



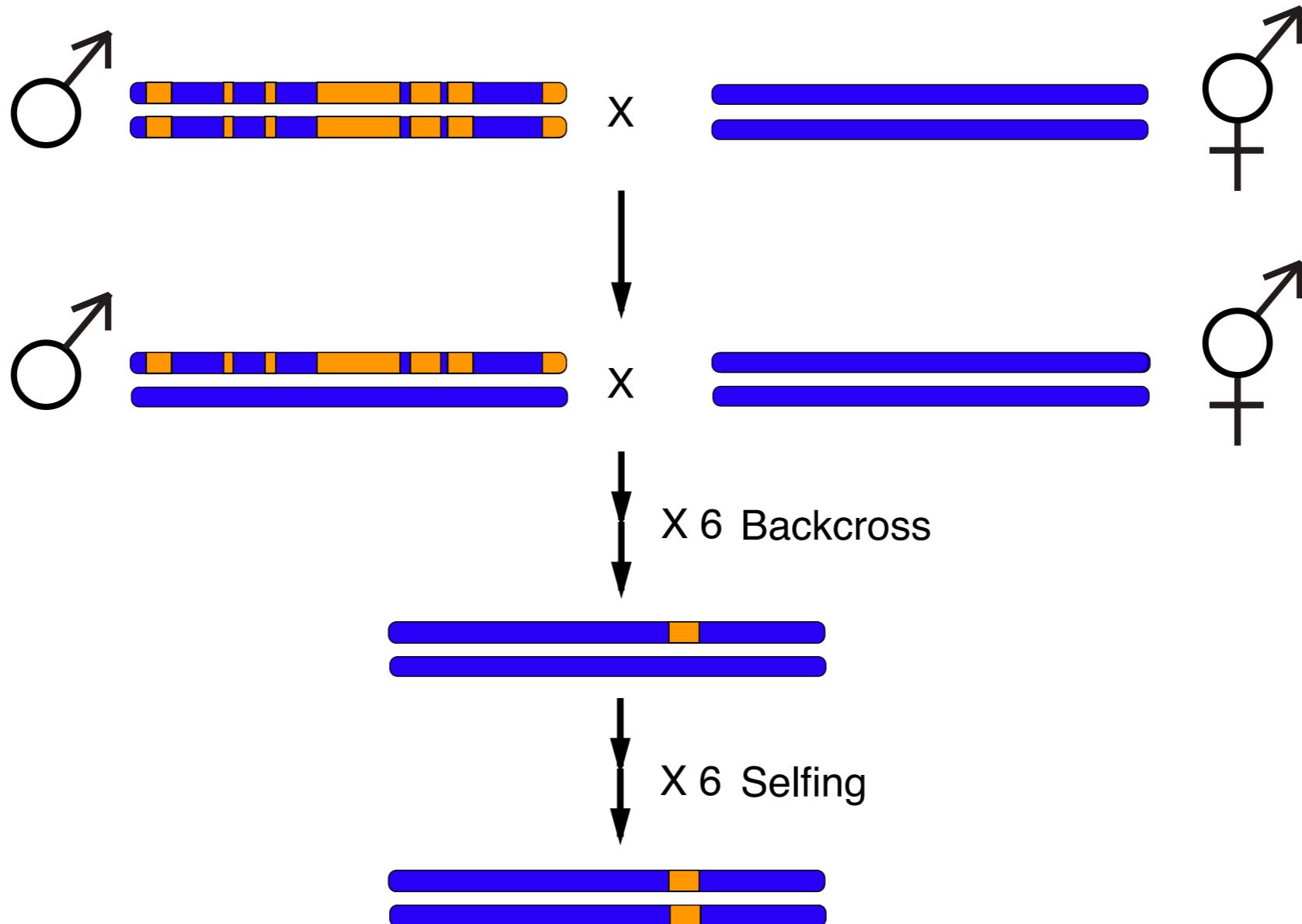
Aim 1

# Nearly Isogenic Lines



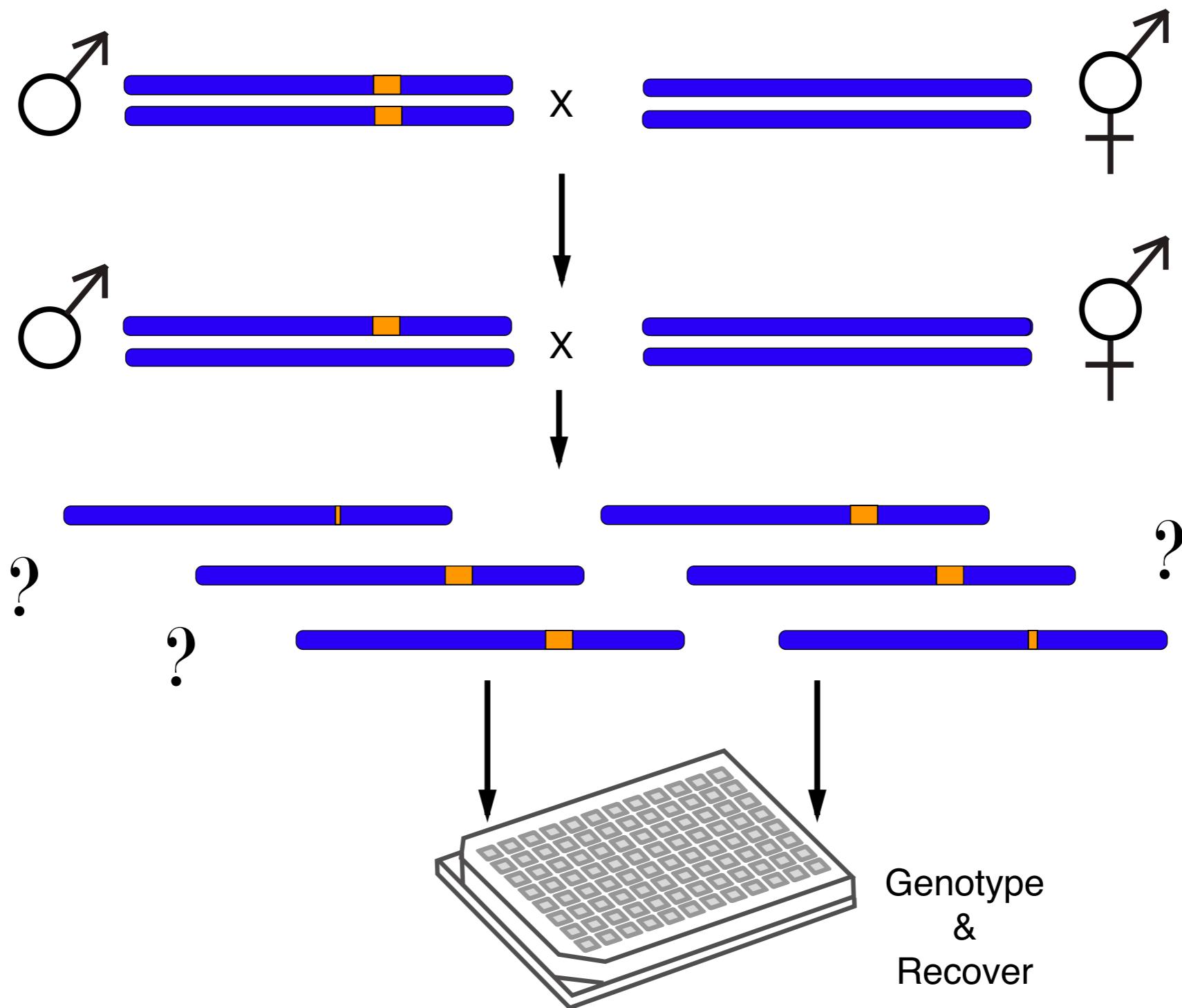
**Aim 1**

# Generating Interval NILs



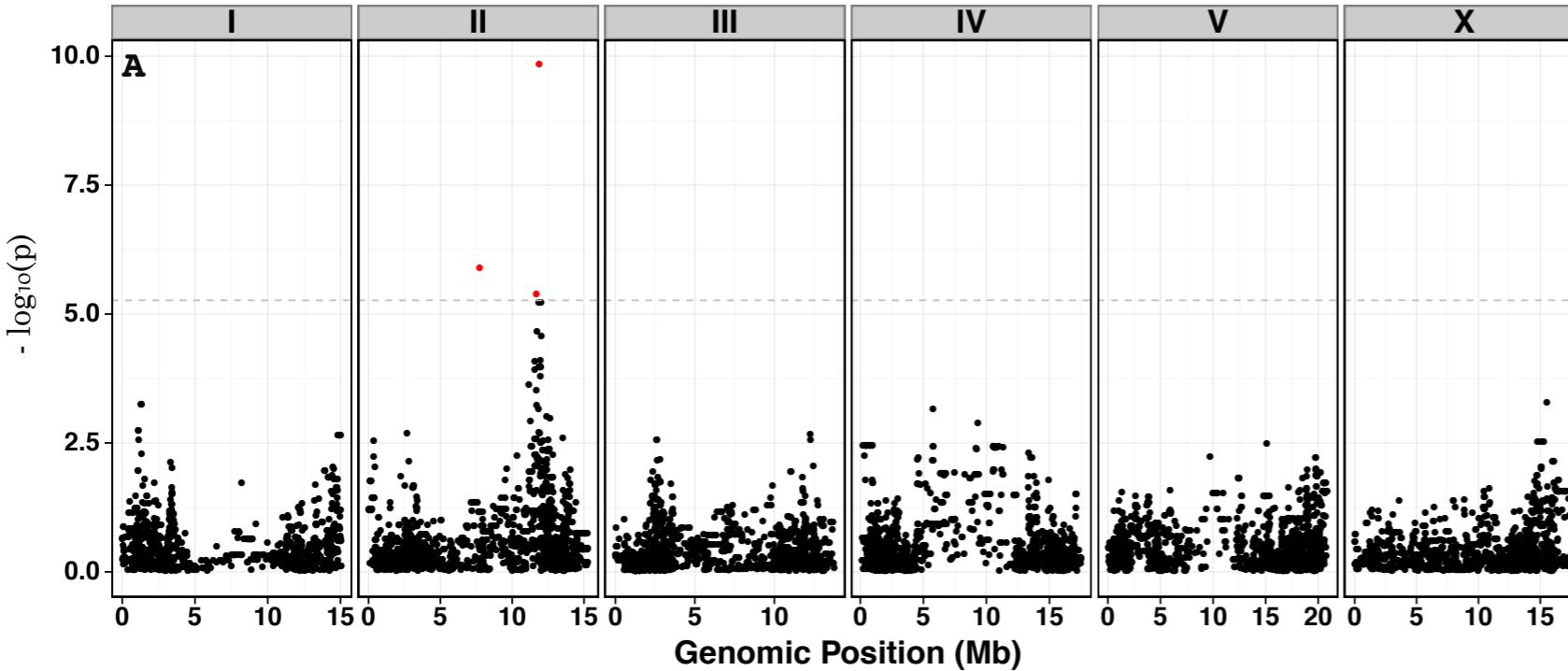
Aim 1

# Generating Sub-interval NILs



Aim 1

# Expedited Interval Narrowing

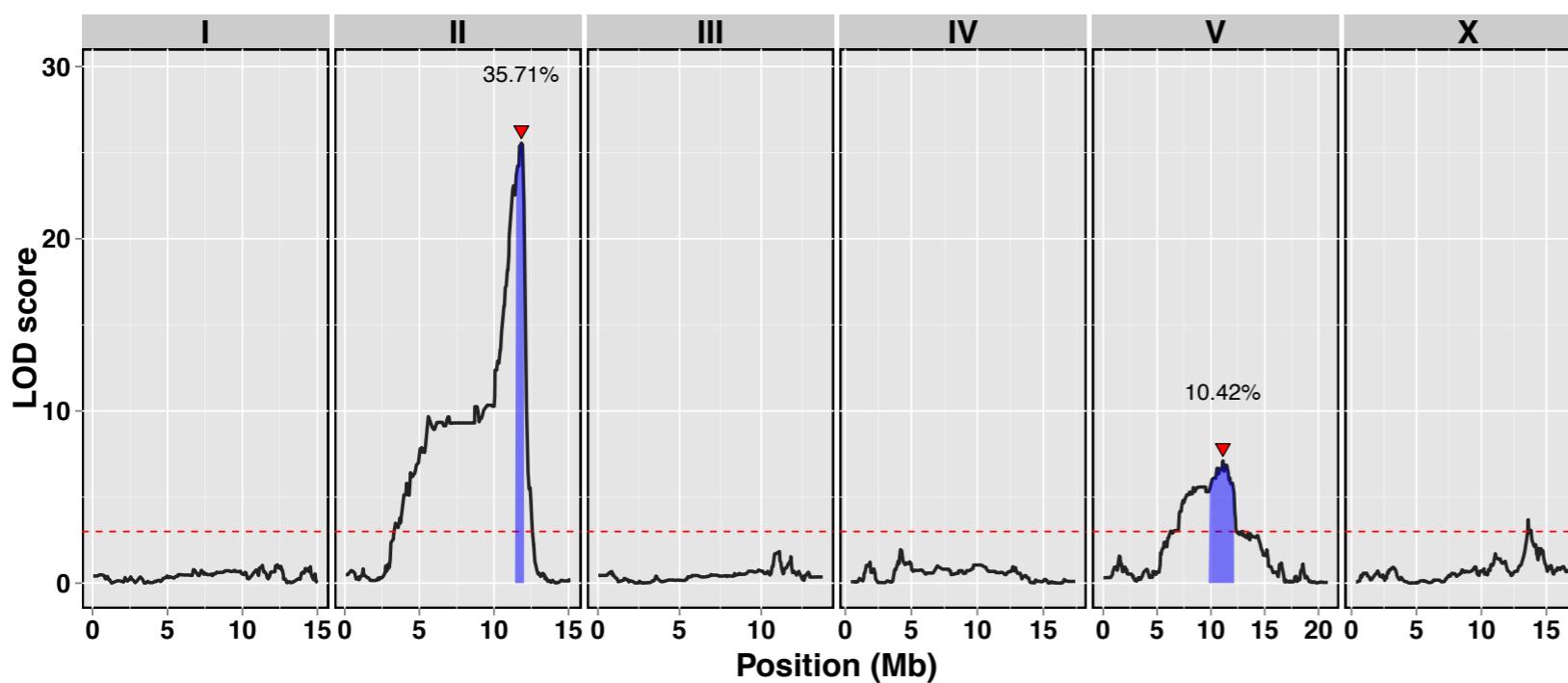


600 Mb interval

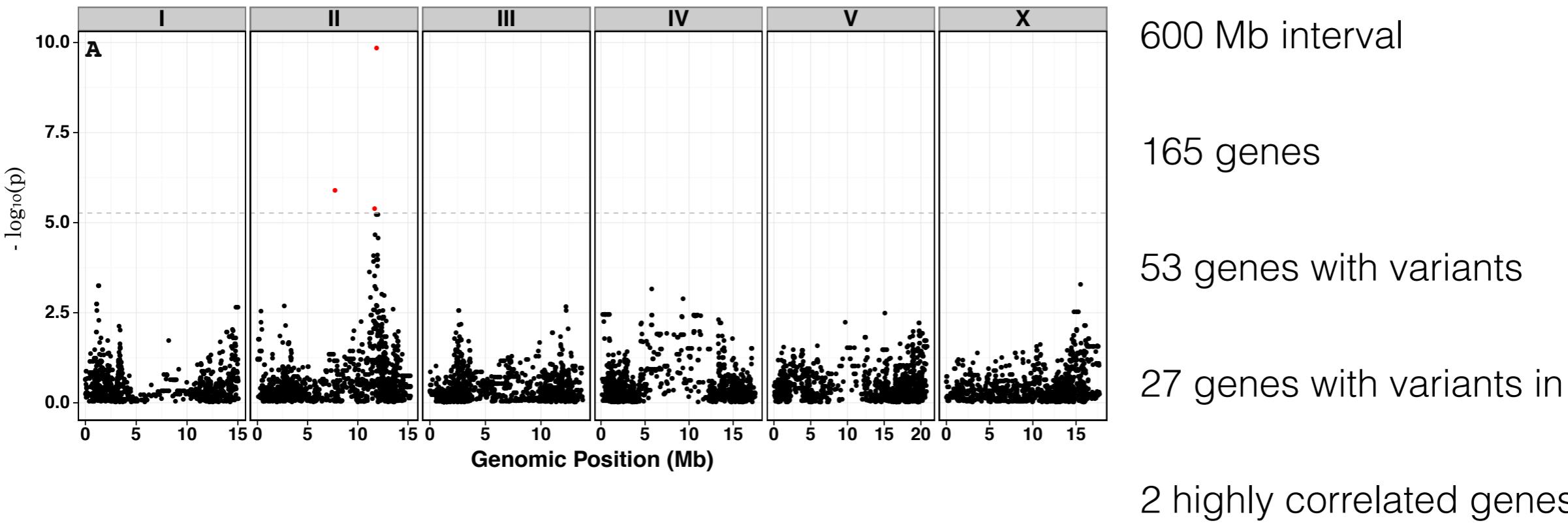
165 genes

53 genes with variants

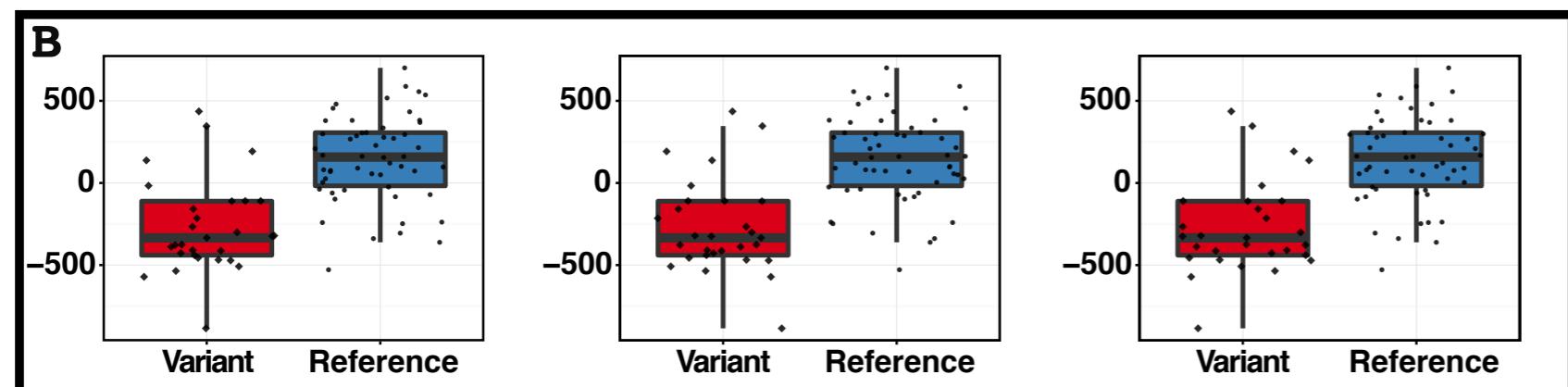
27 genes with variants in CB4856



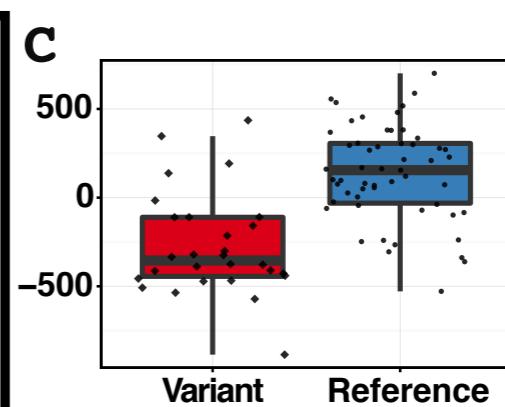
# Expedited Interval Narrowing



*top-2*



*npp-3*



- **Specific Aim 1:** Narrow genomic regions underlying QTL
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# Dominance Test – Design

N2 *top-2*  
N2 *top-2*



*Resistant*

CB4856 *top-2*  
CB4856 *top-2*



*Sensitive*

N2 *top-2*  
CB4856 *top-2*



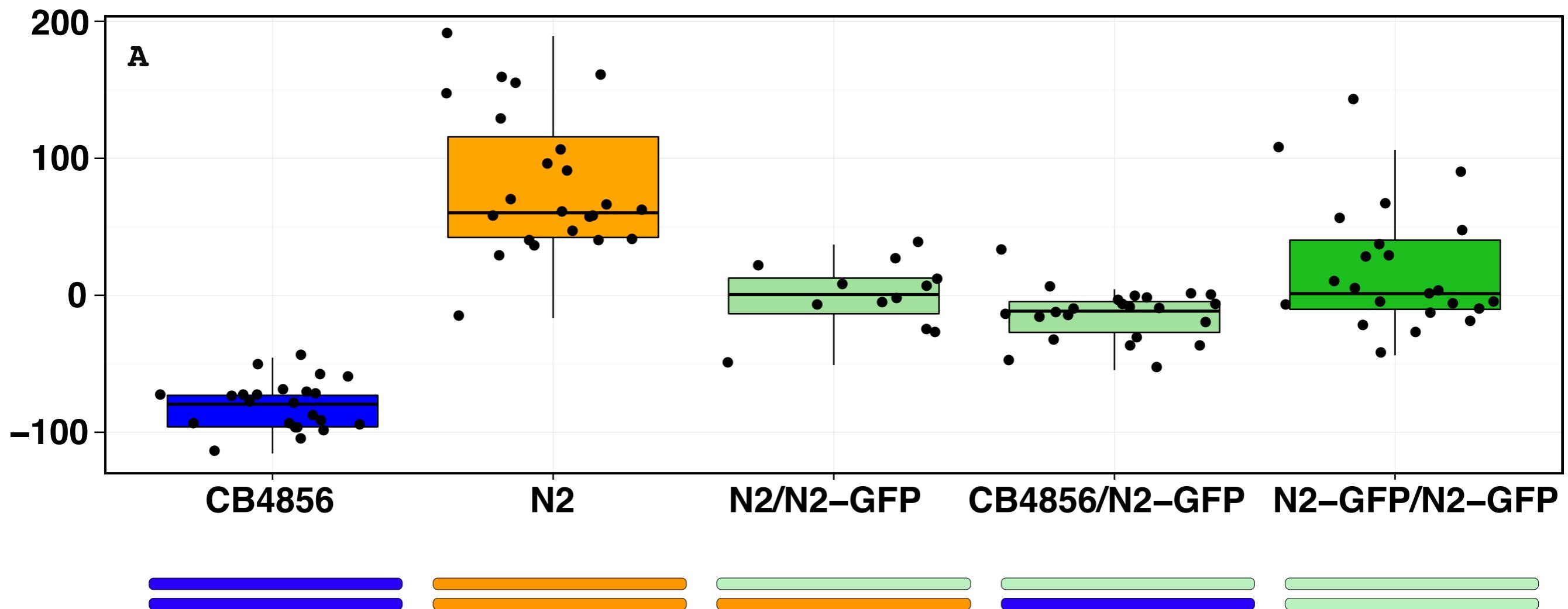
?

**Genotype**

**Phenotype**

# Resistance to Etoposide is Dominant

Brood Size



Aim 2

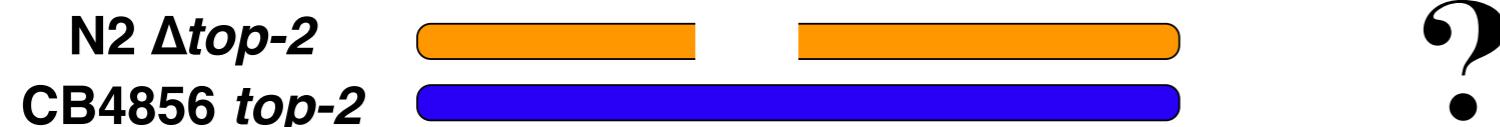
# Complementation Test – Design



*Resistant*



*Resistant*



?

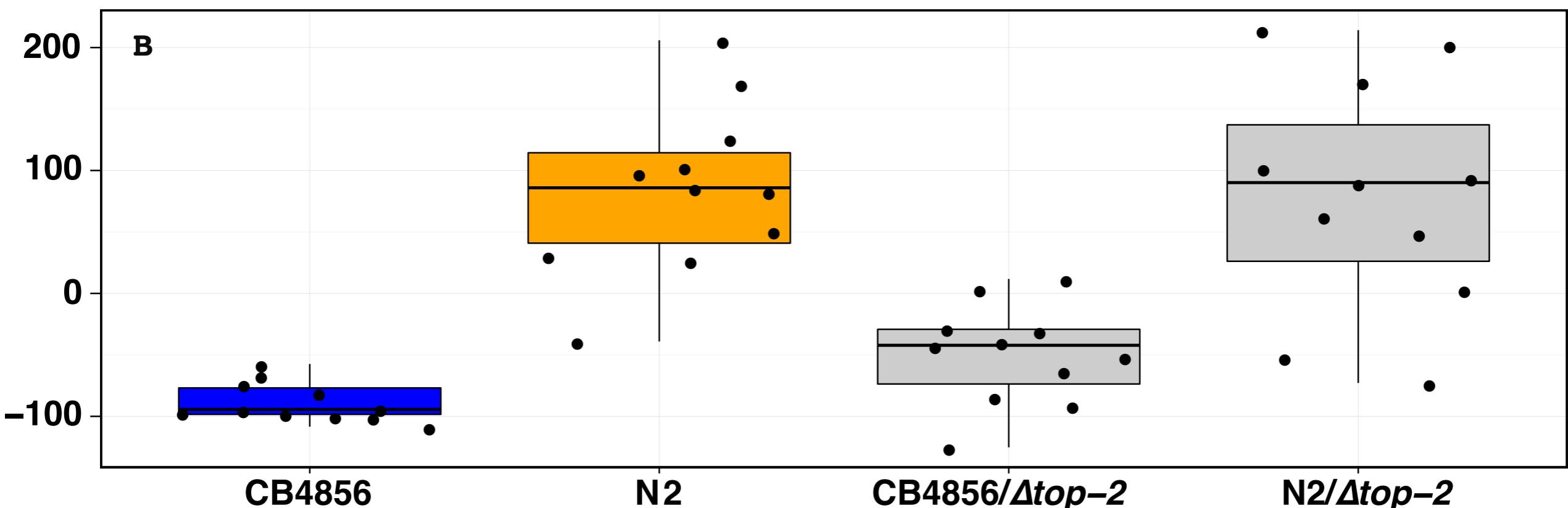
**Genotype**

**Phenotype**

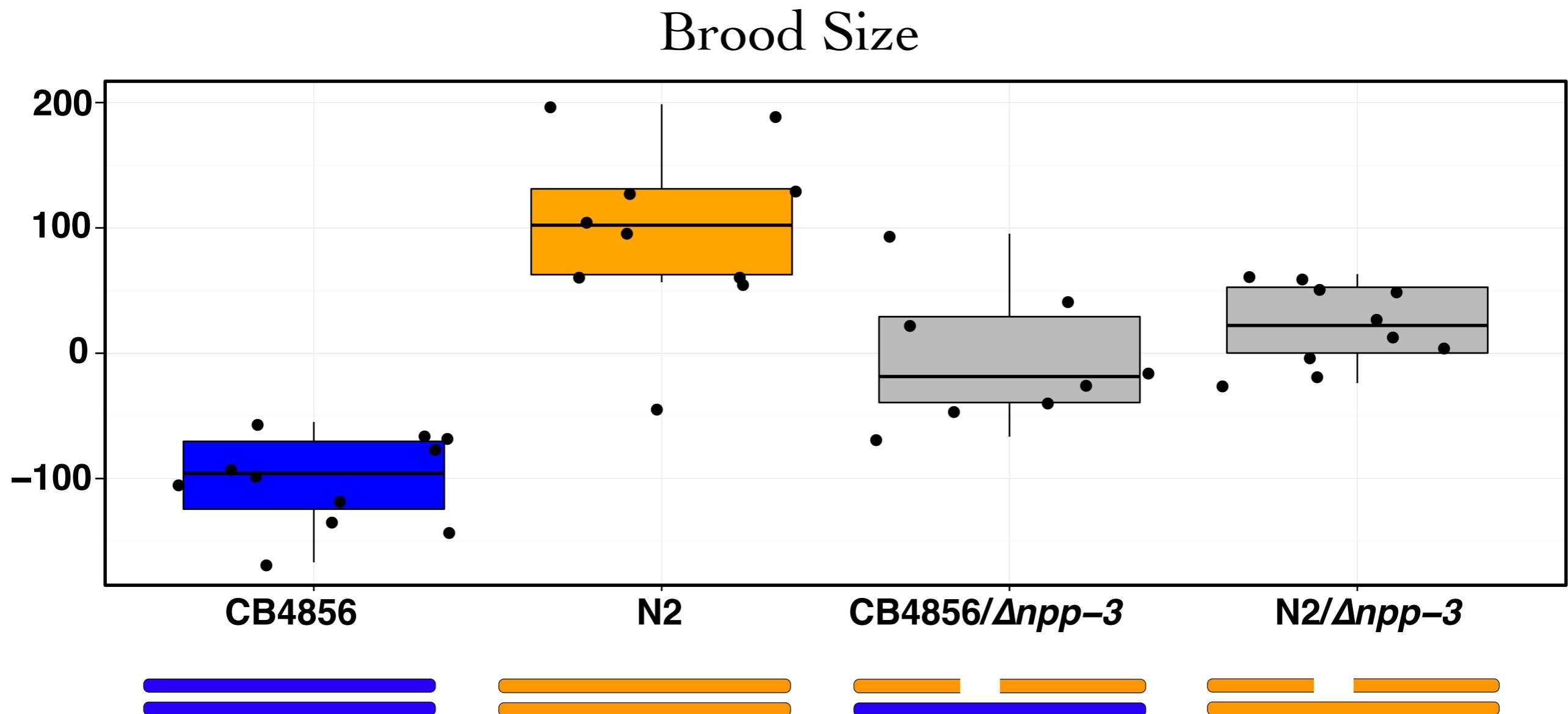
**Aim 2**

# Complementation Test — *top-2*

Brood Size

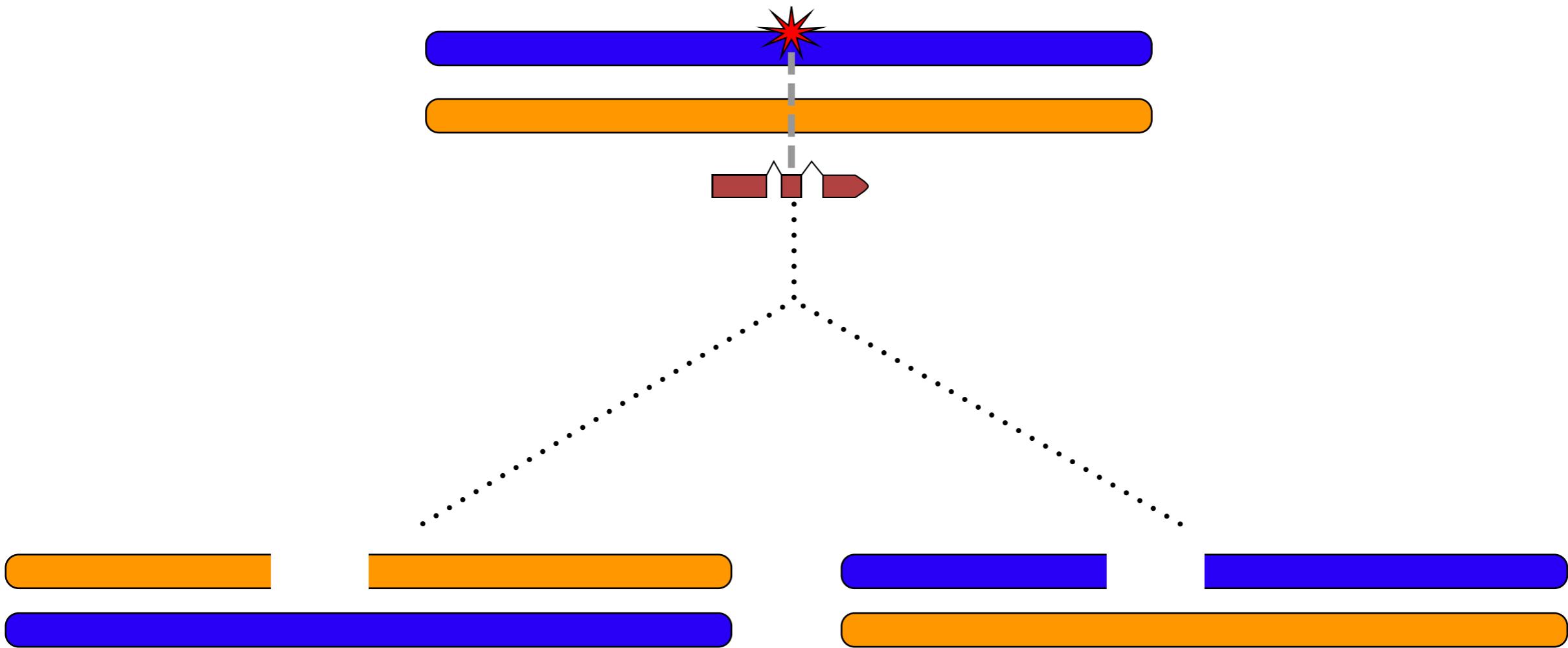


# Complementation Test — *npp-3*



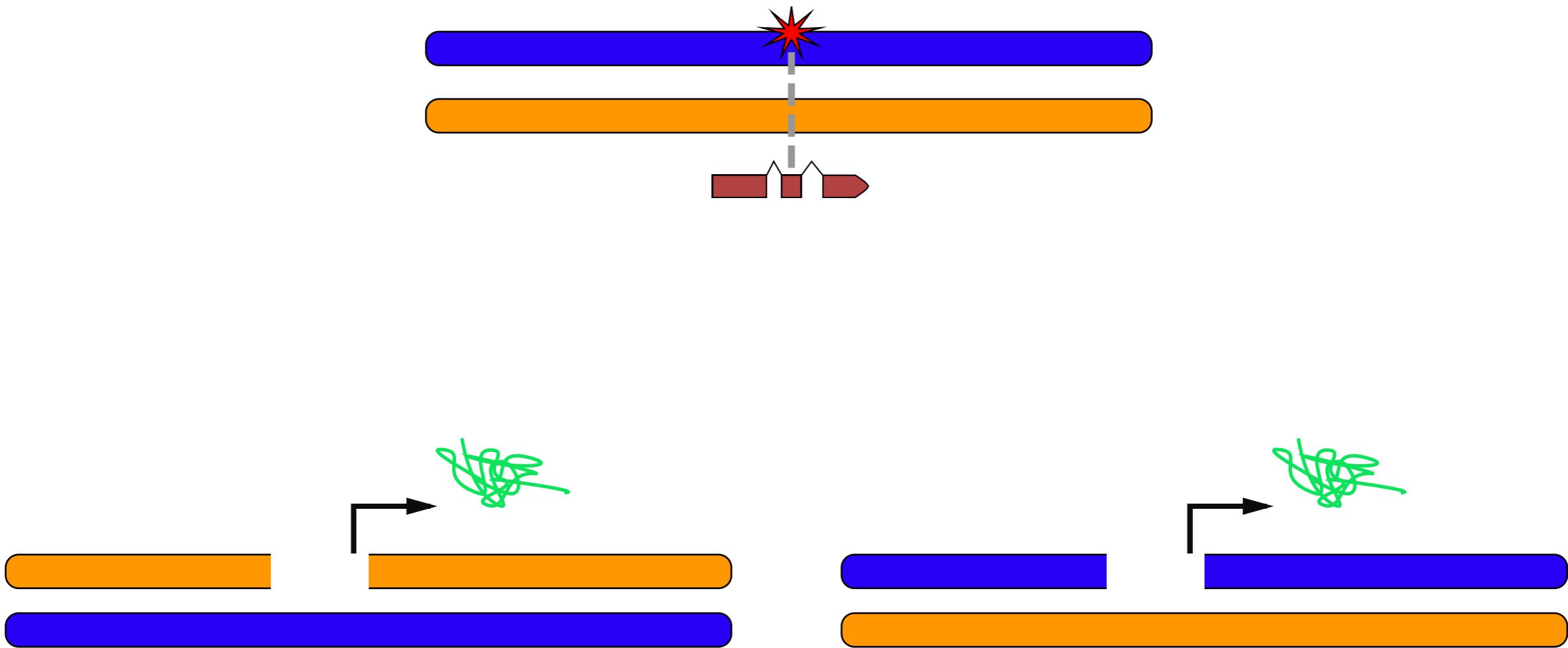
Aim 2

# Reciprocal Hemizygosity



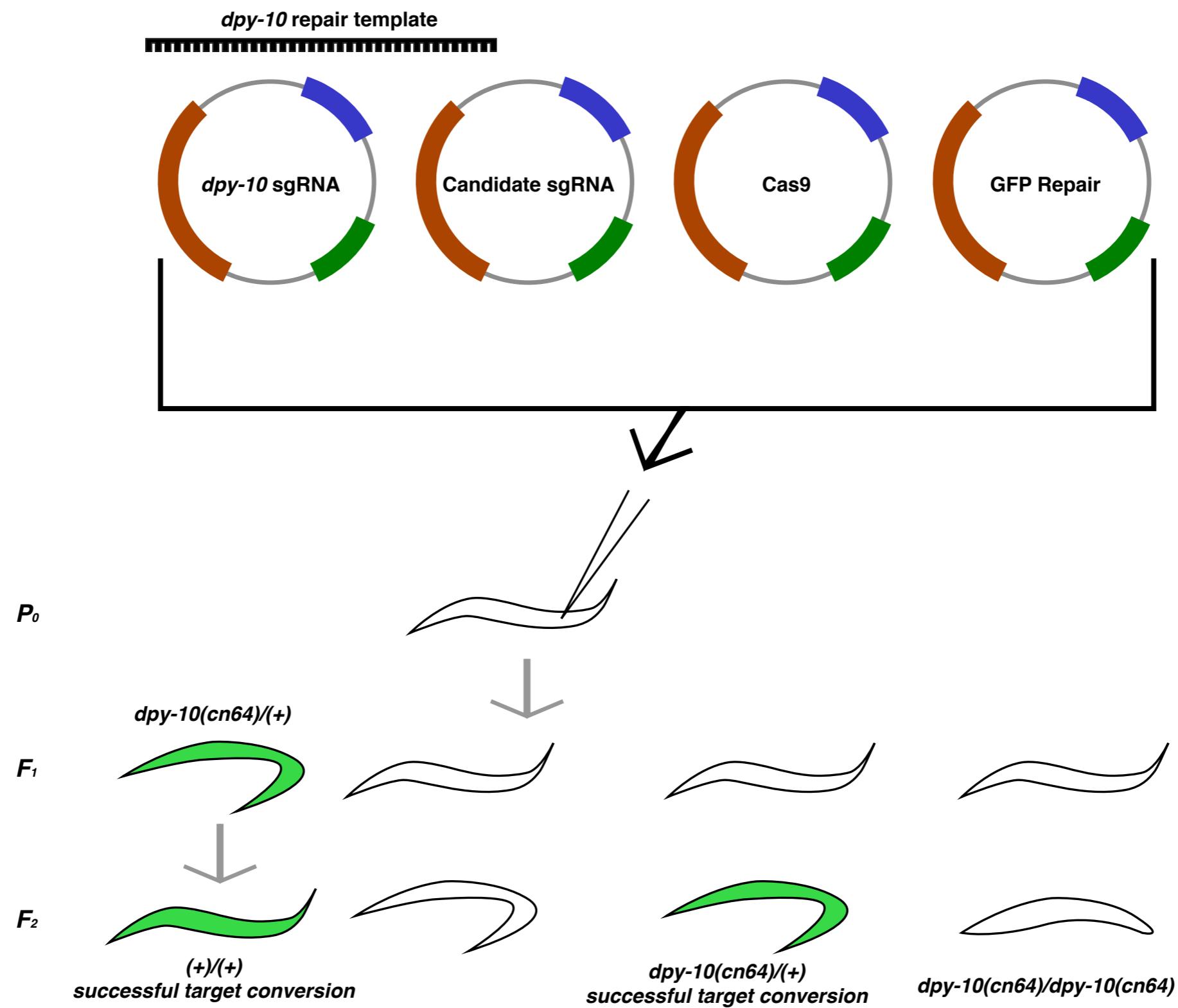
Aim 2

# Reciprocal Hemizygosity



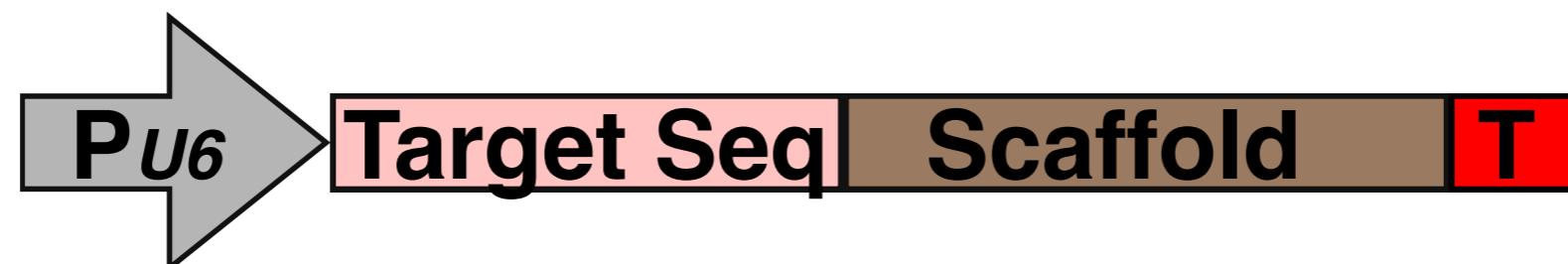
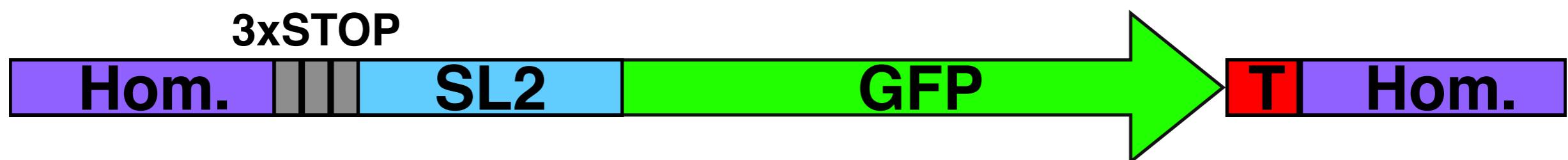
**Aim 2**

# Generating Strains for Reciprocal Hemizygosity



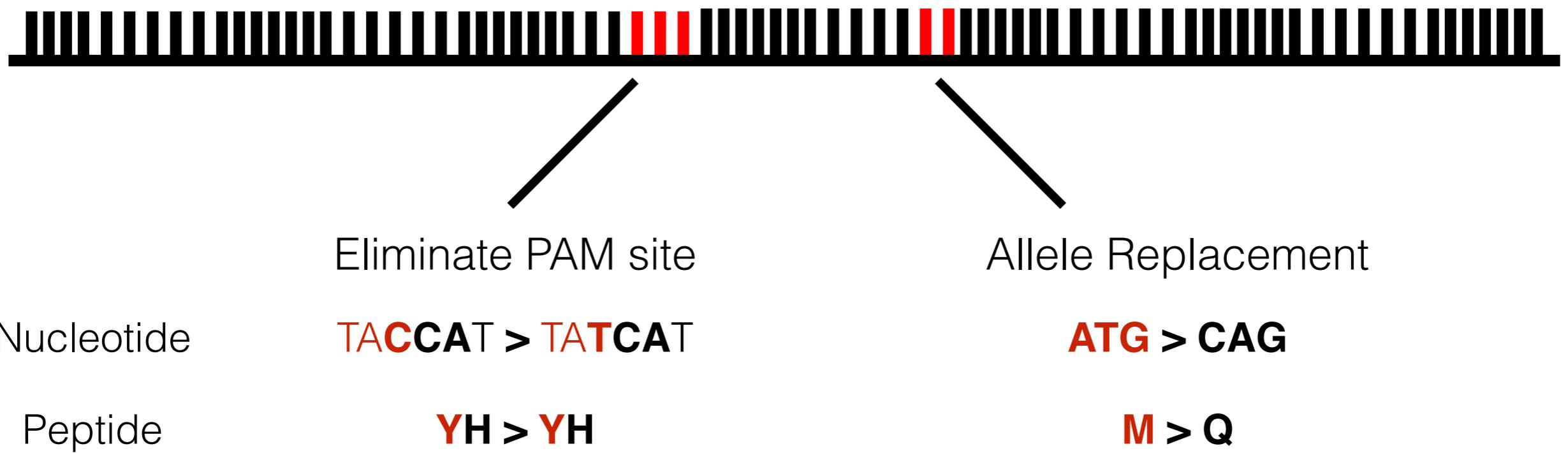
**Aim 2**

# CRISPR/Cas9 Constructs



**Aim 2**

# Allele Replacement Oligonucleotide Design



CB4856 (Sensitive) > N2 (Resistant)

- **Specific Aim 1:** Narrow genomic regions underlying QTL
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- **Specific Aim 3:** Determine the altered molecular mechanisms contributing to phenotypic variation in response to topoisomerase II poisons

# Molecular Mechanism

C.e. TOPOII Resistant	VAEISAYHH <b>GEQSLMGTIVNLAQDYVGSNNINLLLPIGQFGTRLQGGKDSASARYIFT</b>
C.e. TOPOII Sensitive	VAEISAYHH <b>GEMSLMGTIVNLAQDYVGSNNINLLLPIGQFGTRLQGGKDSASARYIFT</b>
Human TOPOIIα	VAEMSSYHH <b>GEMSLMMTIINLAQNFVGSNNLNLLQPIGQFGTRLHGGKDSASPRYIFT</b>
Human TOPOIIβ	VAEMSAYHH <b>GEQALMMTIVNLAQNFVGSNNINLLQPIGQFGTRLHGGKDAASPRYIFT</b>

Drug binding

Catalytic tyrosine

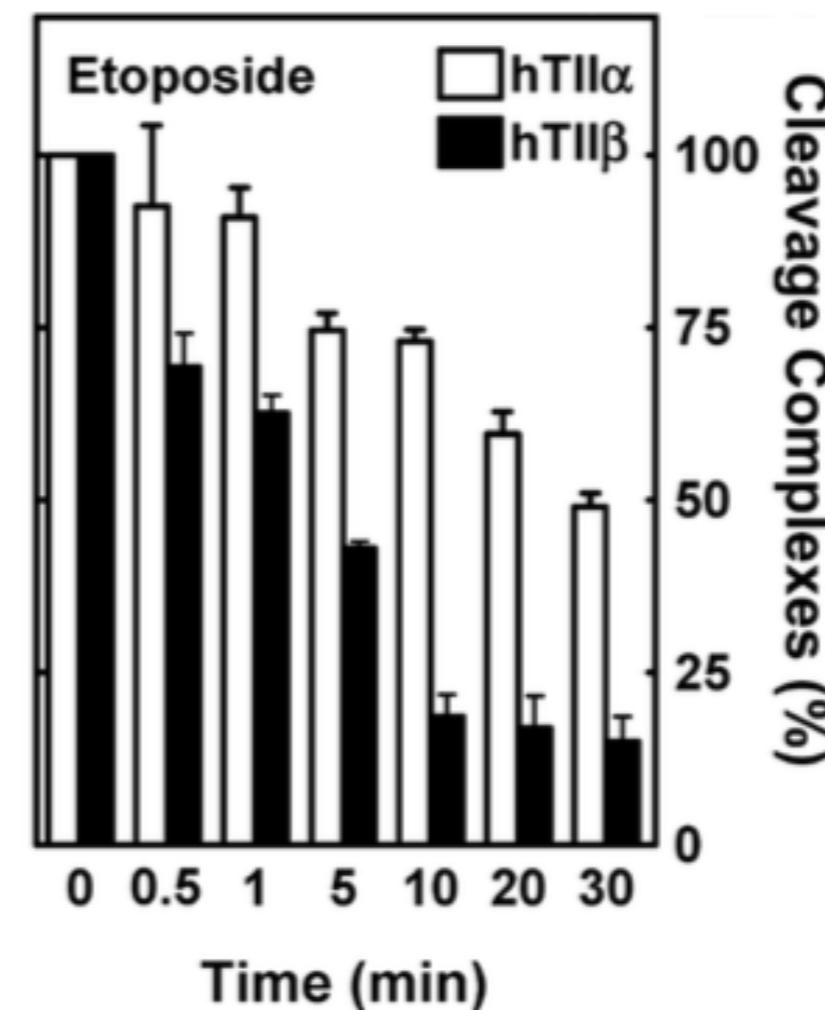
# Molecular Mechanism

C.e. TOPOII Resistant  
C.e. TOPOII Sensitive  
Human TOPOII $\alpha$   
Human TOPOII $\beta$

VAEISAYHH**GEQ**SLMGTIVNLAQDYVGSNNINLLLPIGQFGTRLQGGKDSASARYIFT  
VAEISAYHH**GEM**SLMGTIVNLAQDYVGSNNINLLLPIGQFGTRLQGGKDSASARYIFT  
VAEMSSYHH**GEM**SLMMTIINLAQNFVGSNNLNLLQPIGQFGTRLHGGKDSASPRYIFT  
VAEMSAYHH**GEQ**ALMMTIVNLAQNFVGSNNINLLQPIGQFGTRLHGGKDAASPRYIFT

Drug binding

Catalytic tyrosine



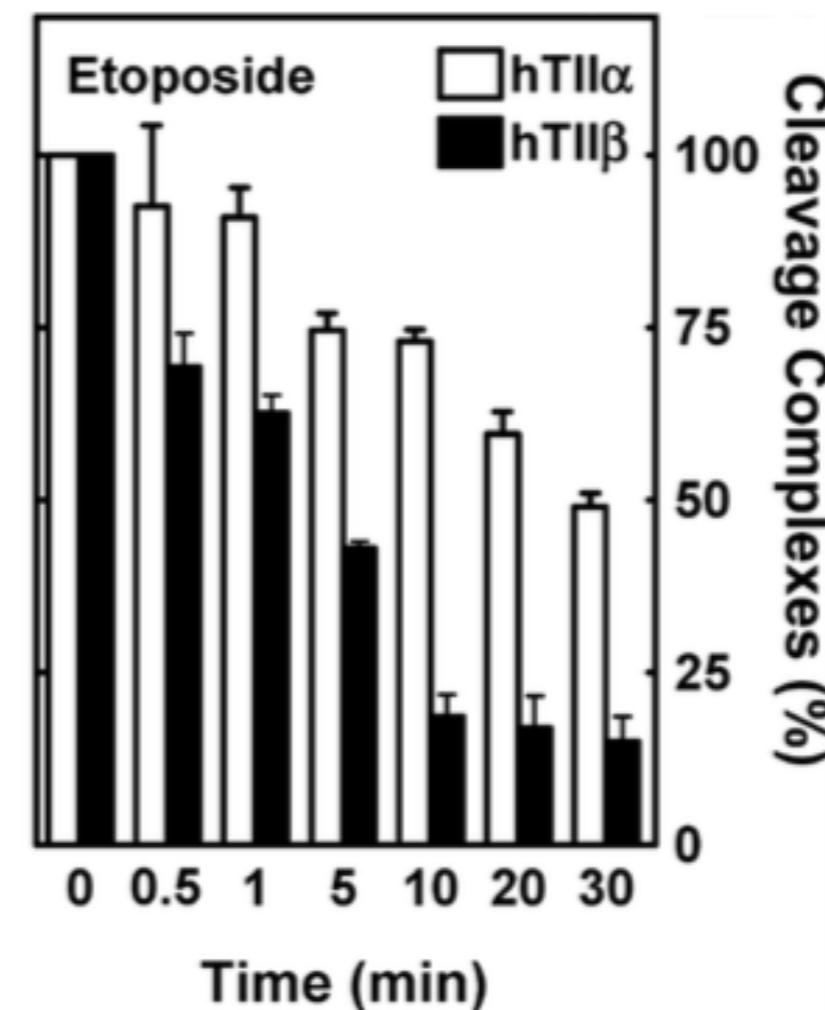
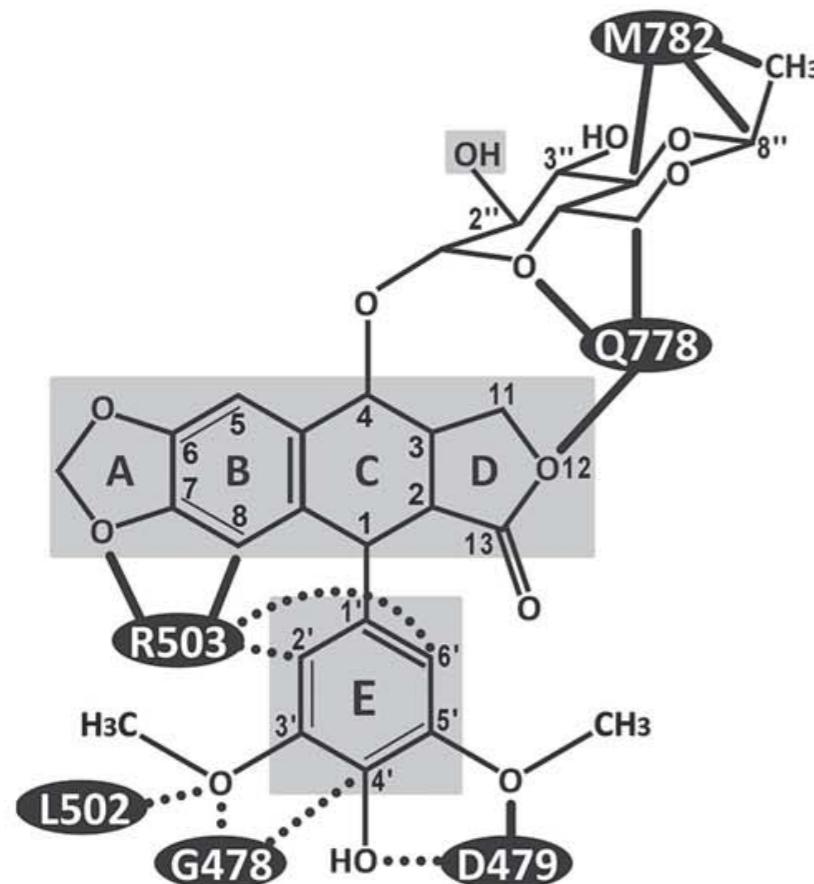
# Molecular Mechanism

C.e. TOPOII Resistant  
 C.e. TOPOII Sensitive  
 Human TOPOII $\alpha$   
 Human TOPOII $\beta$

VAEISAYHH**GEQ**SLMGTIVNLAQDYVGSNNINLLLPIGQFGTRLQGGKDSASARYIFT  
 VAEISAYHH**GEM**SLMGTIVNLAQDYVGSNNINLLLPIGQFGTRLQGGKDSASARYIFT  
 VAEMSSYHH**GEM**SLMMTIINLAQNFVGSNNLNLLQPIGQFGTRLHGGKDSASPRYIFT  
 VAEMSAYHH**GEQ**ALMMTIVNLAQNFVGSNNINLLQPIGQFGTRLHGGKDAASPRYIFT

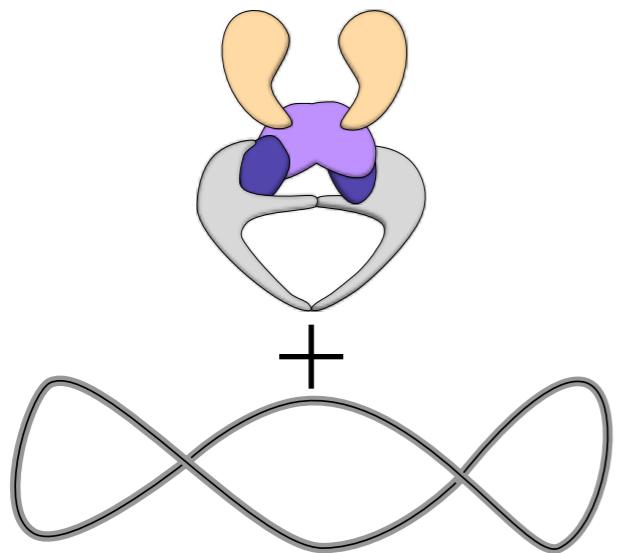
Drug binding

Catalytic tyrosine

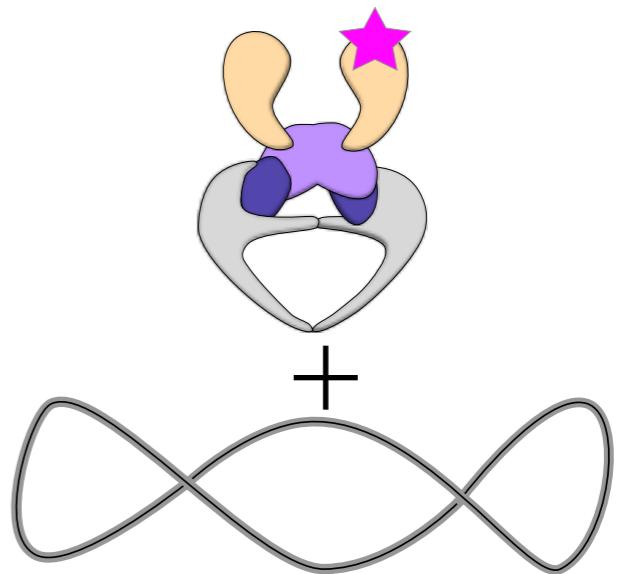


# Top2cc Assay

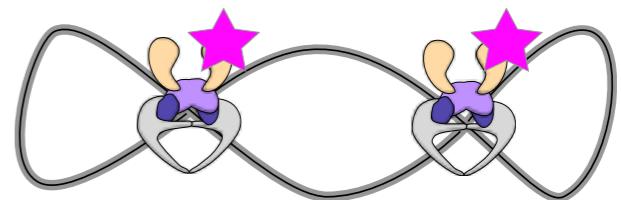
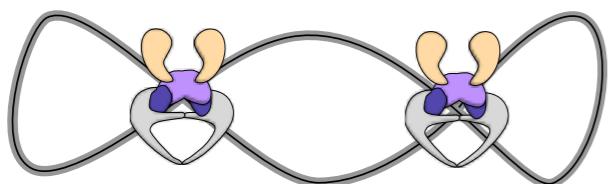
Resistant



Sensitive TOPOII



+Etoposide



**Aim 3**

# Acknowledgements

## **Andersen lab!**

Erik Andersen  
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Zifan Xiang  
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Tyler Shimko



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