



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

Stefan Zdraljevic

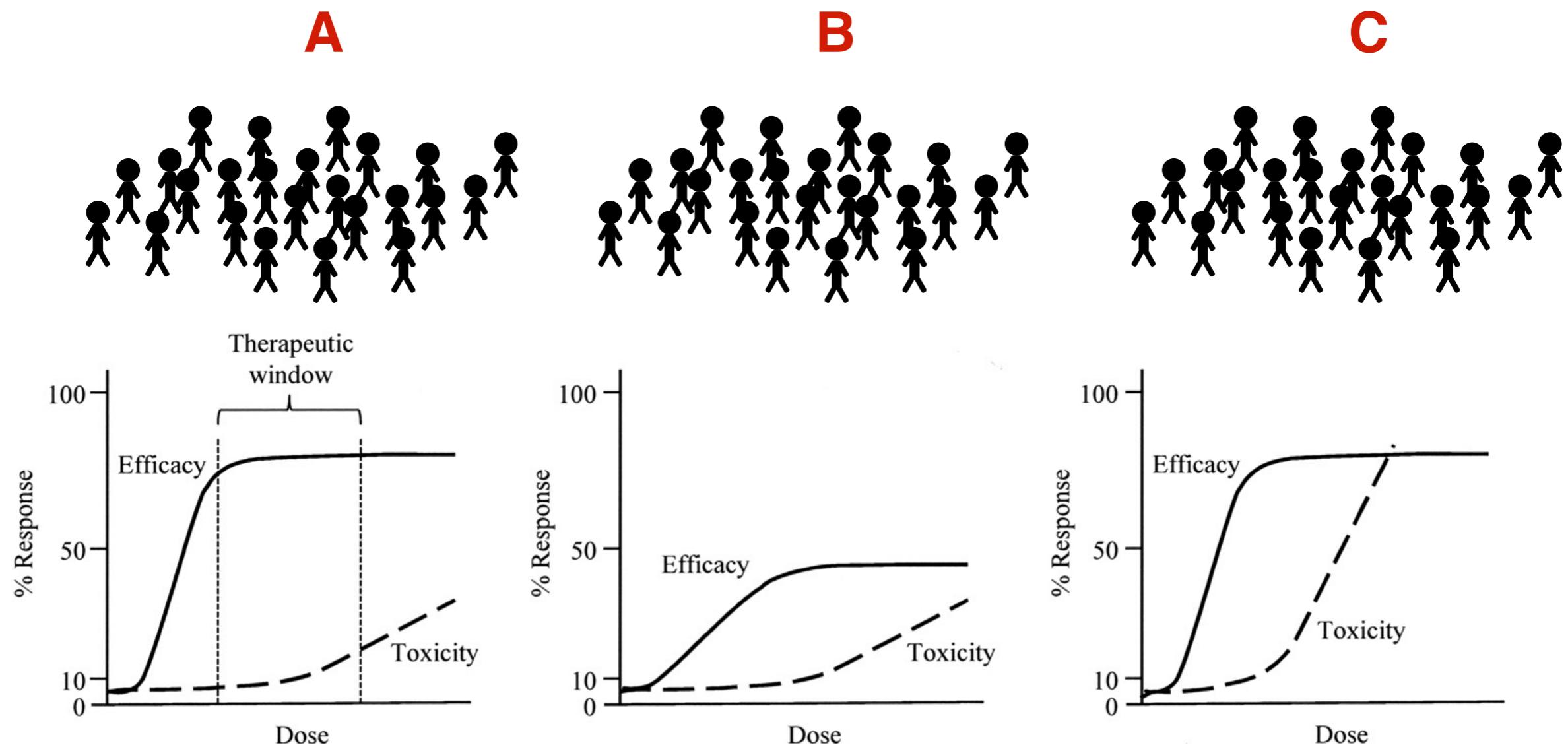
Advisor – Erik C. Andersen, Ph.D.

June 2nd, 2015

# Outline

- Motivation
- Preliminary Results
- Specific Aims

# Identifying an Ideal Therapeutic Window



# Sources of Variation

Adherence to treatment (both patients and doctors)

Medication access

Drug-drug interactions

Drug transport/metabolism

Genetic variation in drug targets

Effect of diet

Environment

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Genetic variation in drug targets

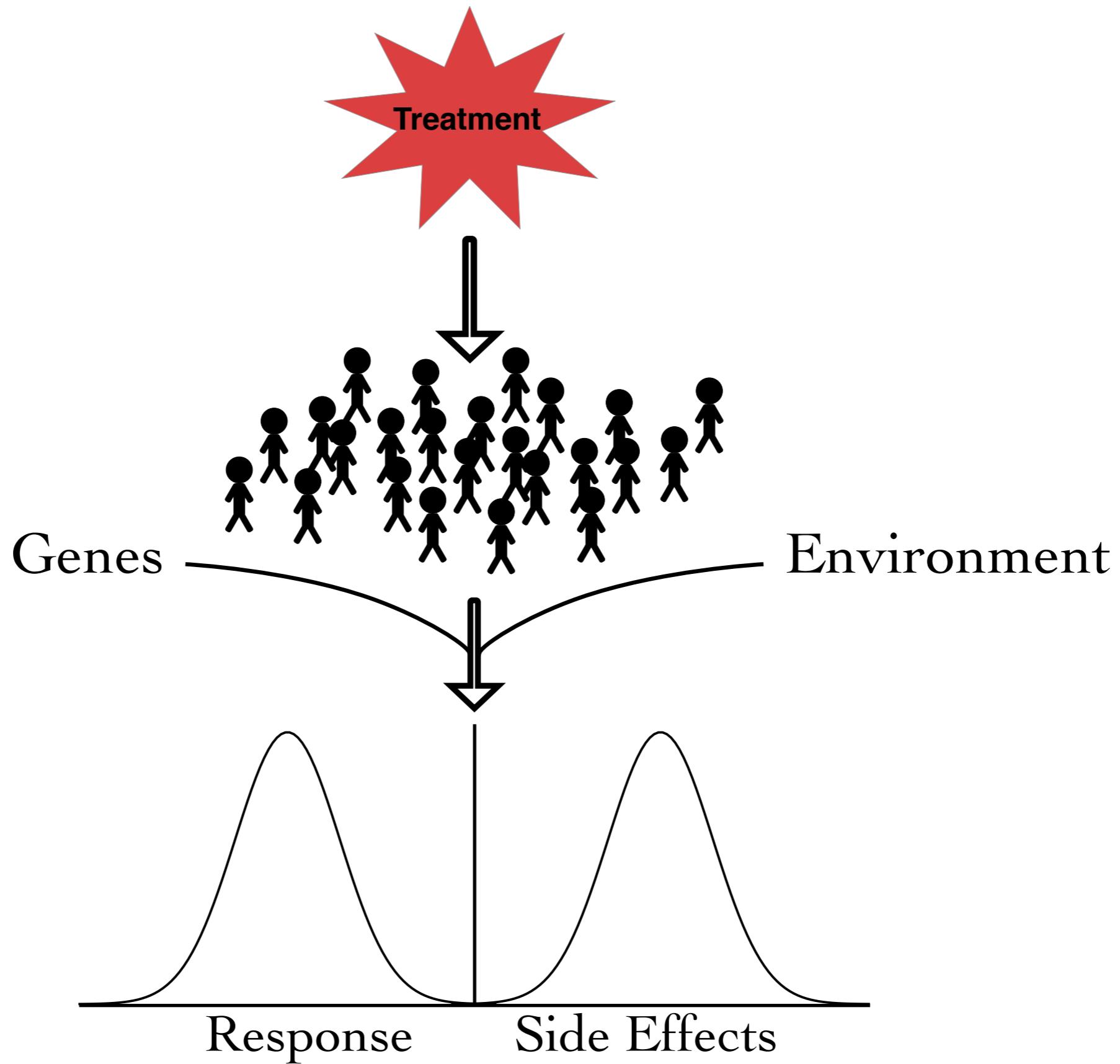
Effect of diet

Environment

**Influenced by genetic factors**

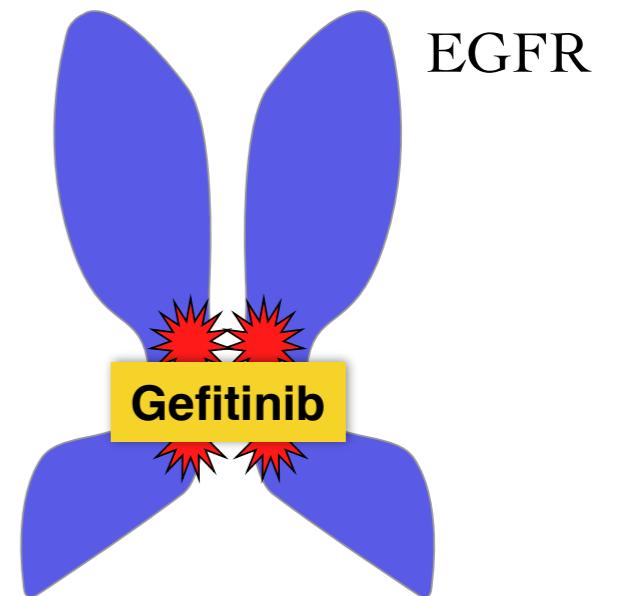
**Difficult to control**

# Genes and Environment Control Response

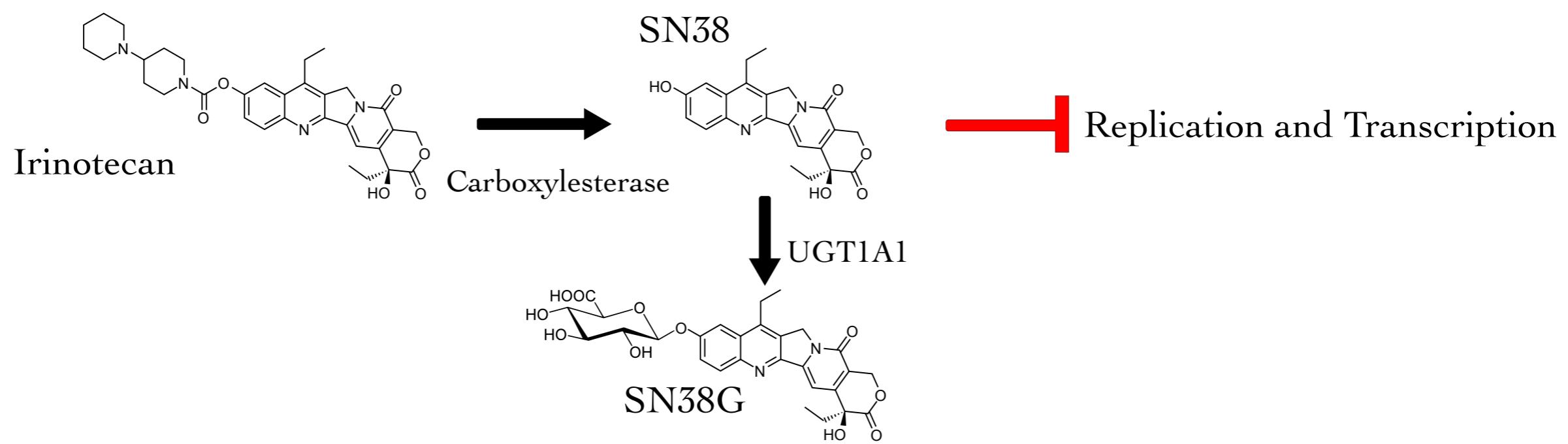


# Examples of Clinically Relevant Variants

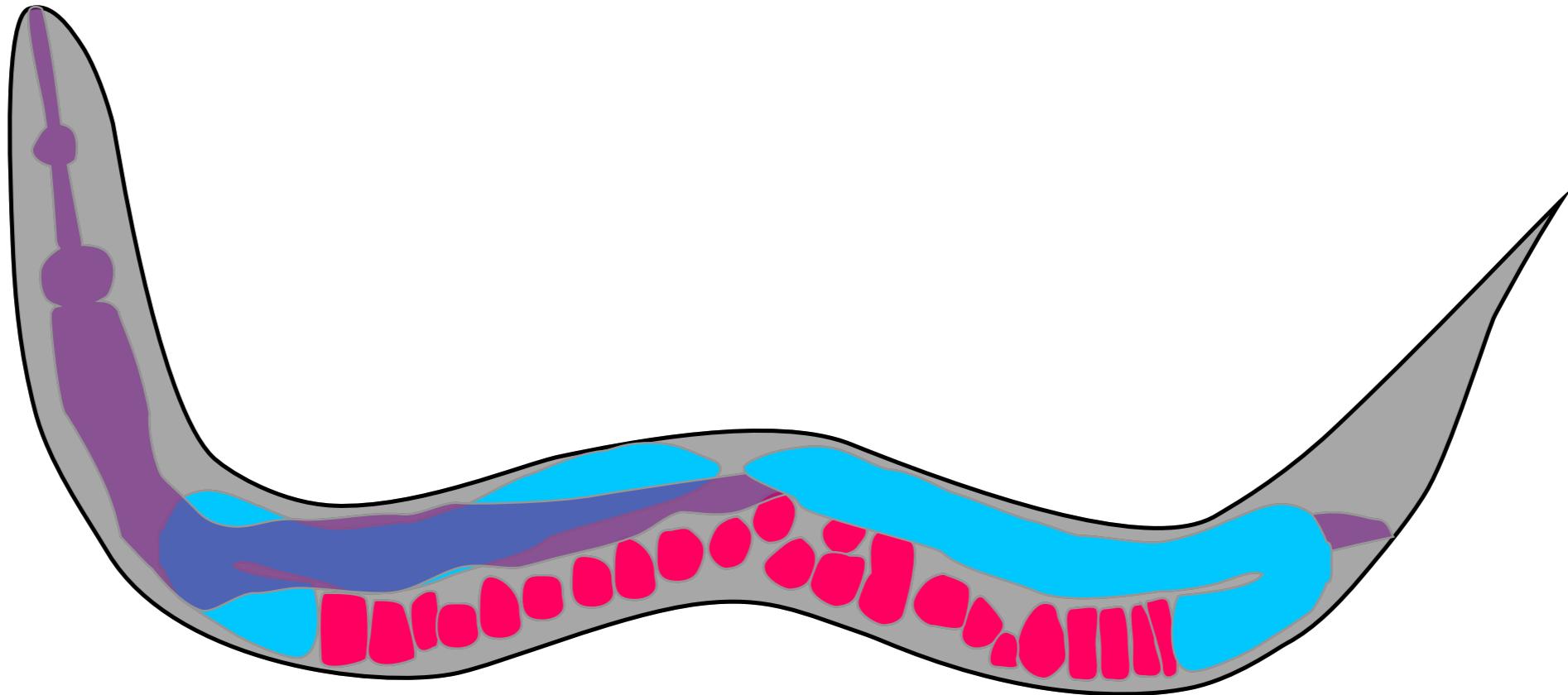
Genetic variation in drug targets



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.”



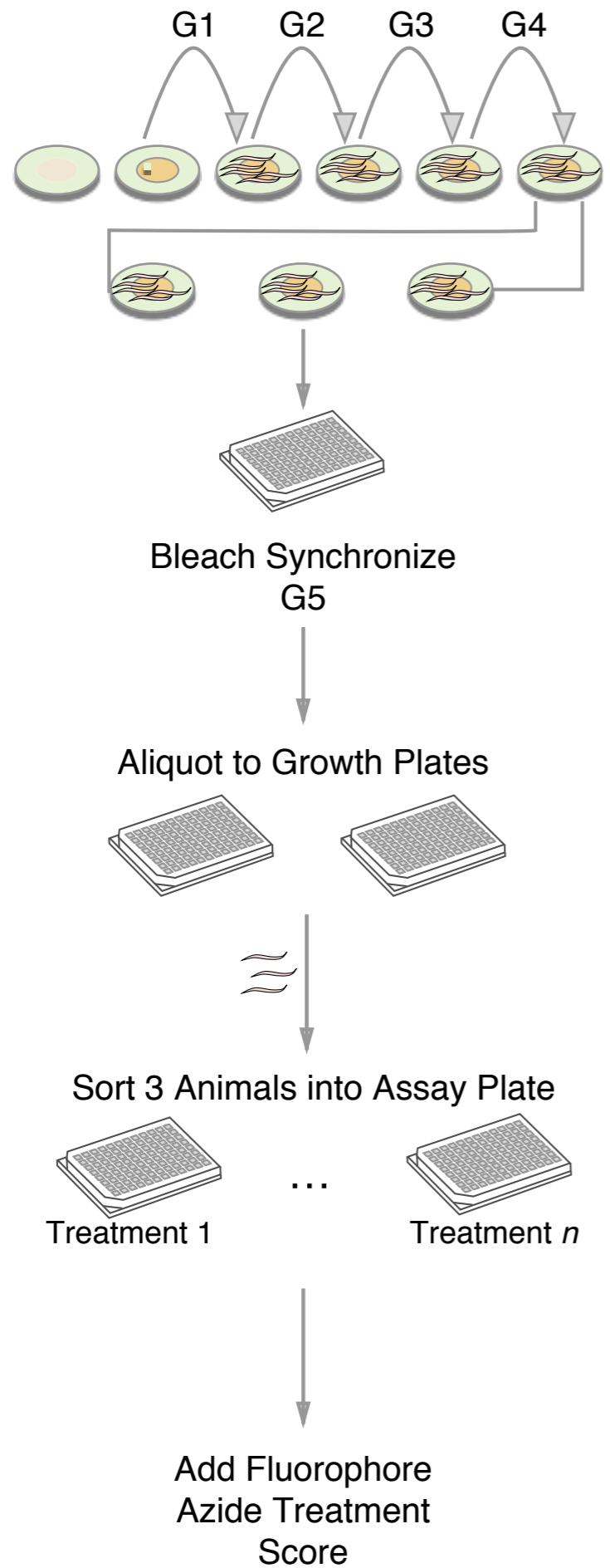
Easy to handle

Low cost

High replication of experiments

Short lifespan

Numerous genetic tools



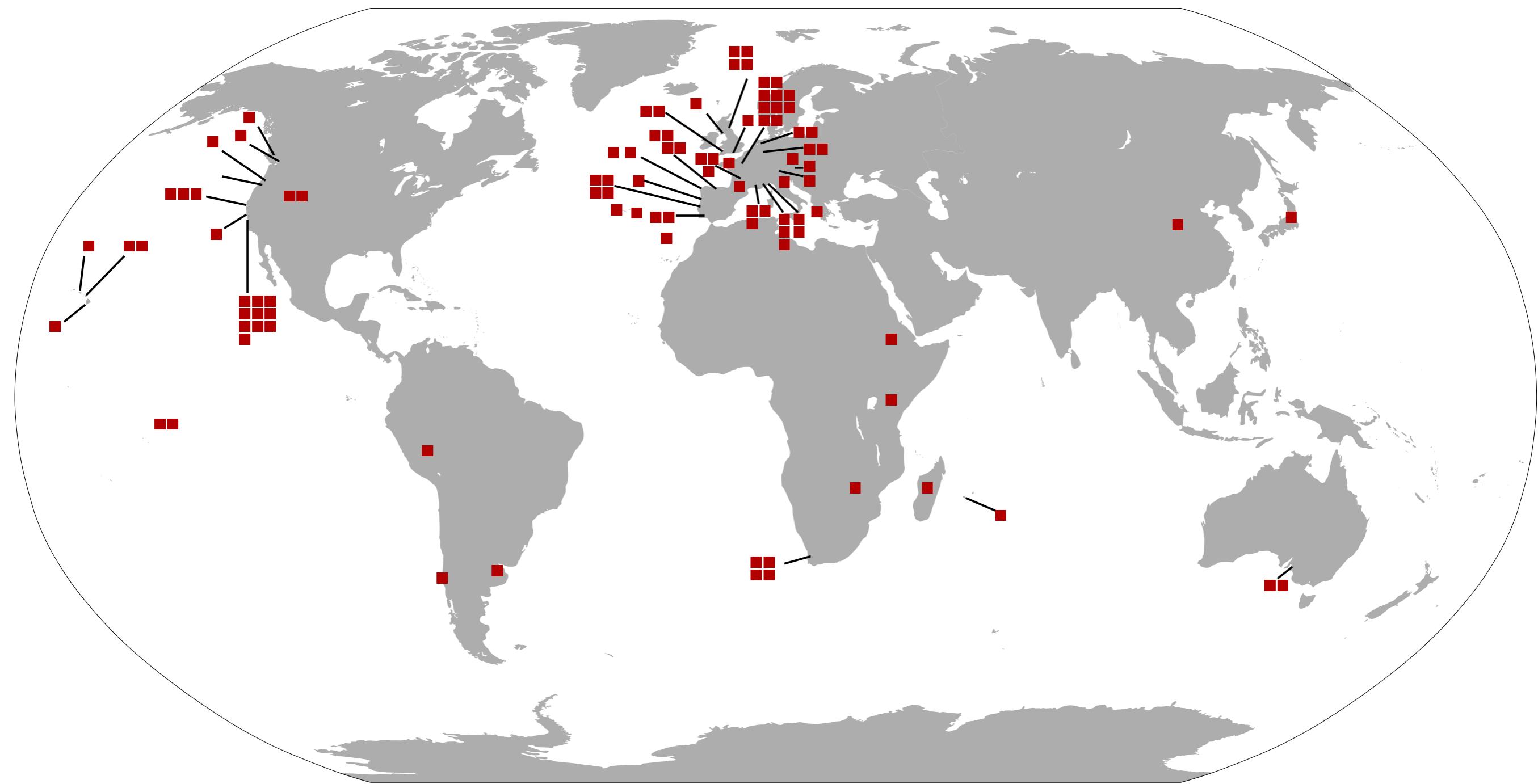
## Fitness Traits:

Brood size

Animal length

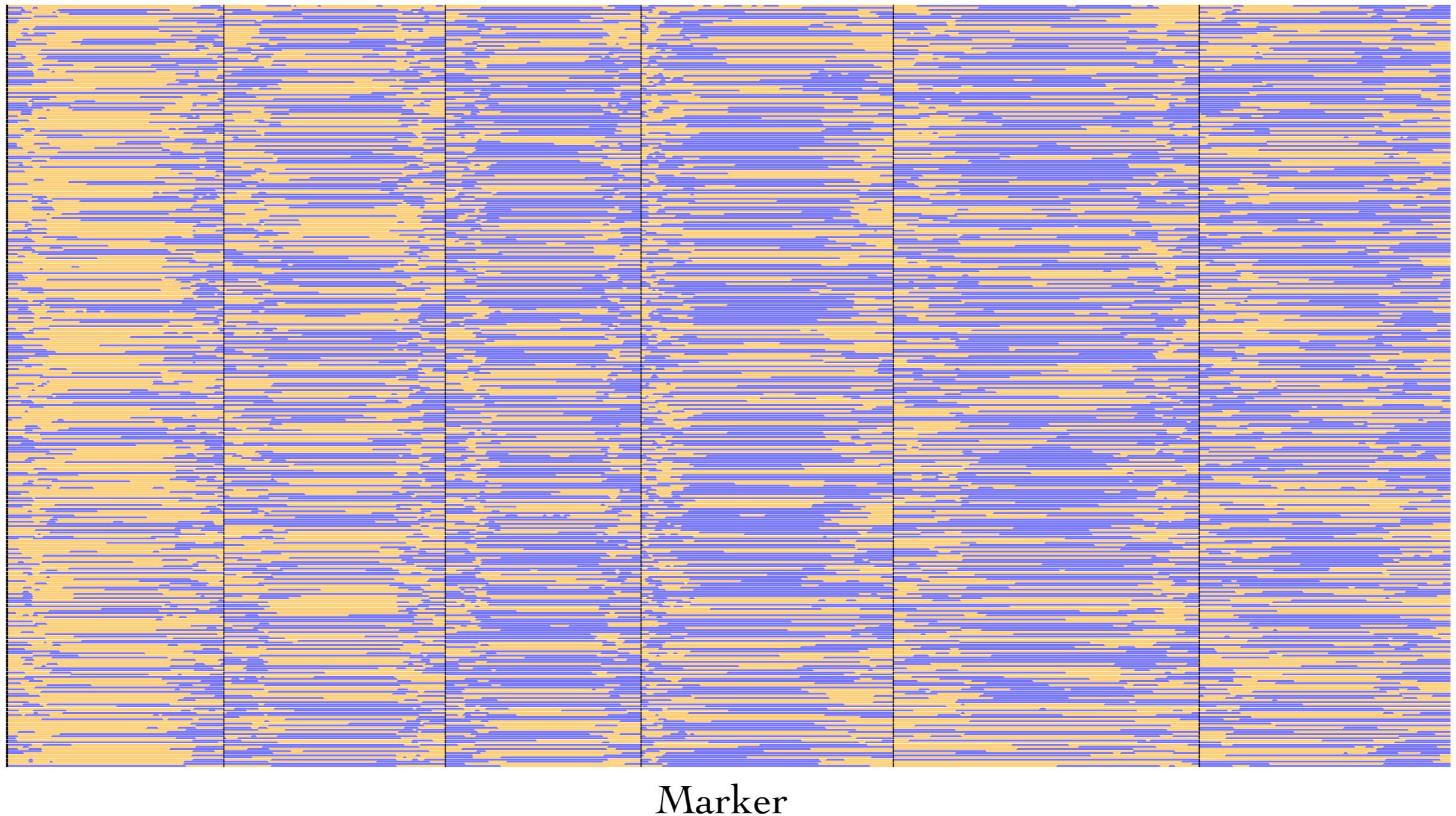
Pharyngeal pumping

# Global Distribution of *C. elegans*



# RIAILs Genotype

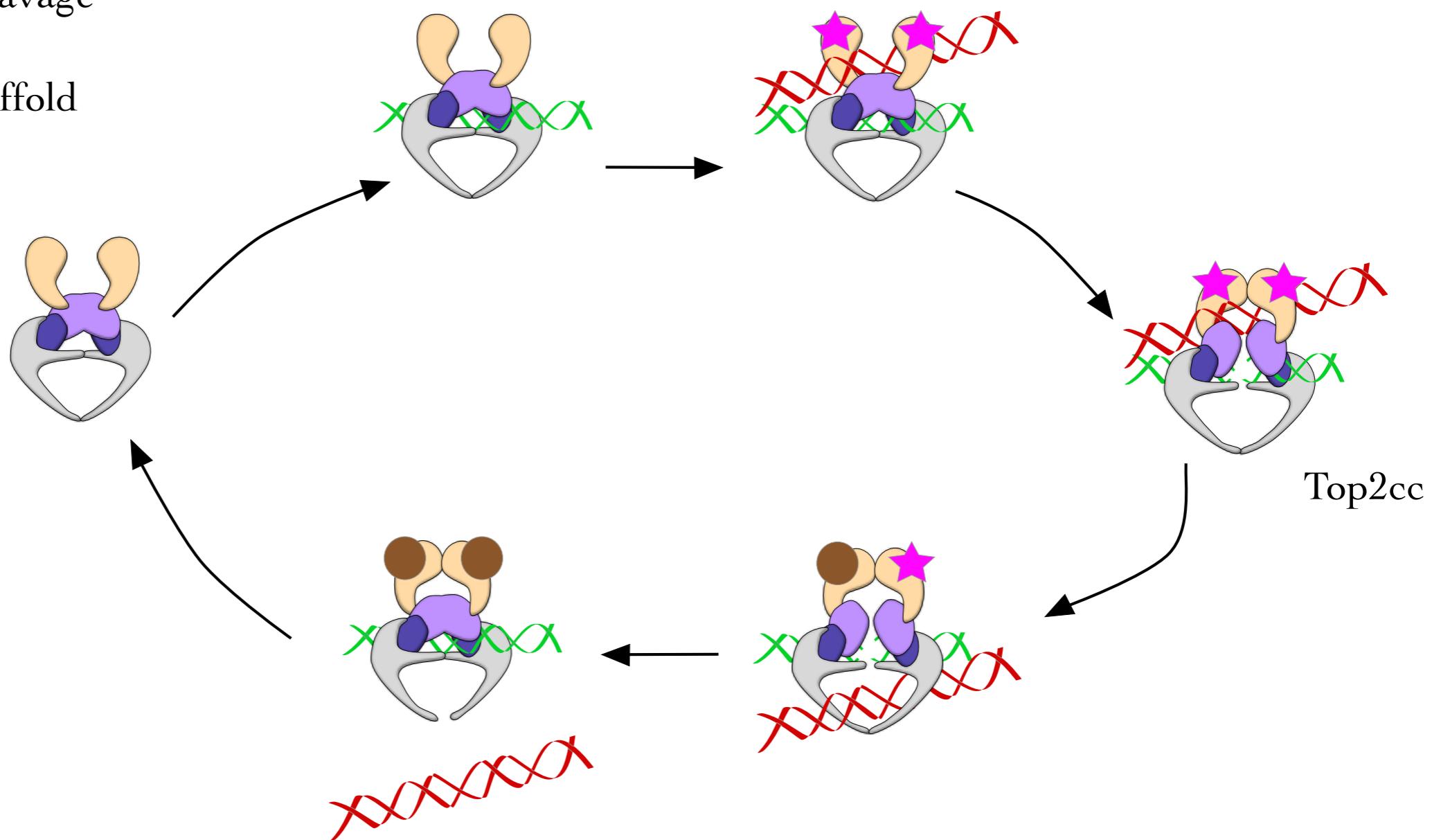
RIAIL



# Topoisomerase II Catalytic Cycle

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

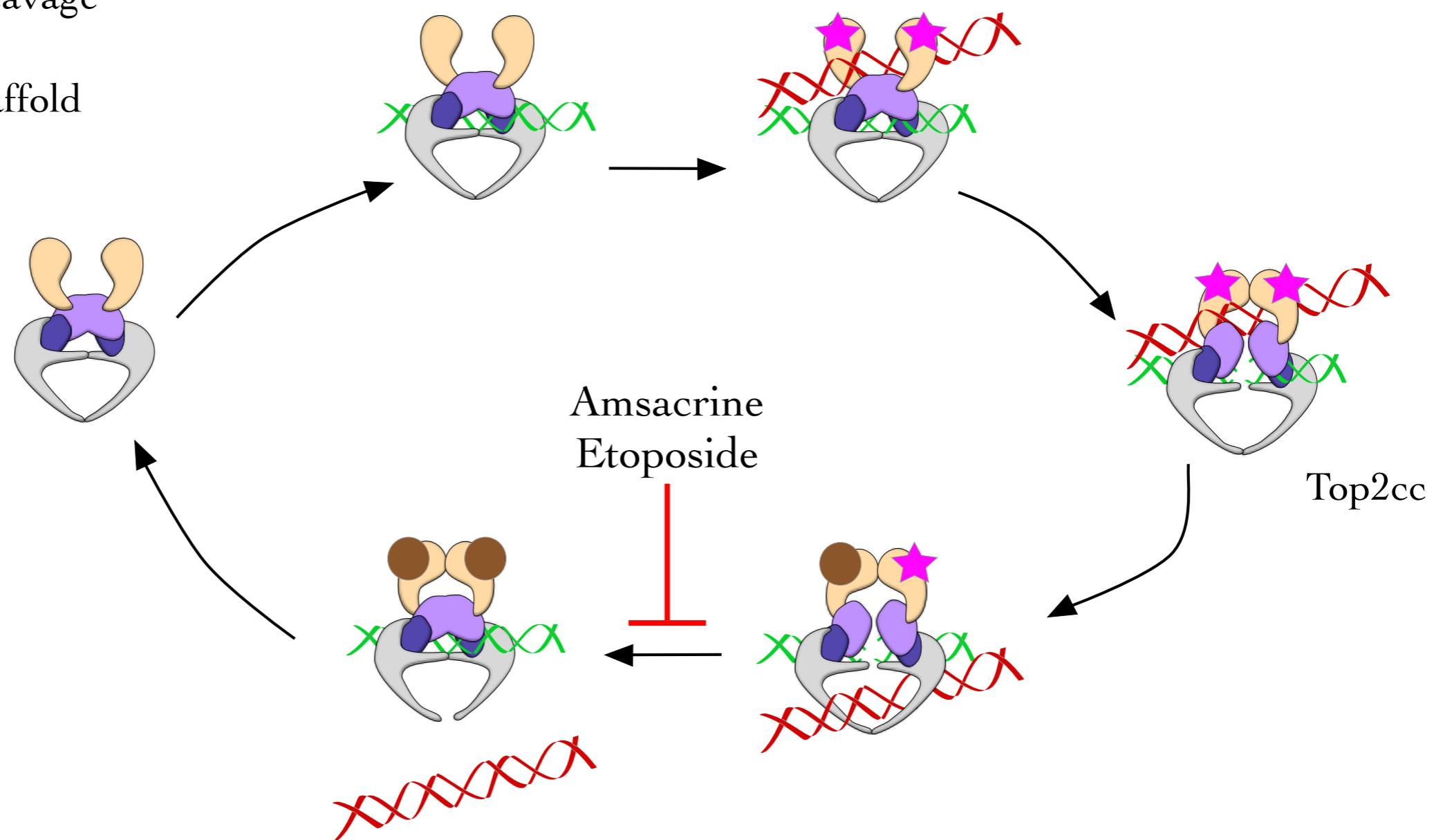
Cleavage  
Scaffold



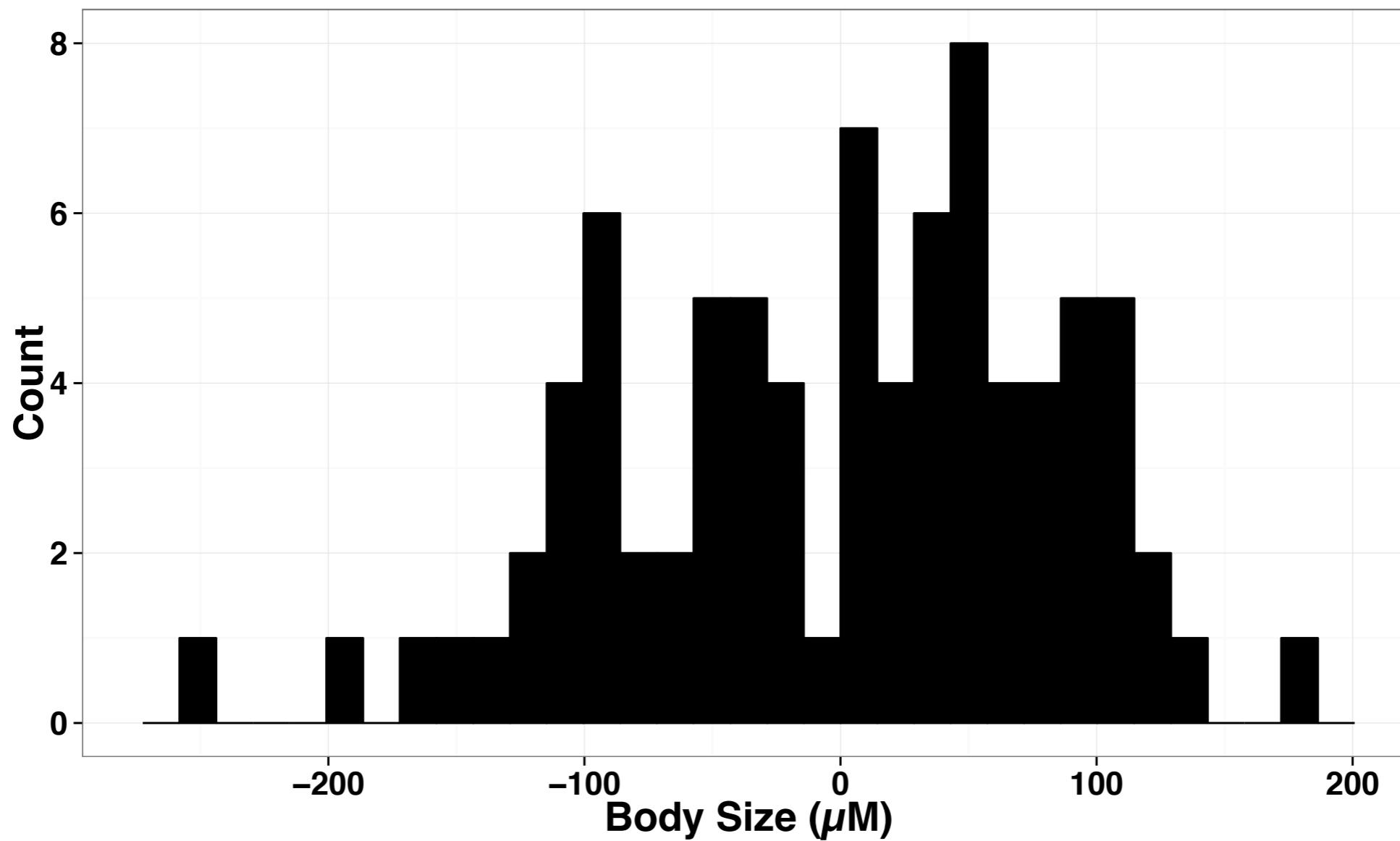
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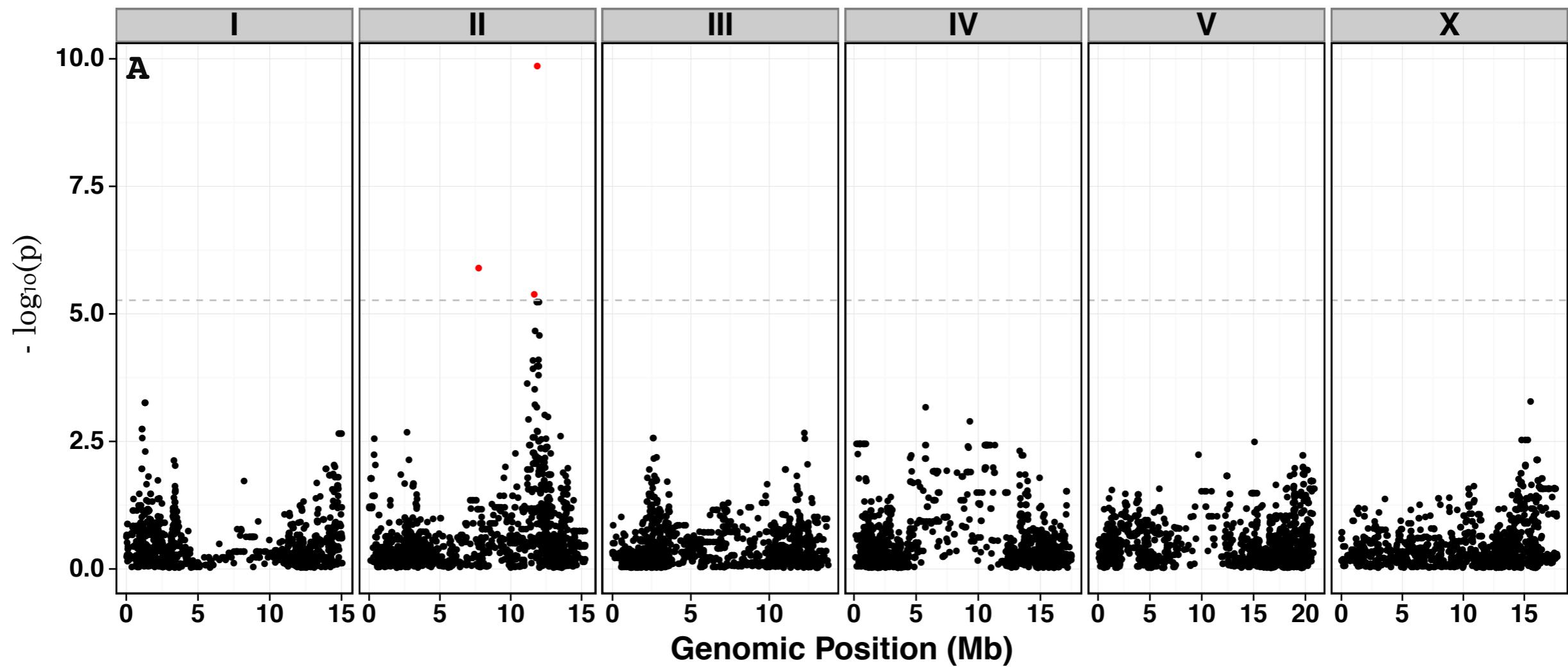


# Variation in Response to Etoposide

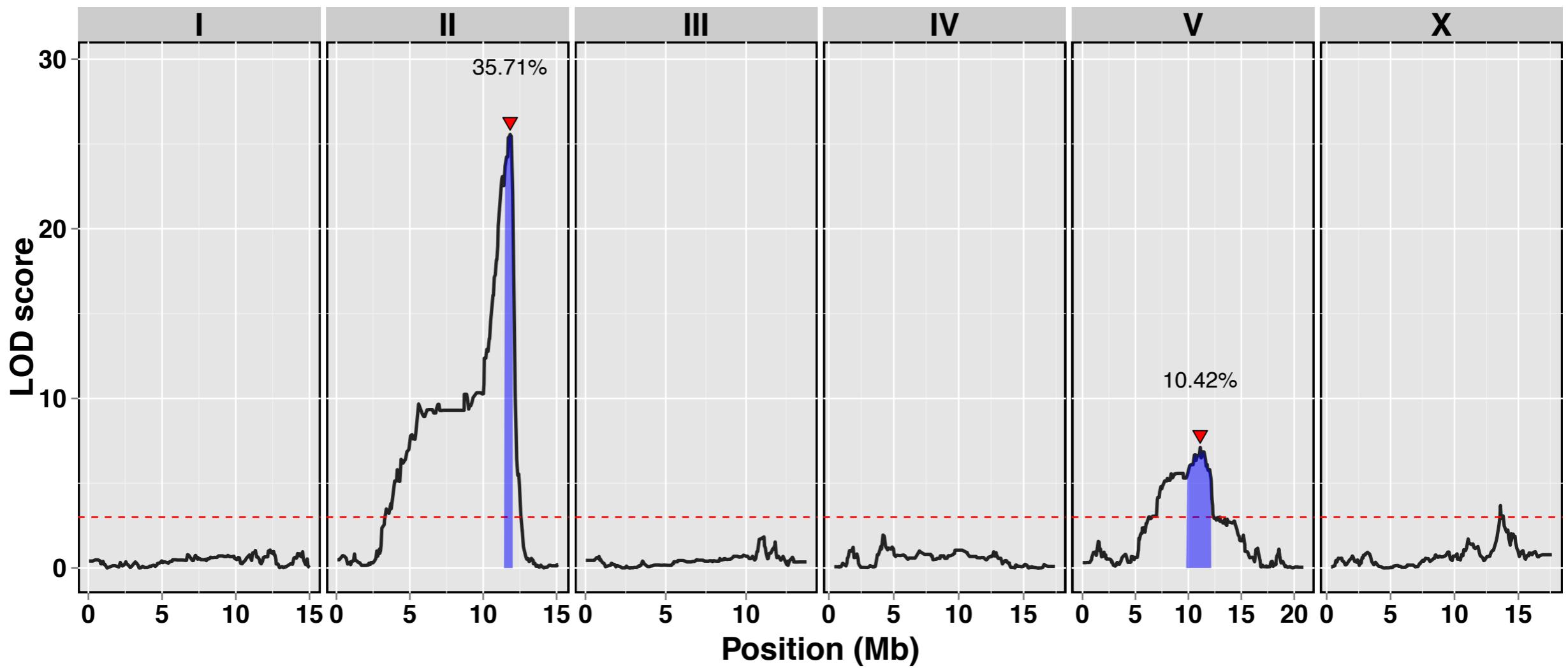


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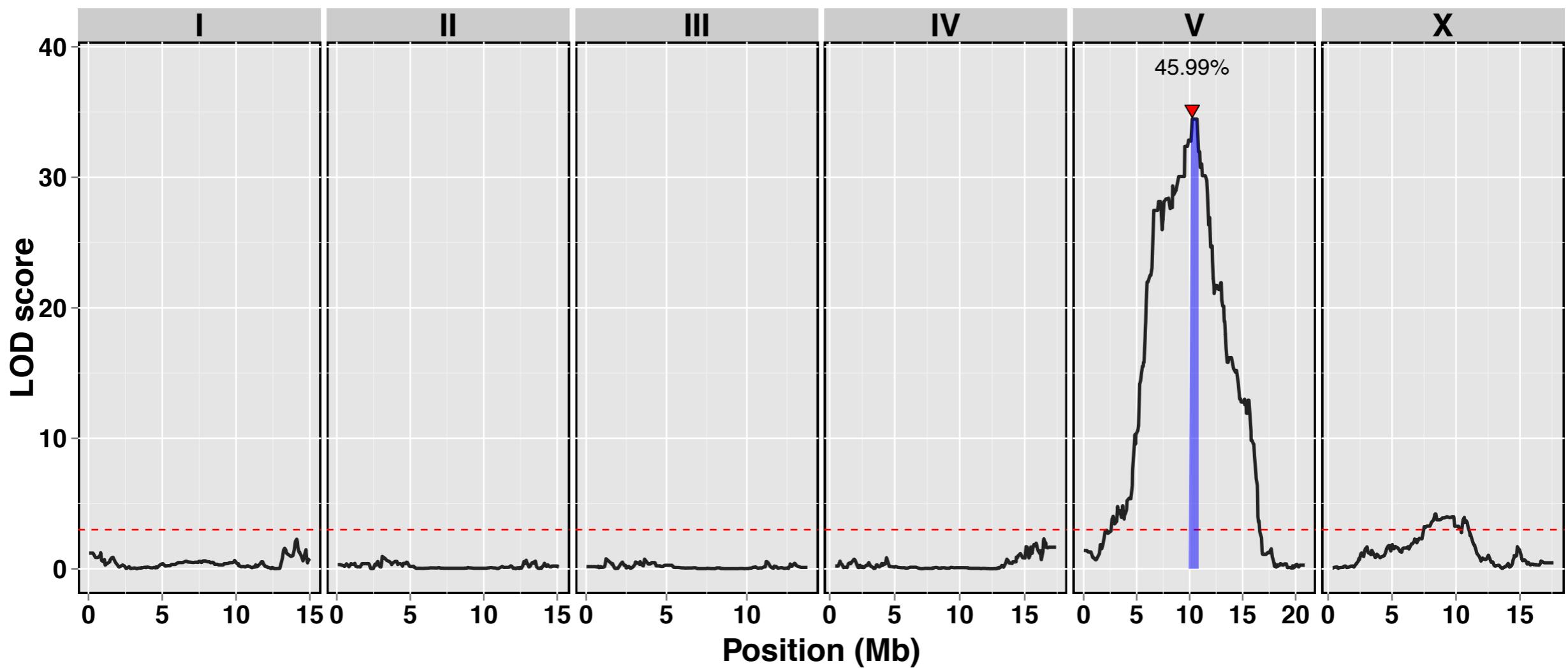
Animal Length



# Variation in Response to Etoposide



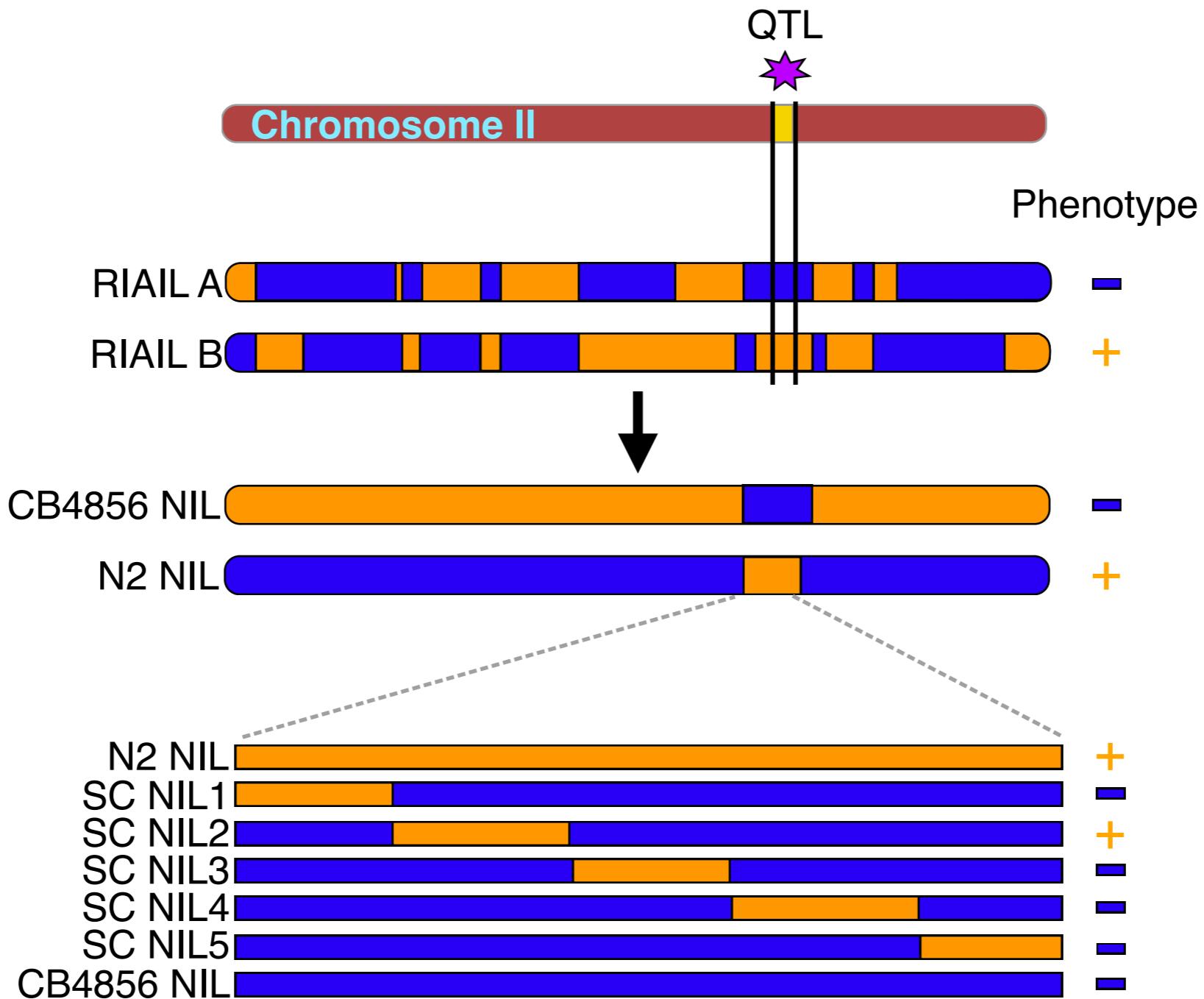
# Variation in Response to Amsacrine



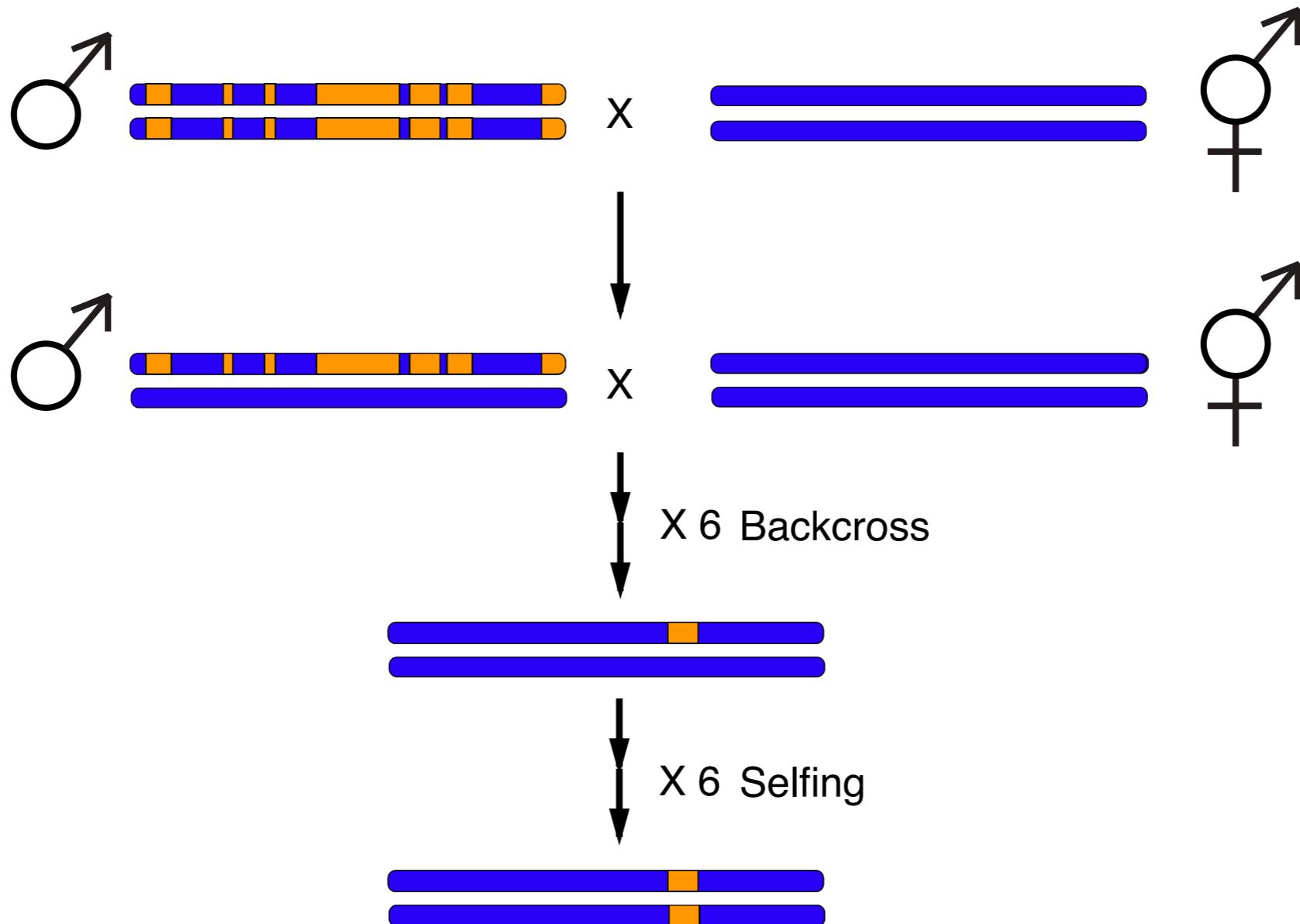
# Specific Aims

- **Specific Aim 1:** Genomic regions underlying QTL will confer resistance to topoisomerase II poisons in an otherwise sensitive genetic background
- **Specific Aim 2:** Specific genetic variants contribute to phenotypic variation in response to topoisomerase II poisons
- **Specific Aim 3:** The Q797M variant topoisomerase II contributes to sensitivity in re- sponse to etoposide treatment

# Narrow Confidence Intervals

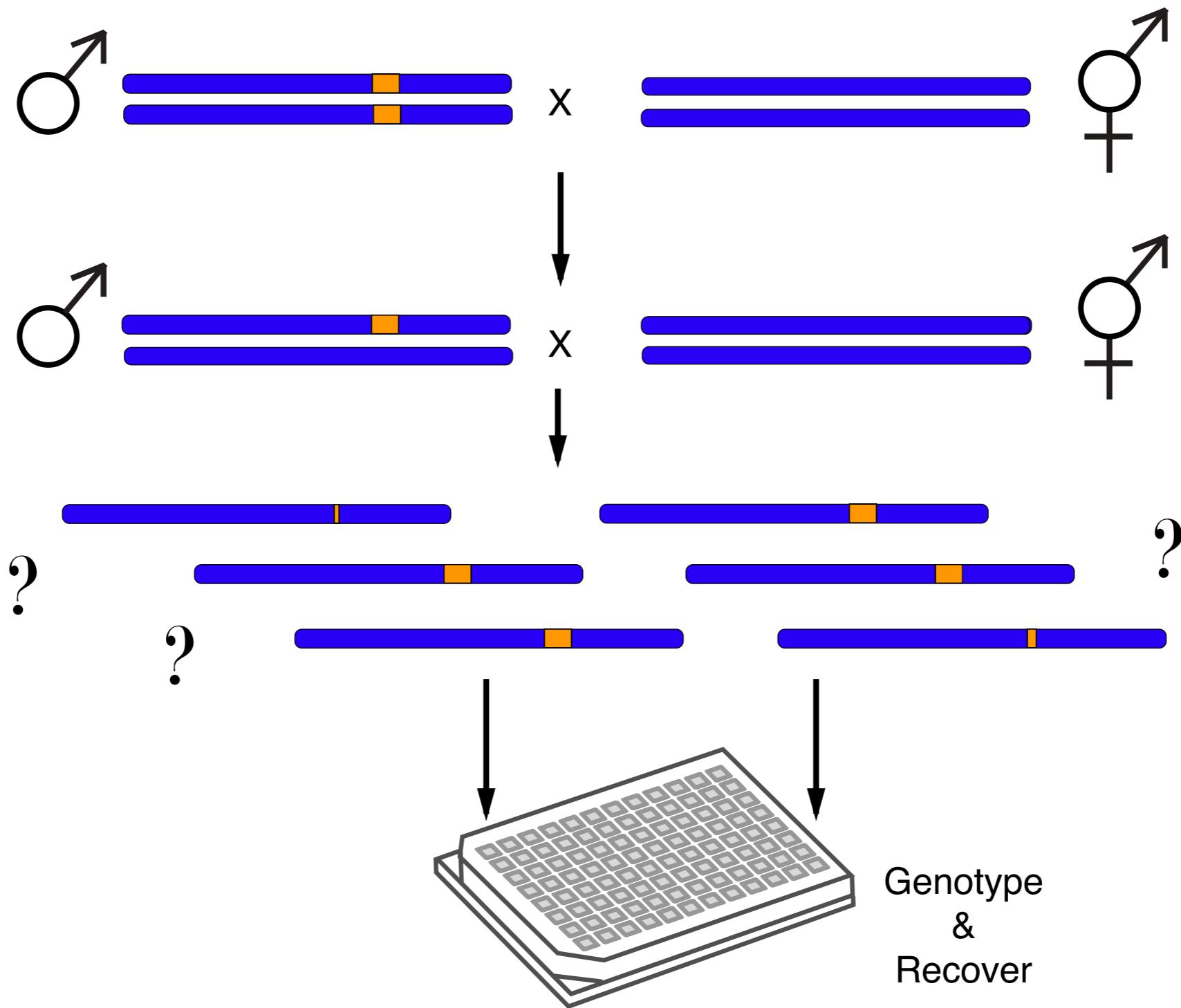


# Generating NILs



Aim 1

# Generating NILs



Aim 1

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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*



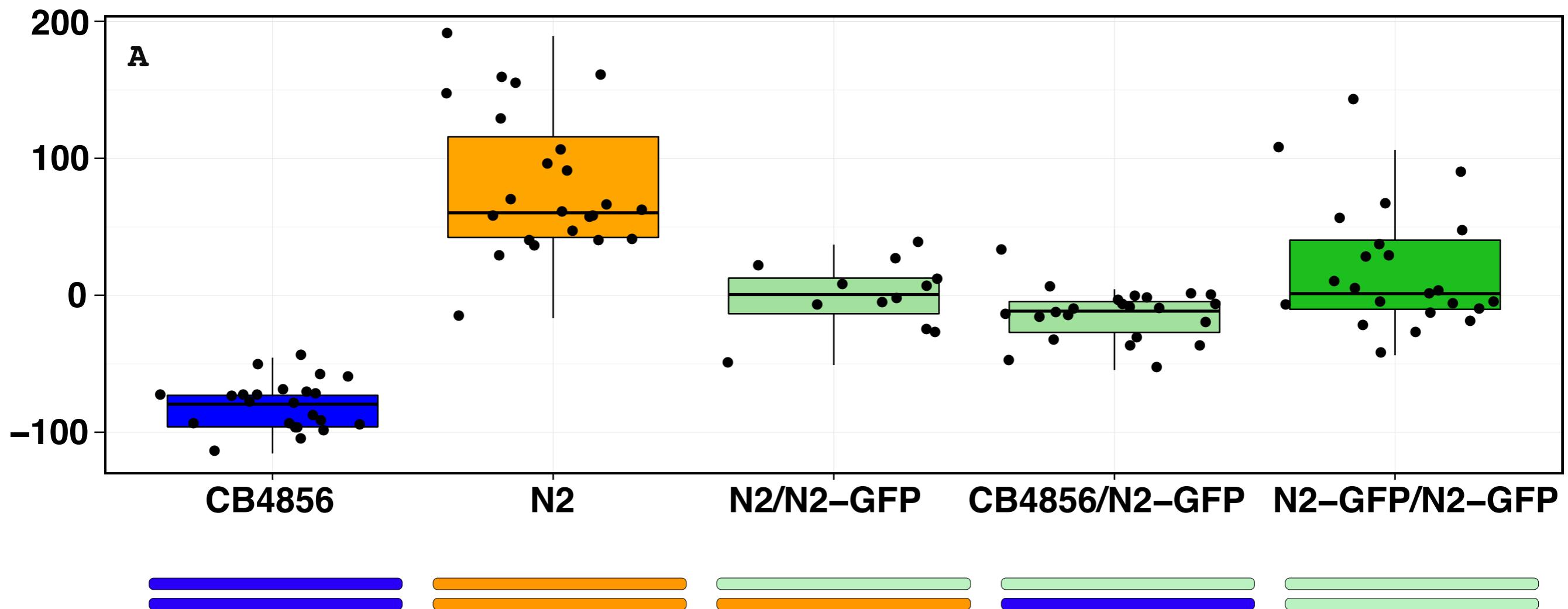
?

**Genotype**

**Phenotype**

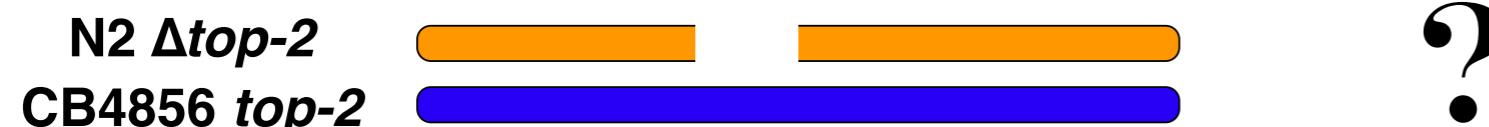
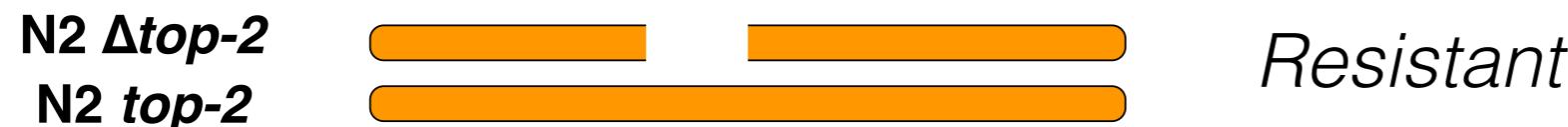
**Aim 2**

# Resistance to Etoposide is Dominant



Aim 2

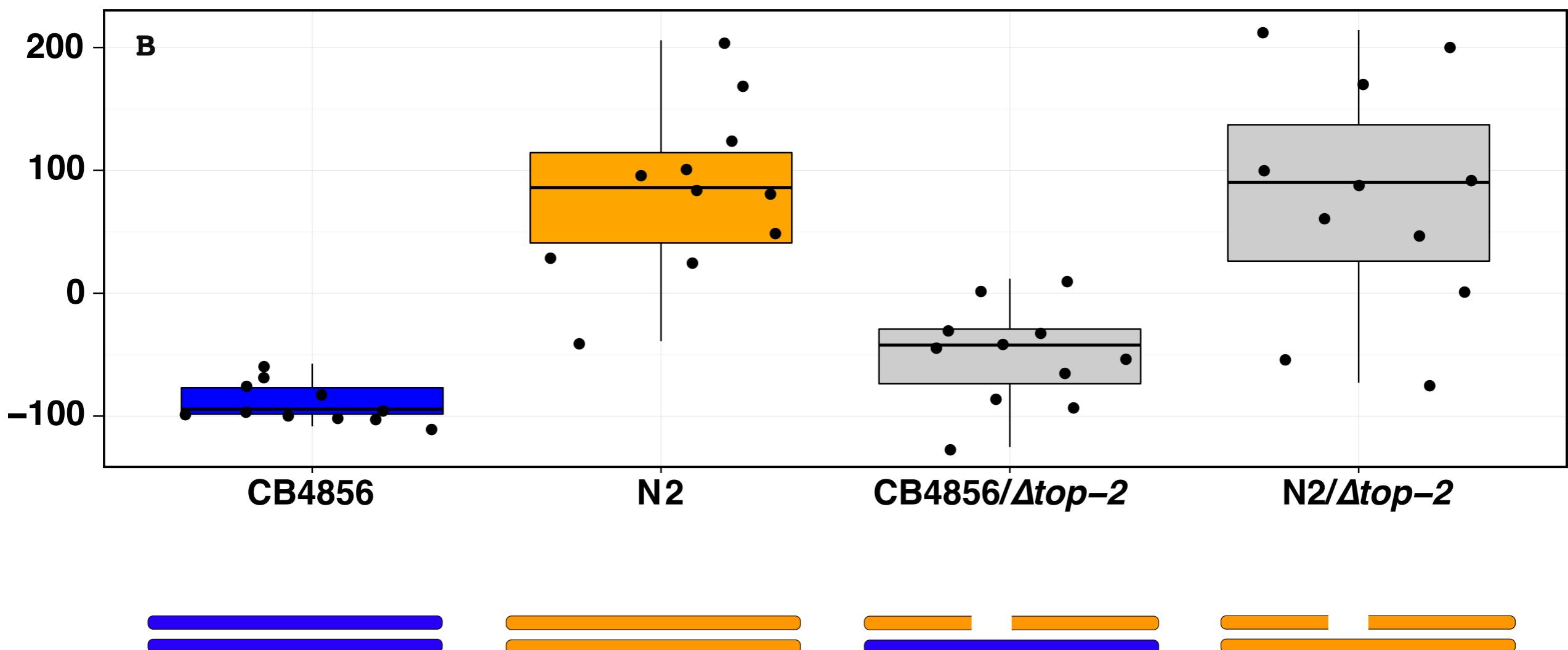
# Complementation Test – Design



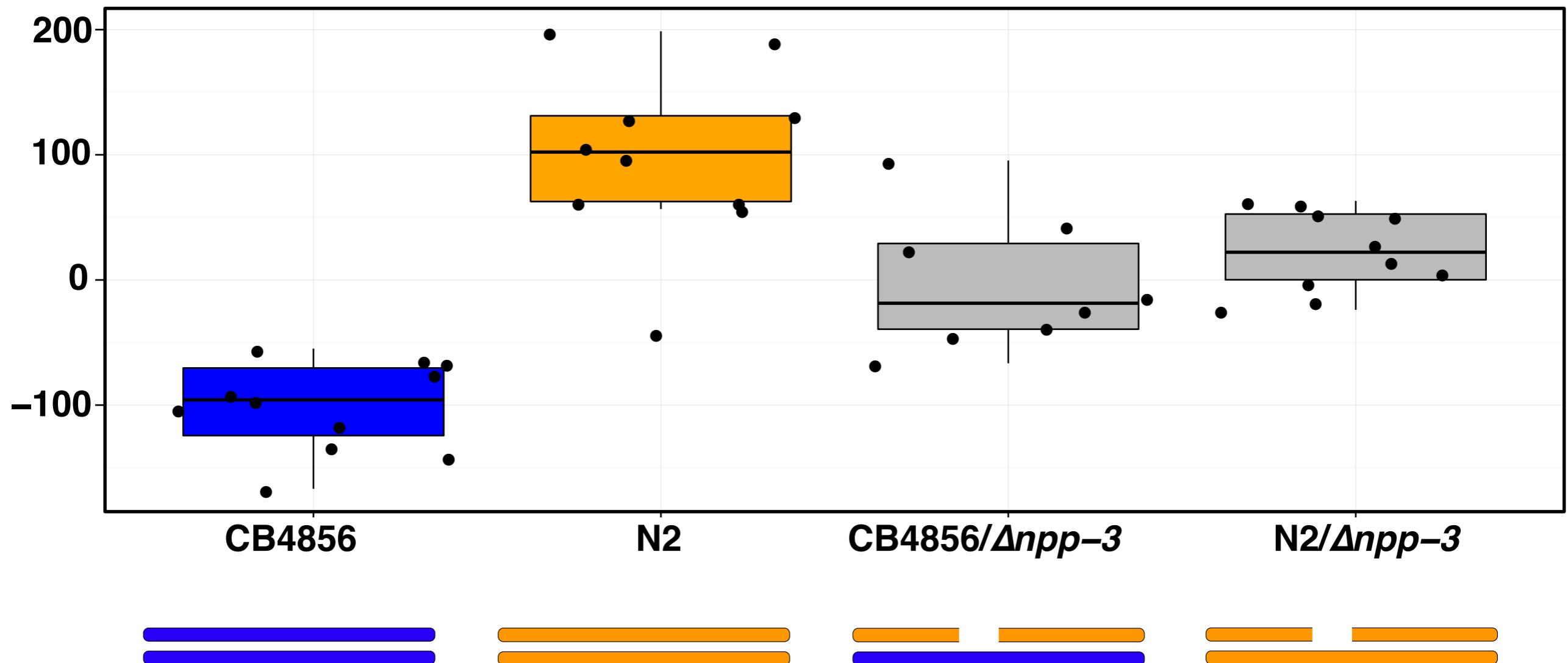
**Genotype**

**Phenotype**

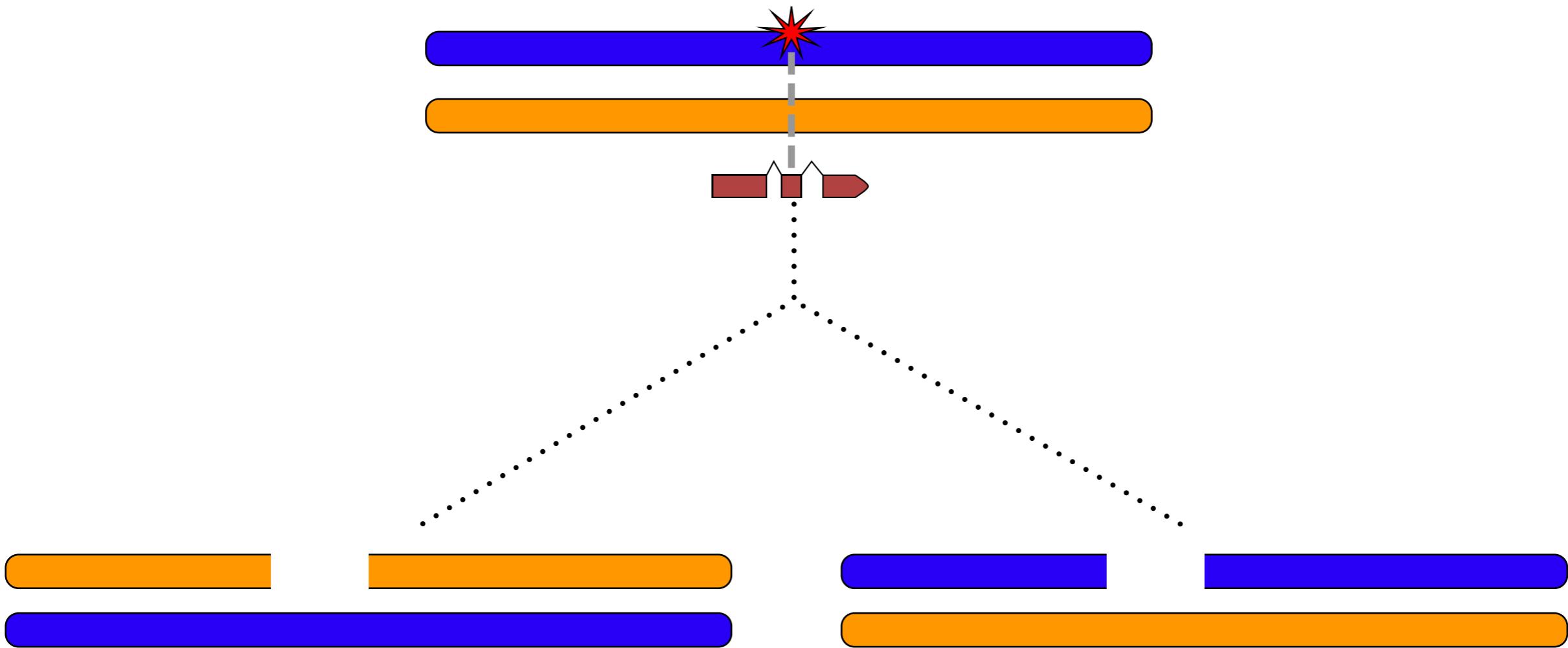
# Complementation Test – *top-2*



# Complementation Test — *npp-3*

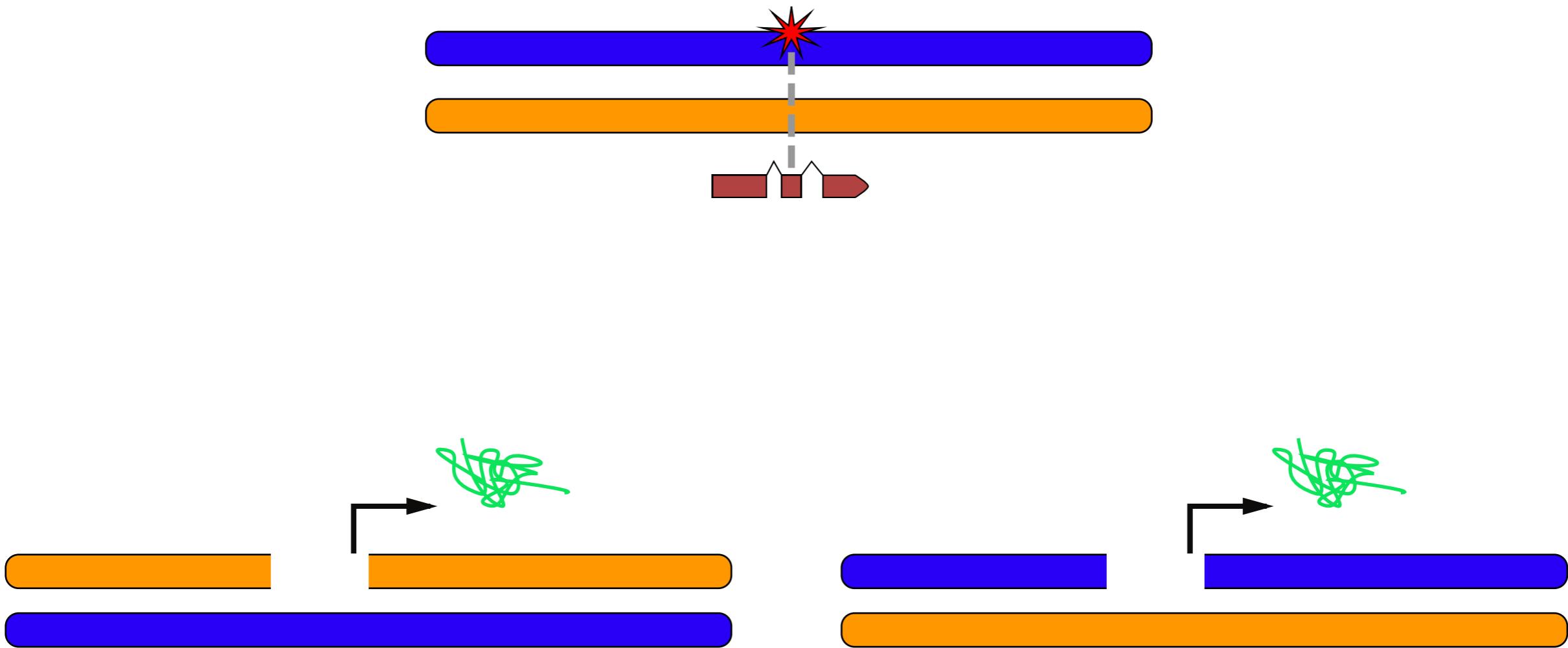


# Reciprocal Hemizygosity



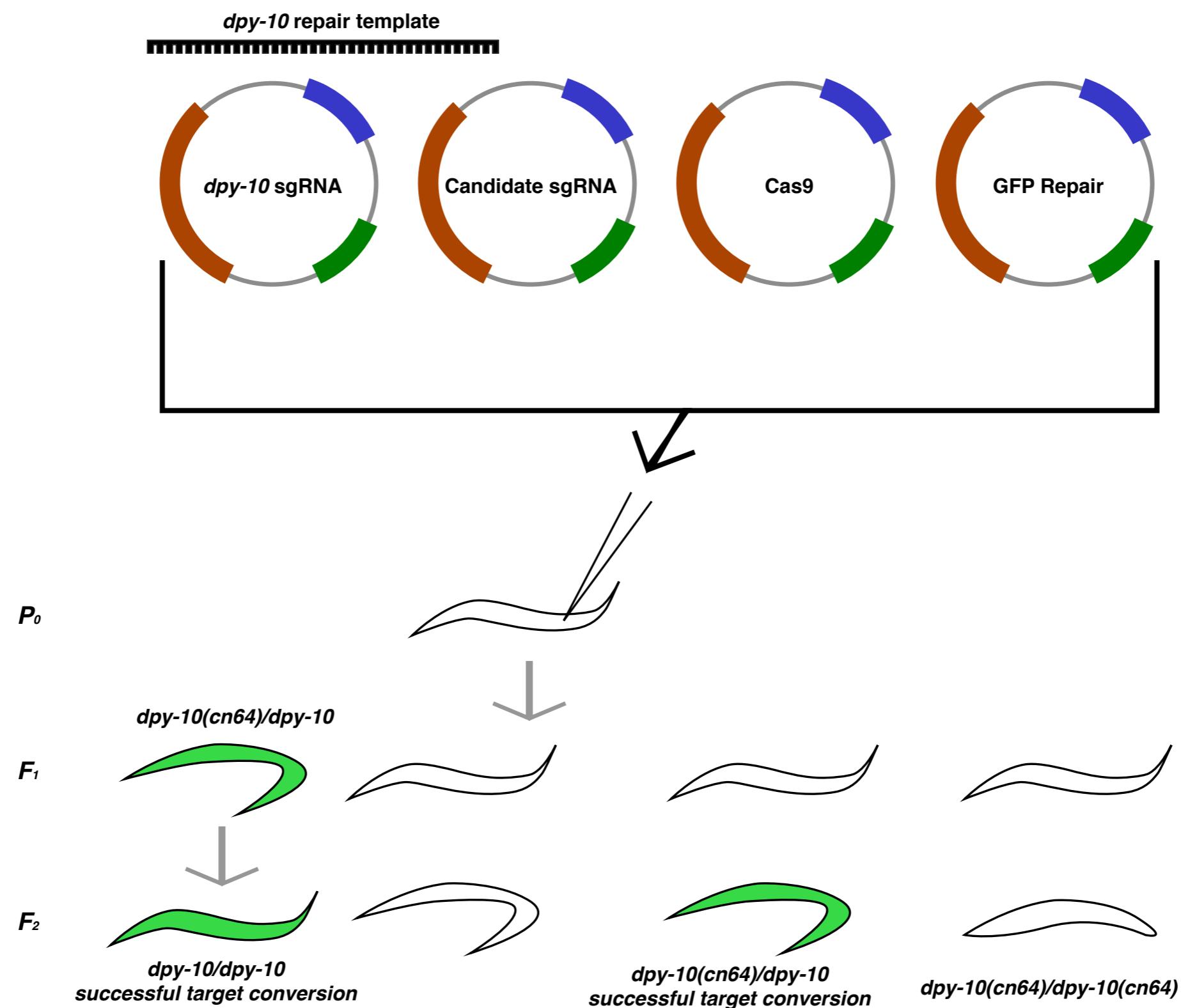
Aim 2

# Reciprocal Hemizygosity



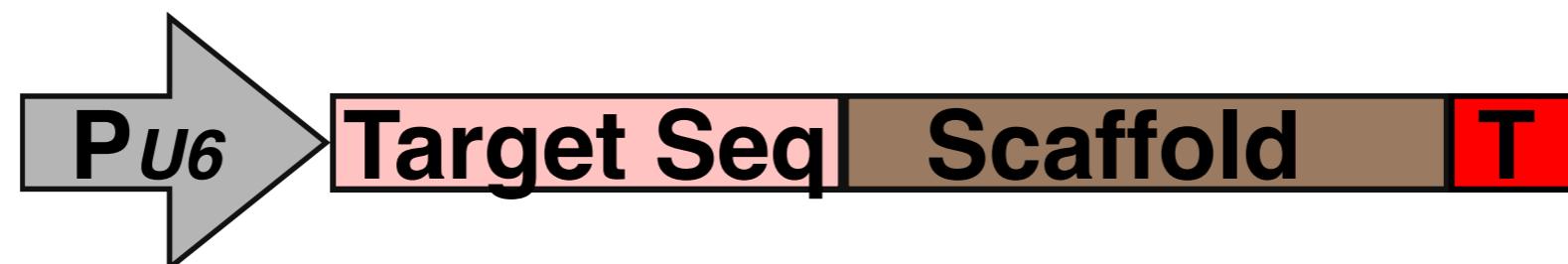
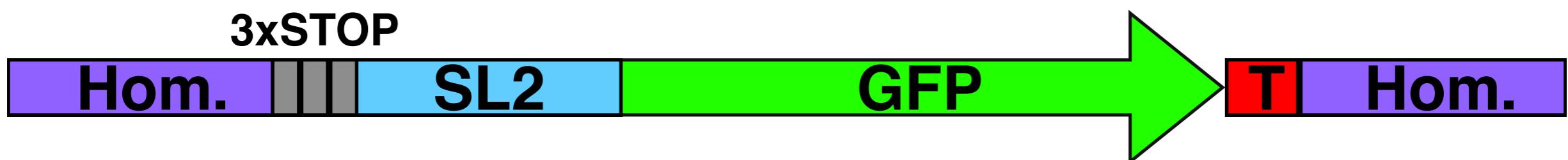
**Aim 2**

# Generating Strains for Reciprocal Hemizygosity



**Aim 2**

# CRISPR/Cas9 Constructs



**Aim 2**

- **Specific Aim 1:** Genomic regions underlying QTL will confer resistance to topoisomerase II poisons in an otherwise sensitive genetic background
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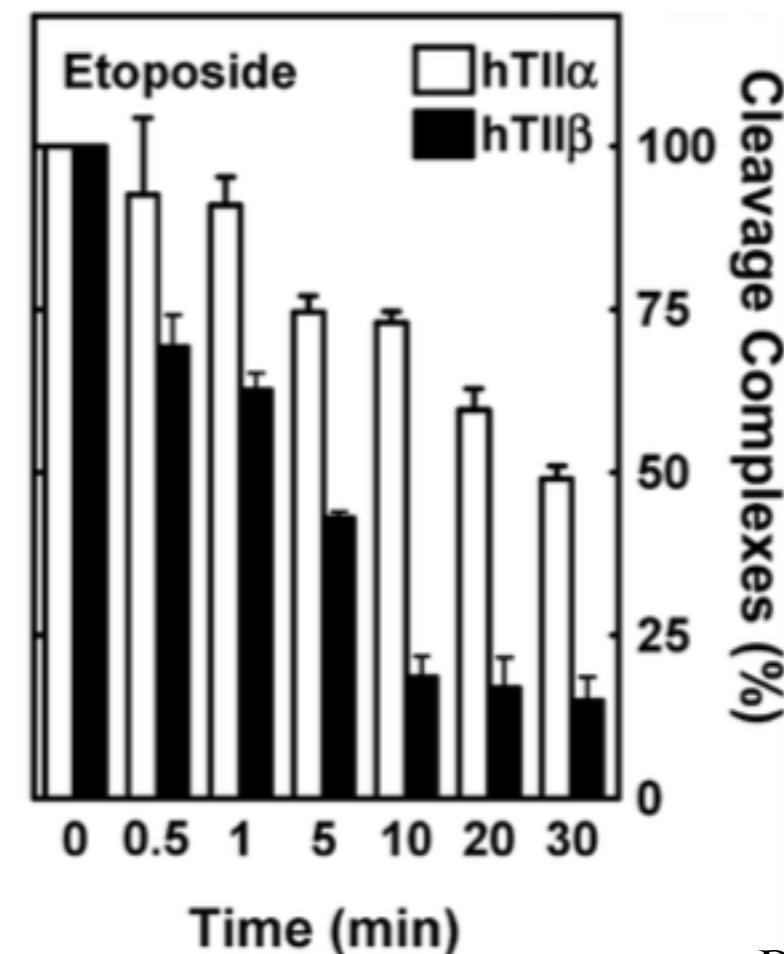
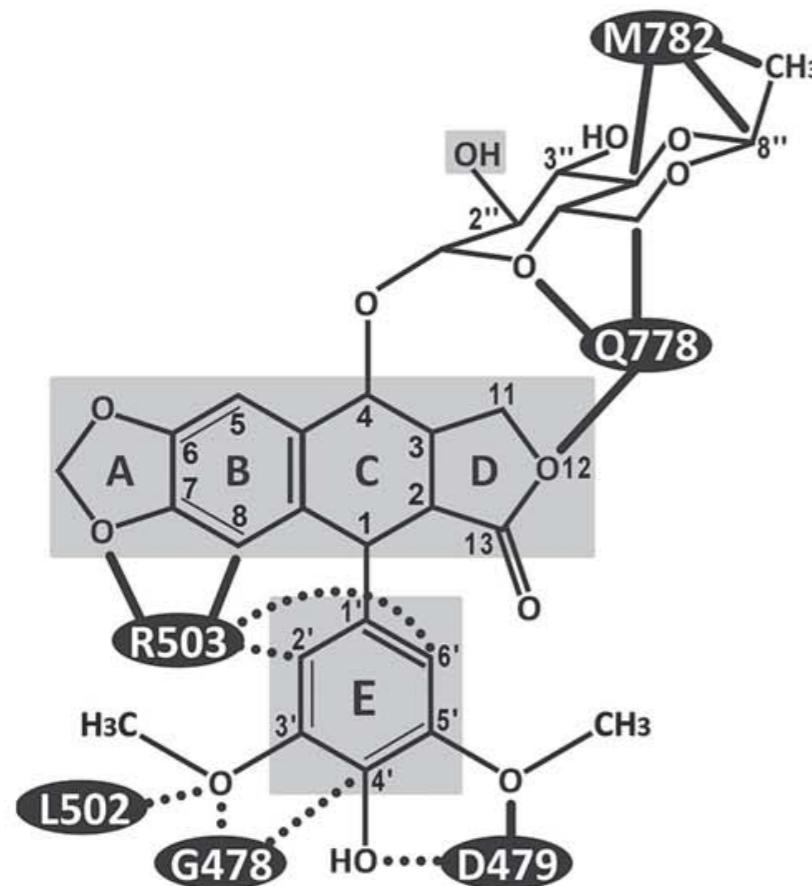
# Molecular Mechanism

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

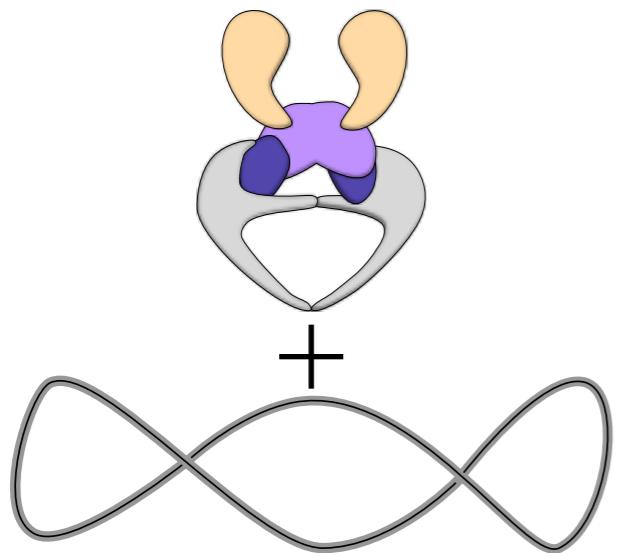
Drug binding

Catalytic tyrosine

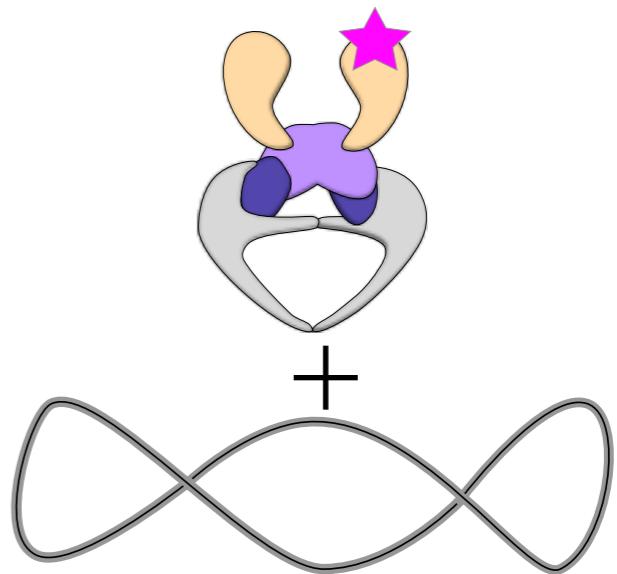


# Top2cc Assay

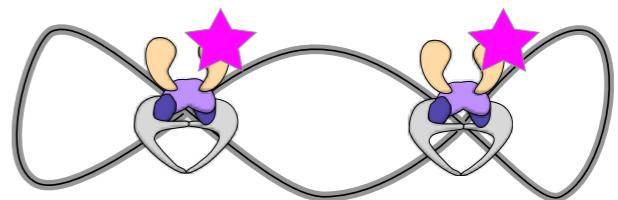
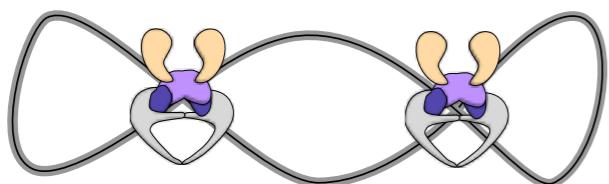
Resistant



Sensitive TOPOII

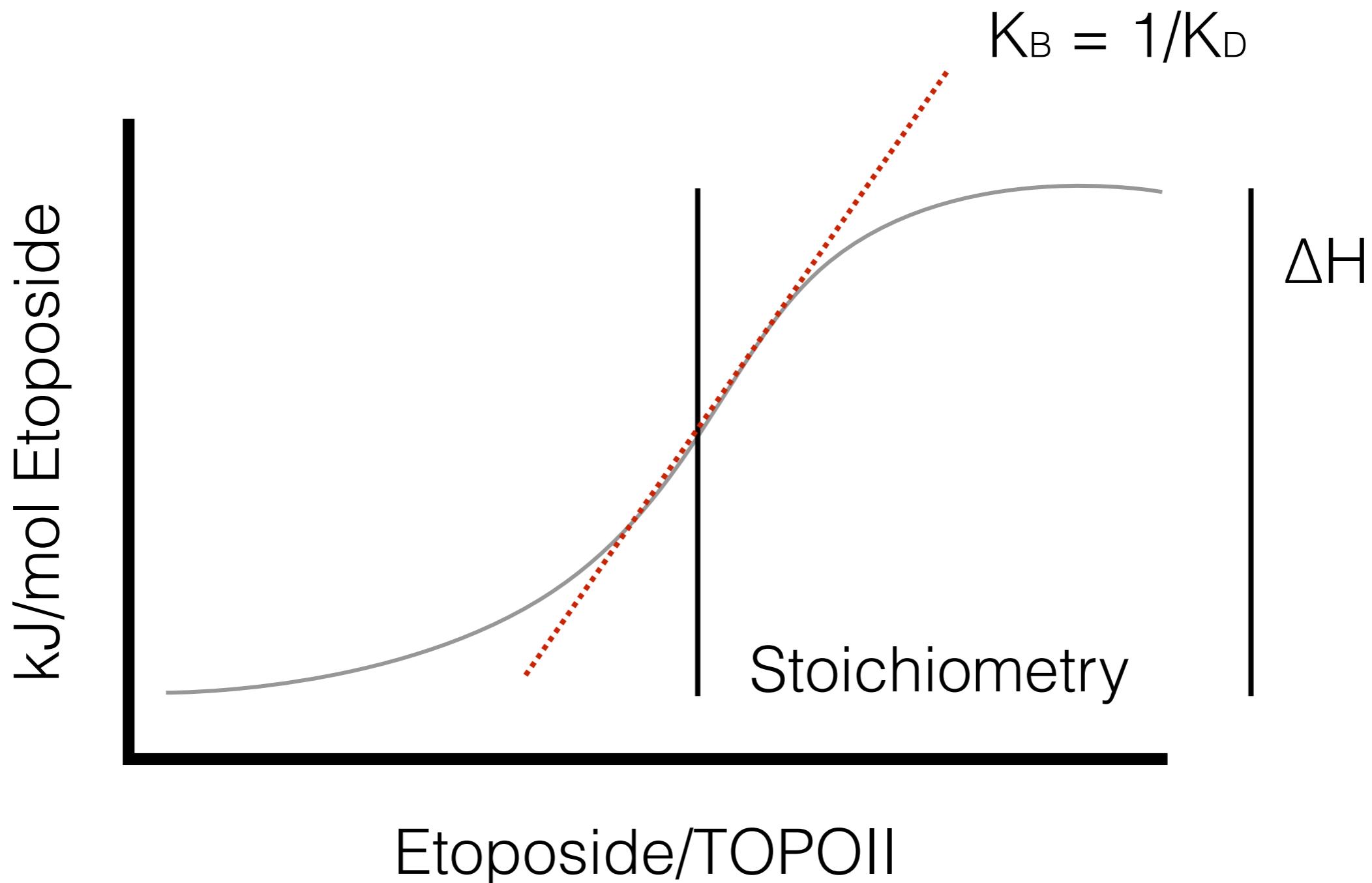


+Etoposide



**Aim 3**

# Isothermal Titration Calorimetry



**Aim 3**

# Acknowledgements

## **Andersen lab!**

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