



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

Stefan Zdraljevic

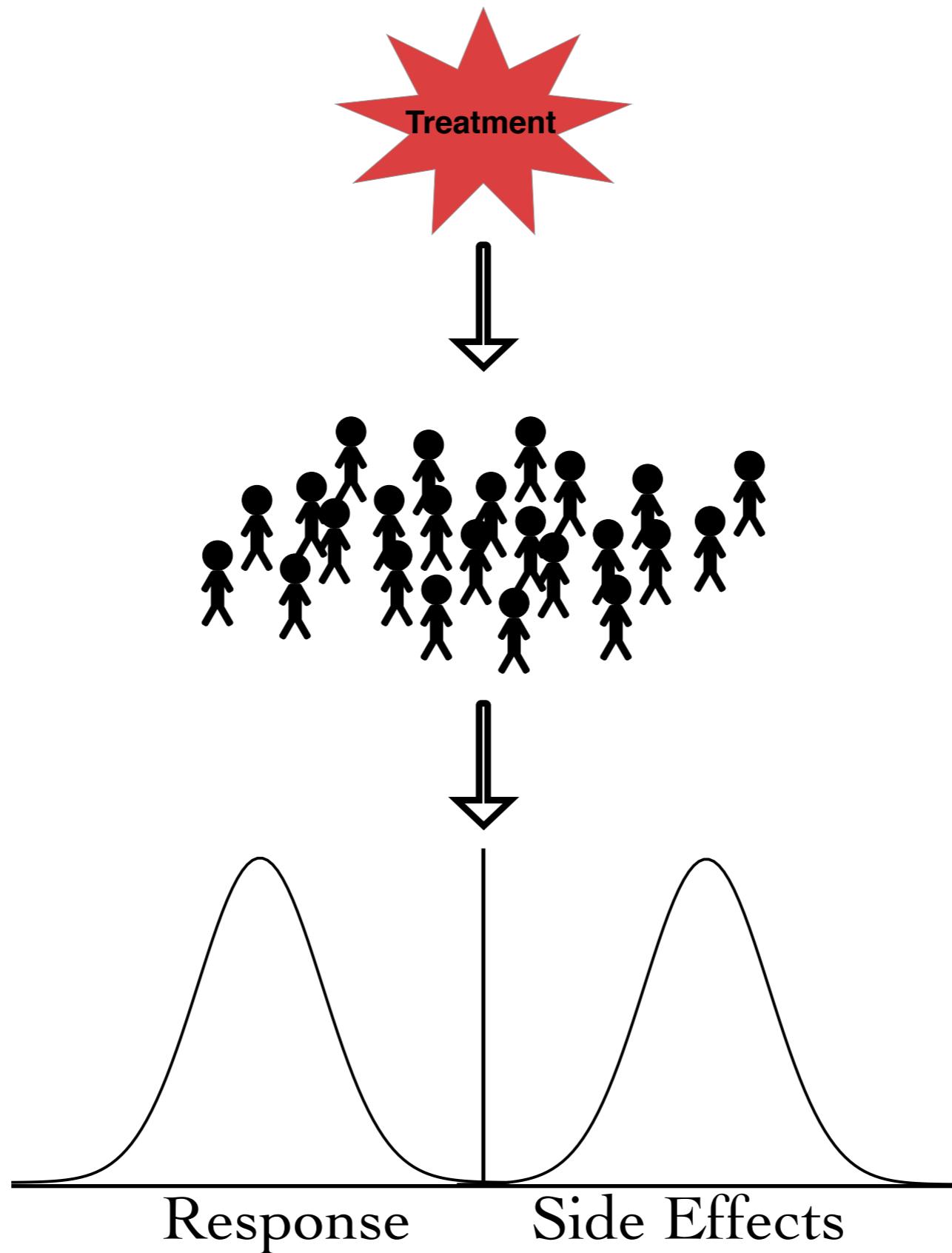
Advisor – Erik C. Andersen, Ph.D.

June 2nd, 2015

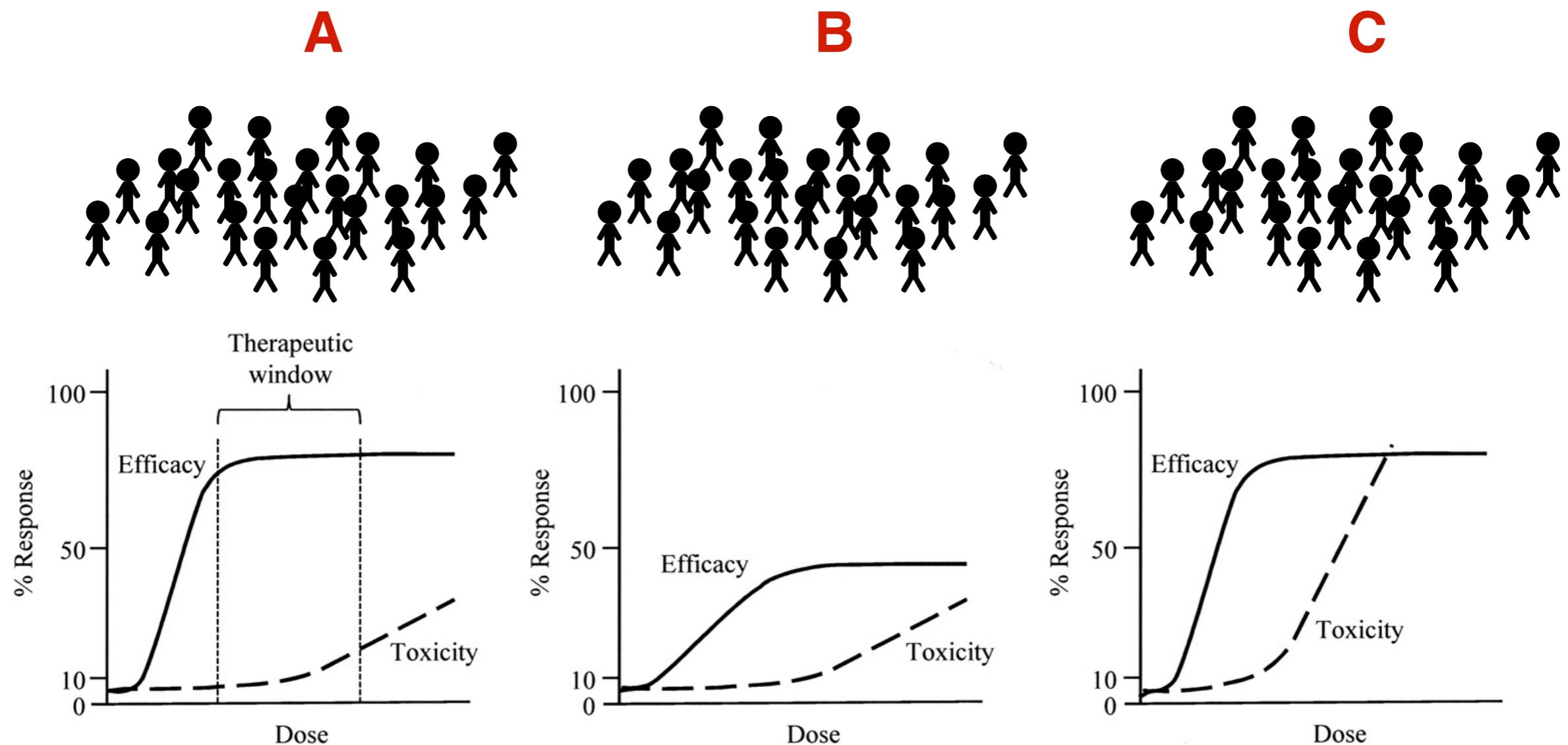
Outline

- Motivation
- Preliminary Results
- Specific Aims

Variable Responses to Therapeutics



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

Sources of Variation

Adherence to treatment (both patients and doctors)

Medication access

Drug-drug interactions

Drug transport/metabolism

Genetic variation in drug targets

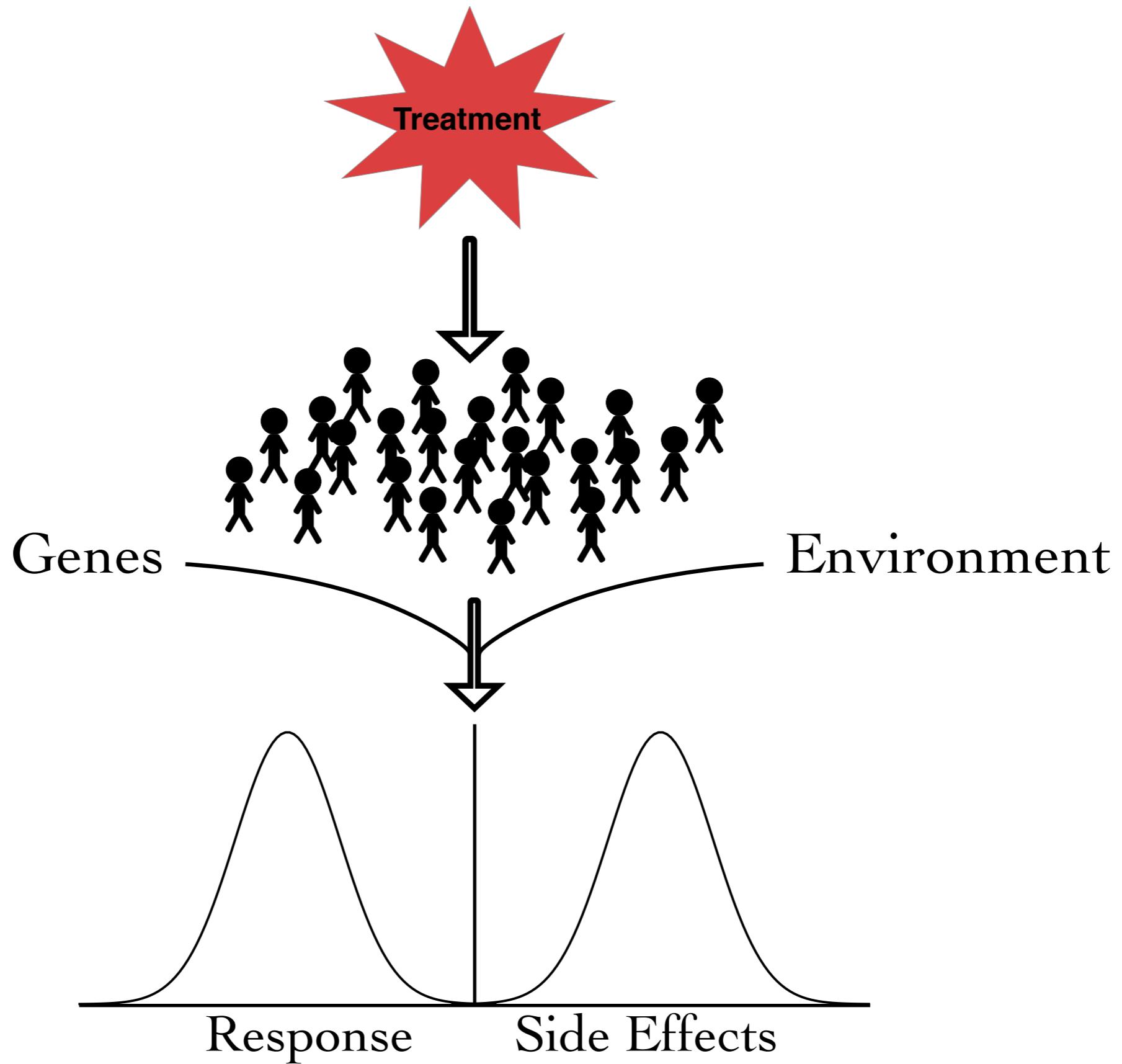
Effect of diet

Environment

Influenced by genetic factors

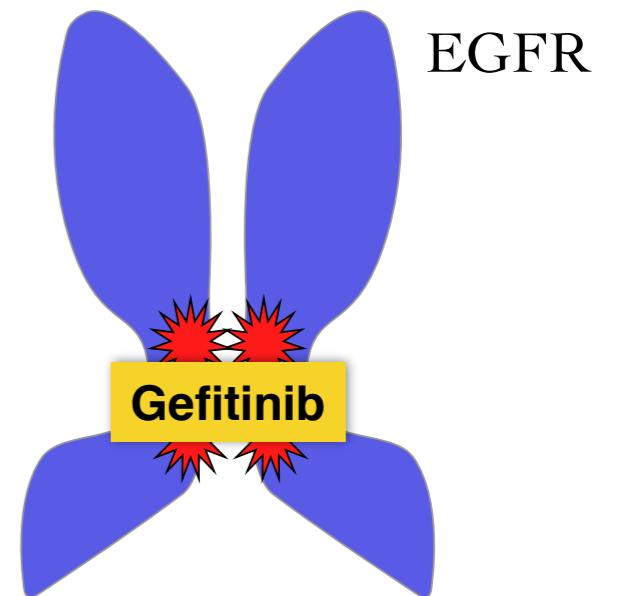
Difficult to control

Genes and Environment Control Response

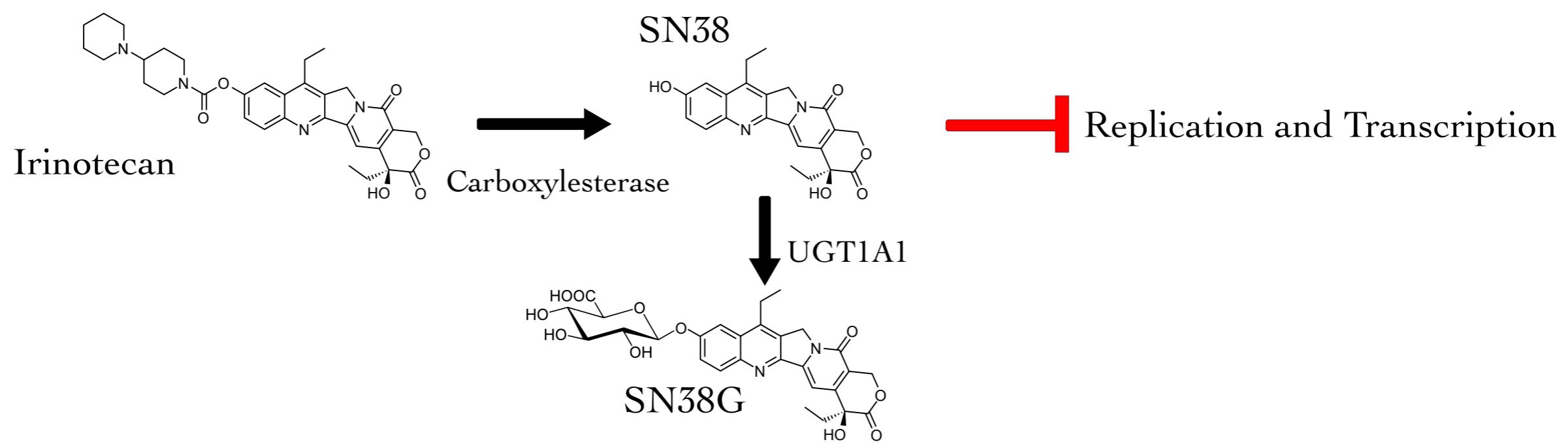


Examples of Clinically Relevant Variants

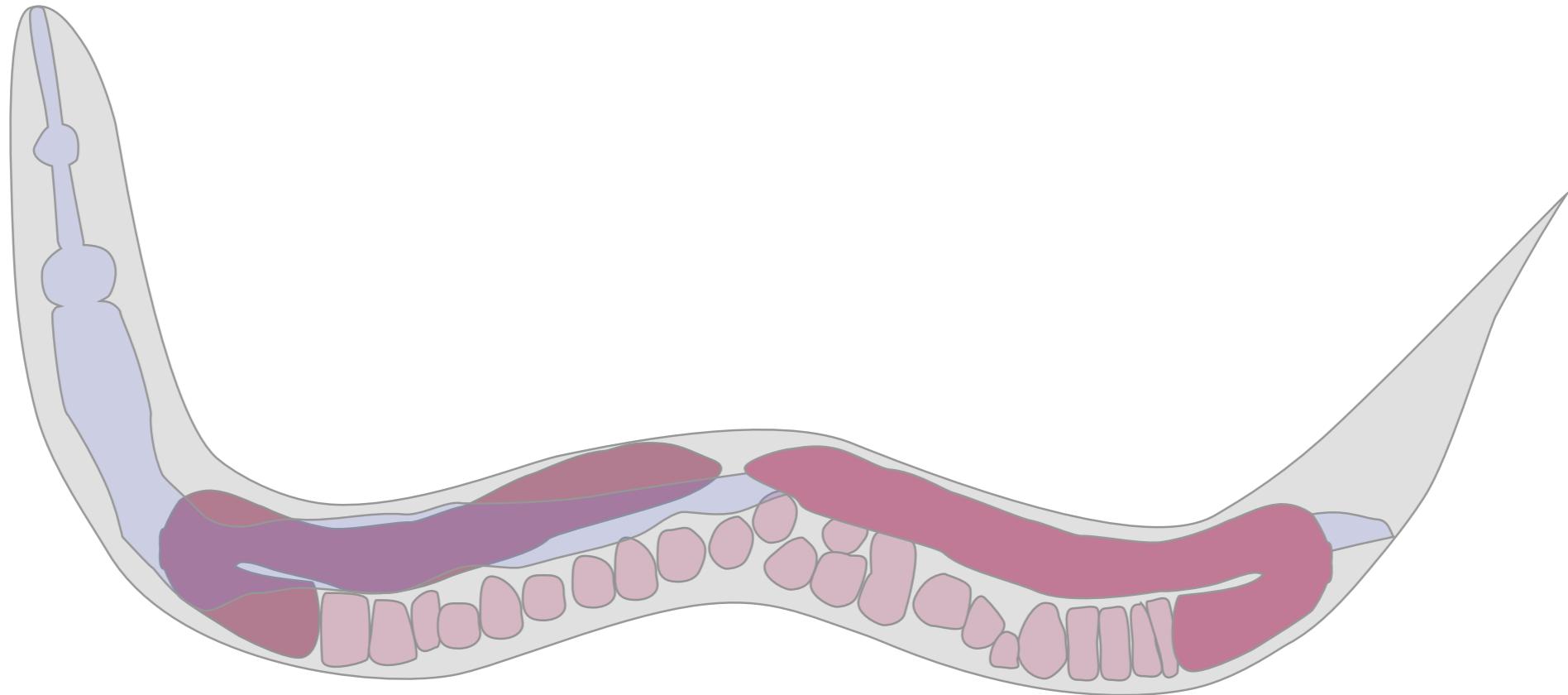
Genetic variation in drug targets



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.”

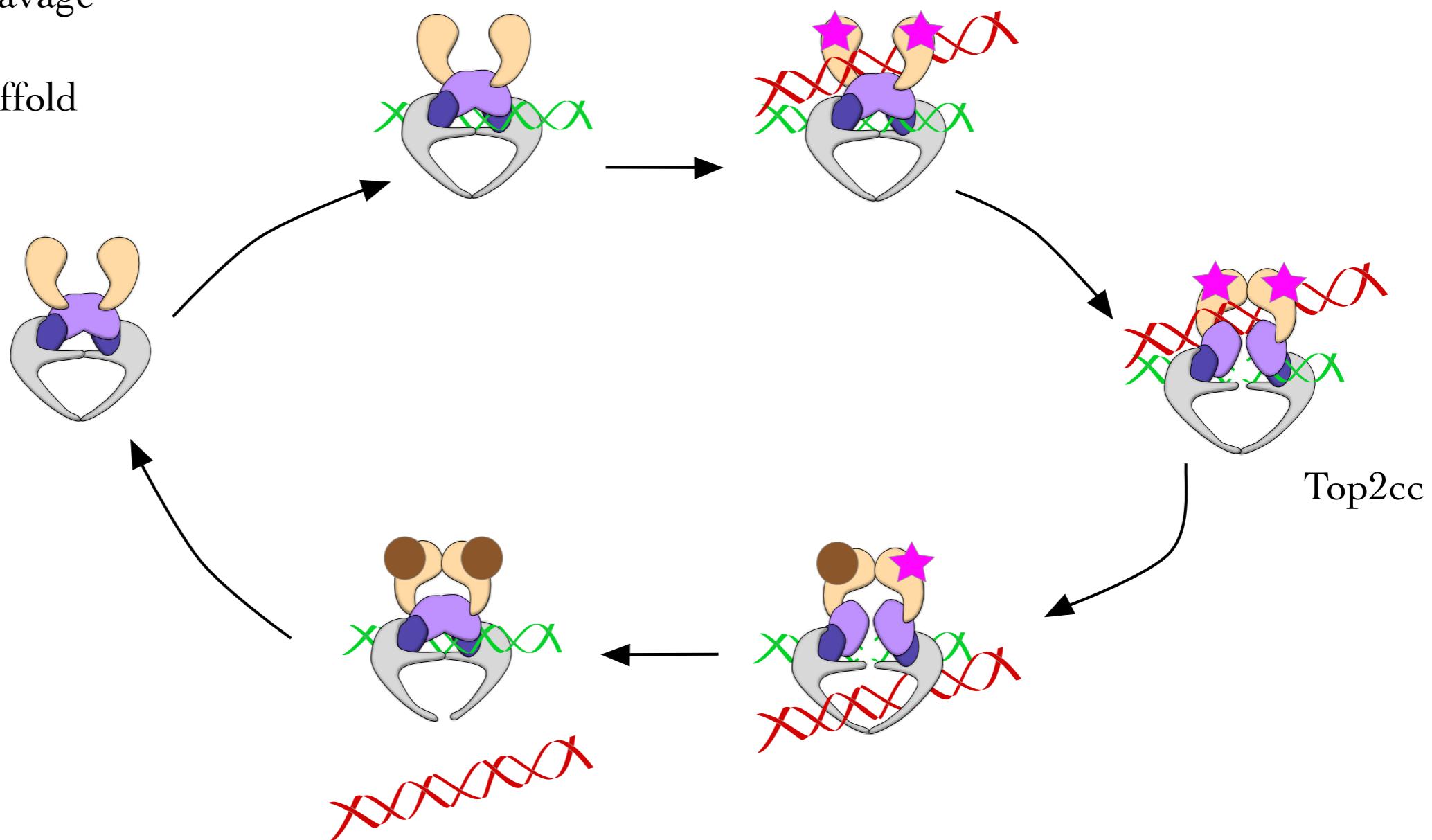


Easy to handle
Low cost
High replication of experiments
Short lifespan
Numerous genetic tools

Topoisomerase II Catalytic Cycle

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

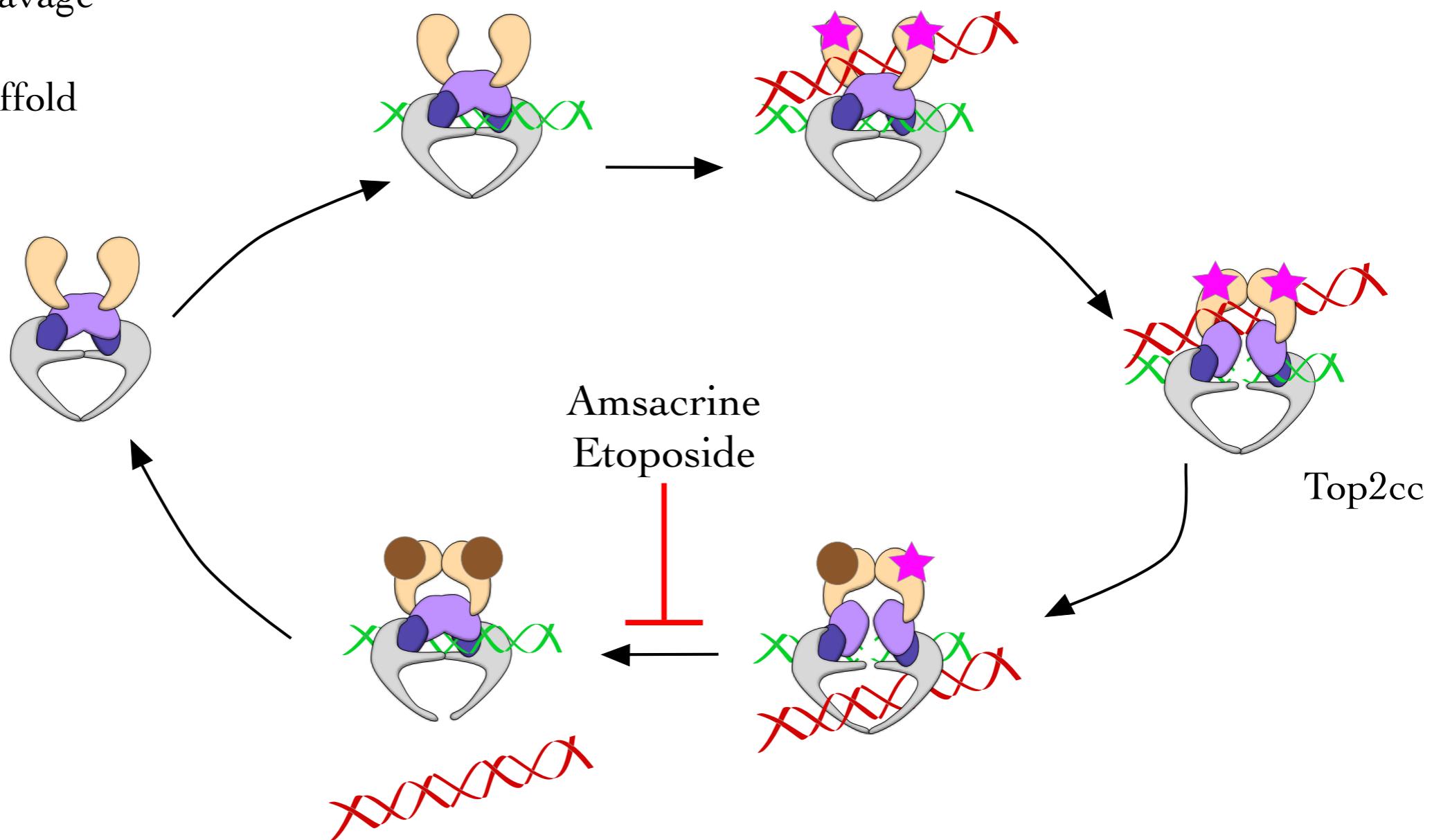
Cleavage
Scaffold

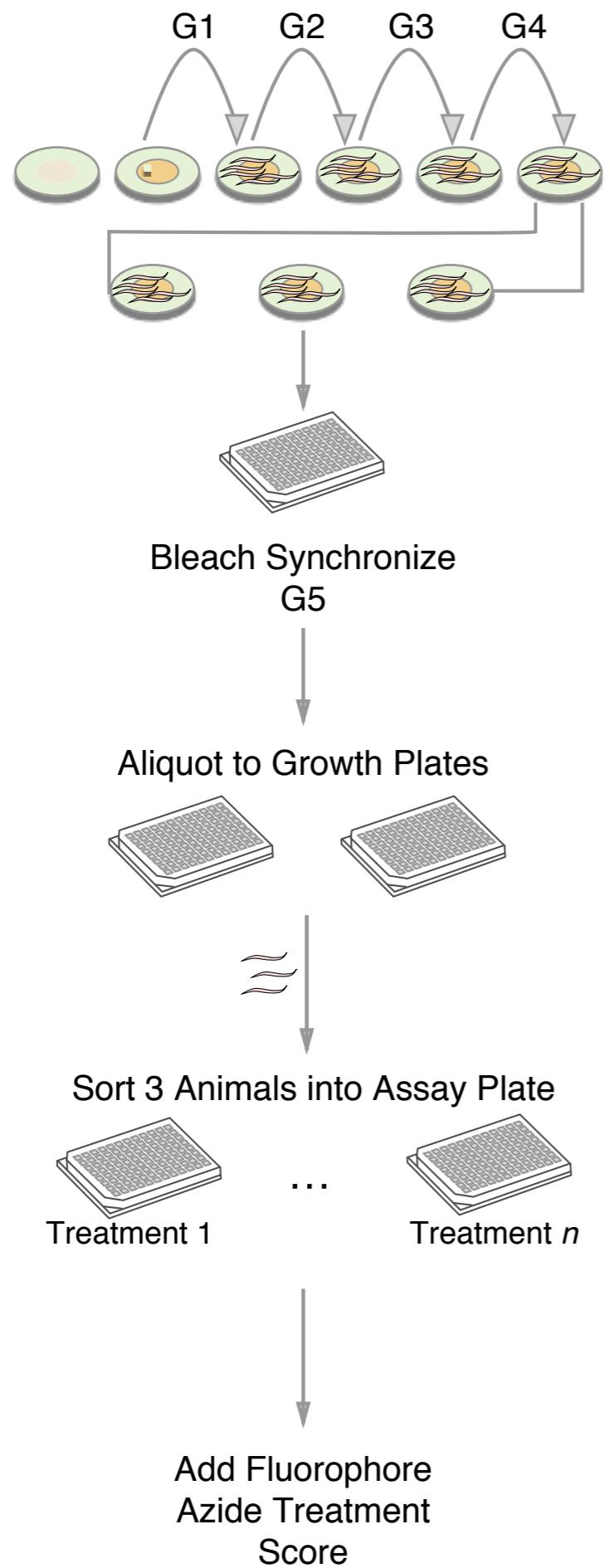


Topoisomerase II Catalytic Cycle

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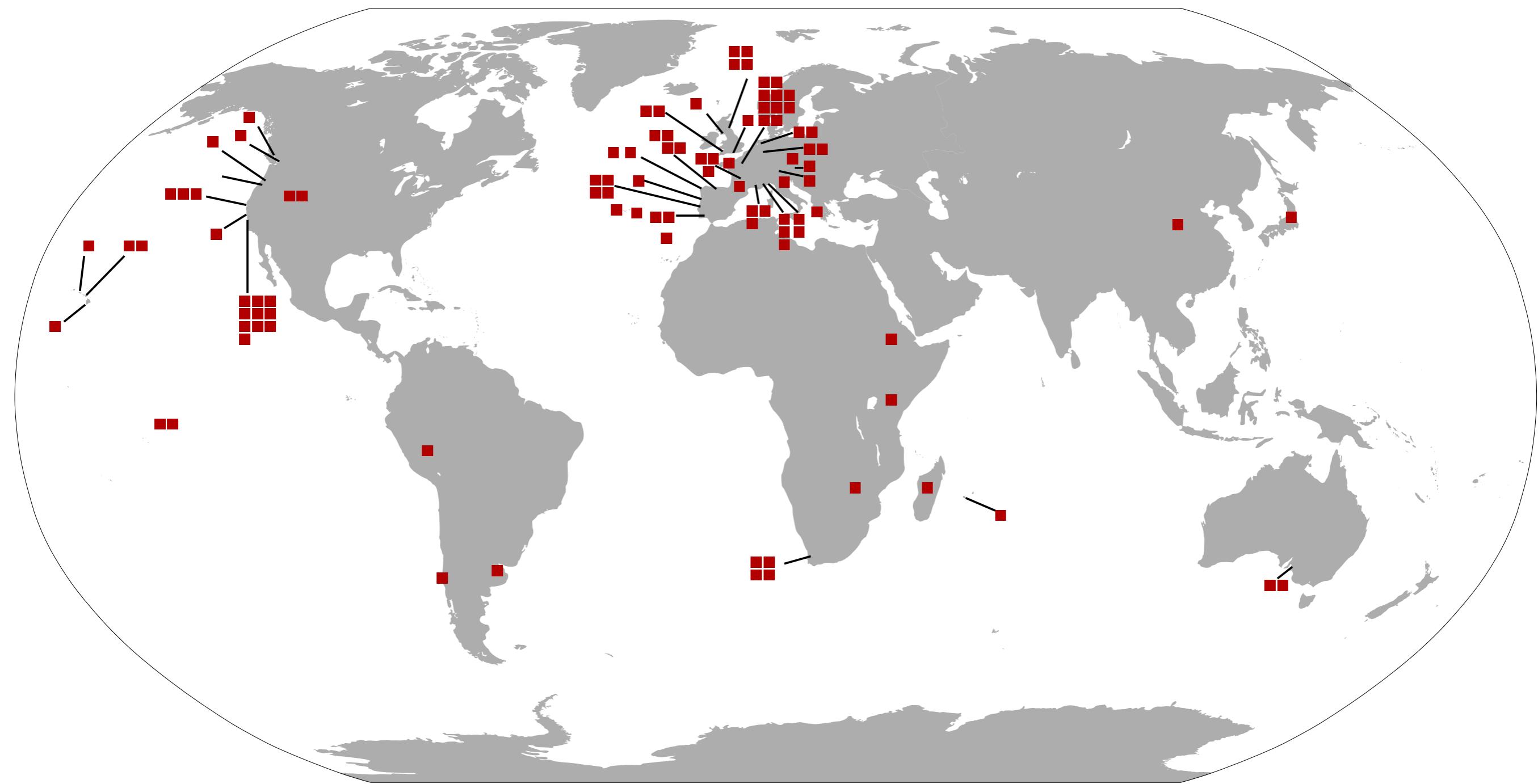
Fitness Traits:

Brood size

Animal length

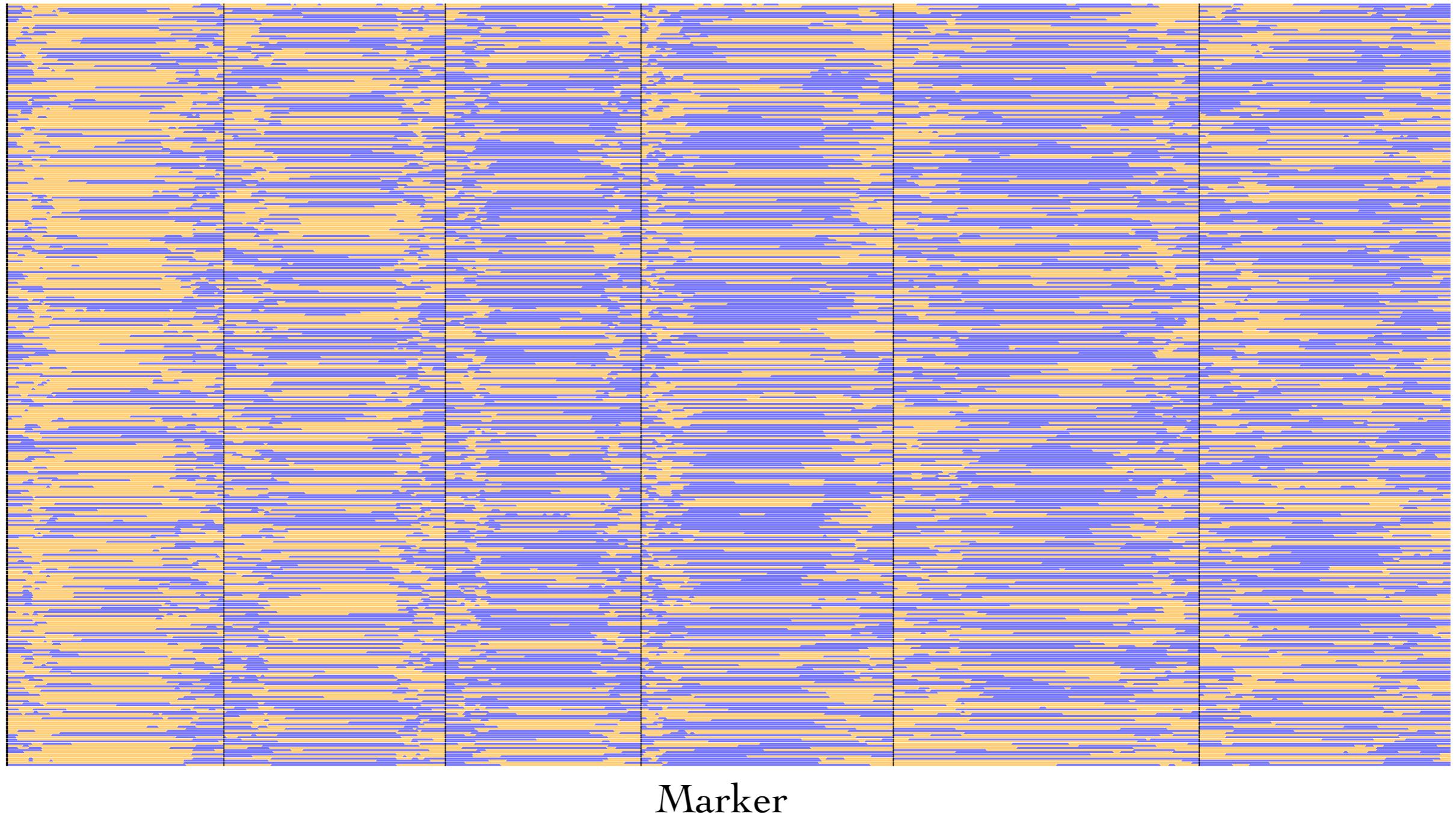
Pharyngeal pumping

Global Distribution of *C. elegans*

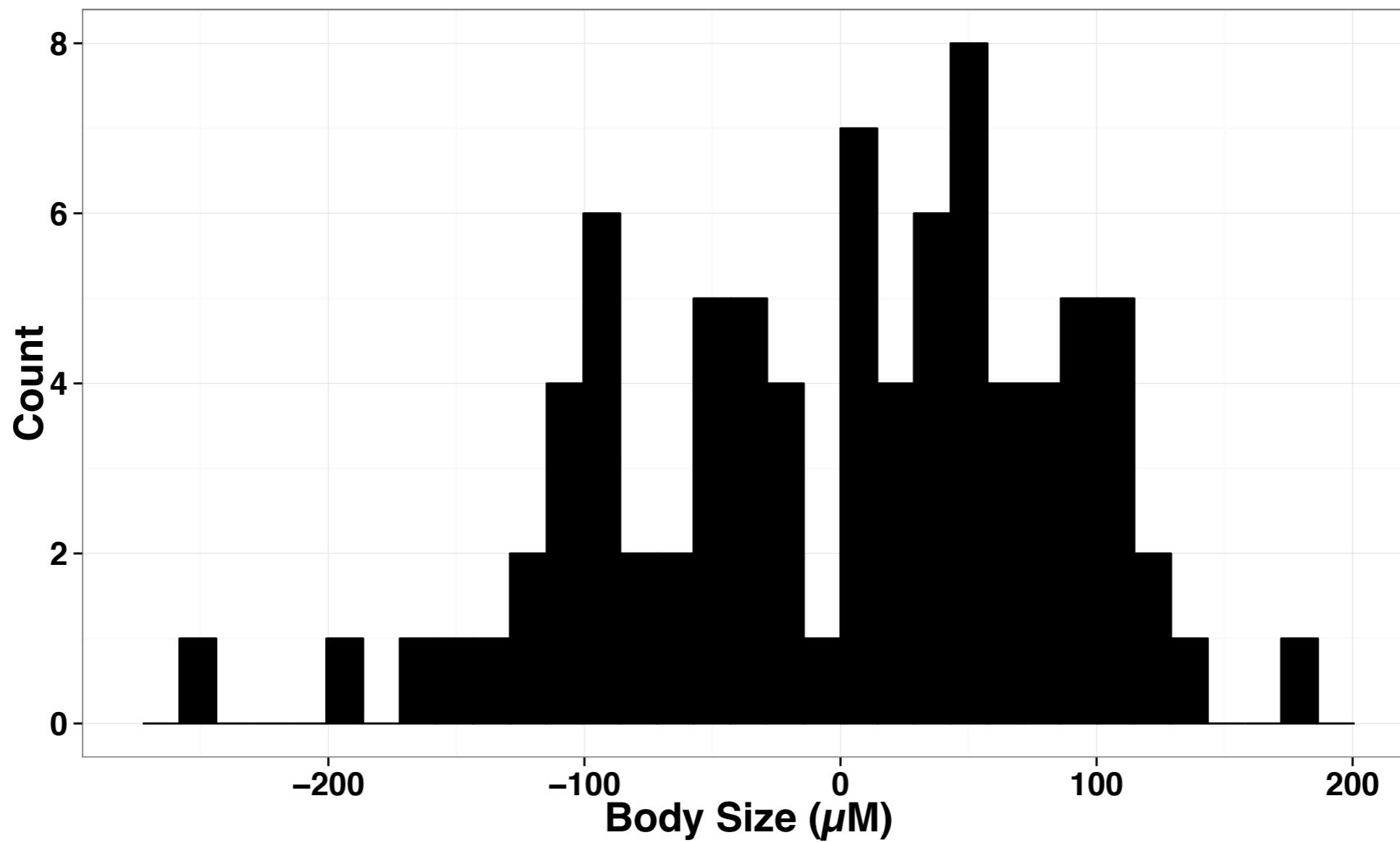


RIAILs Genotype

RIAIL

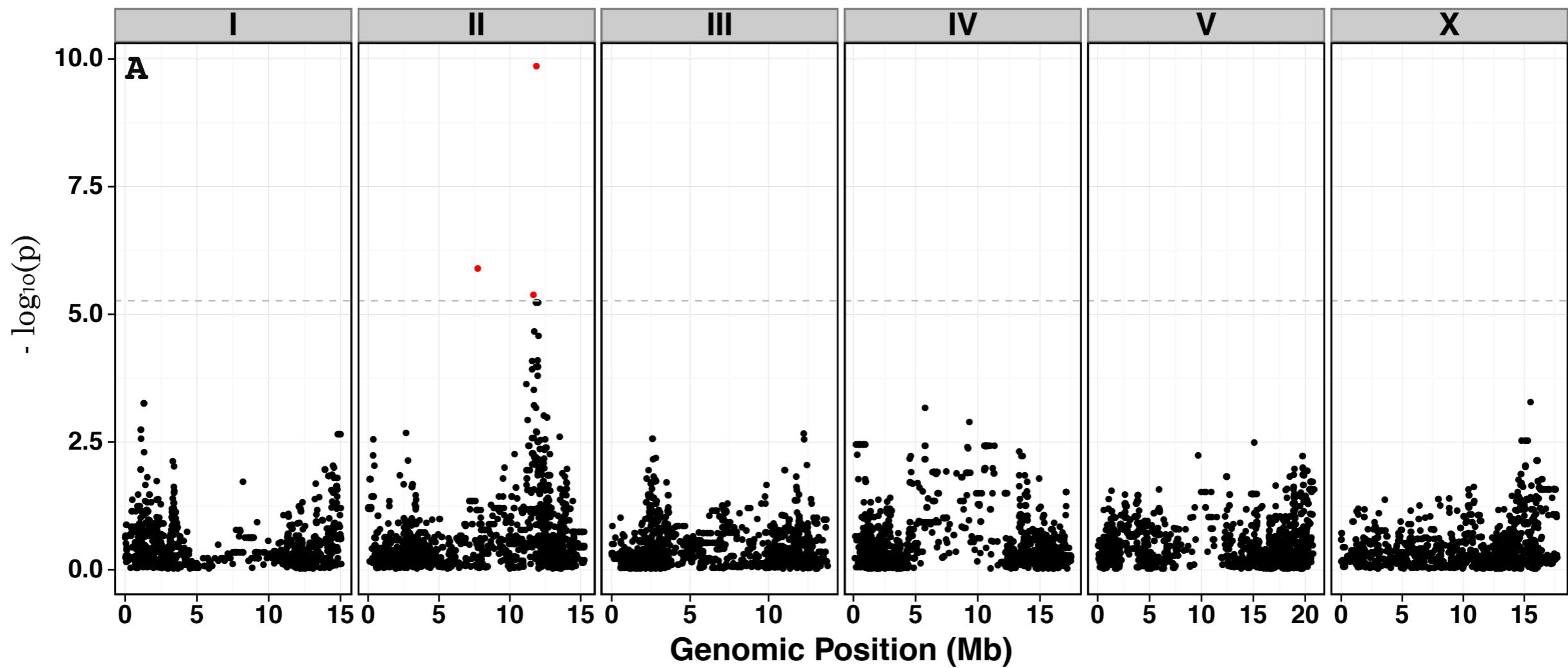


Variation in Response to Etoposide

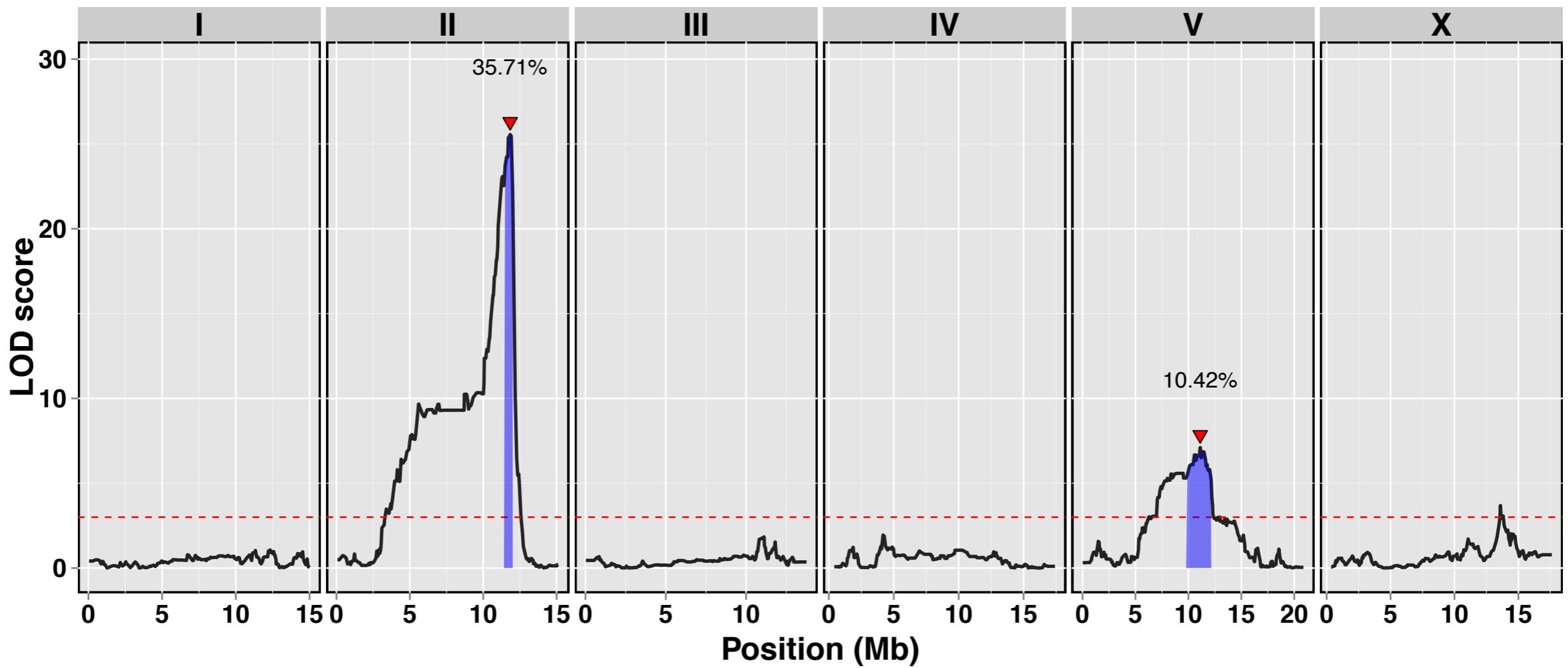


Variation in Response to Etoposide

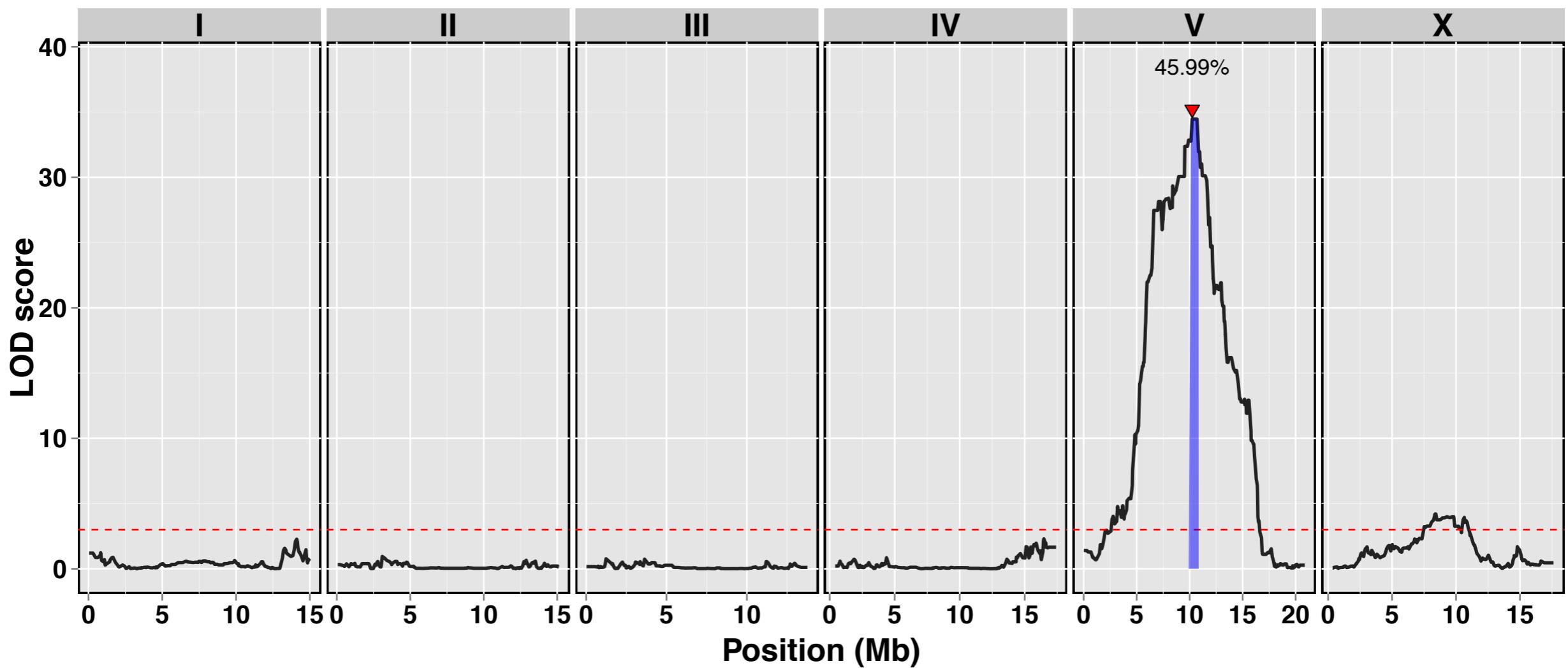
Animal Length



Variation in Response to Etoposide



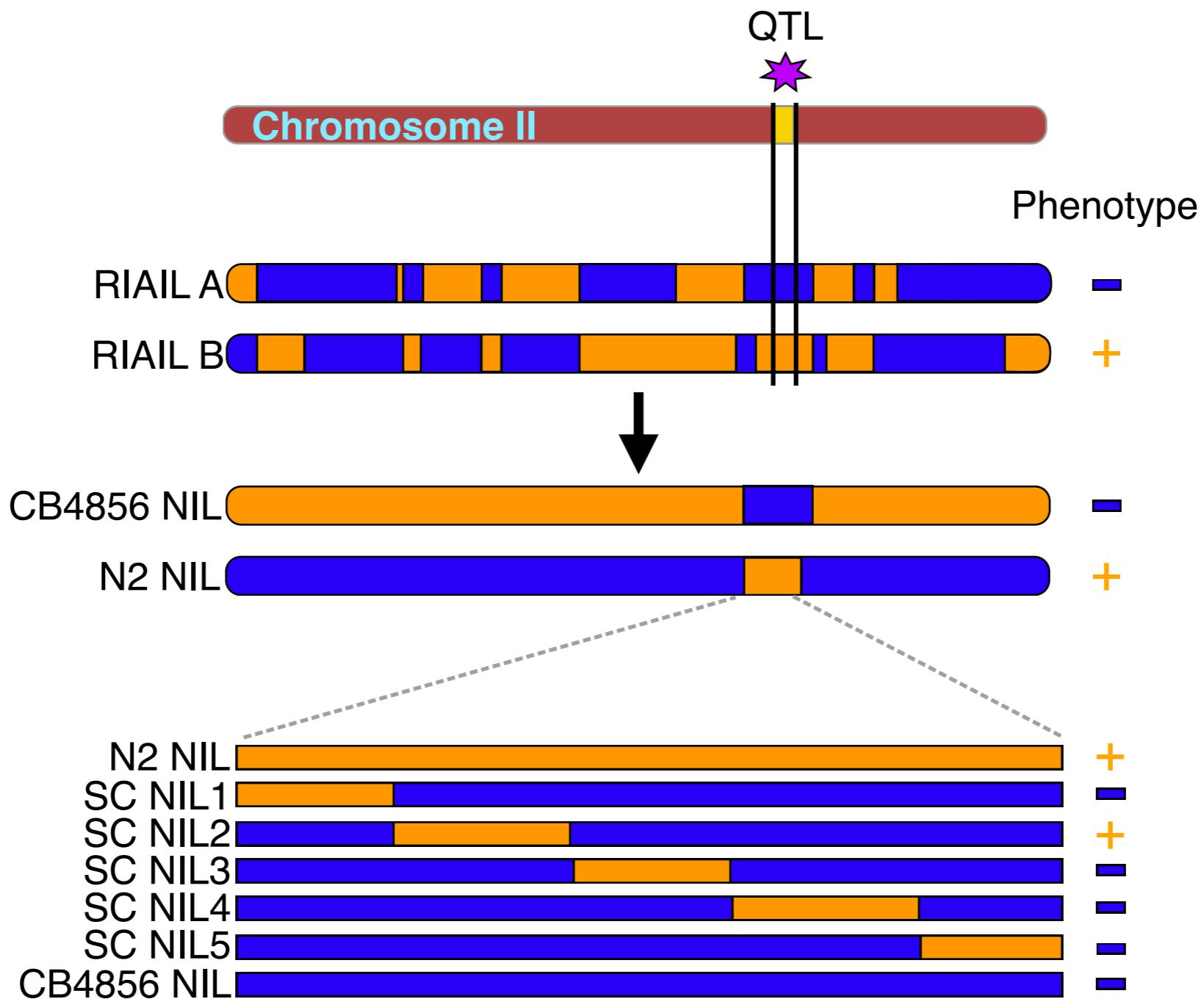
Variation in Response to Amsacrine



Specific Aims

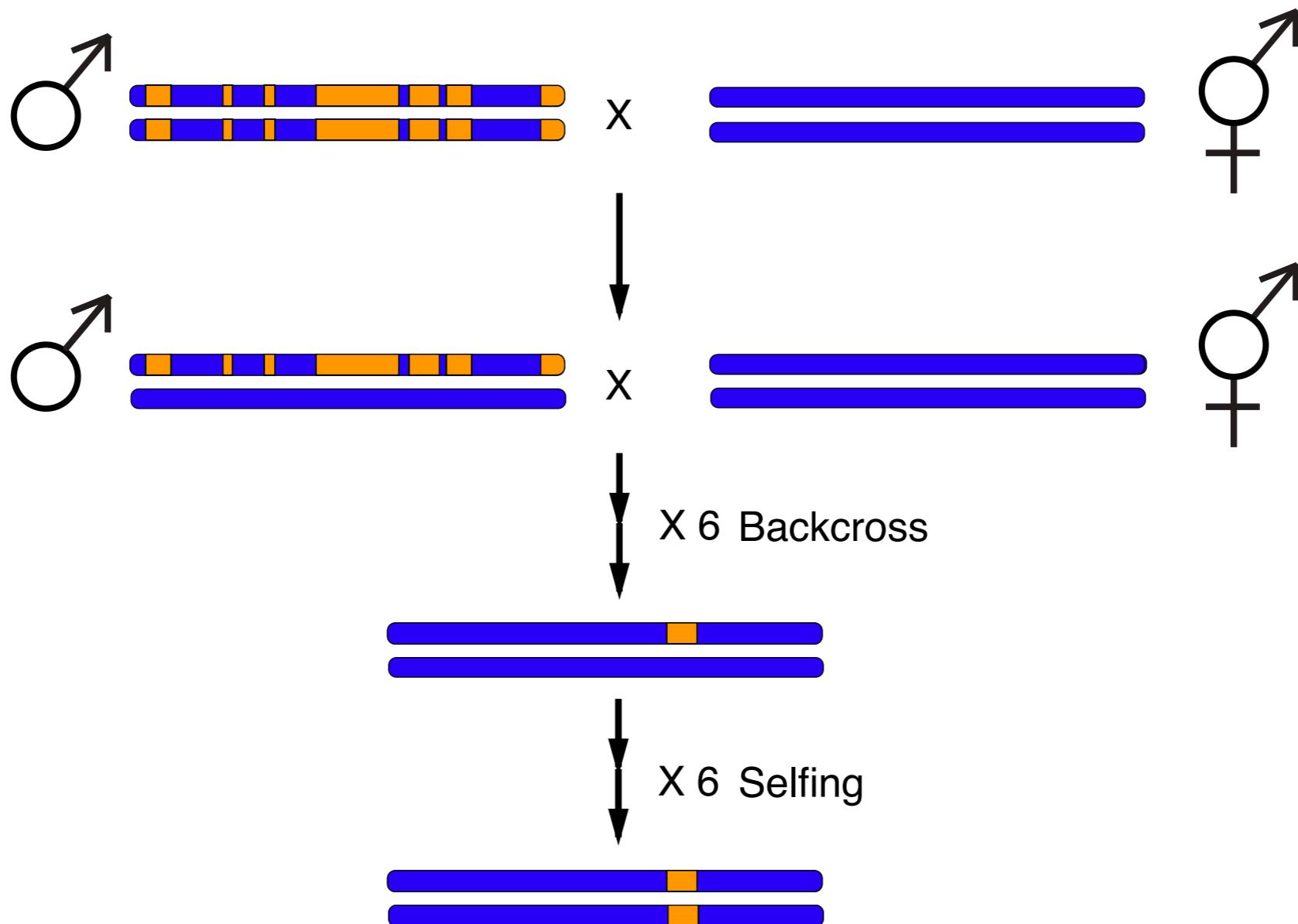
- **Specific Aim 1:** Narrow QTL confidence intervals
- **Specific Aim 2:** Identify causal gene and variant underlying QTL
- **Specific Aim 3:** Determine molecular mechanism associated with causal variants

Narrow Confidence Intervals



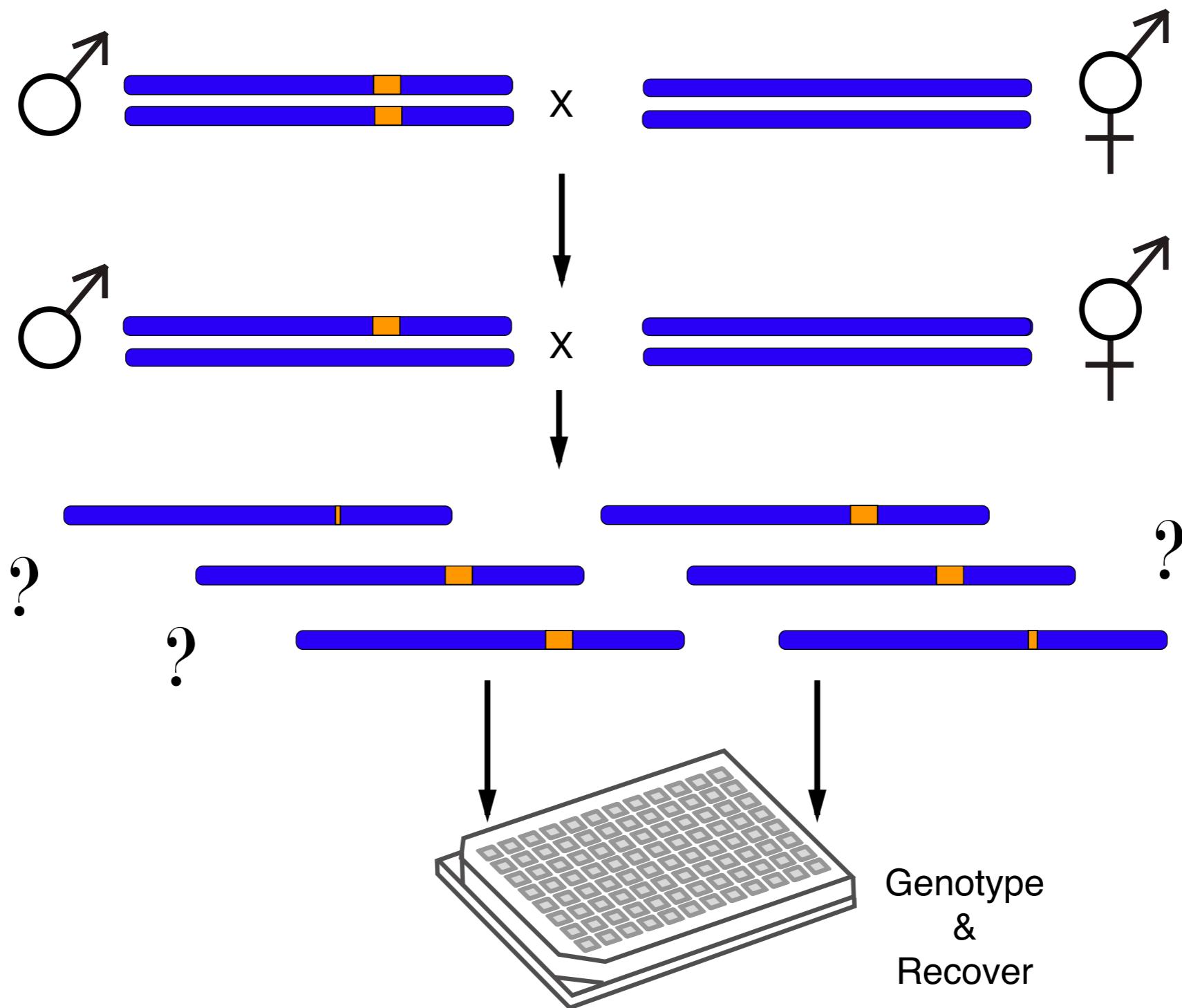
Aim 1

Generating NILs



Aim 1

Generating NILs



Aim 1

- **Specific Aim 1:** Narrow QTL confidence intervals
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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*



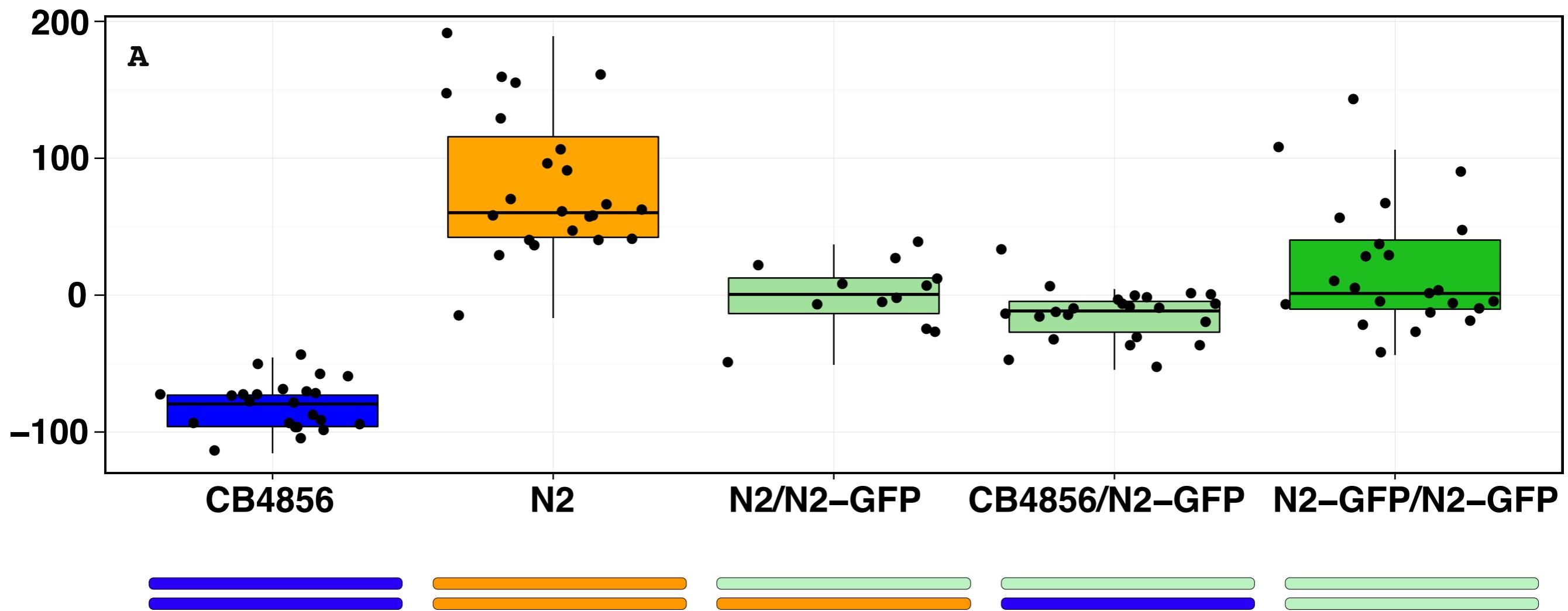
?

Genotype

Phenotype

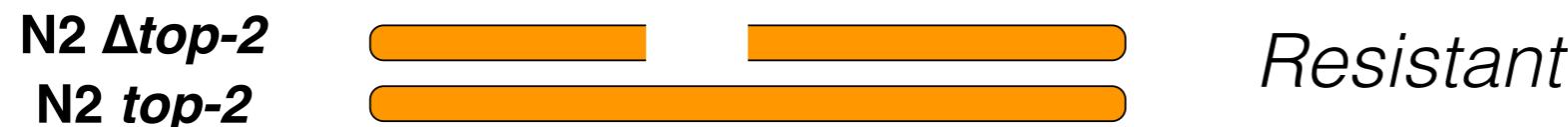
Aim 2

Resistance to Etoposide is Dominant



Aim 2

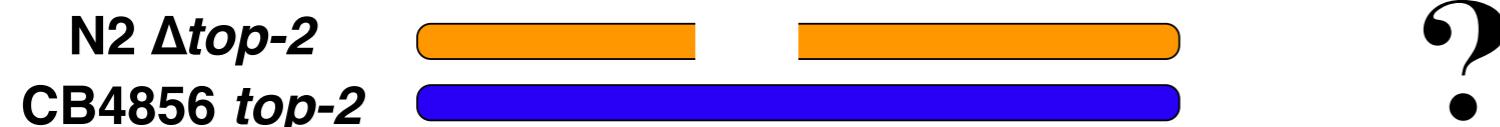
Complementation Test – Design



Resistant



Resistant



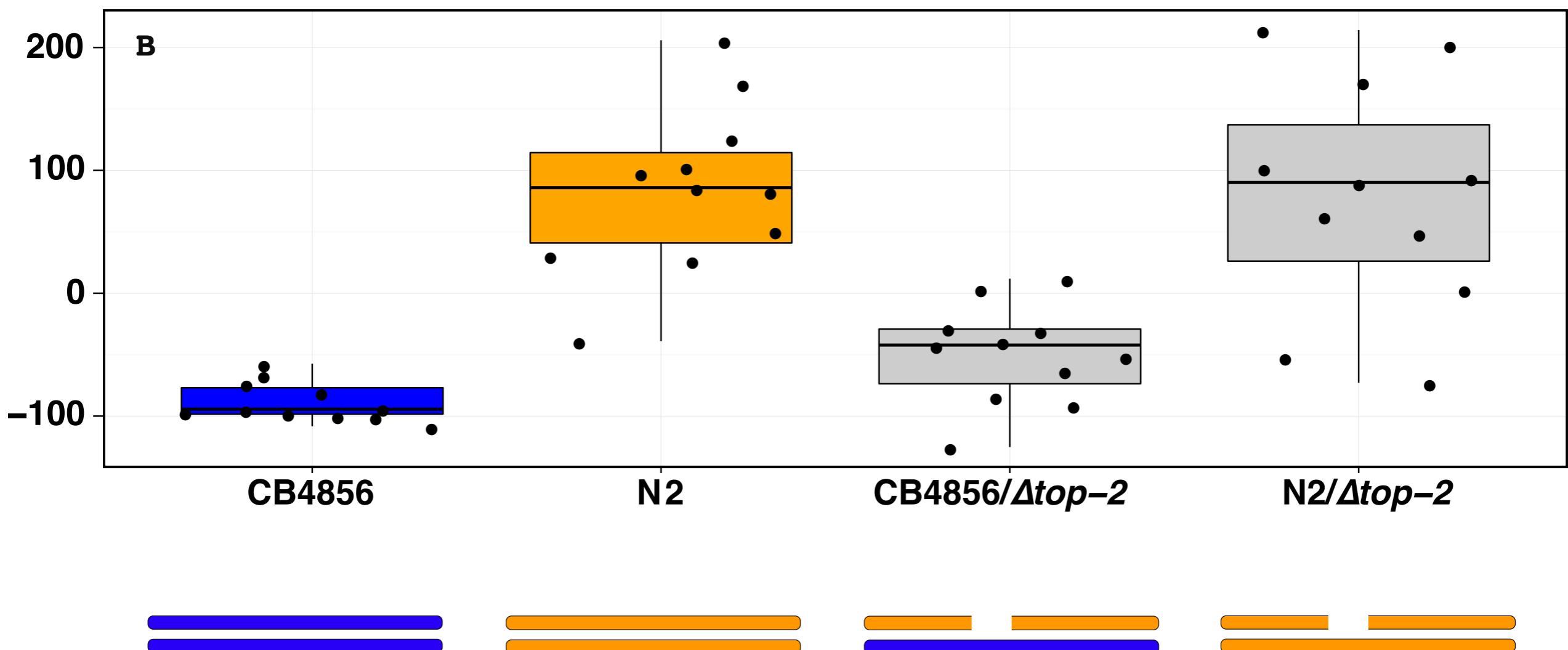
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Genotype

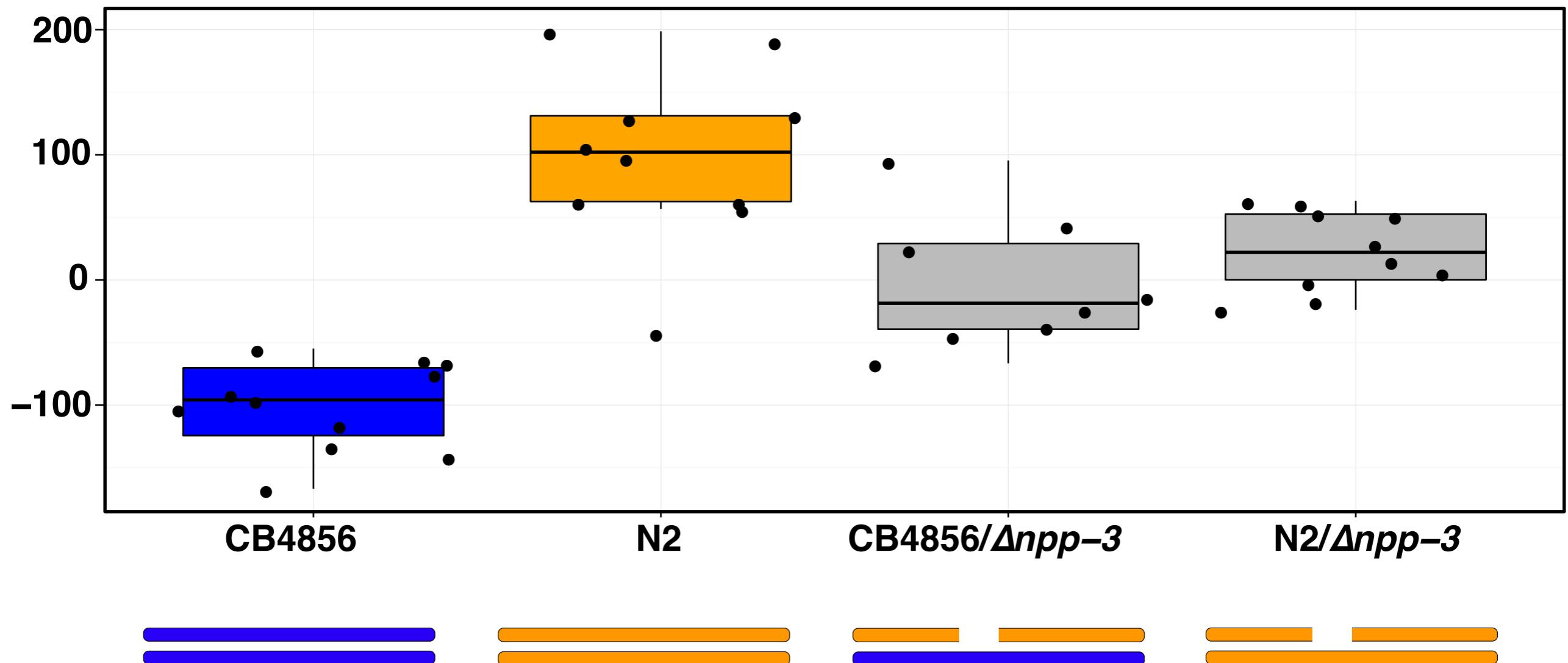
Phenotype

Aim 2

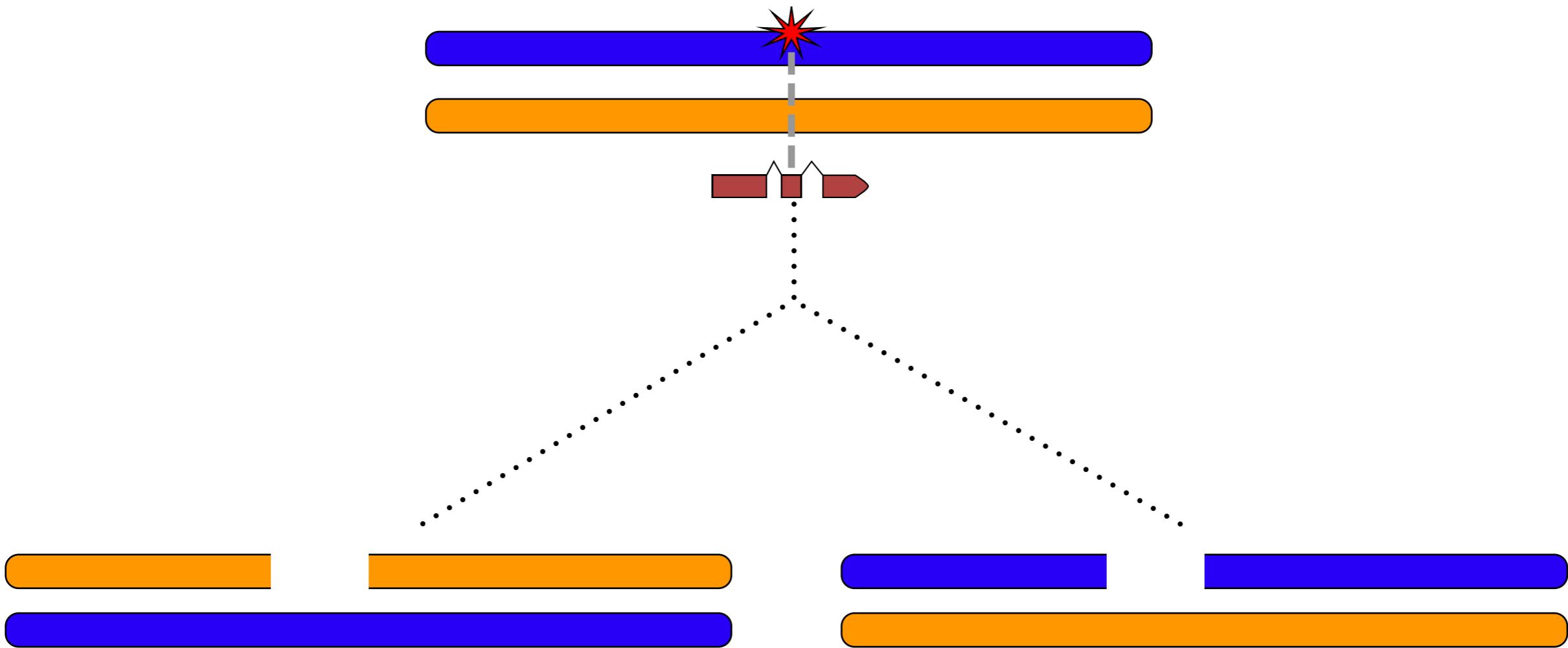
Complementation Test – *top-2*



Complementation Test — *npp-3*

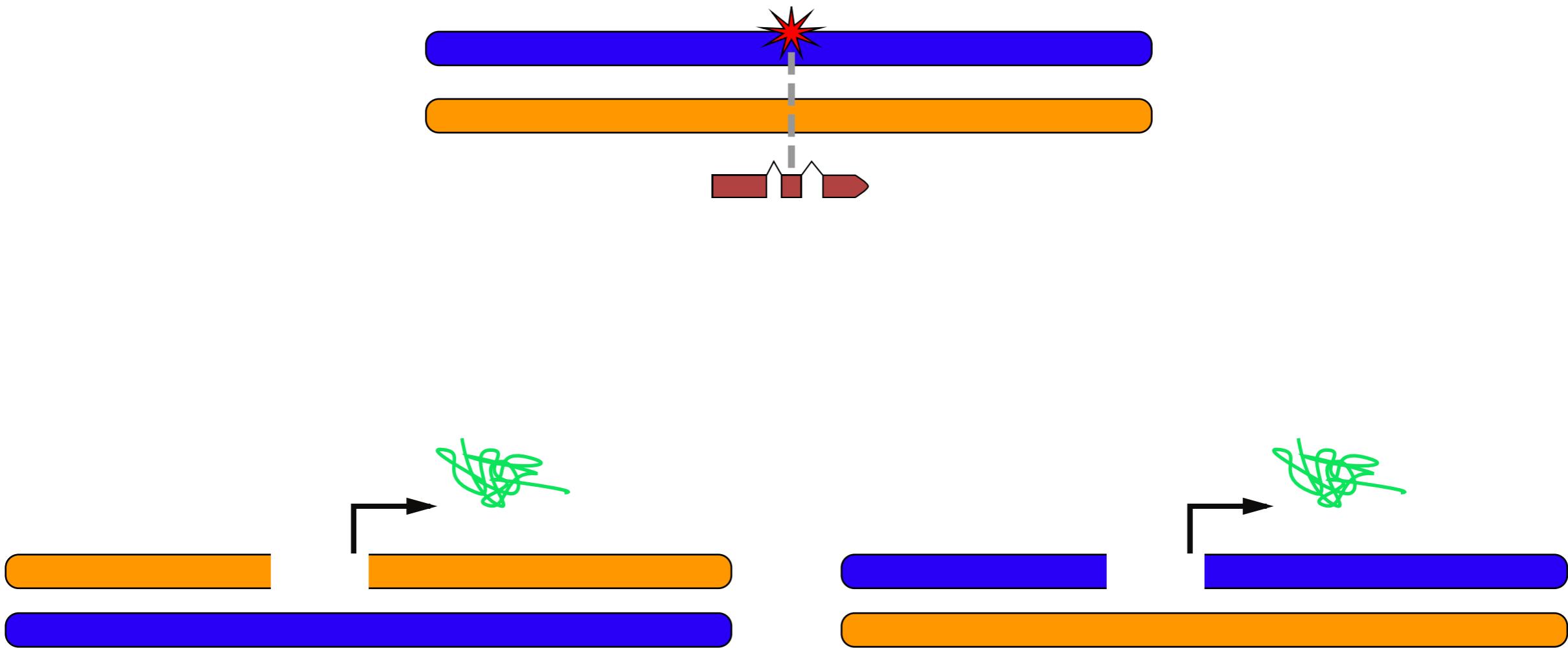


Reciprocal Hemizygosity



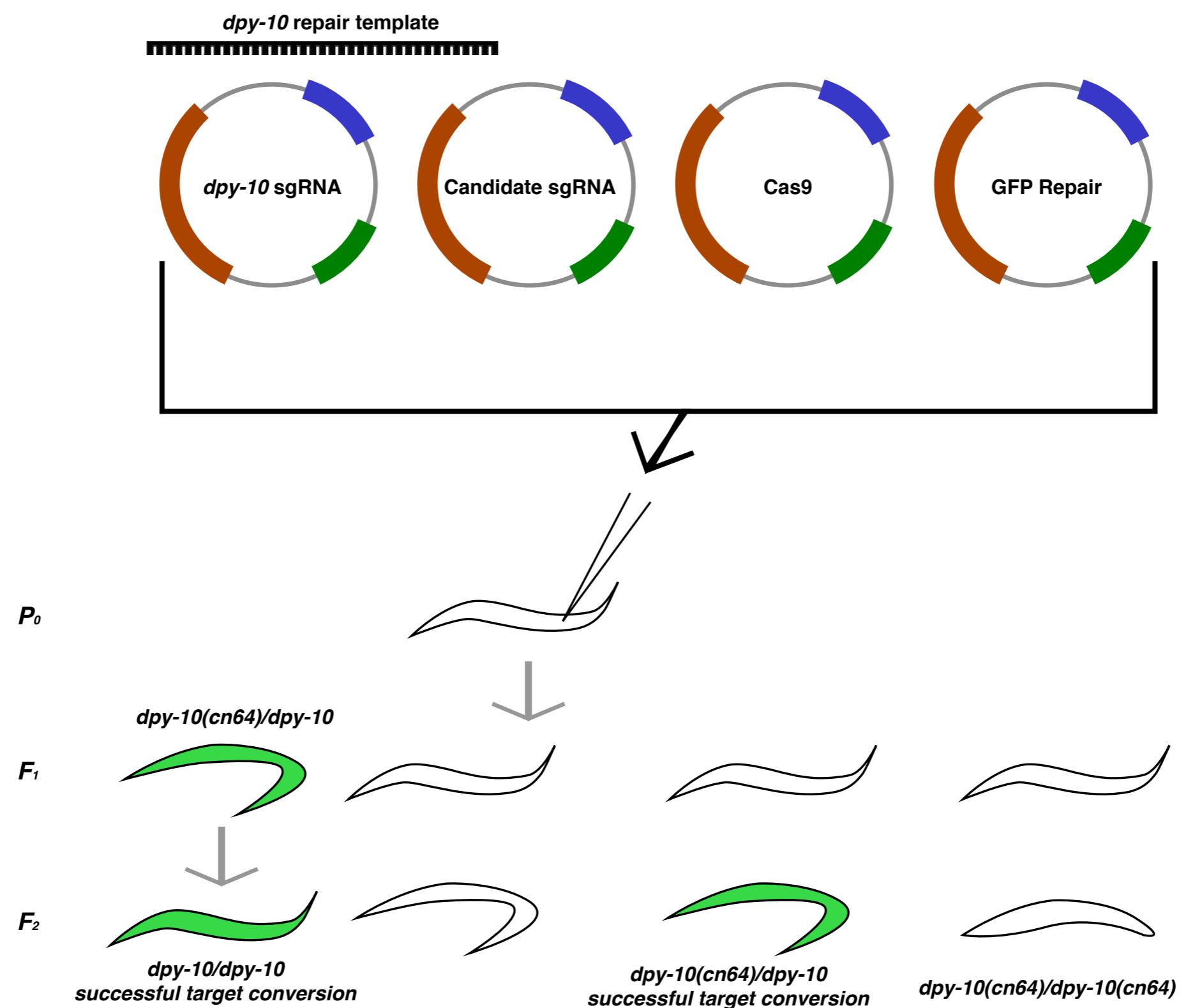
Aim 2

Reciprocal Hemizygosity



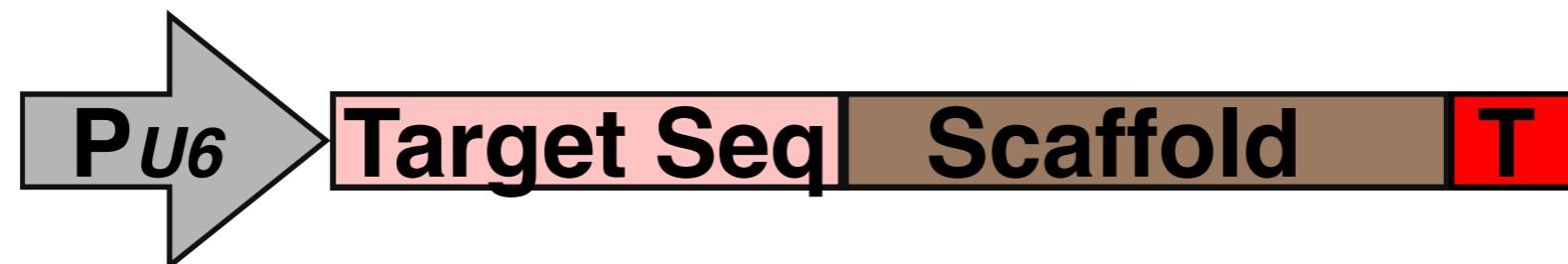
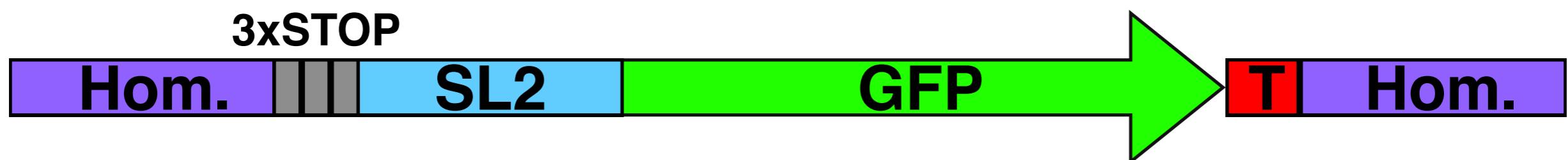
Aim 2

Generating Strains for Reciprocal Hemizygosity



Aim 2

CRISPR/Cas9 Constructs



Aim 2

- **Specific Aim 1:** Narrow QTL confidence intervals
- **Specific Aim 2:** Identify causal gene and variant underlying QTL
- **Specific Aim 3:** Determine molecular mechanism associated with causal variants

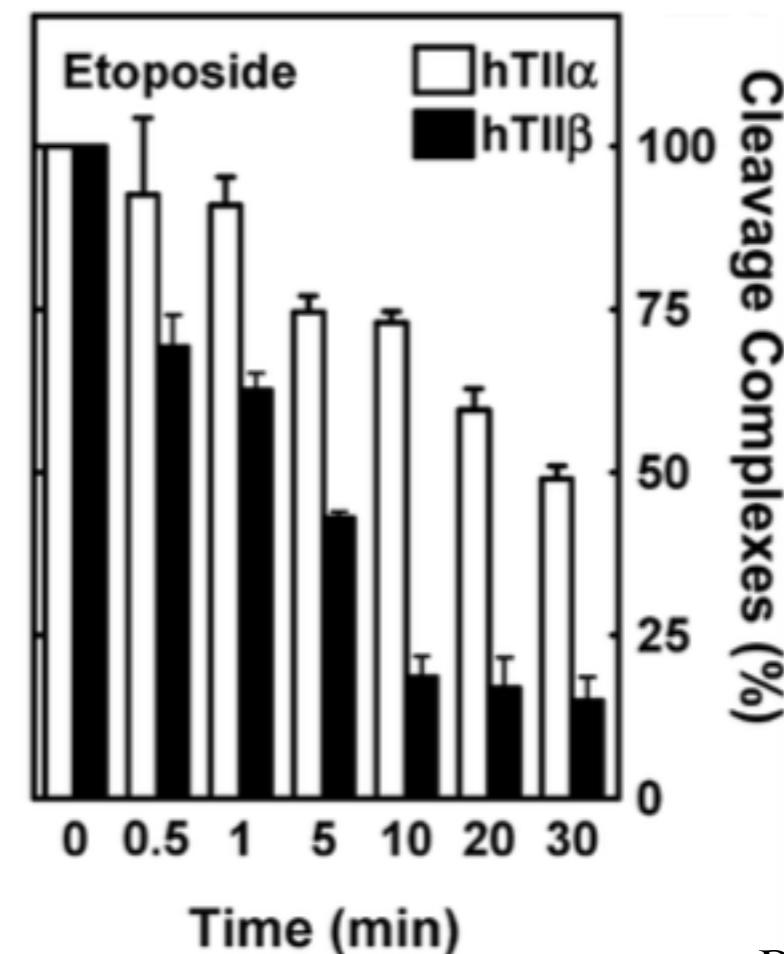
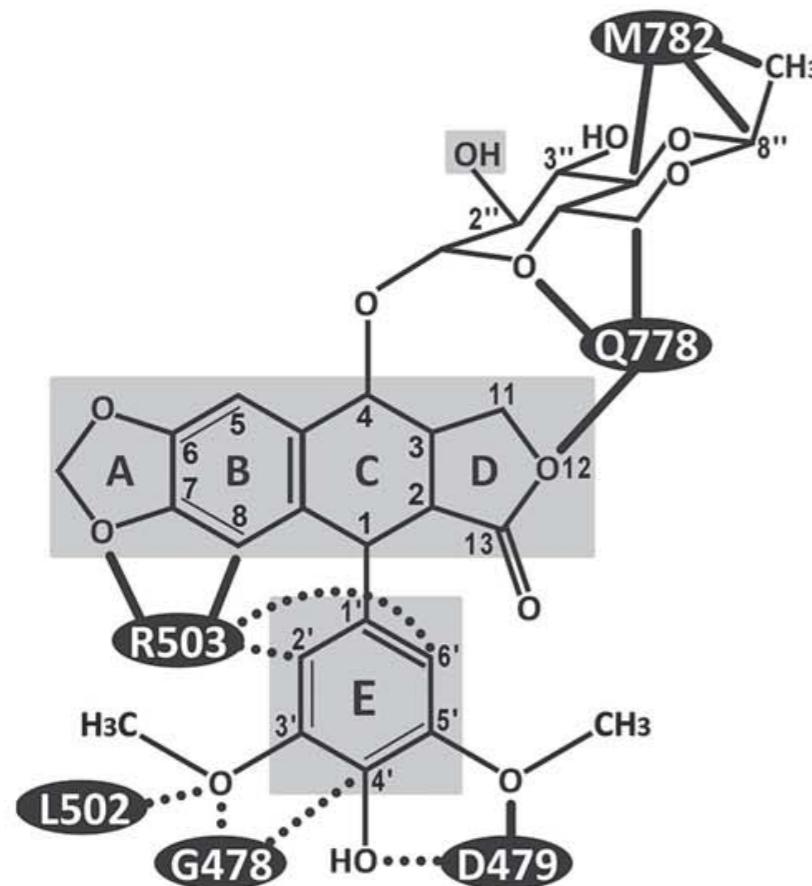
Molecular Mechanism

C.e. TOPOII Resistant
 C.e. TOPOII Sensitive
 Human TOPOII α
 Human TOPOII β

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

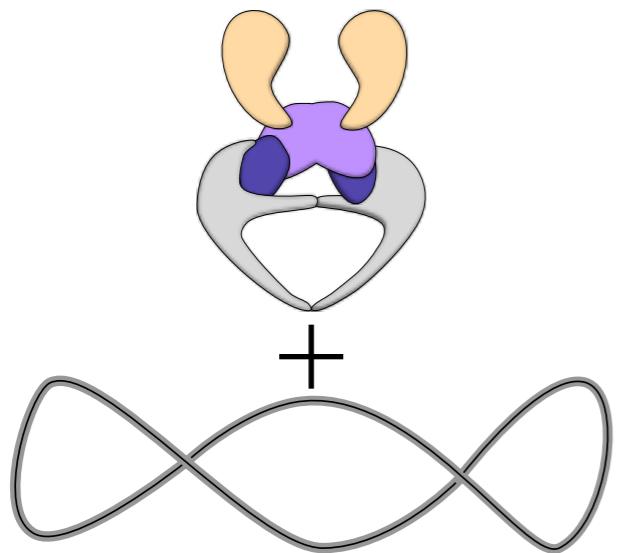
Drug binding

Catalytic tyrosine

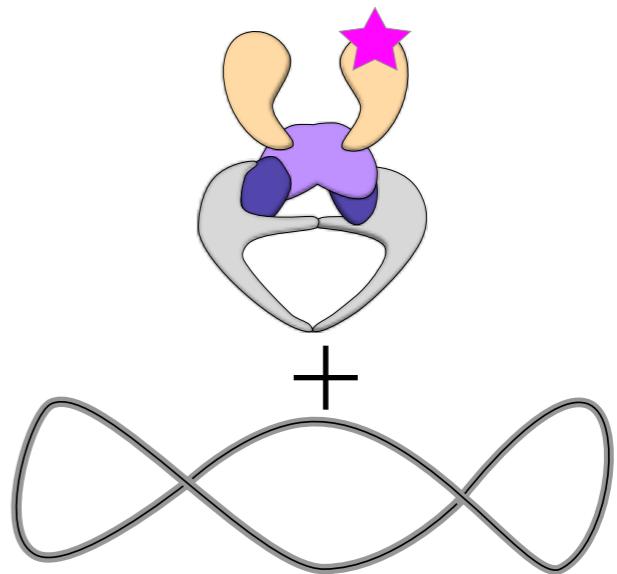


Top2cc Assay

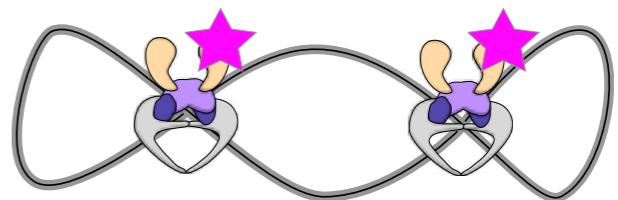
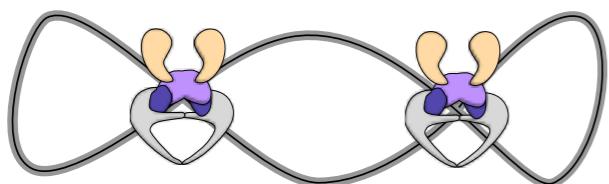
Resistant



Sensitive TOPOII

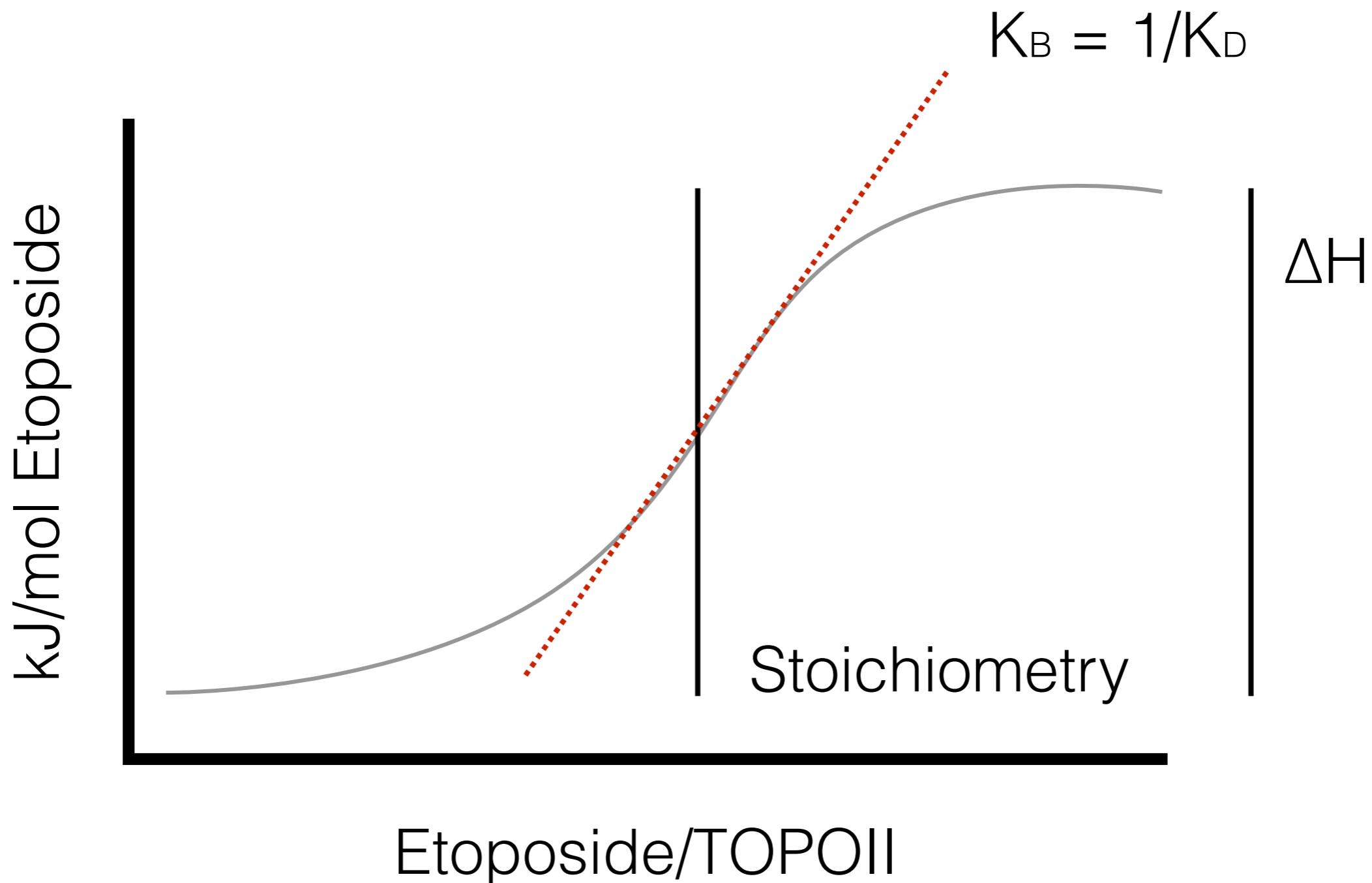


+Etoposide



Aim 3

Isothermal Titration Calorimetry



Aim 3

Acknowledgements

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