

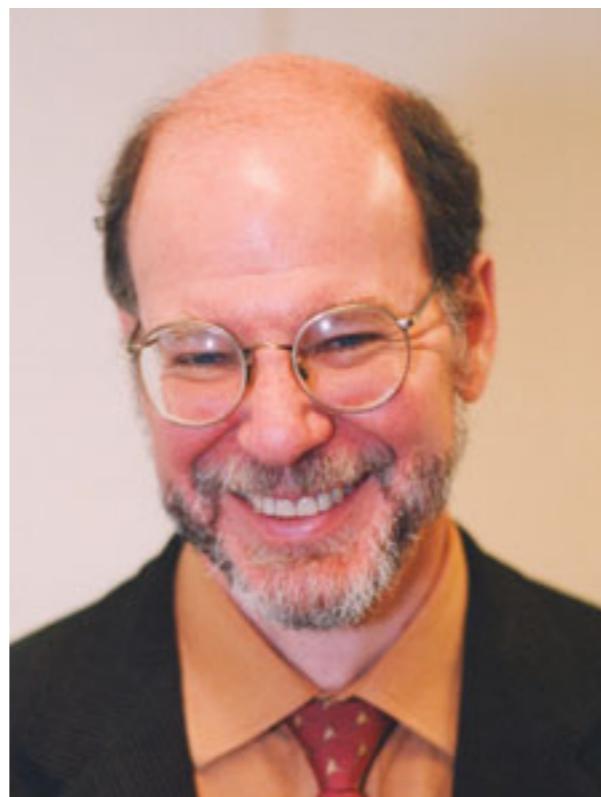
Changes to course...

More office hours: Thursdays 3-5 PM, starting 2/15

Problems closer to exams? More problems?

Bio393: Genetic Analysis

Step-wise genetic analysis

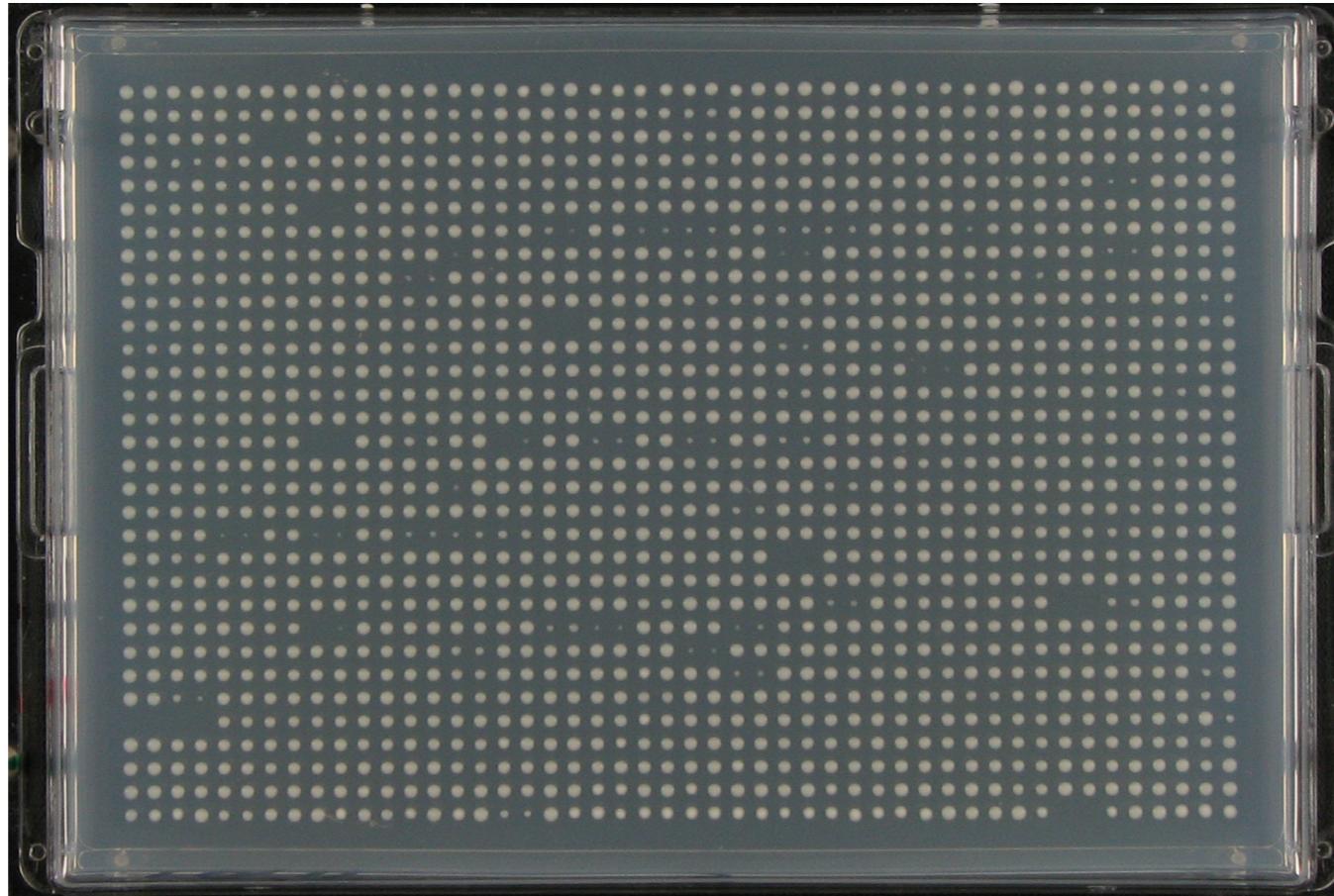


Bob Horvitz

14. Clone the gene

1. Clone by complementation
2. Clone by phenocopy
3. Clone by sequencing

Most model organisms have libraries of strains where each strain has a unique loss-of-function mutation



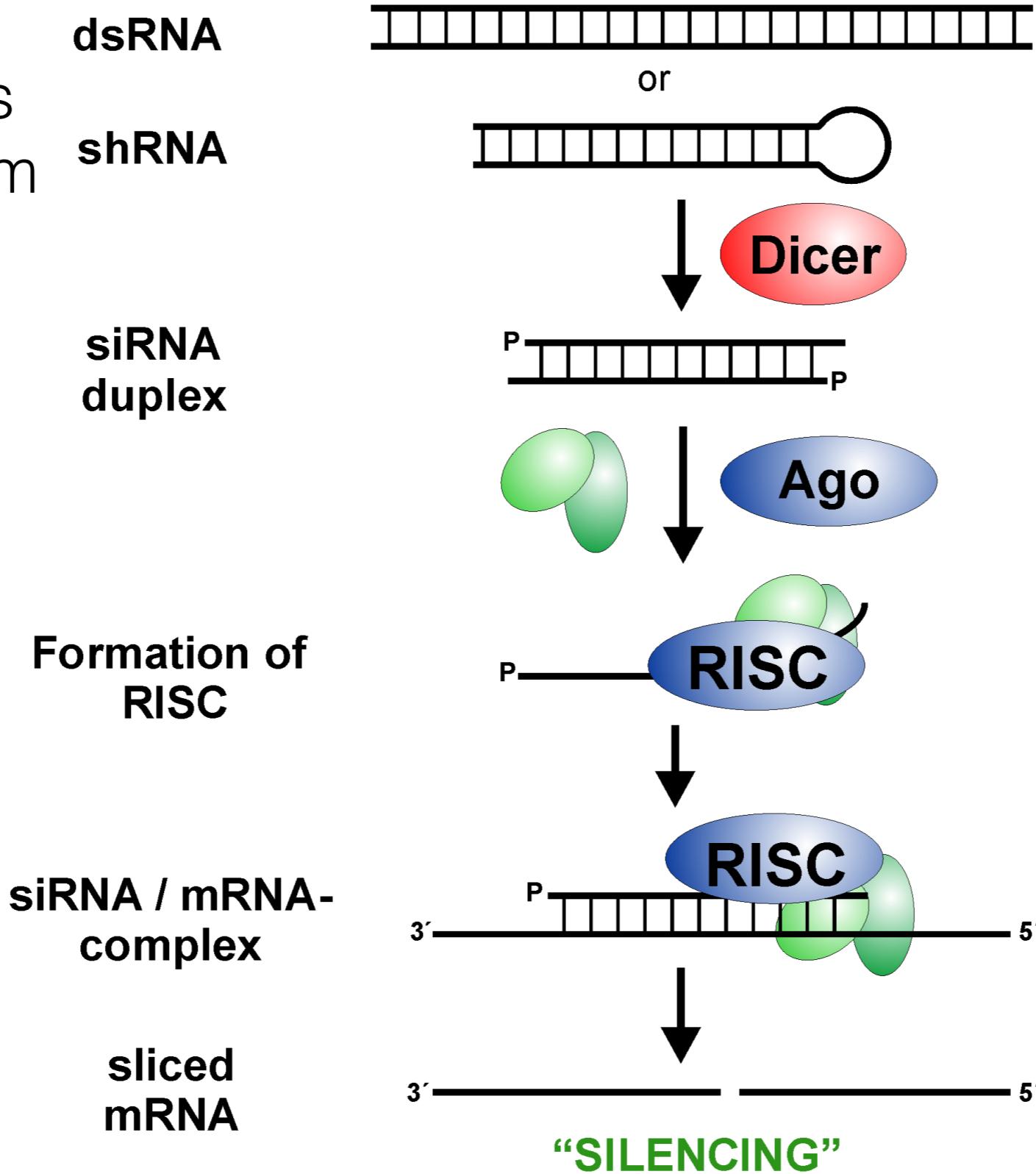
Score them all!!!

Phenotype is everything!

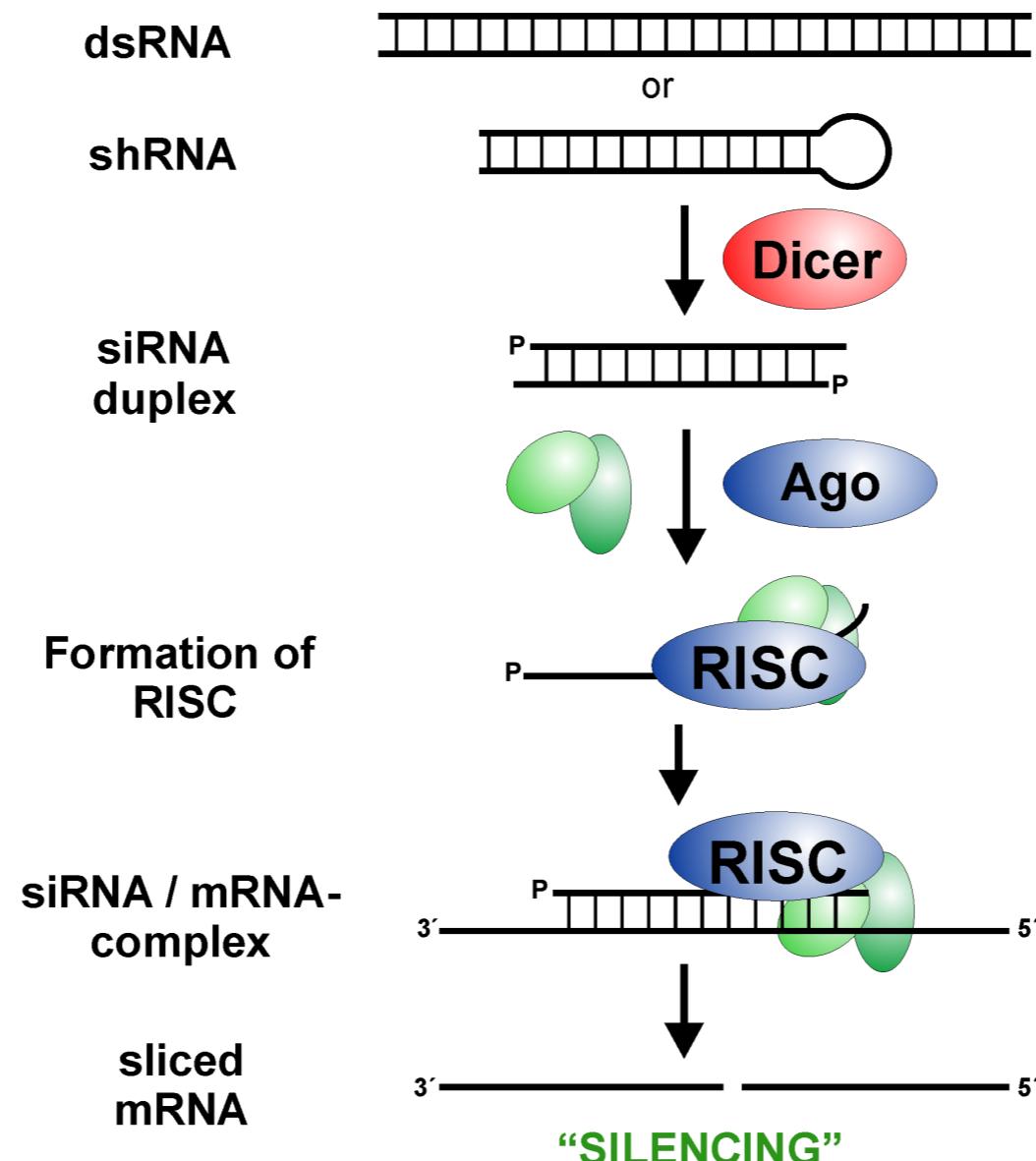
It has to be scalable, quantitative, reproducible, and accurate

RNA interference (RNAi) can be used to inactivate most genes in a variety of organisms

You have to
get these RNAs
into the organism



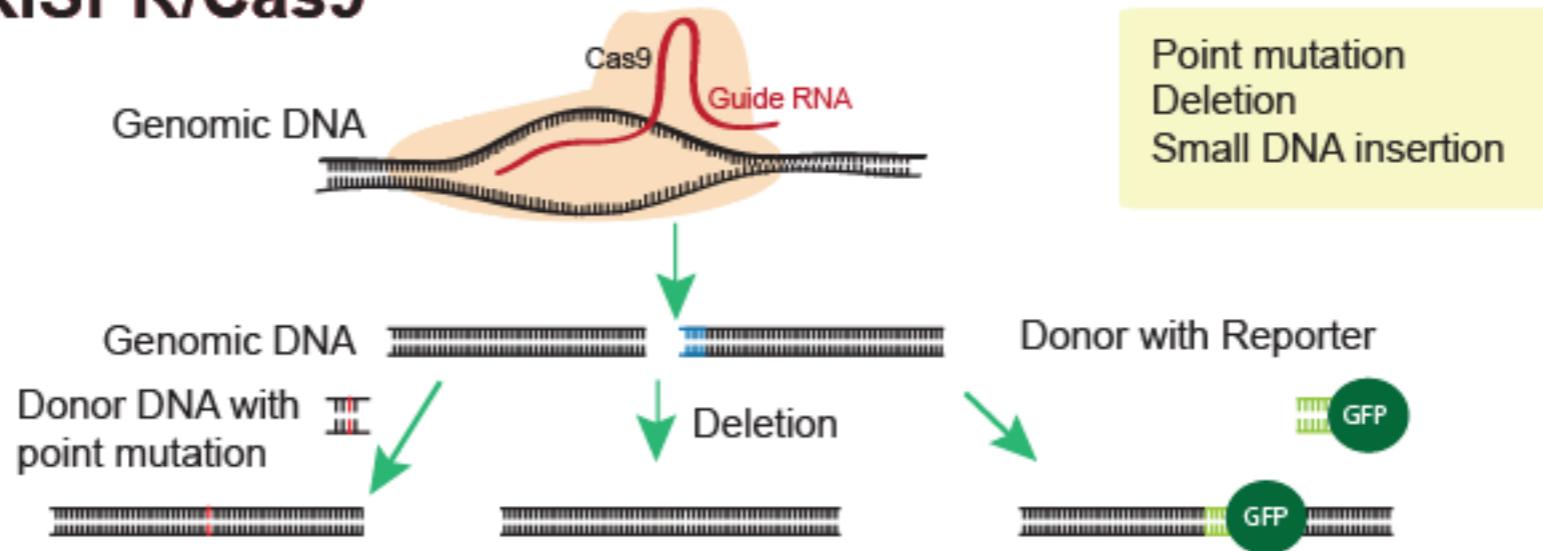
RNA interference (RNAi) can be used to inactivate most genes in a variety of organisms



Caveat: Delivery is problematic, highly variable effects, not heritable, not loss-of-function, not specific

CRISPR/cas9 can be used to create heritable loss-of-function mutations in a variety of organisms

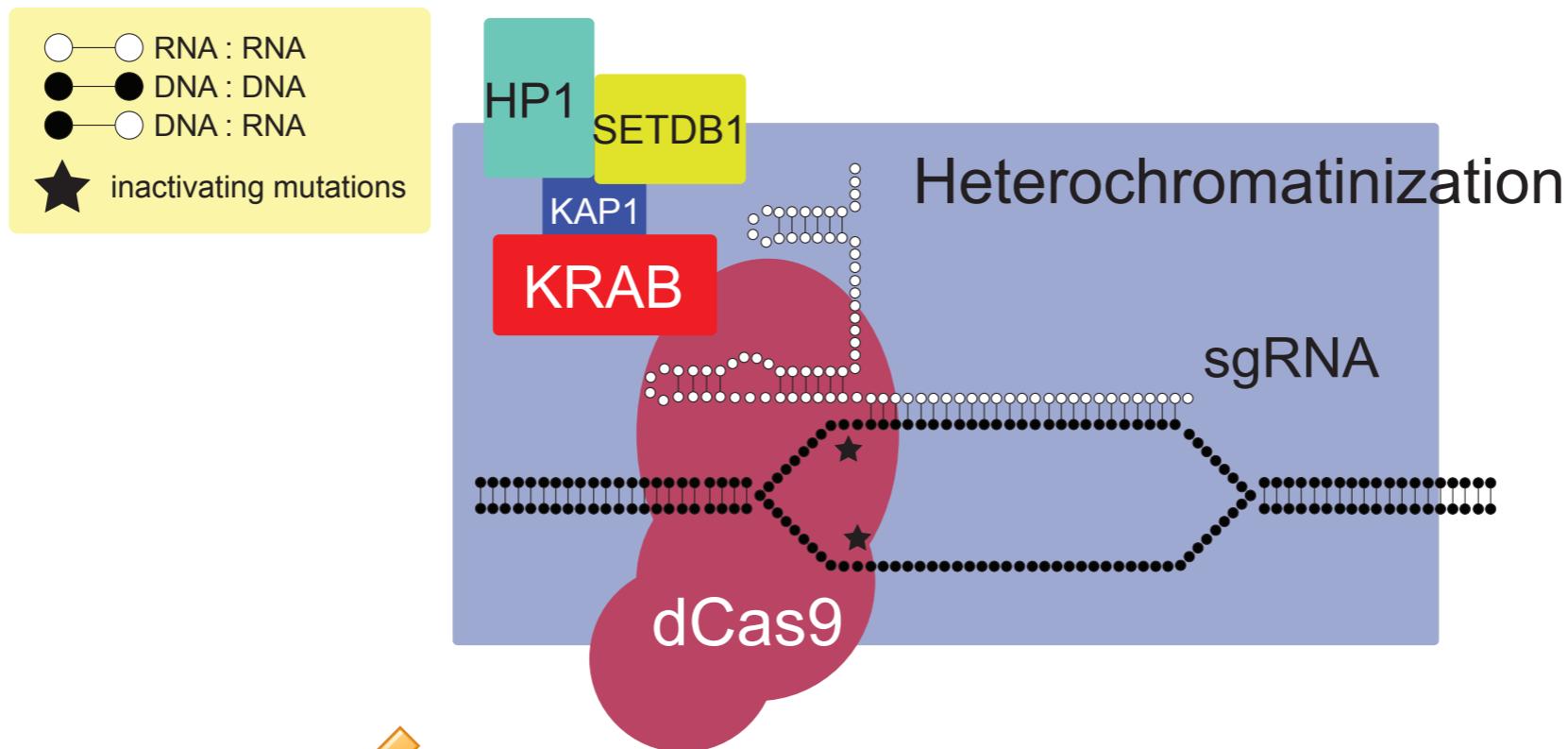
CRISPR/Cas9



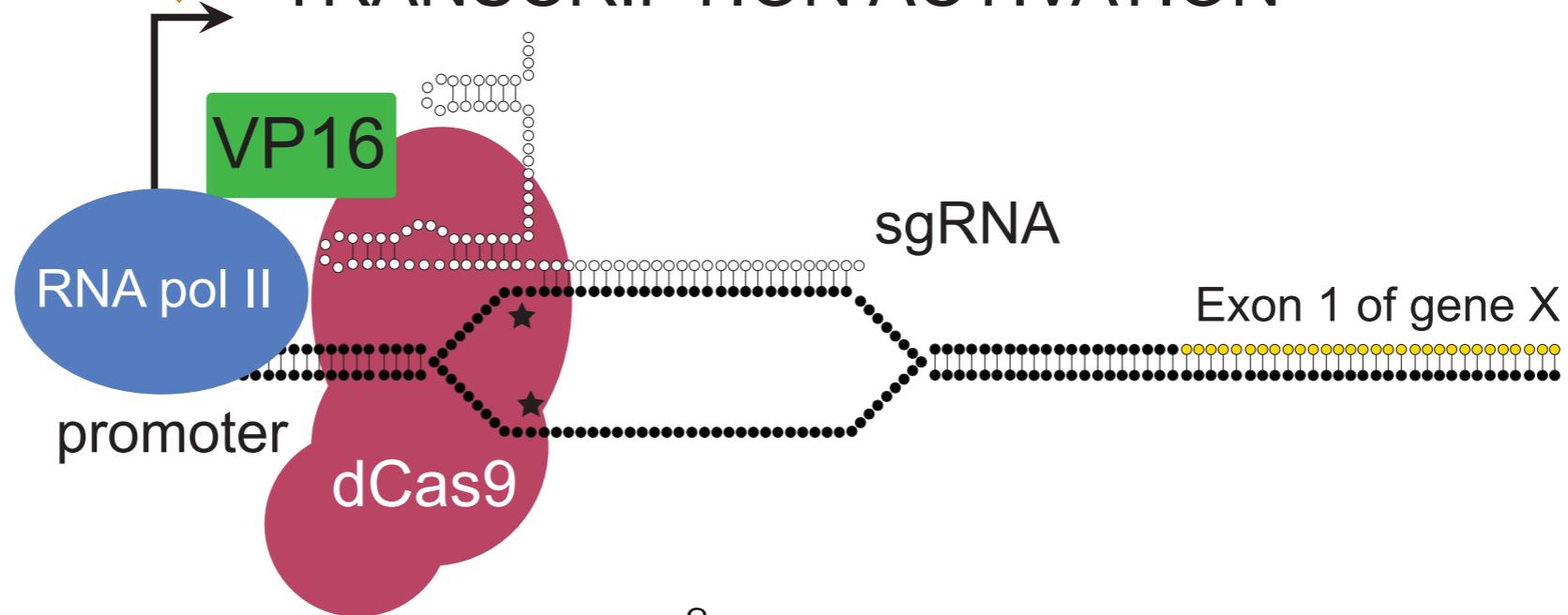
Caveat: Sometimes not specific (off-target effects)

CRISPRi and CRISPRa can test gene function

TRANSCRIPTION REPRESSION



TRANSCRIPTION ACTIVATION



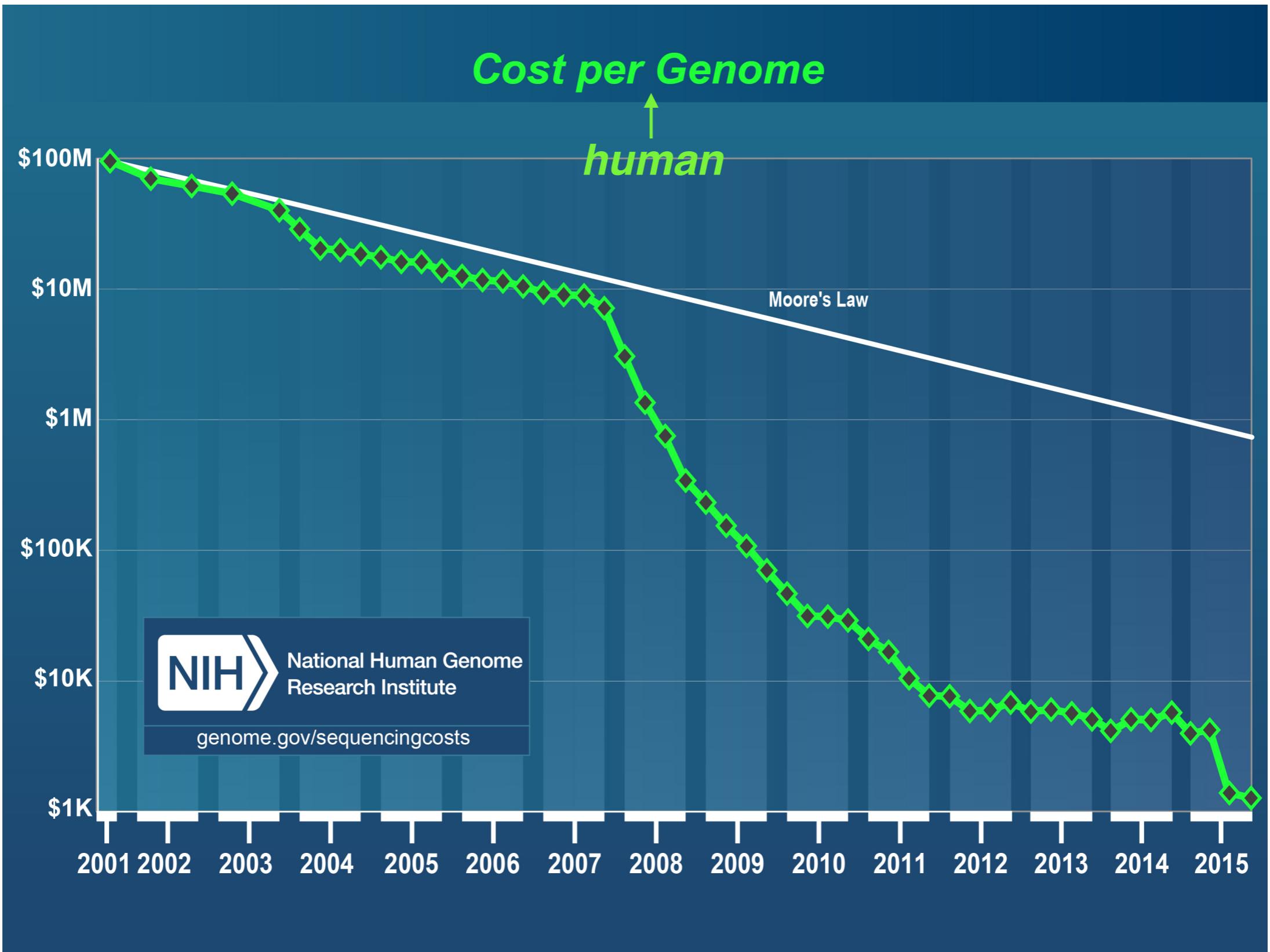
Generate “mutations” by reverse genetic RNAi or CRISPR for “all” genes in an organism

Organism	Approx. # of genes
Yeast (<i>S. cerevisiae</i>)	6,000
Fly (<i>D. melanogaster</i>)	15,000
Worm (<i>C. elegans</i>)	21,000
Zebrafish (<i>D. rerio</i>)	26,000
Chicken (<i>G. gallus</i>)	17,000
Mouse (<i>M. musculus</i>)	23,000
Mustard plant (<i>A. thaliana</i>)	28,000
Human (<i>H. sapiens</i>)	25,000

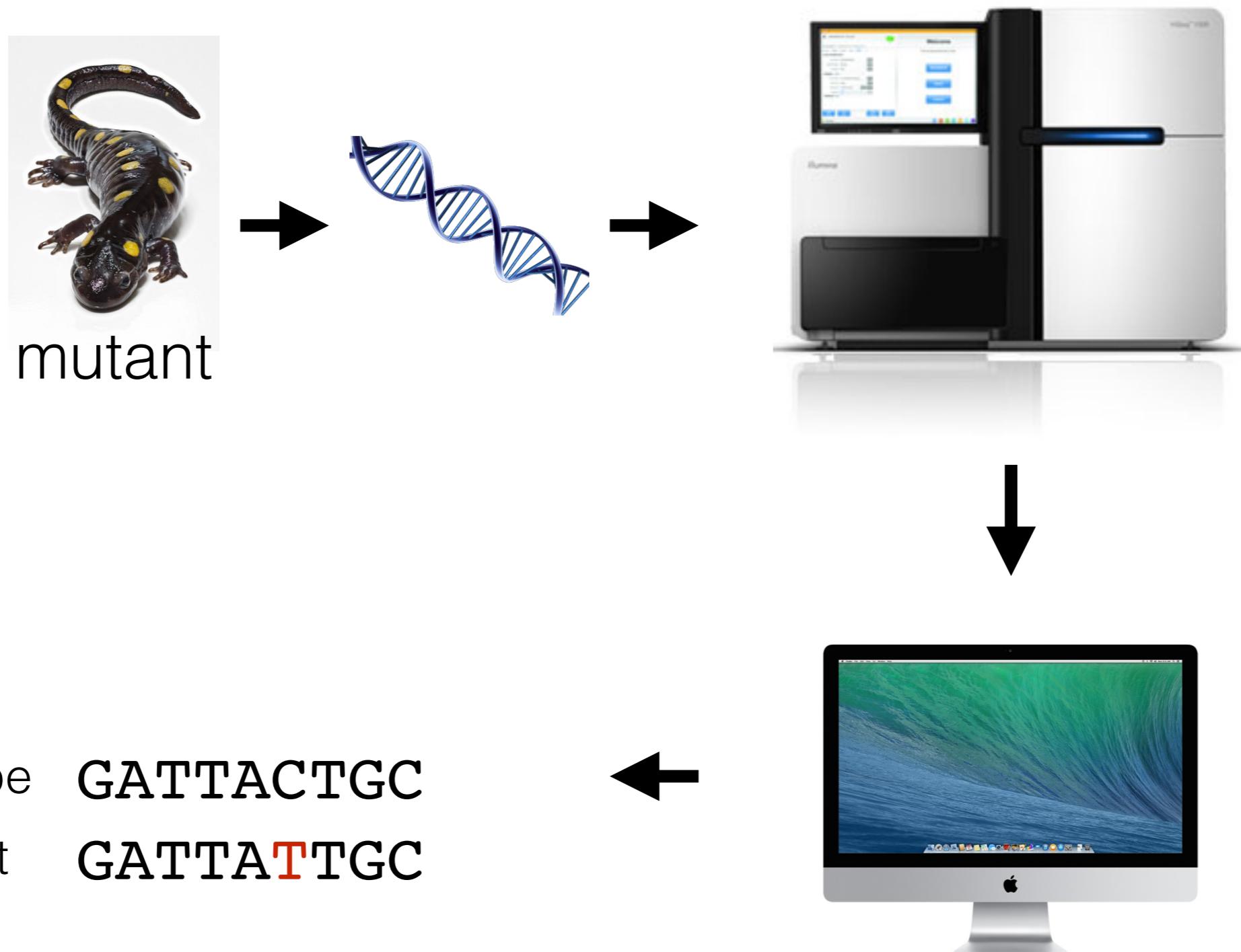
14. Clone the gene

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3. Clone by sequencing

Clone by sequencing

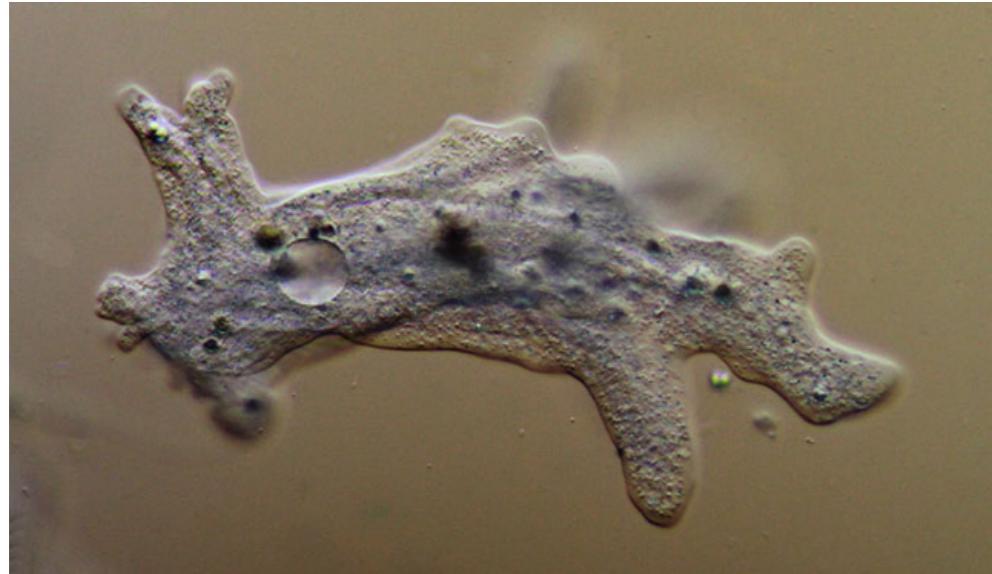


Clone by sequencing



Need multiple non-complementing alleles and mapping

Necessary and sufficient in the logic of cloning genes



Amoeba proteus

We find a mutant that doesn't engulf yeast.

It has a mutation in a signaling component, gene X.

Is gene X mutation responsible for the yeast engulfment defect?

1. RNAi of gene X has same phenotype as our mutant
2. Mutation in gene X with rescue of mutant phenotype in gene X
3. Independent screens for engulfment mutants get multiple alleles of gene X

Proof requires:
independent alleles or RNAi,
failure to complement original mutant,
phenotypic rescue

Step-wise genetic analysis

- 1. Define the problem**
- 2. Choose an organism**
- 3. Perform a mutant hunt**
- 4. Screen until saturation?**
- 5. Establish a strain**
- 6. Backcross and/or outcross**
- 7. Test for dominance**
- 8. Single-gene phenotype?**
- 9. Mapping and complementation**
- 10. Characterize the phenotype**
- 11. Define the nature of the mutant allele(s): gene dosage**
- 12. Perform non-complementation screens**
- 13. Define the null phenotype**
- 14. Clone the gene**

With a physical copy of the gene...

Add in wild-type copy to complement mutant copy

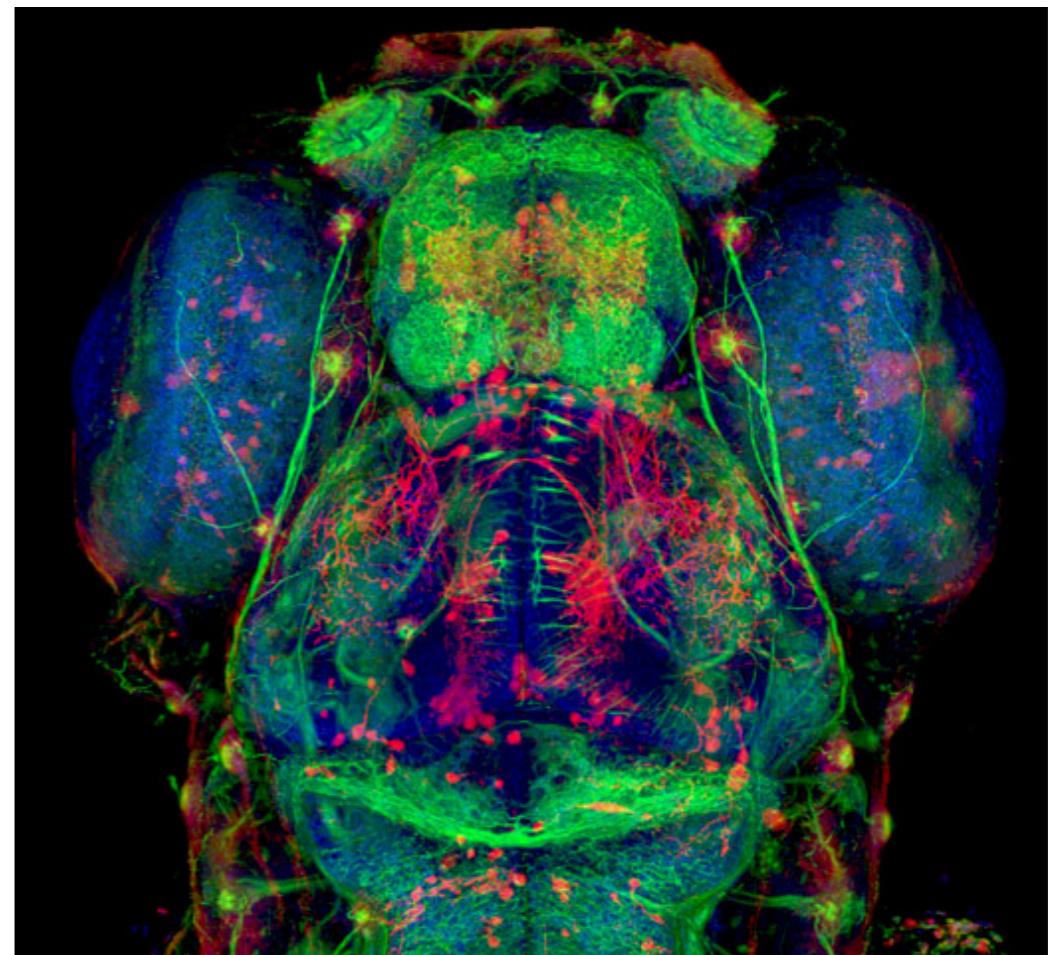
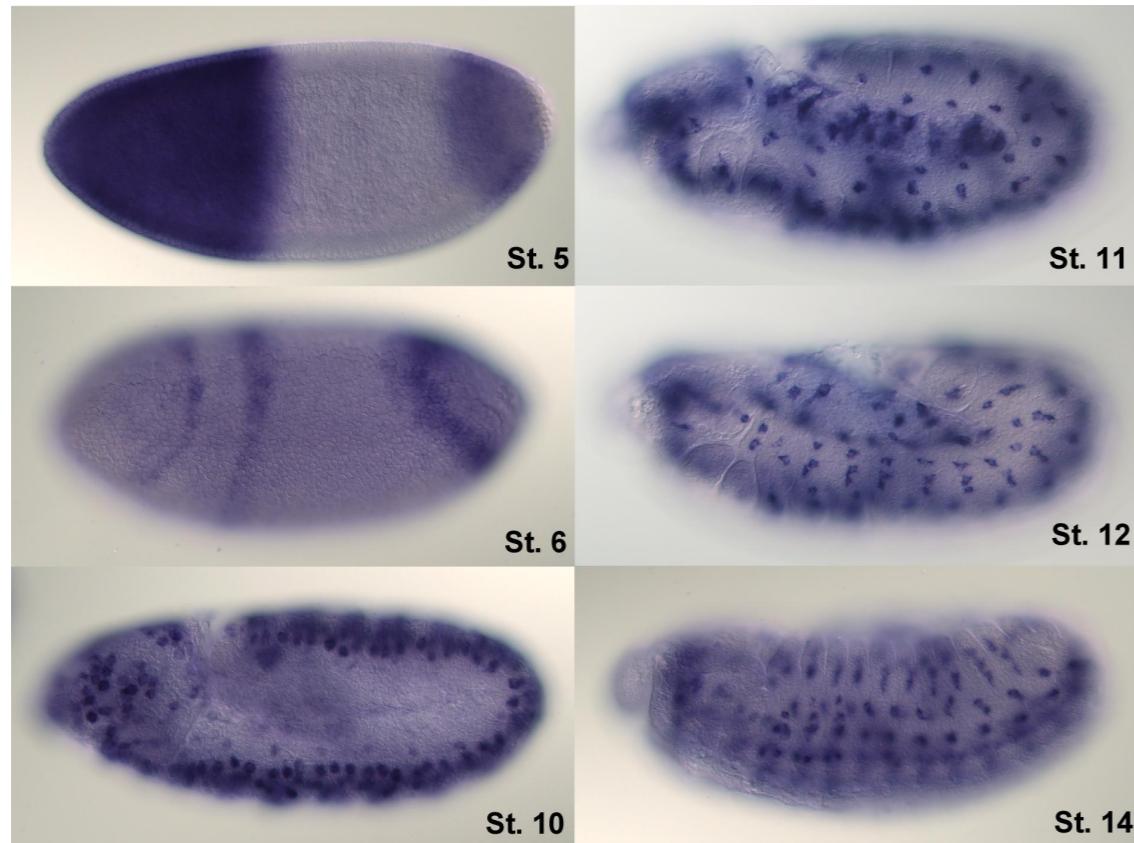
m + normal gene = Rescue of mutant
m mutated in m phenotype
(complementation)

m + mutant gene = **No rescue** of mutant
m mutated in m phenotype

15. Determine where gene is expressed

With no transgenesis:

1. *in situ* hybridization (RNA localization)
2. Antibody immunofluorescence (protein localization)



How do we know we have the right expression pattern?

15. Determine where gene is expressed

With transgenesis:

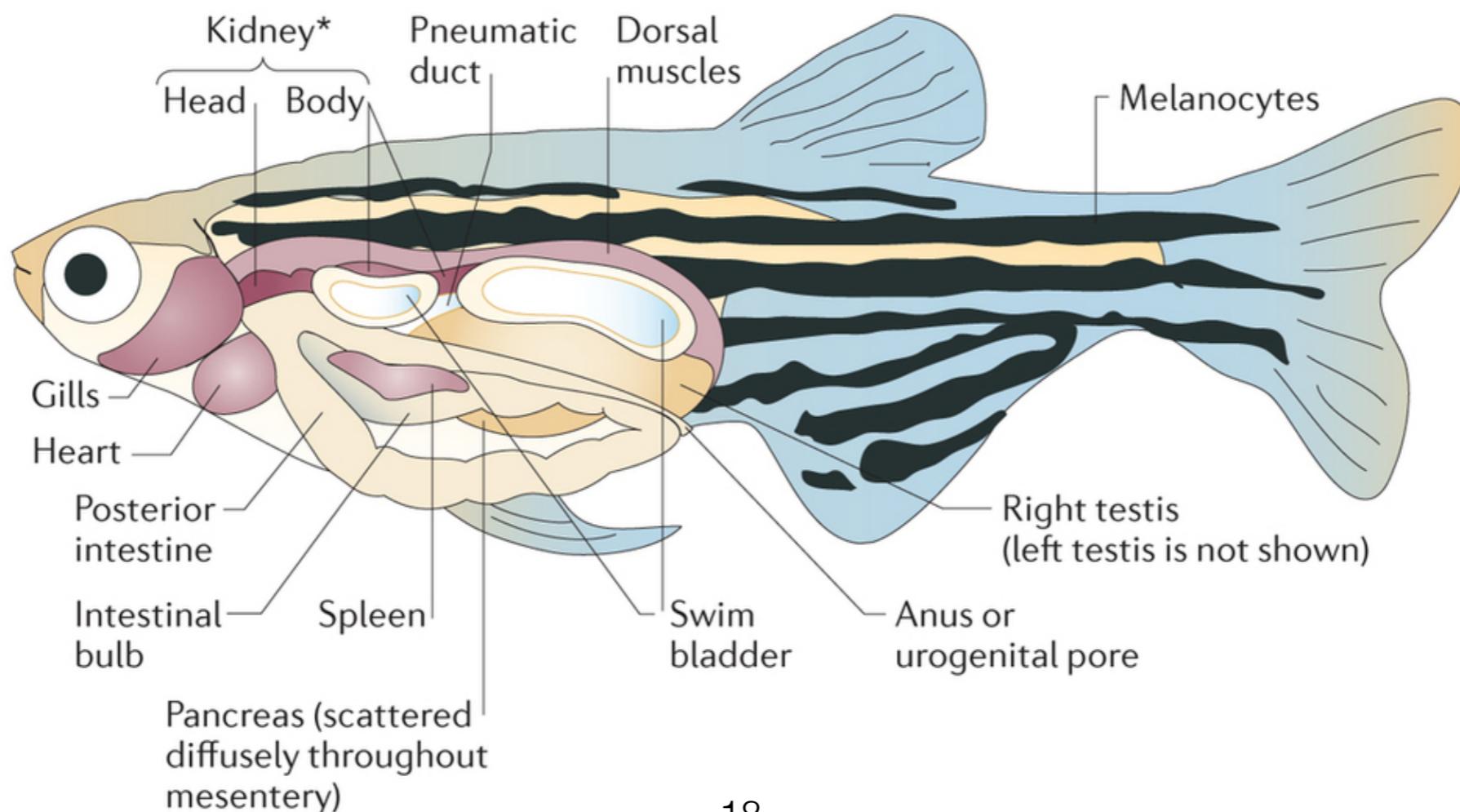


Where a gene is expressed might not be where it acts?

16. Determine site of gene action

What is the cell, organ, and/or tissue where the gene functions?

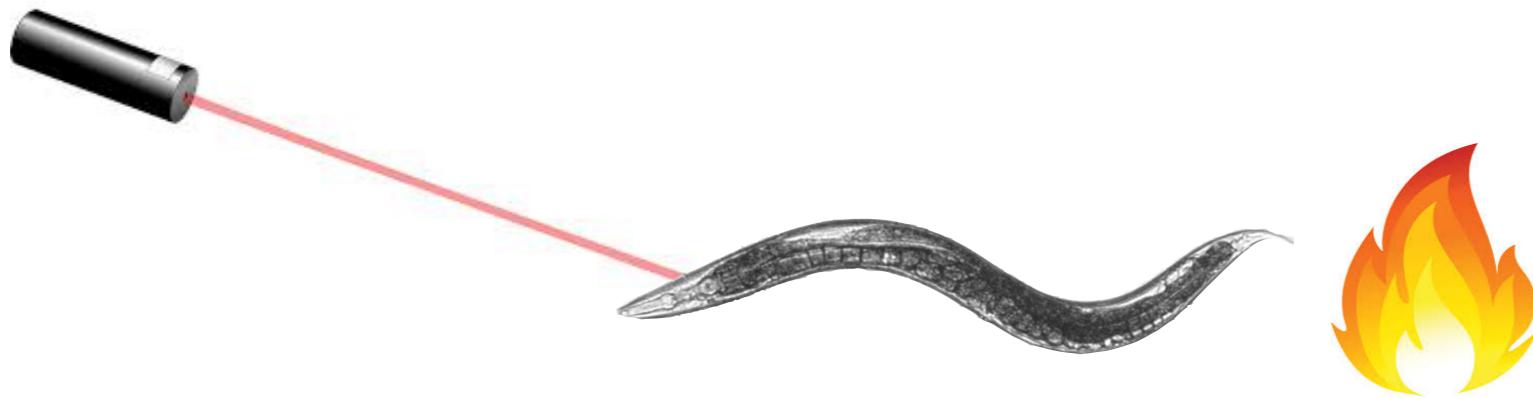
1. Rescue a mutant phenotype in a specific cell, organ, or tissue
2. Mosaic analysis (cell autonomy experiments)



17. Determine time of gene action

When does the gene function?

1. Induce expression to rescue a mutant phenotype at a specific time

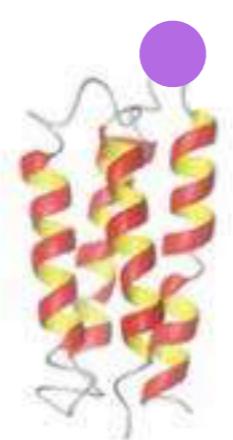
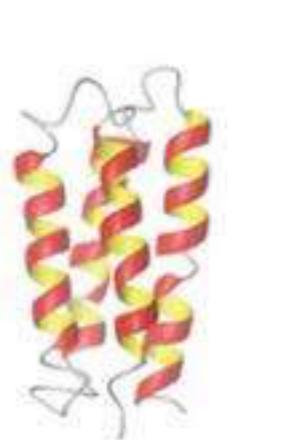


Heat-inducible gene expression in certain tissues
at specific times

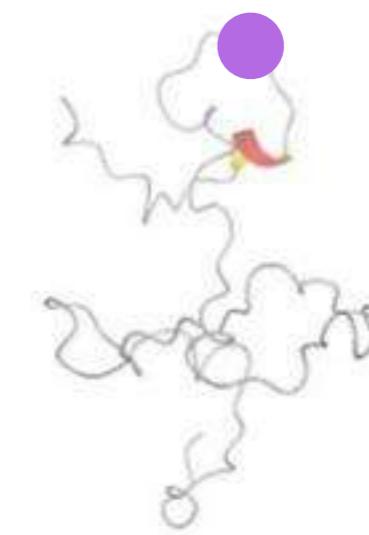
17. Determine time of gene action

When does the gene function?

1. Induce expression to rescue a mutant phenotype at a specific time
2. Use temperature-labile mutants to define the temperature-sensitive period

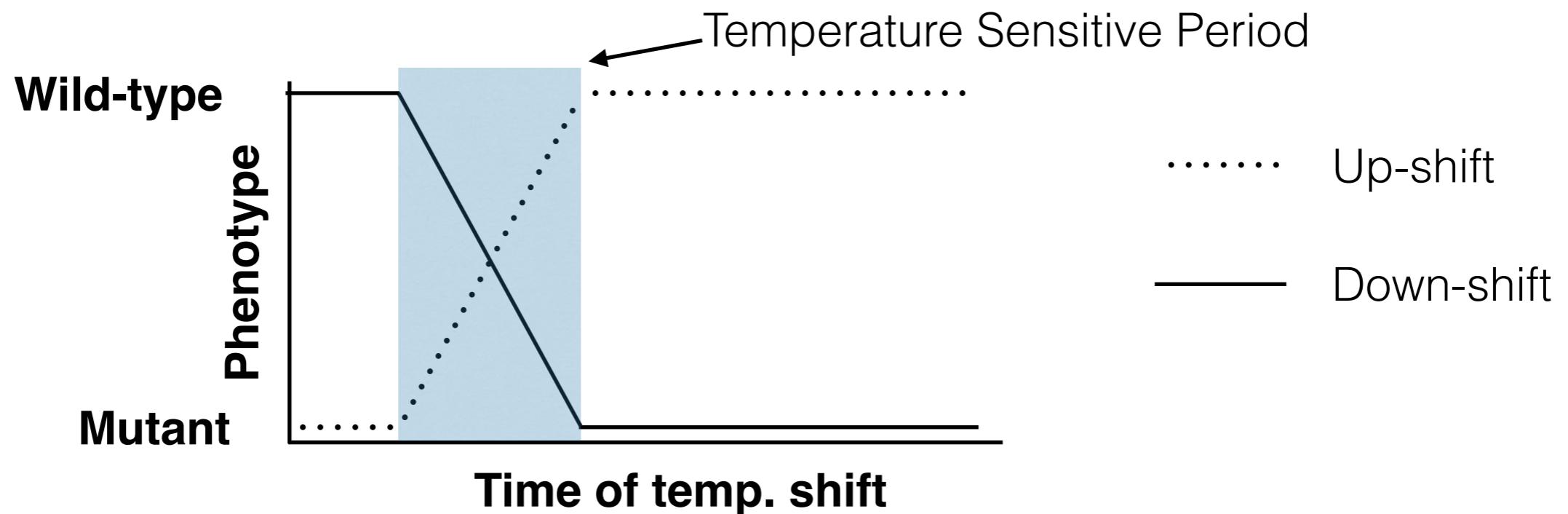


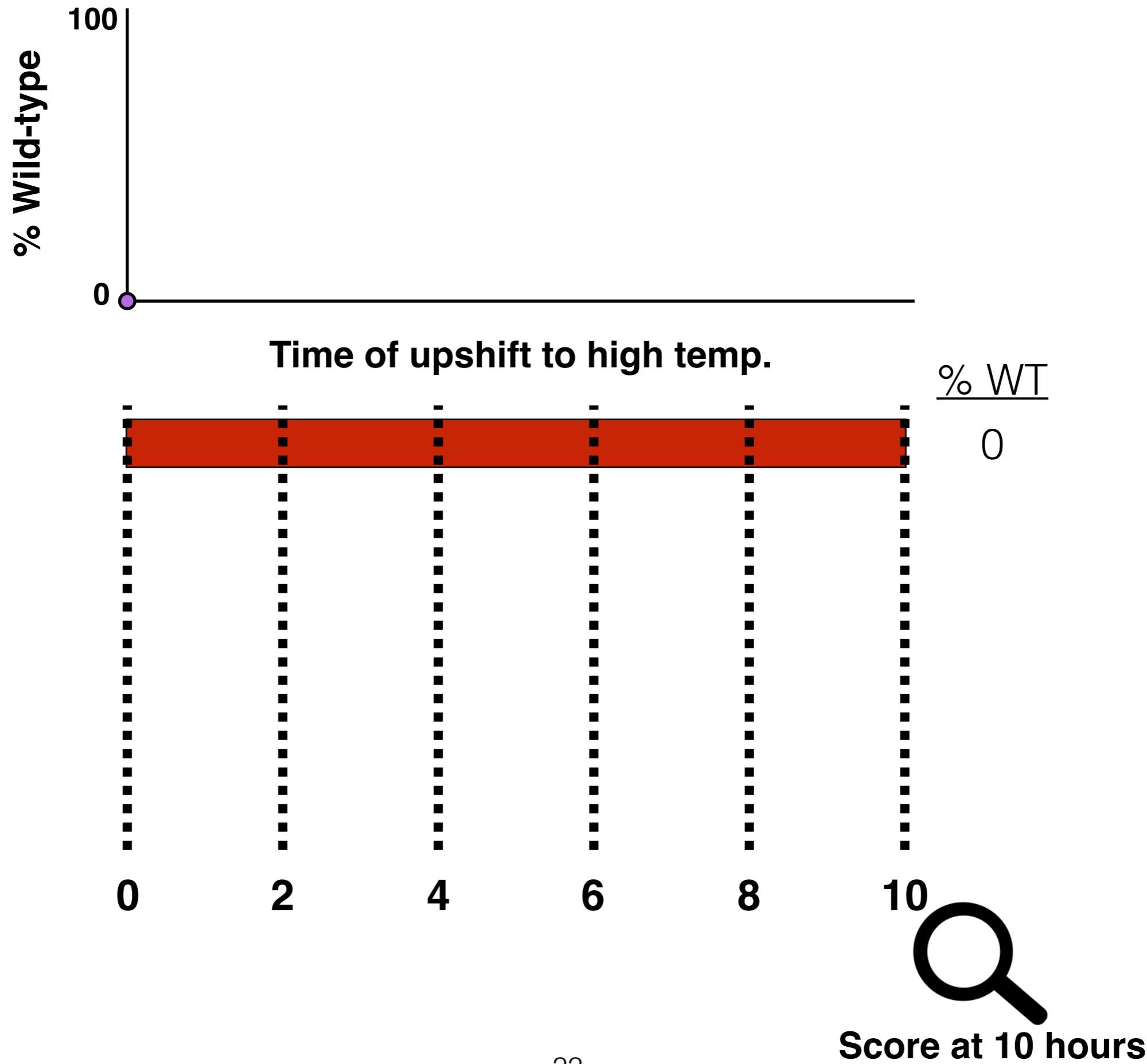
Permissive temperature

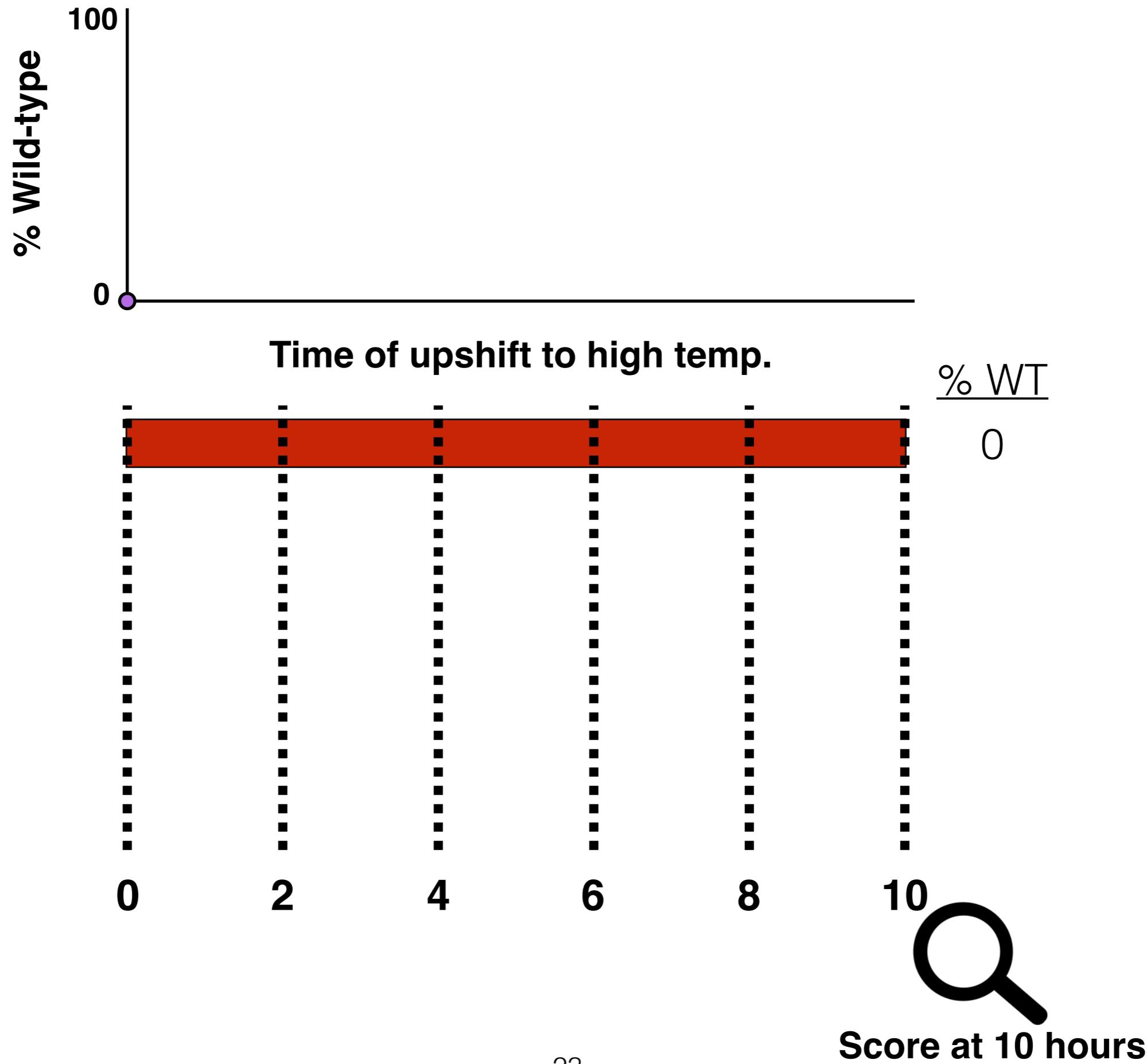


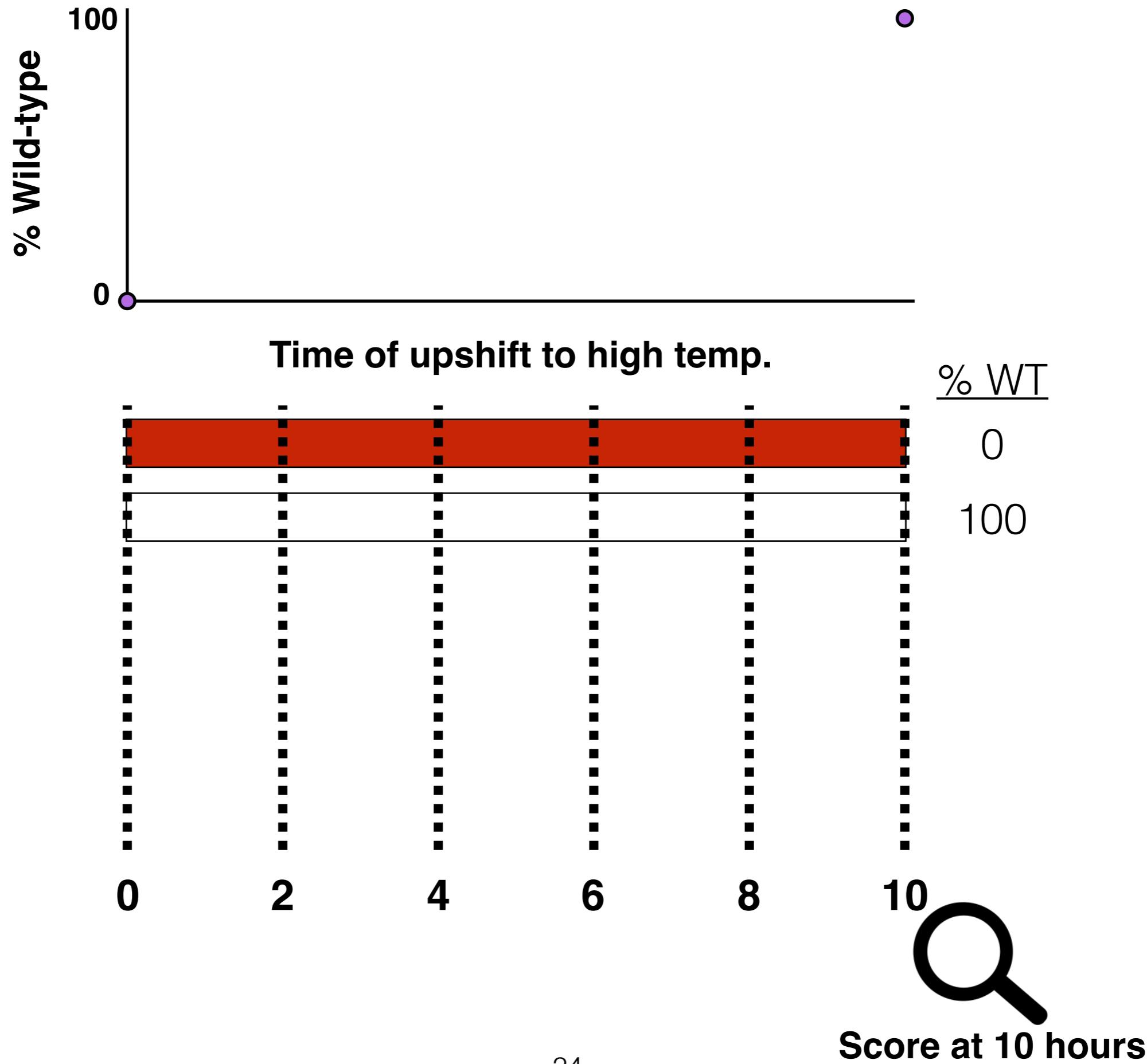
Restrictive temperature

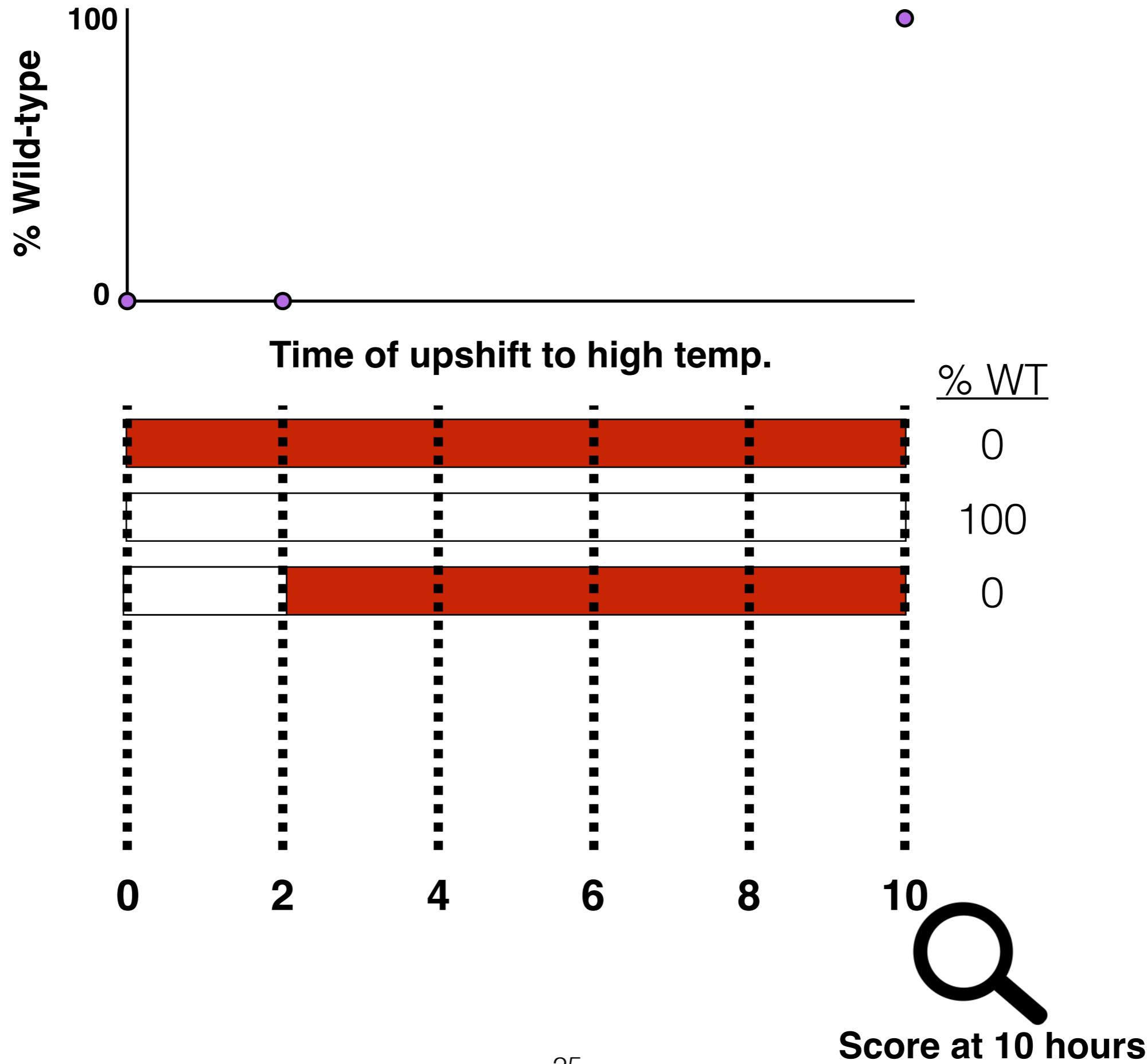
17. Determine time of gene action

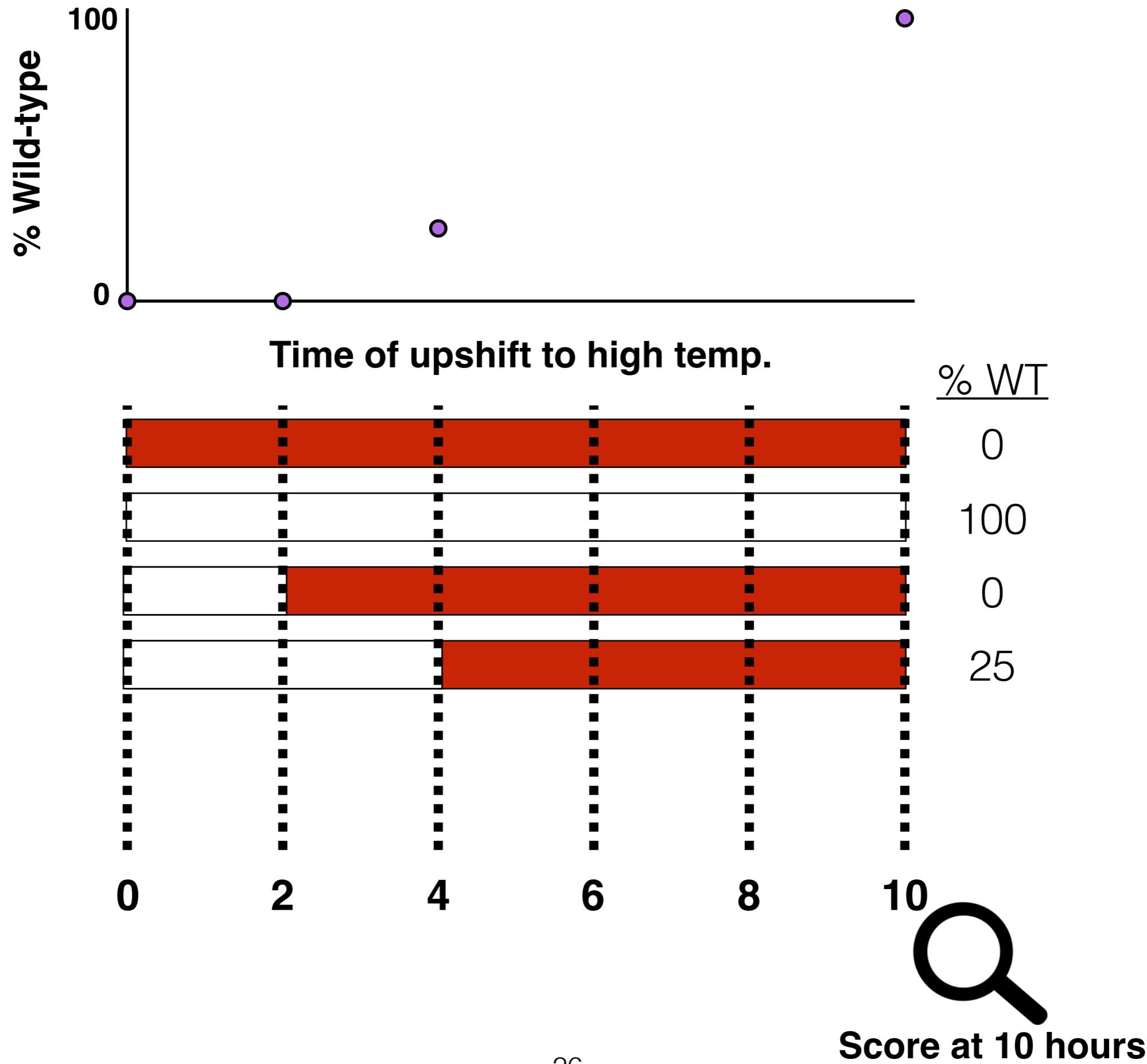


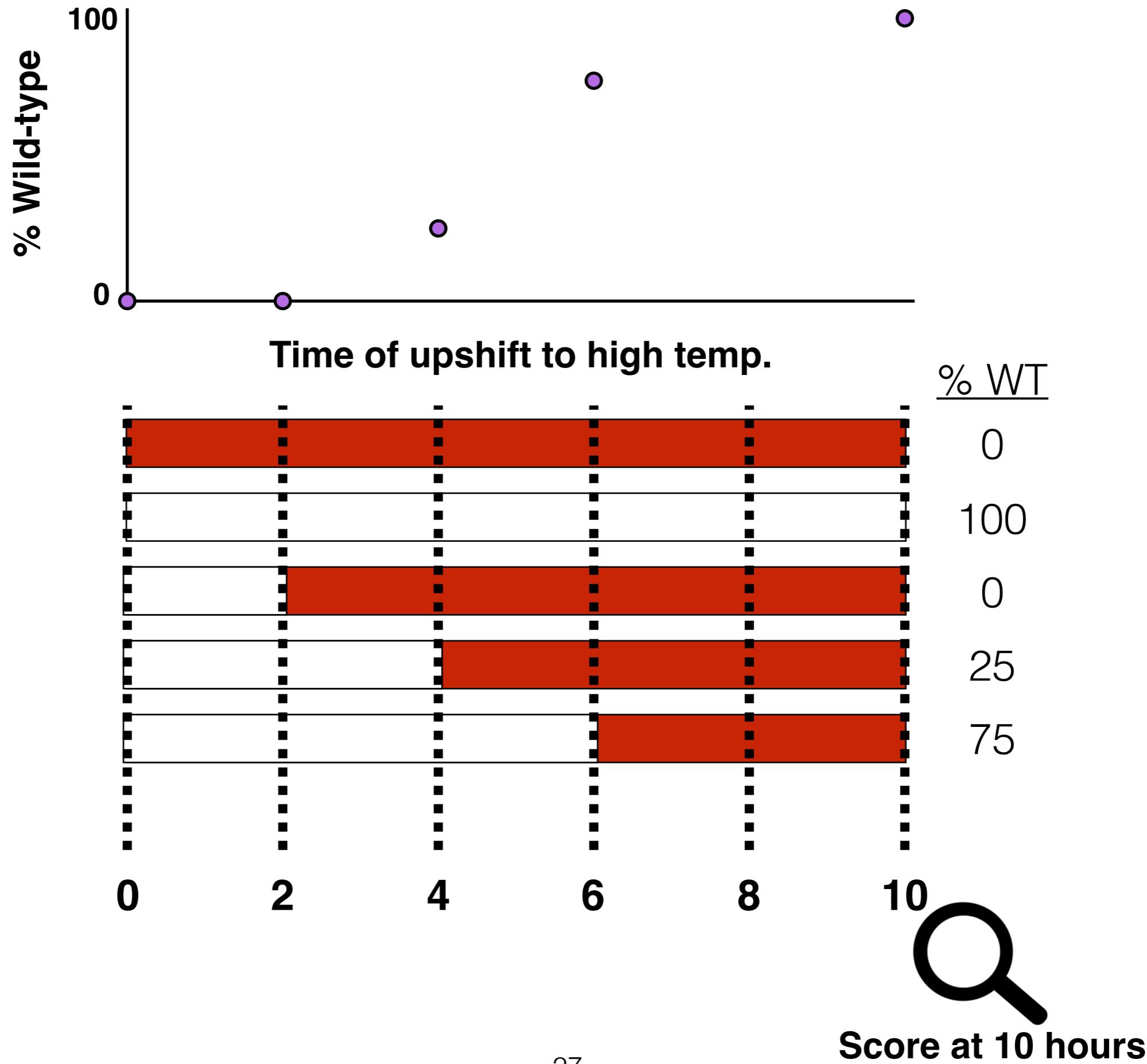


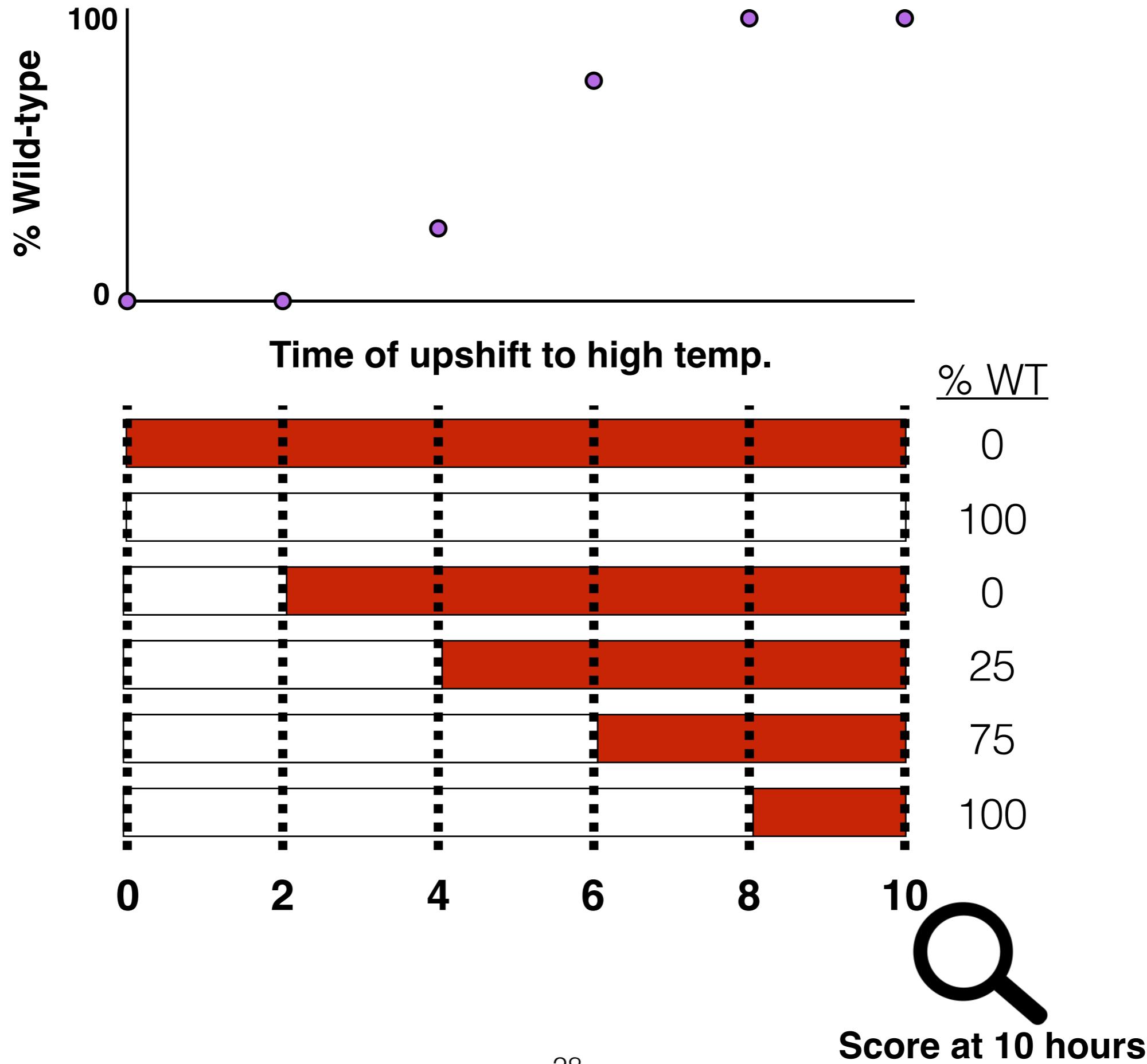


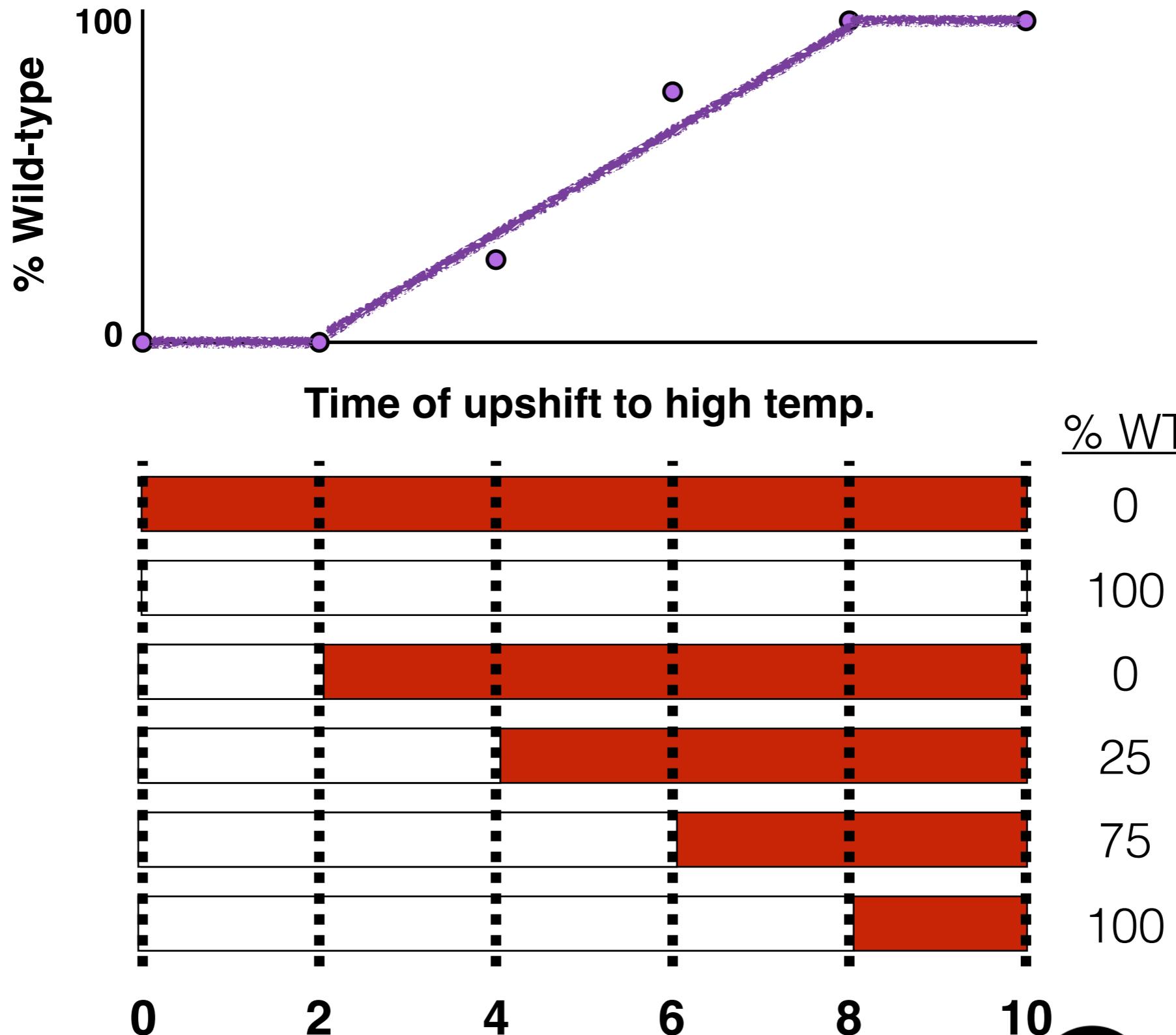


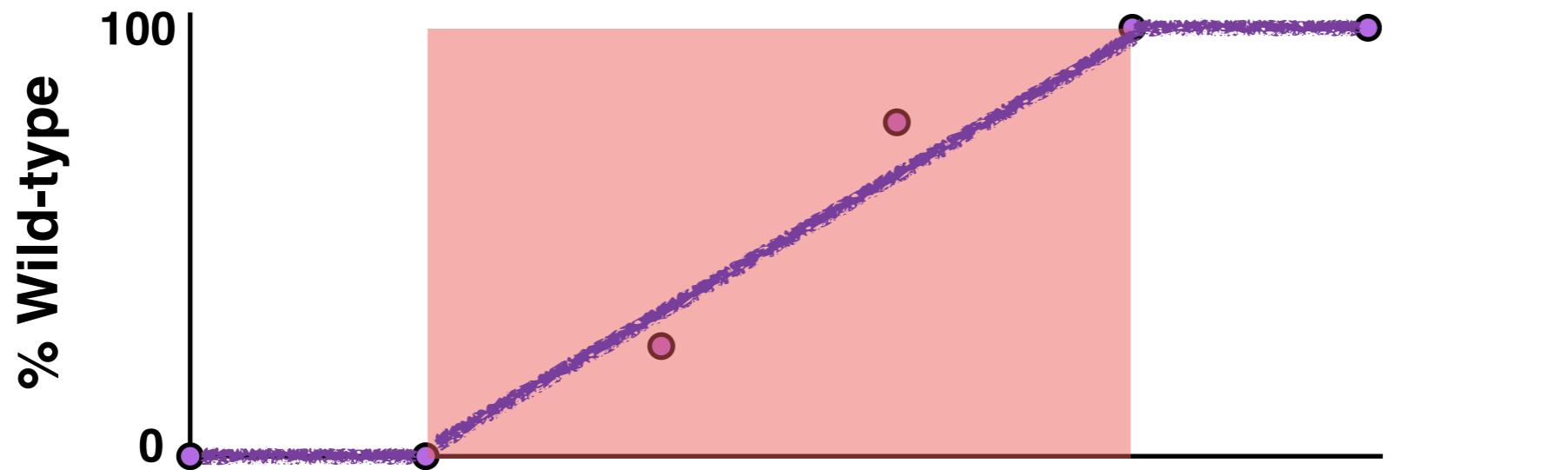






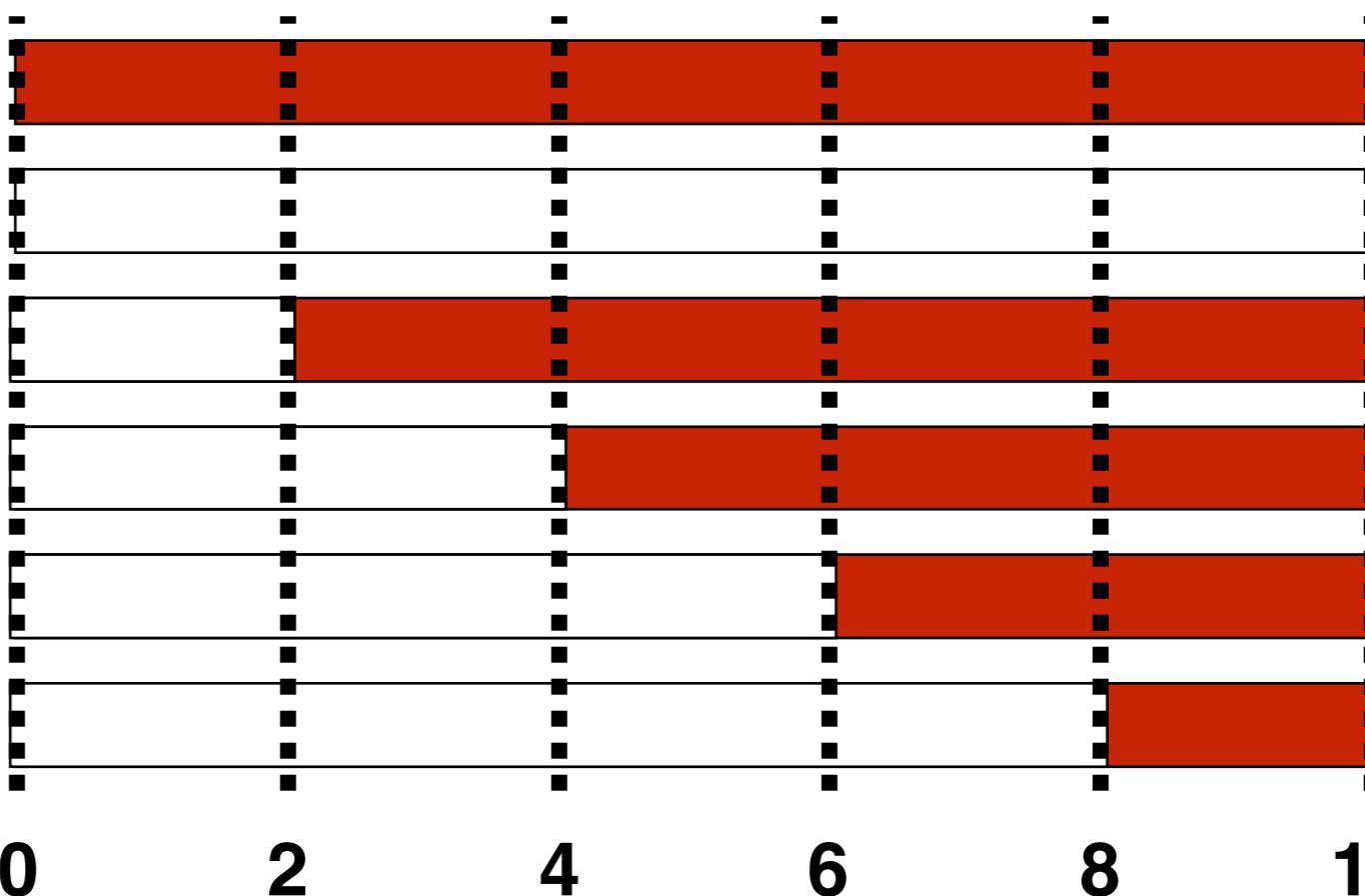




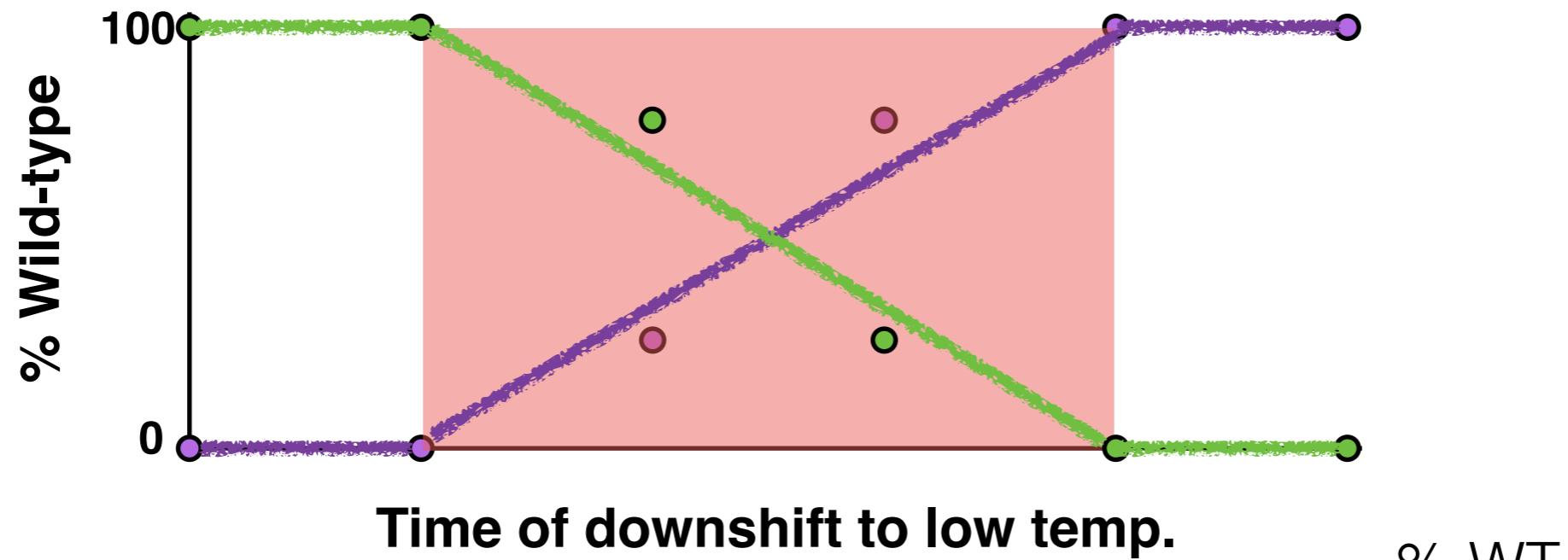


Time of upshift to high temp.

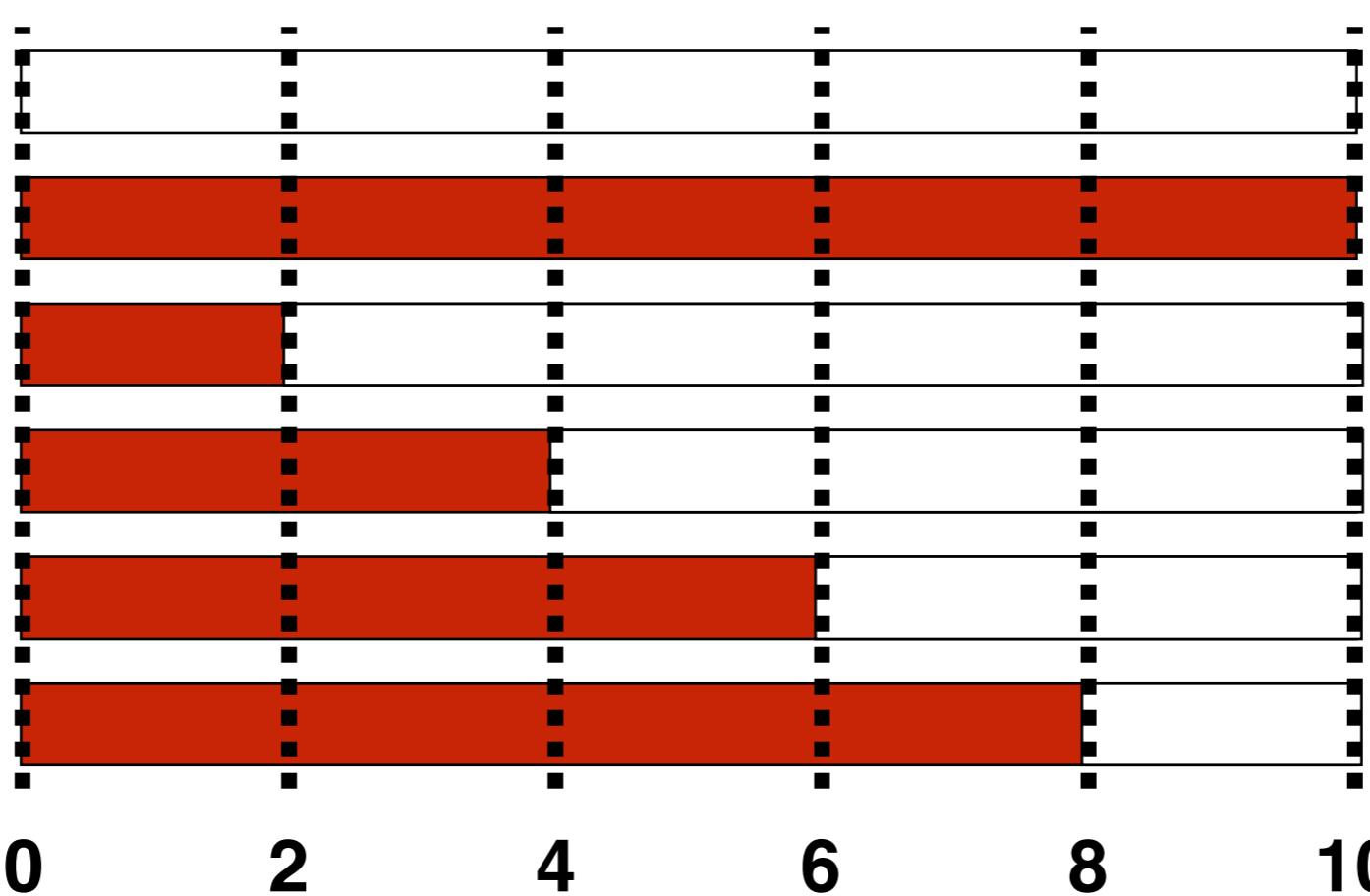
% WT



Score at 10 hours

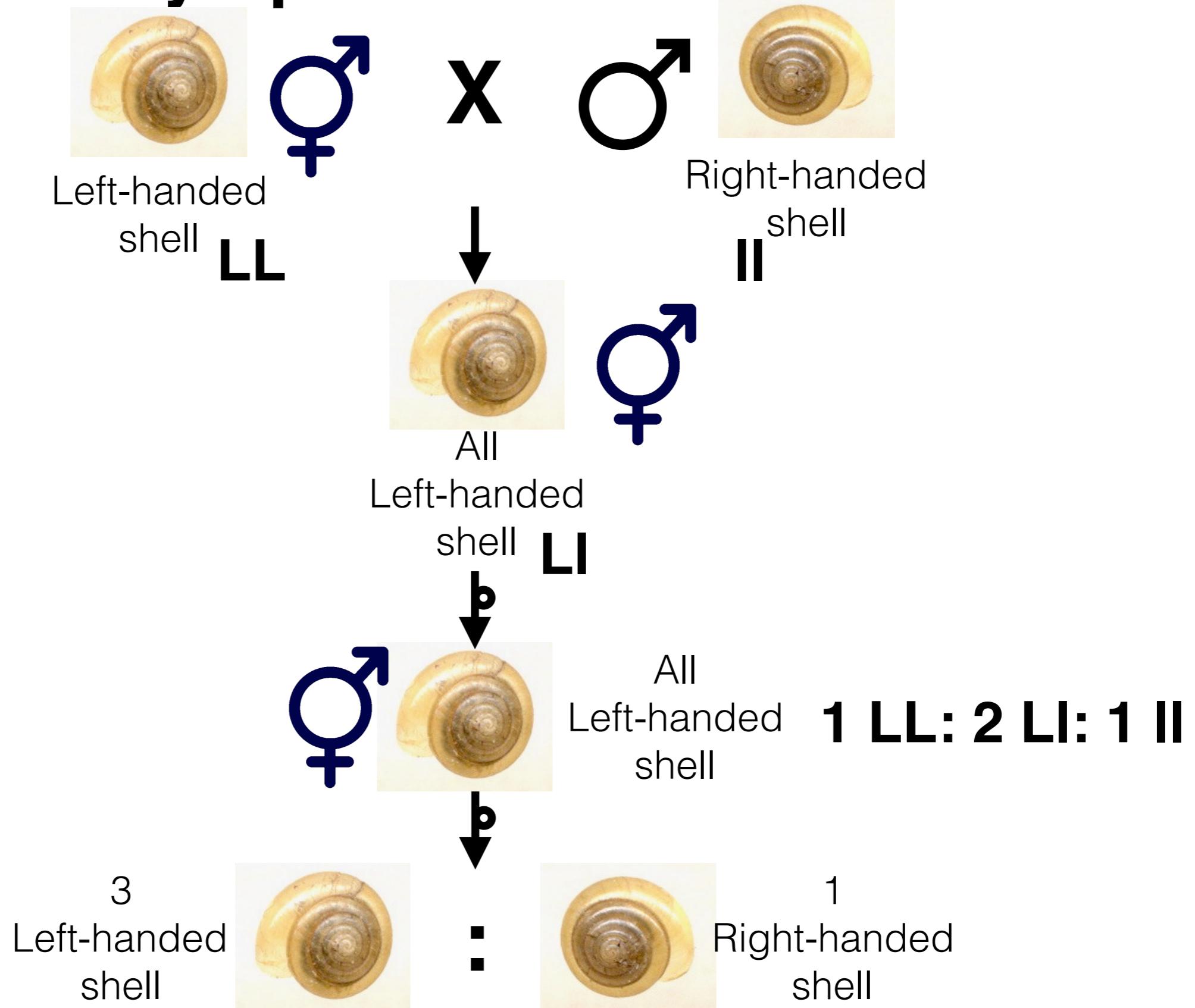


Time of downshift to low temp.



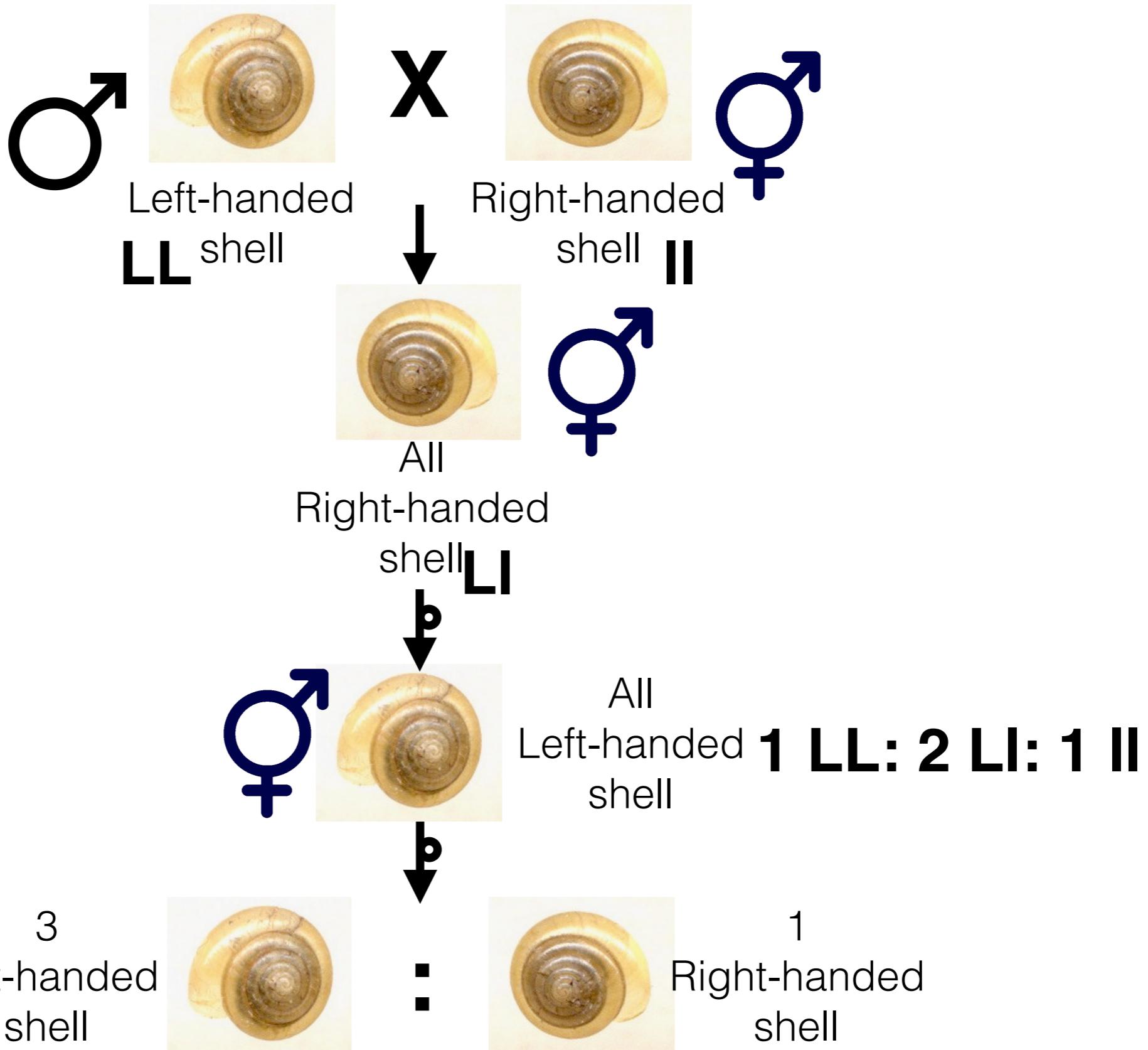
Score at 10 hours

18. Determine if there are maternal effects or cytoplasmic inheritance



18. Determine if there are maternal effects or cytoplasmic inheritance

Reciprocal cross



18. Determine if there are maternal effects or cytoplasmic inheritance



The egg and sperm have different compositions.