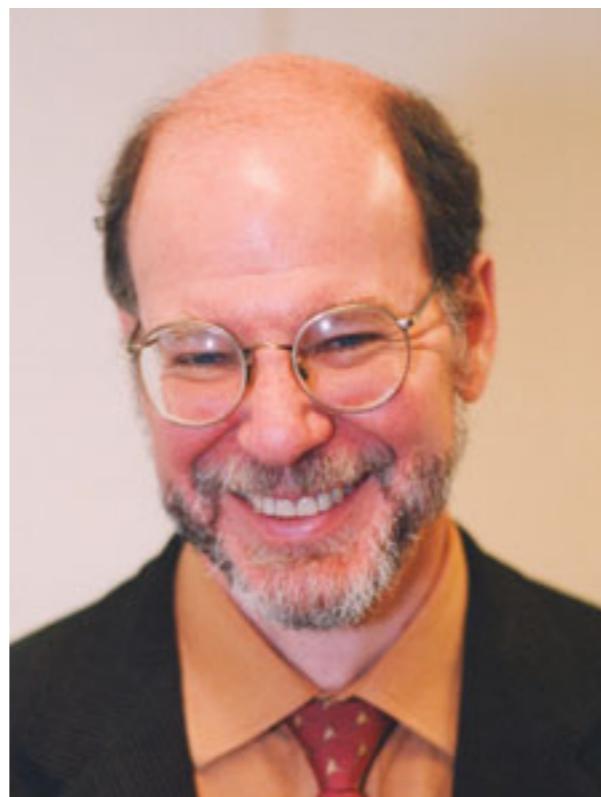


Bio393: Genetic Analysis

Step-wise genetic analysis



Bob Horvitz

Step-wise genetic analysis

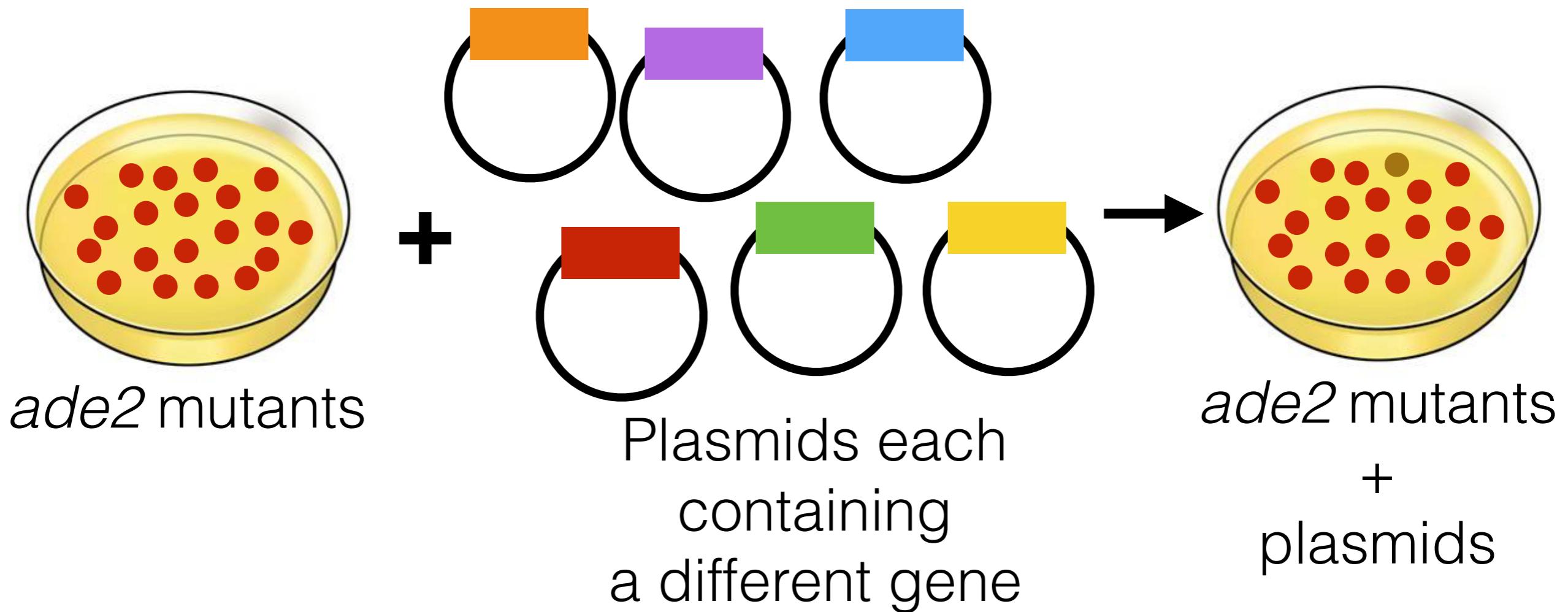
- 1. Define the problem**
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- 13. Define the null phenotype**

14. Clone the gene

***Identify a DNA sequence that contains
your gene of interest***

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

Cloning by complementation in bacteria and yeast



Caveat: overexpression bypass suppressors

Cloning by complementation in worms and flies

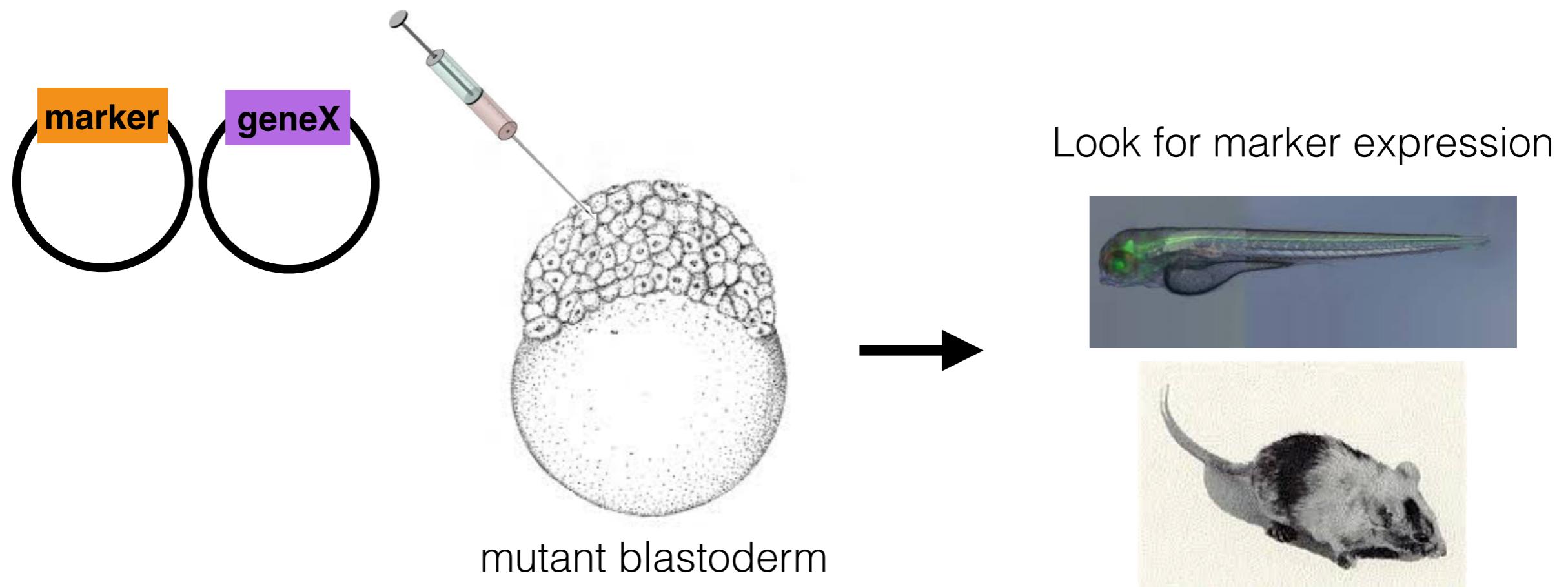


Caveat: overexpression bypass suppressors and not stable



Caveat: overexpression bypass suppressor and variable expression

Cloning by complementation in fish and mice

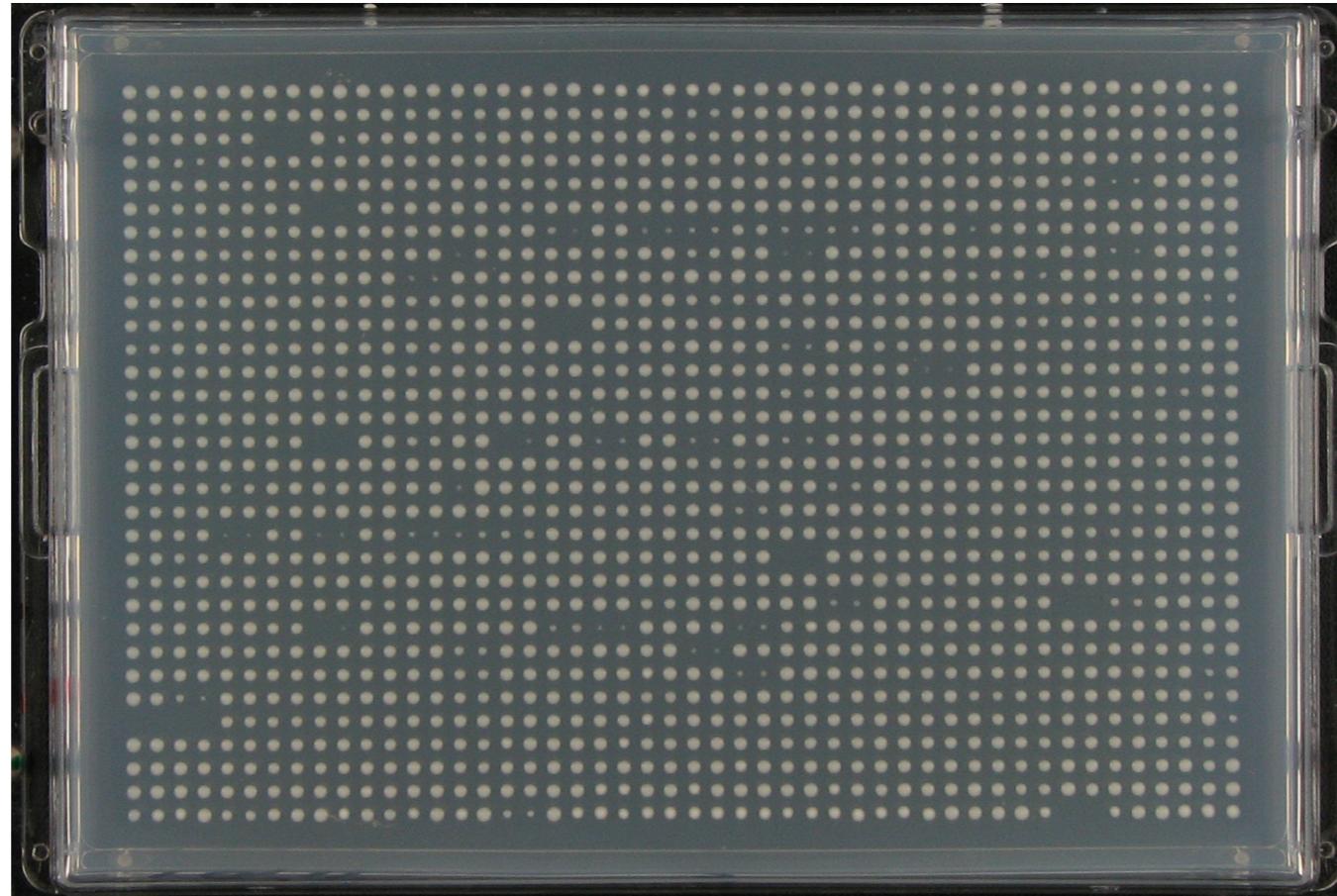


Caveat: overexpression bypass suppressors
and variable expression

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**



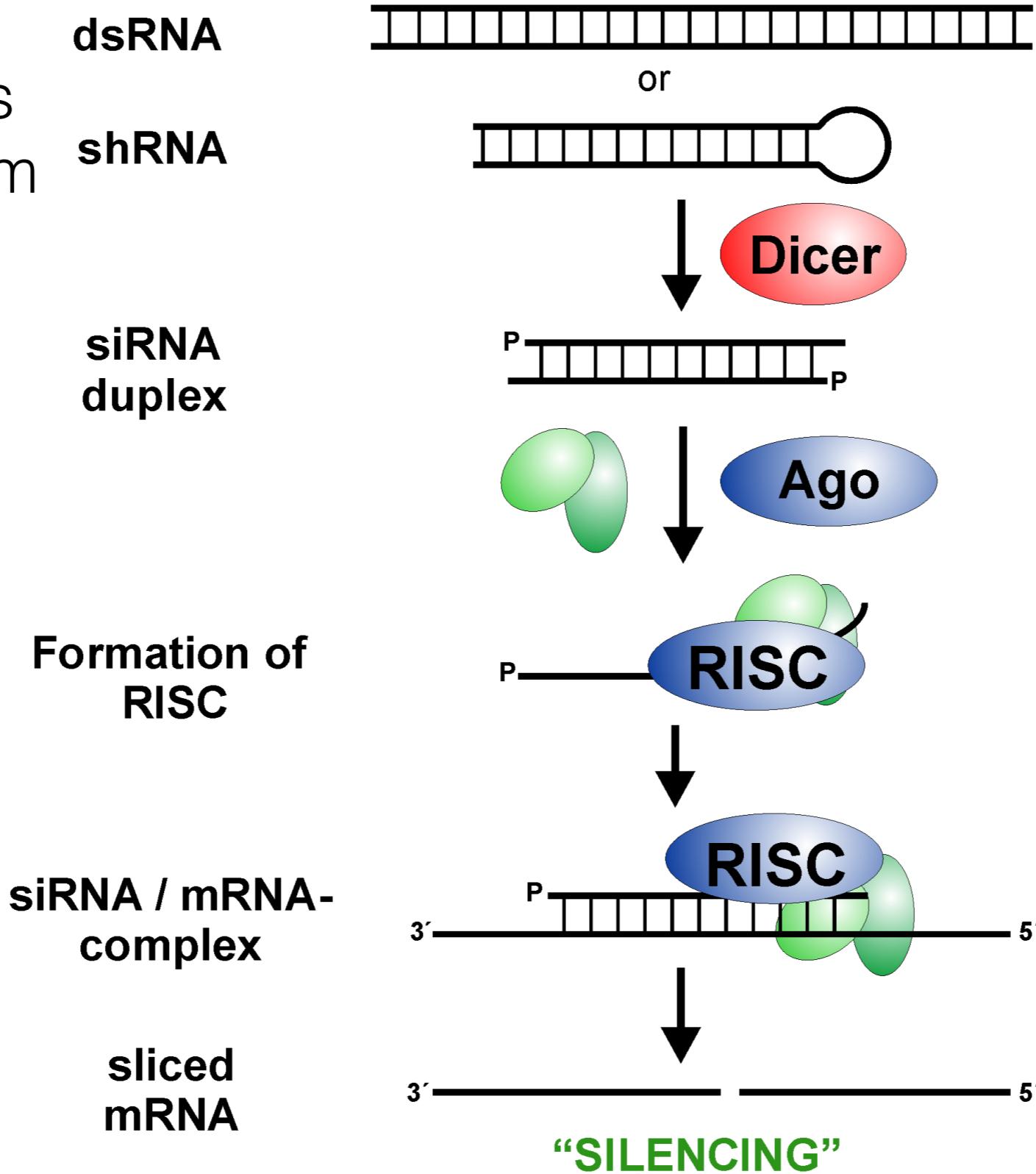
Score them all!!!

Phenotype is everything!

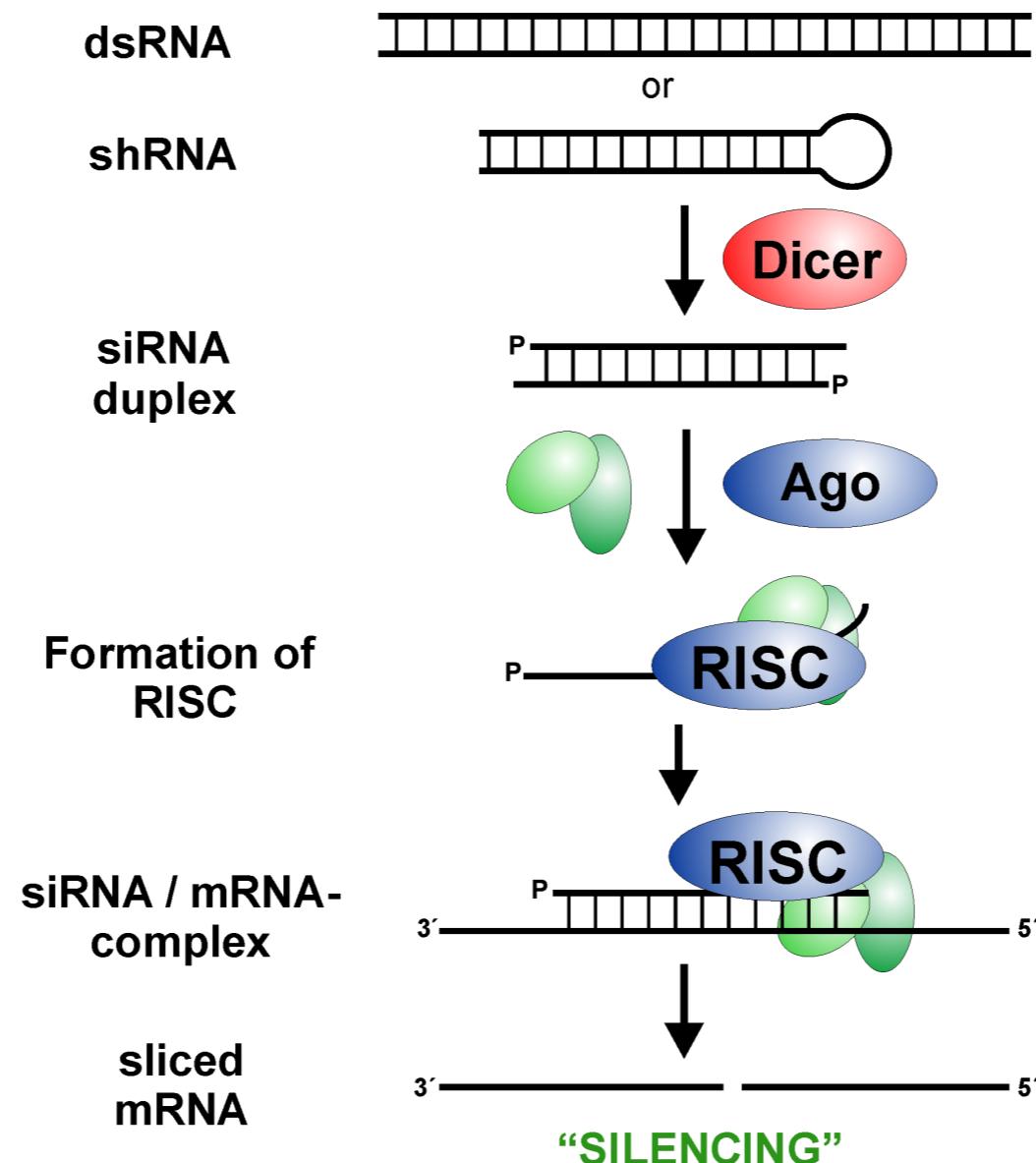
It has to be scalable, quantitative, reproducible,
and accurate

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

You have to
get these RNAs
into the organism



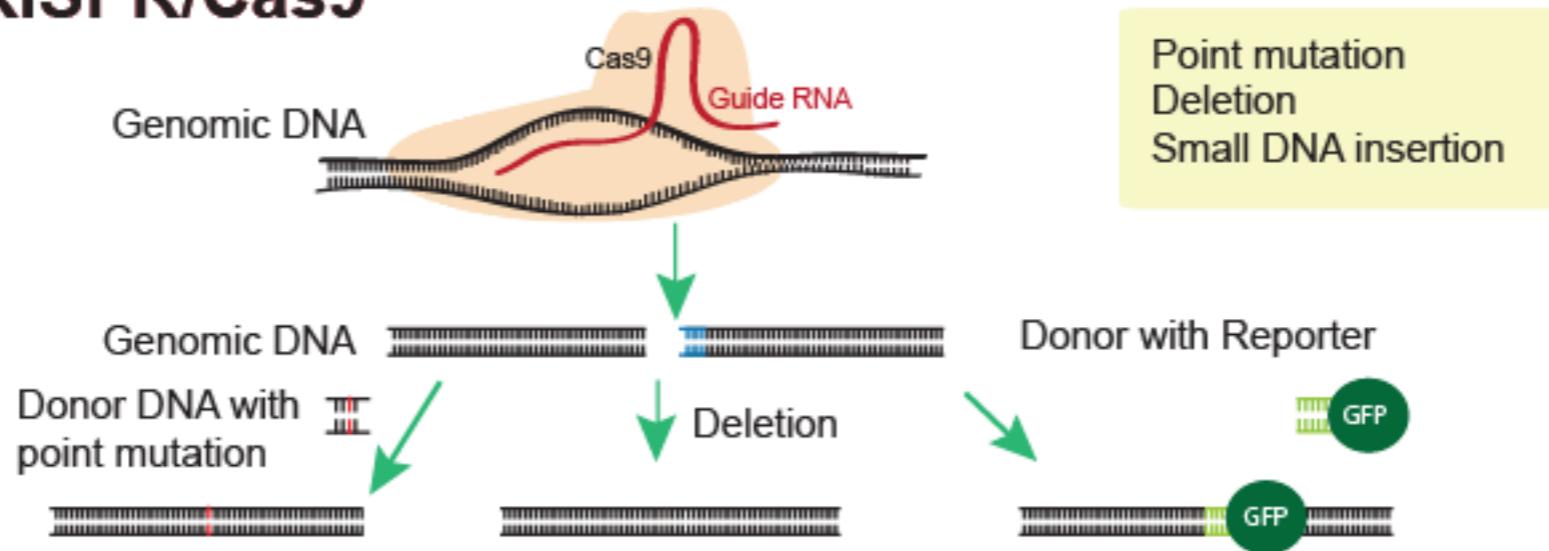
RNA interference (RNAi) can be used to inactivate most genes in a variety of organisms



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)

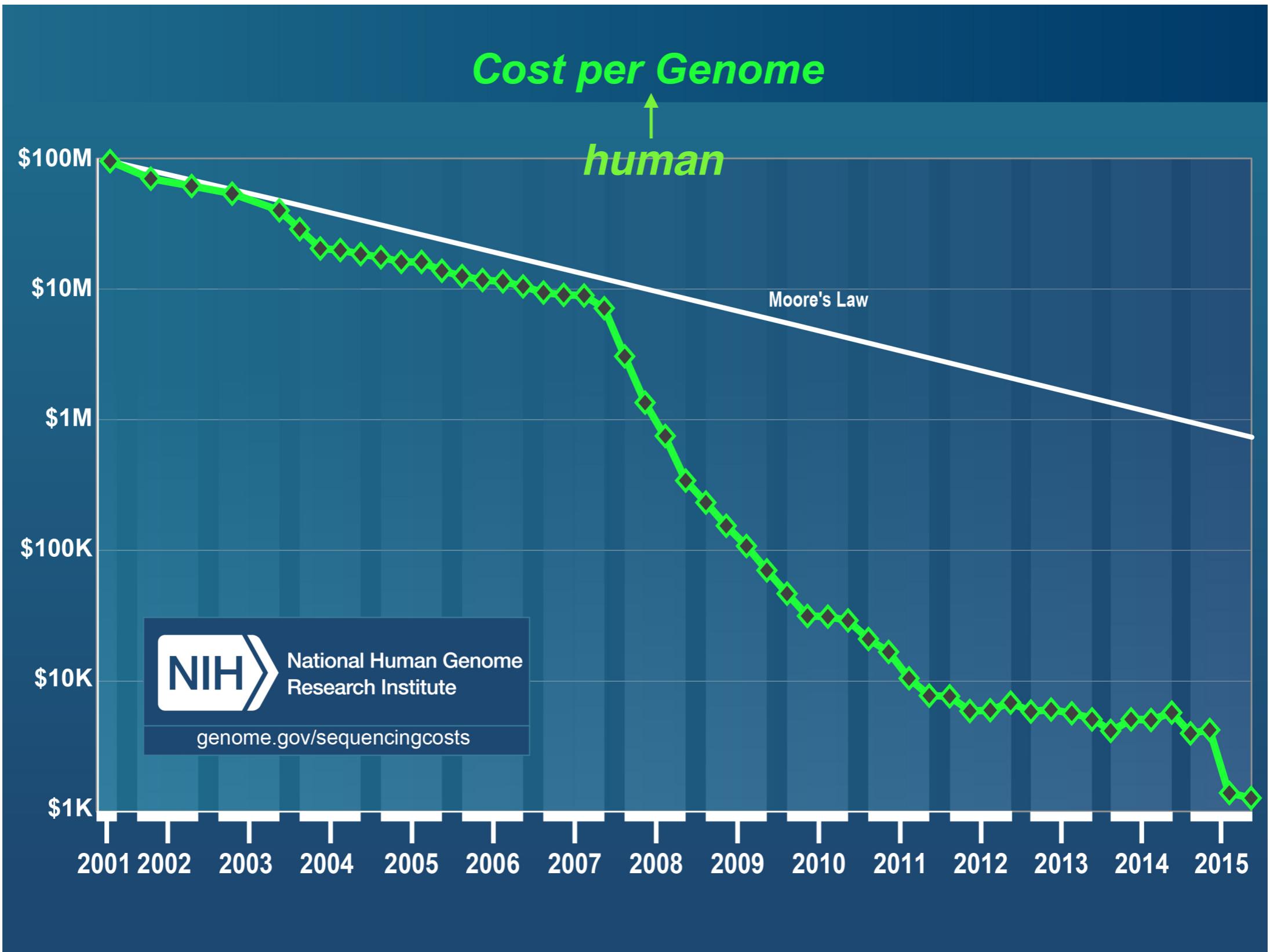
Generate “mutations” by reverse genetic RNAi or CRISPR for “all” genes in an organism

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

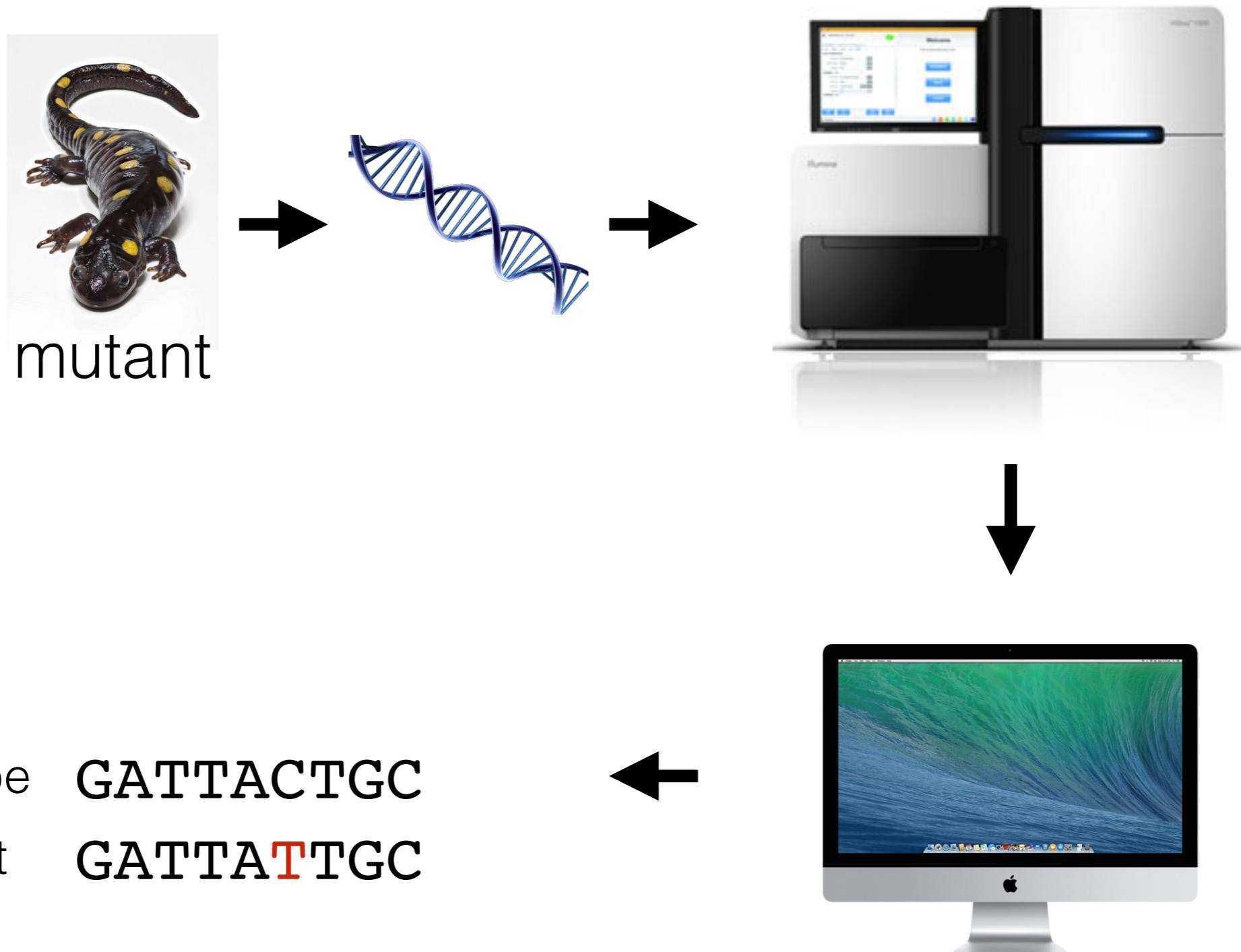
14. Clone the gene

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Clone by sequencing

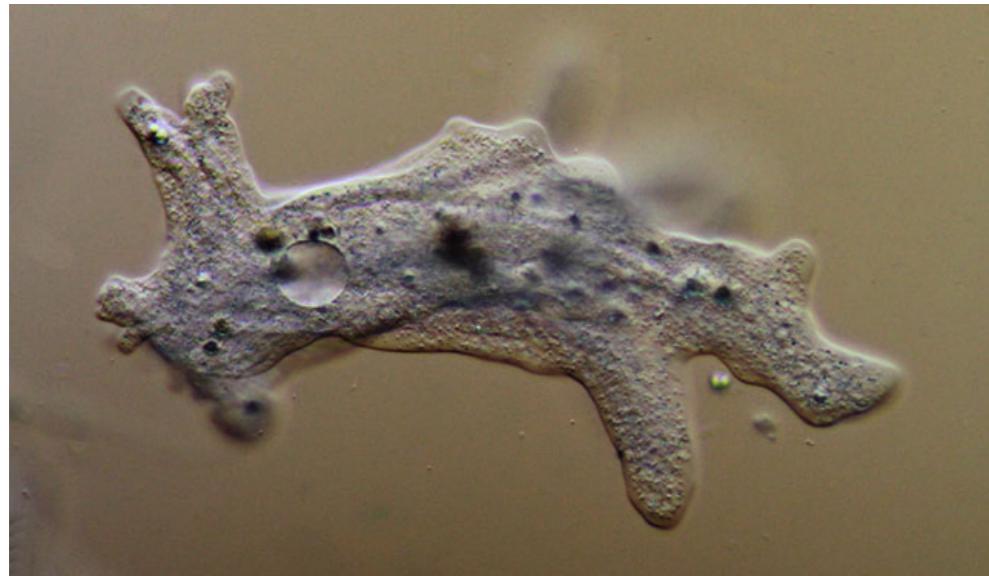


Clone by sequencing



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?

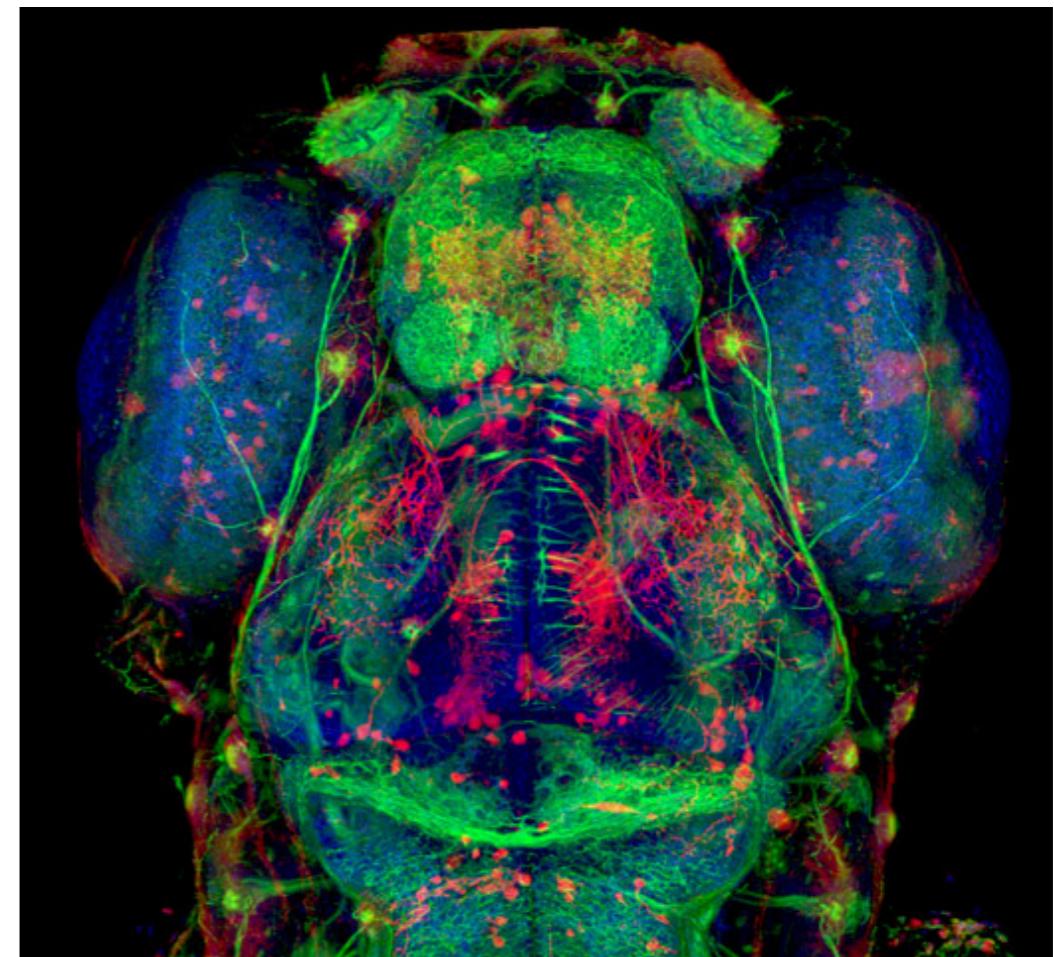
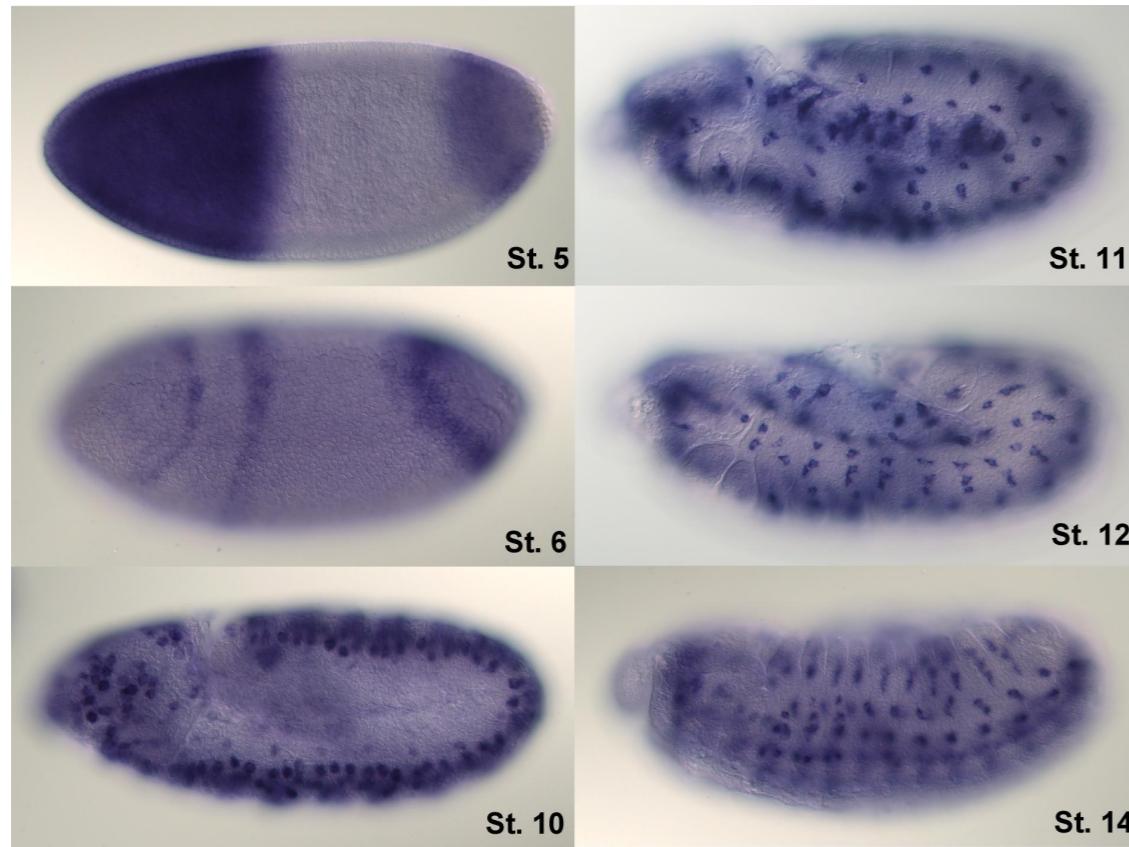
1. RNAi of gene X has same phenotype as our mutant
2. Mutation in gene X with rescue of mutant phenotype in gene X
3. Independent screens for engulfment mutants get multiple alleles of gene X

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

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:

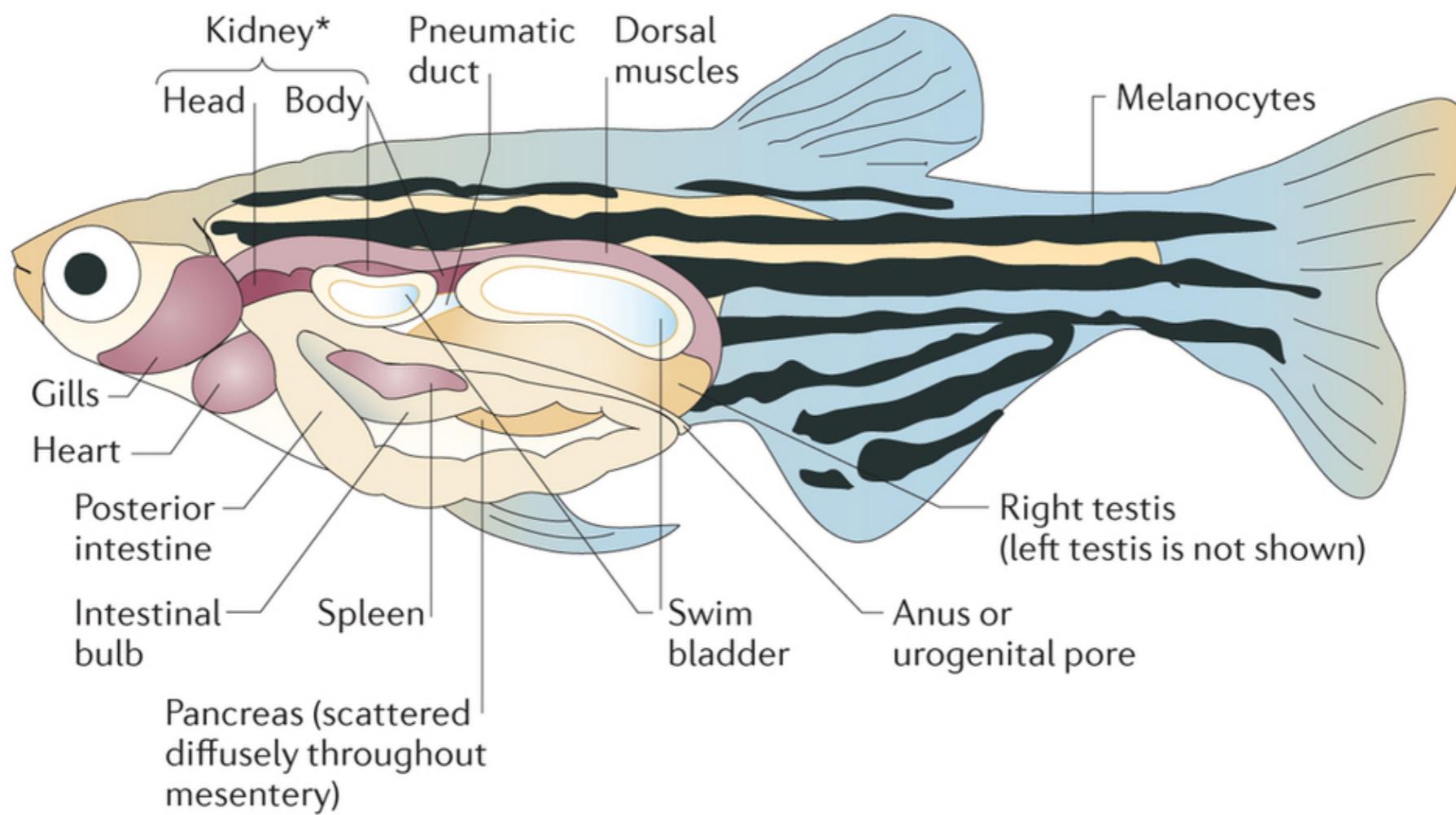


Where a gene is expressed might not be where it acts?

16. Determine site of gene action

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

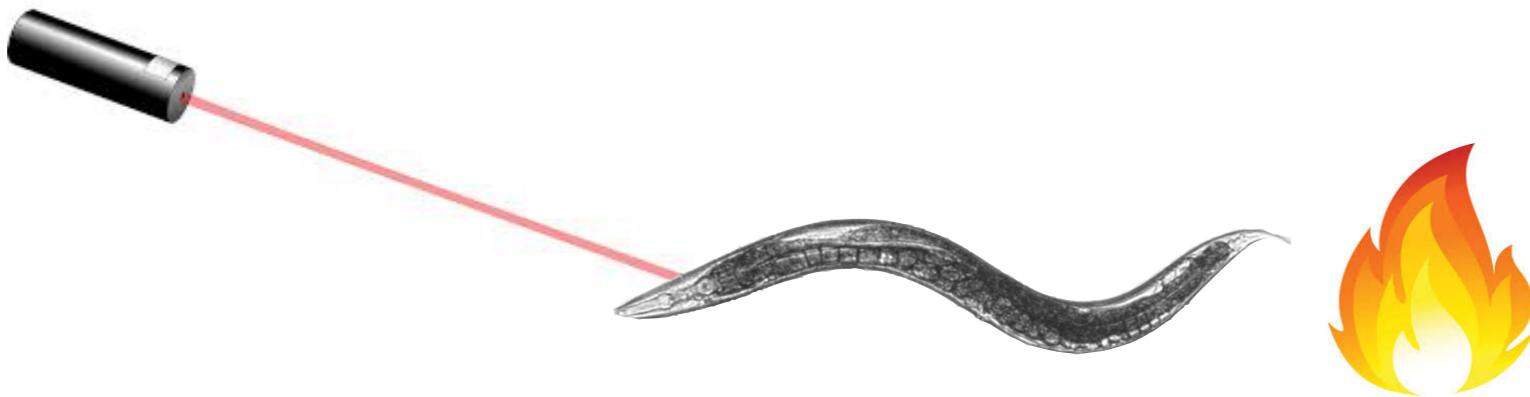
1. Rescue a mutant phenotype in a specific cell, organ, or tissue
2. Mosaic analysis (cell autonomy experiments)



17. Determine time of gene action

When does the gene function?

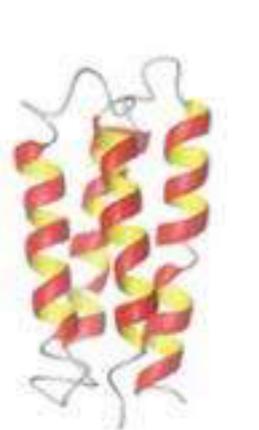
1. Induce expression to rescue a mutant phenotype at a specific time



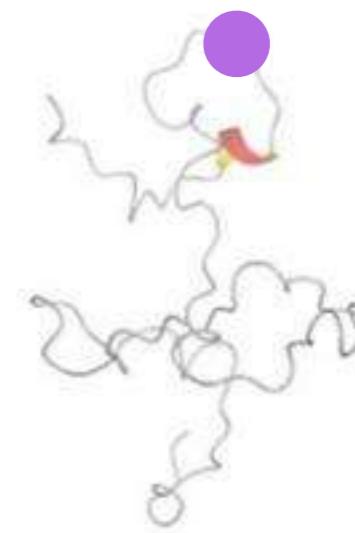
17. Determine time of gene action

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

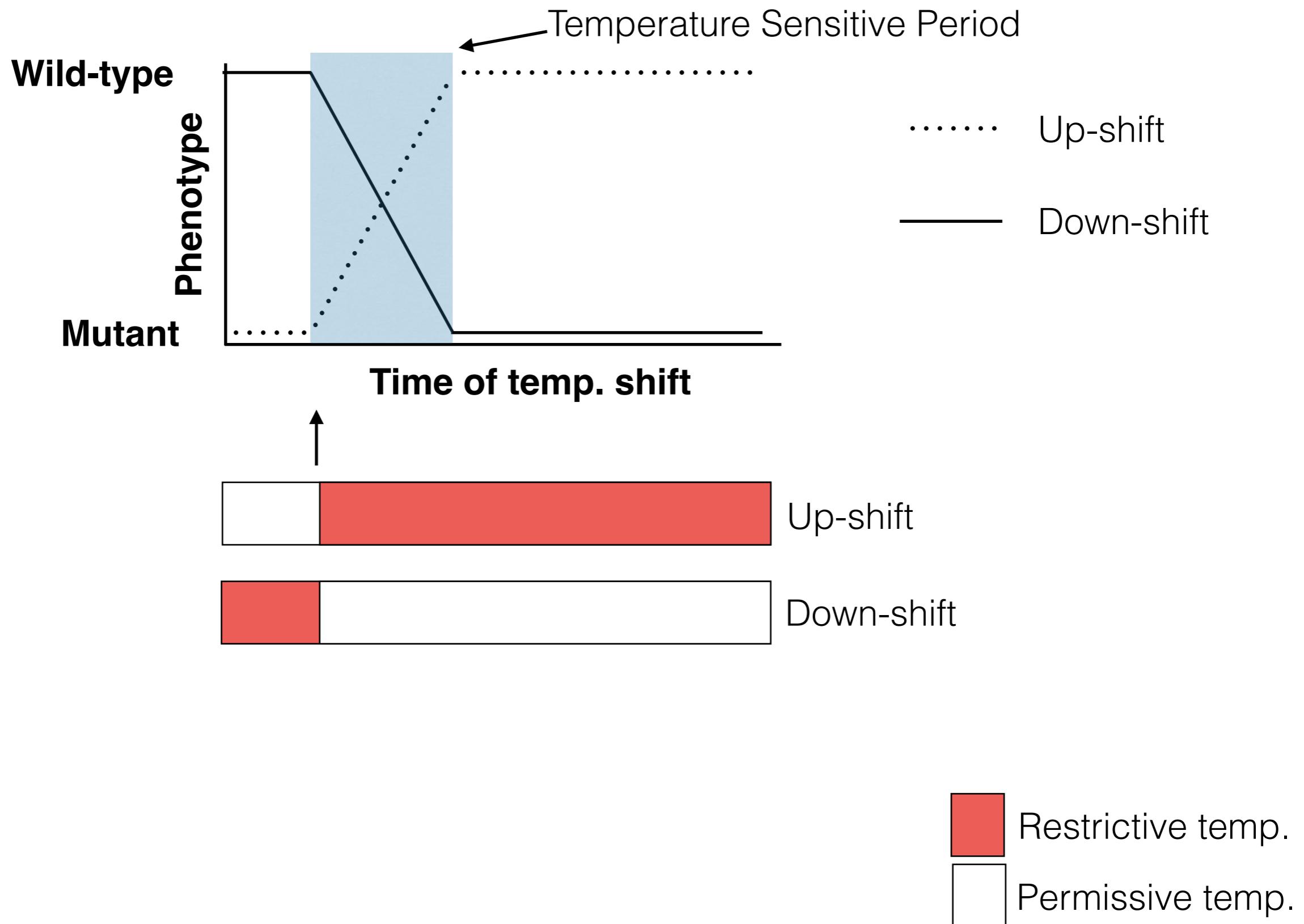


Permissive temperature

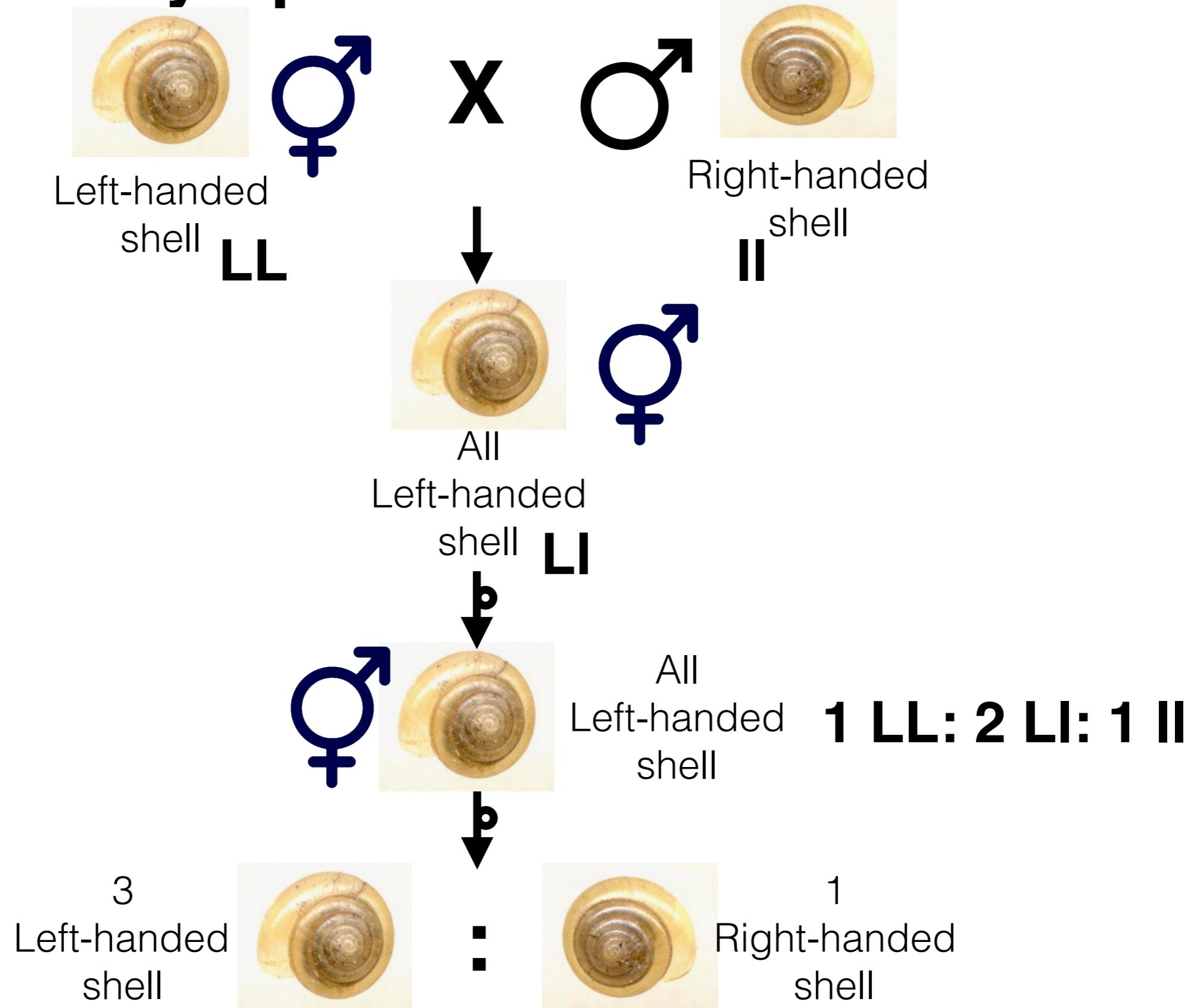


Restrictive temperature

17. Determine time of gene action

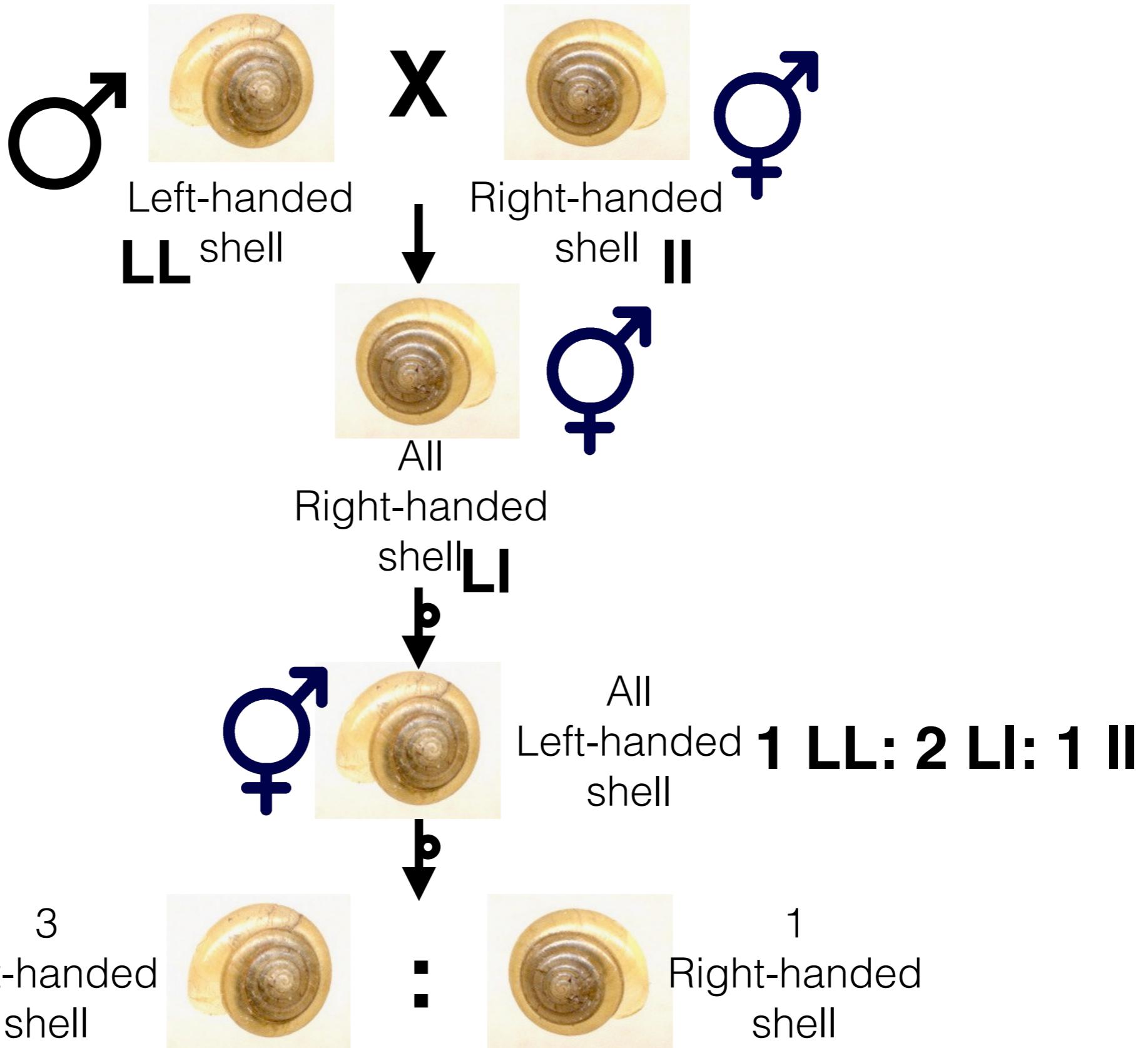


18. Determine if there are maternal effects or cytoplasmic inheritance



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.

18. Determine if there are maternal effects or cytoplasmic inheritance



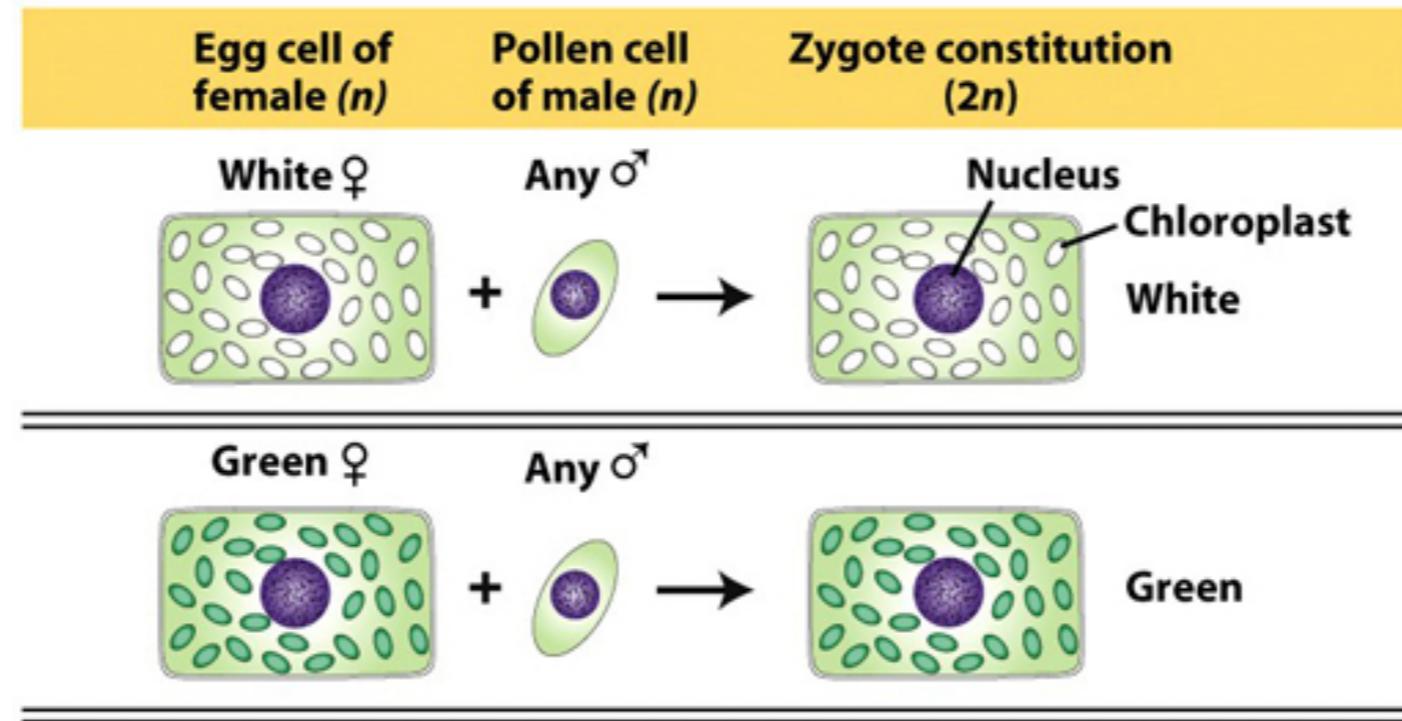
Plastid inheritance in
Mirabilis jalapa

Only the color
of the stem (mother)
matters

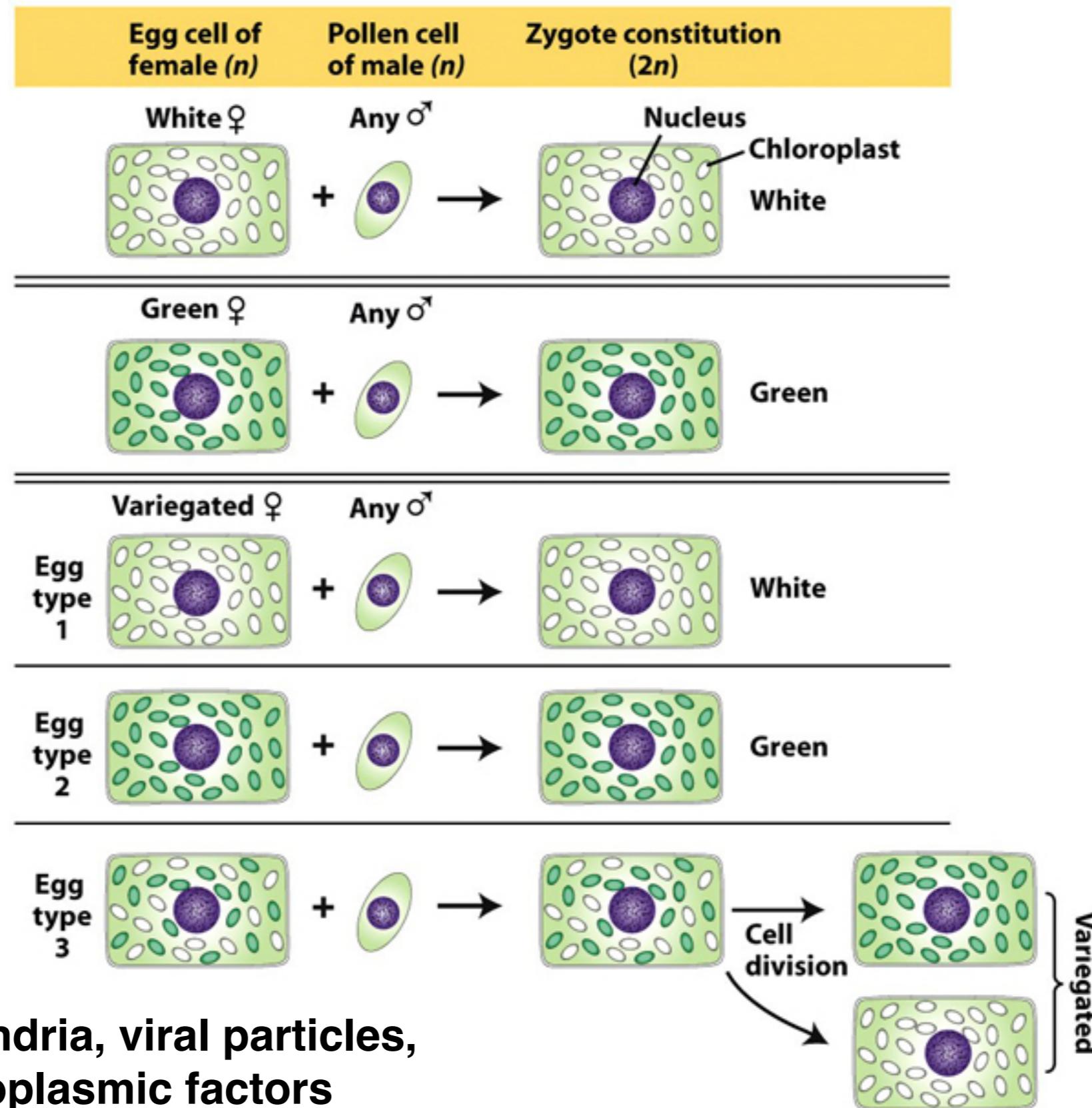
White, green, or
variegated



18. Determine if there are maternal effects or cytoplasmic inheritance



18. Determine if there are maternal effects or cytoplasmic inheritance



Plastids, mitochondria, viral particles, and other cytoplasmic factors

19. Determine the overexpression phenotype

What happens when the wild-type individual has too much of gene X?



Overexpression *might* be useful for investigating genetic interactions

20. Perform an overexpression screen for additional modifiers

1. Screen for dominant phenotypes similar to your mutant phenotype
2. Inducible overexpression of specific genes
3. Transposon-mediated overexpression screens

Find more genes by making hypermorphs

21. Isolate enhancers and suppressors of your mutant phenotype

22. Investigate pathways (measure genetic interactions or epistasis)

Step-wise genetic analysis

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