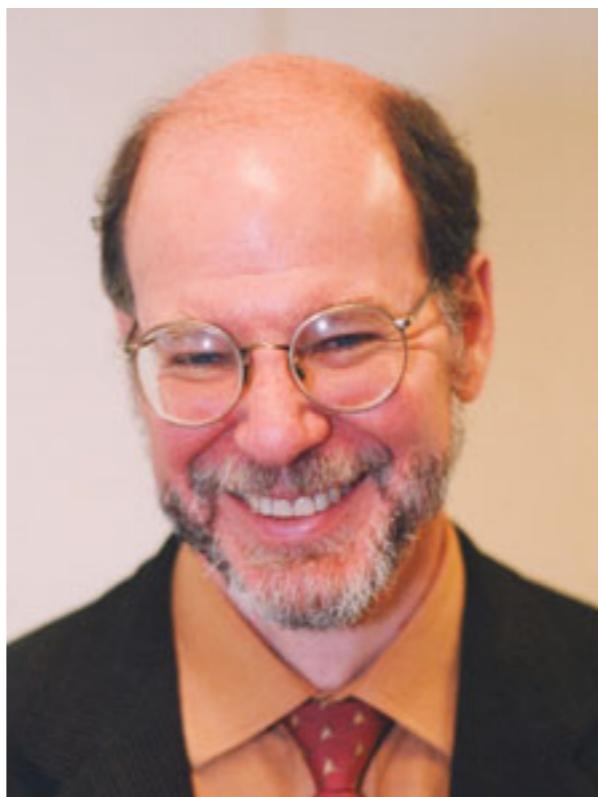


Bio393: Genetic Analysis

Step-wise genetic analysis



Bob Horvitz

Step-wise genetic analysis

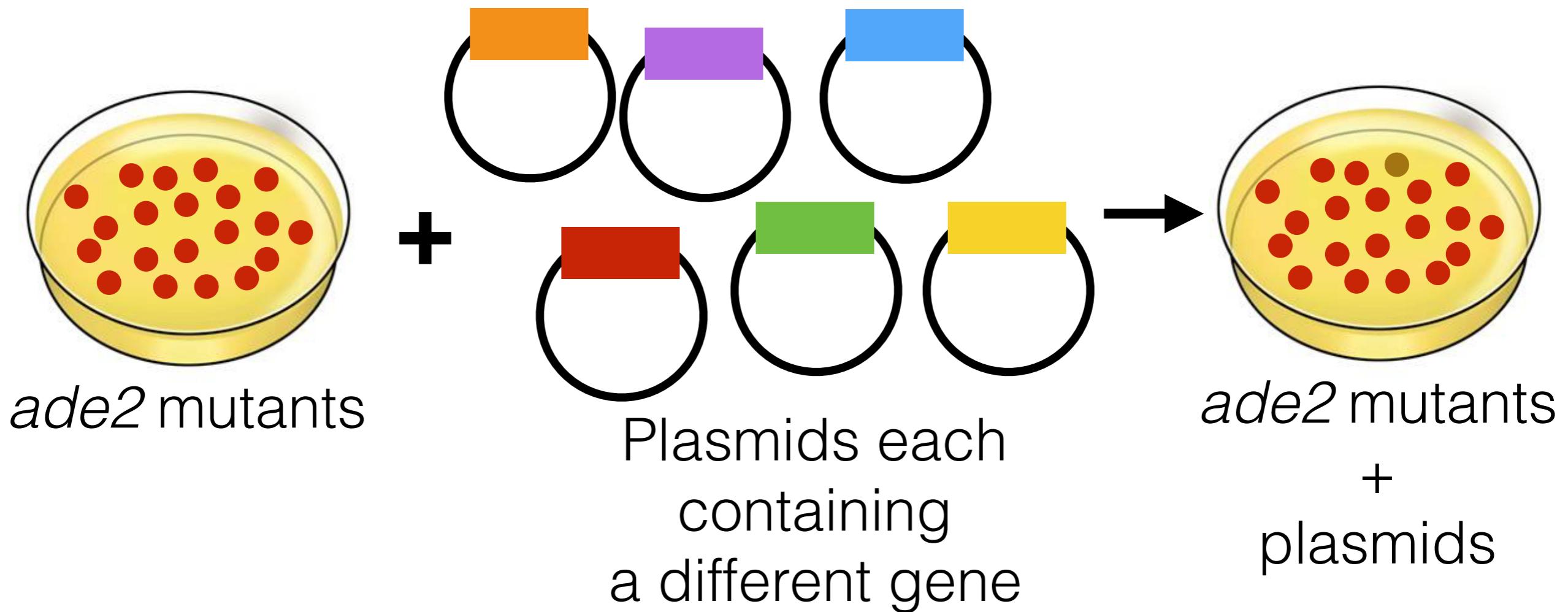
- 1. Define the problem**
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- 8. Single-gene phenotype?**
- 9. Mapping and complementation**
- 10. Characterize the phenotype**
- 11. Define the nature of the mutant allele(s): gene dosage**
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- 13. 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

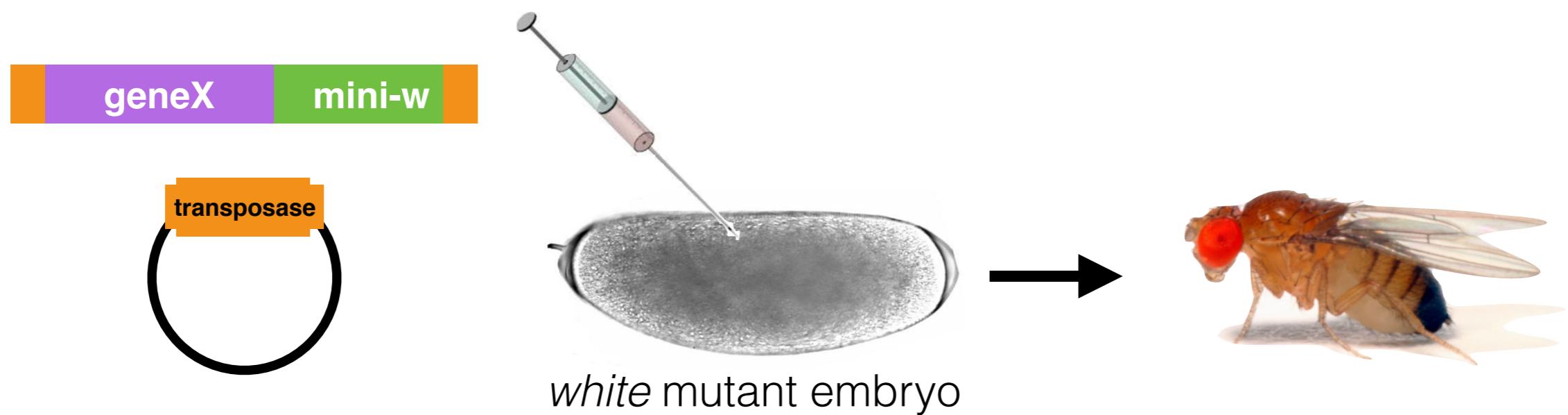


Caveat: overexpression bypass suppressors

Cloning by complementation in worms and flies

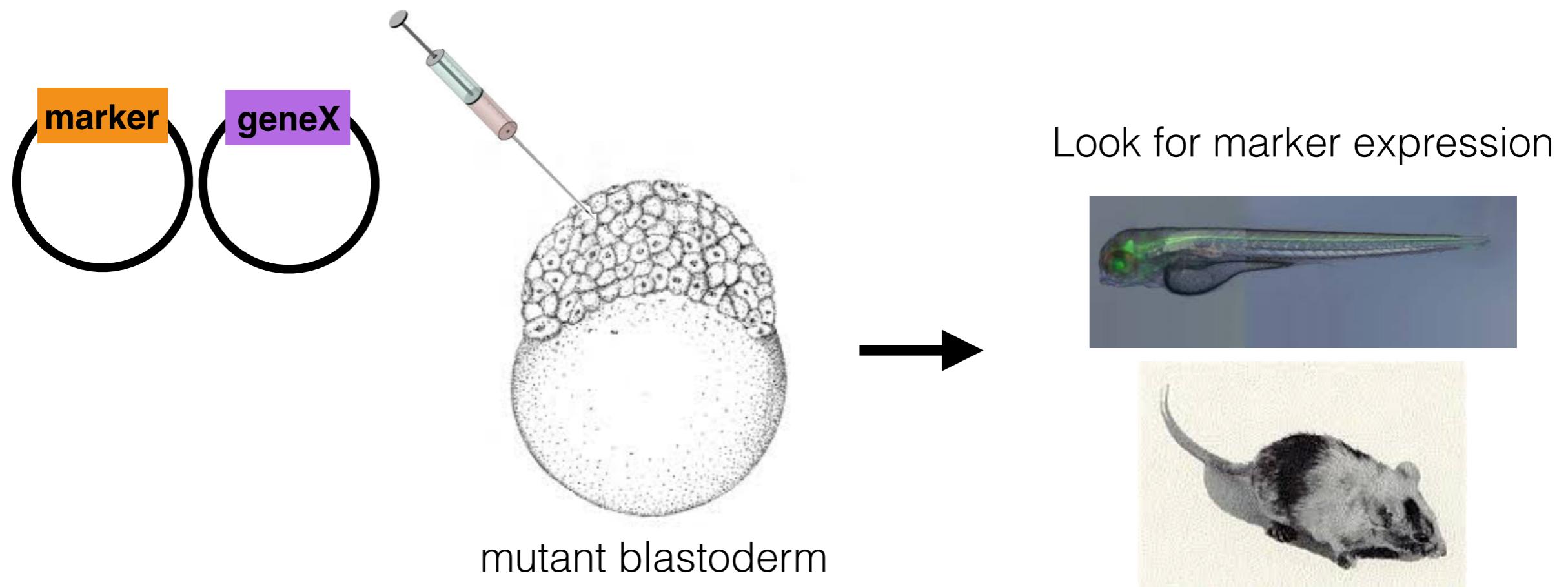


Caveat: overexpression bypass suppressors and not stable



Caveat: overexpression bypass suppressor and variable expression

Cloning by complementation in fish and mice

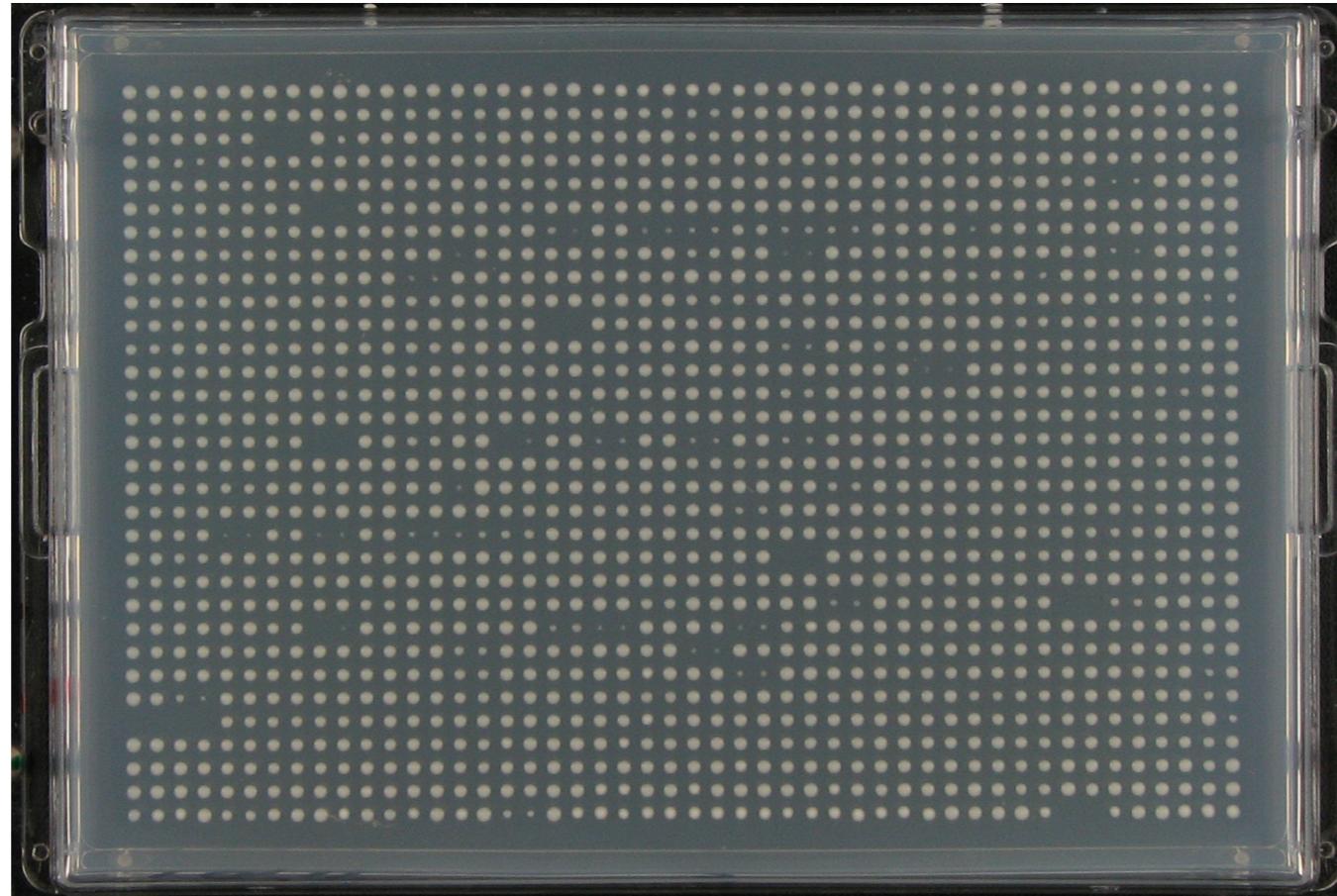


Caveat: overexpression bypass suppressors
and variable expression

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**



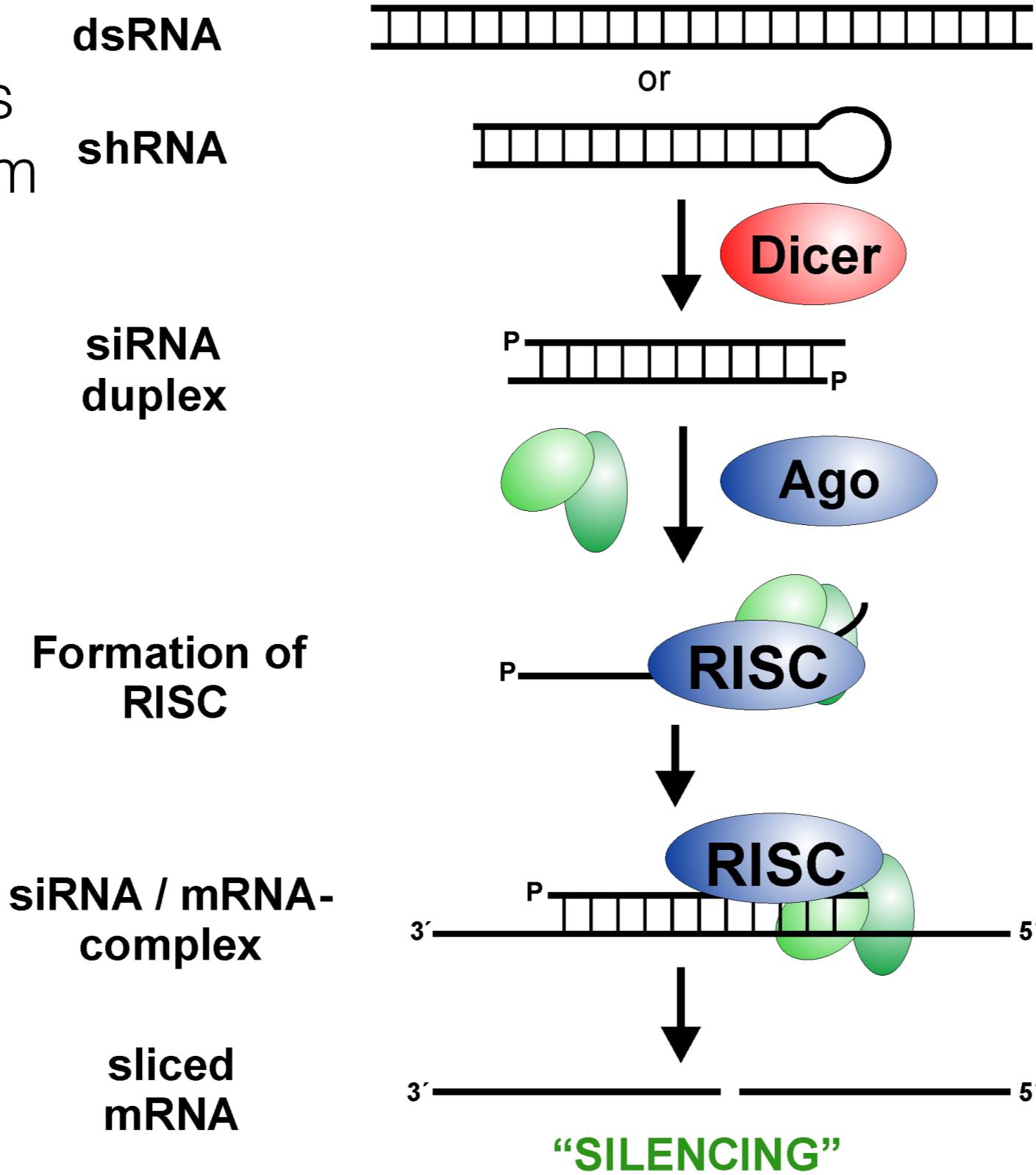
Score them all!!!

Phenotype is everything!

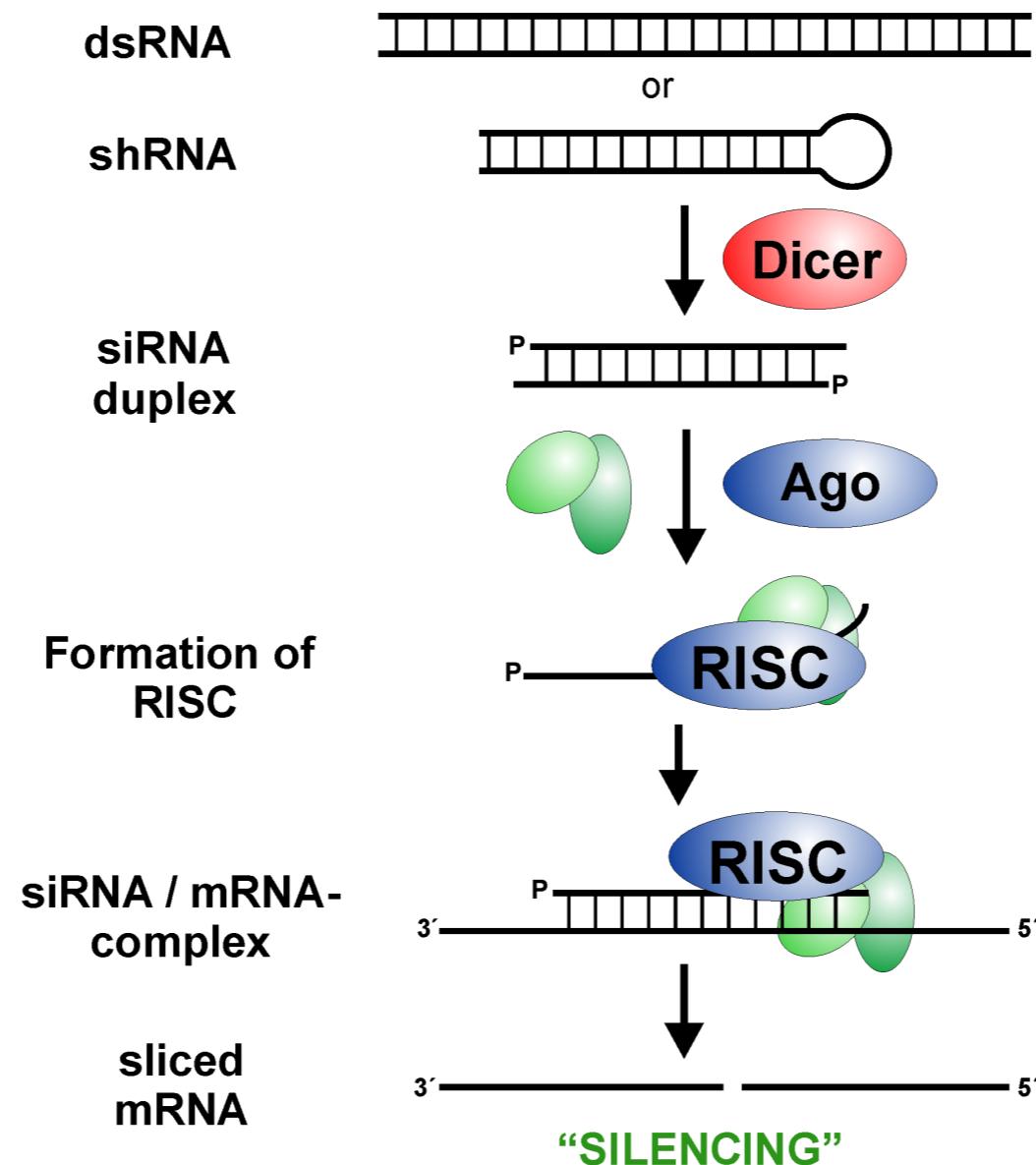
It has to be scalable, quantitative, reproducible,
and accurate

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

You have to
get these RNAs
into the organism



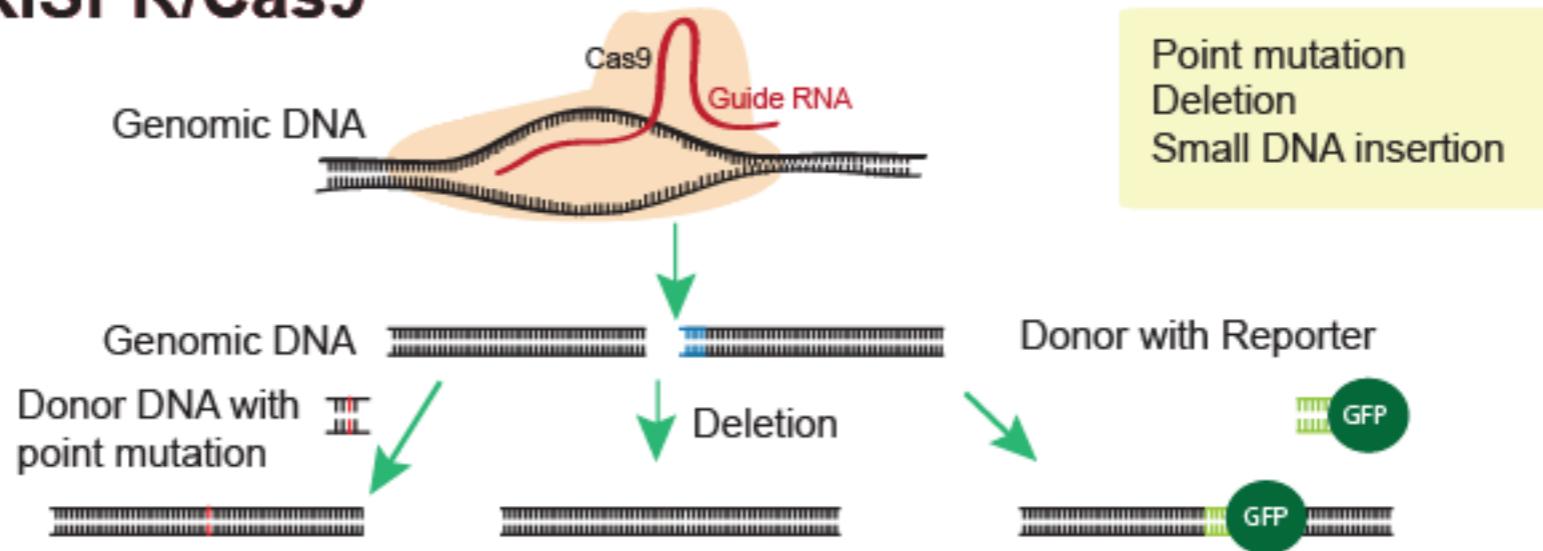
RNA interference (RNAi) can be used to inactivate most genes in a variety of organisms



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)

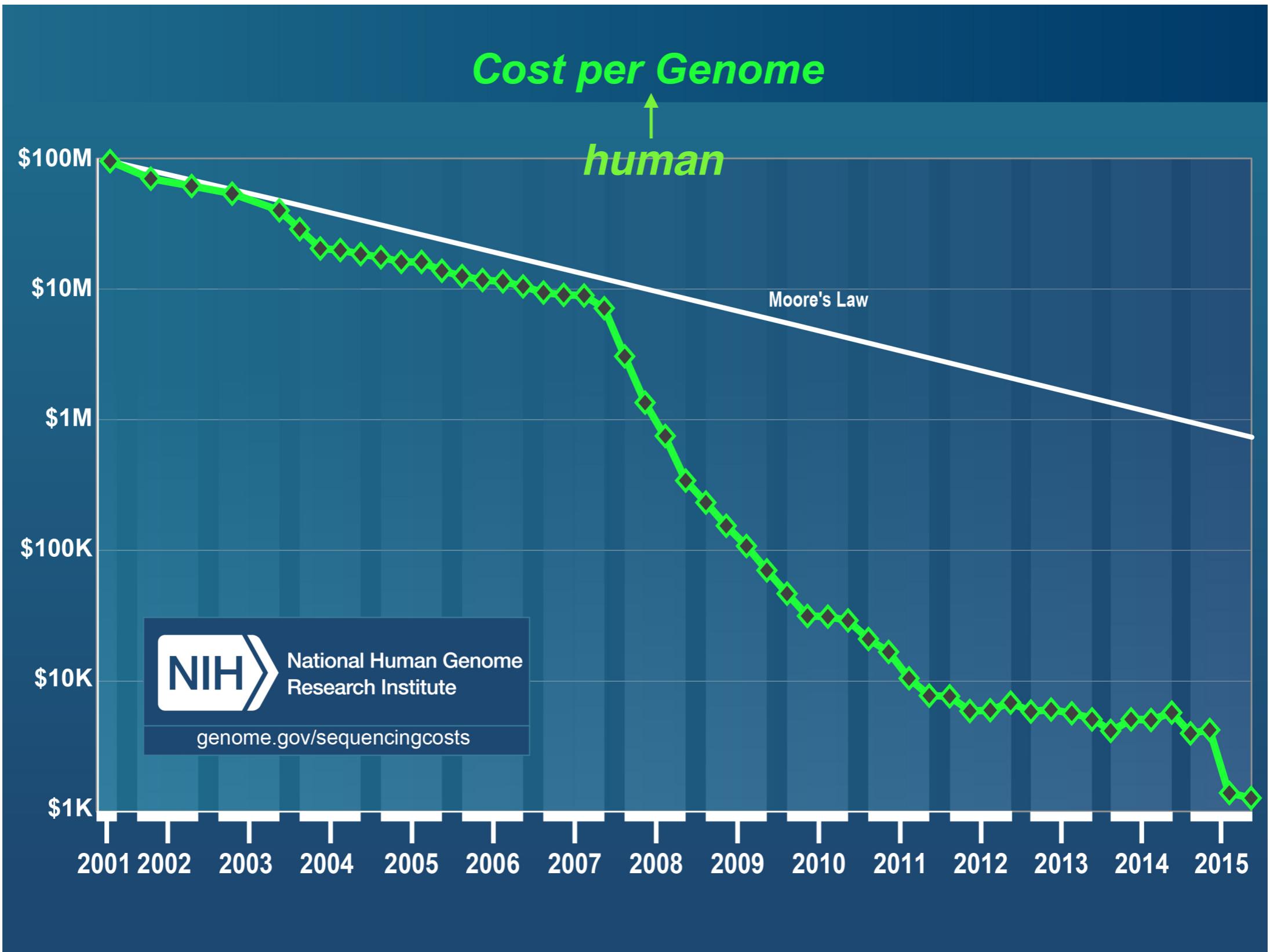
Generate “mutations” by reverse genetic RNAi or CRISPR for “all” genes in an organism

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

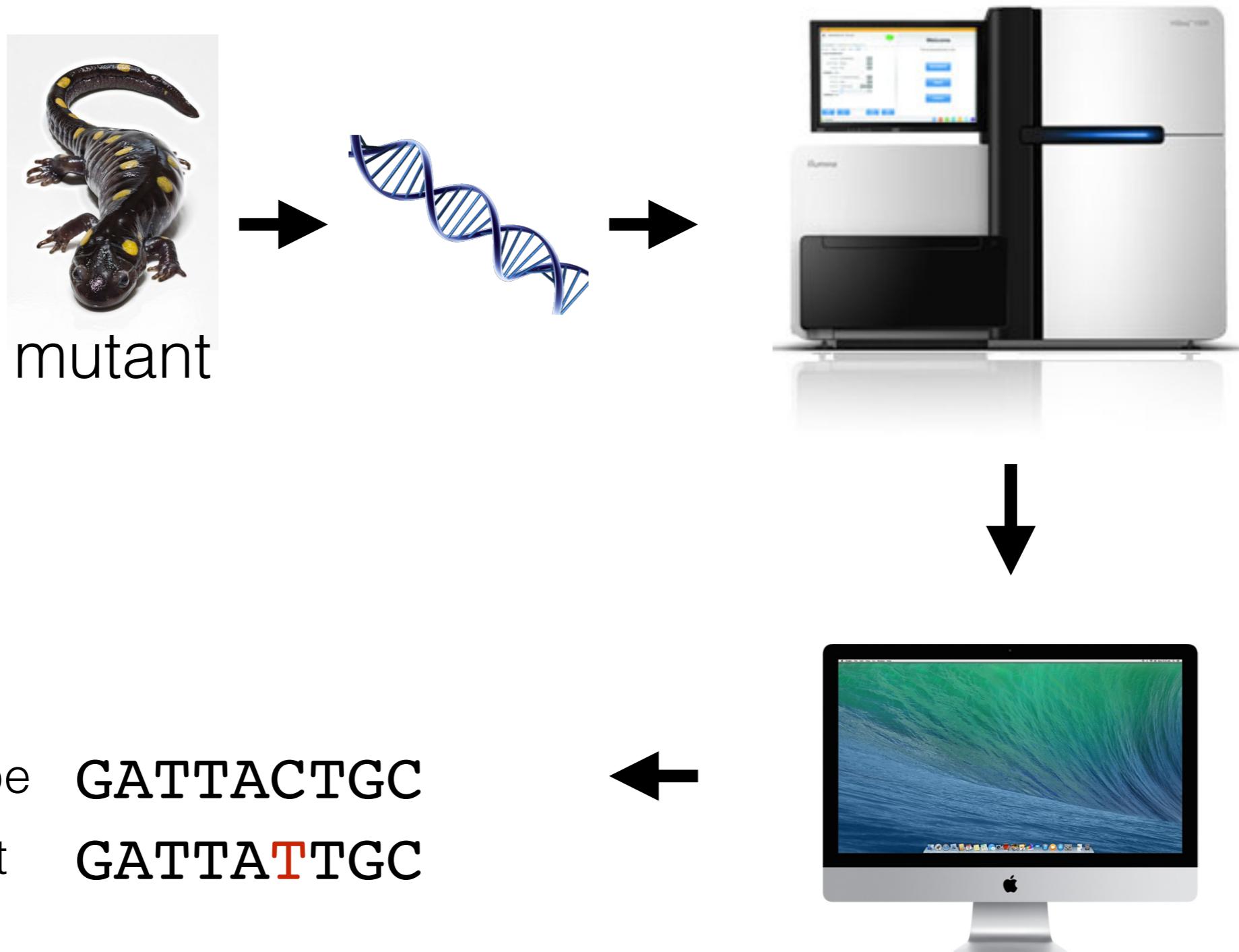
14. Clone the gene

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Clone by sequencing

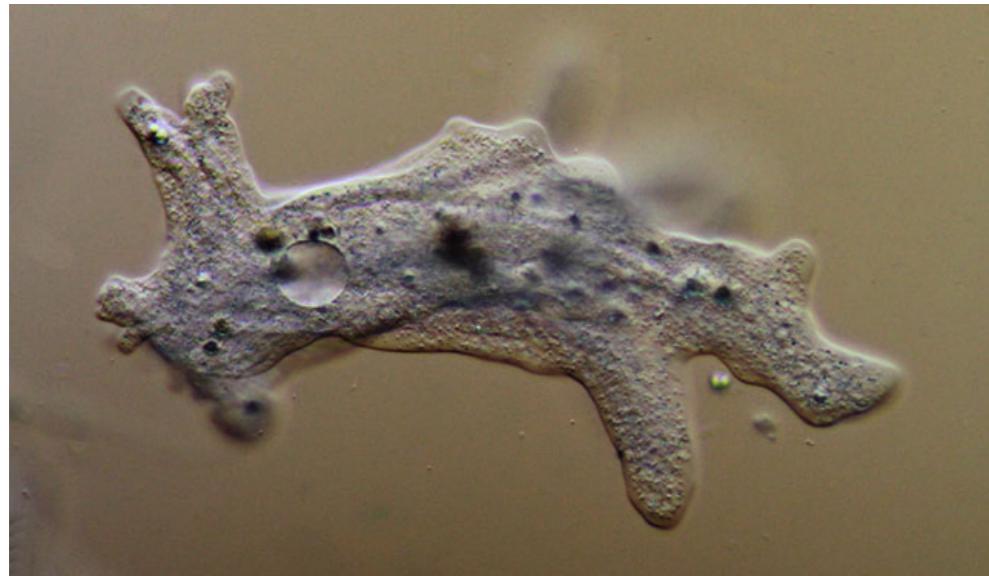


Clone by sequencing



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?

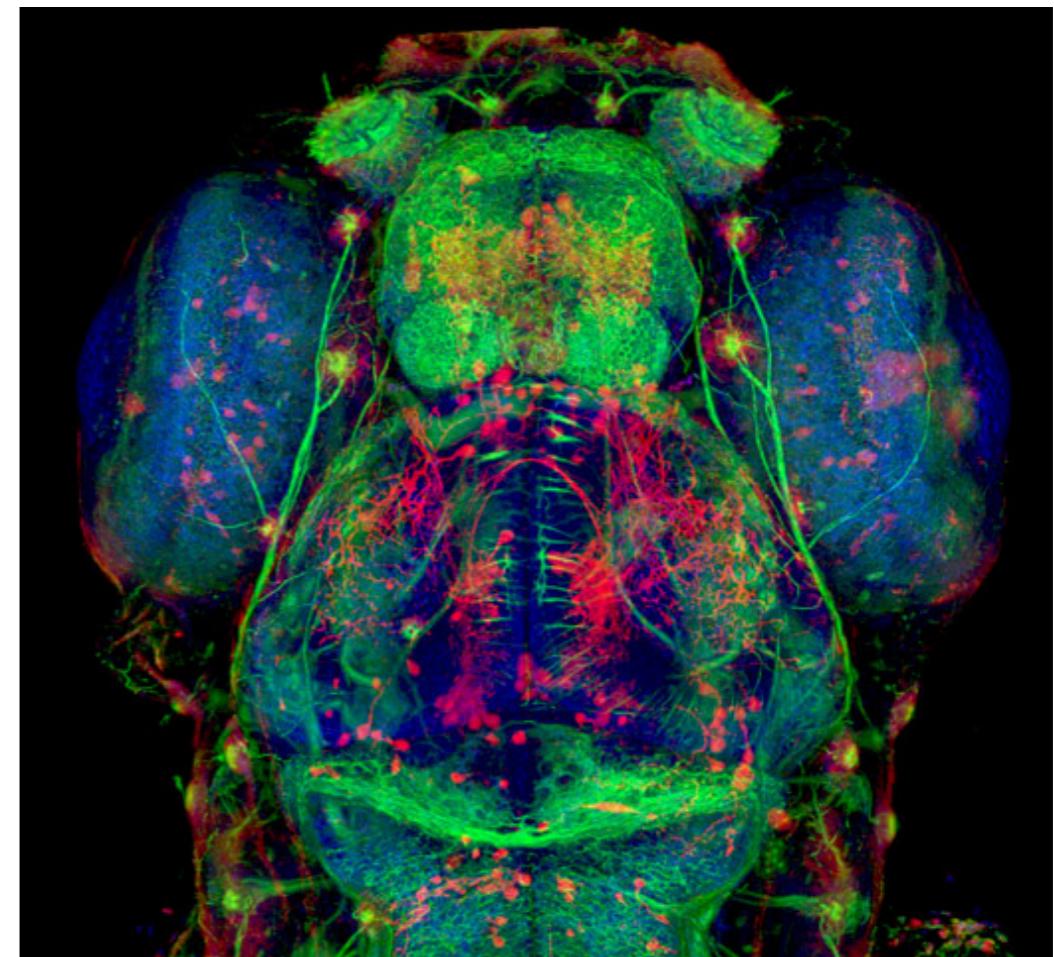
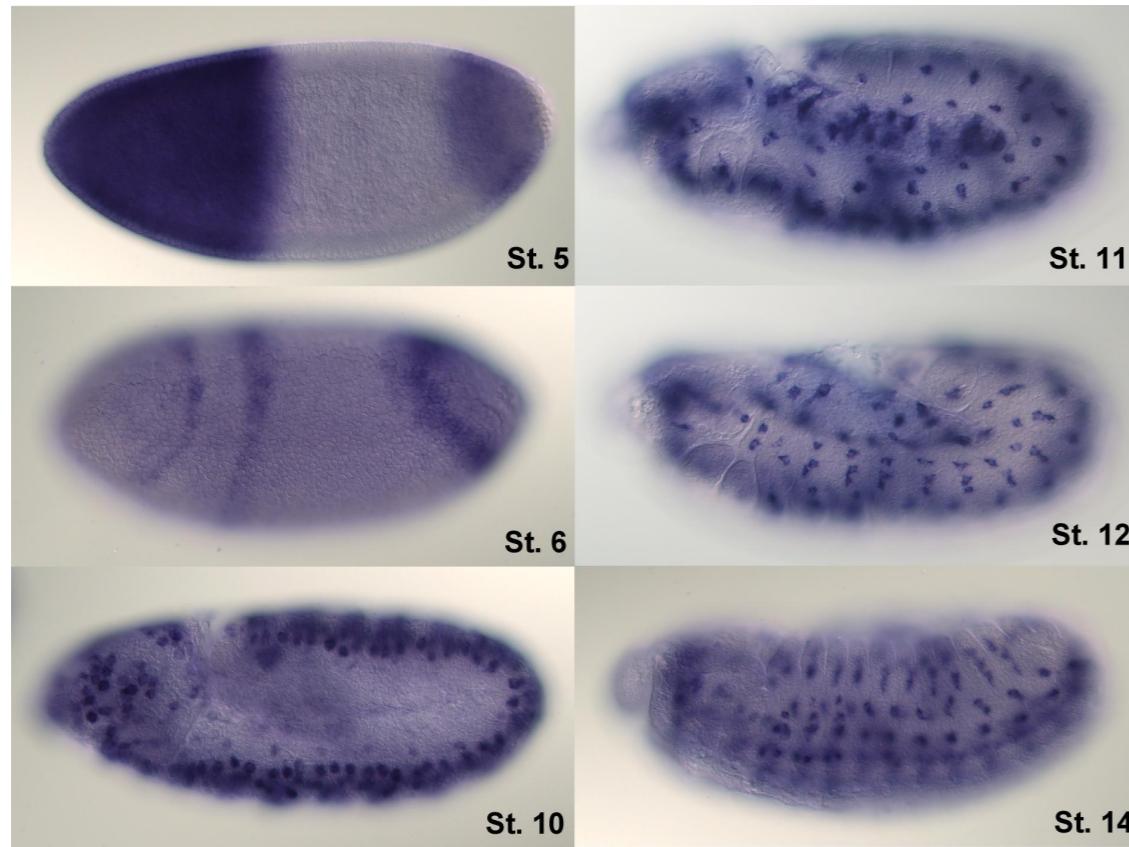
1. RNAi of gene X has same phenotype as our mutant
2. Mutation in gene X with rescue of mutant phenotype in gene X
3. Independent screens for engulfment mutants get multiple alleles of gene X

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:

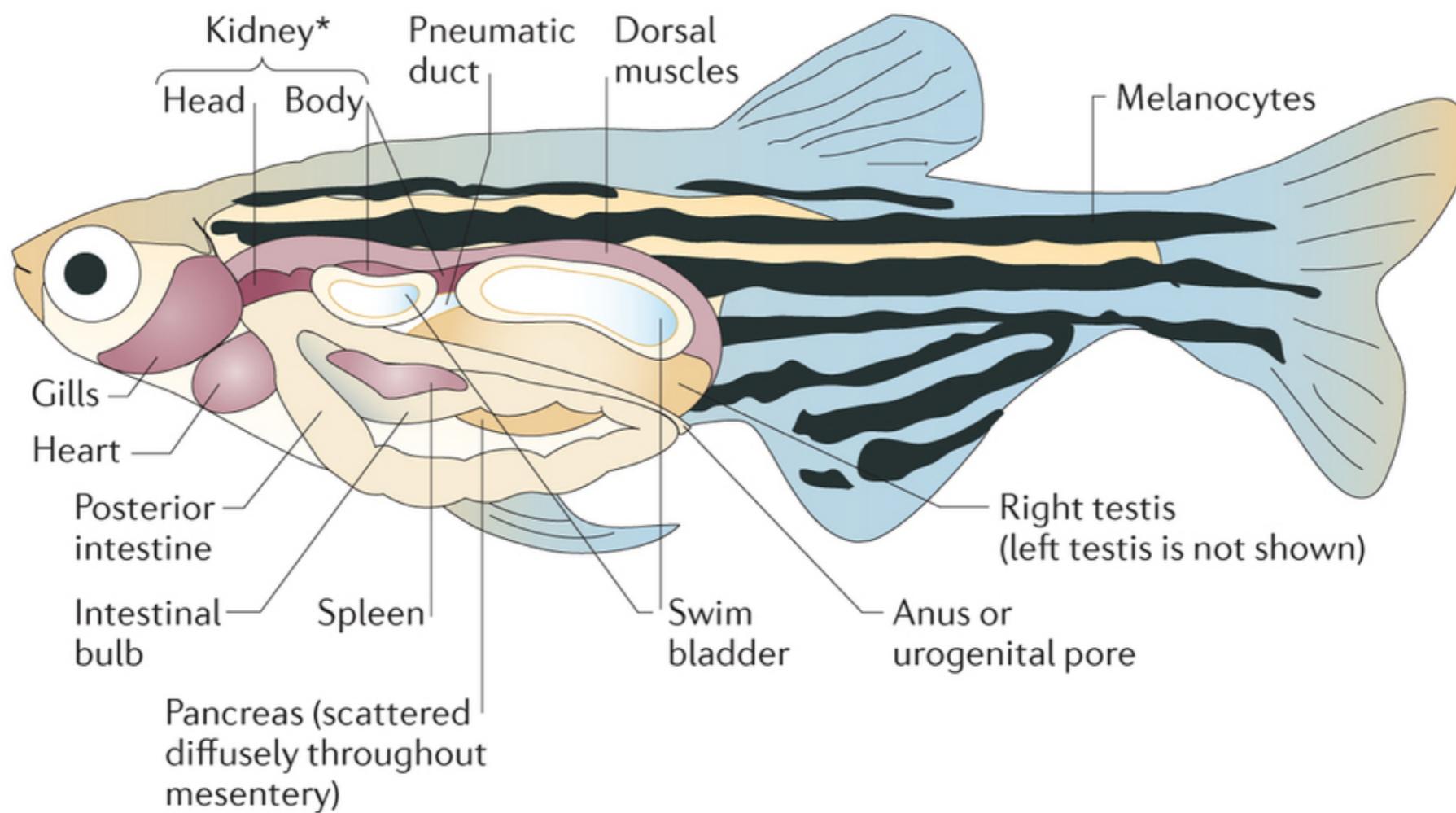


Where a gene is expressed might not be where it acts?

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)



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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