

Bio393: Genetic Analysis

Complementation



Lexus CT 200h





Lexus CT 200h mutant 1



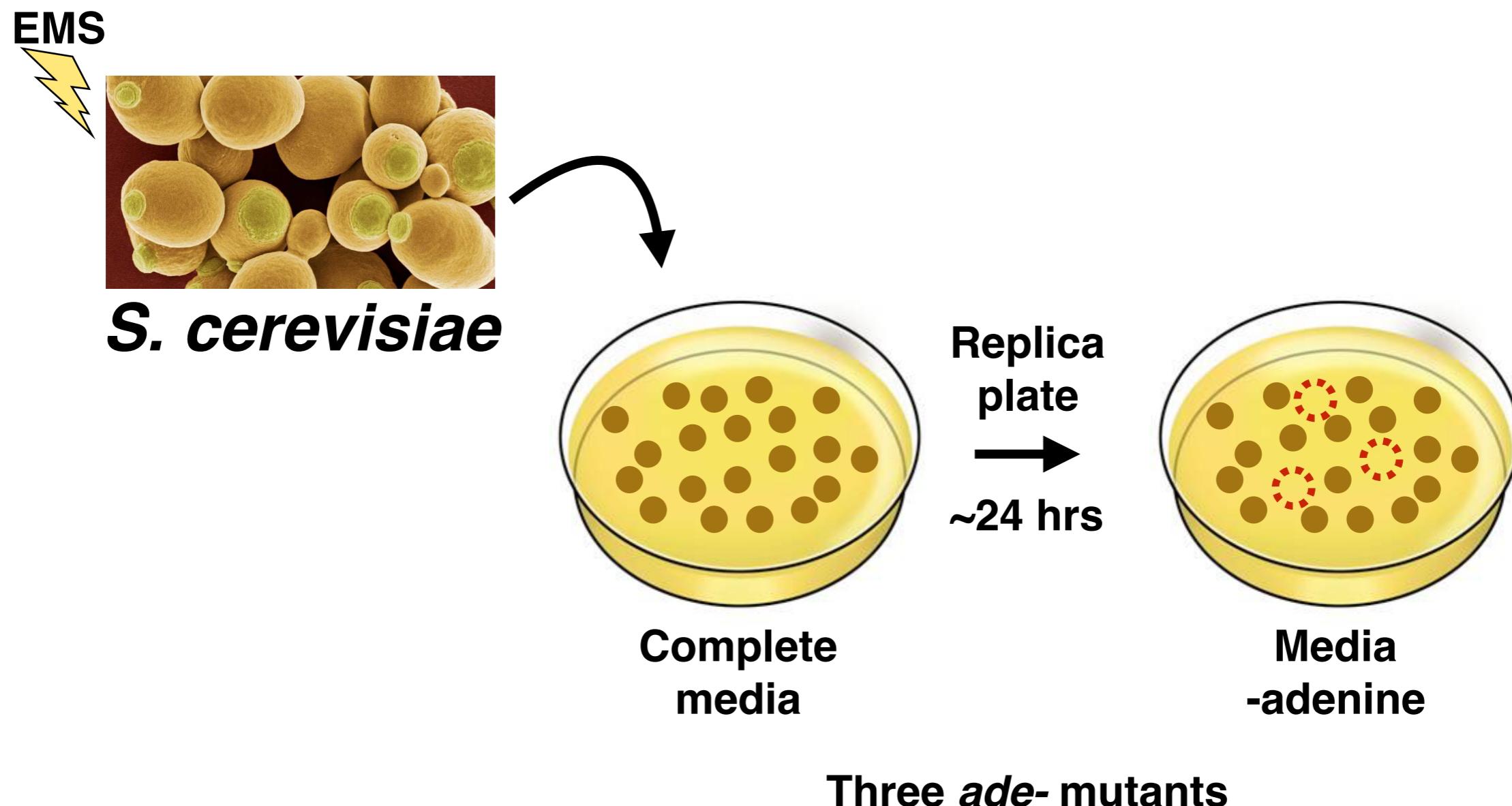
Lexus CT 200h mutant 1



Lexus CT 200h mutant 2

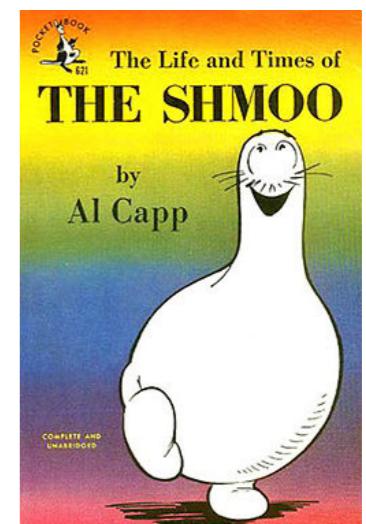
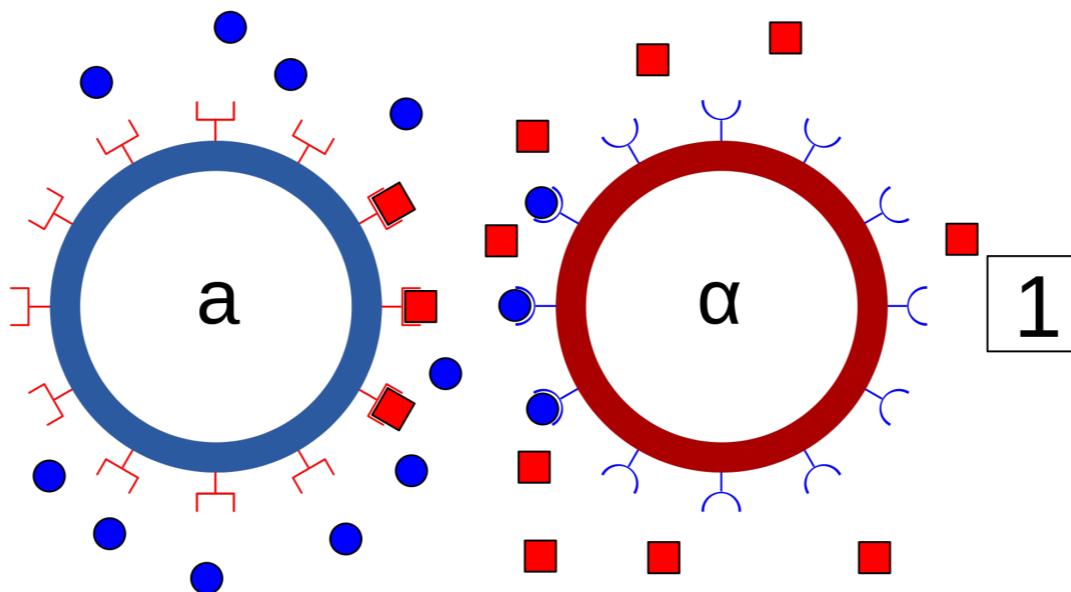
Screen for mutants that require adenine to grow

Screen:

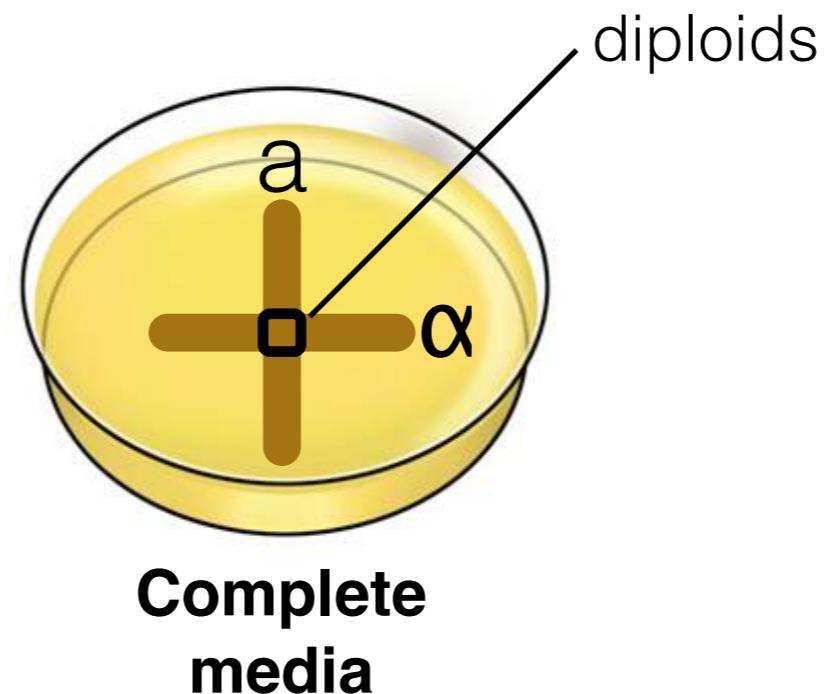


Are all three mutants deficient in the same function
(i.e. mutants in the same gene)?

Yeast mating



Yeast mating tests

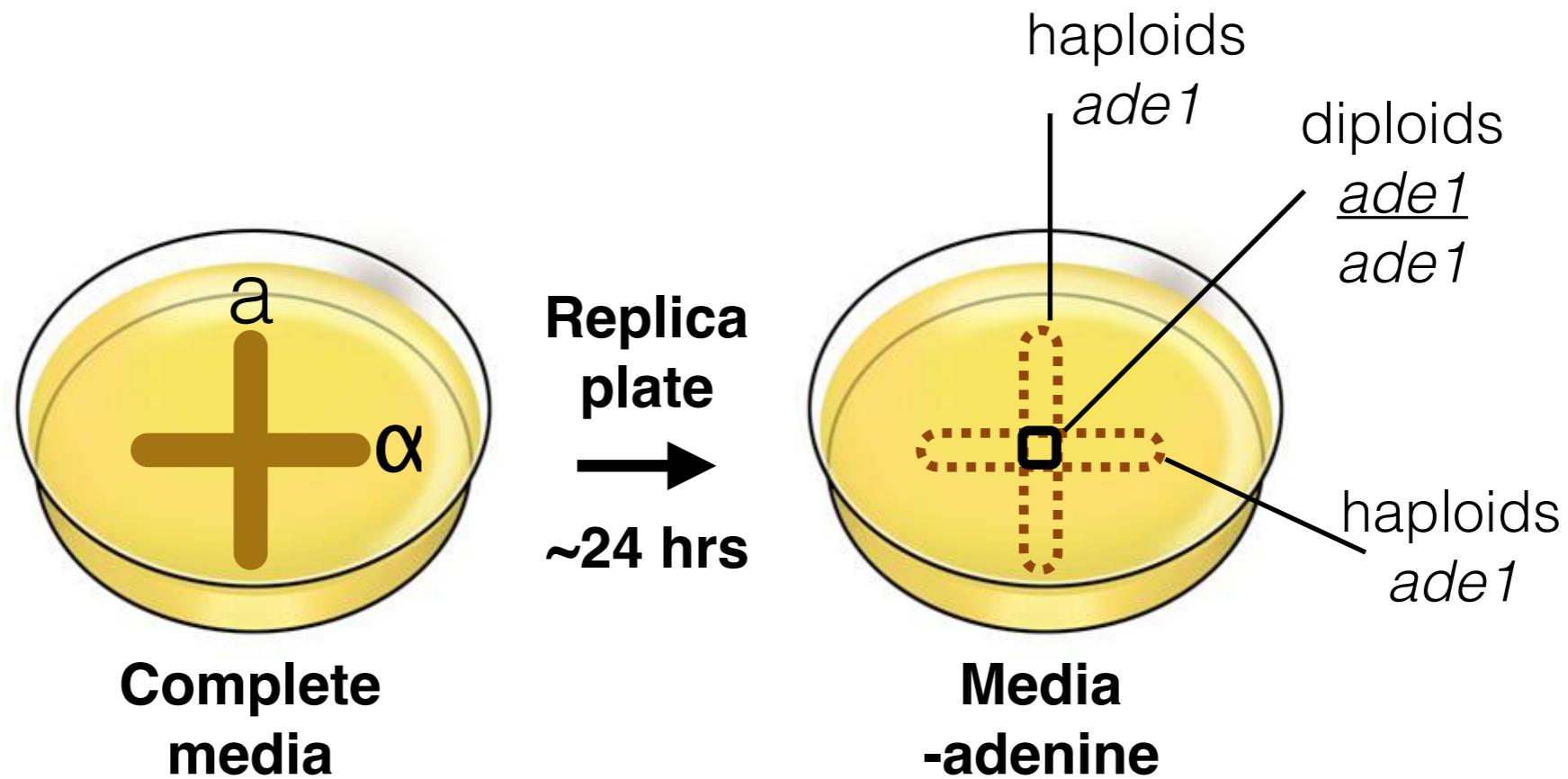


ade1 mutant

a cells are *ade1* mutant

α cells are *ade1* mutant

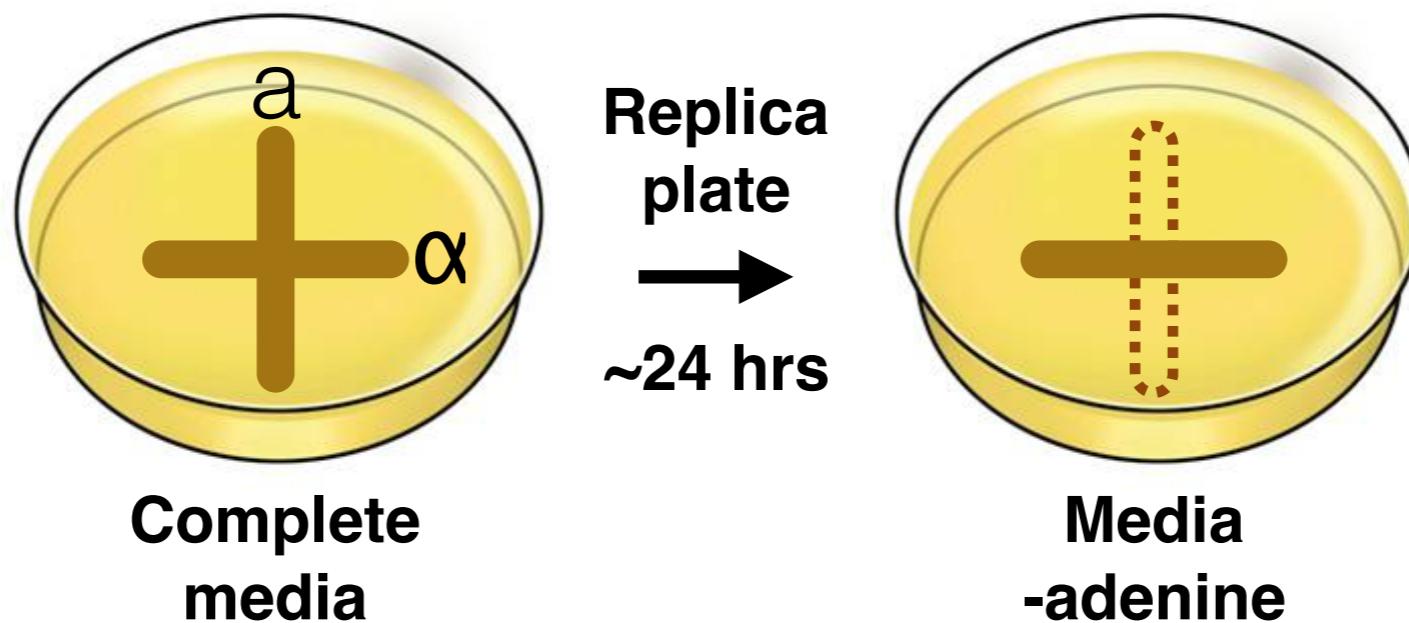
Yeast mating tests



a cells are *ade1* mutant

α cells are *ade1* mutant

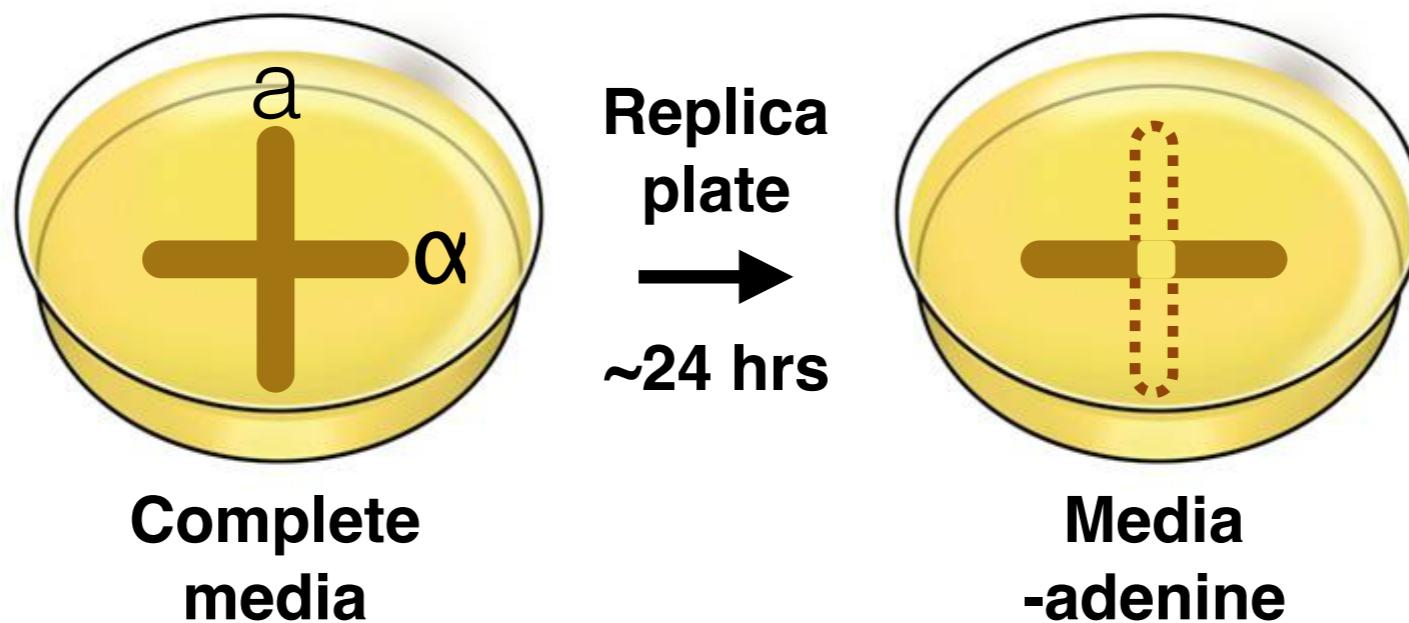
Dominance testing comes before complementation testing



a cells are *ade1* mutant
α cells are wild type

Is the mutant phenotype dominant?

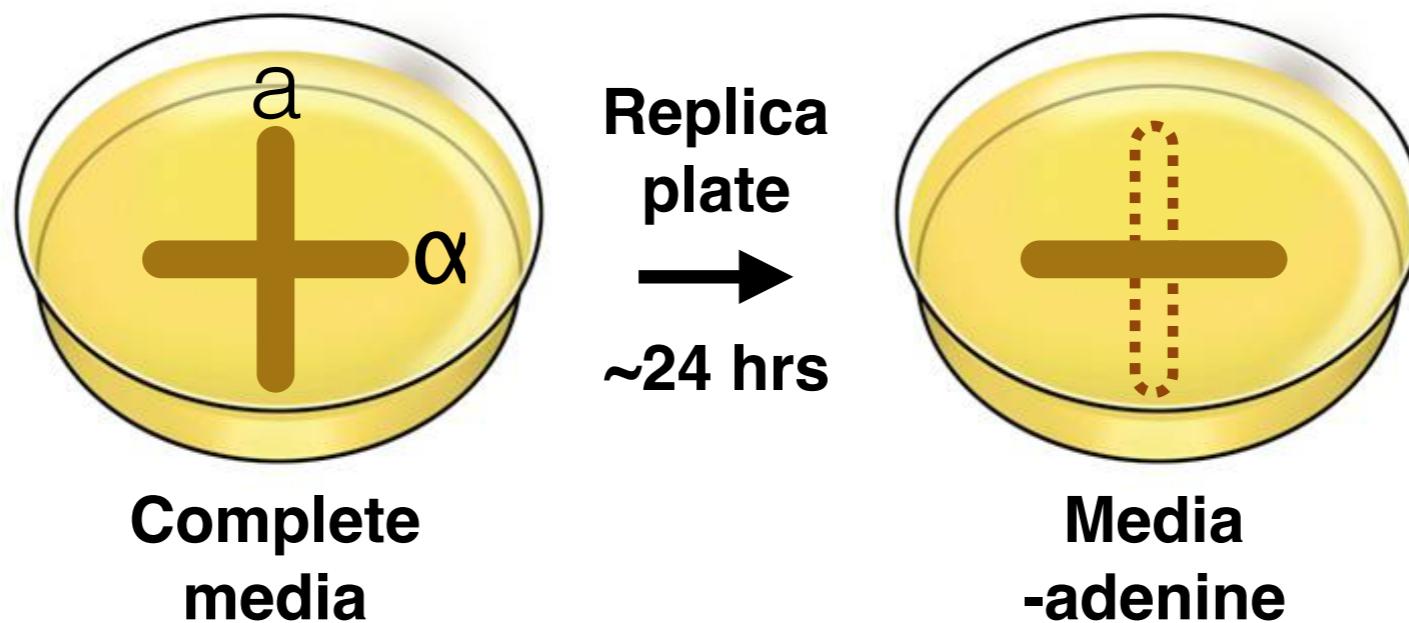
Dominance testing comes before complementation testing



a cells are *ade2* mutant
α cells are wild type

Is the mutant phenotype dominant?

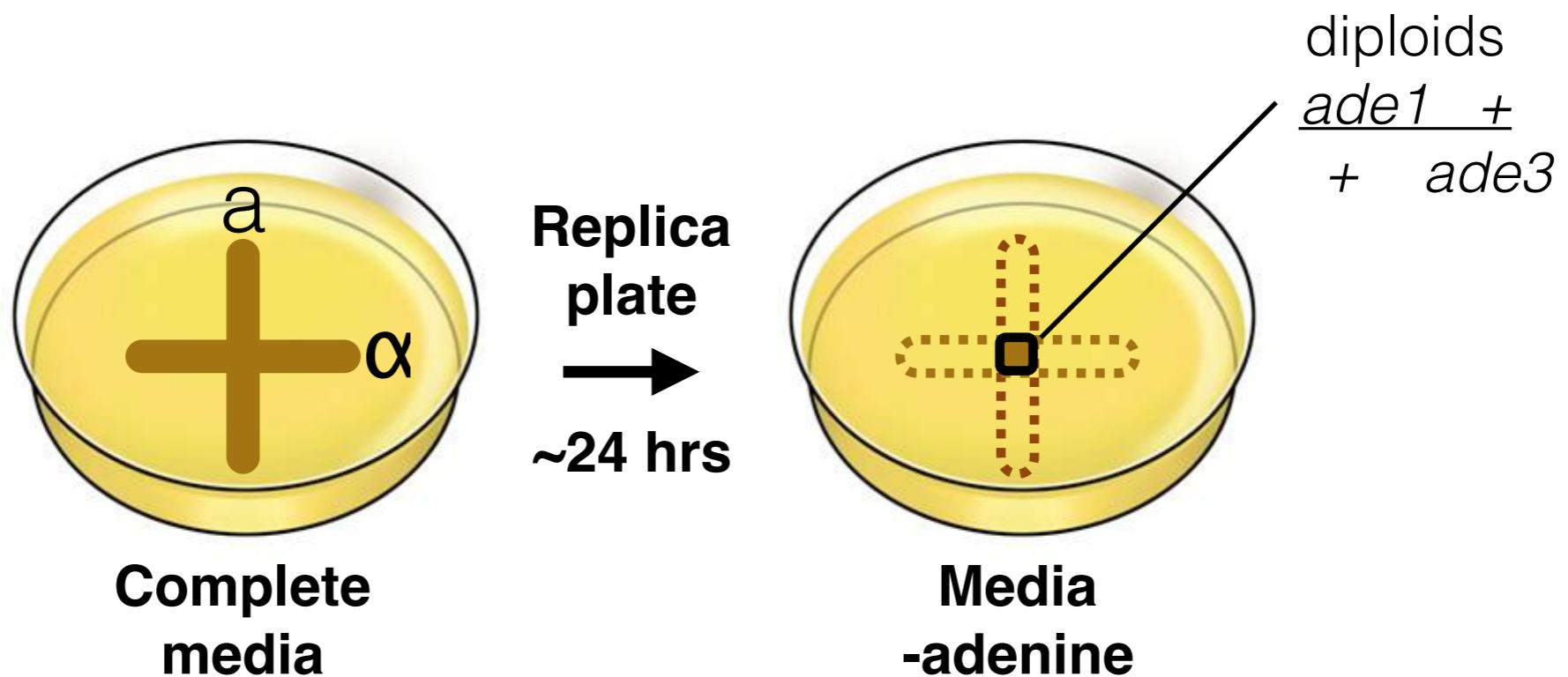
Dominance testing comes before complementation testing



a cells are *ade3* mutant
α cells are wild type

Is the mutant phenotype dominant?

The complementation test



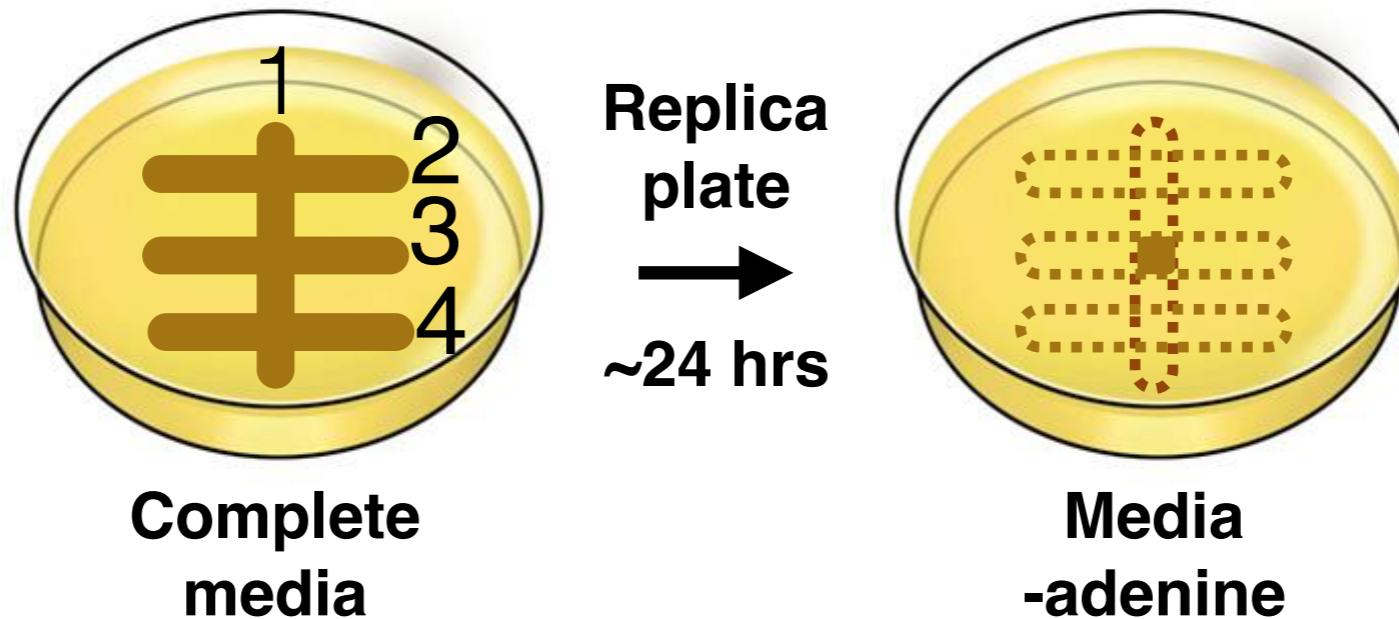
a cells are *ade1* mutant

α cells are *ade3* mutant

***ade1* and *ade3* mutations are in different genes**

They are deficient in different functions!

The complementation test



α cells are *ade1* mutant

α cells are *ade2* mutant

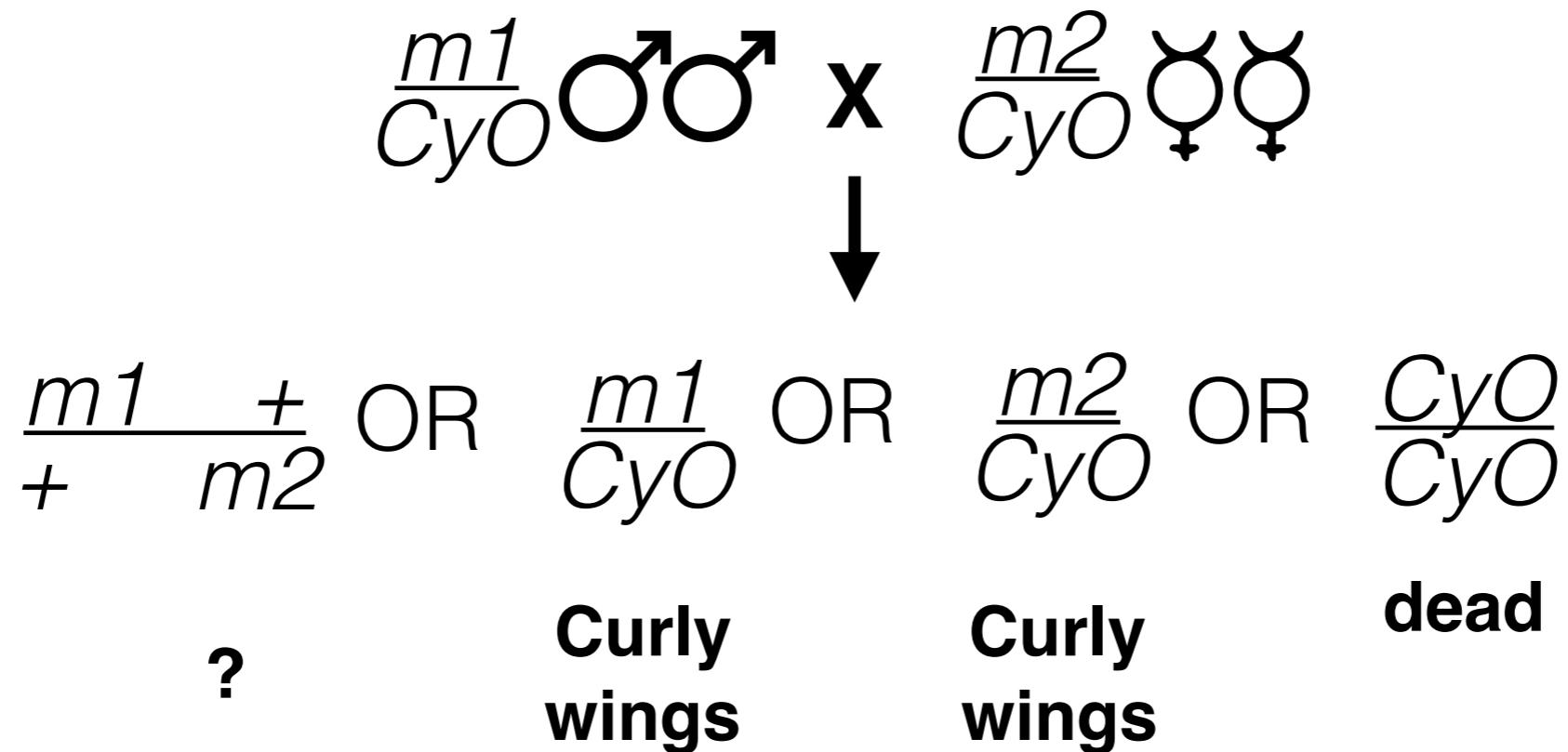
α cells are *ade3* mutant

α cells are *ade4* mutant

***ade1* and *ade4* mutations are in the same gene**

They are deficient in the same function

The complementation test - *Drosophila*



It is easy to control crosses using obligate outcrossers (e.g. males and females)

The complementation test - *C. elegans*



$\frac{m1}{m1}$ ♂ x $\frac{m2}{m2}$ ♀



$\frac{m1}{+}$ +
+ $m2$

The complementation test - *C. elegans*



$\frac{m1}{m1}$ ♂ x $\frac{m2}{m2}$ ♀
↓ ↓

$\frac{m1}{+}$ $\frac{m2}{m2}$

The complementation test - *C. elegans*



$\frac{m1}{m1}$ ♂ x $\frac{dpy-5}{dpy-5}$; $\frac{m2}{m2}$ ♀



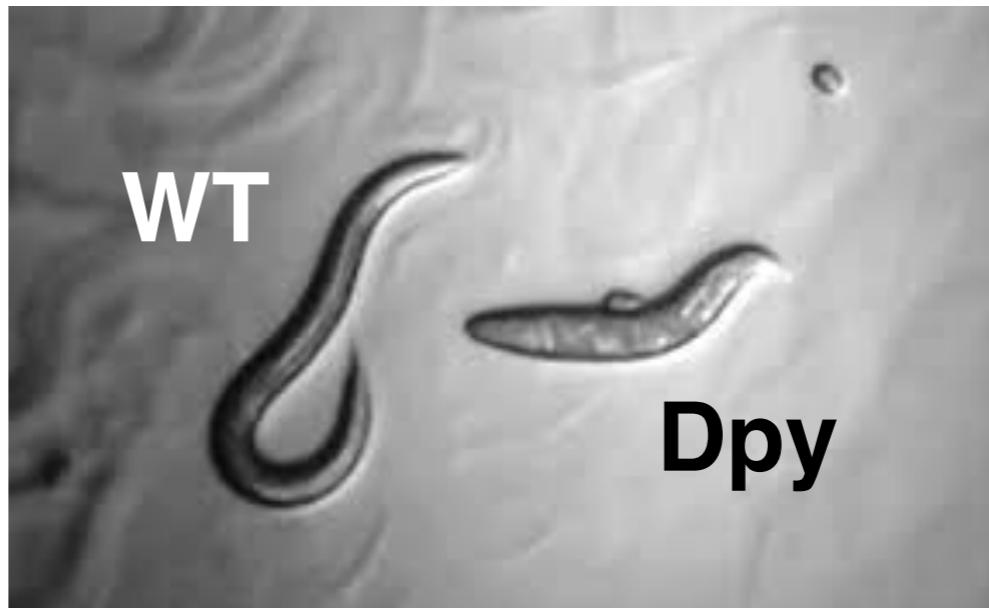
The complementation test - *C. elegans*



$\frac{m1}{m1}$ ♂ x $\frac{dpy-5}{dpy-5}$; $\frac{m2}{m2}$ ♀



$\frac{dpy-5}{+}$; $\frac{m1}{+}$ + $\frac{m2}{m2}$ $\frac{dpy-5}{dpy-5}$; $\frac{m2}{m2}$

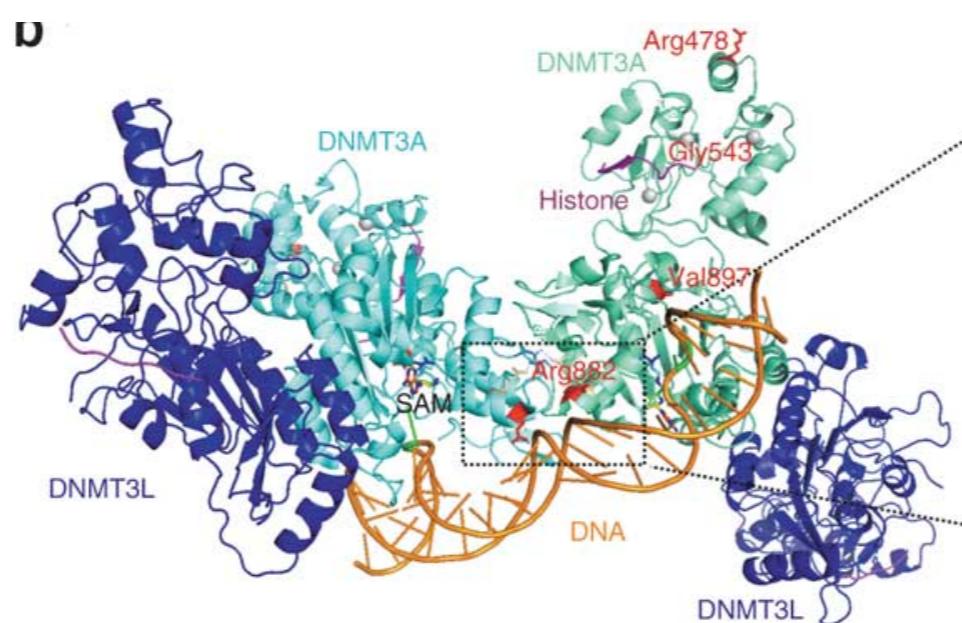


Why do we care about complementation?

- Multiple alleles of a gene allow us to probe gene function



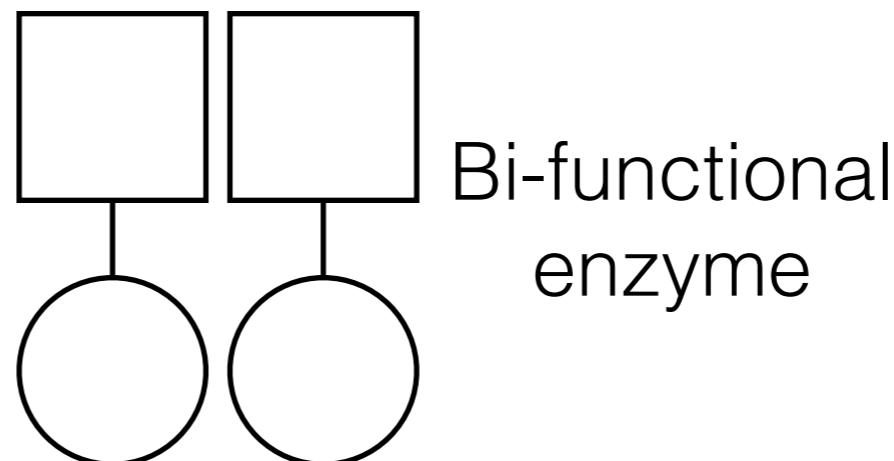
- Different alleles affect different parts of gene



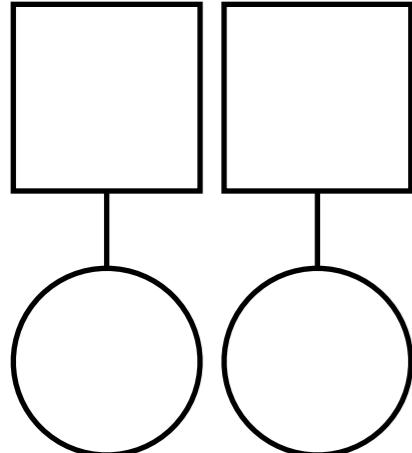
Exceptions to complementation of function

Intragenic complementation

same gene but different functions

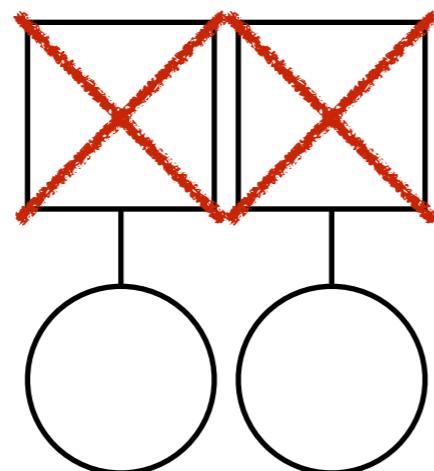


Wild-type



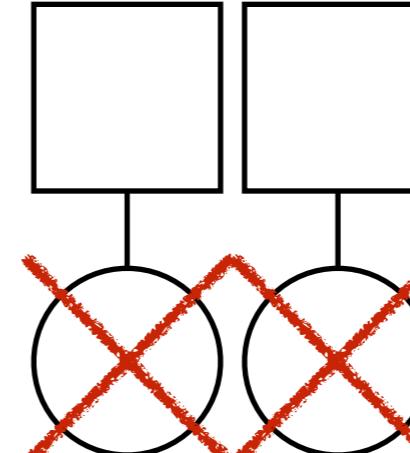
±
+

Mutant



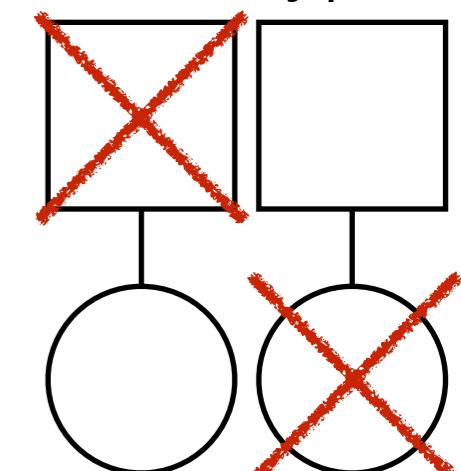
mutation1
mutation1

Mutant



mutation2
mutation2

Wild-type

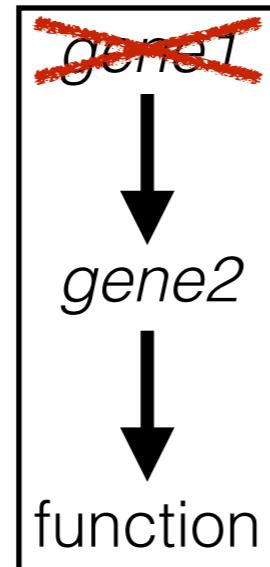
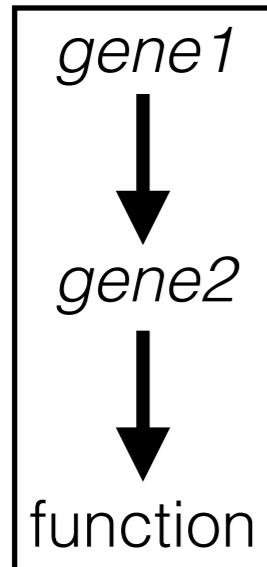
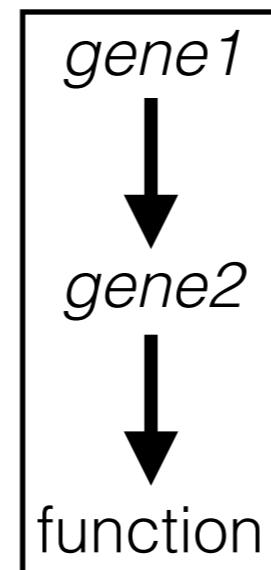


mutation1
mutation2

Exceptions to complementation of function

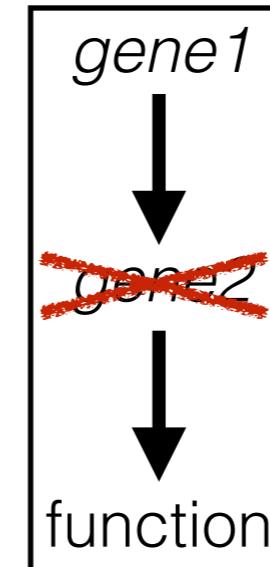
Intergenic noncomplementation

different gene but same function

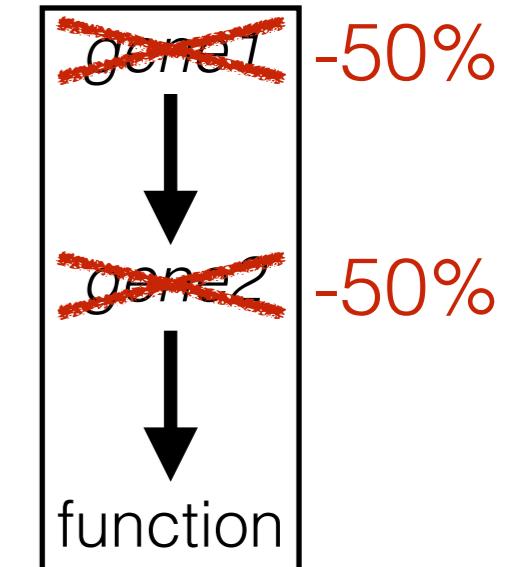


±
+

mut. g1
mut. g1



mut. g2
mut. g2

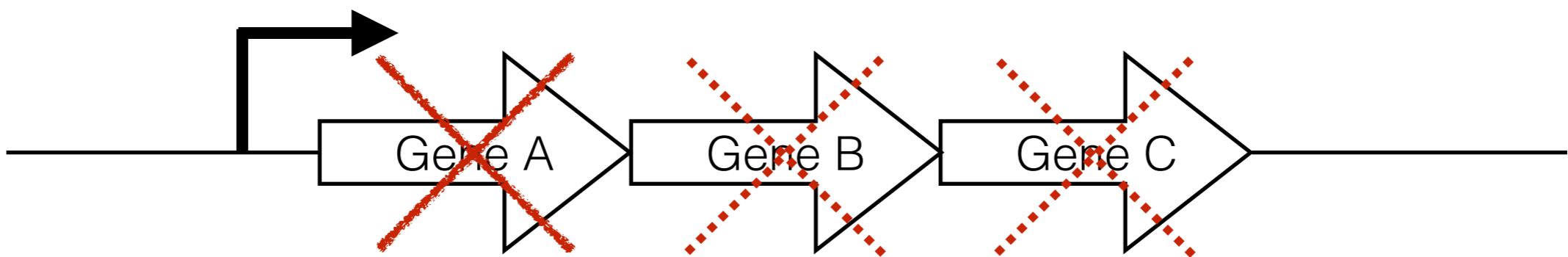
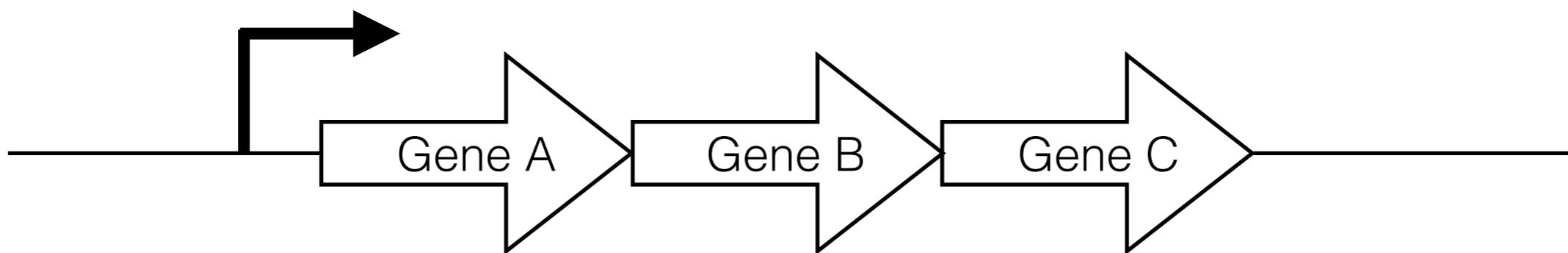


mut. g1 +
+ mut. g2

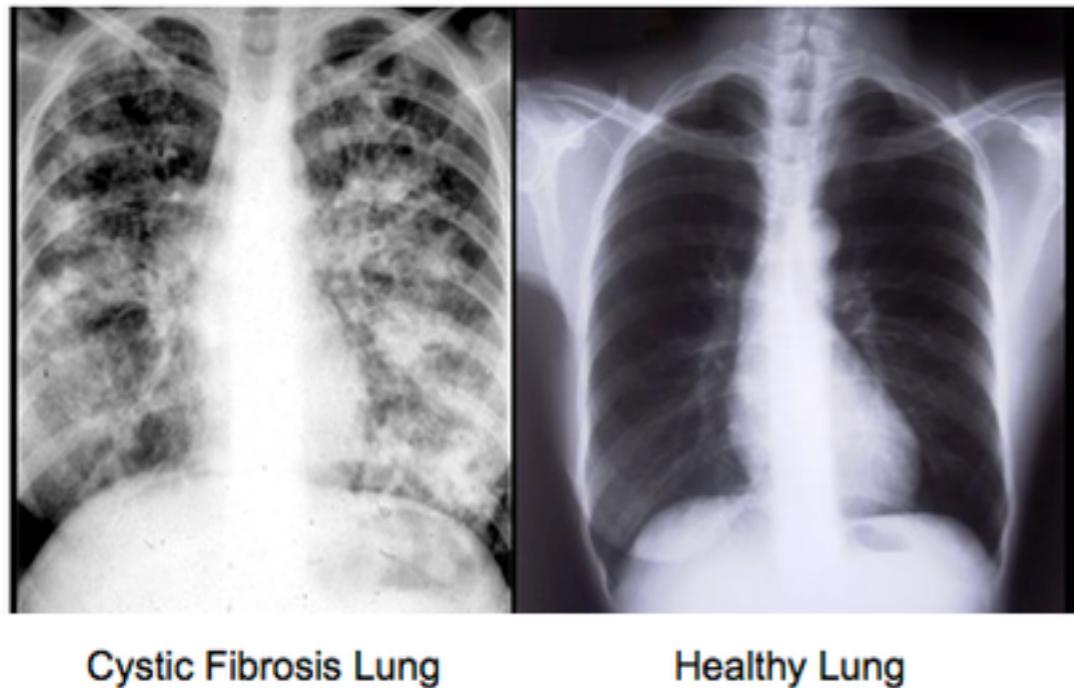
Exceptions to complementation of function

Polar effects

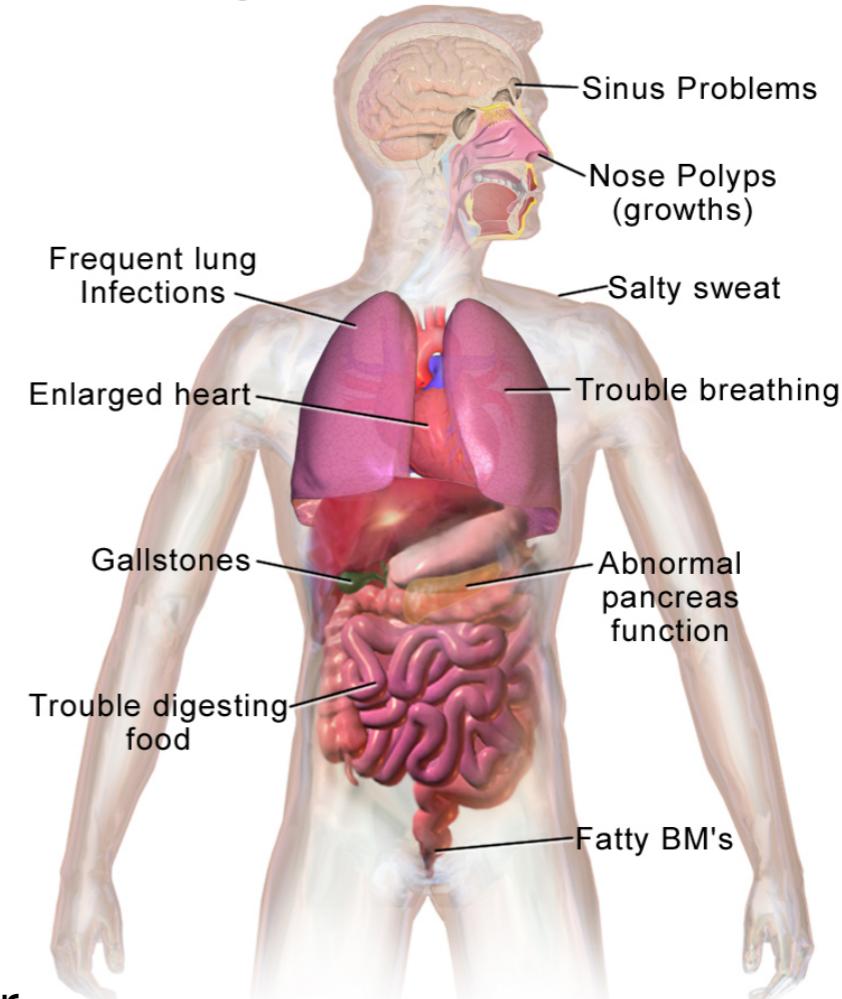
one mutation affects multiple genes/functions



What about cystic fibrosis and today's topic?

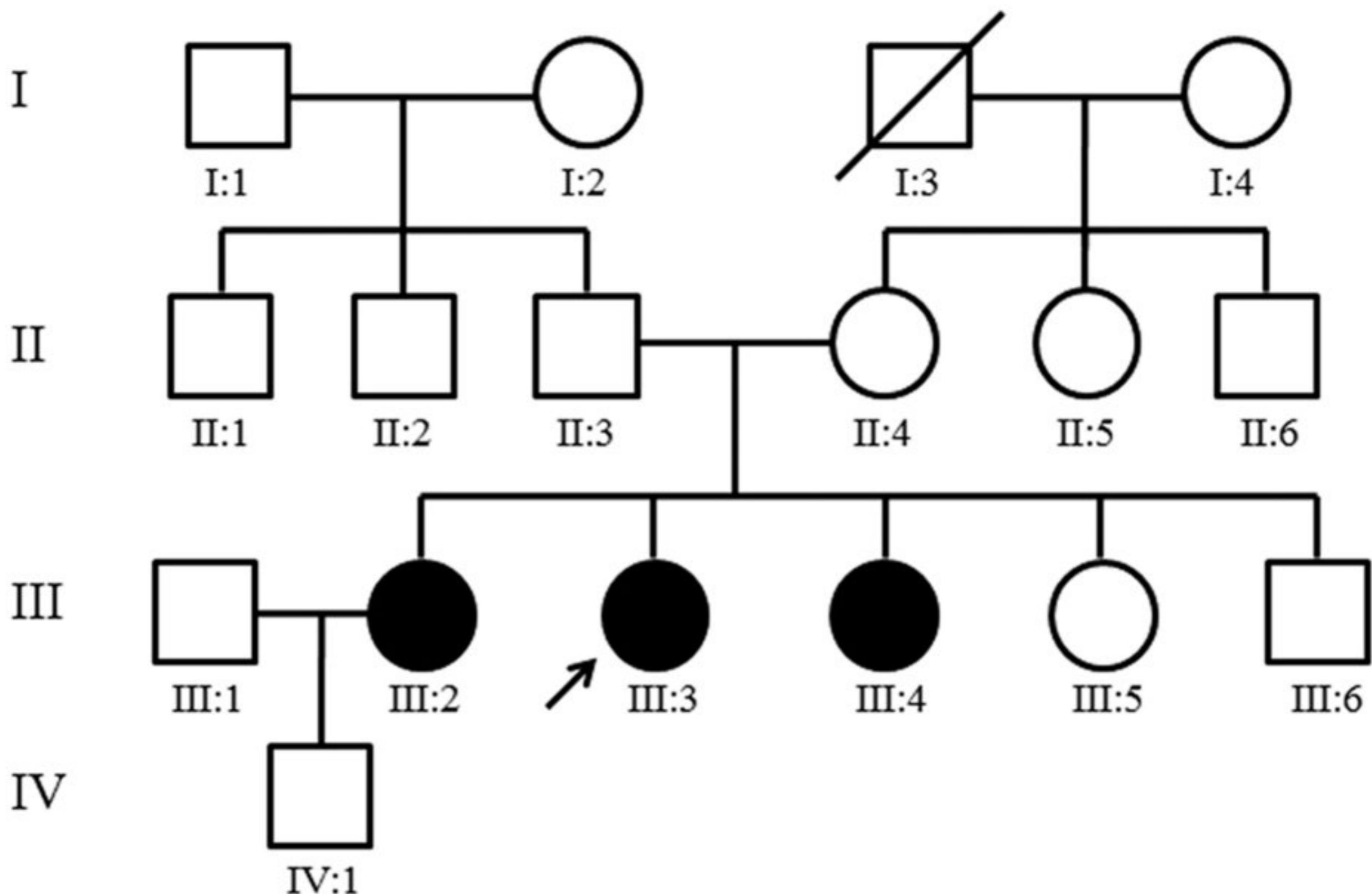


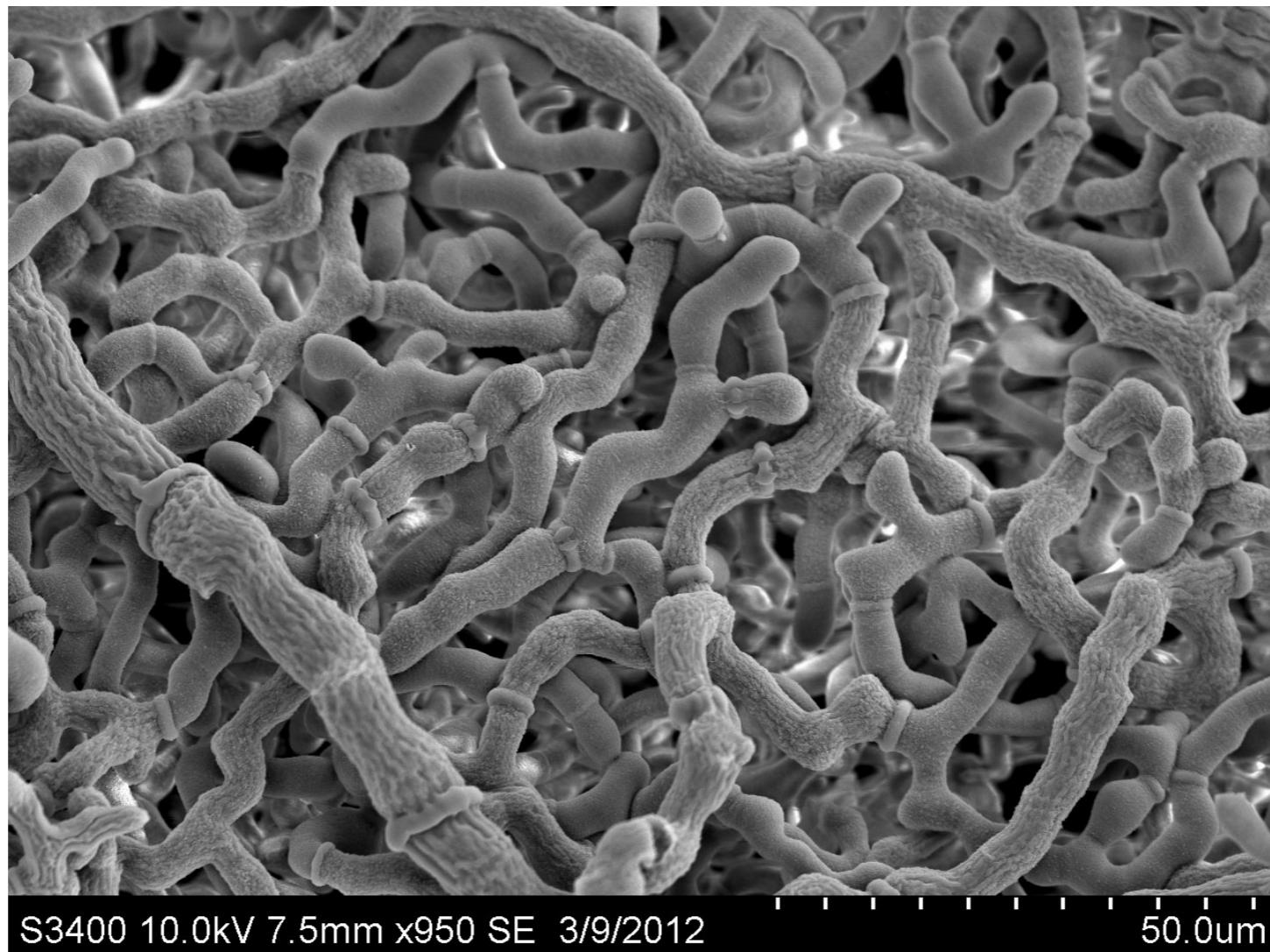
Health Problems with Cystic Fibrosis



1. Autosomal recessive disorder
2. Not caused by chromosomal aberrations or meiotic NDJ
3. Mapped to chromosome 7
4. Mutations in CF gene are null or hypomorphs

Compound heterozygosity - failure to complement





Neurospora crassa

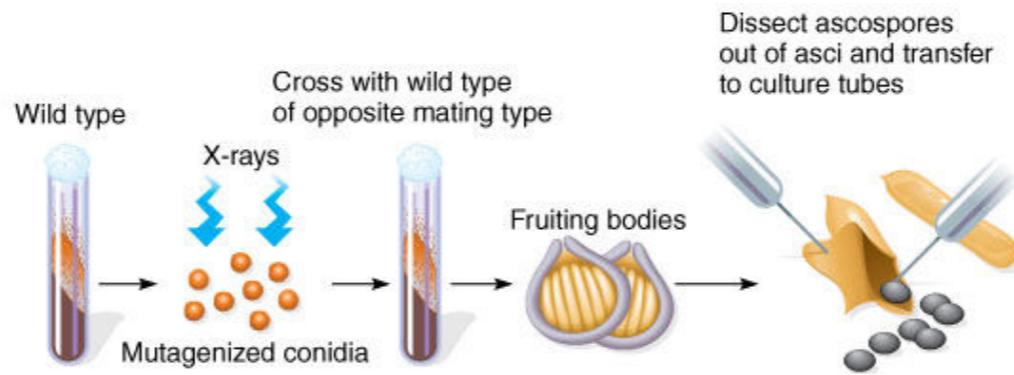


George Beadle

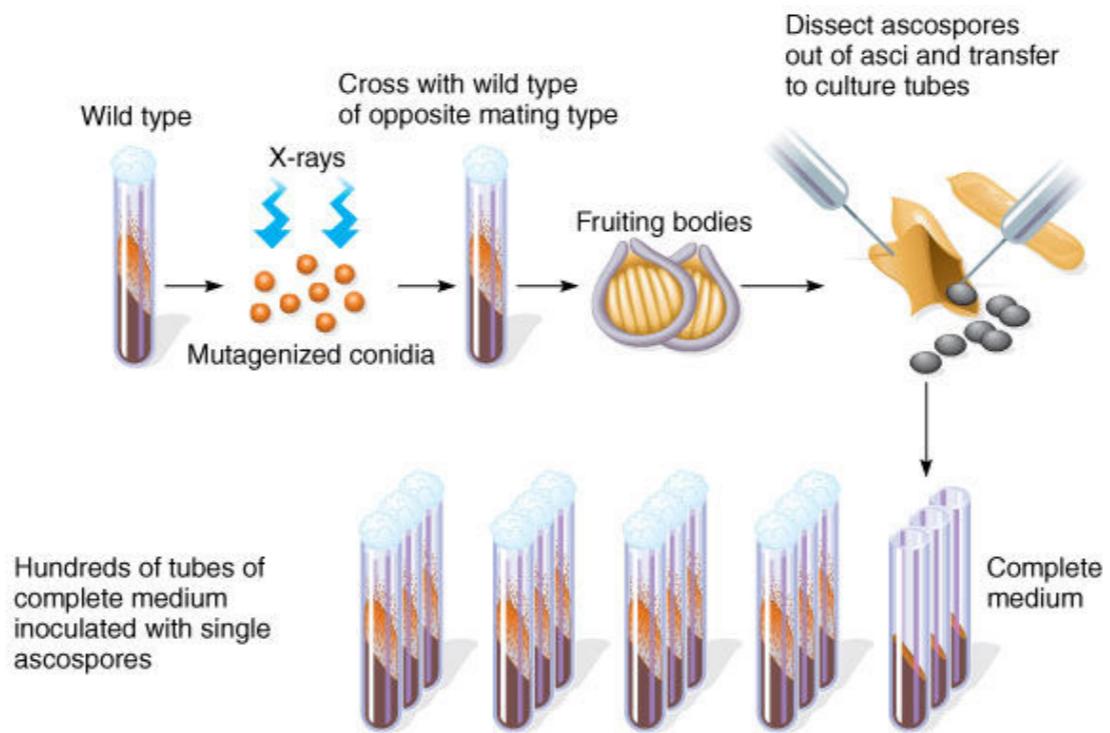


Ed Tatum

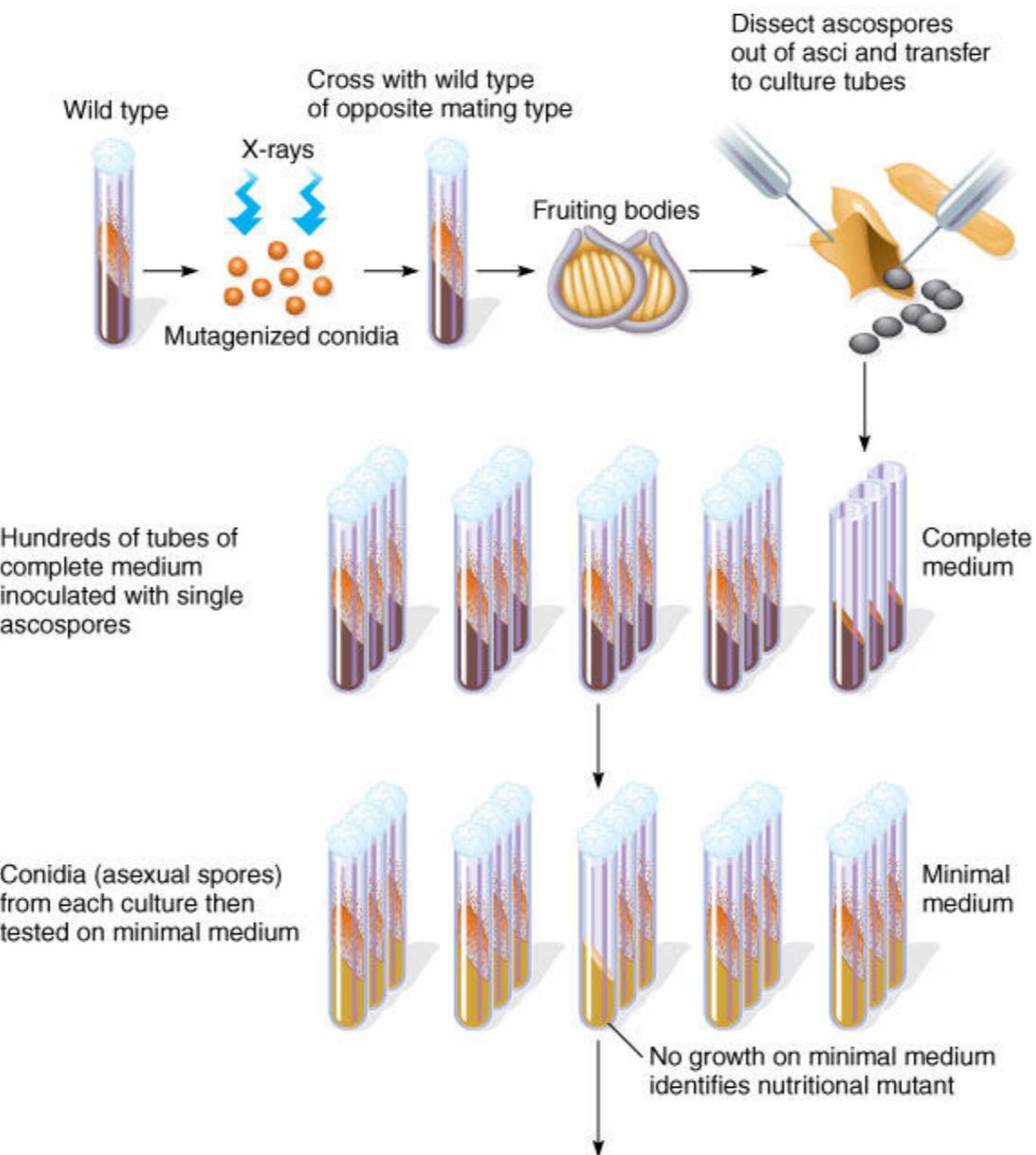
The Beadle- Tatum Experiment



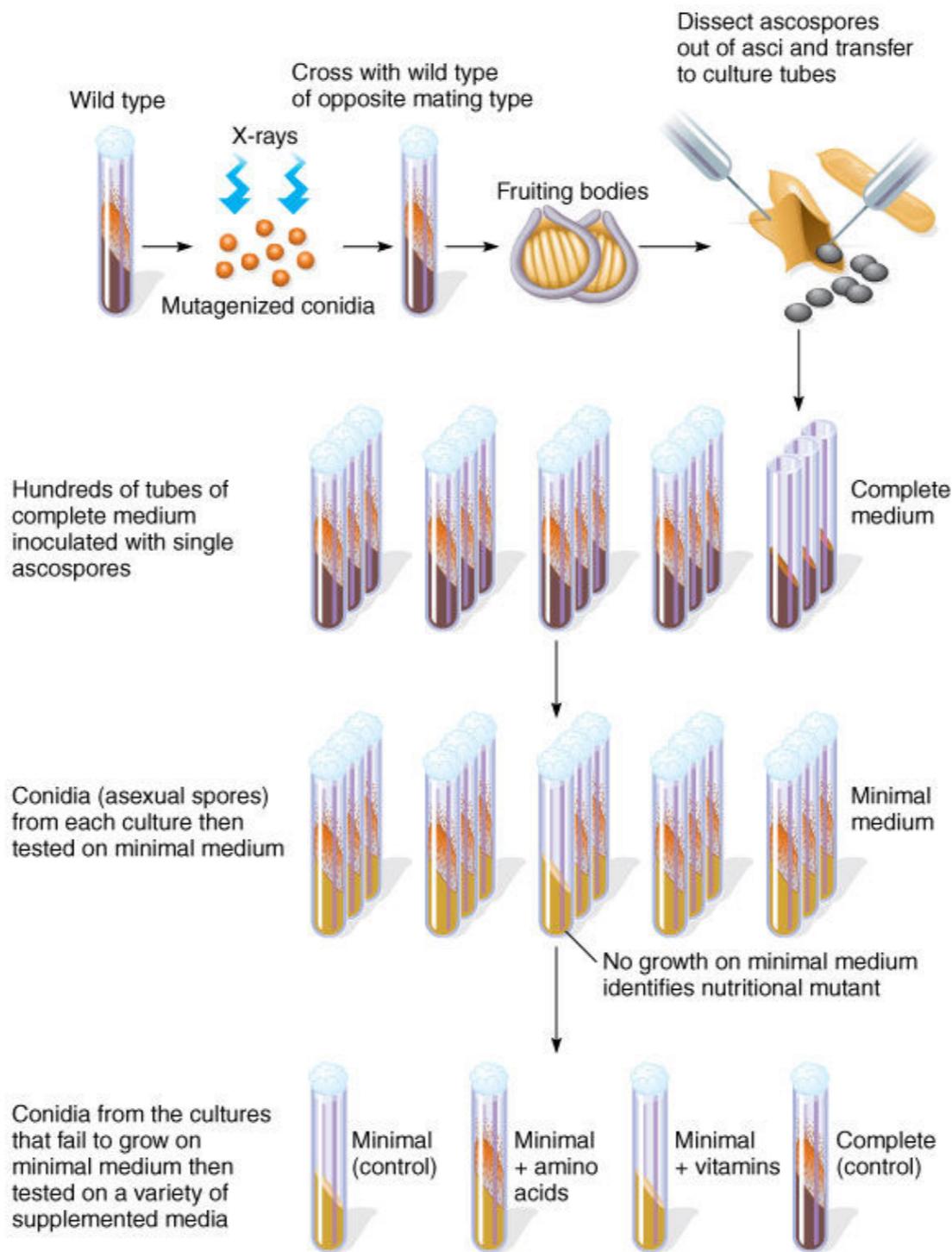
The Beadle- Tatum Experiment



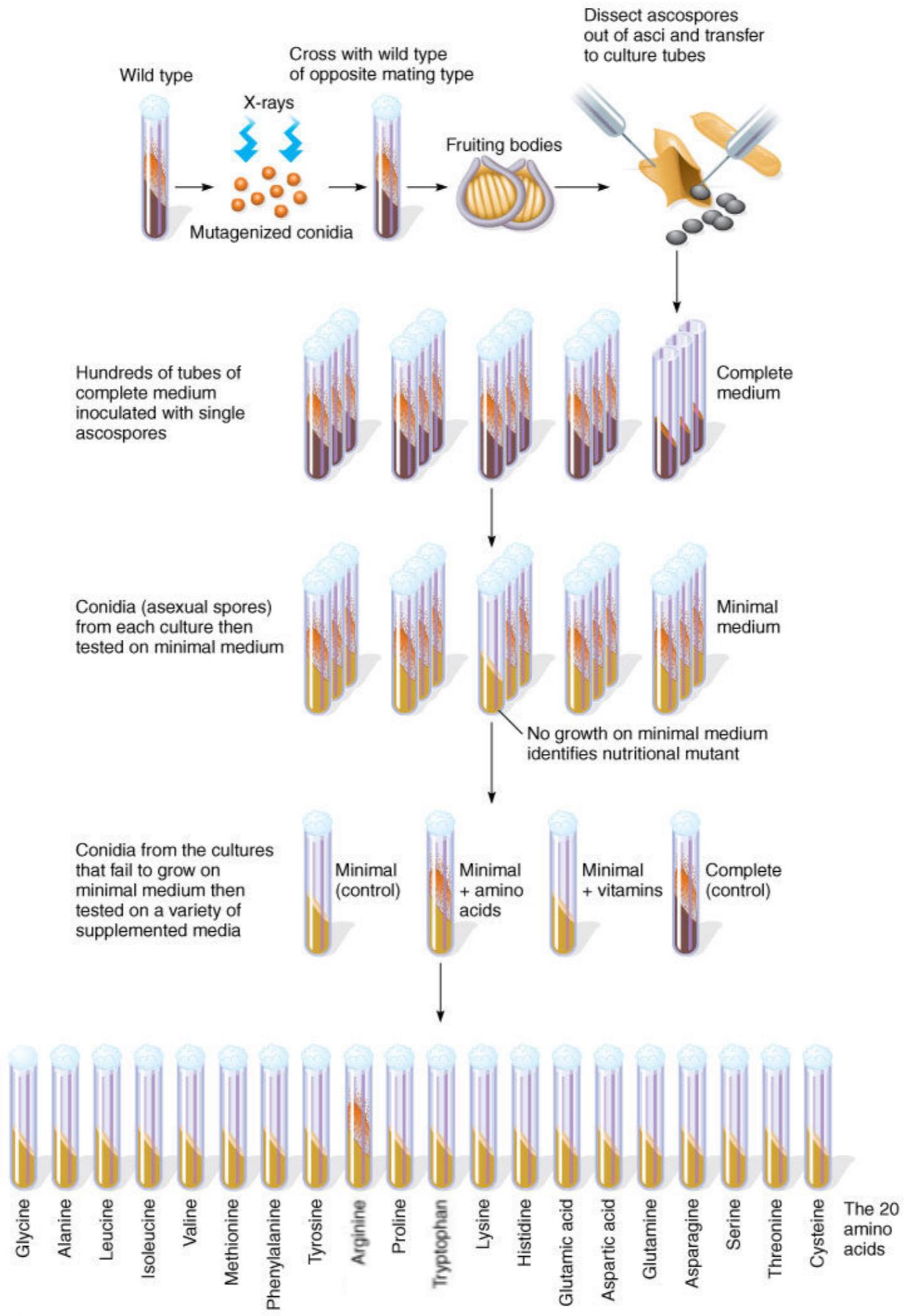
The Beadle- Tatum Experiment



The Beadle- Tatum Experiment

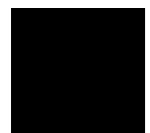


The Beadle-Tatum Experiment



Arginine mutant complementation experiment

	<i>arg-a</i>	<i>arg-b</i>	<i>arg-c</i>	<i>arg-d</i>	<i>arg-e</i>	<i>arg-f</i>	<i>arg-g</i>	<i>arg-h</i>	<i>arg-i</i>
<i>arg-a</i>									
<i>arg-b</i>									
<i>arg-c</i>									
<i>arg-d</i>									
<i>arg-e</i>									
<i>arg-f</i>									
<i>arg-g</i>									
<i>arg-h</i>									
<i>arg-i</i>									



Growth = wild type



No growth = mutant

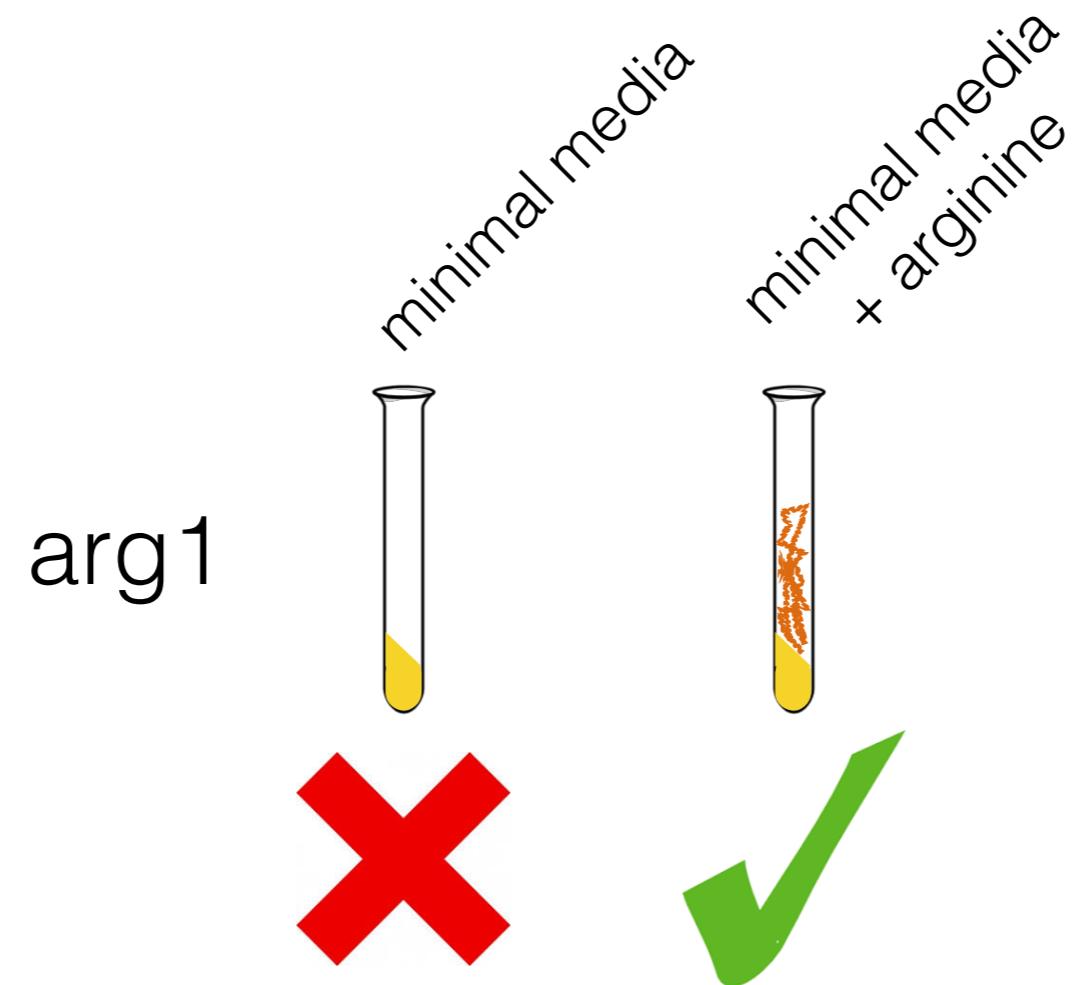
Arginine mutant complementation experiment

Three genes

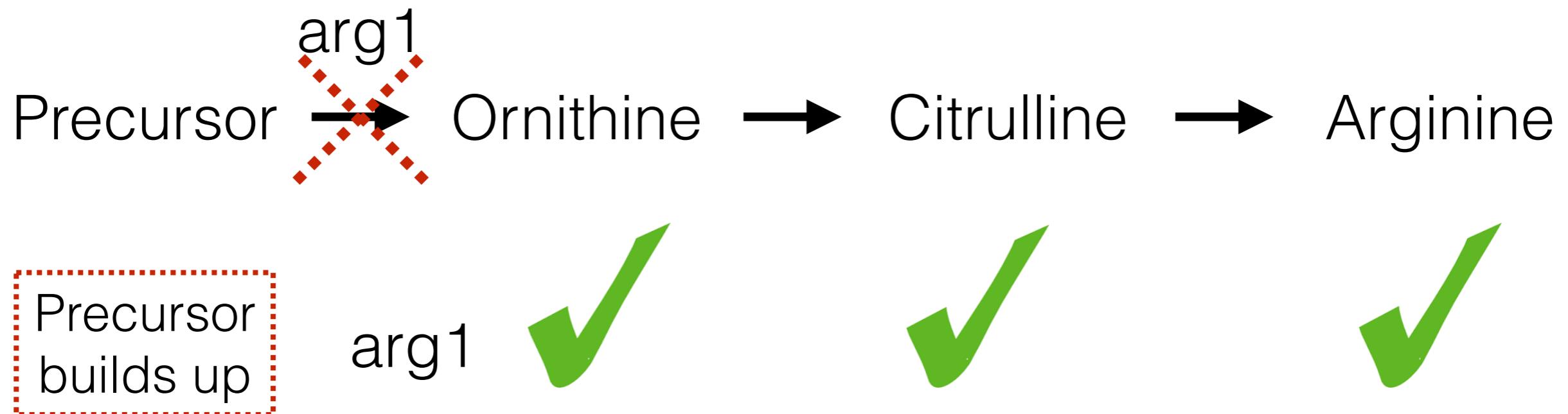
arg1 = [a, d, f, g]

arg2 = [b, c]

arg3 = [e, h, i]

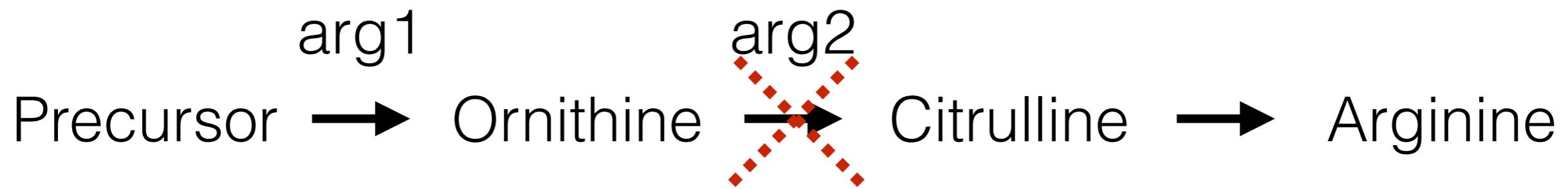


One gene - one enzyme hypothesis



Mutants accumulate precursor for previous step

One gene - one enzyme hypothesis



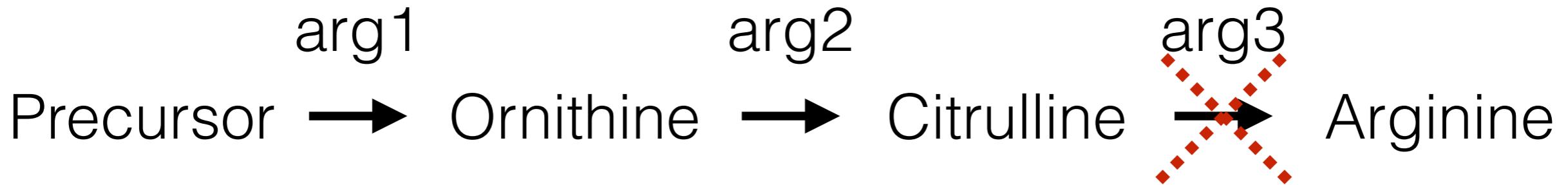
Precursor
builds up



Ornithine
builds up

Mutants accumulate precursor for previous step

One gene - one enzyme hypothesis



Precursor builds up

Ornithine builds up

Citrulline builds up



Mutants accumulate precursor for previous step

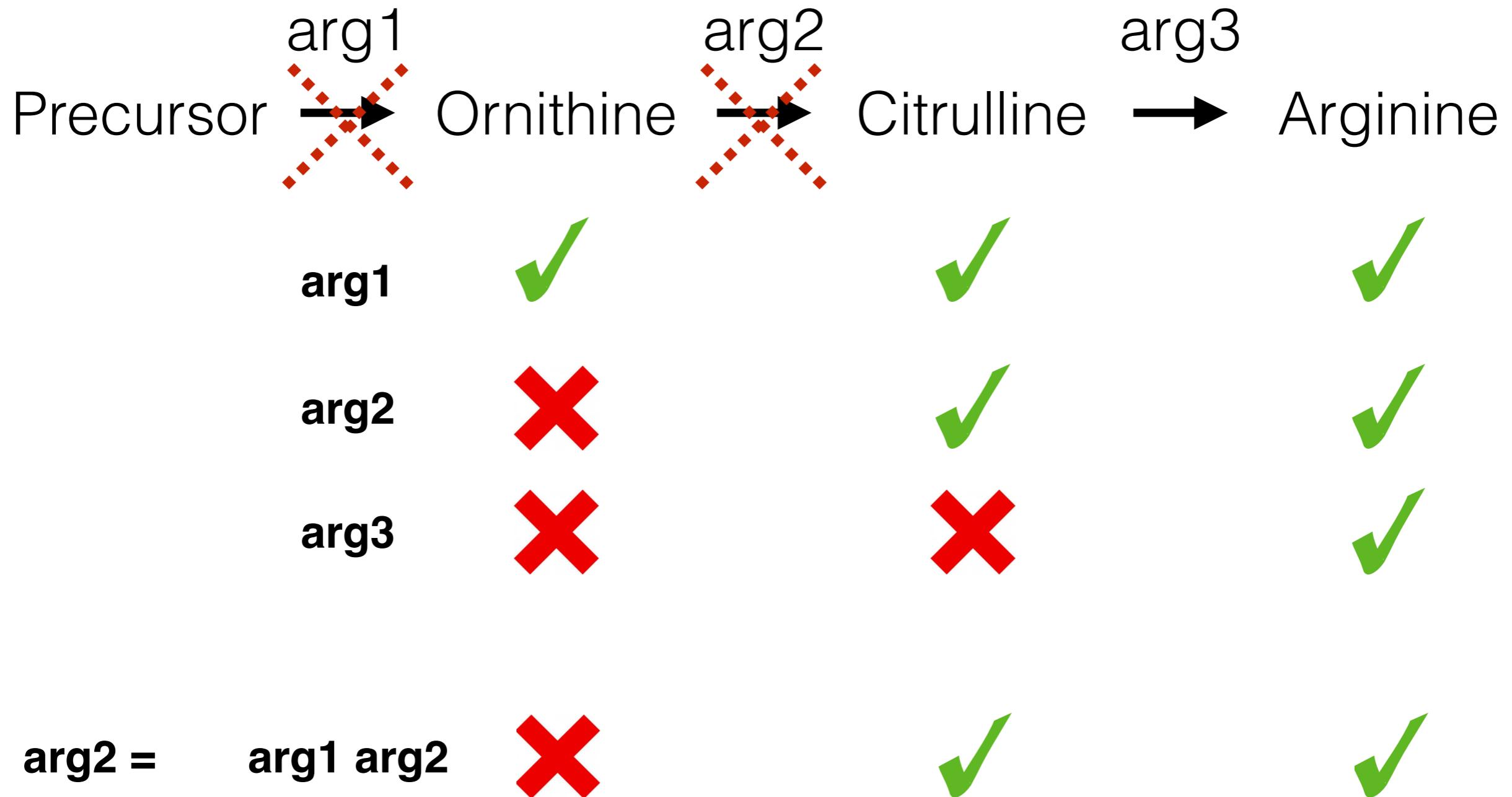
Epistasis

the effect of one gene is dependent on another gene



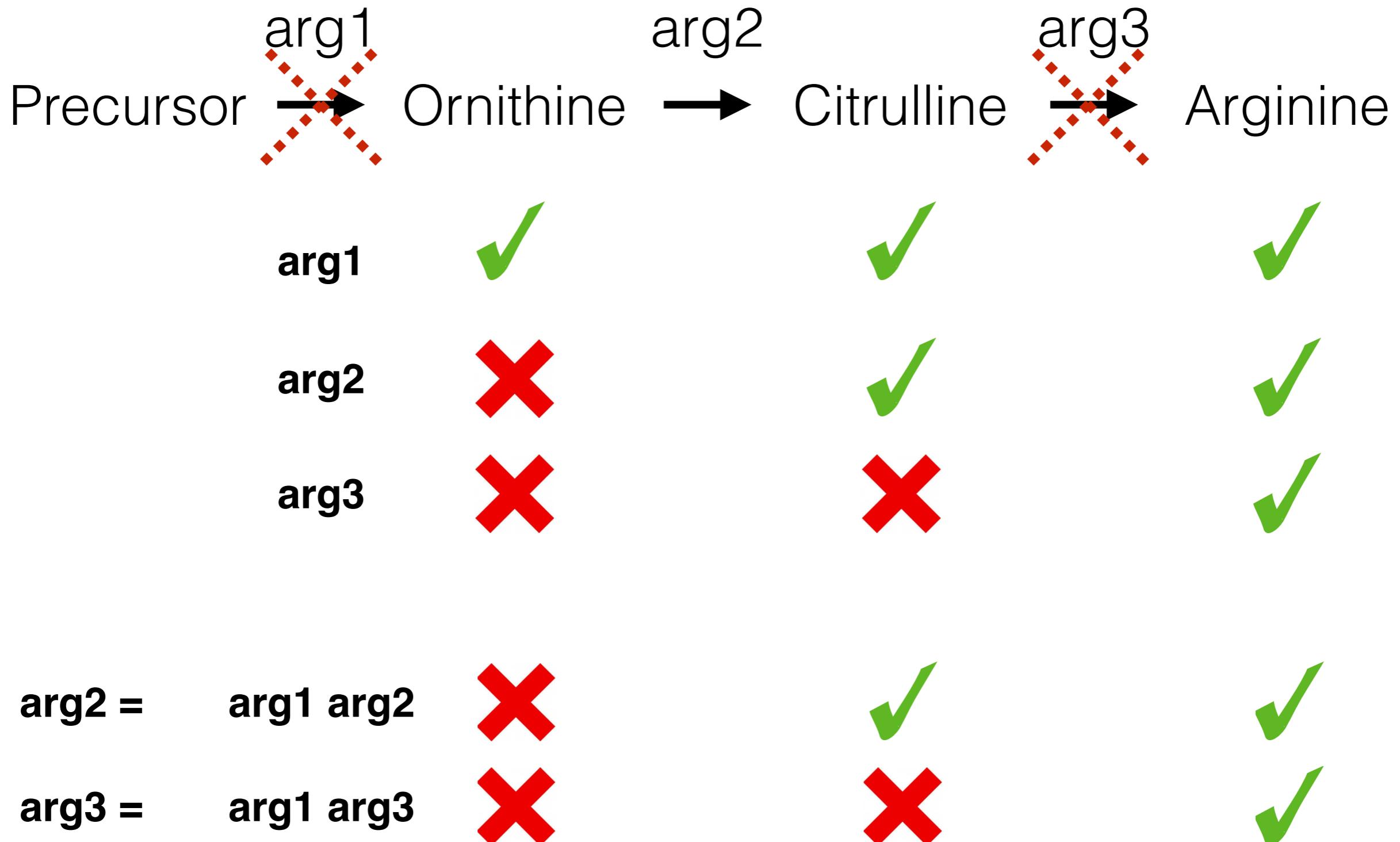
William Bateson

Biochemical epistasis



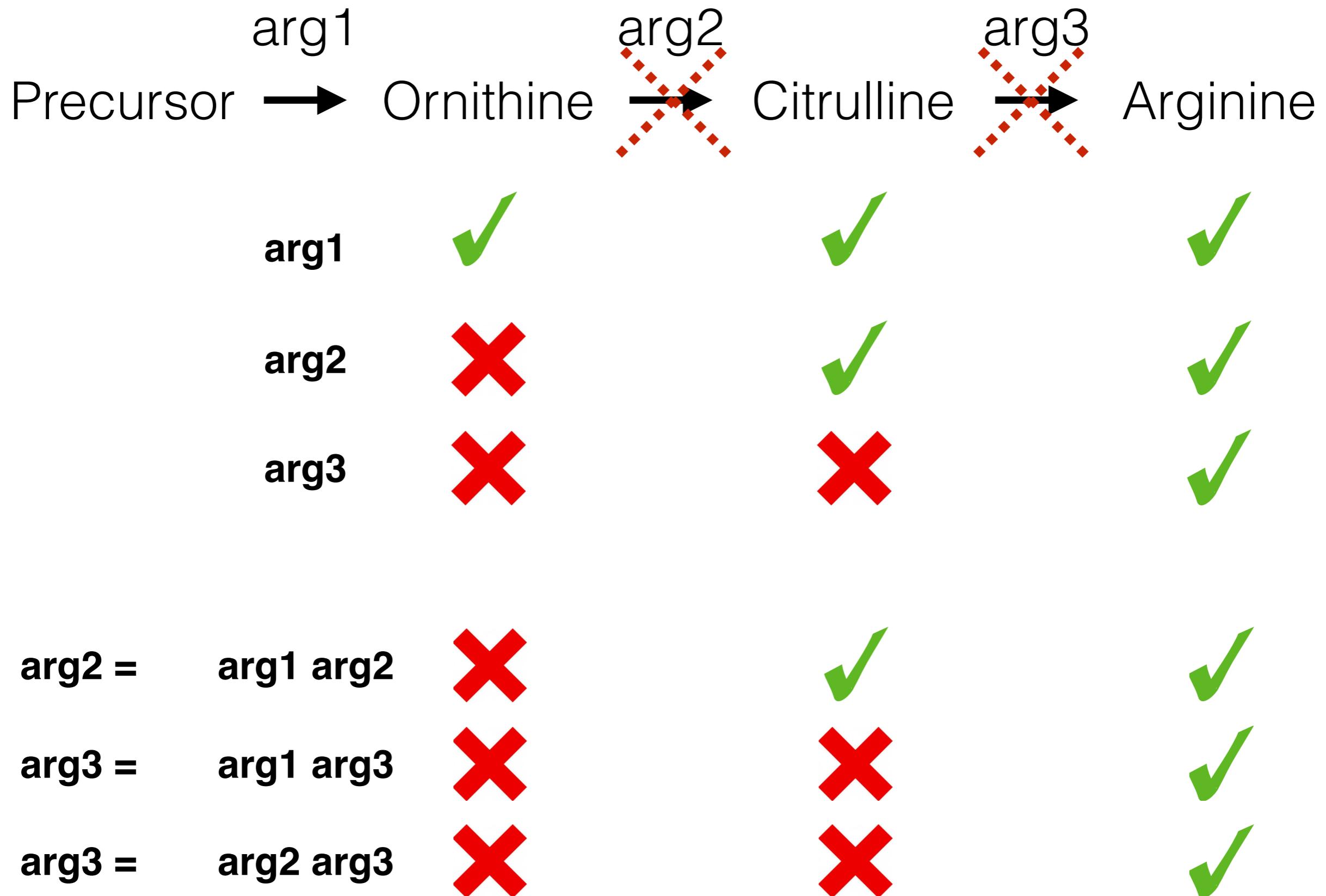
arg2 is epistatic to arg1, arg2 is downstream (or in parallel) to arg1 **Lecture 5**

Biochemical epistasis



arg3 is epistatic to arg1, arg3 is downstream (or in parallel) to arg1 **Lecture 5**

Biochemical epistasis



arg3 is epistatic to arg2, arg3 is downstream (or in parallel) to arg1 **Lecture 5**

Approach to understanding biochemical epistasis



1. Single mutants fail in a step in a biosynthetic pathway
2. Double mutants fail in two steps. The phenotype dictates the most downstream gene in the pathway.
3. What will the single and double mutants accumulate?
4. Pathways can be branched