

Chapter 7: T4 Phage Genetics

T4: lytic phage of *E. coli*

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- All of the cytosine residues in the T4 DNA are replaced by hydroxymethylcytosine, which can be glucosylated (a glucose molecule is attached)

- modification prevents restriction of the phage DNA by the host

- 3 phases of T4



➤ **Immediate early**

- **Host cell recognizes promoters for DNA replication genes and a special sigma factor**



➤ **Delayed early**

- **Alternative sigma factor from immediate early recognizes genes for other sigma factors**

➤ **Late lysis**

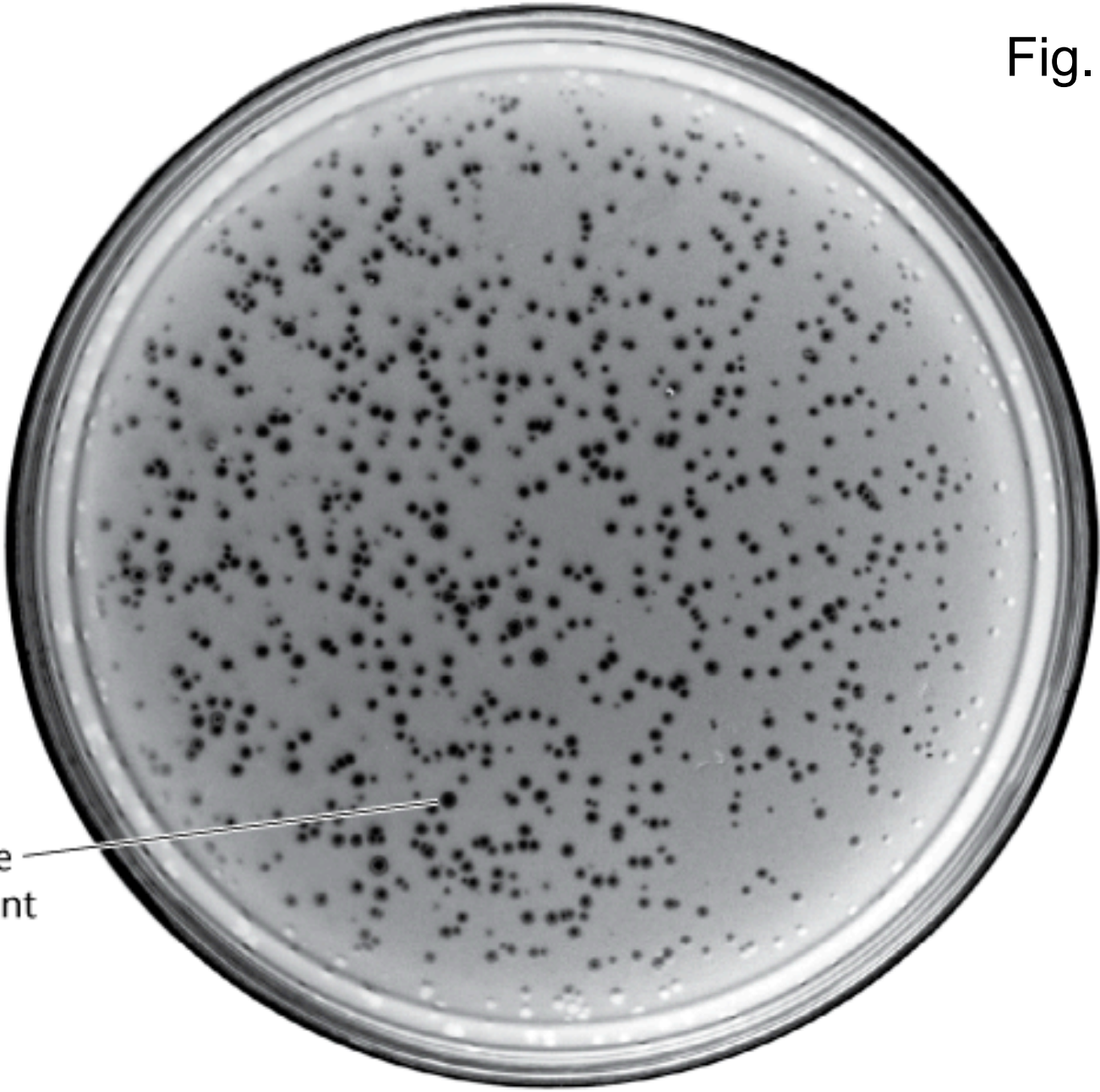
- **Alternative sigma factors from delayed early recognize promoters for lysis genes**

T4 Phage Genetic Crosses

Seymour Benzer:

- - r^+ : wild type T4, plaques have blurry edges b/c of lysis inhibition caused by multiple infections
 - r^- : rapid lysis mutants produced plaques w/ sharp edges
- **Specifically used the conditional lethal mutant *rII***
 - *rII* mutants will grow and produce large plaques on strains of *E. coli* B, but not on lysogenic strains of *E. coli* K12 (lambda).

Fig. 7.23



r-type
mutant

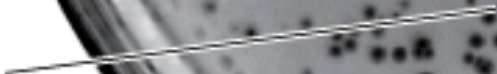
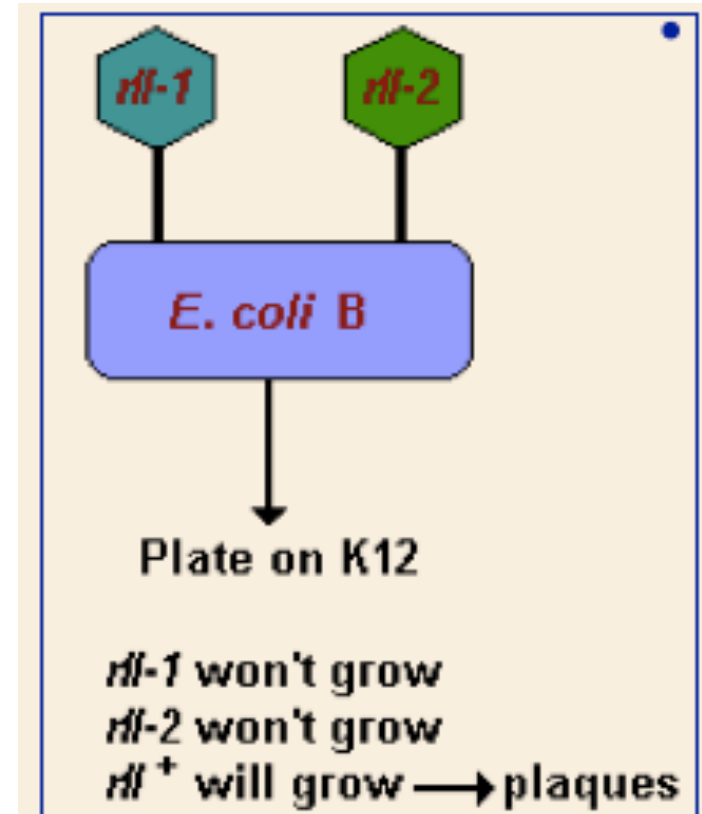


Table 1.		
T4 phage strain	<i>E. coli</i> strain	
	B	K12
<i>rII</i> (mutant)	+ (smooth plaque)	- (no plaque)
<i>rII</i> ⁺ (wild-type)	+ (blurry plaque)	+ (blurry plaque)

Neither *rII-1* or *rII-2* mutants will infect strain K12, but both will infect strain B

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- Plaques that form on K12 are the result of recombination b/w the 2 mutant phage to form a wild-type



How were rII^+ progeny produced?

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rII-1 genotype + + + * + + + + + + + + + +

rII-2 genotype + + + + + + + + + + * + + +

A cross between *rII-1* and *rII-2* would look like:

rII-1 genotype + + + * + + + + + + + + + +

X

rII-2 genotype + + + + + + + + + + * + + +

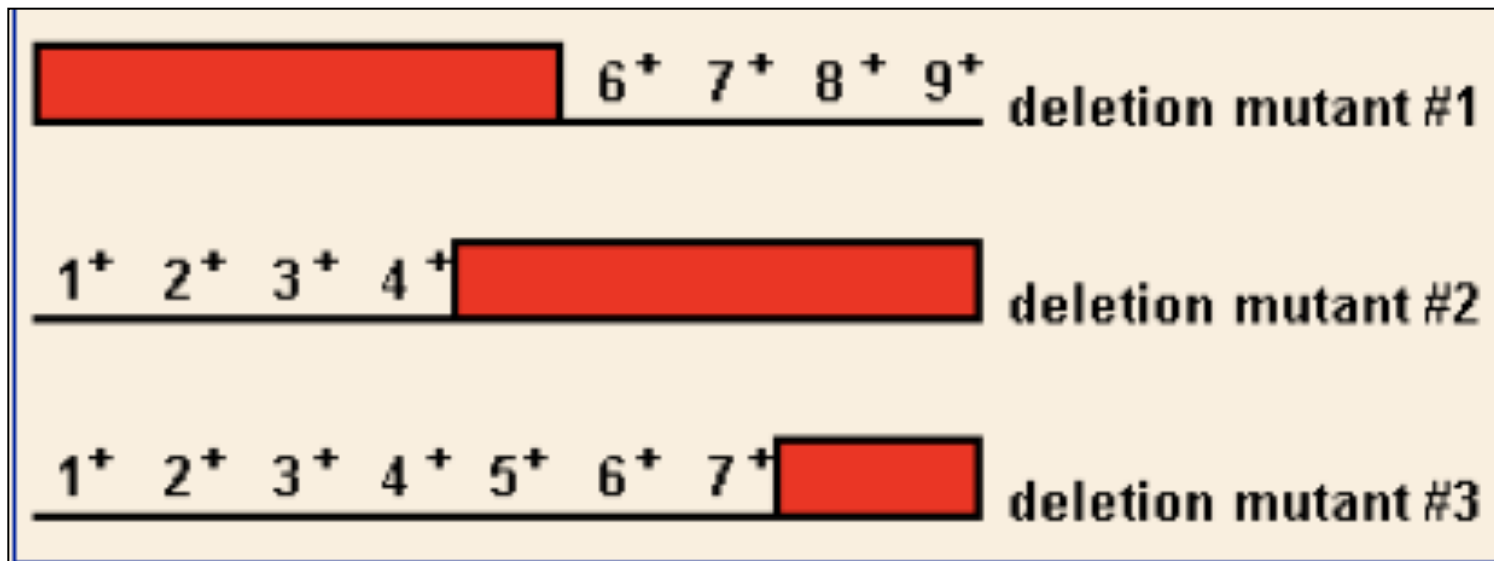
This cross could easily give rise to a wild-type (rII^+) phage.

- Benzer also discovered that the closer two mutations were in the same gene, the less likely crossover will generate a wild type gene

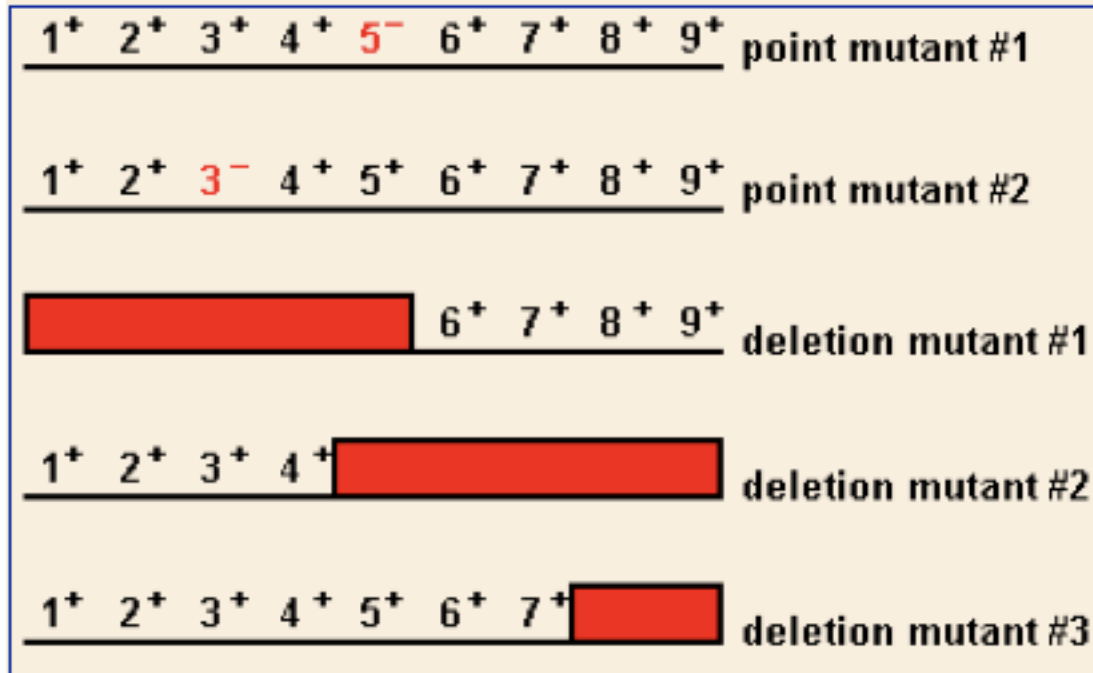
Benzer also discovered deletion mapping while performing phage crosses

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- If two deletions do NOT overlap- possible to get wild type recombinants



Deletion mapping and genetic crosses were used to determine the locus where point mutations occurred



- If know region absent in the deletion mutants, can determine location of point mutations



From deletion mapping Benzer determined:

- Farther apart two deletions (mutations) are, the more WT recombinants will be produced

- Muton -

- Recon -



- Functional alleles - occurring in same cistron and have same phenotype
- Structural alleles - occurring at same site. If cross two point mutants and never get wild-type, must be structural alleles.
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Benzer also discovered complementation with phage mutants

- **Complementation:**

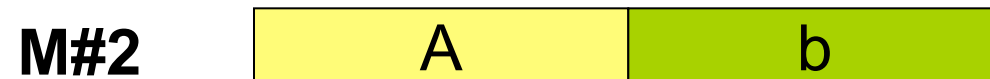


cis-trans test: must have A and B products for progeny

Cis:

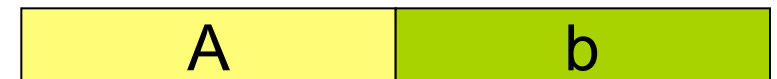


progeny



progeny

Trans:



From his complementation tests, Benzer:

- Came up with the term cistron

Cistron:



- Determined that there were two cistrons involved in his *rII* mutants

Gene *rIIA* --> A product necessary for progeny

Gene *rIIB* --> B product necessary for progeny

T4 Example Problems

You are studying a newly discovered virus and have isolated a number of mutants that you call flop (fuzzy little opaque plaque) mutants. You perform a series of cis-trans tests on ten different point mutants and obtain the following results:

	f1	f2	f3	f4	f5	f6	f7	f8	f9	f10
f1	-	+	-	-	+	+	+	+	+	+
f2	+	-	+	+	+	+	+	+	+	+
f3	-	+	-	-	+	+	+	+	+	+
f4	-	+	-	-	+	+	+	+	+	+
f5	+	+	+	+	-	-	+	+	-	-
f6	+	+	+	+	-	-	+	+	-	-
f7	+	+	+	+	+	+	-	-	+	+
f8	+	+	+	+	+	+	-	-	+	+
f9	+	+	+	+	-	-	+	+	-	-
f10	+	+	+	+	-	-	+	+	-	-

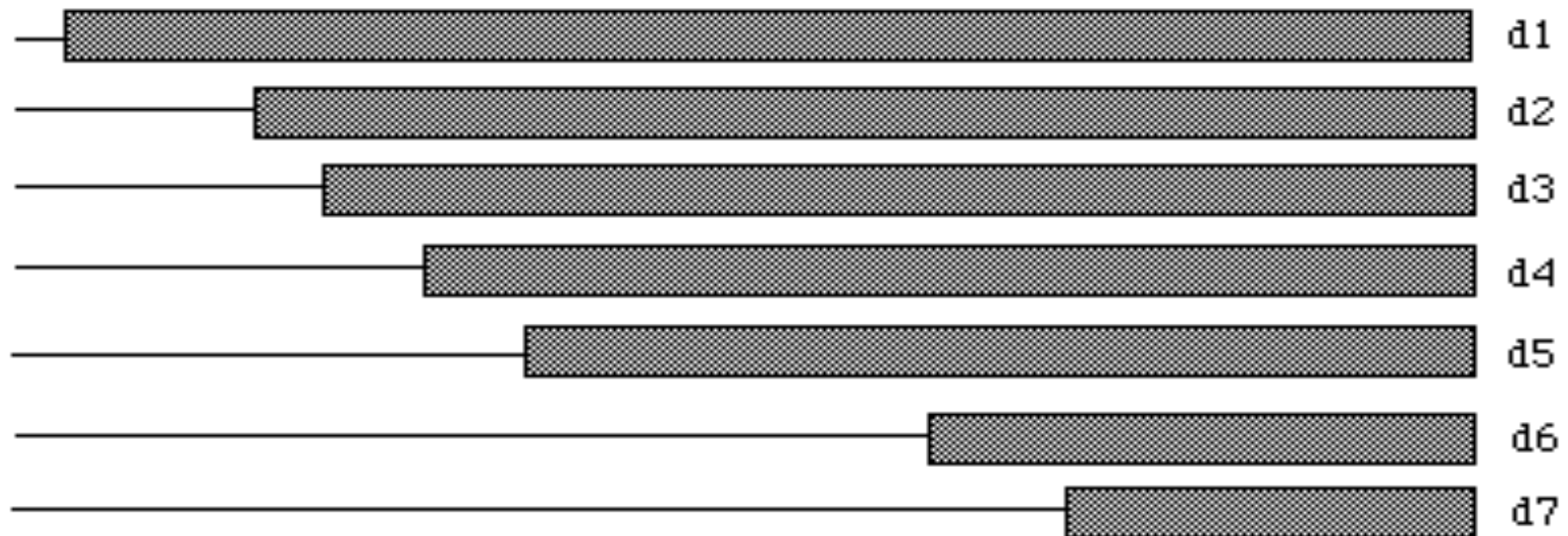
How many genes control this trait?

	f1	f2	f3	f4	f5	f6	f7	f8	f9	f10
f1	-	+	-	-	+	+	+	+	+	+
f2	+	-	+	+	+	+	+	+	+	+
f3	-	+	-	-	+	+	+	+	+	+
f4	-	+	-	-	+	+	+	+	+	+
f5	+	+	+	+	-	-	+	+	-	-
f6	+	+	+	+	-	-	+	+	-	-
f7	+	+	+	+	+	+	-	-	+	+
f8	+	+	+	+	+	+	-	-	+	+
f9	+	+	+	+	-	-	+	+	-	-
f10	+	+	+	+	-	-	+	+	-	-

Identify which point mutations are on the same gene

You now perform deletion mapping on 4 of the point mutants (f5, f6, f9 and f10) using the 7 deletion mutants illustrated below. From the results shown, identify the approximate locations of these four point mutants on the line map.

	d1	d2	d3	d4	d5	d6	d7
f5	-	-	+	+	+	+	+
f6	-	-	-	-	-	+	+
f9	-	+	+	+	+	+	+
f10	-	-	-	+	+	+	+



At last you are ready to perform phage genetic crosses on three of the point mutants: f1, f3, and f4. You obtain the following results.

Cross	# Wildtype	Total # phage	% recombination
f1 x f3	28	217	
f1 x f4	15	326	
f3 x f4	54	309	

Fill in the table by determining the percent recombination

Draw a map of these three point mutations, indicating order and distance (use the recombination frequency as map distance)