

Complementation Test, the rII Locus & Benzer's Experiment

Fine-Structure Analysis of the Gene | Molecular Genetics Study Notes

1. The Question Benzer Set Out to Answer

By the early 1950s, geneticists knew mutations mapped to positions along a chromosome and that recombination could occur between them — but nobody knew the fine internal anatomy of a gene. Was a gene an indivisible bead on a string, or could recombination happen *within* a gene, between two mutations sitting just a few base pairs apart? And when two mutations both gave the same mutant phenotype, how could you tell whether they were two hits on the *same* functional gene, or hits on two *different* genes that both happen to be needed for the same trait? **Seymour Benzer** answered both questions between 1955 and 1961 using an exceptionally clever genetic system built around a single locus of bacteriophage T4 — the **rII** locus — and in doing so essentially invented modern fine-structure gene mapping.

Two separate tools, one system

Benzer used the **same** rII/phage system to ask two different questions with two different genetic tests: the **complementation test** asks whether two mutations lie in the same gene (functional units), while **recombination / deletion mapping** asks exactly where within the DNA each mutation sits (physical/structural units).

2. The Complementation (Cis–Trans) Test

The complementation test is the classic operational definition of “what is a gene”: two recessive mutations are tested together in the same cell, and you ask whether the wild-type phenotype is restored. If it is, the two mutations **complement** each other and are considered to lie in different genes (each chromosome supplies what the other lacks). If the mutant phenotype persists, the mutations **fail to complement** and are considered allelic — different mutant sites within the *same* gene.

2.1 Cis vs Trans Configuration

The test compares two arrangements of the same two mutations, a and b. In the **trans configuration**, the two mutations sit on two separate chromosomes (one phage genome carries mutation a with a wild-type copy of the other gene; a second phage genome carries mutation b with a wild-type copy of the first gene) that co-infect one bacterial cell. In the **cis configuration** — the essential control — both mutations sit together on one chromosome, paired with a fully wild-type chromosome. The cis arrangement must always give a wild-type phenotype (the fully wild-type chromosome rescues everything); if it doesn't, the test system itself is flawed. The biologically informative comparison is the trans result.

The Complementation (Cis-Trans) Test

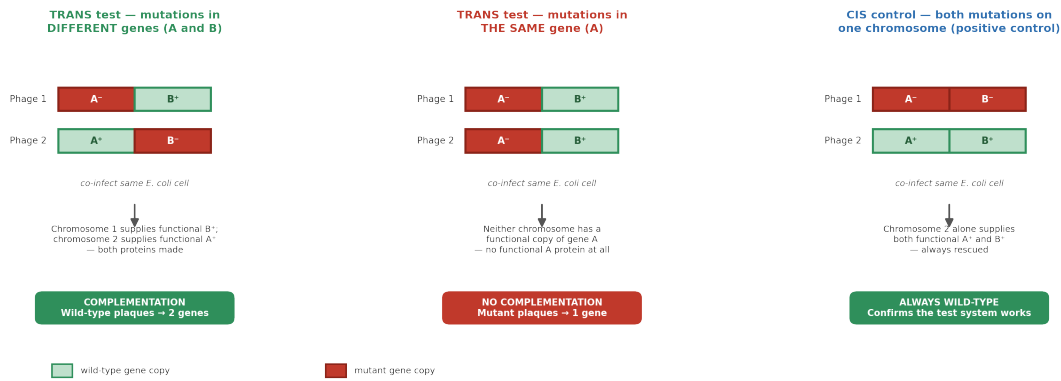


Fig. 1 — The complementation test. When *trans* mutations affect different genes, each chromosome rescues what the other lacks (complementation, wild-type). When they affect the same gene, no chromosome has a working copy (no complementation, mutant). The *cis* arrangement is always wild-type and serves as the control.

2.2 The Functional Gene = the “Cistron”

Benzer coined the term **cistron** for the unit of function defined by this test — operationally, a cistron is the group of mutations that all fail to complement one another. “Cistron” and “gene” are used almost interchangeably today, but the term captures exactly what the cis-trans test measures: a single unit within which mutations interact (intragenic, no complementation) as opposed to across units (intergenic, complementation).

3. Benzer's Experimental System: T4 and the rII Locus

Benzer worked with **bacteriophage T4**, a virus that infects *E. coli*, because phages reproduce in hours, can be grown in astronomically large numbers (billions of particles from a single small culture), and produce visible, countable **plaques** (clear zones of lysis in a bacterial lawn) that report a phage's genotype directly as a phenotype.

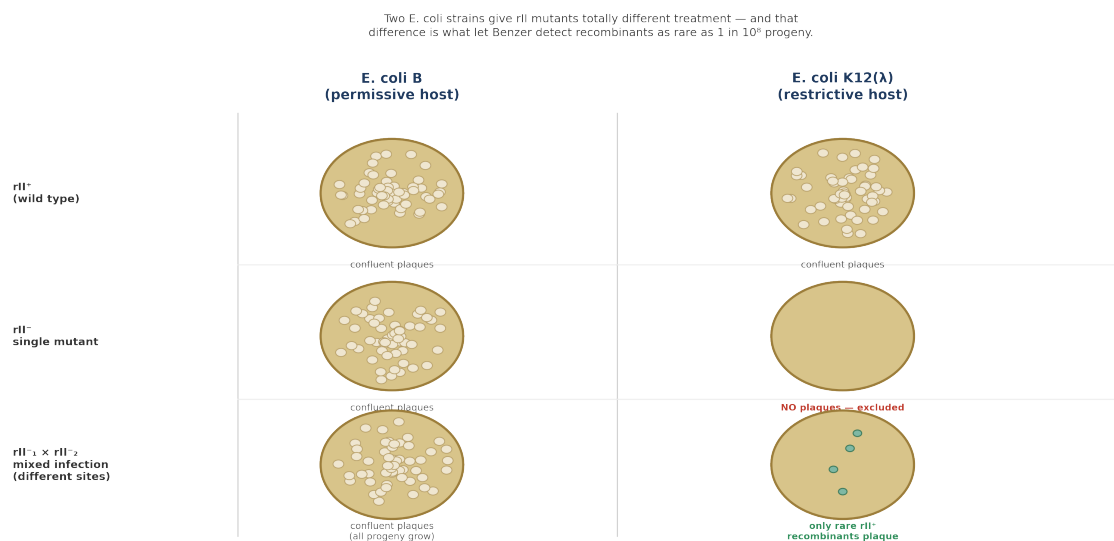
3.1 rII Mutants and Plaque Morphology

Wild-type T4 produces small, turbid-edged plaques on *E. coli* B. A class of mutants, called **rII** ("rapid lysis"), produces distinctively large, sharp-edged plaques instead — an easily scored, visually obvious phenotype. Genetic mapping showed all rII mutations clustered in one region of the T4 genome, organised into **two adjacent cistrons**, **rIIA and rIIB**, both of which must function for normal plaque morphology.

3.2 The Selective Host Trick

The feature that made Benzer's system so powerful was a second bacterial host, ***E. coli* K12(λ)** (lysogenic for phage λ). Wild-type (rII⁺) T4 grows and forms plaques on both *E. coli* B and K12(λ). But **rII⁻ mutants grow on B, yet are completely unable to grow on K12(λ)** — they produce no plaques there at all. This all-or-nothing restriction turned K12(λ) into an extraordinarily sensitive selective screen: mix two different rII⁻ mutants together to co-infect cells, plate on K12(λ), and the only plaques that can possibly appear are from rare wild-type recombinants generated by crossing-over between the two mutant sites during co-infection. Every non-recombinant parental genome is silently excluded, so even a recombination frequency as low as **1 in 10⁸ progeny** stands out as a visible, countable plaque.

Benzer's Selective Host System for the rII Locus



Because K12(λ) gives an absolute NO-GROWTH background for single rII⁻ mutants, even one rare wild-type recombinant plaque stands out perfectly — no need to screen huge numbers of plaques by eye.

Fig. 2 — *E. coli* B tolerates any rII genotype, but *E. coli* K12(λ) excludes single rII⁻ mutants outright. In a mixed infection with two different rII⁻ mutants, only the rare wild-type recombinants can plaque on K12(λ) — giving Benzer a zero-background assay sensitive enough to detect recombination between adjacent base pairs.

3.3 Same System, Two Different Questions

- **To test complementation:** co-infect a K12(λ) cell with two rII⁻ mutants and simply look at cell lysis/plaque formation on a lawn — if the cell lyses normally (functions restored), the mutations complement (different cistrons, A vs B); if the cell fails to lyse normally, they do not complement (same cistron).

- **To test recombination (for mapping):** co-infect an *E. coli* B cell (permissive, so all progeny are produced) with two rII^- mutants, then plate the progeny on K12(λ) — only wild-type recombinants form plaques there, letting Benzer directly count recombination frequency between any two mutations, no matter how close together.

4. Deletion Mapping — Pinpointing Mutations Rapidly

Testing thousands of point mutations against each other pairwise by recombination frequency would have taken millions of crosses. Benzer instead used a set of **deletion mutants** — rII mutants missing a defined stretch of DNA — as fixed rulers against which any new point mutation could be localised in only a handful of crosses.

4.1 The Logic

A point mutation is crossed against a deletion strain and the progeny are plated on K12(λ). If the point mutation lies **outside** the deleted segment, the two genomes can recombine to reconstruct a wild-type sequence, and rare wild-type plaques appear. If the point mutation lies **inside** the deleted segment, no amount of recombination can ever restore a wild-type sequence — the information is simply missing from the deletion chromosome — so *zero* wild-type recombinants are ever seen, however many progeny are screened. By using a graded series of deletions of shrinking, overlapping extent, a point mutation's position can be narrowed down step by step, the same way you'd localise a broken streetlight by asking which overlapping neighbourhoods still have their power on.

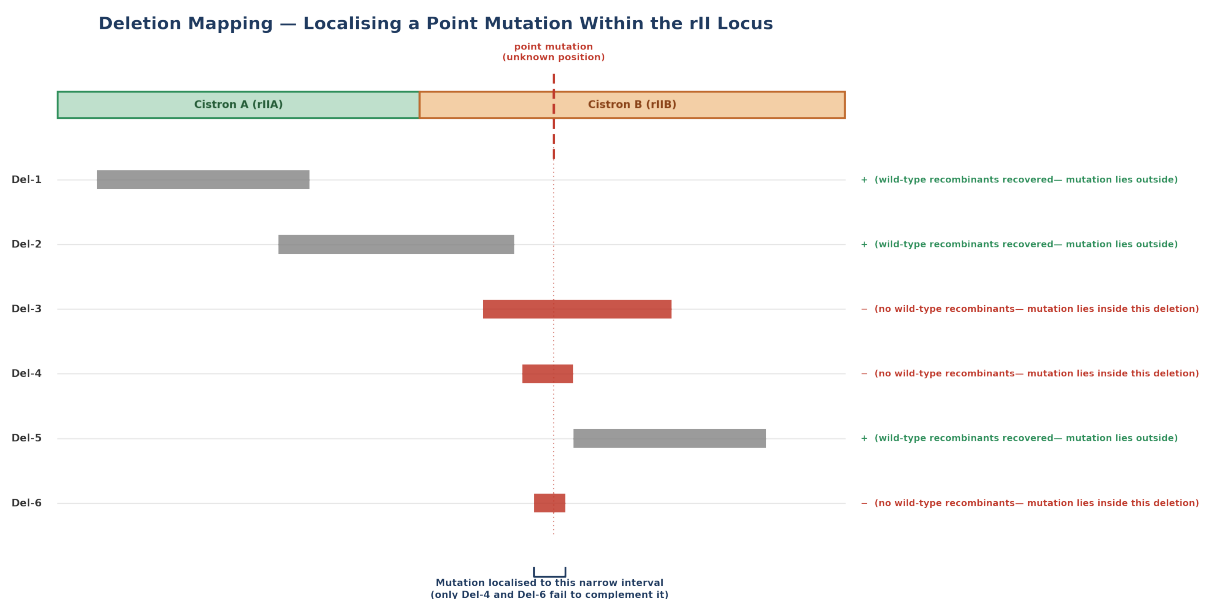


Fig. 3 — Deletion mapping. A point mutation gives wild-type recombinants when crossed against a deletion that does not cover it (+), and none when crossed against a deletion that does (–). Testing against a graded, overlapping series of deletions narrows the mutation's position to a small interval in only a few crosses.

4.2 Two-Tier Strategy

In practice, Benzer first crossed a new mutation against a small set of large deletions spanning the whole rII region to identify which broad sub-segment it fell in, then repeated the process with a nested set of smaller deletions confined to that sub-segment — rapidly zeroing in on a tiny interval. This hierarchical, divide-and-conquer strategy let his lab map thousands of independent mutations across $rIIA$ and $rIIB$ in a fraction of the crosses pairwise recombination testing would have required.

5. What Benzer's Work Established

- **The gene is divisible:** recombination can occur between mutations separated by as little as a single base pair, disproving the older idea of the gene as an indivisible unit of recombination.
- **The gene is linear:** mapping thousands of rII mutations produced a strictly linear, non-branching order — consistent with DNA's linear chemical structure and, later, colinear with the linear sequence of amino acids in the encoded protein.
- **Three distinct units, three different sizes:** Benzer distinguished the **cistron** (unit of function, defined by the complementation test — typically hundreds to thousands of base pairs), the **recon** (the smallest unit that can be separated by recombination — as small as a single base pair), and the **muton** (the smallest unit that can change to cause a mutation — also a single base pair).
- **Non-random mutation distribution:** some sites within rII (“hotspots”) mutated far more frequently than others, an early clue that different DNA sequences have different intrinsic mutability.

Cistron, recon, muton — sizes compared

Think of a gene as a paragraph of text. The **cistron** is the whole paragraph (the unit that must be complete and correct for the sentence's meaning — its function — to come through). The **recon** is the smallest unit you could cut between (down to a single letter) when splicing two paragraphs together. The **muton** is the smallest change (a single misspelled letter) that can alter the meaning. Benzer showed recon and muton are both as small as one DNA base pair, while a cistron spans hundreds to thousands of them.

6. Summary Table

Tool / Concept	Question it Answers	Readout
Complementation test (cis-trans test)	Are two mutations in the same gene (cistron) or different genes?	Trans: wild-type (complement, different genes) or mutant (same gene)
E. coli B	Permissive control host	All rII genotypes form plaques
E. coli K12(λ)	Selective host — detects rII ⁺ function or rare recombinants	Only rII ⁺ phage (or rare recombinants) form plaques
Recombination mapping	How far apart are two mutations physically?	Frequency of wild-type recombinant plaques on K12(λ)
Deletion mapping	Where exactly does a point mutation sit?	Presence (+) or absence (–) of wild-type recombinants vs a deletion strain

7. Quick Revision Points

- Complementation test: **trans** arrangement is diagnostic; **cis** arrangement is the always-wild-type control.
- Complementation (wild-type in trans) → different genes. No complementation (mutant in trans) → same gene.
- rII locus of phage T4 has two cistrons: **rIIA** and **rIIB**.
- *E. coli* B = permissive (grows any rII genotype). *E. coli* K12(λ) = restrictive (excludes rII[–] single mutants; only rII⁺ or rare recombinants plaque).

- This selective host let Benzer detect recombination frequencies as low as 1 in 10^8 , resolving distances down to a single base pair.
- Deletion mapping: no recombinants ever appear if the point mutation lies **inside** the deletion; recombinants appear normally if it lies **outside**.
- Key units, smallest to largest: **muton** (smallest mutable unit) $\approx$ **recon** (smallest recombining unit) $\approx$ 1 base pair, much smaller than a **cistron** (unit of function, hundreds to thousands of base pairs).
- Benzer's work proved the gene is linear, divisible by recombination, and colinear with its protein product.

Study notes compiled for revision purposes — cross-check with your prescribed textbook (e.g. Griffiths et al., An Introduction to Genetic Analysis; Benzer, S. (1961) "On the Topography of the Genetic Fine Structure," PNAS) before an examination.