Global Viral Diversity

60 known families 20 affect humans (50 distinct types of adenoviruses infect humans!).
With about 10 million species of bacteria and maybe 10 host specific phage/species (E. coli has 50) = 100 million phage species!!

Classification

1) DNA vs RNA

2) Single stranded (ss) vs double stranded (ds)

3) For RNA, RNA replicase/RNA dependent RNA polymerase (RNA as template for RNA)

vs.

Reverse transcriptase (RNA as a template for DNA)

Table 1. Viral Diversity

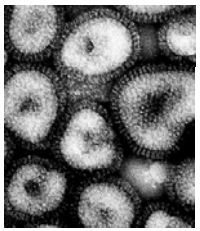
Genetic Material	Families	Example Families	Example Genus	Example Viruses
dsDNA	20	*Herpesviridae*	*Rhadinovirus*	Human herpesvirus 8
ssDNA	5	*Parvoviridae*	*Dependovirus*	Adeno-associated virus 2
RNA/DNA[a]	5	*Retroviridae*	*Lentivirus*	HIV-1
dsRNA	6	*Reoviridae*	*Rotavirus*	Human rotavirus group A
−ssRNA	7	*Orthomyxoviridae*	*Influenzavirus A*	Influenza A virus
		Paramyxoviridae	*Morbillivirus*	Measles virus
		Filoviridae	*Ebolavirus*	Zaire Ebola virus
+ssRNA	18	*Picornaviridae*	*Enterovirus*	Poliovirus

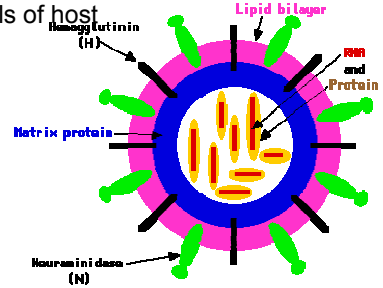
[a]Viruses that reverse transcribe RNA into DNA.

Influenza virus: -ssRNA of 8 short segments (genes)

Epidemics for 1000s of years, but the influenza A (flu) versions are continuously introduced from animal reservoirs.

Antigen surface drifts (gradual small adaptive changes in surface proteins **hemagglutinin, H** and **neuraminidase, N**, selected by Natural Selection): Hemagglutinin binds to glycoproteins on epithelial cells of host





- **HA encodes hemagglutinin** (about 500 molecules of hemagglutinin are needed to make one virion)
- **NA encodes neuraminidase** (about 100 molecules of neuraminidase are needed to make one virion).
- **NP encodes nucleoprotein**.
- **M encodes two matrix proteins** (the M1 and the M2) by using different reading frames from the same RNA segment (about 3000 matrix protein molecules are needed to make one virion).
- **NS encodes two distinct non-structural proteins** (NS1 and NEP) by using different reading frames from the same RNA segment.
- **PA encodes an RNA polymerase**.
- **PB1 encodes an RNA polymerase and PB1-F2 protein (induces apoptosis)** by using different reading frames from the same RNA segment.
- **PB2 encodes an RNA polymerase**.

Influenza virus:

Flu shots are designed for annual drifts

Antigen surface shifts (new version from bird reservoirs), 1957 Asian flu **H2N2** (differs from **H1N1** by 35% and 58% of the amino acids); 1968 Hong Kong flu **H3N2,** 1997 Hong Kong **H5N1***). Recombination important.

The worst epidemic was Spanish flu 1918 **H1N1** with 20-40 million killed (600,000 Americans)!!!
1aa change in H receptor shifted the virus from recognizing α2,3 sialic acid receptor in birds to α2,6 sialic acid receptor in humans!

Date	Strain	Subtype	Notes
1918	Spanish Flu	H1N1	pandemic of Spanish Flu
1957	A/Singapore/57	H2N2	pandemic of "Asian"
1962	A/Japan/62	H2N2	epidemic
1964	A/Taiwan/64	H2N2	epidemic
1968	A/Aichi/68	H3N2	pandemic of "Hong Kong"
1976	A/New Jersey/76	H1N1	swine flu in Army recruits
1977	Russian/77	H1N1	age restricted <25 years old

Recombination and jumping hosts creates "shifts" and pandemics

Mutations within a host strain creates "drifts" and

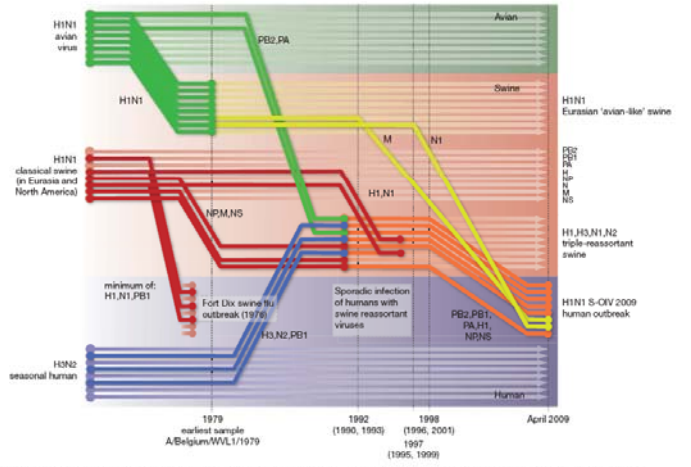


Figure 1 | Reconstruction of the sequence of reassortment events leading up to the emergence of S-OIV. Shaded boxes represent host species; avian divergence of the S-OIV genes from corresponding virus lineages. Reassortment events not involved with the emergence of human disease are

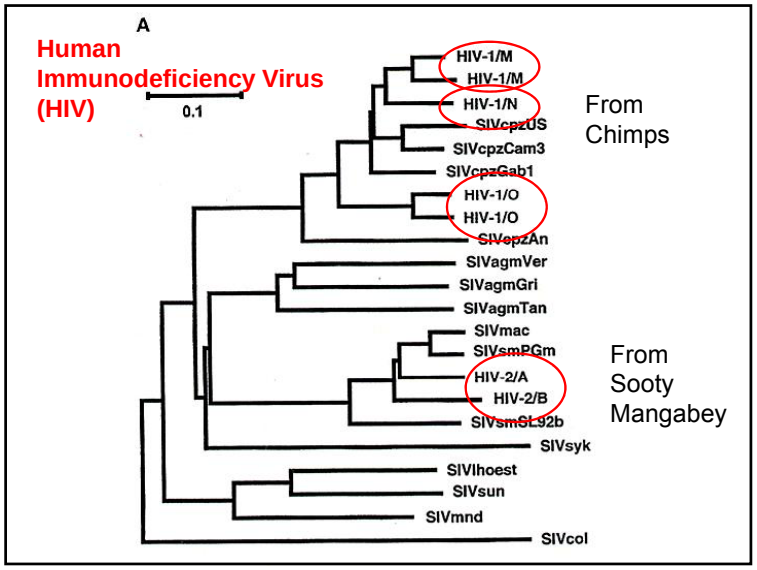
Origin of H1N1/09 Swine Smith et al. 2009 Nature 459: 1122

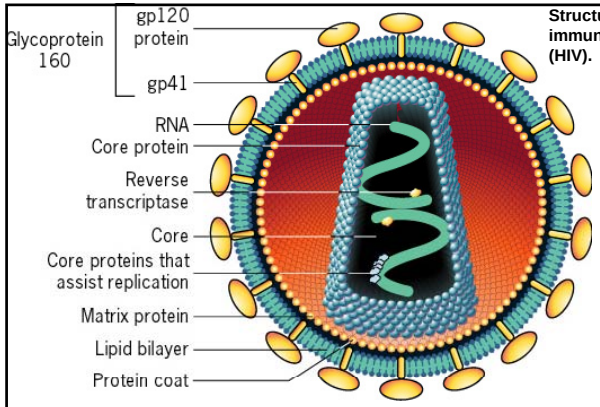
HA	**Hemagglutinin**	**swine (H1)**	**North America**
NA	**Neuraminidase**	**swine (N1)**	**Europe**
PA	**RNA polymerase subunit PA[43][44]**	**avian**	**North America**
PB1	**RNA polymerase subunit PB1[45]**	**human**	**1993 H3N2 strain**
PB2	**RNA polymerase subunit PB2[46]**	**avian**	**North America**
NP	**Nucleoprotein[47]**	**swine**	**North America**
M	**Matrix protein M1, M2**	**swine**	**Eurasia**
NS/NEP	**Non-structural proteins NS1, NEP (Nuclear Export Protein)[48][49]**	**swine**	**North America**

Case Studies of Human Viruses

Herpes viruses: dsDNA, huge 100kb with 70 genes including recent human genes!!!
Extremely old (millions of years) present in common ancestors of mammals and most are "benign". Only very rare cases of jumping species (one or two possible case in all of mammals).
Mutation rate is slow 3 x 10^{-8} (10^5 or 10^6 times slower than HIV).

Ebola: -ssRNA
First appeared in 1976 in Sudan and Zaire. Reappeared in same or neighboring areas1979, 1995, 2000, 2002, 2003. "Hemorrhagic Fever"
Threatens chimp and gorilla populations (1000s died). Maybe originated in duiker (antelope) populations.
Four separate versions each from different reservoir.
Fatality rates of 50%-90%!!!

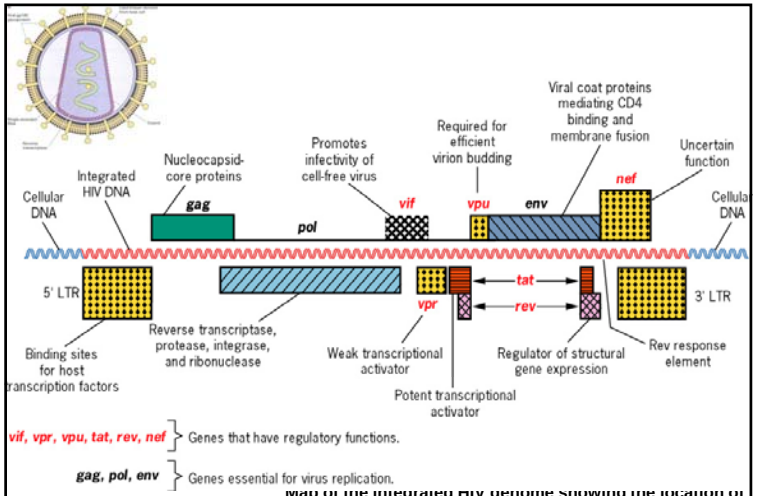




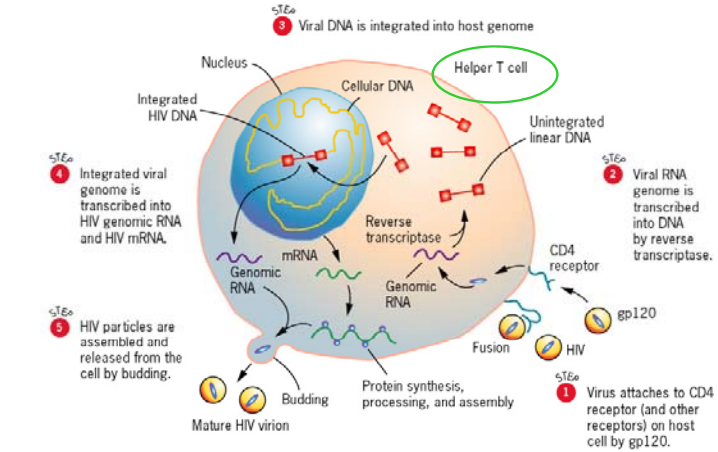
Structure of the human immunodeficiency virus (HIV).

HIV: Retrovirus with RNA to DNA replication

***HIV-1 from Simian IV in Chimps in central Africa. (Three transfers M,N,O).**
***HIV-2 from SIV in sooty mangabeys in west Africa with 4+ separate cross species jumps.**
***Oldest strain is in 1959 blood sample and probably made the jump to humans around 1930.**
***Fast rate of evolution (million x faster than your genes, 1x10^{-2} amino acid substitutions/site/year).**



Map of the integrated HIV genome showing the location of regulatory genes and genes encoding important viral proteins.



Overview of the life cycle of HIV.

Why is HIV so difficult to control?

1) High mutation rates, RT has 10x error rate of normal polymerases.

2) Hotspot for errors is the ***env*** gene that makes the gp120 proteins on viral surface. These are constantly changing and avoid detection.

3) Favorite target of HIV for incorporation in to host genome is the T helper cells. This compromises immune response.

4) HIV favors integrating into active genes once in cell. This guarantees transcription and avoidance of degradation.

We discussed the reasons that bacteria evolve so quickly.

Do we see some similar reasons that explain why viruses also evolve quickly?

Huge numbers, high mutation rates and strong selection.

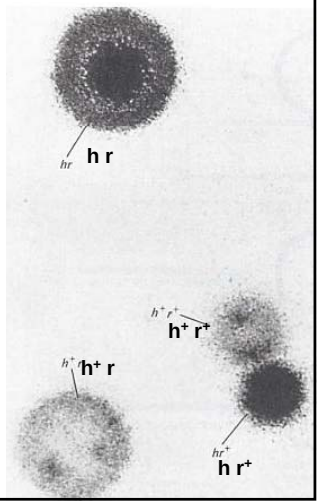
How about exchanging genes (recombination) in viruses?

Recombination and mapping in Viruses (Hershey 1946)

T2 viruses

hr^+ x $h^+ r$

Combine different strains or mutant lines into a single bacterium and see if the viral genomes recombine



r = rapid lysis (big plaques)

r+ = normal lysis

h = extended host range (dark center) E. coli B & B2

h+ = normal host range; *E. coli* B strain only

1) Mixed infections put different mutant viruses into the same host cell:

hr⁺ x h⁺ r

Genotype	Plaques	
hr⁺	42	76% parental
h⁺ r	34	
h⁺ r⁺ recombinant	12	24%
h r	12	

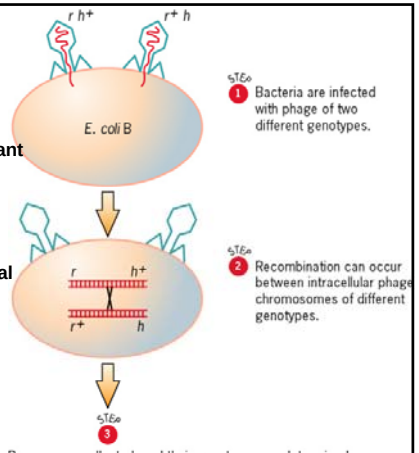


Fig 16.6 A cross between *r h+* and *r+h* bacteriophage.

Benzer's T4 mutants

1) Generate mutants with UV, X-ray or mutagenic chemicals

2) Dilute and grow on a "lawn" of E.coli strain B.

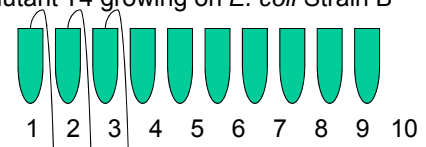
3) Test each colony on Strain K12.

Any line that can not grow on K12, has a mutation that prevented its use of this host.

Benzer generated 1000s of lines that could grow on Strain B but not K12. He performed complementation tests to determine if these mutants were for the same gene or different genes

Mutant T4 growing on *E. coli* Strain B

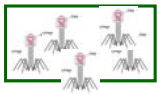
Complementation Test



None of these mutants can grow on K12 by themselves

K12 → No Cell lysis

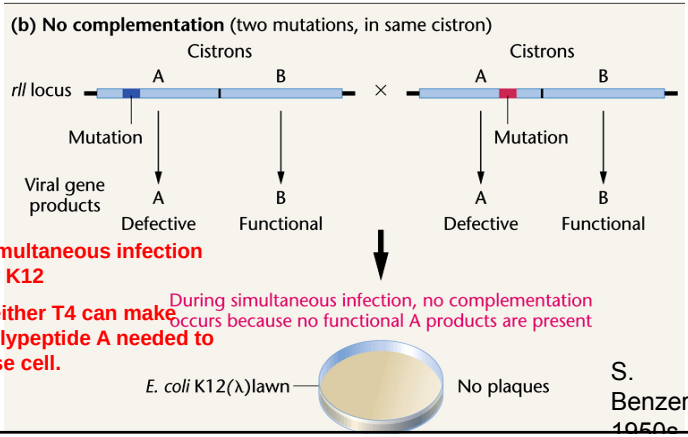
Conclusion: mutants are in the same gene



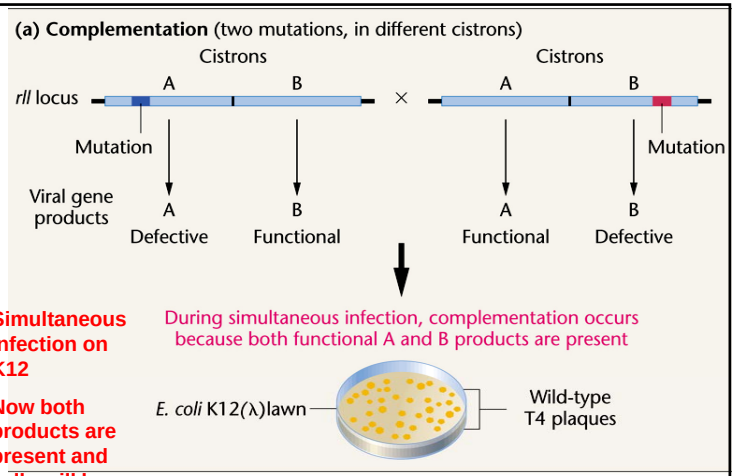
Conclusion: mutants are different genes

Benzer screened 1000s and concluded that he had 2 complementation groups and thus 2 different genes

rII mutants A or B of T4 phage can grow on and lyse *E.coli* strain B, but not strain K12



S. Benzer 1950s



Simultaneous infection on K12

Now both products are present and cells will be lysed!

Rapid Evolution in Viruses

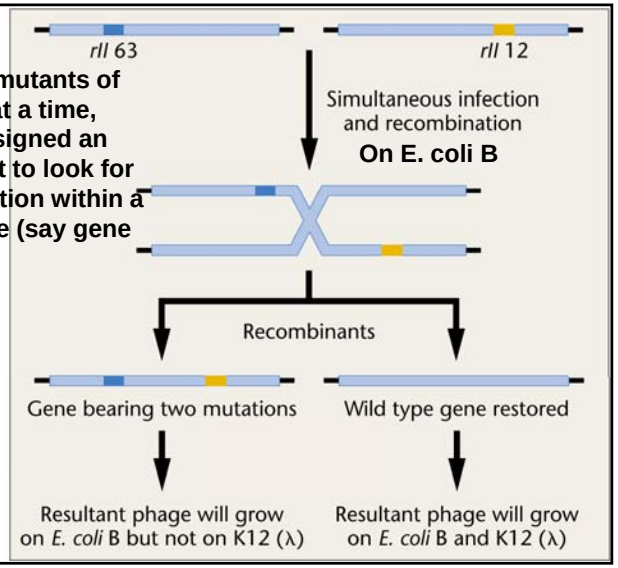
1) We have seen that if different genotypes simultaneously infect the same host cell, intergenic recombination can occur, e.g. Hershey experiment with T2 phage.
   hr^+ x h+ r → $h^+ r^+$ and hr recombinants

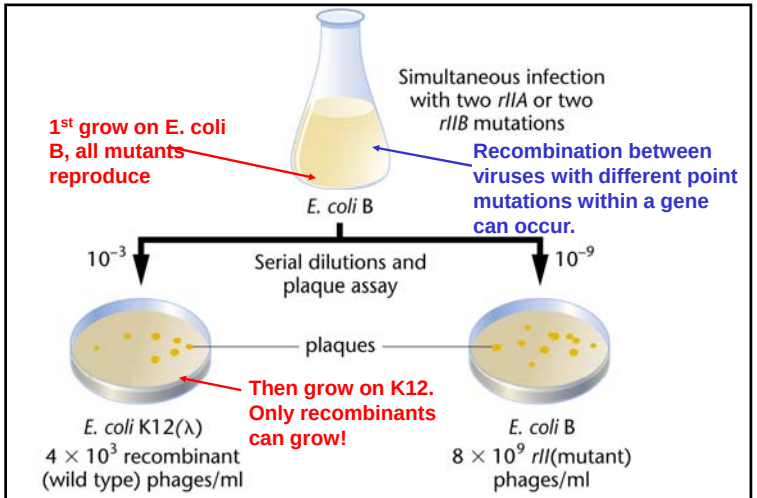
2) We will also see that, for the first time, recombination can occur within a gene (intragenic recombination).

To do this Benzer created and isolated 1000s of mutant T4 phage that could not grow on or lyse E. coli strain K12 (they could grow fine on typical stain B).

Benzer used a complementation test to see whether his mutants were in one or more different genes. He found that there were two genes (*rIIA* and *rIIB*), both required for lysis of K12.

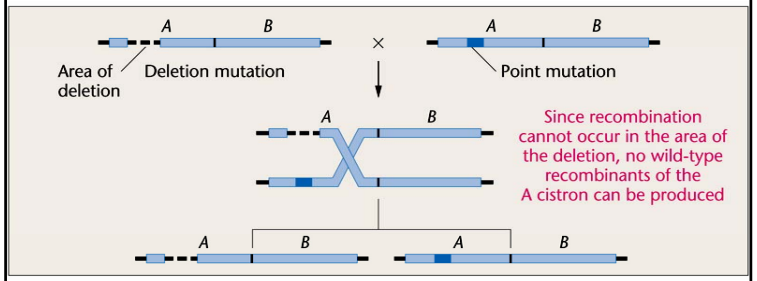
Using the mutants of one gene at a time, Benzer designed an experiment to look for recombination within a single gene (say gene rIIA)?



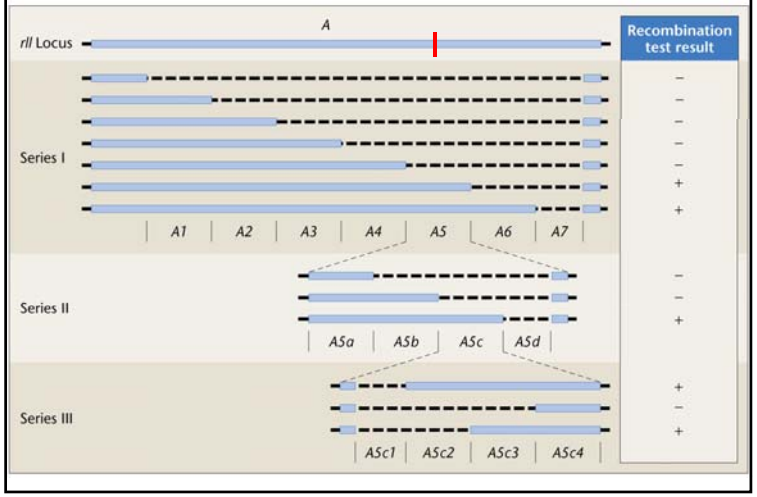


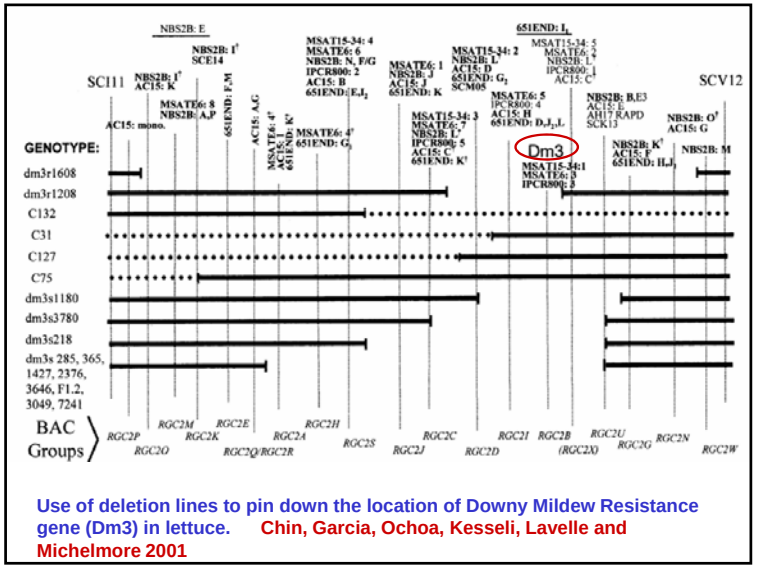
2(recombinants/total) = map distance: $2(4x10^3/8x10^9) = 1x10^{-6}$

Benzer also generated deletion mutations and used these to order point mutations and dissect the genes.

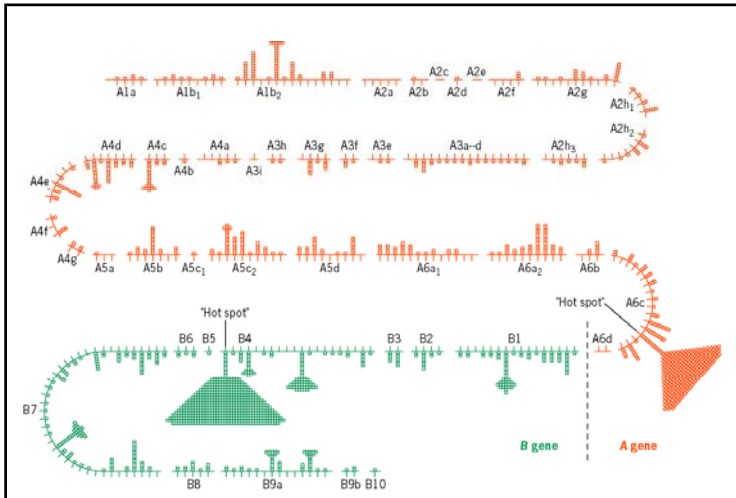


Deletion Maps allowed for rapid placement of single base mutations within a gene.





Use of deletion lines to pin down the location of Downy Mildew Resistance gene (Dm3) in lettuce. Chin, Garcia, Ochoa, Kesseli, Lavelle and Michelmore 2001



The genetic map of the *rII* locus of phage T4.

Complementation Test

Purpose:

To screen unknown mutants that give the same phenotype (can not lyse K12) to see if they are in the same gene

Procedure:

Simultaneous infection of 2 T4 mutants on *E. coli* K12; Neither can grow or lyse cells of K12 on its own.

Results:

1) If phage can not lyse cells, the mutations must be in same gene

2) If phage can lyse cells, mutations in different genes

Recombination, Fine Mapping

Purpose:

To map location of mutations to a known gene.

Procedure:

Simultaneous infection of 2 T4 mutants on *E. coli* B; both can grow on this strain.

Recombination can occur between the T4 mutants while they are in strain B.

Results:

Screen for recombinants on strain K12. Mutants far apart will show more recombinants than close ones

In the analysis of rII mutants of T4 phage, complementation testing yielded the following results:

Simultaneous Infection of 2 mutants on E.coli K12	Results (lysis = +)
1, 2	+
1, 3	+
1, 4	-
1, 5	-

Predict the results for the following:

	A	B	C	D	E
2, 3	-	-	+	-	+
2, 4	-	+	-	+	+
3, 4	-	+	-	-	-