

# RECOMBINATION IN BACTERIA

rvsd 2/2/94, 1/30/95, 1/31/96, 31 Jan 00, 5 Feb 03, 8 Feb 08, 9Feb09, 31Jan11  
 SGML, p 219-) GMSLG 7th, pp 208-220), 181-213,

Working with bacteria: aseptic techniques: spreading, top agar, streaking, media selection, (p 184)

**Conjugation** (together, join, process): **F = fertility factor:** (p 187)

1. replicates independently to host chromosome
2. codes for F pili production
3. Enables transfer of newly synthesized copy of F
4. Occasionally recombines with host chromosome (If stable termed Hfr)

<http://www.youtube.com/watch?v=IYW6wwEAnqs>

<http://www.youtube.com/watch?v=eRvfhjxA4s>

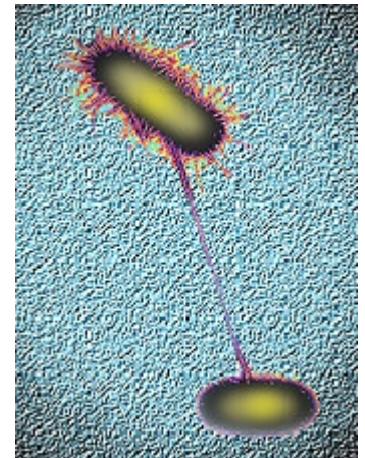
1946: Lederberg and Tatum: mixed:

male:	A:	met-	bio-	thr+	leu+	thi+
female:	B:	met+	bio+	thr-	leu-	thi-

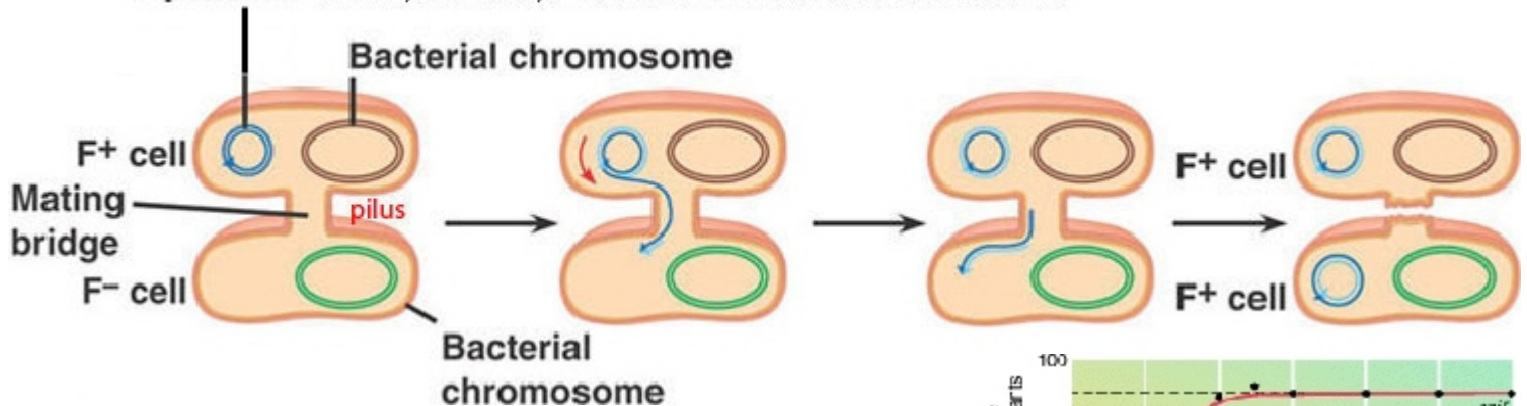
Plated out on MM, mixture got one prototroph per 10,000,000 cells (no colonies/ $10^8$  on A or B alone)  
 cross feeding? (in fine filter U tube) not the reason, required direct contact

1953: Hayes modified expt: *strep* treatment of one or other strains (prevents cell division, but permits mating for short time), washed out. When treated one strain, prevented recombinants, but other did not: thus  
**transfer is not reciprocal: donor** not stopped by strep; **recipient** inhibited by strep

**Conjugation:** Luca Cavalli-Sforza found strain which made 1000x as many recombinants,  
 Termed **Hfr** (high frequency recombination: F factor integrated into the chromosome)



## F plasmid - small, circular, extrachromosomal DNA molecule



**Transfer is linear:** (p 190)

1957, Wollman and Jacob **interrupted mating:**

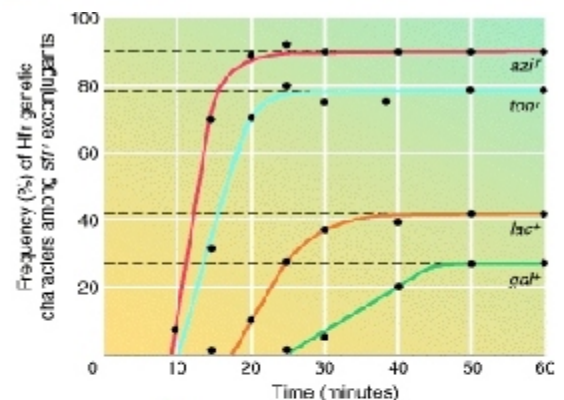
$str^r$  multiply auxotrophic recipient,  $str^s$  prototrophic donor, blend to disrupt mating, plate on strep medium, score for *azi* r, *gal* +, *lac* +, *ton* r.

Found *azi* first, then *ton*, then *lac*, then *gal*.

interpreted linear transfer, linkage (p. 190)

F factor finally transferred after 2 hrs

Tried several different Hfr, got different time of entry for markers.



Concluded that **bacterial chromosome is circular** (p 191)

Episome: F which can either be integrated or free  
 plasmid: only found in free state, RTF, etc

Not used:

Then plated on medium supplemented with one of the five requirements, (requiring recombination of other four markers) then scored colonies for +/- for that unselected, found linkage as shown on p 224

could see that selection for *thr*, *leu* and *thi* pos, brought *bio*+ 60/70 time, therefore linked

