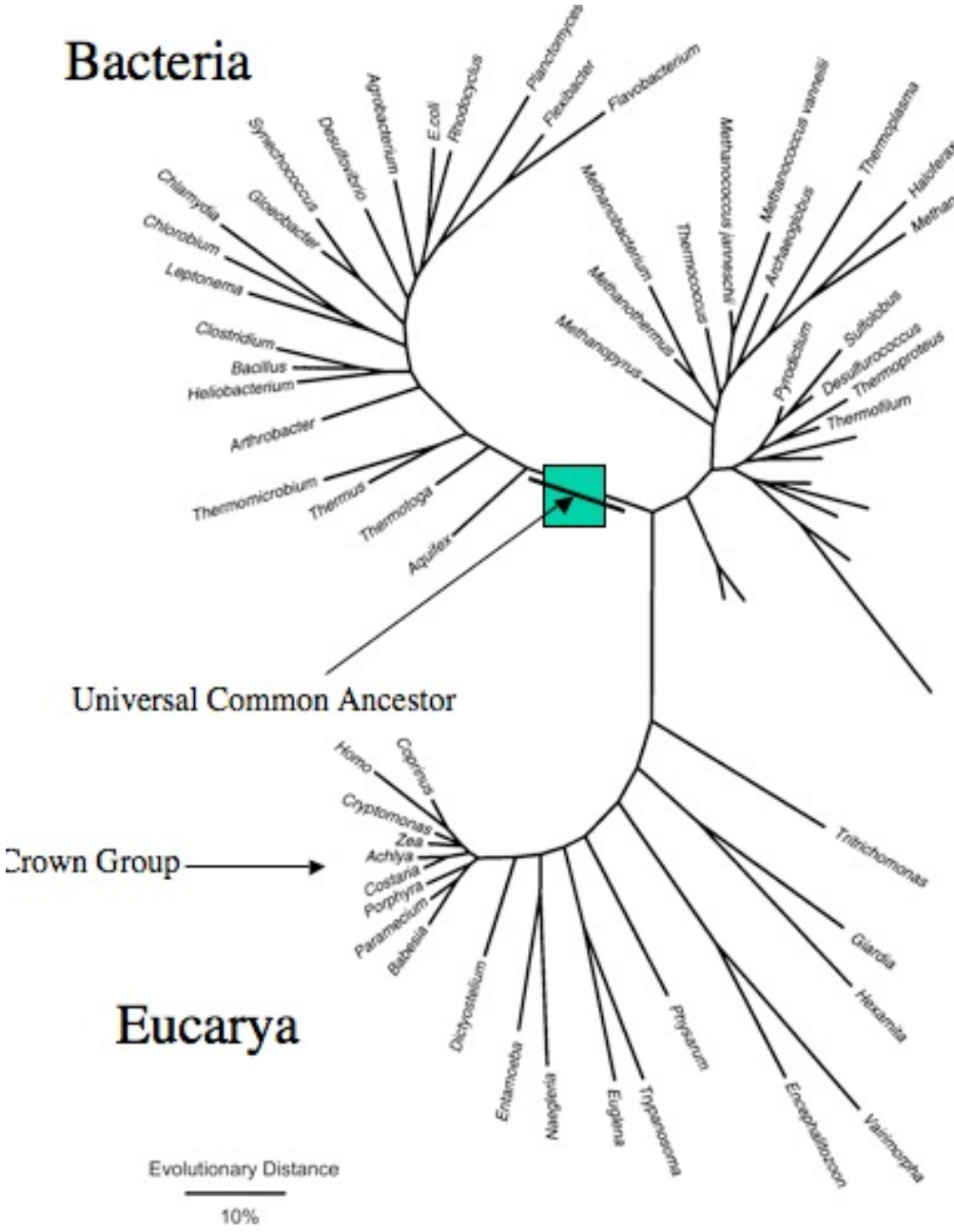
Prokaryotes

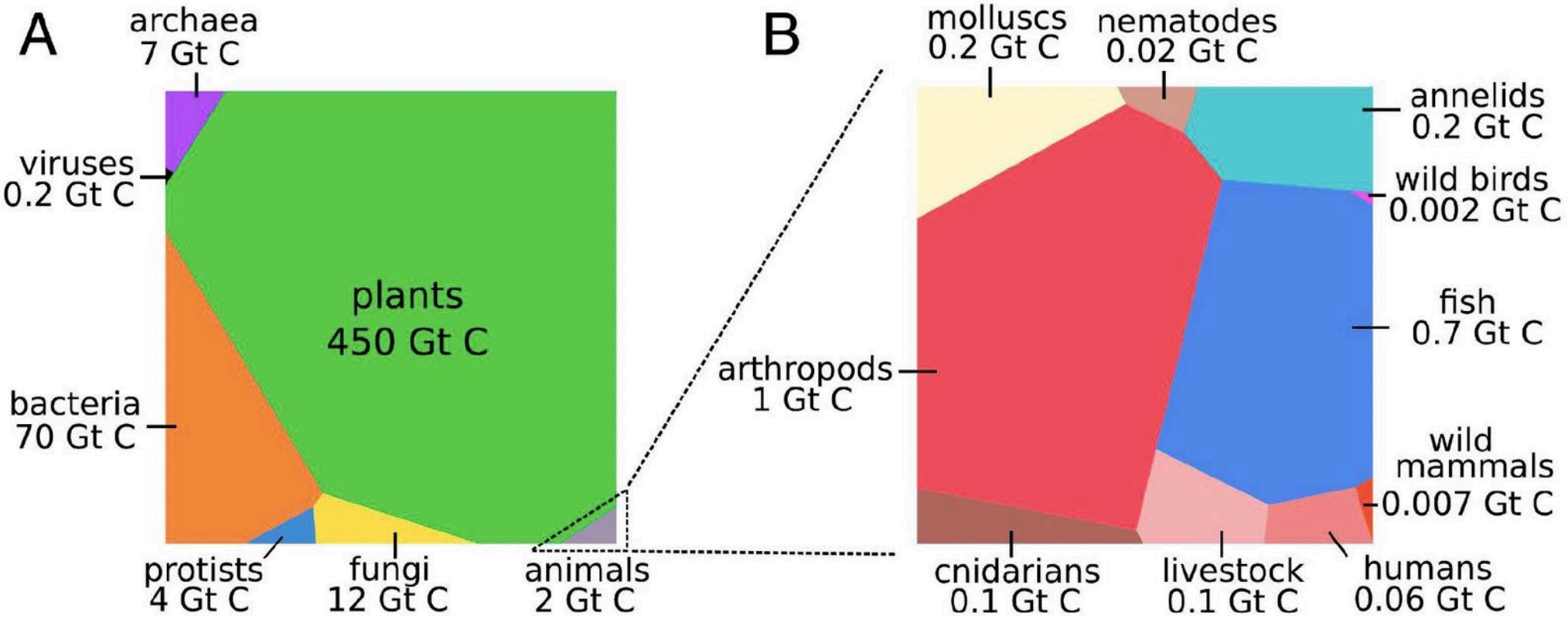
- 200 nm - 500 µm in size,
- soil, water, air, symbionts,
- have adapted to aquatic and terrestrial extremes,

100 grams/person, 10^{14} bacteria.

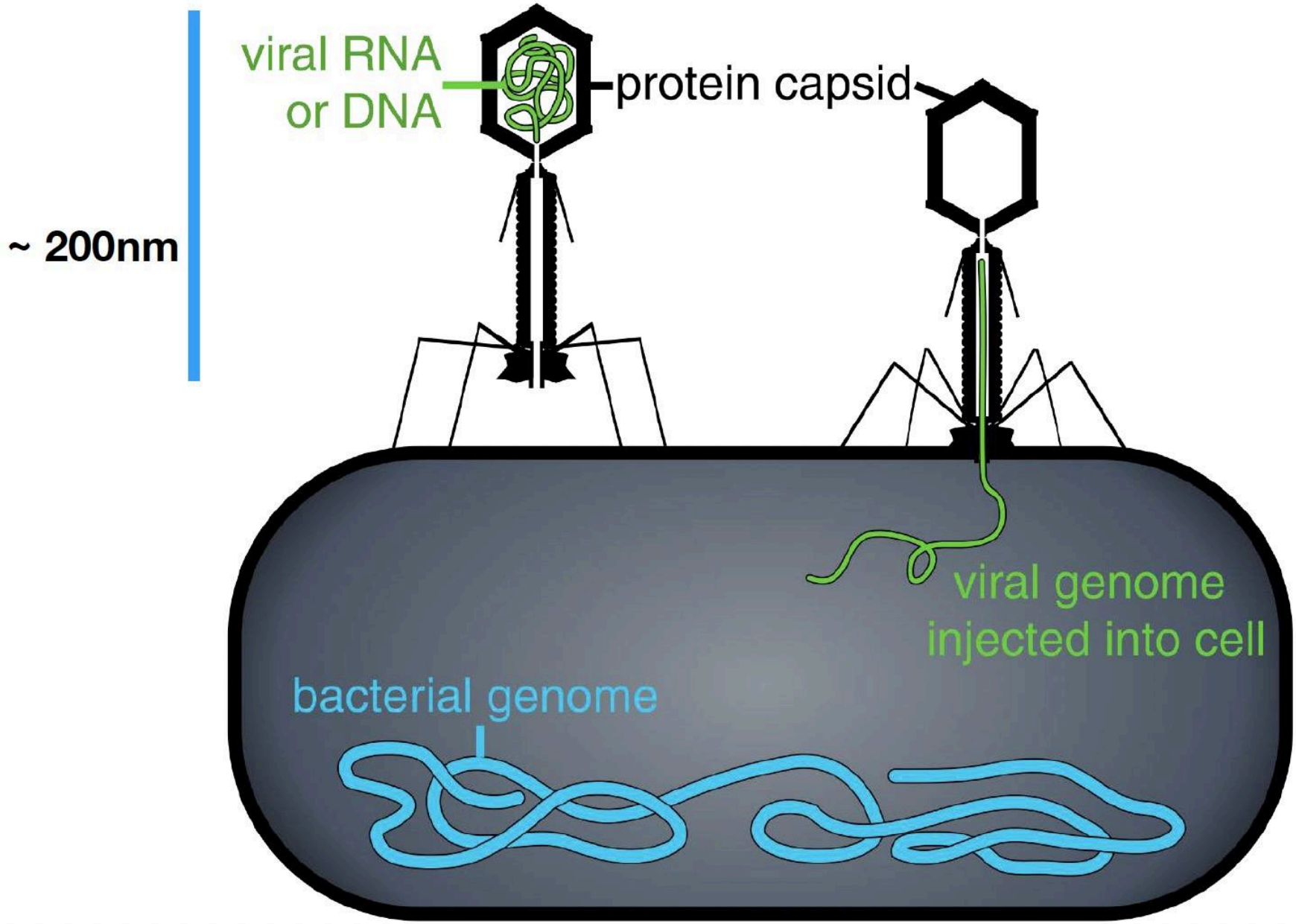
Up to 50% of the carbon on Earth



The sum of the biomass across all taxa on Earth is ≈550 Gt C, of which ≈80% (≈450 Gt C) are plants, dominated by land plants (embryophytes). The second major component is bacteria (≈70 Gt C), constituting ≈15% of the global biomass. Other groups, in descending order, are fungi, archaea, protists, animals, and viruses, which together account for the remaining <10%.



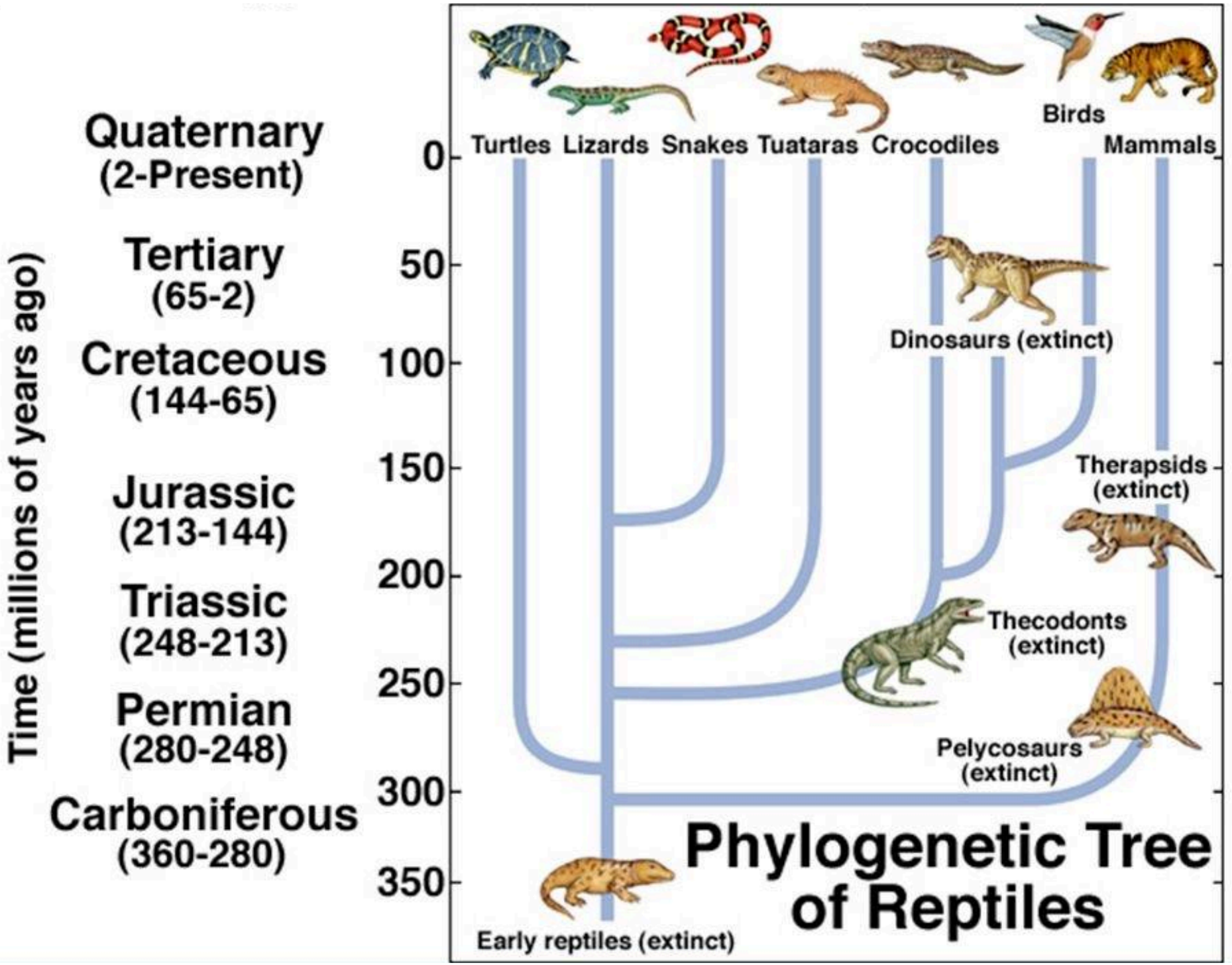
Virus


~ 200nm
viral RNA
or DNA
protein capsid
viral genome
injected into cell
bacterial genome

Bacteria and Viruses by the (Rough) Numbers

"if all the 1×10^{31} viruses on earth were laid end to end, they would stretch for 100 million light years".

- Bacteria on Earth: ~3×10^{30} (Nature 27 August 2012 - revised number)
- Bacteria in oceans: 1.3×10^{29} (=number of stars in the known universe)
- Viruses on Earth 1×10^{31}
- Virus infections in the ocean per day = 1×10^{23} (removes 20-40% of all bacterial cells per day)
- Single SV40 virus has 958,980 atoms
- Bacteria per teaspoon (~5ml) of soil 1×10^{9} (= number of humans in Africa)
- Bacteria per one gram of dental plaque 1×10^{11} (= number of humans who have ever lived)
- 8% of human genome is remnants of previous viral infections
- Only 1% of bacterial species are human pathogens (but there are ~1,000 that are pathogens)
- Viable bacteria have been extracted from amber after >30,000 years


Quaternary
(2-Present)
Tertiary
(65-2)
Cretaceous
(144-65)
Jurassic
(213-144)
Triassic
(248-213)
Permian
(280-248)
Carboniferous
(360-280)
Time (millions of years ago)
0
50
100
150
200
250
300
350
Turtles
Lizards
Snakes
Tuataras
Crocodiles
Birds
Mammals
Dinosaurs (extinct)
Therapsids
(extinct)
Thecodonts
(extinct)
Pelycosaurs
(extinct)
Early reptiles (extinct)
Phylogenetic Tree
of Reptiles

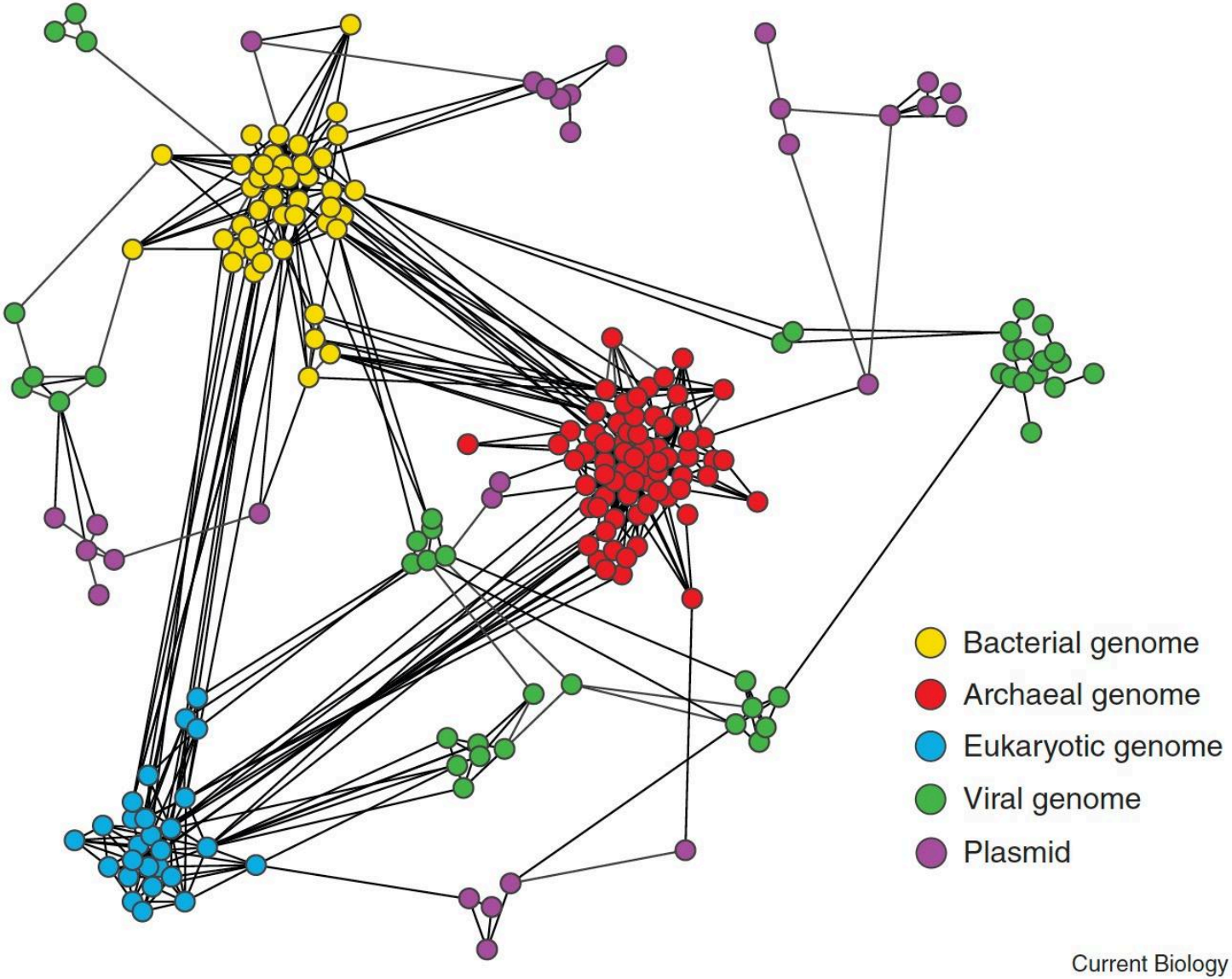
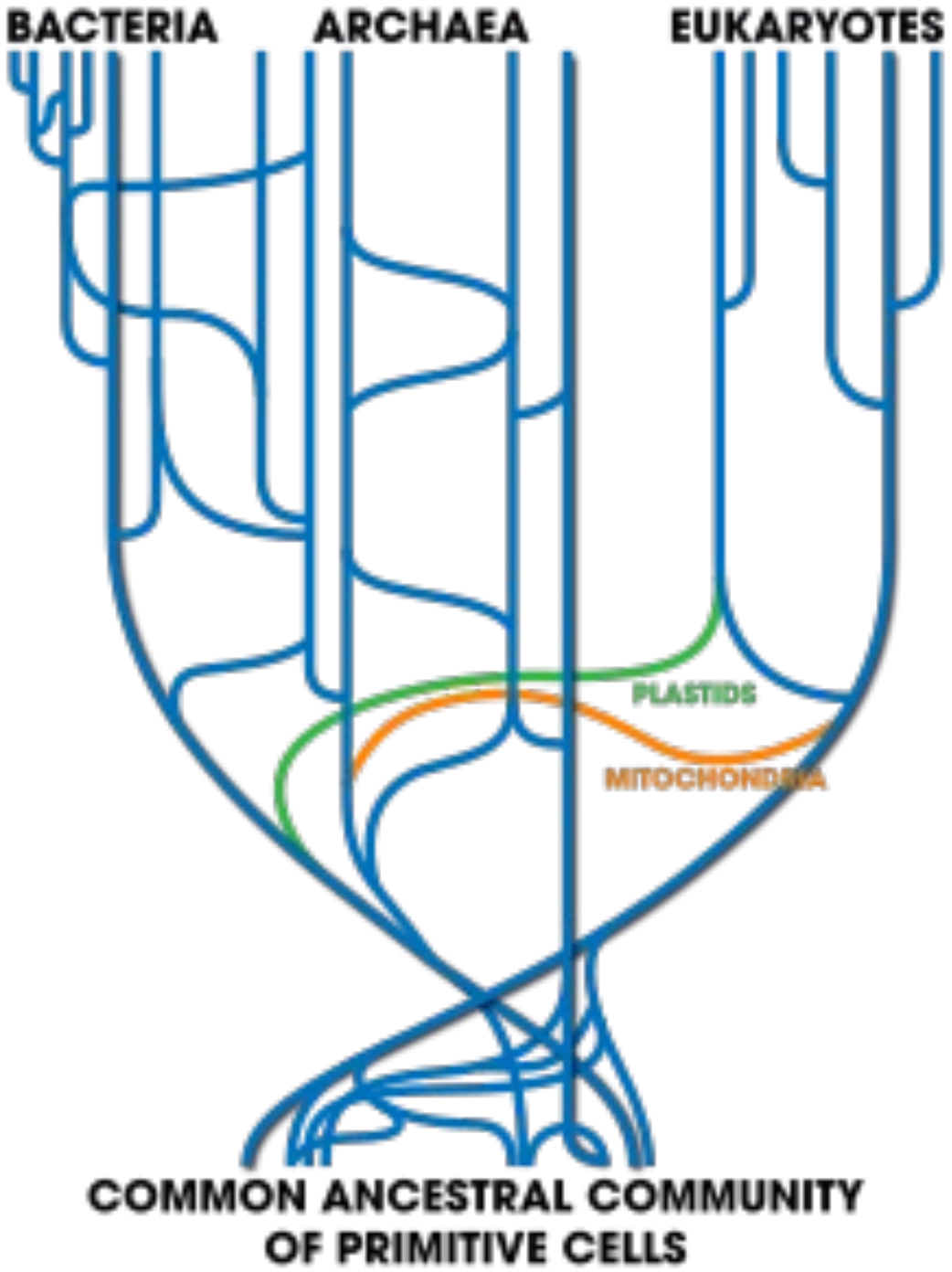


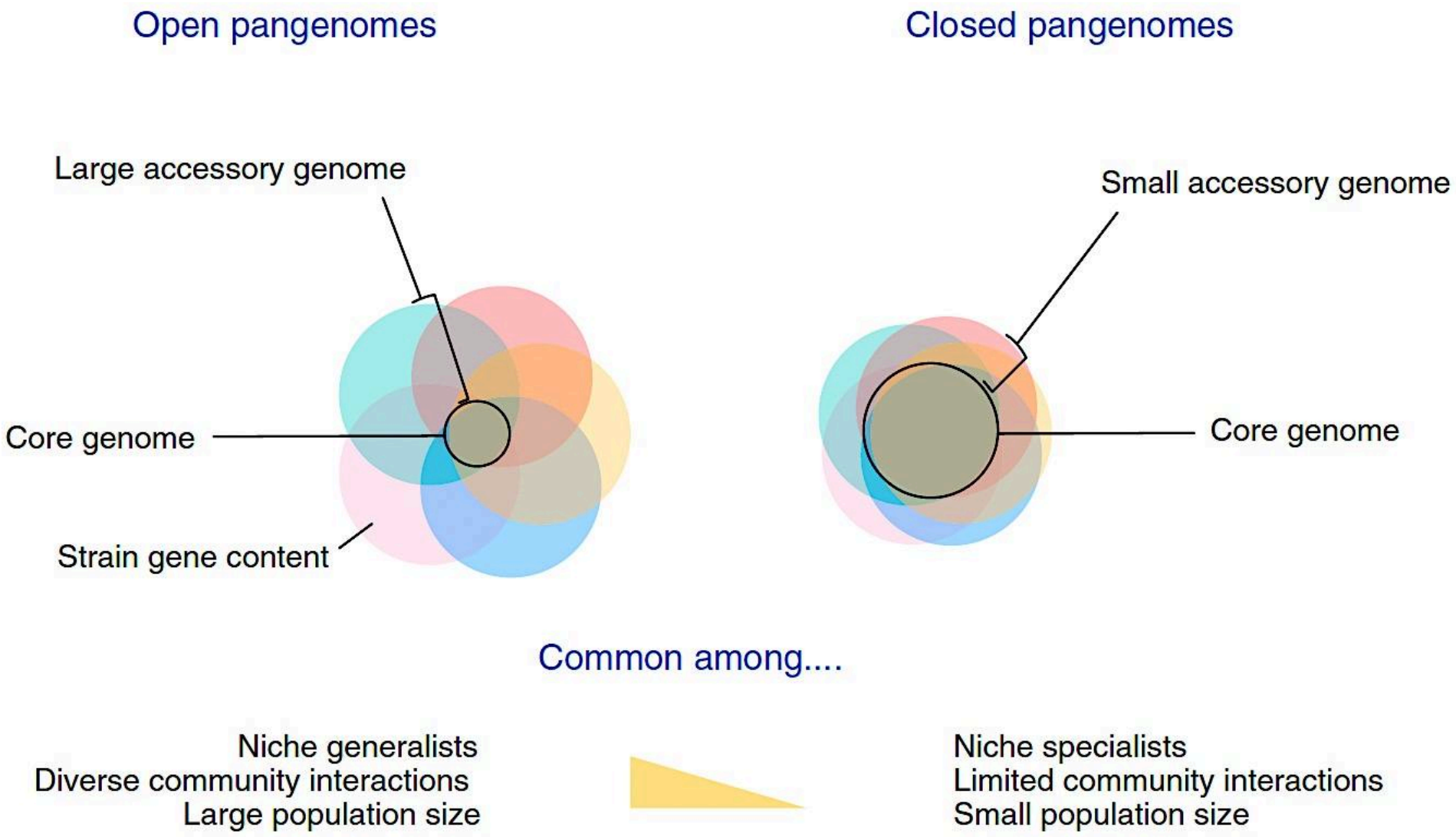
Figure 4. Genetic worlds revealed by networks.
Qualitative rendition of Halary *et al.*'s network of genetic diversity[59]. Nodes represent genomes and two nodes are connected if they share homologous DNA sequences. The genomes of cellular life forms (archaea, bacteria and eukaryotes) and viruses, as well as plasmids and other mobile genetic elements, cluster into discrete genetic worlds defined by frequent intra-world lateral and vertical gene inheritance. Connections between these worlds organize them into an overarching network.

**…All in all,
it appears that only a small fraction of prokaryote genes
are actually consistent with a universal phylogenetic tree a
life [13], thereby suggesting that most prokaryote genes are eventually exchanged over long evolutionary time scales [1]….**



Gene shuffling without sex

The pangenome: The set of all genes



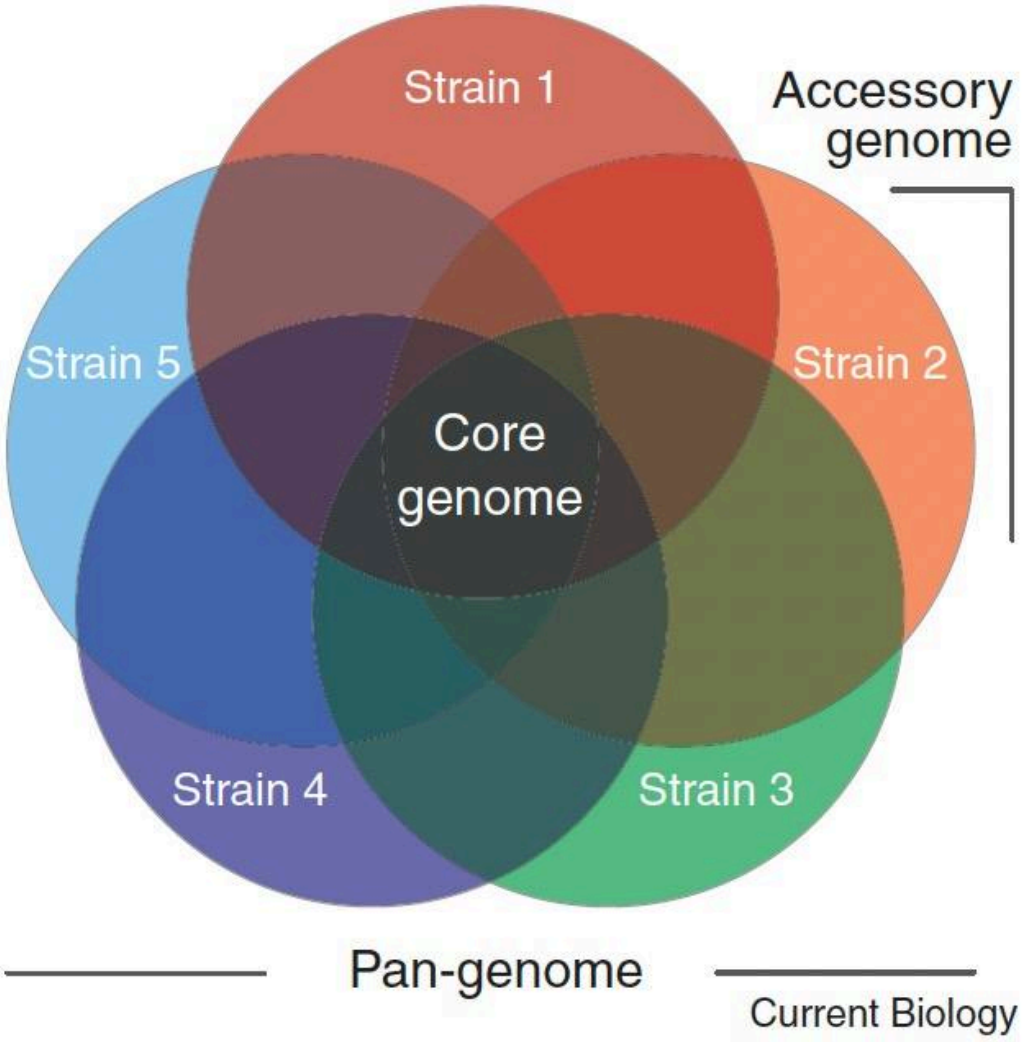


Figure 3. The pan-genome.
The complete set of genes found in members of a given species can be represented as a Venn diagram. Genes shared by all (or most) strains form the core genome, whereas genes found only in some comprise the accessory genome.

Genome size in free-living prokaryotes is tightly constrained

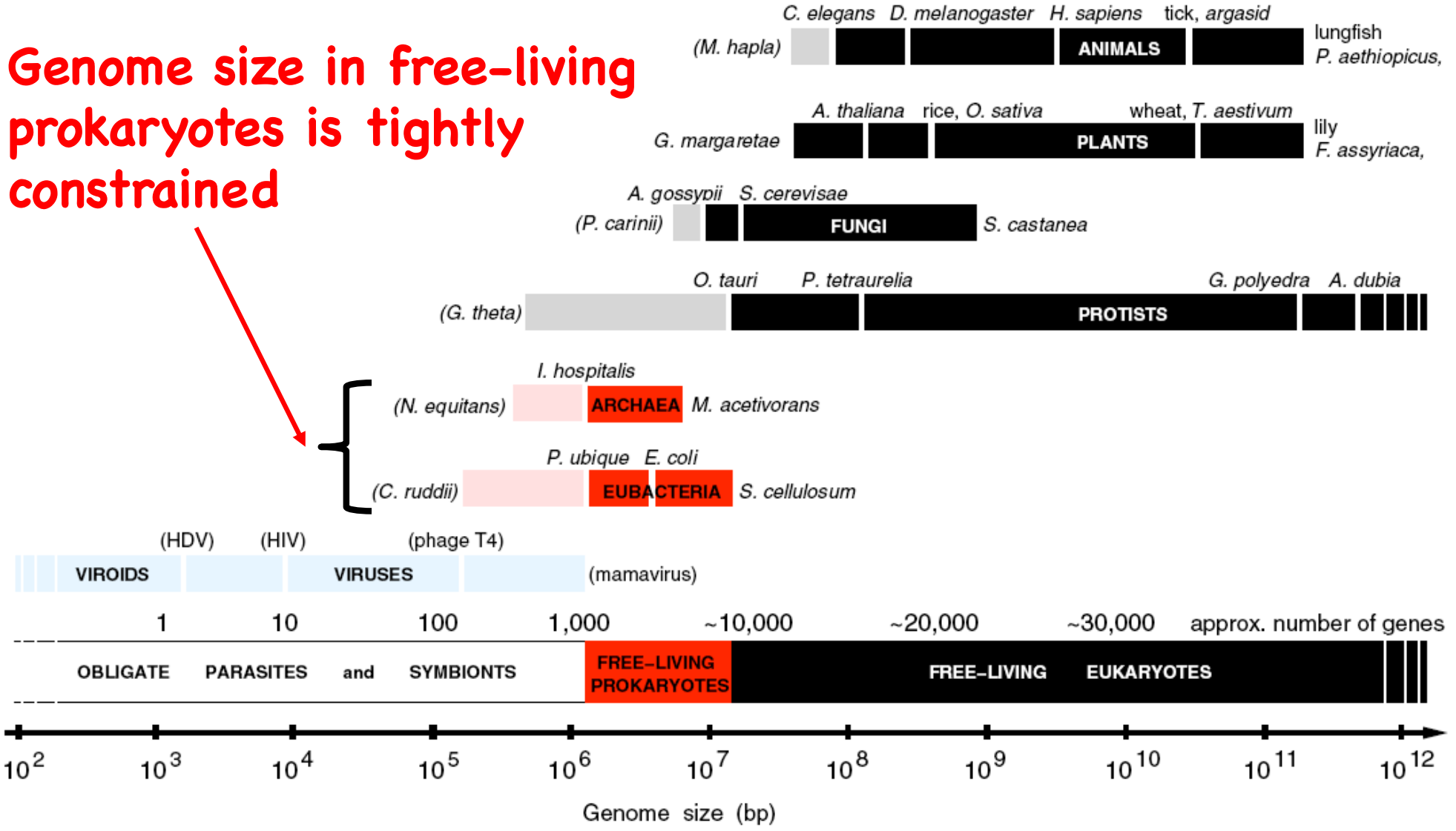


Figure 1

Genome size restriction of free-living prokaryotes. The genomes of free-living prokaryotes (archaea and eubacteria, in red) appear to be restricted to a mere 10-fold range in size from 1.3 Mbp to 13 Mbp, while the sizes of free-living eukaryote genomes (in black) span almost 10^5 folds from 10 Mbp to near 10^6 Mbp. The lower ranges of obligate parasite or symbiont genomes, shown as light pink and grey bars, can be much smaller than free-living prokaryote and eukaryote genomes, respectively, due to the progressive loss of dispensable genes. Viruses and gene-free viroids (in light blue) further reduce the size of parasitic "genomes" down to a few hundred nucleotides only.

Why???

There must be strong selection pressure for minimal genome size: carrying only what you need to survive in the current environment. Carrying extra DNA requires energy that could be used for growth

The ability to take up and incorporate DNA from the environment gives prokaryotes a way to evolve. DNA from those around you is, by definition, coming from survivors--others who have found a way to thrive in the current environment.

But…..those who thrive can also be your antagonists, which requires counter measures. Stay tuned….

Genetics without Sex: Gene transfer in Prokaryotes

- **Transformation** - cells take up DNA directly from the environment
- **Conjugation** - cell-to-cell transfer of genetic material
- **Transduction** - Transfer of genetic material by phage or phage-like particles

Prokaryotic Features

Unicellularity

-Most are single-celled

-Some can form complex **biofilms**

Cell size

-Most are less than 1 μm in diameter

Chromosome

-Single circular double-stranded DNA

-Found in the **nucleoid**

Prokaryotic Features

Internal compartmentalization

-No membrane-bounded organelles

Flagella

-Simple in structure; spin like propellers

Cell division

-Most divide by binary fission

Genetic recombination

-Occurs through horizontal gene transfer

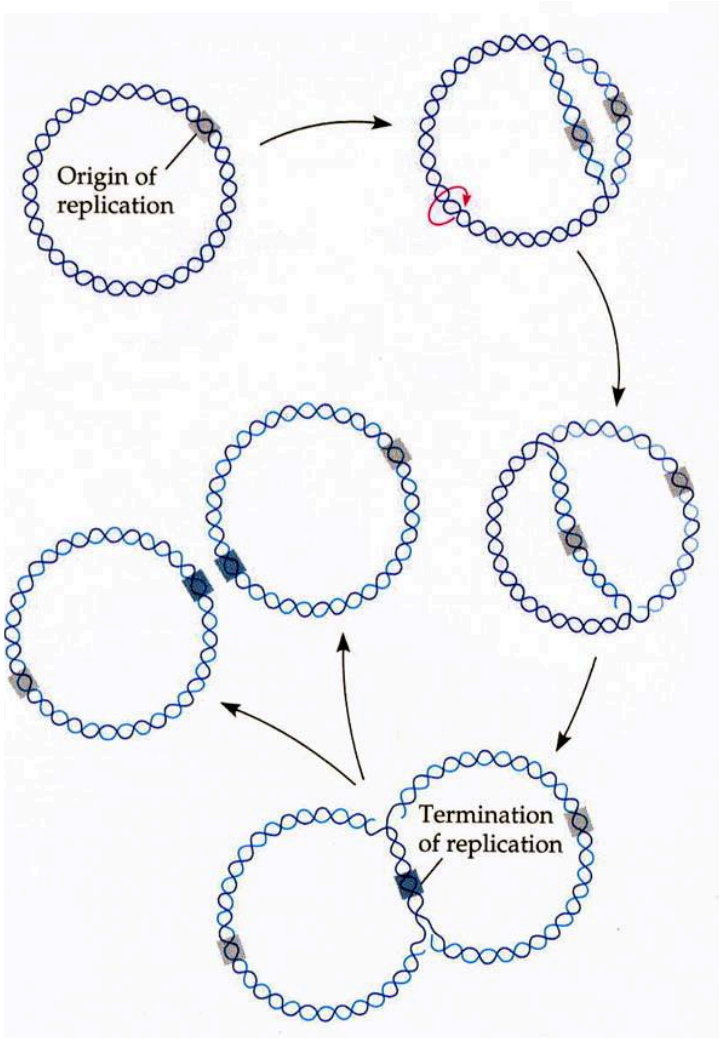
Bacterial Chromosome

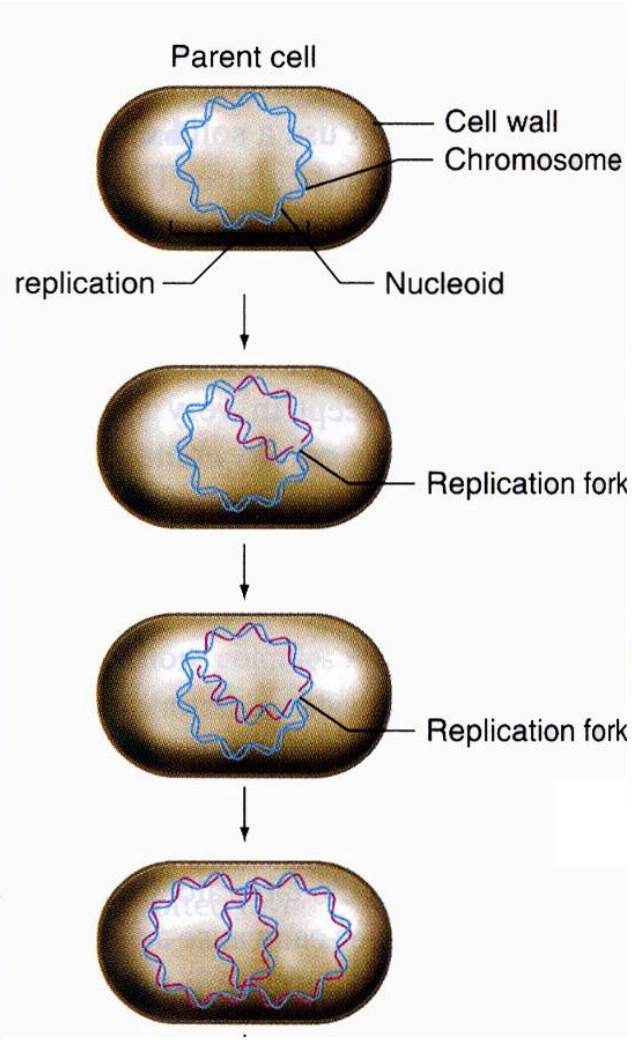
...a circular molecule of double helical DNA,

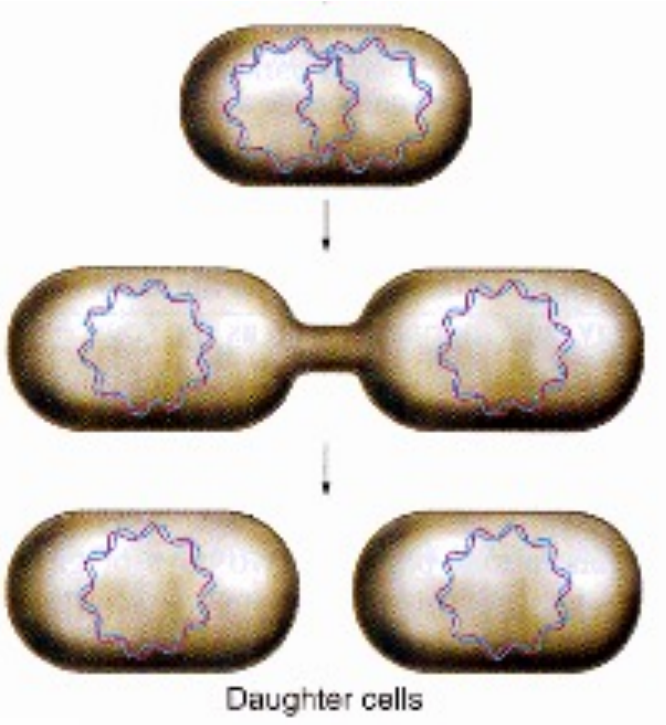
- 4 - 5 Mb long in most species studied,
- 1.6 mm long if broken and stretched out.

- Inside the cell, the circular chromosome is condensed by supercoiling and looping into a densely packed body termed the **nucleoid**.

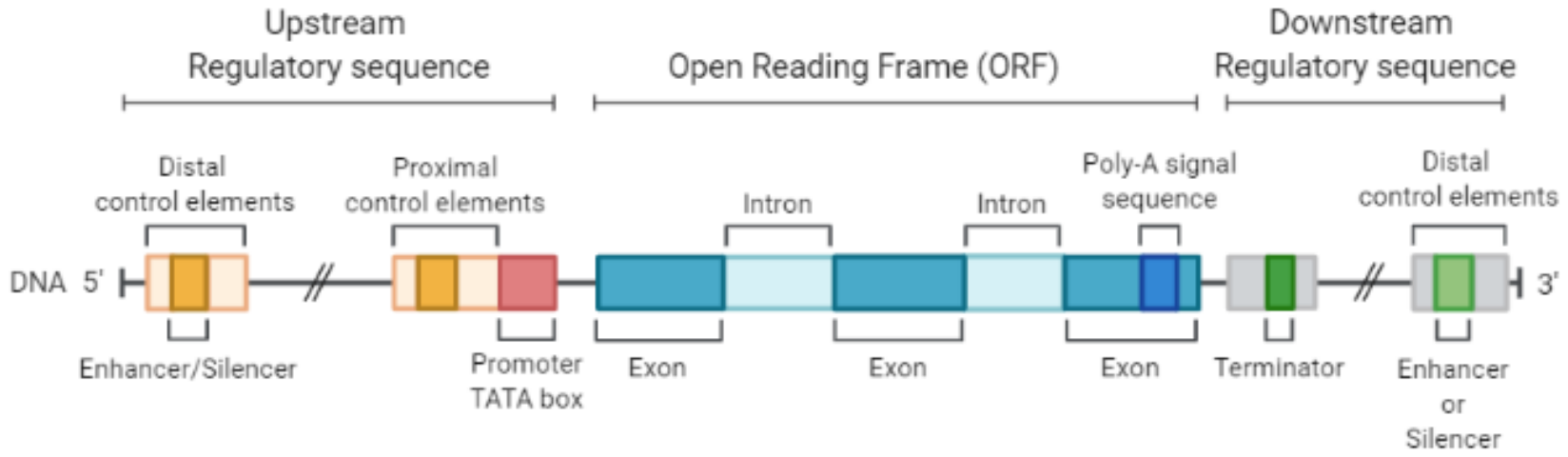
DNA Replication



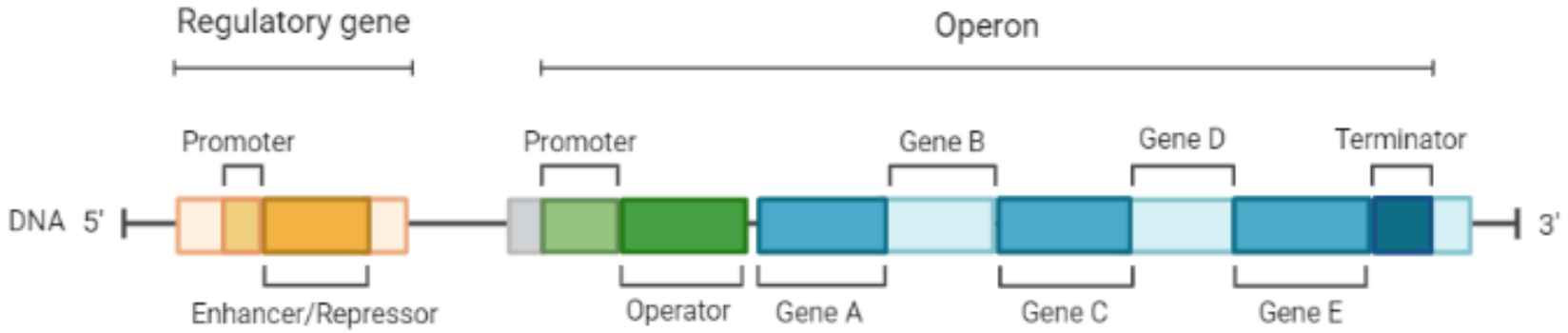




Eukaryotic Gene Structure

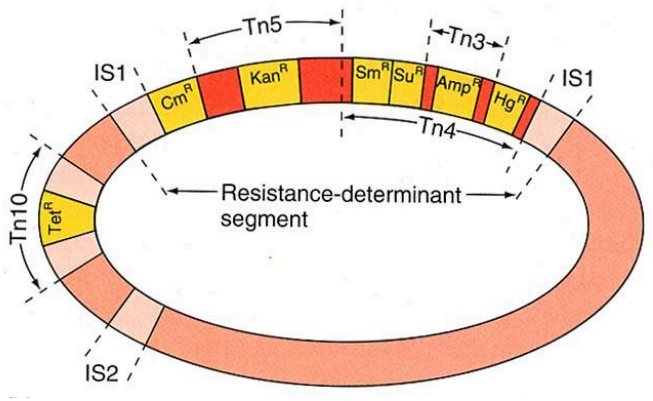


Prokaryotic Gene Structure



Extra Chromosomal DNA

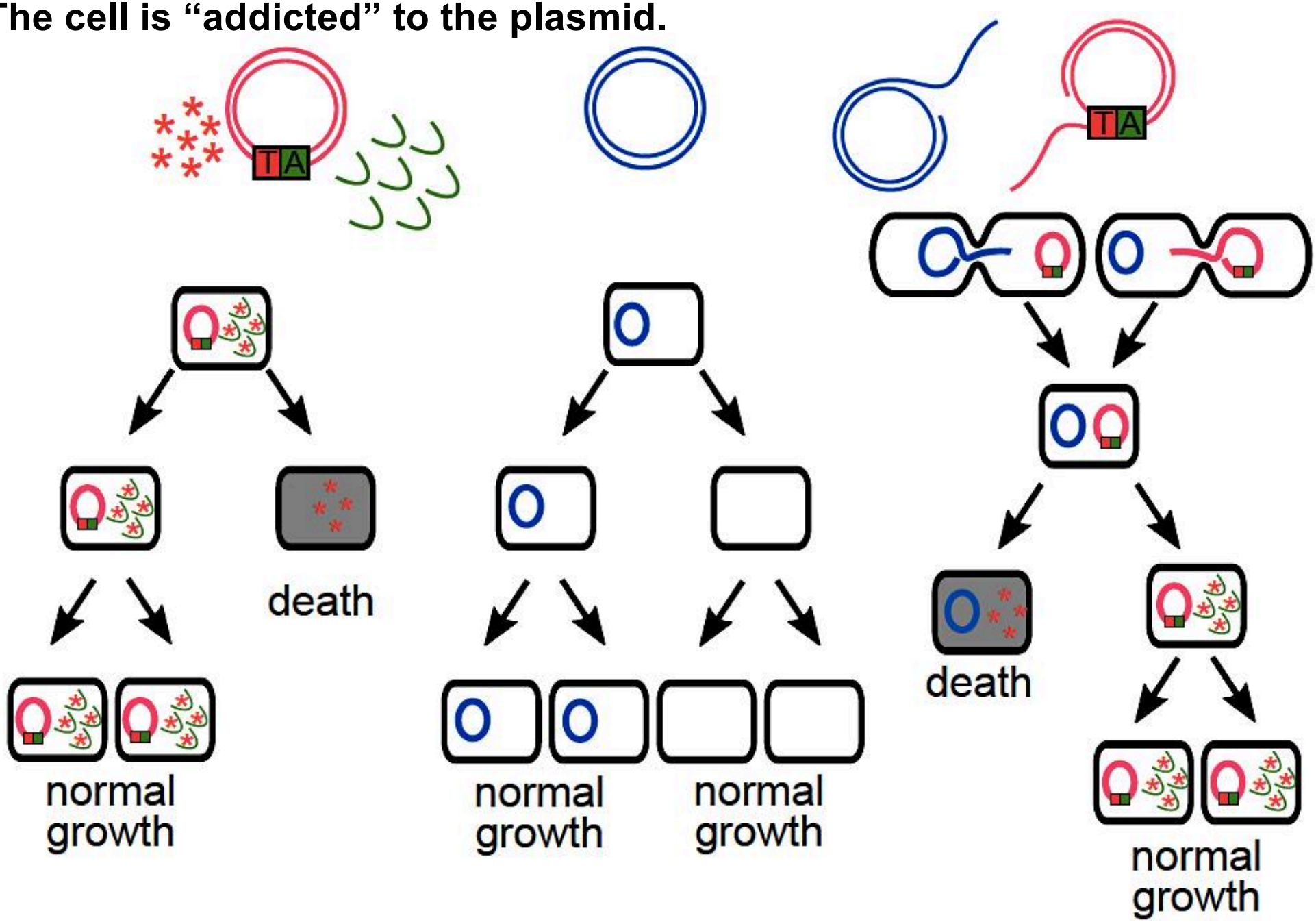
- **Plasmids:** circular double stranded DNA molecule that replicates independently,
  - containing one or more (non-essential) genes, smaller than the bacterial chromosome,
  - may carries genes for pathogenicity,
  - may carry genes for adaptation to the environment, including drug resistance genes.

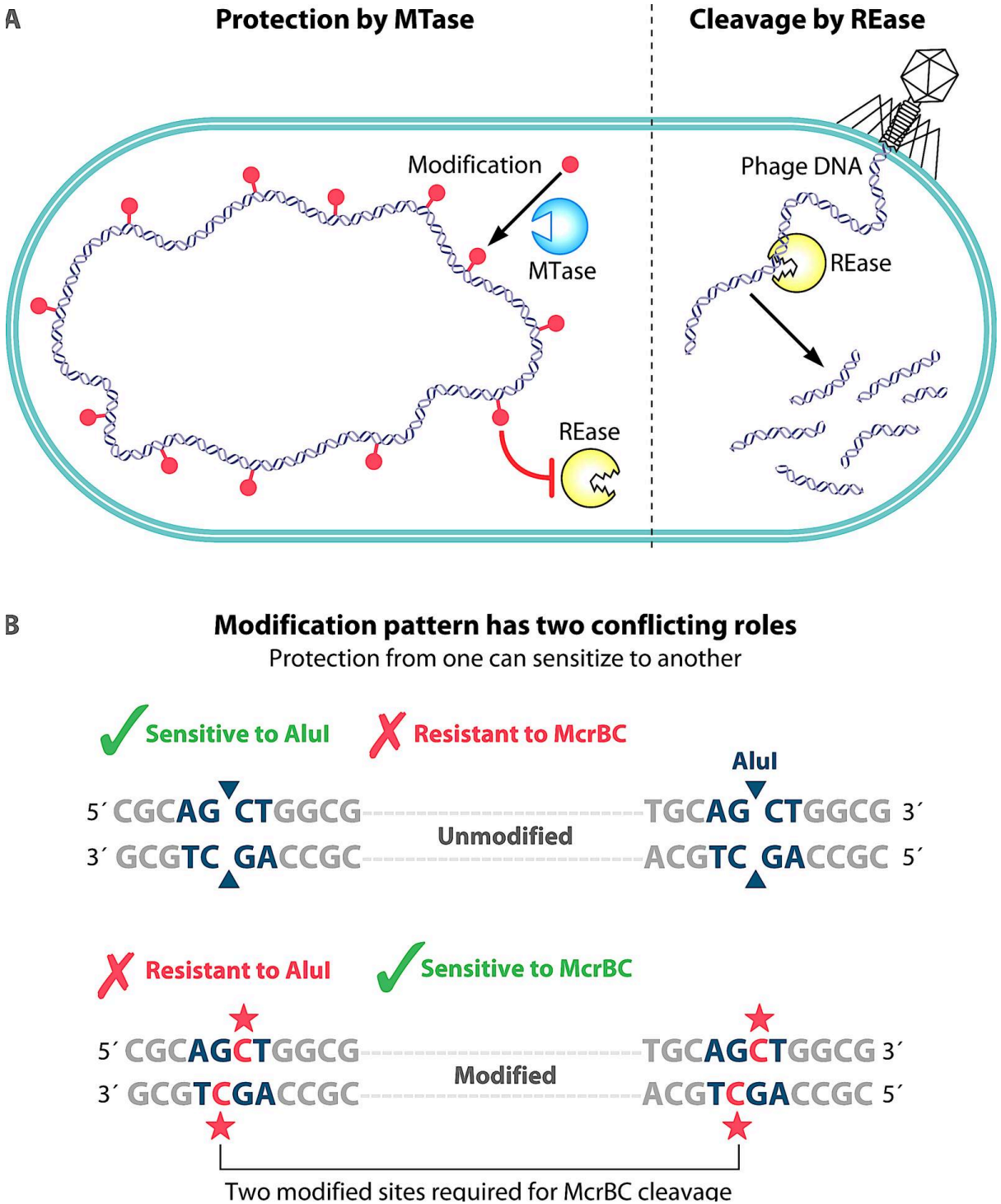


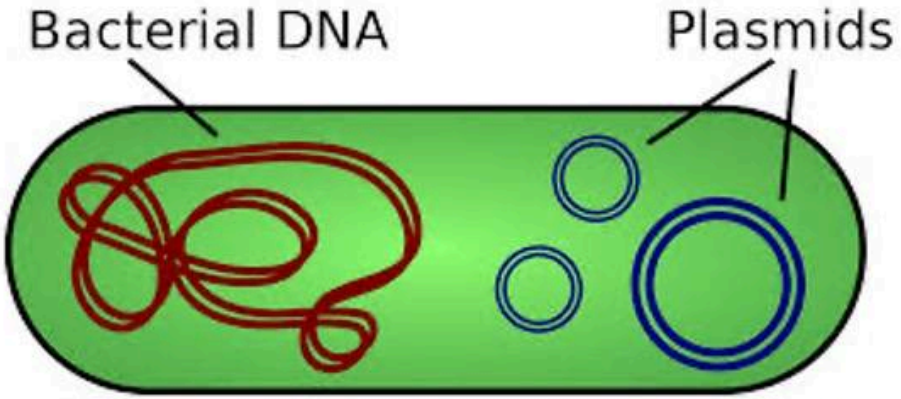
Plasmid Replication

- Each plasmid carries an origin of replication
- Origins determine host specificity
- Each cell can only replicate a single plasmid with each origin type (called an incompatibility group). Two different plasmids of the same IG cannot be maintained in the cell together
- A cell can host multiple plasmids with different origins of replication - *Borrelia burgdorferi* (lyme disease) carries 21 different plasmids

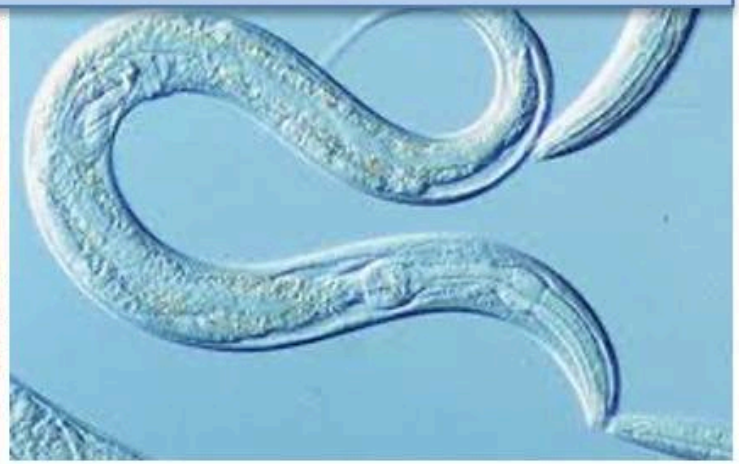
TA cassettes "encourage" their own transmission and kick plasmids of the same incompatibility group that lack them out of the population
The cell is "addicted" to the plasmid.



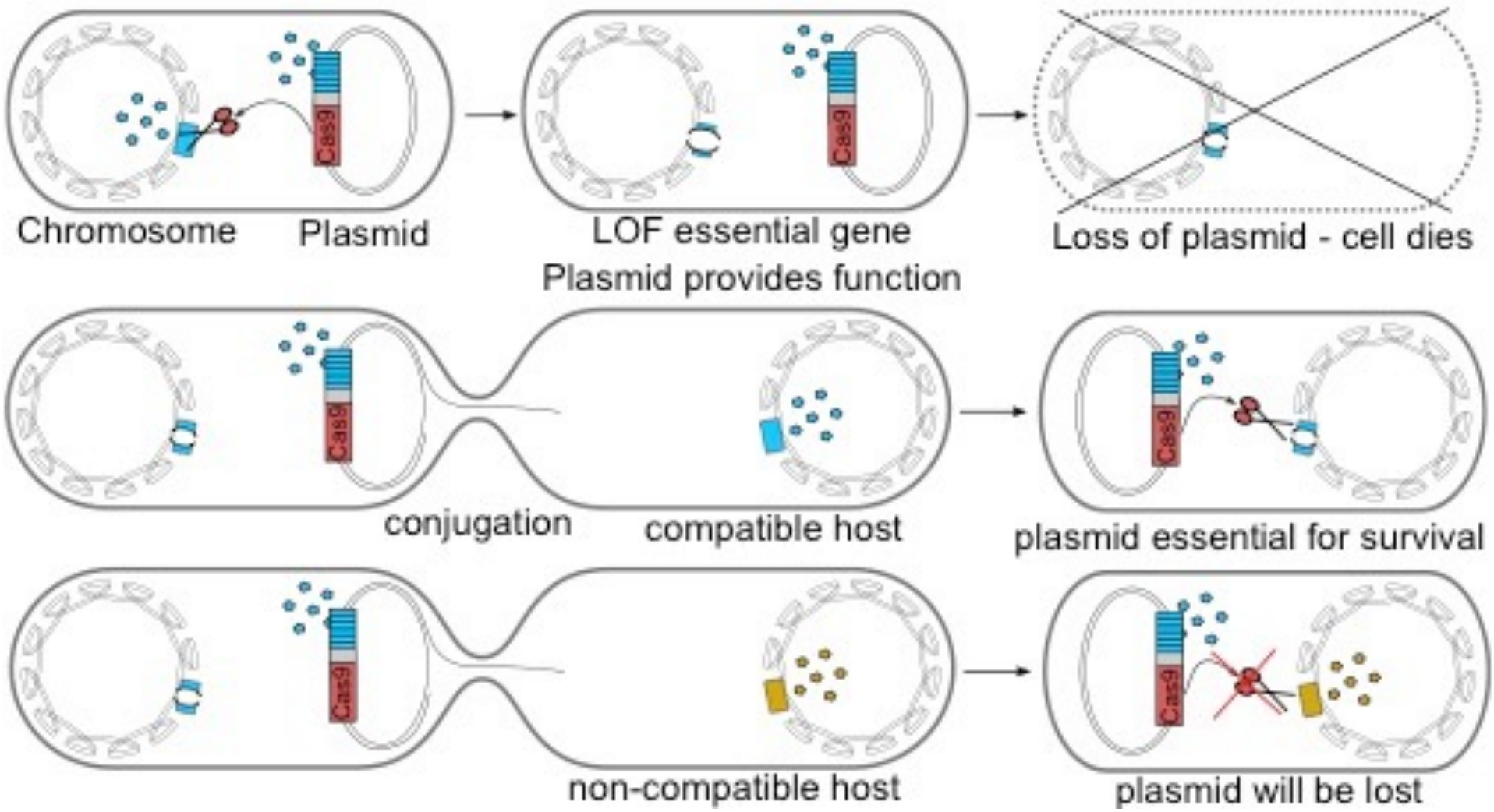

A
Protection by MTase
Cleavage by REase
Modification
MTase
REase
Phage DNA
REase
B
Modification pattern has two conflicting roles
Protection from one can sensitize to another
Sensitive to AluI
Resistant to McrBC
AluI
5′ CGCAG CTGGCG
3′ GCGTC GACCGC
Unmodified
TGCAG CTGGCG 3′
ACGTC GACCGC 5′
Resistant to AluI
Sensitive to McrBC
5′ CGCAGCTGGCG
3′ GCGTCGACCGC
Modified
TGCAGCTGGCG 3′
ACGTCGACCGC 5′
Two modified sites required for McrBC cleavage



Toxin-Antidote Elements Across the Tree of Life.Burga A, Ben-David E, Kruglyak L
Annu Rev Genet. 2020 Nov 23;54:387-415. doi: 10.1146/annurev-genet-112618-043659.



Broad host range plasmids can deliver DNA to many types of organisms. How can we guarantee that only certain species retain the plasmid? Make retention sequence specific, and essential for survival



Bacteria Phenotypes

- *colony morphology*,
  - large, small, shiny, dull, round or irregular,
- resistance to bactericidal agents,
- auxotrophs,
  - unable to synthesize raw materials from minimal media,
- cells unable to break down complex molecules, or use certain molecules as energy sources
- essential genes, usually studied as conditional mutants.

Auxotrophs

…a cell that requires a substance for growth that can be synthesized by a wild-type cell,

his$^-$ ...can't synthesize histidine (*his*$^+$ = wt)

leu$^-$ ...can't synthesize leucine (*leu*$^+$ = wt)

arg$^-$ ...can't synthesize arginine (*arg*$^+$ = wt)

bio$^-$ ...can't synthesize biotin (*bio*$^+$ = wt)

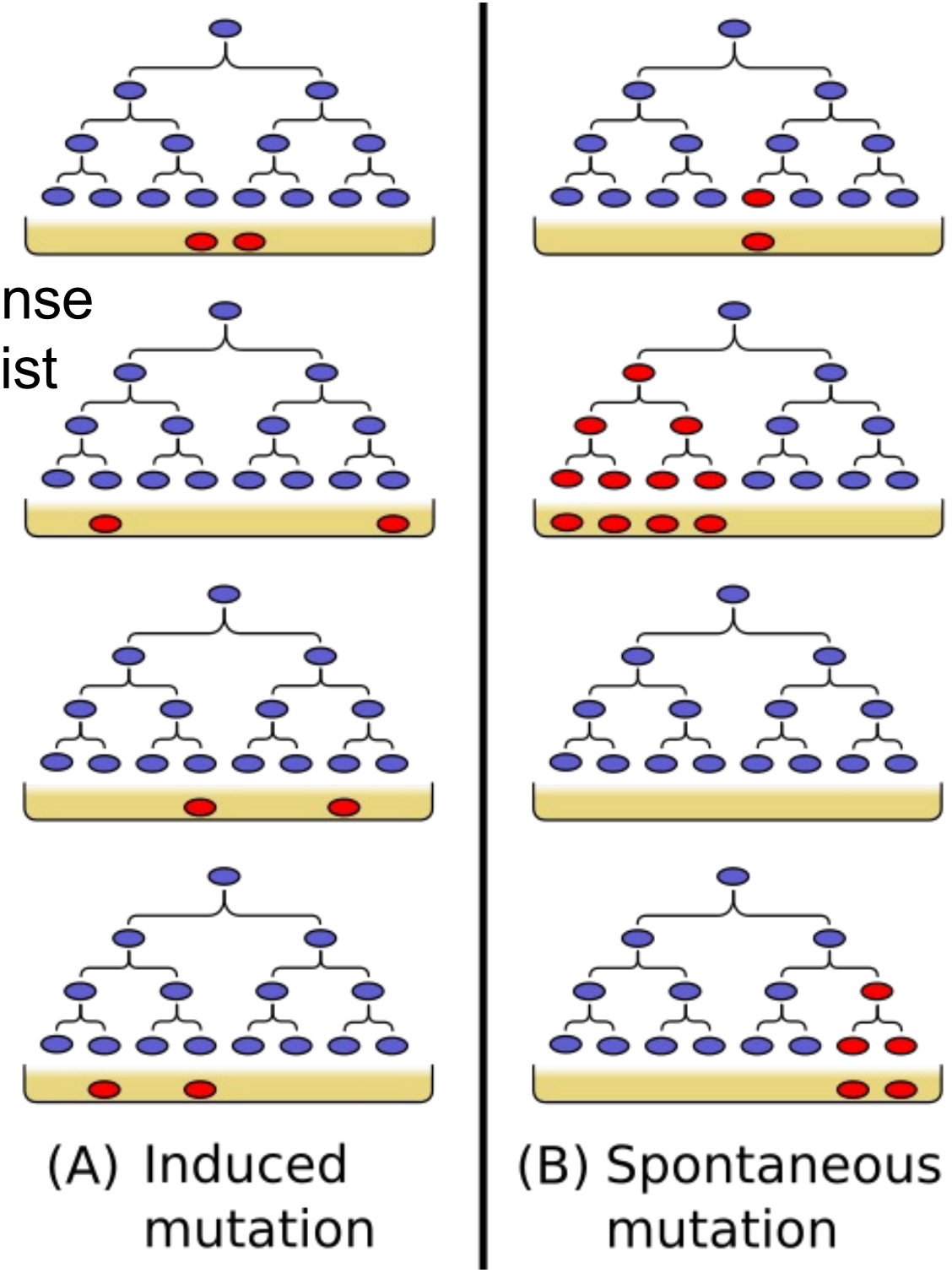
Prototroph

…a cell that is can grow on a defined, minimal media,

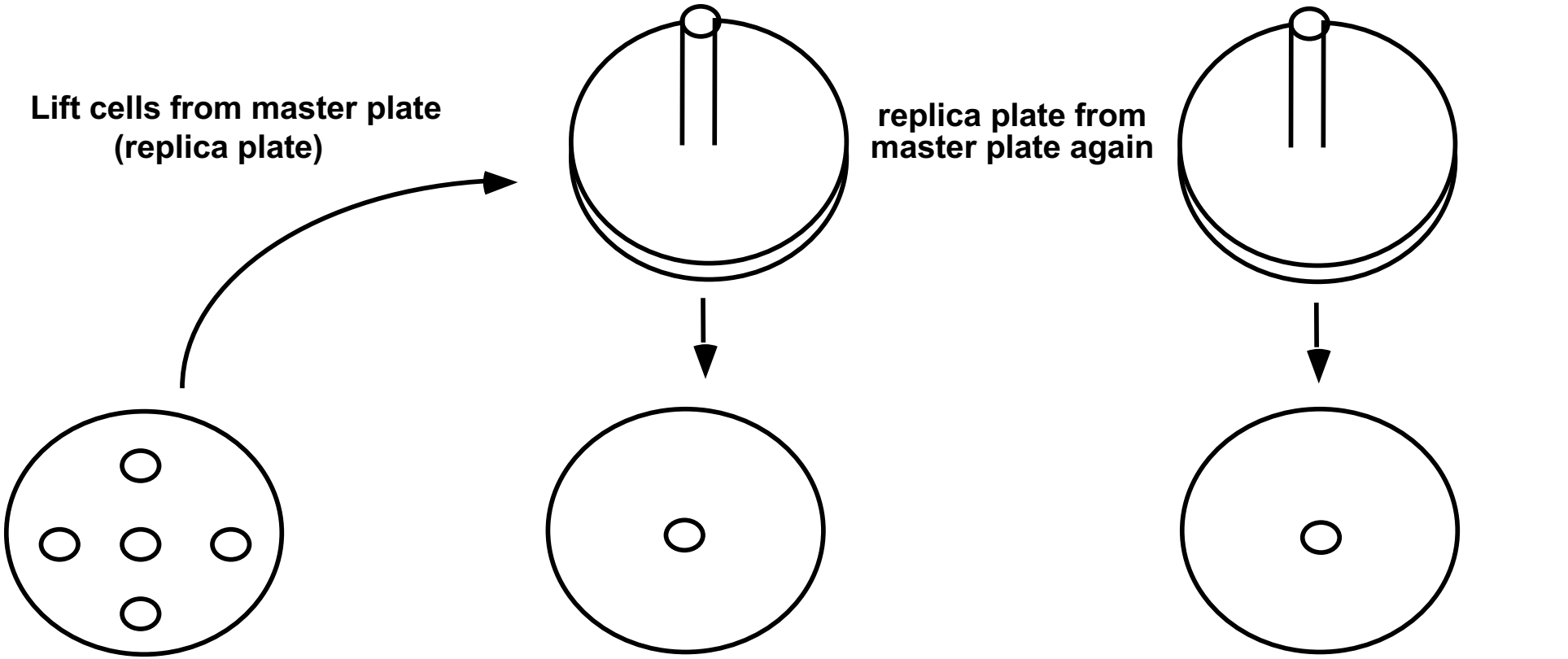
- can synthesize all essential organic compounds,
- usually considered the ‘wild-type’ strain.

Do mutations arise in response tot stress, or do they pre-exist

Luria-Delbruck fluctuation test



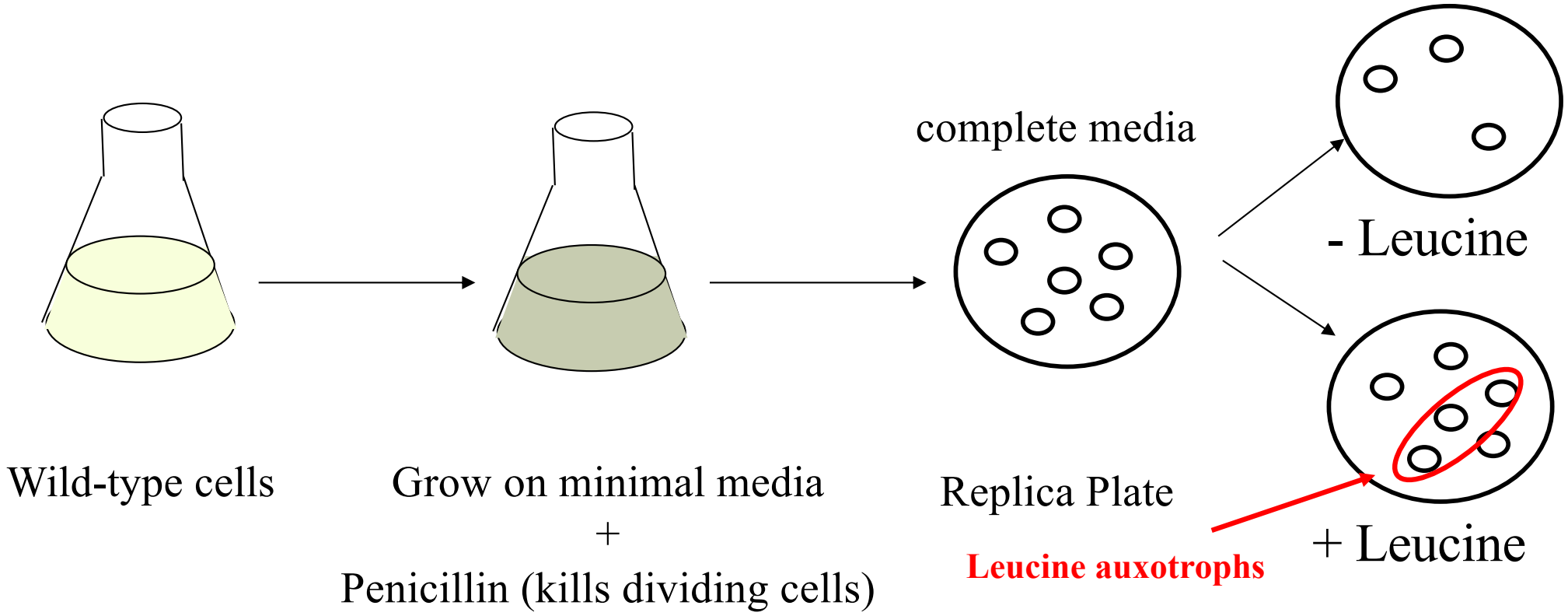
Replica plating (Ledergerg and Lederberg) provides a very straightforward demonstration that mutations preexist in a population



colonies that grow on phage are mutant

Isolation of Auxotrophs

- Isolating rare, less-fit auxotrophs



Bacterial Nomenclature I

- genes not specifically referred to are considered wild-type,

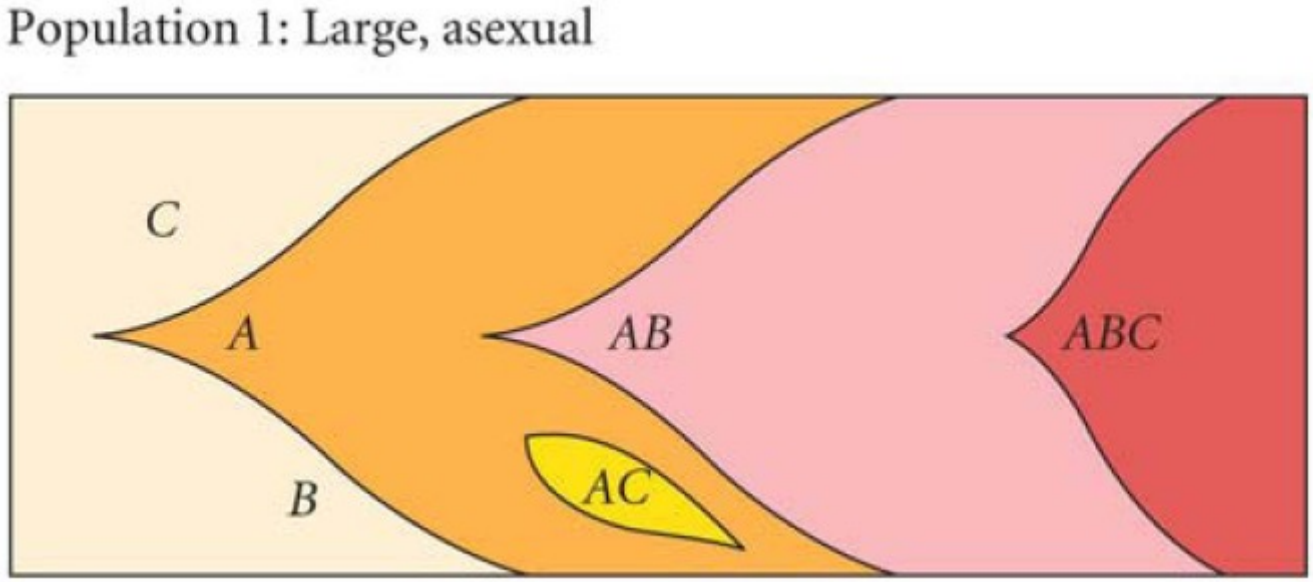
Strain A: met^- bio^- thr^+ leu^+ thi^+

x

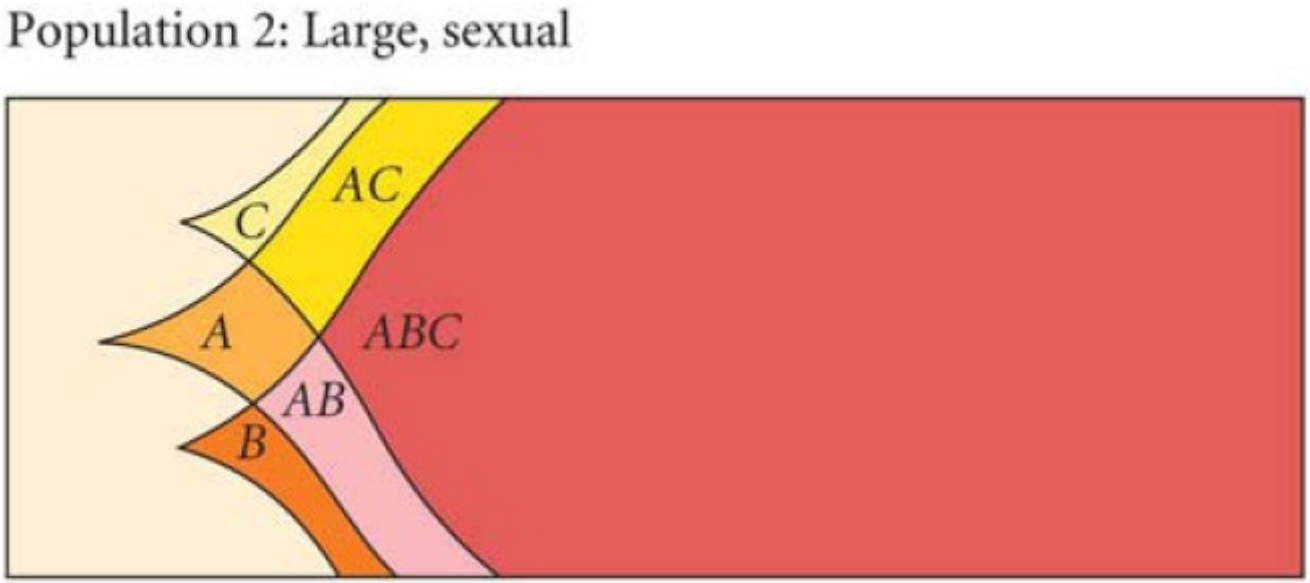
Strain B: thr^- leu^- thi^- met^+ bio^+

Sex and recombination bring together rare, advantageous gene combinations in the same individual

No sex and recombination



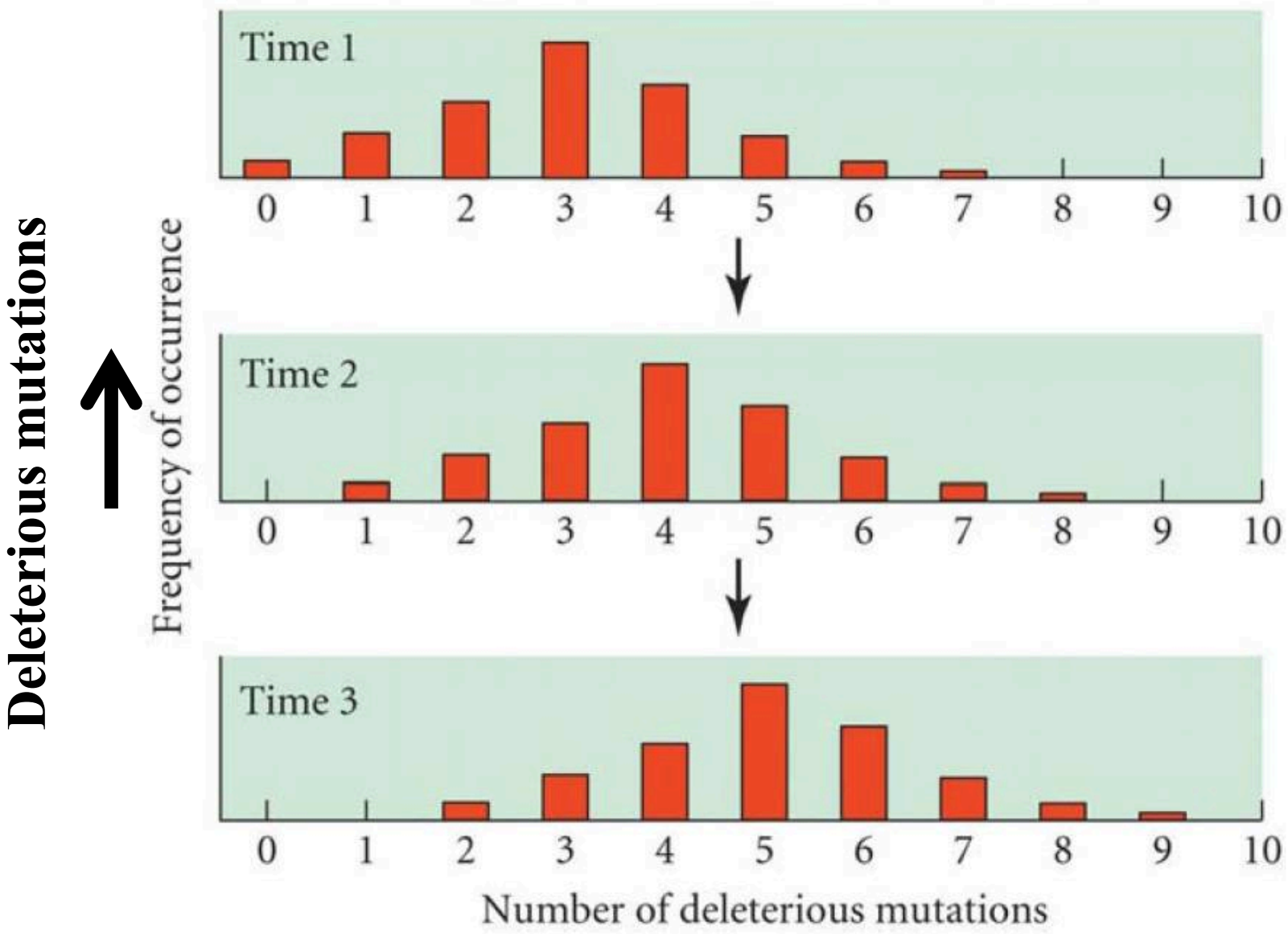
Sex and Recombination



Muller's ratchet

In the absence of recombination genomes that acquire deleterious mutations cannot lose them through recombination

ALL asexuals will suffer the effects of "Muller's Ratchet"



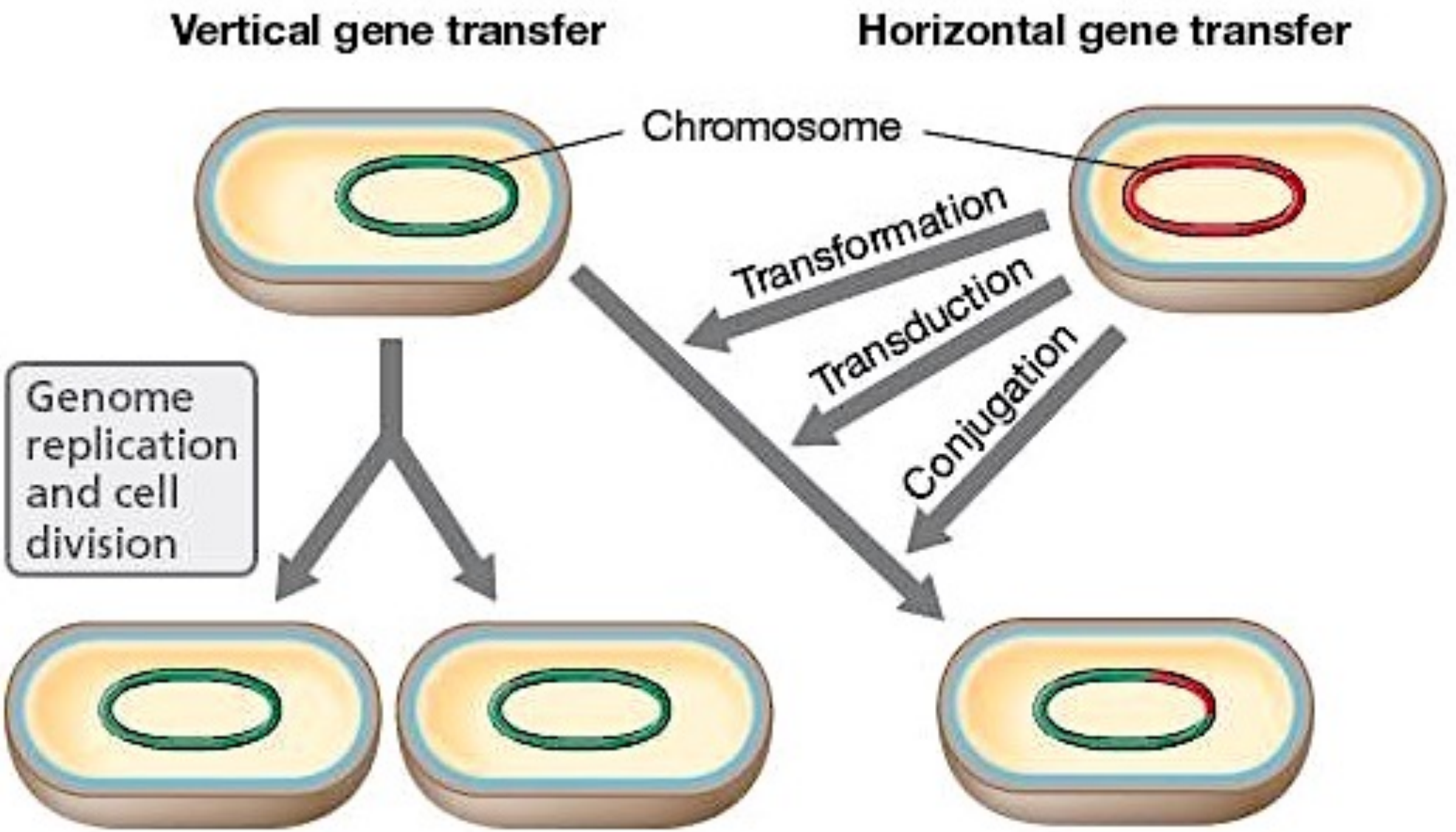
The Red Queen Hypothesis: evolution never stops because competing species are also evolving

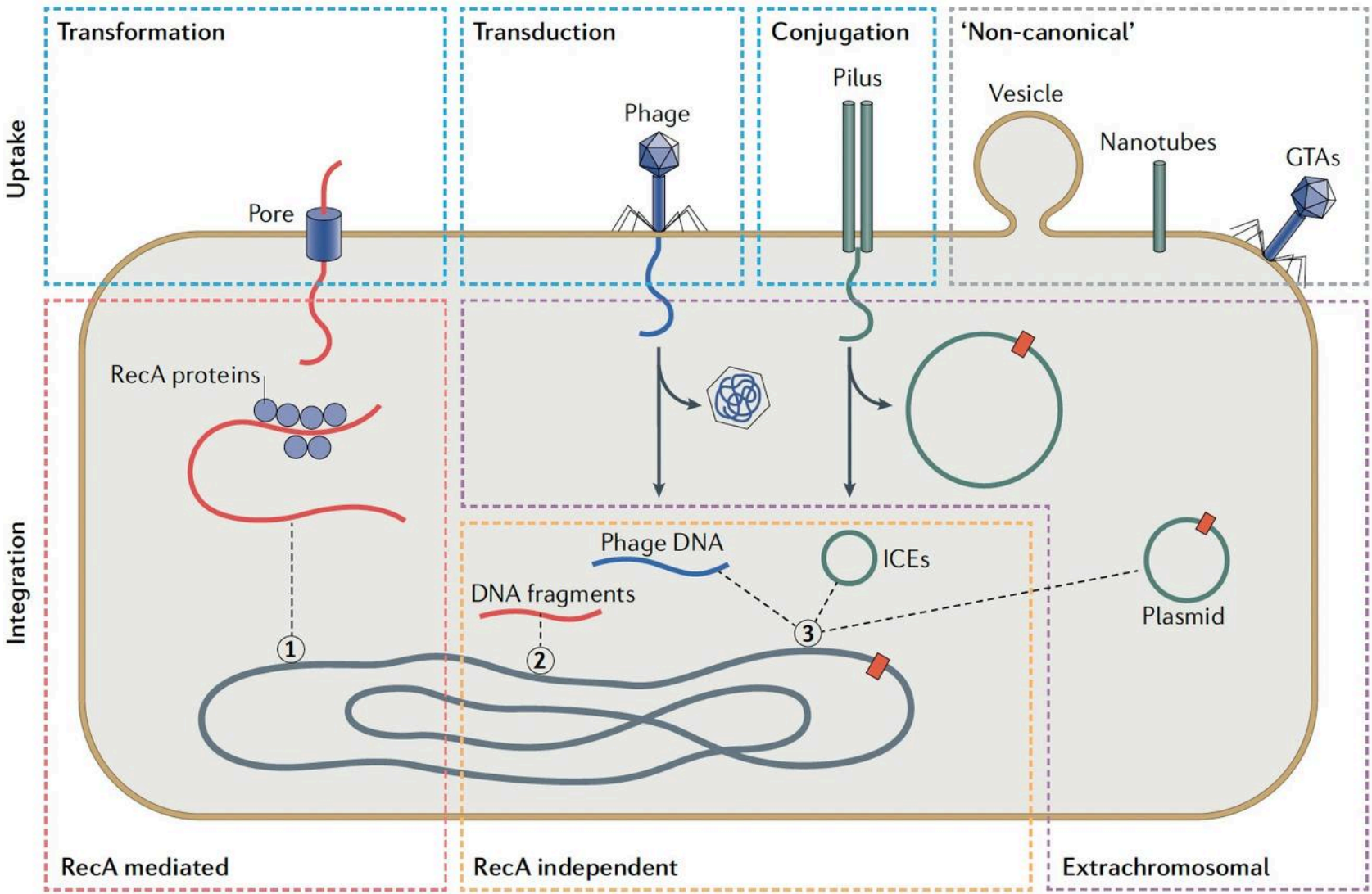
“Now, here, you see, it takes all the running you can do to keep in the same place. If you want to get to somewhere else, you must run twice as fast as that”

https://www.youtube.com/watch?v=y6rqugj_mVk

Brief description of red queen dynamics in the context of virus-host interactions

Vertical and horizontal gene transfer





1 RecA-mediated recombination, includes preprocessing by RecA proteins

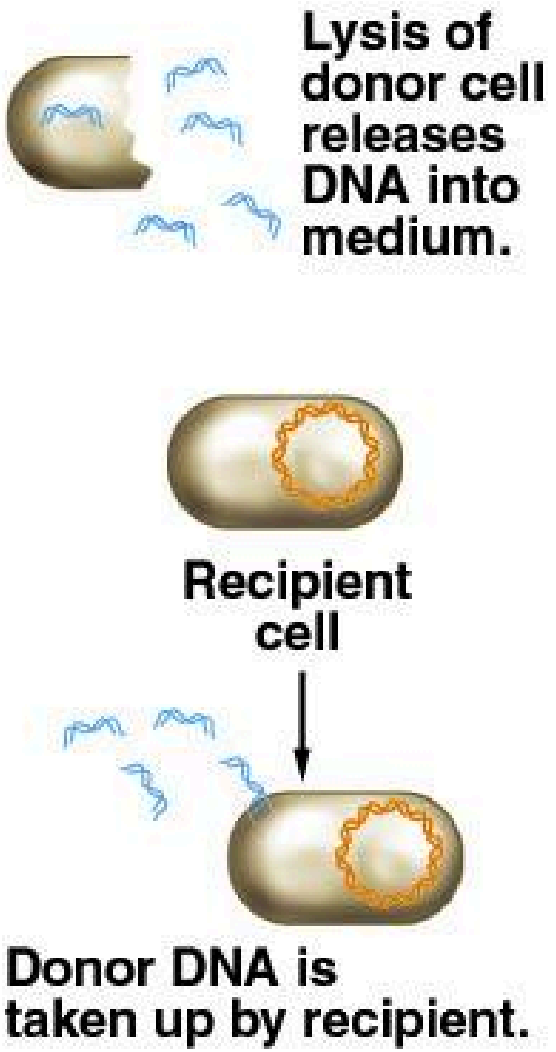
2 RecA-independent recombination: for example, illegitimate recombination from microhomologies or non-homologous end joining

3 Site-specific recombination via integrases, recombinases or transposases

Genetics without Sex: Gene transfer in Prokaryotes

- **Transformation** - cells take up DNA directly from the environment
- **Conjugation** - cell-to-cell transfer of genetic material
- **Transduction** - Transfer of genetic material by phage or phage-like particles

Transformation

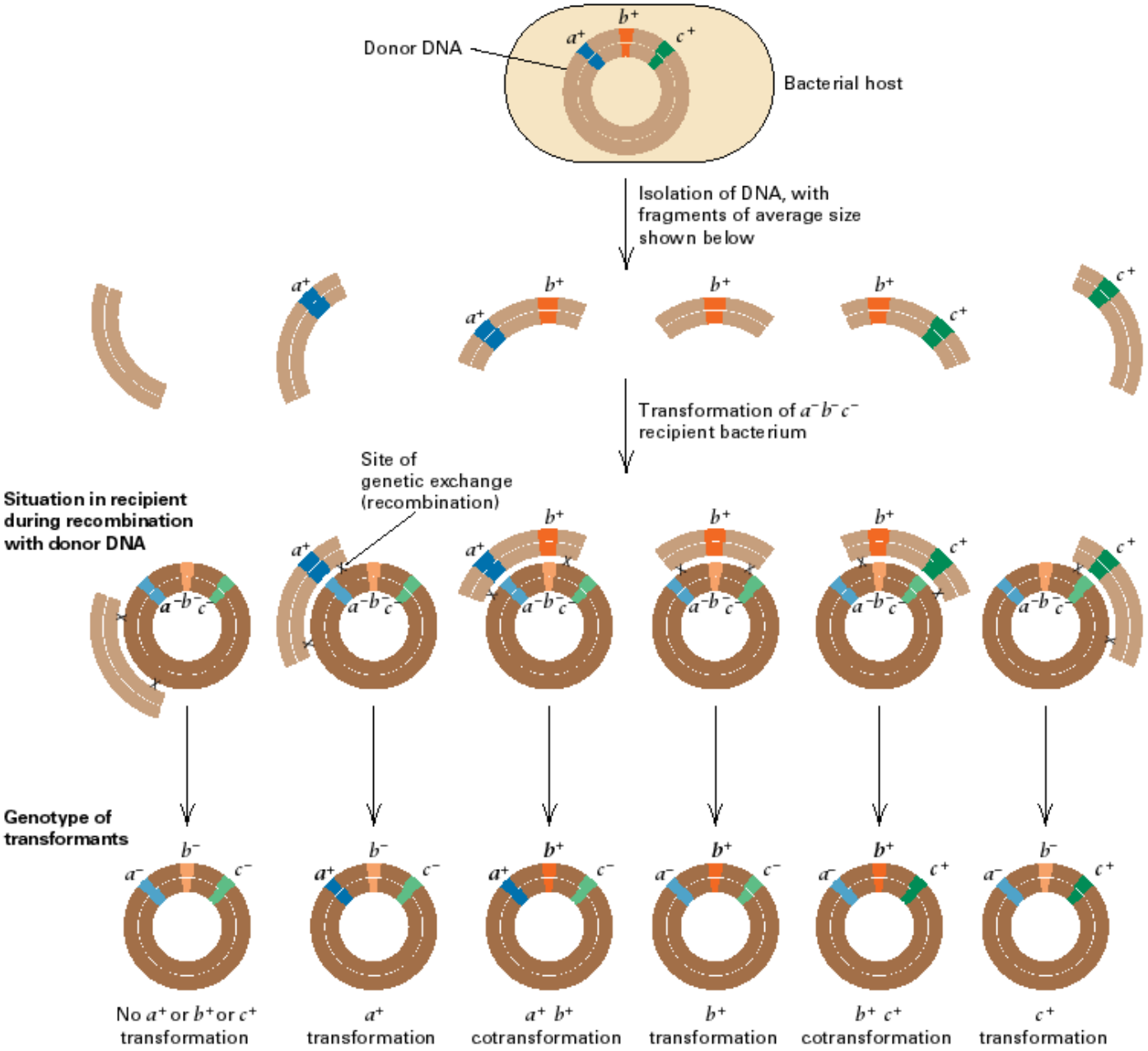


The ability to take up extracellular DNA is referred to as competence

A few bacterial species allow this under natural conditions (naturally competent) - *Bacillus, Acinetobacter, Streptococcus*

Treatments that briefly interrupt membrane integrity can be used to make other types of cells competent (*E. coli*)

Transformation and Mapping

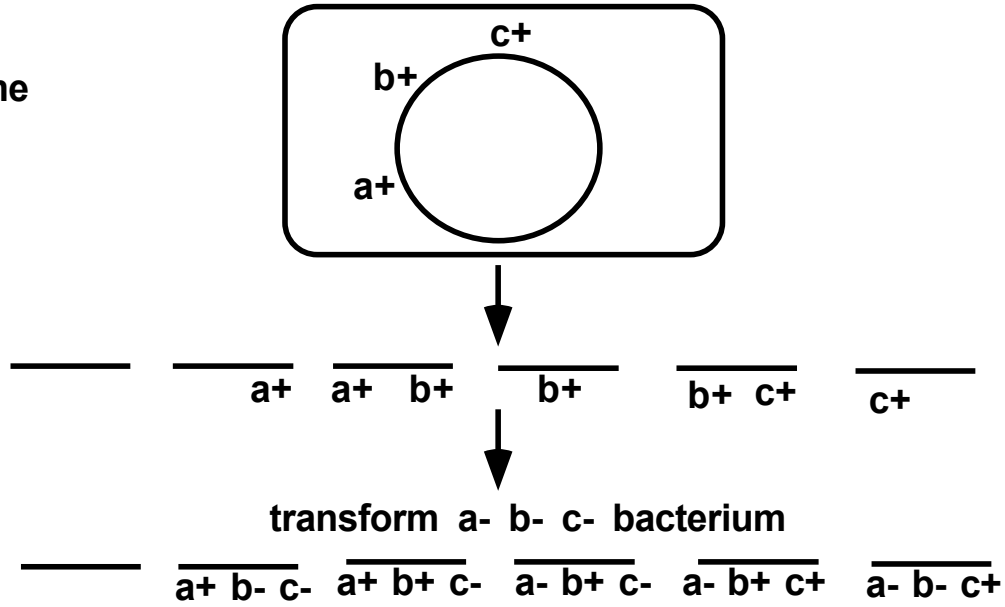


No $a^+ b^+ c^+$ or $a^+ b^- c^+$ cotransformation occurs because the distance between *a* and *c* is too great.

transformation and mapping

DNA undergoes shearing that leads to different average size DNA molecules

$$\frac{\text{7,500 bp fragments}}{\text{4 X } 10^6 \text{ bp/genome}} = \text{1/500 genome}$$



No a+ b+ c+ or a+ b- c+ tranformants

If the transfer of genes are independent events the probability of transfer of both genes = (P1) (P2).

An increased probability of joint transformation reflects linkage.

$$\text{Cotranformation index} = R\ \frac{\text{a+ b+}}{\text{a+ b+, a+ b-, a- b+}}$$

$$\text{Recombination frequency} = Q = 1 - R = \frac{\text{a+ b-, a- b+}}{\text{a+ b+, a+ b-, a- b+}}$$

Transformation mapping only gives meaningful results over short distances. For long distances genes are never transformed together on the same molecule, but as we will see below, linkage maps can still be derived using conjugation or viral transduction.

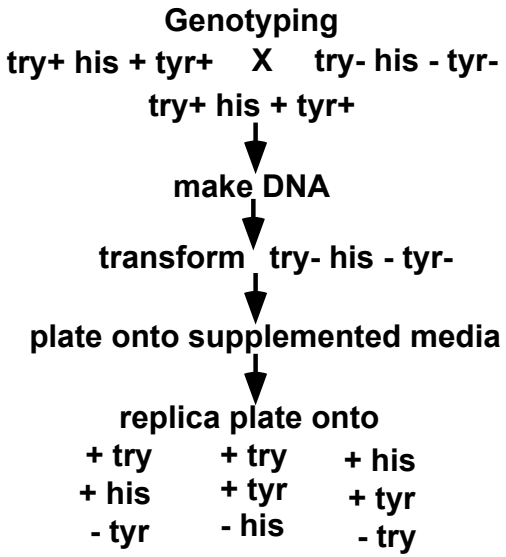


Table 19–2

Number of colonies in different transformant classes from a cross of try_2^+ his_2^+ tyr_1^+ as the donor with try_2^- his_2^- tyr_1^- as the recipient

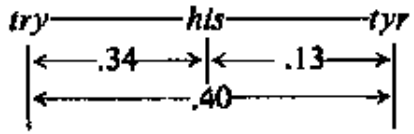
try^-	try^-	try^-	try^+	try^+	try^+	try^+
his^-	his^+	his^+	his^-	his^-	his^+	his^+
tyr^+	tyr^-	tyr^+	tyr^-	tyr^+	tyr^-	tyr^+
685	418	3,660	2,600	107	1,180	11,940

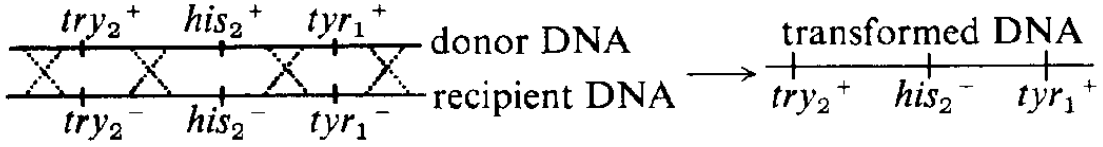
From Nester, Schafer, and Lederberg, 1963.

Table 19–3

Calculation of linkage distances and a linkage map between the three loci try_2, his_2, and tyr_1, on the basis of transformant classes given in Table 19–2

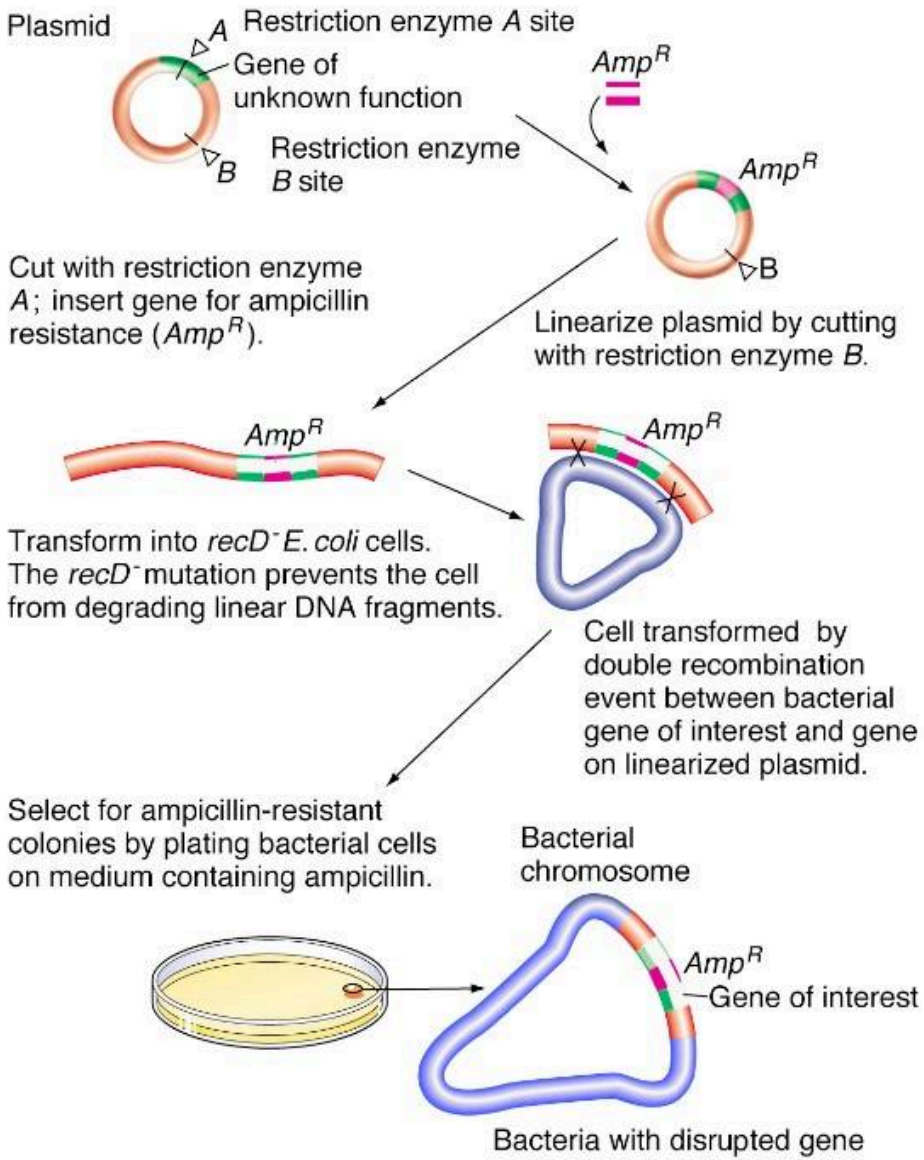
Distance	Transformants	No. colonies	q
try_2- -his_2	try^+ his^-	2,600 + 107	
	try^- his^+	418 + 3,660	6,785/19,905 = .34
	try^+ his^+	1,180 + 11,940	
try_2- -tyr_1	try^+ tyr^-	2,600 + 1,180	
	try^- tyr^+	685 + 3,660	8,125/20,172 = .40
	try^+ tyr^+	107 + 11,940	
his_2- -tyr_1	his^+ tyr^-	418 + 1,180	
	his^- tyr^+	685 + 107	2,390/17,990 = .13
	his^+ tyr^+	3,660 + 11,940	





Making use of transformation to study gene function in the modern age

1. Preparing specific knock-outs in bacteria



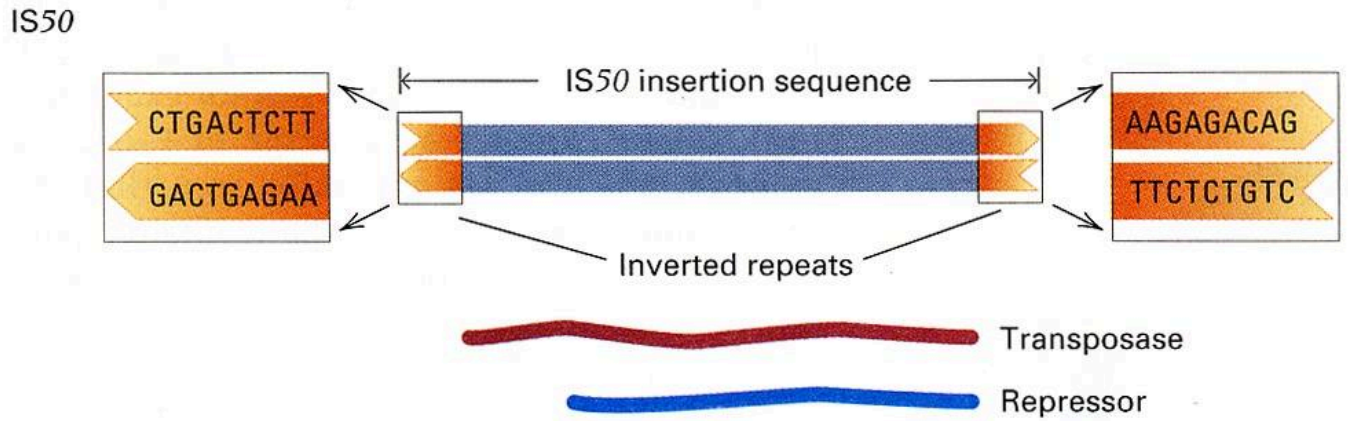
Reverse genetics: making mutants in a known gene and looking for phenotypes (what does the gene do?)

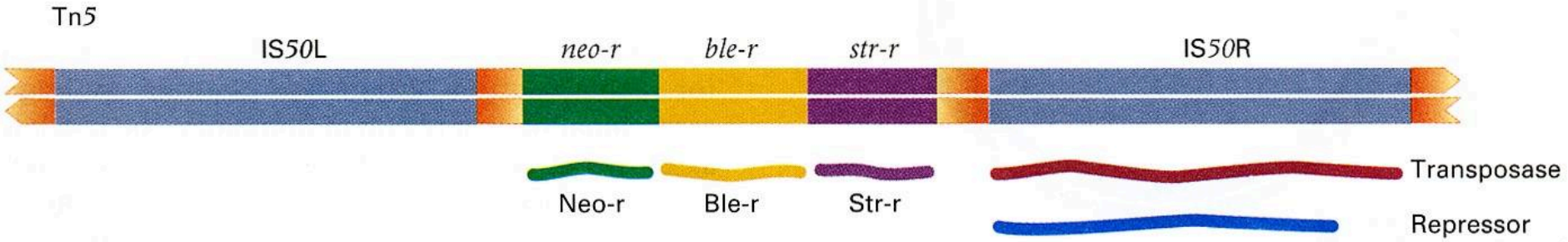
An ampicilin resistance gene (Ampr) is inserted into the gene of interest, located on a plasmid. The linear plasmid is transformed into a recD- E. coli strain where it recombines with the E. coli chromosome to leave a disrupted gene.

Transposon mutagenesis: An example of forward genetics

Transposable Elements

mobile DNA

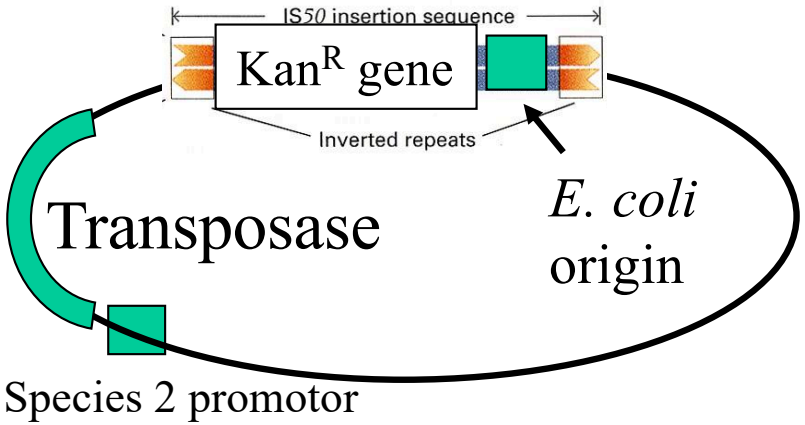




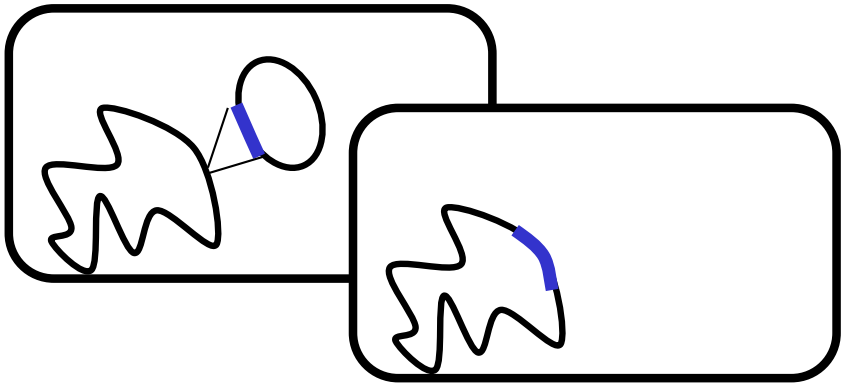
Transposon: carries one or more genes.
Excision and re-integration requires transposase

Use of transposons to identify novel genes in diverse species: forward genetics

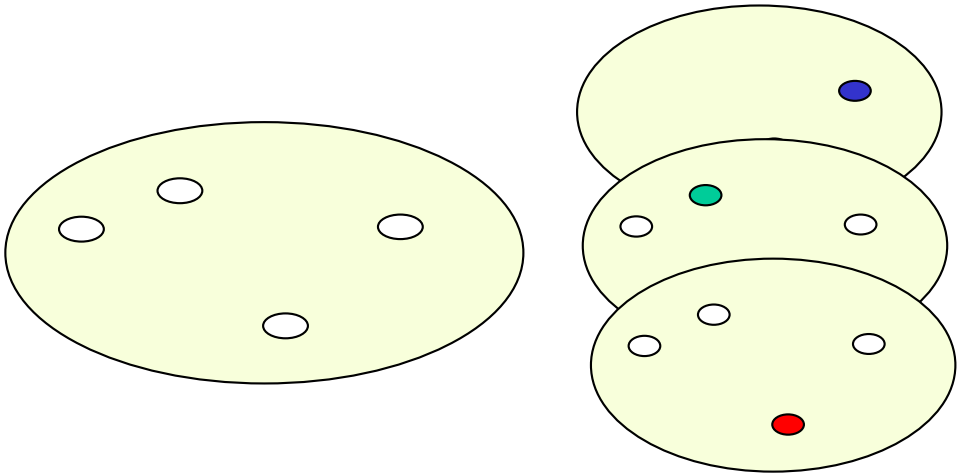
Transposon, with antibiotic resistance gene, located in species 1



1. Transposon with antibiotic resistance gene

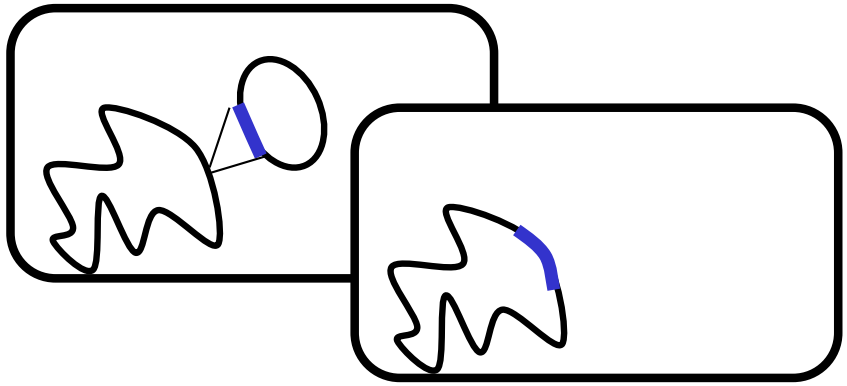


2. Transformation and insertion into genome 2 (plasmid lost because origin does not work in these species)

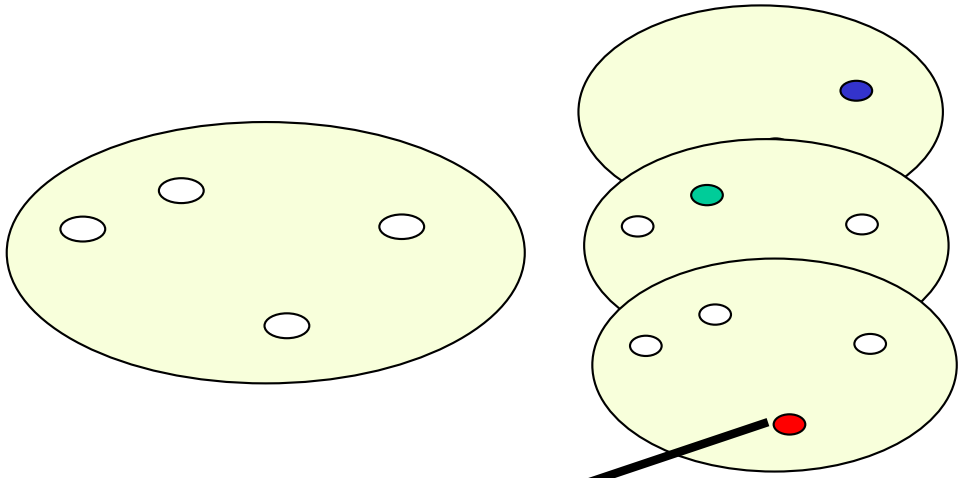


3. Plate on antibiotic media, screen for phenotypes

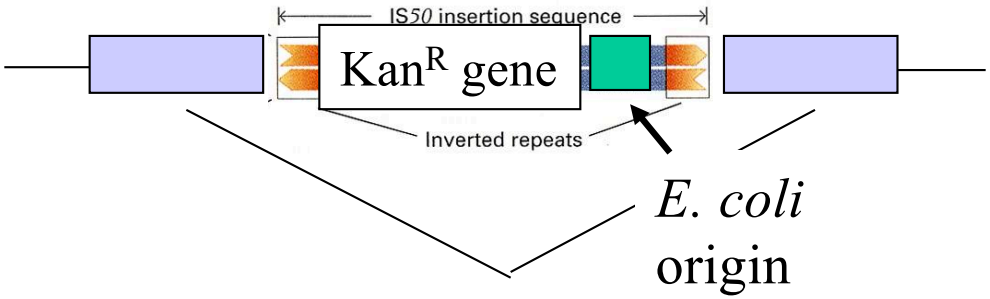
Use of transposons to identify novel genes



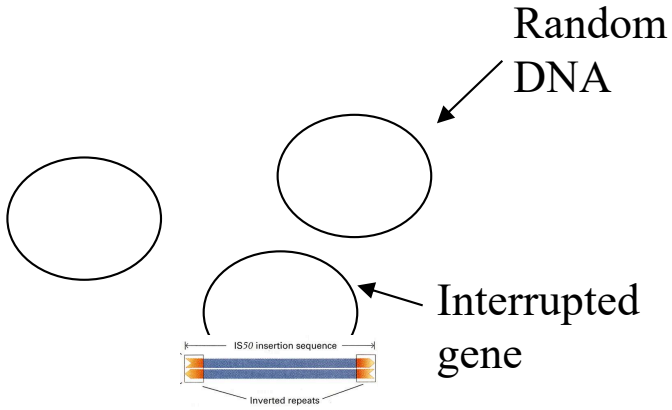
2. Transform into species 2, insertion into genome



3. Plate on antibiotic media, screen for phenotypes



4. Extract genomic DNA from mutants, circularize



5. Transform into *E. coli* - The genomic DNA with your gene will have origin, replicate (with Kan^R)

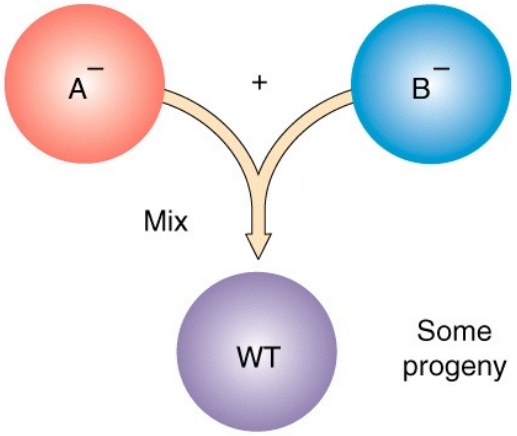
Genetics without Sex: Gene transfer in Prokaryotes

- **Transformation** - cells take up DNA directly from the environment
- **Conjugation** - cell-to-cell transfer of genetic material
- **Transduction** - Transfer of genetic material by phage or phage-like particles

Conjugation: Lederberg and Tatum, 1946

A- B+ X A+ B- ⟶ A+ B+

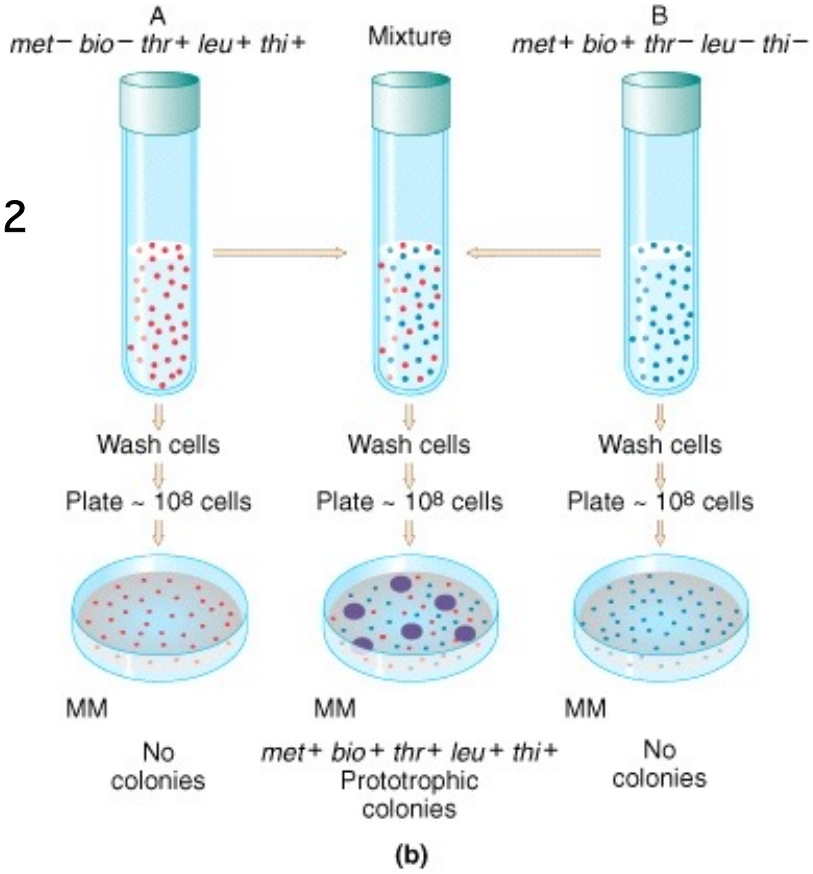
1. Recombination
2. Reversion (10^{-6})



A- B- C+ D+ X A+ B+ C- D- ⟶ A+ B+ C+ D+

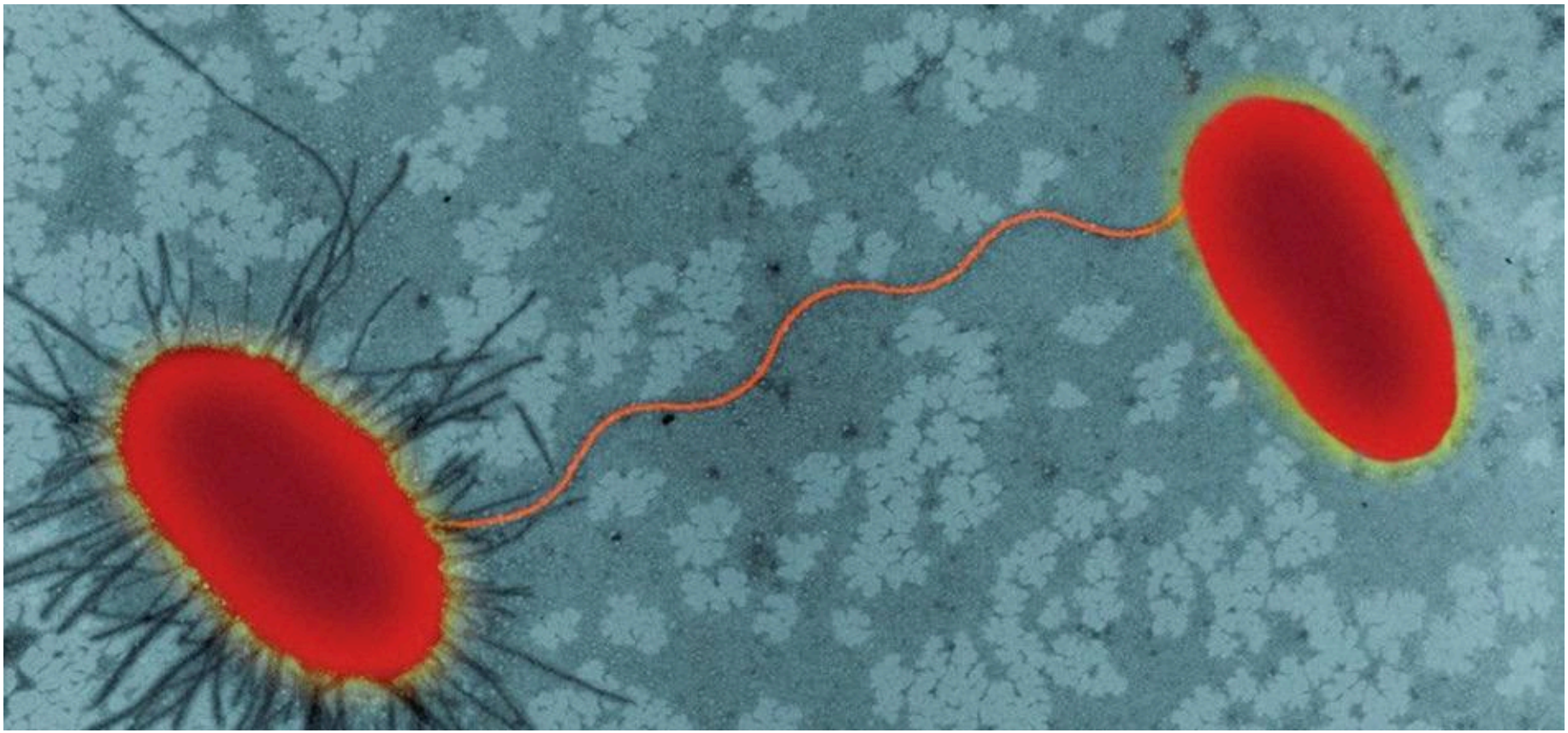
by reversion

$(10^{-6})(10^{-6}) = 10^{-12}$



The presence of a high frequency (relatively speaking) of prototrophs argues that these are not due to reversion, but instead the transfer and recombination of multiple donor traits.

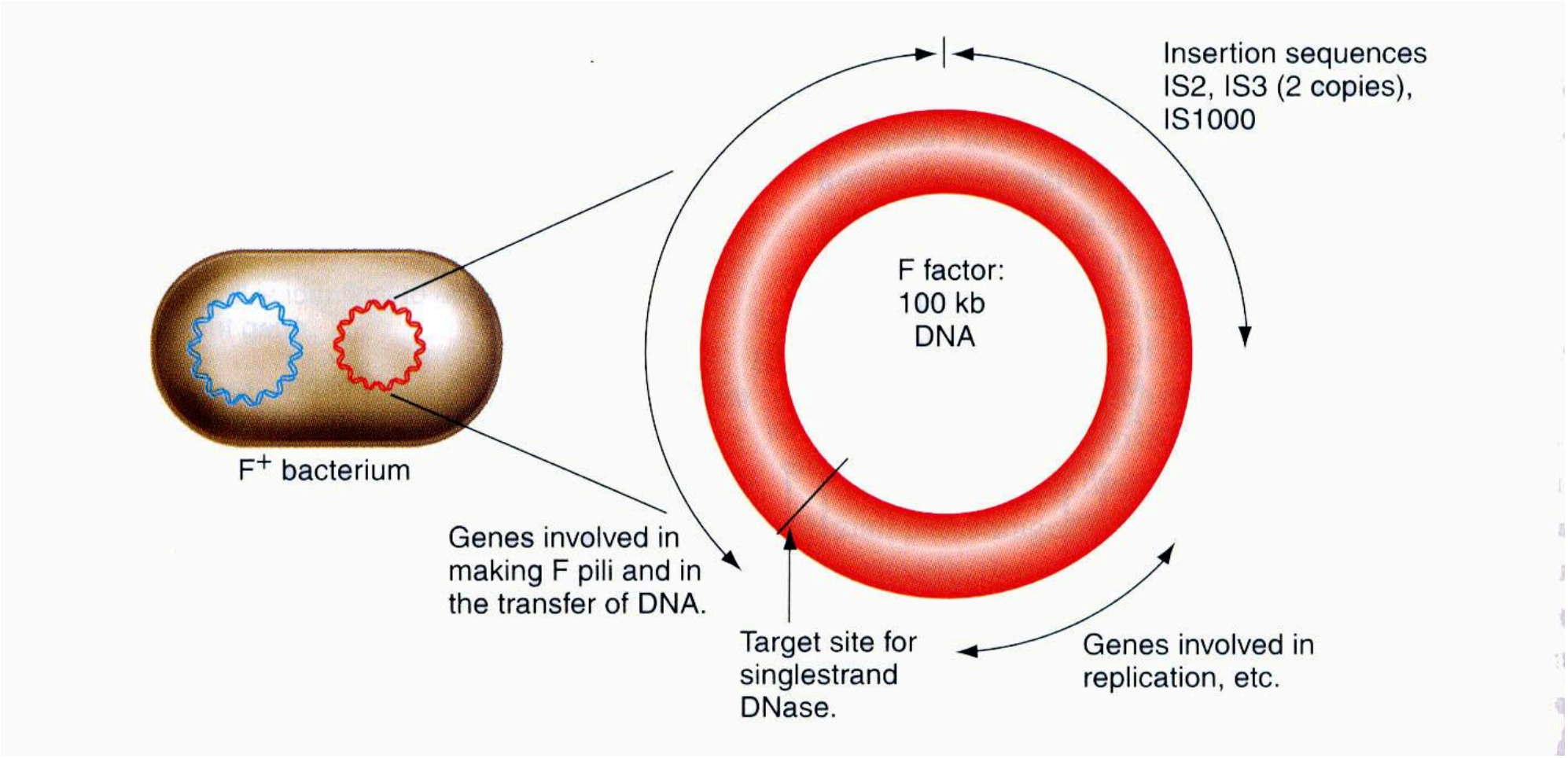
F Pilus



…a filament-like projection from the surface of a bacterium.

F Factor

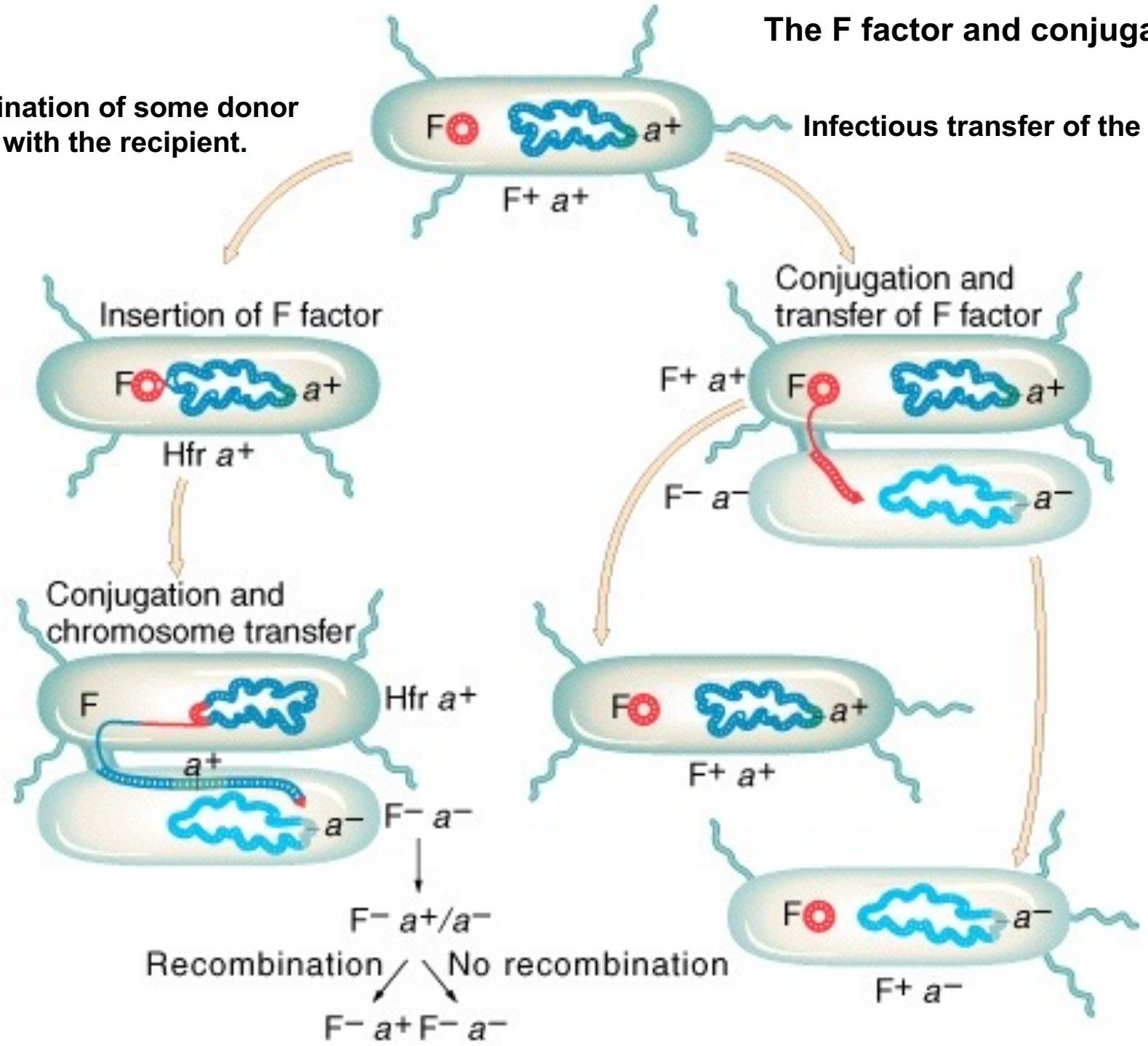
…a plasmid whose presence confers F^+, or donor ability.



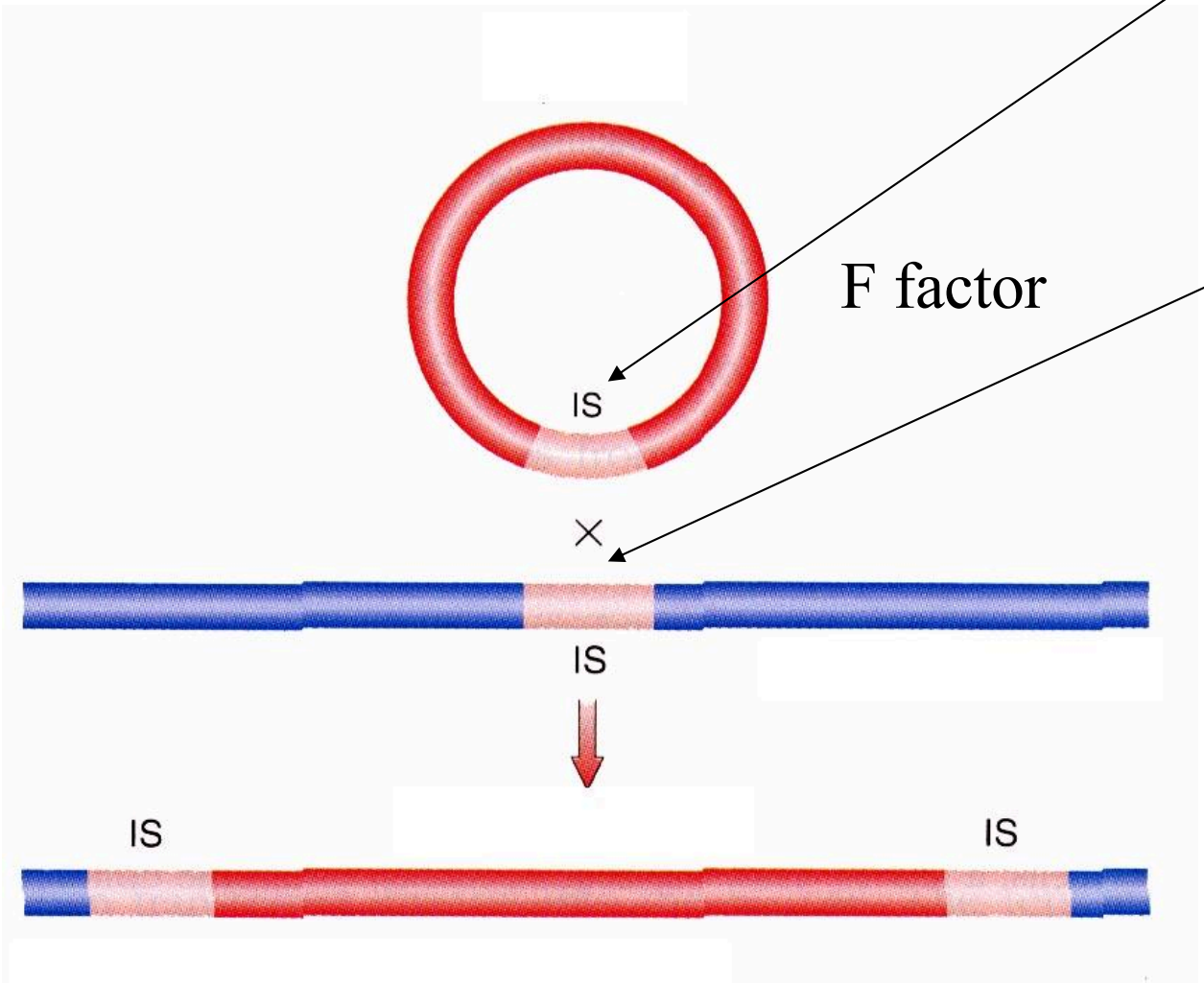
The F factor and conjugation

Recombination of some donor markers with the recipient.

Infectious transfer of the F plasmid



Hfr Cells



...F factor integration site,

F factor

...host (bacteria chromosome) integration site.

Bacterial Chromosome

Inserted F plasmid

High Frequency of Recombination

(Hfr)

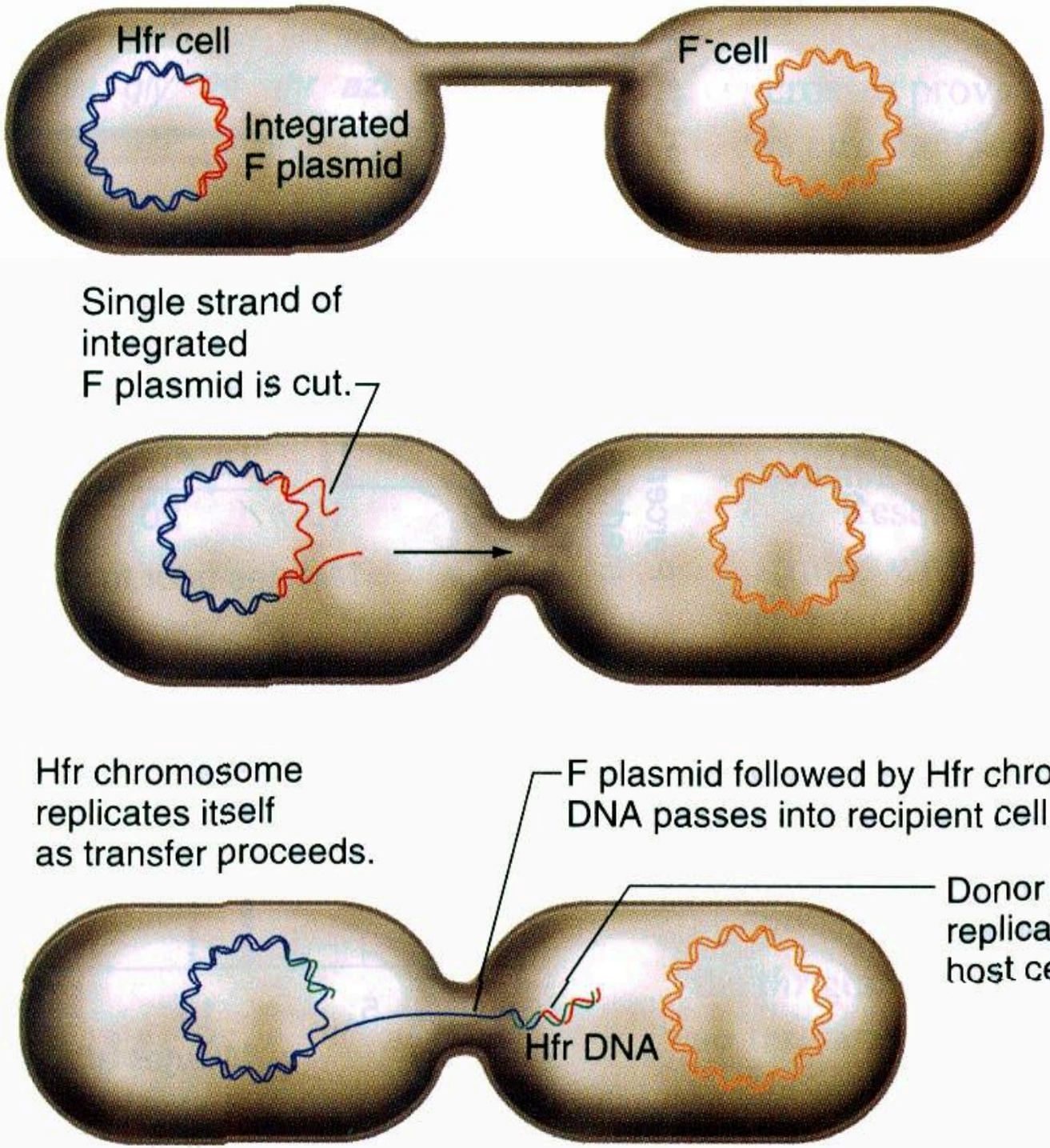
...bacteria exhibiting a high frequency of **recombination**,

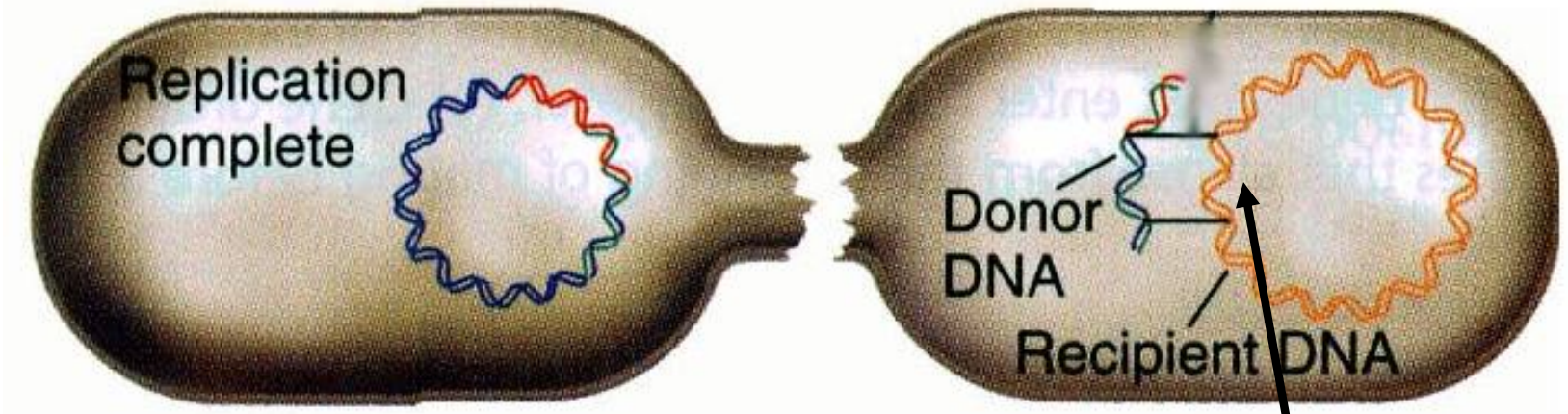
- an alteration DNA sequence such that the genotype of subsequent individuals differs from the parent,

…HFR cell specifically; strains with a chromosome integrated F factor that is able to mobilize and transfer part of the chromosome to the F^- cell.

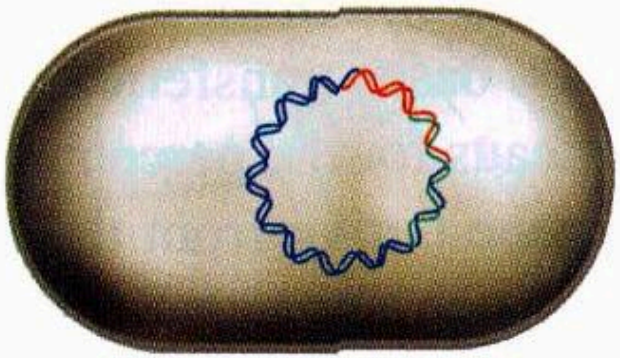
Hfr to F^- Cells

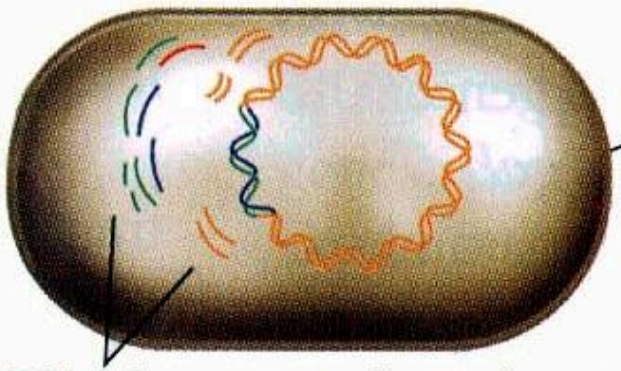
- Hfr transfer begins in the middle of the F factor,
- Only 1/10,000 cells transfer the complete chromosome, including the remainder of the F factor,
- Thus, most recipient cells remain F^-.


Hfr cell
Integrated
F plasmid
F⁻cell
Single strand of
integrated
F plasmid is cut.
Hfr chromosome
replicates itself
as transfer proceeds.
F plasmid followed by Hfr chromosomal
DNA passes into recipient cell.
Donor DNA is
replicated in
host cell.
Hfr DNA



Double Crossover





Cell remains F^- but carries some genes from Hfr chromosome.

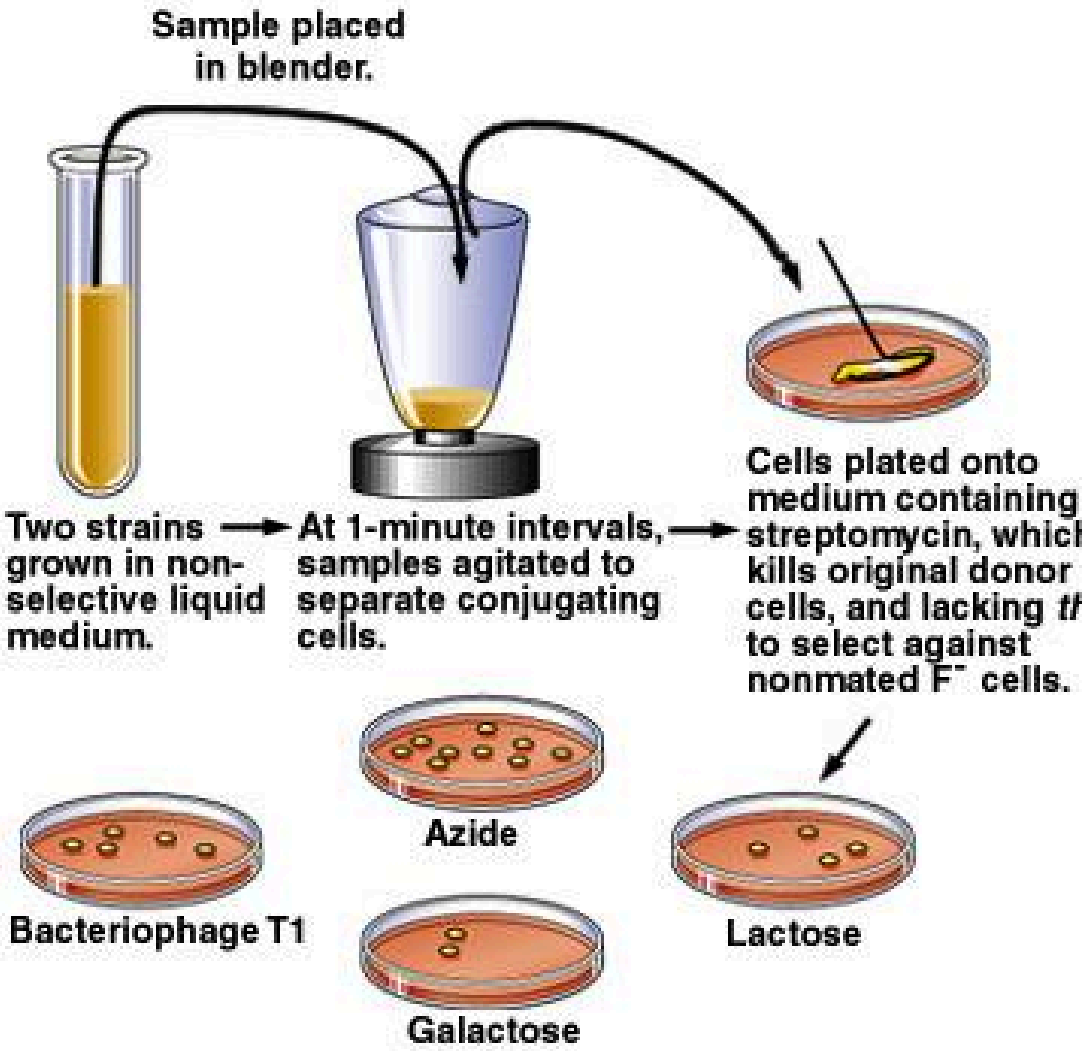
DNA fragments from donor and recipient cells are digested by nucleases.

Interrupted mating experiments

Donor is T+ L+ Azi^r Ton^r lac+ gal+ trp+ Str^s

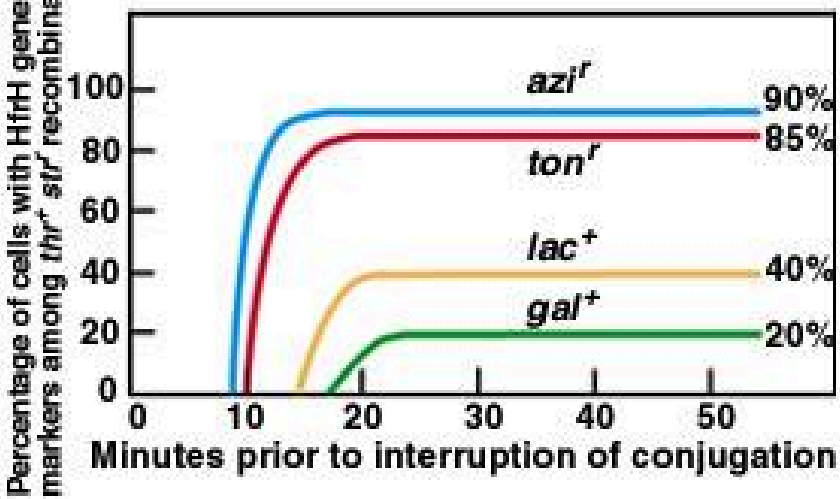
Recipient is T- L- Azi^s Ton^s lac- gal- trp- Str^r

(a)

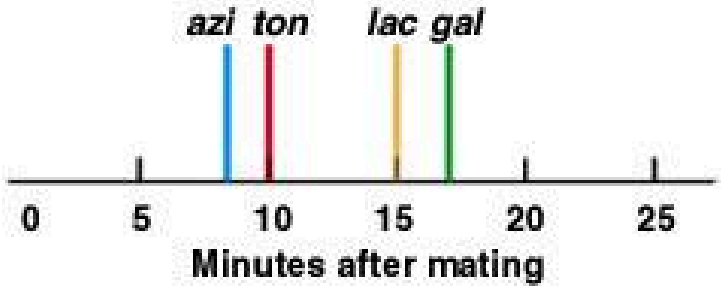


Replica plating tranfers each colony to media that select for four donor markers other than streptomycin.

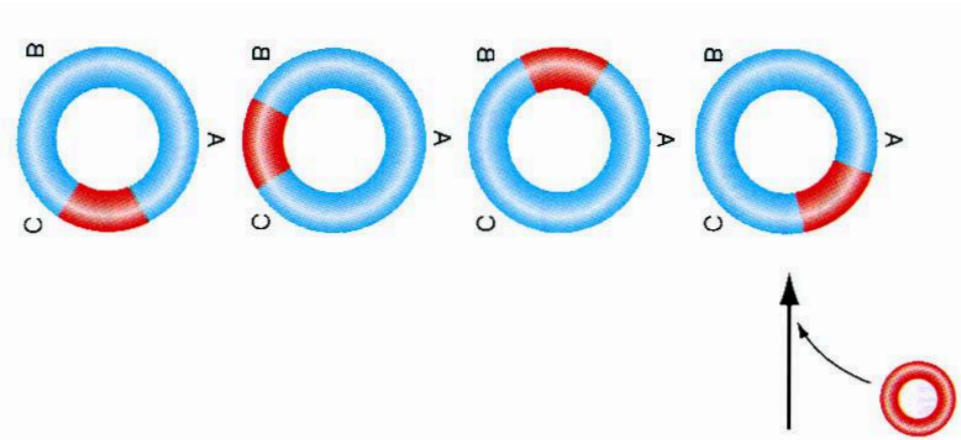
(b)



(c) Map based on mating results

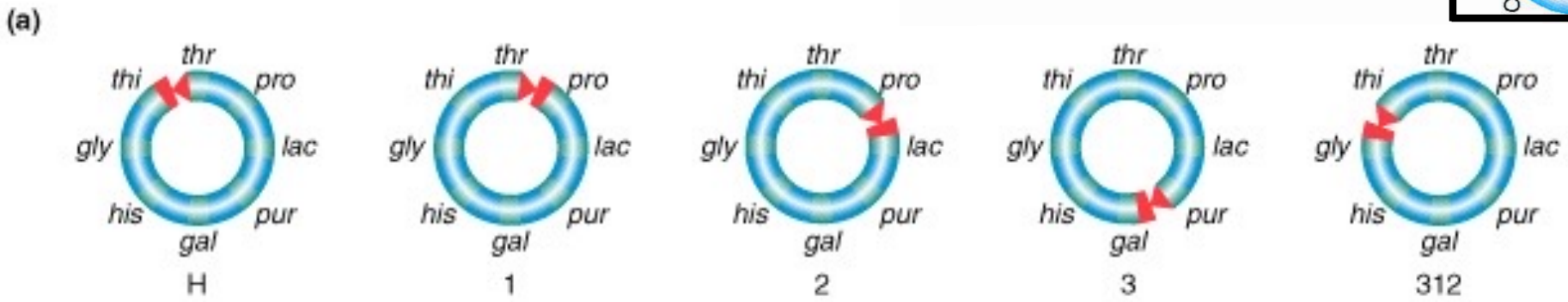


...the bacterial chromosome contains many integration sites,

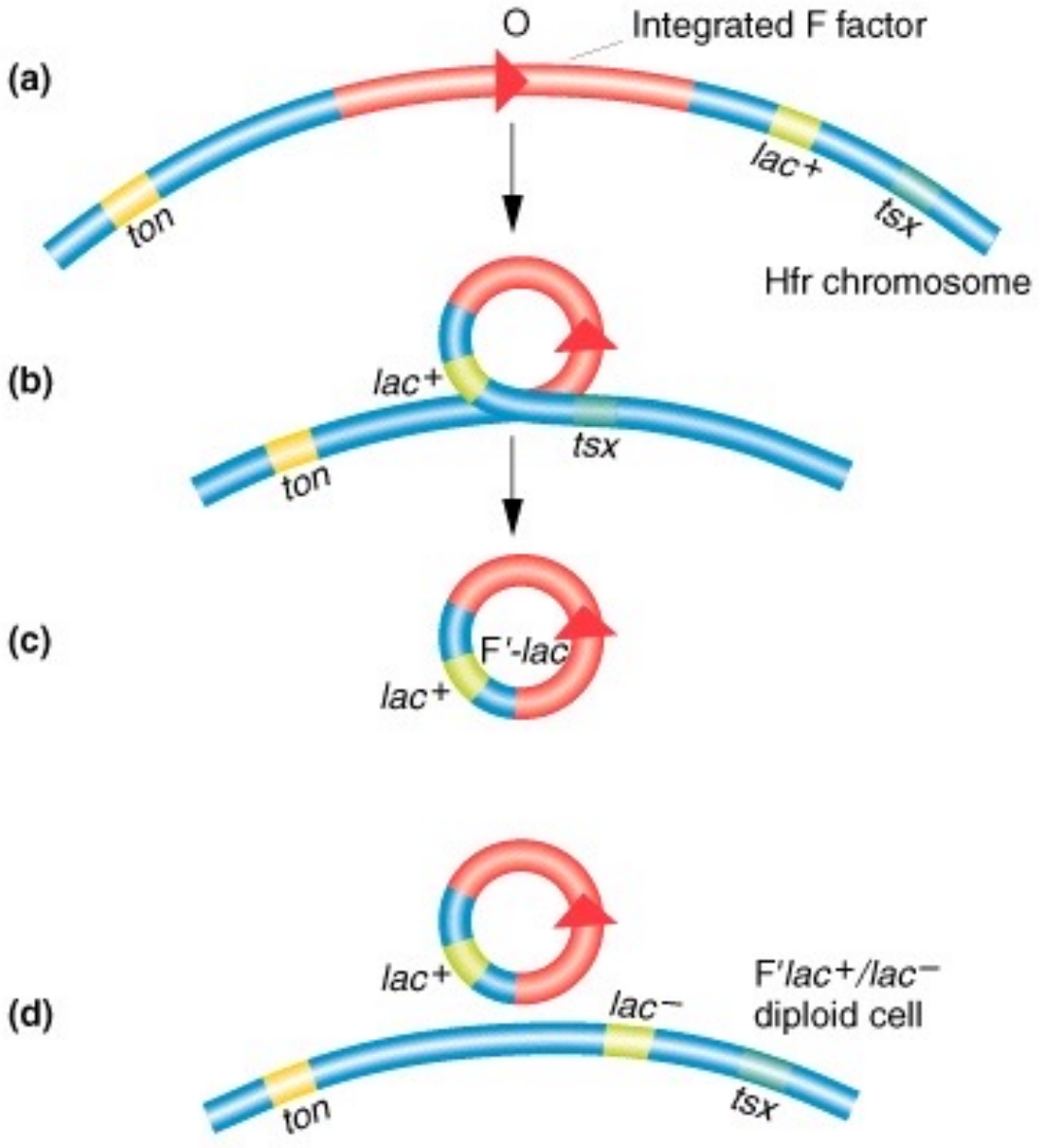


...thus, the F factor inserts in different regions of the bacterial chromosome.

The order of gene transfer using Hfr's with different points of insertion shows that the E.coli chromosome is circular.



The F’. Infectious transfer of an F element that has picked up a small number of cellular genes during excision from the bacterial chromosome.



1. Conjugation allows rapid transfer of plasmid genes between cells

2. Integration (Hfr) followed by transfer of genomic DNA to recipient allows transfer of chromosomal information.

3. F'-like elements allow infectious transfer of neighboring genomic DNA.

Pili-receptor interactions determine which species a bacteria can conjugate with

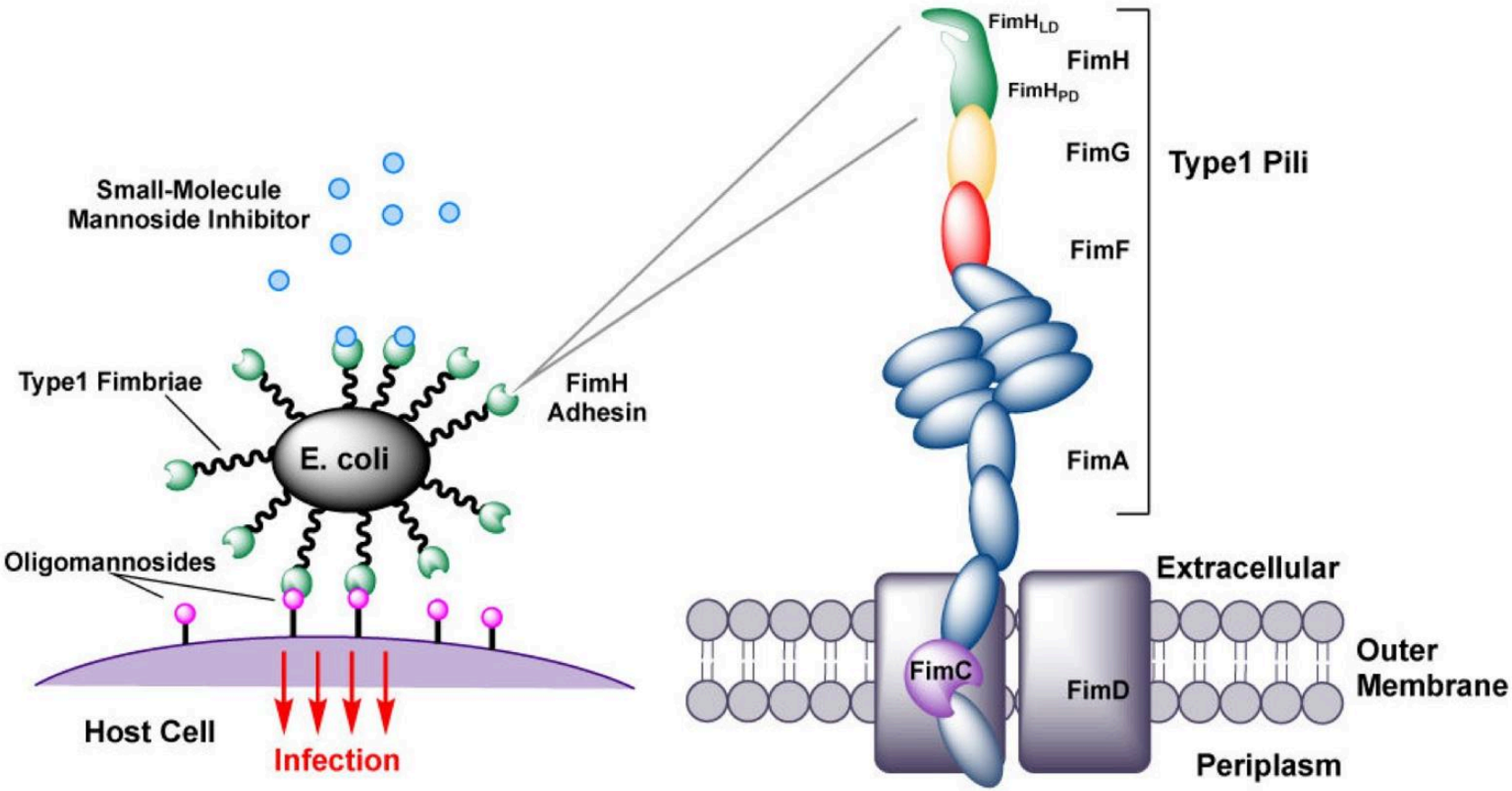


Figure 1.

Molecular recognition of mannosylated receptors on the bladder surface by FimH adhesin of UPEC, residing on the outer tips of type 1 pili. Therapeutic rationale for FimH mannoside ligands in UTI and CD is to block adherence and invasion of bacteria.

The ISME Journal (2015) 9, 934–945

www.nature.com/ismej

ORIGINAL ARTICLE

Broad host range plasmids can invade an unexpectedly diverse fraction of a soil bacterial community

Uli Klümper[1], Leise Riber[2], Arnaud Dechesne[1], Analia Sannazzarro[2], Lars H Hansen[2,3], Søren J Sørensen[2] and Barth F Smets[1]

[1]*Department of Environmental Engineering, Technical University of Denmark, Kgs. Lyngby, Denmark;* [2]*Section for Microbiology, Department of Biology, University of Copenhagen, Copenhagen, Denmark and* [3]*Department of Environmental Science, Aarhus University, Roskilde, Denmark*

Conjugal plasmids can provide microbes with full complements of new genes and constitute potent vehicles for horizontal gene transfer. Conjugal plasmid transfer is deemed responsible for the rapid spread of antibiotic resistance among microbes. While broad host range plasmids are known to transfer to diverse hosts in pure culture, the extent of their ability to transfer in the complex bacterial communities present in most habitats has not been comprehensively studied. Here, we isolated and characterized transconjugants with a degree of sensitivity not previously realized to investigate the transfer range of IncP- and IncPromA-type broad host range plasmids from three proteobacterial donors to a soil bacterial community. We identified transfer to many different recipients belonging to 11 different bacterial phyla. The prevalence of transconjugants belonging to diverse Gram-positive Firmicutes and Actinobacteria suggests that inter-Gram plasmid transfer of IncP-1 and IncPromA-type plasmids is a frequent phenomenon. While the plasmid receiving fractions of the community were both plasmid- and donor- dependent, we identified a core super-permissive fraction that could take up different plasmids from diverse donor strains. This fraction, comprising 80% of the identified transconjugants, thus has the potential to dominate IncP- and IncPromA-type plasmid transfer in soil. Our results demonstrate that these broad host range plasmids have a hitherto unrecognized potential to transfer readily to very diverse bacteria and can, therefore, directly connect large proportions of the soil bacterial gene pool. This finding reinforces the evolutionary and medical significances of these plasmids.

The ISME Journal (2015) **9,** 934–945; doi:10.1038/ismej.2014.191; published online 21 October 2014

Broad host range conjugative plasmids can enter and replicate in many different bacterial species

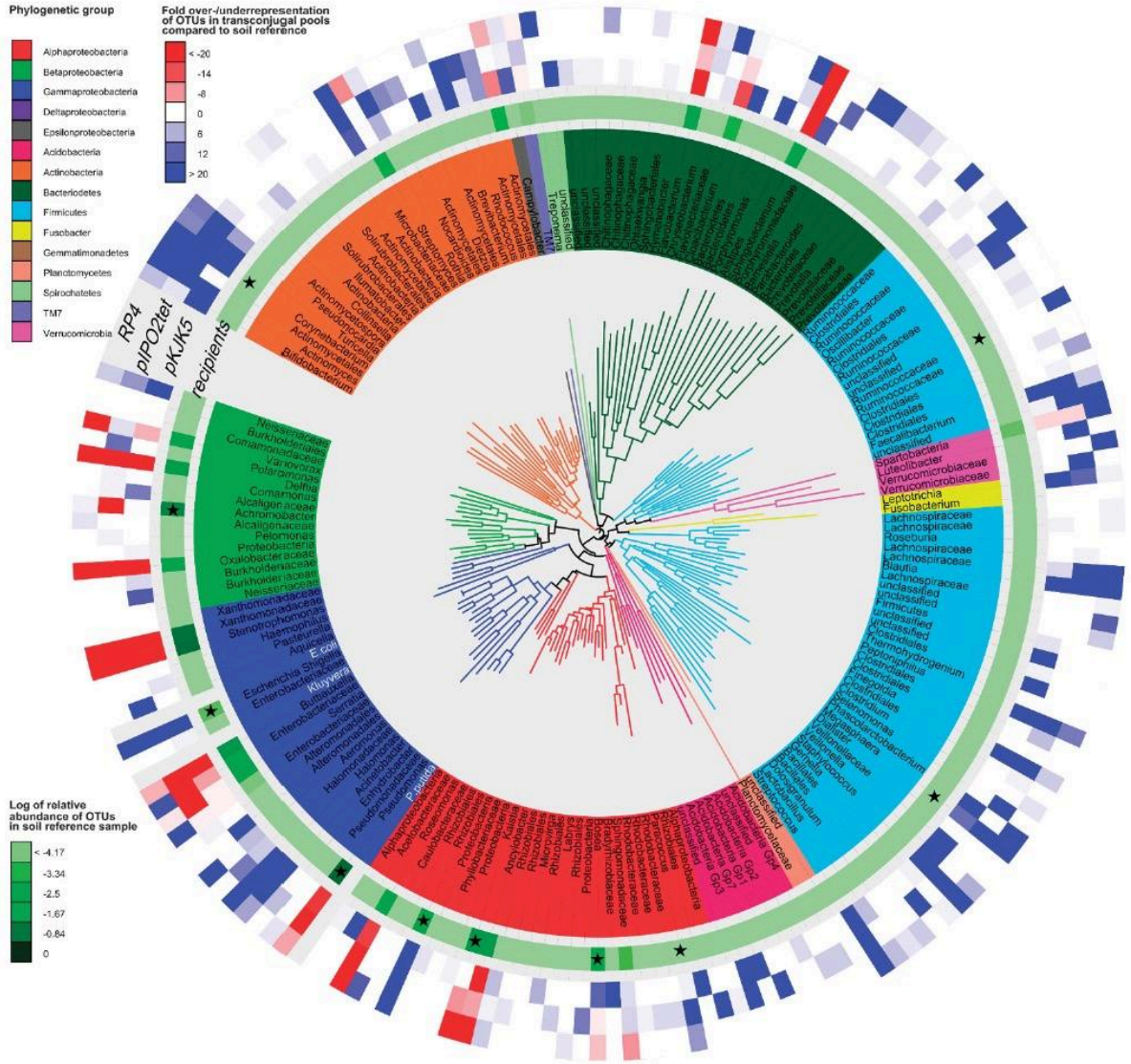
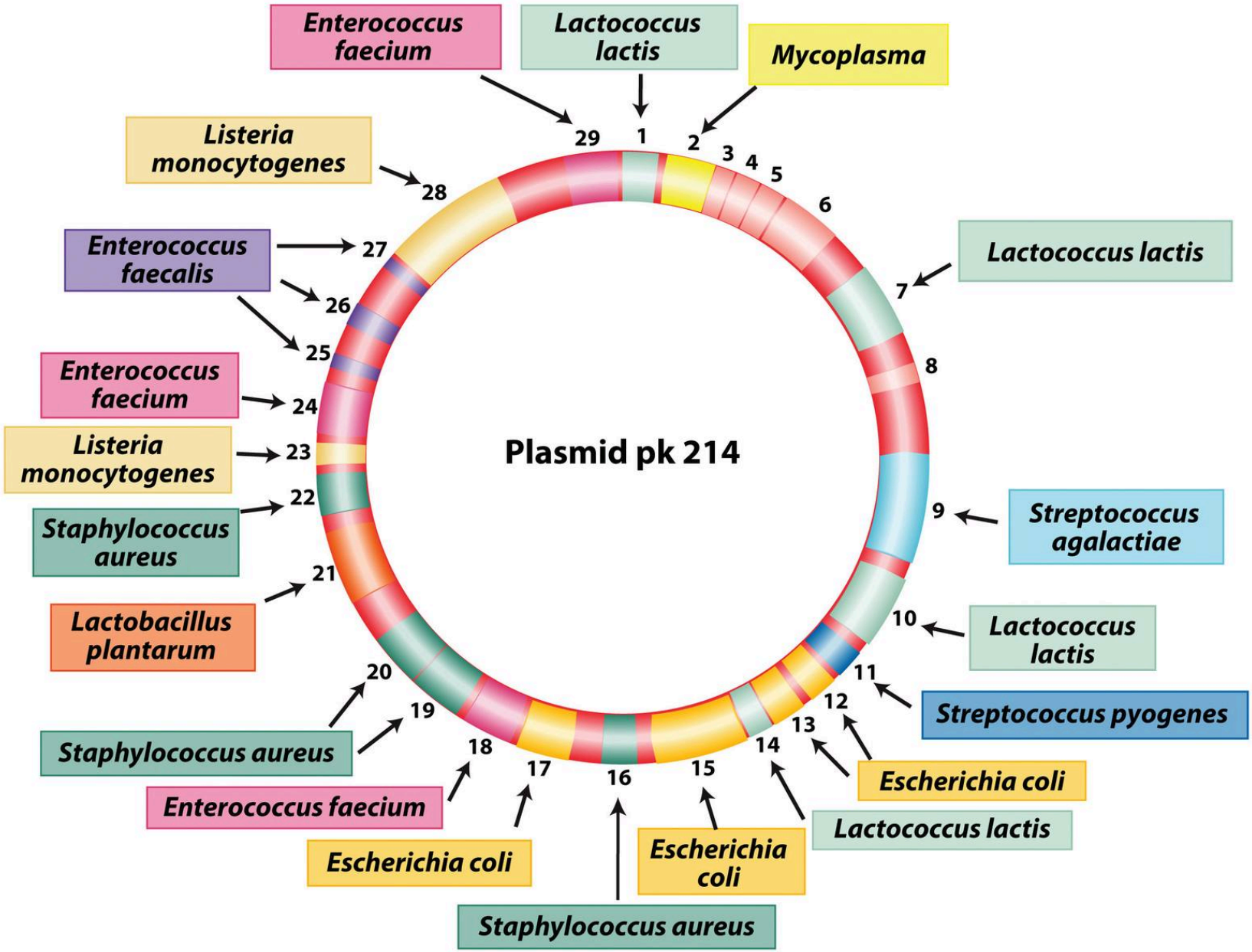


Figure 4 Phylogenetic tree showing all identified transconjugant OTUs for three different plasmids (pKJK5, RP4 and pIPO2tet) from the same donor (*P. putida*). The colors of the branches mark different phylogenetic groups. The three donor strains are shown in white letters in the trees. One green heatmap circle around the tree represents the log-transformed relative OTU abundance in the soil reference-recipient community. Three heatmap circles in blue and red display the *x*-fold over- and underrepresentation of the OTU in the respective transconjugal pool in comparison with the abundance in the reference soil sample. Stars mark the shared (present in all three transconjugal pools) and abundant (present at more than 1% relative sequence abundance) transconjugant OTUs, which constitute the core super-permissive community fraction. Sample size was normalized to 30 000 sequences per transconjugal pool.

A broad host range plasmid picks up genomic DNA from many former bacterial hosts; these are common in our microbiome, in soil, in hospitals



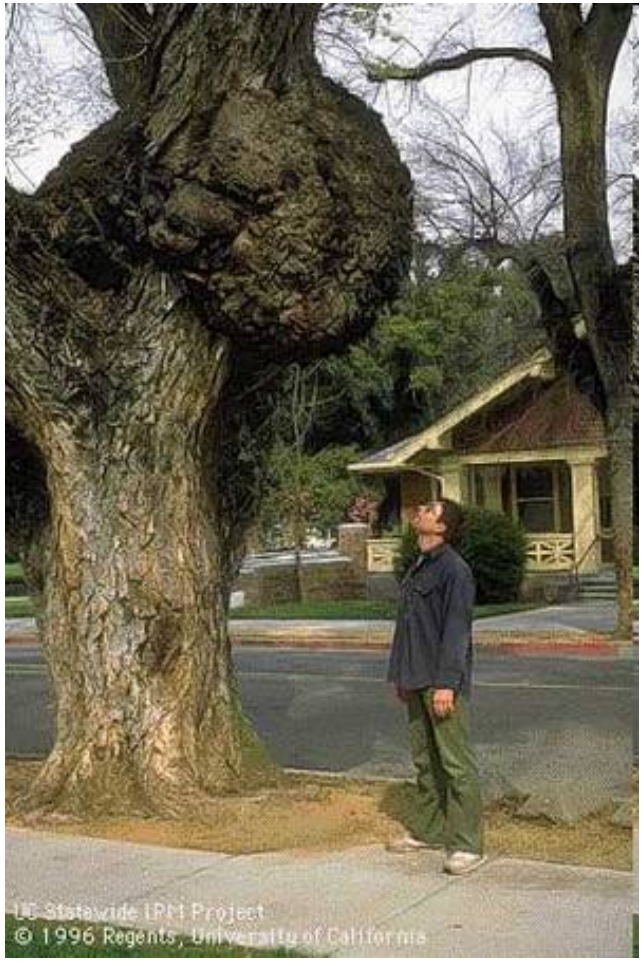
Data from Table 1 in V. Perreten, F. Schwarz, L. Cresta, M. Boeglin, G. Dasen, and M. Teuber, *Nature* 389, 1997, 801–802

Figure 6-19
Introduction to Genetic Analysis, Twelfth Edition

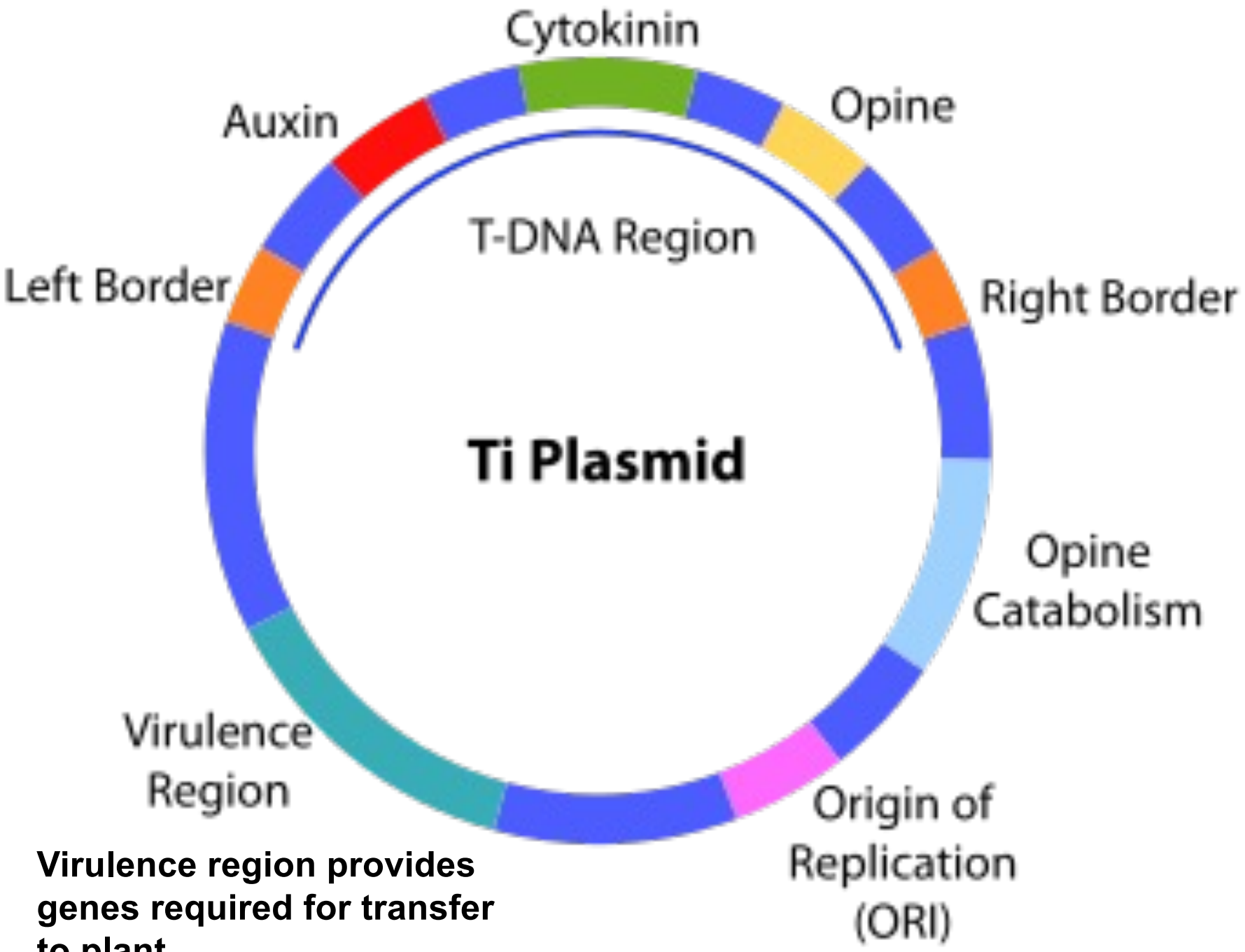
Agrobacterium

Turning a tumor forming bacteria into a powerful tool for plant genetic engineering

Fig. 1. Crown gall tumor on an oak tree.



Agrobacterium

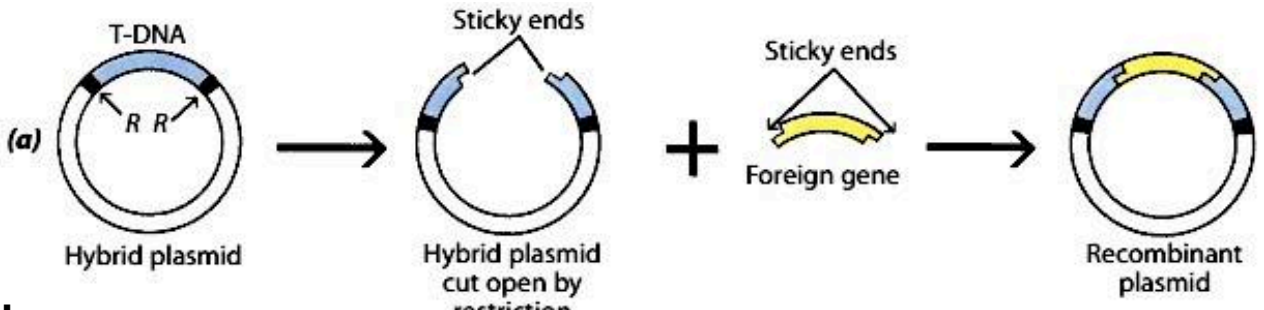


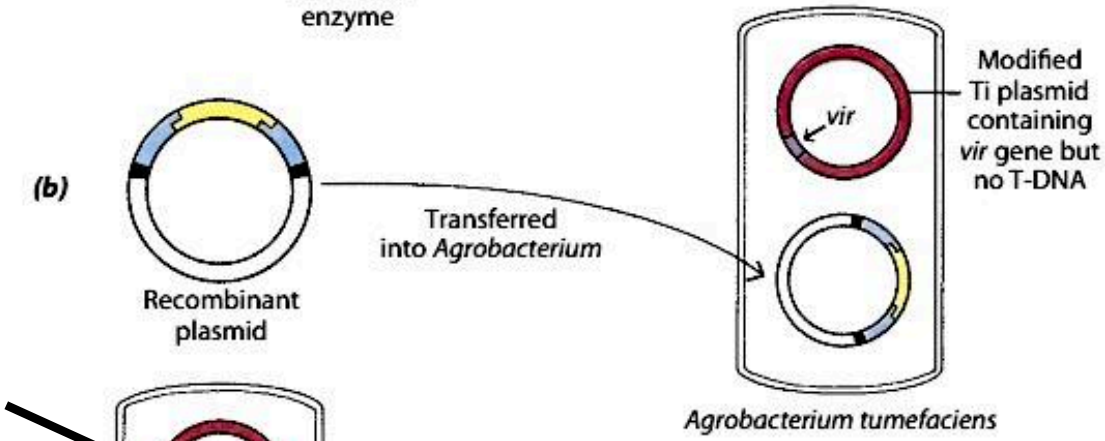
Virulence region provides genes required for transfer to plant

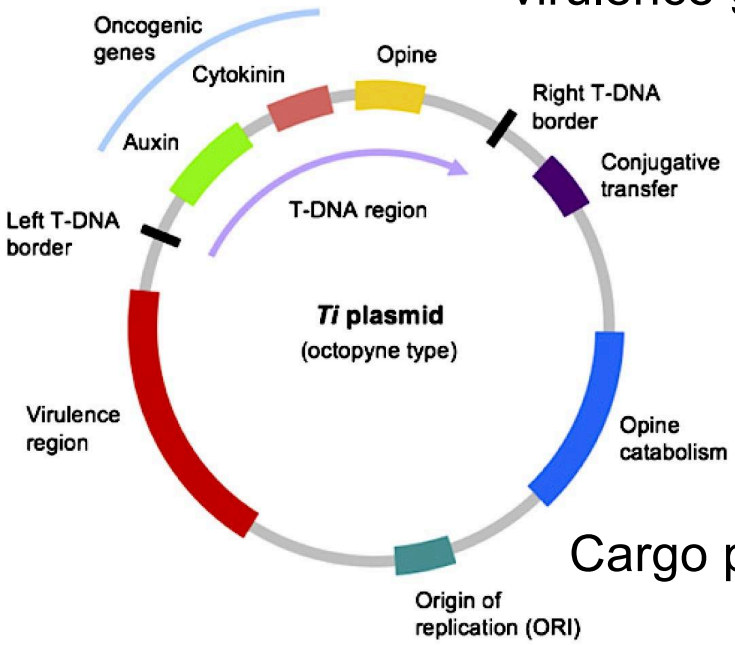
T-DNA can be mobilized from the first plasmid. It carries your gene of interest.

The second plasmid carries virulence genes that mediate transfer of T-DNA from the first plasmid.

When both plasmids are present in agrobacterium the T-DNA is transfered to the plant

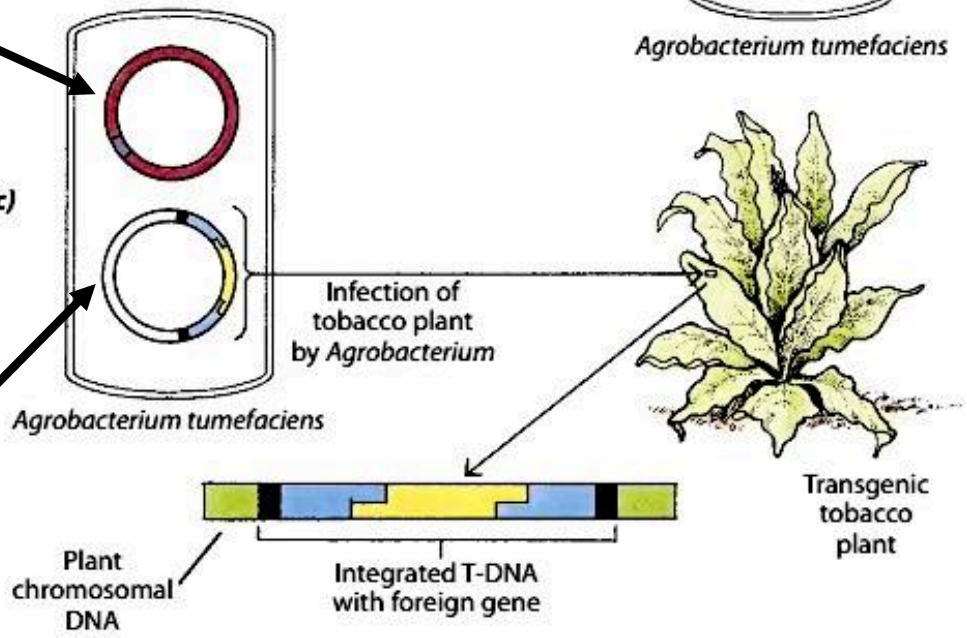






virulence genes

Cargo plasmid



Arabidopsis Floral Dip

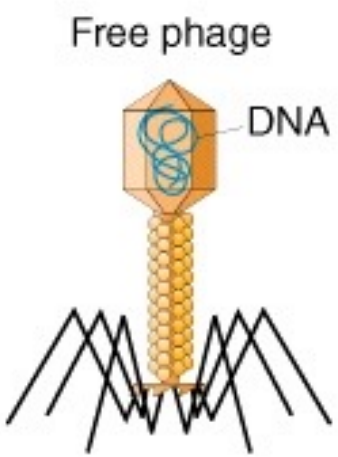
Papaya ringspot virus

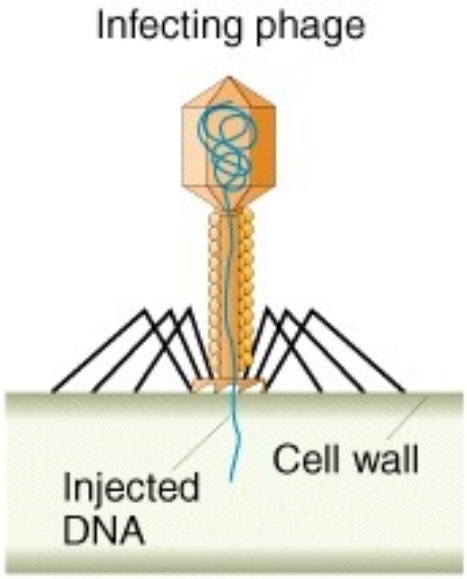
transgenic

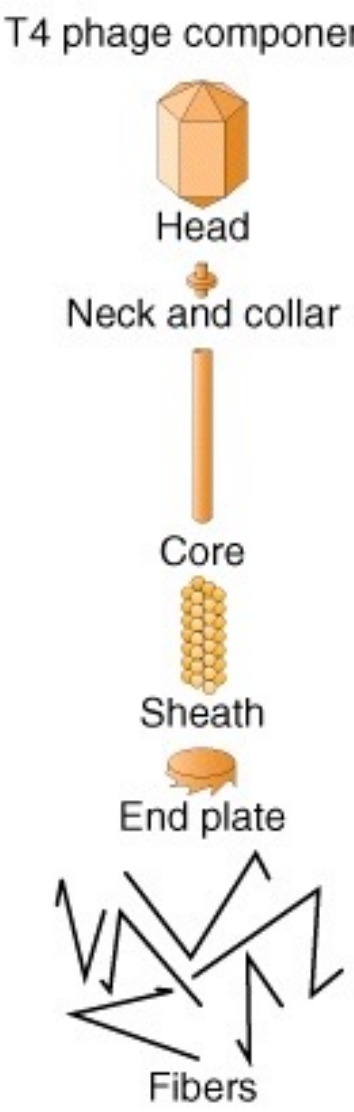
Non-transgenic

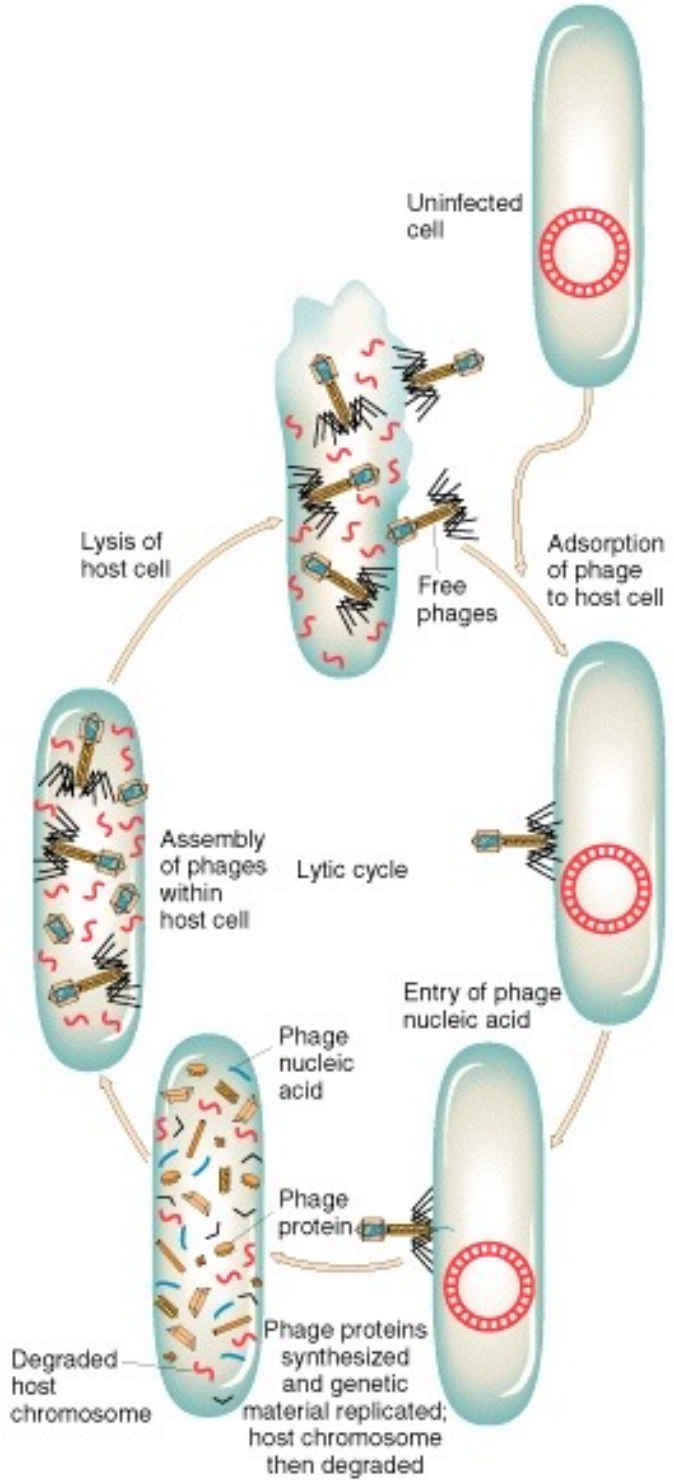
Genetics without Sex: Gene transfer in Prokaryotes

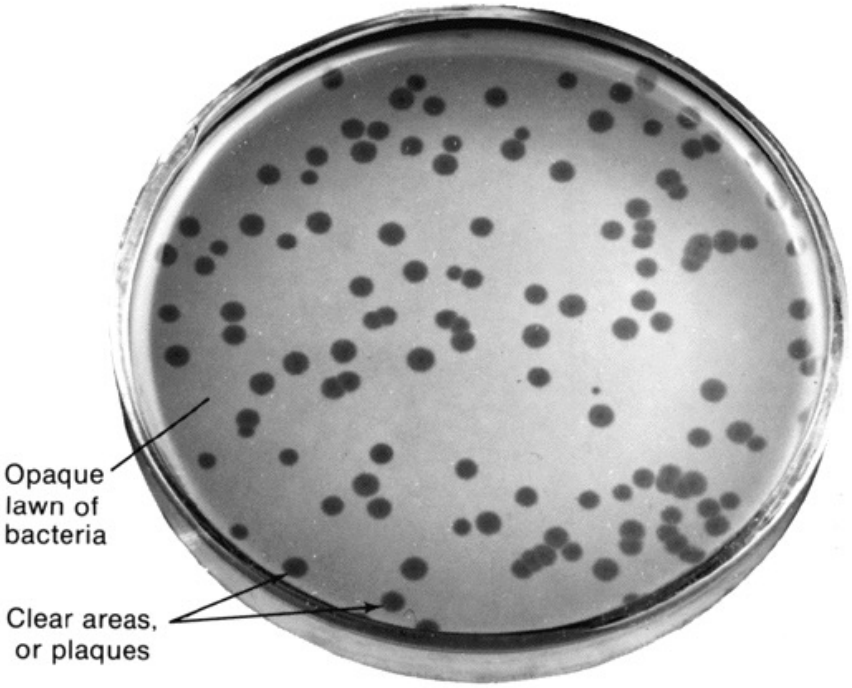
- **Transformation** - cells take up DNA directly from the environment
- **Conjugation** - cell-to-cell transfer of genetic material
- **Transduction** - Transfer of genetic material by phage or phage-like particles

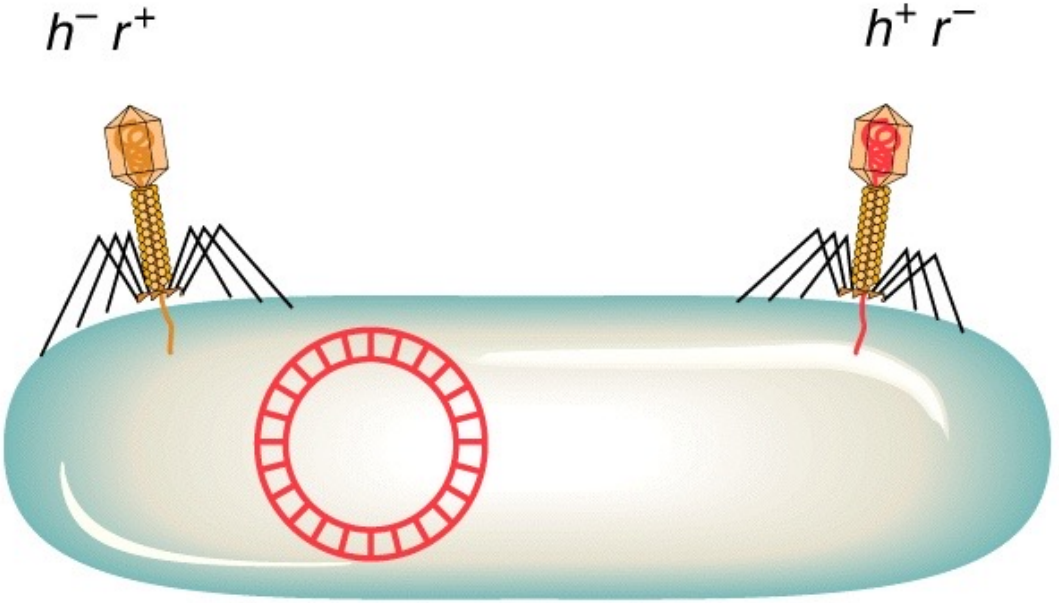

Free phage
DNA


Infecting phage
Injected DNA
Cell wall


T4 phage components
Head
Neck and collar
Core
Sheath
End plate
Fibers

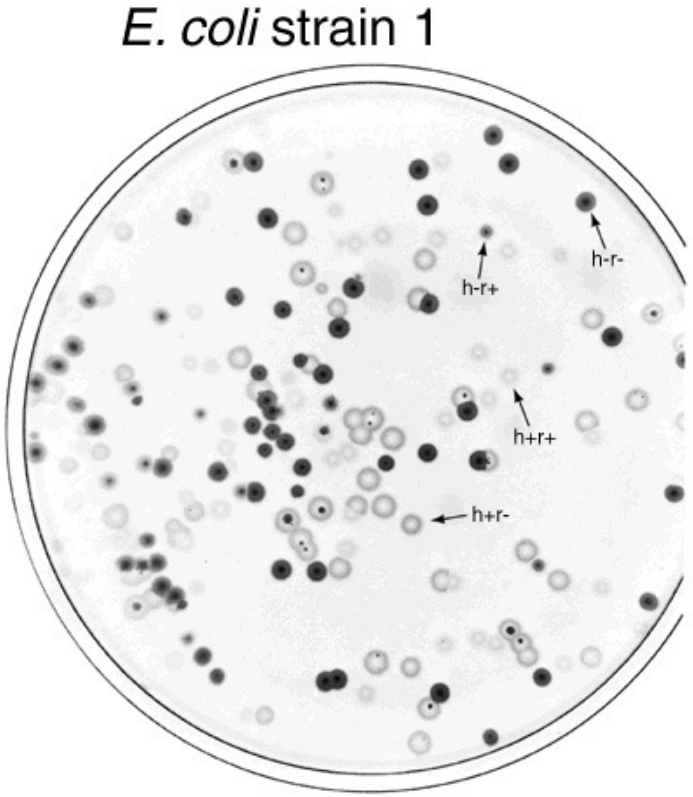

Uninfected cell
Adsorption of phage to host cell
Free phages
Lysis of host cell
Assembly of phages within host cell
Lytic cycle
Entry of phage nucleic acid
Phage nucleic acid
Phage protein
Phage proteins synthesized and genetic material replicated; host chromosome then degraded
Degraded host chromosome





1. **Phage have a single chromosome.**
2. **Thus they are haploid and genotype = phenotype.**
3. **Multiple phage can infect the same cell.**
4. **Recombination can occur between these genomes.**

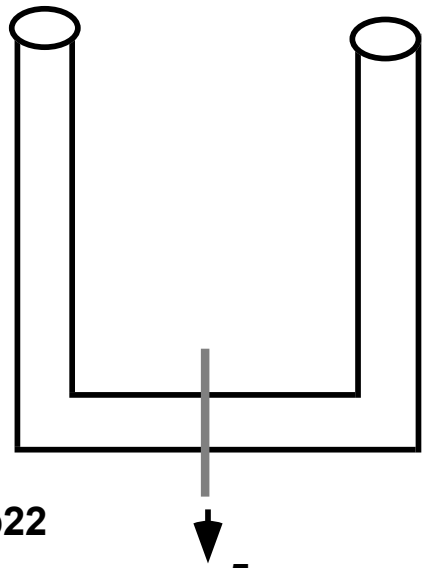
$$RF = \frac{(h+r+) + (h-r-)}{\text{Total plaques}}$$



Generalized transduction

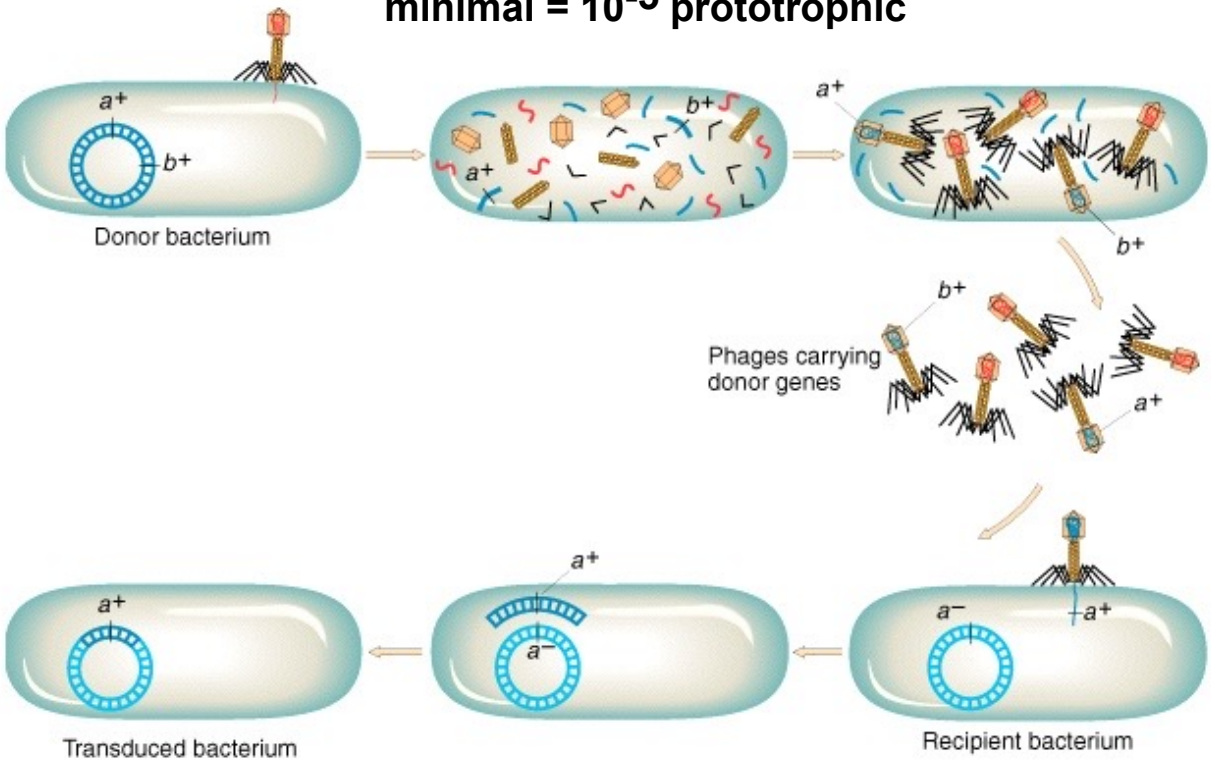
m+ h+ phe- trp- tyr-

m- his- phe + trp + tyr+

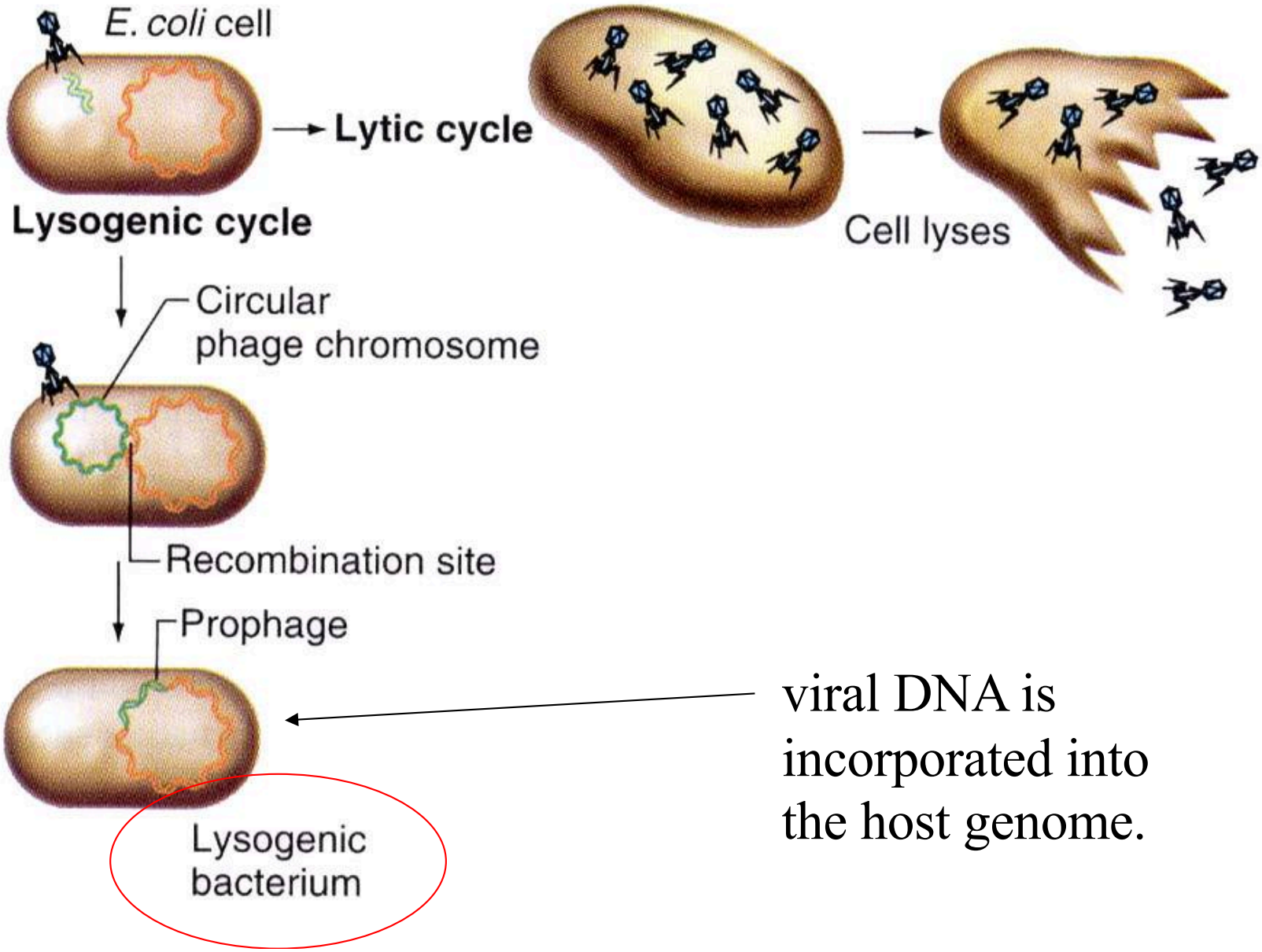


1. can pass through a filter
2. unidirectional
3. not inactivated by DNAse
4. Strain on left lysogenic for phage p22

minimal = 10^{-5} prototrophic



Lytic vs Lysogenic



viral DNA is incorporated into the host genome.

Lysogeny

…the integration of viral DNA into the bacterial genome,

- a virus that can integrate into the genome is termed **temperate**,
- an integrated phage is termed a **prophage**.

Prophage

…non-virulent units that are inserted in the host chromosome, and multiply via binary fission along with the host DNA,

…prophage can re-enter the lytic cycle to complete the virus life cycle.

Phage Induction

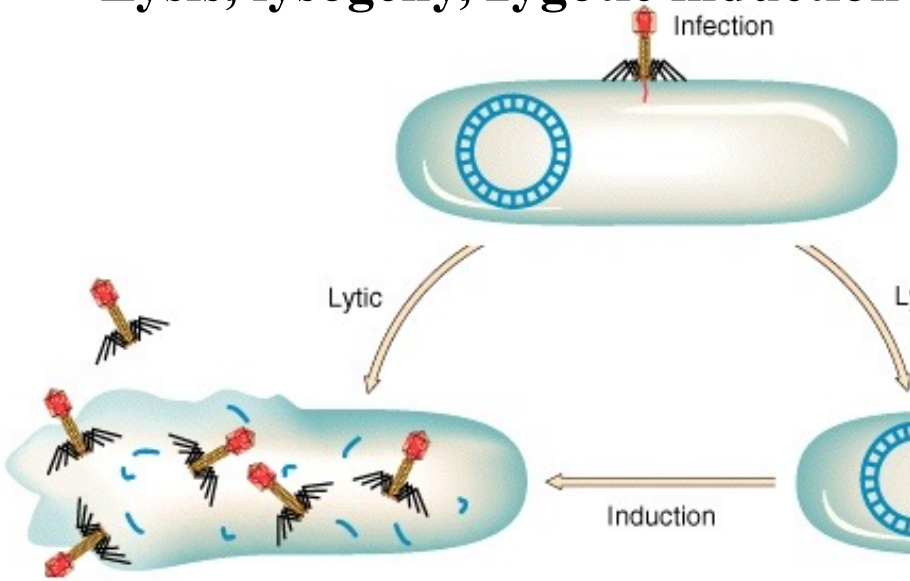
…prophage express a repressor protein that inhibits further infection,

- **also inhibits prophage DNA excision genes, and genes used during the lytic cycle,**

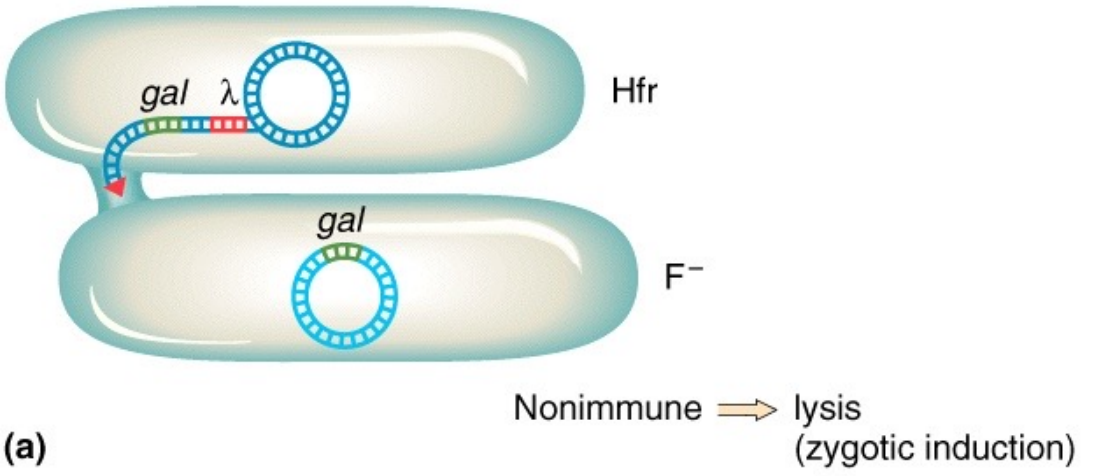
…environmental cues (especially events that damage DNA) block the expression of the repressor protein,

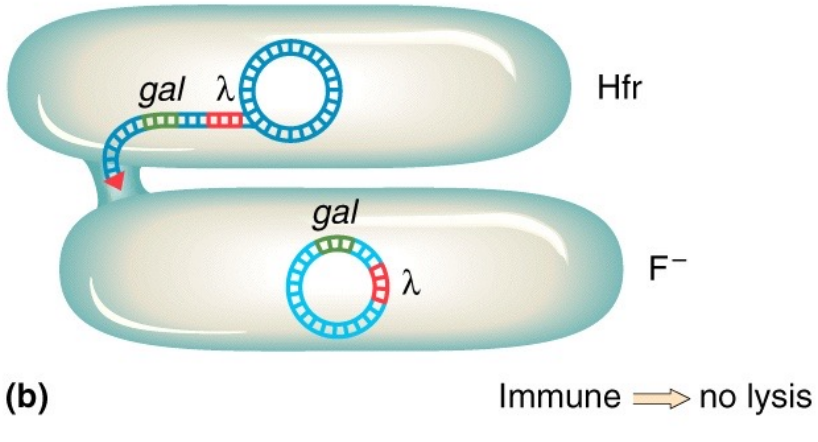
- **prophage excises and enters a lytic cycle.**

Lysis, lysogeny, zygotic induction and immunity

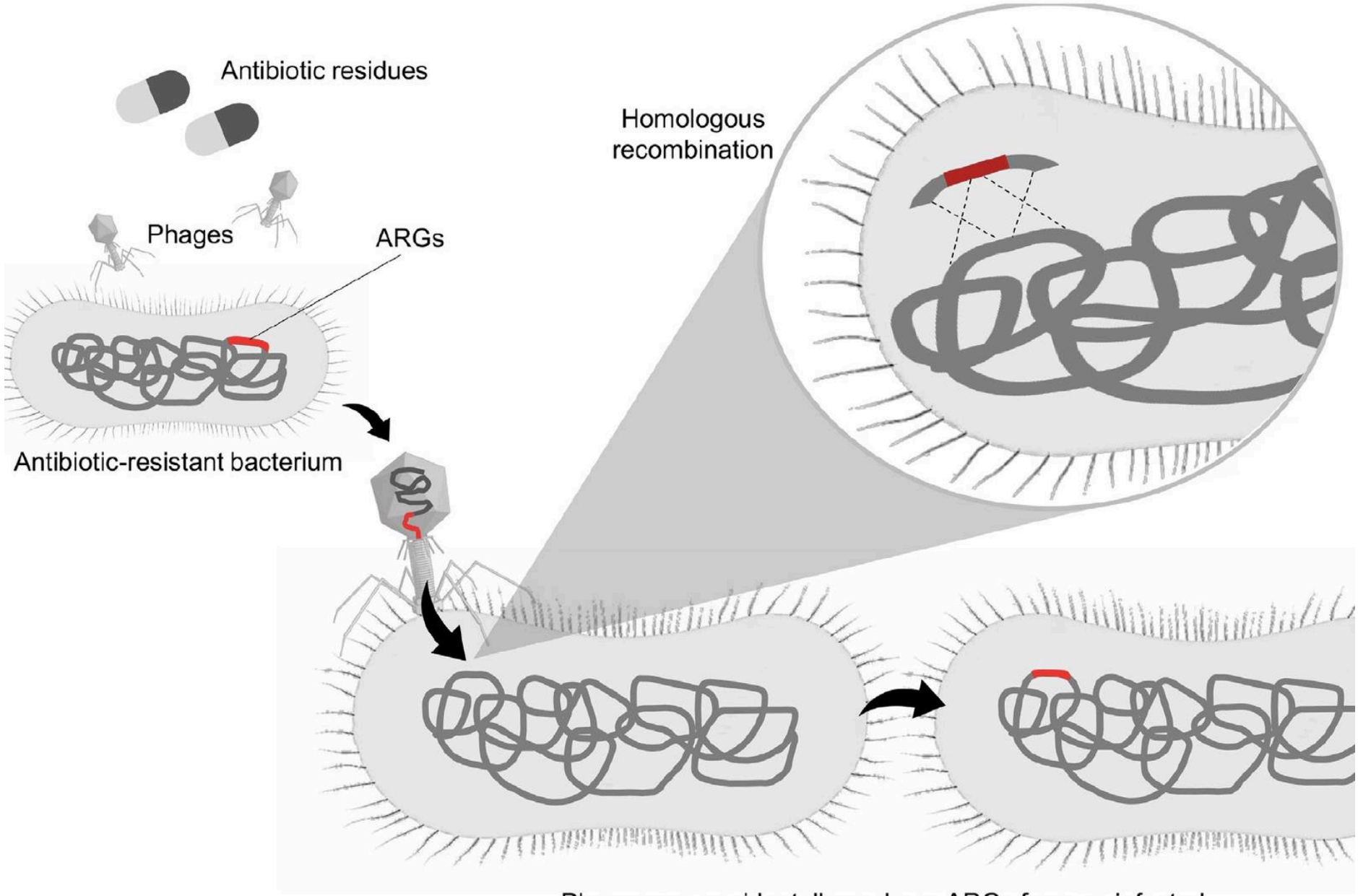


Cells that carry a lysogenic phage express a repressor protein that makes these cells immune to lytic infection by a phage of the same type.





Phage can also transfer antibiotic resistance between bacteria



Phages can accidentally package ARGs from an infected bacterial cell and transduce them into new recipient hosts

Phage therapy for antibiotic resistant infections

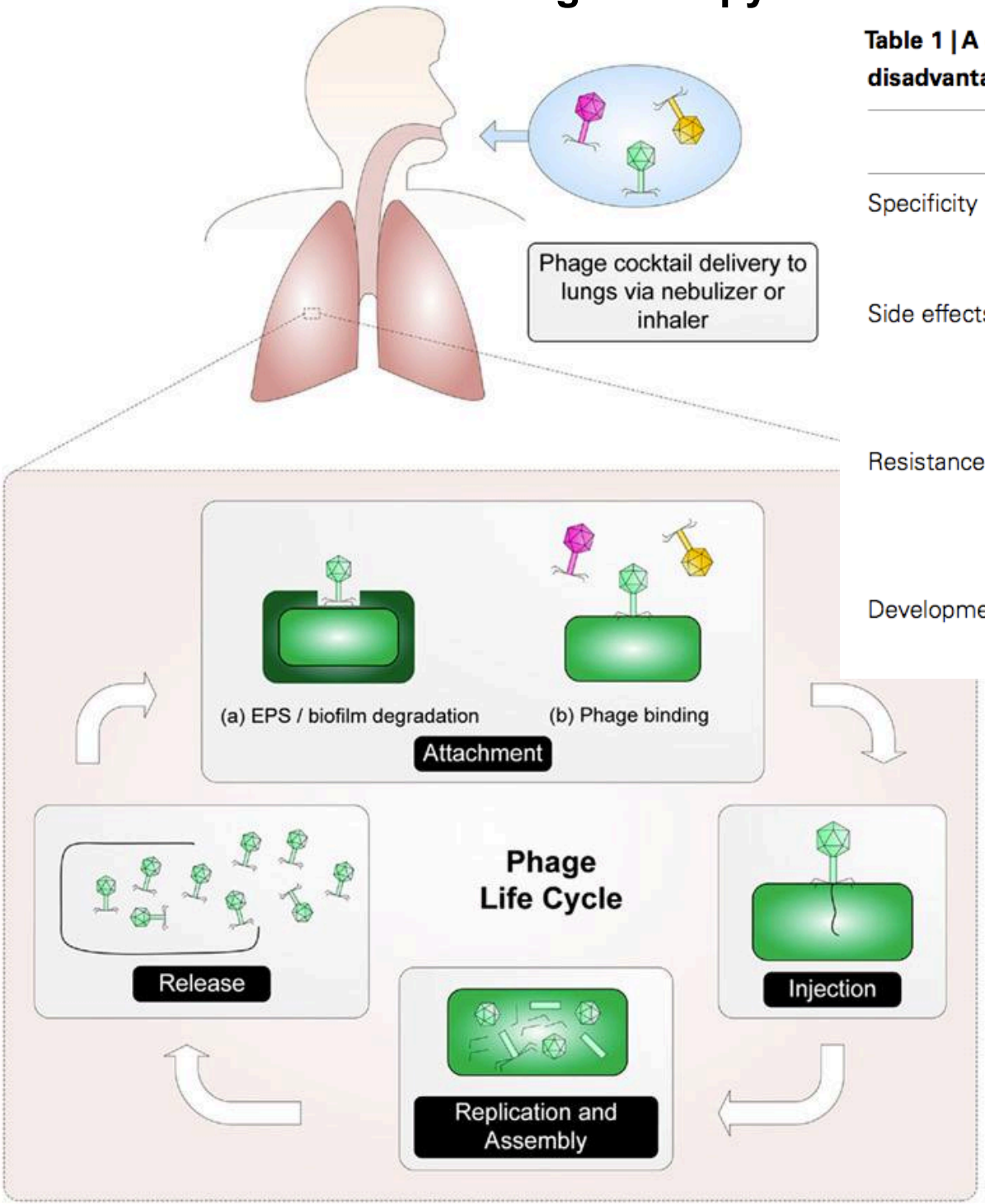
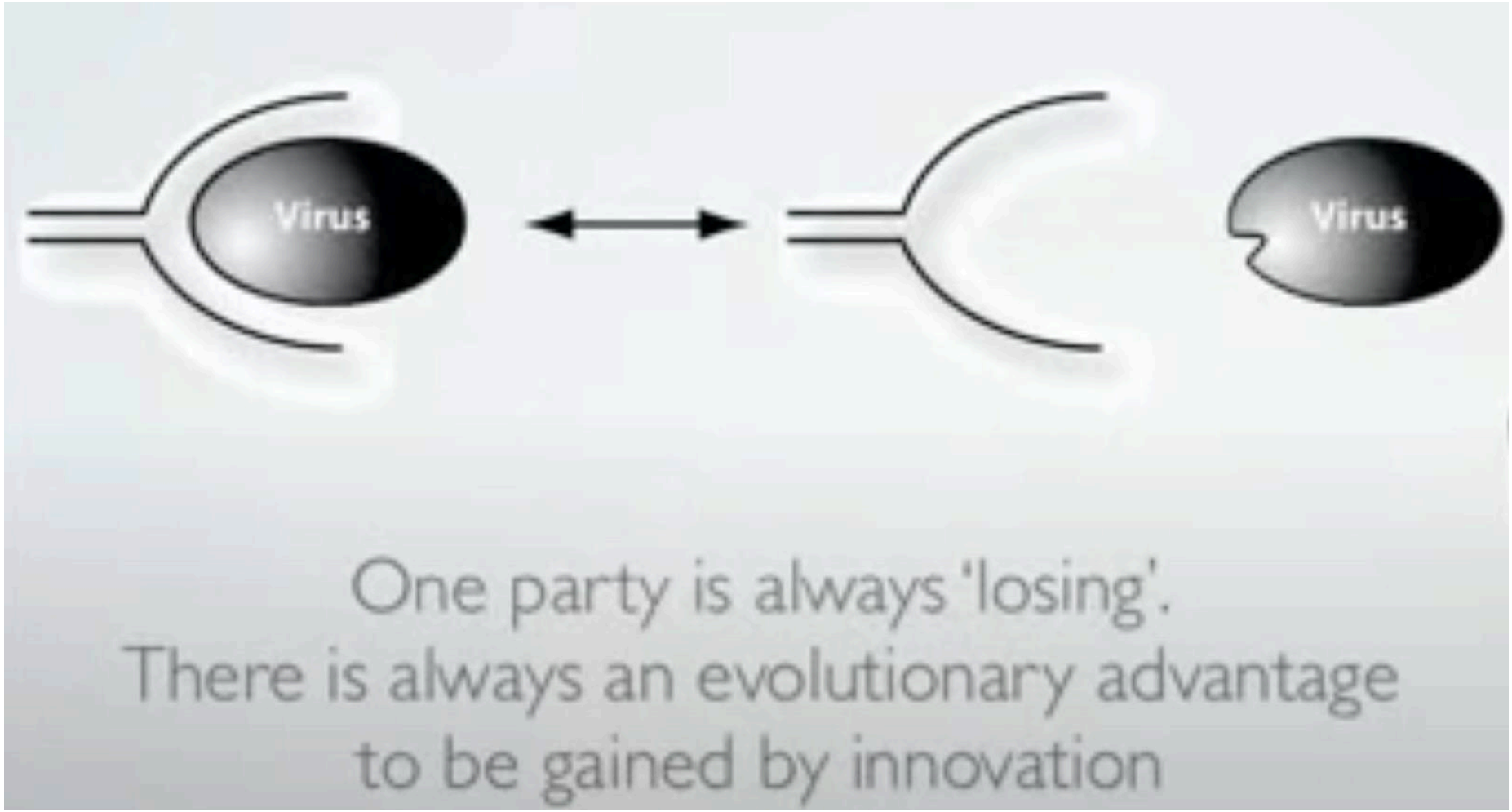


Table 1 | A comparison of some of the main advantages and disadvantages of antibiotics and phage therapy.

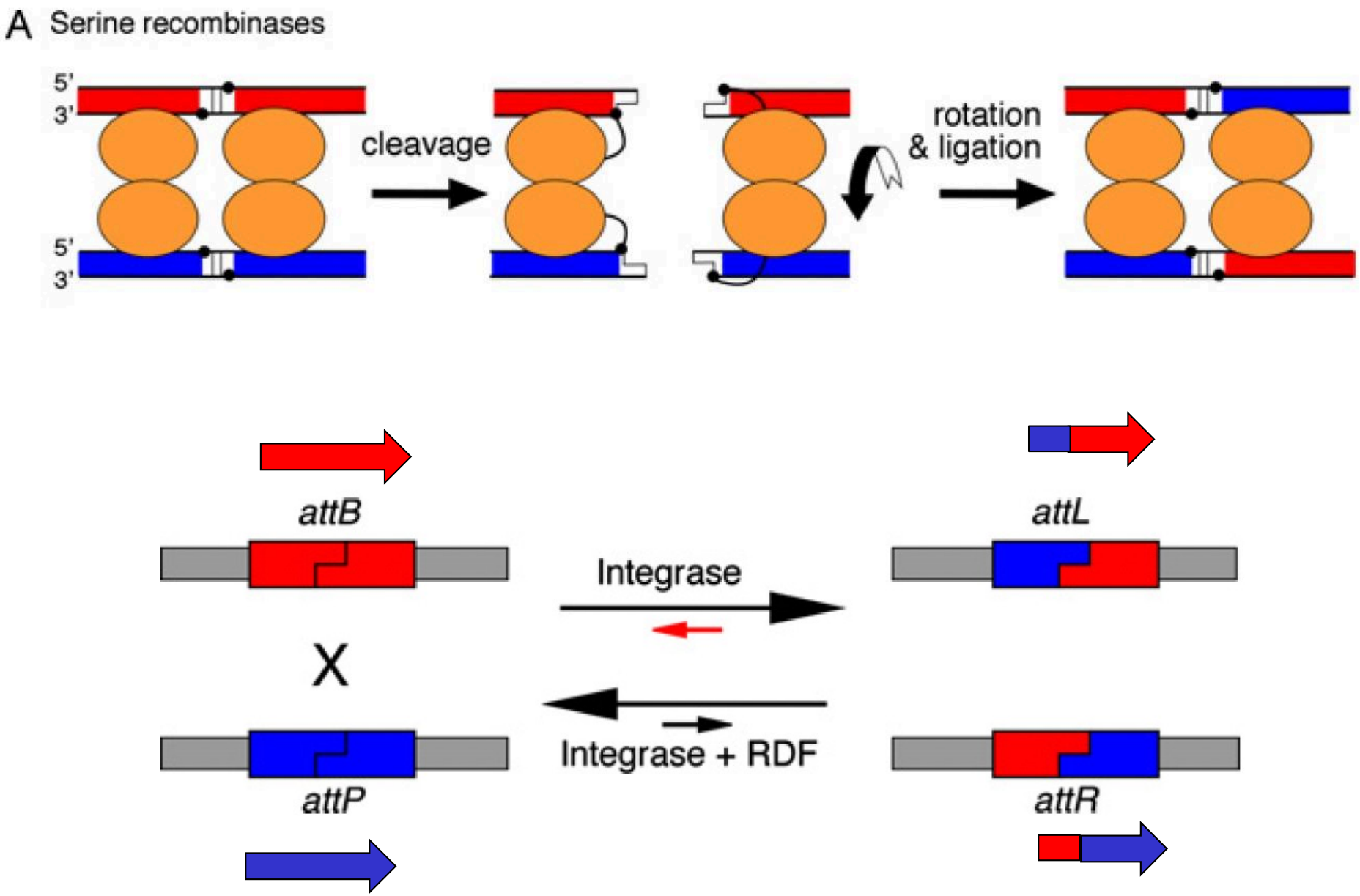
	Antibiotics	Phage therapy
Specificity	Broad spectrum, affecting more than the targeted organism	Generally species or strain specific
Side effects	Many, including allergies and intestinal disorders	No side effects (Bruttin and Brüssow, 2005; Merabishvili et al., 2009; Rhoads et al., 2009; Wright et al., 2009)
Resistance	Occurs and is not limited to targeted bacteria	Occurs, but can be linked to host virulence attenuation (Zahid et al., 2008). Also, phage can co-evolve with host
Development	Time-consuming and expensive	Rapid

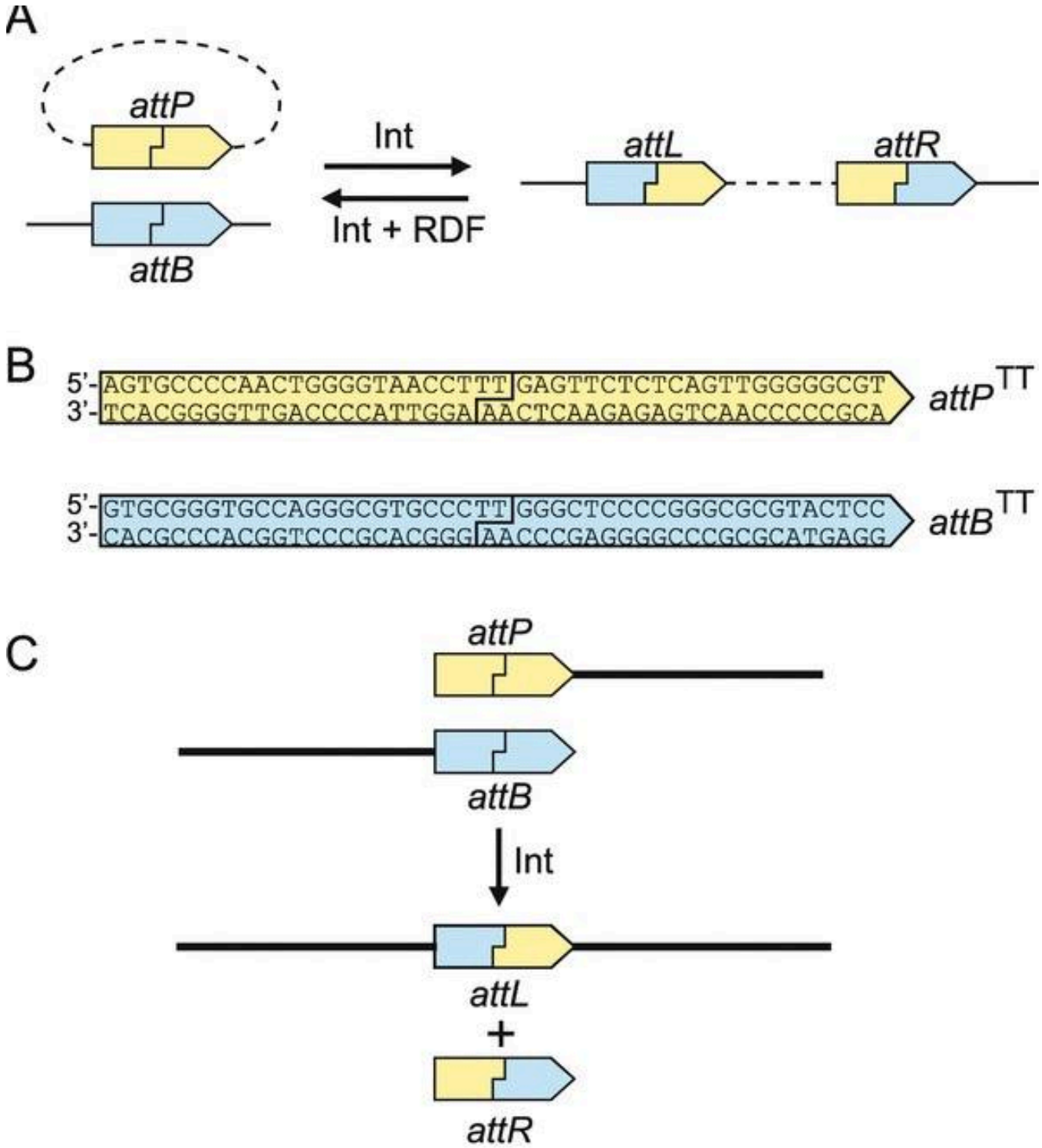
How do species keep winning? Specialized mechanisms for creating genetic diversity at specific loci (genes)



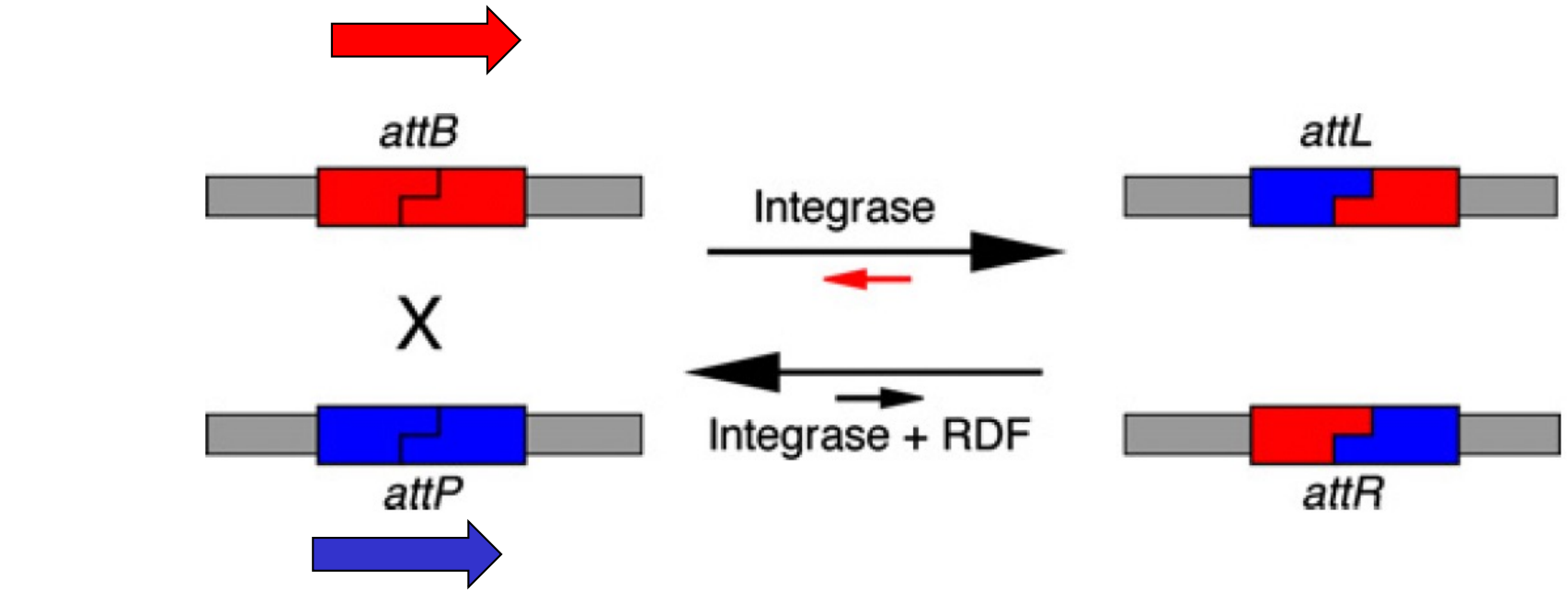
https://www.youtube.com/watch?v=y6rqugj_mVk

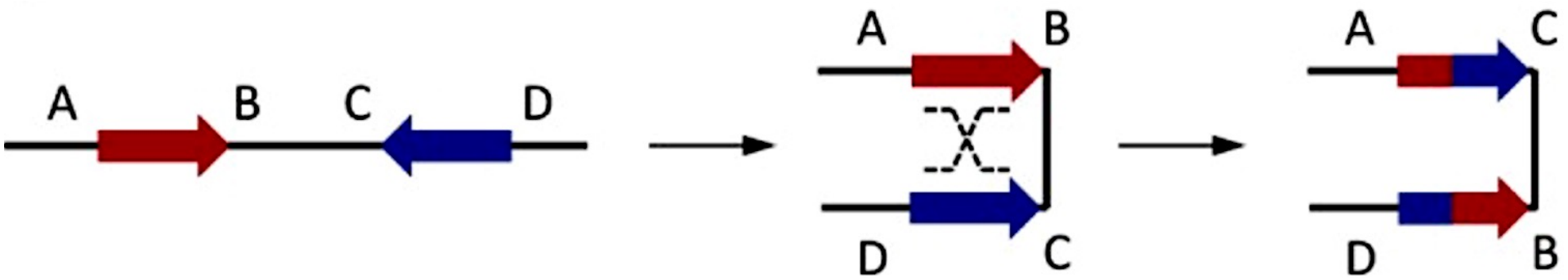
Site-specific recombination between two sequences to change DNA orientation

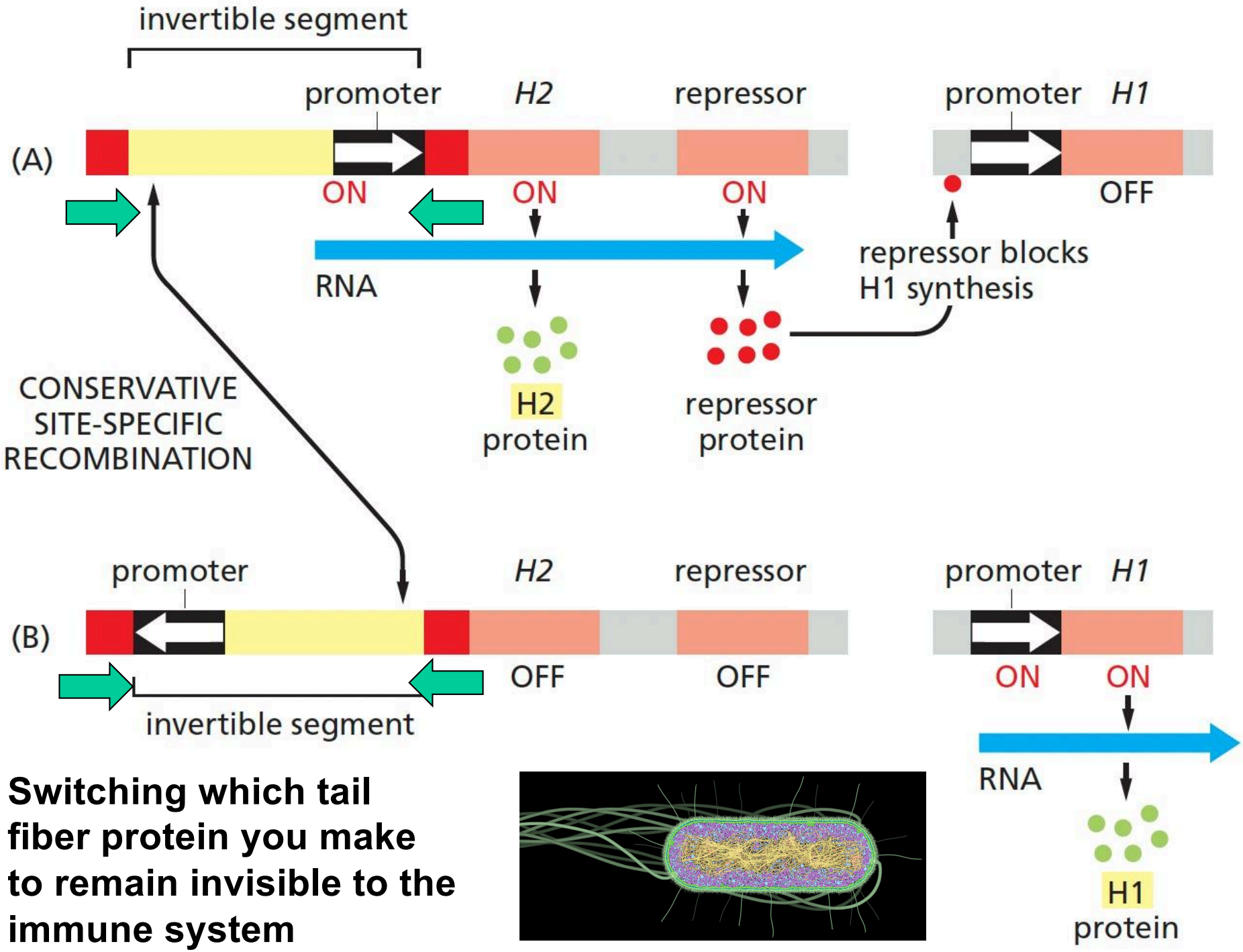



A
attP
attB
Int
Int + RDF
attL
attR
B
5'-AGTGCCCCAACTGGGGTAACCTTT GAGTTCTCTCAGTTGGGGGCGT
3'-TCACGGGGTTGACCCCATTGGA AACTCAAGAGAGTCAACCCCCGCA
attP TT
5'-GTGCGGGTGCCAGGGCGTGCCCTT GGGCTCCCCGGGCGCGTACTCC
3'-CACGCCCACGGTCCCGCACGGG AACCCGAGGGGCCCGCGCATGAGG
attB TT
C
attP
attB
Int
attL
+
attR

When recombinase target sites are inverted with respect to each other the intervening DNA is inverted

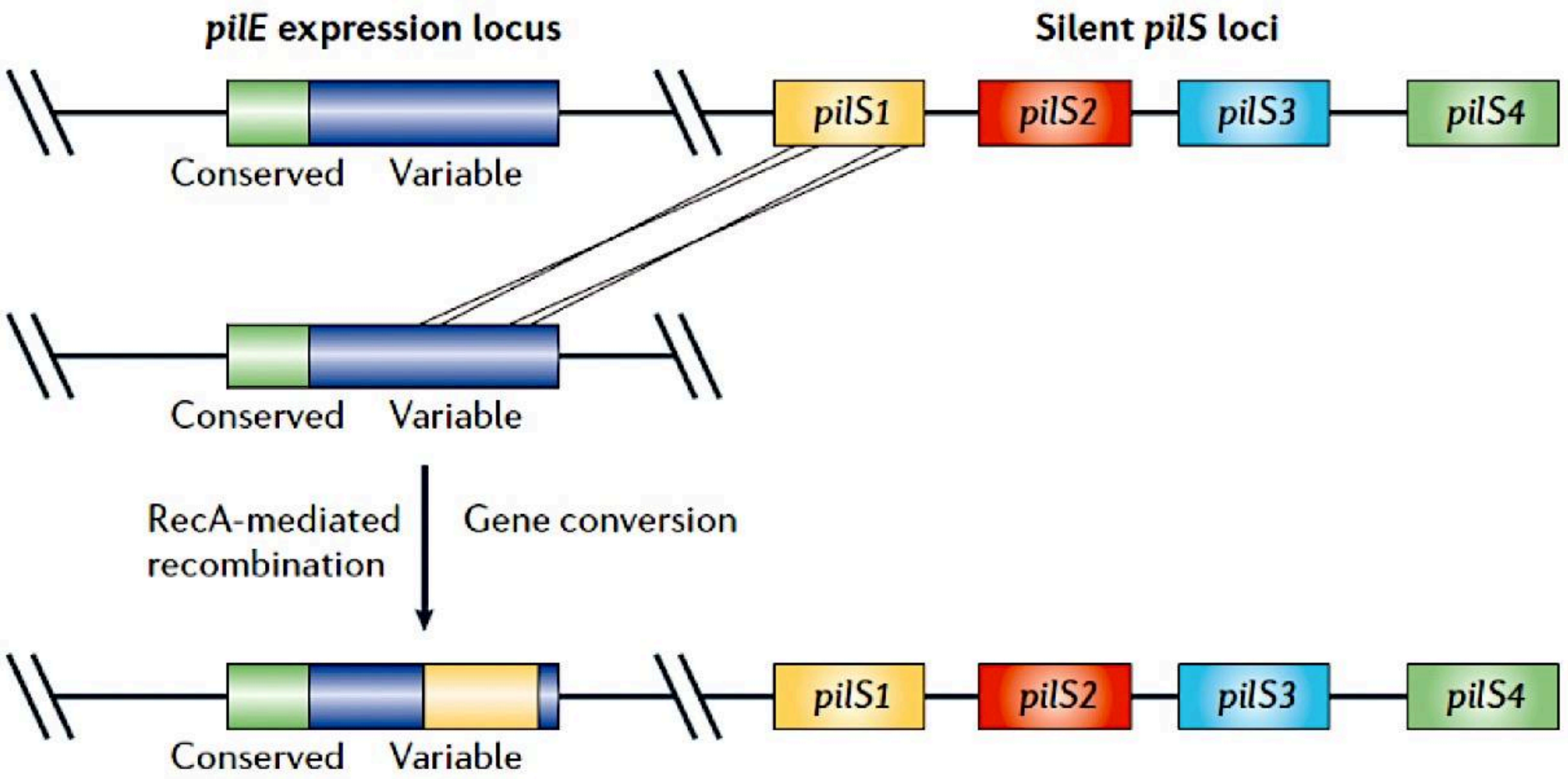






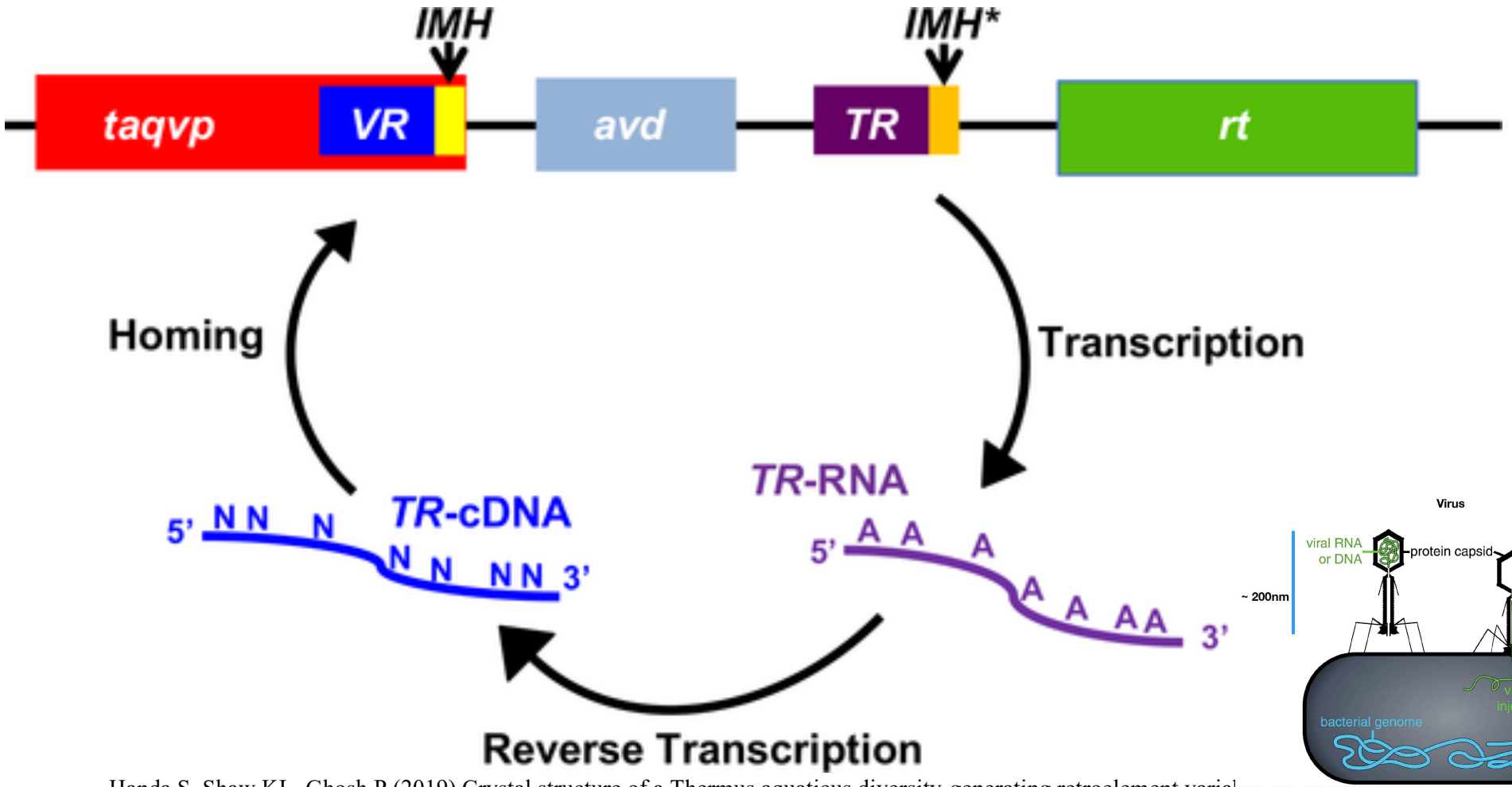
Switching which tail fiber protein you make to remain invisible to the immune system

Swapping between a number of possible protein sequences using homologous recombination



Diversity generating retroelement

Reverse transcriptase copies the TR-RNA into DNA. I takes every A residue and randomizes it. This generates the TR c (copy) DNA. The TR cDNA inserts itself in place of the VR region, creating a gene that carries a novel protein sequence that can bind to other receptors. 100 positions randomized over 20 amino acids. More diversity than stars in the universe. Infinite diversity (targeted mutagenesis) in a very small package



Handa S, Shaw KL, Ghosh P (2019) Crystal structure of a Thermus aquaticus diversity-generating retroelement variable protein. PLOS ONE 14(1): e0205618. https://doi.org/10.1371/journal.pone.0205618

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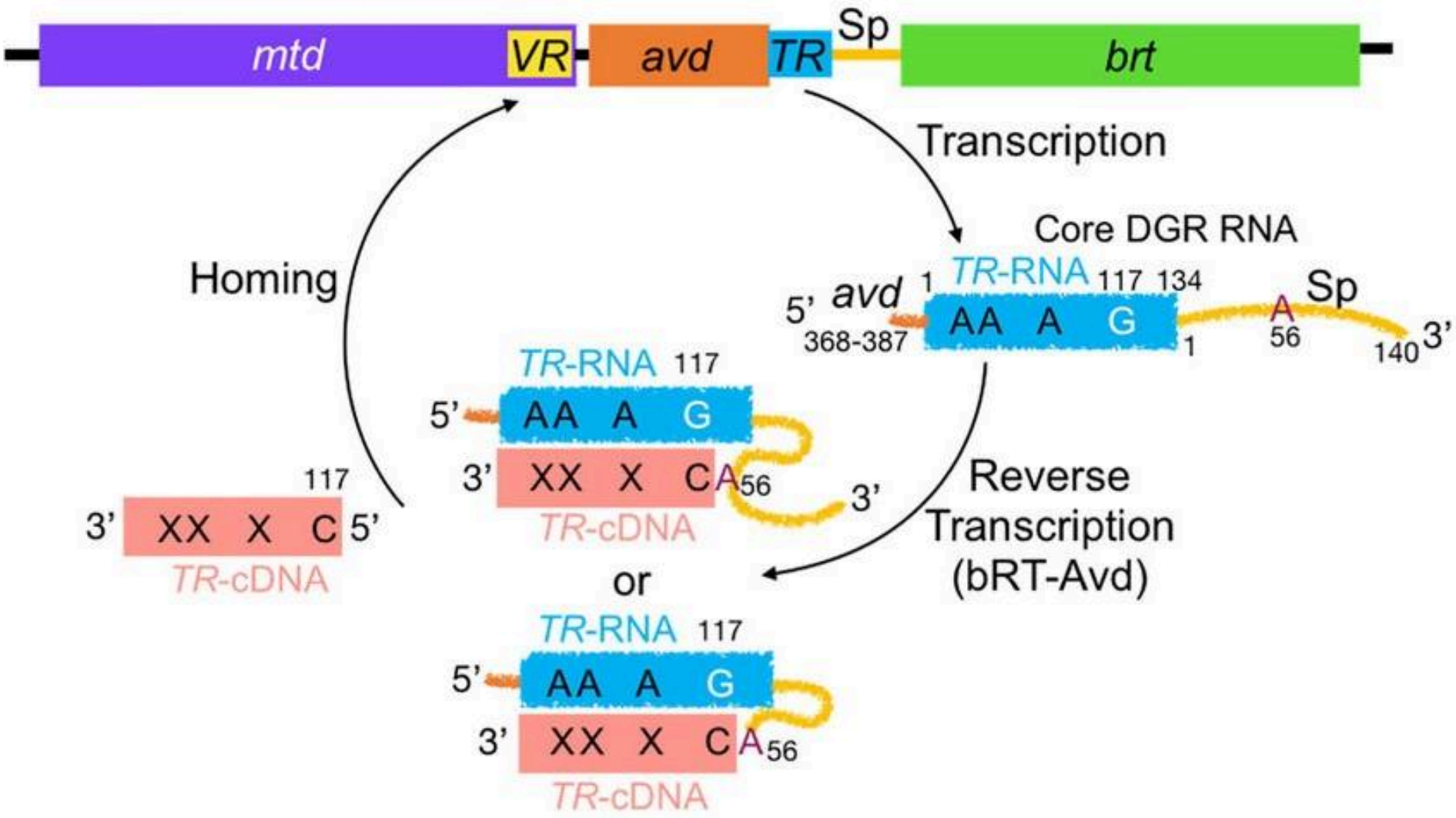
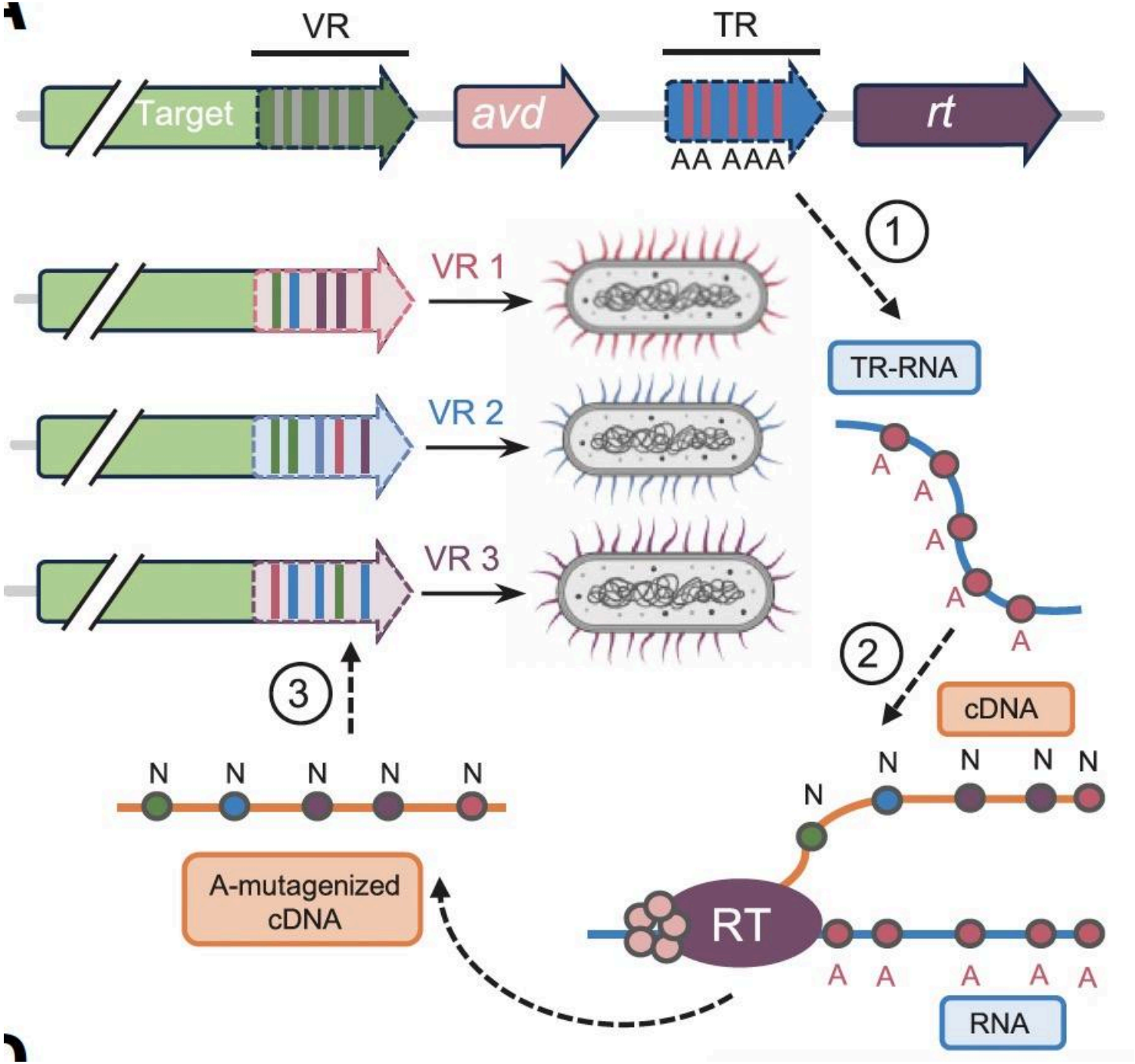
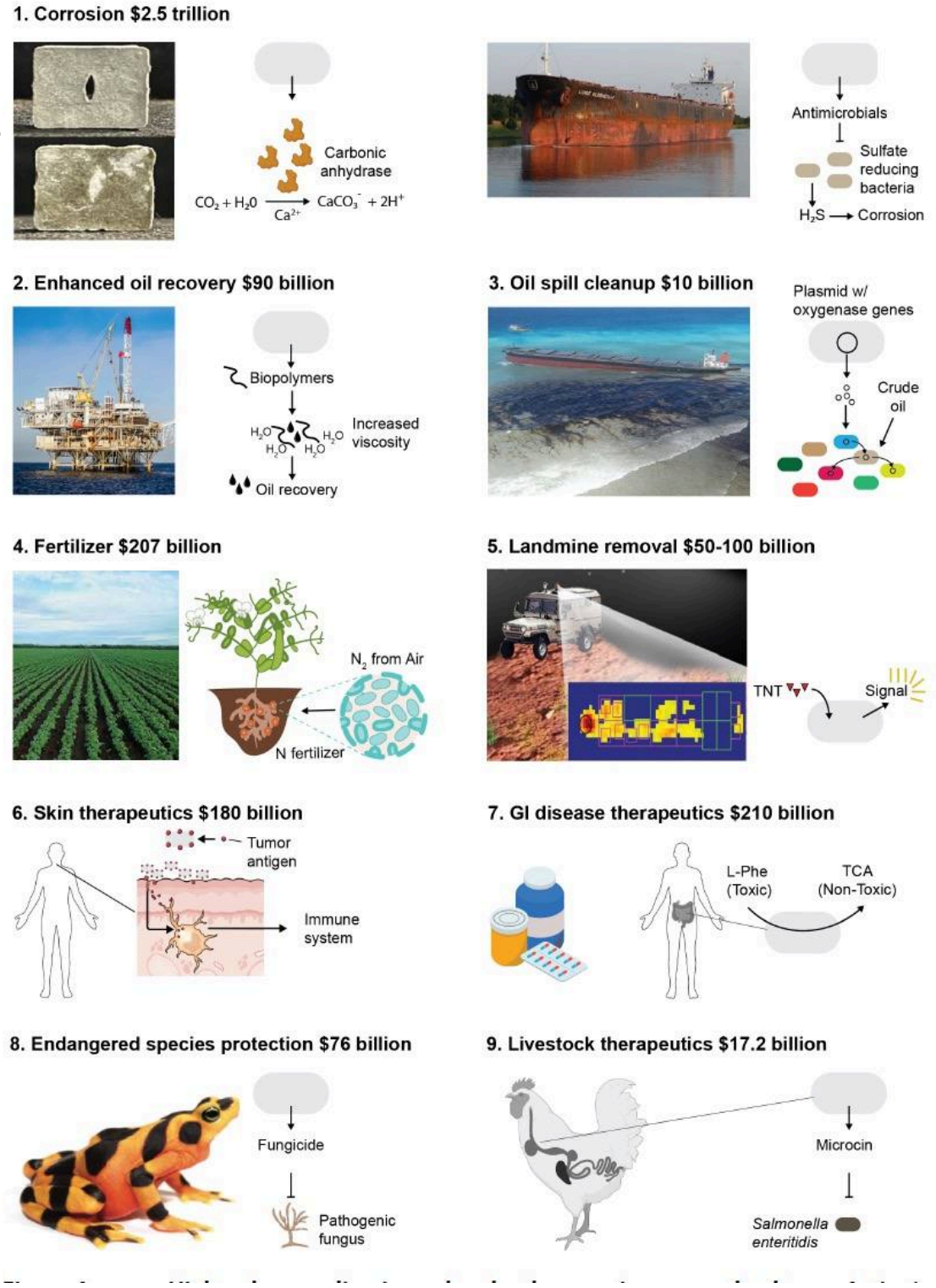



VR
TR
Target
avd
rt
AA AAA
1
VR 1
VR 2
VR 3
TR-RNA
A
A
A
A
A
2
cDNA
N
N
N
N
N
N
N
N
N
N
3
A-mutagenized
cDNA
RT
A
A
A
A
A
RNA

Engineered microbes for environmental release (EMER)



Policy Recommendations for the Regulation of Engineered Microbes for Environmental Release

June 2024

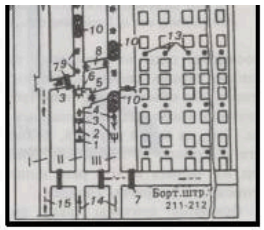
Engineering Bacteria for Environmental Release: Regulatory Challenges and Design Strategies

Yonatan Chemla[1], Connor J. Sweeney[1], Christopher A. Wozniak[2], and Christopher A. Voigt[1]

[1]Synthetic Biology Center, Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

[2] (Retired) Office of Pesticide Programs, U.S. Environmental Protection Agency, Alexandria, VA, USA

Phenol pollution remediated with *Pseudomonas putida*. Phenol is a potent carcinogen that was released due to an 81-day fire at the world's largest oil shale mine in the U.S.S.R. (now Estonia) in 1988. Engineered *P. putida* containing a recombinant phenol-degrading pathway was spread to remediate the pollution[32,33]. The bacteria were inoculated into mine enclosures where it reduced phenol levels 700-fold. Image showing the mine fire diagram (6; location where fire started), adapted from ref[34].

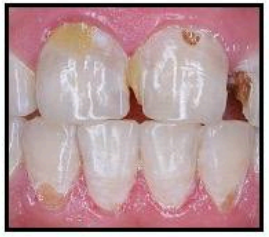


Bio-insecticides based on *Bacillus thuringiensis* with targeted pest control. *B. thuringiensis* produces proteins that kill insects and wild-type strains are the most used bio-insecticide globally[35,36]. These strains disrupt different pests through variations in the endotoxins that they produce (Cry proteins), but a wild-type strain may not have an optimal combination of toxins for a particular pest[36]. Mycogen Corporation developed M-Cap by cloning the Bt toxin from *B. thuringiensis* into *Pseudomonas fluorescens*, to facilitate its use in maize crops[37]. The engineered bacteria are killed before use on the field. M-Cap was released for commercial use in 1990. Other similar products have been commercially developed, including products where the bacteria are not killed prior to release[22,38-40]. Image of Stem borers, sensitive to M-Cap, by the International Maize and Wheat Improvement Center, creative commons license.

Nitrogenous fertilizer delivery to maize with consortium. Nitrogenous fertilizer consumes 2% of global energy and is a major contributor to runoff pollution and greenhouse gases[41]. Microbes do not produce fixed N when used in conjunction with synthetic fertilizer unless they are genetically engineered. In the 1990s, recombinant *Rhizobium meliloti* got EPA approval for legumes, but was never commercialized. In 2019, Pivot Bio commercialized an engineered *Klebsiella variicola* to deliver nitrogen to corn, and later a consortium by adding engineered *Kosakonia sacchari*. Their product is used on millions of acres and replaced an estimated 32,000 tons of synthetic fertilizer in 2022[42]. Image courtesy of Pivot Bio.

Engineered *Bacillus subtilis* probiotic for treating hangovers. Enzyme-based therapeutics often suffer from inactivation when stored or consumed. Activity could be improved if produced at the site needed in the body. Zbiotic is an over-the-counter liquid drink that contains a genetically engineered *Bacillus subtilis* designed to degrade gut-derived acetaldehyde that builds up in the colon after consuming alcohol. The product was released for sale in 2019, and nearly 4 million bottles containing their genetically engineered bacteria are in circulation as of April 2024. Image courtesy of Zbiotics.

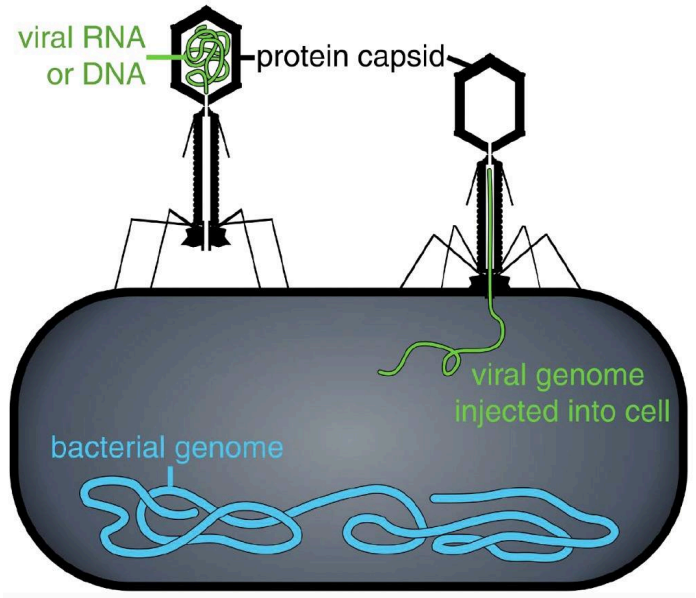
Oral cavity-reducing *Streptococcus mutans* engineered to not produce lactic acid. Biofilms can cause dental cavities by producing lactic acid. An isolate of *S. mutans* was found to produce the antibiotic mutacin 1140, increasing its ability to outcompete native strains and colonize teeth[43]. In this strain, the lactate dehydrogenase gene was replaced with an alcohol dehydrogenase gene from *Zymomonas mobilis*, using allelic exchange. The resulting strain stopped producing lactic acid[44]. It is used by one-time application to teeth to reduce dental cavity formation. While trying to approve the product in the early 2000s as an oral therapeutic, the FDA put constraints on the trial which never completed. In 2023, Latern Bioworks brought the product to market as a "cosmetic" to avoid the need for an FDA permit. Image of dental decay by bacteria. By Shaimaa Abdellatif, under creative commons license.



Bacteria in oceans 1.3 x10^{29}

Virus infections in ocean each day 1x10^{23}/second

Evolutionary arms race



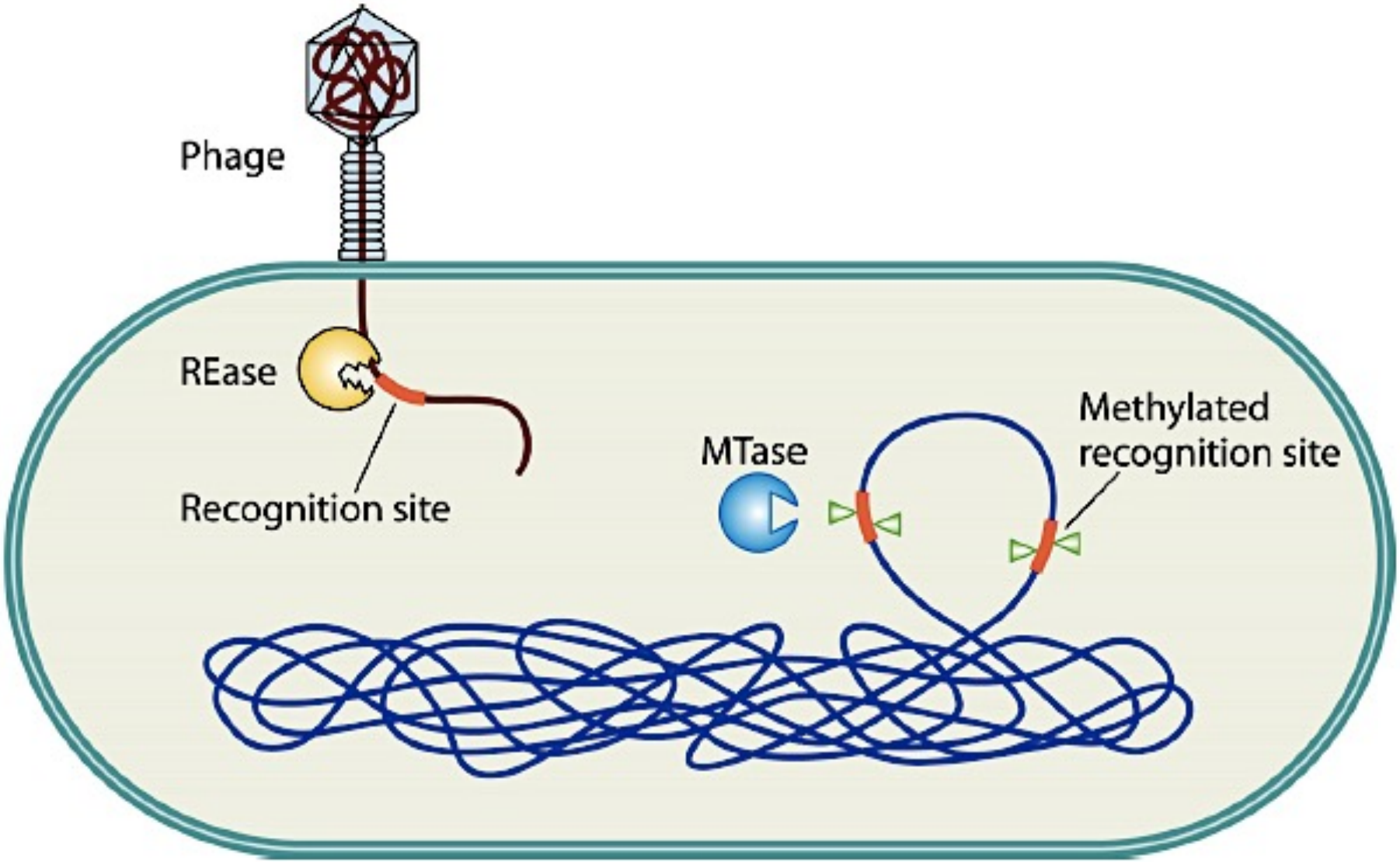
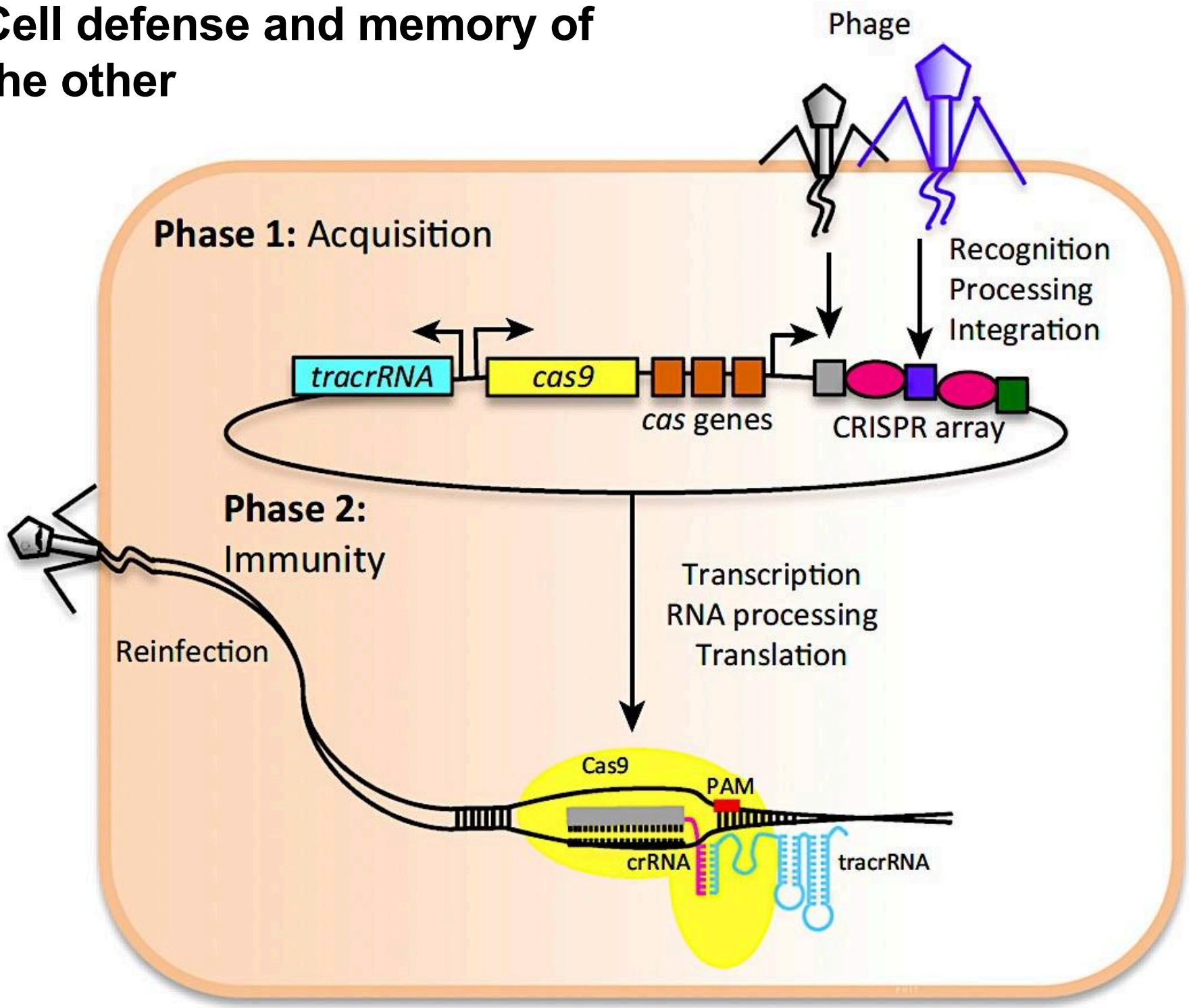
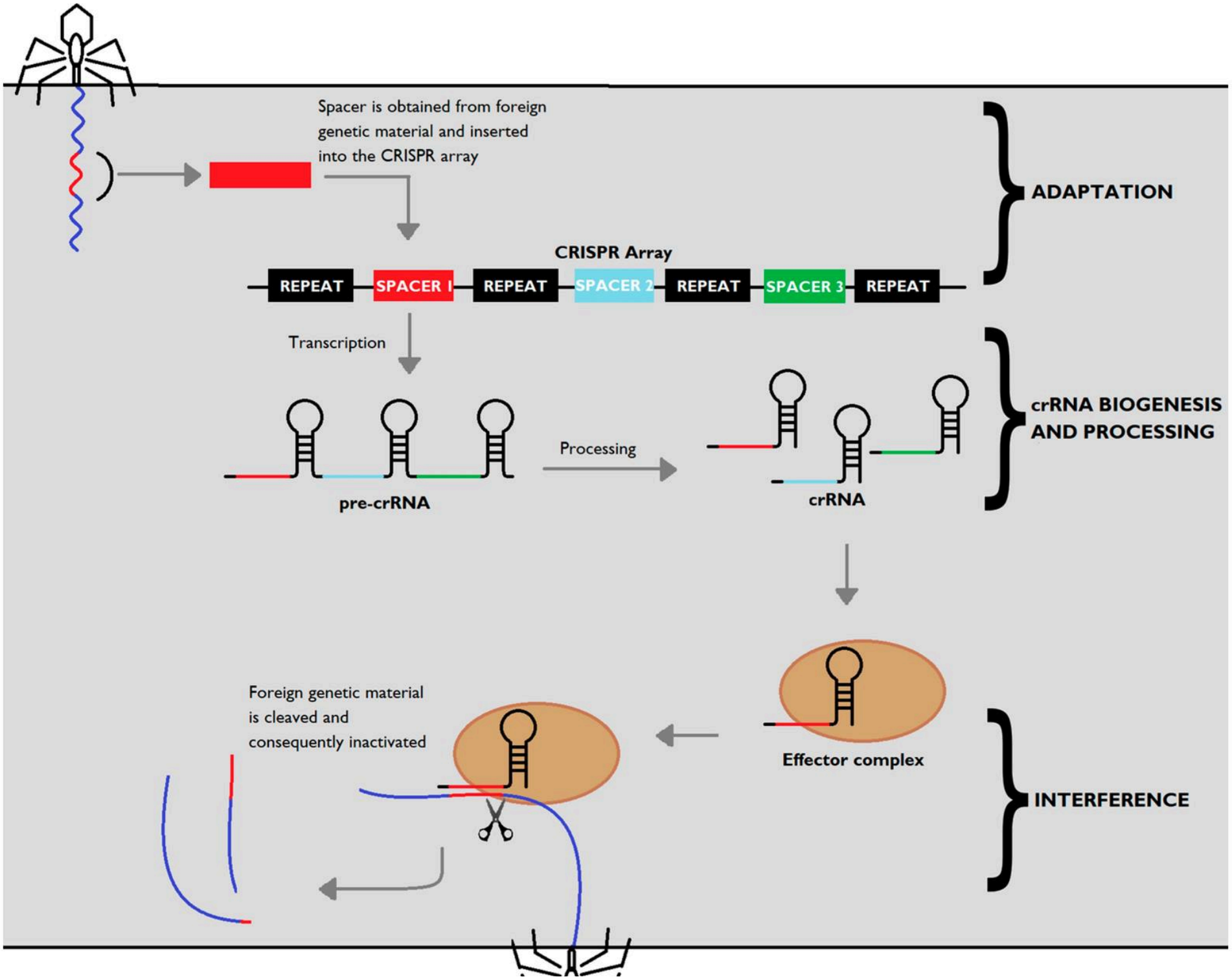


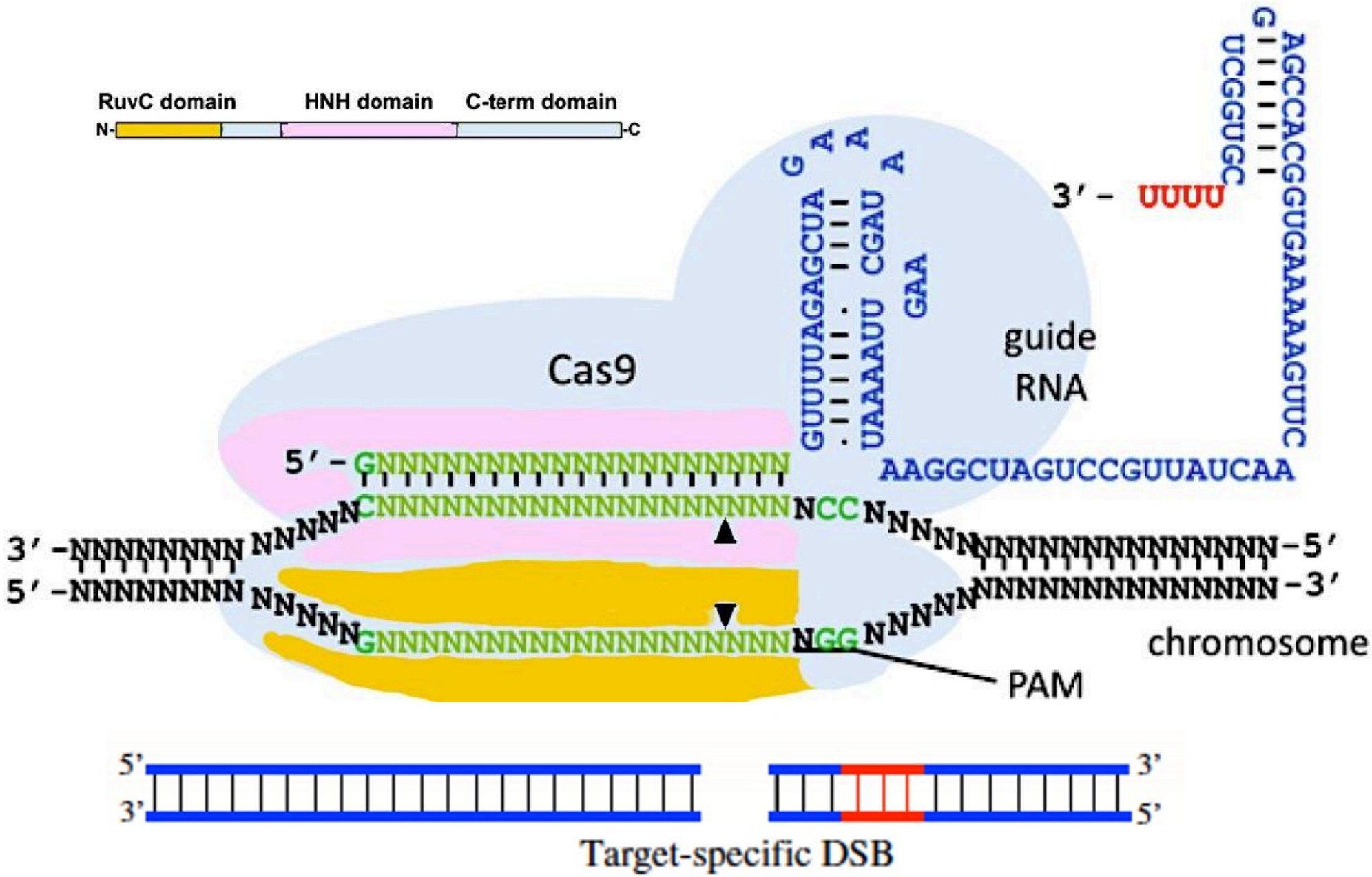
FIG 1 Restriction-modification (R-M) systems as defense mechanisms. R-M systems recognize the methylation status of incoming foreign DNA, e.g., phage genomes. Methylated sequences are recognized as self, while recognition sequences on the incoming DNA lacking methylation are recognized as nonself and are cleaved by the restriction endonuclease (REase). The methylation status at the genomic recognition sites is maintained by the cognate methyltransferase (MTase) of the R-M system.

Cell defense and memory of the other




Spacer is obtained from foreign genetic material and inserted into the CRISPR array
ADAPTATION
CRISPR Array
REPEAT
SPACER 1
REPEAT
SPACER 2
REPEAT
SPACER 3
REPEAT
Transcription
pre-crRNA
Processing
crRNA
crRNA BIOGENESIS AND PROCESSING
Effector complex
Foreign genetic material is cleaved and consequently inactivated
INTERFERENCE

Programmable DNA cleavage using Cas9 and one or more guide RNAs



Some phage encode anti-CRISPR proteins that prevent CRISPR-based destruction of incoming phage (the arms race continues)