Mobile genetic elements as tools for biotechnology

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Mobile Genetic Elements (MGEs)

Learning outcomes. Be able to…

- **Define, compare & contrast five different types of MGEs: insertion sequence, transposon, intron, plasmid, ICE**

- **Give three examples of MGE's or their enyzmes that are useful for biotechnology, and explain their applications**

What is the role of MGEs in microbial life?

- **Bacteria do not reproduce sexually (no meiosis, no genders, a single unpaired chromosome)**
- **Sex provides genetic diversity to drive evolution**
- **<u>Why are bacteria still so abundant & successful</u>?**

→ evolution can act on other sources of diversity eg. random mutations

But more importantly,

→Bacteria *can* exchange DNA via HORIZONTAL GENE TRANSFER, (HGT) usually via <u>MGEs</u>

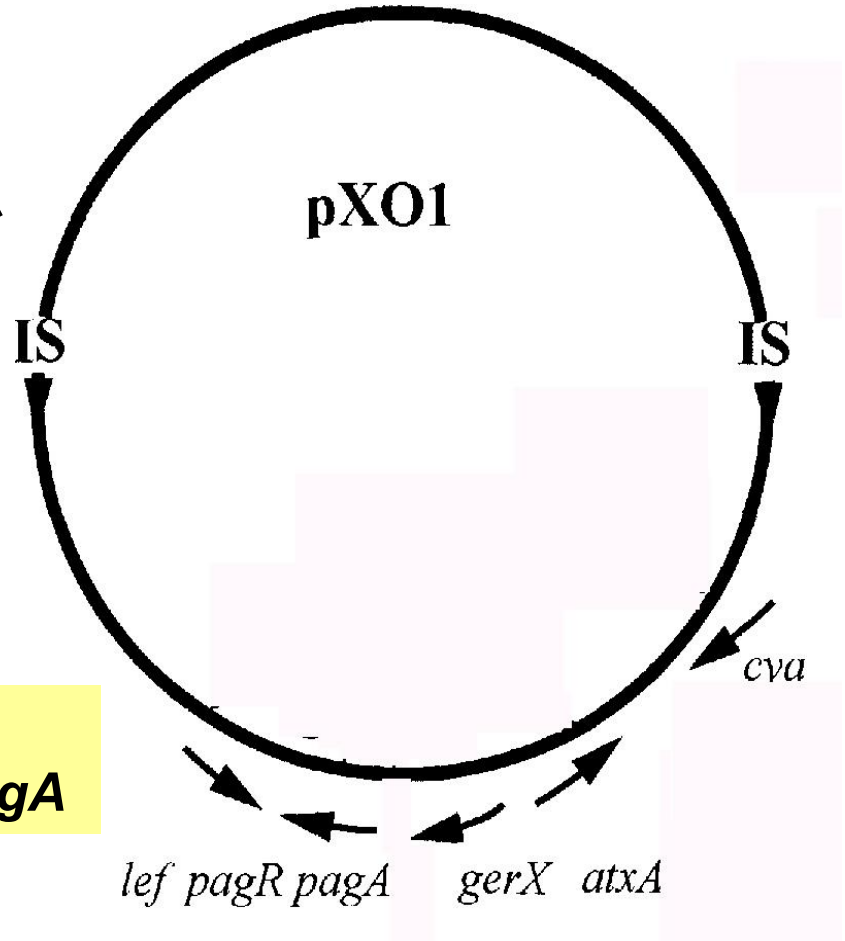
What is the role of MGEs in microbial life?

- **DNA movement can be intercellular (between different cells), or intracellular (between parts of same genome)**
- **IntERcellular DNA movement**
  - **TRANSFORMATION : naked DNA**
  - **CONJUGATION : plasmids**
  - **TRANSDUCTION : phage**
- **IntRAcellular DNA movement:**
  - **HOMOLOGOUS RECOMBINATION : host encoded**
  - **SITE-SPECIFIC RECOMBINATION : phage, integrons**
  - **TRANSPOSITION : insertion sequences, transposons**

What is the impact of MGEs on humans?

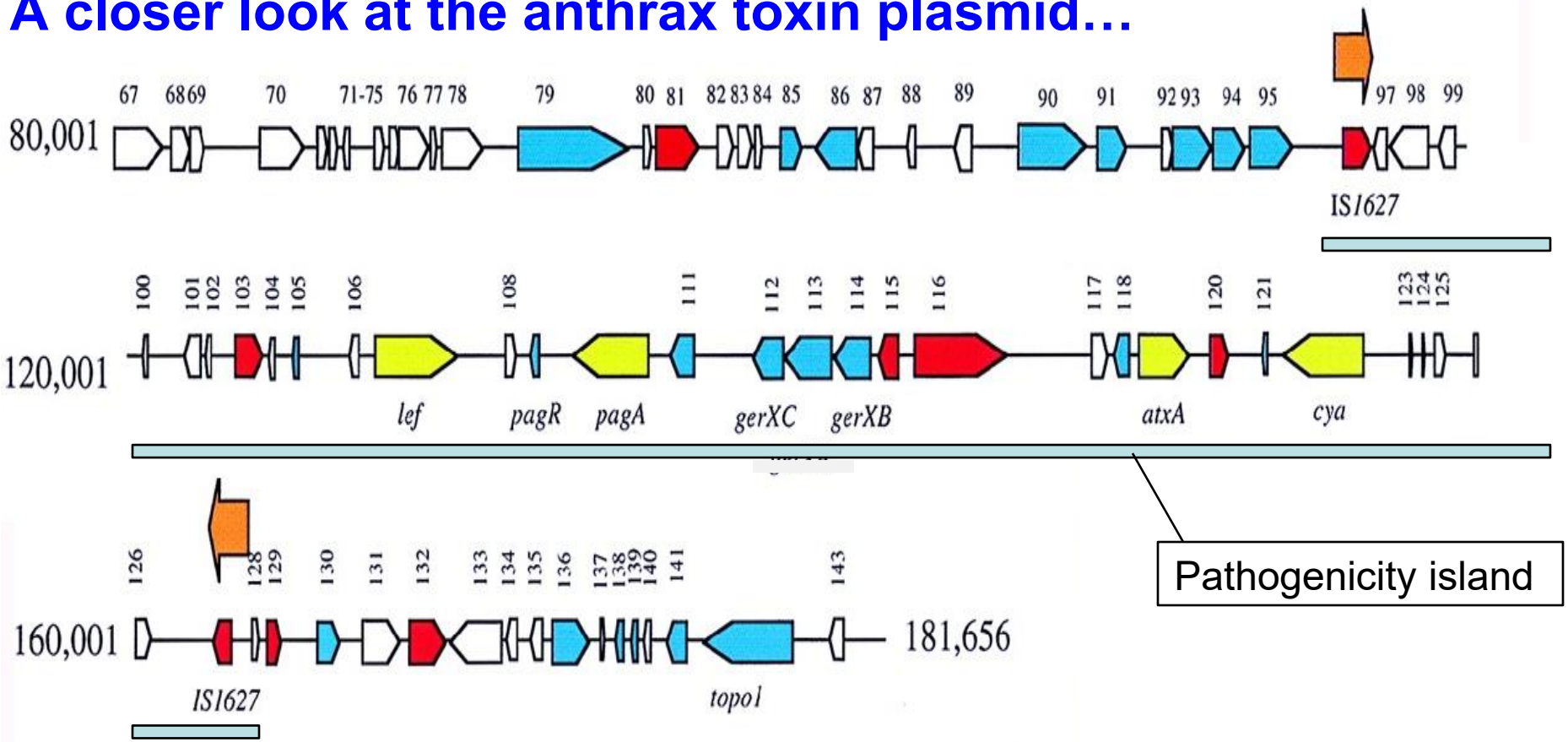
- **Phenotypes encoded on microbial MGEs are important to medicine, agriculture, environmental science.**

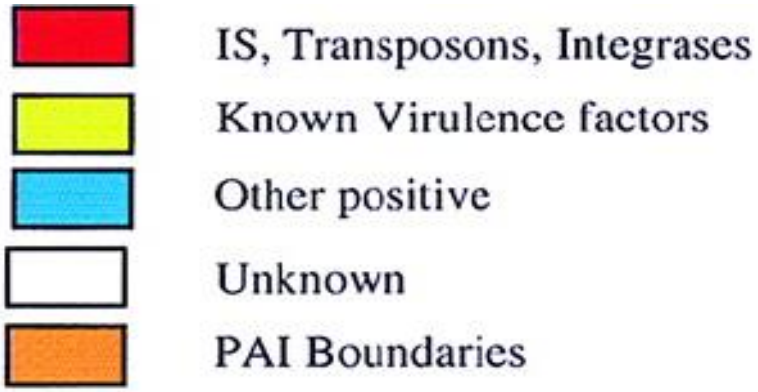
- **Antibiotic resistance ! ! !**
- **<u>Toxins & virulence factors</u>**
- **Pollutant biodegradation**
- **Interactions with plants**



pXO1 plasmid in Bacillus anthracis carries anthrax toxin genes *cya* - *lef* - *pagA*

A closer look at the anthrax toxin plasmid…



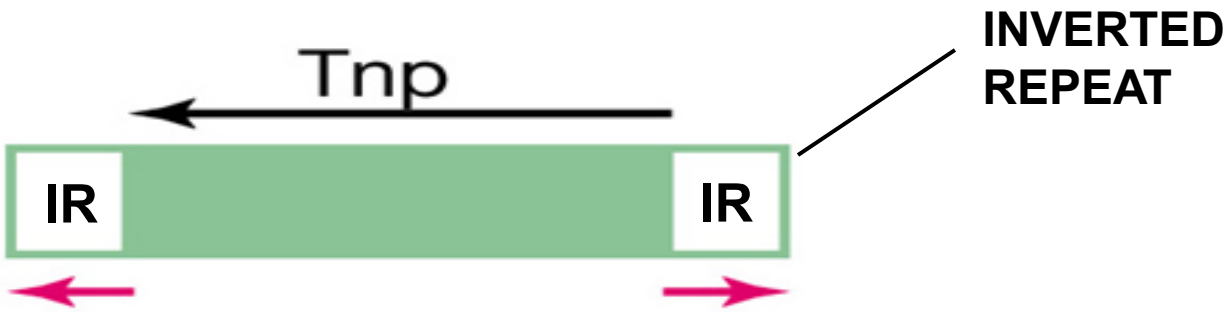


Pathogenicity island (PAI)

Includes toxin genes and their regulators. Located between two identical insertion sequences (IS). The PAI is a MGE: a composite transposon.

Insertion sequences (IS)

- Very simple MGEs: a transposase gene flanked by inverted repeats – usually ~1 kb in size

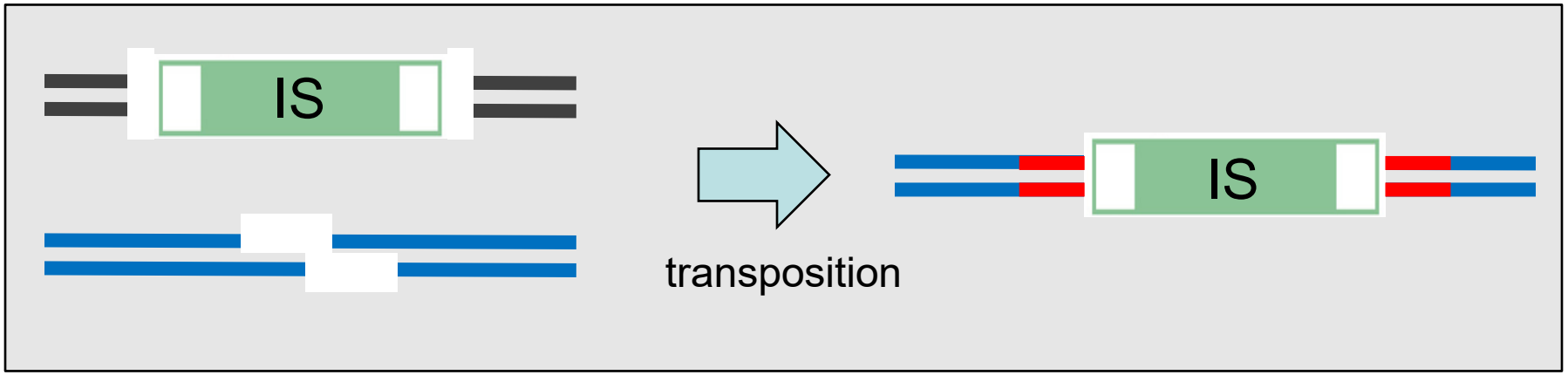


- Code for their own transposition – no other phenotype *
- Dependent on parent replicon (eg. chromosome) for inheritance, but can also 'replicate' by transposition
- IntRAcellular movement only unless associated with another MGE that is conjugative

Transposition of IS elements

- Transposase enzyme cuts inverted repeats of IS element and cuts DNA at target site, moves IS to target site

- Transposition leaves small direct repeats on each side. Why? Blunt cut, joined to staggered-cut, then filled in



- Two modes of transposition are possible: 'Cut and paste' (conservative) or 'copy and paste' (replicative)

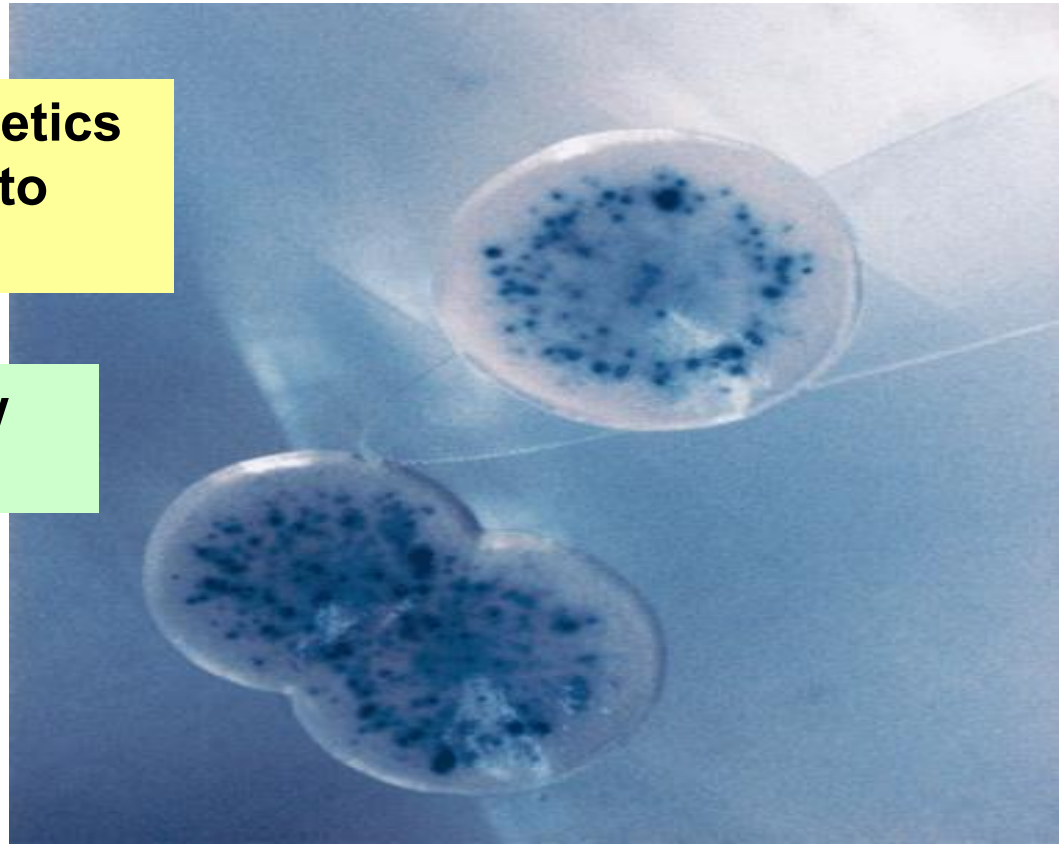
Transposition of IS elements

- **Transposition process is *mostly* random.**
  Some IS's show preference for certain types of seq's

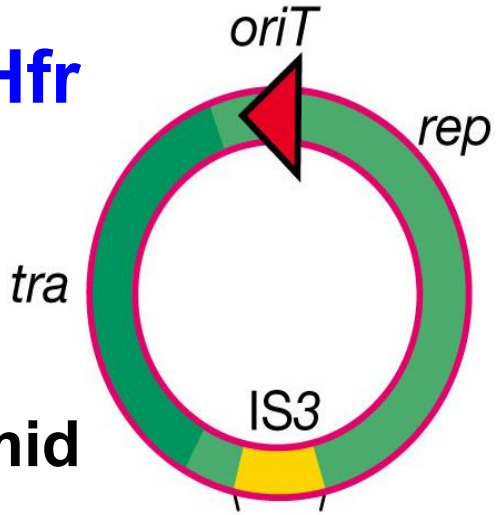
- **IS's can cause mutations – knockout (if IS interrupts gene), or activation (if IS inserts upstream of a gene)**

A LOT of work in bacterial genetics has depended on IS elements to make mutations, esp. in *E.coli*

Papillae on colonies here show transposition of IS903-*lacZ*



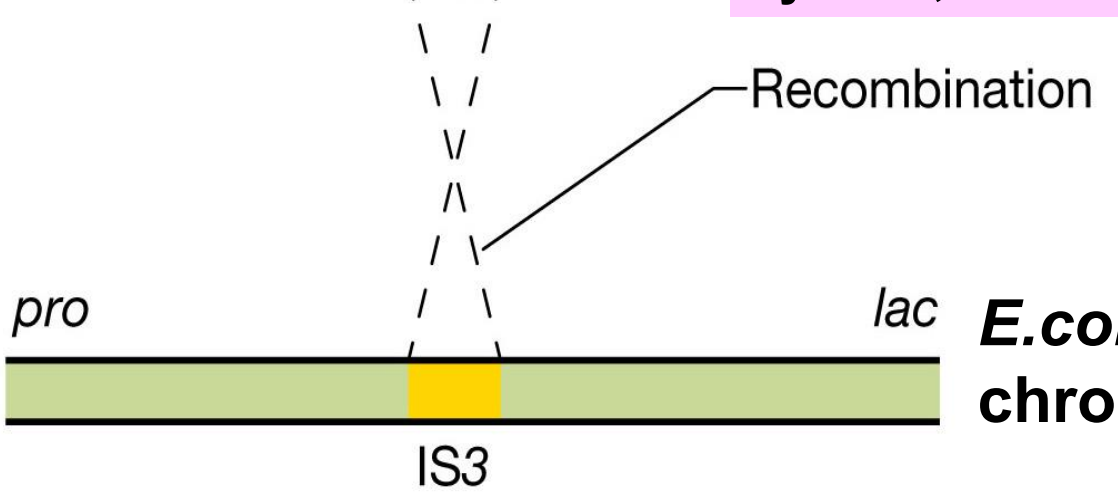
IS3 and Hfr strains



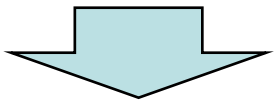
F plasmid

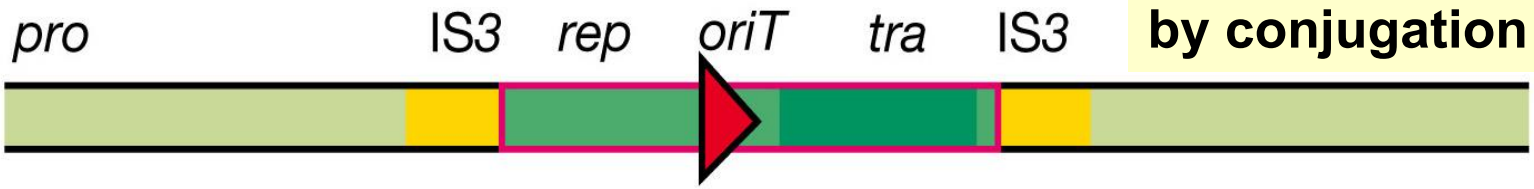
Homologous recombination between two copies of IS3 gives Hfr (High Frequency of Recombination) strains.

note: mediated by host RecA system, not the IS3 transposase.



E.coli **chromosome**





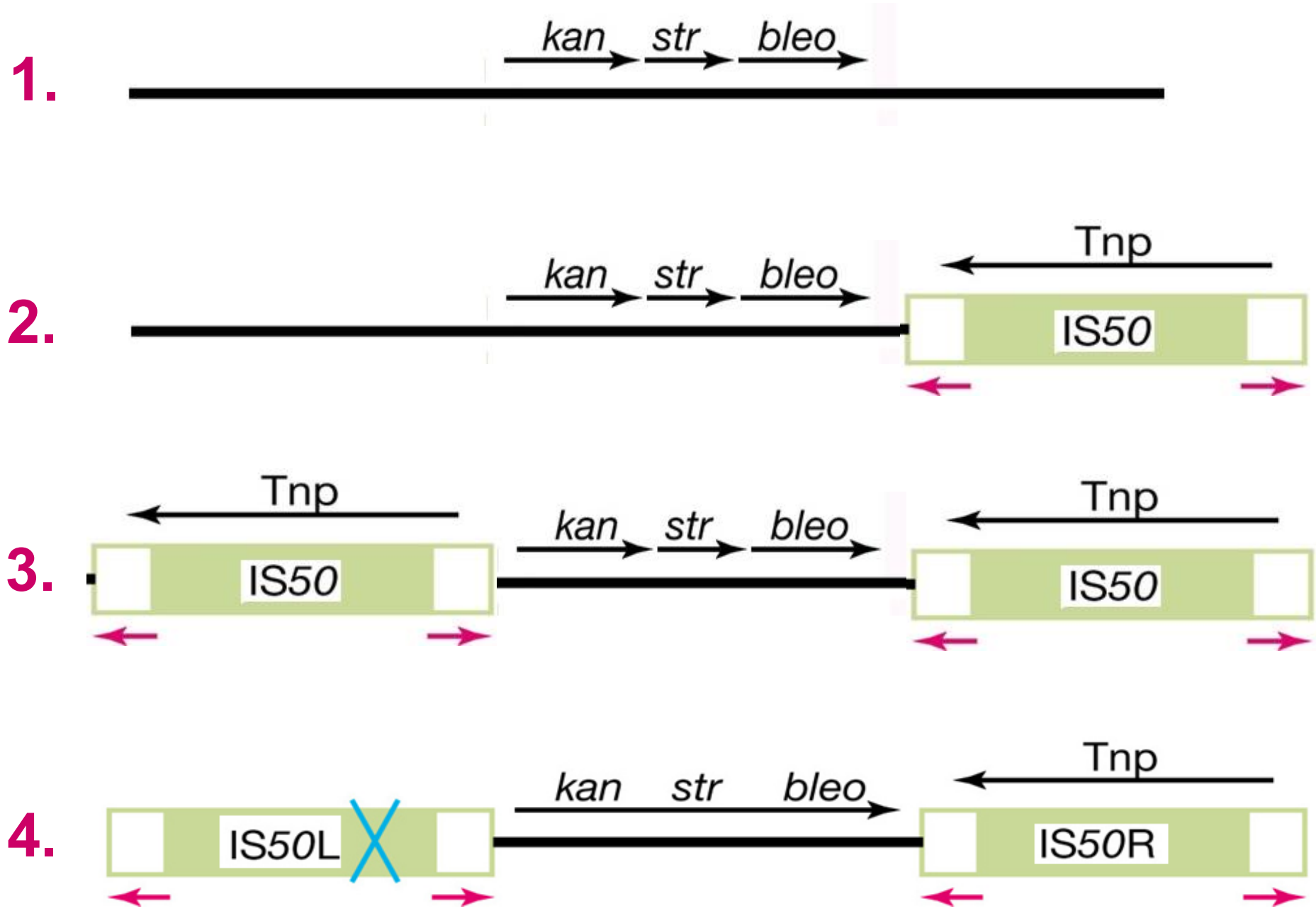
Hfr *E.coli*: can transfer chromosome segments by conjugation

Transposons (Tn)

Similar to IS, but carry accessory genes

- **Composite Tn**
  - **Two IS, on each side of the accessory genes**
  - **Evolve from one IS after a nearby 'copy and paste'**
  - **Usually conservative transposition**
- **Tn3 type**
  - **Have resolvase in addition to transposase.**
  - **Only one copy of transposase gene and IRs present**
  - **Usually replicative transposition via a cointegrate intermediate: joined donor and target replicons**

Evolution of composite transposon Tn5

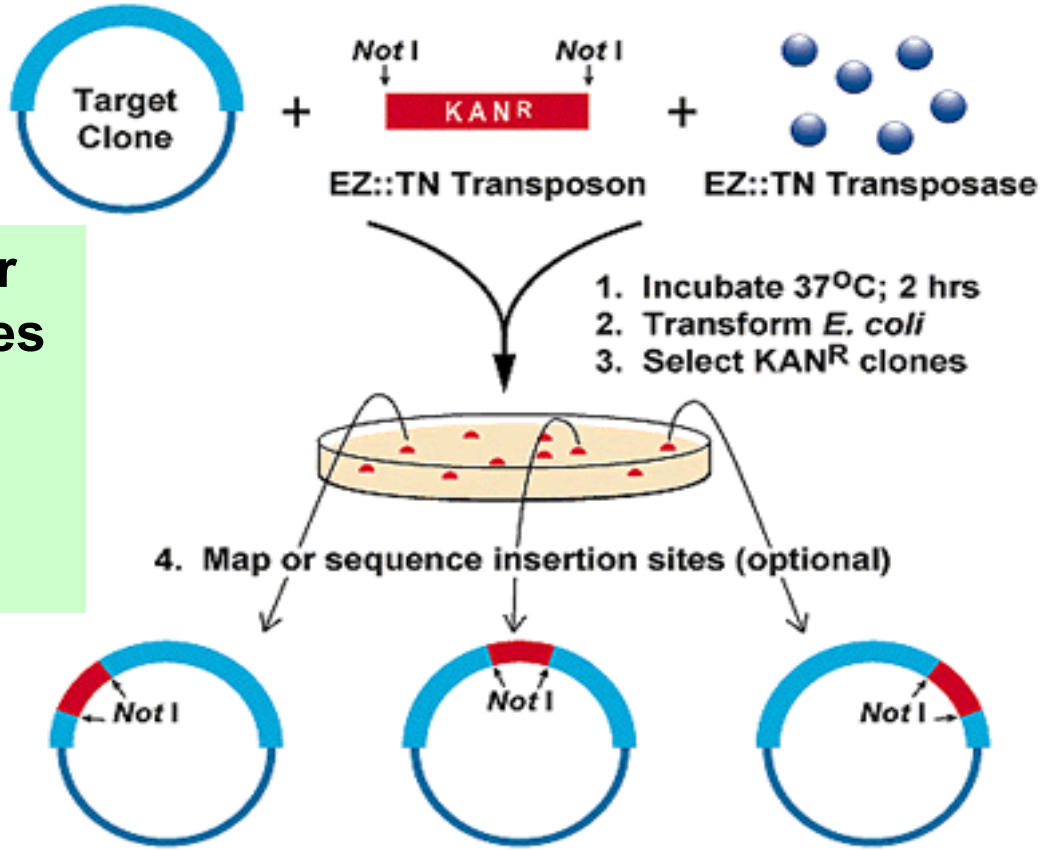


Transposon mutagenesis with Tn5

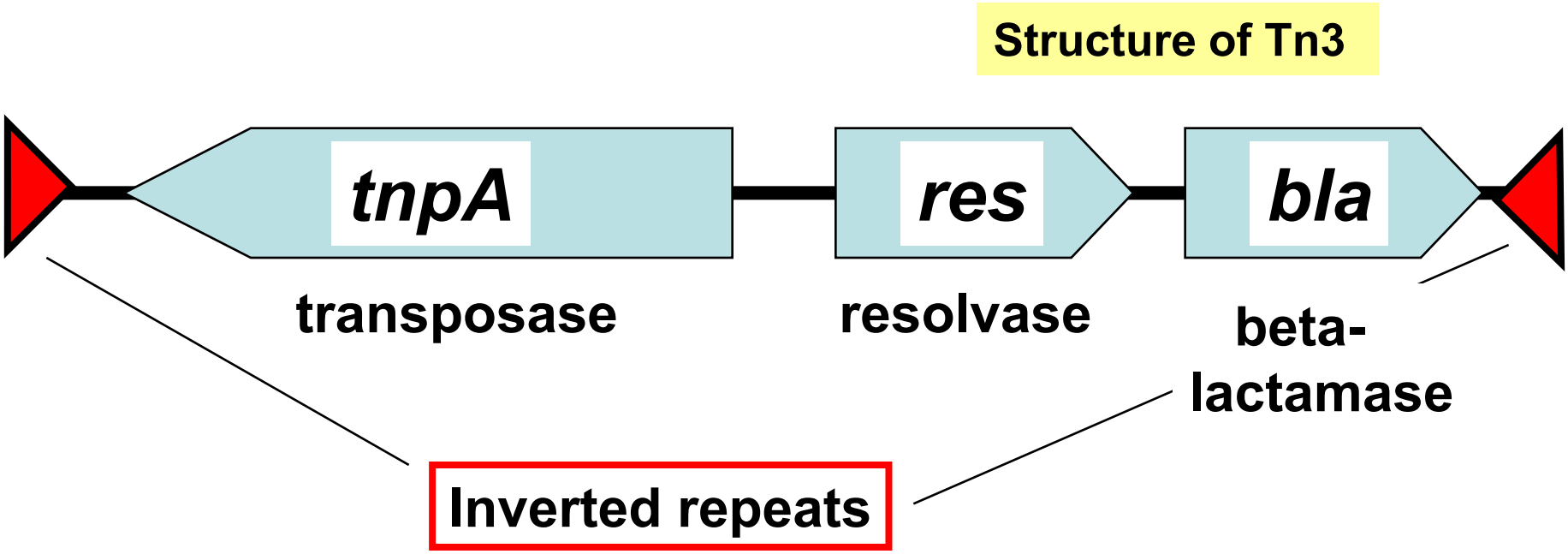
- **Tn5 is a very useful agent for mutagenesis in bacteria**
  - **Transposes in a near-random fashion**
  - **Transposes at high frequencies**
  - **KmR works in many bacteria**

Tn5 based "Transposome" kit for making mutants in plasmid clones

<u>Transposome</u> consists of of the transposon DNA bound to pre-formed transposase enzyme.



Tn*3*-type transposons



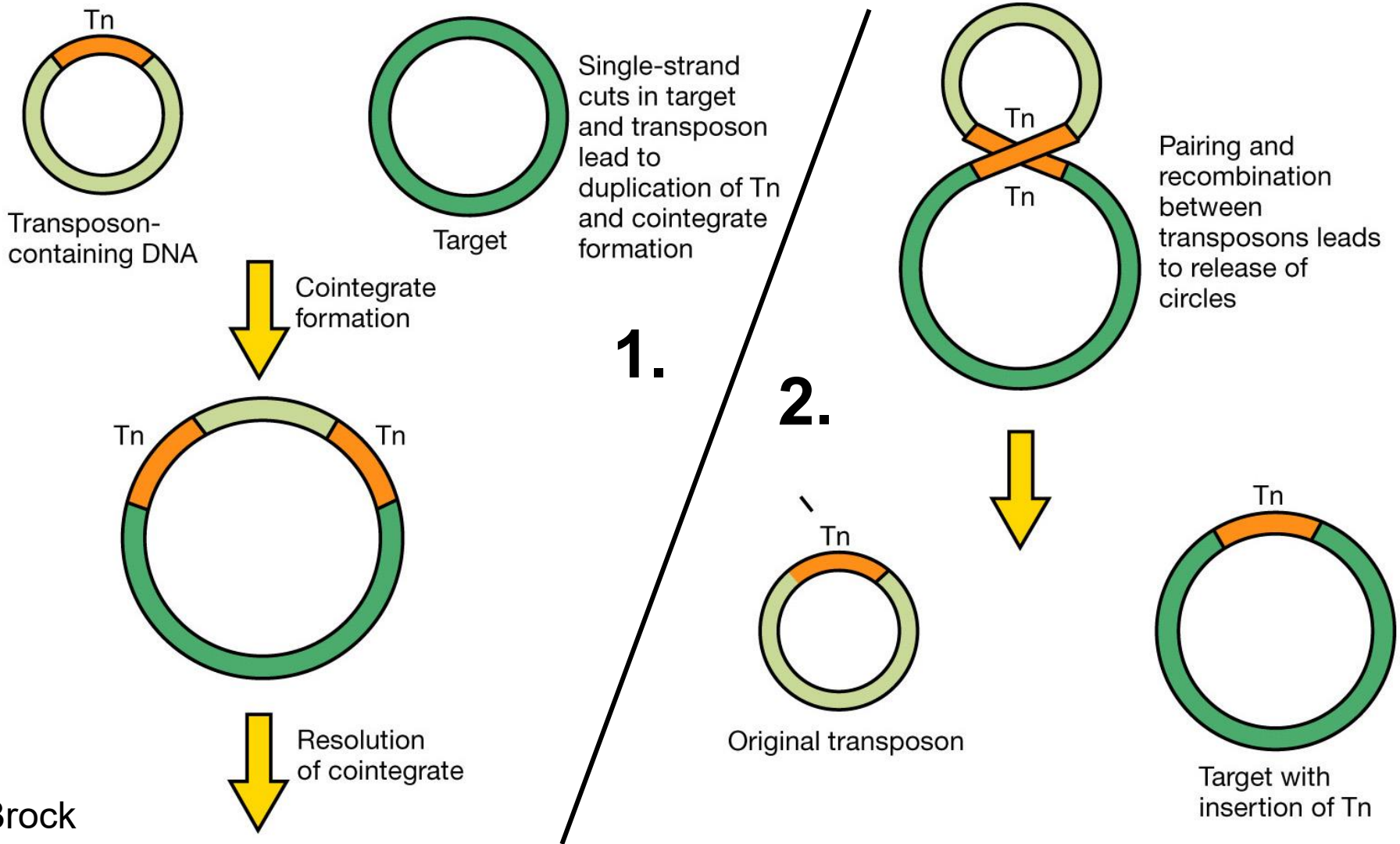
Tn3-like transposons can encode

- **antibiotic resistance (eg. Tn*3*, Tn*21*)**
- **heavy metal resistance (eg. Tn*501*)**
- **metabolic genes (eg. Tn*4651*)**

Tn3 types replicate via a cointegrate intermediate

Cointegrate is converted to final products by resolvase

Also see: www.biostudio.com/d_%20Replicative%20Transposition.htm



Brock

Self-splicing group II introns

- **Intron: Internal segment of a gene that is transcribed into mRNA but removed before translation into protein**

- **Eukaryotic introns (group I):**

  → common, excised by host, non-mobile

- **Prokaryotic introns (group II):**

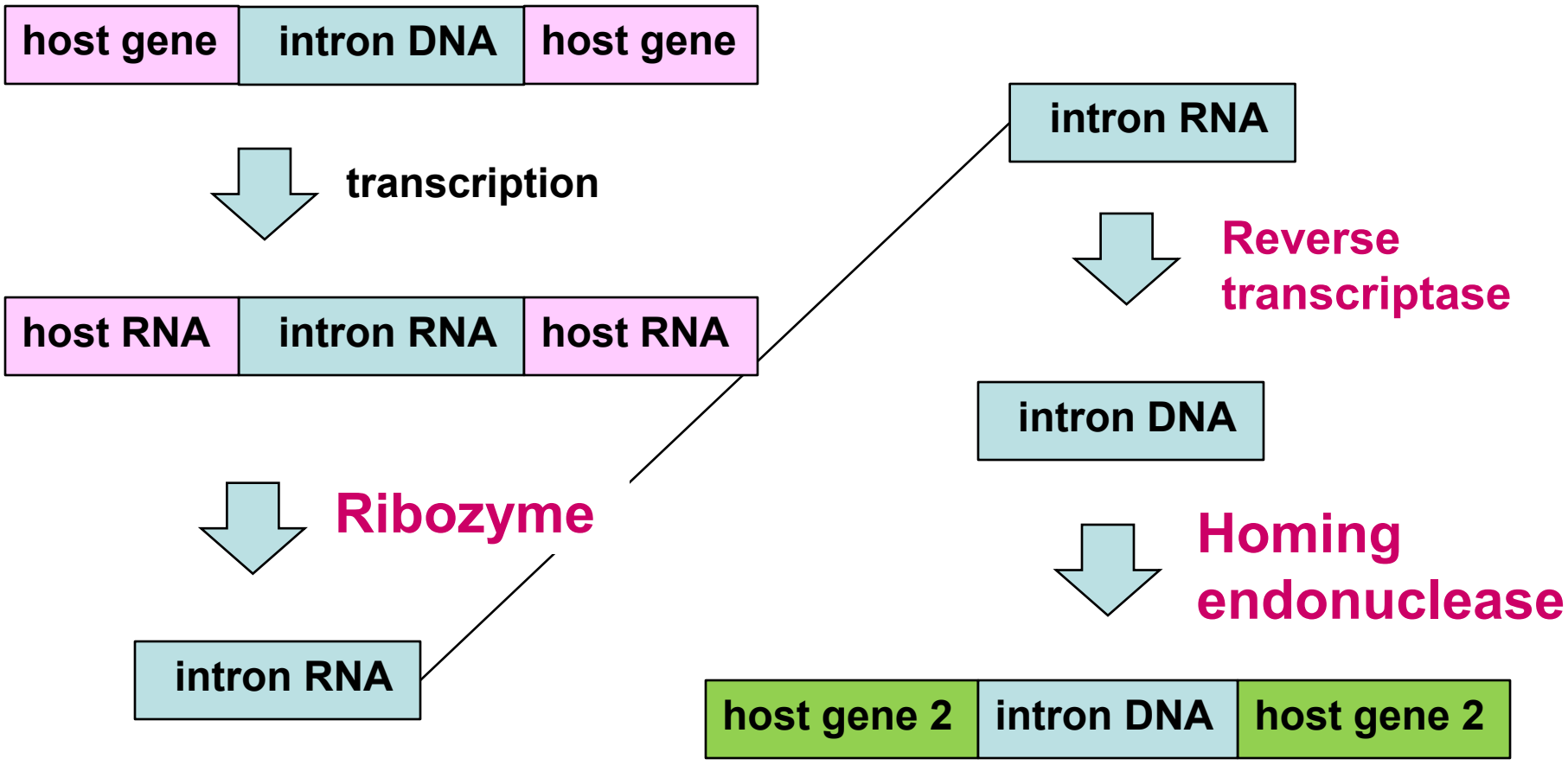
  → rare, self-splicing, mobile

RIBOZYME :
Splices intron RNA out of precursor

HOMING ENDONUCLEASE:
Copies intron to new genomic location

Self-splicing group II introns

- Life cycle of a self-splicing intron is shown below.
- Roles of ribozyme, reverse transcriptase, and homing endonuclease are highlighted.

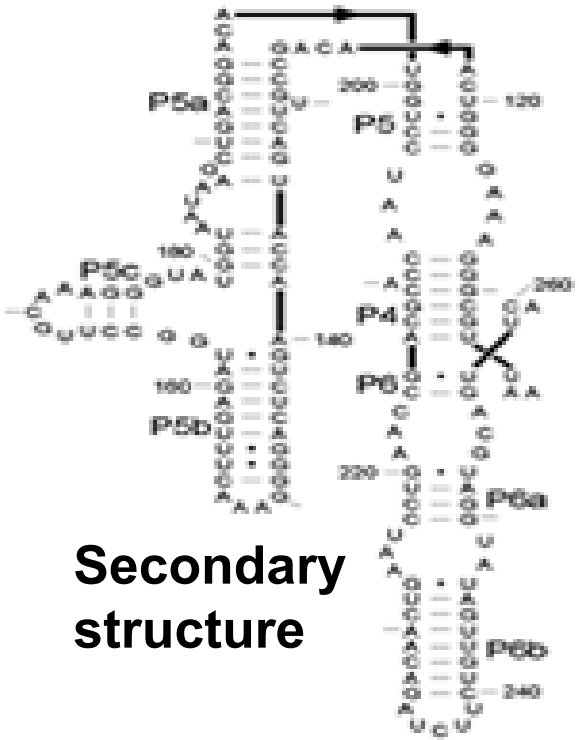


Ribozymes

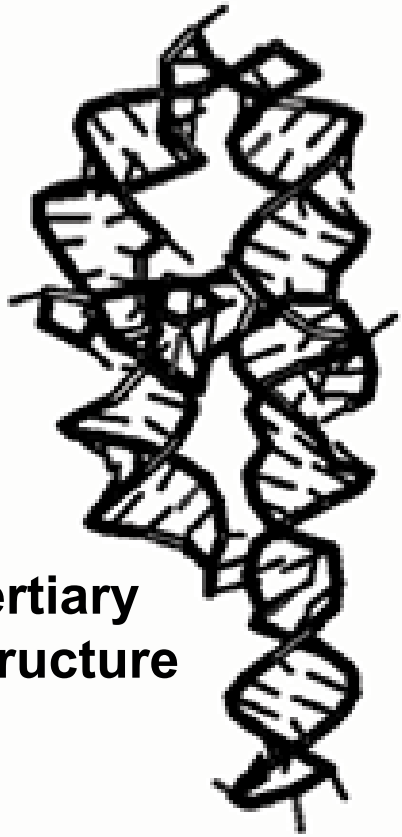
- Catalytic RNA. Acts like enzyme, but is not a protein
- RNA tertiary structure is important for catalysis

AAUUGCGGGAAAGGGGUCAA
CAGCCGUUCAGUACCAAGUC
UCAGGGGAAACUUUGAGAUG
GCCUUGCAAAGGGUAUGGUA
AUAAGCUGACGGACAUGGUC
CUAACCACGCAGCCAAGUCC
UAAGUCAACAGAUCUUCUGU
UGAUAUGGAUGCAGUUCA

primary sequence



Secondary structure



Tertiary structure

Intron ribozymes have been proposed as novel antiviral agents, that attack RNA-genome viruses

Plasmids

- **Self-replicating circular or linear ds. DNA elements, replicate independently of the chromosome**

- **Plasmid replication requires a DNA sequence known as the origin of replication (ORI)**

~20 bp direct repeats in a *Lactococcus* plasmid ORI region

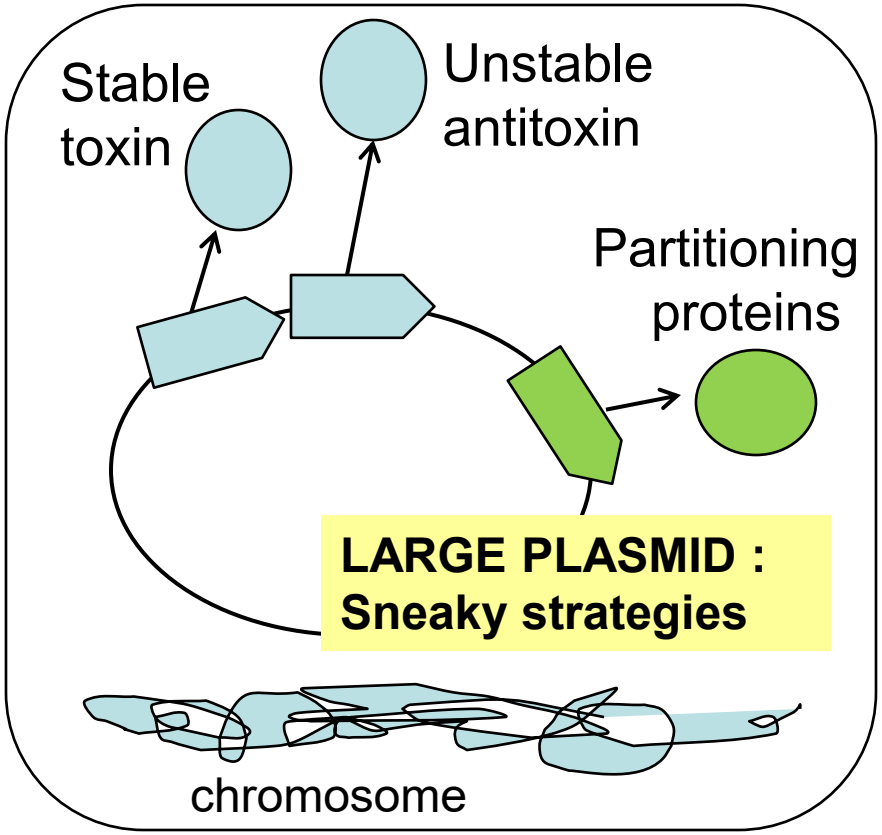
- **All plasmids use host DNA polymerase; some also have replication proteins (REP) which regulate replication**

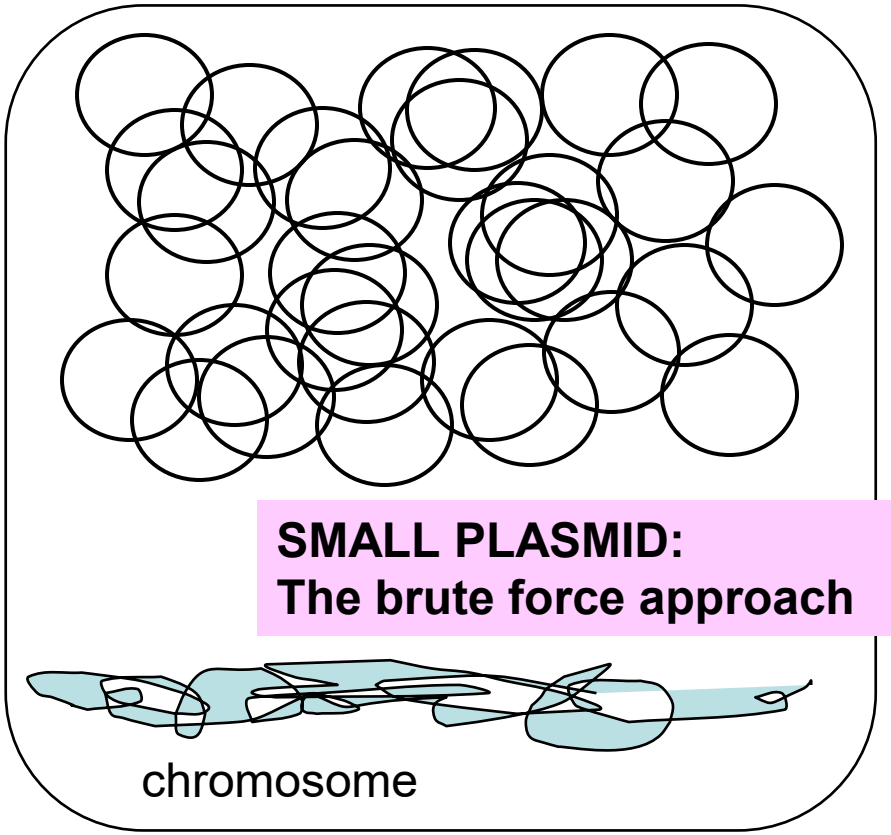
- **Plasmids can be large, so how to distinguish from a chromosome? Plasmids lack essential genes eg. rRNA**

Plasmids

- **Plasmids have highly variable sizes (~ 2 - 1000 kb) and copy number (~1 to 200).**

- **Usually, small plasmids are high copy number, while big plasmids are low copy number. Why?**





IntERcellular transfer of plasmids

- **Different categories of plasmid mobility:**

  1. **self-transferable (conjugative)**
  2. **transferable by other plasmids (mobilisable)**
  3. **non-transferable**

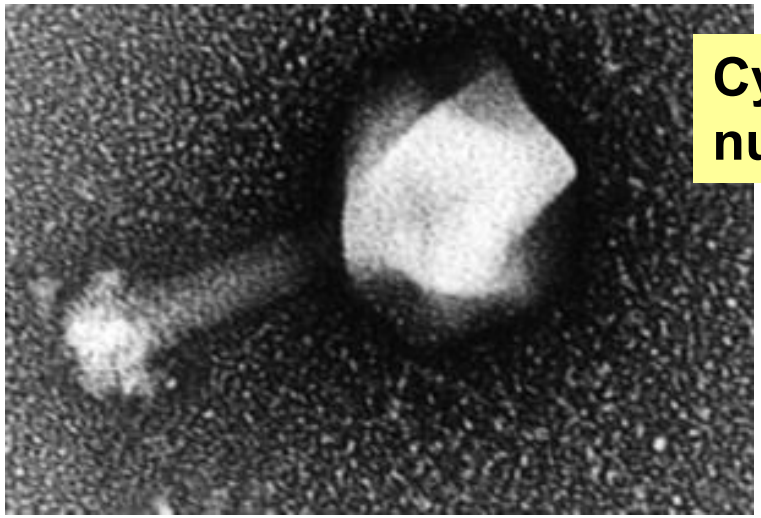
- **The host range of plasmids can differ greatly:**
  - **Broad host range: replicate in many genera**
  - **Narrow host range: specific to a few genera**

- **The host range for transfer may be different to the host range for replication → eg. 'suicide plasmid'**

Bacteriophage

Really need their own lecture series !

- Viruses that infect bacteria – genome can be single or double stranded, and can be DNA or RNA
- Different from all other MGEs: phage have protein coat that protects genetic material → can survive outside host
- Can self-replicate OR integrate into chromosome
- Phage integration is site-specific, not like IS/transposons



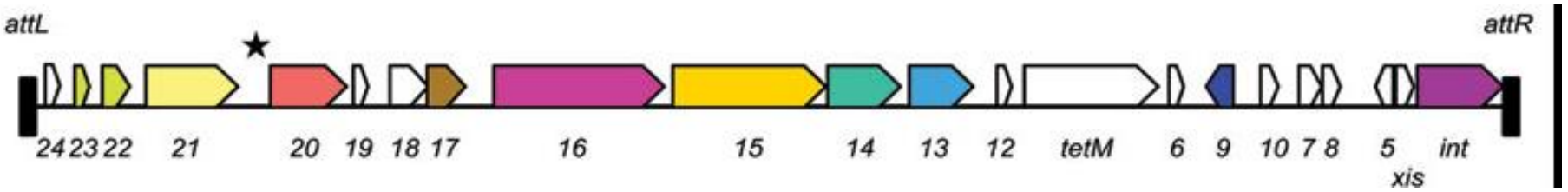
Cyanophage – one of the most numerous 'organisms' on Earth

Bacteriophage of interest to biotechnology: lambda, T4, T7

Integrative conjugative elements (ICEs)

1. Conjugative Transposons

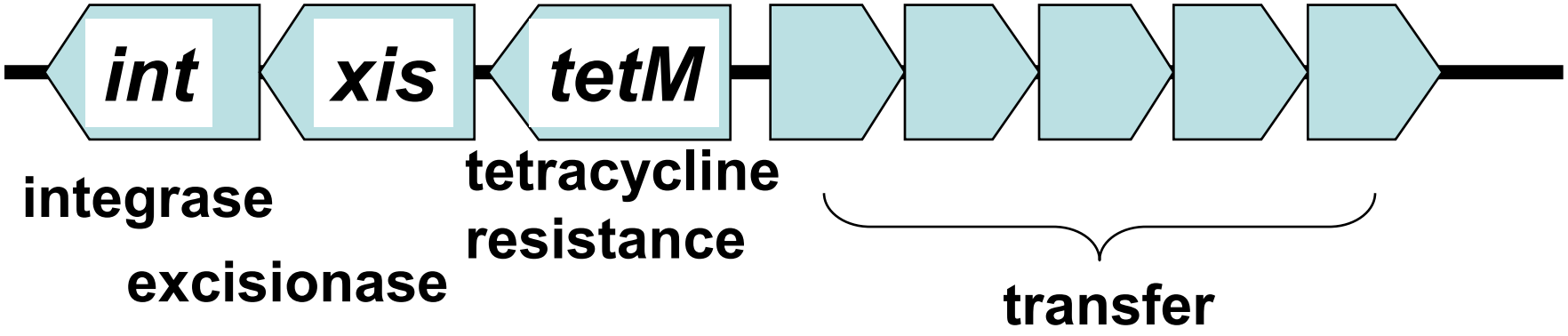
- Transposons that can transfer between bacteria
- Insert randomly into genome, like other Tn's
- Have transfer functions (Tra, Mob) similar to plasmids:
  - Tra: construction of bridge between cells (pilus)
  - Mob: production of ssDNA for transfer



Tn916 – 18 kb conjugative transposon

Why are conjugative Tn's not plasmids?

ICE: Tn*916*, a conjugative transposon from *Enterococcus*



- **Identification and definition of Tn916 was difficult. Should it be categorised as a transposon or a plasmid ?**
- **Integrase / excisionase system, <u>not</u> transposase**
- **Presence of transfer genes**
- **Antibiotic resistance gene**

Integrative conjugative elements (ICEs)

2. Genomic islands

- **Chromosome regions that are highly variable between closely-related strains → indicates HGT**
- **Differ from conjugative transposons in site-specificity of insertion: often downstream of tRNA gene**

Features of diverse genomic islands

tRNAGly--*int*--------Variable part----------Conjugation and transfer----Regulation---18 bp

clc-type genomic island, *Pseudomonas* sp. strain B13

(105 kb)

prfC--------*int*----------AB resistance, unknown----*tra*-like genes------Reg.n of transfer-Repeat

SXT of *Vibrio cholerae* (100 kb); R391 of *Providencia rettgeri* (88 kb)

tRNA^{Phe}--*int*------*fix*---*trb/tra*-like genes, *nod*-----Metabolic, transport functions-----Repeat

Symbiosis island, *Mesorhizobium loti* R7A (500 kb)

van der Meer and Sentchilo (2003) Current Opinion in Biotech.

Genomic islands – significance to biotechnology

- **Environmental biotech: biodegradation genes are often associated with genomic islands eg. chlorocatechol**

- **Agricultural biotech: genes for plant symbiosis are carried by large symbiosis islands or plasmids**

- **Medical biotech: genomic islands often carry antibiotic resistance and virulence genes – how can we control their spread / remove them ?**

Picture credits

http://www.sciencehumor.org/category/cartoons/

http://jb.asm.org/content/vol187/issue13/cover.dtl

Brock textbook "Biology of Microorganisms" 6th ed.

http://www.ebiotrade.com/buyf/productsf/epicentre/readfile.asp?urln=./EZI04KN.htm

http://mic.sgmjournals.org/content/153/5/1664/F2.expansion.html