GENE TRANSFER AND GENETIC ENGINEERING

CHAPTER 8

Craig Venter

Courtesy J. Craig Venter Institute

Man's Failing Heart Heals Itself on Day of Emergency TransplantA miraculous thing happened the day Michael Crowe was set to receive a potentially life-saving heart transplant. Doctors had determined the surgery would be ineffective — but his heart suddenly started beating again. Crowe, a 23-year-old pharmacy student from Omaha, had been diagnosed with **acute myocarditis, or inflammation of the heart muscle, likely caused by a viral infection**. When his mother brought him to the emergency room at his local hospital on Aug. 14, doctors found his heart was only functioning at about 25 percent efficiency. The hospital referred him to the Nebraska Medical Center, and by the time he was admitted to the intensive care unit there, his heart's efficiency had dropped below 10 percent. Doctors hooked Crowe up to a heart-lung machine that would essentially act as his heart for him, pumping blood throughout his body. Crowe was immediately placed on a list for an emergency heart transplant, and remained on the heart-lung machine in a medically induced coma until an appropriate donor heart became available.

After nearly three weeks, a heart was found. The good news was followed by bad, though: tests revealed he had contracted a blood infection. Doctors said he probably would not survive the transplant surgery. About an hour later, one of his doctors noticed something strange — his blood pressure was going up, something that would be impossible if his body was only receiving blood through the machine.

"His heart started working again on its own," Dr. Um told ABC. "The left side of his heart was pumping blood again. The right side was still weak, so we slowly eased him off the machine. At this point, he was in pretty good shape." **In the simplest terms, Dr. Um explained, the heart got sick, triggering an immune response that shut the heart down to fight the infection, and eventually healed itself. Technology kept Crowe's body alive while his heart healed**.

Virus Could Be New Weapon Against Zits Zits begone: It might be possible some day to apply a cream that contains a virus that kills acne-causing bacteria to ward off zits, a new study suggests. The study, published Tuesday in the journal mBio, analyzed the genomes of viruses that attack the skin bacteria linked to acne problems from 11 volunteers. Using over-the-counter pore cleaning strips, the researchers peeled off samples of phages -- viruses that attack bacteria -- from the noses of pimply and unblemished individuals. The researchers were astounded to find that these viruses were remarkably similar genetically from patient to patient, said corresponding author Graham Hatfull, professor of biotechnology and biological sciences at the University of Pittsburgh. The fact that there was so little difference between these viruses from nose to nose suggests that their bacterial prey -- in this case, the bacteria that lead to acne -- are ill-equipped to defend themselves. The increase in antibiotic-resistant strains of the skin bacteria linked to acne highlights the need for new and better acne treatments,…The increase in antibiotic-resistant strains of the skin bacteria linked to acne highlights the need for new and better acne treatments, the study authors wrote. Dr. Doris Day, clinical assistant professor of dermatology at NYU Langone Medical Center and author of "100 Questions and Answers about Acne," explained how the common skin bacteria, **Propionibacterium acnes -- P. acnes for short --** helps pimples develop. "You have a follicle, which is a pore," said Day, who was not involved with the study. "For [some] reason, the skin cells that line it don't slough off as they're supposed to. Once the opening gets blocked, then the oil and skin cells behind it start to build up. That's your whitehead."How could future anti-zit treatments work? There are two ways, said study author Hatfull. **One method would be to create a virus-containing cream that patients could someday slather on pimply areas to kill off P. acnes**. Since this virus is harmless to humans and already lives on our skin, he said, there would be no worry of side effects. A second **potential acne treatment is to use endolysins, a special enzyme produced by the virus that kills bacteria contact, Hatfull said. Endolysins have been shown to be safe and to work well in other types of infections, said Vince Fischetti, professor and head of the Laboratory of Bacterial Pathogenesis and Immunology at Rockefeller University in New York City, who was not involved with the study. The pressure inside a bacterial cell is 10 to 20 times higher than atmospheric pressure, he said. Upon contact, endolysins drill holes in the cell wall that cause a bacterium to explode like a balloon. And Fischetti has the dramatic videos to prove it.**

The types of significance of gene transfer

In bacteria, gene transfer is not an essential part of the life cycle. When it does take place usually only some of the genes of the *donor* cell are transferred to the recipient cell. This combining of genes (DNA) is called **recombination,** and the resulting cell is referred to as a recombinant.

There are three mechanisms of gene transfer in bacteria

1. Transformation-naked DNA into recipient
2. Transduction-DNA transfer via bacterial virus (i.e.,bacteriophage)
3. Conjugation-DNA transfer via conjugal pilus

Transformation - History

- Griffith (1920s)
- Killed versus un-killed strains
- "Transforming principle"

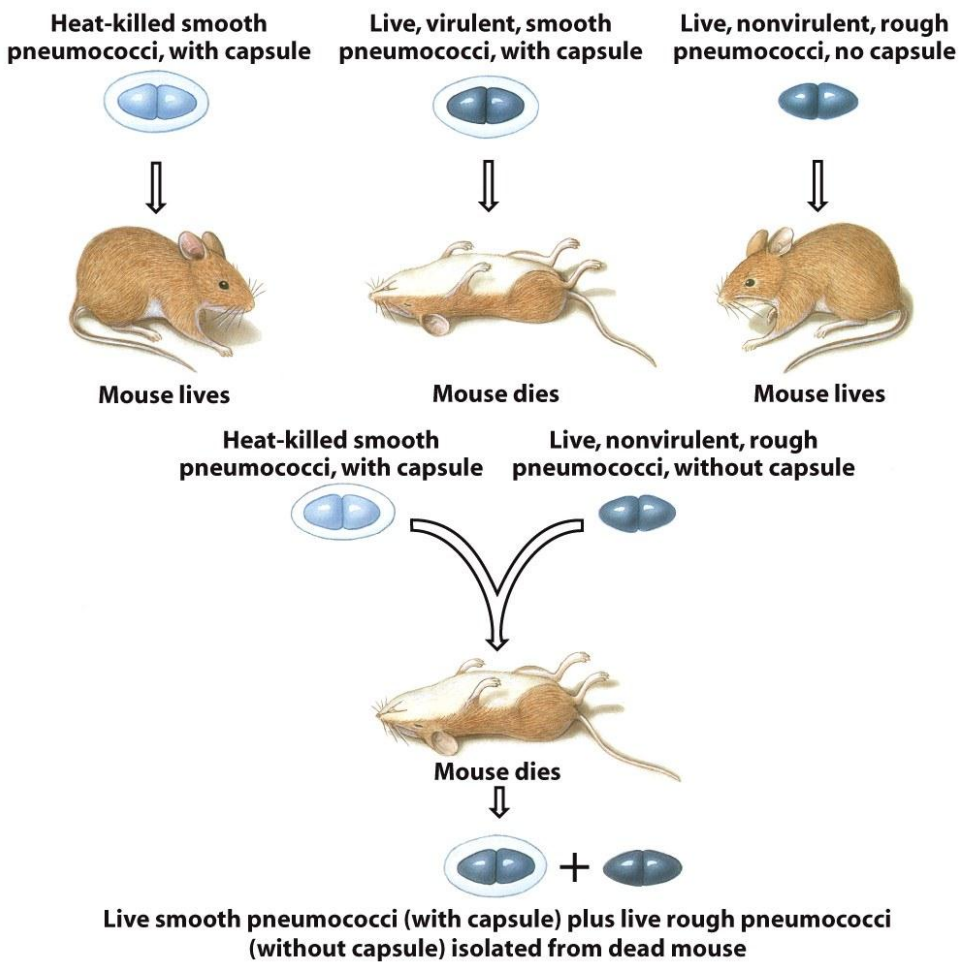
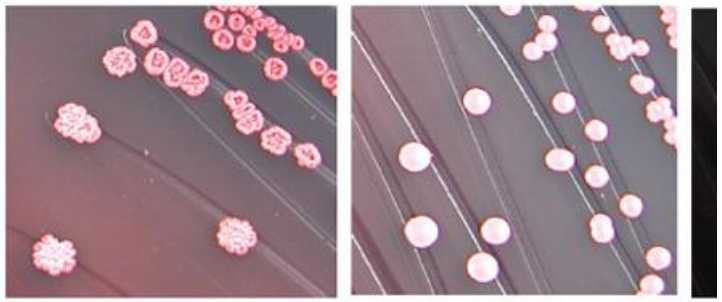


Fig. 8.1 **The discovery of transformation- Griffith's experiments in 1928.**

Figure 8.1 The discovery of transformation: Griffith;s experiment with pneumococcal infections in mice.

Discovered by Frederick Griffith in 1928-while studying pneumococcal infections in mice. He observed that living avirulent pneumococci could be tranformed to virulent pneumococci following exposure to killed virulent pneumococci. Griffith hypothesized that the genetic information was contained in a tranforming factor (which was subsequently shown by Avery MacLeod, and McCarty to be DNA).

In 1944 Avery, MacLeod, and McCarty in 1944 hypothesized that the tranforming factor was DNA and they performed similar experiments to those of Griffith except they used purified DNA from virulent bacteria and exposed the DNA to avirulent pneumococci . Using purified DNA they witnessed a transformation from avirulent to virulent form.



Rough

Smooth

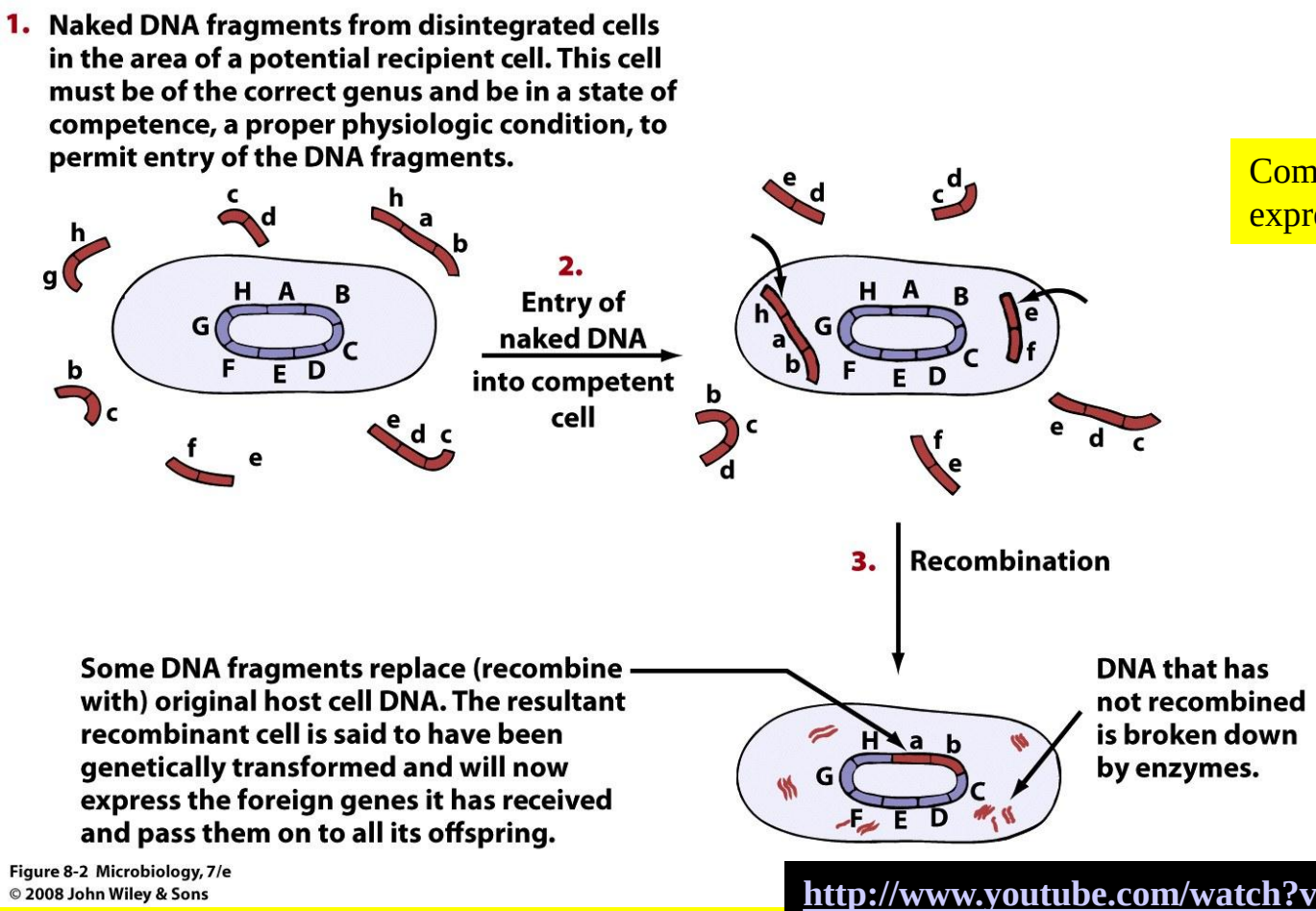


Figure 8-2 Microbiology, 7/e

Competency appears to involve expression of a surface receptor

Fig. 8.2 **The mechanism of bacterial transformation**

http://www.youtube.com/watch?v=n9KZLrYQiFM

1. DNA is released from lysed cells

2. At a certain stage (e.g, late logarithmic stage) a protein called **competence factor** is released into the medium. This factor facilitates entrance of the "naked" DNA into the recipient cell providing the cell has specific receptor sites which can recognize DNA from the same or closely related species but not "foreign" DNA (i.e., DNA from another species).

3.Once DNA reaches the entry sites, endonucleases cut double-stranded DNA into smaller pieces. The strands become separated and only one strand enters the cell. Nucleases that would normally break down the single stranded DNA are inactivated during this process

4. Once inside the cell the donor DNA matches up with an identical loci of the recipient cell and the donor cell recombines (is spliced into) with the recipient DNA. Splicing involves breaking the recipient strand, removing a segment and replacing the segment with the donor DNA.

The significance of transformation

Transformation is utilized in the laboratory to "load" bacteria with foreign DNA to be used to propagate and mutate that DNA in production or experimentation. In the laboratory the recipient cell is "made" competent by "heat shock", electric shock or other treatments (e.g., freeze-thaw).

It is not clear what role transformation plays in genetic diversity in bacteria is not known. Although some antibiotic resistance genes are suspected of being transferred across species barriers by transformation.

Transduction

Unlike transformation, transduction involves the transfer of DNA by bacteriophage (bacterial viruses).

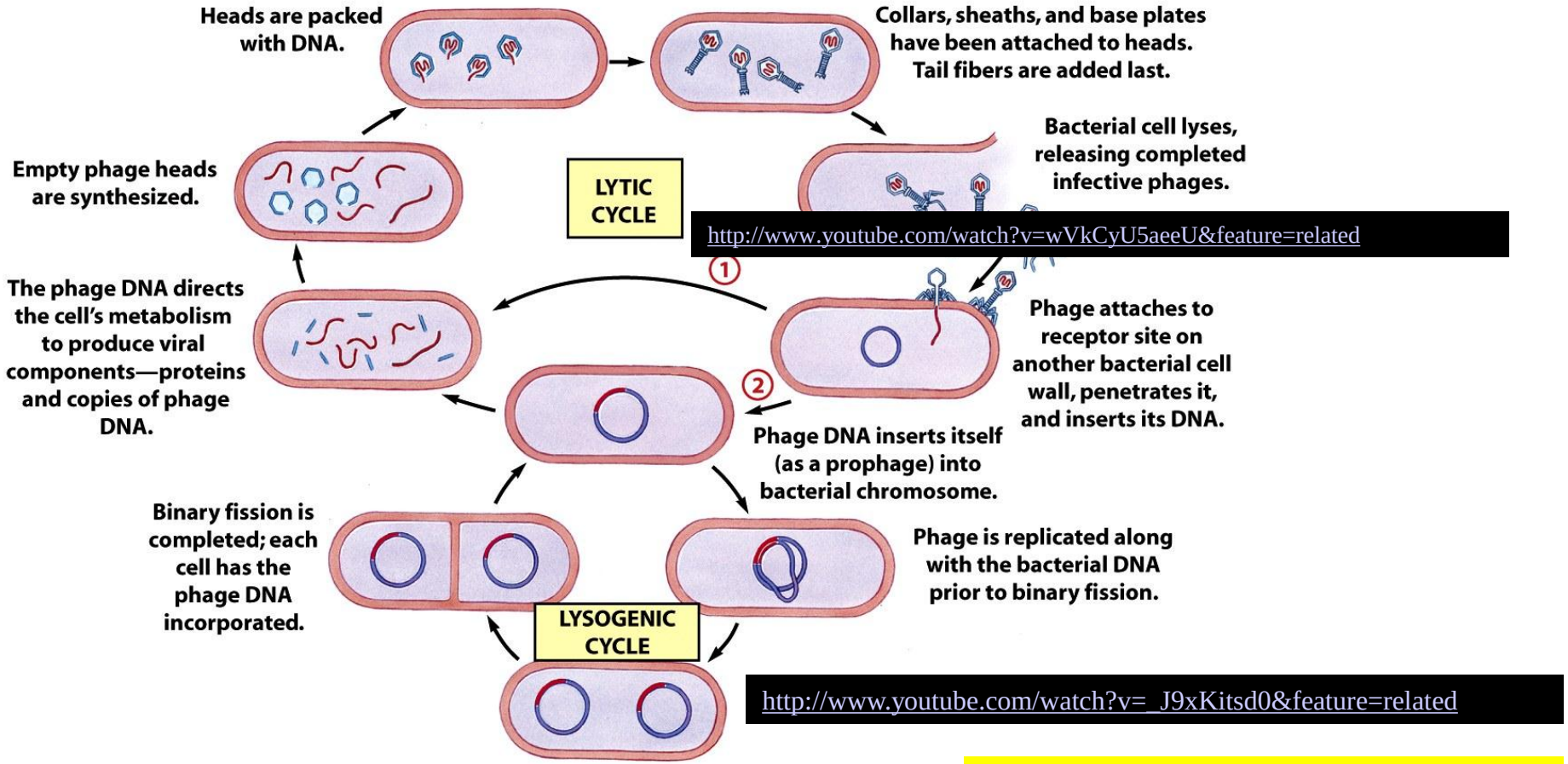


Figure 8-3 Microbiology, 7/e

Lysogeny served as an important model for integration of human viruses into host genome. Viruses like HIV or herpes.

Bacteriophage life cycles- When a bacteriophage injects its viral DNA into a host bacterial cell, at least two different outcomes are possible: i) lytic or ii) lysogenic.
In the lytic cycle the phage replicates and lysis the cell in the lysogenic cycle the phage gets incorporated into the host DNA and is termed a "prophage" and replicates along with the host DNA until such time as it reverts to a lytic phage.

Fig. 8.3 **Bacteriophage life cycles**

New virus can cause fever, cough, breathing problems LONDON (Reuters) - The World Health Organisation (WHO) has issued a global alert about the emergence of a new virus that was previously unknown in humans and can cause a potentially fatal acute respiratory infection. ere is an at-a-glance guide to the virus:

The virus belongs to a family called coronaviruses and has so far been confirmed in only two cases globally. Both occurred between July and September 2012. The first case was in a 60-year-old man in Saudi Arabia and proved fatal. The second is in a 49-year-old Qatari man who recently visited Saudi Arabia. He had the infection diagnosed after travelling to London in early September. The WHO has not yet given the virus a name, but scientists at Britain's Health Protection Agency (HPA) refer to it as **"London1_novel CoV 2012**". Human coronaviruses were first identified in the mid-1960s and are named for the crown-like projections on the surface of the virus.

The family includes viruses that cause the **common cold and SARS, or Severe Acute Respiratory Syndrome,** which emerged in China in 2002 and killed about 10 percent of approximately 8,000 people infected worldwide. Coronaviruses are typically spread like other respiratory infections, such as flu, travelling in airborne droplets when an infected person coughs or sneezes.

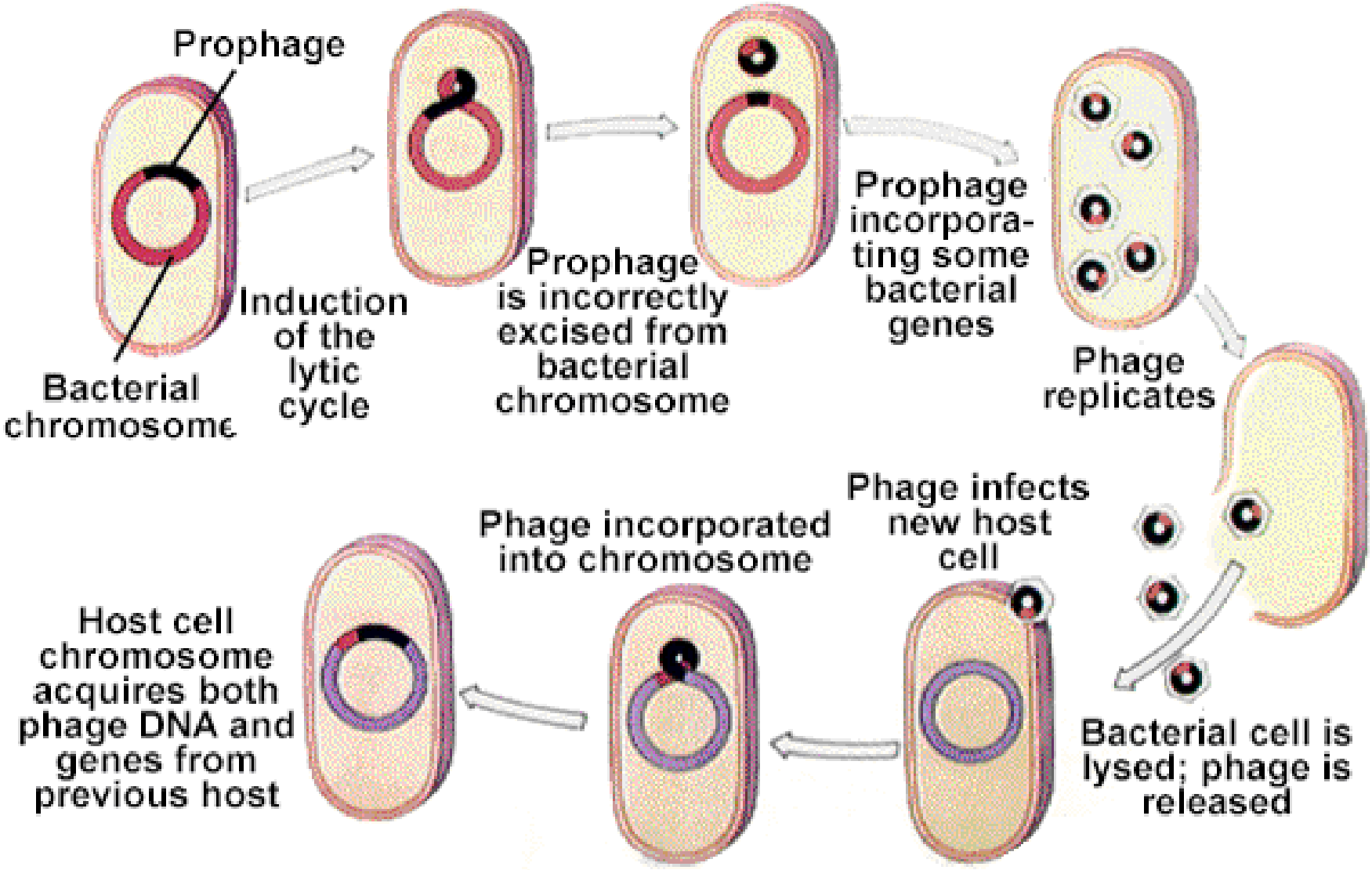
Bizarre tumor case may lead to custom cancer care It's a medical nightmare: a 24-year-old man endures 350 surgeries since childhood to remove growths that keep coming back in his throat and have spread to his lungs, threatening his life. Now doctors have found a way to help him by way of a scientific coup that holds promise for millions of cancer patients. **The bizarre case is the first use in a patient of a new discovery: how to keep ordinary and cancerous cells alive indefinitely in the lab.** The bizarre case is the first use in a patient of a new discovery: how to keep ordinary and cancerous cells alive indefinitely in the lab. The discovery allows doctors to grow "mini tumors" from each patient's cancer in a lab dish, then test various drugs or combinations on them to see which works best. It takes only a few cells from a biopsy and less than two weeks to do, with materials and methods common in most hospitals. **The new technique may reveal in advance whether a person would be helped by a specific chemotherapy, without risking side effects and lost time if the drug doesn't work. "Pretty nifty," Daley wrote.** In the case of the 24-year-old, described in Thursday's New England Journal of Medicine, **lab-dish tests suggested that a drug used to treat a type of blood cancer and some other unrelated conditions might help.**

It's not a drug that doctors would have thought to try, because the man technically does not have cancer. But his lung tumor shrank after a few months of treatment, and he has been stable for more than a year. He still has to have operations to remove throat growths that keep coming back, but only about once every five months. A similar approach could let doctors screen drugs for cancer patients.

"What could be more personalized than taking this person's cell, growing it in culture, finding a drug to treat them and then treat them?" said Doug Melton, co-director of the Harvard Stem Cell Institute. The Georgetown method gives an answer quickly enough that it could save lives, he said.

Specialized transduction-

Figure 8.4 Specialized transduction by lambda phage in *E. coli*. In this process, phage DNA always inserts itself into the bacterial host chromosome at a particular site. When the page DNA replicates it takes bacterial genes from either side of the site and packages them with its own DNA into new phages.



In specialized transduction phage DNA always inserts itself at a particular site

Fig. 8.4 **Specialized transduction by lambda phage in *E.coli***

Generalized transduction-

Figure 8.5-Generalized transduction. Bacteriophage infection of a host bacterium initiates the lytic cycle which results in the breakdown of infected cell DNA. Any fragment of that DNA can be incorporated into the newly "packaged" phage and transferred into a newly infected host bacterial cell.

http://www.youtube.com/watch?v=qGJ0mmXovmc&feature=related

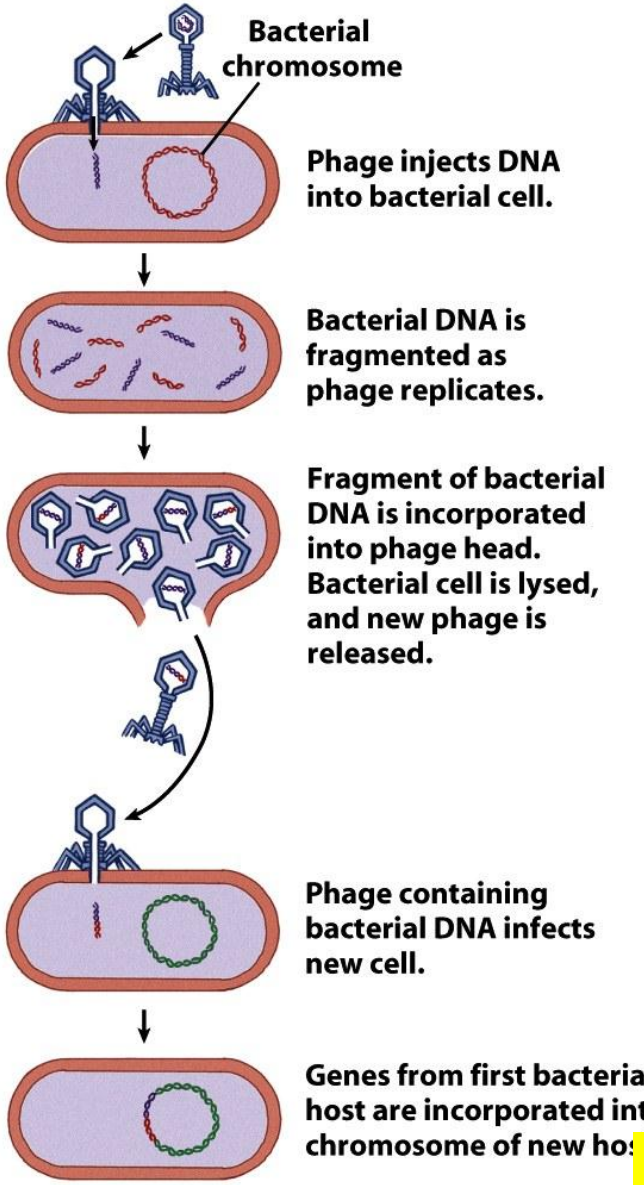


Figure 8-5 Microbiology, 7/e

Phage injects DNA into bacterial cell

Bacterial DNA is fragmented as phage replicates

Fragment of bacterial DNA is incorporated into phage head. Bacterial cell is lysed, and new phage is released

Phage containing bacterial DNA infects new cell

Genes from first bacterial host are incorporated into chromosome of new host during a lysogenic cycle.

Fig. 8.5 **Generalized transduction (phage DNA integrates at random sites in the host genome).**

Bacterial Conjugation-

Conjugation differs from transformation and transduction in two ways:

i) requires contact between cells via a pilus and

ii) it transfers much larger quantities of DNA

http://www.youtube.com/watch?v=AObOs8GYLDY&feature=related

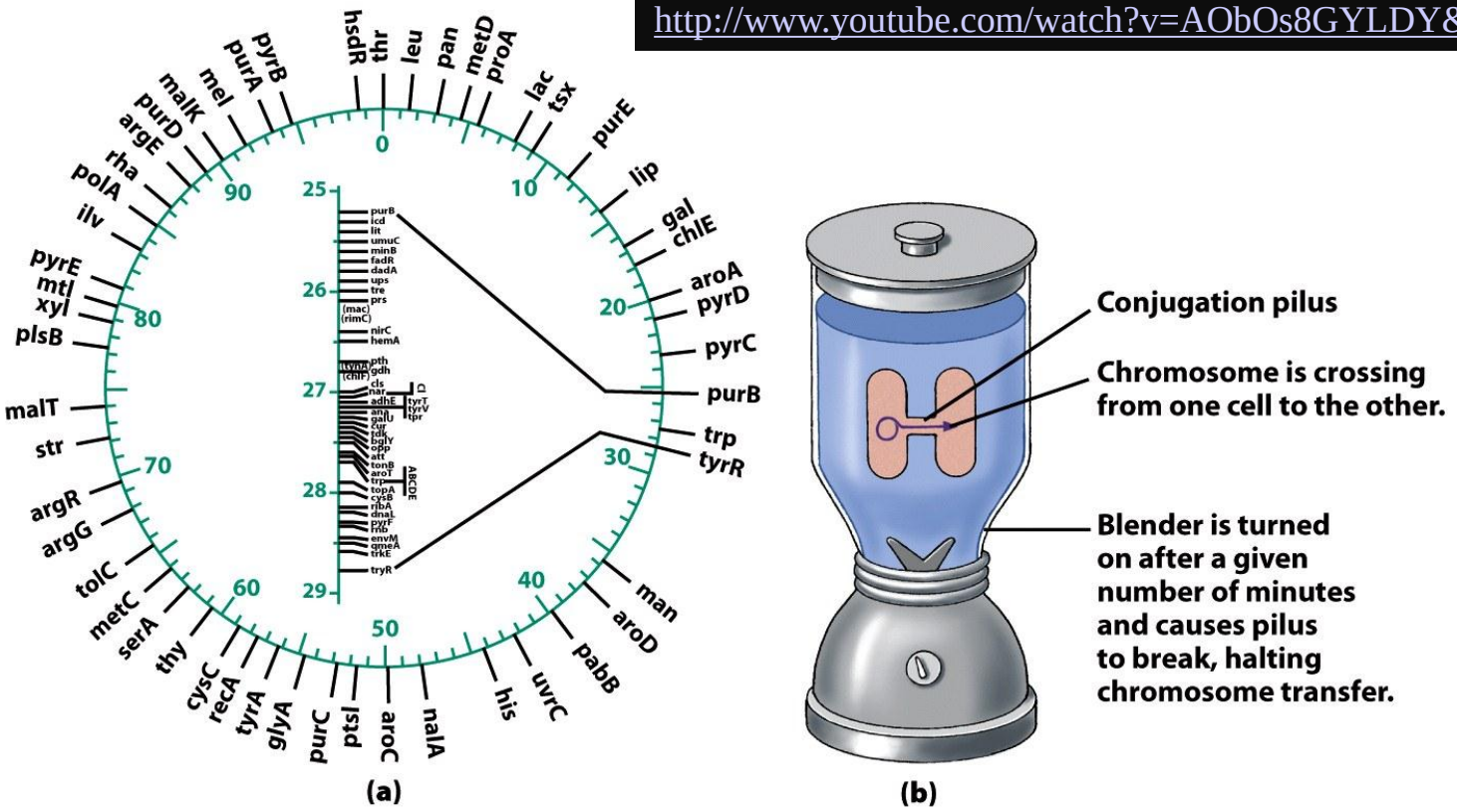


Figure 7-2 Microbiology, 7/e
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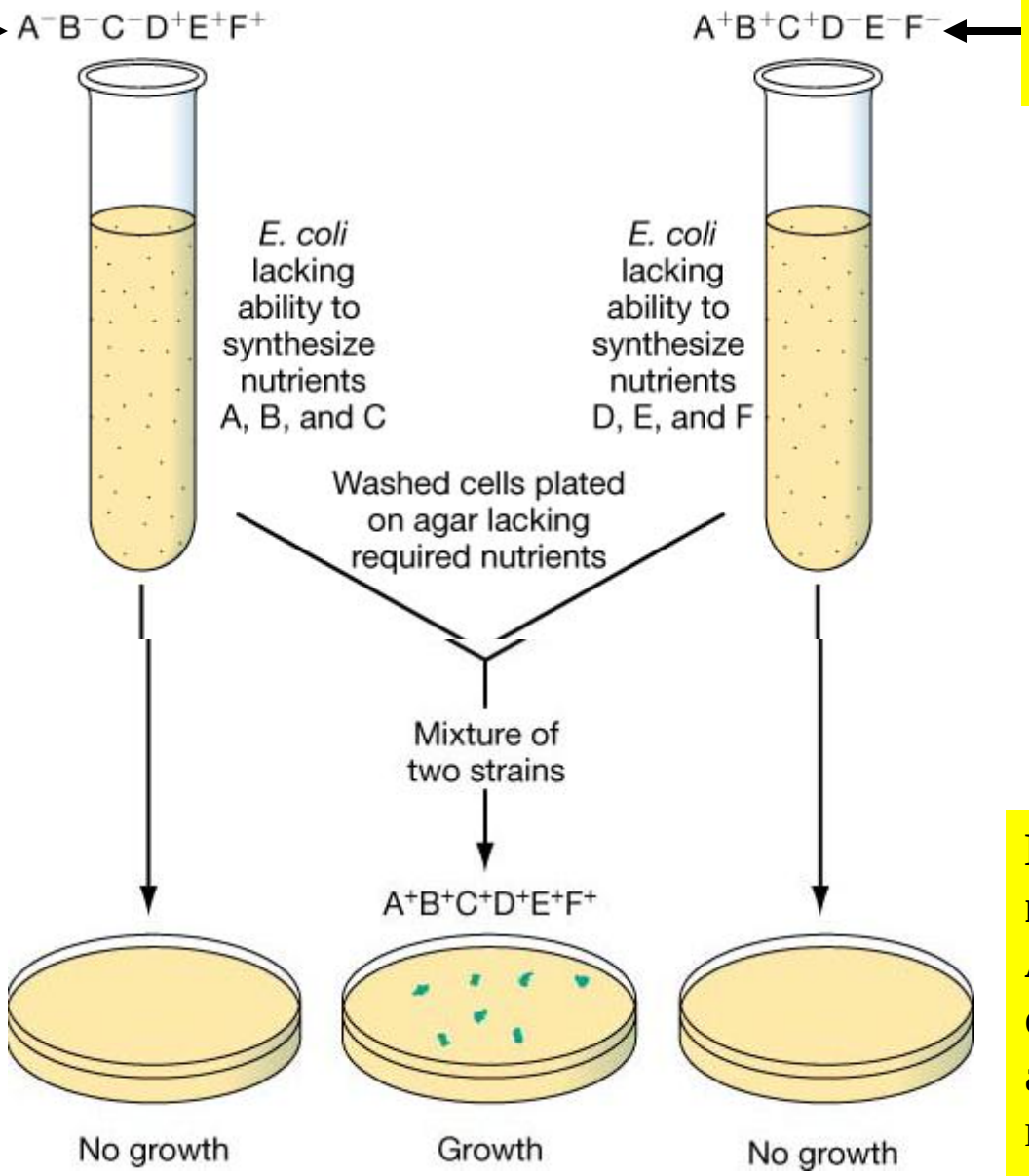
Fig. 7.2 a partial chromosome map of *E. coli*

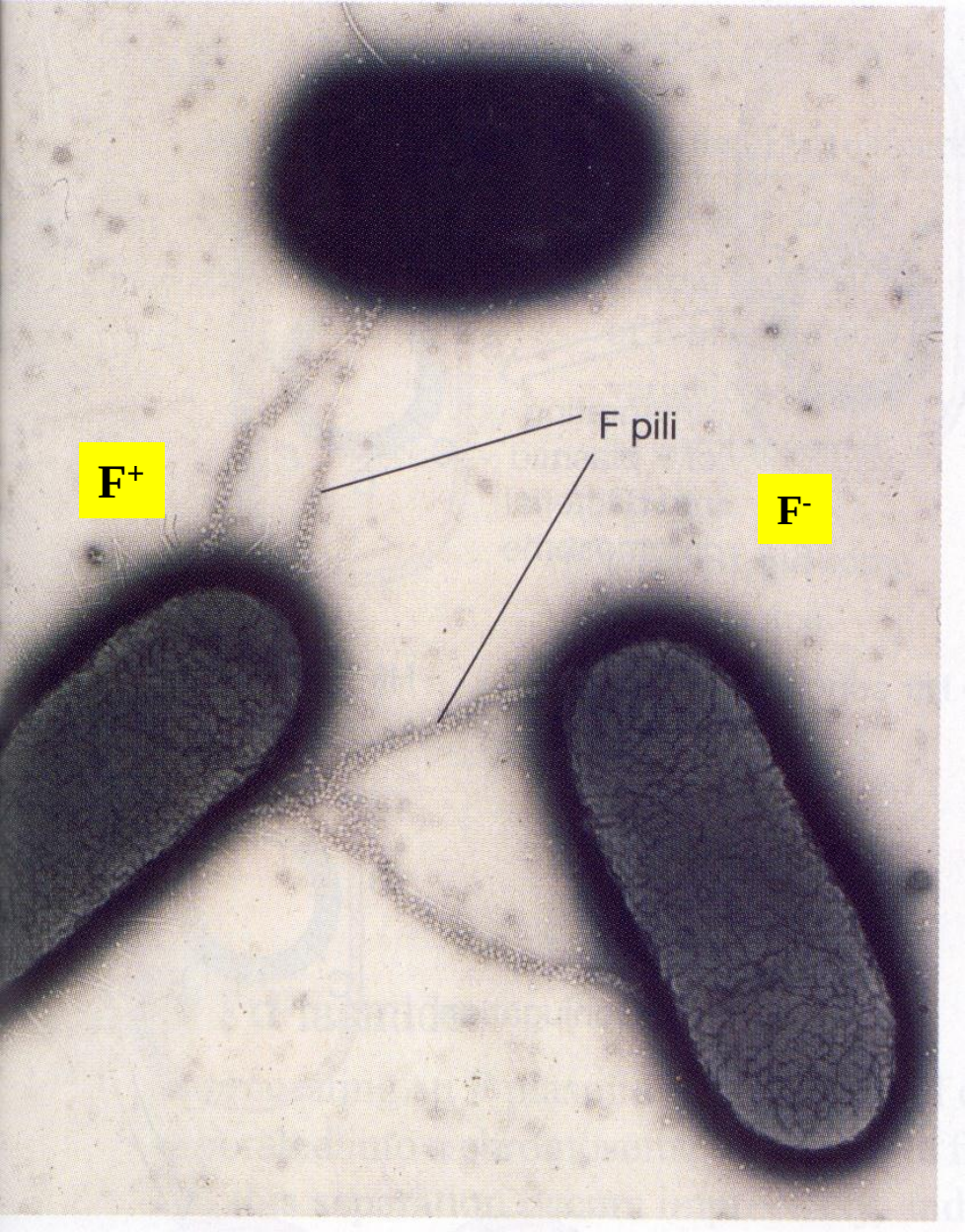
This strain can make DEF but not ABC

This strain can make ABC but not DEF



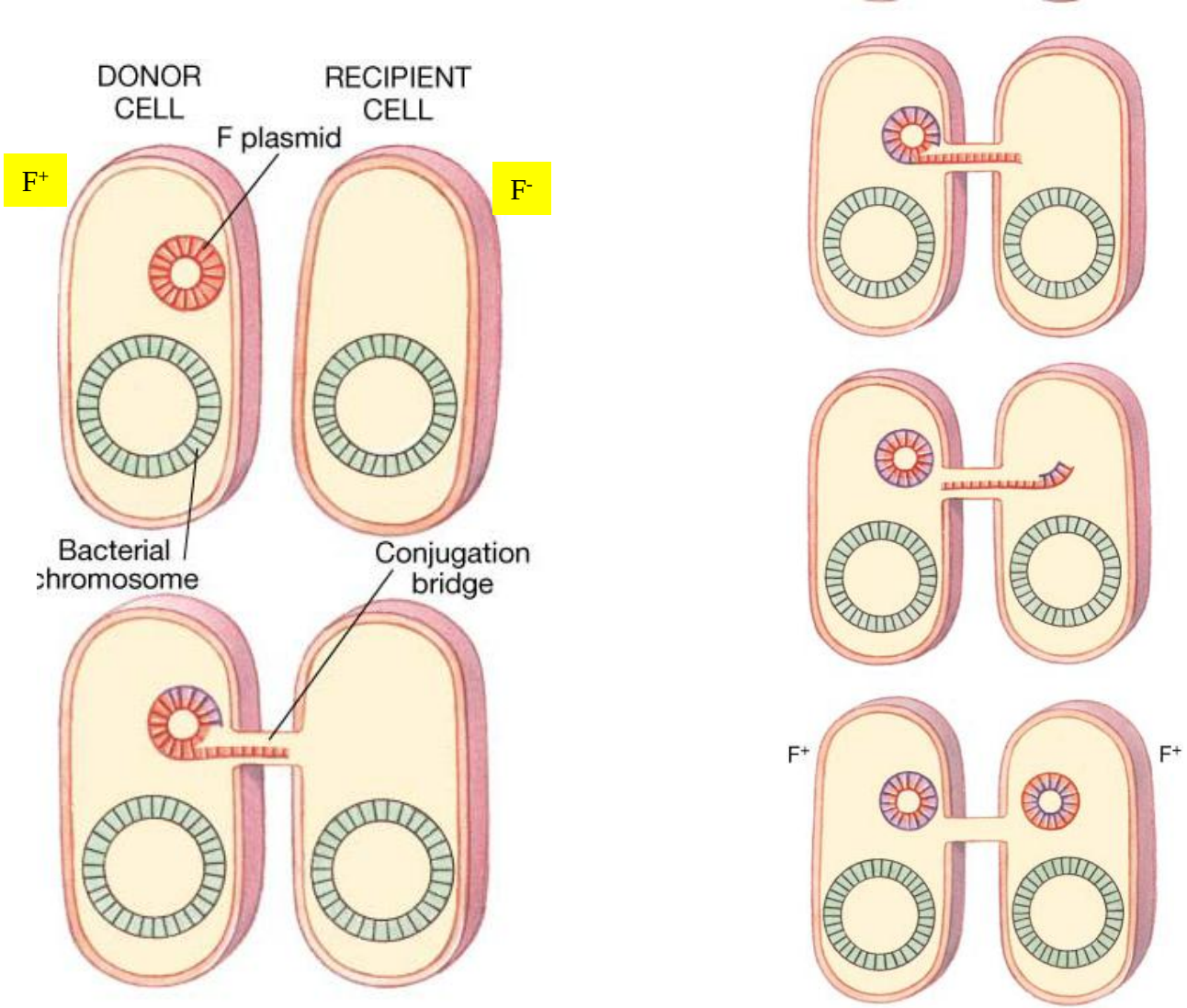
Medium lacks nutrients
A, B, C, D, E, and F
only bacteria that have acquired the ability to make all 6 nutrients will grow

Fig. 8.6 **The discovery of conjugation: Lederberg's experiment**



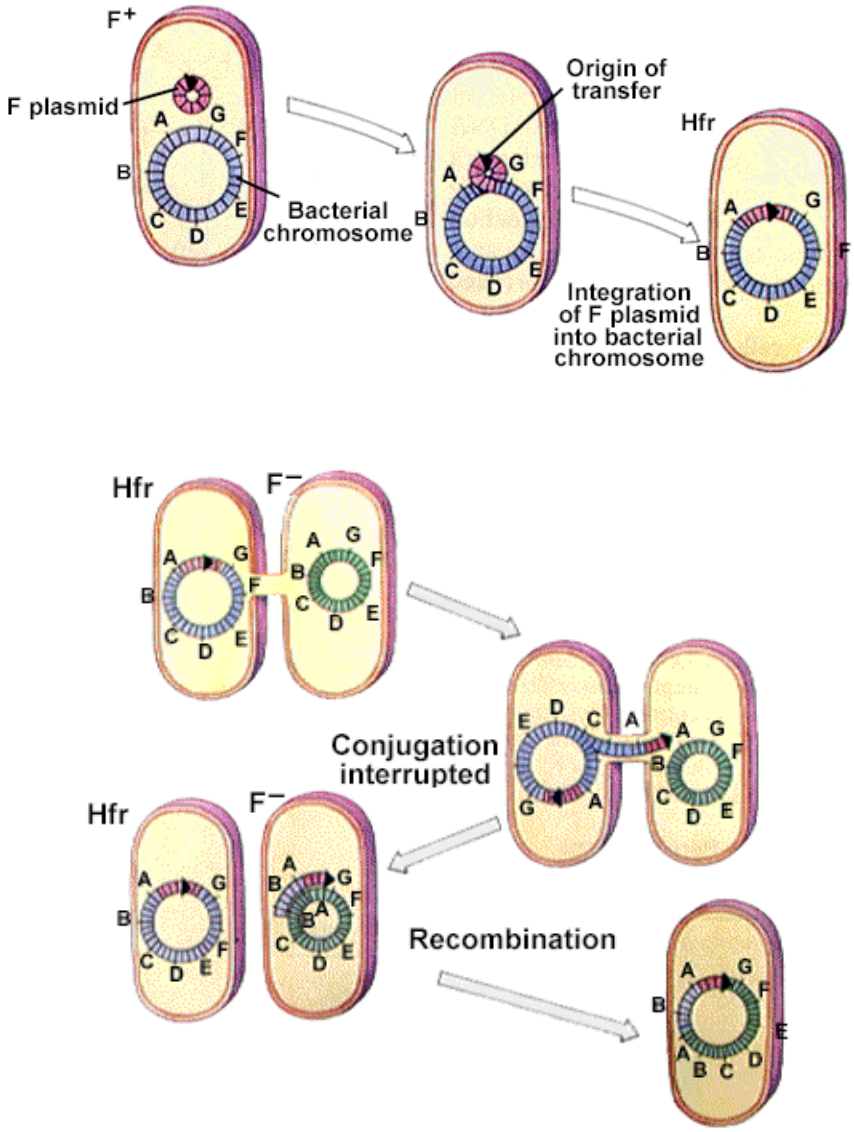
Unlike the shorter attachment pili (fimbriae), this long type of pilus is used for transfer of genes in conjugation and is often called a sex pilus.

Fig. 8.7 transmission electron micrograph of F pilli of *E. coli*



An F^+ x F^- mating- The F+ cell transfers one strand of DNA from its plasmid to the F^- cell via the conjugation bridge. As this occurs, the complementary strands of F plasmid DNA are synthesized.

Fig. 8.8 **An F^+ x F^- mating.**



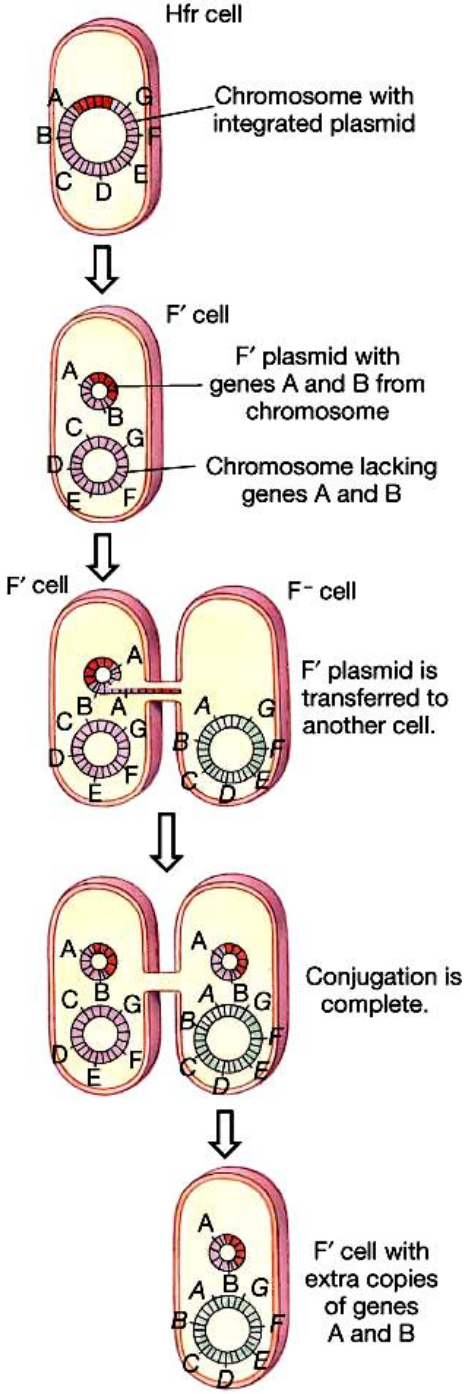
Conversion of F^+ into the Hfr condition

During conjugation the (pink) initiating site of the F plasmid and adjacent genes are transferred to a recipient cell.

However, the entire F^+ plasmid information is almost never transferred to the F^- strain. Hence although Hfr recombinations occur at a high frequency the F^- strain is almost never converted to F^+

Genes are transferred in a linear sequence and the number of genes transferred depends on the duration of conjugation and whether the DNA strand breaks or remains intact.

Fig. 8.9 **High-frequency recombinations**



F', formed by the excision of the F factor plasmid from the Hfr strain contains some host genes in addition to the gene for F plasmid.

The F' transfers its genetic material like an F^+ strain

Fig. 8.10 **The formation and transfer of F' plasmids**

TABLE 8.1

Results of Selected Conjugations

Donor	Recipient	Molecule(s) Transferred	Product
F^+	F^-	F plasmid	F^+ cells
Hfr	F^-	Initiating segment of F plasmid and variable quantity of chromosomal DNA	F^- with variable quantity of chromosomal DNA
F′	F^-	F′ plasmid and some chromosomal genes it carries with it	F′ cell with some duplicate gene pairs: one on chromosome one on plasmid

Table 8-1 Microbiology, 6/e

TABLE 8.2

Summary of the Effects of Various Transfers of Genetic Information

Kind of Transfer	Effects
Transformation	Transfers less than 1 percent of cell's DNA. Requires competence factor. Changes certain characteristics of an organism, depending on which genes are transferred.
Transduction	Transfer is effected by a bacteriophage.
Specialized	Only genes near the prophage are transferred to another bacterium.
Generalized	Fragments of host bacterial DNA of variable length and number are packed into the head of a virus.
Conjugation	Transfer is effected by a plasmid.
F^+	A single plasmid is transferred.
Hfr	An initiating segment of a plasmid and a linear sequence of bacterial DNA that follows the initiating segment are transferred.
F′	A plasmid and whatever bacterial genes adhere to it when it leaves a bacterium are transferred.

Table 8-2 Microbiology, 6/e

Plasmids

Characteristics of plasmids

F plasmid was first to be discovered, subsequently many others have been found. Most plasmids are circular, double-stranded extra-chromosomal DNA. They are self replicating and have been identified by virtue of characteristics they give to a bacterium:

1. F plasmids (fertility factors)
2. *Resistance (R) plasmids* carry genes that proved resistance to various antibiotics, e.g, chloramphenicol and tetracycline and to heavy metals such as arsenic and mercury
3. Bacteriocins- plasmids that direct the production of bacteriocidal proteins
4. Virulence plasmids- cause disease signs and symptoms
5. Catabolic enzyme plasmids
6. Tumor-inducing (Ti) plasmids

Plasmids

http://www.youtube.com/watch?v=GNMJBMtKKWU

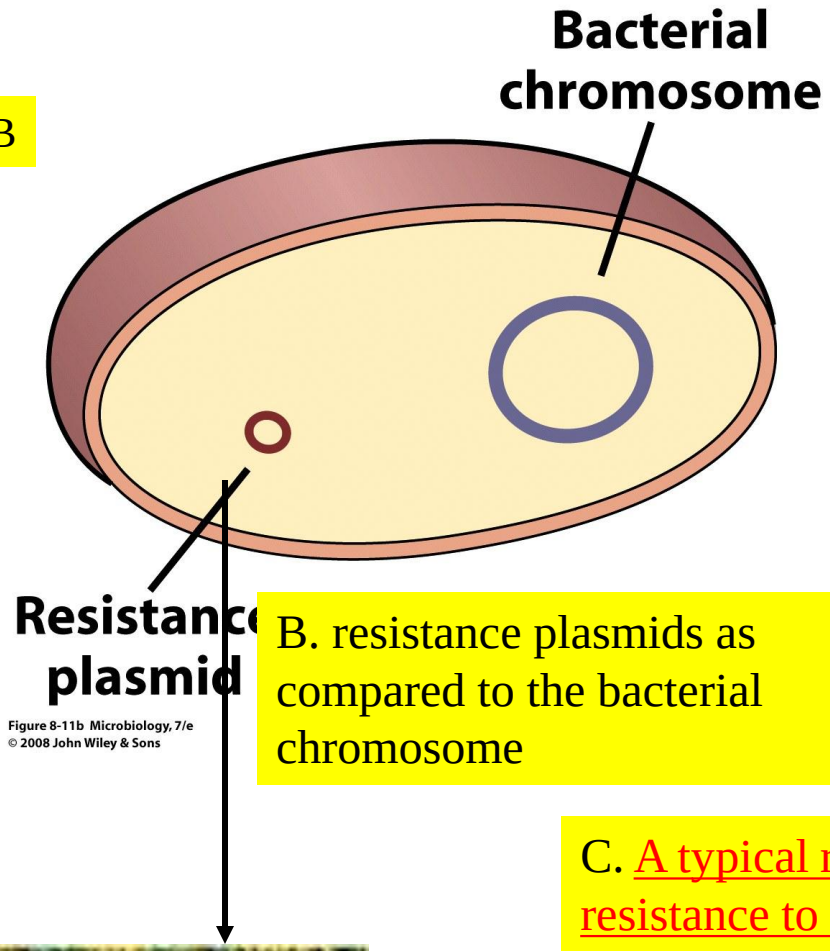
Resistance plasmids

Resistance plasmids generally contain two components

i.) resistance transfer factor (RTF)

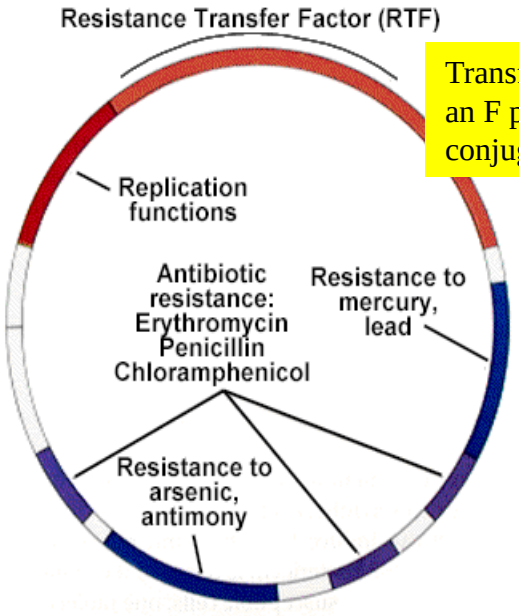
ii) one or more resistance (R) genes

B



B. resistance plasmids as compared to the bacterial chromosome

C



Transfers much like an F plasmid via conjugation

C. A typical resistance plasmid can carry genes for resistance to various antibiotics and to inorganic toxic substances, sometime used in disinfectants. The resistance transfer factor includes genes needed for the plasmid to undergo conjugation

Resistance plasmids generally contain two components

i.) resistance transfer factor (RTF)

ii) one or more resistance (R) genes

Fig. 8.11 **Resistance plasmids**

Bacteriocinogens-(Bacterial plasmid's responsible for the elaboration of bacteriocins) proteins that inhibit growth of other strains of the same or a closely related species. The mechanisms include:

1) **Some enter the cell and destroy DNA.**

2) **Others arrest protein synthesis by disrupting the molecular structure of ribosomes.**

3) **Still others act on cell membranes by inhibiting active transport or increasing membrane permeability to ions.**

Bacteriocins are of interest in medicine because they are made by non-pathogenic bacteria that normally colonize the human body. Loss of these harmless bacteria following antibiotic use may allow opportunistic pathogenic bacteria to invade the human body.

This year's flu vaccine guards against new strains WASHINGTON (AP) — Time to get your flu vaccine — and a surprising new report shows babies and toddlers seem to be getting protected better than the rest of us. Last year's flu shot won't shield you this year: **Two new strains of influenza have begun circling the globe, and the updated vaccine appears to work well against them,** government officials said Thursday. Just because last year was the mildest flu season on record doesn't mean the virus might not bounce back to its usual ferocity this winter. People cannot become complacent this year," said Dr. Howard Koh, assistant secretary of the Department of Health and Human Services, who received his own flu shot Thursday. A yearly vaccination now is recommended for nearly everybody, but new figures released Thursday show that last year 52 percent of children and just 39 percent of adults were immunized. Best protected: Three-quarters of tots ages 6 months to 23 months were vaccinated. That's a significant jump from the previous year, when 68 percent of those youngsters were immunized. Older adults got a little lost in the recent public health push to explain that flu vaccine benefits all ages — and it's time to target them again, said Dr. Daniel Jernigan, a flu specialist with the Centers for Disease Control and Prevention. Flu specialists can't say how bad this winter's flu season might be. Influenza strains constantly evolve, and some cause more illness than others. But strains from **the H3N2 family tend** to be harsher than some other flu types, and a new H3N2 strain is included in this year's vaccine because it is circulating in parts of the world. **Only one ingredient in this year's flu vaccine was retained from last year's, protection against the H1N1 strain that caused the 2009 swine flu pandemic and has been the main kind of influenza circulating since. Also new in this year's shot is protection against a different Type B strain**.

Vomiting virus hits thousands of German children BERLIN (AP) — German health authorities say the number of children that have fallen ill with vomiting and diarrhea after eating food from school cafeterias and daycare centers has risen from about 4,500 to 8,400. uthorities in Berlin and the surrounding eastern German states reported the new gastroenteritis cases Saturday, while laboratory investigations to determine the exact cause of the outbreak were still under way.
Berlin's health department says the sicknesses are moderate and most children recover within two days without requiring to be hospitalized.
In Saxony state, **at least 16 cases of norovirus, a mostly food- or water-borne illness,** were proven, according to German news agency dapd.
The government-affiliated Robert Koch Institute said Friday that all facilities where the illness occurred likely received food from a single supplier.

Five techniques of genetic engineering:

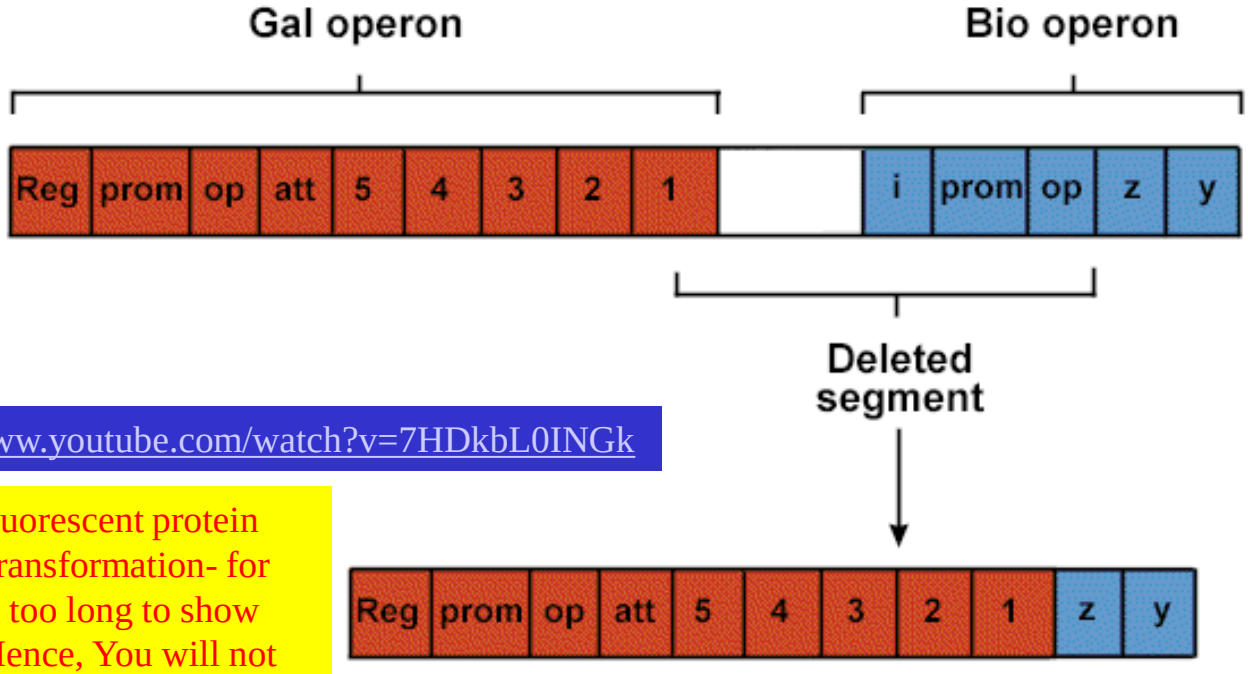
i) **gene fusion**

ii) **protoplast fusion**

iii) **gene amplification**

iv) **recombinant DNA technology**

v) **creation of hybridomas**



Prom = promoter
Z and Y are the structural genes for GFP

http://www.youtube.com/watch?v=7HDkbL0INGk

Inserting green fluorescent protein into bacteria by transformation- for you to watch it is too long to show during lecture. Hence, You will not be quizzed on this video

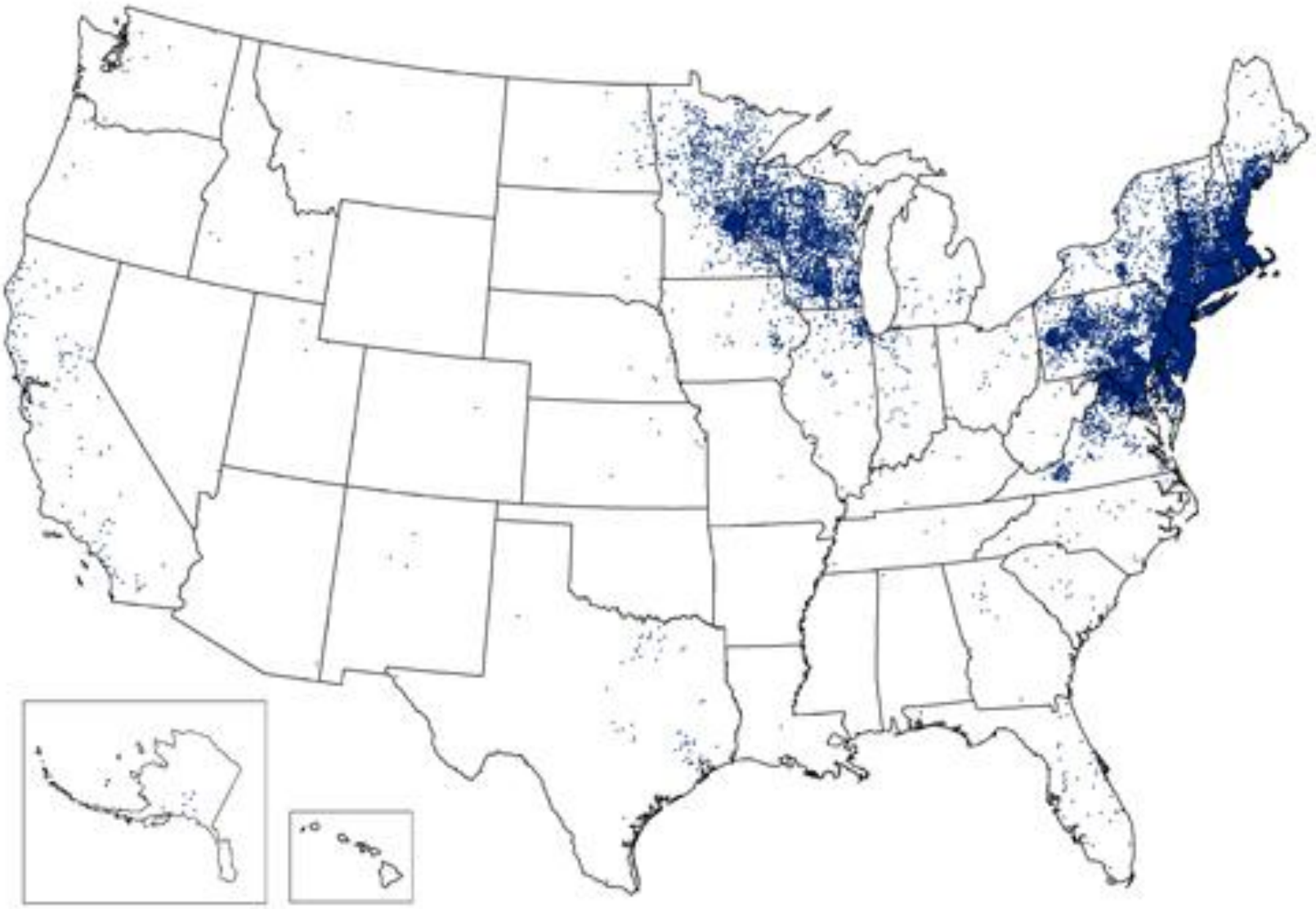
An example of gene fusion: One can have two genes under the control of a single regulator (for example fuse a fluorescent protein with your protein of interest and when the bacterial colony (or plant) lights up under fluorescent light you know your gene was transferred. Or one can use DNA fusion to test for promoter activity (fuse a detector gene (e.g., fluorescent protein or beta-galactosidase) to a brain specific promoter and inject into a worm embryo and follow the development of the brain by observing which cells light up and where they go)

Fig. 8.13 **Genetic fusion**

Green fluorescent Axolotls- these animals are developed at the University of Kentucky by Dr. Randal Voss's laboratory

http://www.youtube.com/watch?v=Uleb3MlZ4JU

Reported Cases of Lyme Disease -- United States, 2010



1 dot placed randomly within county of residence for each confirmed case

Recombinant DNA technology.- One of the most useful of all techniques of genetic engineering is the production of recombinant DNA-DNA that contains information from two different species of organisms.

If these genes integrate permanently into the egg or sperm cells such that the genes can be transferred to offspring, the resulting organism is said to be a transgenic, or recombinant, organism.

Making recombinant DNA involves three processes:

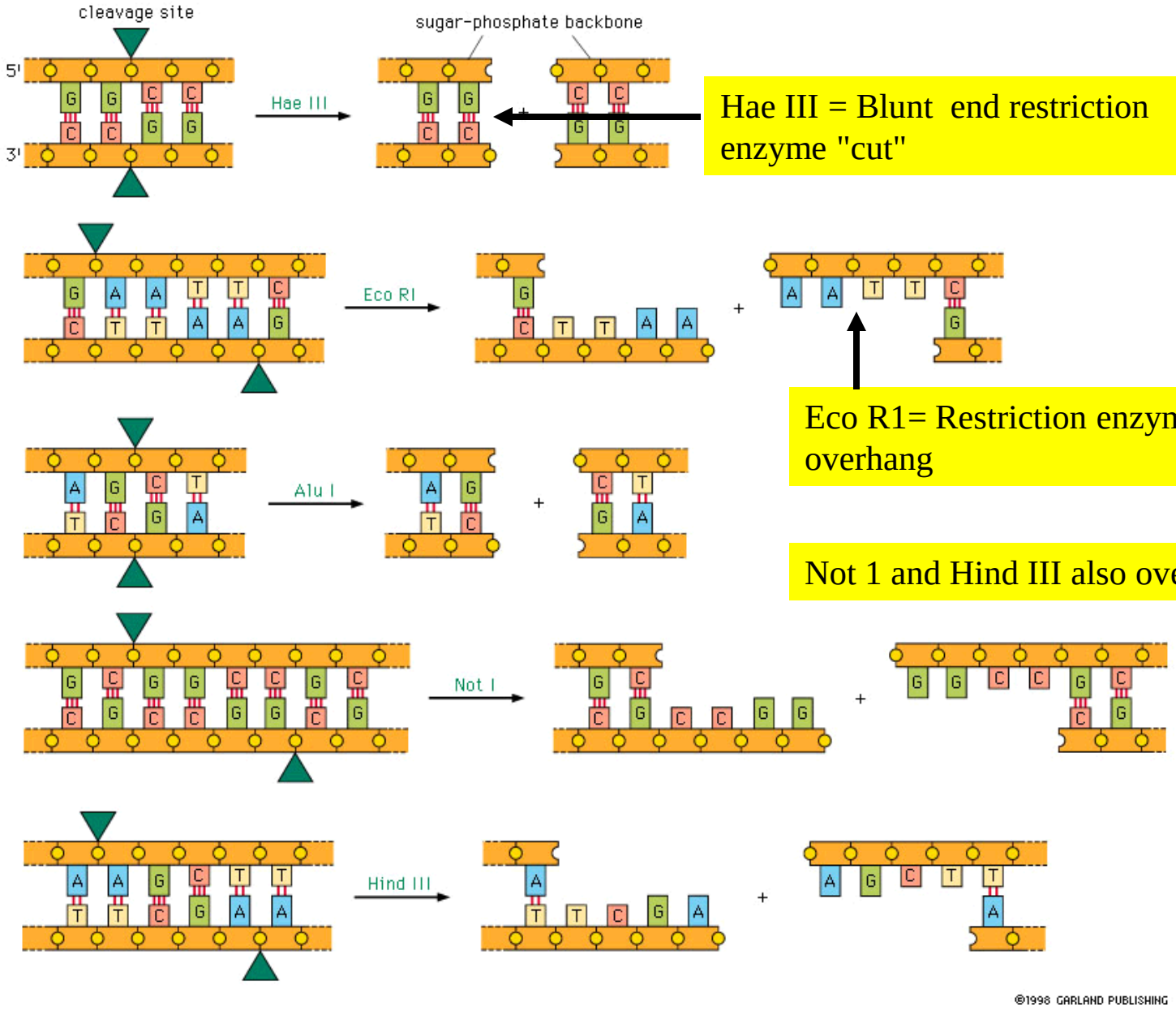
i. The manipulation of DNA *in vitro*-

ii. The recombination of another organism's DNA with bacterial DNA in a phage or a plasmid

iii. The *cloning* , or production of many genetically identical progeny, of phages or plasmids that carry foreign DNA.

http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120078/bio37.swf::Restriction Endonucleases

Restriction endonuclease



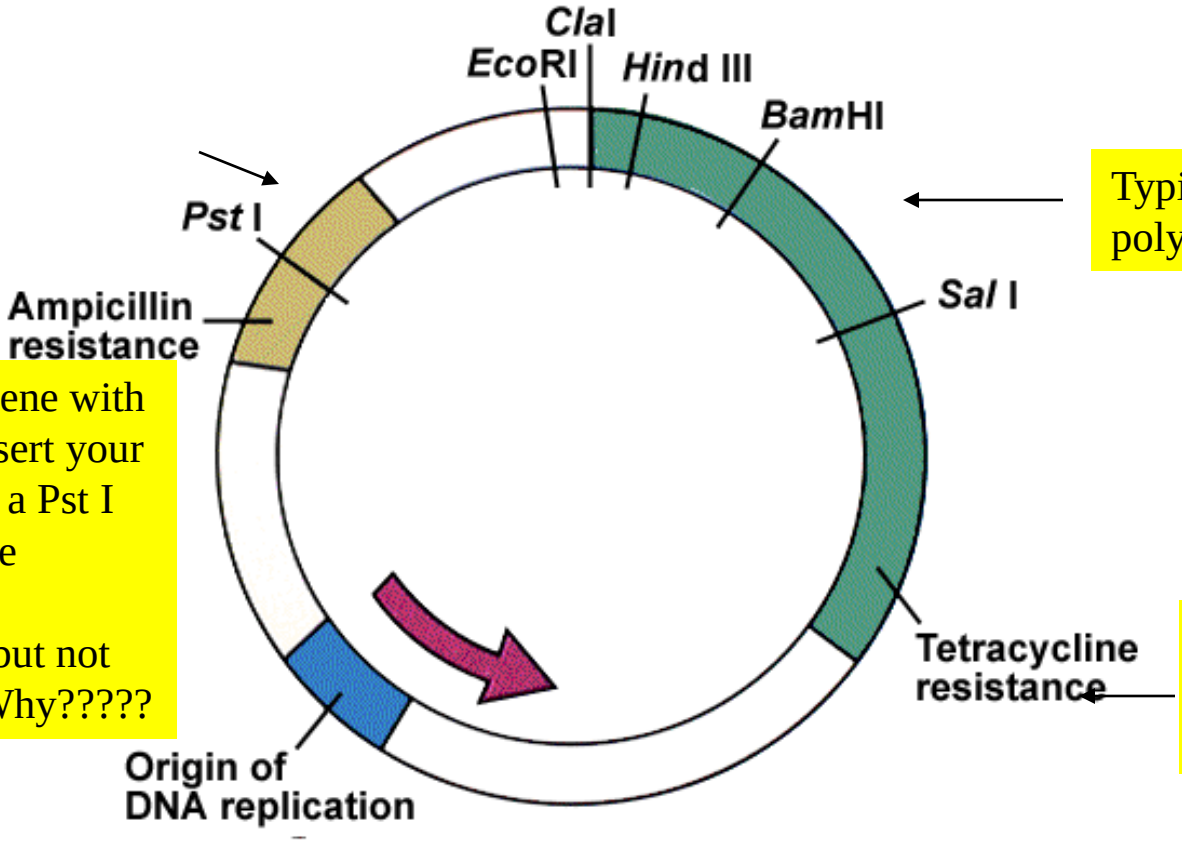
Hae III = Blunt end restriction enzyme "cut"

Eco R1= Restriction enzyme "cut" with overhang

Alu 1 blunt end

Not 1 and Hind III also overhang

Restriction endonucleases (not in your text)



Typically contains a polycloning site

antibiotic resistance gene with cloning site. If you insert your gene in this site using a Pst I restriction enzyme, the resulting cells will be tetracycline resistant but not ampicillin resistant. Why?????

antibiotic resistance gene with cloning site

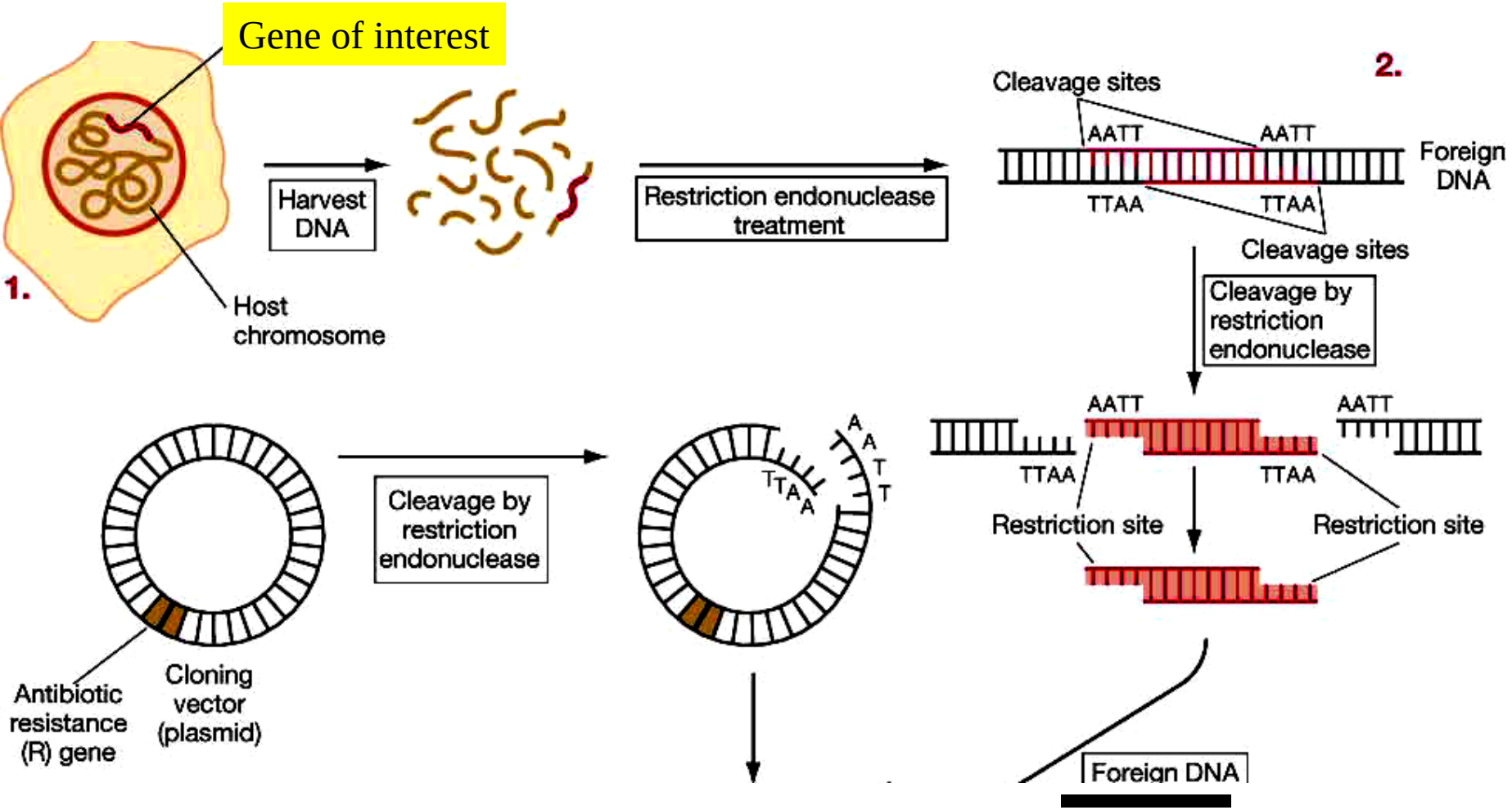
<u>Typical cloning vector</u>

Antibiotic resistance important to help select for organisms that are transformed with the plasmid

http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120078/micro10.swf::Steps in Cloning a Gene

Cloning a gene

http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter16/animations.html



It is best to use the same restriction endonuclease to "cut" the cloning vector and the host chromosome. Why?? You would try to use a restriction enzyme that had overhangs. Why? If not

Fig. 8.15 Method for amplifying and obtaining genes from bacteria with plasmid

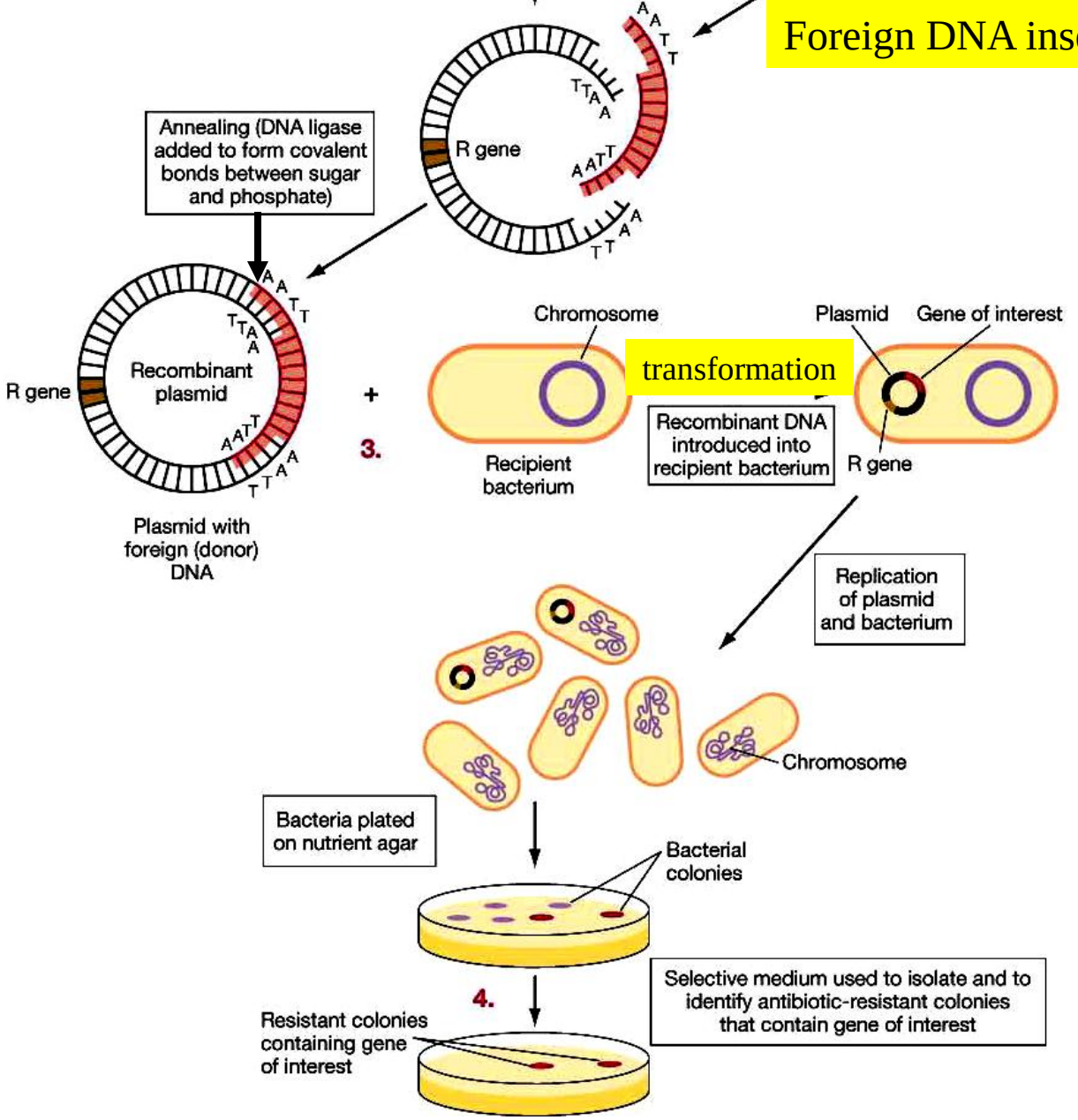
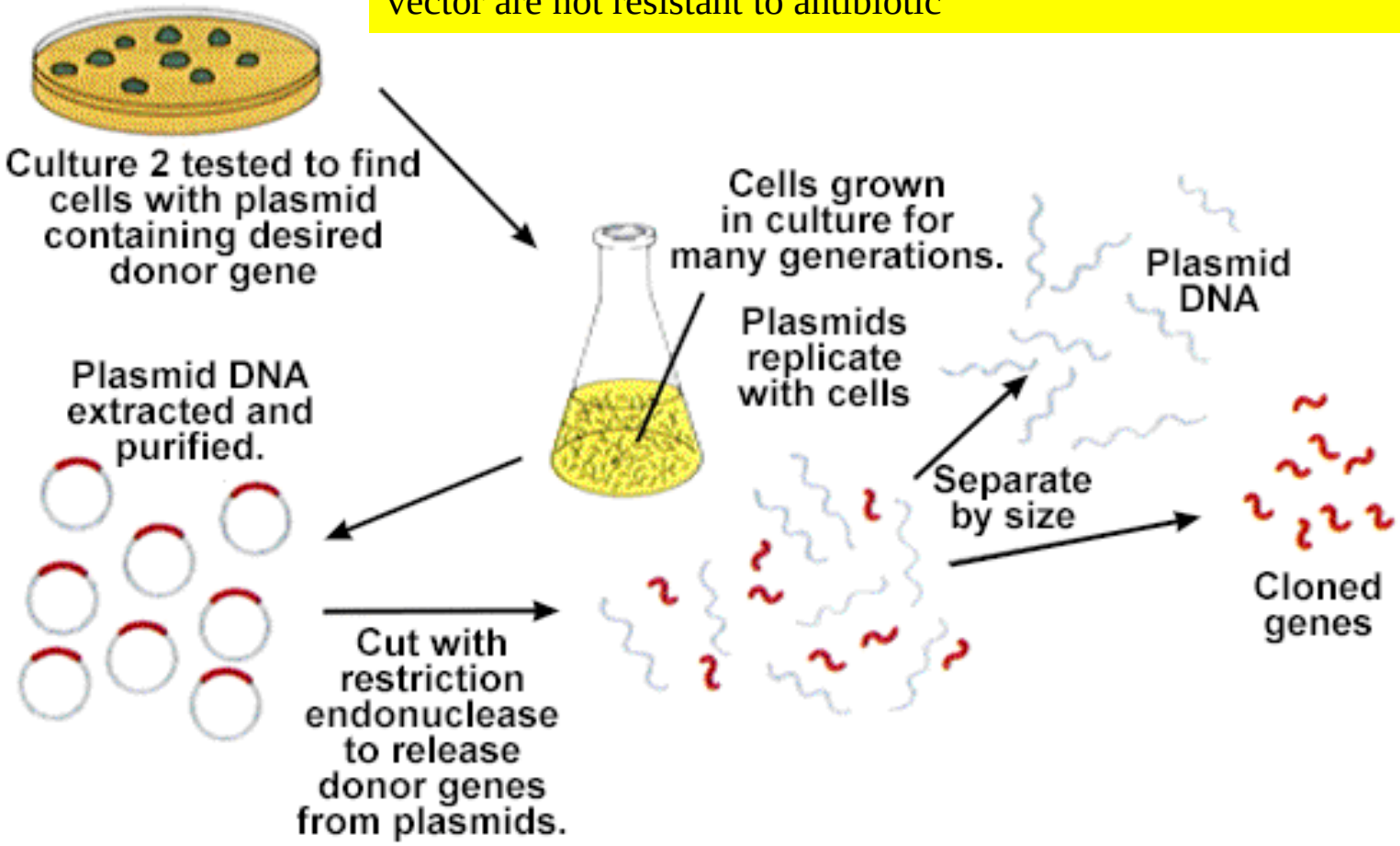


Fig. 8.15 Method for amplifying and obtaining genes from bacteria with plasmid

presence of antibiotic resistance in cloning vector allows for selection on agar containing that antibiotic. Cells that do not contain the cloning vector are not resistant to antibiotic



Method for amplifying and obtaining genes from bacteria with plasmid (not in your text).