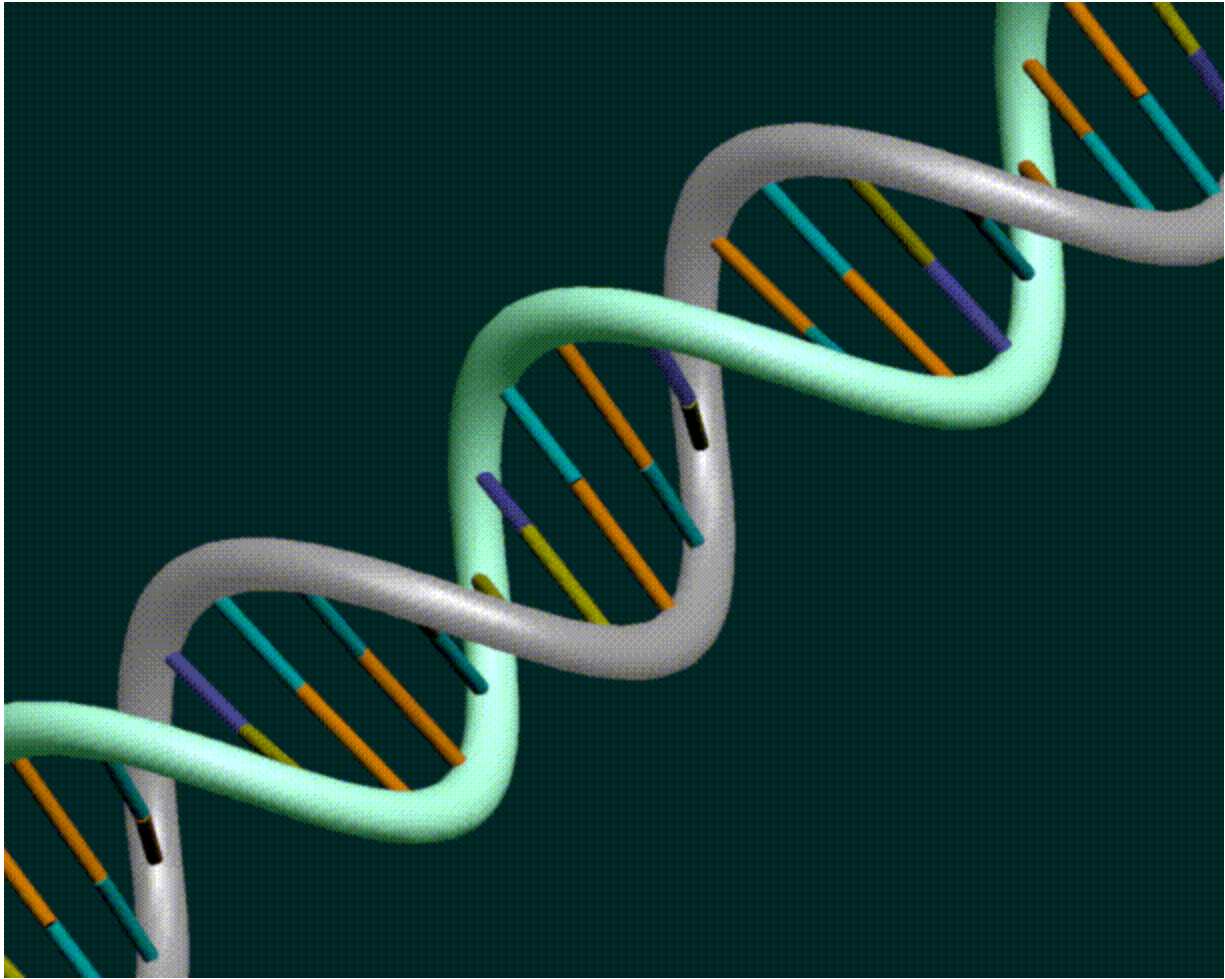
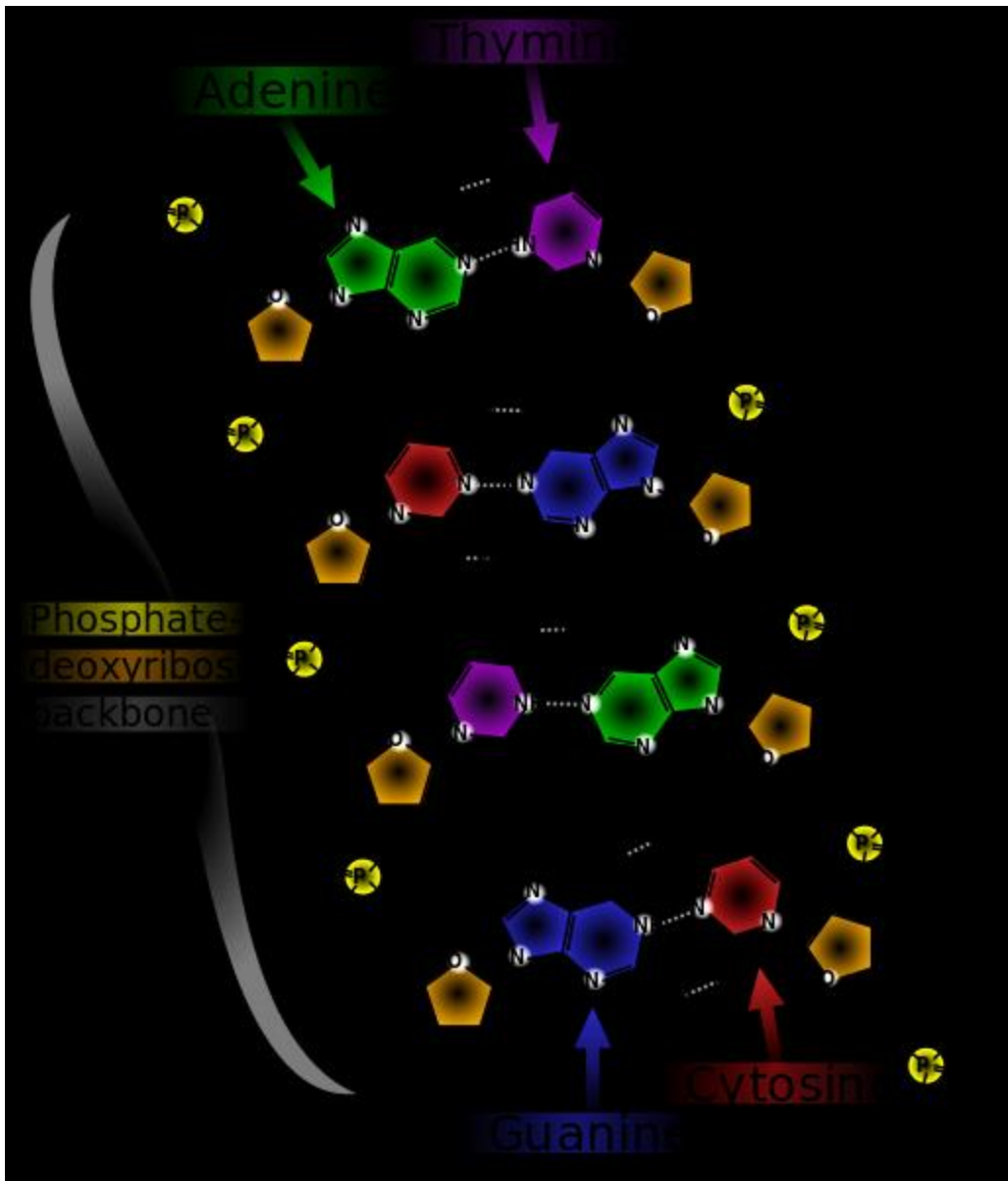
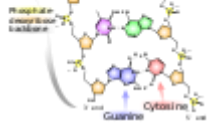


BACTERIAL GENETICS

DNA

- Storehouse of genetic information
- Most wonderful substance on earth.
- Double helix
- Nucleotide chain consists of backbone with alternating deoxyribose and phosphate. Sugar is linked to bases.
- Purine –adenine(A) and guanine(G)
- Pyrimidine –thymine(T) and cytosine(C)





- Codon -triplet of bases that codes for a single amino acid
- More than one triplet may code for the same amino acid.
- UAA , UGA, UAG –nonsense codon
- Segment of DNA carrying codons specifying for a particular polypeptide is called GENE

		Second Base								
		U	C	A	G					
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U	
	UUC		UCC		UAC		UGC		C	
	UUA	Leu	UCA	UAA	Stop	UGA	Stop	A		
	UUG		UCG	UAG	Stop	UGG	Trp	G		
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U	
	CUC		CCC		CAC		CGC		C	
	CUA		CCA		CAA	Gln	CGA		A	
	CUG		CCG		CAG	CGG	G			
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U	
	AUC		ACC		AAC		AGC		C	
	AUA		ACA		AAA	Lys	AGA	Arg	A	
	AUG	Met / Start	ACG		AAG	AGG		G		
G	GUU	Val	GCU	Ala	CAU	Asp	GGU	Gly	U	
	GUC		GCC		GAC		GGC			C
	GUA		GCA		GAA	Glu	GGA			A
	GUG		GCG		GAG		GGG			G

- Genotype -sum total of genetic capacity
- Phenotype –expressed part of the genotype

- Genotypic variation –due to change in the gene structure –heritable ,environment independent,stable
- Phenotypic variation –phenol agar,lactose fermentation - influenced by environment,not inherited,temporary

MUTATION

- Random, heritable, undirected variation due to a change in the nucleotide sequence of DNA
- Addition, deletion or substitution of a base pair
- Spontaneous mutation
- Induced mutation -mutagens
- Lethal mutation

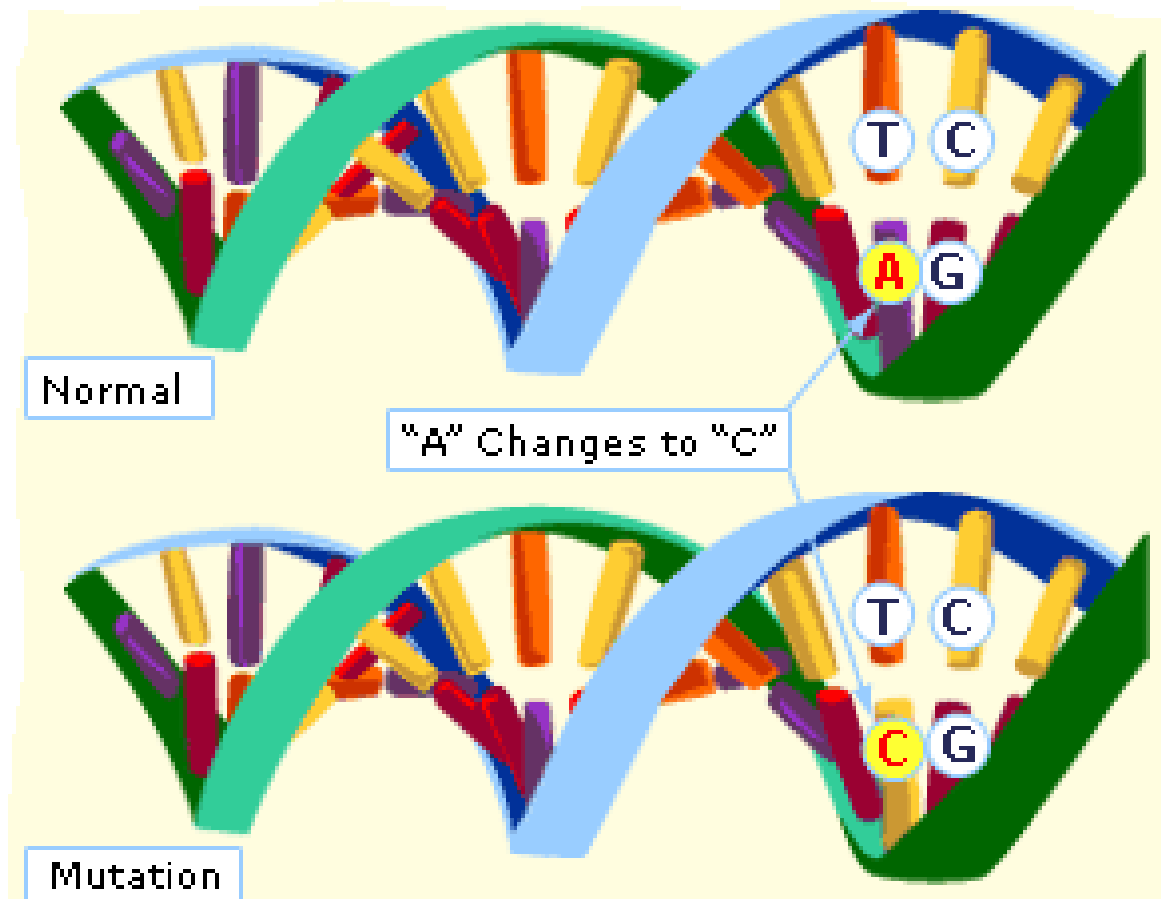
Silent Mutations

ATG	GAA	GCA	CGT
Met	Glu	Ala	Gly



ATG	GAG	GCA	CGT
Met	Glu	Ala	Gly

Point Mutation



Frameshift Mutation

ATG	GAA	GCA	CGT
Met	Glu	Ala	Gly

ATG	AAG	CAC	GT
Met	Lys	His	



Gene Transfer Facilitates the Spread of Drug Resistance

Resistant and non-resistant bacteria exist

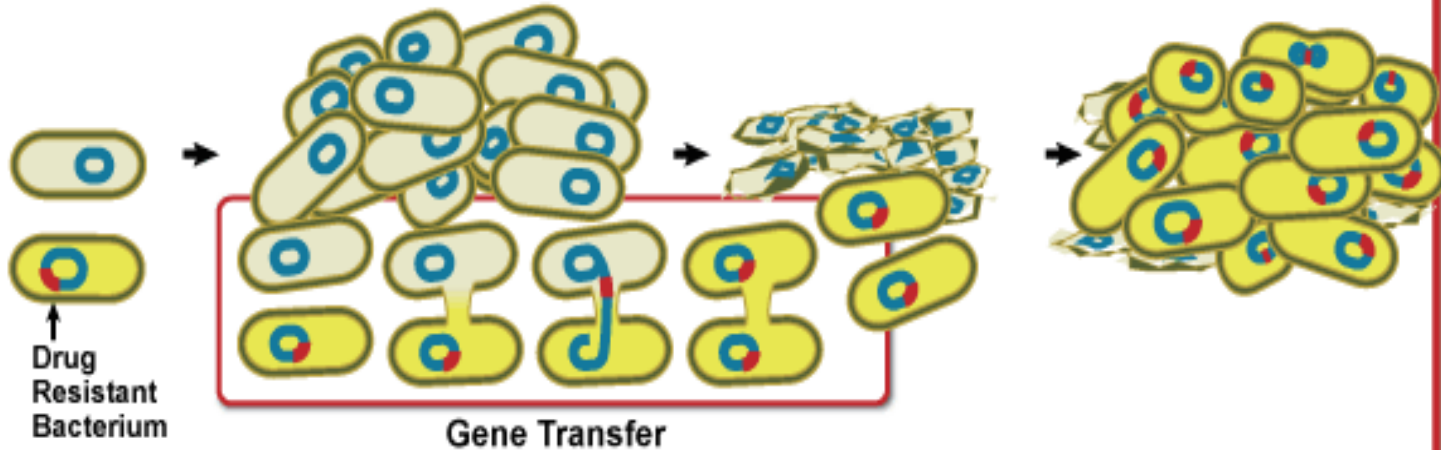
Bacterium multiply by the billions

Non-resistant bacteria receive new DNA.

Drug resistant bacteria multiply and thrive.

Bacteria that have drug resistant DNA may transfer a copy of these genes to other bacteria.

Non-resistant bacteria become resistant. In the presence of drugs, only drug-resistant bacteria survive.



GENE TRANSFER

- Transformation
- Conjugation
- Transduction
- Lysogenic conversion
- Transposition

TRANSFORMATION

- Transfer of genetic information through free DNA
- Griffith in 1928 conducted the first genetic experiment.
- Pneumococci injected to mice
- Capsulated –smooth (S) –virulent
- Noncapsulated-(rough)- avirulent

rough strain
(nonvirulent)



mouse lives

smooth strain
(virulent)



mouse dies

heat-killed
smooth strain

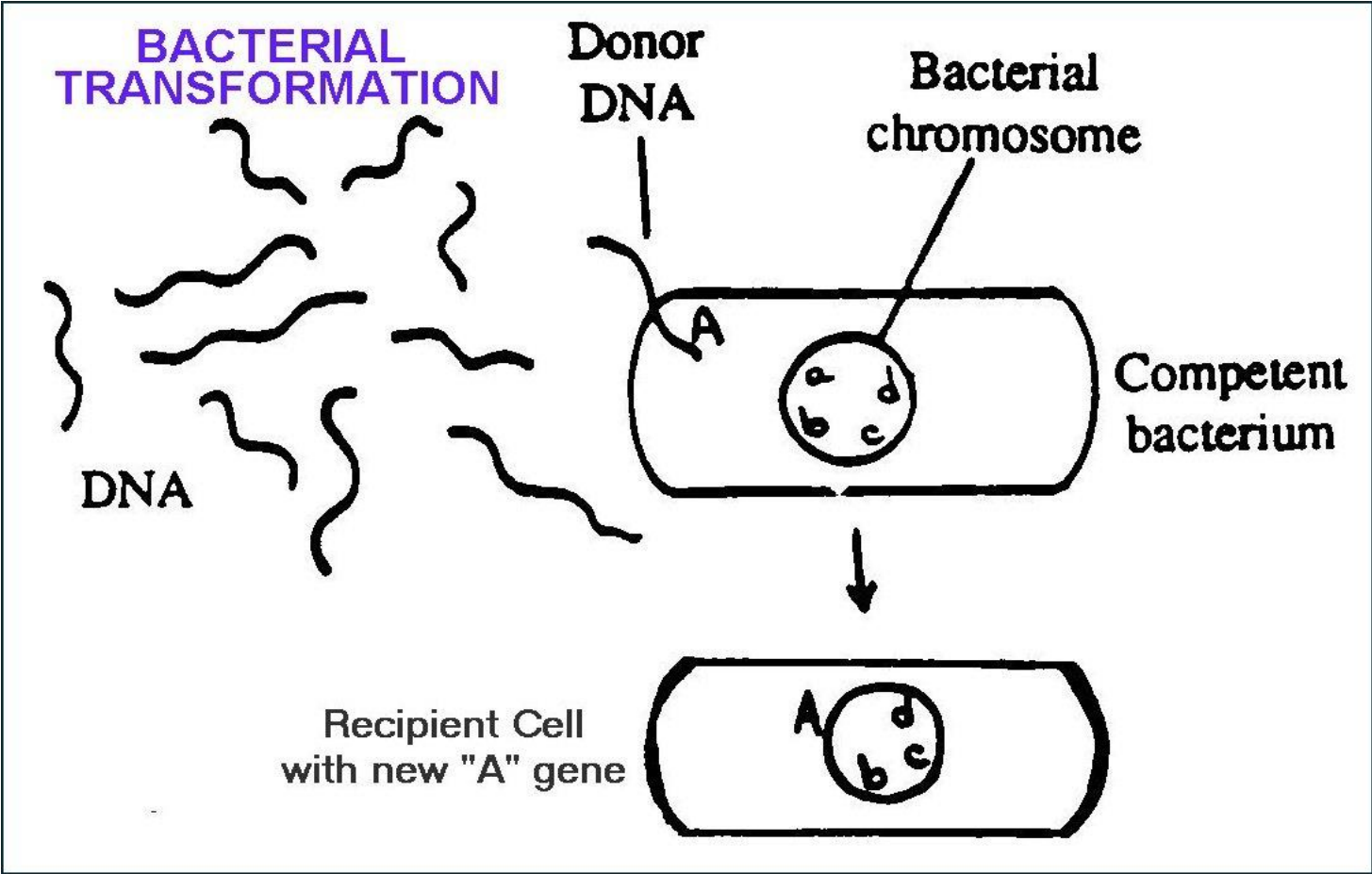


mouse lives

rough strain &
heat-killed
smooth strain



mouse dies

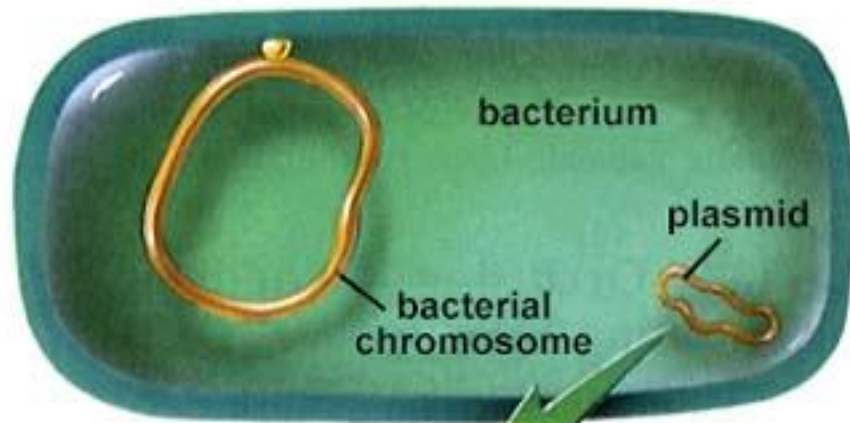


CONJUGATION

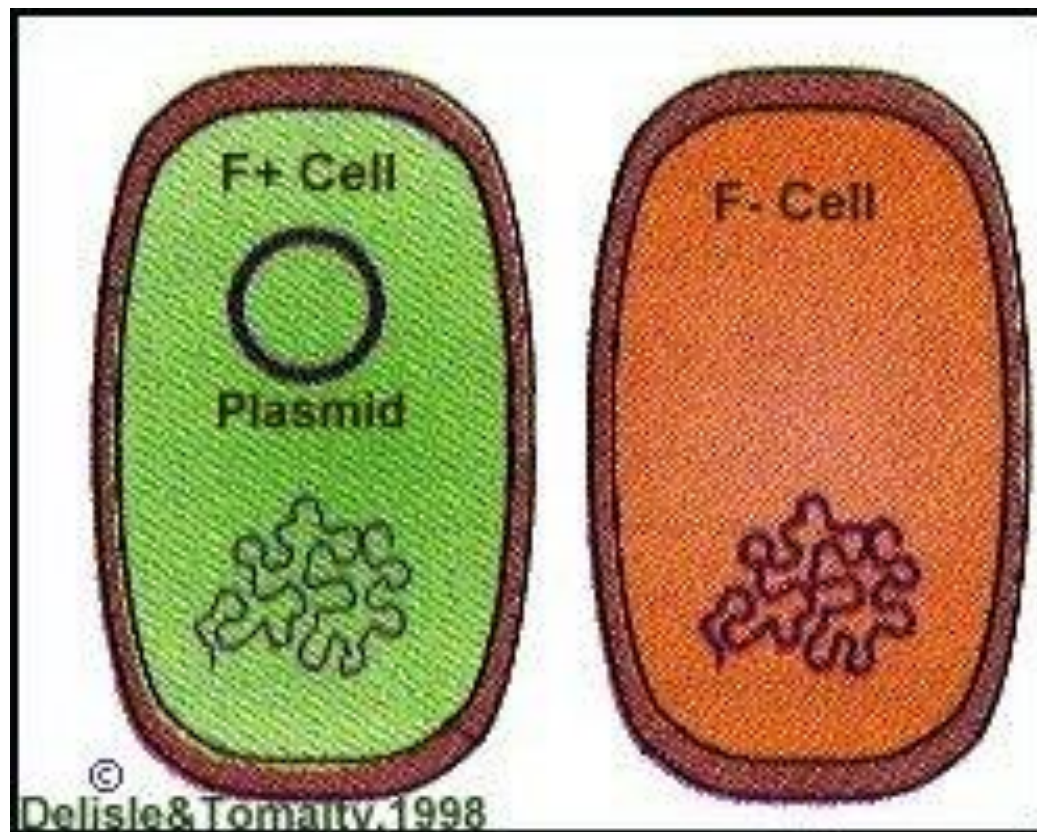
- Genetic material transferred from donor to the recipient by establishing physical contact through tube(in 1946).
- Equivalent to sexual polarity in bacteria.
- Studied in *E. coli* K12 strain.

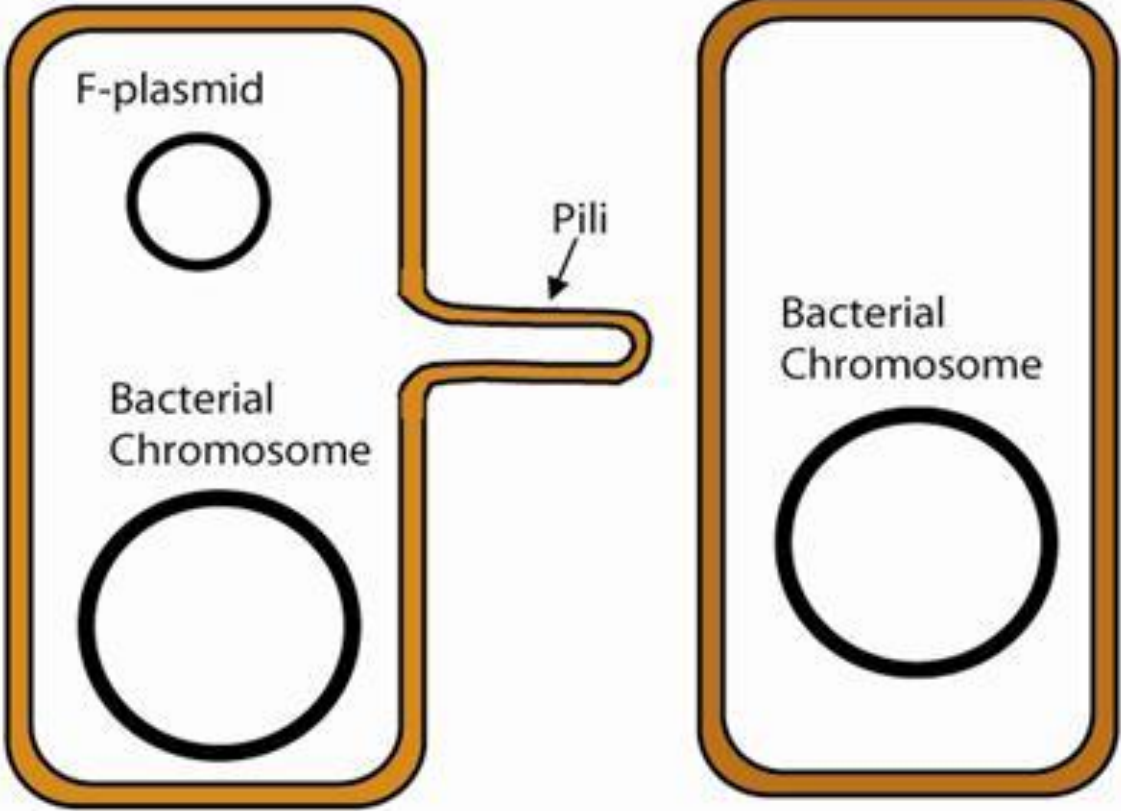
PLASMID

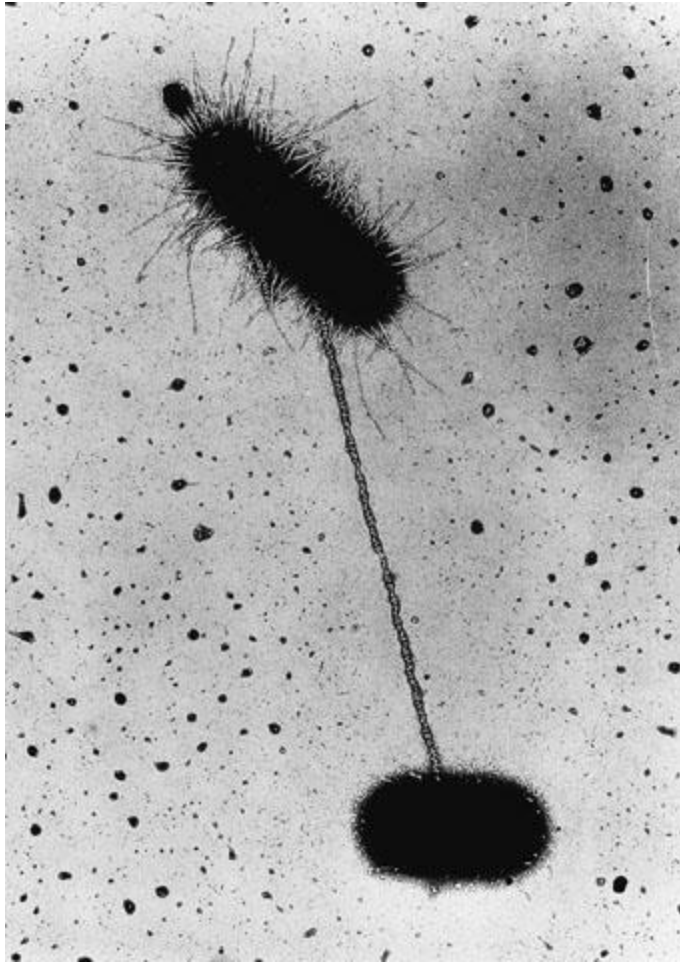
- Extrachromosomal cytoplasmic genetic determinant capable of autonomous replication.
- F factor- fertility factor
- R factor- resistance factor.
- Col factor- colicinogenic factor
- Ent factor- enterotoxigenic factor.

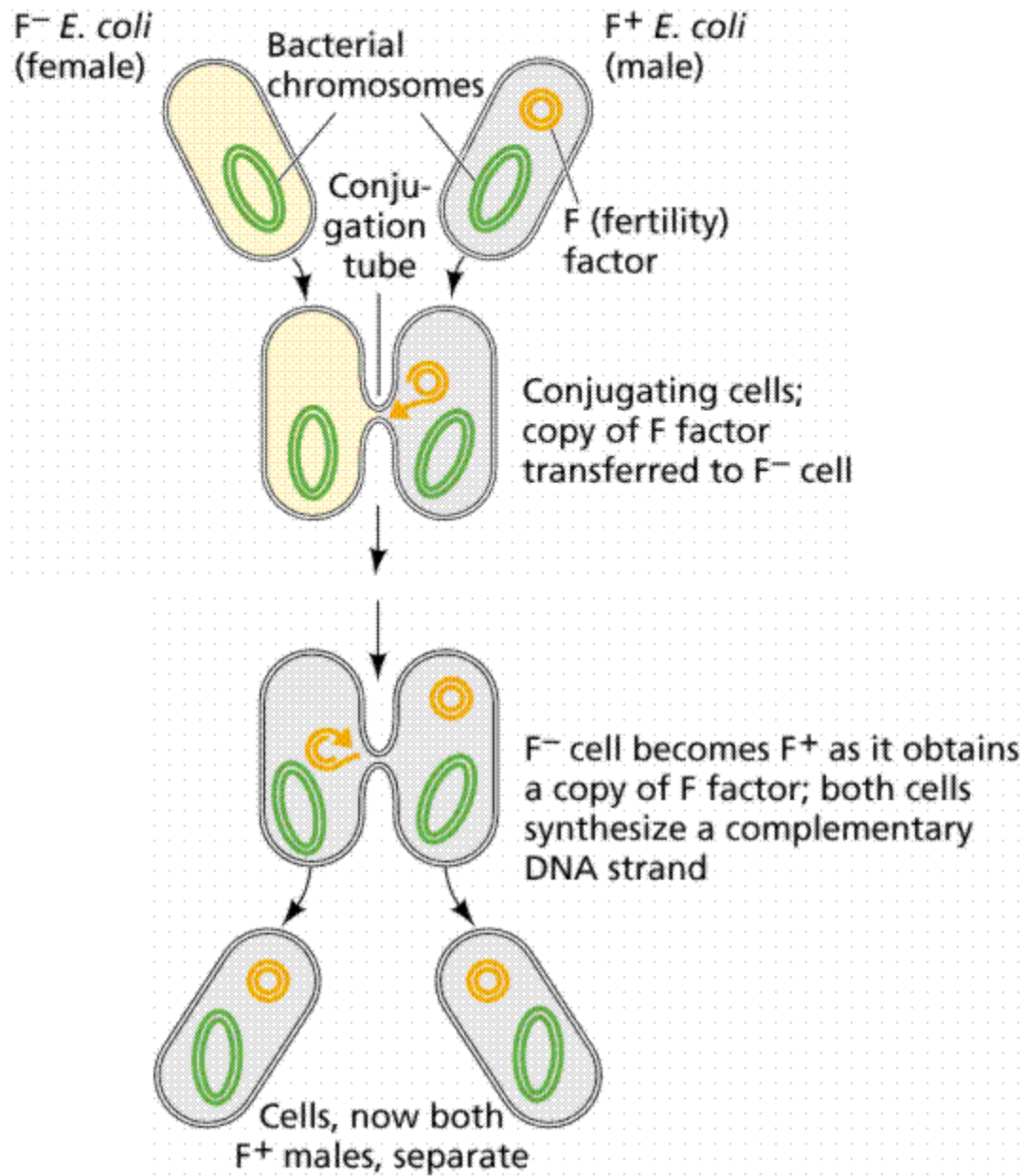


1 μm





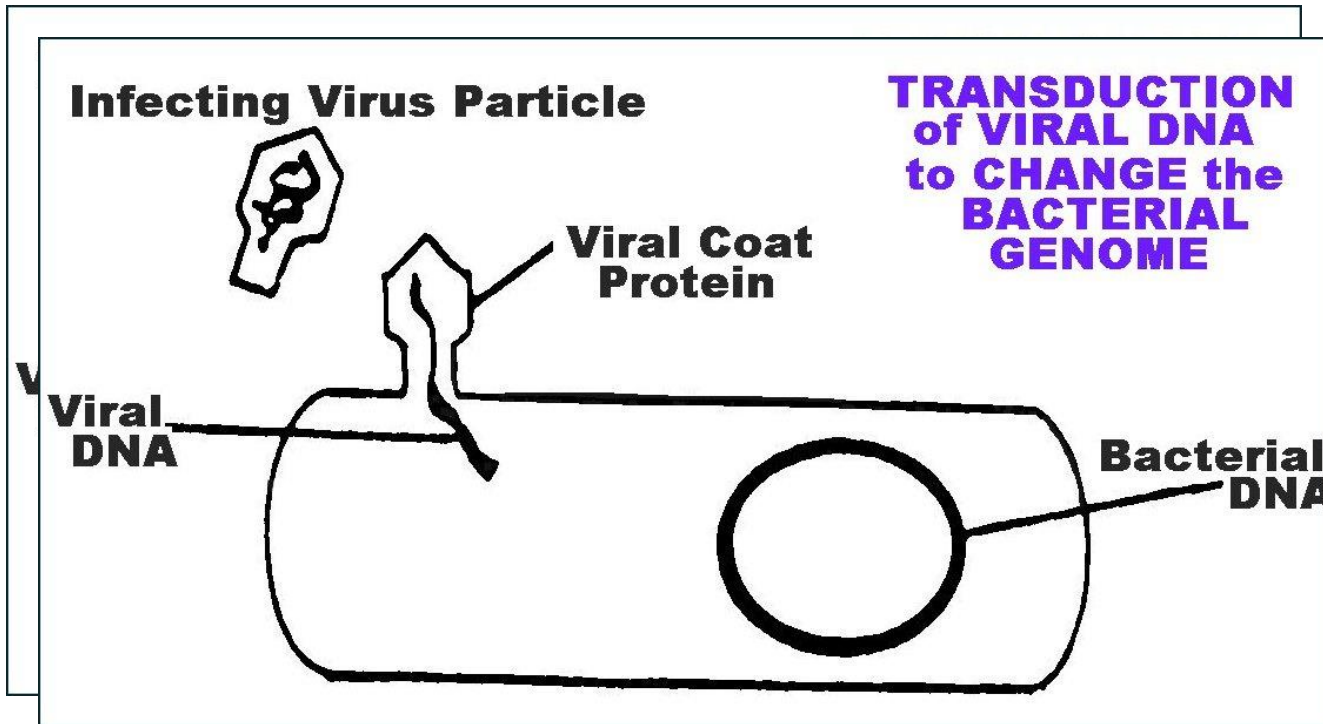


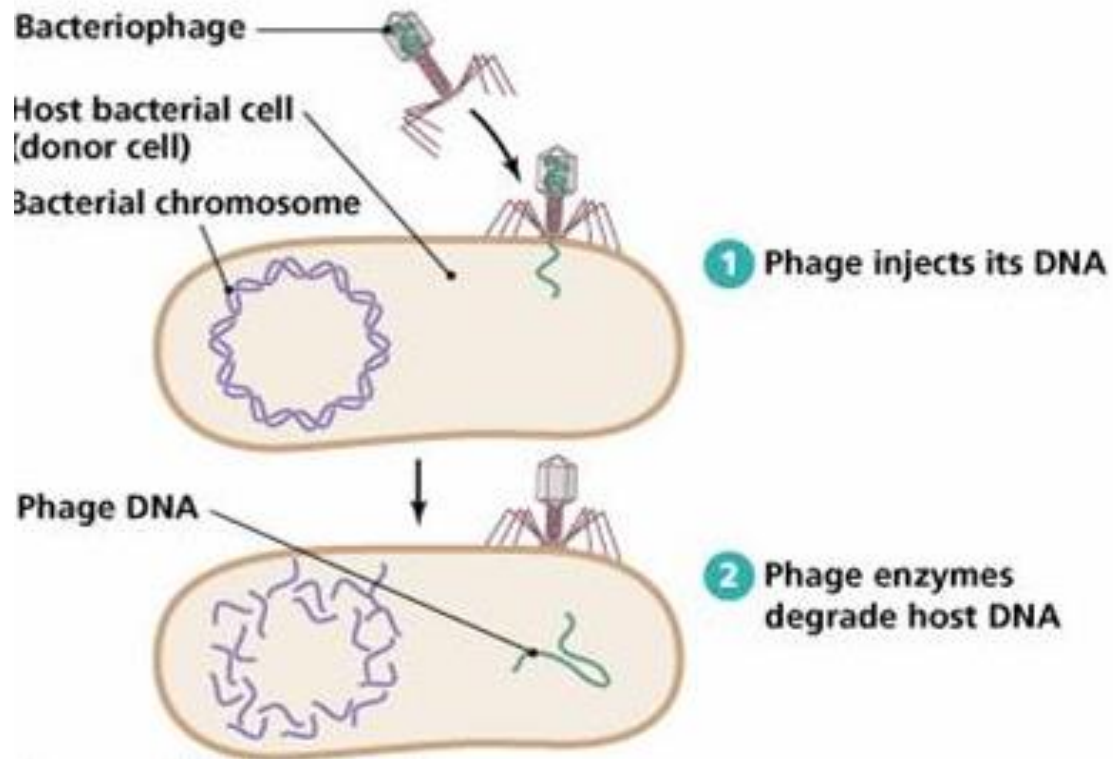


- R factor- resistance factor
- Watanabe –japanese worker observed that *Shigella* that causes bacillary dysentery developed multiple drug resistance at once to many drugs like tetracycline, sulphonamides, streptomycin and chloramphenicol.
- R factor transferred from *E.coli* to *shigella*
- Indiscriminate use of antibiotics in veterinary practice leads to drug resistance.

TRANSDUCTION

- Bacteriophage mediated transfer of genetic material from one bacteria to another.
- Penicillinase production in Staphylococci.
- Ex. Lambda phage in *E.coli* transfers a particular gene.





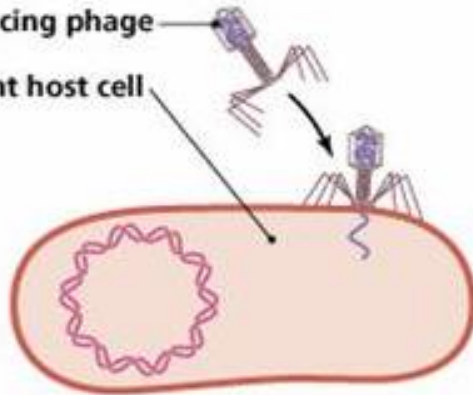
Phage with donor DNA
(transducing phage)



3 Cell synthesizes new phages that incorporate phage DNA and, mistakenly, some host DNA

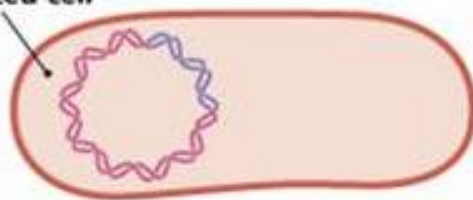
Transducing phage

Recipient host cell



4 Transducing phage injects donor DNA

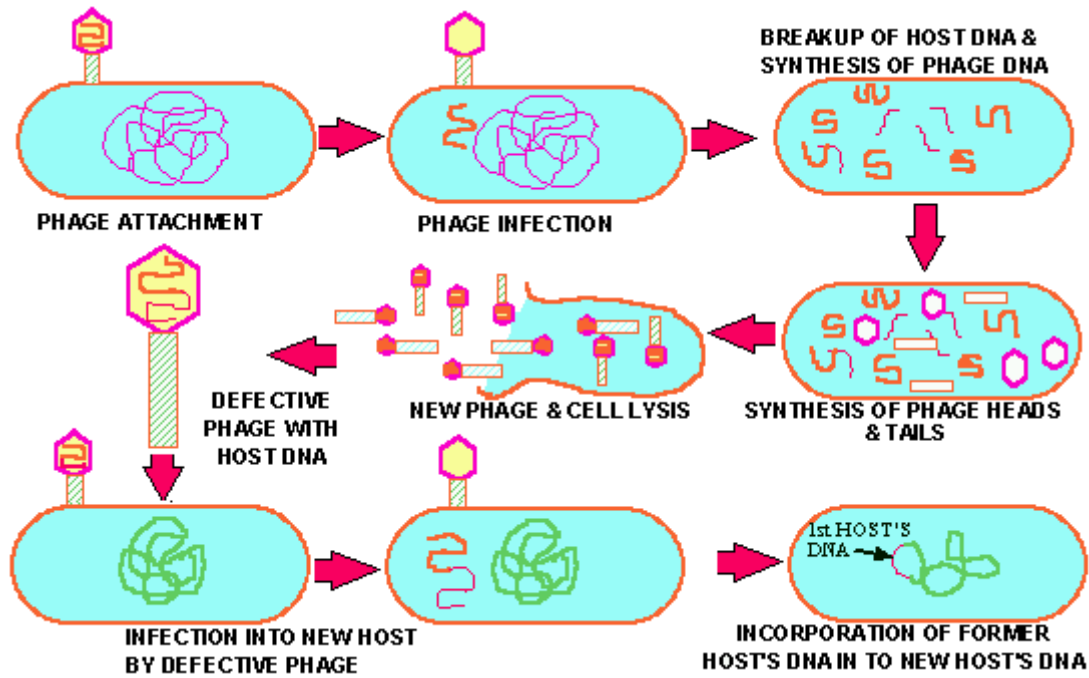
transduced cell



5 Donor DNA is incorporated into recipient's chromosome by recombination

LYSOGENIC CONVERSION

- Lysogenisation- when phage infects bacteria the phage DNA gets incorporated into bacterial chromosome conferring a new property.
- The beta phage in the chromosome of diphtheria bacilli gives the property of toxigenicity.
- Toxigenicity is lost if the phage is removed.



Genetic mechanisms of drug resistance in bacteria.

- Mutation
- Transformation
- Conjugation –R factor
- Transduction

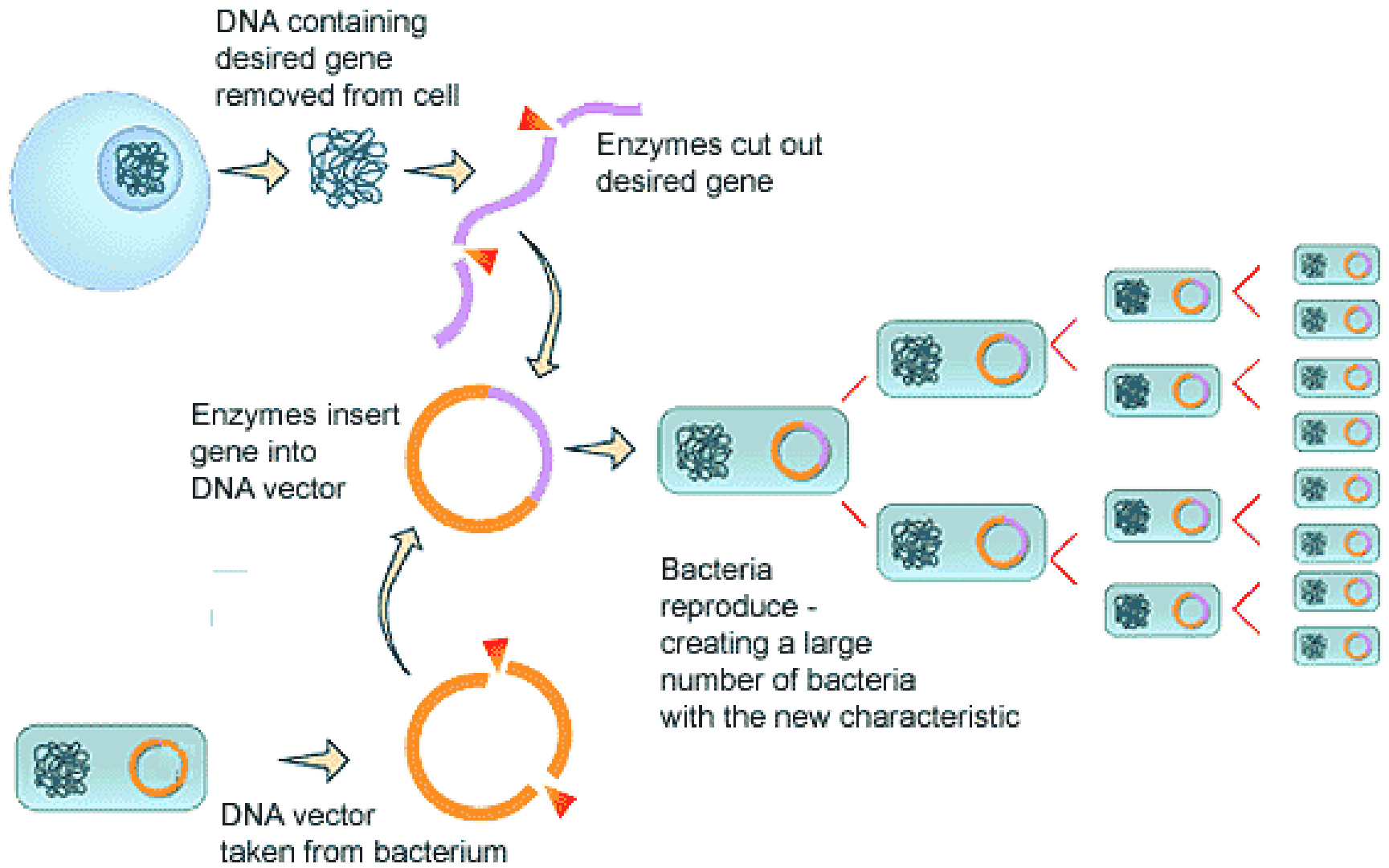
GENETIC ENGINEERING

RECOMBINANT DNA TECHNOLOGY

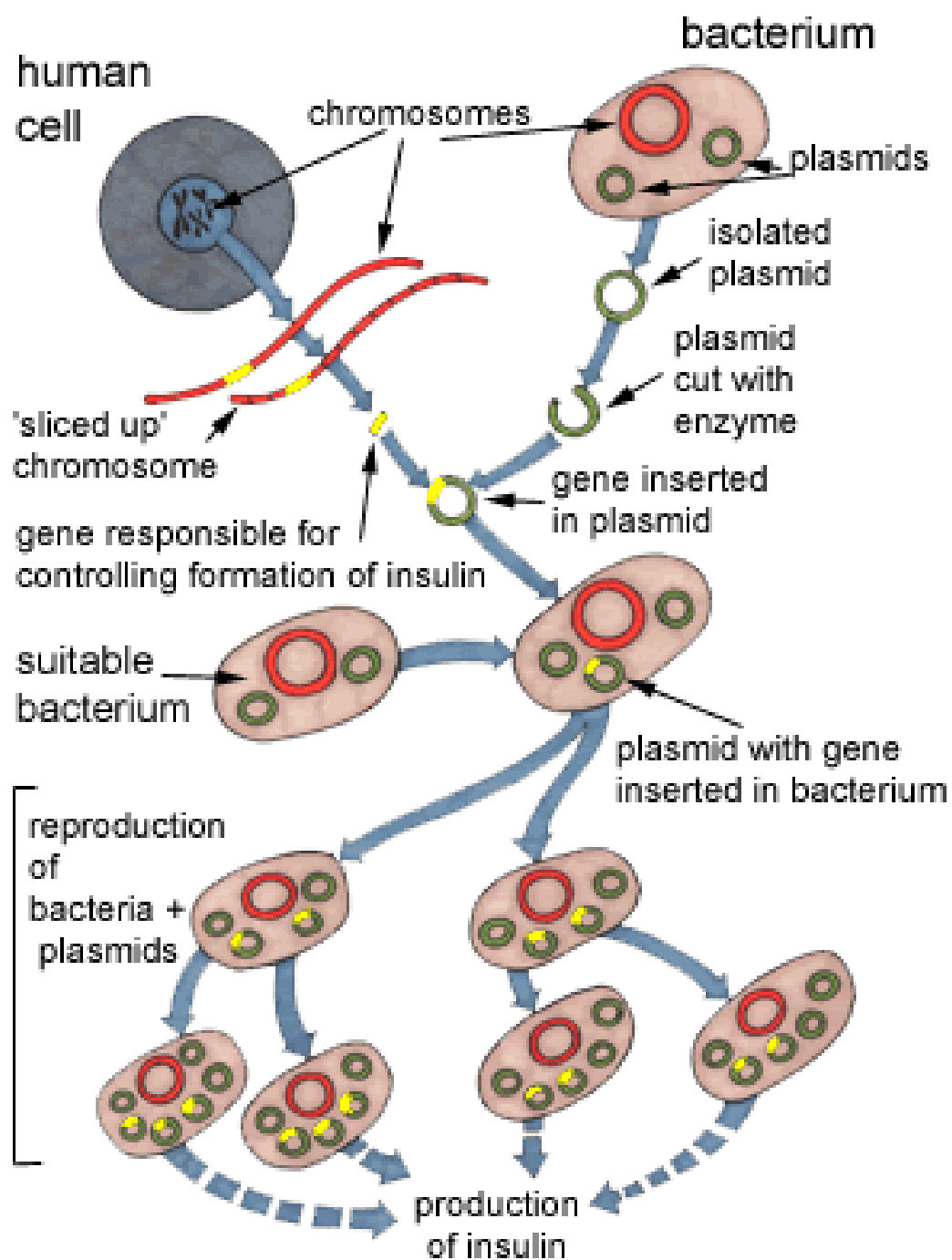
(rDNA)

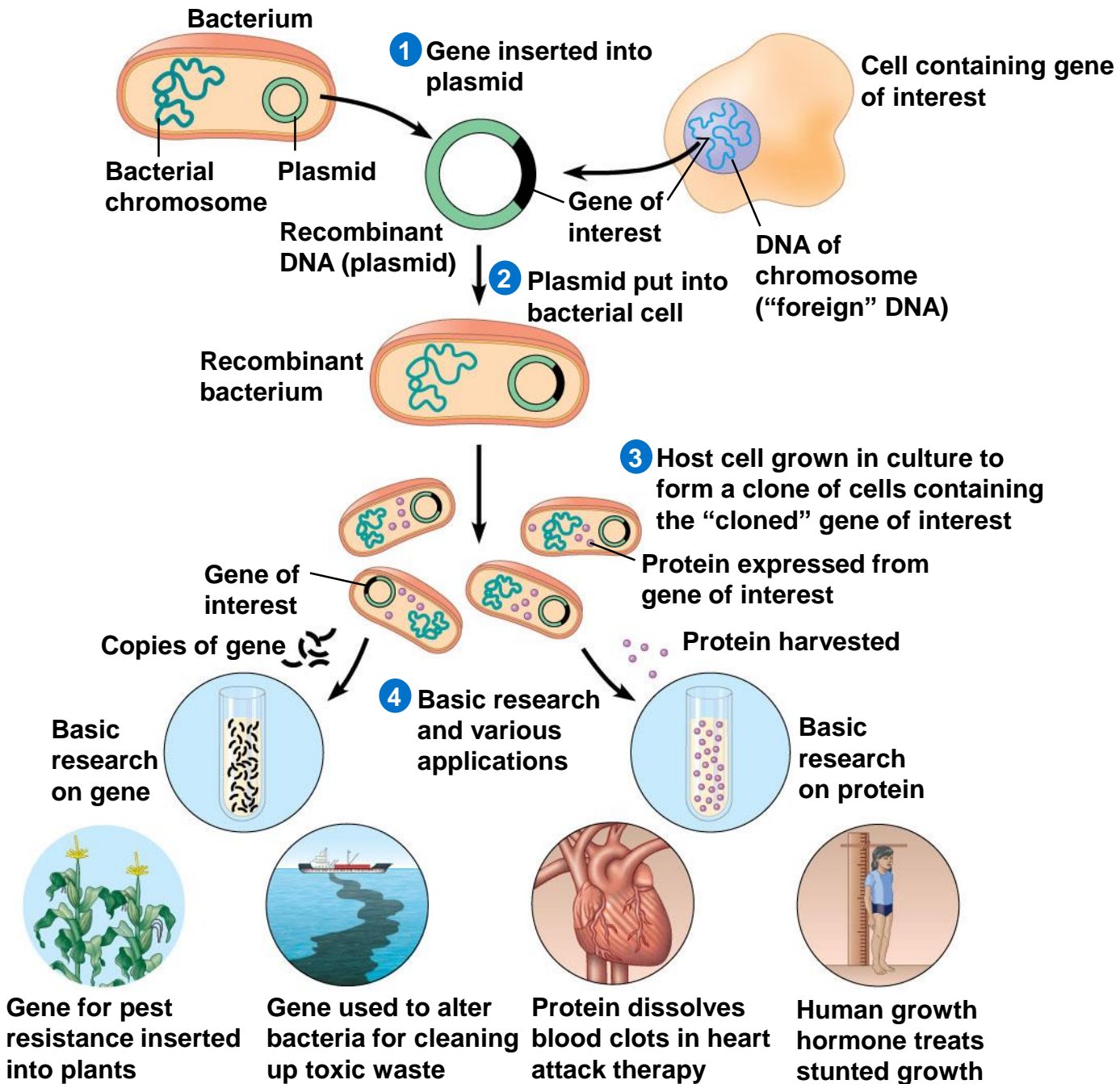


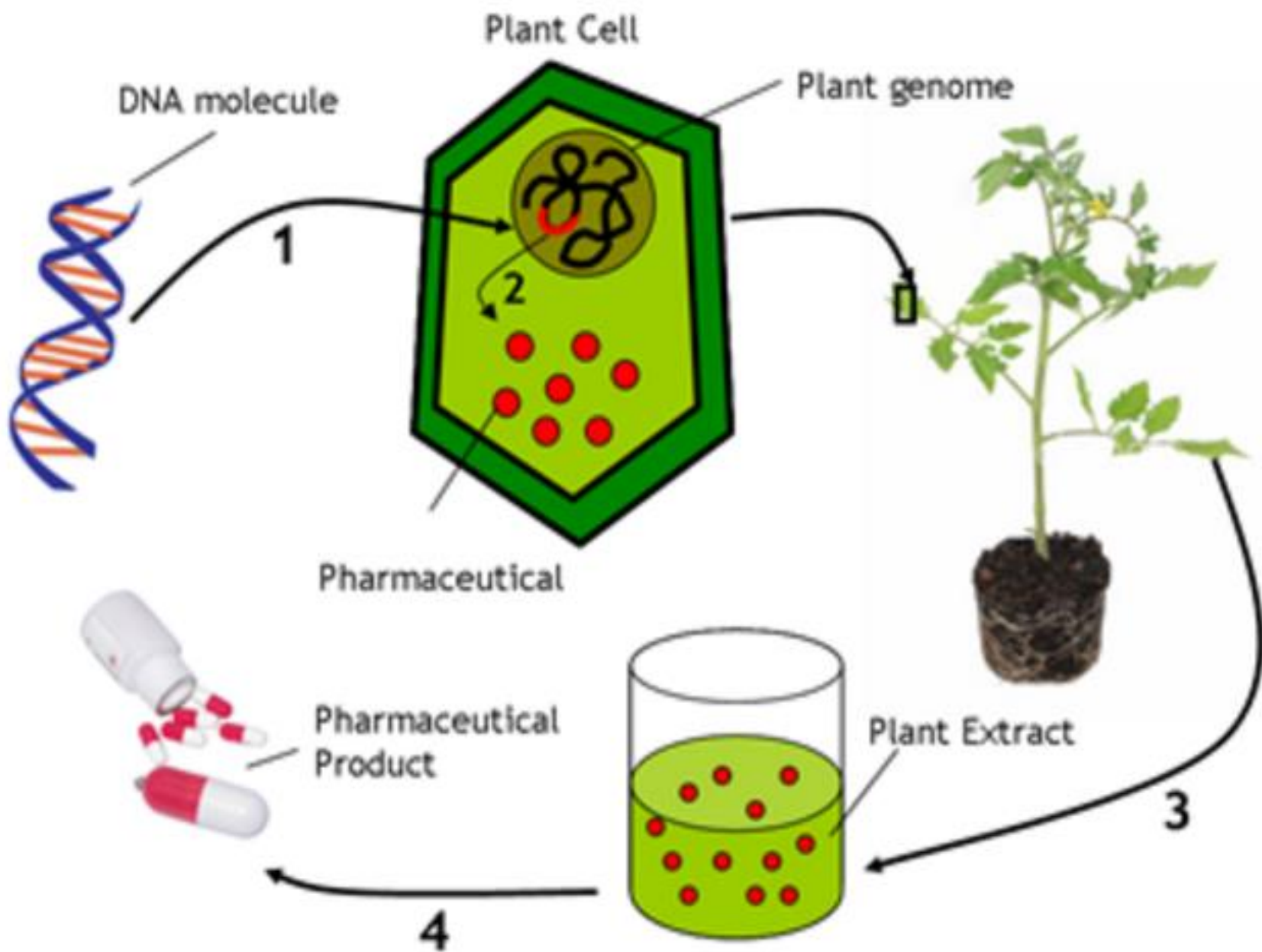
- Artificially introducing a desired gene into the bacteria or yeast
- Isolation of gene of interest, by cutting it at the specific desired sites using molecular scissors called restriction endonucleases.
- Plasmid or phage used as vector to carry the DNA segment.
- Cut and paste technique
- Ligase enzyme used to paste the DNA into the genome of *E.coli*.



- Production of insulin,interferon,interleukin,somatostatin
- Vaccine- hepatitis B vaccine,rabies vaccine
- Gene therapy.
- Restriction enzymes
Eco RI Hind III





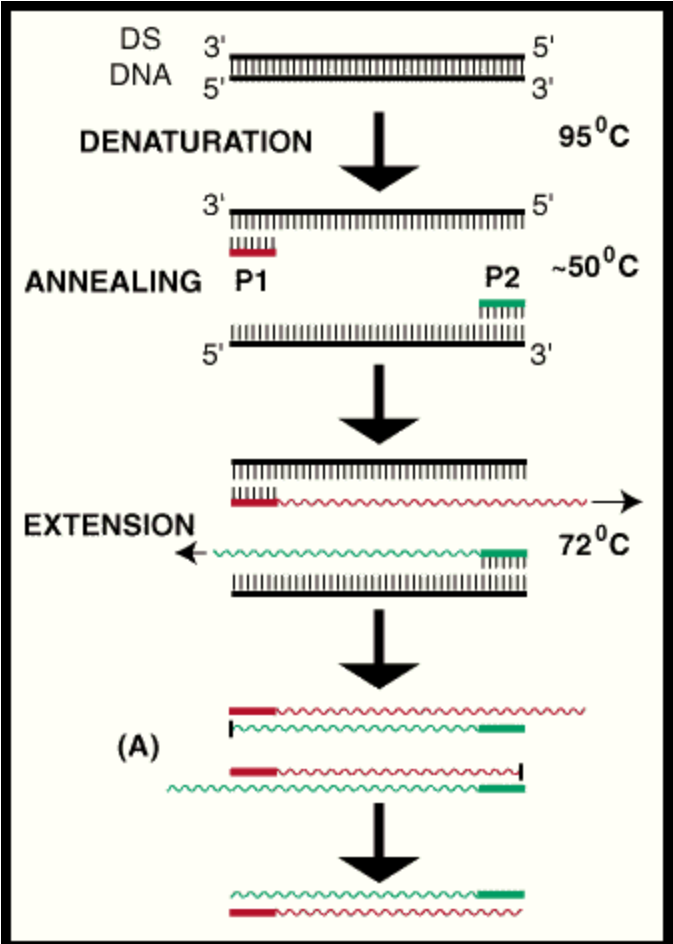


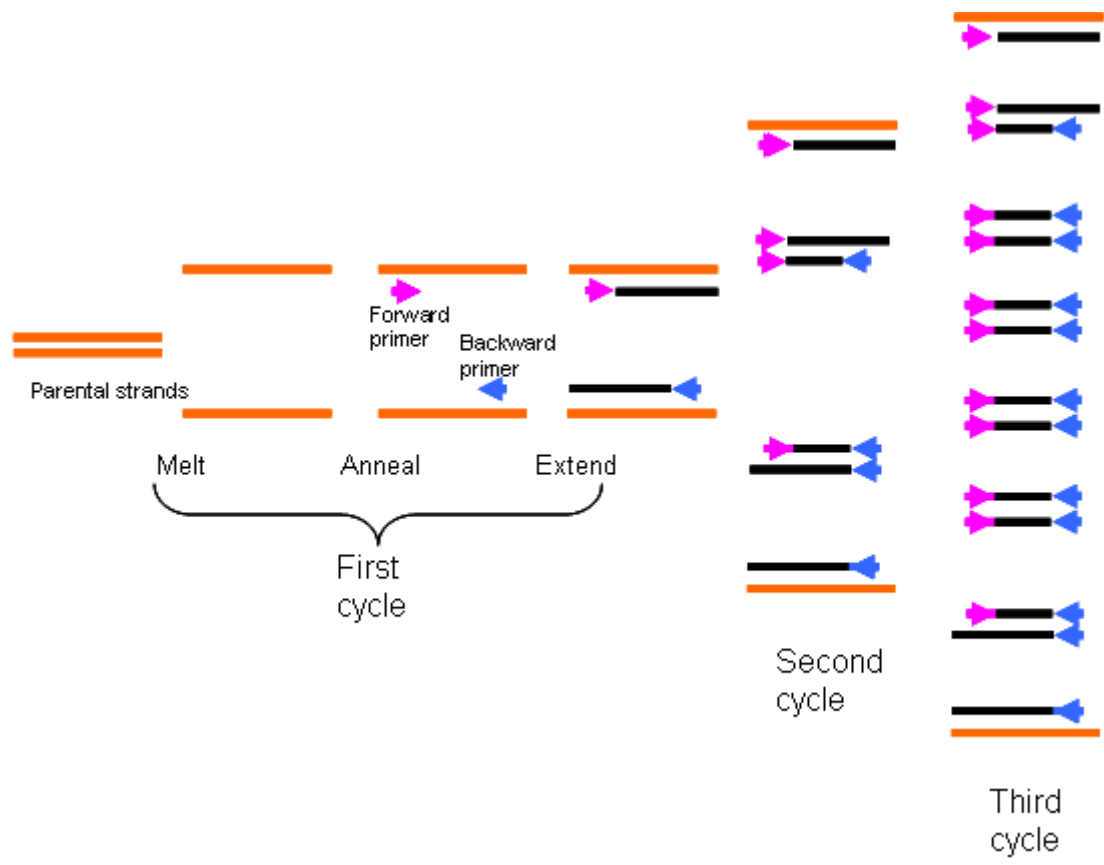
Polymerase Chain Reaction (PCR)

- Amplification of DNA or genes in the lab
- Carried out in thermocyclers
- Each cycle consists of
 - denaturation
 - annealing
 - extension
- After 20-30 cycles millions of copies formed
- Product of one cycle becomes template of next cycle.



PCR machine or thermocycler





- Target DNA identified
- Two oligonucleotide primers complimentary to sequence of target DNA
- Heat stable polymerase Taq 1 used.
- Amplified DNA detected by electrophoresis

- Applications of PCR
- Bacterial disease- tb, H.pylori, chlamydiae, mycoplasma
- Viral disease –hepatitis, HIV, herpes, cytomegalo virus
- Fungal disease –candida, cryptococcus
- Parasitic disease- toxoplasma, trypanosoma, plasmodium.