

Allele Specific PCR

ALLELE SPECIFIC PCR

- Uses primers whose 3' ends encompass the SNP.
- PCR amplification under stringent conditions is much less efficient in the presence of a mismatch between template and primer.

Allele Specific PCR

AMPLIFICATION WITH SNP SPECIFIC PRIMER

- Successful amplification with an SNP-specific primer signals presence of the specific SNP in a sequence.

Allele Specific PCR

ALLELE SPECIFIC PCR

- This diagnostic or cloning technique is used to identify or utilize single-nucleotide polymorphisms (SNPs).

Essentials of Genetics

Lesson No.17

COLONY PCR

Colony PCR

COLONY PCR

- The screening of bacterial or yeast clones for correct ligation or plasmid products.

Colony PCR

COLONY PCR METHODOLOGY

- The screening of bacterial or yeast clones for correct ligation or plasmid products.
- Pick a bacterial colony with an autoclaved toothpick, swirl it into 25 μ l of TE autoclaved dH₂O in an microfuge tube.
- Heat the mix in a boiling water bath (90-100°C) for 2 minutes
- Spin sample for 2 minutes high speed in centrifuge.

Colony PCR

COLONY PCR

- Transfer 20 μ l of the supernatant into a new microfuge tube.
- Take 1-2 μ l of the supernatant as template in a 25 μ l PCR standard PCR reaction.

Colony PCR

COLONY PCR

- The screening of bacterial or yeast clones for correct ligation or plasmid products.

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IN SITU PCR

In Situ PCR

IN SITU PCR

- In Situ PCR (ISH) is a polymerase chain reaction that actually takes place inside the cell on a slide.
- In situ PCR amplification can be performed on fixed tissue or cells .

In Situ PCR

IN SITU PCR

- Applies the methodology of hybridization of the nucleic acids.
- Allows identification of cellular markers
- Limited to detection of non-genomic material such as RNA.

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INVERSE PCR

Inverse PCR

INVERSE PCR

- Inverse PCR uses standard PCR primers oriented in the reverse direction of the usual orientation.

Inverse PCR

INVERSE PCR

- The template for the reverse primers is a restriction fragment that has been self-ligated
- Inverse PCR functions to clone sequences flanking a known sequence.
- Flanking DNA sequences are digested and then ligated to generate circular DNA.

Inverse PCR

IDENTIFICATION OF SEQUENCES FLANKING TRANSPOSABLE ELEMENTS

- Amplification and identification of sequences flanking transposable elements, and the identification of genomic inserts.

Inverse PCR

INVERSE PCR

- A method used to allow PCR when only one internal sequence is known.
- Especially useful in identifying flanking sequences to various genomic inserts.

Essentials of Genetics

Lesson No.20

AFLP PCR

AFLP PCR

AFLP PCR - METHODOLOGY

- Genomic DNA is digested with one or more restriction enzymes. tetracutter (MseI) and a hexacutter (EcoRI).
- Ligation of linkers to all restriction fragments. Pre-selective PCR is performed using primers which match the linkers and restriction site specific sequences.
- Electrophoretic separation and amplicons on a gel matrix, followed by visualization of the band pattern.

AFLP PCR

AFLP PCR

- AFLP is a highly sensitive PCR-based method for detecting polymorphisms in DNA.
- AFLP can be also used for genotyping individuals for a large number of loci.

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**BIO203 Complete MERGED
PPT Slides 1 TO 66**

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Merged File date 2021

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ASSEMBLY PCR

Assembly PCR

ASSEMBLY PCR

- Assembly PCR used to assemble two or more pieces of DNA into one piece.

Assembly PCR

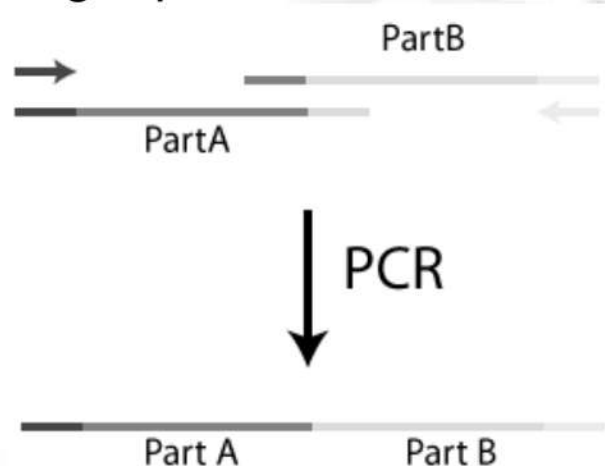
ASSEMBLY PCR - PRINCIPLE

- Assembly PCR is the synthesis of long DNA structures by performing PCR on a pool of long oligonucleotides with short overlapping segments, to assemble two or more pieces of DNA into one piece.

Assembly PCR

ASSEMBLY PCR - METHODOLOGY

- It involves an initial PCR with primers that have an overlap and a second PCR using the products as the template that generates the final full-length product.



Assembly PCR

ASSEMBLY PCR

- Assembly PCR used to assemble two or more pieces of DNA into one piece.

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SUICIDE PCR

Suicide PCR

SUICIDE PCR - PRINCIPLE

- Suicide PCR is typically used in paleogenetics or other studies where avoiding false positives and ensuring the specificity of the amplified fragment is the highest priority.
- The method prescribes the use of any primer combination only once in a PCR, which should never have been used in any positive control PCR reaction.

Suicide PCR

SUICIDE PCR

- Primers should always target a genomic region never amplified before in the lab using this or any other set of primers.
- This ensures that no contaminating DNA from previous PCR reactions is present in the lab, which could otherwise generate false positives.

Suicide PCR

SUICIDE PCR

- Use of any primers combination only once in a PCR.

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Lesson No.23

METHYLATION SPECIFIC PCR

Methylation Specific PCR

METHYLATION SPECIFIC PCR

- Methylation-specific PCR is used to identify patterns of DNA methylation at CpG islands in genomic DNA.

Methylation Specific PCR

METHYLATION SPECIFIC PCR

- Target DNA is first treated with sodium bisulfite, which converts unmethylated cytosine bases to uracil, which is complementary to adenosine in PCR primers.
- Two amplifications are then carried out on the bisulfite-treated DNA: One primer set anneals to DNA with cytosines (corresponding to methylated cytosine), and the other set anneals to DNA with uracil (corresponding to unmethylated cytosine).

Methylation Specific PCR

MSP

- MSP used in quantitative PCR provides information about methylation state of a given CpG island.

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Lesson No.24

INTER SEQUENCE SPECIFIC PCR

InterSequence Specific PCR

INTER SEQUENCE SPECIFIC PCR

- A method for DNA fingerprinting that uses primers selected from segments repeated throughout a genome to produce a unique fingerprint of amplified product lengths.
- The use of primers from a commonly repeated segment is called Alu-PCR, and can help to amplify sequences adjacent (or between) these repeats.

InterSequence Specific PCR

PCR

- PCR used to produce a unique fingerprints of amplified product lengths.

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LIGATION MEDIATED PCR

Ligation-mediated PCR

METHODOLOGY

- Uses small DNA oligonucleotide 'linkers' (or adaptors) that are first ligated to fragments of the target DNA.
- PCR primers that anneal to the linker sequences are then used to amplify the target fragments.

Ligation-mediated PCR

PCR

- DNA sequencing
- Genome walking
- DNA footprinting

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WHOLE GENOME AMPLIFICATION PCR

Whole Genome Amplification PCR

WHOLE GENOME AMPLIFICATION PCR

- Primers can be designed to be 'degenerate'
 - able to initiate replication from a large number of target locations.
- Whole genome amplification (WGA) is a group of procedures that allow amplification to occur at many locations in a genome.

Whole Genome Amplification PCR

PCR

- Whole genomes can be amplified by WGA.

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MINI-PRIMER PCR

Mini Primer PCR

MINI PRIMER PCR

- Mini Primer PCR uses a thermostable polymerase (S-Tbr) that can extend from short primers as short as 9 or 10 nucleotides.
- This method permits PCR targeting to smaller primer binding regions, and is used to amplify conserved DNA sequences, such as the 16S (or eukaryotic 18S) rRNA gene.

Mini Primer PCR

MINI PRIMER PCR

- PCR that can extend from short primers.

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Lesson No.28

ADVANTAGES AND LIMITATIONS OF PCR

Advantages & Limitations

ADVANTAGES

- Specific
- Simple, rapid, relatively inexpensive
- Amplifies from low quantities
- Works on damaged DNA
- Sensitive
- Flexible

Advantages & Limitations

LIMITATIONS

- Contamination risk
- Primer complexities
- Primer-binding site complexities
- Amplifies rare species
- Detection methods

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Lesson No.29

APPLICATIONS OF PCR

Applications of PCR

APPLICATIONS

- Detection of Infectious diseases: AIDS, TB, CMV, H1N1, etc Viral, Bacterial and fungal infections,
- Diagnosis of latent viruses.
- Forensic applications: DNA finger printing

Applications of PCR

APPLICATIONS

- Detection of Mutations: Inherited disorders & carriers
Track DNA abnormalities
- Prenatal diagnosis of genetic disorders.

Applications of PCR

APPLICATIONS

- Detection of pathogens
Pre-natal diagnosis
DNA fingerprinting
Gene therapy
Mutation screening
- Drug discovery
- Classification of organisms
Genotyping

Applications of PCR

APPLICATIONS

- Molecular Archaeology
- Molecular Epidemiology
- Molecular Ecology
- Bioinformatics
- Genomic cloning
- Site-directed mutagenesis
- Gene expression studies

Applications of PCR

CLINICAL MICROBIOLOGY

- Identification of bacteria:
- Slow growing bacteria
- Bacterial antibiotic resistance genes.
- VIRUS: almost all viruses identification and viral load.

Applications of PCR

CLINICAL MICROBIOLOGY

- FUNGUS:
- Aspergillus spp
- Candida spp
- Plasmodium spp
- Trypanosoma spp
- Leishmania spp
- Babesia spp

Polymerase chain reaction (PCR)

Lesson No.30

Applications of PCR

- PCR has widespread applications in various fields of life sciences including genetic engineering, medical, forensic, agriculture, environment etc.

Polymerase chain reaction (PCR)

PCR-Gene cloning and expression

- PCR has been used in gene cloning and screening of genomic libraries

Polymerase chain reaction (PCR)

Lesson No.31

PCR-Medicine

- PCR has major impact on medicine especially in the field of clinical microbiology or diagnosis
- Molecular tools have also allowed to perform prenatal genetic diagnosis

Polymerase chain reaction (PCR)

Lesson No.32

PCR-Forensic sciences

- Forensic science is the application of scientific procedures to solve criminal and legal matters
- Molecular methods are used to establish the filiations of a person or to obtain evidence from minimal samples of saliva, semen or other tissues

Polymerase chain reaction (PCR)

PCR-DNA profiling

- DNA profiling or DNA fingerprinting is a forensic technique used to identify individuals by characteristics of their DNA

Polymerase chain reaction (PCR)

Lesson No.33

PCR-Agricultural sciences and environment

- PCR has also facilitated research in detection of pathogens in plants, animals and environment

Polymerase chain reaction (PCR)

Lesson No.34

PCR-Molecular paleontology

- Molecular paleontology refers to the recovery and analysis of DNA and protein from ancient human, animal and plant remains

Cutting DNA molecules

Lesson No.35

Types of restriction and modification (R-M) system

- At least four R-M systems are known
- Type I
- Type II
- Type III
- Type IIs

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Cutting DNA molecules

Type I

- Type I systems were the first to be characterized from *E. coli* K12
- The active enzyme consists of two restriction subunit, two modification subunit and one recognition subunit
- Type I systems are of little value for gene manipulation

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Cutting DNA molecules

Type II

- Most of the useful R-M system is Type II
- Type II enzymes recognize a defined sequence and cut within it

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Cutting DNA molecules

Type III

- Type III enzymes have symmetrical recognition sequences but otherwise resemble type I systems and are of little value

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Cutting DNA molecules

Type IIs

- Type IIs systems have similar cofactors and structure to type II but restriction occurs at a distance from recognition site that limits their usefulness

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Cutting DNA molecules

Lesson No.36

Nomenclature

- A suitable system was proposed by Smith and Nathans (1973)
- The species name of host organisms is identified by the first letter of genus and first two letters of specific epithet
- *E. coli* = Eco
- *H. influenzae* = Hin

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Cutting DNA molecules

Nomenclature

- Strain identification is written as *EcoK*
- In case, host strain has several restriction and modification systems, these are identified by roman numerals, for example, in case of *H. influenzae*
- *HindI*, *HindII*, *HindIII* etc

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Cutting DNA molecules

Nomenclature

- All restriction enzymes have general name endonuclease R and modification-methylase M followed by the system name, for example, in case of *H. influenzae*
- R. *HindIII* or M. *HindIII*

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Examples of restriction endonuclease nomenclature

Enzyme	Enzyme source	Recognition sequence
<i>SmaI</i>	<i>Serratia marcescens</i> , 1st enzyme	CCCGGG
<i>HaeIII</i>	<i>Haemophilus aegyptius</i> , 3rd enzyme	GGCC
<i>HindII</i>	<i>H. influenzae</i> , strain d, 2nd enzyme	GTPyPuAC
<i>HindIII</i>	<i>H. influenzae</i> , strain d, 3rd enzyme	AAGCTT
<i>HamHI</i>	<i>Bacillus amyloliquefaciens</i> , strain H, 1st enzyme	GGATCC

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Cutting DNA molecules

Lesson No.37

Target sites

▪ Type II endonucleases recognize and cleave DNA within particular sequences of four to eight nucleotides which have a twofold axis of rotational symmetry i.e. referred as *palindromes*

5'-GAATTC-3'

5'-CTTAAG-3'

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5'-G A A T T C-3'
3'-C T T A A G-5'

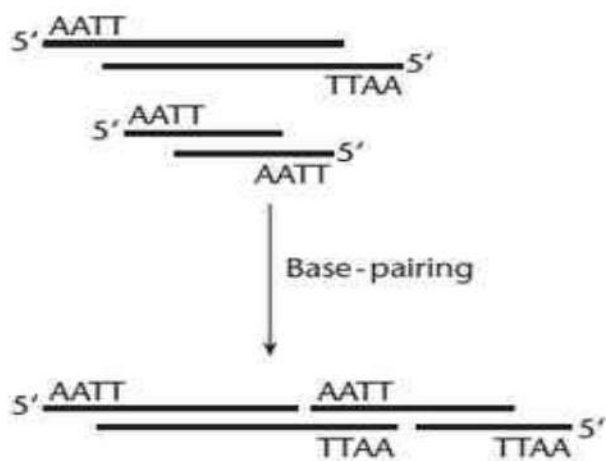
5'-G/ A A* T T C-3'
3'-C T T A* A/ G-5'

5'-G 5'- A A T T C-3'
3'-C T T A A-5' G-5'

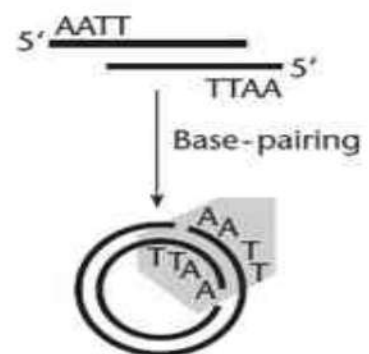
Single stranded breaks by *EcoR*1

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Intermolecular association



Intramolecular association



Cohesive fragments of DNA produced by digestion with *EcoR*1

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Cutting DNA molecules

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Number and size of restriction fragments

- The number and size of fragments generated by restriction enzyme depend on the frequency of occurrence of the target site in the DNA to be cut

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Cutting DNA molecules

Number and size of restriction fragments

- Four base recognition site occurs every 4^4 (256) bp
- Six base recognition site occurs every 4^6 (4096) bp
- Eight base recognition site occurs 4^8 (65,536) bp

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Average fragment size (bp) produced by different enzymes

Enzyme	Target	Arabidopsis	E. Coli	Human
<i>Apal</i>	GGGCCC	25000	15000	2000
<i>Bam</i> HI	GGATCC	6000	5000	5000
<i>Spe</i> I	ACTAGT	8000	60000	10000

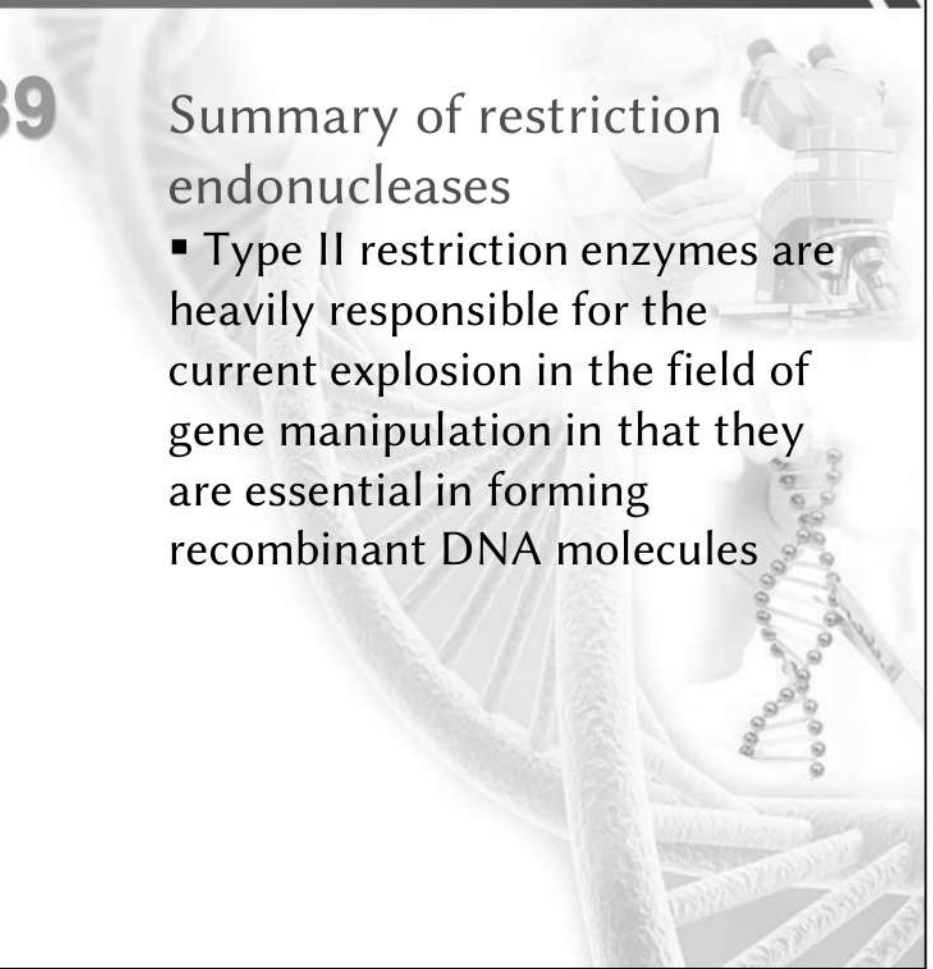
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Cutting DNA molecules

Lesson No.39

Summary of restriction endonucleases

- Type II restriction enzymes are heavily responsible for the current explosion in the field of gene manipulation in that they are essential in forming recombinant DNA molecules



Joining DNA molecules

Lesson No.40

DNA modifying enzymes

- Nucleases
- DNA Polymerase
- Reverse transcriptases
- DNA ligases

Joining DNA molecules

Nucleases

- Nucleases or DNases are the enzymes that degrade DNA
- Two broad classes of nucleases
 - i). Exonucleases
 - ii). Endonucleases

Joining DNA molecules

DNA Polymerase

- An enzyme that catalyzes template-dependent synthesis of DNA

Joining DNA molecules

Reverse transcriptases

- An RNA-directed DNA polymerase in retroviruses; capable of making DNA complementary to an RNA

Joining DNA molecules

DNA ligase

- An enzyme that creates a phosphodiester bond between the 3' end of one DNA segment and the 5' of another

Joining DNA molecules

Lesson No.41

Methods of joining DNA fragments

- Mainly three methods are used for joining DNA in vitro
 - i). Joining covalently annealed cohesive ends by DNA ligase
 - ii). Joining blunt-ended fragments by DNA ligase from phage T4 infected *E. coli*

Joining DNA molecules

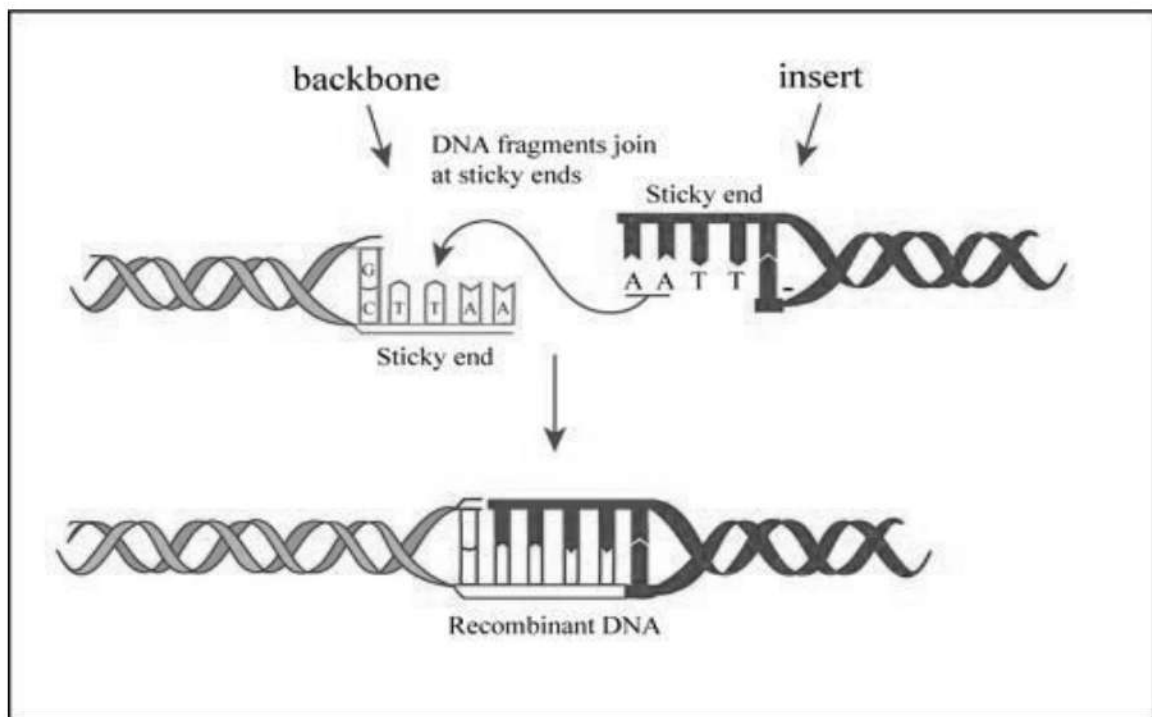
Methods of joining DNA fragments

iii). The third utilizes the enzyme terminal deoxynucleotidyltransferase to synthesize homopolymeric 3' single stranded tails at the ends of fragments

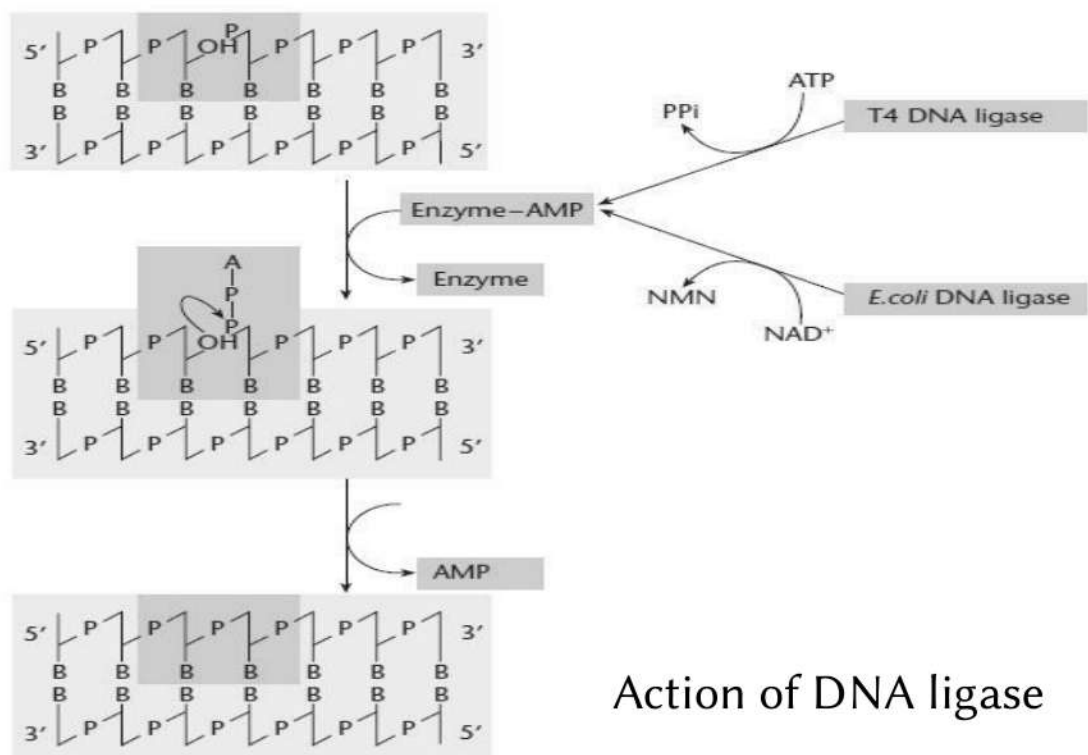
Joining DNA molecules

DNA ligase

- *E. coli* and phage T4 encode enzyme, DNA ligase, which seals single stranded nicks between adjacent nucleotides in a duplex DNA chain



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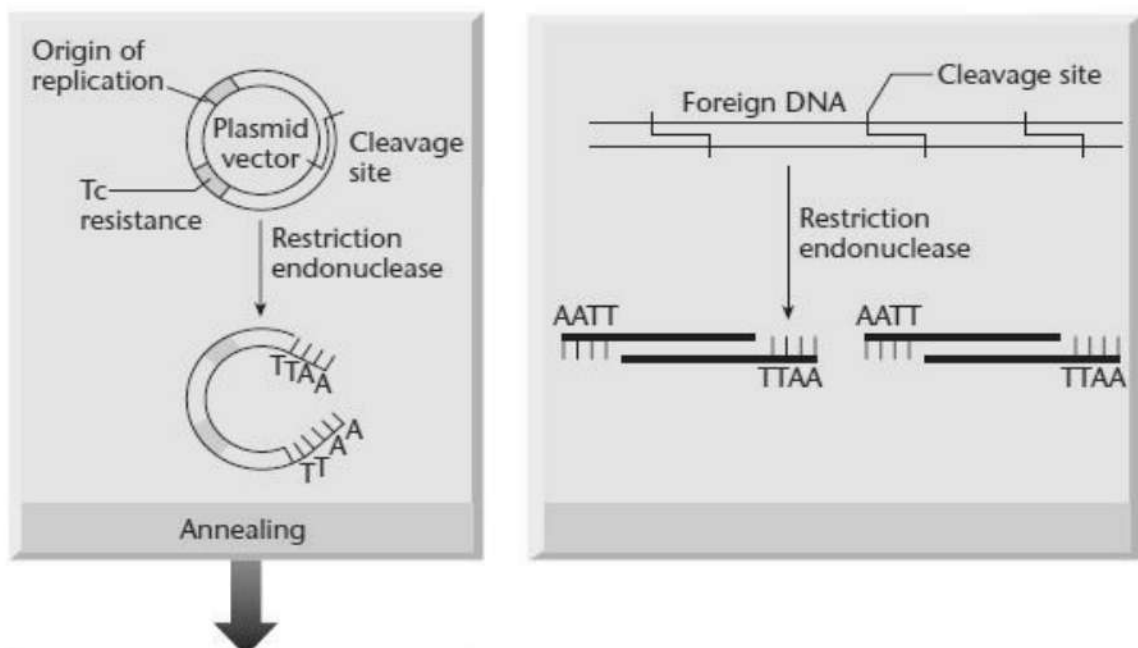
Joining DNA molecules

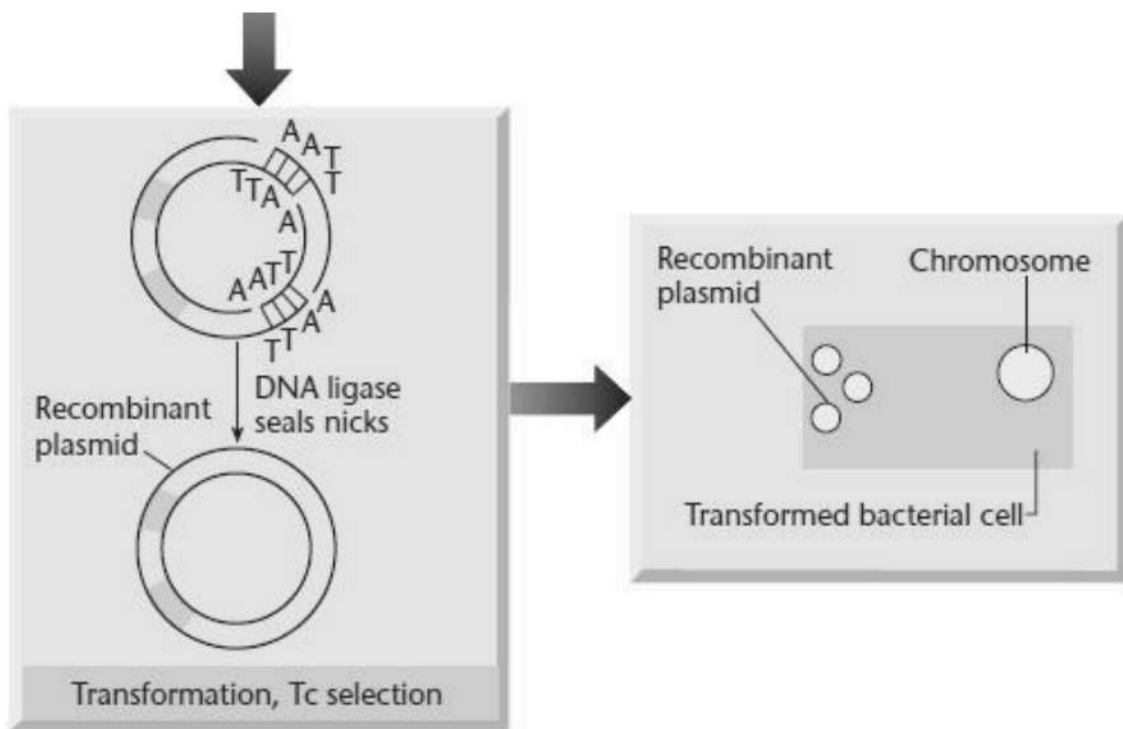
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DNA ligase to create covalent recombinant DNA

- DNA ligase can repair the nicks produced after the association of cohesive ends of DNA strands

Use of DNA ligase to create a covalent DNA recombinant





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Joining DNA molecules

Lesson No.43

Alkaline Phosphatase

- An enzyme responsible for removing phosphate groups from many types of molecules including DNA
- Treatment of linearized plasmid vector DNA with alkaline phosphatase to remove 5'-terminal phosphate groups will prevent recircularization and plasmid dimer formation