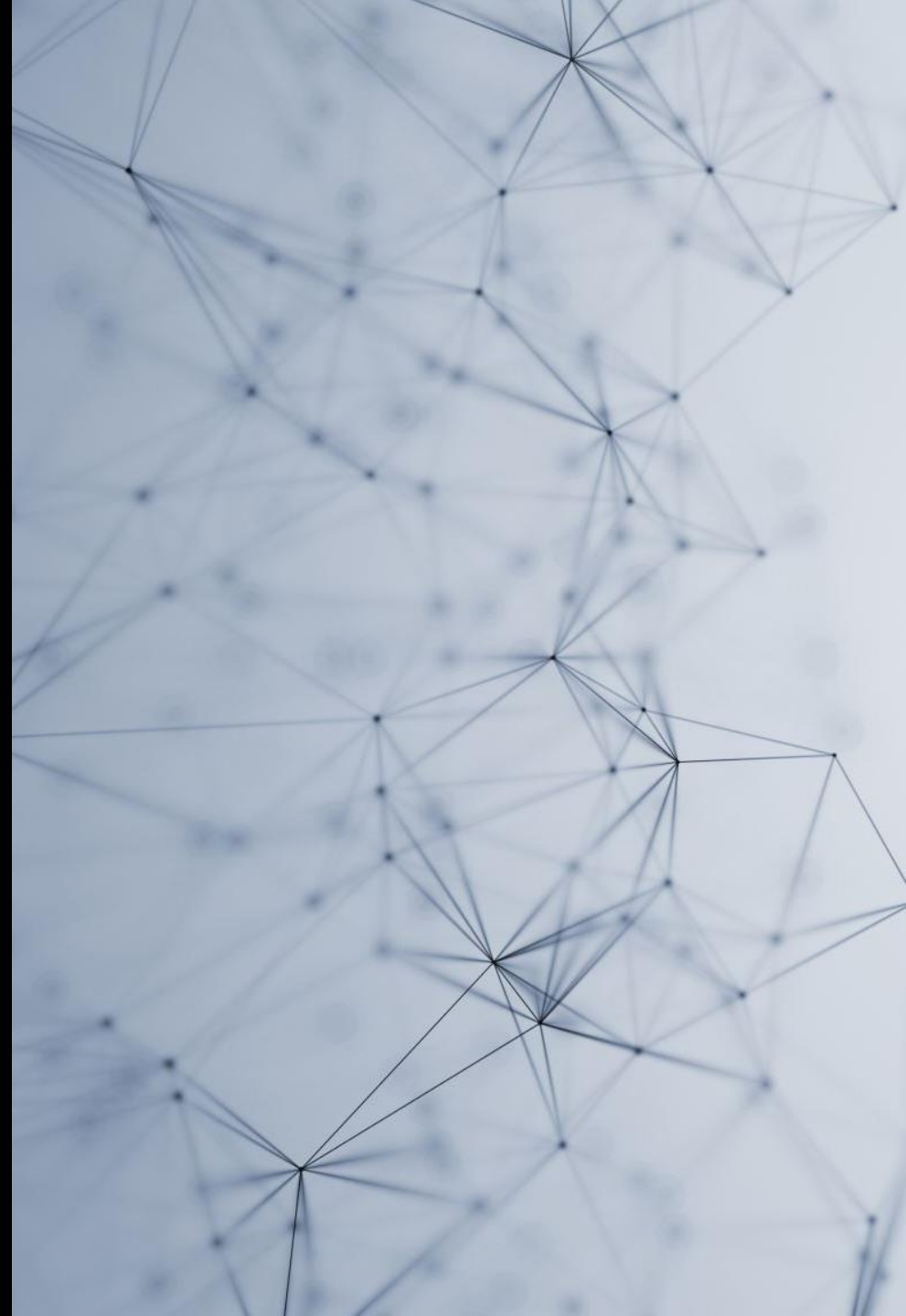
# The main enzymes used in DNA recombination technologies

**Lecturer**: Senior Lecturer, Department of Molecular Biology and Genetics, PhD, Smekenov I.T.
**Subject**: Genetic engineering

*(Lecture 2)*



# Lecture Goal:
To explore the main enzymes used in DNA recombination technologies, their roles, and mechanisms of action.

**Tasks:**

1. Discuss restriction enzymes, their classification, and the types of DNA fragment ends they produce.
2. Explain the function of DNA ligase, phosphatase (FastAP), and protein kinases in DNA manipulation.
3. Describe the roles of T4 DNA polymerase, Klenow fragment, DNA polymerase I, and reverse transcriptase in DNA recombination and synthesis.

***Keywords:*** *Restriction enzymes, blunt ends, sticky ends, DNA ligase, phosphatase, FastAP, protein kinases, T4 DNA polymerase, Klenow fragment, DNA polymerase I, reverse transcriptase*

# Summary

**1. Restriction enzymes**

- Recognize specific DNA sequences and cut both strands at defined positions.
- Generate either **blunt ends** (straight cuts) or **sticky ends** (overhanging single-stranded regions) useful for ligation.

**2. Blunt and sticky ends**

- **Blunt ends** have no overhangs and can be joined to any other blunt-ended DNA, though less efficiently.
- **Sticky ends** have short single-stranded overhangs that promote specific base pairing between compatible fragments.

**3. DNA ligase**

- Joins DNA fragments by catalyzing the formation of **phosphodiester bonds** between the 3′-hydroxyl and 5′-phosphate groups.
- Essential for sealing nicks and constructing recombinant DNA molecules.

**4. Phosphatase (e.g., FastAP alkaline phosphatase)**

- Removes 5′-phosphate groups from DNA to **prevent self-ligation** of vectors.
- Used during cloning to ensure that only DNA fragments with inserts can be ligated.

**5. Protein kinases (e.g., T4 polynucleotide kinase)**

- Add phosphate groups to the 5′ ends of DNA or RNA molecules.
- Enable the ligation of fragments that lack 5′-phosphates.

**6. T4 DNA polymerase**

Has **3′→5′ exonuclease activity** and can remove overhangs to create blunt ends.

Also fills in recessed 3′ ends, making it useful for end repair in cloning and sequencing.

**7. Klenow fragment (from DNA polymerase I)**

A large fragment of *E. coli* DNA polymerase I lacking 5′→3′ exonuclease activity.

Used for filling in recessed ends, synthesizing complementary strands, or labeling DNA.

**8. DNA polymerase I**

Performs DNA synthesis and repair; possesses both 5′→3′ polymerase and exonuclease activities.

Replaces RNA primers with DNA during replication and can be used for nick translation.

**9. Reverse transcriptase**

Synthesizes complementary DNA (cDNA) from an RNA template.

Used in cDNA library construction and in RT-PCR for studying gene expression.

# Control Questions

- ✓ What are the main differences between sticky ends and blunt ends?
- ✓ How does DNA ligase contribute to the formation of recombinant DNA molecules?
- ✓ Why is alkaline phosphatase used during vector preparation?
- ✓ What role do protein kinases play in DNA end modification?
- ✓ How does T4 DNA polymerase assist in end repair during cloning?
- ✓ What are the main applications of the Klenow fragment in molecular biology?
- ✓ How does reverse transcriptase differ from DNA polymerase I in function and substrate?

# Restriction enzymes

- A **restriction enzyme**, restriction endonuclease, or *restrictase* is an enzyme that cleaves DNA into fragments at or near specific recognition sites within molecules known as restriction sites.
- Over **3,000 restriction enzymes** have been studied in detail, and more than 600 of these are available commercially.
- These enzymes are routinely used for DNA modification in laboratories, and they are a vital tool in molecular cloning.

**Summary of the discovery of restriction enzymes**

| Year | Restrictase | Prototype |
|---|---|---|
| 1976 | 84 | 22 |
| 1980 | 227 | 54 |
| 1984 | 503 | 103 |
| 1986 | 710 | 126 |
| 1988 | 839 | 152 |
| 2002 | 3516 | 211 |

## TYPES AND ACTIVITIES OF RESTRICTION ENZYMES

**Type I**
Cleaves DNA at random sites far from its recognition sequence

**Type II**
Cleaves DNA at defined positions close to or within its recognition sequence

**Type IIG**
Cleaves outside its recognition sequence with both REase and MTase enzymatic activities in the same protein

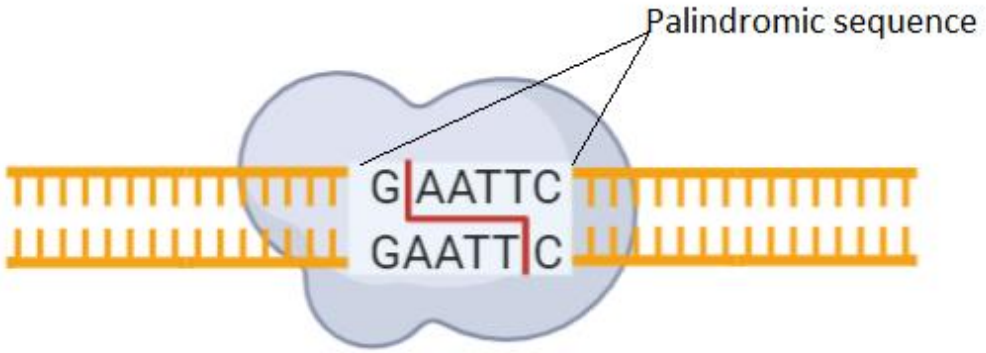
**Type IIP**
Cleaves symmetric targets and cleavage sites

**Type IIS**
Recognizes asymmetric sequences

**Type III**
Cleaves outside its recognition sequence and require two sequences in opposite orientations within the same DNA

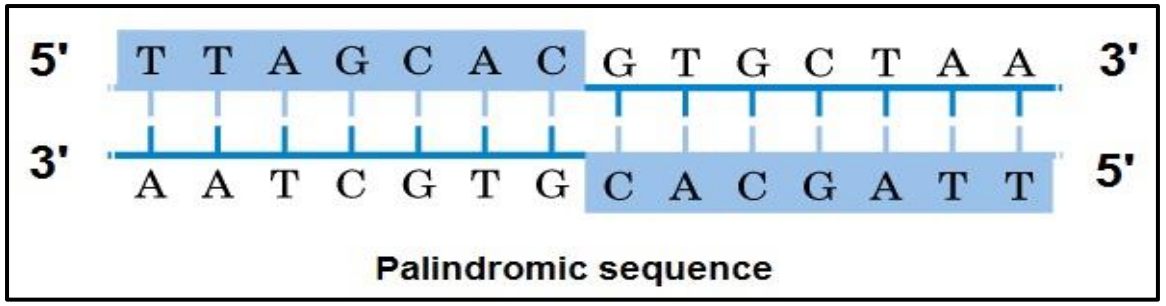
**Type IV**
Cleaves modified (e.g., methylated) DNA

| | Cleavage site | Location of methylase | Examples |
|---|---|---|---|
| Type I | Random<br>Around 1000bp away from recognition site | Endonuclease and methylase located on a single protein molecule | EcoK I<br>EcoA I<br>CfrA I |
| Type II | Specific<br>Within the recognition site | Endonuclease and methylase are separate entities | EcoR I<br>BamH I<br>Hind III |
| Type III | Random<br>24-26 bp away from recognition site | Endonuclease and methylase located on a single protein molecule | EcoP I<br>Hinf III<br>EcoP15 I |

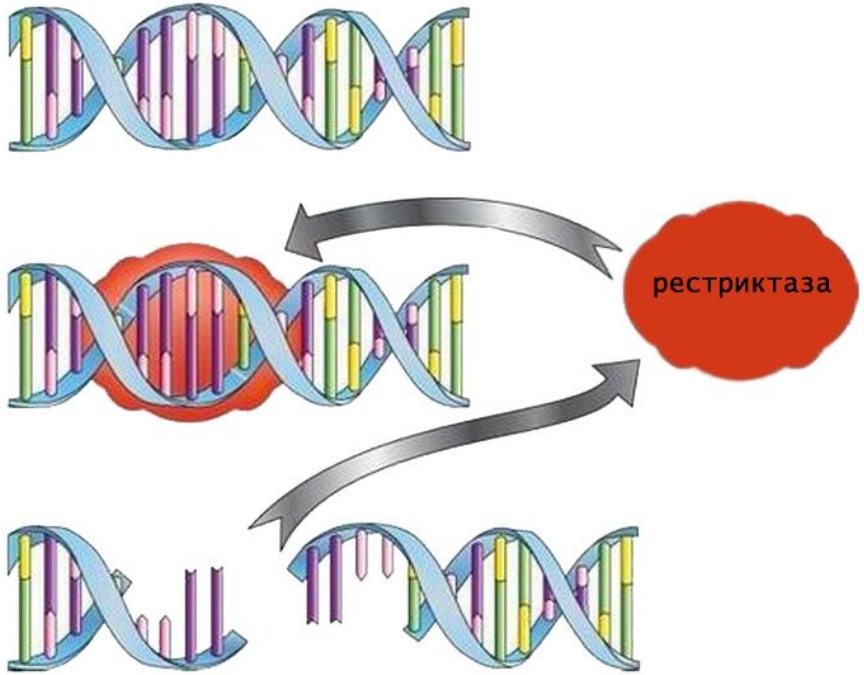



Restriction Endonuclease enzyme

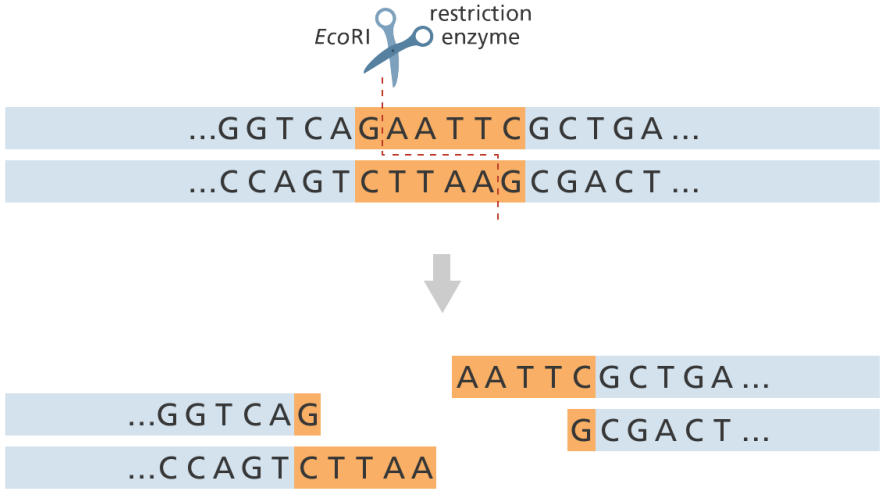
| | Type I | Type II | Type III | Type IV |
|---|---|---|---|---|
| **Nuclease structure** | Multimer; heterotrimer | Homodimer | Homodimer | |
| **Recognition site pattern** | Two sites, in any orientation | Small (4–8 bp); usually palindromic | Two sites, in head-to-head orientation; non-palindromic | Weak specificity |
| **Cleavage site** | Variable distance from recognition site; non-specific cleavage | Cleavage within (Type IIP) or outside (Type IIS) the recognition site | Cleavage of one strand (nicking activity) 24–25 bp from recognition site | Methylated only |
| **Cofactor** | ATP, Mg2+, SAM | Mg2+ | ATP, Mg2+, SAM | ATP, GTP |



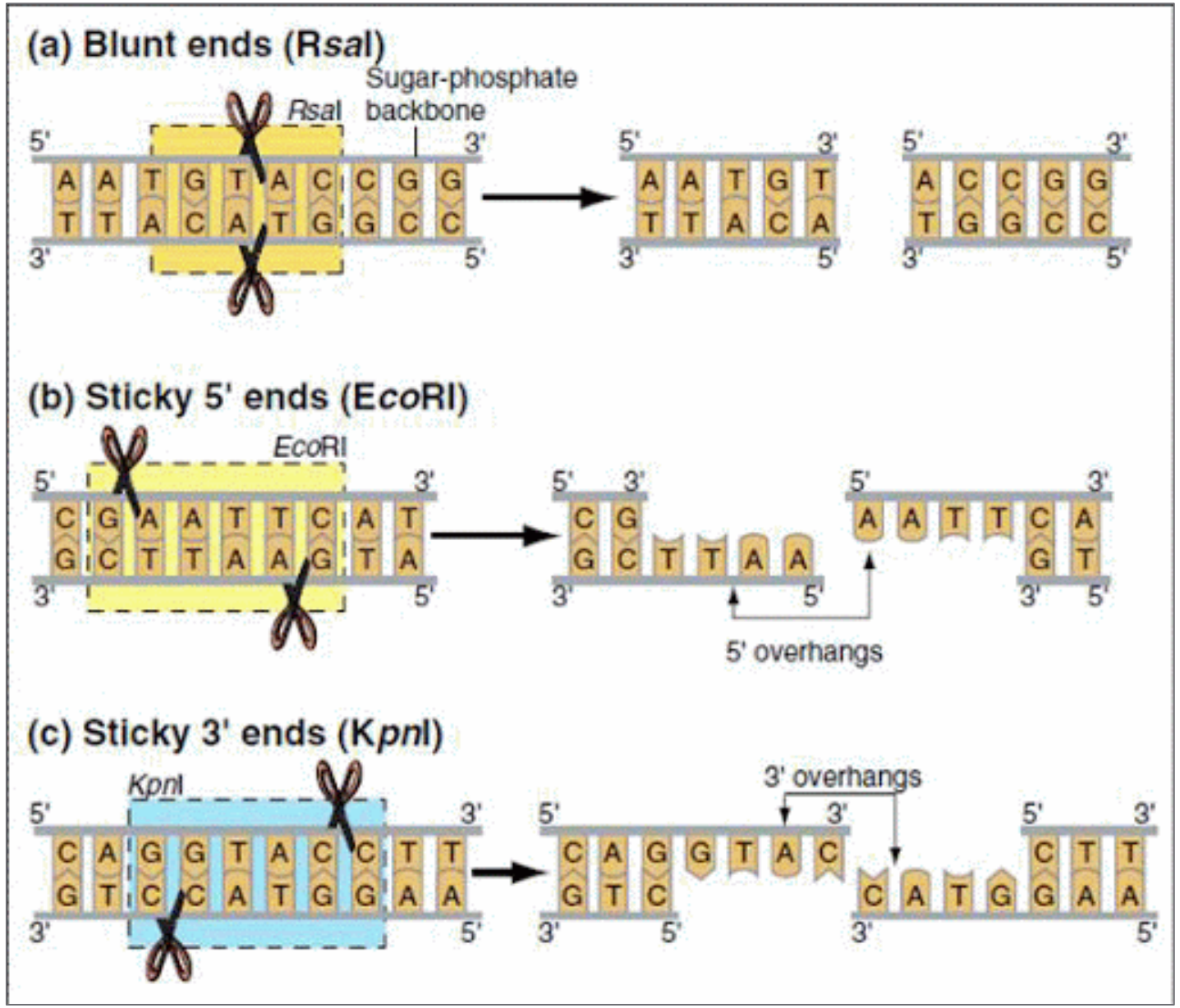

A **palindromic recognition site** reads the same on the reverse strand as it does on the forward strand when both are read in the same orientation




- **In 1968** M. Mezolson and R. Yuan for the first time isolated the restriction enzyme from E. coli K12 strain (EcoK and EcoB).
- **In 1970**, X. Smith and K. Wilvox isolated the HindII enzyme specifically for recognizing and cutting DNA for the first time.
- **In 1970** X. Smith and D. Nathans discovered EcoRI and EcoRII



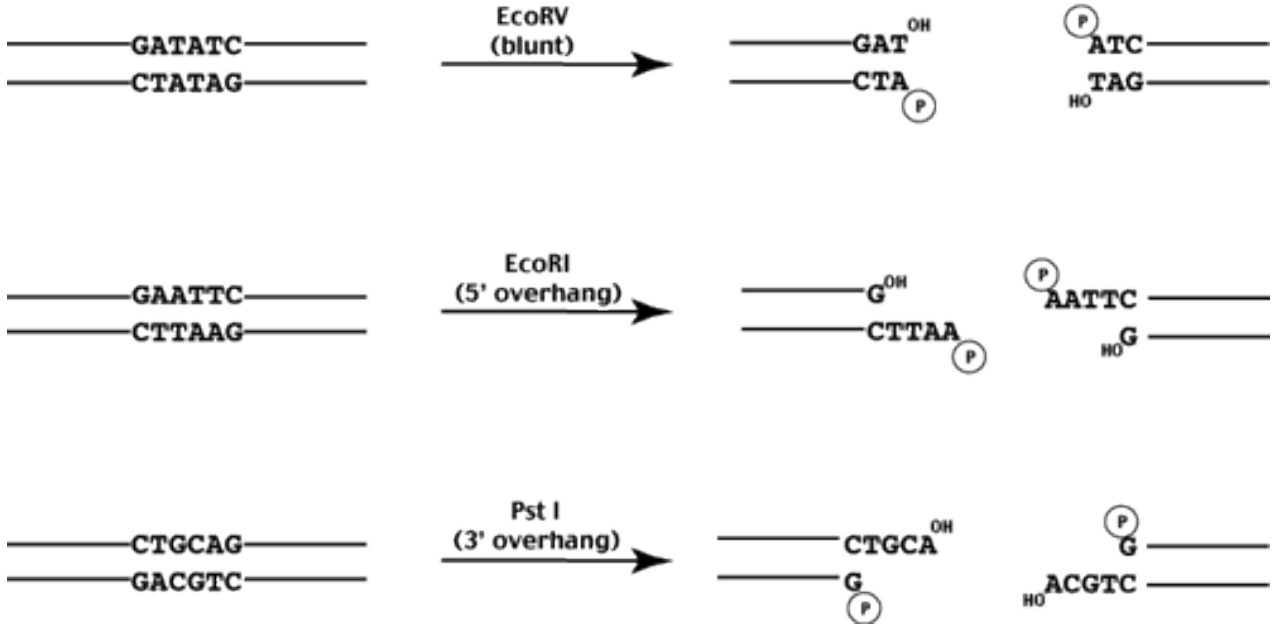
# Types of fragment ends

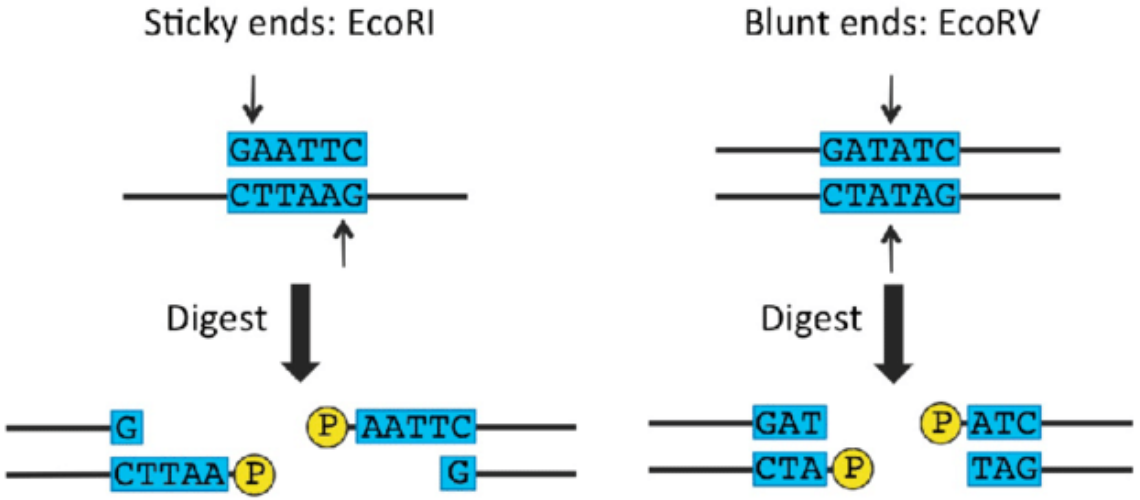


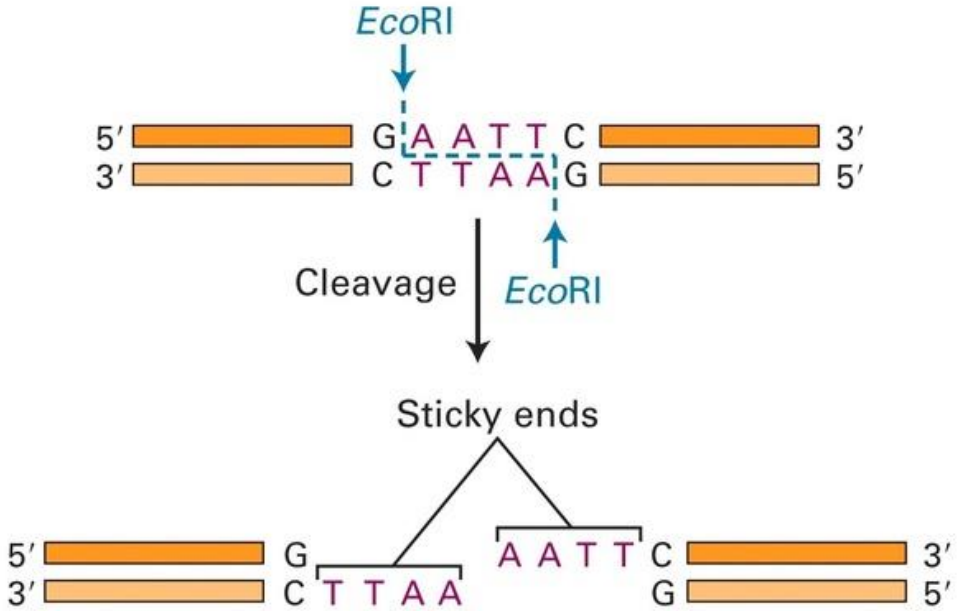

**The prototype** is the first discovered enzyme that recognizes a new sequence.

**Isoschisomers** are pairs of restriction endonucleases that have specificity for recognizing the same sequences, but sometimes differing in the presence of methylated nucleotide residues, and cut these sequences in the same places.

# Examples of restrictase



## Derivation of the EcoRI name

| Abbreviation | Meaning | Description |
|---|---|---|
| E | *Escherichia* | Genus |
| co | *Coli* | species |
| R | RY13 | Strain |
| I | First identified | order of identification |

| Microorganism of origin | Enzyme | Recognition site | After restriction enzyme digestion |
|---|---|---|---|
| Escherichia coli | *Eco*RI | 5′-GAATTC-3′<br>3′-CTTAAG-5′ | 5′-G AATTC-3′<br>3′-CTTAA G-5′ |
| Serratia marcescens | *Sma*I | 5′-GGGCCC-3′<br>3′-CCCGGG-5′ | 5′-GGG CCC-3′<br>3′-CCC GGG-5′ |
| Arthrobacter luteus | *Alu*I | 5′-AGCT-3′<br>3′-TCGA-5′ | 5′-AG CT-3′<br>3′-TC GA-5′ |
| Streptomyces albus | *Sal* I | 5′-GTCGAC-3′<br>3′-CAGGTG-5′ | 5′-G TCGAC-3′<br>3′-CAGGT G-5′ |
| Haemophilus influenzae | *Hin*dIII | 5′-AAGCTT-3′<br>3′-TTCGAA-5′ | 5′-A AGCTT-3′<br>3′-TTCGA A-5′ |

| Microorganism | Restriction Enzyme Name | Restriction Site |
|---|---|---|
| *Bacillus amyloliquefaciens* H | *Bam*HI | G\|G A T C C<br>C C T A G\|G |
| *Brevibacterium albidum* | *Bal*I | T G G\|C C A<br>A C C\|G G T |
| *Escherichia coli* RY13 | *Eco*RI | G\|A A T T C<br>C T T A A\|G |
| *Haemophilus aegyptius* | *Hae*II | Pu G C G C\|Py<br>Py\|C G G C Pu |
| *Haemophilus aegyptius* | *Hae*III | G G\|C C<br>C C\|G G |
| *Haemophilus influenzae* $R_d$ | *Hin*dII | G T Py\|Pu A C<br>C A Pu\|Py T G |
| *Haemophilus influenzae* $R_d$ | *Hin*dIII | A\|A G C T T<br>T T C G A\|A |
| *Haemophilus parainfluenzae* | *Hpa*I | G T T\|A A C<br>G A A\|T T G |
| *Haemophilus parinfluenzae* | *Hpa*II | C\|C G G<br>G G C\|C |
| *Providencia stuartii* 164 | *Pst*I | C T G C A\|G<br>G\|A C G T C |
| *Streptomyces albus* G | *Sal*I | G\|T C G A C<br>C A G C T\|G |

Table 2.1: *Restriction enzymes*

# Restriction nomenclature

**In 1973, Smith and Nathans proposed a nomenclature for restrictases, including the following items:**

1. The abbreviation of the name of each enzyme is derived from the binary name of the microorganism containing this methylase-restriction system. Compose according to the rule: the first two lowercase letters of the species are added to the first capital letter of the genus name.

   *Streptomyces albus* - Sal, *Escherichia coli* – Eco.

2. If necessary, add a serotype or strain designation, e.g. Eco B.

3. Various restriction systems - modifications encoded by one bacterial cell are indicated by Roman numerals: Hind II, Hind I, Hind III (*Haemophilus influenzae*).

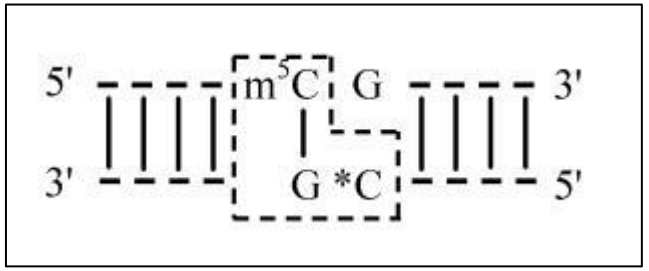
4. Restriction enzymes are denoted by the letter R (R Hind III), метилазы - M (M Hind III).

The discovery of new restrictases forced Roberts in 1978 to make additions to the system of rational designations of enzymes: if the abbreviated name is the same for several enzymes, then the first 2 letters of the abbreviation remain unchanged, and the third is taken from the subsequent letters of the species name:
*Haemophilus parainfluenzae* - Hpa I *Haemophilus parahaemolyticus* - Hph I.

**DNA methyltransferases** are a group of enzymes that catalyze the methylation of nucleotide residues in DNA.The activity of methyltransferases, which consists in the transfer of methyl (CH3-) groups to the nitrogenous base cytosine in DNA, leads to a change in the properties of DNA, while the activity, functions of the corresponding genes, as well as the spatial structure of the nucleic acid (conformation) change

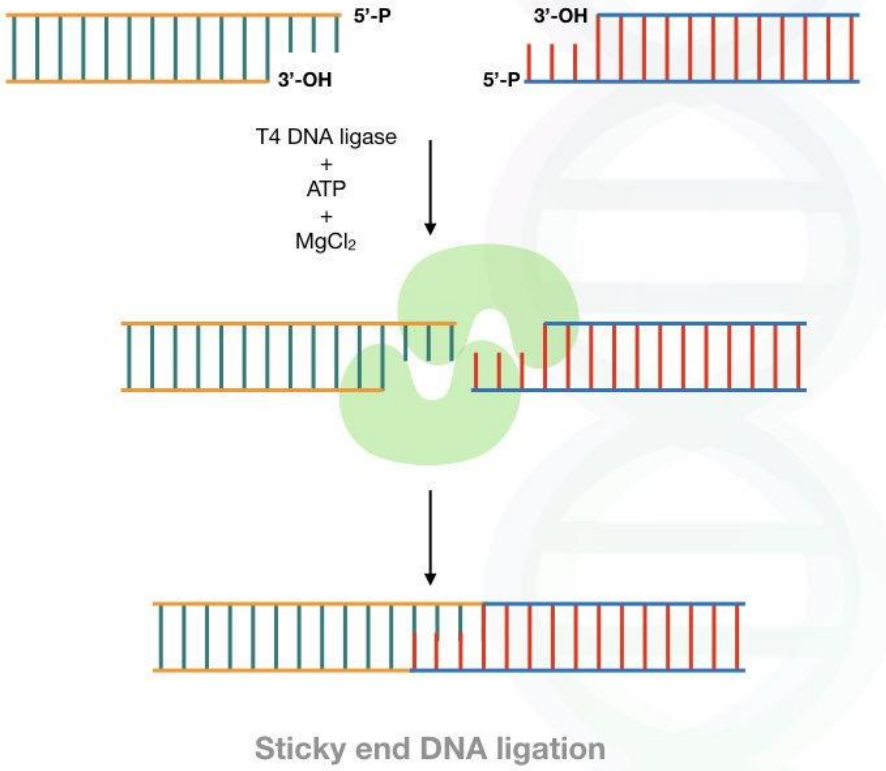
A quick review of relevant DNA methylation restriction enzymes.

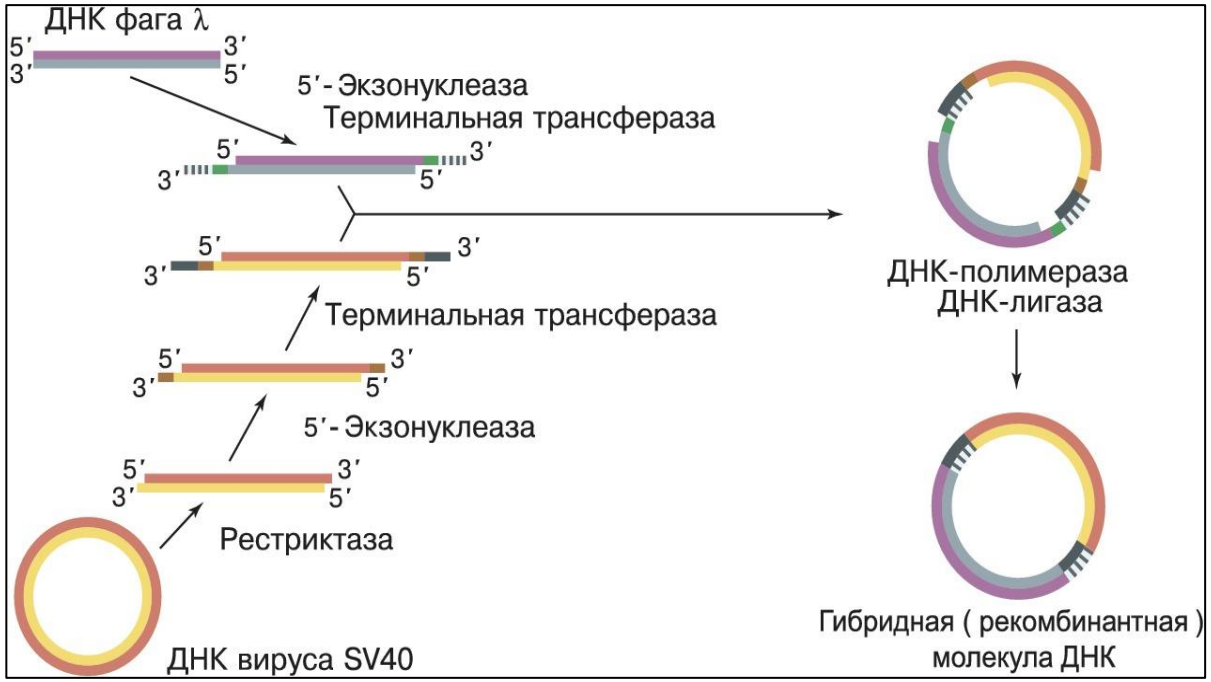
| Product | DNA Methylation Application | Recognition Site |
|---|---|---|
| MspJI | Identify 5-hmC and 5-mC | 5′... $^{m}$CNNR(n)$_{9}$ ▼...3′<br>3′... GNNY(N)$_{11}$▲...5′ |
| LpnPI | Identify 5-hmC and 5-mC | 5′... C$^{m}$CDG(N)$_{10}$ ▼...3′<br>3′... GGHC(N)$_{14}$▲...5′ |
| FspEI | Identify 5-hmC and 5-mC | 5′... C$^{m}$C(N)$_{12}$ ▼...3′<br>3′... GG(N)$_{16}$▲...5′ |
| DpnI | Identify 5-mA; used with DpnII | CH$_3$<br>5′... GA▼TC...3′<br>3′... CT▲AG...5′<br>CH$_3$ |
| DpnII | Identify 5-mA; used with DpnI | 5′... ▼GATC ...3′<br>3′... CTAG▲...5′ |
| McrBC | Identify 5-mC | 5′...Pu$^{m}$C(N$_{40\text{-}3000}$)Pu$^{m}$C...3′ |
| MspI | Identify 5-mC; used with HpaII | 5′... C▼CGG ...3′<br>3′... GGC▲C...5′ |
| HpaII | Identify 5-mC; used with MspI | 5′... C▼CGG ...3′<br>3′... GGC▲C...5′ |



# DNA ligase

**DNA ligases (68 kDa)** are enzymes (EC 6.5.1.1) that catalyze the covalent fusion of DNA strands in a duplex during replication, repair and recombination. They form phosphodiester bridges between the 5'-phosphoryl and 3'-hydroxyl groups of adjacent deoxynucleotides at DNA breaks or between two DNA molecules (discovered in 1967).



Thermo Scientific T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA. The enzyme repairs single-strand nicks in duplex DNA, RNA, or DNA/RNA hybrids. It also joins DNA fragments with either cohesive or blunt termini, but has no activity on single-stranded nucleic acids.

T4 DNA Ligase requires ATP as a cofactor.

**Highlights**

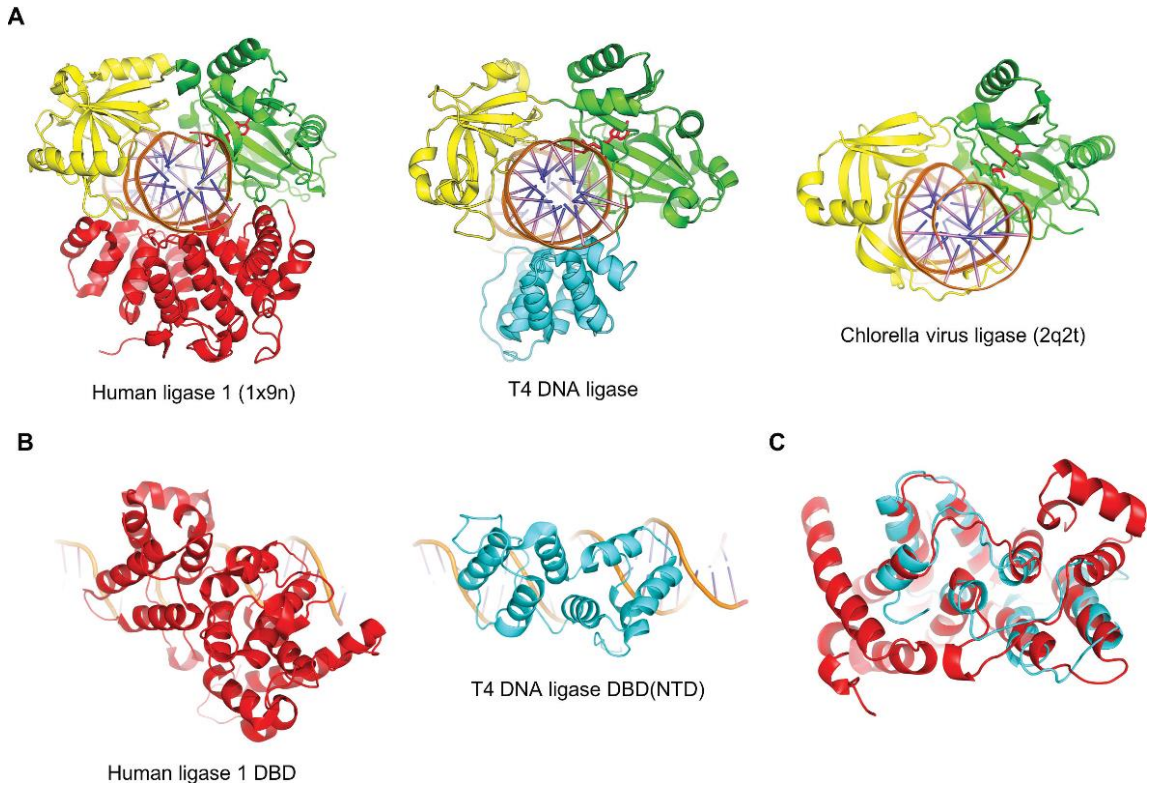
• Active in Themo Scientific restriction enzyme, PCR, and RT buffers (when supplemented with ATP)
• Fast—sticky-end ligation is completed in 10 minutes at room temperature
• Supplied with PEG solution for efficient blunt-end ligation

**Applications**

• Cloning of restriction enzyme generated DNA fragments
• Cloning of PCR products
• Joining of double-stranded oligonucleotide linkers or adaptors to DNA
• Site-directed mutagenesis
• Amplified fragment length polymorphism (AFLP)
• Ligase-mediated RNA detection (*see* Reference 3)
• Nick repair in duplex DNA, RNA or DNA/RNA hybrids
• Self-circularization of linear DNA.

**Includes**

• T4 DNA Ligase
• 10X T4 DNA Ligase Buffer
• 50% PEG Solution

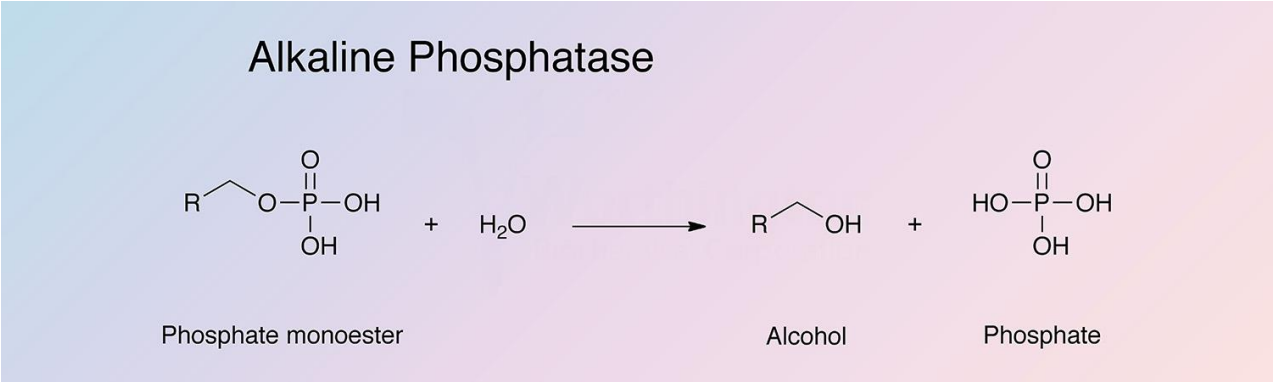


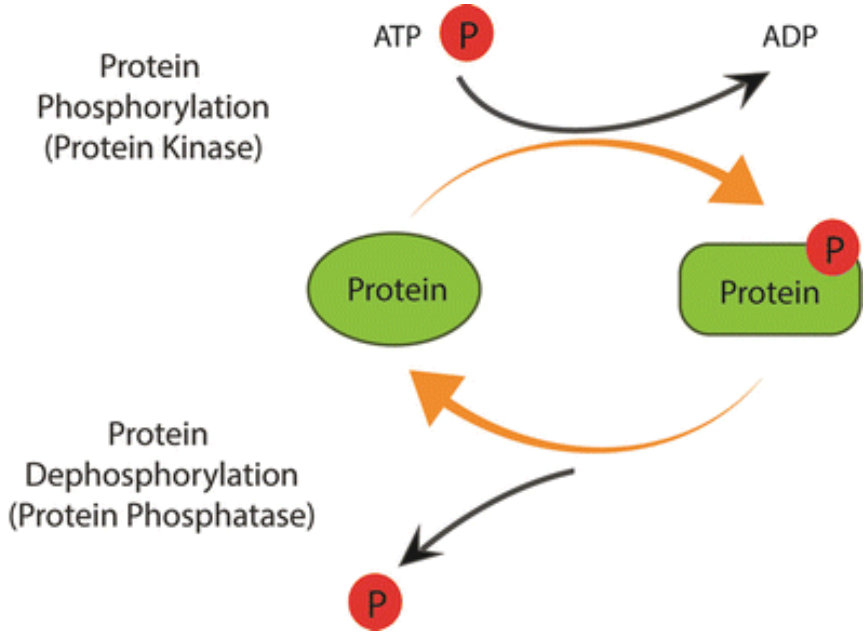

## Specifications

| | |
|---|---|
| **Concentration** | 5 U/µL |
| **Enzyme** | DNA Ligase |
| **Compatible Buffer** | 10X T4 DNA Ligase Buffer |
| **Quantity** | 200 U |
| **Product Type** | T4 DNA Ligase |
| **Unit Size** | 200 units |

# Additional Enzymes

- **Phosphatase** is an enzyme that catalyzes the dephosphorylation of a substrate (usually another protein) by hydrolysis of the phosphoric acid ester bond. In this case, a phosphate anion and a product molecule with a hydroxyl group are formed. According to its catalytic and physiological action, phosphatase is an antagonist of phosphorylase and kinase, which attach a phosphate group to the substrate.



# FastAP Thermosensitive Alkaline Phosphatase (1 U/µL)

Thermo Scientific FastAP Thermosensitive Alkaline Phosphatase catalyzes the release of 5'- and 3'-phosphate groups from DNA, RNA, and nucleotides. This enzyme also removes phosphate groups from proteins.

FastAP is a novel alkaline phosphatase, which is active in all Thermo Scientific restriction enzyme buffers as well as in PCR buffers. It dephosphorylates all types of DNA ends (blunt, 5'- and 3'-overhangs) in 10 minutes at 37°C. The enzyme is inactivated in 5 minutes at 75°C (*see* Figure 1 in Supporting Data). Therefore, removal of alkaline phosphatase is not required prior to ligation.

**Highlights**

- **Recombinant enzyme**
- **Fast dephosphorylation**—10 minutes at 37°C
- **Fast and complete inactivation**—5 minutes at 75°C
- **Simultaneous digestion and dephosphorylation of vector DNA**
- **100% active** in restriction enzyme and PCR buffers
- **PCR clean-up in conjunction with Exo I**
- **Protein dephosphorylation**

**One protocol for all types of DNA ends:**

- 5'-overhangs
- 3'-overhangs
- blunt-ends
- single nucleotides

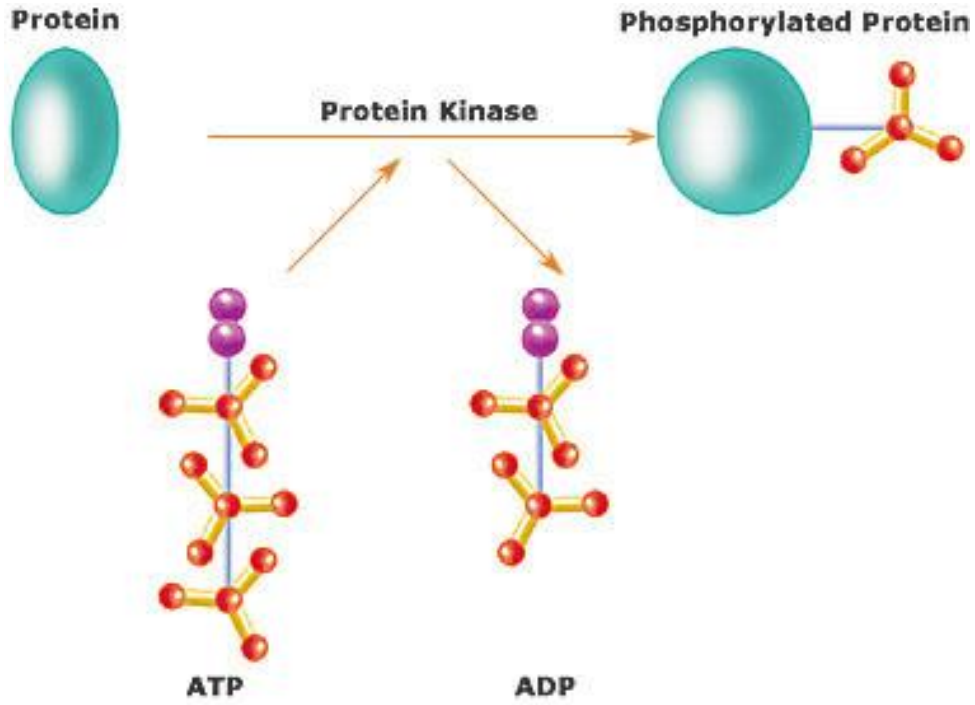
**Specifications**

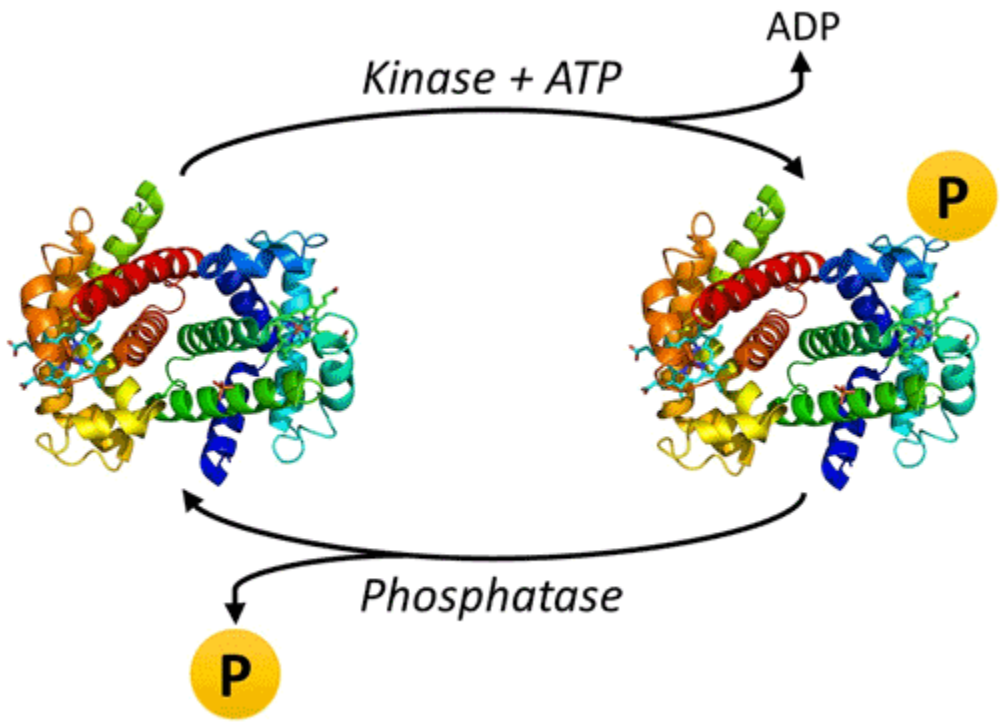
| | |
|---|---|
| **Concentration** | 1 U/µL |
| **Enzyme** | Phosphatase |
| **Compatible Buffer** | PCR Buffer, Enzyme Buffer |
| **Quantity** | 300 U |
| **Product Type** | Alkaline Phosphatase |
| **Unit Size** | 300 units |

**Applications**

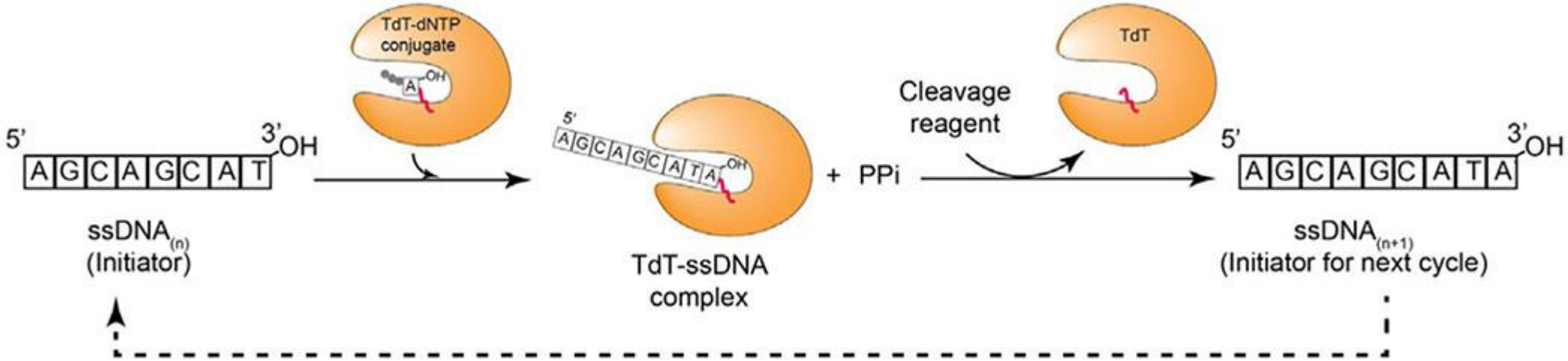
- Dephosphorylation of cloning vector DNA to prevent recircularization during ligation
- Simultaneous digestion and dephosphorylation of vector DNA
- PCR product clean-up: nucleotide degradation prior to sequencing of PCR product
- Dephosphorylation of nucleic acid 5'-termini prior to labeling with T4 Polynucleotide Kinase
- Other applications where dephosphorylation of DNA and RNA substrates is necessary
- Protein dephosphorylation

- **Protein kinases** are a subclass of kinase enzymes (phosphotransferases). Protein kinases modify other proteins by phosphorylation of amino acid residues that have hydroxyl groups (serine, threonine, and tyrosine) or the heterocyclic amino group of histidine.



- **Terminal deoxynucleotidyl transferase (TdT),** also known as DNA nucleotidyl exotransferase (DNET) or terminal transferase, is a specialized DNA polymerase expressed in immature, pre-B, pre-T lymphocytes, and in acute lymphoblastic leukemia.



Terminal Deoxynucleotidyl Transferase, Recombinant (rTdT) is a DNA polymerase that catalyzes the addition of deoxynucleotides to the 3fi hydroxyl terminus of DNA. A TdT Technical Bulletin is available.

**Applications:** Homopolymer tailing of vector and insert for cloning. Labeling oligonucleotides with biotin (1,2), $^{32}$P- or $^{35}$S-label (3), or in apoptosis (TUNEL) (4,5).

**Source:** Purified from E. coli clone of calf thymus TdT.

**Performance and Quality Testing:** Endonuclease, 3´ and 5´ exodeoxyribonuclease, and levels of incorporation tested.

**Unit Definition:** One unit incorporates 1 nmol dATP into acid-precipitable material in 1 h at 37°C, using d(pA)$_{50}$ as a primer.

**Hazard Warning:** Toxic; potassium cacodylate contained in reaction buffer. Also contains cobalt chloride, a highly toxic chemical. See MSDS.

**Unit Reaction Conditions:** 0.2 M potassium cacodylate (pH 7.2), 10 mM $MgO_4$ $C_4$ $H_6$ , 1 mM 2-mercaptoethanol, 0.5 mg/ml BSA, 100 flM d(pA)$_{50}$, 1 mM [$^3$H]dATP, and enzyme in 0.15 ml for 1 h at 37°C.

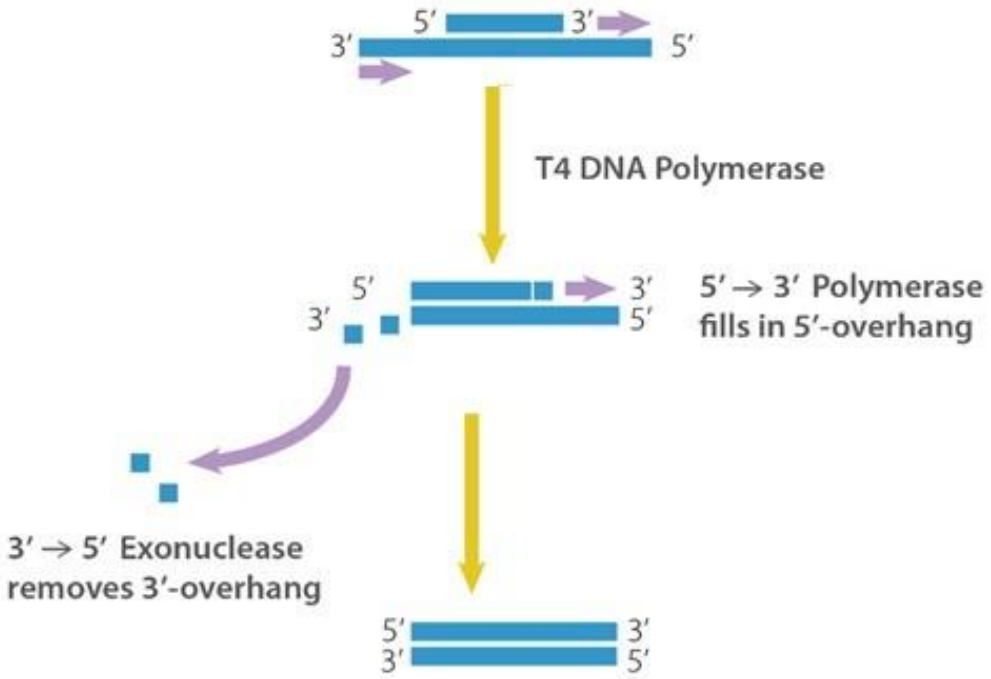
## Specifications

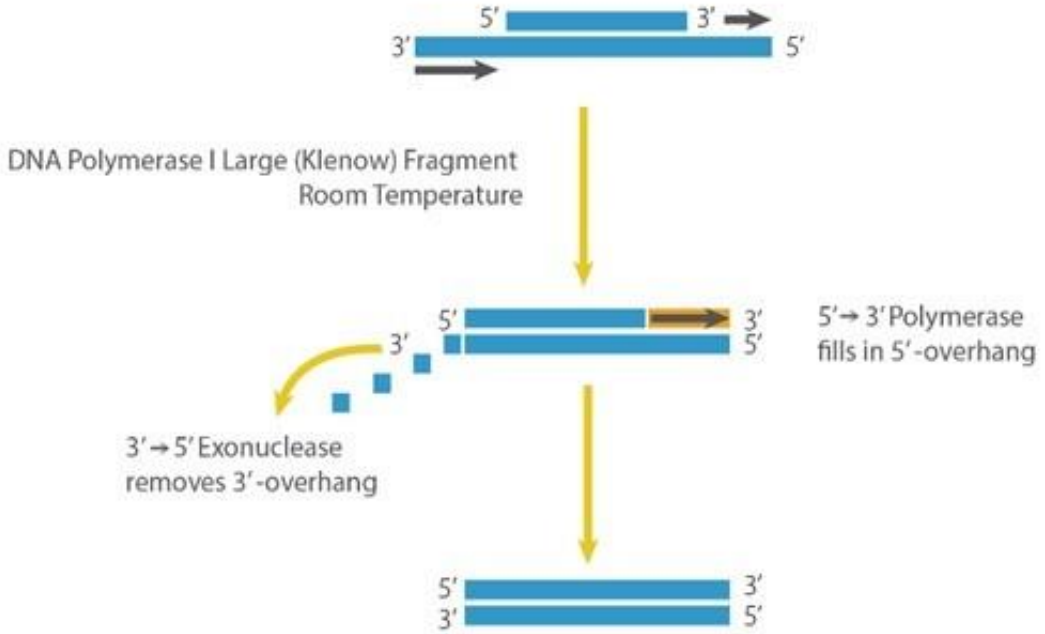
| | |
|---|---|
| **Shipping Condition** | Approved for shipment on Wet or Dry Ice |
| **Enzyme** | Terminal Deoxynucleotidyl Transferase |
| **Compatible Buffer** | 5X Buffer, Reaction Buffer |
| **Quantity** | 500 U |
| **Product Type** | TdT |
| **Unit Size** | 500 units |

## Contents & Storage

Terminal Deoxynucleotidyl Transferase, Recombinant (rTdT) is supplied with vial of 5X buffer [500 mM potassium cacodylate (pH 7.2), 10 mM $CoCl_2$ , 1 mM DTT]. Store at -20°C.

- **T4 DNA polymerase and Klenov fragment** are DNA blunting enzymes.



Thermo Scientific T4 DNA Polymerase is a template-dependent DNA polymerase that catalyzes 5'-3' synthesis from primed single-stranded DNA. The enzyme has a 3'-5' exonuclease activity, but lacks 5'-3' exonuclease activity.

**Highlights**

- **Stronger 3'-5' exonuclease activity** on single-stranded than on double-stranded DNA and greater (more than 200 times) than DNA polymerase I, *E. coli,* and Klenow fragment
- Active in Thermo Scientific restriction enzyme, PCR, RT and T4 DNA Ligase buffers

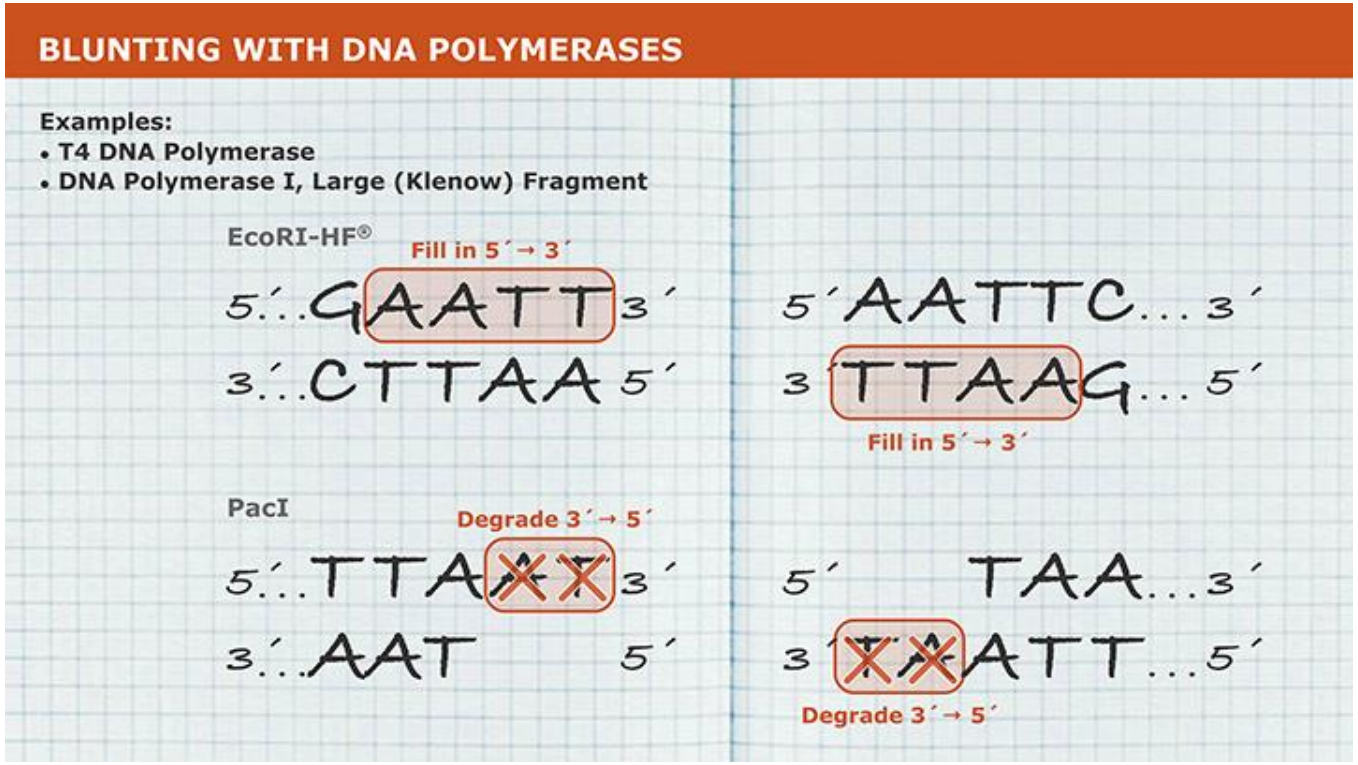
**Applications**

- Blunting of DNA ends: fill-in of 5'-overhangs or/and removal of 3'-overhangs(*see* References1, 2)
- Blunting of PCR products with 3'-dA overhangs
- Synthesis of labeled DNA probes by the replacement reaction(*see* Reference3)
- Oligonucleotide-directed site-specific mutagenesis(*see* Reference4)
- Ligation-independent cloning of PCR products

# DNA Polymerase I, Large (Klenow) Fragment

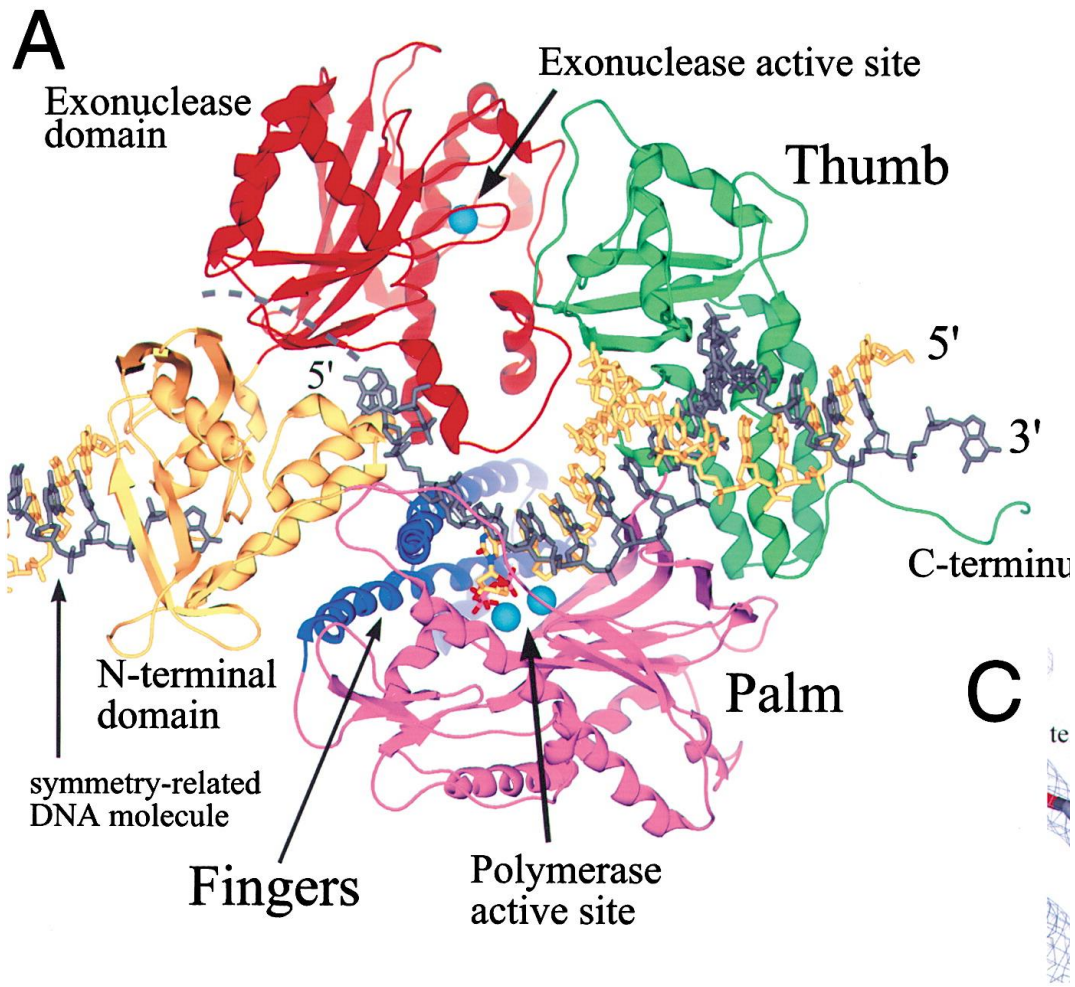
DNA Polymerase I, Large (Klenow) fragment was originally derived as a proteolytic product of *E.coli* DNA polymerase that retains polymerase and 3' —> 5' exonuclease activity

- Removal of 3' overhangs or fill-in of 5' overhangs to form blunt ends
- Lacks 5' —> 3' exonuclease activity
- Generates probes using random primers
- Second strand cDNA synthesis



# DNA polymerase I (103 kDa) - polI

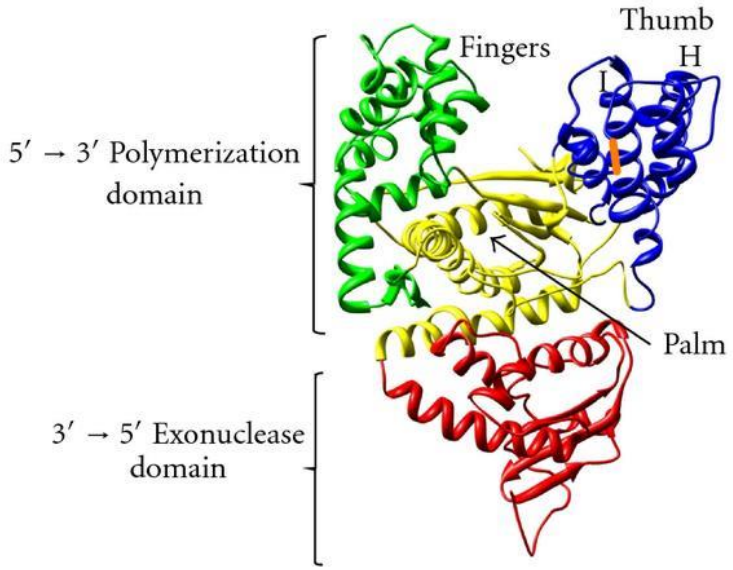
- 1958 Kornberg discovered this enzyme in E. coli.
- **Domains**:
- N-terminus: 5'-3' - exoculase activity
- C-terminus: 5'-3' - polymerase activity
- Middle domain: - 3'-5' - exonuclease activity



Thermo Scientific *Taq* DNA Polymerase is a highly thermostable DNA polymerase from the thermophilic bacterium *Thermus aquaticus*. The enzyme catalyzes 5' to 3' synthesis of DNA, has no detectable 3' to 5' exonuclease (proofreading) activity, and possesses low 5' to 3' exonuclease activity. In addition, *Taq* DNA Polymerase exhibits deoxynucleotidyl transferase activity, which frequently results in the addition of extra adenines at the 3'-end of PCR products. Recombinant *Taq* DNA Polymerase is the ideal tool for standard PCR of templates 5 kb or shorter.

**Features**
- Thermostable—half life is more than 40 min at 95°C
- Generates PCR products with 3'-dA overhangs
- Supplied with two buffers: 10X *Taq* Buffer with KCl and 10X *Taq* Buffer with $(NH_4)_2SO_4$
- Incorporates modified nucleotides (e.g., biotin-, digoxigenin-, fluorescent-labeled nucleotides)

**Applications**
- Routine PCR amplification of DNA fragments up to 5 kb
- High throughput PCR
- DNA labeling




## Specifications

| | |
|---|---|
| **GC-Rich PCR Performance** | Low |
| **Polymerase** | *Taq* DNA Polymerase |
| **Reaction Speed** | Standard |
| **Product Type** | *Taq* DNA Polymerase (Recombinant) |
| **Quantity** | 100 Units |
| **Shipping Condition** | Dry Ice |
| **For Use With (Application)** | Standard PCR |
| **Concentration** | 5 U/µL |
| **Fidelity (vs. Taq)** | 1X |
| **Hot Start** | No |
| **Overhang** | 3'-A |
| **Reaction Format** | Separate Components |
| **Unit Size** | 100 units |

- Thermo Scientific **Phusion high quality DNA polymerases** set the gold standard for high throughput PCR. With an error rate 50 times lower than Taq and 6 times lower than Pfu , Phusion's high fidelity DNA polymerase is an excellent choice for cloning and other high precision applications.
- Phusion DNA polymerases provide reliable performance with short protocol times even in the presence of PCR inhibitors and provide higher yields with lower enzyme levels than other DNA polymerases.• Fast PCR due to short expansion time (15-30 s/kb)

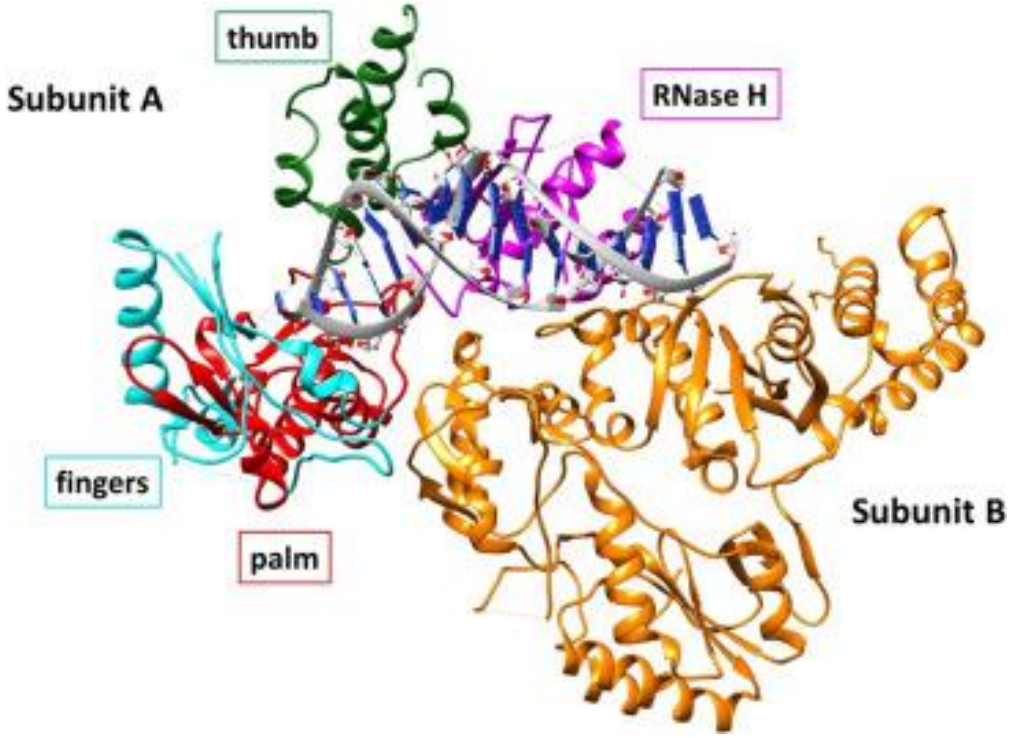
**Features**
•High fidelity (52X *Taq*)
•No non-specific amplification and primer degradation during reaction setup
•Fast PCR due to short extension times (15–30 s/kb)
•Increased product yields with minimal enzyme amounts
**Applications**
•Standard PCR
•qPCR
•High fidelity and long PCR
•LAMP-PCR
•cDNA synthesis
•RT-PCR, RDA, MDA
•DNA labeling and sequencing

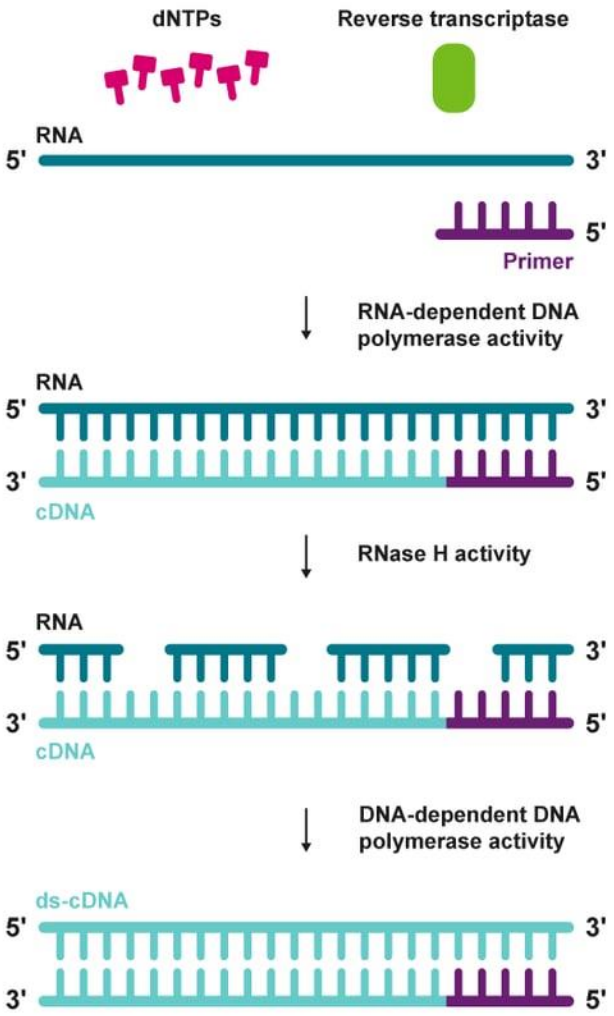
## Specifications

| | |
|---|---|
| **Detection Method** | Primer-probe |
| **GC-Rich PCR Performance** | High |
| **Polymerase** | Phusion Hot Start II DNA Polymerase |
| **Reaction Speed** | Fast |
| **Product Type** | Hot Start DNA Polymerase and dNTP Mix |
| **Shipping Condition** | Dry Ice |
| **For Use With (Application)** | Hot-start PCR, High-fidelity PCR |
| **Concentration** | 2 U/µL |
| **Fidelity (vs. Taq)** | 52X |
| **Hot Start** | Built-In Hot Start |
| **No. of Reactions** | 2000 Reactions |
| **Overhang** | Blunt |
| **Reaction Format** | Separate Components |
| **Size (Final Product)** | 20 kb or less |
| **Unit Size** | 2000 reactions |

# Reverse transcriptase

- **Description**
- RevertAid (RT) reverse transcriptase has lower RNase H activity compared to AMV reverse transcriptase.
- The enzyme remains active at 42-50°C and is suitable for the synthesis of cDNA up to 13 kb.



- The Thermo Scientific RevertAid RT Kit is a complete system for efficient synthesis of first strand cDNA from mRNA or total RNA templates. The kit uses RevertAid Reverse Transcriptase (RT), which has lower RNase H activity compared to AMV reverse transcriptase. The enzyme maintains activity at 42–50°C and is suitable for synthesis of cDNA up to 13 kb. The recombinant RiboLock RNase Inhibitor, supplied with the kit, effectively protects RNA from degradation at temperatures up to 55°C.

  First strand cDNA synthesized with this system can be directly used as a template in PCR or real-time PCR. It is also ideal as a first step for second strand cDNA synthesis or linear RNA amplification. Radioactively and non-radioactively labeled nucleotides can be incorporated into first strand cDNA for use as a probe in hybridization experiments, including microarrays.

  **Features of the RevertAid RT Kit include:**
  • Full-length first strand cDNA up to 13 kb
  • Optimum reaction temperature 42°C
  • Complete kit includes all the components for RT reactions

  **Applications**
  • First strand cDNA synthesis for RT-PCR and RT-qPCR
  • Construction of full length cDNA libraries
  • Antisense RNA synthesis

## Specifications

| | |
|---|---|
| **Format** | Kit |
| **Reaction Speed** | 60 min. |
| **Technique** | Reverse Transcription |
| **Optimal Reaction Temperature** | 42°C |
| **Reverse Transcriptase** | RevertAid |
| **Ribonuclease H Activity** | Yes |
| **Shipping Condition** | Dry Ice |
| **For Use With (Application)** | Real Time PCR (qPCR) |
| **Final Product Type** | First-Strand cDNA |
| **No. of Reactions** | 500 Reactions |
| **Reaction Format** | Separate components |
| **Reagent Type** | Reverse Transcription |
| **Size (Final Product)** | Up to 13 kb |
| **Starting Material** | RNA |
| **Unit Size** | 500 reactions |

# References


1) Rae, P. M. M., Barnett, T. R. & Babbitt, D. G. Factors influencing the yield of satellite DNA in extractions from Drosophila virilis and Drosophila melanogaster adults and embryos. BBA Sect. Nucleic Acids Protein Synth. (1976) doi:10.1016/0005-2787(76)90157-X.

2) Patrinos, G. P., Danielson, P. B. & Ansorge, W. J. Molecular Diagnostics: Past, Present, and Future. in Molecular Diagnostics: Third Edition (2016). doi:10.1016/B978-0-12-802971-8.00001-8.

3) Ali, N., Rampazzo, R. D. C. P., Costa, A. Di. T. & Krieger, M. A. Current Nucleic Acid Extraction Methods and Their Implications to Point-of-Care Diagnostics. BioMed Research International (2017) doi:10.1155/2017/9306564.

4) Vogelstein, B. & Gillespie, D. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. U. S. A. (1979) doi:10.1073/pnas.76.2.615.

5) Green, M. R. & Sambrook, J. Molecular Cloning, 3-Volume Set : A Laboratory Manual. Cold Spring Harbour Laboratory Press (2012).