

Table 1 Main DNA polymerases used in molecular biology

| Enzyme | Requirements | Activity | Main applications/notes |
|---------------------------------------------|--------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|
| <i>Prokaryotic DNA polymerases</i> | | | |
| Pol I | Template | 5'→3' polymerase | DNA replication |
| | Primer | 3'→5' exonuclease (proofreading) | Nick translation |
| | dNTPs | 5'→3' exonuclease | Removal of 3' protruding DNA ends (without dNTPs) Primer removal |
| Pol II | Template | 5'→3' polymerase | DNA replication |
| | Primer | 3'→5' exonuclease | |
| | dNTPs | | |
| Pol III | Template | 5'→3' polymerase | DNA replication |
| | Primer | 3'→5' exonuclease | |
| | dNTPs | | |
| Klenow (large fragment of Pol I) | Template | 5'→3' polymerase | DNA replication when exonuclease activity in 3' needs to be avoided (fill in large gaps) |
| | Primer | 3'→5' exonuclease | |
| | dNTPs | | |
| T4 DNA Pol | Template | 5'→3' polymerase (with dNTPs) | DNA replication when exonuclease activity in 3' needs to be avoided (fill in large gaps) |
| | Primer | 3'→5' exonuclease (without dNTPs) | |
| | dNTPs | | |
| Modified T7 DNA Pol | Template | 5'→3' polymerase | Amplification of large DNA fragments. Preparation of radioactive probes |
| | Primer | 3'→5' exonuclease | |
| | dNTPs | Thioredoxin connects Pol to template to limit dissociation | |
| | | | |
| Mutated modified T7 DNA Pol | Template | 5'→3' polymerase | Sequencing |
| | Primer | Thioredoxin connects Pol to template to limit dissociation | |
| | dNTPs | | |
| Terminal deoxynucleotidyl transferase (TdT) | DNA primer | Addition of a homopolymer tail to 3'-OH ends of DNA using single-stranded DNA primer (with Mg ²⁺) or double-stranded DNA primer with (Co ²⁺) | Labeling DNA 3'ends with modified nucleotides Addition of a homopolymer tail to 3'-OH ends of DNA |
| | Mg ²⁺ or Co ²⁺ | | |
| | No template | | |

Thermostable DNA polymerases without proofreading activity

| | | |
|----------------|-------------------------------------------------------------------------|---------------------------------------------|
| Bst polymerase | Template, MgCl ₂ | 5'→3' polymerase |
| | Optimal Temp of 65°C | 5'→3' exonuclease |
| Taq polymerase | Template, MgCl ₂ | 5'→3' polymerase |
| | Optimal Temp of 80°C | 5'→3' exonuclease |
| Tth polymerase | Template (DNA or RNA, with Mg ²⁺ or Mn ²⁺) | DNA Pol in the presence of Mg ²⁺ |
| | Optimal temp of 74°C | RT in the presence of Mn ²⁺ |

Thermostable DNA polymerases with proofreading activity

| | | |
|---------------------|------------------------------------------|---------------------------------------------------------------------------|
| Pfu, Pow, Vent, Pab | Template, dNTPs, MgCl ₂ | 5'→3' polymerase 5'→3' exonuclease 3'→5' exonuclease (proofreading) |
|---------------------|------------------------------------------|---------------------------------------------------------------------------|

PCR from DNA template

Error rates: Taq (8.0×10^{-4}) < Tth (7.7×10^{-5})
< Bst (1.5×10^{-5})

Caution: for PCR reaction, annealing temperature is = or > to the lower T_m of primers; primers T_m should be compatible with enzyme stability.

PCR or RT-PCR, depending on buffer composition

High-fidelity PCR from DNA template.

Error rates: Pfu (1.3×10^{-6}) < Deep Vent (2.7×10^{-6}) < Vent (2.8×10^{-6}) < Pow (7.4×10^{-7})

Table 2 Main reverse transcriptases used in molecular biology

| Enzyme | Requirements | Activity | Main applications/notes |
|--------------------------------------------|----------------------------------------|---------------------------------------------------------------------------------------|------------------------------------------------|
| <i>Reverse transcriptases</i> | | | |
| AMV/MAV RT | RNA Template Primer | RT RNase H | Synthesis of cDNA from RNA (RT-PCR) |
| MuLV RT | RNA Template Primer | RT RNase H | RT-PCR for long transcripts |
| <i>Thermostable reverse transcriptases</i> | | | |
| Tth | Template Primer Mn ²⁺ | RT in the presence of Mn ²⁺ DNA Pol in the presence of Mg ²⁺ | PCR or RT-PCR, depending on buffer composition |
| MonsterScript™ RT | Template Primer | RT, no RNase H activity | RT-PCR for long transcripts |
| Klenow fragment of <i>C. therm</i> | Template Primer Mg ²⁺ | RT | RT-PCR |

Table 3 Main ligases used in molecular biology

| Enzyme | Requirements | Activity | Main applications/notes |
|--------------------------------------------|--------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| <i>DNA ligases</i> | | | |
| T4-DNA Ligase | Mg ²⁺ ATP | Connects blunt and cohesive ends in duplex DNA, RNA or DNA/RNA hybrids | Most frequently used for cloning |
| E. Coli DNA ligase | Mg ²⁺ NAD ⁺ | Connects preferentially cohesive double-stranded DNA ends, active on blunt ends DNA in the presence of Ficoll or polyethylene glycol | Ligation when blunt end or RNA/DNA ligation needs to be avoided |
| Thermostable DNA ligases (various sources) | | Ligation at high temperature | Not a substitute for T4 or E. Coli ligases, but used for specific techniques like LCR. |
| <i>RNA ligases</i> | | | |
| T4 RNA ligase 1 | ATP | Ligates single stranded nucleic acids and polynucleotides to RNA molecules | RNA labeling, primer extension |
| T4 RNA ligase 2 (T4 Rnl-2) | ATP | Ligates double-stranded RNA or connects dsRNA to dsDNA | Repair nicks in dsRNA |
| Truncated T4 RNL2 | | Ligates pre-adenylated 5' end of DNA or RNA to 3' end of RNA molecules | Optimized linker ligation for cloning of microRNAs |

Table 4 Main enzymes used for phosphate transfer or removal in molecular biology

| Enzyme | Requirements | Activity | Main applications/notes |
|---------------------------------------|-------------------------------------------|---------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|
| <i>Phosphate removal and transfer</i> | | | |
| Alkaline Phosphatase | Zn ²⁺ , Mg ²⁺ | Removes 5'-phosphate groups from nucleic acids | To prevent recircularization of DNA vectors in cloning experiments |
| T4 Polynucleotide Kinase | ATP, Mg ²⁺ , reducing agent | Transfers a phosphate group from ATP to 5'-OH terminus of a nucleic acid (neutral pH) 3'-phosphatase (acidic pH) | Nucleic acid labeling |
| Mutant T4 Polynucleotide Kinase | ATP, Mg ²⁺ , reducing agent | Transfers a phosphate group from ATP to 5'-OH terminus of a nucleic acid (neutral pH) | Same as T4 polynucleotide kinase, but no 3'-phosphatase activity |
| Tobacco Acid Pyrophosphatase | | Hydrolyzes pyrophosphate bonds in cap's triphosphate bridges | Removes cap of mRNA (First step in 5' mRNA labeling) |

Table 5 Main nucleases used in molecular biology

| Enzyme | Requirements | Activity | Main applications/notes |
|---------------------------|-------------------------------------------------|---------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|
| <i>Deoxyribonucleases</i> | | | |
| DNase I | Divalent cation (nature affects specificity) | Single or double-stranded DNA, isolated or incorporated in chromatin | Nick translation of DNA, Dideoxy sequencing, elimination of DNA in RNA or protein preparations, DNase footprinting |
| Exonuclease III | Mg ²⁺ or Mn ²⁺ | 3' exonuclease on double stranded DNA RNase H Phosphatase (pH<7.4) Endonuclease (pH>7.6) | DNA labeling (used with Klenow) or DNA length reduction (with nuclease S1) |
| Bal31 | Ca ²⁺ , Mg ²⁺ | Shorten duplex DNA (both ends) Endonuclease on single stranded DNA | Restriction mapping and shortening of DNA or RNA. |
| Exonuclease VII | | Degrades single-stranded DNA (both ends) No activity on RNA or DNA/ RNA hybrids | Removal of protruding ends from DNA Removal of primers from a completed PCR reaction |

Ribonucleases

| | | | |
|-----------------------------------|---------------------------------------------------|---------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|
| Pancreatic ribonuclease (RNase A) | | Degrades RNA in 3' Cleaves RNA in DNA/RNA hybrids at site of single mismatch | RNA elimination from DNA preparation Mutation mapping |
| Ribonuclease H (RNase H) | Reducing agents 7.5<pH<9.1 Mg ²⁺ | Degrades RNA in DNA/RNA hybrids | Removal of RNA probes Removal of polyA tails of mRNA |
| Phy I | | Cleaves RNA at G, A, and U, but not at C residues. | RNA sequencing |
| CL3 | | Cleaves RNA adjacent to cytidilic acid | RNA sequencing |
| Cereus | | Cleaves RNA at U and C residues | RNA sequencing |
| Phy M | | Cleaves RNA at U and A residues | RNA sequencing |
| RNase T1 | | Cleaves single-stranded RNA at G residues | RNA sequencing |
| RNase T2 | | Cleaves all phosphodiester bonds in RNA | 3'-terminal analysis of RNA and for RNase protection assays |
| RNase U2 | | Cleaves the 3'-phosphodiester bond adjacent to purines (standard conditions). Cleaves adenine residues (50°C) | RNA sequencing, in complement with RNase T1 |

DNA/RNA Nucleases

| | | | |
|--------------------|------------------------------------|-------------------------------------------------------------|------------------------------------------------|
| Nuclease S1 | Zn ²⁺ 4.0<pH<4.3 | Degrades RNA or single stranded DNA into 5' mononucleotides | Removes single-stranded protruding ends of DNA |
| Mung Bean Nuclease | Zn ²⁺ Reducing agent | Degrades single stranded DNA and RNA | Removes single-stranded protruding ends of DNA |

Nucleasas → DNAsas

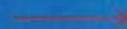
Endonucleasas inespecíficas.

Enzima

Molde

Aplicaciones

DNAsa I
(páncreas bovino)



Degrada DNA dc, sc. A bajas concentraciones genera nicks



Sondas, Nick translation
Dnasa footprinting

RQ I



Degrada DNA dc, sc
Libre de Rnasas



Extracciones de RNA

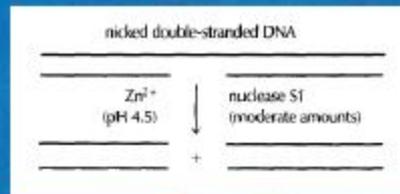
S I



Degrada DNA y RNA sc



Reparación de extremos DNA
Análisis estructura de híbridos
DNA:RNA



Mung Bean



Degrada DNA y RNA sc



Reparación de extremos DNA

Nucleasas → DNAsas

Endonucleasas sitio específicas – Enzimas de restricción.

Molde DNA dc

| | <i>Tipo I</i> | <i>Tipo II</i> | <i>Tipo III</i> |
|--------------------------------|---------------------------------------------|------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|
| Cofactor | ATP, Mg +2 | Mg +2 | ATP, Mg +2 |
| Sitio de reconocimiento | Se pega en sitio específico y corta al azar | Se pega en sitio específico, corta y se disocia De 4-8 nt | Se pega en sitio específico, corta y se disocia De 4-8 nt |
| Palindrómico | No | Si | No |
| Metilación | Mismo Polipéptido | Diferente Polipéptido | Mismo Polipéptido |
| Ejemplos | EcoAI GAG(Nx7)GTCA BI TGA(Nx8)TGCT | Bgl II A*GATCT BamH I G*GATCC N de I CATATG EcoRV GAT*ATC Kpn I GGTAC*C Sau3A I GATC Not I GC*GGCCGC | |

Metilasas

Molde

Aplicaciones

Dam metilasas

Metila dsDNA en la posición N6 de la Adenina en la secuencia GATC.

Bloquea y protege la acción de algunas ER que reconocen GATC, Ej: MboI (GATC) sensible, sSau3AI (GATC) no.
Digestión por enzimas que reconocen dam metilación, Ej: DpnI.

Dcm metilasas

Metila dsDNA en la posición C5 de la Citocina interna en la secuencia CCAGG o CCTGG.

Bloquea y protege la acción de algunas ER que reconocen CCAGG o CCTGG.