Table 1 Main DNA polymerases used in molecular biology

Enzyme	Requirements	Activity	Main applications/notes
Prokaryotic DNA polymerases			
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)	
Modified T7 DNA Pol	Template	5′→3′ polymerase	Amplification of large DNA fragments.
	Primer	3′→5′ exonuclease	Preparation of radioactive probes
	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 Mg^{2+} or Co^{2+}	Addition of a homopolymer tail to 3′-OH ends of DNA using single-stranded DNA primer (with Mg^{2+}) or double-stranted DNA primer with (Co^{2+})	Labeling DNA 3′ends with modified nucleotides
	No template		Addition of a homopolymer tail to 3′-OH ends of DNA

Thermostable DNA polymerases without proofreading activity

Bst polymerase	Template, $MgCl_2$	5′→3′ polymerase	PCR from DNA template
	Optimal Temp of 65°C	5′→3′ exonuclease	Error rates: Taq (8.0×10^{-4}) < Tth (7.7×10^{-5}) < Bst (1.5×10^{-5})
Taq polymerase	Template, $MgCl_2$	5′→3′ polymerase	Caution: for PCR reaction, annealing temperature is = or > to the lower Tm of primers; primers Tm should be compatible with enzyme stability.
	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+}	PCR or RT-PCR, depending on buffer composition
	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)	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 transctiptases used in molecular biology

Enzyme	Requirements	Activity	Main applications/notes
Reverse transcriptases			
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
Thermostable reverse transcriptases			
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 *C. therm*	Template Primer Mg^{2+}	RT	RT-PCR

Table 3 Main ligases used in molecular biology

Enzyme	Requirements	Activity	Main applications/notes
DNA ligases			
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.
RNA ligases			
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
Phosphate removal and transfer			
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
Deoxyribonucleases			
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

	Tipo I	Tipo II	Tipo III
Cofactor	ATP, Mg +2	Mg +2	ATP, Mg +2
Sitio de reconocimiento	Se pega en sitio especifico y corta al azar	Se pega en sitio especifico , corta y se disocia De 4-8 nt	Se pega en sitio especifico , 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.