Core course
BMS361N
Genetic Engineering

Manipulation of Purified DNA

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Manipulation of Purified DNA

DNA Manipulative Enzymes

DNA Manipulative Enzymes

- There are five broad classess
- Depending on type of reaction that they catalyze
  1. Nulcease
  2. Ligases
  3. Polymerases
  4. Modifying enzymes
  5. Topoisomerases

ENZYMES USED IN MOLECULAR BIOLOGY

Alkaline phosphatase	Removes phosphate groups from 5' ends of DNA (prevents unwanted re-ligation of cut DNA)
DNA ligase	Joins compatible ends of DNA fragments (blunt/blunt or complementary cohesive ends). Uses ATP
DNA polymerase I	Synthesises DNA complementary to a DNA template in the 5'-to-3'direction. Starts from an oligonucleotide primer with a 3' OH end
Exonuclease III	Digests nucleotides progressiviely from a DNA strand in the 3' -to-5' direction
Polynucleotide kinase	Adds a phosphate group to the 5' end of double- or single-stranded DNA or RNA. Uses ATP
RNase A	Nuclease which digests RNA, not DNA
Taq DNA polymerase	Heat-stable DNA polymerase isolated from a thermostable microbe (*Thermus aquaticus*)

The Nuclease

1. Exonucleases
2. Endonucleases

Nucleases

- Exonucleases
  - Remove nucleotides one at a time from the end of a DNA molecules
- Endonucleases
  - Able to break internal phosphodiester bonds within DNA molecules

Nuclease and Source	Substrates, Activity and Uses
Exonuclease III (E. coli)	Removes mononucleotides from the 3' termini of duplex DNA. The preferred substrates are DNAs with blunt or 5' protruding ends. It will also extend nicks in duplex DNA to create single-stranded gaps. In works inefficiently on DNA with 3' protruding ends, and is inactive on single-stranded DNA. Used most commonly to prepare a set of nested deletions of the termini of linear DNA fragments.
Mung Bean Nuclease (Mung bean sprouts)	Digests single-stranded DNA to 5'-phosphorylated mono or oligonucleotides. High concentrations of enzyme will also degrade double-stranded nucleic acids. Used to remove single-stranded extensions from DNA to produce blunt ends.
Nuclease BAL 31 (*Alteromonas*)	Functions as an exonuclease to digest both 5' and 3' ends of double-stranded DNA. It also acts as a single-stranded endonuclease that cleaves DNA at nicks, gaps and single stranded regions. Does not cleave internally in duplex DNA. Used for shortening fragments of DNA at both ends.
Nuclease S1 (*Aspergillus*)	The substrate depends on the amount of enzyme used. Low concentrations of S1 nuclease digests single-stranded DNAs or RNAs, while double-stranded nucleic acids (DNA:DNA, DNA:RNA and RNA:RNA) are degraded by large concentrations of enzyme. Moderate concentrations can be used to digest double-stranded DNA at nicks or small gaps. Used commonly to analyze the structure of DNA:RNA hybrids (S1 nuclease mapping), and to remove single-stranded extensions from DNA to produce blunt ends.
Ribonuclease T1 (*Aspergillus*)	An endonuclease that cleaves RNA at 3' phosphates of guanine residues, producing oligonucleotides terminal guanosine 3' phosphates. Used to remove unannealed regions of RNA from DNA:RNA hybrids.

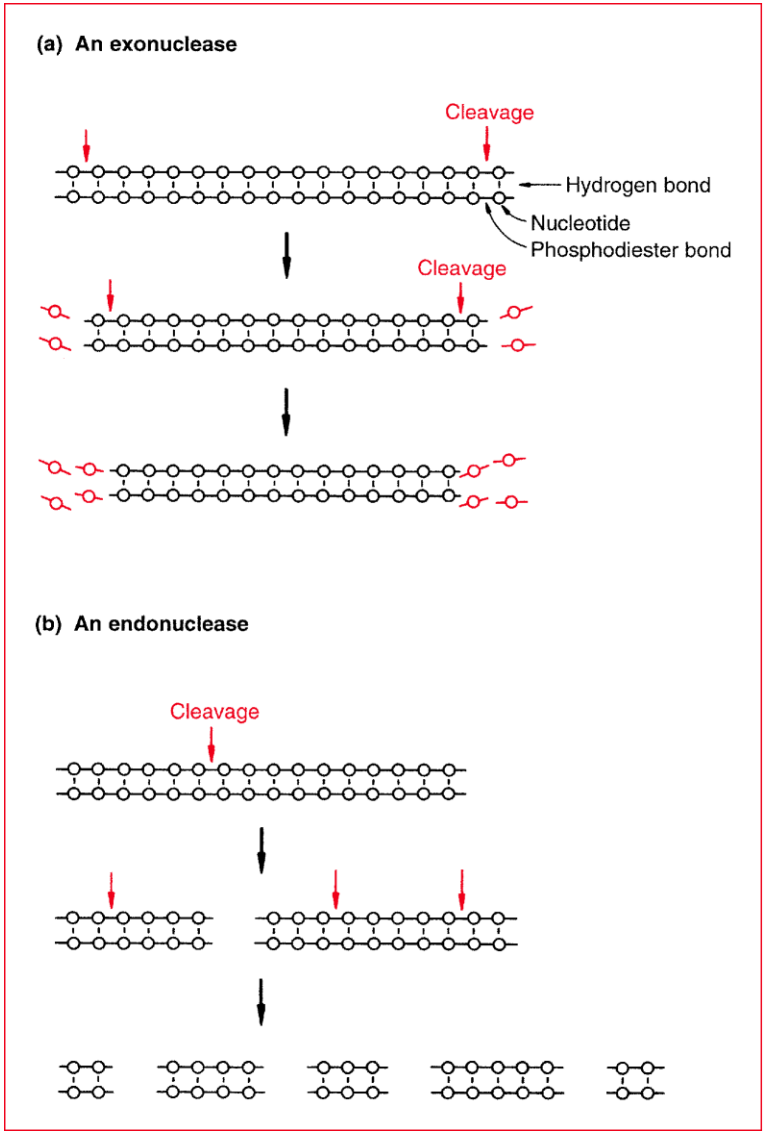
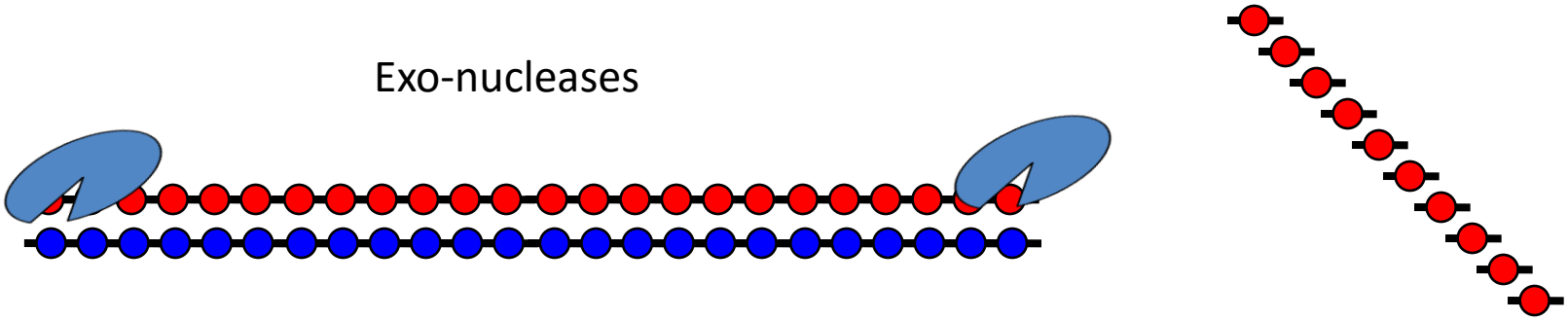
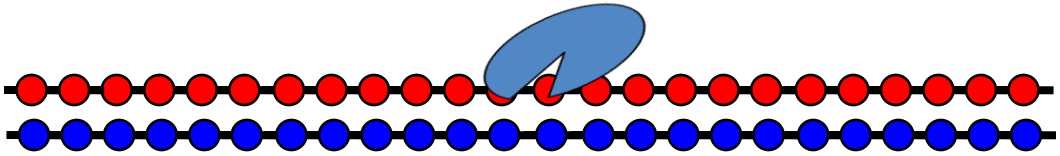


Figure 4.1 The reactions catalysed by the two different kinds of nuclease. (a) An exonuclease, which removes nucleotides from the end of a DNA molecule. (b) An endonuclease, which breaks internal phosphodiester bonds.

Exo-nucleases

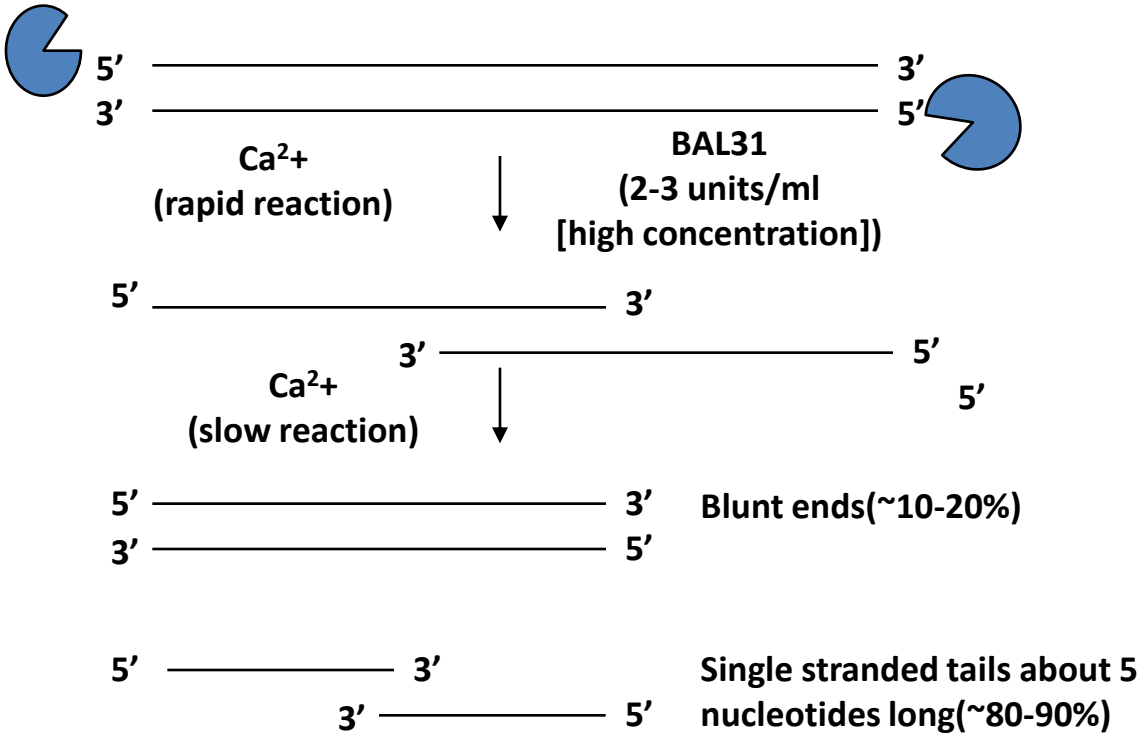


Endo-Nucleases



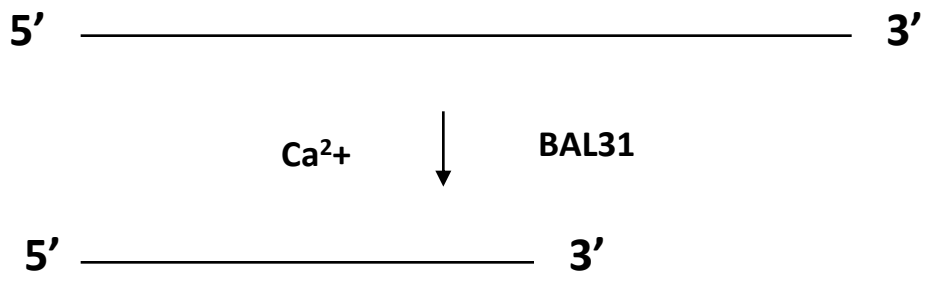
BAL 31 Nuclease(*Alteromonas espejiana BAL 31);*

- Activity: Exonuclease/endonuclease
- Substrate: ds DNA sequentially from both termini
- Reaction:



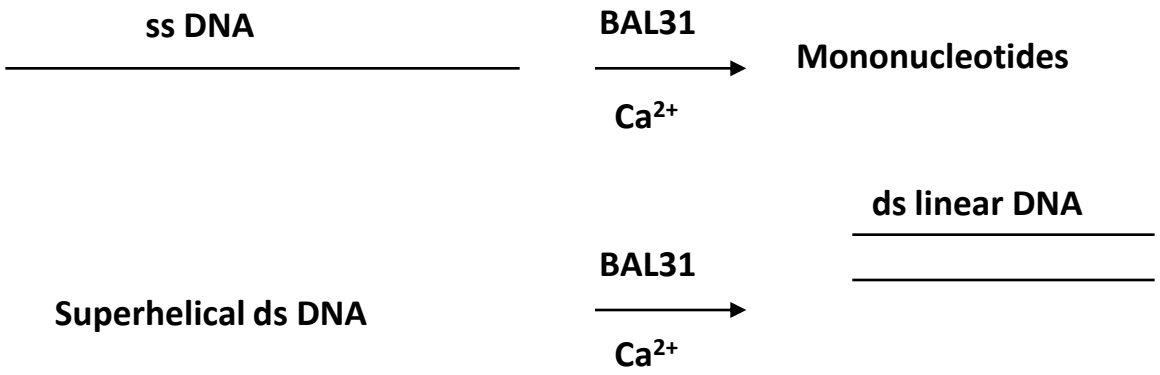
BAL31 Nuclease(1)

- Activity: Exonuclease(shortens ss DNA)
- Substrate: ss DNA with 3'-hydroxyl termini
- Reaction



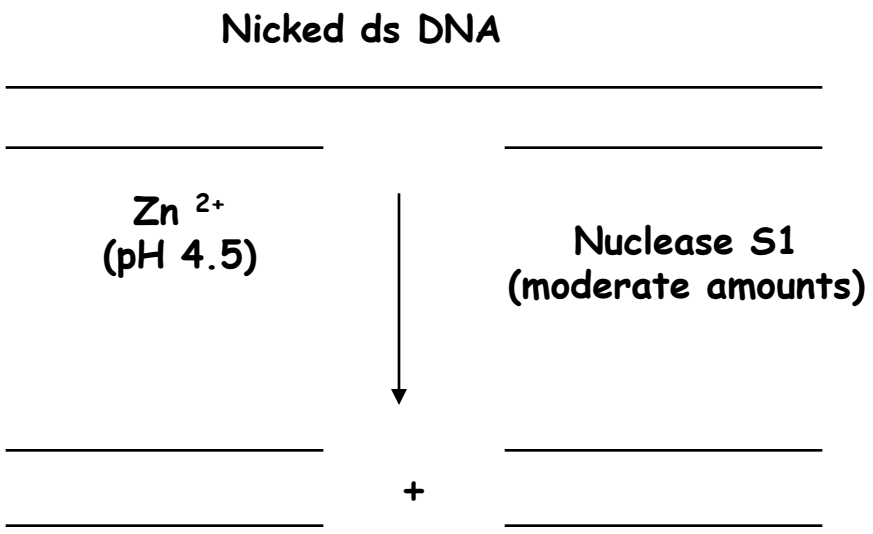
BAL31 Nuclease(2)

- Activity: Endonuclease
- Substrate: ss DNA; supercoiled DNA; DNA with B-DNA, Z-DNA junctions and other non-B DNA Conformations
- Reaction:



Nuclease S1

- Activity: single strand specific nuclease
- Substrate: ss DNA or RNA/ more active on DNA than on RNA
- Reaction: ss DNA or RNA → $^{5'}_{p}$dN or $^{5'}_{p}$rN



Mung Bean Nuclease (Mung bean sprouts)

Mung Bean Nuclease belongs to a class of enzymes that demonstrate a preference for single-stranded nucleic acids, lack sugar specificity and hydrolyze single-stranded substrates to products with 5'-phosphoryl and 3'-hydroxyl termini.

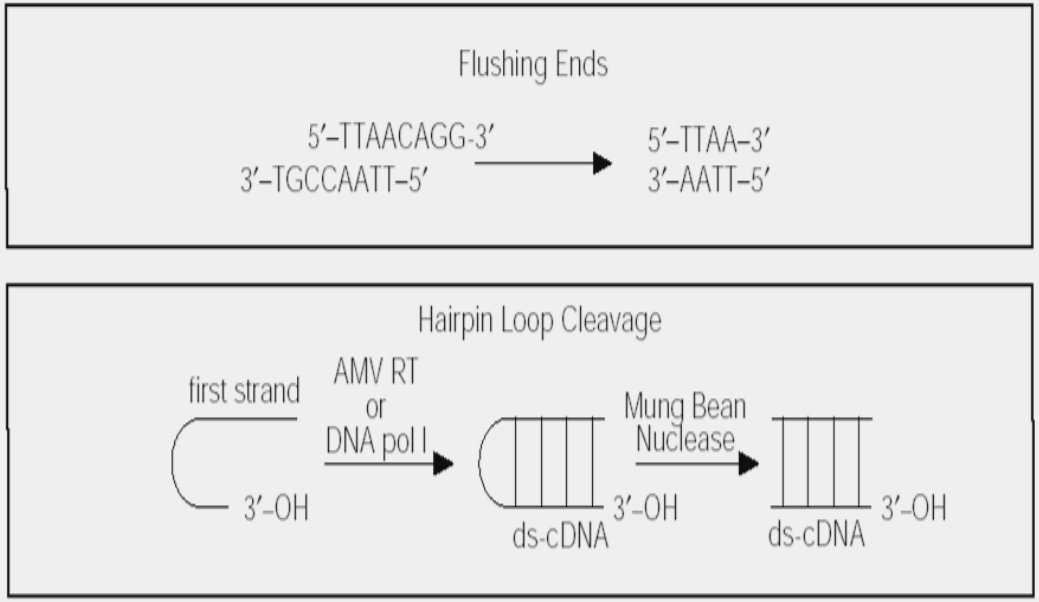


Figure 1. Mung Bean Nuclease catalyzes the generation of blunt-ended DNA and also cleaves hairpin loops to separate cDNA strands.

Exonuclease III (*E.coli*) (1-2)

A double-strand specific, nonprocessive 3´→5´ exodeoxyribonuclease activity; however, 3´-overhangs of ≥4 bases are protected from Exo III activity (1).

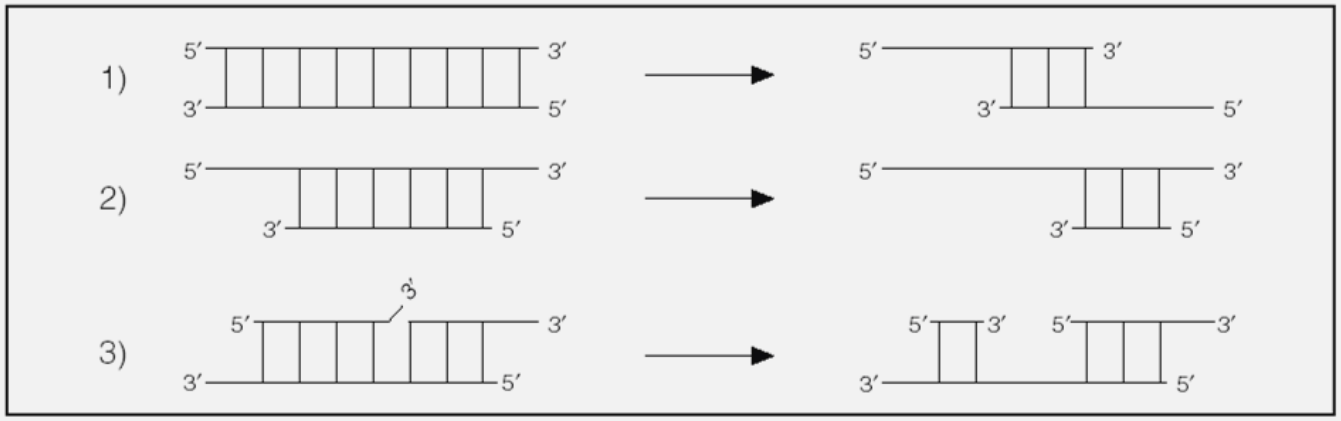
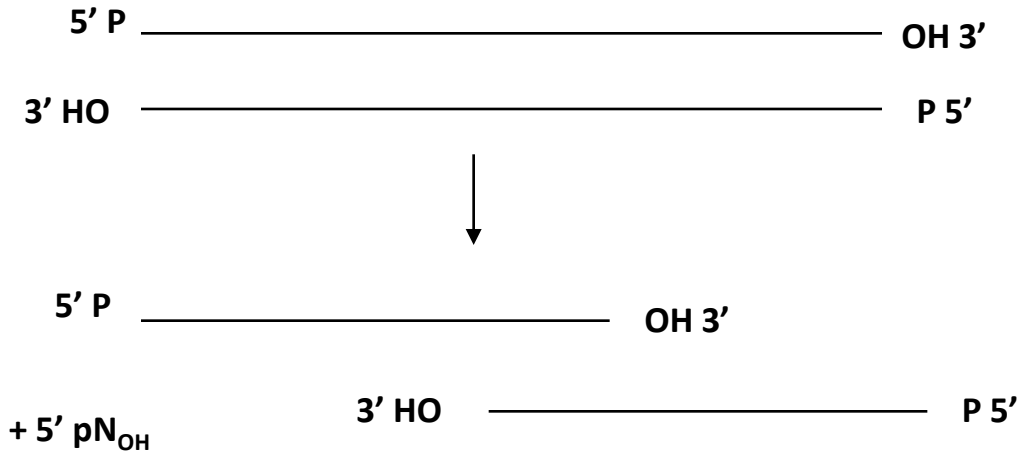


Figure 1. Exonuclease III catalyzes the stepwise removal of mononucleotides starting from a 3´-OH at: 1) blunt ends, 2) recessed ends and 3) nicks. Exonuclease III will also act on 3´-overhangs of less than 4 bases (not shown). Note that the 3´-overhangs shown in 3) are ≥4 bases and therefore not susceptible to Exonuclease III activity.

Exonuclease III (*E.coli*) (1)

- Activity: 3′ Exonuclease
- Substrate:
  - active on 3′-hydroxyl termini of ds DNA with blunt ends or with ends containing unpaired 5′-termini and recessed 3′-termini.
  - 3′-hydroxyol termini at nicks in ds DNA
  - The DNA must contain phosphodiester bonds; thioester are not cleaved
- Reaction:



Exonuclease III (*E.coli*) (2)

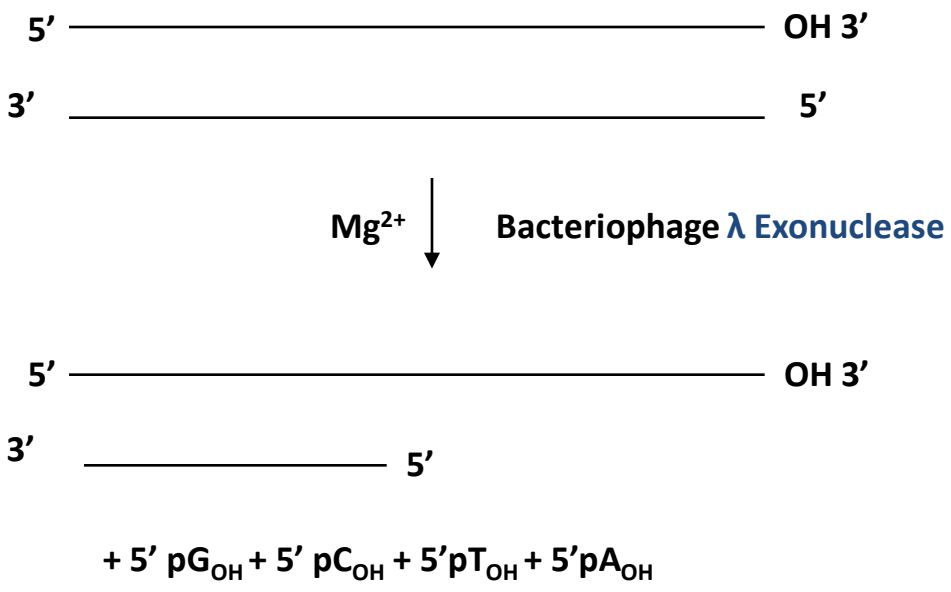
- Activity: 3′ phosphatase
- Substrate: ds or ss DNA with a 3′-phospate terminus; internal phosphodiester bonds are not cleaved.

- Reaction:

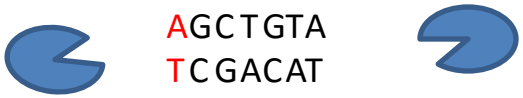
Bacteriophage λ Exonuclease

(Bacteriophage λ-infected *E.coli*)

- Activity: 5′ Exonuclease
- Substrate: ds DNA with 5′-phosphate termini or with protruding 5′-termini
- Reaction:



Exonucleases

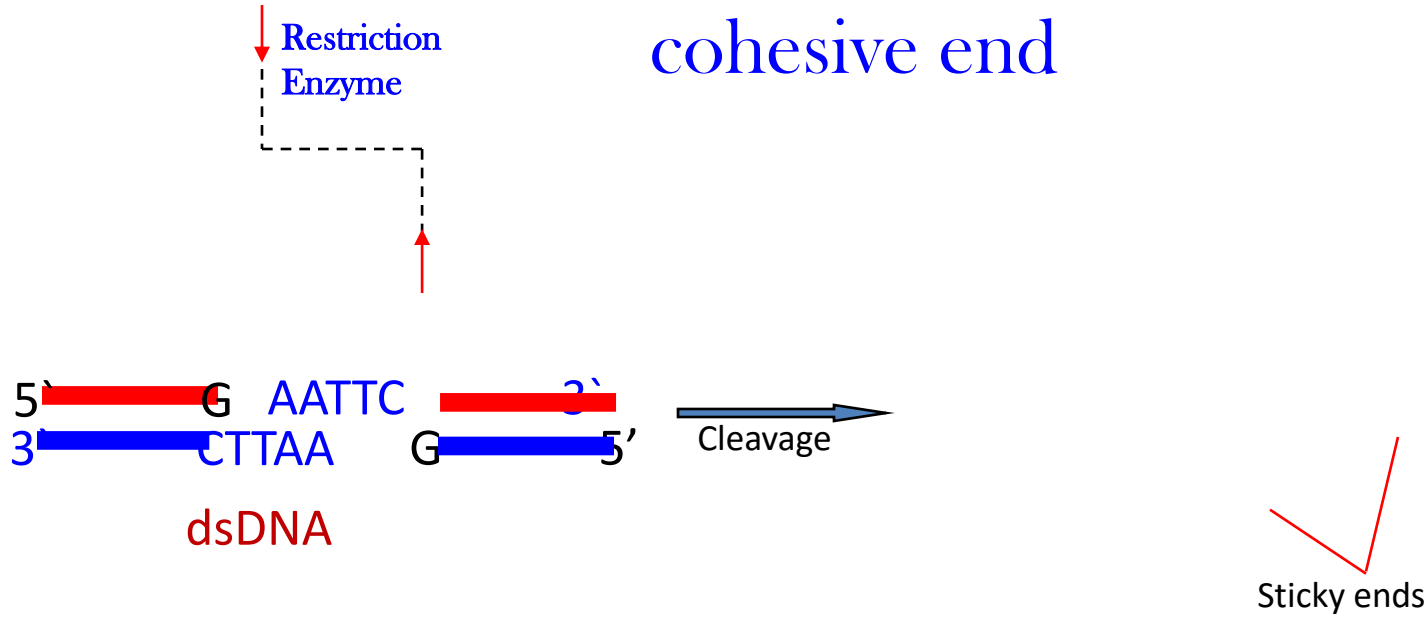


CGATGCT

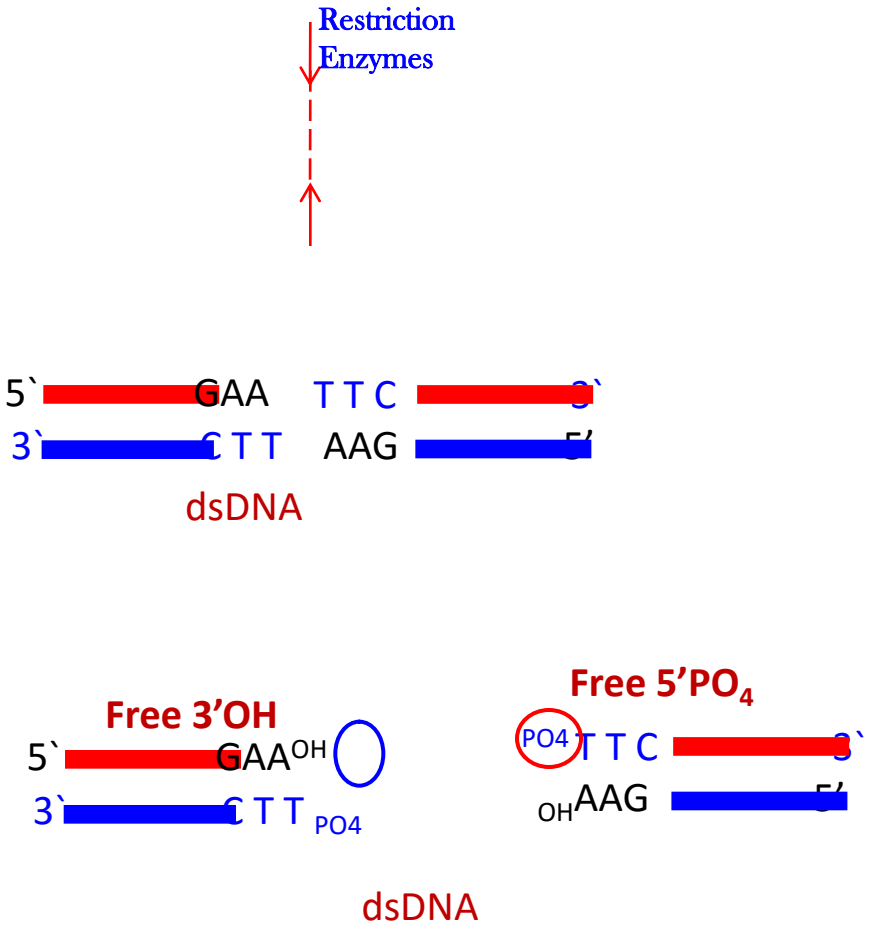
EndoNucleases

TACGTCCGTGAC GTGATCGACGTGCA
ATGCAGGCACTGCGATGCTCACTAGCTGCACGT

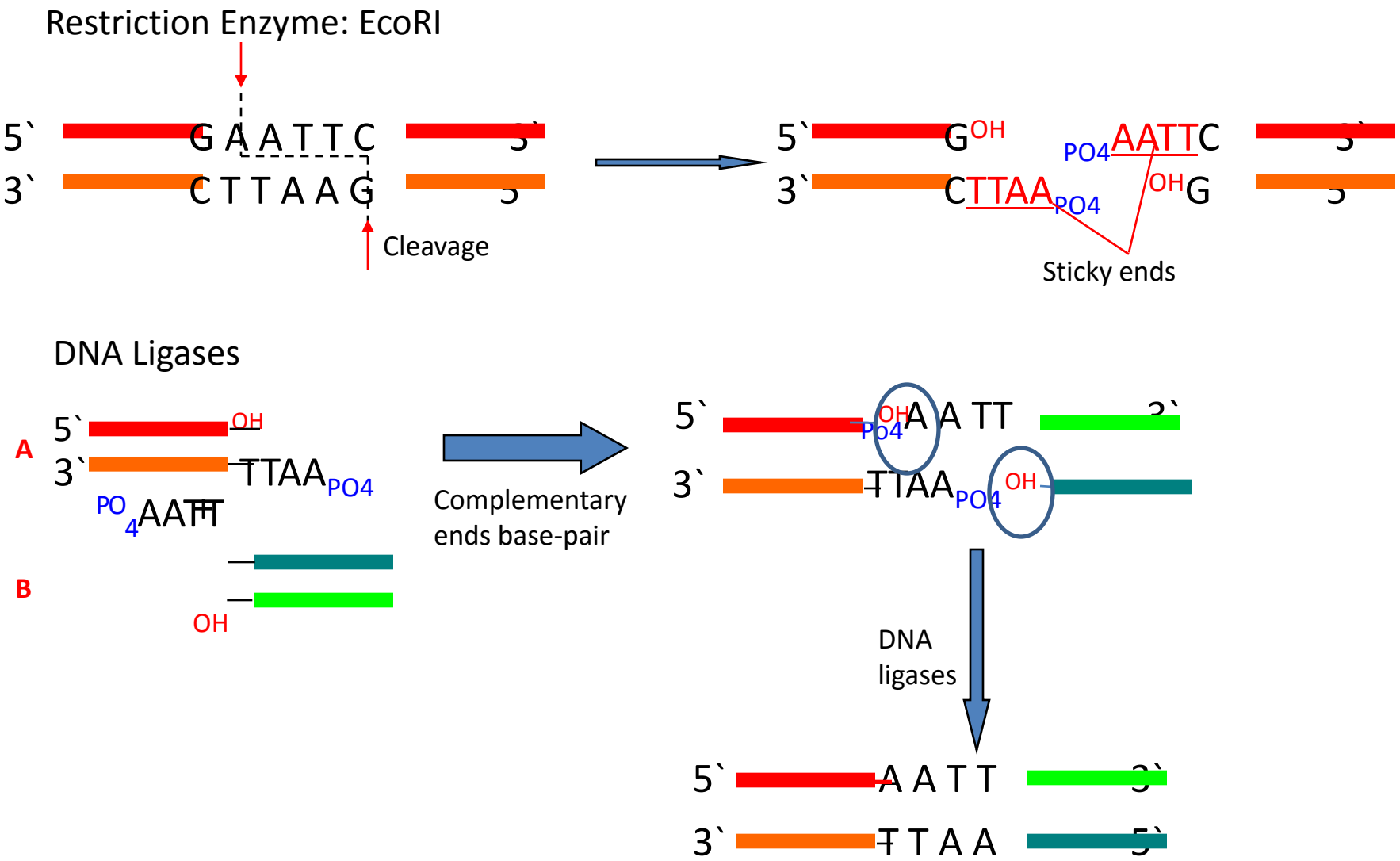
Restriction Enzyme which gives rise to cohesive end



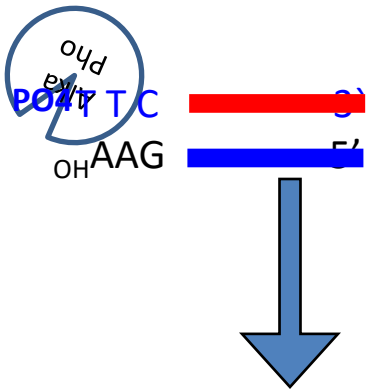
Restriction Enzyme which gives blunt end

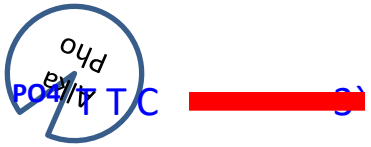


Mechanism


Restriction Enzyme: EcoRI
5`
G A A T T C
3`
C T T A A G
Cleavage
5`
G OH
3`
C TTAA PO4
PO4 AATTC
OH G
Sticky ends
DNA Ligases
A
5`
OH
3`
TTAA PO4
PO4 AATT
B
OH
Complementary ends base-pair
5`
OH A A TT
PO4
3`
TTAA PO4
OH
DNA ligases
5`
A A T T
3`
T T A A

Alkaline phosphatase:



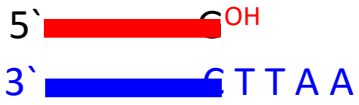


CIP enzyme cleaves the free 5' PO_4 group in dsDNA, ssDNA or RNA

ALP enzyme cleaves the 5' PO_4 group of both dsDNA and ssDNA

Poly Nulceotide Kinase

AT P

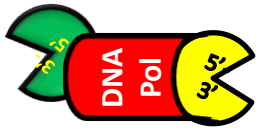


Free 5'OH

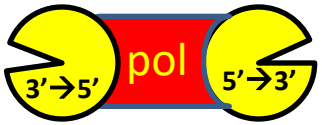
Free 5'PO_4

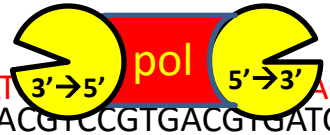
Klenow enzyme

AATT

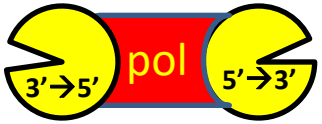



pol
3’→5’
5’→3’
ATGCAGGCACTGCACT
ACGCATGCAGGCACTGCACTAGCTGCACGT
TACGTCCGTGACGTGA
TGCGTACGTCCGTGACGTGATCGACGTGCA



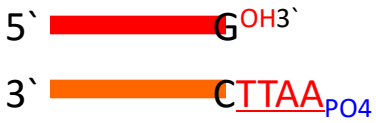


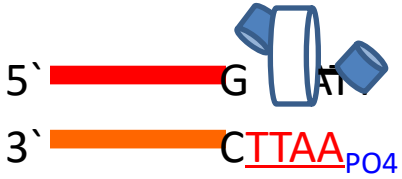
ATAGCTGCACGTTTGACGCATGCAGGCACTGCACTAGCTGCACGT
TACGTCCGTGACGTGATCGACGTGCAAACTGCGTACGTCCGTGACGTGATCGACGTGCA



ATGCAGGCACTGCACTAGCTGCACGT GTGACGCATGCAGGCACTGCACTAGCTGCACGT
TACGTCCGTGACGTGATCGACGTGCAAACTGCGTACGTCCGTGACGTGATCGACGTGCA

Klenow enzyme action





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The End

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