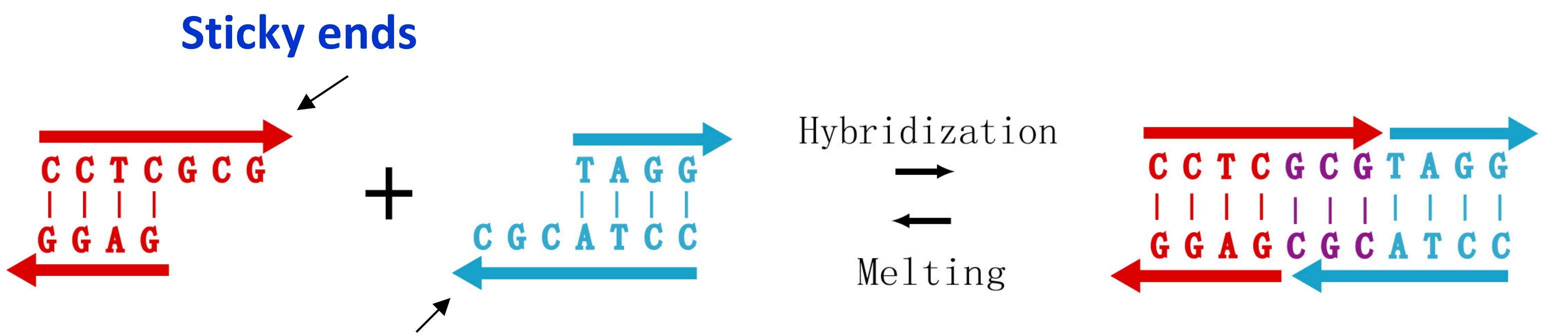
DNA Enzymes

- Ligation enzymes
- Restriction enzymes
- Polymerase enzyme
- Strand-displacing polymerases
- Helicase enzymes

Discovery and Obtainability:

- **Most enzymes are proteins discovered in cells.**
- **But DNAzymes were discovered by protocols using In vivo-evolution**
- **Obtainable from Scientific supply companies.**

DNA Hybridization



DNA Hybridization:

- **Two single-stranded complementary DNA form a double-stranded DNA.**

- **Is not an enzymic reaction**

www.cs.duke.edu/~reif/paper/peng/.../talk.WalkerDesign.**ppt**

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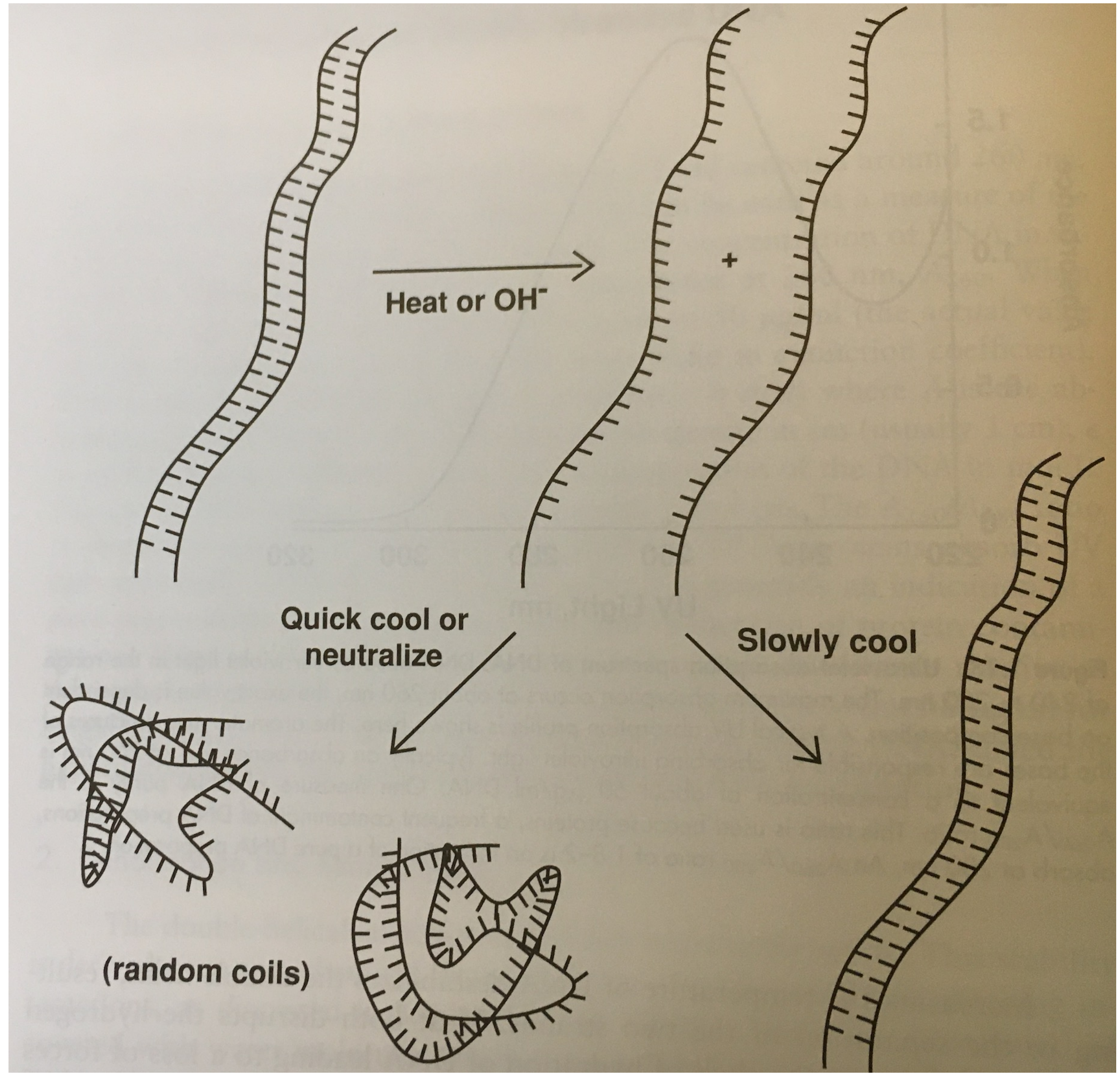
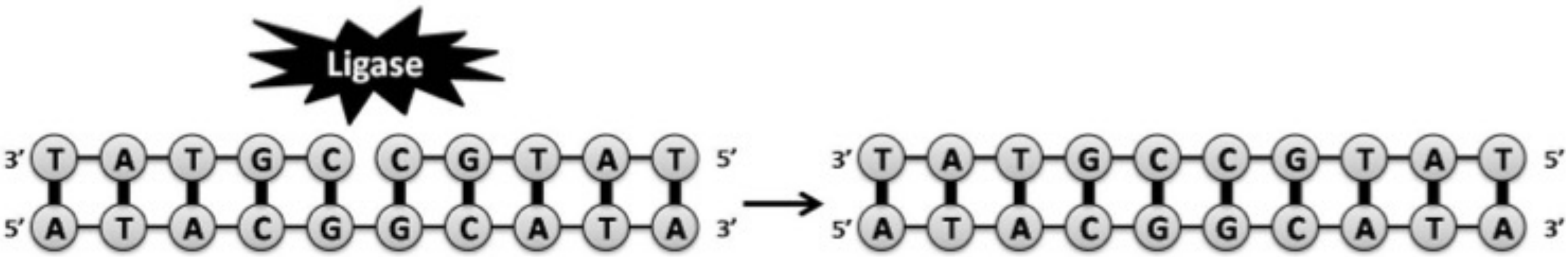
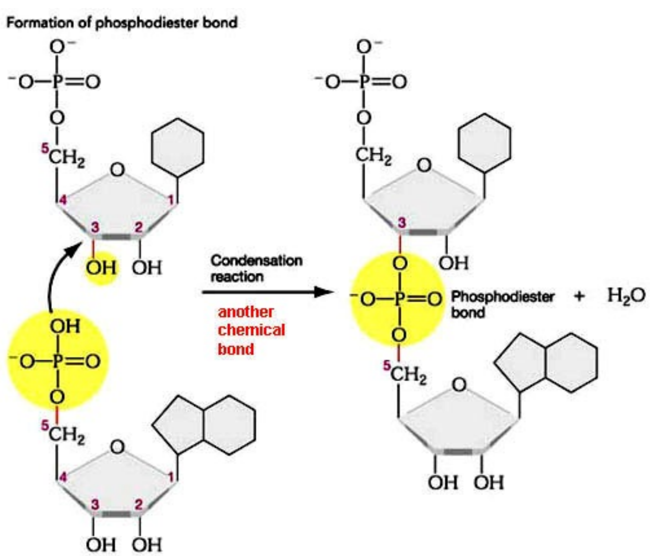


Ligation: Ligase – “to bind” or “to glue together”



Ligase healing a single stranded nick. *Note that the two parts are bound to the same template*

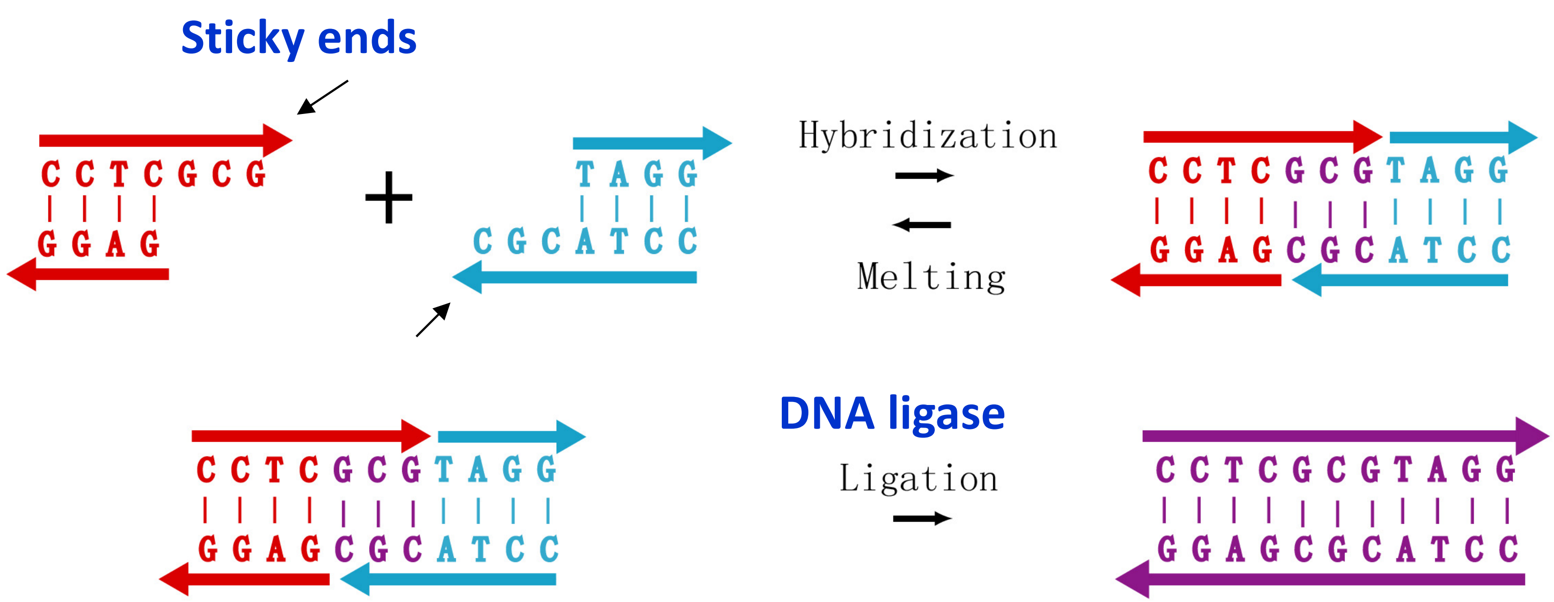


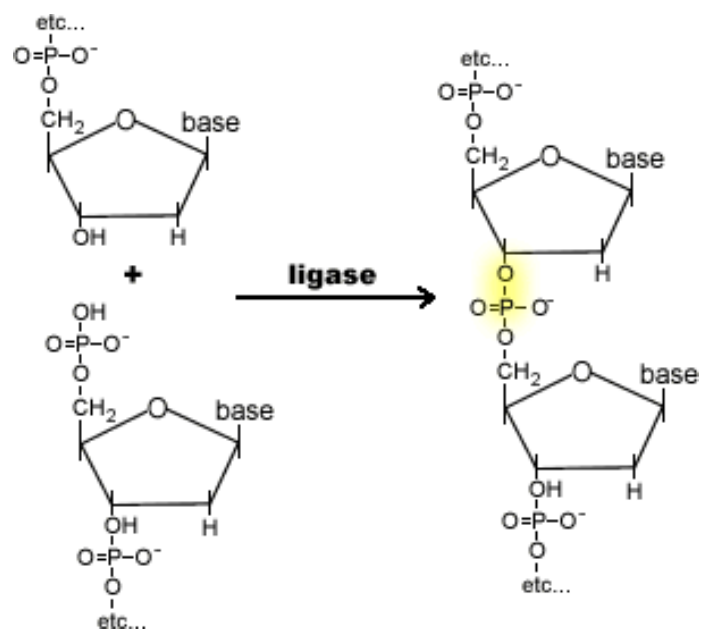
T4 DNA Ligase – a single polypeptide, MW ~ 86,000 daltons, (pH 7.5 – 8.0, 10 mM Mg2+, DTT, NaCl 200 mM to stop reaction)

[Self-assembled DNA Nanostructures and DNA Devices, Nanofabrication Handbook, Taylor and Francis 2012, with Nikhil Gopalkrishnan, Thom LaBean and John Reif]

http://www.bio.miami.edu/dana/pix/phosphodiester.jpg

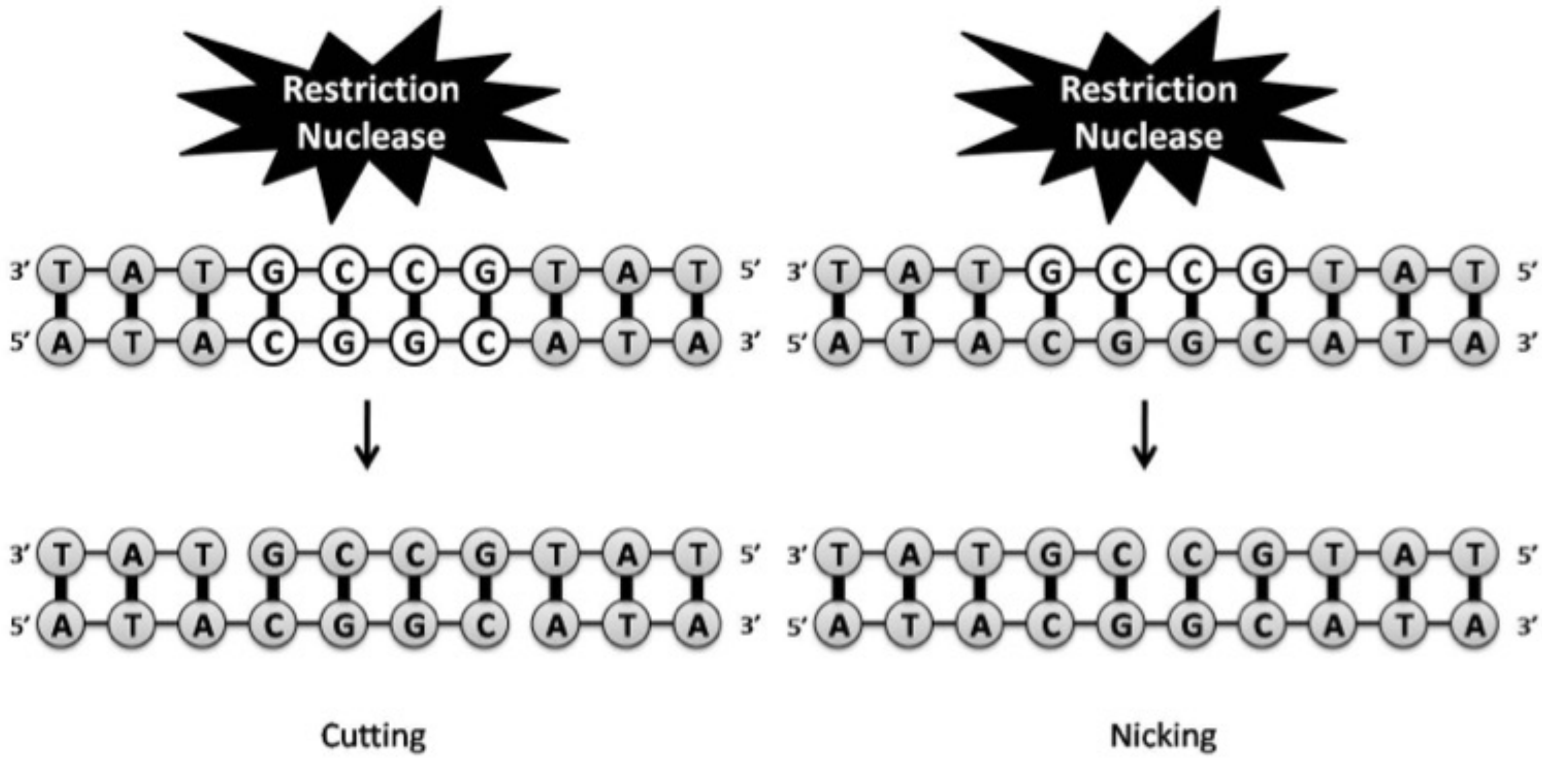
DNA Hybridization & Enzyme Ligation activity





www.cs.duke.edu/~reif/paper/peng/.../talk.WalkerDesign.**ppt**
http://www.angelfire.com/sc3/toxchick/molbiolab/molbiolab01.html

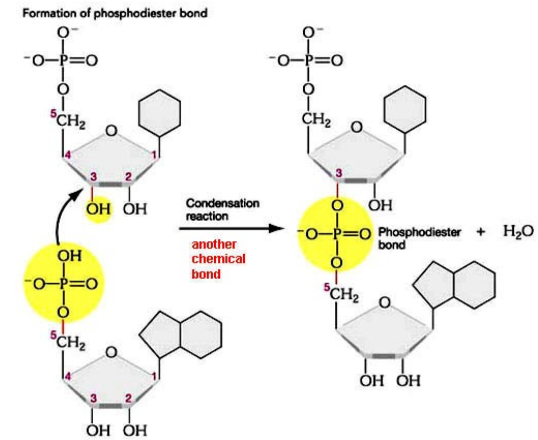
Restriction Enzymes

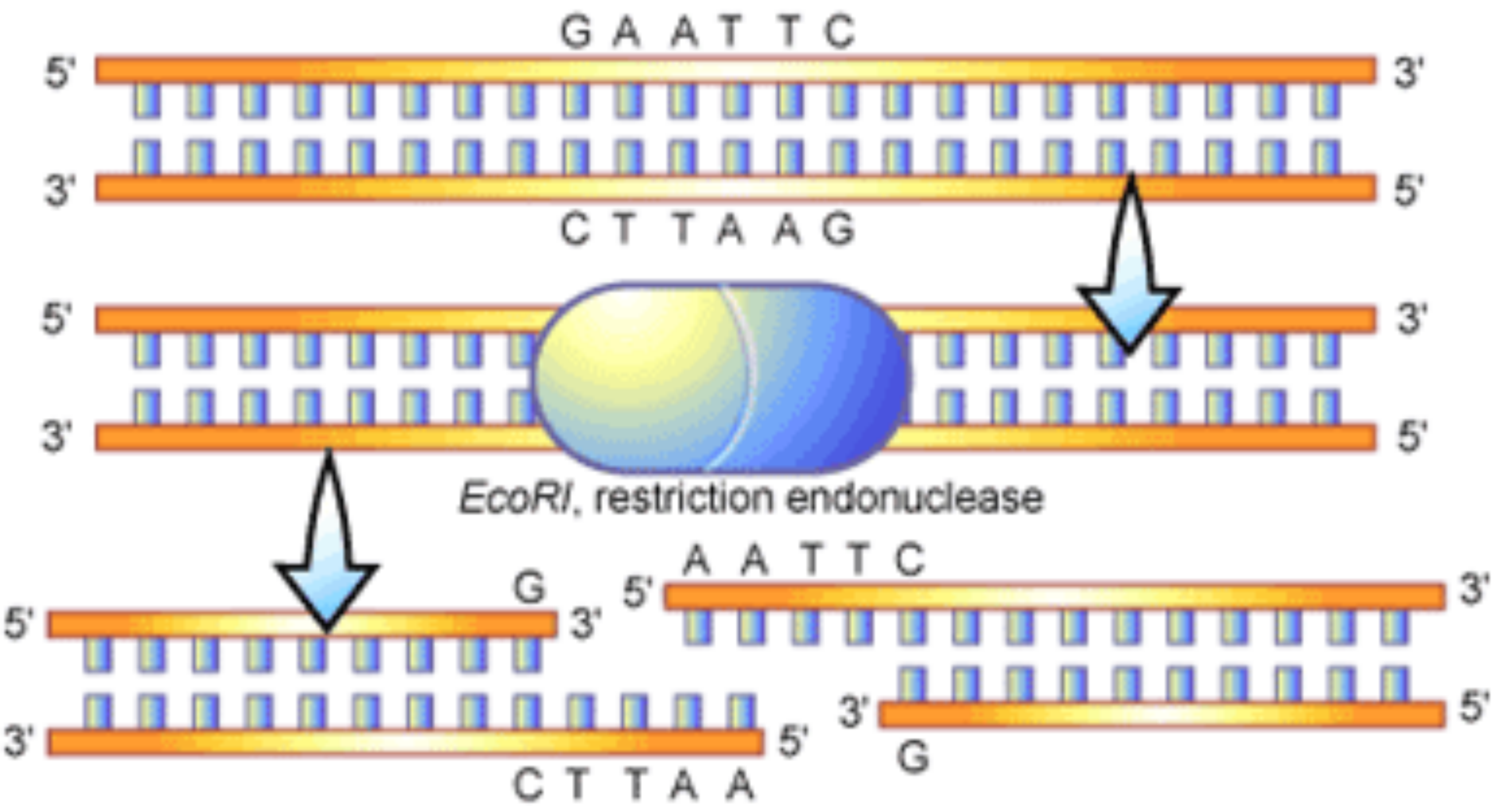


Example of restriction enzyme cuts of a single stranded DNA sequence. *The subsequence recognized by the nuclease is unshaded*

Self-assembled DNA Nanostructures and DNA Devices, Nanofabrication Handbook, Taylor and Francis 2012, with Nikhil Gopalkrishnan, Thom LaBean and John Reif

Restriction Enzymes

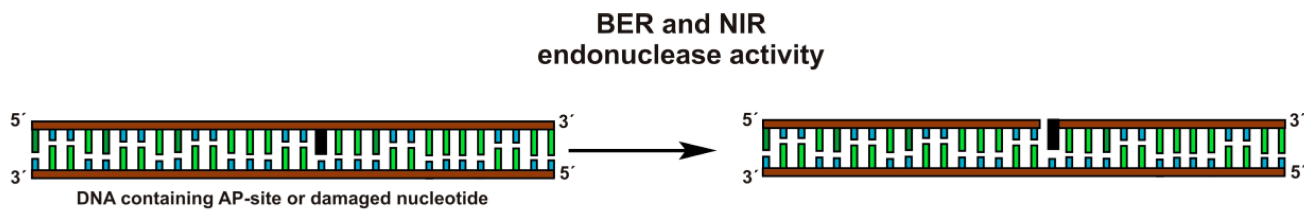




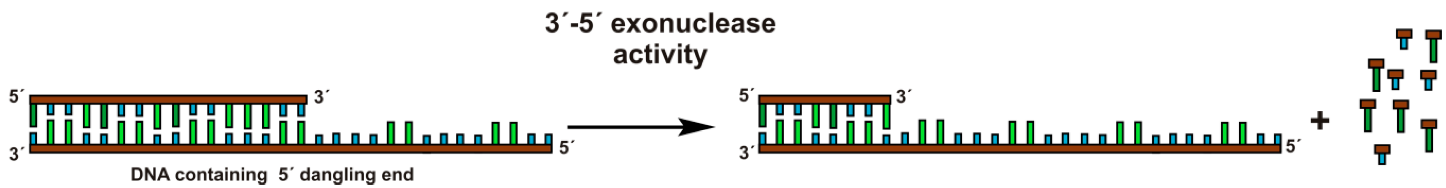
http://www.scq.ubc.ca/restriction-endonucleases-molecular-scissors-for-specifically-cutting-dna/

Exonucleases & Endonucleases

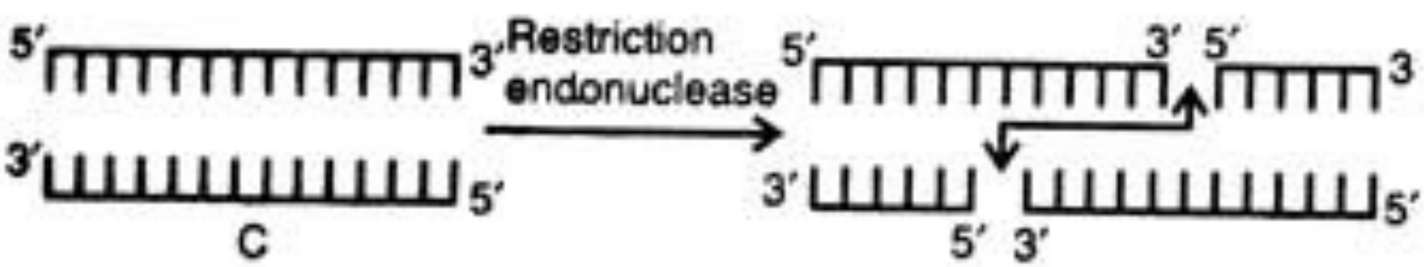
- Endonuclease



- Exonuclease

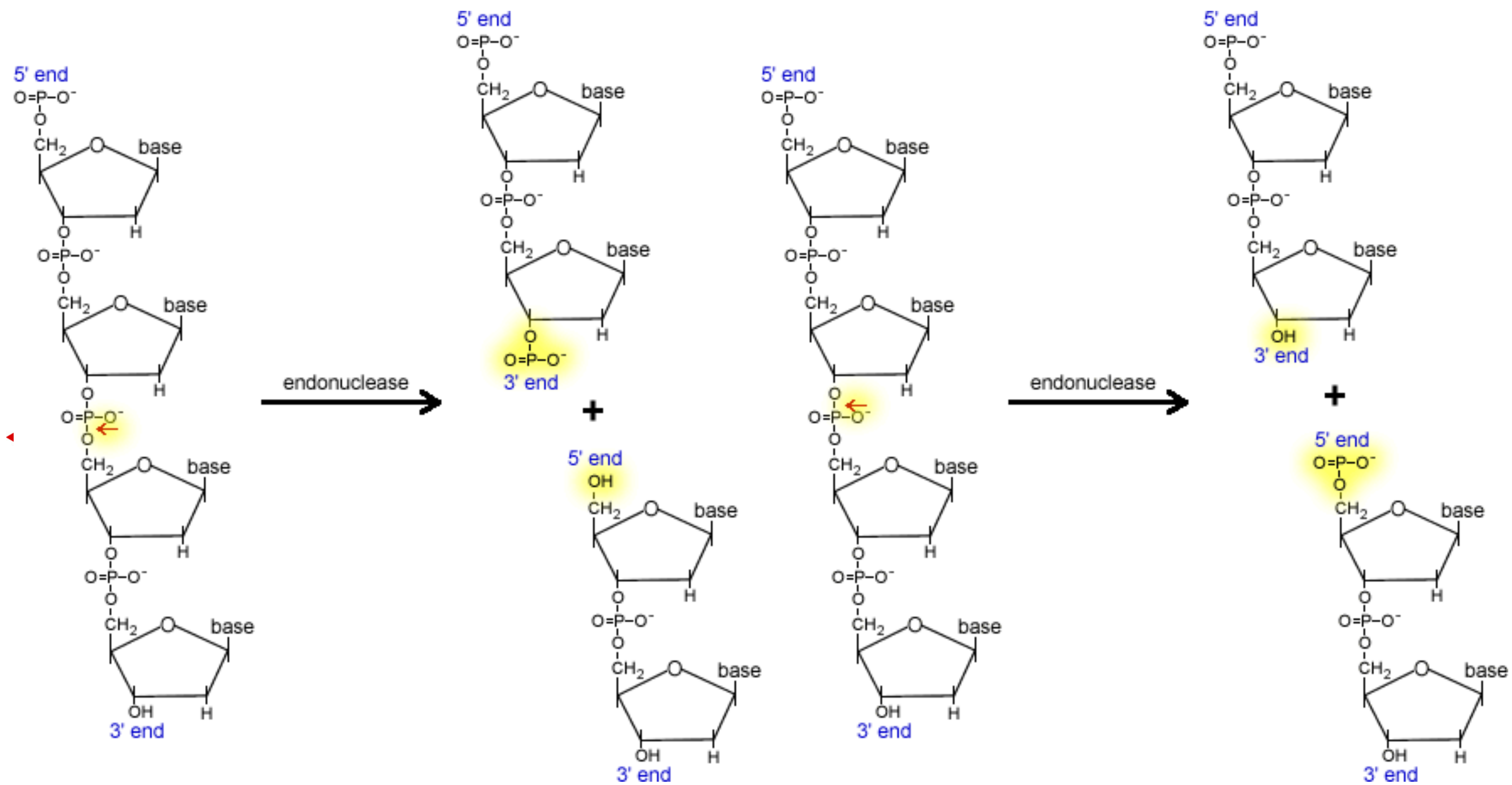


- Restriction Endonucleases
  - Type I – cut elsewhere of recognition sites
  - Type II – cut within recognition sites

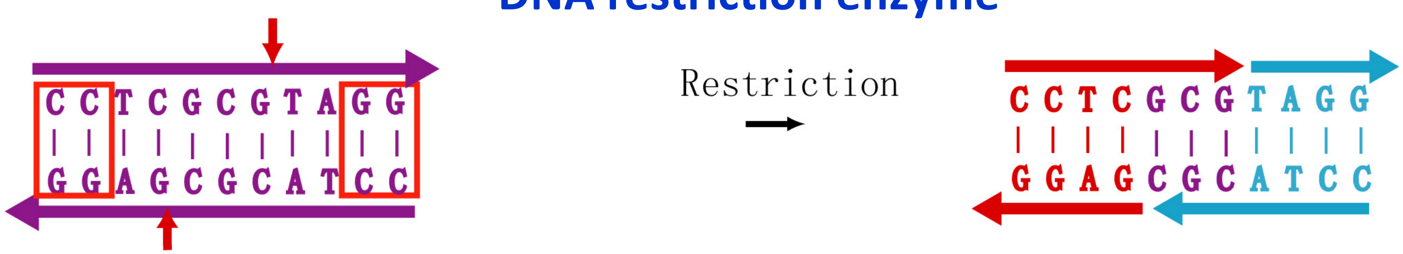


http://www.eplantscience.com/index_files/biotechnology/Genes%20&%20Genetic%20Engineering/Tools%20of%20Genetic%20Engineering/biotech_enzymes.php

Restriction enzyme action



DNA restriction enzyme



[www.cs.duke.edu/~reif/paper/peng/.../talk.WalkerDesign.**ppt**](www.cs.duke.edu/~reif/paper/peng/.../talk.WalkerDesign.ppt)

http://www.angelfire.com/sc3/toxchick/molbiolab/molbiolab01.html

Restriction Enzymes

Some restriction enzymes produce "sticky" ends:

Other restriction enzyme's cleavage produces "blunt" ends:

http://www.scq.ubc.ca/restriction-endonucleases-molecular-scissors-for-specifically-cutting-dna/

Restriction Endonucleases

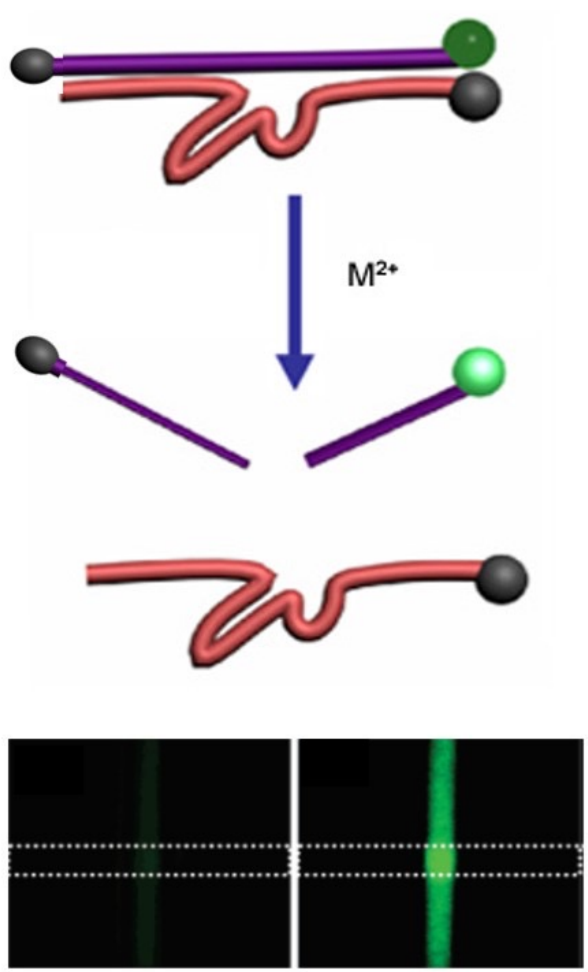
- Nicking Enzymes
- Restriction Enzymes

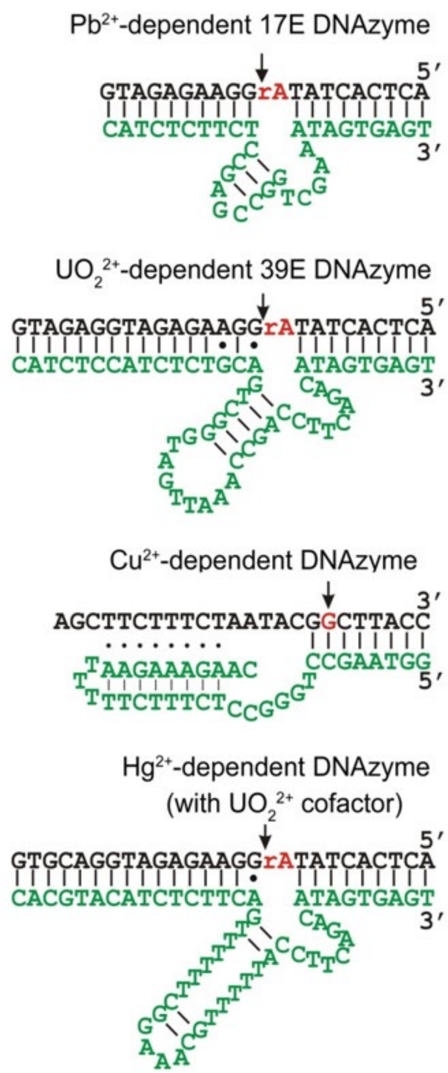
Enzyme	Sequence	Cut Site	Overhang	Properties (NEB Enzymes Only)
BstUI	CGCG	C G/C G G C/G C	blunt	NEB4 60° No

Enzyme	Sequence	Cut Site	Overhang	Properties (NEB Enzymes Only)
BfaI	CTAG	C/T A G G A T/C	5´ - TA	NEB4 37° Yes
CviQI	GTAC	G/T A C C A T/G	5´ - TA	RR NEB3 BSA 25° No

Enzyme	Sequence	Cut Site	Overhang	Properties (NEB Enzymes Only)
AciI	CCGC	C/C G C G G C/G	5´ - CG	RR NEB3 37° Yes
BmgBI	CACGTC	C A C/G T C G T G/C A G	blunt	RR NEB3 BSA 37° Yes

DNAzymes: DNA strands with a few reactive RNA bases





Pb^{2+}: Detection limit: 1 nM
EPA MCL: 75 nM

UO_2^{2+}: Detection limit: 45 pM
EPA MCL: 126 nM
ICP-MS: 420 pM

Cu^{2+}: Detection limit: 35 nM
EPA MCL: 20 μM

Hg^{2+}: Detection limit: 2.4 nM
EPA MCL: 10 nM

DNAzymes are found using in-vivo evolution protocols

Montypython.scs.uiuc.edu

Programmable Restriction Enzymes from CRISPR Systems

Cas proteins *[Jansen et al 2002]:*

Programmable Restriction Enzymes from CRISPR systems.

- **CRISPR are natural systems for cellular repair: They fix the breaks in the cell's nucleic acids, allowing for gene insertion, deletion, or modification. They act like molecular scissors for gene editing.**
- **They use guide RNA (gRNA) to find and cut specific DNA or RNA sequences.**
- **The gRNA base-pairs with the target sequence of a nucleic acid, guiding the Cas enzyme to create a double-strand break (DSB) or other modifications**

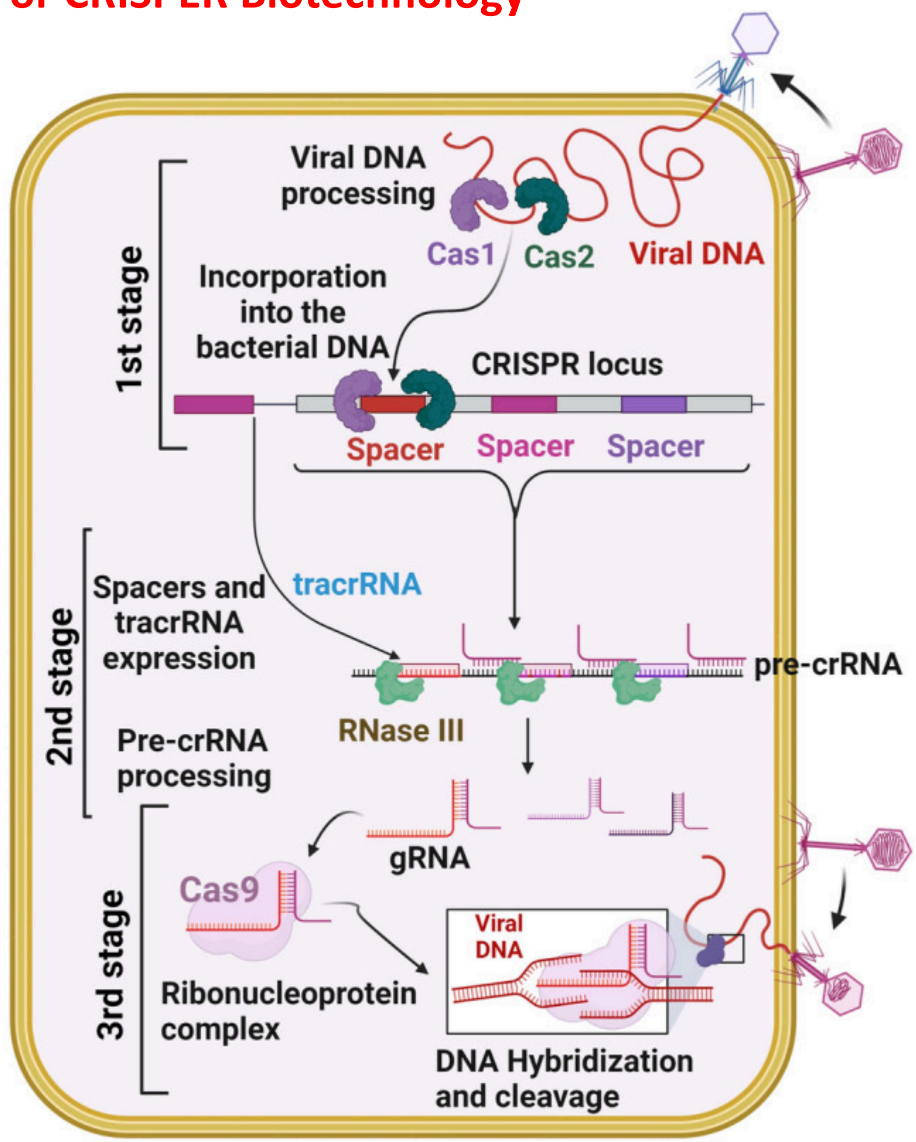
Example Cas proteins:

- **Cas9 cuts targeted sites of DNA**
- **Cas13 cuts targeted sites of RNA**

Significance: [2005]

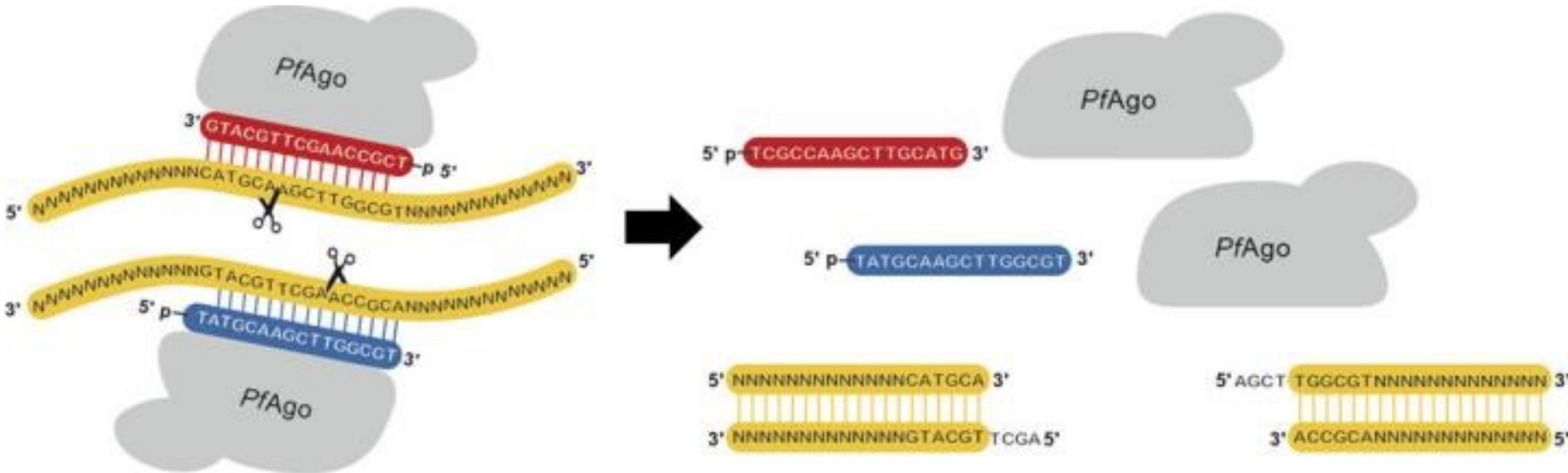
- **Restriction enzymes have fixed recognition sites,**
- **Cas systems can be programmed with any gRNA to target virtually any DNA sequence.**
- **Enables precise gene editing for research, diagnostics, and potential medical therapies.**

2020 Nobel Prize in Chemistry: awarded to Emmanuelle Charpentier and Jennifer Doudna For CRISPER Biotechnology



Edwin Hillary 1, S Antony Ceasar, A Review on the Mechanism and Applications of CRISPR/Cas9/Cas12/Cas13/Cas14 Proteins Utilized for Genome Engineering V 1, Mol Biotechnol, 2022

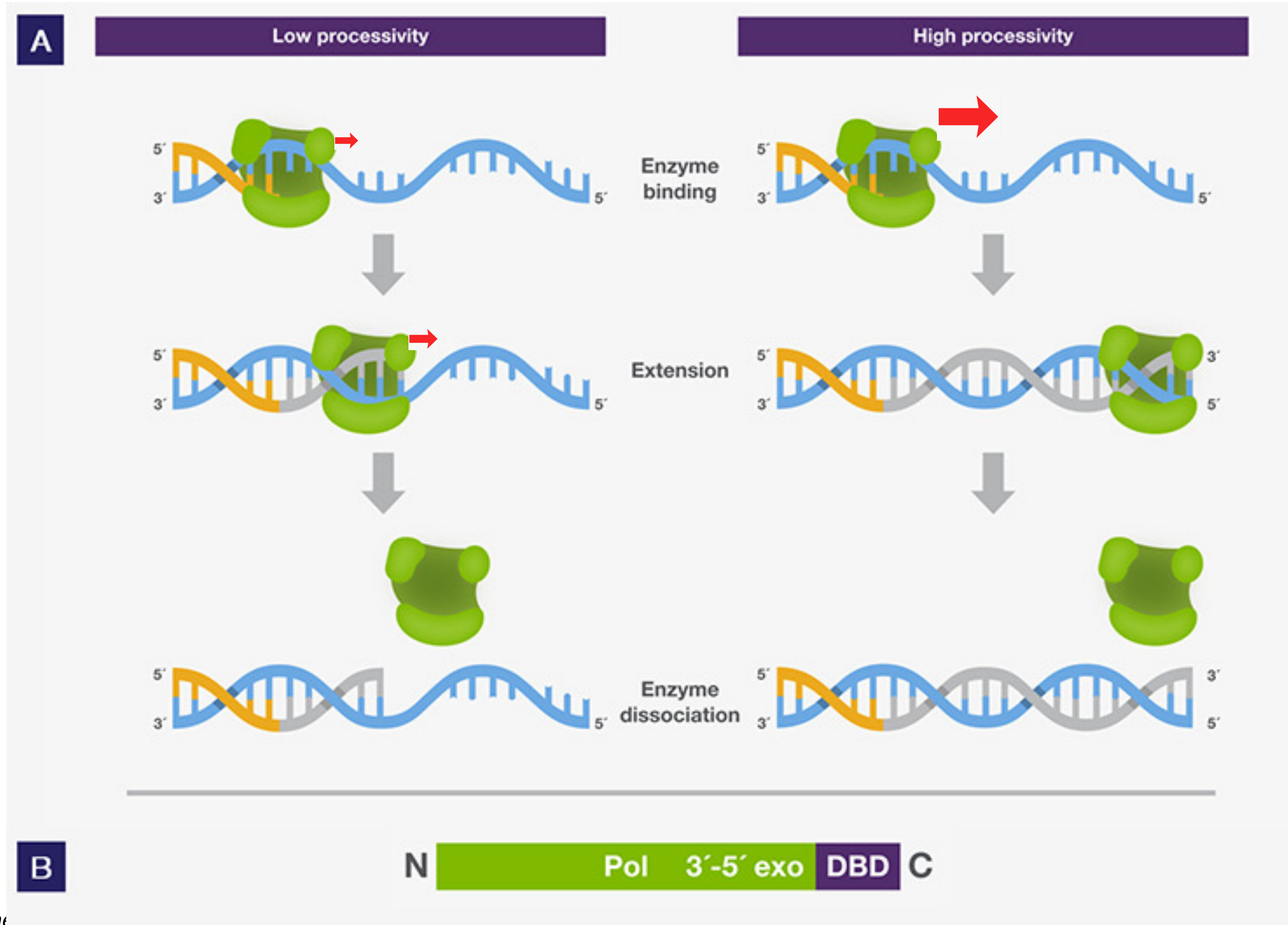
Artificial Restriction Enzymes (AREs)



- **The Pyrococcus furiosus Argonaute (PfAgo)-based platform provides generation of artificial restriction enzymes (AREs) which can be programmed to:**
  - **Recognize and cleave DNA sequences at arbitrary sites and**
  - **Generate defined sticky ends of varying length.**

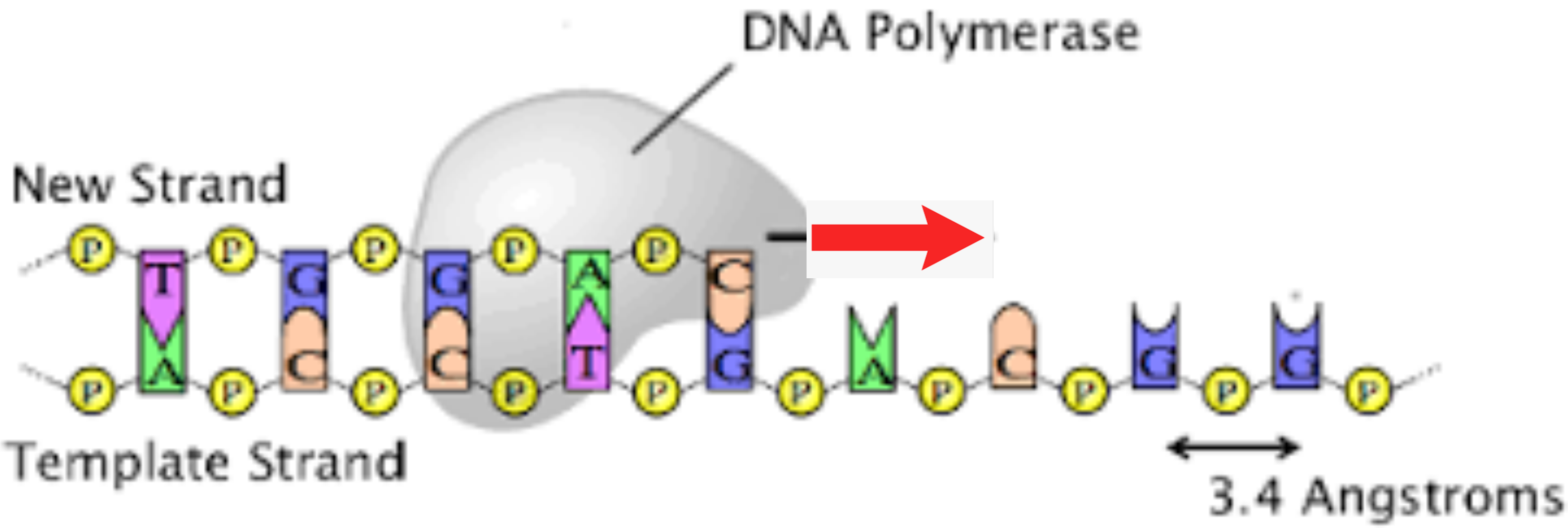
Behnam Enghiad and Huimin Zhao, "Programmable DNA-Guided Artificial Restriction Enzymes", ACS Synthetic Biology,2017

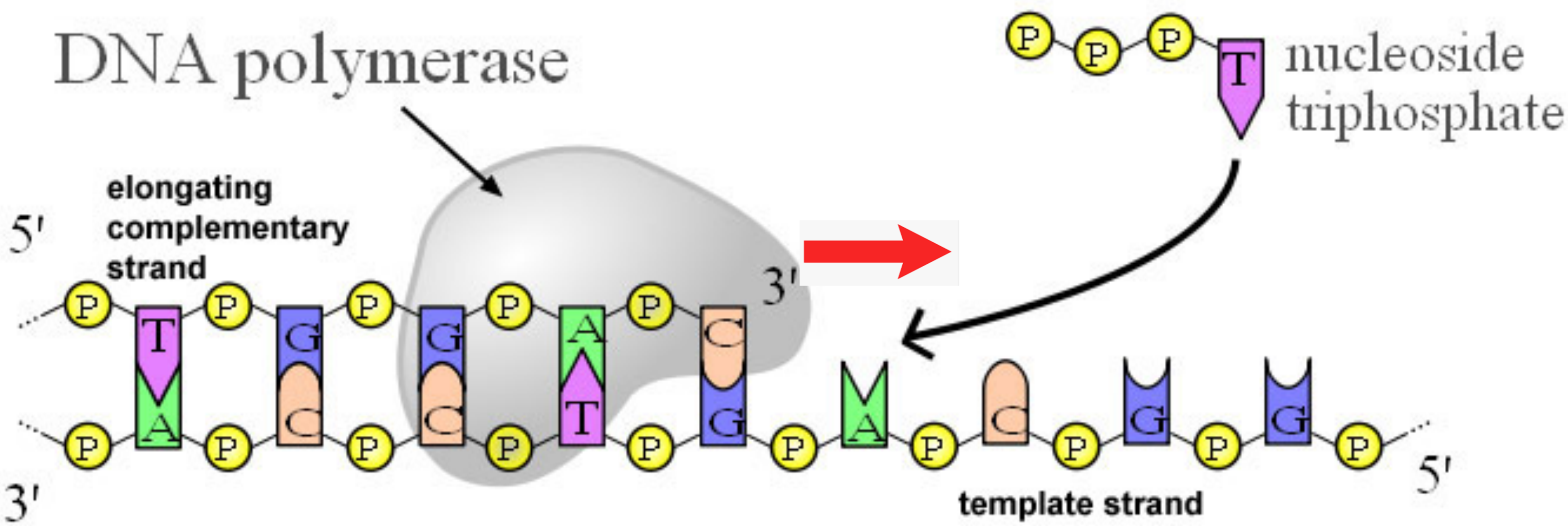
Polymerization



The

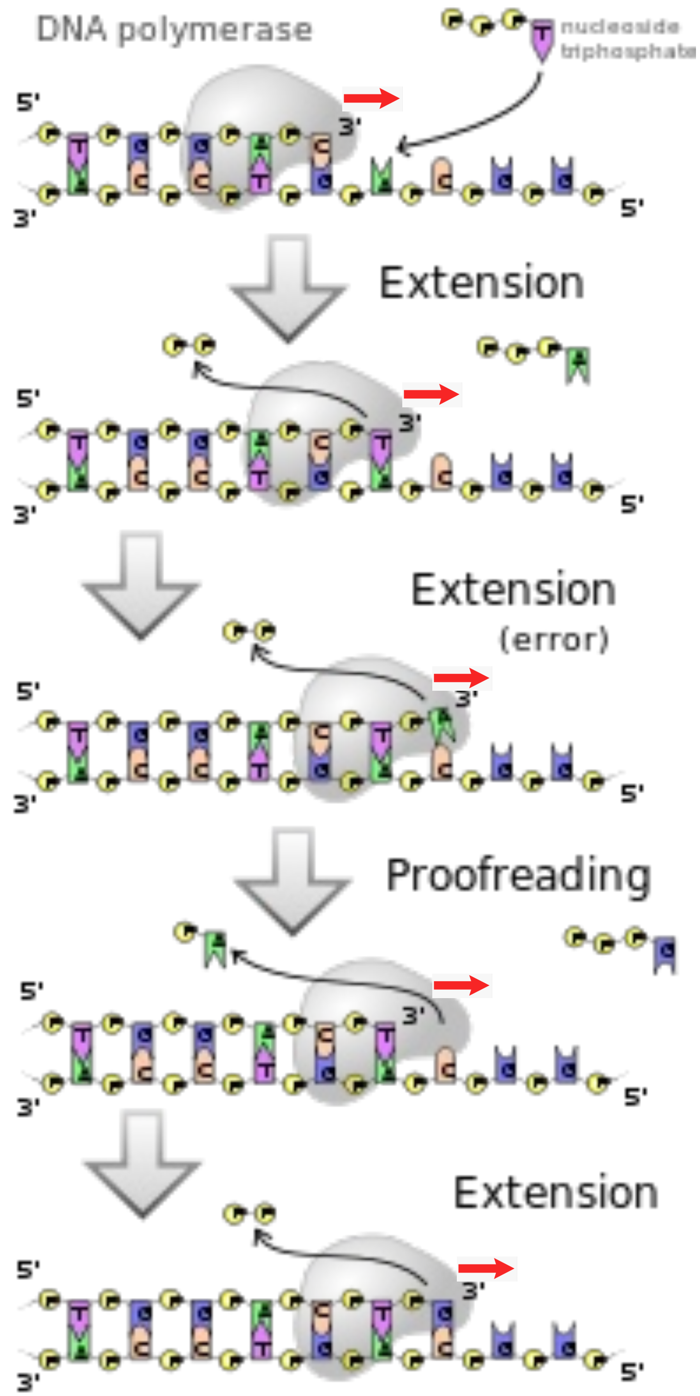
Polymerization





Khan Acadamy

Polymerization



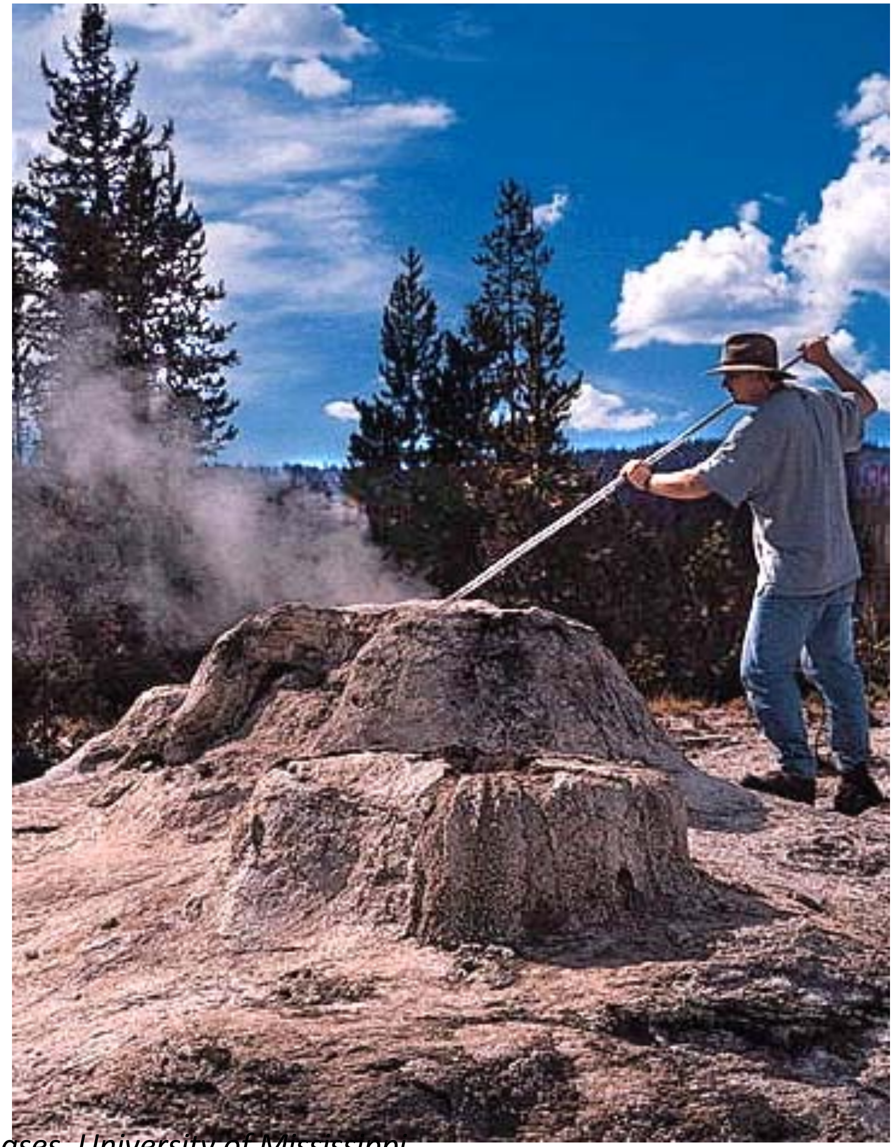
Khan Acadamy

Polymerization

- Denaturation of target (template)
  - Usually 95^{o}C
- Annealing of primers
  - Temperature of annealing is dependent on the G+C content
  - May be high (no mismatch allowed) or low (allows some mismatch) stringency
- Extension (synthesis) of new strand

Discovery of Thermostable DNA Polymerases:

At hot springs Yellowstone National Park



Donna C. Sullivan, Division of Infectious Diseases, University of Mississippi

Discovery of Thermostable DNA Polymerases: Deep Sea Vents

Donna C. Sullivan, Division of Infectious Diseases, University of Mississippi

Thermostable Polymerases

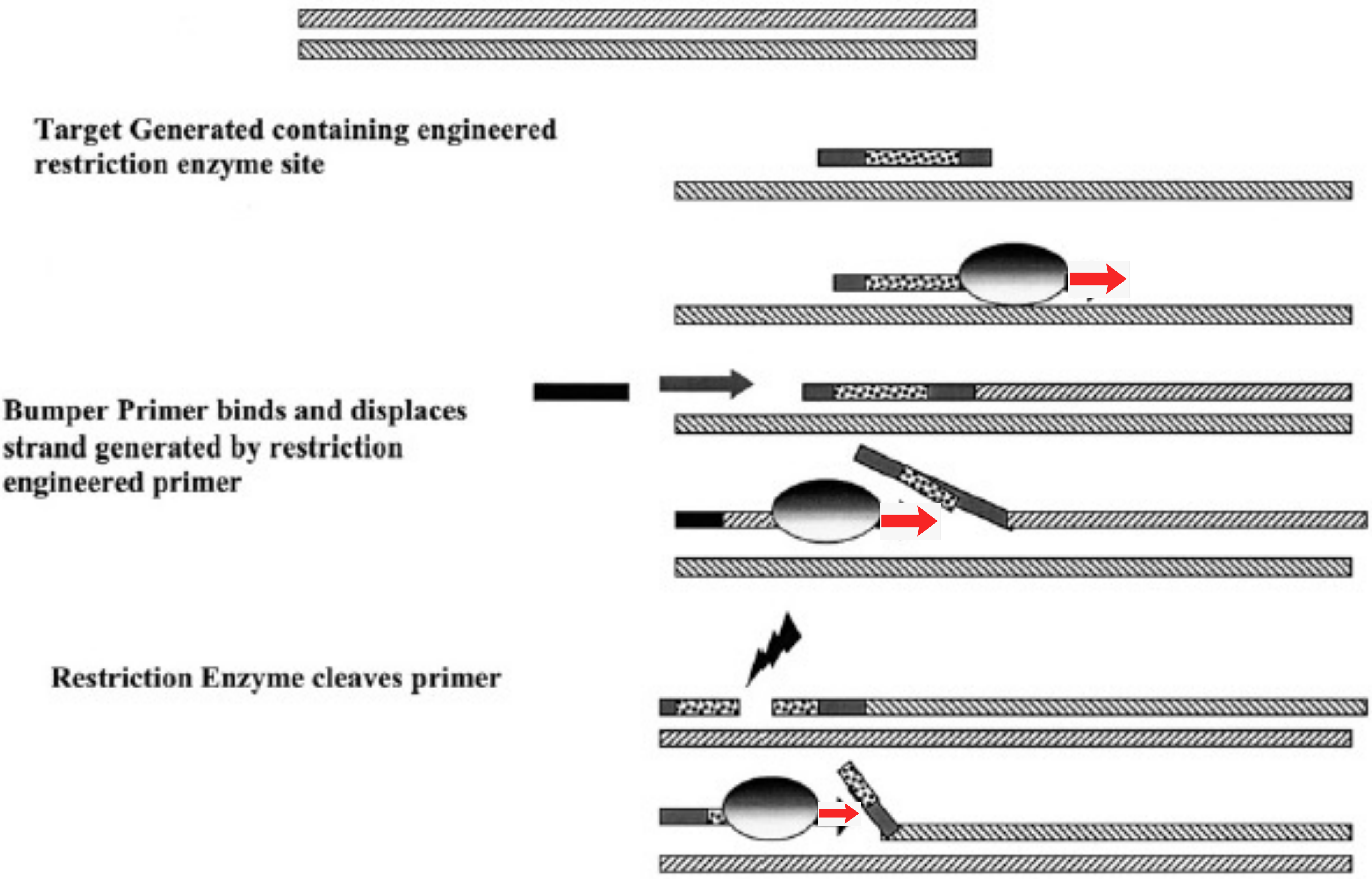
Polymerase	T ½, 95ºC	Extension Rate (nt/sec)	Type of ends	Source
Taq pol	40 min	75	3'A	*T. aquaticus*
Amplitaq (Stoffel fragment)	80 min	>50	3'A	*T. aquaticus*
Vent*	400 min	>80	95% blunt	*Thermococcus litoralis*
Deep Vent*	1380 min	?	95% blunt	*Pyrococcus GB-D*
Pfu	>120 min	60	Blunt	*Pyrococcus furiosus*
Tth* (RT activity)	20 min	>33	3'A	*T. thermophilus*

*Have proof-reading functions and can generate products over 30 kbp

Thermostable Polymerases

- *Taq*: *Thermus aquaticus* (most commonly used)
  - Sequenase: *T. aquaticus* YT-1
  - Restorase (*Taq* + repair enzyme)
- *Tfl*: *T. flavus*
- *Tth*: *T. thermophilus* HB-8
- *Tli*: *Thermococcus litoralis*
- Carboysothermus hydrenoformans (RT-PCR)
- *P. kodakaraensis* (Thermococcus) (rapid synthesis)
- *Pfu*: *Pyrococcus furiosus* (fidelity)
  - Fused to DNA binding protein for processivity

Strand Displacement Polymerases



Donna C. Sullivan, Division of Infectious Diseases, University of Mississippi

Example
Strand Displacement Polymerases

- Phi20 (active 20-37°C)
- Bst (active 65°C)

Helicase Enzymes

- Helicase enzymes are motor proteins that moving along a DNA double helix to denature its structure (unwind the double helix) independent of temperature.
- In particularly, helicase enzymes directionally break hydrogen bonds between base pairing in DNA double helix.
- [Animation of Helicase Unwinding the DNA Double Helix](https://study.com/academy/lesson/how-helicase-unwinds-the-dna-double-helix-in-preparation-for-replication.html):

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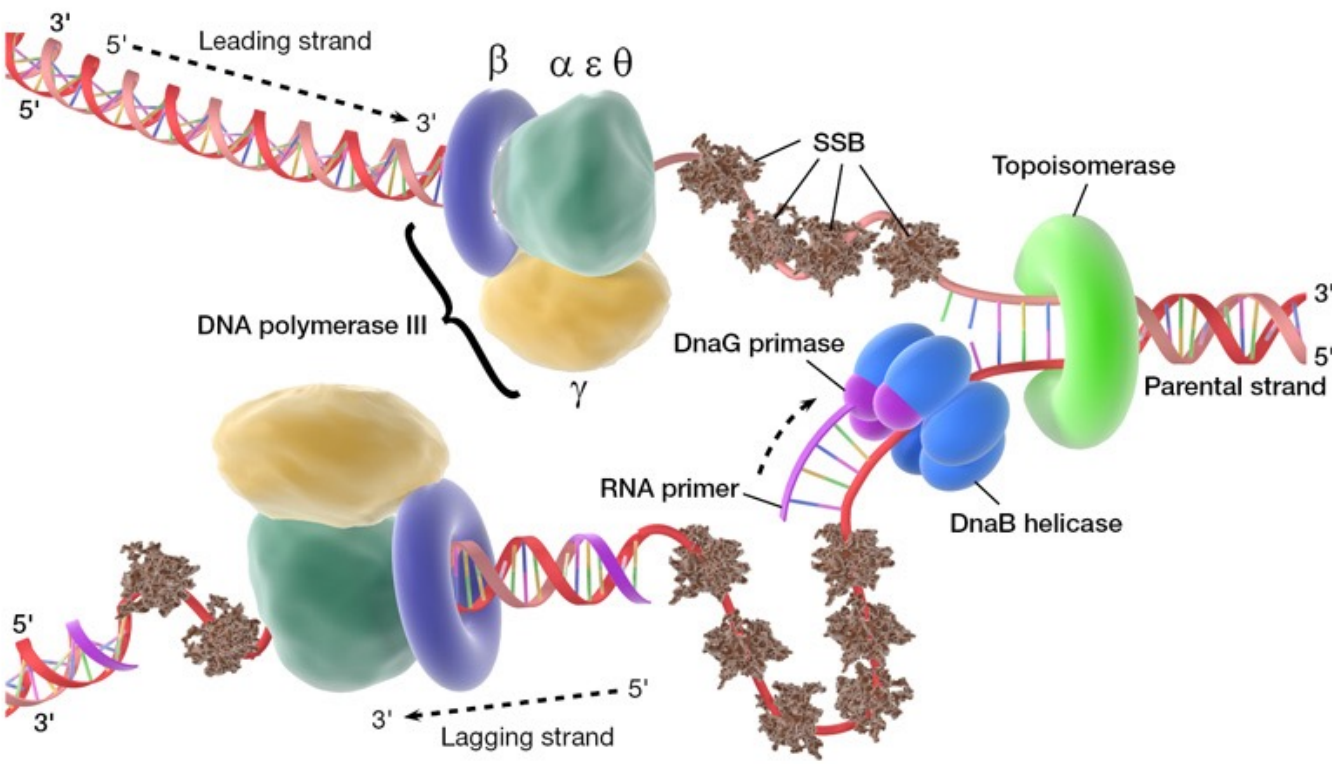

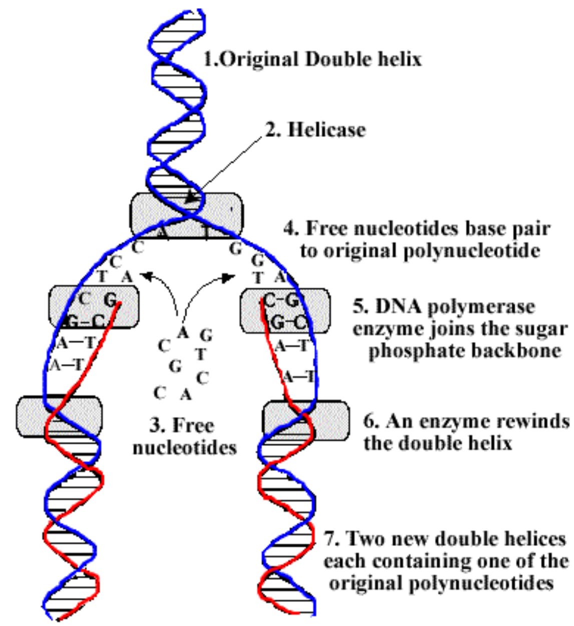

http://click4biology.info/c4b/3/chem3.4.htm
http://www.pdbj.org/eprots/index_en.cgi?PDB%3A3BEP