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:

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

- Is not an enzymic reaction
DNA Hybridization:

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

- Is not an enzymic reaction
Ligation: Ligase – “to bind” or “to glue together”

**T4 DNA Ligase** – a single polypeptide, MW \(~86,000\) daltons, (pH 7.5 – 8.0, 10 mM Mg\(^{2+}\), 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

Sticky ends

Hybridization

Melting

DNA ligase

Ligation

Restriction Enzymes

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

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Restriction enzyme action

DNA restriction enzyme

Restriction Enzymes

Some restriction enzymes produce "sticky" ends:

```
GAATTC
CTTAAG
```

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

```
CCCGGGG
GGGC CCC
```
Restriction Endonucleases

- Nicking Enzymes
- Restriction Enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Sequence</th>
<th>Cut Site</th>
<th>Overhang</th>
<th>Properties (NEB Enzymes Only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BstUI</td>
<td>CGCG</td>
<td>C/G/C/G</td>
<td>blunt</td>
<td><img src="neb4.png" alt="Image" /> 60° No!</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Sequence</th>
<th>Cut Site</th>
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</thead>
<tbody>
<tr>
<td>BfaI</td>
<td>CTAG</td>
<td>C/T/A/G</td>
<td>5’ - TA</td>
<td>NEB4 37° Yes!</td>
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<tr>
<td></td>
<td></td>
<td>G/A/T/C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CviQ1</td>
<td>GTAC</td>
<td>G/T/A/C</td>
<td>5’ - TA</td>
<td>NEB3 BSA 25° No!</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/A/T/G</td>
<td></td>
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</tbody>
</table>

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<th>Enzyme</th>
<th>Sequence</th>
<th>Cut Site</th>
<th>Overhang</th>
<th>Properties (NEB Enzymes Only)</th>
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<tbody>
<tr>
<td>AcI</td>
<td>CCGC</td>
<td>C/G/C/G</td>
<td>5’ - CG</td>
<td>NEB3 37° Yes!</td>
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<tr>
<td></td>
<td></td>
<td>G/G/C/G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BmgBI</td>
<td>CACGTC</td>
<td>C/A/C/G</td>
<td>blunt</td>
<td>NEB3 BSA 37° Yes!</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G/T/G/C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DNAzymes: DNA strands with a few reactive RNA bases

**Pb$$^{2+}$$-dependent 17E DNAzyme**

- Detection limit: 1 nM
- EPA MCL: 75 nM

**UO_2^{2+}-dependent 39E DNAzyme**

- Detection limit: 45 pM
- EPA MCL: 126 nM
- ICP-MS: 420 pM

**Cu$$^{2+}$$-dependent DNAzyme**

- Detection limit: 35 nM
- EPA MCL: 20 μM

**Hg$$^{2+}$$-dependent DNAzyme (with UO_2^{2+} cofactor)**

- Detection limit: 2.4 nM
- EPA MCL: 10 nM

DNAzymes are found using in-vivo evolution protocols.
Polymerization
Polymerization

DNA polymerase

3.4 Angstroms

Template Strand

New Strand
Polymerization
Polymerization

• Denaturation of target (template)
  – Usually 95°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

<table>
<thead>
<tr>
<th>Polymerase</th>
<th>$T_{1/2}$, 95°C</th>
<th>Extension Rate (nt/sec)</th>
<th>Type of ends</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>Taq pol</td>
<td>40 min</td>
<td>75</td>
<td>3’A</td>
<td>T. aquaticus</td>
</tr>
<tr>
<td>Amplitaq (Stoffel fragment)</td>
<td>80 min</td>
<td>&gt;50</td>
<td>3’A</td>
<td>T. aquaticus</td>
</tr>
<tr>
<td>Vent*</td>
<td>400 min</td>
<td>&gt;80</td>
<td>95% blunt</td>
<td>Thermococcus litoralis</td>
</tr>
<tr>
<td>Deep Vent*</td>
<td>1380 min</td>
<td>?</td>
<td>95% blunt</td>
<td>Pyrococcus GB-D</td>
</tr>
<tr>
<td>Pfu</td>
<td>&gt;120 min</td>
<td>60</td>
<td>Blunt</td>
<td>Pyrococcus furiosus</td>
</tr>
<tr>
<td>Tth* (RT activity)</td>
<td>20 min</td>
<td>&gt;33</td>
<td>3’A</td>
<td>T. thermophilus</td>
</tr>
</tbody>
</table>

*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 hydreniformans* (RT-PCR)
- *P. kodakaraensis* (Thermococcus) (rapid synthesis)
- *Pfu*: *Pyrococcus furiosus* (fidelity)
  - Fused to DNA binding protein for processivity
Strand Displacement Polymerases

Target Generated containing engineered restriction enzyme site

Bumper Primer binds and displaces strand generated by restriction engineered primer

Restriction Enzyme cleaves primer

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:


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