DNA Enzyme reactions

• Ligation
• Restriction enzymes
• Helicase enzymes
• Polymerization
• Strand-displacing polymerases
DNA Hybridization

Sticky ends

Ligation

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

Figure 4: 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}
DNA Hybridization & Enzyme Ligation activity

Sticky ends

DNA ligase

Exonucleases & Endonucleases

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

Restriction Enzymes

**Figure 3:** 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 enzyme action

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 G G C/G C</td>
<td>blunt</td>
<td>![NEB 4] ![60°] ![No]</td>
</tr>
<tr>
<td>BfaI</td>
<td>CTAG</td>
<td>C T A G G A T / C</td>
<td>5’ - TA</td>
<td>![NEB 4] ![37°] ![Yes]</td>
</tr>
<tr>
<td>AcI</td>
<td>CCGC</td>
<td>C / C G C G G C / G</td>
<td>5’ - CG</td>
<td>![RR] ![NEB 3] ![37°] ![Yes]</td>
</tr>
<tr>
<td>BmgBI</td>
<td>CACGTC</td>
<td>C A C / G T C G T G / C A G</td>
<td>blunt</td>
<td>![RR] ![NEB 3] ![BSA] ![37°] ![Yes]</td>
</tr>
</tbody>
</table>
Restriction Enzymes

http://www.scq.ubc.ca/restriction-endonucleases-molecular-scissors-for-specifically-cutting-dna/
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 particular, helicase enzymes directionally break hydrogen bonds between base pairing in DNA double helix.

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

• DNAzymes for sensing, nanobiotechnology and logic gate applications – Willner et. al.
• Functional DNA nanotechnology: emerging applications of DNAzymes and aptamers – Liu et. al.
• DNAzymes: From Creation In Vitro to Application In Vivo – Li et. al.
• FRET Study of a Trifluorophore-Labeled DNAzyme – Lu et. al.