3.2: Overview of Recombinant DNA Technology

**Recombinant DNA technology** utilizes the power of microbiological selection and screening procedures to allow investigators to isolate a gene that represents as little as 1 part in a million of the genetic material in an organism. The DNA from the organism of interest is divided into small pieces that are then placed into individual cells (usually bacterial). These can then be separated as individual colonies on plates, and they can be screened through rapidly to find the gene of interest. This process is called **molecular cloning**.

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**Joining DNA in vitro to form recombinant molecules**

**Restriction endonucleases** cut at defined sequences of (usually) 4 or 6 bp. This allows the DNA of interest to be cut at specific locations. The physiological function of restriction endonucleases is to serve as part of a system to protect bacteria from invasion by viruses or other organisms. (See Chapter 7)

*Table 3.1. List of restriction endonucleases and their cleavage sites. A ' means that the nuclease cuts between these 2 nucleotides to generate a 3’ hydroxyl and a 5’ phosphate.*

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Site</th>
<th>Enzyme</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alu</em>I</td>
<td>AG’CT</td>
<td><em>Not</em>I</td>
<td>GC’GGCCGC</td>
</tr>
<tr>
<td><em>Bam</em>HI</td>
<td>G’GATCC</td>
<td><em>Pst</em>I</td>
<td>CTGCA’G</td>
</tr>
<tr>
<td><em>Bgl</em>II</td>
<td>A’GATCT</td>
<td><em>Pvu</em>II</td>
<td>CAG’CTG</td>
</tr>
<tr>
<td><em>Eco</em>RI</td>
<td>G’AATTCC</td>
<td><em>Sal</em>I</td>
<td>G’TCGAC</td>
</tr>
<tr>
<td><em>Hae</em>III</td>
<td>GG’CC</td>
<td><em>Sau</em>3AI</td>
<td>’GATC</td>
</tr>
<tr>
<td><em>Hha</em>I</td>
<td>GCG’C</td>
<td><em>Sma</em>I</td>
<td>CCC’GGG</td>
</tr>
</tbody>
</table>

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HincII  GTY'RAC  SpeI  A'CTAGT
HindIII  A'AGCTT  TaqI  T'CGA
HinfI  G'ANTC  XbaI  T'CTAGA
HpaII  C'CGG  XhoI  C'TCGAG
KpnI  GGTAC'C  Xmal  C'CCGGG
MboI  'GATC

N = A,G,C or T
R = A or G
Y = C or T
S = G or C
W = A or T

a. Sticky ends

(1) Since the recognition sequences for restriction endonucleases are pseudopalindromes, an off-center cleavage in the recognition site will generate either a 5' overhang or a 3' overhang with self-complementary (or "sticky") ends.

e.g. 5' overhang EcoRI G'AATTC
BamHI G'GATCC
3' overhangPstI CTGCA'G

(2) When the ends of the restriction fragments are complementary,

e.g. for EcoRI 5'---G AATTC---3'
3'---CTTAA G---5'

the ends can anneal to each other. Any two fragments, regardless of their origin (animal, plant, fungal, bacterial) can be joined in vitro to form recombinant molecules (Figure 3.3).

Figure 3.3.
b. Blunt ends

(1) The restriction endonuclease cleaves in the center of the pseudopalindromic recognition site to generate blunt (or flush) ends.

(2) E.g. HaeIII GG’CC

HincII GTY’RAC

**T4 DNA ligase** is used to tie together fragments of DNA (Figure 3.4). Note that the annealed “sticky” ends of restriction fragments have **nicks** (usually 4 bp apart). Nicks are breaks in the phosphodiester backbone, but all nucleotides are present. **Gaps** in one strand are missing a string of nucleotides.

T4 DNA ligase uses ATP as source of adenylyl group attached to 5’ end of the nick, which is a good leaving group after attack by the 3’ OH. (See Chapter 5 on Replication).

At high concentration of DNA ends and of ligase, the enzyme can also ligate together blunt-ended DNA fragments. Thus any two blunt-ended fragments can be ligated together. Note: Any fragment with a 5’ overhang can be readily converted to a blunt-ended molecule by fill-in synthesis catalyzed by a DNA polymerase (often the Klenow fragment of DNA polymerase I). Then it can be ligated to another blunt-ended fragment.
Linkers are short duplex oligonucleotides that contain a restriction endonuclease cleavage site. They can be ligated onto any blunt-ended molecule, thereby generating a new restriction cleavage site on the ends of the molecule. Ligation of a linker on a restriction fragment followed by cleavage with the restriction endonuclease is one of several ways to generate an end that is easy to ligate to another DNA fragment.

Annealing of homopolymer tails are another way to joint two different DNA molecules.

The enzyme terminal deoxynucleotidyl transferase will catalyze the addition of a string of nucleotides to the 3’ end of a DNA fragment. Thus by incubating each DNA fragment with the appropriate dNTP and terminal deoxynucleotidyl transferase, one can add complementary homopolymers to the ends of the DNAs that one wants to combine. E.g., one can add a string of G’s to the 3’ ends of one fragment and a string of C’s to the 3’ ends of the other fragment. Now the two fragments will join together via the homopolymer tails.
Figure 3.5. Use of linkers (left) and homopolymer tails (right) to make recombinant DNA molecules.