

## Restriction Enzymes

**Directions:** Identify the restriction sites for each of the examples given. Show the cuts, sticky ends or blunt, number of DNA fragments produced and the number of base pairs in each (count the top row). (If there are three nucleotides on either side of the dash it is a blunt cut. If there are fewer than three bases on either side of the dash it produces "sticky ends" cut.)

Remember: Analyze in the 5' → 3' direction

1. *HindII* --- 5' GTC ↓ GAC 3'

5' ACGACGTAGTCGACTTATTAT GTCGACCCGCCGCGTGTGACCATCA 3'  
 3' TGCTGCATCAGCTGAATAATACAGCTGGGCGGCGCACAGCTGGTAGT 5'

Number of pieces of DNA 4

2. *EcoRI* --- 5' G ↓ AATTC 3'

5' ACG ACGTATTAGAATTCTTAT CCGCCGCCGGAATTCT CATCA 3'  
 3' TGC TGCATAATCTTAA GAATAGGCGGCGGCCTTAAGAGTAGT 5'

Number of pieces of DNA 3

3. *HaeIII* --- 5' CC ↓ GG 3'

5' ACGCCGGCCGTATTAT CCGGATCCGCCG CCGGCTGTCCCGGATCA 3'  
 3' TGCGGCCGGCATAATAGGCTAGGCGGCGGCCGACAGGGCCTAGT 5'

Number of pieces of DNA 5

4. *BamI* --- 5' CCTAG ↓ G 3'

5' ACGCCTAGGACGTATTATCCTAGGTAT CCGCCGCCGT CATCA 3'  
 3' TGCGGATCCTGCATAATAGGATCCATAGGCGGCGGCAGTAGT 5'

Number of pieces of DNA 3

5. *HindII* --- 5' GTC ↓ GAC 3' and *HaeIII* --- 5' CC ↓ GG 3'

5' ACGGTCGACACGTATTATTAGTCGACTCCGCCGCCCGGTCATCA 3'  
 3' TGCCAGCTGTGCATAATAATCAGCTGAGGCGGCGGCGGCCAGTAGT 5'

Number of pieces of DNA 4

6. *HindII* --- 5' GTC ↓ GAC 3', *HaeIII* --- 5' CC ↓ GG 3' and *BamI* --- 5' CCTAG ↓ G 3'

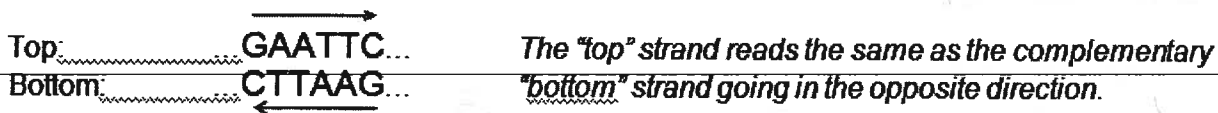
5' ACGCCGGACGTACCTAGGTTTAGTCGACTC CGCCG CCCCTAGGGTCATCA 3'  
 3' TGCGGCCTGCATGGATCCAAATCAGCTGAGGCGGCGGGGATCCCAGTAGT 5'

Number of pieces of DNA 5

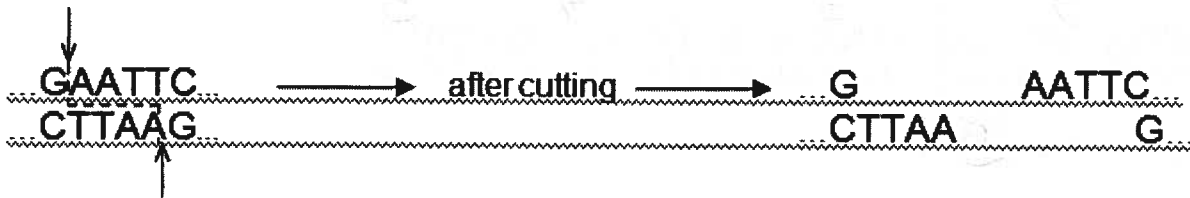
# RESTRICTION ENZYMES: DNA SCISSORS

**Background:** DNA fingerprinting is made possible in part by special enzymes that cut DNA. These enzymes are called restriction enzymes. Restriction enzymes are proteins that bacteria use to cut up DNA that doesn't belong to them. If a bacterium senses that a virus is trying to invade, or a different species of bacterium represents a threat, it can use a restriction enzyme to cut up the foreigner's DNA.

Restriction enzymes don't just cut DNA randomly – that would lead to the destruction of the bacterium's own DNA. (That's a *bad* thing!) Restriction enzymes recognize specific segments of bases called restriction sites. Each different restriction enzyme (and there are hundreds, made by different species of bacteria) has its own particular restriction site where it will cut DNA. Most restriction sites are 4 to 6 bases long and are DNA palindromes. A DNA palindrome is a sequence where the "top" strand reads the same as the "bottom" strand running in the opposite direction. For example:



This sequence happens to be the restriction site for the enzyme called *EcoR1*. Its name comes from the bacterium in which it was discovered – *Escherichia coli* strain RY 13 (that's the *EcoR* part). The "1" comes from the fact that it was the first restriction enzyme found in this strain of *E. coli*. The enzyme *EcoR1* cuts between the A and G in each strand of the DNA. (The arrows in the diagram below show where *EcoR1* snips the DNA.) After the cuts are made, the two strands are held together only by weak hydrogen bonds between complementary bases, which separate easily, like a broken zipper.



You may notice that the *EcoR1* enzyme does not cut straight across the DNA molecule. When *EcoR1* cuts DNA, it leaves single stranded "tails" called sticky ends, because they could stick (although not very tightly) to other segments of DNA that have been cut with the same enzyme. (Remember this trait of restriction enzymes, because these sticky ends can be useful for re-joining the cut DNA – even if the DNA pieces originally came from different species.) Not all restriction enzymes make sticky ends when they cut; some cut straight across the DNA to leave blunt ends that won't stick to each other.

The restriction sites for four other restriction enzymes are shown below. The arrows show where they cut the DNA strands:

