Plasmid Modeling: Technology-Based Version

Problem: How do you get a gene with a desirable trait into another organism?

DNA Sequence with Desired Gene:
TGGGCCTAGGCACAGGGCCCGGAGATTCTTAAGTCAAGCAGGTTCGAAGG**TACATAACGTCTCTTC-GTCATGTGCCTTTTAAATGTAATATT**CCTCCTTAAGAATTTCGAACGGGGCCCTAGGACC

Bold text – Representing the Desired Gene – This area cannot be cut!

Plasmid Sequence:
GCCCAGAGTTTCTTAAGGTCTCGAGTTAACCTAGGAGGGCCCTGGTG**GGGG**CAAGGTTATACTTAAG-CCGTAGGTTCGAACGCC

Bold text – Representing the Plasmid Replication Sequence – This area cannot be cut!

Restriction Enzyme Possibilities	Plasmid Cuts	DNA Cuts	Distance from Gene – Front	Distance from Gene – Back
1: CCTGG				
2: TTCGAA				
3: CCTAGG				
4: TCTAGA				
5: GGCC				
6: CTTAAG				
7:CTCGAG				
8: GGGCCC				
9: AAGCG				

To fill in the table, type "Control F" and then type the enzyme sequence into the Navigation Dialog box. This lets Microsoft Word work as a Genome Analyzer, by highlighting the areas in the sequences where the specific sequences coded for the restriction enzyme sequences are located.

Remember if the highlight is in the **bold text** area it does not count as a "cut" because you cannot cut in those areas.

Which restriction enzyme do you recommend to use in this case? Remember the goal is to cut the DNA sequence as closely as possible to the desired gene without cutting into the gene sequence itself.

Plasmid Modeling: Technology-Based Version (Continued)

What is the sequence for the resulting plasmid DNA?

There is one thing this model does not show in regards to restriction enzymes. A restriction enzyme cuts the DNA at a certain location through both strands of DNA. This model only shows you the sequence on one side of the helix, not both antiparallel strands. Restriction enzymes work on both strands simultaneously. They cut both horizontally through the back bone of the helix, and they also cut vertically through the hydrogen bonds, which hold together the base pairs. Ultimately, leaving one side of the helix slightly longer than the other side. This is what we call "sticky ends". If it is cutting the plasmid, the restriction enzyme leaves a tail of exposed bases on the plasmid DNA that must match up perfectly with the opposing tail from the cut on the DNA with the desired gene. Creating two "sticky ends", as opposed to blunt ends, is of major importance. What would happen if the restriction enzyme were to create "blunt ends" instead of sticky ones?