# Paper Plasmid Modeling

| #1 | #2 | #3 | #4 | #5 | P1 | P2 | P3 | P4 | RE3 | RE7 |
|---|---|---|---|---|---|---|---|---|---|---|
| T A | T A | T A | C G | A T | G C | T A | G C | T A | C G | C G |
| G C | T A | A T | T A | G C | C G | T A | C G | C G | C G | T A |
| G C | C G | C G | T A | A T | C G | A T | A T | G C | T A | C G |
| G C | T A | A T | T A | A T | C G | A T | A T | A T | A T | G C |
| C G | T A | T A | T A | T A | A T | C G | G C | A T | G C | A T |
| C G | A T | A T | A T | T A | G C | C G | G C | C G | G C | G C |
| T A | A T | A T | A T | T A | A T | T A | T A | G C | RE4 | |
| A T | G C | C G | A T | C G | G C | A T | T A | C G | T A | RE8 |
| G C | T A | G C | T A | G C | T A | G C | A T | C G | C G | G C |
| G C | C G | T A | G C | A T | T A | G C | T A | | T A | G C |
| C G | A T | C G | T A | A T | T A | A T | A T | RE1 | A T | G C |
| A T | A T | T A | A T | C G | C G | G C | C G | C G | G C | C G |
| C G | G C | C G | A T | G C | T A | G C | T A | C G | A T | C G |
| A T | C G | T A | T A | G C | T A | G C | T A | T A | RE5 | C G |
| G C | A T | T A | A T | G C | A T | C G | A T | G C | G C | |
| G C | G C | C G | T A | G C | A T | C G | A T | G C | G C | RE9 |
| G C | G C | G C | T A | C G | G C | C G | G C | | C G | A T |
| C G | T A | T A | C G | C G | G C | T A | C G | RE2 | C G | A T |
| C G | T A | C G | C G | C G | T A | G C | C G | T A | RE6 | C G |
| C G | C G | A T | T A | T A | C G | G C | G C | T A | C G | G C |
| G C | G C | T A | C G | A T | T A | T A | T A | C G | T A | |
| G C | A T | G C | C G | G C | C G | G C | A T | G C | T A | |
| A T | A T | T A | T A | G C | G C | G C | G C | A T | A T | |
| G C | G C | G C | T A | A T | A T | G C | G C | A T | A T | |
| A T | G C | C G | A T | C G | G C | G C | T A | | G C | |

# Paper Plasmid Modeling (Continued)

1. Cut out each column.
2. Tape together P1, P2, P3, P4, in order, the top of P2 onto the bottom of P1 and continue, until all the strips are taped together.
3. Now tape the end of P4 to the beginning of P1 to form one continuous loop, creating your plasmid DNA sequence.
4. Cut out and tape the other sequences labeled #1, #2, #3, #4, #5. Tape these together in numerical order as before but **do no**t tape into a circle. This represents your DNA sequence with the desired gene. The desired gene sequence is indicated by the **bold text** and horizontal line between the base pairs.
5. Cut out the restriction enzyme cards, represented by RE1… Cut out all 9 individually.
6. The goal is to determine what restriction enzyme will work to cut both the Desired gene and the plasmid. In order to insert the gene into the plasmid you will want to cut the plasmid once, but the desired gene twice.
    - The trick is you don't want to cut into the gene as it might cause a mutation and change the trait you are trying to convey.
    - You also cannot cut into the part of the plasmid that causes the DNA to be expressed. This is represented in the plasmid DNA the same way, **bold text** and a vertical line.
7. Compare the restriction enzymes to the plasmid DNA, mark on the sequences where each enzyme would cut the sequence. **Do not** cut the sequence now.
    - Make sure you show the "sticky ends" when you mark on the plasmid DNA
    - You may find it helpful to use a different colored pencil for restriction enzyme to help you keep track of each location.
    - Some restriction enzymes may not cut the plasmid. Anything that doesn't cut the sequence, can be thrown out.
    - We are looking for enzymes that cut the sequences on both side of the desired gene as closely as possible.
8. Compare the restriction enzymes left to the source DNA, mark on the sequences where each enzyme would cut the sequence. **Do not** cut the sequence now.
    - Make sure you show the "sticky ends" when you mark on the plasmid DNA.
    - We are looking for enzymes that cut the sequences on both side of the desired gene as closely as possible.
    - Anything that does not cut the plasmid and the source DNA can be thrown out.
9. You should be down to one successful enzyme! Use this enzyme to cut both the plasmid and the source DNA.
    - Tape the source gene into the plasmid matching up the sticky ends.

10. Congratulations – you have successfully engineered your plasmid!