

Creating a “Plug and Play” Antibody in SnapGene

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We created a plug-and-play method of antibody BioBrick production that can be achieved through four phases of 3A assembly. We recommend the use of the RFC23 prefix and suffix for proper in-frame assembly. We also recommend the use of different PCR product vectors with different antibiotic markers for each part. Each part was created for optimal use in *Escherichia Coli* (*E. coli*). The sequences were optimized using Reverse Translate software from The Sequence Manipulation suite. Restriction sites were removed using SnapGene software to identify suitable alternative codons. For plasmid design in SnapGene, you may use the “Restriction and Insertion Cloning – Insert Fragment” function under “Actions” in the application. This will allow you to cut your selected plasmids with the desired enzymes for each part. You may test for the proper resulting sequence using the “Simulate Agarose Gel” function under “Tools”.

Part 1

- 1) Cut your variable light chain BioBrick with EcoRI and SpeI.
- 2) Cut your constant light chain BioBrick with XbaI and PstI.
- 3) Cut your uncut PCR product vector with EcoRI and PstI.
- 4) Mix and ligate.

** Please note that this part may be skipped if you have a BioBrick with the complete light chain sequence available for use.*

Part 2

- 5) Cut your Part 1 product with EcoRI and SpeI.
- 6) Cut BioBrick J61100 with XbaI and PstI.
- 7) Cut your uncut PCR product with EcoRI and PstI.
- 8) Mix and ligate.

Part 3

- 9) Cut your Part 2 product with EcoRI and SpeI.
- 10) Cut your variable heavy chain region BioBrick with XbaI and PstI.
- 11) Cut your uncut PCR product with EcoRI and PstI.
- 12) Mix and ligate.

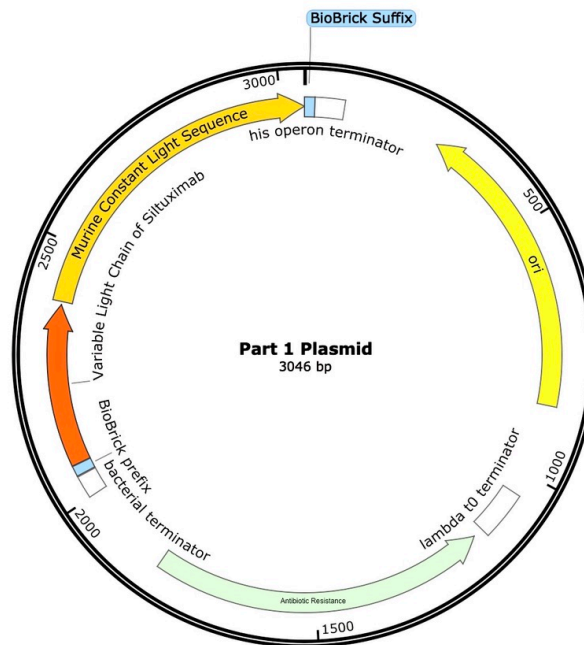
Part 4

- 13) Cut your Part 3 product with EcoRI and SpeI.
- 14) Cut our mutated variable constant chain region BioBrick with XbaI and PstI.
- 15) Cut your uncut PCR product with EcoRI and PstI.
- 16) Mix and ligate.

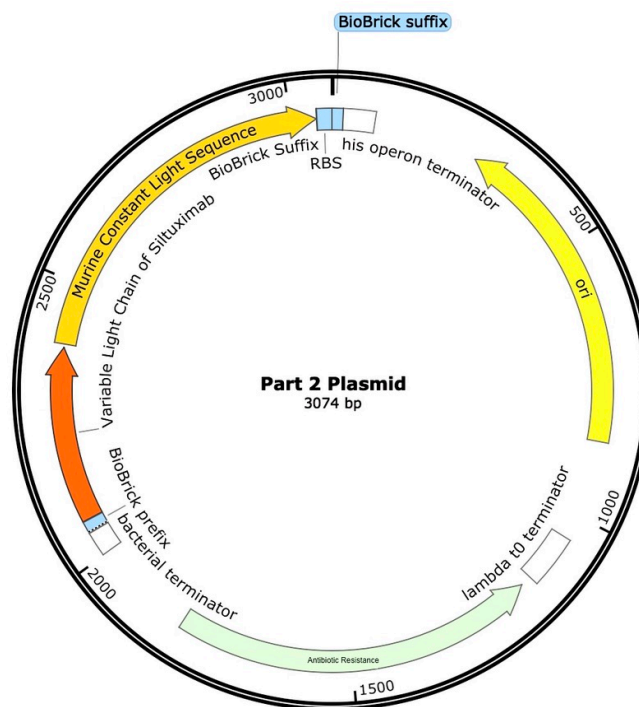
NOTE: The final product may be kept in a standard BioBrick such as pSB1C3 or it may be ligated into an expression vector with a T5 promoter and strong RBS. Please view next page for diagrams of resulting plasmids for each part. Each part was constructed in the pSB1C3 backbone for this manual.

Plasmids for SnapGene Design of Antibody Production Plasmids

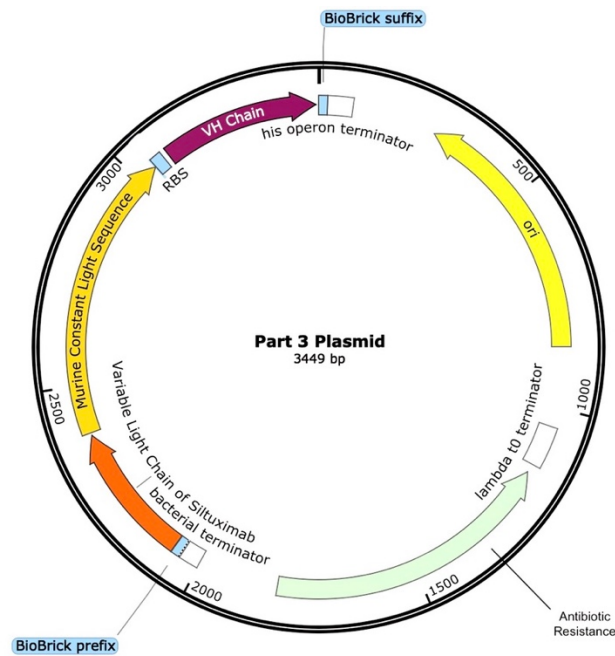
Part 1 Plasmid with light chain region of desired antibody.



Part 2 Plasmid with light chain region and strong ribosomal binding site (J61100)



Part 3 Plasmid with light chain region, strong RBS, and heavy chain region.



Part 4 Plasmid with light chain region, strong RBS, heavy chain region, and constant chain.

