Figure 2B: Primer design for PCR-generated vector and insert using an in silico created final DNA sequence file.

I. Create a final sequence file displaying both DNA strands:

Vector Left Arm | Insert | Vector Right Arm

II. Mark the junctions between the vector and the insert:

III. Choose 15-25 nt overlap region between vector and insert:

IV. Design overlapping primers starting from the first 5' nucleotide of the overlap region:

   Primer FP1
   Primer RP2

V. Amplify insert with primers FP1 + RP1 and Vector with primers FP2 + RP2: