Designing Oligos for Universal Knock-in Vectors using Type 2 Restriction Enzymes:

Note In the following document when orientation words are used, they are used in the context of the reading frame of your genetic loci of interest. e.g. A 5' Reverse CRISPR means that the target site for the CRISPR is on the reverse strand at your locus and is toward the 5' end of the gene. Upstream Homology Domains are 5' of the CRISPR cut and Downstream Homology Domains are 3' of the cut. Also note: Upper case and lower case bases are the same; they are typed the way they are as a visual marker of the different parts of the homology domains.

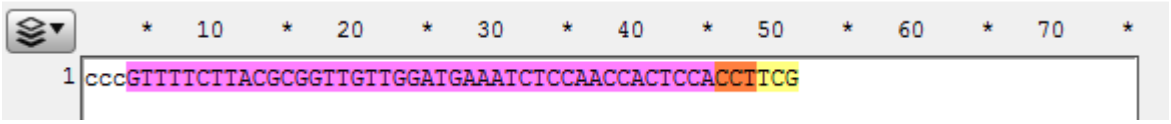
For the 5' Homology Domain:

1) Open the sequence file for the gene of interest and identify the CRISPR site. (In this example it is a Reverse CRISPR target in Yellow, the PAM is in Orange)

Copy the 48 bp 5' of the CRISPR cut into a new sequence file; this is the 5' homology.

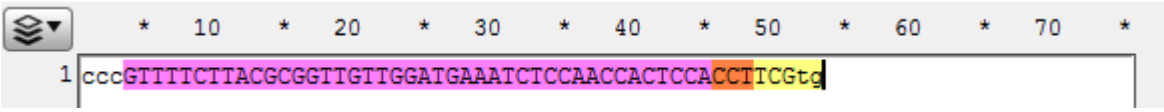
2) Observe the next three bases immediately upstream of the 48 bp of 5' homology, and pick a base not present to be the 3 bp spacer between the homology and the Universal PAM in the vector. (Here the three bases are "GGA" so "ccc" was chosen for the spacer)

Add the spacer to the new file 5' (in front) of the homology.

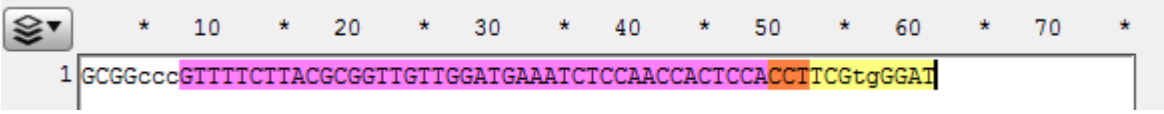


3) Determine where the last codon is in the 5' homology. Here the 3' G in the homology domain is the first base in the last codon upstream of the CRISPR cut.

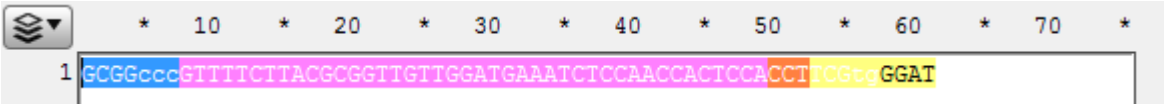
Complete the codon by adding the remaining bases for that codon from the gene sequence to ensure the integration event will be in frame.



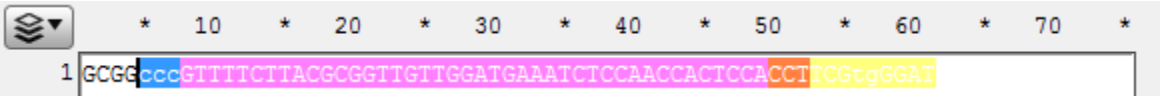
4) Add the BfuAI overhang sequences for cloning, to the ends of the 5' homology domain. 5'GCGG and 3'GGAT (or 3'CTTC for the pPRISM series). (Here both overhangs are set to prevent errors in copying sequence for the oligos in the next two steps.)



5) The 5' Homology Oligo A will be this sequence from the beginning to the end of the last codon. Copy and paste this into a new file and save it. (In this example, this oligo sequence is 5'GCGGcccGTTTTCTTACGCGGTTGTTGGATGAAATCTCCAACCACTCCACCTTCGtg3')



6) The 5' Homology Oligo B will be the reverse compliment of this sequence from beginning of the spacer to the end of the sequence. Copy the reverse compliment, paste it into a new file, and save it. (In this example, this oligo sequence is 5'ATCCcaCGAAGGTGGAGTGGTTGGAGATTTCATCCAACAACCGCGTAAGAAAACggg3')



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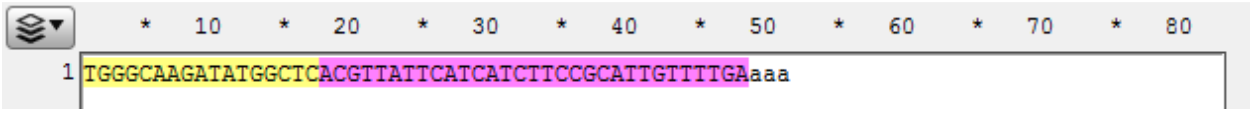
For the 3' Homology Domain:

7) Open the sequence file for the gene of interest and identify the CRISPR site. (Reverse CRISPR target in Yellow, PAM in Orange)

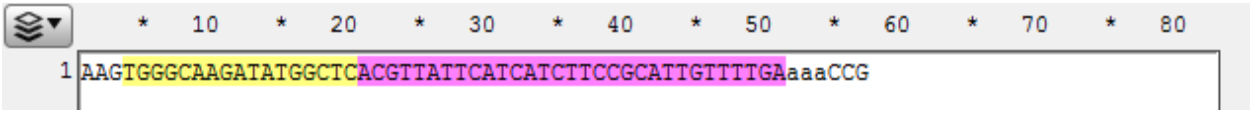
Copy the 48 bp 3' of the CRISPR cut into a new sequence file; this is the 3' homology.

8) Observe the next three bases downstream of the 48 bp of homology, and pick a base not present to be the 3 bp spacer between the homology and the Universal PAM in the vector. (Here the bases are "CTG" so "aaa" was chosen for the spacer.)

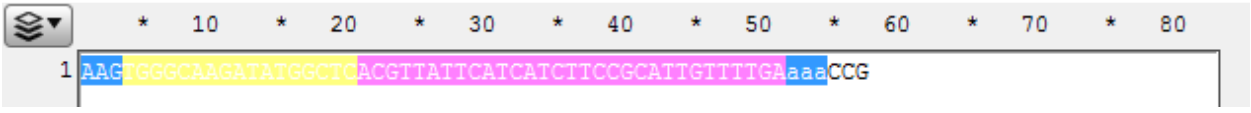
Add the spacer to your new file 3' of (after) your homology.



9) Add the BspQI overhang sequences for cloning, to the ends of the 3' homology domain. 5'AAG and 3'CCG. (Here both overhangs are set to prevent errors in copying sequence for the oligos in the next two steps.)



10) The 3' Homology Oligo A will be this sequence from the beginning of the sequence to the end of the spacer. (Here = 5'AAGTGGGCAAGATATGGCTCACGTTATTCATCATCTTCCGCATTGTTTTGAaaa3')



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11) The 3' Homology Oligo B will be the reverse compliment of this sequence from the beginning of the homology to the end of the sequence. (Here = 5'CGGtttTCAAAACAATGCGGAAGATGATGAATAACGTGAGCCATATCTTGCCCA3')