Designing Oligos for BRED Gene Deletion

OBJECTIVE

To design oligonucleotides for gene deletion with BRED.

BACKGROUND

Bacteriophage recombineering with electroporated DNA (BRED) a system commonly used for deletion genes or regions of interests in a phage's genome. While a separate protocol outlines the experimental procedures, here the procedures need to design deletion and extender oligos are described in a simplified fashion.

APPROXIMATE TIME NEEDED

~1 – 1.5 Hours

MATERIALS NEEDED

Software

- DNA Strider (Mac or PC. Downloadable here: http://sourceforge.net/projects/dnastrider/)
- DNA Master (PC only. Downloadable here: http://cobamide2.bio.pitt.edu/)

HELPFUL TIPS

- In this protocol, DNA Strider for Mac 1.59.2 is used for demonstration. The design of oligos for deleting Adephagia gp73 is used as an example.

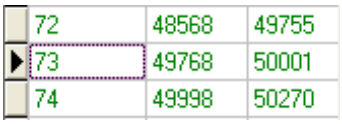
PROCEDURES

1. Visit the phage's information page on www.phagesdb.org, select the gene to be deleted. To do so, click on "Click to View" by "Gene List" under "Characterization."

Characterization	
Cluster	K
Other Cluster Members	Click to View
Subcluster	K1
Morphotype	Siphoviridae
Has been Phamerated?	Yes
Has been Annotated?	Yes
Gene List	Click to View

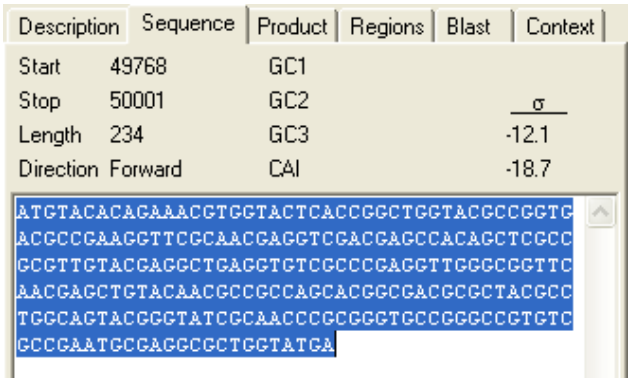
2. Record the genome position and direction under the "Gene View."

3. Download the final DNA Master file on the phage's information page, under "Available Multimedia" if haven't already done so. Open the file and select the gene to be deleted.

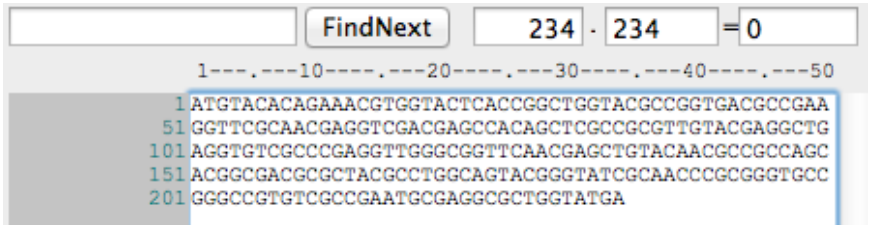


72	48568	49755
73	49768	50001
74	49998	50270

4. Make sure the coordinates in DNA Master are the same as what was listed under "Gene View" on PhagesDB. Click on the "Sequence" tab under "Features" and copy the whole sequence.



5. Open DNA Strider, go to "File," select "New File." Paste the sequence. Make sure the length is the same as what was listed in DNA Master and "Gene View" on PhagesDB. (In this case, 234bp)



6. Save the file and name with phage's name and gene number. (e.g. Adephagia_gp73)

7. Some calculations have to be done at this point. Recall the coordinates of the gene to be deleted. Subtract/add 700 to both ends.

   a. For example, the coordinates of gp73 in Adephagia is 49768 – 50001.

   b. 49768 – 700 = 49068
      50001 + 700 = 50701

   c. The range to be used later is 49068 – 50701.

8. Go back to DNA Master. Click on the main "Sequence" tab. (NOT the "Sequence" tab under a specific feature) Highlight the range determined in step #7. In this case, select 49068 – 50701. Selected positions would be displayed on the top toolbar. Copy the sequence.

9. Go back to DNA Strider. Select "File," then "New File." Paste the sequence. Select the entire sequence in DNA Strider. Under "Format" of the top toolbar, select "lowercase." This will be very helpful later.

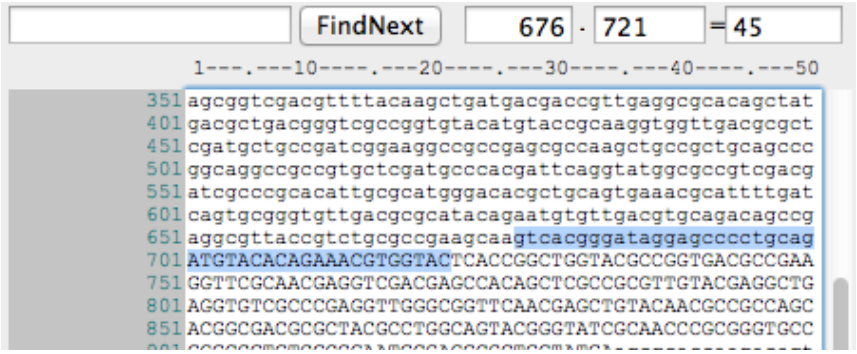
10. Now open the DNA Strider file containing JUST the gene to be deleted (in this case, the file named "Adephagia_gp73.") Copy the sequence of the entire gene. This window can be closed.

11. In the new DNA Strider file created (the one with 700bp added to both ends and all lowercase), paste the gene sequence in the top field, and then click "FindNext."

12. The gene should now be highlighted. Under "Format," select "UPPERCASE." If everything is done correctly, the gene to be deleted is now in upper case, and regions upstream and downstream of the gene are in lower case letters.

13. Save this DNA Strider file with the phage's name, gene number, and +700. (e.g. Adephagia_gp73+700) Keep the file opened.

14. Select 24bp upstream of the start codon of the gene and 21bp into the gene. This will result in a 45bp of the upstream portion for the first part of the deletion oligo (DO). YOU MUST ADHERE TO THESE NUMBERS.



For example, in this case the following sequence will result:
gtcacgggataggagcccctgcagATGTACACAGAAACGTGGTAC

Copy the sequence and paste into a new DNA Strider file.

15. In this new file, after the 45bp, a 9bp TAG sequence will be inserted. The TAG sequence is a barcode-like sequence that will be used later to screen plaques to find the mutant.

    a. Successful TAG sequences in phage Giles have been:

       atc ttg ata; atc ttg atc; ttc ttg ata; ttc ttg atc; cag gtc aga

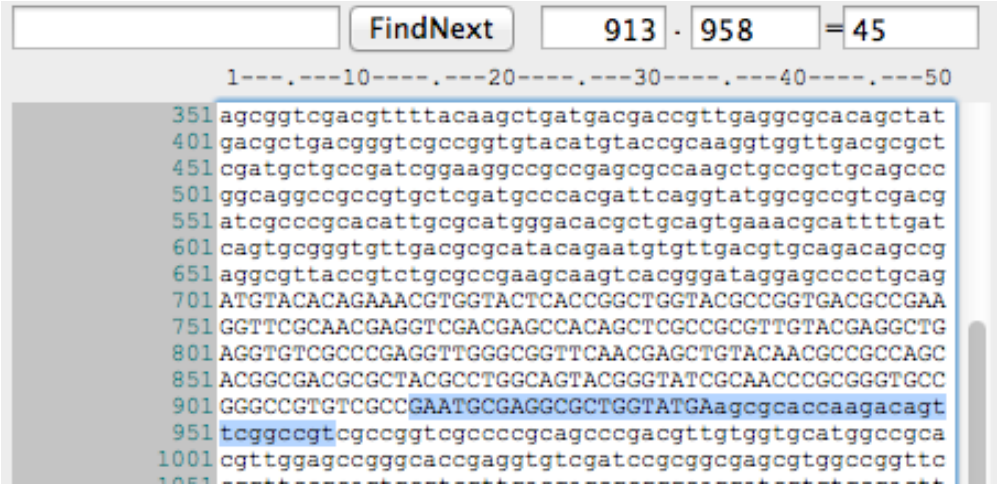
    b. The key to a good TAG sequence is to select codons that are commonly used in the phage, but that do not occur within and near the gene (hence 700bp upstream and downstream).

    c. CHECK by finding the TAG sequence in the phage_gene+700 DNA Strider file. If the sequence is not present in that file, that it is fine to use.

    d. In this example, the TAG sequence *atc ttg atc* will be used. Add this to the new file created with 45 upstream base pairs.

In this case, the sequence will now be:
gtcacgggataggagcccctgcagATGTACACAGAAACGTGGTACatcttgatc

16. Now 45 base pairs of the downstream must be needed. Select the last 21bp of the gene and 24bp downstream of this. This will again result in a 45bp sequence for the last part of the oligo.



In this case, the sequence will now be:
gtcacgggataggagcccctgcagATGTACACAGAAACGTGGTACatcttgatcGAATGCGAGGC
GCTGGTATGAagcgcaccaagacagttcggccgt

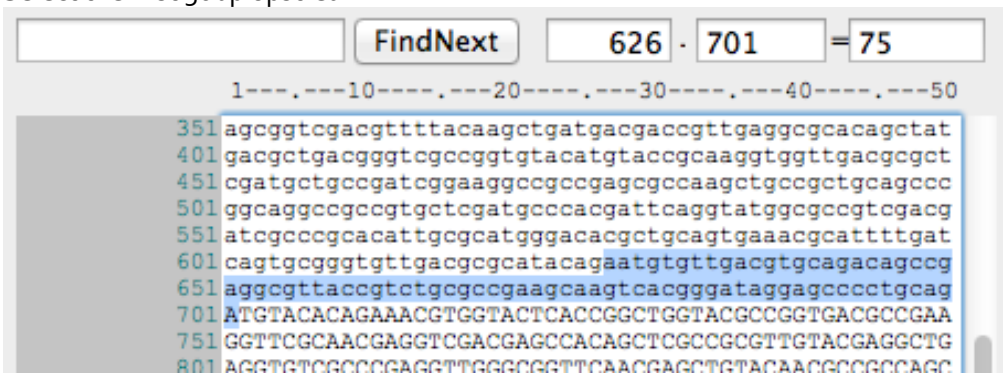
17. Save this 99bp file as phage_gene_DO. (e.g. Adephagia_gp73_DO).

18. Now the Extenders will be prepared. They are designed to add homology to the deletion oligo, generating a 200bp substrate made by PCR.

19. The first extender should include the first 25bp of the DO and 50bp upstream of those 25bp.

    a. One way is to copy the first 25bp of the DO and find them in the phage_gene+700 file.

    b. Select the first 50bp upstream.



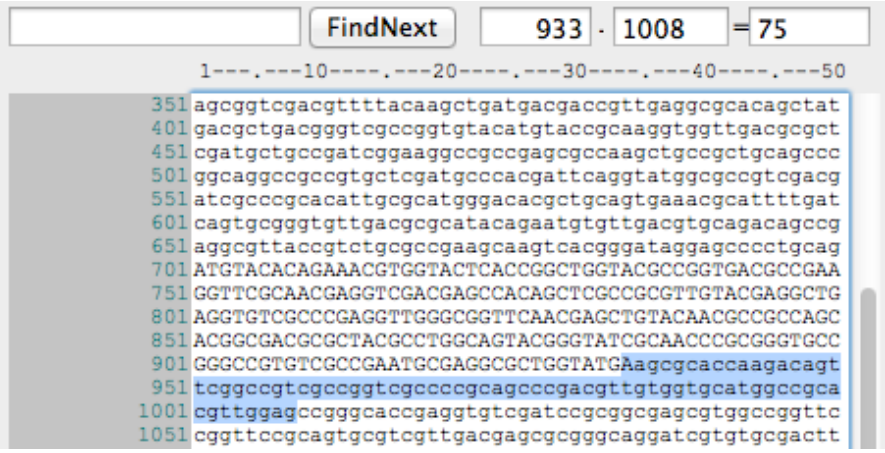
In this case, the sequence will be:
aatgtgttgacgtgcagacagccgaggcgttaccgtctgcgccgaagcaagtcacgggataggag
cccctgcagA

20. Paste the sequence in a new DNA Strider file, and save this new DNA Strider file. (e.g. Adephagia_gp73_Extender1)

21. The second extender should include the last 25bp of the DO and 50bp downstream of those 25bp.

    a. A way is to copy the last 25bp of the DO and find them in the phage_gene+700 file.

    b. Select the 50bp downstream.



In this case, the sequence will be:
`Aagcgcaccaagacagttcggccgtcgccggtcgccccgcagcccgacgttgtggtgcatggccg cacgttggag`

22. Paste the sequence in a new DNA Strider file, but don't save yet! Because Extender2 will amplify in the reverse direction, the sequence must be reversed and complement.

23. Highlight the Extender2 sequence, under "File," select "Reverse and Complement."

24. In this case, the reversed and complemented sequence will be:
`CTCCAACGTGCGGCCATGCACCACAACGTCGGGCTGCGGGGCGACCGGCGACGGCCGAACTGTC TTGGTGCGCTT`

25. Save this DNA Strider file. (e.g. Adephagia_gp73_Extender2)

26. Oligos are very, very expensive. Make sure all files are checked and verified by someone experienced before ordering them!