🛞 Phagehun	ting Program				(Phagehunting
	१९१२ २ २ २			A C C C T A A A C C C C C C C C C C C C		
PREPARATION ISOLATION	PURIFICATION AMPLIFICATION	EXTRACTION	CHARACTERIZATION	SEQUENCING	ANNOTATION	PHAMERATION FURTHER DISCOVERY
		Deletier				

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: <u>http://sourceforge.net/projects/dnastrider/</u>)
- DNA Master (PC only. Downloadable here: <u>http://cobamide2.bio.pitt.edu/</u>)

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 <u>www.phagesdb.org</u>, select the gene to be deleted. To do so, click on "Click to View" by "Gene List" under "Characterization."

Characterization	
Cluster	К
Other Cluster Members	Click to View
Subcluster	К1
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
Þ	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.

Description	Sequence	Product	Regions B	last Context
Start 49768		GC1		
Stop 50001		GC2		<u> </u>
Length 2	Length 234			-12.1
Direction F	orward	CAI		-18.7
ATGTACAC.	AGAAACGTG(GTACTCA	CGGCTGGT	ACGCCGGTG 📐
ACGCCGAA	GGTTCGCAA(CGAGGTC	GACGAGCCAG	CAGCTCGCC
GCGTTGTA	CGAGGCTGA	GETETCE	CCCGAGGTT	GGGCGGTTC
AACGAGCT	GTACAACGC	COCCAGCI	CCCCCCACCO	CGCTACGCC
TGGCAGTA	CGGGTATCG	CAACCCG	COCOTOCCO	GGCCGTGTC
GCCGAATG	CGAGGCGCT	GGTATGA		

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)

FindNext 234 234 = 0	
110304050	D
1 ATGTACACAGAAACGTGGTACTCACCGGCTGGTACGCCGGTGACGCCGAA 51 GGTTCGCAACGAGGTCGACGACGACGCCACAGCTCGCCGCGTTGTACGAGGCTG 101 AGGTGTCGCCCGAGGTTGGGCGGTTCAACGAGCTGTACAACGCCGCCGAGG 151 ACGGCGACGCGCTACGCCTGGCAGTACGGGTATCGCAACCCGCGGGGTGCC 201 GGGCCGTGTCGCCGAATGCGAGGCGCTGGTATGA	

- 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.

Overview	Features References	Sequence	Documen	itation							
Feature		• F 🕅	- 翻 Po	osition : 49068	8 - 50701 (1634	bases)	Raw 🕨	BLASTN	BLASTP	Add Feature	1 71.7 2 62.6 3 63.8
48961	TGGCGACTGCGCCGTC	GGTGCCTGA	GCCGCTG	GGGCGTTGC	TTGCATTGTT	CGGCGCCTGCGCA	GACGTTTCI	GTGCTGGT	CGTGTGT	GGGCATGTTG	CG 🔼
49046	CCGCCAGCTCGTCGAG	GTGCCTTGG	CTGTTGC	GTCGTCTGC.	AGGAGTCGGC	GTACGGCGAGGCGA	AAGGTCGCC	CGTAAGGG	TGGGCCT	CGGGTGTCGA	CG 👘
49131	GGGGAGCGGCTGCCGT	CGTTGCCGI	TGAACAC	TCGCGCGGC	CGACATGCTG	CGCGACGCTGCGC	GTCTGGTGI	CGTGGTGG	GAGCAGG	TGGGTGGCGT	CG
49216	ACCAGGGCGGCCCGCA	TGATGCTGC	GCGCGTC	GAGTCCGCG	GCCCGCTGGC	FGGCCGCCGAGCC	GGG <mark>CGCGAT</mark>	GATGGCGC	ACCCGTG	GGCACCTGAC	GC
49301	GCTGGGTTGGGTGTTG	CAGTGGCGC	CAGGACG	CTGAGCGTG	TGATEGACTT	GCCGCCGGATACG	CAGTACGCO	GGGCCGTG	CCAGAAC	GTCGTGCAGC	CG
49386	CCGAGTGCATCCGACG	CCGGTACGC	CGCTGCC	GCCTCGTGA	GTGCGGCACG	CCGCTGTATGTCG	ACGCCGAGO	CCCTGGTC	GCCGAGT	GCTACCGCTG	CG
49471	GCTGCTCGTGGCGGGT	CGAGGATTI	GCAGCGG	CAGGCCCTC	GATEGEATEG	ACGAGGCCGCGCCC	CCGCACGGO	CGCCGATA	TGTGGCG	GCTGCTCAAG	TT
49556	CGCGGGCCGCGACGTT	AAGCGGTCG	ACGTTTT	ACAAGCTGA'	TGACGACCGT	rgaggcgcacagc:	TATGACGCI	GACGGGTO	GCCGGTG	TACATGTACC	GC
49641	AAGGTGGTTGACGCGC	TCGATGCTG	CCGATCG	GAAGGCCGC	CGAGCGCCAA	GCTGCCGCTGCAG	CCCGGCAGO	CCGCCGTG	CTCGATG	CCCACGATTC	AG
49726	GTATGGCGCCGTCGAC	GATEGECEG	CACATTG	CGCATGGGA	CACGCTGCAG	(GAAACGCATTTT)	GATCAGTGO	GGGTGTTG	ACGCGCA	TACAGAATGT	GT
49811	TGACGTGCAGACAGCC	GAGGCGTTA	CCGTCTG	CGCCGAAGC.	AAGTCACGGG	ATAGGAGCCCCTG	CAGATGTAC	CACAGAAAC	GTGGTAC	TCACCGGCTG	GT
49896	ACGCCGGTGACGCCGA	AGGTTCGCA	ACGAGGT	CGACGAGCC.	ACAGCTCGCC	GCGTTGTACGAGG	CTGAGGTGT	CGCCCGAG	GTTGGGC	GGTTCAACGA	GC
49981	TGTACAACGCCGCCAG	CACGGCGAC	GCGCTAC	GCCTGGCAG	TACGGGTATCO	GCAACCCGCGGGGT(GCCGGGGCCG	TGTCGCCG	AATGCGA	GGCGCTGGTA	TG
50066	AAGCGCACCAAGACAG	TTCGGCCGT	CGCCGGT	CGCCCCGCA	GCCCGACGTT	GTGGTGCATGGCC	GCACGTTGO	GAGCCGGGG	ACCGAGG	TGTCGATCCG	CG
50151	GCGAGCGTGGCCGGTT	CCGGTTCCC	CAGTGCG	TCGTTGACG.	AGCGCGGGGCA	GGATCGTGTGCGA	CTTCATCGO	GGGCCCTG	CTGGTCA	CGAGACCTGG	CG
50236	GTEGTTETATECEGAC	CGTATCCGC	ACGGTGC.	ACCGTTTGA.	ACCGCACCCG	CGCGAACGCTGCC	GCATAGTCA	CGTGTTGA	CATGCAT	ACAGCGTGAG	GG
50321	GTACTGTATGCATGTC	AACACACAC	CGGGATA	GGAGCCCAC.	AGTGACGATT	FCGACCGCGACCC	GCAACATGA	CGCAGATO	GAAGCTC	ACCAGATCGC	CG
50406	TTGGCCTGATCCGCGA	GCATGGTCT	GATCGGC	TGGACTGTG.	AGCTGGGACA	ACGCCCGTCGTCG	CGCCGGTCA	GTGCCGCT	ACACGTC	GCGCACGATC	AG
50491	CTTGTCAAAGCCGCTG	CTGCGCCAG	CGTTCCT	ACGACGACA	CGATGATGAC	CATTACGCACGAG	ATTGCGCAC	GCCCTGGT	CGGCCCG	AAGCACGGGC	AT
50576	GACGCCGTGTGGGGCGG	CCAAGCACC	GACAGCT	CGGCGGCAA	CGGTCAGCGC	IGCTTTGAGCACC	TCGACGAGI	CGGCGCCG	TGGATGG	GCACGTGCGA	CC
50661	ACGGTAAGAAGTTCGC	GCGGTACCO	GGCACCG	AAGCGCCTC	GACGGGTGGC	GCTGCAAGTGCAC	GGCCGCCGG	TAGCCCCG	TGGTGTG	GGTCAACCAG	CG 🦳
50746	ATAGCGTCGACGCCCC	CAATCTTCC	GAGGTTG	GGGGCGTTT	TEGTETETAT	IGTTGACATGCAT#	ACAGCCCAC	GGGTTACI	GTATGCA	TACCAACAAC	GC
50831	ACTGACCACCTACCGA	TAGGAGCCC	ACAATGT	CGAACATCG	TCGCCGCCGCC	CCCCGCCGCCGGT	CGTTTCAAC	GCCGCTGC	CGCGCTG	AACATGATTC	TC
50916	GGTATCAACCTGTCGG	ACGGGCAGA	AGCGTGC	GCGCCTGCT	CGCGCTGGCG	GTGTCGAATGACG	CTGCCTCTG	GAGTTCAAC	TTGCGCG	CCGCTCGCAA	GG 🔜
	CGCTGGCCGCCGGTCG	CCTGGCCGA	GGCTGAT	CGTTGCGTC	GACGCTGCGG	AGTTCTACAACAA	CCGCGCCAA	GCGCCTGC	GCGACGA	GGCCCGCGCT	AT 🔛

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.

	FindNext	1634 · 1634	= 0
	120	304	050
1	cgcgatgatggcgcacccgtgg	gcacctgacgcgctggg	ttgggtgttgc 📔
51	agtggcgccaggacgctgagcg	tgtgatcgacttgccgc	cggatacgcag
101	tacgccgggccgtgccagaacg	tcgtgcagccgccgagt	gcatccgacgc
151	cggtacgccgctgccgcctcgt	gagtgcggcacgccgct	gtatgtcgacg
201	ccgaggccctggtcgccgagtg	etaccgctgcggctgct	cgtggcgggtc
251	gaggatttgcagcggcaggccc	togatogcatogaogag	geegegeeeeg
301	cacggccgccgatatgtggcgg	ctgctcaagttcgcggg	ccgcgacgtta
351	agcggtcgacgttttacaagct	gatgacgaccgttgagg	cgcacagctat
401	gacgctgacgggtcgccggtgt	acatgtaccgcaaggtg	gttgacgcgct
451	cgatgctgccgatcggaaggcc	geegagegeeaagetge	cgctgcagccc
501	ggcaggccgccgtgctcgatgc	ccacgattcaggtatgg	cgccgtcgacg
551	atcgcccgcacattgcgcatgg	gacacgetgeagtgaaa	cgcattttgat
601	cagtgcgggtgttgacgcgcat	acagaatgtgttgacgt	gcagacagccg
651	aggogttaccgtctgcgccgaa	gcaagtcacgggatagg	ageceetgeag
701	atgtacacagaaacgtggtact	caccggctggtacgccg	gtgacgccgaa
751	ggttcgcaacgaggtcgacgag	ccacagetegeegett	gtacgaggctg
801	aggtgtcgcccgaggttgggcg	gttcaacgagctgtaca	acgeegeeage
851	acggcgacgcgctacgcctggc	agtacgggtatcgcaac	ccgcgggtgcc
901	gggccgtgtcgccgaatgcgag	gcgctggtatgaagcgc	accaagacagt
951	teggeegtegeeggtegeeeeg	cagcccgacgttgtggt	gcatggccgca
1001	cgttggagccgggcaccgaggt	gtcgatccgcggcgagc	gtggccggttc
1051	cggttccgcagtgcgtcgttga	cgagcgcggggcaggate	gtgtgcgactt
1101	catcggcggccctgctggtcac	gagacetggeggtegtt	ctatcccgacc
1151	gtatccgcacggtgcaccgttt	gaaccgcacccgcgcga	acgetgeegea
1201	tagtcacgtgttgacatgcata	cagcgtgaggggtactg	tatgcatgtca
1251	acacacccgggataggagccc	acagtgacgatttcgac	cgcgacccgca
1301	acatgacgcagatggaagctca	ccagatcgccgttggcc	tgatccgcgag
1351	catggtctgatcggctggactg	tgagetgggacaacgee	cgtcgtcgcgc
1401	cggtcagtgccgctacacgtcg	regeacgateagettgte	aaagcegetge
1451	tgcgccagcgttcctacgacga	cacgatgatgaccatta	cgcacgagatt
1501	gcgcacgccctggtcggcccga	agcacgggcatgacgcc	gtgtgggcggc
1551	annananananan annanananan	and and an another the	tanaanataa

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.

FindNext 676 - 721 = 45	
11020304050	
351 agcggtcgacgttttacaagctgatgacgaccgttgaggcgcacagctat 401 gacgctgacgggtcgccggtgtacatgtaccgcaaggtggttgacgcgct	
451 cgatgctgccgatcggaaggccgccgagcgccaagctgccgctgcagccc 501 ggcaggccgccgtgctcgatgcccacgattcaggtatggcgccgtcgacg	
601 cagtgcgggtgttgacgcgcatacagaatgtgtgacgtgcagcagcag 601 cagtgcgggtgttgacgcgcatacagaatgttgacgtgcagacagccg	
701 ATGTACACAGAAACGTGGTACTCACCGGCTGGTACGCCGGTGACGCCGAA 751 GGTTCGCAACGAGGTCGACGACGACGACGCCGCGCTGTACGACGCCGA	١.
801 AGGTGTCGCCCGAGGTTGGGCGGTTCAACGAGCTGTACAACGCCGCCAGC 851 ACGGCGACGCGCTACGCCTGGCAGTACGGGTATCGCAACCCGCGGGTGCC	

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.

	FindNext	913 · 958	= 45
	120		050
351 401 451 501 551 601 651 701 751 801 851 901 951 1001	agcggtcgacgttttacaagct gacgctgacggtcgccggtgt cgatgctgccgatcggaaggcc ggcaggccgccgtgttcgatgg atcgcccgcacattgcgcatgg cagtgcgggtgttgacgcgcat aggcgttaccgtctgcgccgaa ATGTACACAGAAACGTGGTACT GGTTCGCAACGAGGTCGACGAG AGGTGTCGCCCGAGGTTGGGCG ACGCCGACGCGCTACGCCTGGC GGGCCGTGTCGCCCGAATGCGAG tcggccgtcgccggtcgccccg cgttggagccgggcaccgaggt	gatgacgaccgttgaggg acatgtaccgcaaggtgg gccgagcgccaagctgcg gacacgetgcagtgaaag acagatgtgtgacgtg gcaagtcacgggatagga CACCGGCTGGTACGCCGG CCACAGCTCGCCGCGTTC GTTCAACGAGCTGTACAA AGTACGGGTATCGCAACG GCGCTGGTATGAagcgca cagcccgacgttgtggtg	cgcacagctat gttgacgcgct cgctgcagccc cgccgtcgacg cgcattttgat gcagacagccg agcccctgcag GTGACGCCGAA GTACGACGCCGAA GTACGACGCCAGC CCGCGGGGTGCC accaagacagt gcatggccgca gtggccggttc

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.

FindNext 626 · 701 = 75	
11020304050	
351 agcggtcgacgttttacaagctgatgacgaccgttgaggcgcacagctat 401 gacgctgacgggtcgccggtgtacatgtaccgcaaggtggttgacgcgct 451 cgatgctgccgatcggaaggccgccgagcgccaagctgccgctgcagccc 501 ggcaggccgccgtgctcgatgcccacgattcaggtatggcgccgtcgacg 551 atcgcccgcacattgcgcatgggacacgctgcagtgaaacgcattttgat	
601 cagtgcgggtgttgacgcgcatacagaatgtgttgacgtgcagacagccg 651 aggcgttaccgtctgcgccgaagcaagtcacgggataggagcccctgcag 701 ATGTACACAGAAACGTGGTACTCACCGGCTGGTACGCCGGTGACGCCGAA	
751 GGTTCGCAACGAGGTCGACGAGCCACAGCTCGCCGCGTTGTACGAGGCTG 801 AGGTGTCGCCCGAGGTTGGGCGGTTCAACGAGCTGTACAACGCCGCCAGC	

In this case, the sequence will be:

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 5obp 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.

FindNext 933 · 1008 = 75
11020304050
351 agcggtcgacgttttacaagctgatgacgacgttgaggggcacagctat 401 gacgctgacggtgcgccggtgtacatgtaccgcaaggtggtgacggct 451 cgatgctgccgatcggaaggccgccgaggcgcaaggtgcgcgcgtgagc 501 ggcaggccgcgtgctcgatgccacgatcaggtatggggcgcgtcgacg 551 atcgcccgcacattgcgcatgggacacgctgcagtgaaacgcatttgat 601 cagtgcgggtgttgacgcgcaaacagaatgtgttgacgtgcagacagccg 651 aggcgttaccgtctggcgcaagcaagtcagggataggagcccctgcag 701 ATGTACACAGAAACGTGGTACTCACCGGCTGGTACGCCGGTACGCCGAA 751 GGTTCGCAACGAGGTCGACGACGACCACAGCTCGACGCCGCGCGCG

In this case, the sequence will be:

- 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."
- 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!