I have set up a template for you to use with Clustal alignment. You will see the last sequence has already been supplied for you. It comes from pClone Red and contains some of the flanking DNA (teal bases), the left-facing promoter (black bases), and the two sticky ends (bold letters). The regular black font is the original promoter pointing towards GFP. First, find your top oligos (V1 and V2) and replace the appropriate 4 Fs but do not include the sticky ends. Then copy and paste the first ~250 bases from your 4 sequencing reactions to replace the appropriate 4 Zs. Do not include any N bases at the beginning of your sequence. You must format your sequences using the FASTA format which is this:

>name\_of\_sequence

sequence pasted here (limit to < 250 bases)

Remove any spaces between each sequence as shown below.

>your\_intended\_V1promoter\_oligo

FFFF

>your\_intended\_V2promoter\_oligo

FFFF

>your\_V2X1\_cloned\_sequence

ZZZZ

>your\_V2X2\_cloned\_sequence

ZZZZ

>your\_V2X3\_cloned\_sequence

ZZZZ

>your\_V1\_cloned\_sequence

ZZZZ

>pClone\_Red\_promoter

tcttctcctttacgcatctagtatttctcctctttaattactaga**cgac**tgagaccccgggcgctatcatgccataccgcgaaaggtggtgtcaacgtaaatgcatgccgctggtctct**gcgg**gggcccaagttcacttaaaaaggagat

Copy all 7 sequences from above and paste them into the appropriate box on the Clustal Omega site. Make sure you have changed the program from protein to DNA. Under Step 2, choose “More options”. At the bottom of the options, change the ORDER from “aligned” to “input” so the sequences will appear in the same order as above. Once you see these complete results, you may want to submit only a subset of the 7 sequences to produce figures you can use in your final lab report.