

Verifying Bases of Interest: xClone Red

1. Right click on one of your file (.seq and .ab1). On a Mac, you can Get Info and have it do this every time as demonstrated in lab.
2. Go to CLUSTAL Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) web tool. Change the data type to DNA.
3. Copy and paste your designed sequence using FASTA format (see below). You will need to remember the part numbers for v1 and v2 designs. Do the same with your sequencing results. Open a .seq file to get the sequence. Omit the initial Ns and terminal Ns. Use FASTA format to create a name as shown below. Submit using “CLUSTAL w/ numbers”.

STEP 1 - Enter your input sequences

Enter or paste a set of

DNA

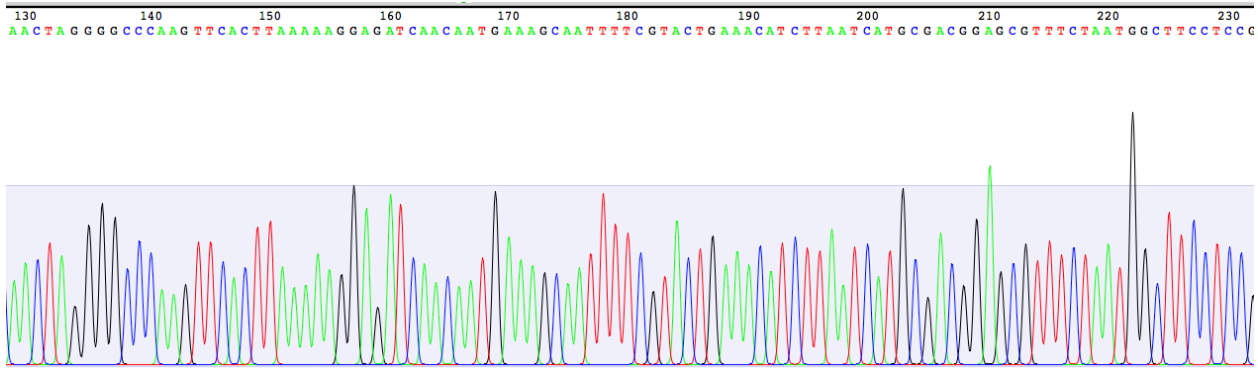
sequences in any supported format:

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>BBa_J100325 Part-only sequence (54 bp)
agagccccacgtcgtctcgacagttaactatgtaatgctaactctcgaaa
>ASL-442 AM pClone v2
TTTACGCATCTAGTATTTCTCCTCTTTAATACTAGAGGTCCTCCGACAGAGCCCCACGTGCGTCTCGACAGTTAAGTATGTAATGCTAACTTCTCGAAAGCGGG
AAGACAAC TAGGGCCCAAGTTCACCTAAAAAGGAGATCAACAATGAAAGCAATTTTCGTAAGTAAACATCTTAATCATGCGACGGAGCGTTTCTAATGGCTTCT
CCGAAGATGTTATCAAAGAGTTCATGCGTTTCAAAGTTCGTATGGAAGGTTCCGTTAACGGTCACGAGTTCGAAATCGAAGGTGAAGGTGAAGGTGTCGGTAC
GAAGGTACCCAGACCGCTAAACTGAAAGTTACCAAAGGTGGTCCGCTGCCGTTCCGTTGGGACATCCTGTCCCGCAGTTCAGTACGGTCCAAAGCTTAC
GTTAAACACCCGGCTGACATCCCGGACTACCTGAAACTGTCCTTCCCGGAAGGTTTCAAATGGGAACGTGTTATGAACTTCGAAGATGGTGGTGTGTTACCGT
TACCCAGGACTCCTCCCTGCAAGACGGTGAGTTCATCTACAAAGTTAAACTGCGTGGTACCAACTTCCCGTCCGACGGTCCGGTTATGAGAAAAAACCATG
GGTTGGGAAGCTTCCACCGAAGTATGTACCCGGAAGATGGTGTCTGAAAGGTGAAATCAAATGCGTCTGAAACTGAAAGACGGTGGTCACTACGACGCTG
AAGTTAAACCACTACATGGCTAAAAAACCGGTTACGCTGCCGGGTGCTTACAAAACCGACATCAAAGTGGACATCACCTCCACAAACGAAGATTACACCATC
GTTGAACAGTACGAACGTGCTGAAGGTGCTCACTCCACCGGTGCTTAATAATACTAGTAGCGGCGGCTGCAGGCTTCCCTCGCTCACTGACTGCTGCGCTCG
GTCGTTCCGGTCCGCGGAGNGNTATCANNTCACTCAANGGNGNNAATACGGTTATCCNNGAATCAGGGGANAACGCAGGAAAGGACTGTGAGCAAAAGGC

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4. If there are bases in your region of interest, then you can open the chromat (.ab1) using ApE. You can verify any bases about which the sequencing software was uncertain (see below).



5. Record the results for each of your 4 sequencing reactions. If your sequencing reaction did not produce usable data, look at the chromat and confirm that the sequence cannot be read.