

Supplement for Laboratory Exercise #17

Automated Nucleotide Sequencing and Electropherogram Evaluation

Automated nucleotide sequencing will be covered in class next week, but the sequences generated for PUNK2 cultures are currently available on the website, and you are encouraged to access them, edit and combine them (as instructed on pages 200-201 of the laboratory manual) to create a contiguous sequence of around 1470-1530 bases in length.

Our nucleotide sequences (electropherograms or chromatograms) are in the form of .ab1 files, and can only be accessed through the use of appropriate software programs. If you are using an Apple computer, the recommended software is called 4Peaks, and can be acquired for free at <http://nucleobytes.com/4peaks/>. This same site offers alternative programs for PC users.

Our nucleotide sequences have been compressed using Stuffit Destinations, and are in the form of .sitx files. These can be accessed using Stuffit Expander also available for free at <https://www.macupdate.com/app/mac/20954/stuffit-expander>.

After you have acquired these software programs, go to Web-based Laboratory Assignments and click on Fall 2017 – PUNK2 Electropherograms (EPGs). This will open a grid with a set of numbers. Make sure you are looking at the Fall 2017 PUNK2 set, because the Spring 2017 set is different.

Click on the number for your PUNK2 to open the linked file folder. If you are using a small monitor, the folder will likely open behind the page showing the number grid, and you will not see it. Do not click multiple times, just close the grid page so you can see the file folder behind it.

Next, click on the folder to view the .ab1 files inside. Most folders contain four files, but some contain only three. If four files are present, two of them are labeled 533, but one is a forward sequence, and one is a reverse sequence (it is very important to note the difference). There are always two forward sequences, 8-forward and 533-forward, and these should be opened first.

Place the 8-forward sequence near the top of your desktop and extend it such that it takes up most of the desktop length. Next open the 533-forward sequence, position it just below the 8-forward and extend it not quite as far as the 8-forward .



Next, open a word file and then copy and paste the two forward sequences into that file.

Do not copy and paste translations, as we are not interested in amino acid sequences. Once you have the two forward sequences visible as word files, edit the 8-forward sequence by inserting two spaces just beyond the sequence GGGCGTAAA (about 500 bases from the start). Note, this sequence may appear as GGGTTTAAA, but this is less common. Since variation is possible (remember, mutation happens) I recommend you look for the sequence using your own visual capabilities rather than asking the computer to find it. Each line of text is about 50 bases in length, so every two lines is approximately equal to 100bp. Count down to 500 and look for the sequence (GGGCGTAAA).

Look for this same sequence in the first line of letters representing the 533-forward sequence; however, in most cases the 533-forward sequence will read GGGCGTAA (there actually are three adenine bases, but because this sequence occurs so close to the start, it does not have proper resolution).

8-forward

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ATGCATCGCAGCTTAMCATGCAGTCGAGGGGTATACTTCTTCGGGAGTAGAGACCGGGCGCACGGGTGCG
TAACGCGTATGCAATCTACCTTTTACAGAGGGATAGCCCAGAGAAATTTGGATTAATACCTCATAGCAT
AGCAACTTCGCATGAAGTCACTATTAAGTCACAACGGTAAAAGATGAGCATGCGTCCCATTAGCTAGT
TGGTAAGGTAACGGCTTACCAAGGCTACGATGGGTAGGGGTCCTGAGAGGGAGATCCCCCACACTGGTAC
TGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATTGGACAATGGGCGCAAGCCTGATC
CAGCCATGCCGCGTGCAGGATGACGGTCTATGGATTGTAAACTGCTTTTATACAGGAAGAAACCCTGGT
TCGTGAACCAGCTTGACGGTACTGTAAGAATAAGGATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATAC
GGAGGATCCAAGCGTTATCCGGAATCATTGGGTTAAAGGGTCCGTAGGCGGTTTAGTAAGTCAGTGGT
GAAAGCCATCGCTCAACGGTGAACGGCCATTGATACTGCTAAACTTGAATTATTAGGAAGTAACTAG
AATATGTAGTGTAGCGGTGAAATGCTTAGAGATTACATGGAATACCAATTGCGAAGGCAGGTTACTACT
AATWKATTGACGCTGATGGACGAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCG
TAAACGATGGATACTAGCTGTTGGGAGCAATCTCAGTGGCTAAGCGAAAGTGATAAGTATCCCACCTGG
GGAGTACGTTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGT
TTAATTCGATGATACGCGAGGAACCTTACCAAGGCTTAAATGTAGTTTGACCGATTTGGAAACAGATTT
TTCGCAAGACAAATTACAAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCAGTAAGTCTAT
ACGAGCGCACCCCTGTGTAGTGCAGCGAGTCATGTCCGACTCTACAGACTGCCAGTGCAACTGTGAG
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533-forward

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AGGCTCGTACCGGATCATTGGGTTAAAGGGTCCGTAGGCGGTTTAGTAAGTCAGTGGTGAAAGCCATCG
CTCAACGGTGAACGGCCATTGATACTGCTAAACTTGAATTATTAGGAAGTAACTAGAATATGTAGTGT
AGCGGTGAAATGCTTAGAGATTACATGGAATACCAATTGCGAAGGCAGGTTACTACTAATWKATTGACG
CTGATGGACGAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGGAT
ACTAGCTGTTGGGAGCAATCTCAGTGGCTAAGCGAAAGTGATAAGTATCCCACCTGGGGAGTACGTTTCG
AAGAATGAAACTCAAAGGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTAATTCGATGAT
ACGCGAGGAACCTTACCAAGGCTTAAATGTAGTTTGACCGATTTGGAAACAGATTTTTCGCAAGACAAA
TTACAAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCAGGTTAAGTCCTATAACGAGCGCA
ACCCCTGTTGTTAGTTGCCAGCGAGTCATGTCCGGAACTCTAACAAGACTGCCAGTGCAAACCTGTGAGGA
AGGTGGGGATGACGTCAAATCATCACGGCCCTTACGCCTTGGGCTACACACGTGCTACAATGGCCGGTAC
AGAGAGCAGCCACTGGGCGACCAGGAGCGAATCTATAAAACCGGTCACAGTTCGGATCGGAGTCTGCAAC
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To combine these sequences, place your cursor behind the GGGCGTAAA in the 8-forward sequence (shown in the alternate form above) and insert two spaces (everything beyond the AAA will be removed). Do the same for the 533-forward sequence, but in that case, after inserting the spaces, remove everything ahead of the final AA.

8-forward

ATGCATCGCAGCTTAMCATGCAGTCGAGGGGTATACTTCTTCGGGAGTAGAGACCGGGCCACGGGTGCG
TAACGCGTATGCAATCTACCTTTTACAGAGGGATAGCCCAGAGAAATTTGGATTAATACCTCATAGCAT
AGCAACTTCGCATGAAGTCACTATTAAGTCAACAACGGTAAAAGATGAGCATGCGTCCCATTAGCTAGT
TGGTAAGGTAACGGCTTACCAAGGCTACGATGGGTAGGGGTCCTGAGAGGGAGATCCCCACACTGGTAC
TGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATTGGACAATGGGCGCAAGCCTGATC
CAGCCATGCCCGTGCAGGATGACGGTCTATGGATTGTAACTGCTTTTATACAGGAAGAAACCCTGGT
TCGTGAACCAGCTTGACGGTACTGTAAGAATAAGGATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATAC
GGAGGATCCAAGCGTTATCCGGAATCATTGGGTTTAAA

GGGTCCGTAGGCGGTTTAGTAAGTCAGTGGTGAAAGCCCATCGCTCAACGGTGGAACGGCCATTGATACT
GCTAAACTTGAATTATTAGGAAGTAACTAGAATATGTAGTGTAGCGGTGAAATGCTTAGAGATTACATG
GAATACCAATTGCGAAGGCAGGTTACTACTAATWKATTGACGCTGATGGACGAAAGCGTGGGTAGCGAA
CAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGGATACTAGCTGTTGGGAGCAATCTCAGTGGC
TAAGCGAAAGTGATAAGTATCCCACCTGGGGAGTACGTTTCGCAAGAATGAAACTCAAAGGAATTGACGG
GGGCCCCGACAAGCGGTGGAGCATGTGGTTTAAATTCGATGATACGCGAGGAACCTTACCAAGGCTTAAAT
GTAGTTTGACCGATTTGGAACAGATTTTTTCGCAAGACAAATTACAAGGTGCTGCATGGTTGTCGTCAG
CTCGTGCCGTGAGGTGTCAGTAAGTCTATACGAGCGCACCCCTGTGTAGTGCAGCGAGTCATGTCCGACTC
TACAGACTGCCAGTGCAACTGTGAG

533-forward

AGGCTCGTACCGGATCATTGGGTTTAA

GGGTCCGTAGGCGGTTTAGTAAGTCAGTGGTGAAAGCCCATCGCTCAACGGTGGAACGGCCATTGATACT
GCTAAACTTGAATTATTAGGAAGTAACTAGAATATGTAGTGTAGCGGTGAAATGCTTAGAGATTACATG
GAATACCAATTGCGAAGGCAGGTTACTACTAATWKATTGACGCTGATGGACGAAAGCGTGGGTAGCGAA
CAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGGATACTAGCTGTTGGGAGCAATCTCAGTGGC
TAAGCGAAAGTGATAAGTATCCCACCTGGGGAGTACGTTTCGCAAGAATGAAACTCAAAGGAATTGACGG
GGGCCCCGACAAGCGGTGGAGCATGTGGTTTAAATTCGATGATACGCGAGGAACCTTACCAAGGCTTAAAT
GTAGTTTGACCGATTTGGAACAGATTTTTTCGCAAGACAAATTACAAGGTGCTGCATGGTTGTCGTCAG
CTCGTGCCGTGAGGTGTCAGGTTAAGTCCTATAACGAGCGCAACCCCTGTTGTTAGTTGCCAGCGAGTCA
TGTCGGGAACTCTAACAAGACTGCCAGTGCAAACTGTGAGGAAGGTGGGGATGACGTCAAATCATCAG
GCCTTACGCCTTGGGCTACACACGTGCTACAATGGCCGGTACAGAGAGCAGCCACTGGGCGACCAGGAG
CGAATCTATAAAACCGGTCACAGTTCGGATCGGAGTCTGCAAC

Delete the highlighted portion of the sequence (as shown), and then remove any spaces left between the two so that you have just one combined sequence.

Next, access your reverse sequence(s). Before you copy and paste these into your word file, you must apply the edit option "Flip sequence". This is because reverse primers bind to DNA strands that are complementary to those the forward primers bind to.

If you have a 533-reverse sequence, this will overlap the 8-forward sequence, and provide you with additional bases at the front end (you can see the 8-forward primer used for the sequencing reaction). Look on page 196 of the lab manual to see the 8-forward primer base sequence. The 1530-reverse and 1492-reverse sequences (you will have one or the other, but not both) overlap the 533-forward sequence rather extensively. The base sequence recommended for editing between the 533-forward and 1530-reverse (or 1492-reverse) sequences is GGGGGCCC (sometimes GGGGACCC). A possible overlap sequence to look for between 533-reverse and 8-forward is also shown below.

533-reverse complement

CCGCSTAGAGTTTGATCCTGGCTCAGGATGAACGCTAGCGGCAGGCTTAACACATGCAAGTCGAGGGGTA
TACTTCTTCGGGAGTAGAGACCGGCGCACGGGTGCGTAACGCGTATGCAATCTACCTTTTACAGAGGGAT
AGCCCAGAGAAATTTGGATTAATACCTCATAGCATAGCAACTTCGCATGAAGTCACTATTAAGTCACA
ACGGTAAAAGATGAGCATGCGTCCCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCTACGATGGG
TAGGGGTCCTGAGAGGGAGATCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCA
GTGAGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGGATGACGGTCCTATGGA
TTGTAAACTGCTTTTATACAGGAAGAAACCCTGGTTCGTGAACCAGCTGACGGTACGTAGATAGTAGCC
GAACC

The sequence shown in red above is shown in the earlier 8-forward sequences as blue. The green portion of the 533-reverse complement is junk (not part of our primer).

Combined 8-forward and 533-forward sequences look like this:

ATGCATCGCAGCTTAMCATGCAGTCGAGGGTATACTTCTTCGGGAGTAGAGACCGGCGCACGGGTGCG
TAACGCGTATGCAATCTACCTTTTACAGAGGGATAGCCCAGAGAAATTTGGATTAATACCTCATAGCAT
AGCAACTTCGCATGAAGTCACTATTAAGTCACAACGGTAAAAGATGAGCATGCGTCCCATTAGCTAGT
TGGTAAGGTAACGGCTTACCAAGGCTACGATGGGTAGGGGTCCTGAGAGGGAGATCCCCACACTGGTAC
TGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATTGGACAATGGGCGCAAGCCTGATC
CAGCCATGCCGCGTGAGGATGACGGTCCTATGGATTGTAAACTGCTTTTATACAGGAAGAAACCCTGGT
TCGTGAACCAGCTTGACGGTACTGTAAGAATAAGGATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATAC
GGAGGATCCAAGCGTTATCCGGAATCATTGGGTTAAAGGGTCCGTAGGCGGTTTAGTAAGTCAGTGGT
GAAAGCCATCGCTCAACGGTGGAACGGCCATTGATACTGCTAAACTTGAATTATTAGGAAGTAACTAG
AATATGTAGTGTAGCGGTGAAATGCTTAGAGATTACATGGAATACCAATTGCCAAGGCAGGTTACTACT
AATWKATTGACGCTGATGGACGAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCG
TAAACGATGGATACTAGCTGTTGGGAGCAATCTCAGTGGCTAAGCGAAAGTGATAAGTATCCCACCTGG
GGAGTACGTTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGCCGCACAAGCGGTGGAGCATGTGGT
TTAATTCGATGATACGCGAGGAACCTTACCAAGGCTTAAATGTAGTTTGACCGATTTGGAAACAGATTT
TTCGCAAGACAAATTACAAGGTGCTGCATGGTTGTGTCAGCTCGTGCCGTGAGGTGTCAGGTTAAGTCC
TATAACGAGCGCAACCCCTGTTGTTAGTTGCCAGCGAGTCATGTCGGGAACCTCTAACAAGACTGCCAGTG
CAAACGTGTGAGGAAGGTGGGGATGACGTCAAATCATCACGGCCCTTACGCCTTGGGCTACACACGTGCTA
CAATGGCCGTTACAGAGAGCAGCCACTGGGCGACCAGGAGCGAATCTATAAAACCGGTCACAGTTCGGAT
CGGAGTCTGCAAC

1530-reverse complement looks like this

TWWTTGGACATTGGGCGCAAGCTGATTCAGCATGCGCKTGACATGACGTCTATGATGTAAACTGCTT
TWTACAGGAGAAATCTGGTCGTGATCAGCTTGACGGTACTGTAAGAATAAGGATCGGCTAACTCCGTGC
CAGCAGCGCGTAATACGGAGGATCAAGCGTTATCCGGAATCATTGGGTTTAAAGGGTCCGTAGGCGGT
TTAGTAAGTCAGTGGTGAAAGCCATCGCTCAACGGTGAACGGCCATTGATACTGCTAAACTTGAATT
ATTAGGAAGTAACTAGAATATGTAGTGTAGCGGTGAAATGCTTAGAGATTACATGGAATACCAATTGCG
AAGGCAGGTTACTACTAATWKATTGACGCTGATGGACGAAAGCGTGGGTAGCGAACAGGATTAGATACC
CTGGTAGTCCACGCCGTAAACGATGGATACTAGCTGTTGGGAGCAATCTCAGTGGCTAAGCGAAAGTGA
TAAGTATCCCACCTGGGGAGTACGTTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGCCGCACAAG
CGGTGGAGCATGTGGTTTAAATTCGATGATACGCGAGGAACCTTACCAAGGCTTAAATGTAGTTTGACCG
ATTTGGAAACAGATTTTTCGCAAGACAAATTACAAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAG
GTGTCAGGTTAAGTCTATAACGAGCGCAACCCCTGTTGTTAGTTGCCAGCGAGTCATGTCGGGAACTCT
AACAAGACTGCCAGTGCAAACGTGAGGAAGGTGGGGATGACGTCAAATCATCACGGCCCTTACGCCTTG
GGCTACACACGTGCTACAATGGCCGGTACAGAGAGCAGCCACTGGGCGACCAGGAGCGAATCTATAAAAC
CGGTACAGTTCGGATCGGAGTCTGCAACTCGACTCCGTGAAGCTGGAATCGCTAGTAATCGGATATCAG
CCATGATCCGGTGAATACGTTCCCGGGCCTTGACACACCGCCCGTCAAGCCATGGAAGCTGGGGGTGCC

TGAAGTCGGTGACCGCAAGGAGCTGCCTAGGGTTAAAAGTGGTAACTAGGGCTAAGTCGTAATCAAGWG
CAMCCC

Insert spaces behind the GGGGCC (shown in red) in both the combined sequence and the 1530 or 1492-reverse (count up 500 bases from the end to find look for this).

Combined 8-forward and 533-forward sequences look like this:

ATGCATCGCAGCTTAMCATGCAGTCGAGGGTATACTTCTTCGGGAGTAGAGACCGGCGCACGGGTGCG
TAACGCGTATGCAATCTACCTTTTACAGAGGGATAGCCCAGAGAAATTTGGATTAATACCTCATAGCAT
AGCAACTTCGCATGAAGTCACTATTAAGTACAACGGTAAAAGATGAGCATGCGTCCCATTAGCTAGT
TGGTAAGGTAACGGCTTACCAAGGCTACGATGGGTAGGGGTCCTGAGAGGGAGATCCCCACACTGGTAC
TGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATTGGACAATGGGCGCAAGCCTGATC
CAGCCATGCCGCGTGCAGGATGACGGTCTATGGATTGTAACTGCTTTTATACAGGAAGAAACCCTGGT
TCGTGAACCAGCTTGACGGTACTGTAAGAATAAGGATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATAC
GGAGGATCCAAGCGTTATCCGGAATCATTGGGTTAAAGGGTCCGTAGGCGGTTTAGTAAGTCAGTGGT
GAAAGCCATCGCTCAACGGTGAACGGCCATTGATACTGCTAACTTGAATTATTAGGAAGTAAGTAG
AATATGTAGTGTAGCGGTGAAATGCTTAGAGATTACATGGAATACCAATTGCGAAGGCAGGTTACTACT
AATWKATTGACGCTGATGGACGAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCG
TAAACGATGGATACTAGCTGTTGGGAGCAATCTCAGTGGCTAAGCGAAAGTGATAAGTATCCCACCTGG
GGAGTACGTTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGCCC

GCACAAGCGGTGGAGCATGTGGTTTAATTTCGATGATACGCGAGGAACCTTACCAAGGCTTAAATGTAGT
TTGACCGATTTGAAACAGATTTTTCGCAAGACAAATTACAAGGTGCTGCATGGTTGTCGTCAGCTCGTG
CCGTGAGGTGTCAGGTTAAGTCTATAACGAGCGCAACCCCTGTTGTTAGTTGCCAGCGAGTCATGTCGG
GAACTCTAACAAGACTGCCAGTGCAAACTGTGAGGAAGGTGGGGATGACGTCAAATCATCACGGCCCTT
ACGCCTTGGGCTACACACGTGCTACAATGGCCGGTACAGAGAGCAGCCACTGGGCGACCAGGAGCGAATC
TATAAAACCGGTCACAGTTCGGATCGGAGTCTGCAAC

1530-reverse complement looks like this

TWWTTGGACATTGGGCGCAAGCTGATTCAGCATGCGCKTGCAGATGACGTCTATGATGTAACTGCTT
TWTACAGGAGAAATCTGGTCGTGATCAGCTTGACGGTACTGTAAGAATAAGGATCGGCTAACTCCGTGC
CAGCAGCGCGTAATACGGAGGATCAAGCGTTATCCGGAATCATTGGGTTTAAAGGGTCCGTAGGCGGT
TTAGTAAGTCAGTGGTAAAGCCATCGCTCAACGGTGAACGGCCATTGATACTGCTAACTTGAATT
ATTAGGAAGTAAGTAAATATGTAGTGTAGCGGTGAAATGCTTAGAGATTACATGGAATACCAATTGCG
AAGGCAGGTTACTACTAATWKATTGACGCTGATGGACGAAAGCGTGGGTAGCGAACAGGATTAGATACC
CTGGTAGTCCACGCCGTAAACGATGGATACTAGCTGTTGGGAGCAATCTCAGTGGCTAAGCGAAAGTGA
TAAGTATCCCACCTGGGAGTACGTTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGCCC

GCACAAGCGGTGGAGCATGTGGTTTAATTTCGATGATACGCGAGGAACCTTACCAAGGCTTAAATGTAGT
TTGACCGATTTGAAACAGATTTTTCGCAAGACAAATTACAAGGTGCTGCATGGTTGTCGTCAGCTCGTG
CCGTGAGGTGTCAGGTTAAGTCTATAACGAGCGCAACCCCTGTTGTTAGTTGCCAGCGAGTCATGTCGG
GAACTCTAACAAGACTGCCAGTGCAAACTGTGAGGAAGGTGGGGATGACGTCAAATCATCACGGCCCTT
ACGCCTTGGGCTACACACGTGCTACAATGGCCGGTACAGAGAGCAGCCACTGGGCGACCAGGAGCGAATC
TATAAAACCGGTCACAGTTCGGATCGGAGTCTGCAACTCGACTCCGTGAAGCTGGAATCGTAGTAATCG
GATATCAGCCATGATCCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCTG
GGGGTGCCTGAAGTCGGTGACCGCAAGGAGCTGCCTAGGGTTAAAAGTGGTAACTAGGGCTAAGTCGTA
ATCAAGWGCAMCCC

Remove the highlighted portion and delete any spaces so that you have just one combined sequence.

Combined 8-forward, 533-forward and 1530-reverse complement sequences looks like this:

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ATGCATCGCAGCTTAMCATGCAGTCGAGGGGTATACTTCTTCGGGAGTAGAGACCGGGCGCACGGGTGCG
TAACGCGTATGCAATCTACCTTTTACAGAGGGATAGCCCAGAGAAATTTGGATTAATACCTCATAGCAT
AGCAACTTCGCATGAAGTCACTATTAAGTCAACAACGGTAAAAGATGAGCATGCGTCCCATTAGCTAGT
TGGTAAGGTAACGGCTTACCAAGGCTACGATGGGTAGGGGTCCTGAGAGGGAGATCCCCCACACTGGTAC
TGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATTGGACAATGGGCGCAAGCCTGATC
CAGCCATGCCGCGTGCAGGATGACGGTCCTATGGATTGTAAACTGCTTTTATACAGGAAGAAACCCTGGT
TCGTGAACCAGCTTGACGGTACTGTAAGAATAAGGATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATAC
GGAGGATCCAAGCGTTATCCGGAATCATTGGGTTAAAGGGTCCGTAGGCGGTTTAGTAAGTCAGTGGT
GAAAGCCCATCGCTCAACGGTGAACGGCCATTGATACTGCTAAACTTGAATTATTAGGAAGTAACTAG
AATATGTAGTGTAGCGGTGAAATGCTTAGAGATTACATGGAATACCAATTGCGAAGGCAGGTTACTACT
AATWKATTGACGCTGATGGACGAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCG
TAAACGATGGATACTAGCTGTTGGGAGCAATCTCAGTGGCTAAGCGAAAGTGATAAGTATCCCACCTGG
GGAGTACGTTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGCCCGCACAAAGCGGTGGAGCATGTGGT
TTAATTCGATGATACGCGAGGAACCTTACCAAGGCTTAAATGTAGTTTGACCGATTTGGAAACAGATTT
TTCGCAAGACAAATTACAAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCAGGTTAAGTCC
TATAACGAGCGCAACCCCTGTTGTTAGTTGCCAGCGAGTCATGTGCGGAACTCTAACAAGACTGCCAGTG
CAAATGTGAGGAAGGTGGGGATGACGTCAAATCATCACGGCCCTTACGCCTTGGGCTACACACGTGCTA
CAATGGCCGGTACAGAGAGCAGCCACTGGGCGACCAGGAGCGAATCTATAAAACCGGTCACAGTTCGGAT
CGGAGTCTGCAACTCGACTCCGTGAAGCTGGAATCGCTAGTAATCGGATATCAGCCATGATCCGGTGAAT
ACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCTGGGGGTGCCTGAAGTCGGTGACCGC
AAGGAGCTGCCTAGGGTTAAACTGGTAACTAGGGCTAAGTCGTAATCAAGWGCAMCCC
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If your folder contains a 533-reverse sequence, use it (the complement) to replace the wobble bases and other junk (inaccurate sequence) at the front end. It will overlap the 8-forward sequence at the front end.

You can also look at the far end of your 533-forward sequence to see if it reads onto the 1530 or 1492-reverse primer sequence.

1530-reverse complement = TGCGGTTGGATCACCTCCT

1492-reverse complement = GTCGTAACAAGGTAGCCGT

Supplement for Laboratory Exercise #18 Genomics, Proteomics and Bioinformatics

After you have edited and combined your sequences (word files), you can use the NCBI BLAST algorithm to determine the identity of your PUNK2 culture. Follow the instructions provided on page 205 of your laboratory manual (you can use whatever browser you like best).

Note – It is not necessary to record information other than the culture’s identity at this point in time. The ultimate goal of this exercise is learning how to edit and combine nucleotide sequences so you will be able to do this with data obtained from your semester project culture.