Assembly:

You should submit a file called assembly.pdf, containing a brief report of your workflow, findings, and any strengths or potential shortcomings of your approach. You may consider reporting intermediate statistics generated during your process, as well as your final inferred sequence.

Workflow:

I took 3 project files and combined them in sequential order to find the final inferred sequence. I first took the reads files and removed any of the reads which contained contamination. I did this by running all the reads through the **contamination.py** file, which returns a list of contaminated reads. Once I removed all the contaminated reads, I determined the reads which I needed to correct. Because we do not know the actual length of the fully sequenced sampleRead.txt, we have to take in all the kmers derived from the reads to sequence to find the longest contig. I place decontaminated reads into the **correction.py** file, which replaces all infrequent kmers with frequent kmers throughout the reads. Now we have a list of decontaminated and corrected reads. Afterward, I generate kmers from this list of a specified length. These kmers are then placed into the **debruijn.py** file that then generates the longest contiguous segment that can be inferred from these kmers.

I chose to do contamination before correction because we do not want to be correcting a contamination region. If we corrected a contamination region by accident, then our contamination algorithm would not be able to determine if the read was contaminated or not because that region would have changed as a result of the correction. It is possible that a contamination region may have been altered and thus, preventing the algorithm from detecting the contamination. In this case, using correction to rectify this alteration would be helpful. However, this case is more unlikely to occur and it would prove to be more harmful if we accidentally introduced more alterations in the contamination regions that would prevent the algorithm from identifying it and removing it.

At the end of the correction phase, I pass all the corrected reads into the de Bruijn file to create the graph and find the longest contig that can be inferred. For the sampleReads.txt file, I used the parameters [10, 30, 17, 2, 2].

- 10 = kmer length to determine seeding in contamination
- 30 = kmer length of reads being used to correct and construct a De Bruijn graph
- 17 = kmer length to determine seeding in correction
- 2 = threshold value to determine the frequency of common kmer values in correction
- 2 = difference value for how much a replaceable kmer can differ by in correction

Strengths:

- A strength is that we implement various algorithms to remove contamination and mutations to generate reads that are most likely the correct values of the original genome. By cleaning up the reads before inputting them into the de Bruijn graph, we reduce the probability of a kmer that will introduce errors into the final inferred sequence. Another strength is that both contamination and mutations use BLAST-like seeding and extension to find the optimal reads. Using this technique allows for the best reads to be produced. The de Bruijn graph also adds another layer of correction because we only

produce the longest reads that can be inferred from the graph. If there happens to be a kmer that is incorrect that has become a node within the graph, it is very likely that it will be part of the inferred sequence because it will be an uncommon node with very few incoming/outgoing edges. Finally, I have tuned the parameters of the assembly.py file to ensure that optimal reads and contigs are produced. More about these parameters can be found in the intermediate statistics section.

Shortcomings:

- A shortcoming is run time. The two longest sections that contribute to a long runtime are correction and de Bruijn. Because we generate so many kmers that need to be corrected, the runtime in which correction performs is very large. Similarly, because we generate a ton of kmers that act as nodes, it takes a long time for the program to perform a depth-first search in finding all the contigs. Another shortcoming is that we do not know if this program is actually getting a sequence close to that of the original genome because we have nothing to compare to. We are using numbers that we believe can obtain this information, but there is no way to truly know if the program is optimally working under the parameters we are using. Another limitation to this program is that we are only using one de Bruijn graph to infer the longest contig. A better implementation would be to create several de Bruijn graphs with different parameters (ie. different lengths of kmers) so that we can gather more information about the sequence. Finally, the program produces a variety of contigs of equal length. As a result, we do not know which contig best represents the true sequence.

Intermediate statistics:

- Total number of reads: 1274
- Number of contaminated reads: 51
- Number of uncontaminated: 1223
- Number of frequent k-mers: 1613 / 8498 = 0.18980936690986114
- Number of corrected reads: 1223

A lot of the parameter tuning came from trial and error

- 10 = kmer length to determine seeding in contamination
  - The length of the contamination kmer value had to be big enough to ensure that the vector does indeed contaminate the reads. However, it can not be too big, or else it would be very unlikely that we will find an exact match for the vector in our reads. If the kmer is too small, all the reads will be marked as contaminated because it is more likely that we can find a smaller read that exactly matches the vector than a larger kmers. Thus, to balance these two, I arrived at the number 10.
- 30 = kmer length of reads being used to correct and construct a De Bruijn graph
  - We want a kmer length that results in a lot of connections in a de Bruijn graph, but also represents a long sequence of correct reads. If we had a kmer value that was too small, it would result in a very large graph and would be super complex to traverse with lots of nodes and edges. On the other hand, if we had a large kmer value, it would result in a very small

and simple graph. Thus to balance these two bounds, I decided to have a value in the middle and as a result, I arrived at the number 30.

- 17 = kmer length to determine seeding in correction
    - The length of the kmers derived from the reads should be similar in length to that of the optimal kmer length we found in pr2. In pr2, we saw that as we increased the kmer length to around 17-20, the higher the accuracy of the true genome was. A larger kmer means that is a lower frequency of that kmer. As a result, any kmer that surpasses a frequency threshold will most likely be a correct kmer of the true read. Thus, to get as close to the genome we are trying to sequence, we should use a number that is similar, which is why I arrived at the number 17. We should not use too large of a number because that may cause no frequent kmers and thus, we have no kmers to replace.
- 2 = threshold value to determine the frequency of common kmer values in correction
    - The threshold value should also be similar in length to that of the optimal kmer length we found in pr2. In pr2, we saw that as we decreased the threshold value to around 2-4, the higher the accuracy of the true genome was. A smaller threshold means that there are more frequent kmers and less infrequent kmers. As a result, there are a lot more options to choose from to replace the infrequent kmers and will most likely result in the correct sequence. Thus, to get as close to the genome we are trying to sequence, we should use a number that is similar, which is why I arrived at the number 2.
- 2 = difference value for how much a replaceable kmer can differ by in correction
    - The difference value should be a small number because we want to be as close to the true sequence as possible. If the difference value was super large, we could replace the kmer with a lot more possible kmers that may be incorrect. However, if we had a really small difference value, we would only have a few options to choose from. These options are most likely to be optimal kmers that can replace the incorrect kmer because they are derived from the most frequent kmer list and are very similar to the current kmer. This indicates that the new kmer used to replace is commonly found throughout the genome and is likely to be correct. Thus, I arrived at the number 2.

Findings:

After applying the De Bruijn algorithm to the kmer reads, I am able to obtain 16 reads of length 1260. An example reads i provided below

ATGTCTGATAATGGACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCTC
AGATTCAACTGGCAGTAACCAGAATGGAGAACGCAGTGGGGCGCGATCAAAACAACGTCG
GCCCCAAGGCTTACCCAATAATACTGCGTCTTGGTTCACCGCTCTCACTCAACATGGCAAG
GAAGACCTTAAATTCCCTCGAGGACAAGGCGTTCCAATTAACACCAATAGCAGTCCAGATGA
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GATCTCAGTCCAAGATGGTATTGCTACTACCTAGGAACTGGGCCAGAAGCTGGACTTCCCTA
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AACAACATTGCCAAAAGGCTTCTACGCAGAAGGGAGCAGAGGCGGCAGTCAAGCCTCTTC
TCGTTCCTCATCACGTAGTCGCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAGGGGA
ACTTCTCCTGCTAGAATGGCTGGCAATGGCGGTGATGCTGCTCTTGCTTTGCTGCTGCTTG
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AAGCATACAATGTAACACAAGCTTTCGGCAGACGTGGTCCAGAACAAACCCAAGGAAATTTT
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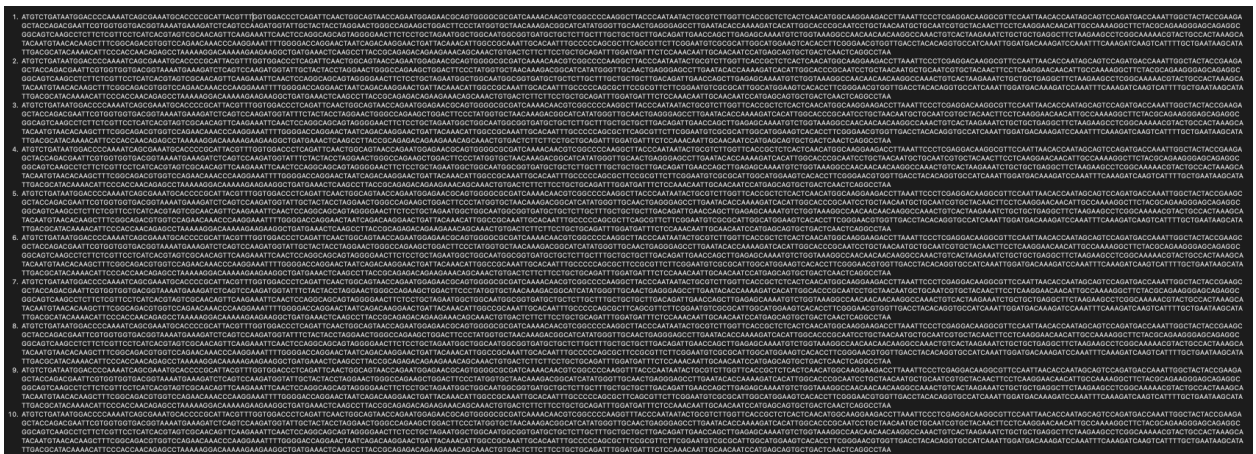
TGCCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGCATTGGCATGGAAGTCACACCTTCG
GGAACGTGGTTGACCTACACAGGTGCCATCAAATTGGATGACAAAGATCCAAATTTCAAAGA
TCAAGTCATTTTGCTGAATAAGCATATTGACGCATACAAAACATTCCCACCAACAGAGCCTAA
AAAGGACAAAAAGAAGAAGGCTGATGAAACTCAAGCCTTACCGCAGAGACAGAAGAAACAG
CAAACTGTGACTCTTCTTCCTGCTGCAGATTTGGATGATTTCTCCAAACAATTGCAACAATCC
ATGAGCAGTGCTGACTCAACTCAGGCCTAA

Google docs was unable to paste all the contigs into the doce, but here is a screenshot of all 16 reads. These reads can be found in the text file sampleReadContigs.txt



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CAAGGAAGACCTTAAATTCCCTCGAGGACAAGGCGTTCCAATTAACACCAATAGCAGTCCAGATGACCAAATTGGCTACTACCGAAGAGC
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ACGCATACAAAACATTCCCACCAACAGAGCCTAAAAAAGGACAAAAAGAAGAAGGCTGATGAAACTCAAGCCTTACCGCAGAGACAGAAG
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CAGGCCTAA

11. ATGTCTGATAATGGACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGAATGGA
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CAAGGAAGACCTTAAATTCCCTCGAGGACAAGGCGTTCCAATTAACACCAATAGCAGTCCAGATGACCAAATTGGCTACTACCGAAGAGC
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GGCCTAA
12. ATGTCTGATAATGGACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGAATGGA
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14. ATGTCTGATAATGGACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGAATGGA
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15. ATGTCTGATAATGGACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGAATGGA
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CAGGCCTAA

16. ATGTCTGATAATGGACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGAATGGA
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