HW2 Construct condensed De Bruijn Graph

Files:
A general python file **hw2.py** is the code I used to process the data and generate the result.
A fasta file **edges.fasta** is the output of edges, with their length and average kmer coverage.
A dot file **edges.dot** is the formatting information of De Bruijn graph and can be visualized with **graphviz** tool.

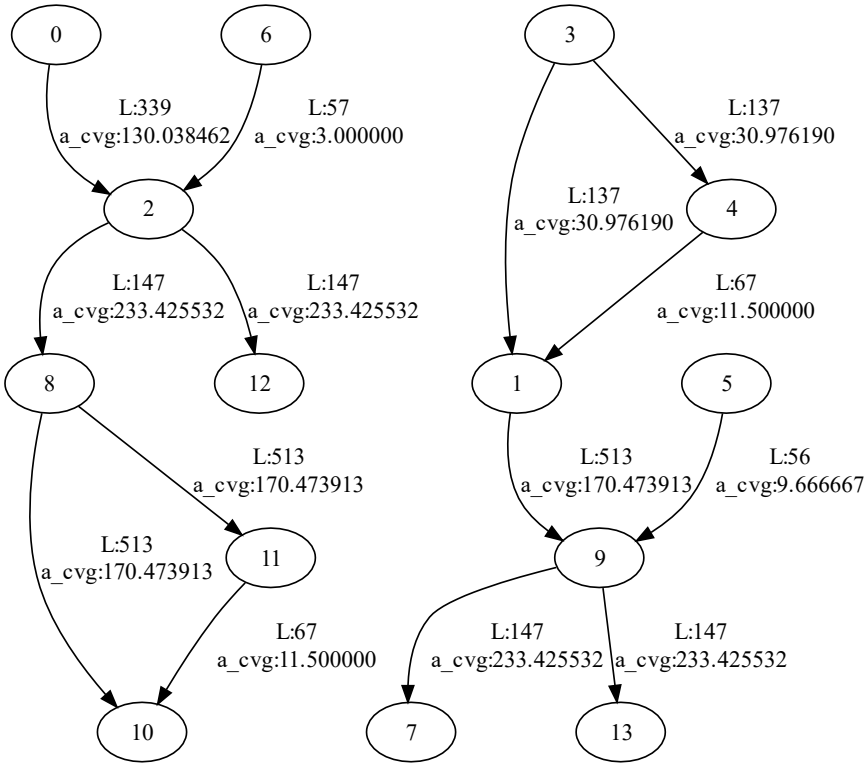
Results:
1. edges.fasta (sequence omitted)

```
>edge0   length: 339    coverage: 130.038462
>edge1   length: 513    coverage: 170.473913
>edge2   length: 147    coverage: 233.425532
>edge3   length: 137    coverage: 30.976190
>edge4   length: 67     coverage: 11.500000
>edge5   length: 56     coverage: 9.666667
>edge6   length: 57     coverage: 3.000000
>edge7   length: 339    coverage: 130.038462
>edge8   length: 513    coverage: 170.473913
>edge9   length: 147    coverage: 233.425532
>edge10  length: 137    coverage: 30.976190
>edge11  length: 67     coverage: 11.500000
>edge12  length: 56     coverage: 9.666667
>edge13  length: 57     coverage: 3.000000
```

There are totally 14 edges assembled from the given fastq file. Noticed that the last 7 edges in fact is the complement sequence of the first 7, there are only 7 contigs I got from my program. The first 3 edges have relative high coverage over 100, while the last 4 is all under 40. Edge3 has a length of 137 which is not short relatively but only has a average kmer coverage of around 30.

2. edges.dot
The formatting information includes all the direction of edges, with their length and coverage.

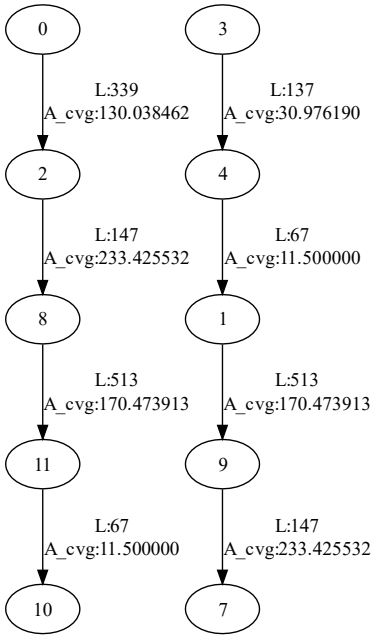
```
digragh dbg {

    0 -> 2 [label="L:339 \nA_cvg:130.038462"]
    3 -> 1 [label="L:137 \nA_cvg:30.976190"]
    4 -> 1 [label="L:67 \nA_cvg:11.500000"]
    1 -> 9 [label="L:513 \nA_cvg:170.473913"]
    6 -> 2 [label="L:57 \nA_cvg:3.000000"]
    2 -> 8 [label="L:147 \nA_cvg:233.425532"]
    2 -> 12 [label="L:147 \nA_cvg:233.425532"]
    3 -> 4 [label="L:137 \nA_cvg:30.976190"]
    5 -> 9 [label="L:56 \nA_cvg:9.666667"]
    9 -> 7 [label="L:147 \nA_cvg:233.425532"]
    8 -> 10 [label="L:513 \nA_cvg:170.473913"]
    8 -> 11 [label="L:513 \nA_cvg:170.473913"]
    9 -> 13 [label="L:147 \nA_cvg:233.425532"]
    11 -> 10 [label="L:67 \nA_cvg:11.500000"]

}
```



The graph is topologically symmetric.

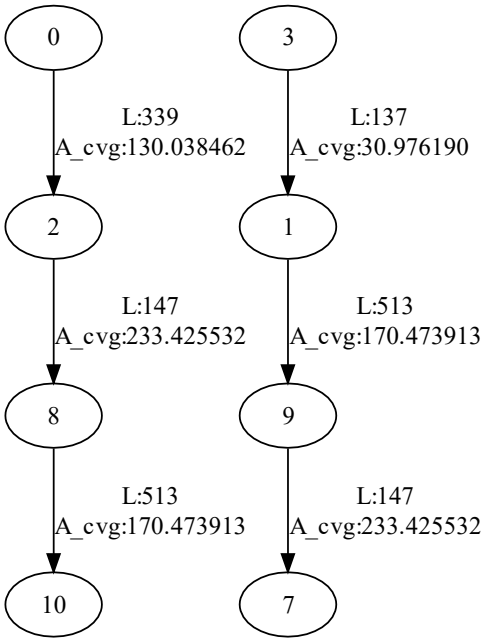
3. Graph simplification

Algorithm1: removing tips with short length and low coverage. If an edge with long length and high coverage is a tip, remove it anyway. whether an edge is a tip depend on whether it is the longest path.



By this algorithm, we always get the longest and still relative complete path in the graph. However, the longest doesn't always mean correct. Sometimes the relative short pathway could actually exist in real situation like isoform of genes, splice variant etc.

Algorithm 2: remove all the short and low coverage edges beforehand. Edge 3 and 10 is retained here.



This algorithm is quite conservative. It can ensure the quality of later assembly, as all the remaining edges are long and with high coverage. However, it would lose a lot of information of the data, especially those possible and potential pathways but getting a low sequencing quality due to system error.

3. Assembler assessment

1) **ABySS**

Use ABySS to assemble file **s_6.first1000.fastq**, command in shell as below:

abyss -k 55 s_6.first1000.fastq -o ./abyss_result

Get result:

>0 754 122487

TTAAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGA
CAGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCAC
CACCATCACCATTACCACAGGTAACGGTGCGGGCTGACGCGTACAGGAAACACAGAA
AAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAGGTAACGAGGTAACA
ACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGC
GTGTTGCCGATATTCTGGAAAGCAATGCCAGGCAGGGGCAGGTGGCCACCGTCCTCTC
TGCCCCCGCCAAAATCACCAACCACCTGGTGGCGATGATTGAAAAAACCATTAGCGGC
CAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTTGACGGG
ACTCGCCGCCGCCCAGCCGGGGTTCCCGCTGGCGCAATTGAAAACTTTCGTCGATCAG
GAATTTGCCCAAATAAAACATGTCCTGCATGGCATTAGTTTGTTGGGGCAGTGCCCGGA
TAGCATCAACGCTGCGCTGATTTGCCGTGGCGAGAAAATGTCGATCGCCATTATGGCCG

GCGTATTAGAAGCGCGCGGTCACAACGTTACTGTTATCGATCCGGTCGAAAAACTGCT
GGCAGTGGGGCATTACCTCGAATCTACCGTCGATATTGCTGAGTCCACCCGCC

2) **minia**
Use minia to assemble the same file, command in shell as below:
minia -in s_6.first1000.fastq -kmer-size 55 -out minia_result

Get result:
ACCATGTGATCAGCCGGAATGCGGCTTGCCGCAATACGGCGGGTGGACTCAGCAATAT
CGACGGTAGATTCGAGGTAATGCCCCACTGCCAGCAGTTTTTCGACCGGATCGATAAC
AGTAACGTTGTGACCGCGCGCTTCTAATACGCCGGCCATAATGGCGATCGACATTTTCT
CGCCACGGCAAATCAGCGCAGCGTTGATGCTATCCGGGCACTGCCCCAACAAACTAAT
GCCATGCAGGACATGTTTTATTTGGGCAAATTCCTGATCGACGAAAGTTTTCAATTGCG
CCAGCGGGAACCCCGGCTGGGCGGCGGCGAGTCCCGTCAAAAGTTCGGCAAAAATAC
GTTCGGCATCGCTGATATTGGGTAAAGCATCCTGGCCGCTAATGGTTTTTTCAATCATCG
CCACCAGGTGGTTGGTGATTTTGGCGGGGGCAGAGAGGACGGTGGCCACCTGCCCCT
GCCTGGCATTGCTTTCCAGAATATCGGCAACACGCAGAAAACGTTCTGCATTTGCCACT
GATGTACCGCCGAACTTCAACACTCGCATGGTTGTTACCTCGTTACCTTTGGTCGAAAA
AAAAAGCCCGCACTGTCAGGTGCGGGCTTTTTTCTGTGTTTCCTGTACGCGTCAGCCC
GCACCGTTACCTGTGGTAATGGTGATGGTGGTGGTAATGGTGGTGCTAATGCGTTTCAT
GGATGTTGTGTACTCTGTAATTTTTATCTGTCTGTGCGCTATGCCTATATTGGTTAAAGTA
TTTAGTGACCTAAGTCAATAAAATTTTAATTTACTCACGGCAGGTAACCAGTTCAGAAG
CTGCTATCAGACACTCTTTTTTTAATCCACACAGAGACATATTGCCCGTTGCAGTCAGA
ATGAAAAGCT

Compare contigs got from the two assemblers using QUAST.
Genome is choice as first 1K in E.coli, and skip contigs shorter than 50bp.

Using ABySS I only got a single assembled sequence and no other contig information was provided. Result showed that only 754bp was assembled in only one contig (75.4%), which is relatively low, and duplication ratio is 1.

While using minia, results showed that 7 contigs were retrieved, which is consistent to my results. Moreover, total alignment is 1200 which more than 1k in reference genome, duplication ratio is 1.2 and even 2 contigs are unaligned to the genome.

GC content in contigs generated by the two assembler is different, for ABySS GC% is 50.4% and for minia is 51.86%, with ABySS result more close to the reference (about 50.6%)

We can conclude that ABySS is more conservative and loses some information while minia is getting redundant information after assembly. The former may be more accurate than the later.

ABySS assembly results:

Genome statistics	abyss_resul
Genome fraction (%)	75.4
Duplication ratio	1
Largest alignment	754
Total aligned length	754
NG50	754
NG75	754
NA50	754
NA75	754
NGA50	754
NGA75	754
LG50	1
LG75	1
LA50	1
LA75	1
LGA50	1
LGA75	1

Statistics without reference	
# contigs	1
# contigs (>= 0 bp)	1
# contigs (>= 1000 bp)	0
# contigs (>= 5000 bp)	0
# contigs (>= 10000 bp)	0
# contigs (>= 25000 bp)	0
# contigs (>= 50000 bp)	0
Largest contig	754
Total length	754
Total length (>= 0 bp)	754
Total length (>= 1000 bp)	0
Total length (>= 5000 bp)	0
Total length (>= 10000 bp)	0
Total length (>= 25000 bp)	0
Total length (>= 50000 bp)	0
N50	754
N75	754
L50	1
L75	1
GC (%)	50.4
Similarity statistics	
# similar correct contigs	0
# similar misassembled blocks	0

minia assembly results:

Genome statistics	minia_results.unitigs
Genome fraction (%)	100
Duplication ratio	1.2
Largest alignment	511
Total aligned length	1200
NG50	511
NG75	340
NA50	340
NA75	147
NGA50	511
NGA75	340
LG50	1
LG75	2
LA50	2
LA75	3
LGA50	1
LGA75	2

Unaligned	
# fully unaligned contigs	2
Fully unaligned length	115
# partially unaligned contigs	0
Partially unaligned length	0

Statistics without reference	
# contigs	7
# contigs (>= 0 bp)	7
# contigs (>= 1000 bp)	0
# contigs (>= 5000 bp)	0
# contigs (>= 10000 bp)	0
# contigs (>= 25000 bp)	0
# contigs (>= 50000 bp)	0
Largest contig	511
Total length	1315
Total length (>= 0 bp)	1315
Total length (>= 1000 bp)	0
Total length (>= 5000 bp)	0
Total length (>= 10000 bp)	0
Total length (>= 25000 bp)	0
Total length (>= 50000 bp)	0
N50	340
N75	147
L50	2
L75	3
GC (%)	51.86
Similarity statistics	
# similar correct contigs	0
# similar misassembled blocks	0