Genomics: Genome Storage and Assembly

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Introduction

- Data size and storage proves to be an obstacle for many genetic researchers
- 3 billion basepair-long genome is broken up into fragments to be sequenced and reassembled
- Fragments of DNA, called "kmers', are represented as k-character strings and stored in De Bruijn graph
- Slight variations of genome between people are responsible for traits can cause disease
- Single nucleotide polymorphisms (SNPs), insertions and deletions, and copy number variations are possible variations
- Determine variations by lack of presence in reference genome
- Variant Graph holds information for long sequences of kmers, helps to find complex variation
- Our project wants to improve upon current methods including Velvet[4] developed by Zerbino and Birney and Cortex[2][3] developed by Iqbal, McVean, and Turner

Methods

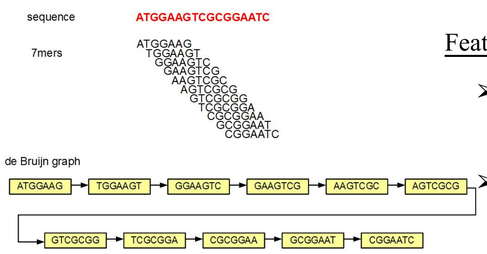


Figure 1: De Bruijn Graph for K = 7[7]

Features of the de Bruijn Graph:

- Large overlap helps with reconstruction of sequence
- Useful to handle repetitive regions

Features of the Variant Graph:

- Holds strings of kmer sequences
- Stores location of variation from reference genome, if found

Figure 2: Example of string variation[1]

Data Source/Format

```
>HSBGPG
GGCAGATTCCCCCTAGACCCGCCCGCACCATGGTCAG
TGGGCACAGCCCAGAGGGTATAAACAGTGCTGGAGGC
AGTCCTGAGCAGCAGCCCAGCGCAGCCACCGAGACAC
CTCGCCCTATTGGCCCTGGCCGCACTTTGCATCGCTG
CCACCTCCCCTCAGGCCGCATTGCAGTGGGGGCTGAG
CACCTCTTCTCACCCCTTTGGCTGGCAGTCCCTTTGC
AGGCTCAATCCATTTGCCCCAGCTCTGCCCTTGCAGA
AGCTGCCCGAGACGCAGGGGAAGGAGGATGAGGGCCC
ACCAGGCTCCCTTTCCTTTGCAGGTGCGAAGCCCAGC
GTGCAGGTATGAGGATGGACCTGATGGGTTCCTGGAC
CCCTCAGTCTCATTCCCCCACTCCTGCCACCTCCTGT
GCCTGCTCCCCACCTGATCCTCCCAAACCCAGAGCCA
CTCCACAGCCTTTGTGTCCAAGCAGGAGGGCAGCGAG
GCTACCTGTATCAATGGCTGGGGTGAGAGAAAAGGCA
```

Figure 3: FASTA format for reference genome

```
@H3GFVCCXX150415:8:2224:9627:35467/1
GGGAATTTTAACTGGCAAAACTCAGAACTCCATCCAAAC
+
AAAFF<<FFAFAFAAAAFFFAFAAAAAAFAAAFAAFAFA
@H3GFVCCXX150415:8:2224:8957:23407/1
CATACTTGATGGTCTCAGATATGTGTGGATTTTGGAATT
+
<FAFAFFAAAA7AAFAAAAFAAAAAAFAFAFFFAAFFFF.
@H3GFVCCXX150415:8:2224:8907:25745/1
GTTAATTAAAAGCCCTTTACGAATGGACTAGATGTACCT
+
AAAFFAFAF/FFAAAA<FAA7FFAAAFAFAF7FAAFAAF
@H3GFVCCXX150415:8:2224:8825:55175/1
GCACCCTGTGTCAACAACCTGAGAGTGGCCTTGAGTTGC
+
AAFAAAFAAAAAFFAFFAAFAFAAAAFAAAFFFAFAFAA
```

Figure 4: FASTQ format for sequenced reads

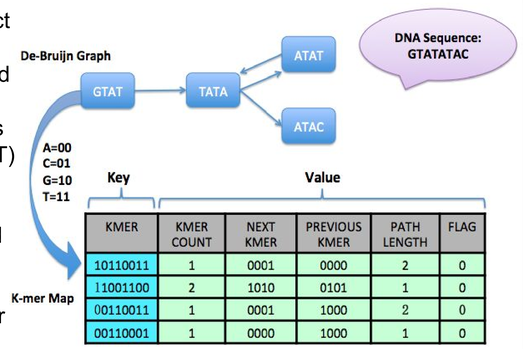
Data Source: Nation Center for Biotechnology Information[5]

Methods

❑Data Structure: KmerGraph

Features:

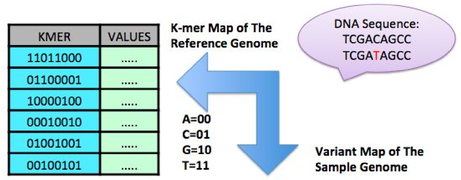
- Kmer sequences act as the keys of bitpacked unordered map
- Encoding four types nucleobases (ACGT) into 2 bit binary numbers.
- Struct can only hold 32 character sequence
- Stores various kmer statistics to assist with queries and assembly



KMER	KMER COUNT	NEXT KMER	PREVIOUS KMER	PATH LENGTH	FLAG
10110011	1	0001	0000	2	0
11001100	2	1010	0101	1	0
00110011	1	0001	1000	2	0
00110001	1	0000	1000	1	0

Figure 5: Data Structure of the Kmer Graph (Map)

❑Data Structure: VariantGraph



KMER	VALUES
11011000	.....
01100001	.....
10000100	.....
00010010	.....
01001001	.....
00100101	.....

KMER	NEXT KMER COUNT OF A	NEXT KMER COUNT OF C	NEXT KMER COUNT OF G	NEXT KMER COUNT OF T
01100011	1	0	0	0
10001100	0	0	1	0
00110010	0	1	0	0
11001001	0	1	0	0

Figure 6: Data Structure of the Variant Graph (Map)

Features:

- Variant Graph holds edge information needed to construct sequences
- Being able to store long sequences allows identification of complex variations

❑Implementation: Functions

Longest Path	Basic Query	Data Storage	String Complement
• Calculate the lengths of non-branching paths starting from every distinct kmers in the KmerGraph in O(N) time. • Print out the lengths and paths of the non-branching sequences starting from any kmer strings. • Store and print the length and the heads of the longest non-branching paths of the graph.	• Most common kmer • Number of nodes (can be split into unique and non-unique nodes) • Lookup of kmer by key	• Insert data from FASTA and FASTQ files into unordered map • Transfer data between binary file and hash table	• Takes in sequence input and returns sequence of complementary strand (can then be used to construct graph for string's complementary strand)

Results

❑Memory Footprint Reduction and Time Efficiency

- Use of bit packed structs reduces memory usage
- Hashing allows for fast access to stored data
- Storage in binary files for compactness
- *O*(n) run time for all functions

❑Constructed De Bruijn graph for reference genome
❑Stored reference graph with k = 32 in 56 GB binary file
❑Implemented function to find longest linear (i.e. non-branching) path through DeBruijn graph

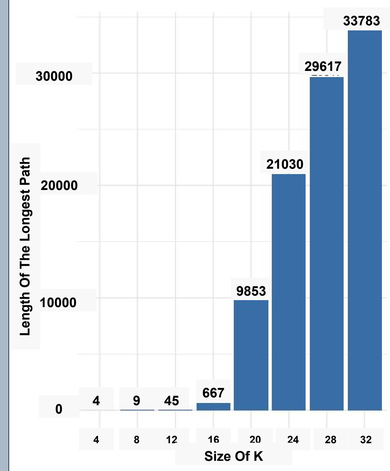


Figure 7: Influence of the size of K on the length of the longest non-branching path

Choosing the right size of K:

- Finding longest path assists in reassembly of genome sequence
- Choice of K has impact on longest path length
- Ideal value of K is uncertain, small k leads to ambiguity in assembly, large k has trouble with sequencing errors

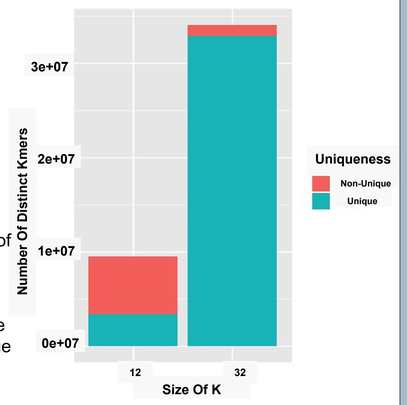


Figure 8: Influence of the size of K on the total number of nodes and the percentage of unique nodes in the de bruijn graph.

Future Work

❑Pruning and error cleaning of variant graph
❑Store 10,000+ genomes as variant graphs to find common variants
❑FM index graph using Burrows-Wheeler Transform to reduce storage size while allowing fast query lookups

References

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