A 20,000 league view of Bioinformatics

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2019 AMD Symposium
May 23, 2019

Slides available at:
www.k-florek.net/talks

https://amd-midwest.github.io
/bioinfo_course/

Bioinformatics: An interdisciplinary field that develops methods and software tools for understanding biological data.

What does the data look like

```
@M03478:141:000000000-C5B4D:1:1101:25956:10945 1:N:0:1
TTCCGTATTCATGCAACCTATGATGAAAGTATTAGTCGGTTACTCAATGTATTTGAGCGC
+
ABBBBCFFFFFFGGGGGGGGGG5GHHHHHGGHHHHHHGGGGFHHHHHHHHHHHHHFGHGG
```

```
@M03478:141:000000000-C5B4D:1:1101:25956:10945 1:N:0:1
TTCCGTATTCATGCAACCTATGATGAAAGTATTAGTCGGTTACTCAATGTATTTGAGCGC
+
ABBBBCFFFFFFGGGGGGGGGG5GHHHHHGGHHHHHHGGGGFHHHHHHHHHHHHHFGHGG
```

- **M03478** - the unique instrument name
- **141** - the run id
- **000000000-C5B4D** - flowcell id

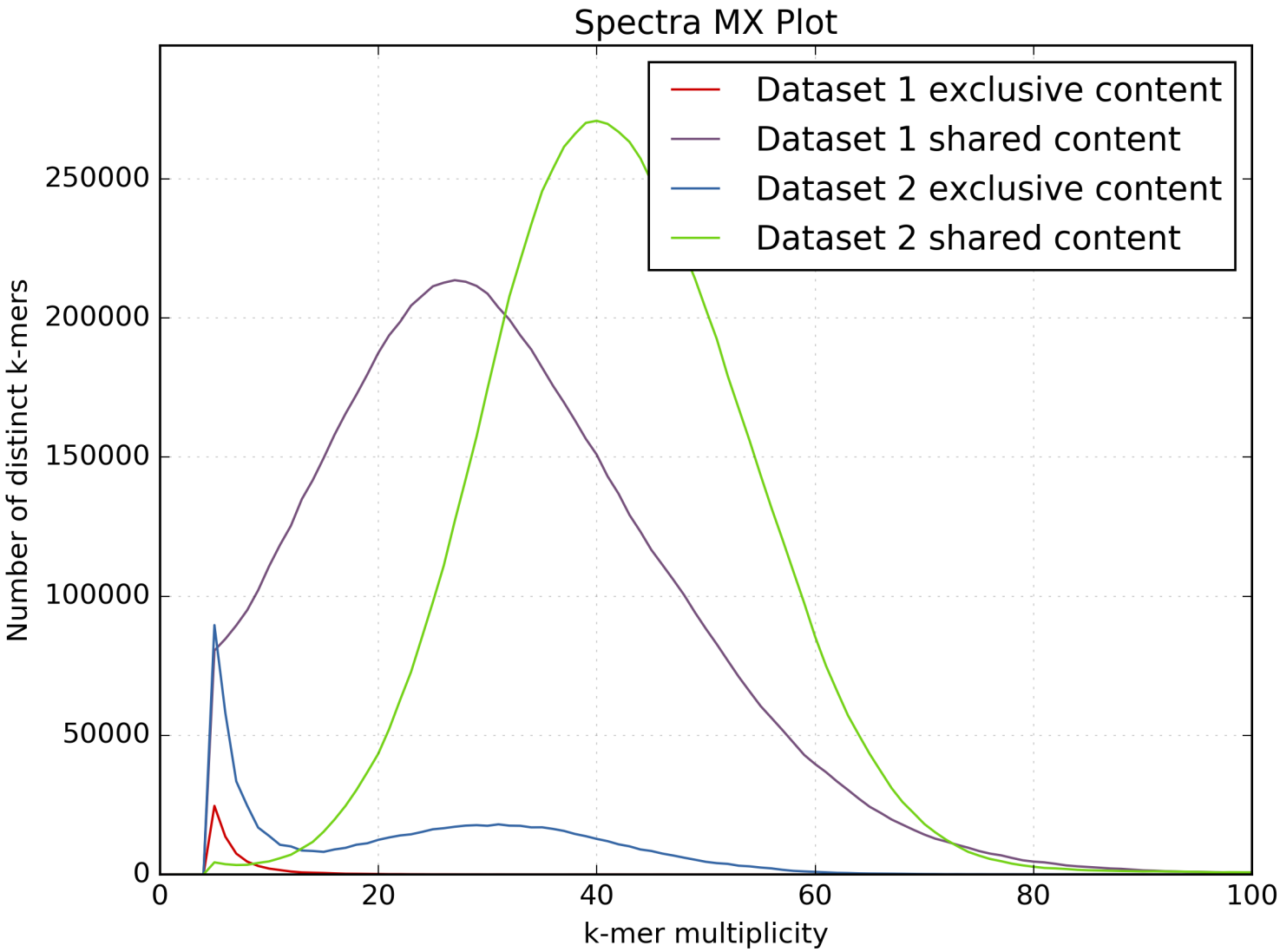
Phred Score

- 10: 1 in 10 90%
- 20: 1 in 100 99%
- 30: 1 in 1000 99.9%
- 40: 1 in 10,000 99.99%
- 50: 1 in 100,000 99.999%
- 60: 1 in 1,000,000 99.9999%

what can you do with fastq / read data

k-mers: all the possible substrings of length k

```
TTCCGTATTCATGCAACCTATGATGAAAGTATTAGTCGGTTACTCAATGTATTTGAGCGC
TTCCG  TTCAT  AACCT  GATGA  GTATT  TCGGT  CTCAA  TATTT
 TCCGT  TCATG  ACCTA  ATGAA  TATTA  CGGTT  TCAAT  ATTTG
  CCGTA  CATGC  CCTAT  TGAAA  ATTAG  GGTTA  CAATG  TTTGA
   CGTAT  ATGCA  CTATG  GAAAG  TTAGT  GTTAC  AATGT  TTGAG
    GTATT  TGCAA  TATGA  AAAGT  TAGTC  TTACT  ATGTA  TGAGC
     TATTC  GCAAC  ATGAT  AAGTA  AGTCG  TACTC  TGTAT  GAGCG
      ATTCA  CAACC  TGATG  AGTAT  GTCGG  ACTCA  GTATT  AGCGC
```



https://kat.readthedocs.io

basic analysis pipeline

- quality trimming
- assembly
  - *de novo* assembly
  - reference mapping
- antibiotic resistance detection

ensuring quality reads

```
@M03478:141:000000000-C5B4D:1:1101:25956:10945 1:N:0:1
TTCCGTATTCATGCAACCTATGATGAAAGTATTAGTCGGTTACTCAATGTATTTGAGCGC
+
ABBBBCFFFFFFGGGGGGGGGG5GHHHHHGGHHHHHHGGGGFHHHHHHHHHHHHHFGHGG
```

- remove sequencing adapters
- trim when quality drops
- specify a minimum length
- scan for contamination

basic analysis pipeline

- ~~quality trimming~~
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  - *de novo* assembly
  - reference mapping
- antibiotic resistance detection

de novo assembly: assembly of read data without the use of a reference sequence

de Brujin graph: a directed graph representing overlaps between sequences of symbols

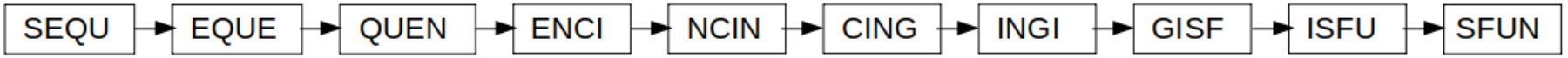
de Brujin graphs

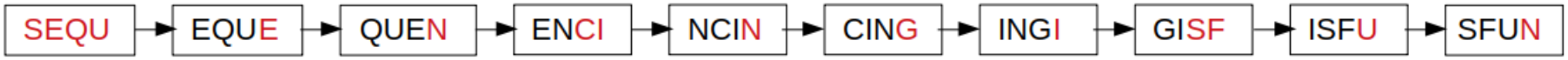
cingi sequen sfun encin cing isfu

all 4-mers: cing ingi sequ eque quen sfun enci ncin cing gisf isfu

unique 4-mers: cing ingi sequ eque quen sfun enci ncin gisf isfu

assembly graph:





sequencingisfun

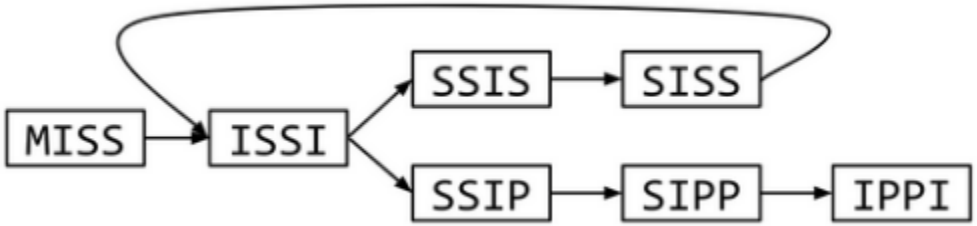
difficult de Brujin graph

missis ssissi ssippi

all 4-mers: miss issi ssis ssis siss issi ssip sipp ippi

uniqe 4-mers: miss issi ssis siss ssip sipp ippi

assembly graph:



mississippi or mississississippi

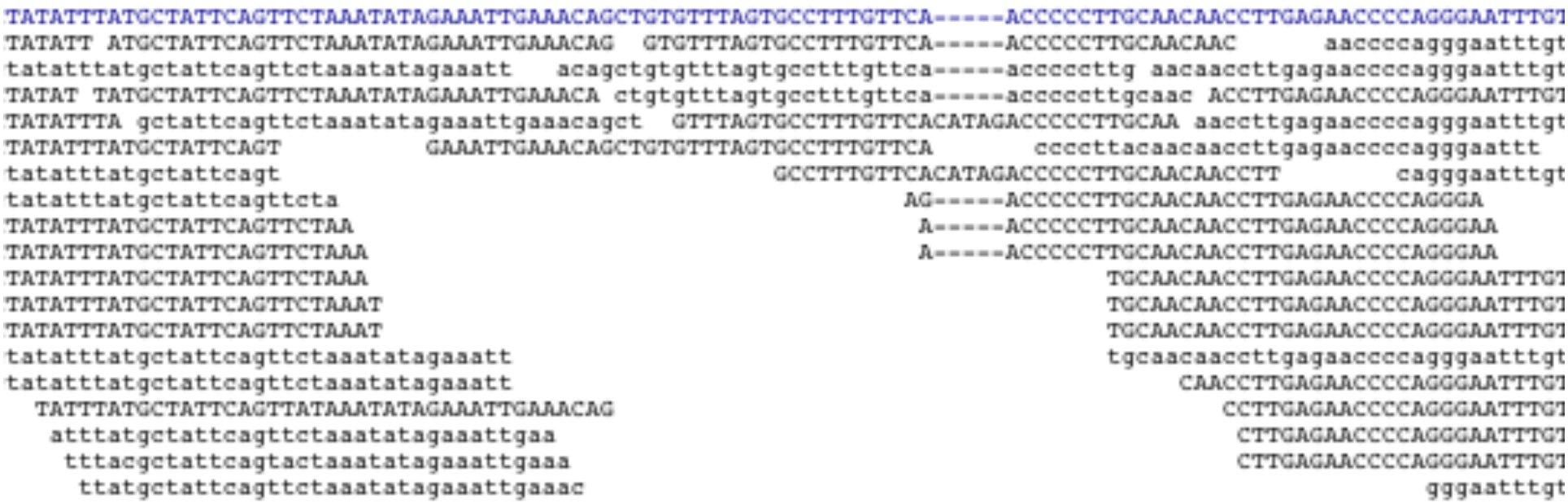
choosing k

- low k
  - more connections
  - higher chance of repeats
  - higher coverage
- high k
  - less connections
  - higher chance of resolving repeats
  - lower coverage

storing genome assemblies (the .fasta file)

>A/Hong_Kong/4801/2014_NP
gttaataatcactcactgagtgacatcaaagtcatggcgt
cccaaggcaccaaacggtcttatgaacagatggaaactga
tggagatcgccagaatgcaactgagattagggcatccgtc
gggaagatgattgatggaattgggagattctacatccaaa

reference mapping: a method of mapping the reads to a reference sequence


TATATTTATGCTATTCAGTTCTAAATATAGAAATTGAAACAGCTGTGTTTAGTGCCTTTGTTCA-----ACCCCCTTGCAACAACCTTGAGAACCCCAGGGAATTTGT
TATATT ATGCTATTCAGTTCTAAATATAGAAATTGAAACAG GTGTTTAGTGCCTTTGTTCA-----ACCCCCTTGCAACAAC aaccccagggaatttgt
tatatttatgctattcagttctaaatatagaaatt acagctgtgtttagtgcctttgttca-----acccccttg aacaaccttgagaaccccagggaatttgt
TATAT TATGCTATTCAGTTCTAAATATAGAAATTGAAACA ctgtgtttagtgcctttgttca-----acccccttgcaac ACCTTGAGAACCCCAGGGAATTTGT
TATATTTA gctattcagttctaaatatagaaattgaaacagct GTTTAGTGCCTTTGTTCACATAGACCCCCTTGCAA aaccttgagaaccccagggaatttgt
TATATTTATGCTATTCAGT GAAATTGAAACAGCTGTGTTTAGTGCCTTTGTTCA ccccttacaacaaccttgagaaccccagggaattt
tatatttatgctattcagt GCCTTTGTTCACATAGACCCCCTTGCAACAACCTT cagggaatttgt
tatatttatgctattcagttcta AG-----ACCCCCTTGCAACAACCTTGAGAACCCCAGGGA
TATATTTATGCTATTCAGTTCTAA A-----ACCCCCTTGCAACAACCTTGAGAACCCCAGGGAA
TATATTTATGCTATTCAGTTCTAAA A-----ACCCCCTTGCAACAACCTTGAGAACCCCAGGGAA
TATATTTATGCTATTCAGTTCTAAA TGCAACAACCTTGAGAACCCCAGGGAATTTGT
TATATTTATGCTATTCAGTTCTAAAT TGCAACAACCTTGAGAACCCCAGGGAATTTGT
TATATTTATGCTATTCAGTTCTAAAT TGCAACAACCTTGAGAACCCCAGGGAATTTGT
tatatttatgctattcagttctaaatatagaaatt tgcaacaaccttgagaaccccagggaatttgt
tatatttatgctattcagttctaaatatagaaatt CAACCTTGAGAACCCCAGGGAATTTGT
TATTTATGCTATTCAGTTATAAATATAGAAATTGAAACAG CCTTGAGAACCCCAGGGAATTTGT
atttatgctattcagttctaaatatagaaattgaa CTTGAGAACCCCAGGGAATTTGT
tttacgctattcagtactaaatatagaaattgaaa CTTGAGAACCCCAGGGAATTTGT
ttatgctattcagttctaaatatagaaattgaaac gggaatttgt

storing read mapping (the .sam file)

- read name / reference name
- position read maps to on the reference sequence
- sequence read and quality information
- many others..

storing the read mappings in a binary format (the .bam file)

provides a faster access to data and tends to use less memory

compression

- gzip
- repetitions in the data are replaced by references to the data
- repetitions in the data are replaced by references to <7,8>
- replaces more frequent characters with variable length encoding
- T : 01010100 ----> T : 11

compression matters

- Uncompressed:
  - **E coli** both set of reads ~900MB
  - **E coli** sequencing run (16 isolates) ~20GB
- Compressed:
  - **E coli** both set of reads ~200MB
  - **E coli** sequencing run (16 isolates) ~4GB

moving data

data moves across the internet in 1,500 byte packets

- ftp
- http
- sftp
- https

basic analysis pipeline

- ~~quality trimming~~
- assembly
  - ~~*de novo* assembly~~
  - ~~reference mapping~~
- antibiotic resistance detection

using the data to find resistance mechanisms

- database
  - multifasta
  - SQL
- search for patterns

NCBI BLAST (basic local alignment search tool)

BLAST finds similar sequences by locating short matches between sequences

after the first match BLAST begins to make local alignments

location: 4377811 - 4378944

gene name: Escherichia_coli_ampC

coverage: 100

identity: 100

database: card

description: A class C ampC beta-lactamase (cephalosporinase) enzyme described in Escherichia coli shown clinically to confer resistance to penicillin-like and cephalosporin-class antibiotics.

review

- quality control / trimming of reads
- assembly
  - *de novo*
  - reference mapping
- AR detection using BLAST

review

- sequencing data storage
- data compression
- transferring data across networks

applied Linux virtual course

https://forms.gle/oKSB5KFKcv5DX4k57

Course Dates: June 10th - June 14th, 2019

Length: 2hr sessions Monday, Wednesday and Friday; Office hours on Tuesday and Thursday

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