RNA virus full genome sequencing and haplotype reconstruction

Sebastian Krautwurst

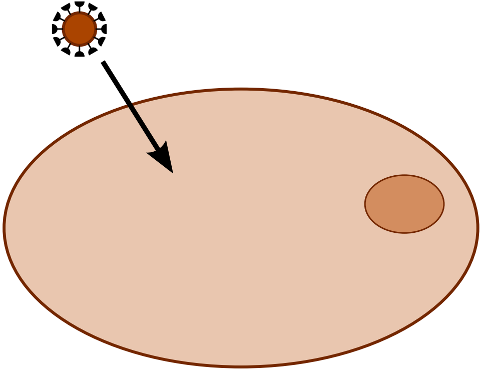
February 13, 2018
33rd TBI Winterseminar in Bled

FRIEDRICH-SCHILLER-UNIVERSITÄT JENA

Background

VIRAL HAPLOTYPES

- One species = one genome?



Viral haplotypes

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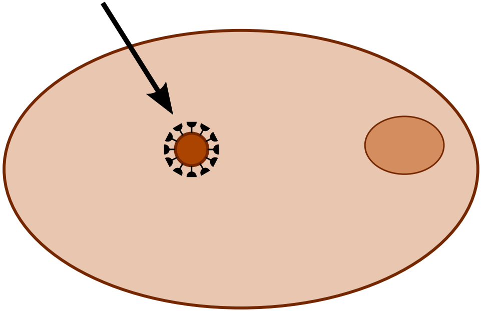

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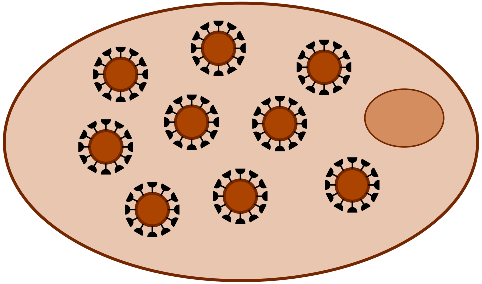

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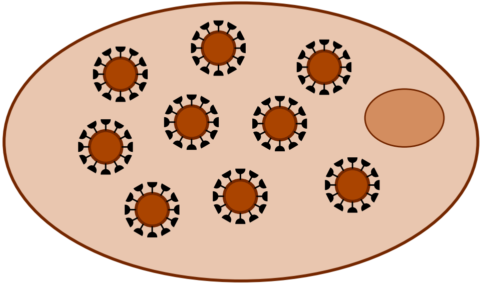

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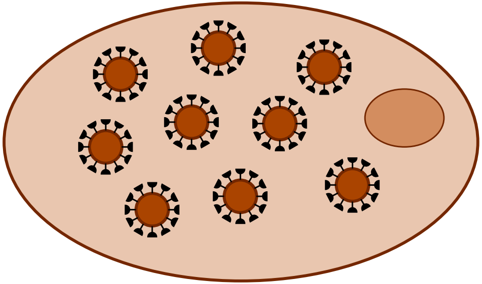
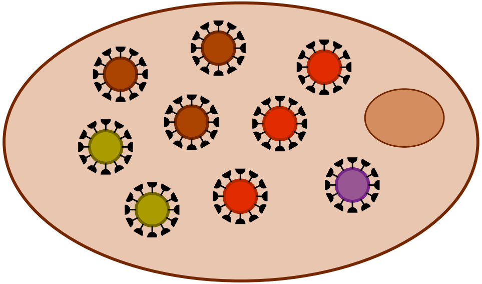


VIRAL HAPLOTYPES

- One species = one genome?
- RNA viruses: error-prone replication
- Mutation, recombination, segment reassortment
- Diverse spectrum of genomes
  ⇒ Quasispecies reconstruction



Nanopore sequencing

- ONT MinION

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nanoporetech.com/sites/default/files/s3/minion-cutout.png

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- Direct RNA sequencing protocol kit

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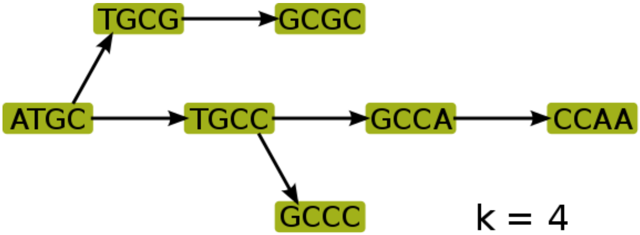
De Bruijn graph

- Constructed from overlapping k-mers



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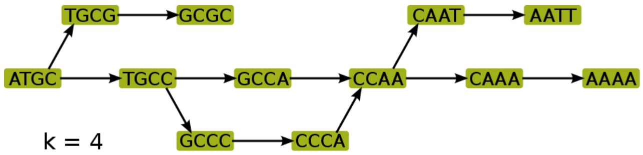

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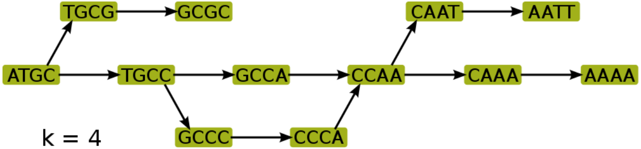

DE BRUIJN GRAPH

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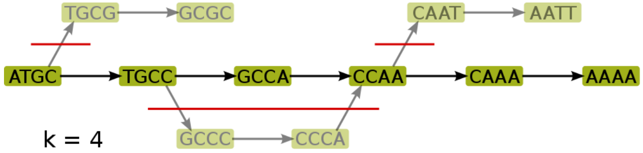

De Bruijn graph

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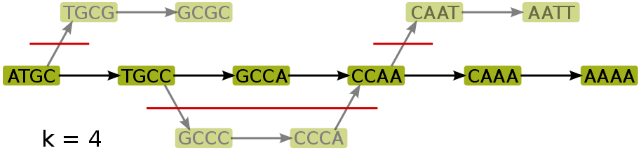
- Captures variants
- Assembly: consensus
- Tip- and bulge removal
- Collapse unambiguous chains



ASSEMBLY BY DE BRUIJN GRAPH

- Established de novo assembly method (Velvet, SPAdes)

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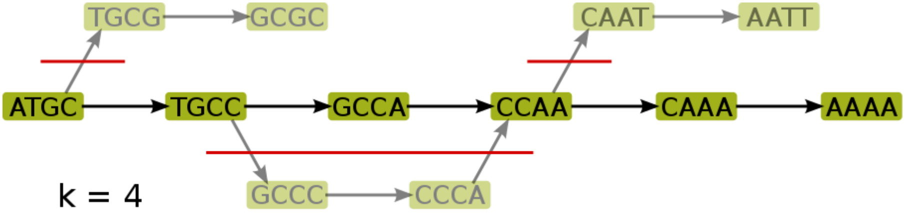

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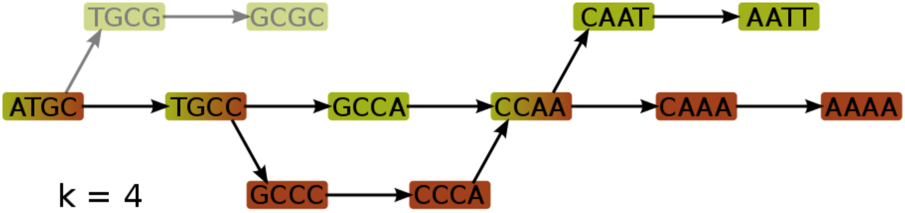

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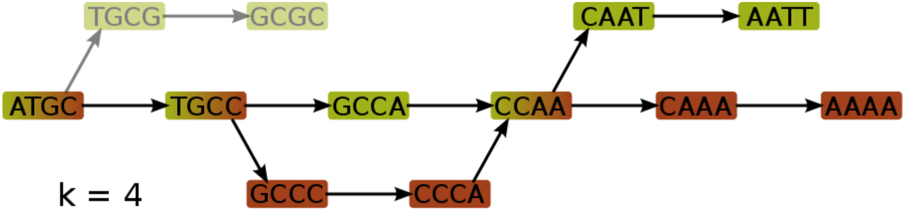
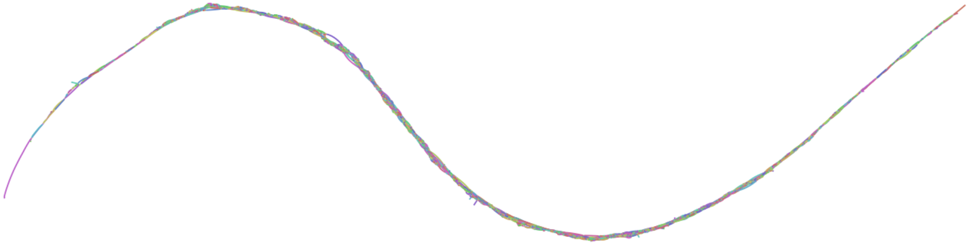
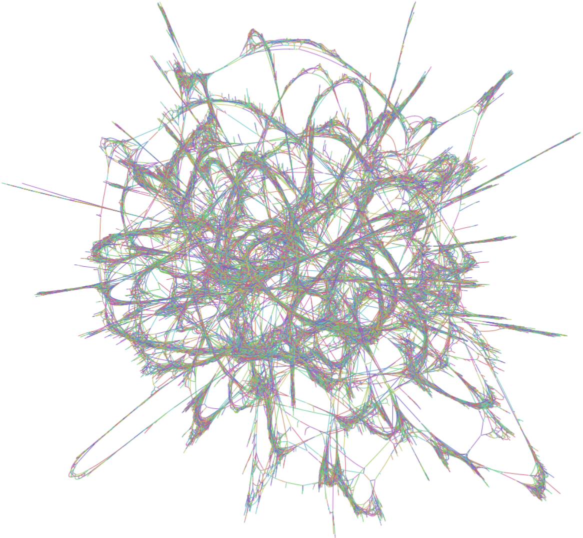
- Separate haplotypes by graph manipulation, long read information
- Assemble haplotype consensus sequences



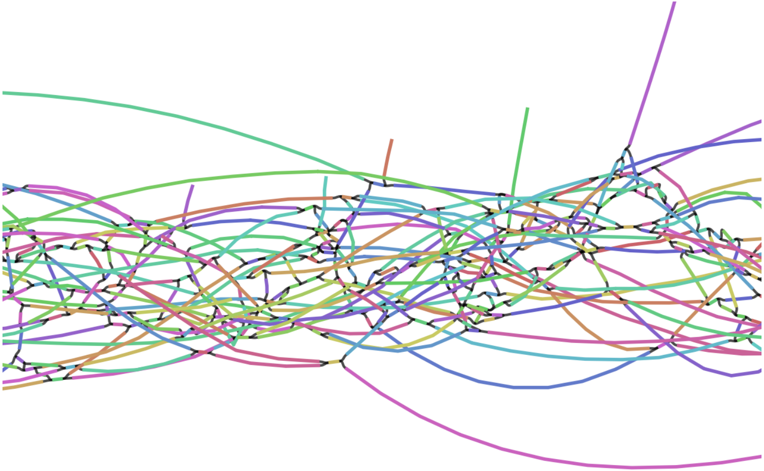
Results so far



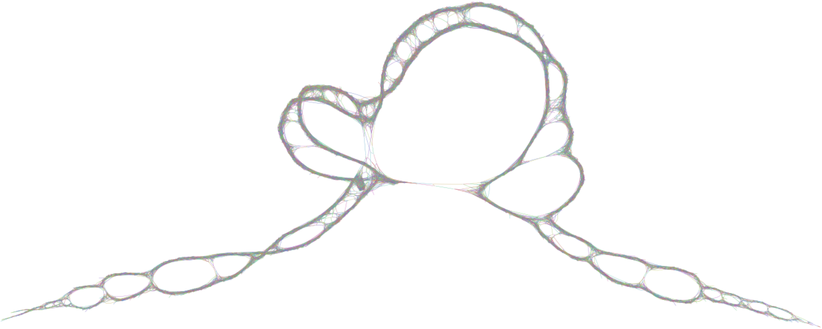
corona simul - 1 genome - 20 reads - 10000 nt - 10 % indels - k=25



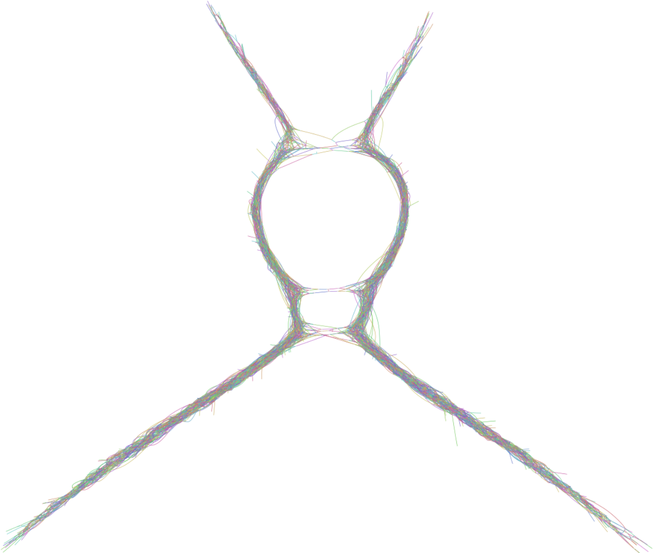
corona simul - 1 genome - 100 reads - 10000 nt - 10 % indels - **k=16**



corona - 1 genome - 40 reads - 10000 nt - **15 % indels** - k=30



bvdv simul - **2 genomes** - 100/100 reads - 6000 nt - 10 % indels - k=20



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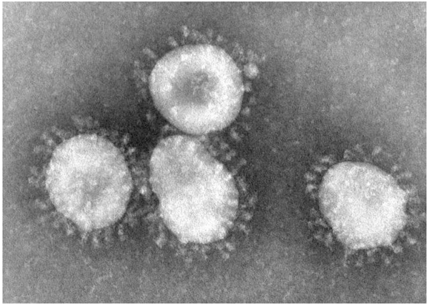
Real coronavirus read data

- HCoV 229E in human cell culture



en.wikipedia.org/wiki/Coronavirus#/media/File:Coronaviruses_004_lores.jpg

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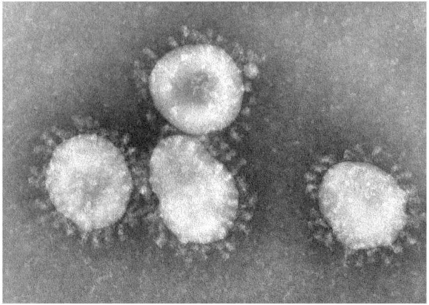

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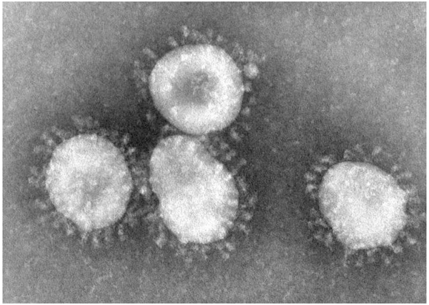
- 293406 reads, 27 % virus, rest human



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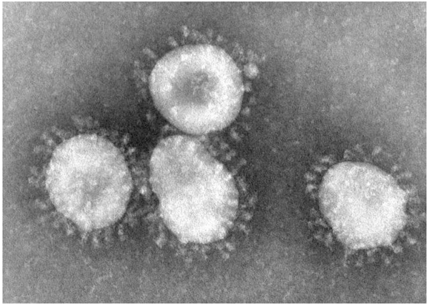

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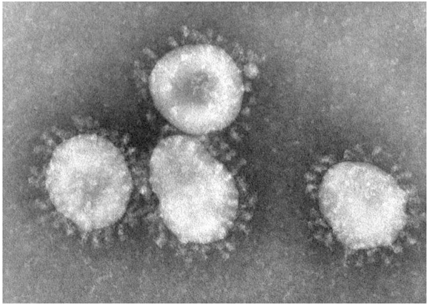

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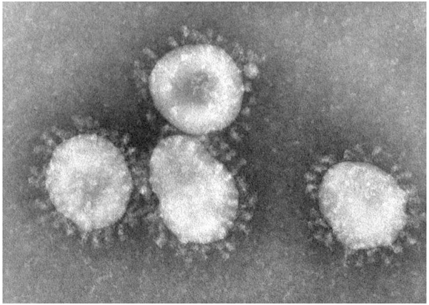

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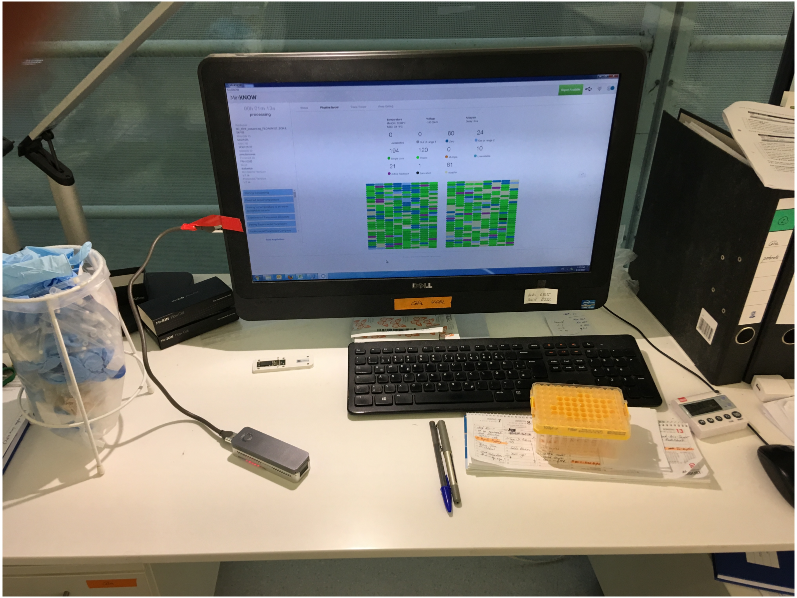
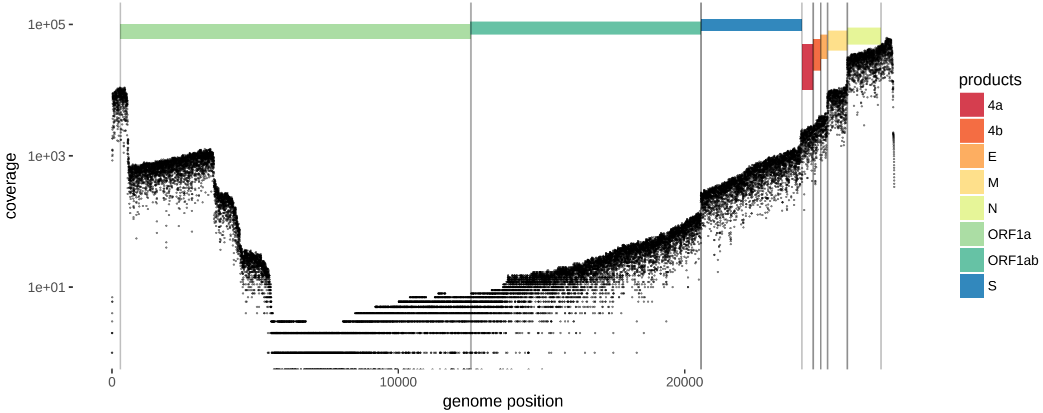
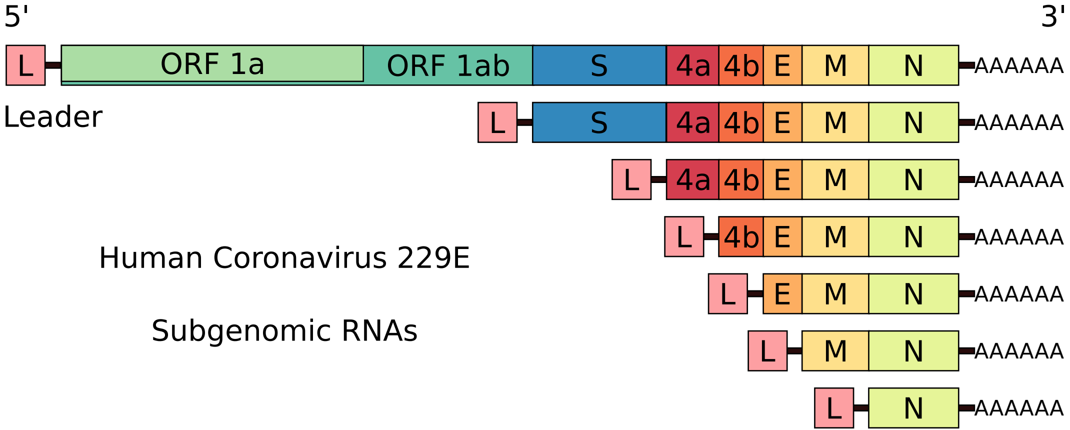
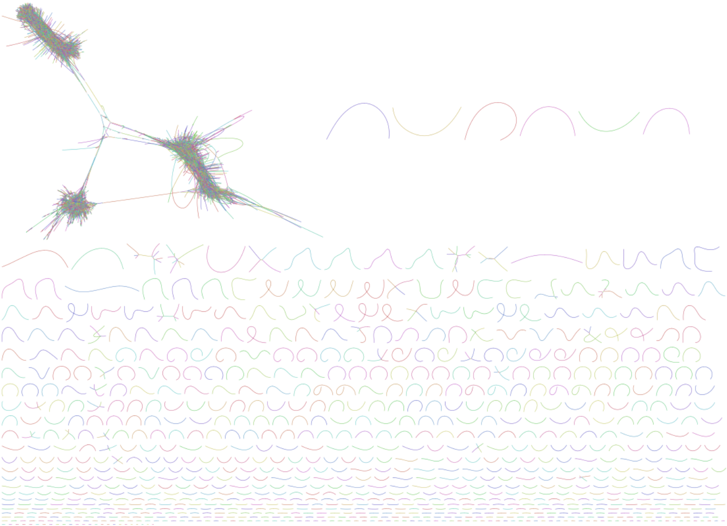

MinKNOW
processing
MinION Flow Cell
MinION Flow Cell
DELL


1e+05
1e+03
1e+01
coverage
0
10000
20000
genome position
products
4a
4b
E
M
N
ORF1a
ORF1ab
S



Human Coronavirus 229E

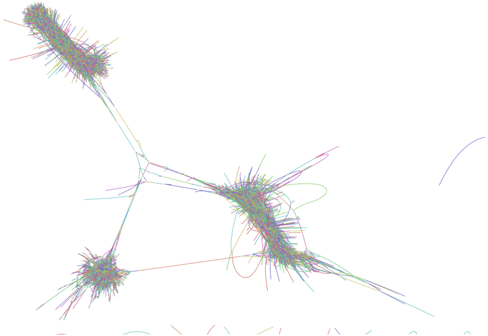
Subgenomic RNAs



corona - sequenced data - **2000 reads** - **k=40**

SUBGRAPH CONSENSUS

- Needed: subgraph separation



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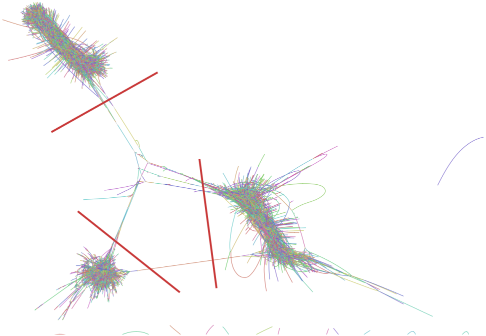

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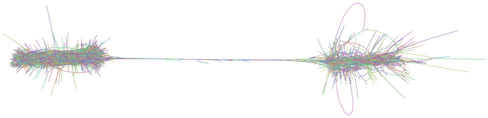

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Subgraph consensus

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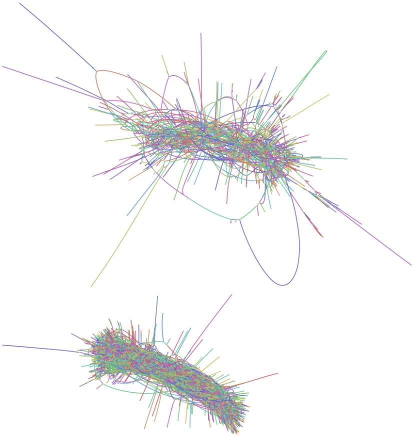

Subgraph consensus

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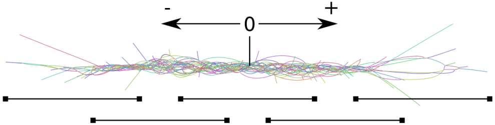
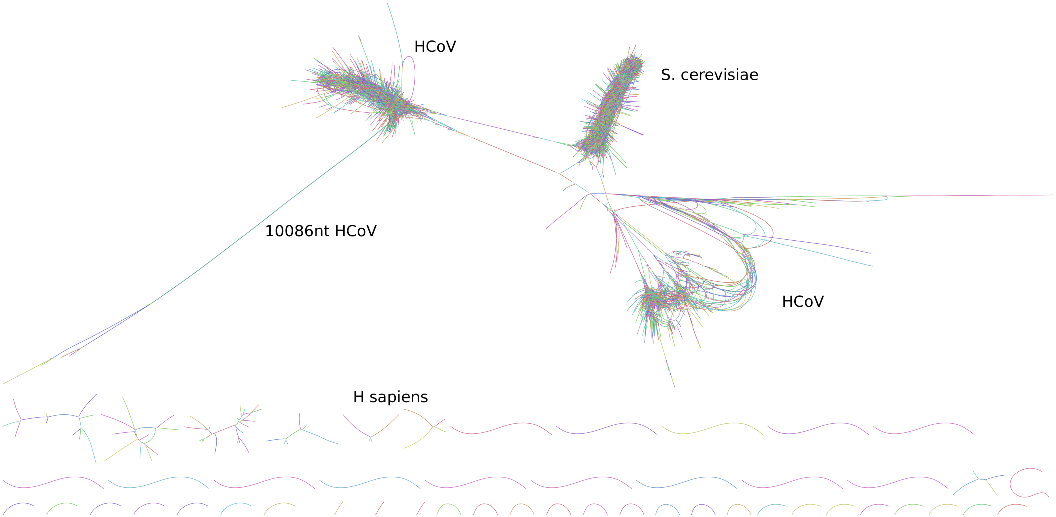
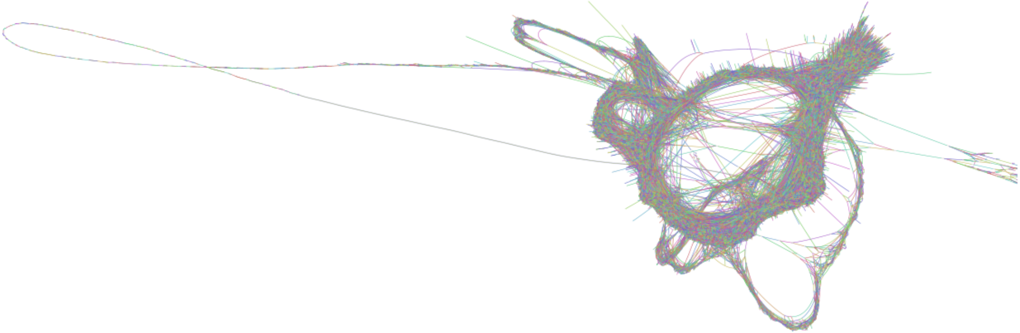
- Separates clusters that are minimally connected
- Subgraph consensus is implemented





Yeast enolase is included in the direct RNA kit as a positive control



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SEQUENCING ERRORS

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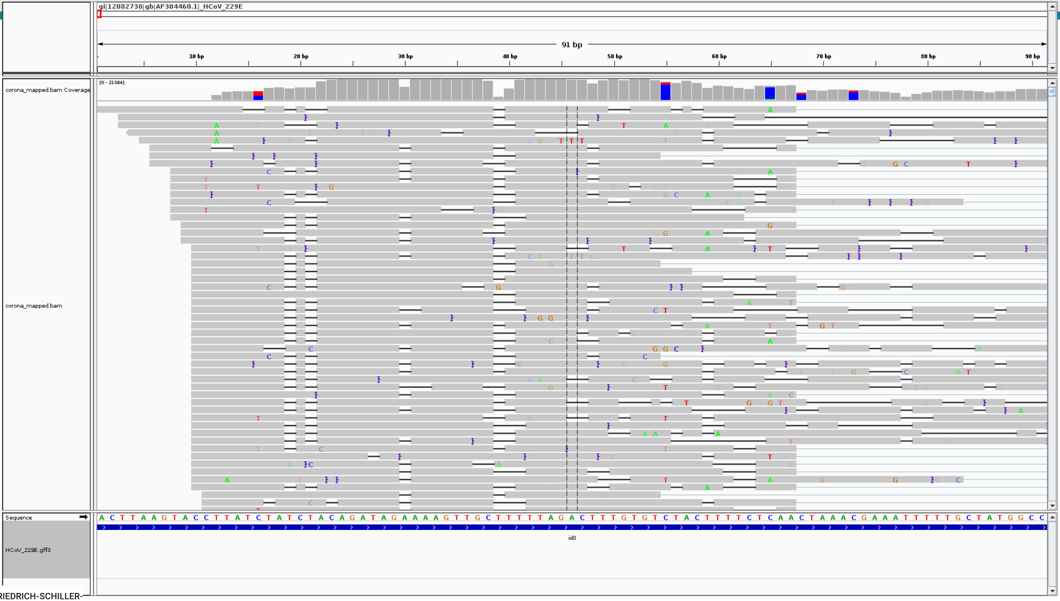
- Deletions happen systematically at homopolymers



gi|12082738|gb|AF304460.1|_HCoV_229E

corona_mapped.bam Coverage

[0 - 21084]

corona_mapped.bam

Sequence

A C T T A A G T A C C T T A T C T A T C T A C A G A T A G A A A A G T T G C T T T T T A G A C T T T G T G T C T A C T T T T C T C A A C T A A A C G A A A T T T T T G C T A T G G C C

HCoV_229E.gff3

Long Read Error Correction

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  Systematic errors are problematic

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- `HG-CoLoR` by P. Morisse et al.
- Longest read (25932 nt)
  Identity to reference: 84% → 99%
  Gap of 407 nt, 90 min runtime

Reference-based Indel Correction

- Reference from `nanopolish` by J. Simpson – RNA NYI

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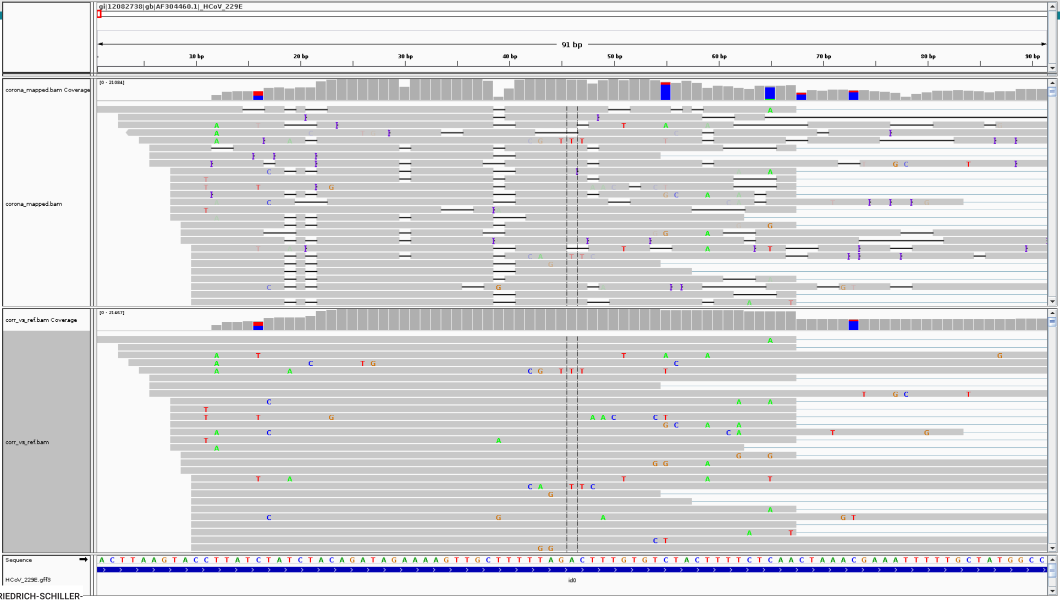
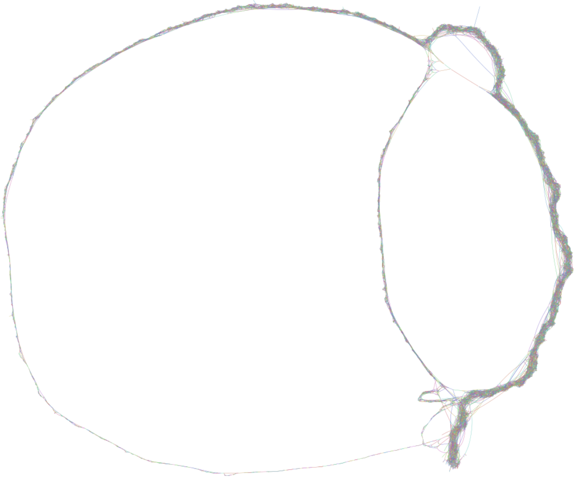
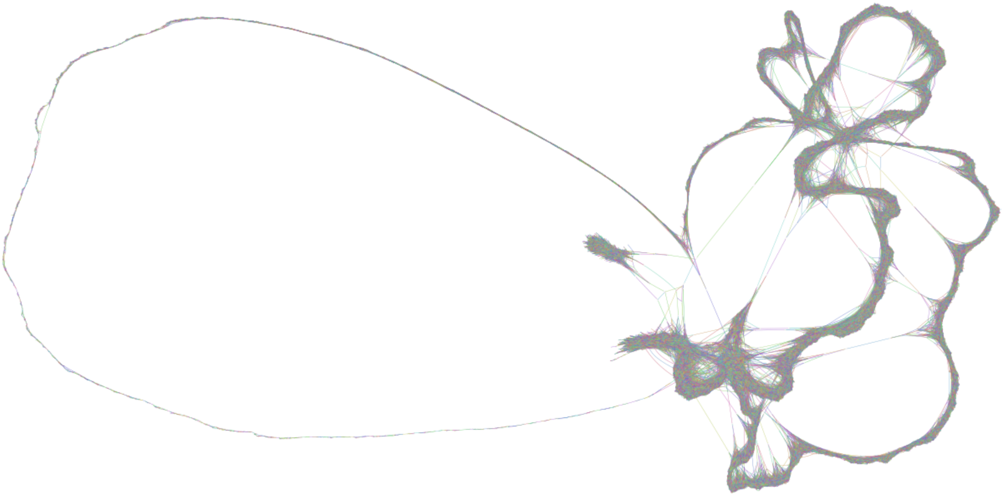
- Parse CIGAR string to remove insertions, fill deletions

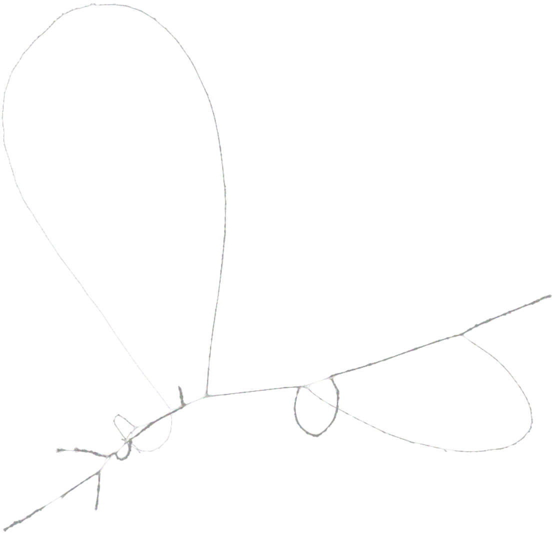

gi|12082738|gb|AF304460.1|_HCoV_229E
91 bp
10 bp
20 bp
30 bp
40 bp
50 bp
60 bp
70 bp
80 bp
90 bp
corona_mapped.bam Coverage
[0 - 21084]
corona_mapped.bam
corr_vs_ref.bam Coverage
[0 - 21467]
corr_vs_ref.bam
Sequence
A C T T A A G T A C C T T A T C T A T C T A C A G A T A G A A A A G T T G C T T T T T A G A C T T T G T G T C T A C T T T T C T C A A C T A A A C G A A A T T T T T G C T A T G G C C
HCoV_229E.gff3
id0



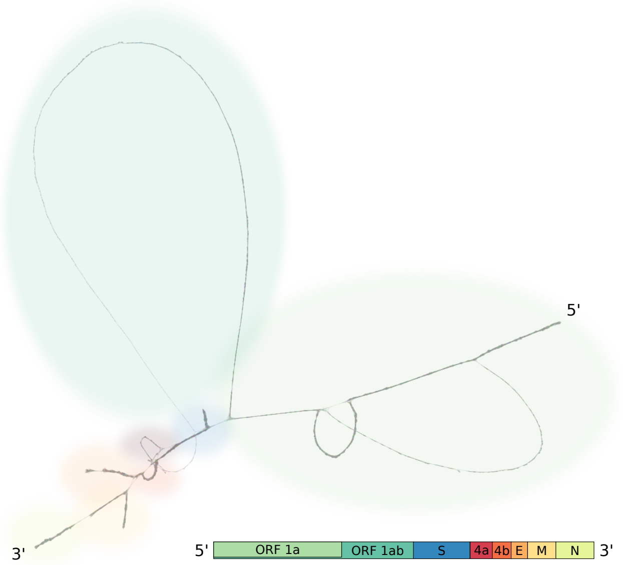
corona - indels corrected - **1% best nucleotides** - **k=40**



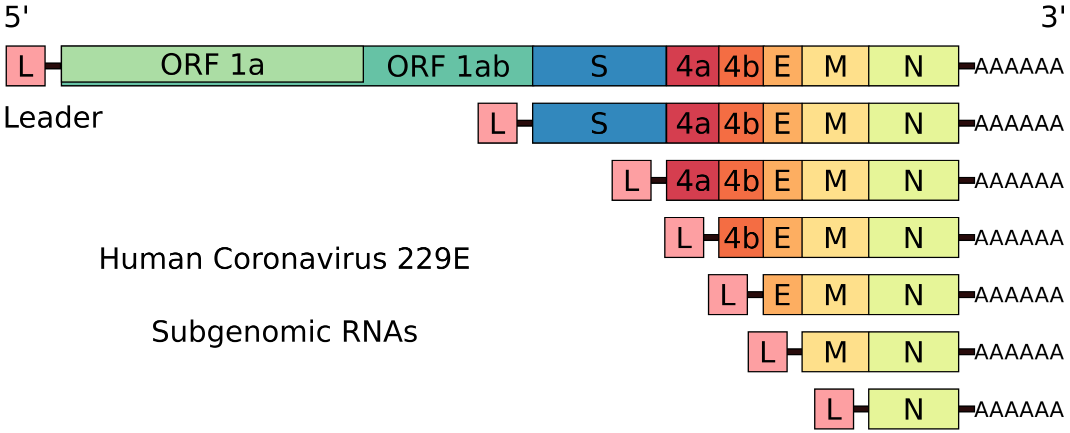
corona - indels corrected - **10% best nucleotides** - **k=30**



corona - indels corrected - **1% best nucleotides** - **k=20**


5'
3'
5'
ORF 1a
ORF 1ab
S
4a
4b
E
M
N
3'

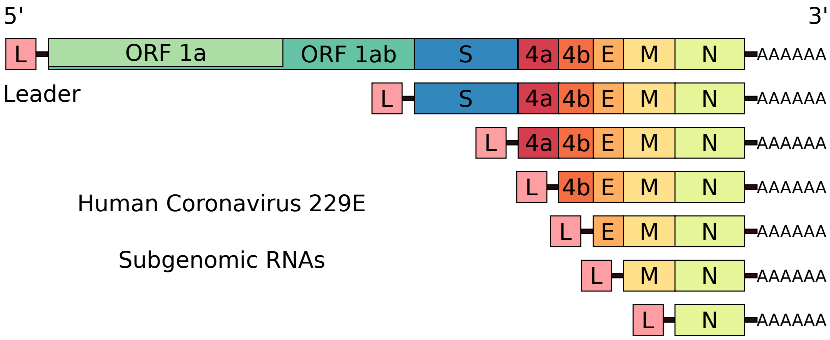
SUBGENOMIC TYPES



Human Coronavirus 229E

Subgenomic RNAs

ANNOTATION BASED CLASSIFICATION



assigned:

2

141

1077

85

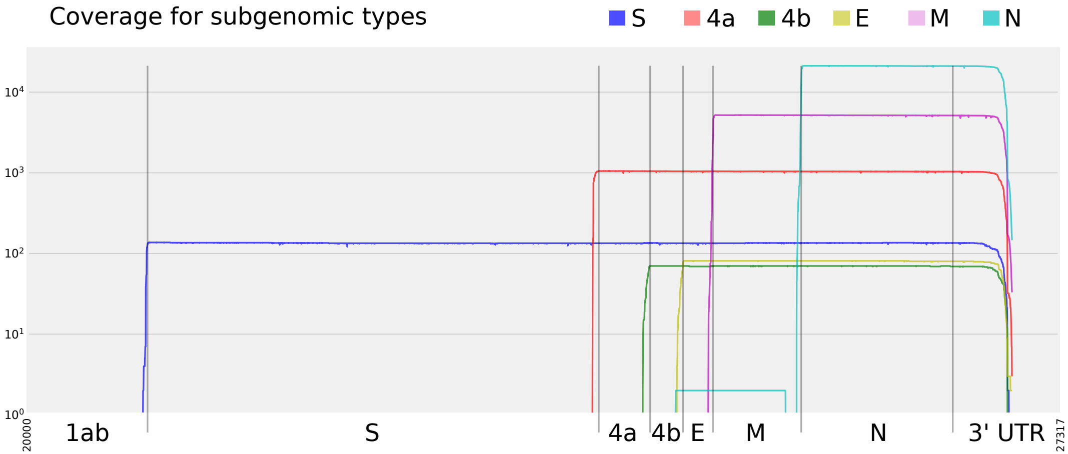
105

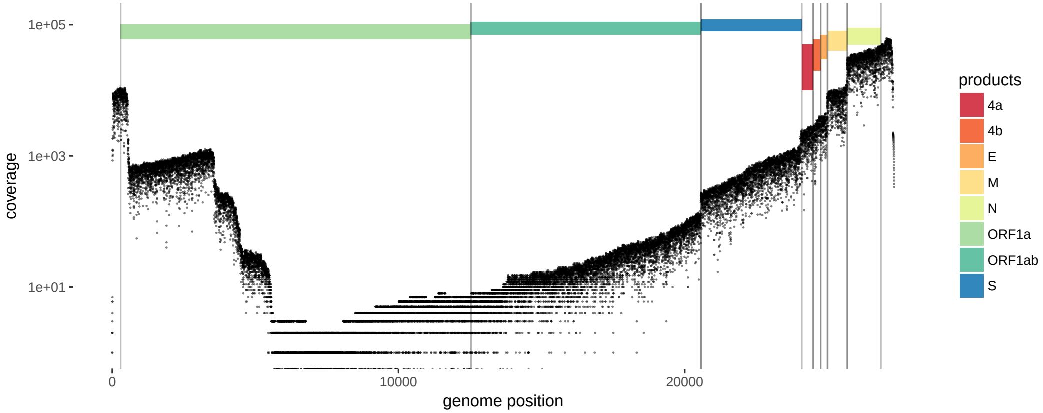
5407

21896

Total: 28711

Unassigned: 44822


Coverage for subgenomic types
S
4a
4b
E
M
N
10^4
10^3
10^2
10^1
10^0
20000
1ab
S
4a
4b
E
M
N
3' UTR
27317



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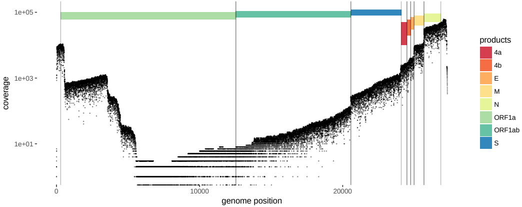
Conclusions

- Viral full genome sequencing



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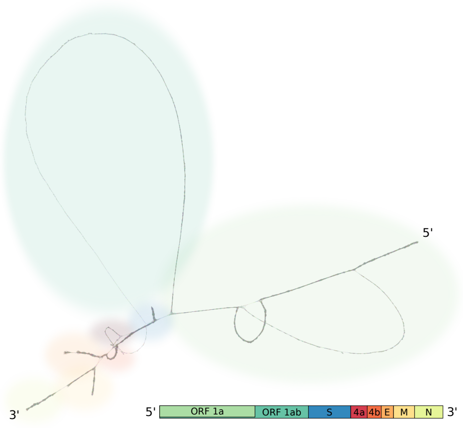

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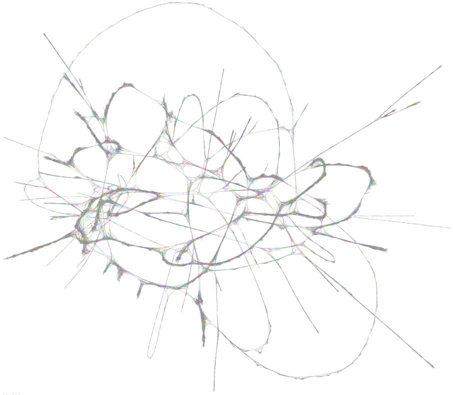

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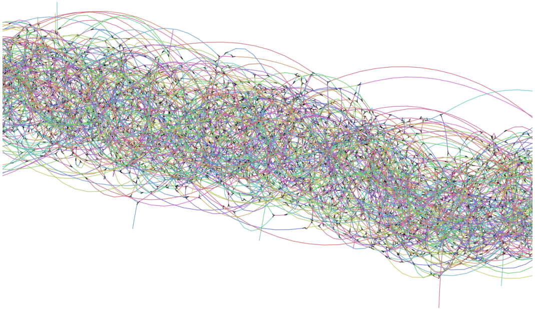
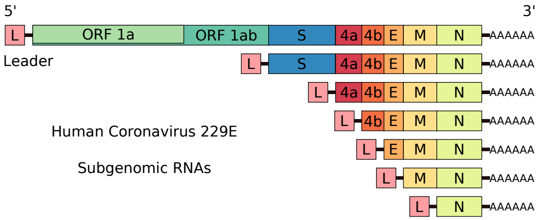
- Importance of k
- Indel correction required



Conclusions

- Viral full genome sequencing
- Structure is visible in graph
- Importance of k
- Indel correction required
- Coronavirus is ...complicated



Outlook

NEXT STEPS

- Test on new data (more HCoV, plum pox virus)

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- Test on new data (more HCoV, plum pox virus)
- Improve error correction
- Find robust way to extract haplotypes
- Utilize long read information

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All of my group!

Thank you!