De novo assembly of short reads using Velvet

Adapted from Nick Loman
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Velvet

- One of the first short read assemblers
- Developed by Daniel Zerbino of EBI
- A *de Bruijn* graph assembler, like:
  - SPAdes
  - SOAPdenovo
  - ABYSS
  - ALLPATHS
  - Etc.

K-mers again

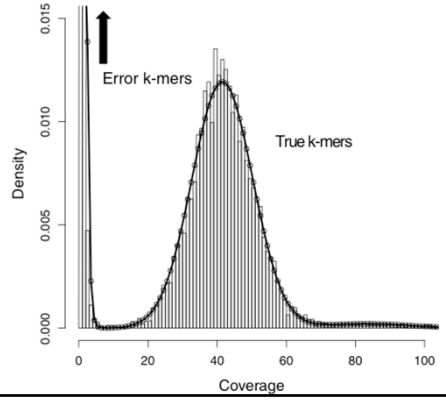
Read GACCTACA

K-mers (K=3)
GAC
ACC
CCT
CTA
TAC
ACA

K-1 bases overlap

de Bruijn graph

Idealised k-mer plot



Counting k-mers

- Plotting k-mer frequencies is a quick and easy way of:
  - Estimating genome size
  - Seeing copy number variation in genome
  - Estimating sequence read error
  - Planning a short-read assembly

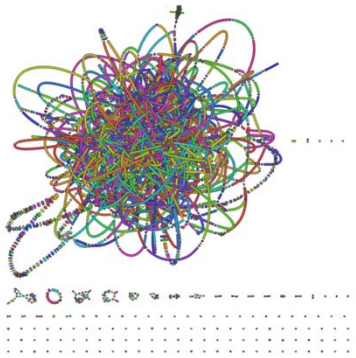
K-mers and K

- No magical value of k
- Depends on
  - read length
  - sequencing error
  - rate of polymorphism
  - coverage
- Some rules:
  - k must be less than the read length
  - k can't be an even number (can produce palindromes)

K-mer value effects

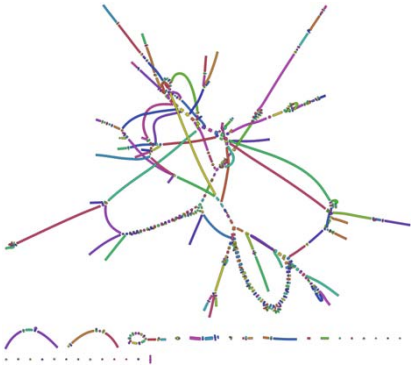
- Bigger K:
  - Solves more repeats
  - Fewer overlaps
  - Lower k-mer coverage
- Smaller k:
  - More overlaps
  - Higher k-mer coverage
- Larger: longer contigs, fewer connections
- Smaller: short contigs with lots of connections

K-mer size effect: Salmonella, 51



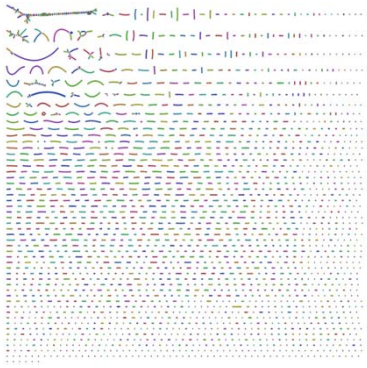
https://github.com/rrwick/Bandage/wiki/Effect-of-kmer-size

K-mer size effect: Salmonella, 71



https://github.com/rrwick/Bandage/wiki/Effect-of-kmer-size

K-mer size effect: Salmonella, 91



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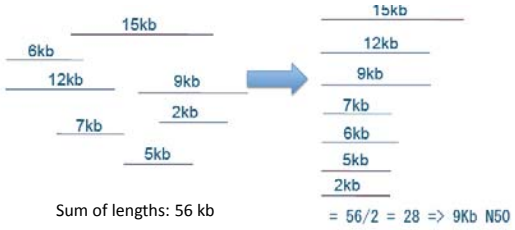
Metrics

- contigs
  - how many
  - total bases
  - N50
- scaffolds
  - how many
  - total bases
  - N50
  - how many gaps
  - total gap bases

N50

•Size of contig such that 50% of total bases are in contigs of this length or more



Thomas Keane and Jan Aerts, Wellcome Trust Sanger

N50

•Size of contig such that 50% of total bases are in contigs of this length or more

•OR

•Shortest of the longest contigs that together make up 50% of the assembly

N50

•Size of contig such that 50% of total bases are in contigs of this length or more

→longer N50 is better

•N50 count:

•number of contigs of at least N50 size

N50 – NG50

•N50:

•Size of contig such that 50% of total bases are in contigs of this length or more

•NG50:

•Replace 'total bases' with 'genome length'

N50

- Minimum contig length influences N50
- Take away shorter contigs → N50 goes up

N50

- High N50 → better assembly

- BUT
- says nothing of quality