
De Novo Illumina
Assemblies with Velvet

Velvet

- Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. D.R. Zerbino and E. Birney. Genome Research 18:821-829. (2008)

- Pebble and Rock Band: Heuristic Resolution of Repeats and Scaffolding in the Velvet Short-Read de Novo Assembler. Zerbino DR, McEwen GK, Margulies EH, Birney E. PLoS One. 2009 Dec 22;4(12):e8407.

- Using the Velvet de Novo Assembler for short-read sequencing technologies. Zerbino DR. Curr Protoc Bioinformatics. Chap 11:Unit 11.5

Overview

- Run velveth
- Background
- Run velvetg
- Details on selecting velvetg parameters
- Post-assembly analyses

Velvet, a short read *de novo* assembler

- Velvet is currently one of the most popular *de novo* assemblers for short (25-150 bp) read data
- Velvet does not use quality scores, instead it uses coverage data for error correction
- You should trim low quality bases from your reads prior to assembly (if necessary after QC).

Getting started

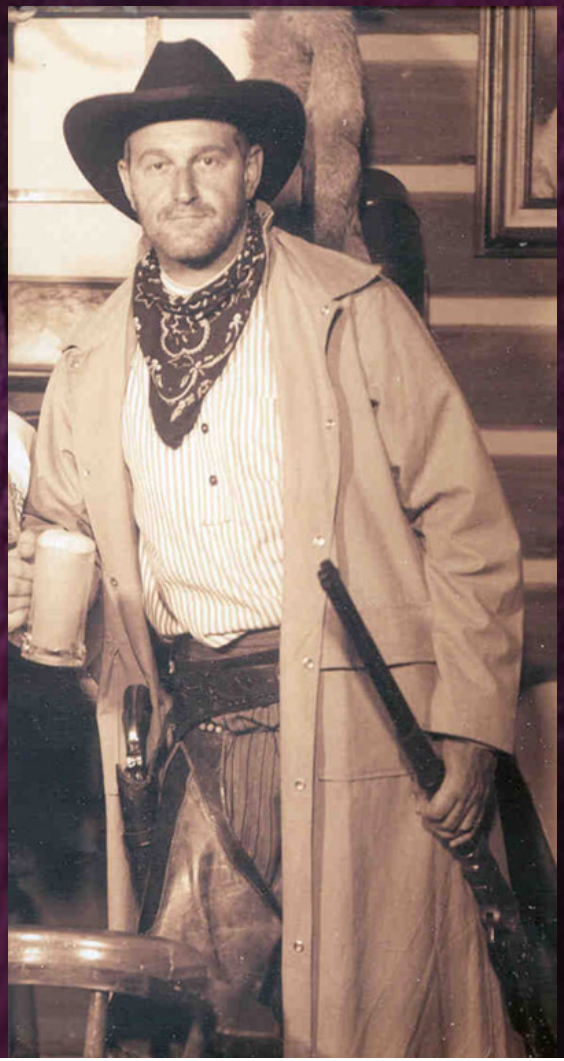
- **CAREFULLY** READ THROUGH THE GETTING STARTED BEFORE YOU BEGIN THE ACTIVITY
- http://evomics.org/learning/assembly-and-alignment/velvet/
- Check your disk space! Clean if necessary
- Complete exercise 1 and start exercise 2

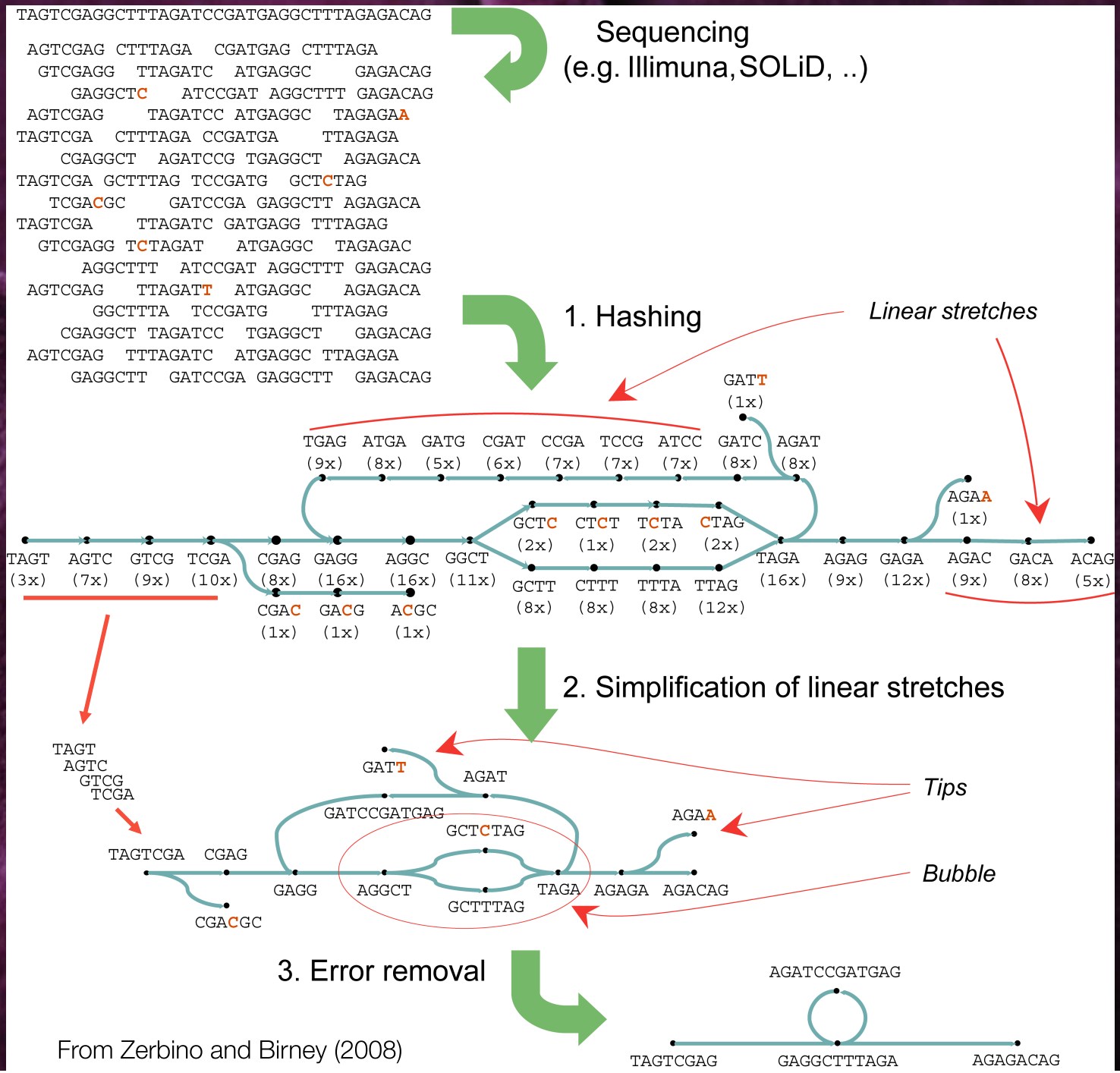
Getting started

- Exercise 2:
- velveth auto 31,45,2 -fastq -shortPaired1….
- 1/2 of the class run velveth with 31,37,2 and the other 1/2 with 39,45,2

Assembly strategies: Eulerian paths on de Bruijn graphs

- Pavel Pevzner 2001 (PNAS)
- k-mers instead of whole reads

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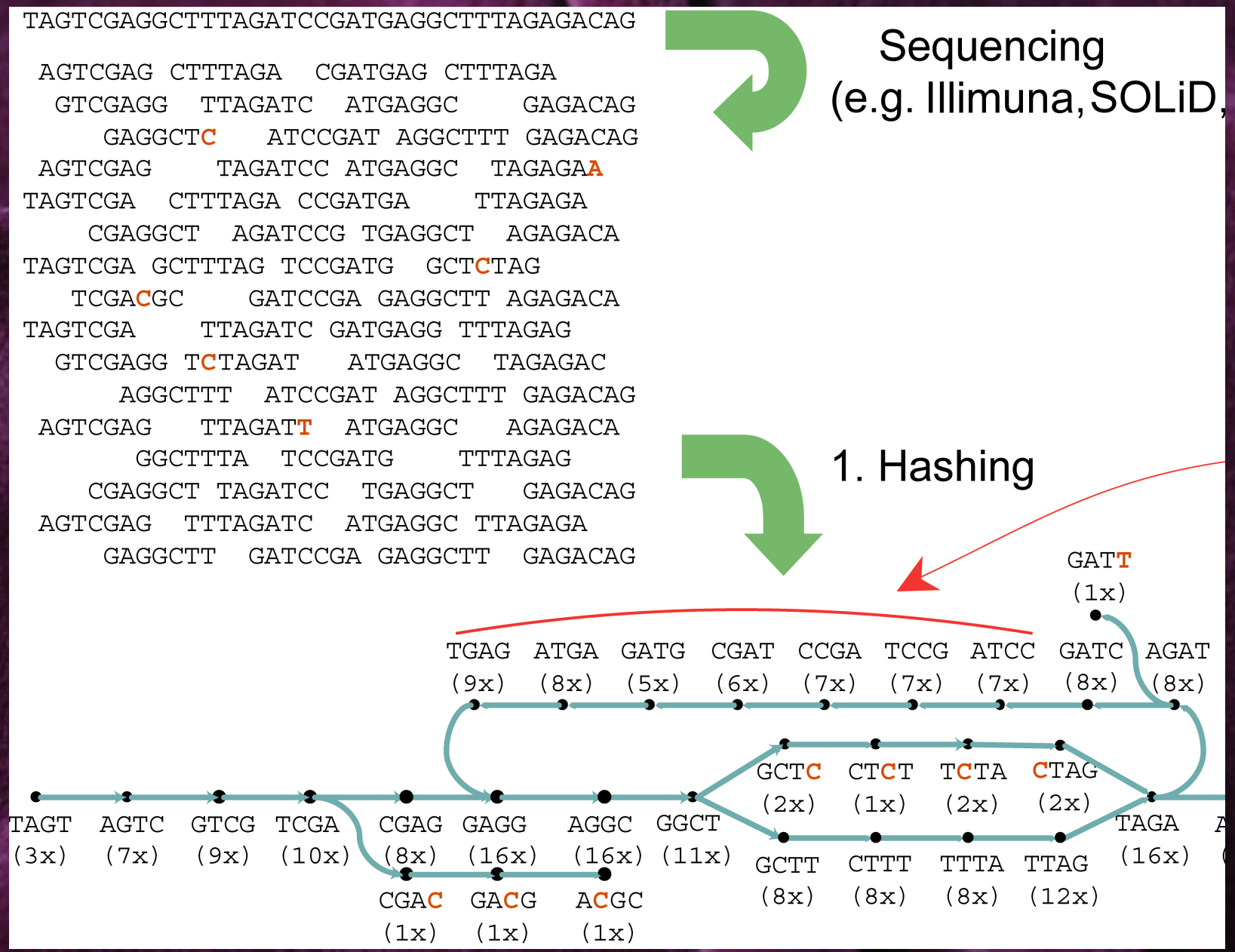
AGTCGAG TAGATCC ATGAGGC TAGAGAA
TAGTCGA CTTTAGA CCGATGA TTAGAGA
CGAGGCT AGATCCG TGAGGCT AGAGACA
TAGTCGA GCTTTAG TCCGATG GCTCTAG
TCGACGC GATCCGA GAGGCTT AGAGACA
TAGTCGA TTAGATC GATGAGG TTTAGAG
GTCGAGG TCTAGAT ATGAGGC TAGAGAC
AGGCTTT ATCCGAT AGGCTTT GAGACAG
AGTCGAG TTAGATT ATGAGGC AGAGACA
GGCTTTA TCCGATG TTTAGAG
CGAGGCT TAGATCC TGAGGCT GAGACAG
AGTCGAG TTTAGATC ATGAGGC TTAGAGA
GAGGCTT GATCCGA GAGGCTT GAGACAG
Sequencing
(e.g. Illimuna, SOLiD, ..)
1. Hashing
Linear stretches
GATT (1x)
TGAG (9x) ATGA (8x) GATG (5x) CGAT (6x) CCGA (7x) TCCG (7x) ATCC (7x) GATC (8x) AGAT (8x)
AGAA (1x)
GCTC (2x) CTCT (1x) TCTA (2x) CTAG (2x)
GCTT (8x) CTTT (8x) TTTA (8x) TTAG (12x)
TAGT (3x) AGTC (7x) GTCG (9x) TCGA (10x) CGAG (8x) GAGG (16x) AGGC (16x) GGCT (11x)
CGAC (1x) GACG (1x) ACGC (1x)
TAGA (16x) AGAG (9x) GAGA (12x) AGAC (9x) GACA (8x) ACAG (5x)
2. Simplification of linear stretches
TAGT
AGTC
GTCG
TCGA
GATT
AGAT
GATCCGATGAG
GCTCTAG
AGAA
Tips
Bubble
TAGTCGA CGAG
GAGG AGGCT TAGA AGAGA AGACAG
GCTTTAG
CGACGC
3. Error removal
AGATCCGATGAG
TAGTCGAG GAGGCTTTAGA AGAGACAG
From Zerbino and Birney (2008)


TAGTCGAGGCTTTAGATCCGATGAGGCTTTAGAGACAG
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AGTCGAG TAGATCC ATGAGGC TAGAGAA
TAGTCGA CTTTAGA CCGATGA TTAGAGA
CGAGGCT AGATCCG TGAGGCT AGAGACA
TAGTCGA GCTTTAG TCCGATG GCTCTAG
TCGACGC GATCCGA GAGGCTT AGAGACA
TAGTCGA TTAGATC GATGAGG TTTAGAG
GTCGAGG TCTAGAT ATGAGGC TAGAGAC
AGGCTTT ATCCGAT AGGCTTT GAGACAG
AGTCGAG TTAGATT ATGAGGC AGAGACA
GGCTTTA TCCGATG TTTAGAG
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TGAG ATGA GATG CGAT CCGA TCCG ATCC GATC AGAT
(9x) (8x) (5x) (6x) (7x) (7x) (7x) (8x) (8x)
GCTC CTCT TCTA CTAG
(2x) (1x) (2x) (2x)
TAGT AGTC GTCG TCGA CGAG GAGG AGGC GGCT TAGA
(3x) (7x) (9x) (10x) (8x) (16x) (16x) (11x) (16x)
GCTT CTTT TTTA TTAG
(8x) (8x) (8x) (12x)
CGAC GACG ACGC
(1x) (1x) (1x)

Creating a hashtable

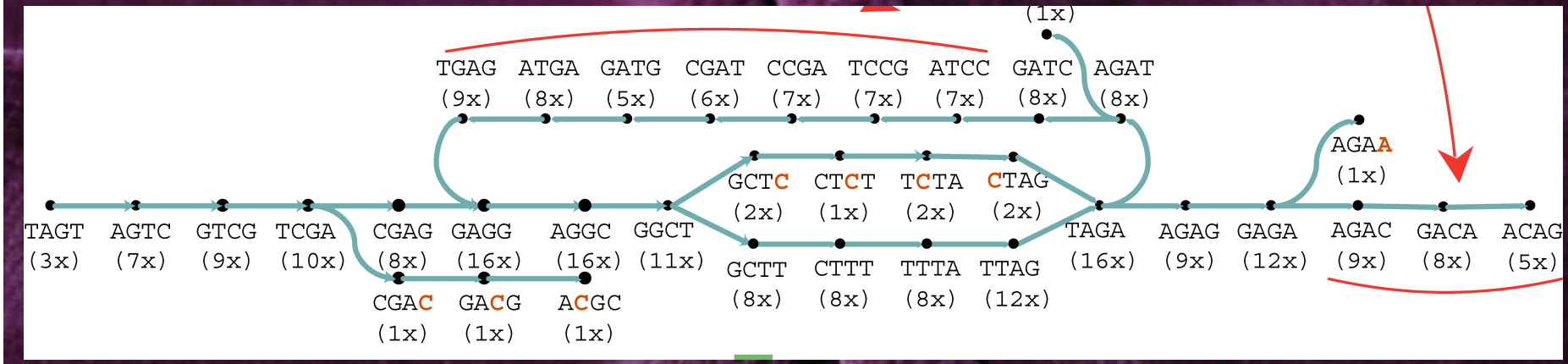
- k=4, read length=7

```
Reads:  AGTCGAG     CTTTAGA      …
kmers:  AGTC        CTTT
          GTCG        TTTA
           TCGA        TTAG
            CGAG        TAGA
```

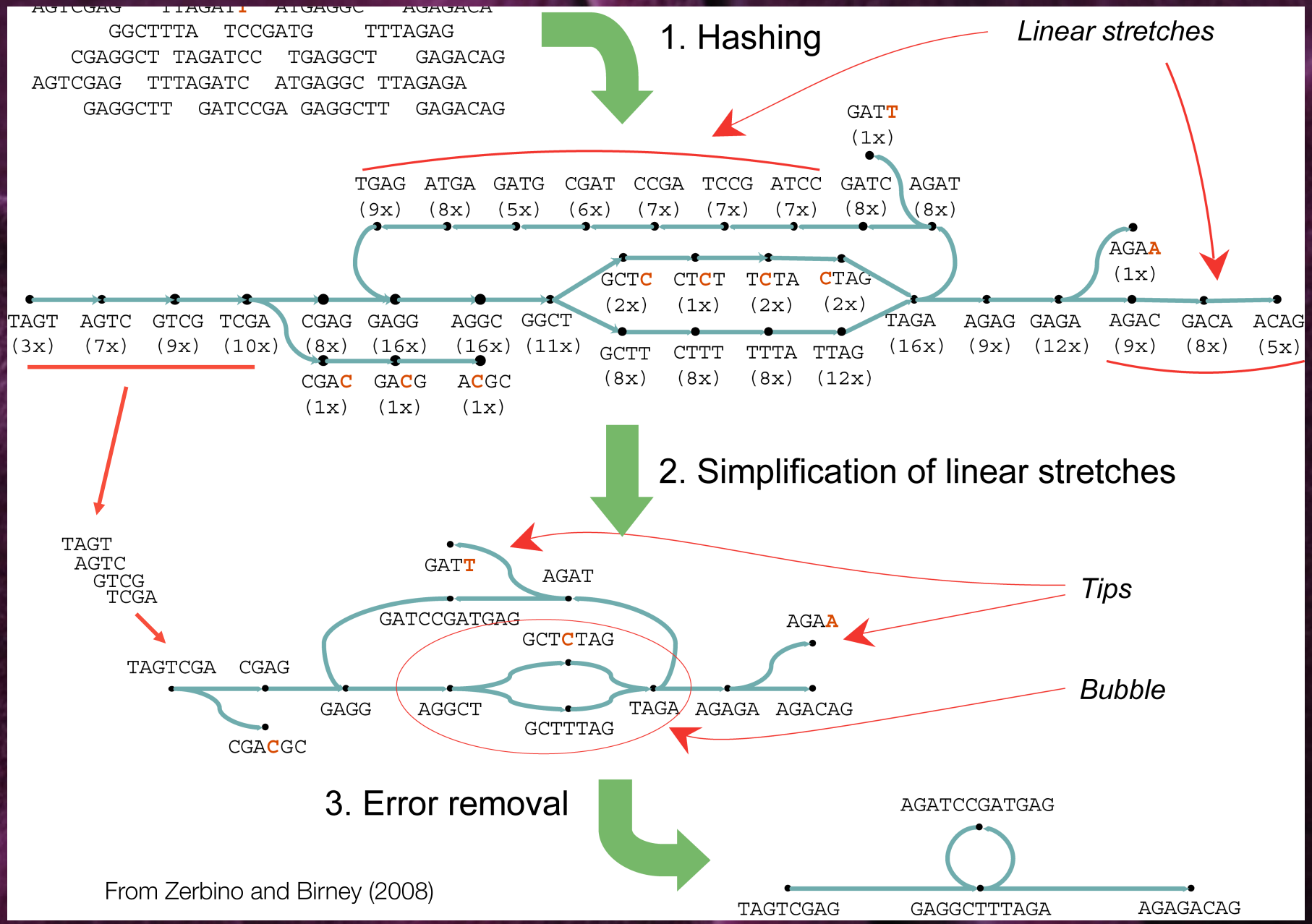
Creating a hashtable

- `AAAA: 8`
- `AAAC: 10`
- `AACT: 9`
- …

Building the graph



- Choose a kmer (educated guess…): AGTC
- Find its extensions: kmers that start with GTC (4 at most
- Iterate


GGCTTTA TCCGATG TTTAGAG
CGAGGCT TAGATCC TGAGGCT GAGACAG
AGTCGAG TTTAGATC ATGAGGC TTAGAGA
GAGGCTT GATCCGA GAGGCTT GAGACAG
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TAGTCGAG GAGGCTTTAGA AGAGACAG
From Zerbino and Birney (2008)

Continue exercises

- Finish exercise 2 and start exercise 3.

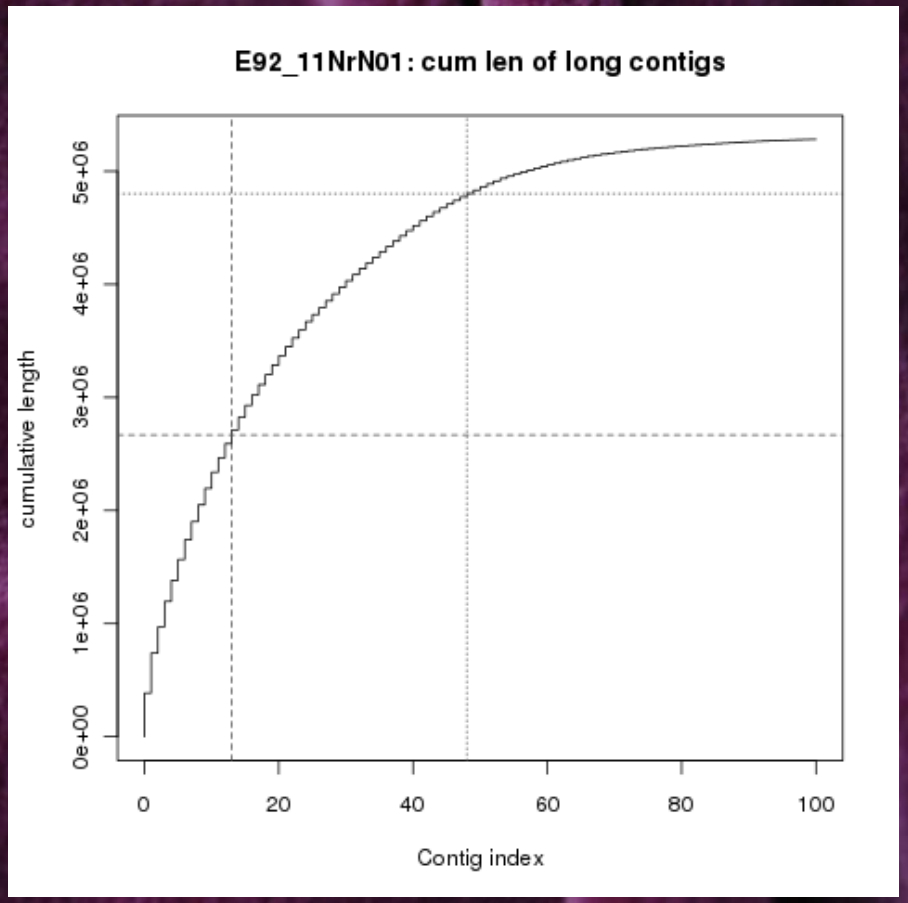
Primary velvetg parameters

- k
  - Short k-mer high accuracy, short contigs
  - Long k-mer lower accuracy, longer contigs
- exp_cov
  - = in k-mer coverage !!!
- ins_length

Assembly statistics: n50 and n90

- "Contig or scaffold N50 is a weighted median statistic such that 50% of the entire assembly is contained in contigs or scaffolds equal to or larger than this value" -seqanswers.com
- Especially helpful compared to the maximum contig size

Assembly statistics: n50 and n90



k-mer coverage (C_k)

- All coverage values in Velvet are in k-mer coverage, not nucleotide coverage
- $C_k = C * (L–k+1)/L$
  - k = *kmer* length, L = read length, C = nucleotide coverage
- k-mer coverage is always lower than the actual bp coverage!
- For example: When k=31 and L=36, your k-mer coverage is 1/6 of the actual coverage
- The longer the kmer, the lower the coverage

Continue activity

- Finish exercises 4-7
- Report results in the Excel spreadsheet on display

VelvetOptimiser (Perl script)

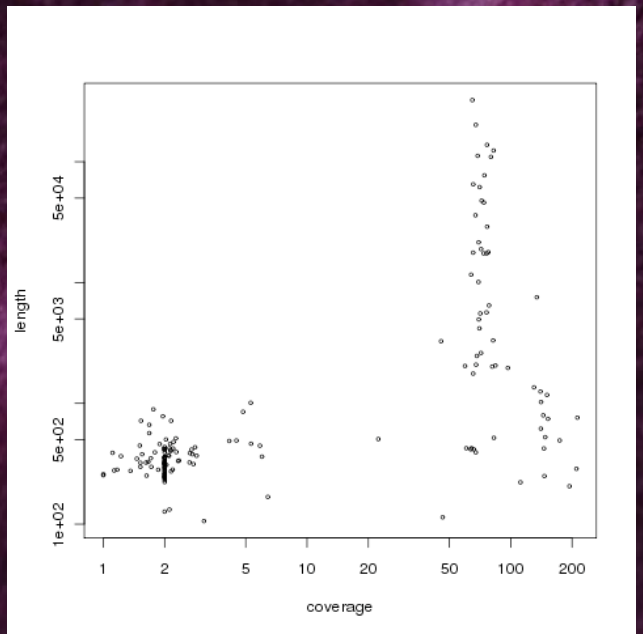
- Simon Gladman
- Runs velvet on a *k-mer* range you specify to determine optimum
- Estimates optimum *exp_cov* and then searches for optimum cov_cutoff
- Pros:
  - Optimization functions (n50, max contig, tbp)
  - Good job of guessing amount of RAM required
- Cons:
  - Can't specify most velvetg parameters directly
- It is recommended to re-run velvetg with VelvetOptimiser parameters and try out additional parameters

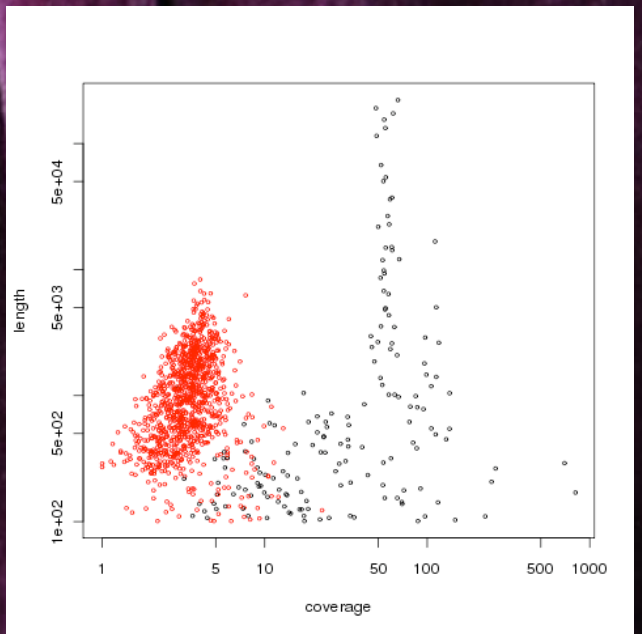
Post-assembly analyses

- Always check your assembly for obvious misassemblies
  - Use tablet and/or a whole genome alignment to reference genome (Mauve, nucmer+ACT, …)
- Contigs that represent adjacent genomic regions may have overlap of < 2k, and sometimes can be merged (larger contigs!)

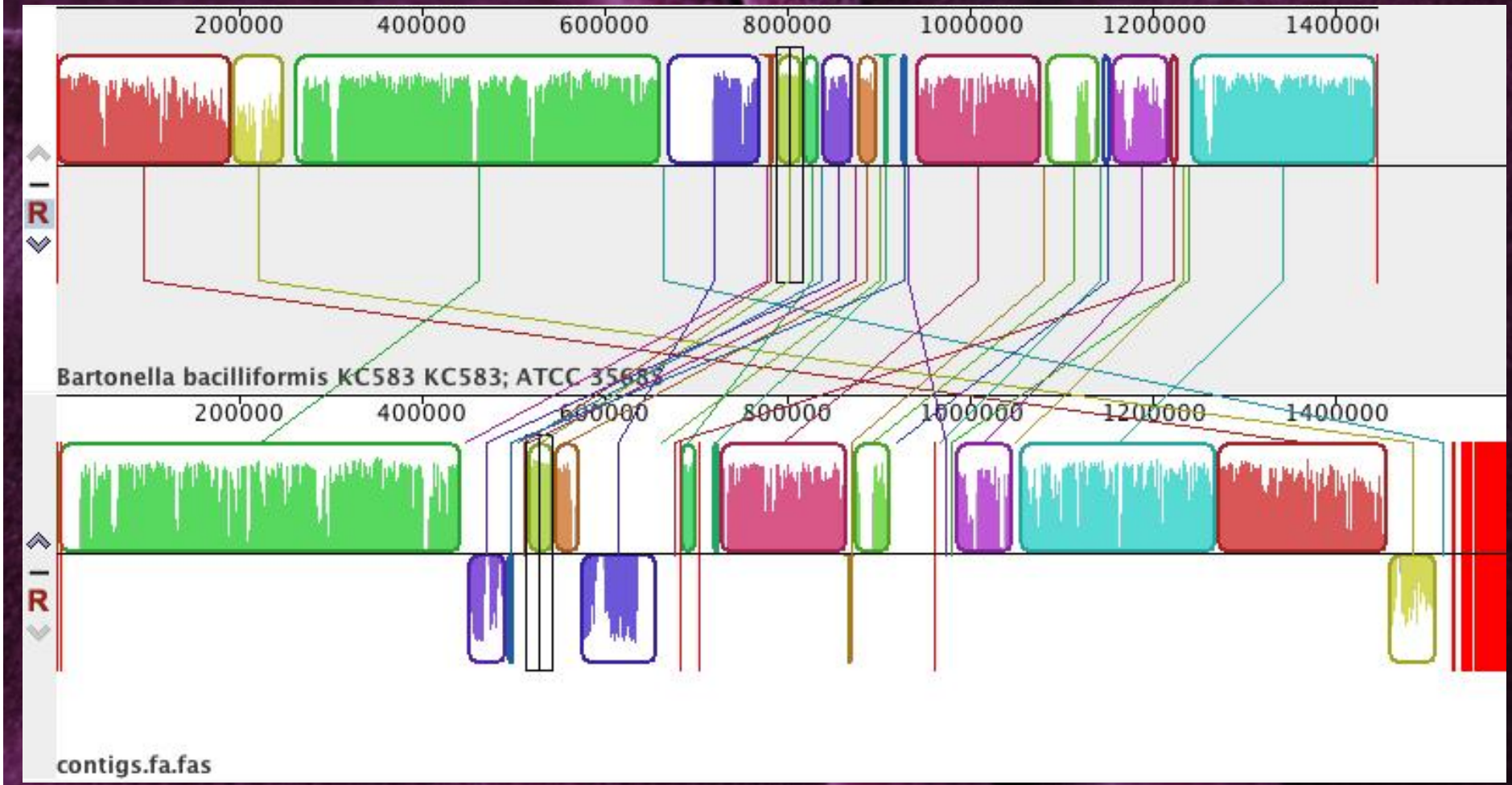
Post-assembly analyses

- Things to consider:
  - Check contamination (BLAST to nr, “metagenomics” approach)
  - Coverage vs contig length



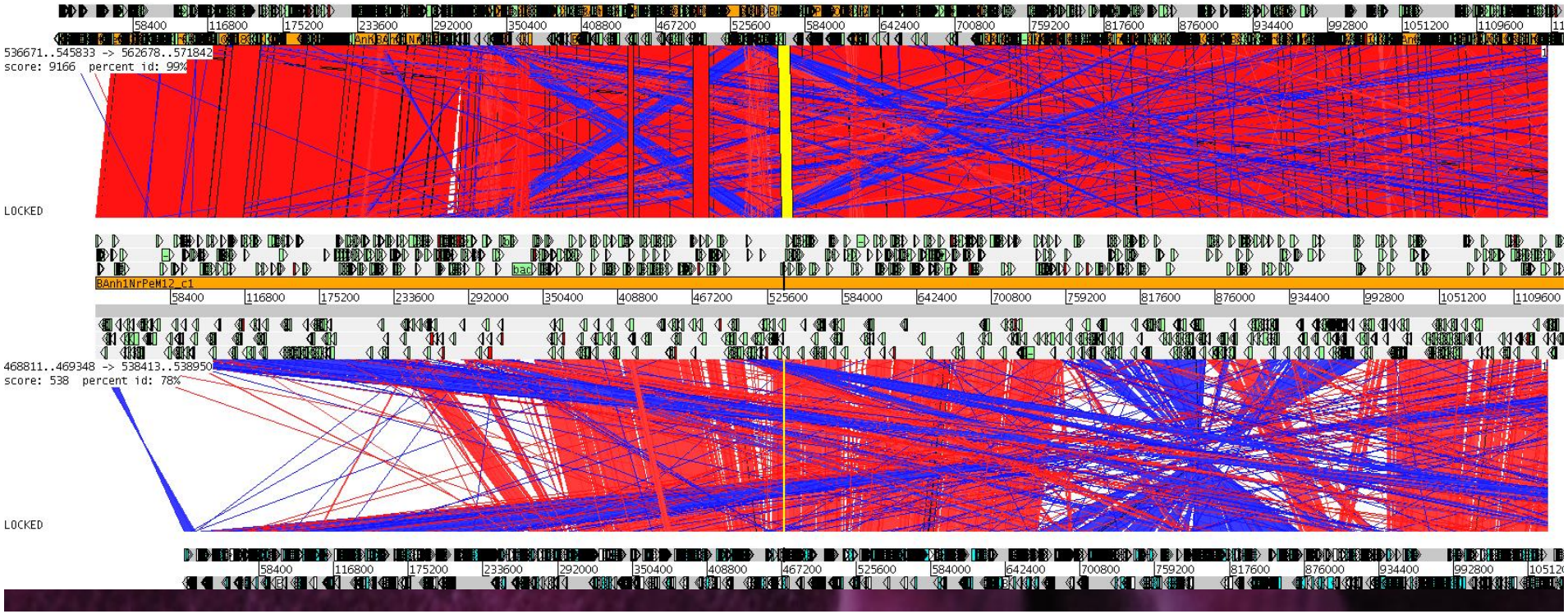


Post assembly analyses (Mauve)



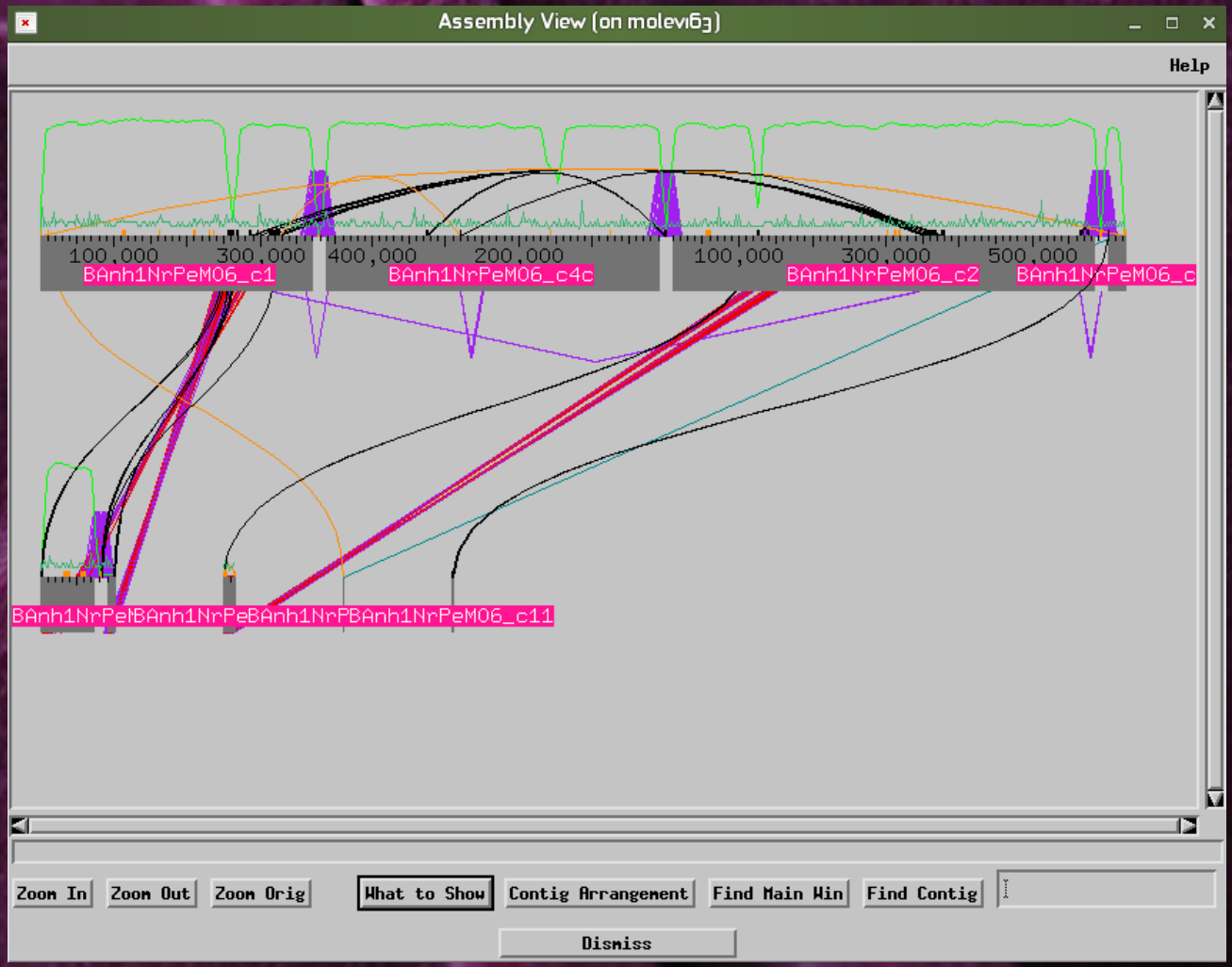
Post-assembly analyses

- Order contigs/scaffolds with respect to a reference assembly or a close genome (nucmer)
- Compare with ACT (Artemis comparison tool)



Post-assembly analyses

- Even coverage?
- Even distribution of good paired-end reads?



Hardware considerations

- A 1GB genome project with a mixture of ~1 billion reads (~73x) is using 300GB+ of RAM
- Some assemblers such as ABySS tend to use less memory
- Opt for a processor with higher L3 cache (24MB) than simply clock speed
- Lots of disk space

Velvet associated software

- Oases: *de novo* transcriptome assembler for short reads
- MetaVelvet: *de novo* assembly of metagenomic data
- Columbus module: allows the assembly process to be assisted by alignment information onto a set of reference sequences
- Curtain: *de novo* assembly of large genomes