CS681: Advanced Topics in Computational Biology

Week 8 Lecture 1

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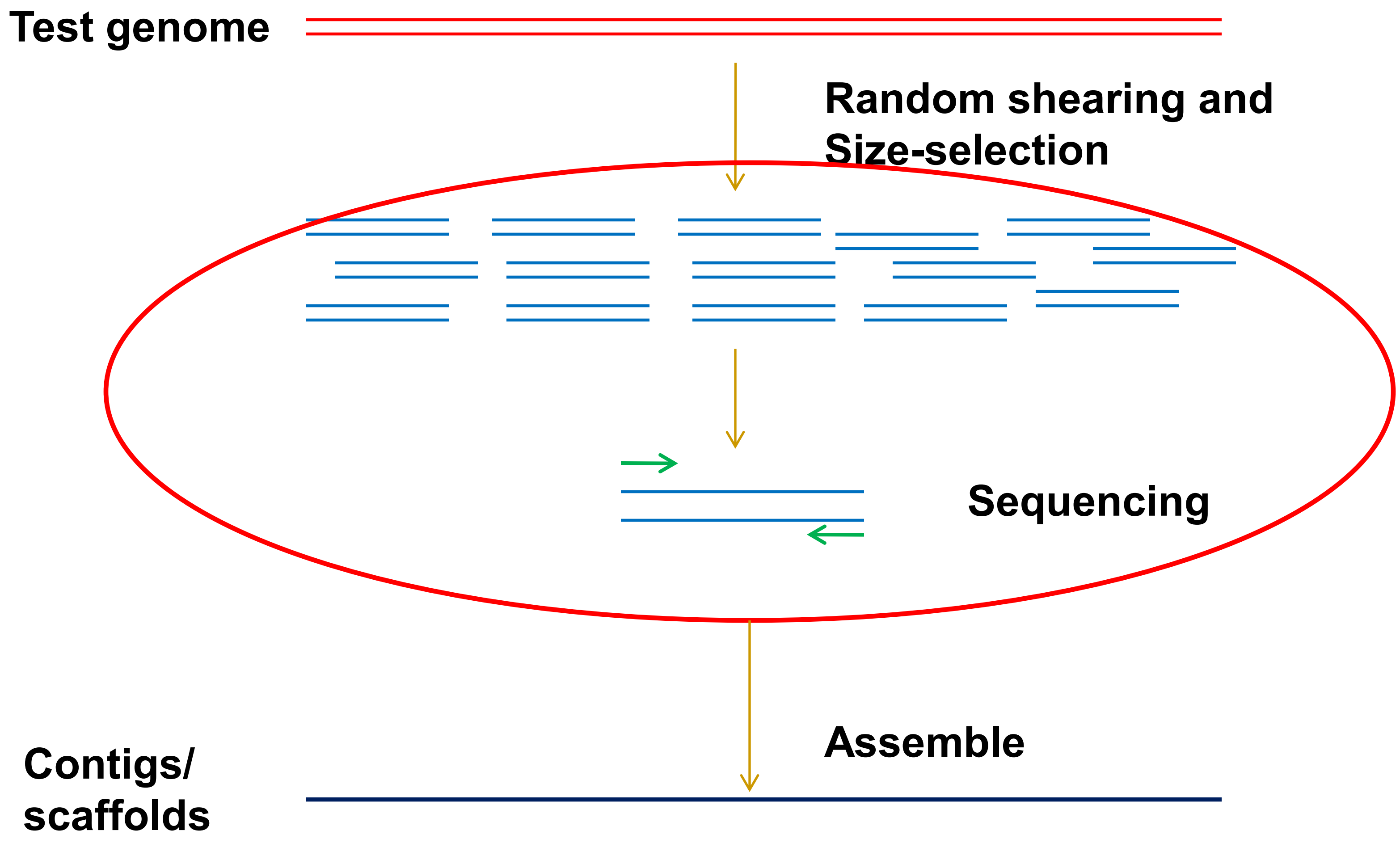
Genome Assembly



De Bruijn Graphs

- *n*-dimensional directed graph of *m* symbols
  - m^n vertices: all possible length-*n* sequences of *m* symbols
  - Edges between vertices *v* and *w* if *sequence(w)* can be generated by *shifting sequence(v)* by one character and add one new character
  - $S = \{s_1, s_2, \ldots, s_m\}$
  - $V = S^n = \{(s_1, \ldots, s_1, s_1), (s_1, \ldots, s_1, s_2), \ldots, (s_m, \ldots, s_m, s_m)\}$
  - $E = \{((v_1, v_2, \ldots, v_n), (w_1, w_2, \ldots, w_n)): v_2=w_1, v_3=w_2, \ldots, v_n=w_{n-1}\}$

De Bruijn Graph for DNA Assembly

- m = 4 (A, C, G, T)
- n = k (k-mer size)
- 4^k *potential* vertices
  - In reality if *k* is sufficiently large, upper bound is genome size
  - Twin vertices: vertices with sequences that are reverse-complement of each other
    - *AAAA* twin of *TTTT*

De Bruijn Assemblers

- Currently the most common for NGS: Euler, ALLPATHS-LG, Velvet, ABySS, SOAPdenovo
- Divide reads into k-mers
  - Build graph from k-mers
    - Put an edge if there is k-1 bp prefix-suffix match
  - Error correction
  - Eulerian path
- The first parts (graph construction & correction) is essentially common to all these assemblers, with a few implementation differences (e.g. parallelization in ABySS)

A quick example

```
TAGTCGAGGCTTTAGATCCGATGAGGCTTTAGAGACAG

 AGTCGAG CTTTAGA  CGATGAG CTTTAGA
  GTCGAGG  TTAGATC  ATGAGGC    GAGACAG
     GAGGCTC   ATCCGAT AGGCTTT GAGACAG
 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
```

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A quick example

```
 AGTCGAG CTTTAGA  CGATGAG CTTTAGA
  GTCGAGG  TTAGATC  ATGAGGC    GAGACAG
     GAGGCTC   ATCCGAT AGGCTTT GAGACAG
 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
```

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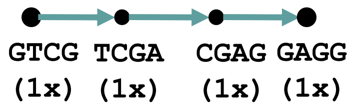
A quick example

First read: GTCGAGG



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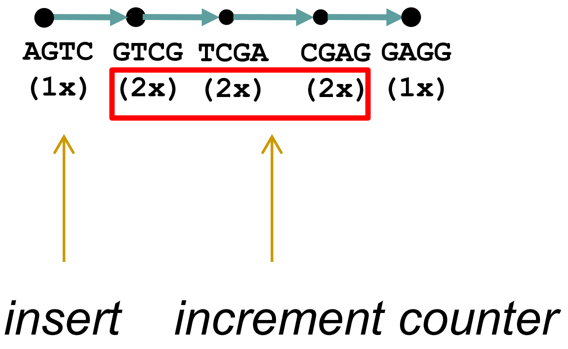
A quick example

First read: GTCGAGG

Second read: AGTCGAG



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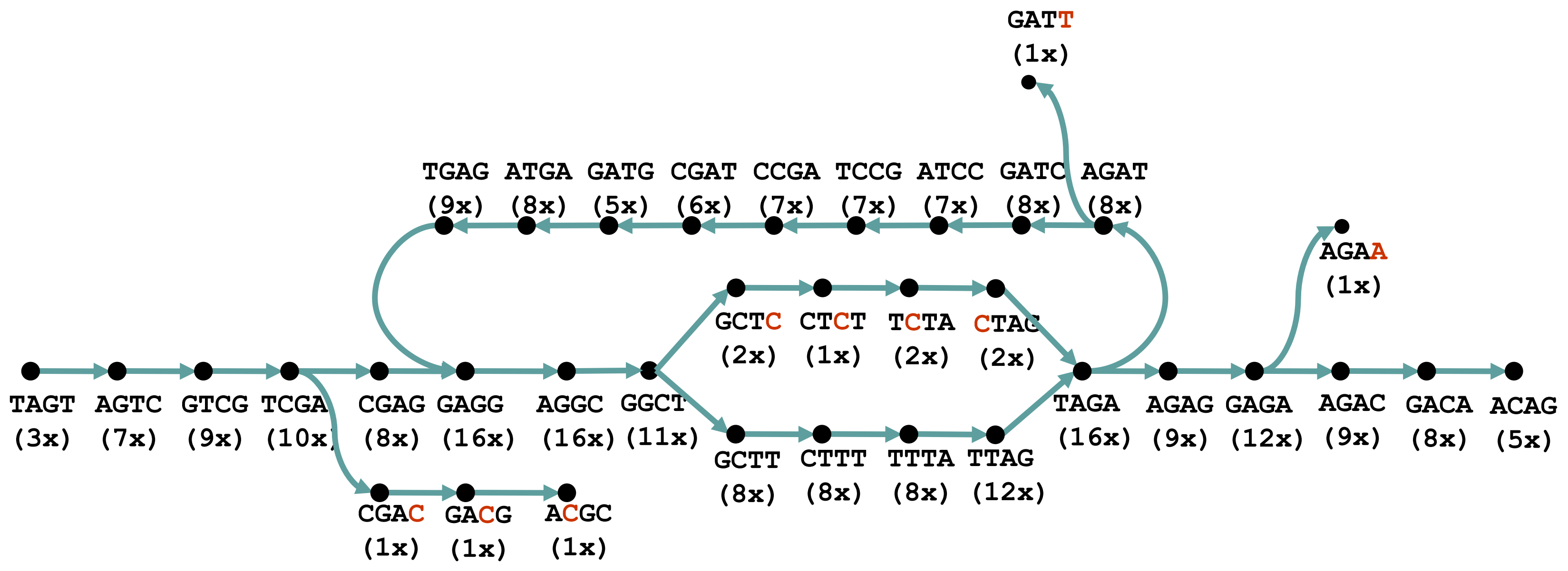
A quick example

All the others…



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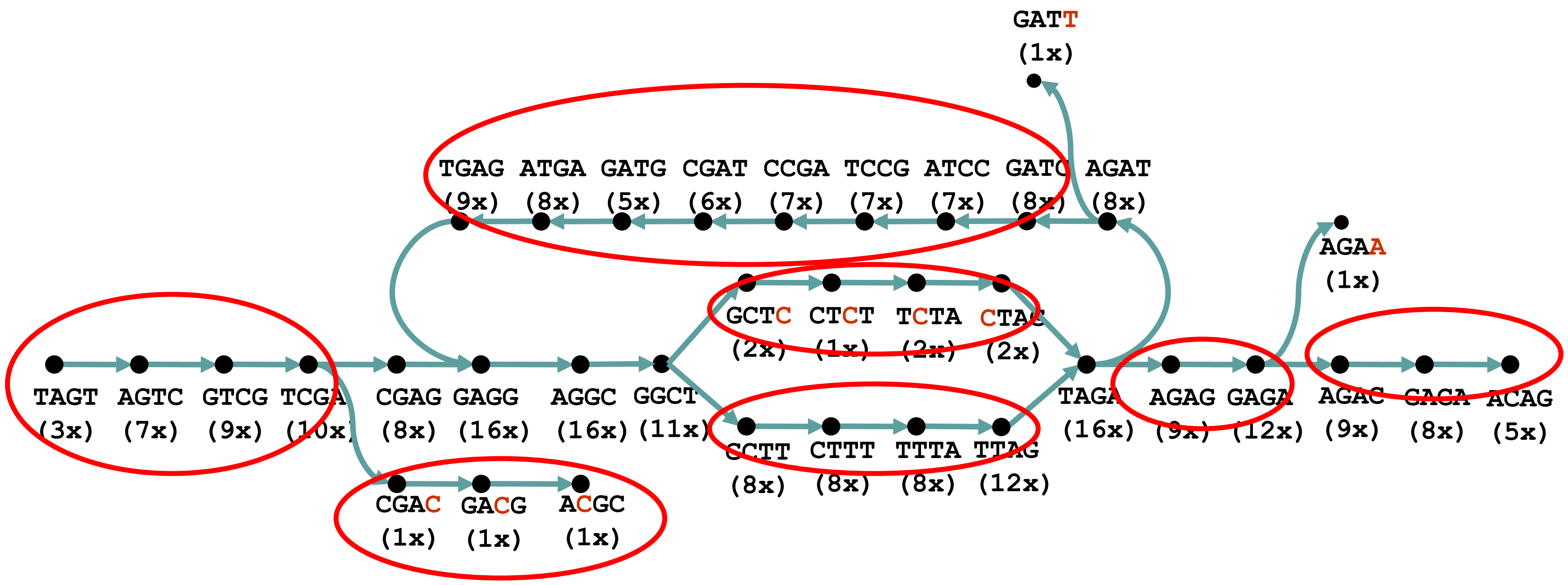
A quick example

All the others…



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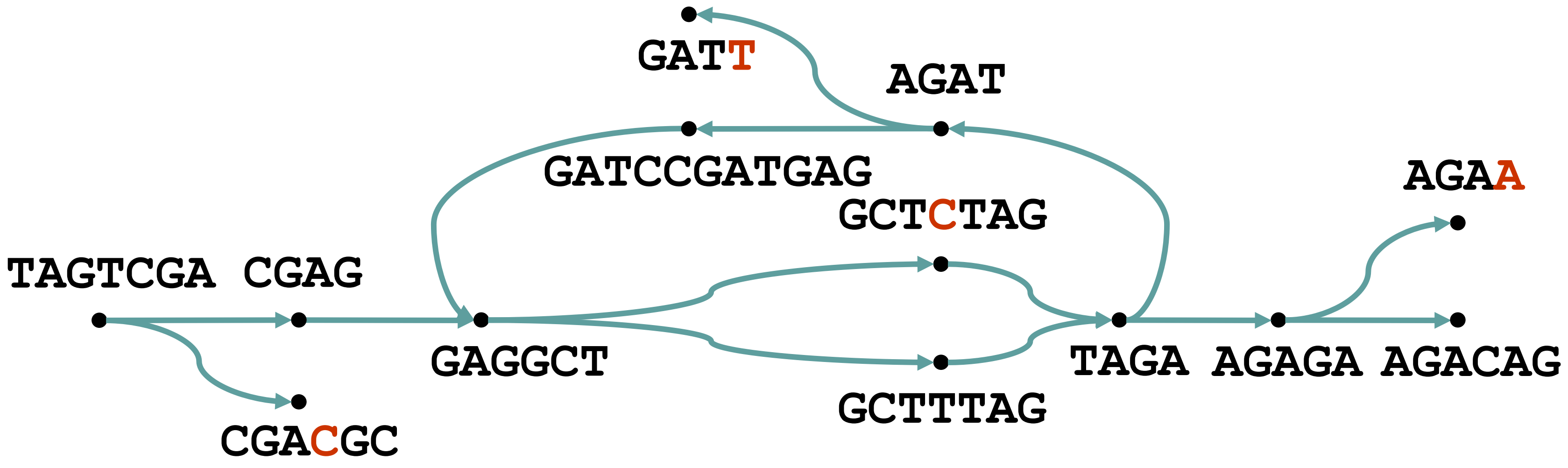
A quick example

After simplification…



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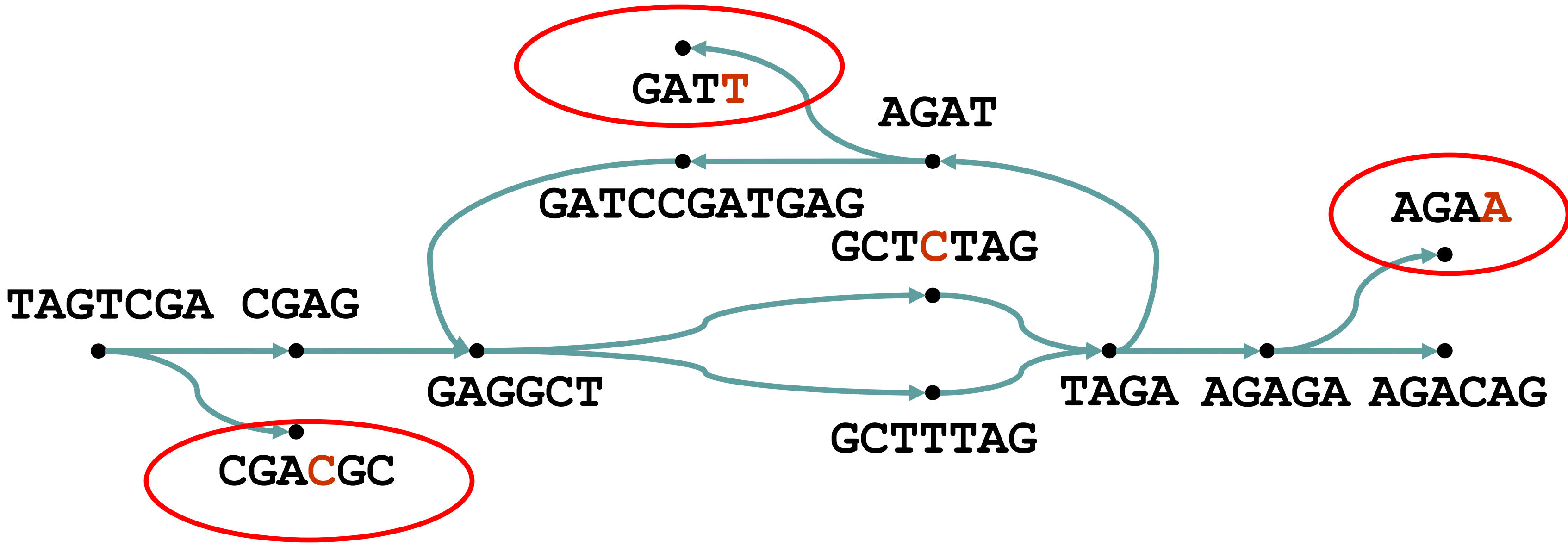
Tips



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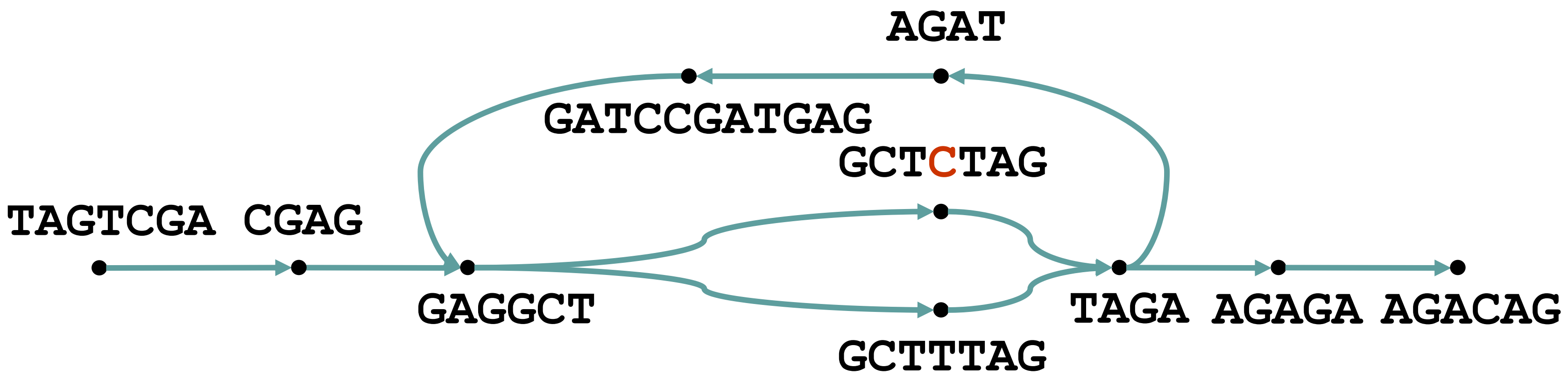
Error removal

Tips removed…



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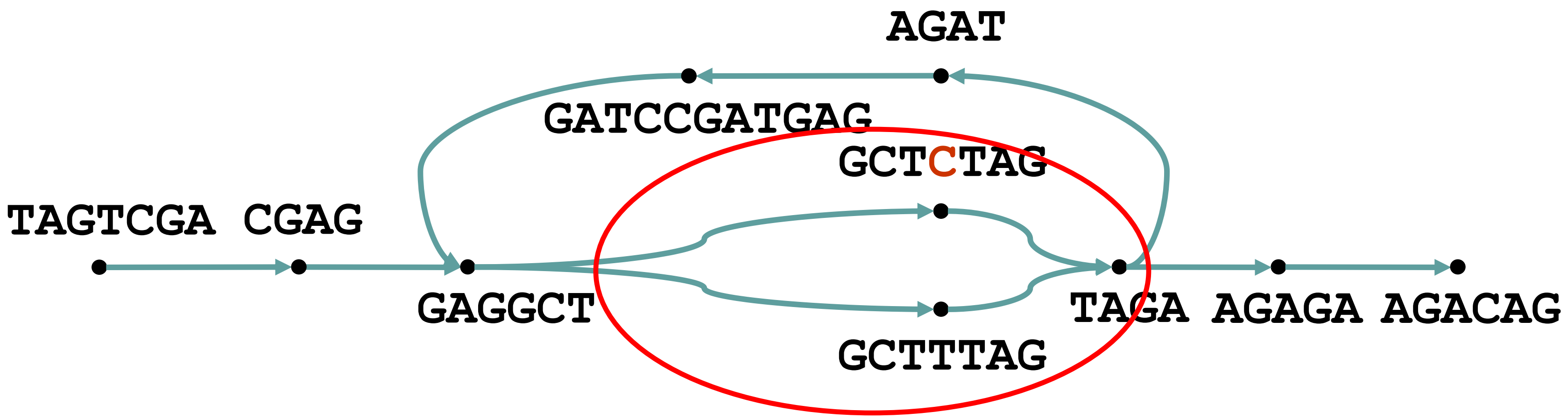
Bubbles



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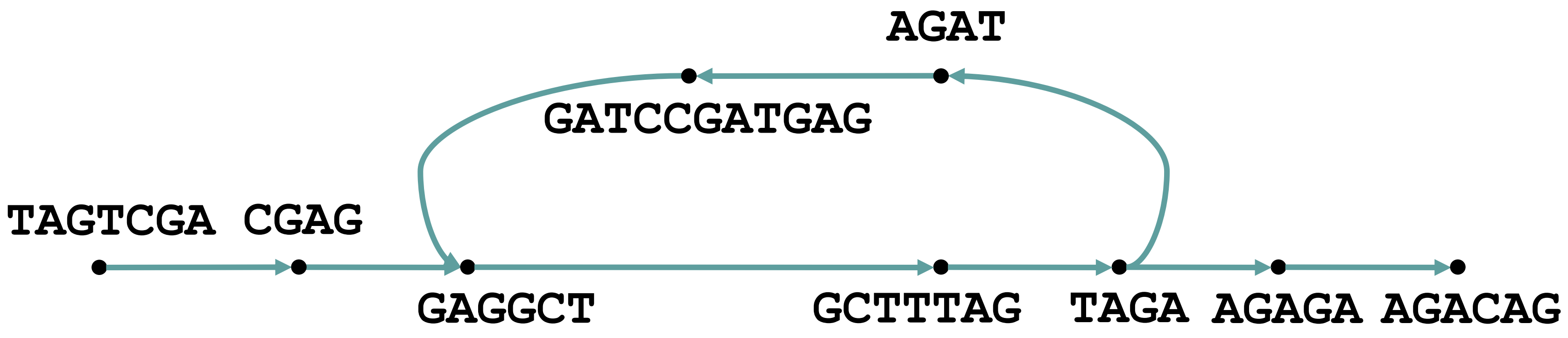
Error removal

Bubbles removed



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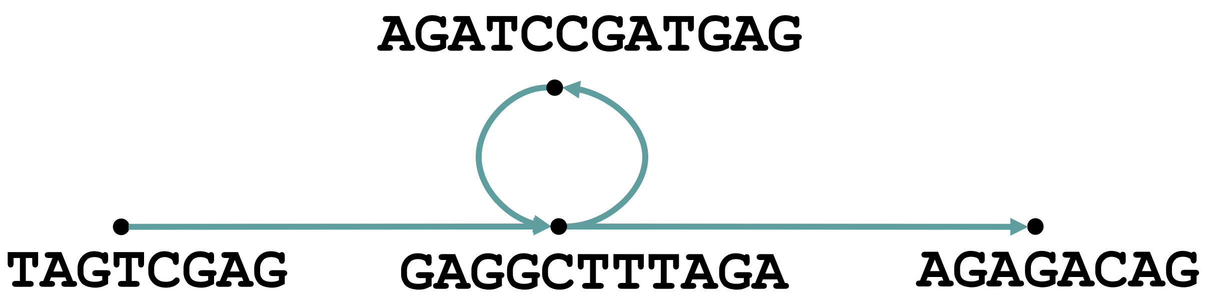
Error removal

Final simplification…



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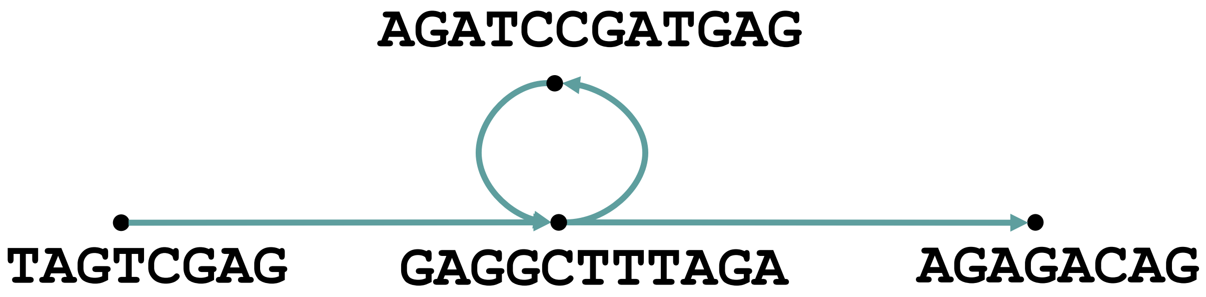
Eulerian path



TAGTCGAG GAGGCTTTAGA AGATCCGATGAG GAGGCTTTAGA AGAGACAG

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Differences: de Bruijn vs Overlap

- Algebraic difference:
  - Reads in the OLC methods are atomic
  - Reads in the DB graph are sequential paths through the graph
- This leads to practical differences:
  - DB graphs allow for a greater variety of overlaps.
  - Overlaps in the OLC approach require a global alignment, not just a shared *k*-mer

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Considerations

- Graph size scales with genome size
  - Increased error rate -> larger graph
- Clipping to short k-mers get rid of sequence errors accumulated at the ends of reads
- k value:
  - Small -> increased connectivity vs. more repeat collapses
  - Large -> increased specificity vs. decreased connectivity

Resolving repeats using long reads or paired-end reads

REPEAT RESOLUTION

Chromosome X

- 548 million Illumina reads were generated from a flow-sorted human X chromosome.
  - Fit in 70GB of RAM.
  - Many contigs: 898,401 contigs
  - Short contigs: 260bp N50 (max 6,956bp)
  - Overall length: 130Mb.
- Moral: there are engineering issues to be resolved but the complexity of the graph needs to be handled accordingly.
  - Reduced representation (Margulies et al.).
  - Combined re-mapping and de novo sequencing (Cheetham et al., Pleasance et al.).
  - Code parallelization (ABySS)
  - Improved indexing (Cortex).
  - Use of intermediate re-mapping

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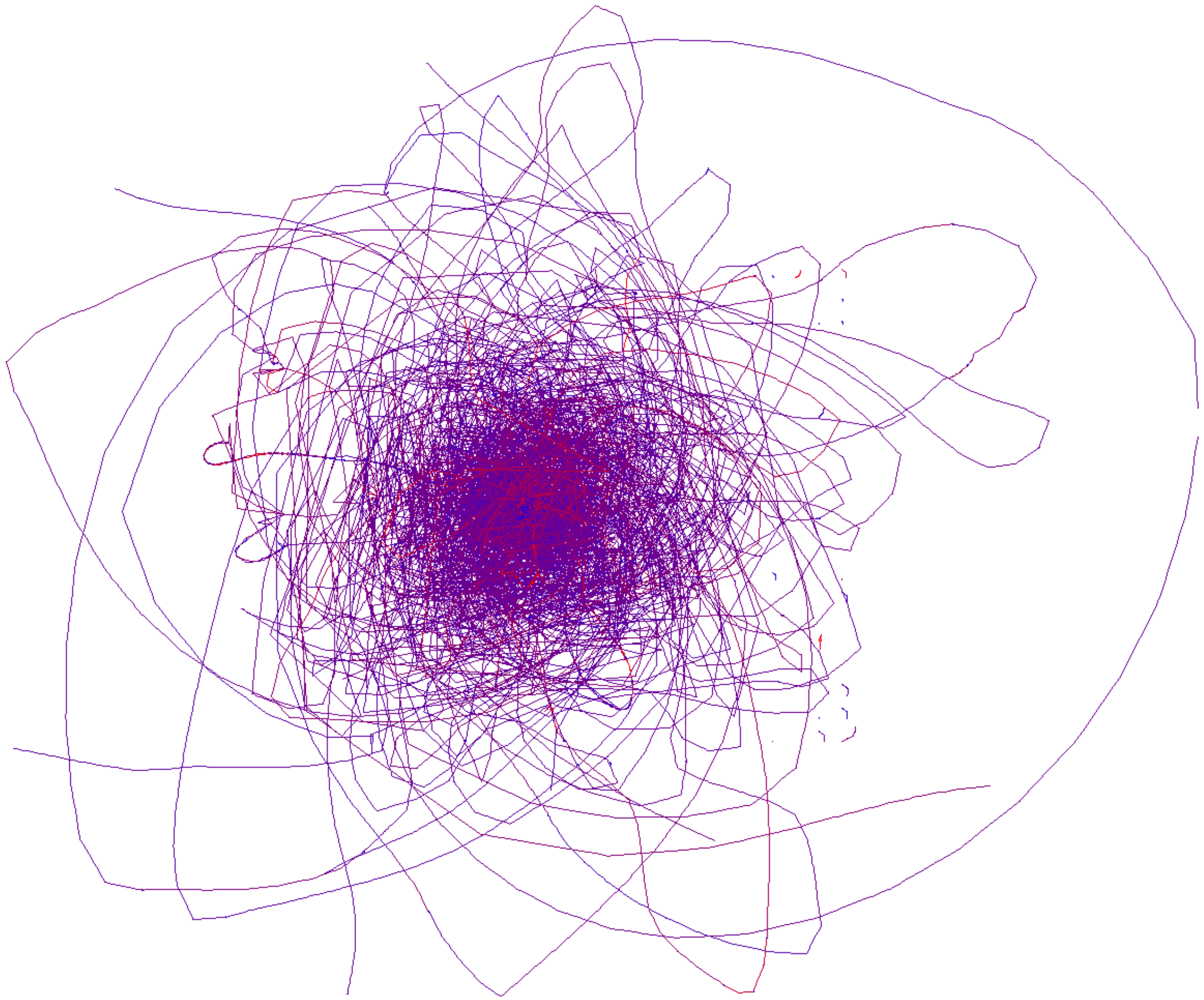
Repeats in a de Bruijn graph



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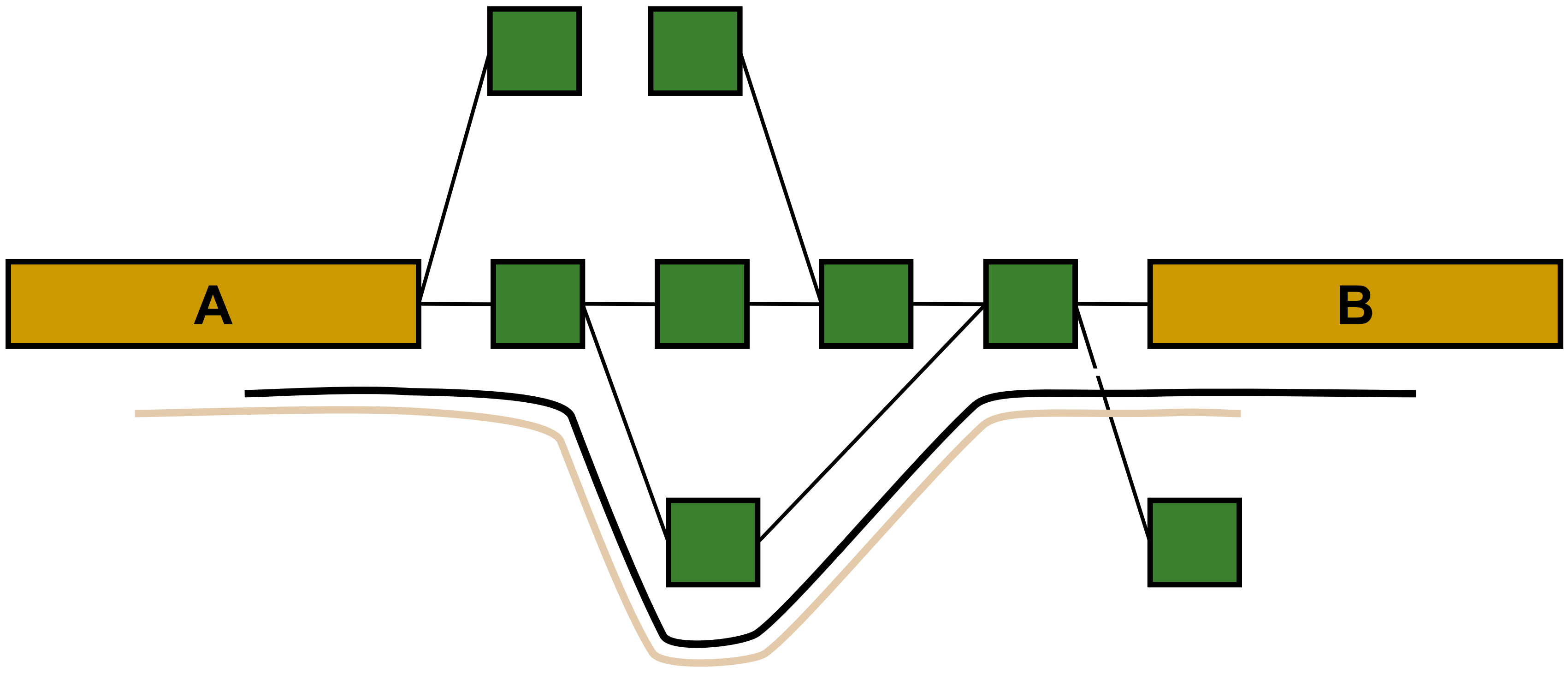
Velvet: RockBand



Use long and short reads together

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Different approaches to repeat resolution

- Theoretical: spectral graph analysis
  - Equivalent to a Principal Component Analysis
  - Relies on a (massive) matrix diagonalization
  - Comprehensive: all the data is integrated at once
  - Robust: small variations don’t disturb the overall result
  - Never used because of the computational cost.

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Different approaches to repeat resolution

- Traditional scaffolding
  - e.g. Arachne, Celera, BAMBUS.
  - Heuristic approach similar to that used in traditional overlap-layout-consensus contigging.
  - Build a big graph of pairwise connections, simplify, extract obvious linear components.

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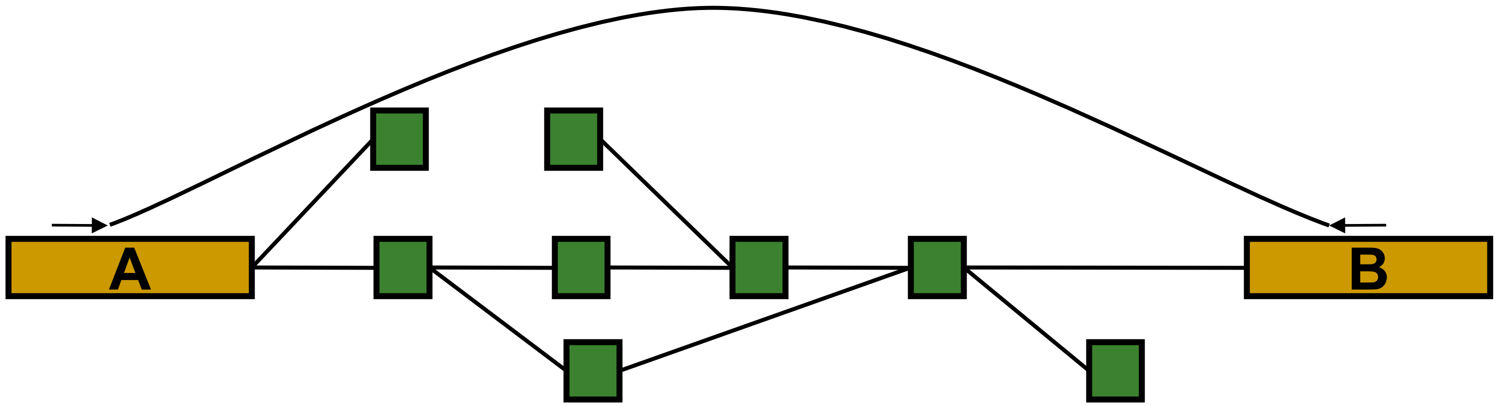
Different approaches to repeat resolution

- In NGS assemblers:
  - EULER: for each pair of reads, find all possible paths from one read to the other.
  - ABySS: Same as above, but the read-pairs are bundled into node-to-node connections to reduce calculations
  - ALLPATHS: Same as above, but the search is limited to localized clouds around pre-computed scaffolds.



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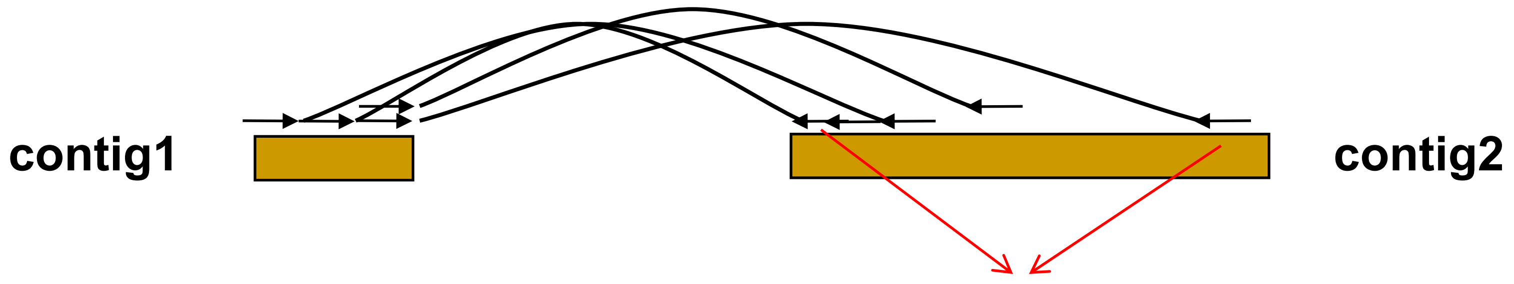
Different approaches to repeat resolution

- Using the differences between insert length
  - The Shorty algorithm uses the variance between read pairs anchored on a common contig on *k*-mer.



Collapsed repeat in contig1 ?

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PRACTICAL CONSIDERATIONS

Colorspace

- Di-base encoding has a 4 letter alphabet, but very different behavior to sequence space
  - Different rules for complementarity
- Direct conversion to sequence-space is simple but erroneous
  - One error messes up all the remaining basepairs
- Conversion must therefore be done at the very end of the process, when the reads are aligned
  - You can then use the transition rules to detect errors

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Different error models

- When using different technologies, you have to take into account different technologies
  - Easy for OLC assembly
  - Much more tricky for de Bruijn assembly, since k-mers are not assigned to reads.
  - Different assemblers have different settings

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Pre-filtering the reads

- Some assemblers have built-in filtering of the reads (e.g. Euler) but not a generality.
  - Low phred quality
  - Reads with N characters
- Efficient filtering of low quality bases can cut down on the computational cost (memory & time)
- Some assemblers require reads of identical lengths.

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