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De novo genome assembly versus mapping to a reference genome

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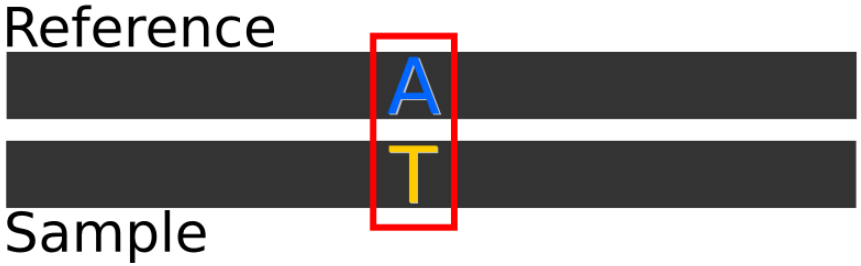
Outline

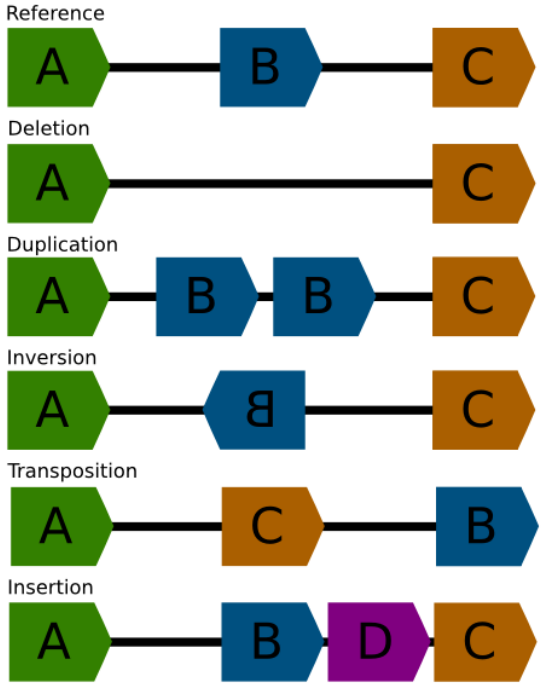
- Genetic variations & sequencing
- De novo sequence assembly
- Reference based mapping/alignment
- Comparison
- Conclusion

Variation types

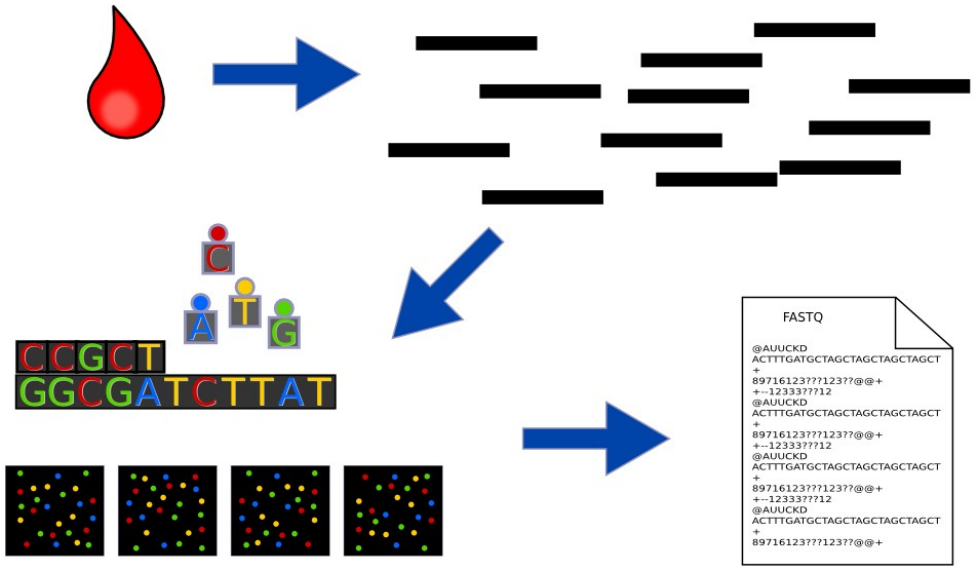
SNV (Single nucleotide variation)



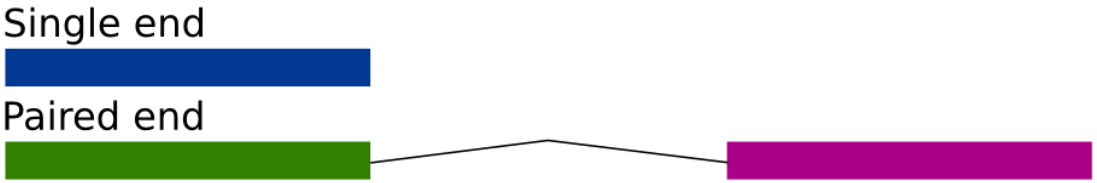
Structural variations



Sequencing technologies



- Different read lengths, 36 – 10'000bp (150-500bp is typical)
- Different sequencing technologies produce different data

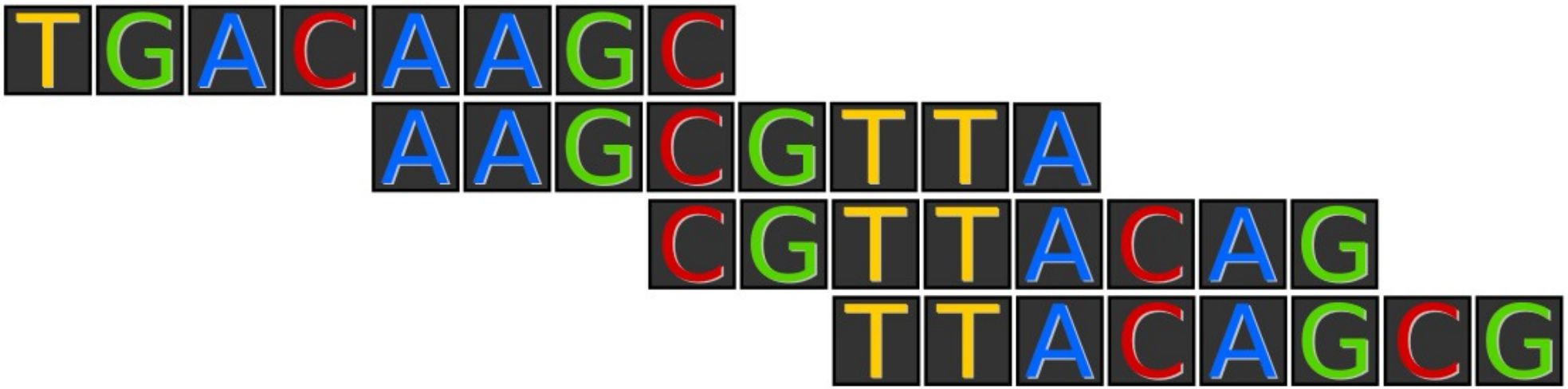


Recreating the genome

- The problem:
  - Recreate the original patient genome from the sequenced reads
    - For which we dont know where they came from and are noisy
- Solutions:
  - Recreate the genome with no prior knowledge using de novo sequence assembly
  - Recreate the genome using prior knowledge with reference based alignment/mapping

De novo sequence assembly

- Ideal approach
- Recreate **original genome** sequence through overlapping sequenced reads

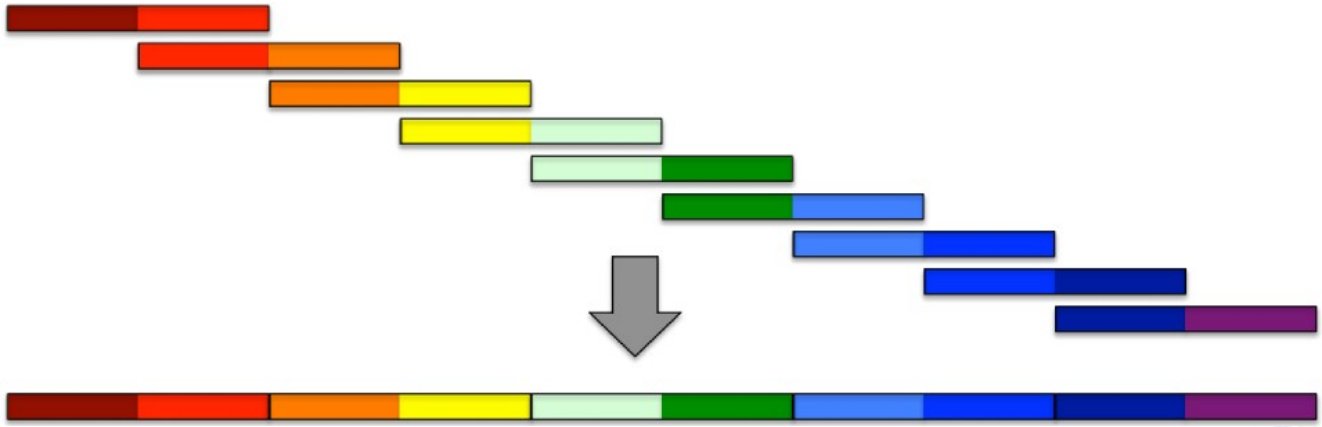


De novo sequence assembly

- Construct assembly graph from overlapping reads

...AGCCTAGGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGTTTGGTCAACCTCGGACGGAC
CAACCTCGGACGGACCTCAGCGAA...

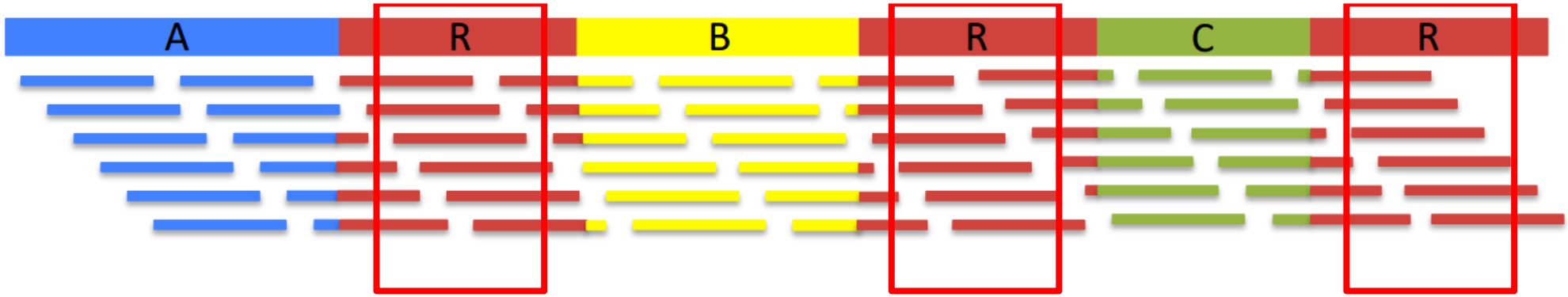
- Simplify assembly graph



Modified from: De novo assembly of complex genomes using single molecule sequencing, Michael Schatz

De novo sequence assembly

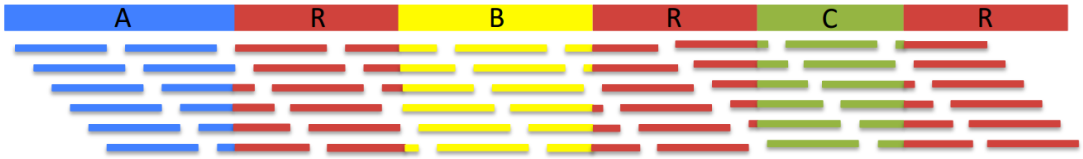
- Genome with repeated regions

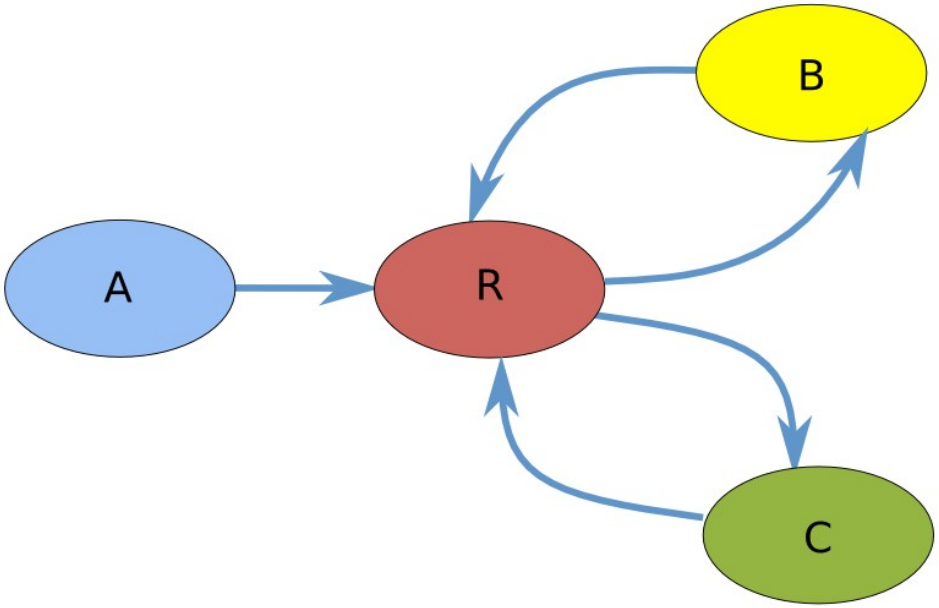


Modified from: De novo assembly of complex genomes using single molecule sequencing, Michael Schatz

De novo sequence assembly

- Graph generation

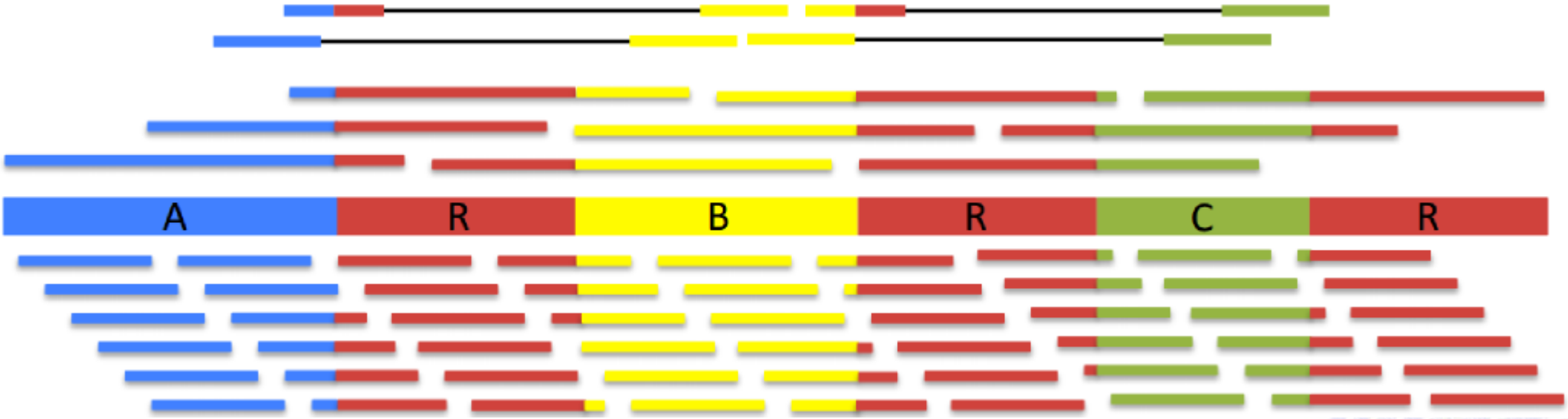




Modified from: De novo assembly of complex genomes using single molecule sequencing, Michael Schatz

De novo sequence assembly

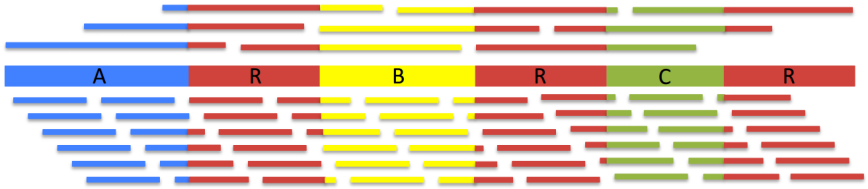
- Double sequencing, once with short and once with long reads (or paired end)

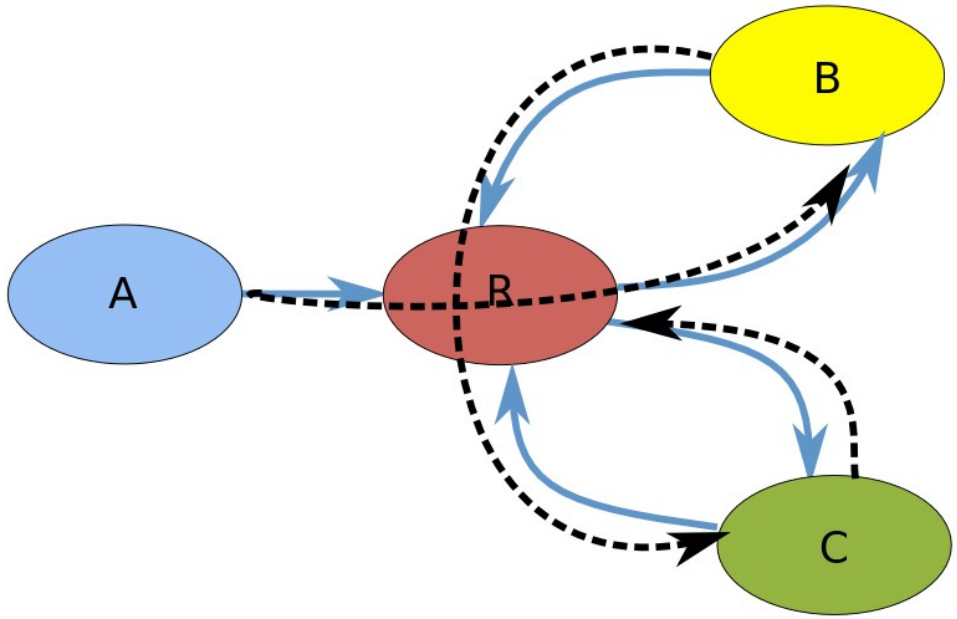


Modified from: De novo assembly of complex genomes using single molecule sequencing, Michael Schatz

De novo sequence assembly

- Finding the correct path through the graph with:
  - Longer reads
  - Paired end reads





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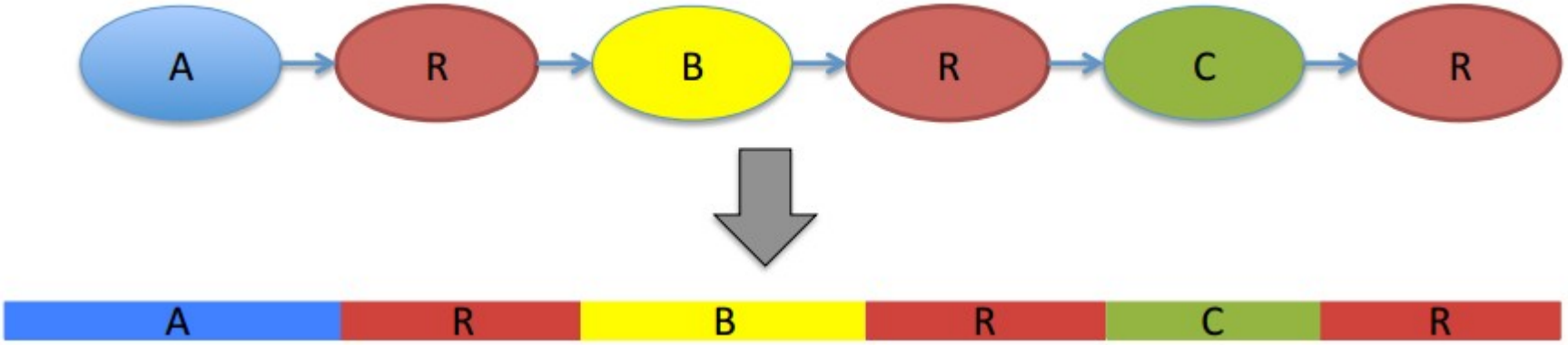
De novo sequence assembly



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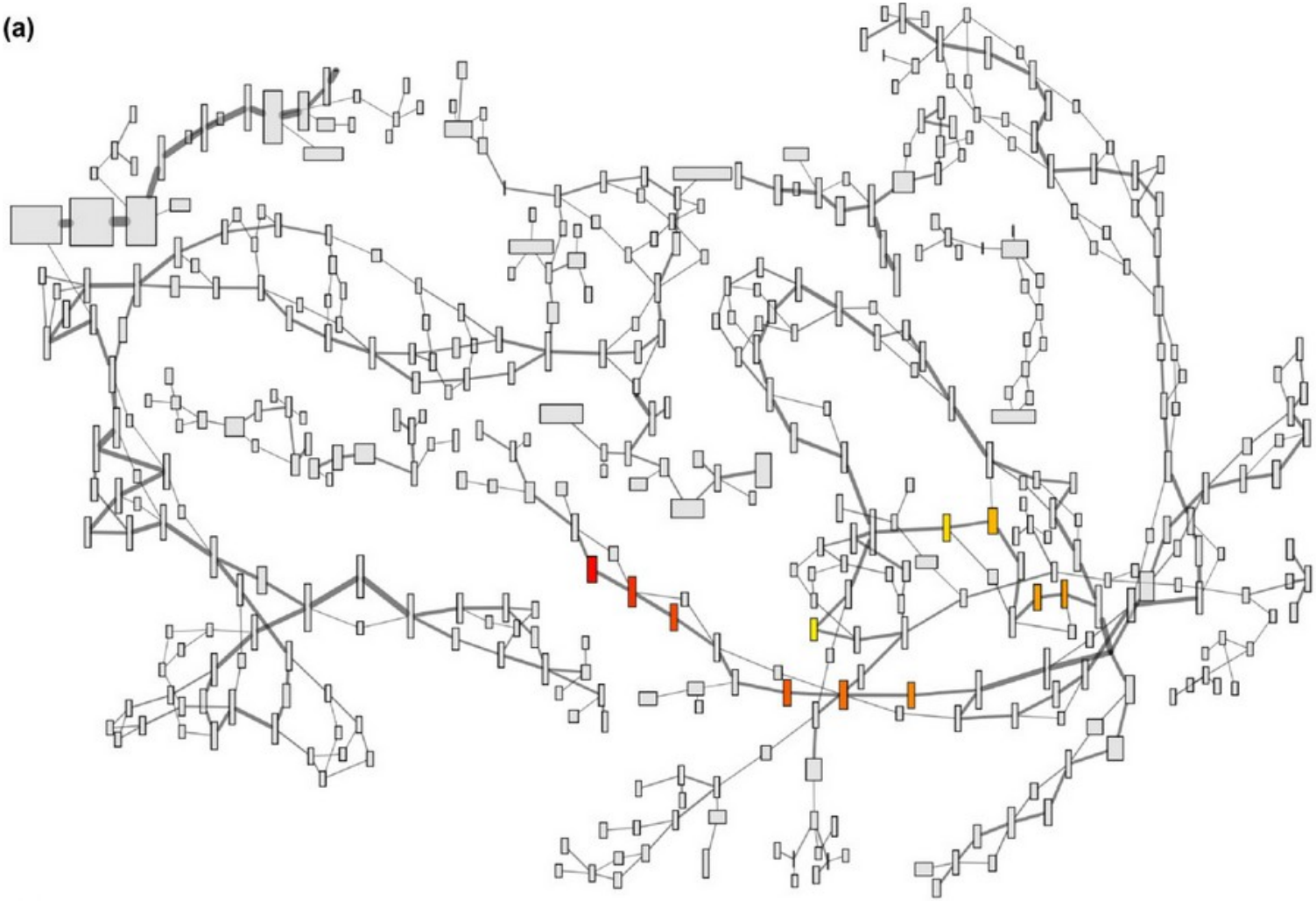
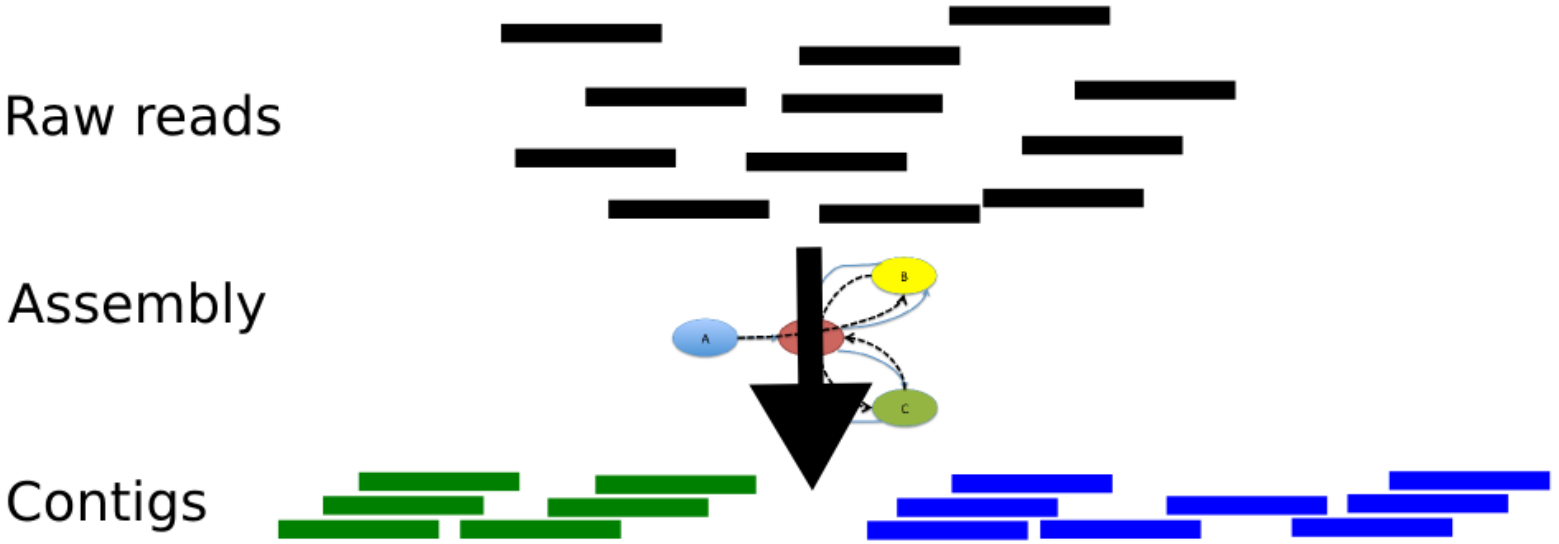
De novo sequence assembly



Modified from: EMIRGE: reconstruction of full-length ribosomal genes from microbial community short read sequencing data, Miller et al.

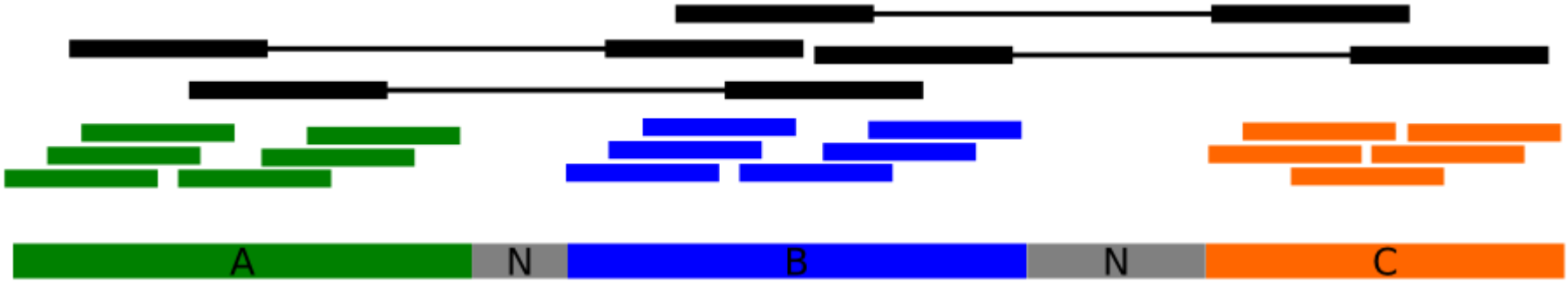
De novo sequence assembly

- Overlapping reads are assembled into groups, so called contigs



De novo sequence assembly

- Scaffolding
  - Using paired end information, contigs can be put in the right order



De novo sequence assembly

- Final result, a list of scaffolds
  - In an ideal world of the size of a chromosome, molecule, mtDNA etc.

Scaffold 1

Scaffold 2

Scaffold 3

Scaffold 4

De novo sequence assembly

- What is needed for a good assembly?
  - High coverage
  - High read lengths
  - Good read quality
- Current sequencing technologies do not have all three
  - Illumina, good quality reads, but short
  - PacBio, very long reads, but low quality

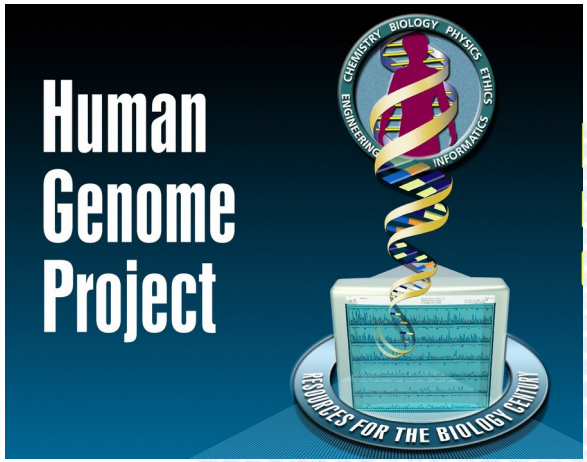
De novo sequence assembly

- Combined sequencing technologies assembly
  - High quality contigs created with short reads
  - Scaffolding of those contigs with long reads

- Double sequencing means
  - High infrastructure requirements
  - High costs

Human reference sequence

- Human Genome project
  - Produced the first „complete“ human genome
- Human genome reference consortium
  - Constantly improves the reference
    - GRCh38 released at the end of 2013



Reference based alignment

- A previously assembled genome is used as a reference
- Sequenced reads are independently aligned against this reference sequence
- Every read is placed at its most likely position
- Unlike sequence assembly, no synergies between reads exist

Reference based alignment

- Naive approach:
  - Evaluate every location on the reference

- Too slow for billions of reads on a big reference

Reference based alignment

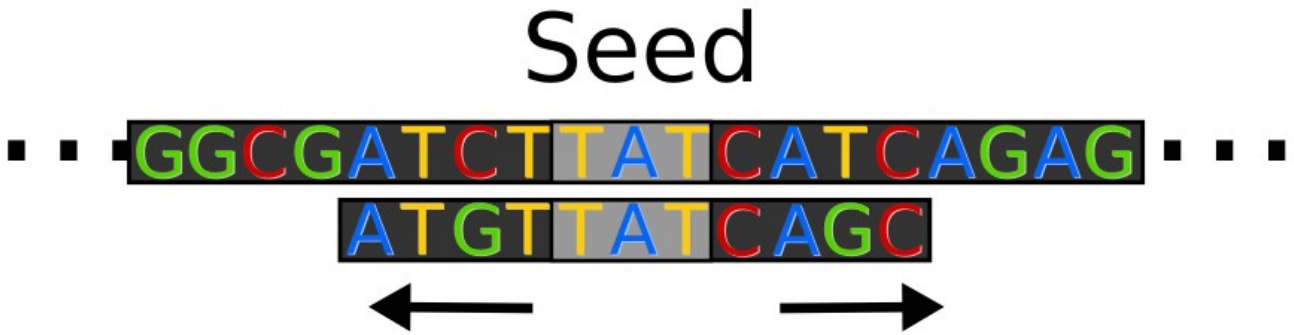
- Speed up with the creation of a reference index

- Fast lookup table for subsequences in reference

Reference based alignment

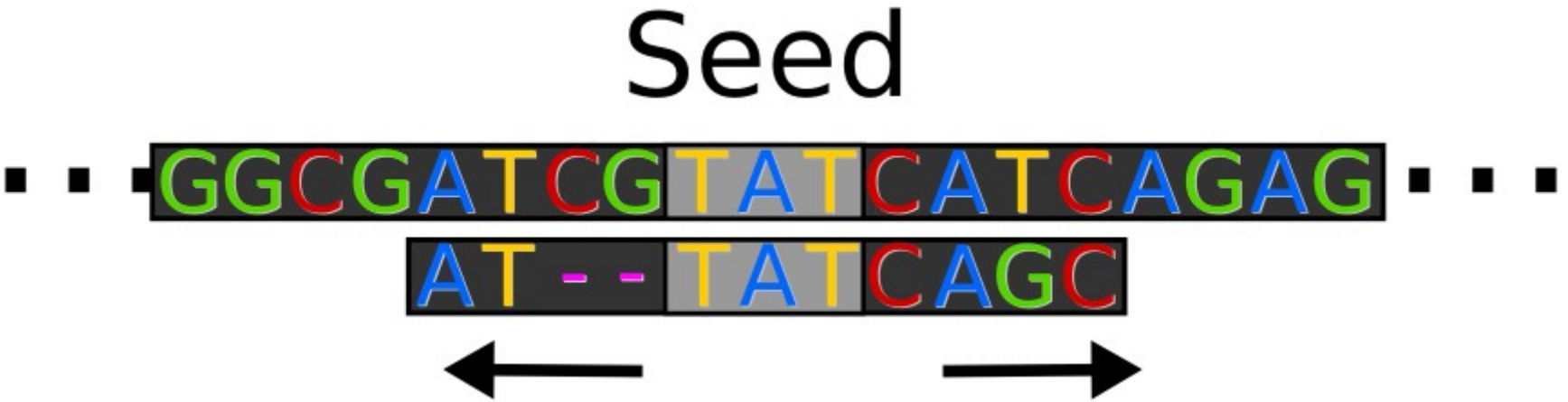
- Find all possible alignment positions
  - Called seeds

- Evaluate every seed



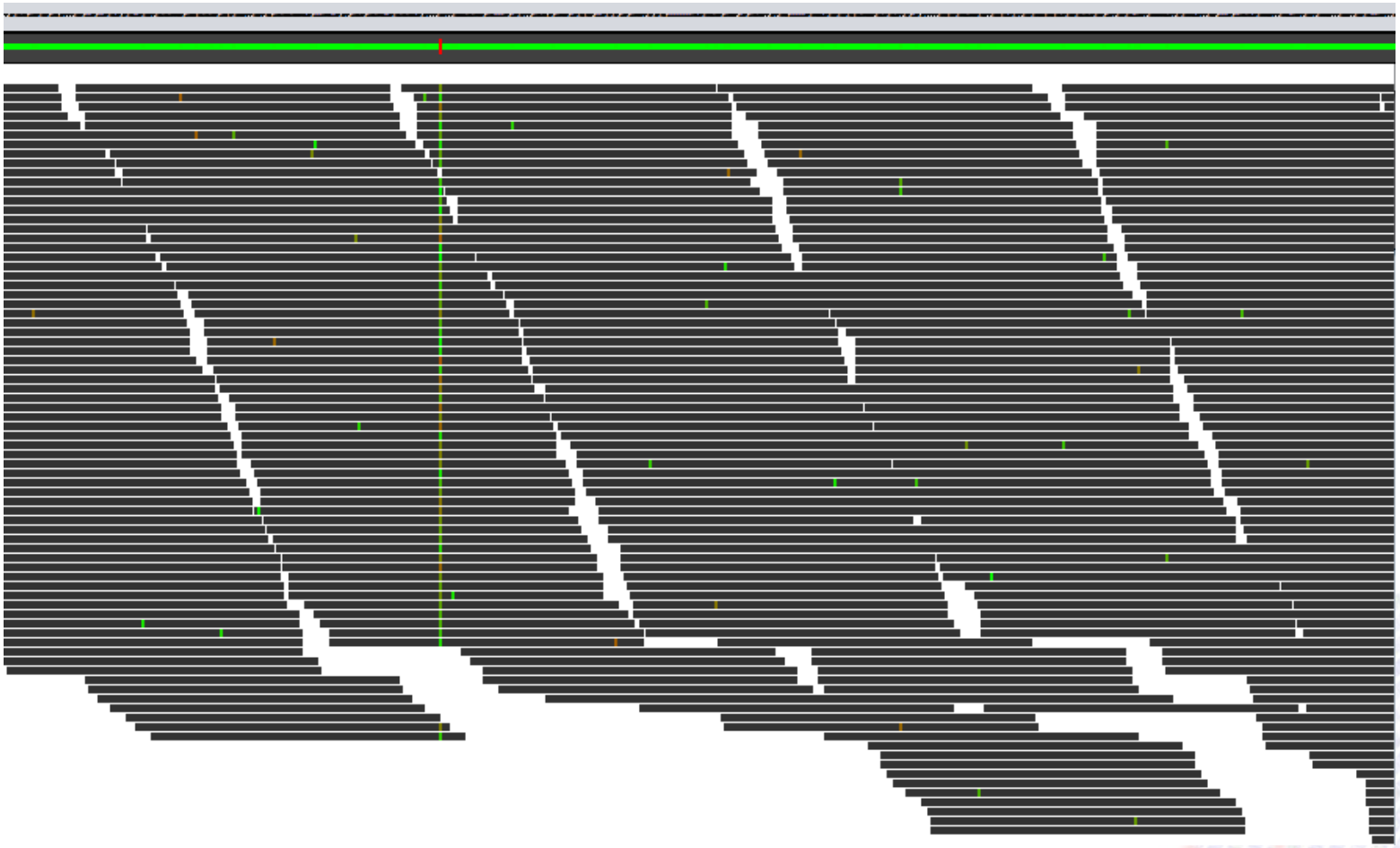
Reference based alignment

- Determine optimal alignment for the best candidate positions
- Insertions and deletions increase the complexity of the alignment



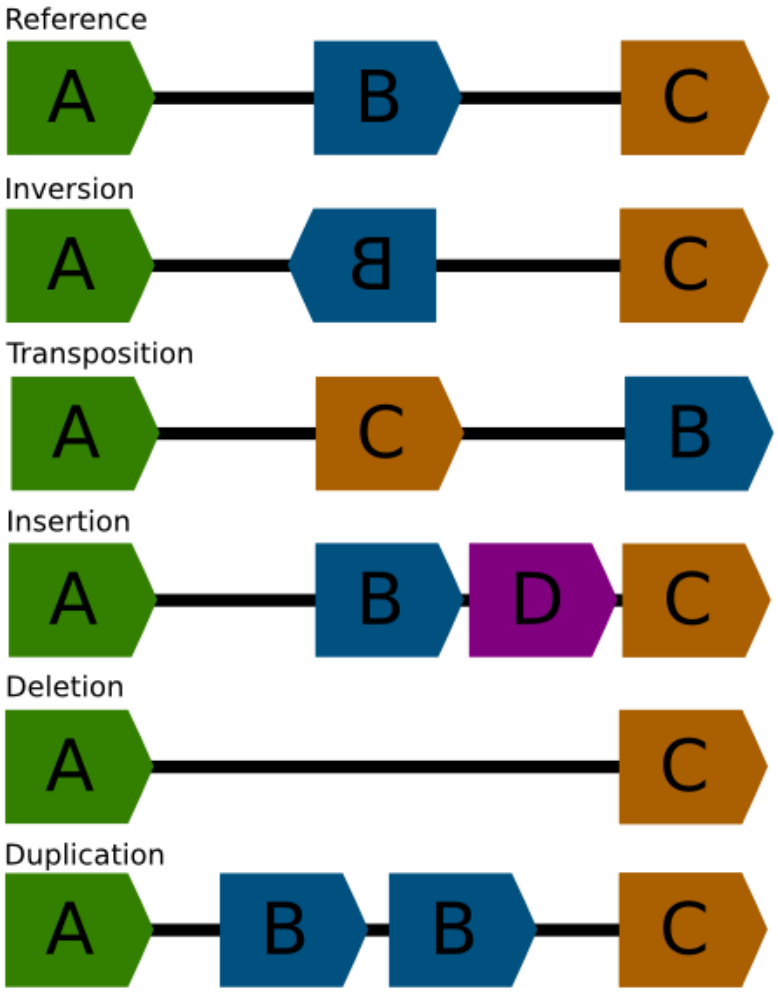
Reference based alignment

- Final result, an alignment file (BAM)



Alignment problems

- Regions very different from reference sequence
  - Structural variations
    - Except for deletions and duplications



Alignment problems

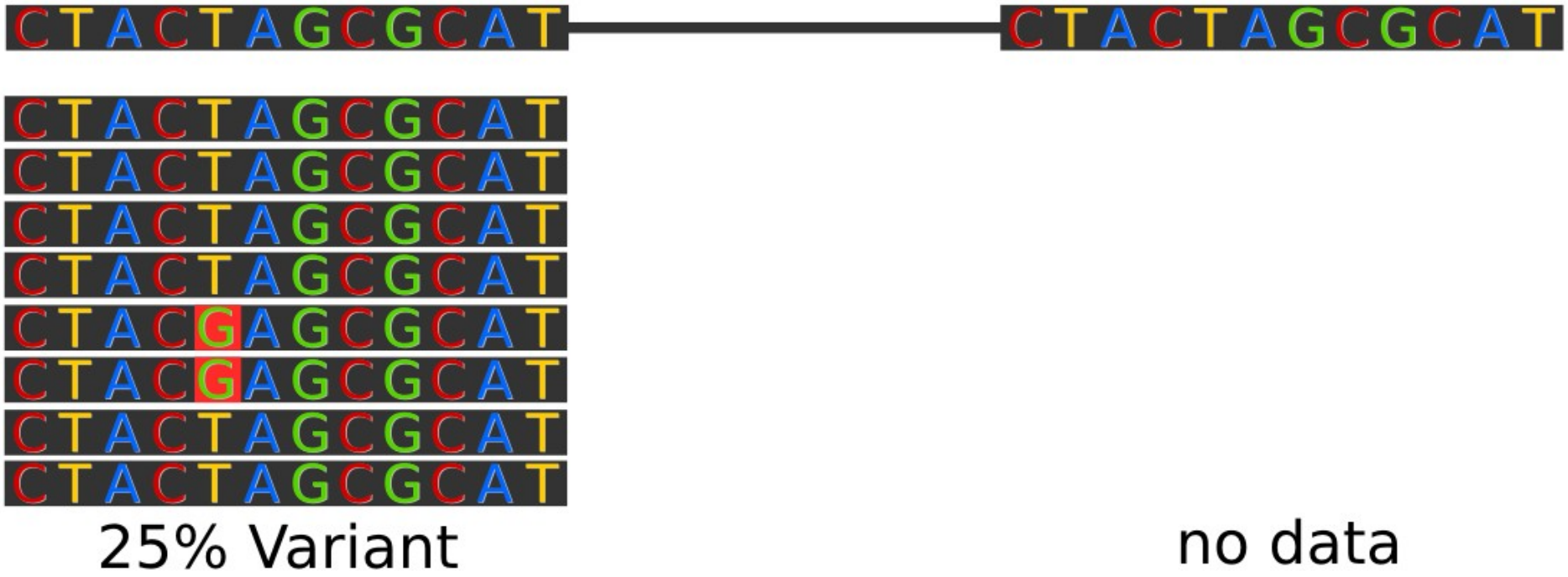
- Reference which contains duplicate regions
- Different strategies exist if multiple positions are equally valid:
  - Ignore read
  - Place at multiple positions
  - Choose one location at random
  - Place at first position
  - Etc.

Alignment problems

- Example situation
  - 2 duplicate regions, one with a heterozygote variant

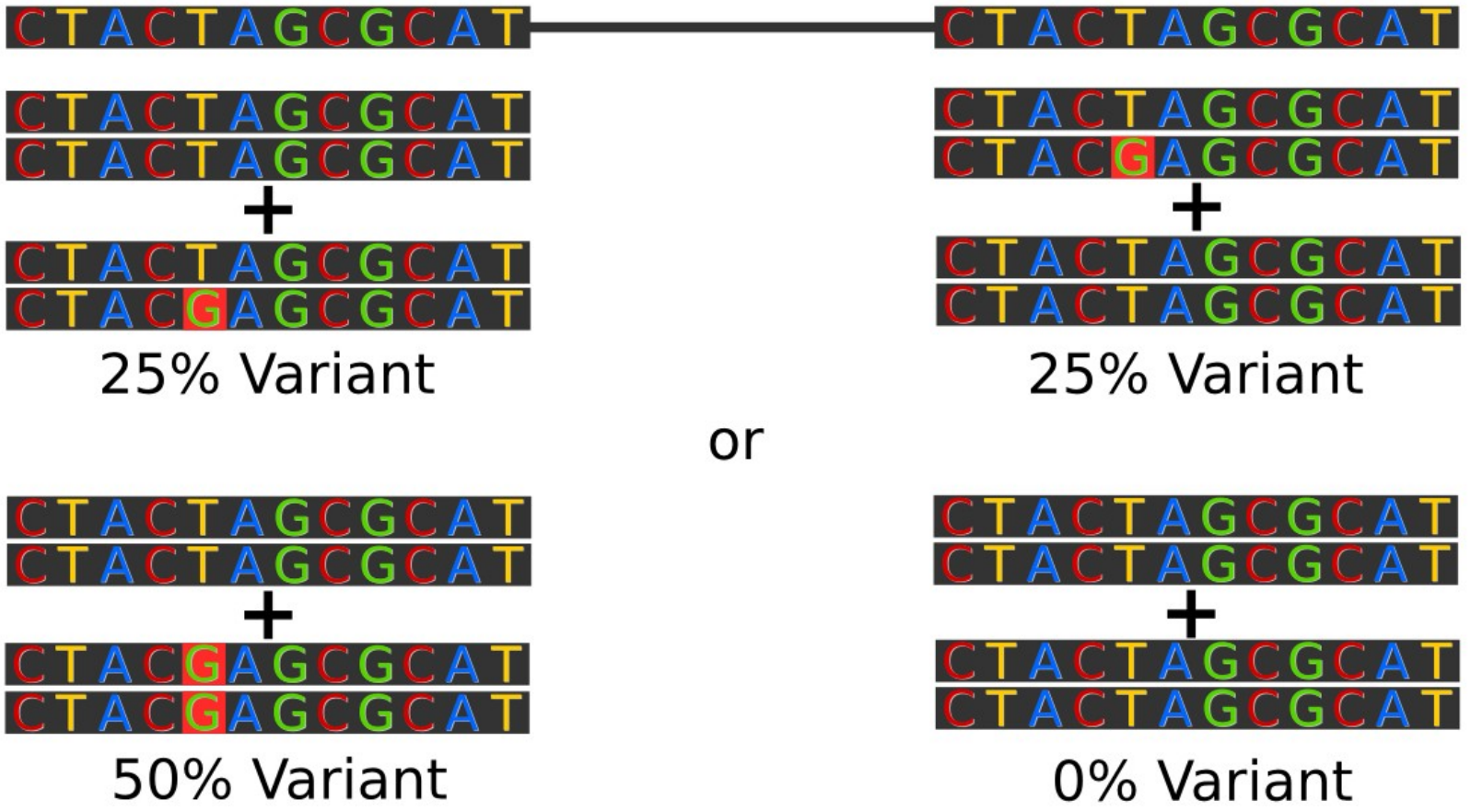
Alignment problems

- Map to first position



Alignment problems

- Map to random position



Alignment problems

- To dustbin

deletion

deletion

Dustbin

- Sequences that are not aligned can be recovered in the dustbin
  - Sequences with no matching place on reference
  - Sequences with multiple possible alignments
- Several strategies exist to handle them
  - De novo assembly
  - Realigning with a different aligner
  - Etc.
- Important information can often be found there

De novo vs. reference

- Reference based alignment
  - Good for SNV, small indels
  - Limited by read length for feature detection
  - Works for deletions and duplications (CNVs)
    - Using coverage information
  - Alignments are done “quickly“
  - Very good at hiding raw data limitations
  - The alignment does not necessarily correspond to the original sequence
  - Requires a reference that is close to the sequenced data

De novo vs. reference

- De novo assembly
  - Assemblies try to recreate the original sequence
  - Good for structural variations
  - Good for completely new sequences not present in the reference
  - Slow and high infrastructure requirements
  - Very bad at hiding raw data limitations

De novo vs. reference

- Unless necessary, stick with reference based alignment
  - Easier to use
  - More tools to work with the results
  - Easier annotation and comparison
  - Current standard in diagnostics
  - Can still benefit from de novo alignment through local de novo realignment
  - Analyze dustbin if results are inconclusive

Conclusion

- Reference based alignment is the current standard in diagnostics
- Assemblies can be used if reference based alignment is not conclusive
- Assembly will become much more important in the future when sequencing technologies are improved

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Thank you for your attention

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Further resources

Next Generation Variant Calling:
http://blog.goldenhelix.com/?p=1434
De novo alignment:
http://schatzlab.cshl.edu/presentations/
Structural variation in two human genomes mapped at single-nucleotide resolution by whole genome de novo assembly:
http://www.nature.com/nbt/journal/v29/n8/abs/nbt.1904.html

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