De novo genome assembly versus mapping to a reference genome as the method to use to identify the variants

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Outline

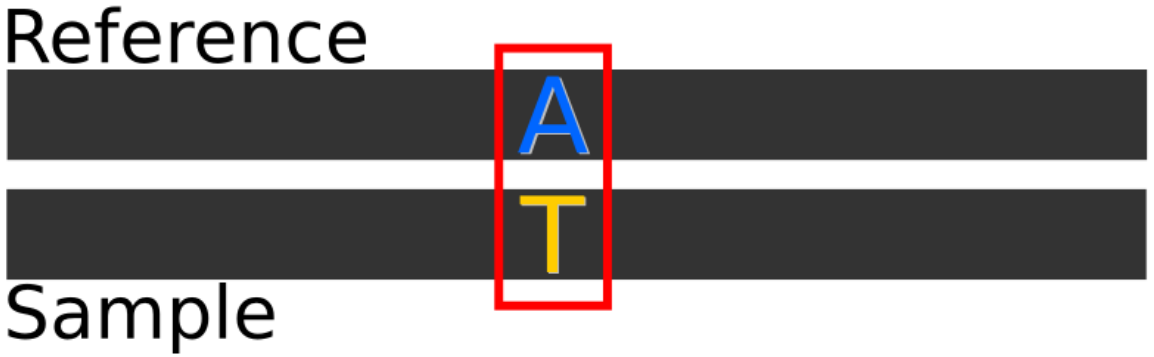
- Genetic variations
- De novo sequence assembly
- Reference based mapping/alignment
- Variant calling
- Comparison
- Conclusion

What are variants?

- Difference between a sample (patient) DNA and a reference (another sample or a population consensus)
- Sum of all variations in a patient determine his genotype and phenotype

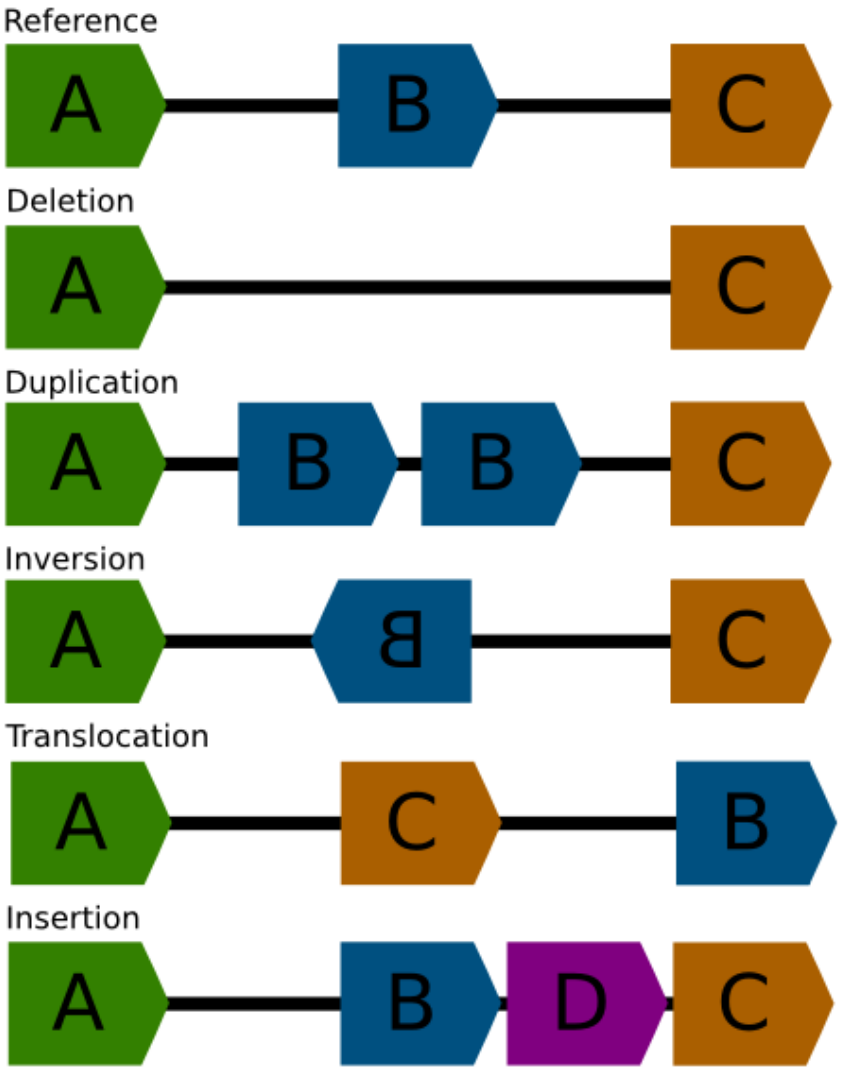
Variation types

- Small variations (< 50bp)
  - SNV (Single nucleotide variation)



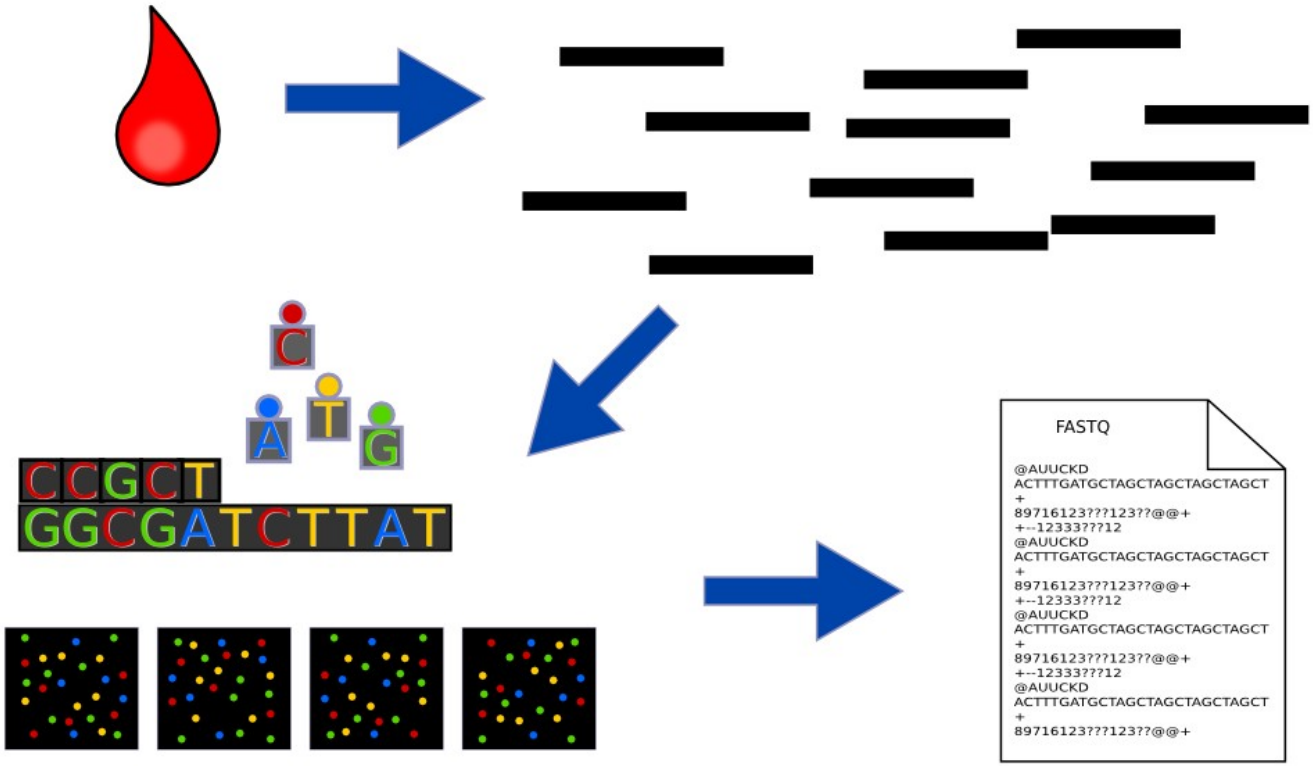
  - Indel (insertion/deletion)

Structural variations



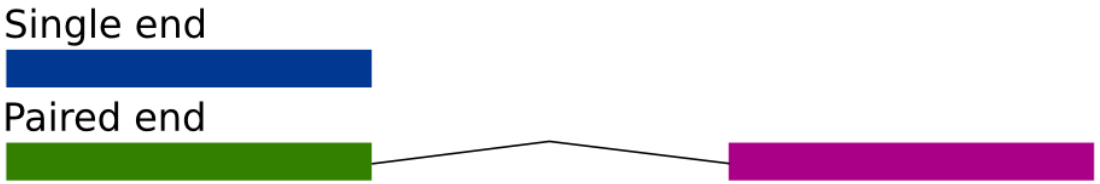
Sequencing technologies

- Sequencing produces small overlapping sequences



Sequencing technologies

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



And different kinds of errors

- Substitutions (Base replaced by other)
- Homopolymers (3 or more repeated bases)
  - AAAAA might be read as AAAA or AAAAAA
- Insertion (Non existent base has been read)
- Deletion (Base has been skipped)
- Duplication (cloned sequences during PCR)
- Somatic cells sequenced

Sequencing technologies

- Standardized output format: **FASTQ**
  - Contains the read sequence and a quality for every base

```
@9WV6Z:791:946
GCTCTTCCGATCTATGGATGCACCAAGATATATGACCCTGTCTGTGGGACTGATGGAA
+
774399222034221774399222034221774399222034221774399222034
```

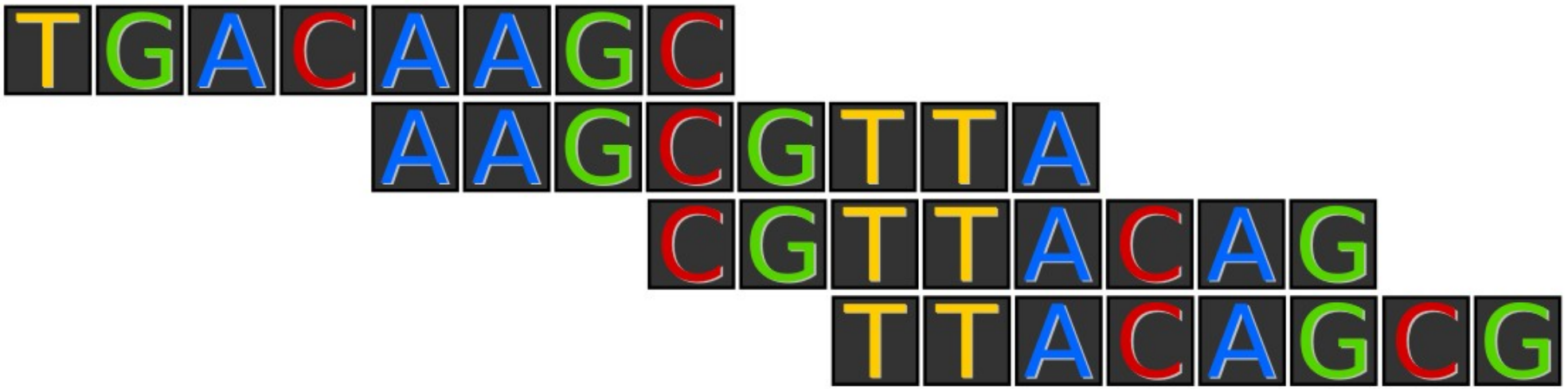
http://en.wikipedia.org/wiki/FASTQ_format

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
- Solution:
  - 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

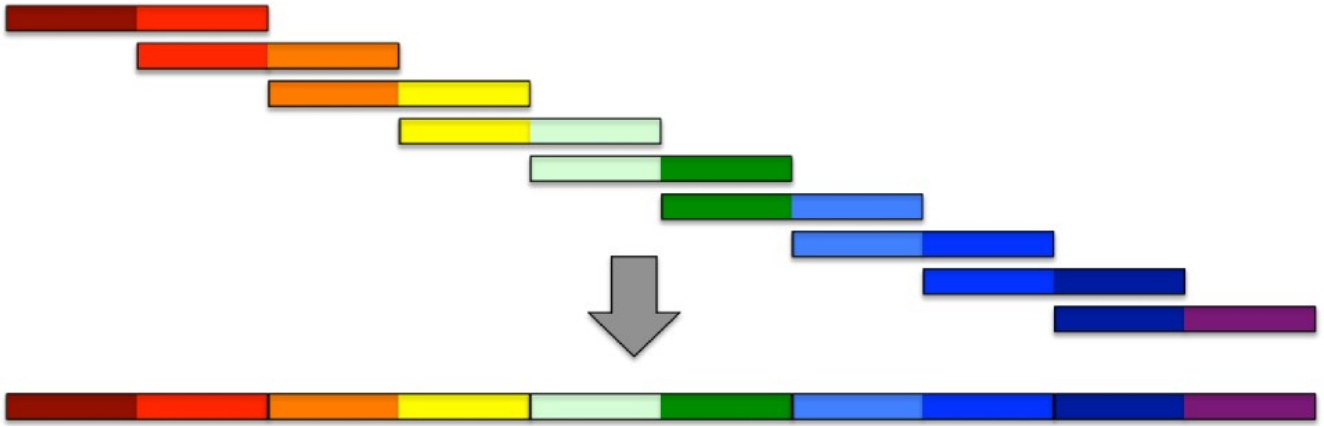


De novo sequence assembly

- Construct assembly graph from overlapping reads

...AGCCTAGGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGTTTGGTCAACCTCGGACGGAC
CAACCTCGGACGGACCTCAGCGAA...

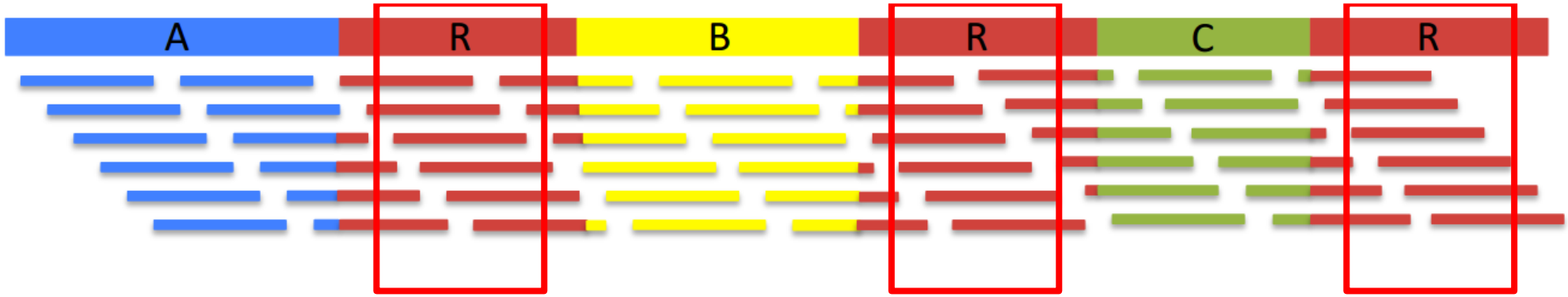
- Simplify assembly graph



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

De novo sequence assembly

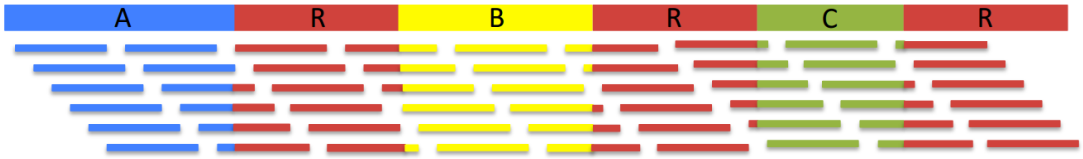
- Genome with repeated regions

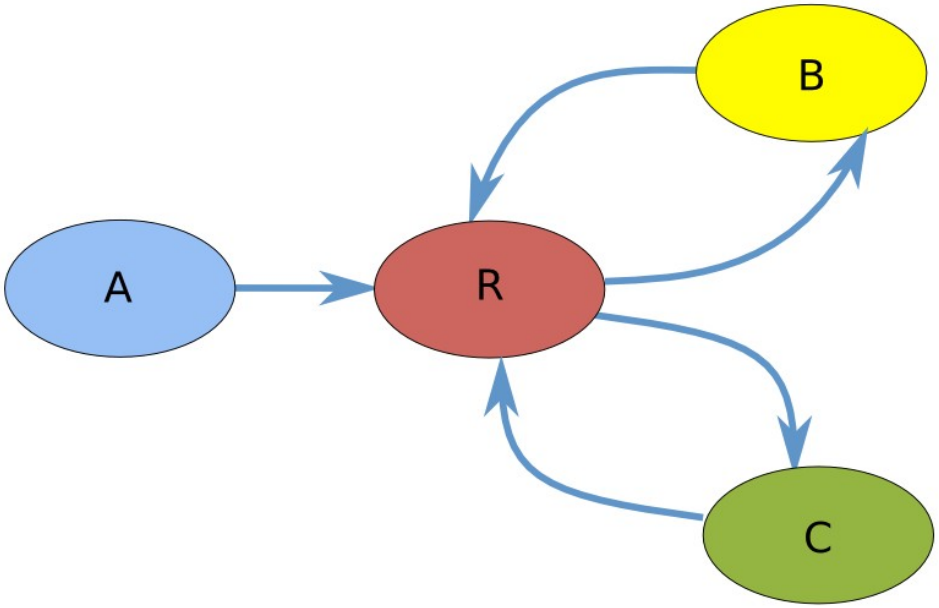


Modified from: De novo assembly of complex genomes using single molecule sequencing, Michael Schatz

De novo sequence assembly

- Graph generation

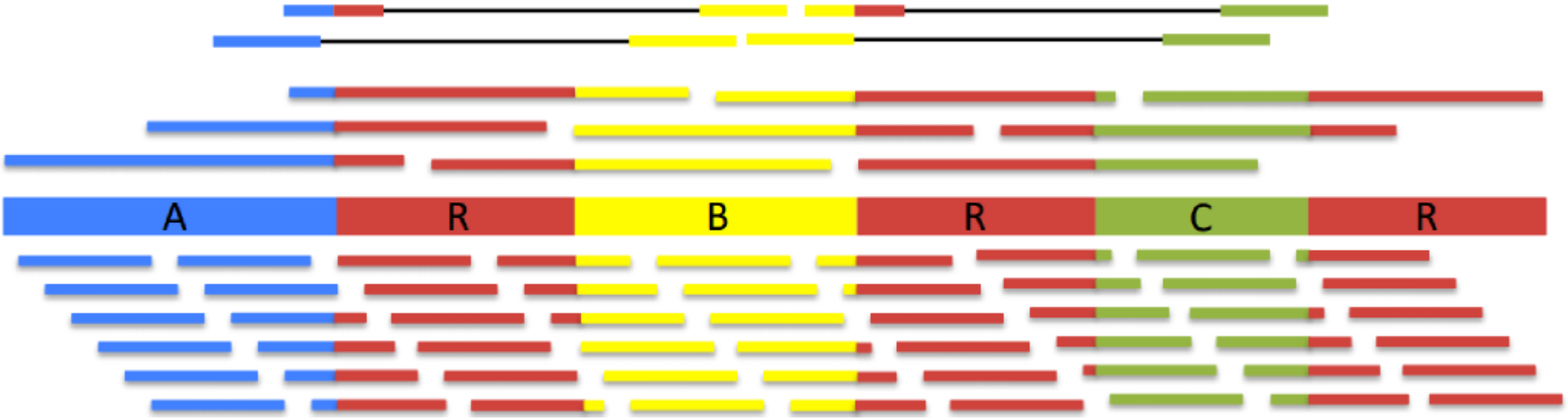




Modified from: De novo assembly of complex genomes using single molecule sequencing, Michael Schatz

De novo sequence assembly

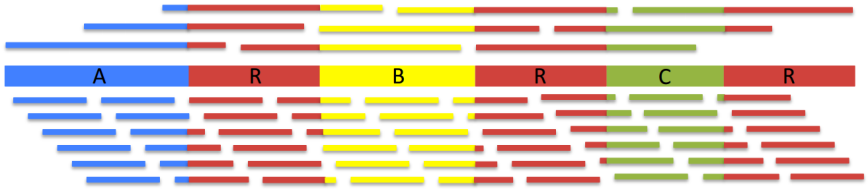
- Double sequencing, once with short and once with long reads (or paired end)

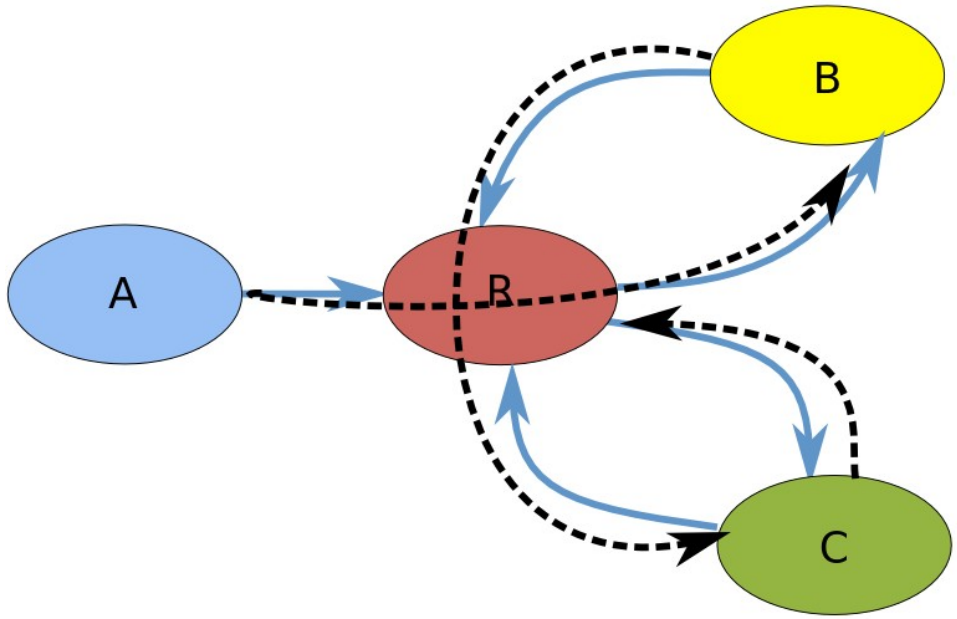


Modified from: De novo assembly of complex genomes using single molecule sequencing, Michael Schatz

De novo sequence assembly

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





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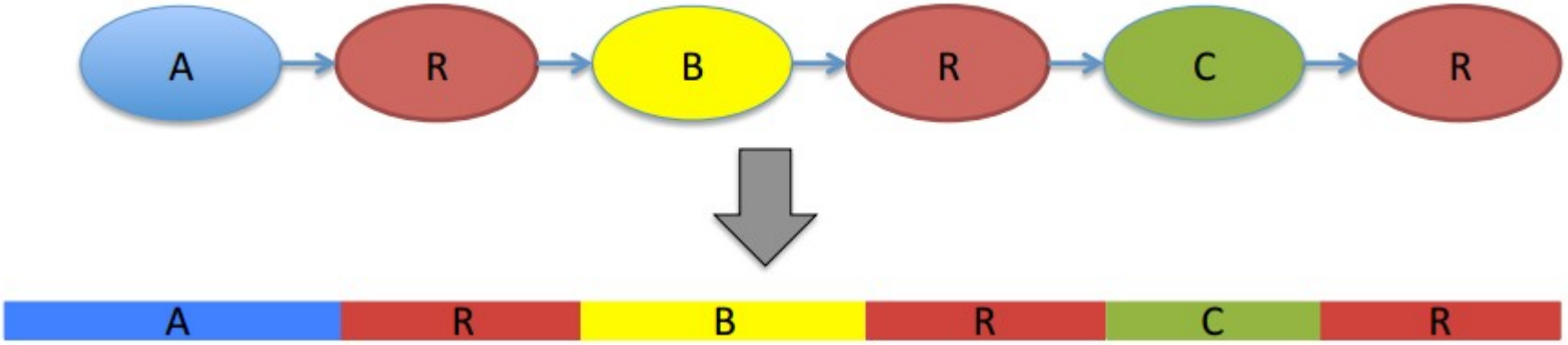
De novo sequence assembly



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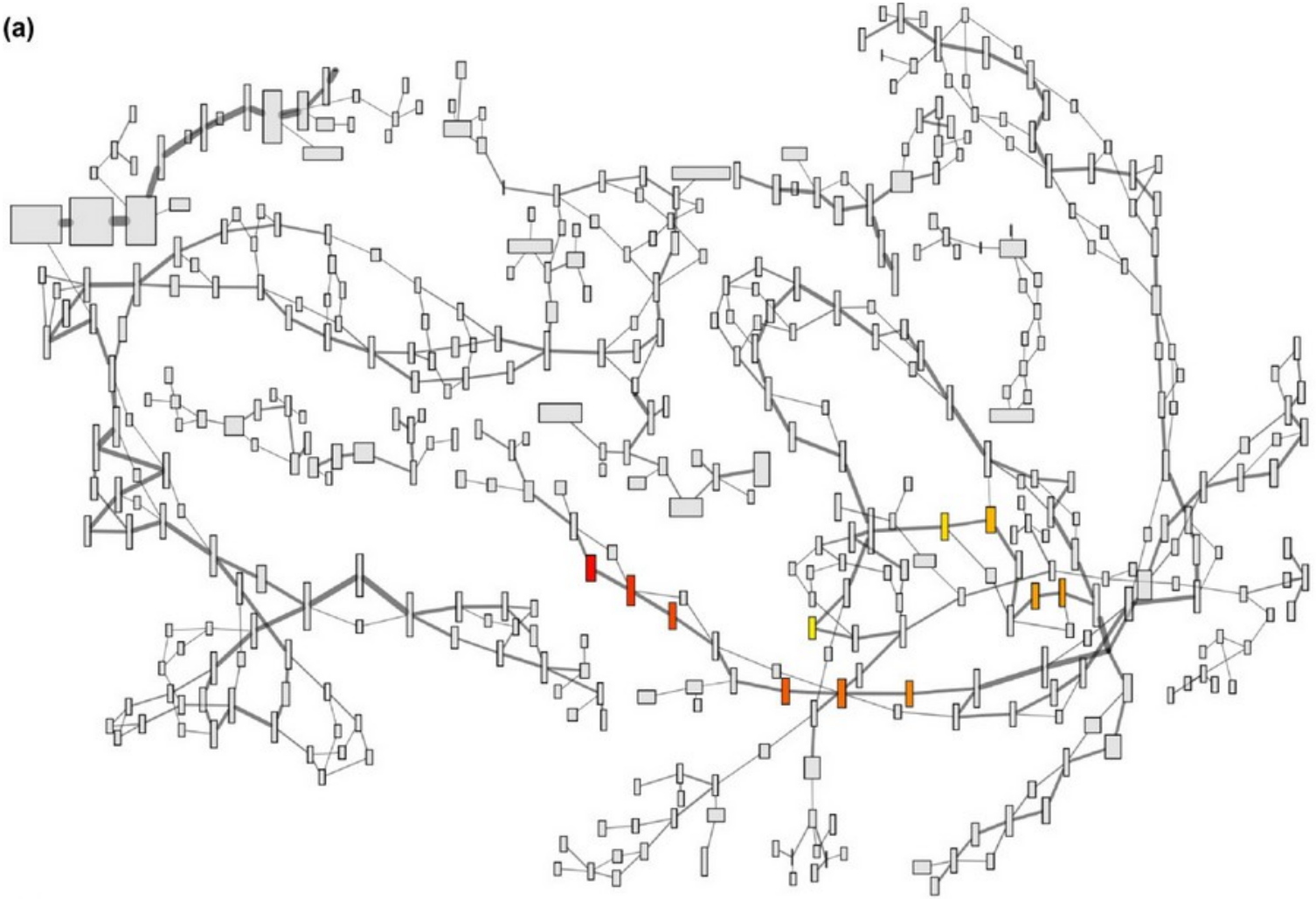
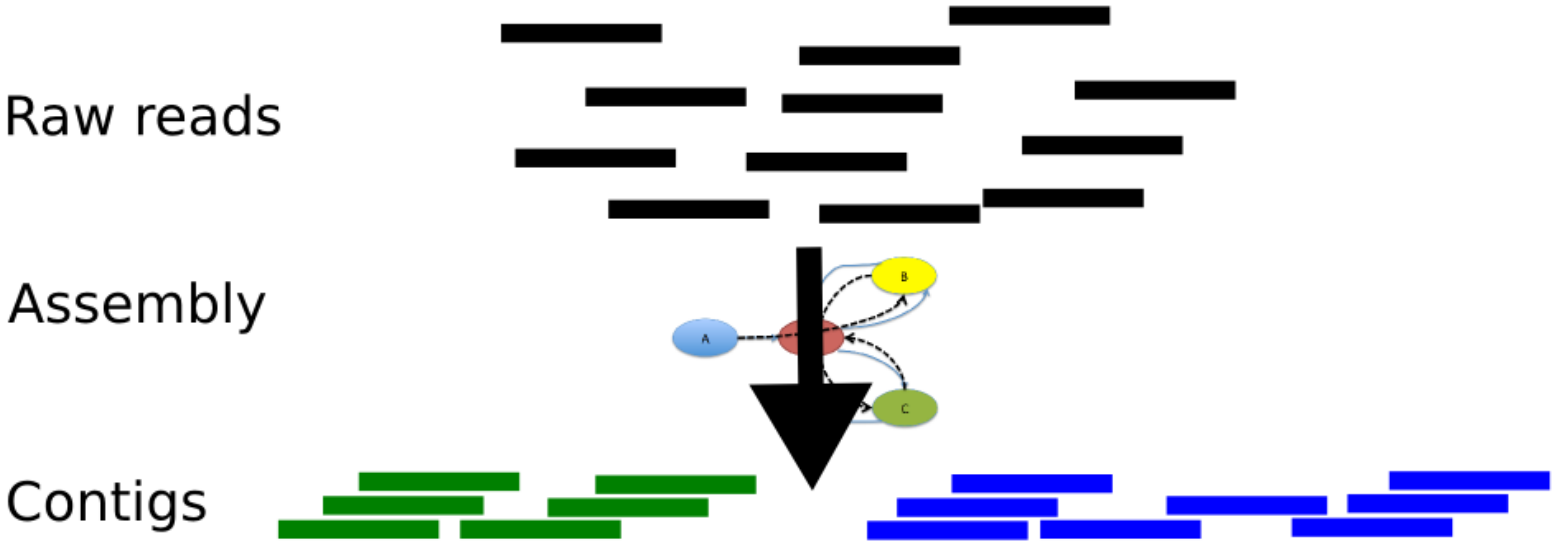
De novo sequence assembly



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

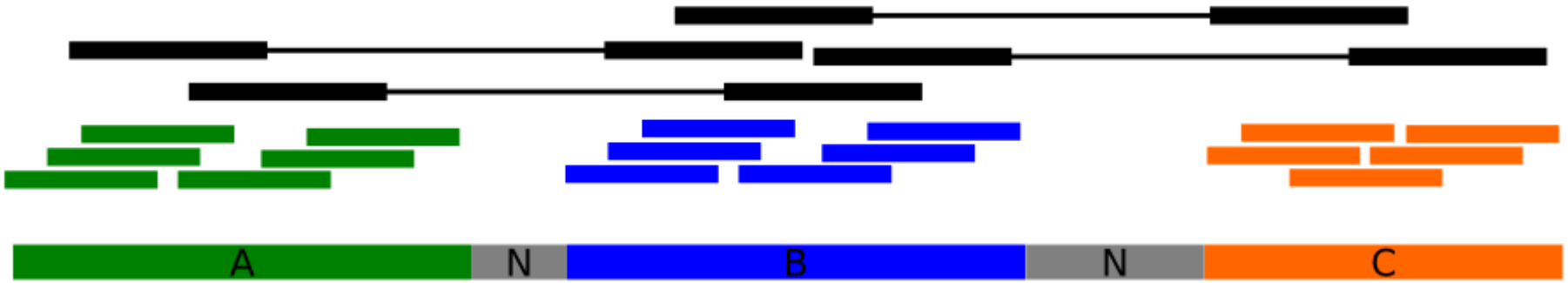
De novo sequence assembly

- 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

De novo sequence assembly

- Field of assemblers is constantly evolving
  - Competitions like Assemblathon 1 + 2 exist

    https://genome10k.soe.ucsc.edu/assemblathon
- The results vary greatly depending on datatype and species to be assembled
- High memory and computational complexity

De novo sequence assembly

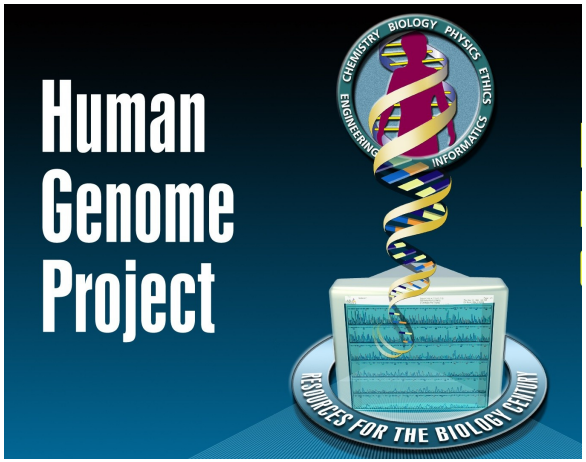
- Short list of assemblers
  - ALLPATHS-LG
  - Meraculous
  - Ray
- Software used by winners of Assemblathon 2:

  SeqPrep, KmerFreq, Quake, BWA, Newbler, ALLPATHS-LG, Atlas-Link, Atlas-GapFill, Phrap, CrossMatch, Velvet, BLAST, and BLASR

- Creating a high quality assembly is complicated

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

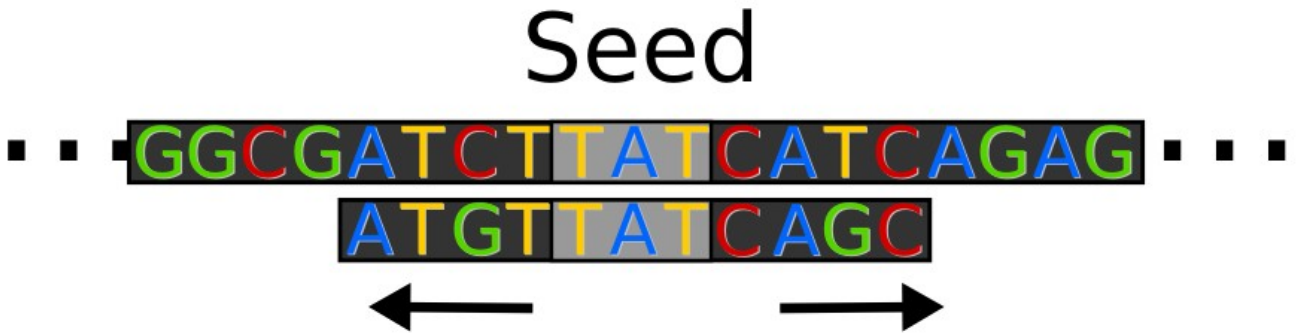
- Speed up with the creation of a reference index

- Fast lookup table for subsequences in reference

Reference based alignment

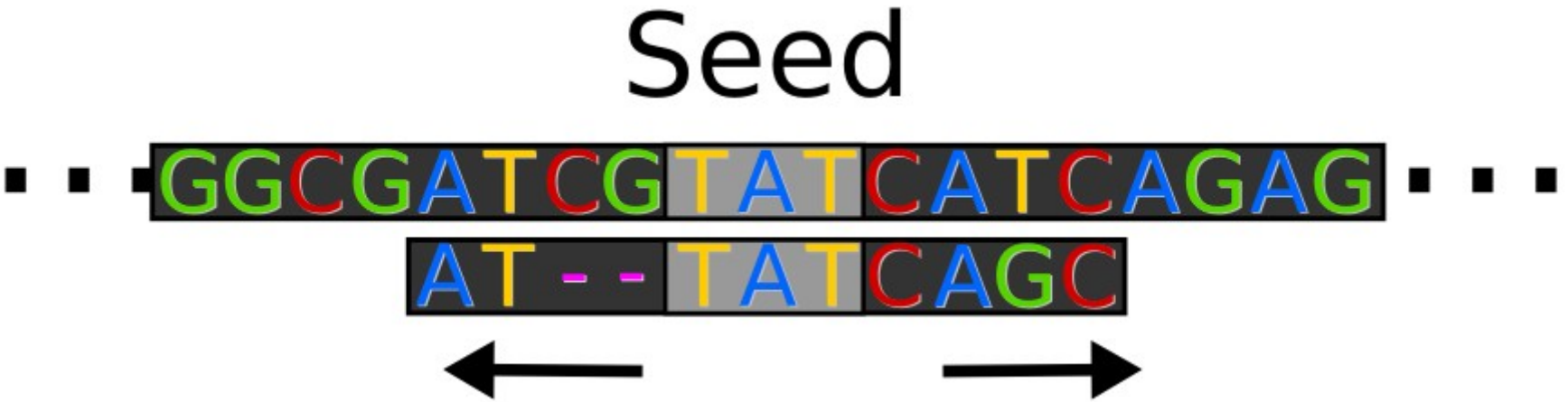
- Find all possible alignment positions
  - Called seeds

- 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

- Most common technique, dynamic programming
- Smith-Watherman, Gotoh etc. are common algorithms

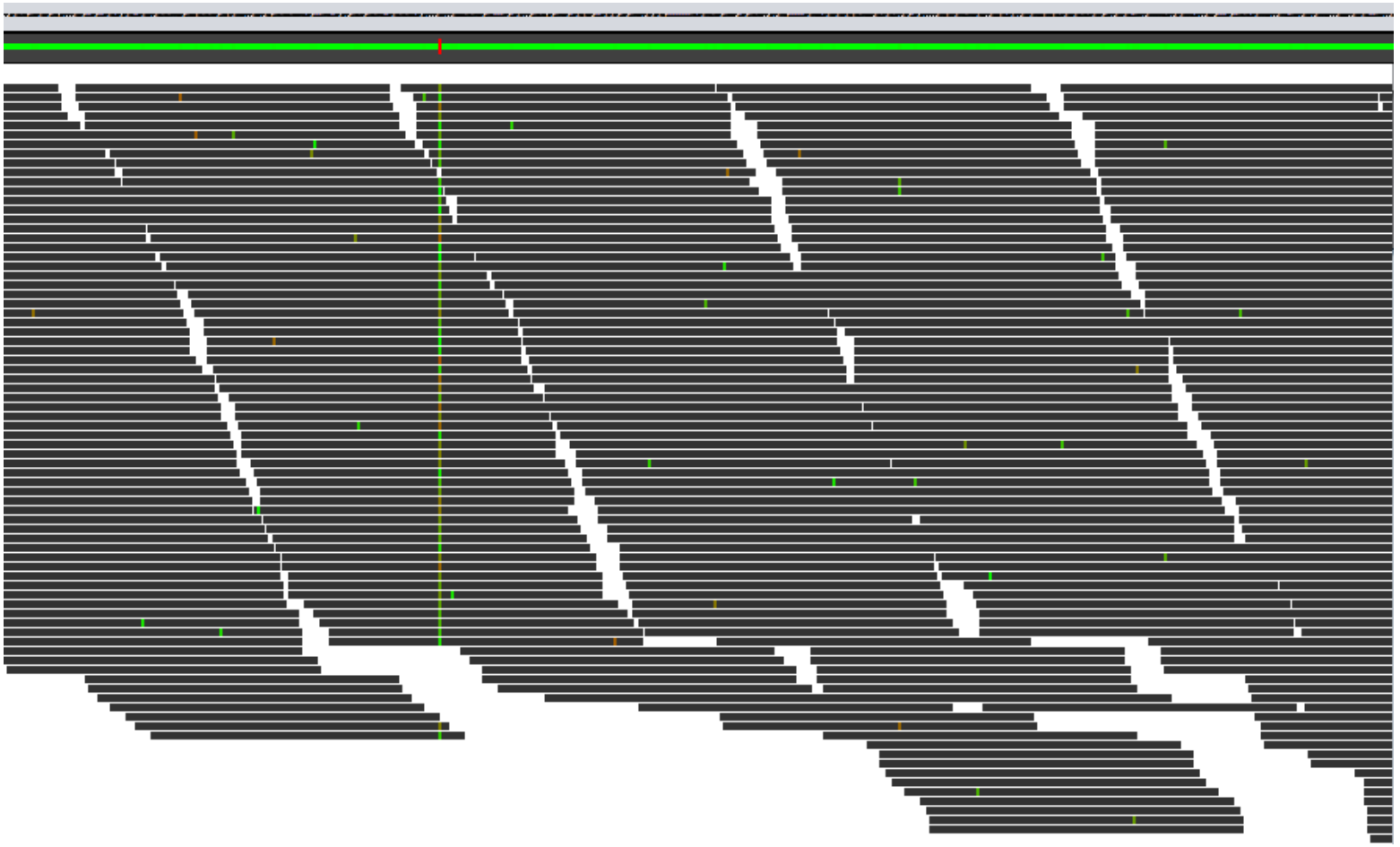
$$H = \begin{pmatrix} & - & A & C & A & C & A & C & T & A \\ - & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ A & 0 & 2 & 1 & 2 & 1 & 2 & 1 & 0 & 2 \\ G & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 0 & 1 \\ C & 0 & 0 & 3 & 2 & 3 & 2 & 3 & 2 & 1 \\ A & 0 & 2 & 2 & 5 & 4 & 5 & 4 & 3 & 4 \\ C & 0 & 1 & 4 & 4 & 7 & 6 & 7 & 6 & 5 \\ A & 0 & 2 & 3 & 6 & 6 & 9 & 8 & 7 & 8 \\ C & 0 & 1 & 4 & 5 & 8 & 8 & 11 & 10 & 9 \\ A & 0 & 2 & 3 & 6 & 7 & 10 & 10 & 10 & 12 \end{pmatrix}$$

$$T = \begin{pmatrix} & - & A & C & A & C & A & C & T & A \\ - & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ A & 0 & \nwarrow & \leftarrow & \nwarrow & \leftarrow & \nwarrow & \leftarrow & \leftarrow & \nwarrow \\ G & 0 & \uparrow & \nwarrow & \uparrow & \nwarrow & \uparrow & \nwarrow & \nwarrow & \uparrow \\ C & 0 & \uparrow & \nwarrow & \leftarrow & \nwarrow & \leftarrow & \nwarrow & \leftarrow & \leftarrow \\ A & 0 & \nwarrow & \uparrow & \nwarrow & \leftarrow & \nwarrow & \leftarrow & \leftarrow & \nwarrow \\ C & 0 & \uparrow & \nwarrow & \uparrow & \nwarrow & \leftarrow & \nwarrow & \leftarrow & \leftarrow \\ A & 0 & \nwarrow & \uparrow & \nwarrow & \uparrow & \nwarrow & \leftarrow & \leftarrow & \nwarrow \\ C & 0 & \uparrow & \nwarrow & \uparrow & \nwarrow & \uparrow & \nwarrow & \leftarrow & \leftarrow \\ A & 0 & \nwarrow & \uparrow & \nwarrow & \uparrow & \nwarrow & \uparrow & \nwarrow & \nwarrow \end{pmatrix}$$

http://en.wikipedia.org/wiki/Smith-Waterman_algorithm

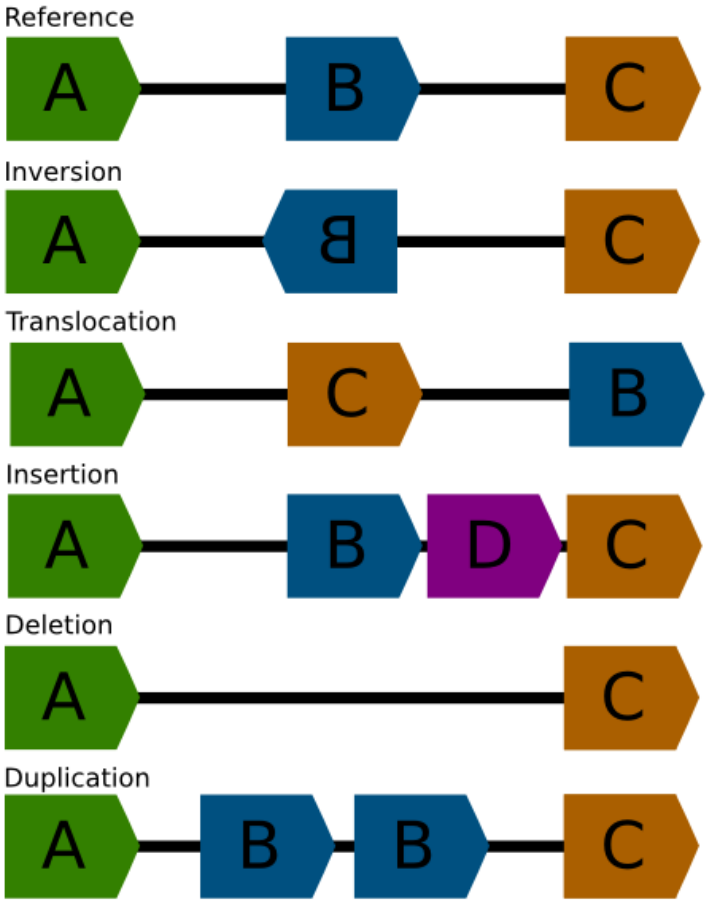
Reference based alignment

- 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

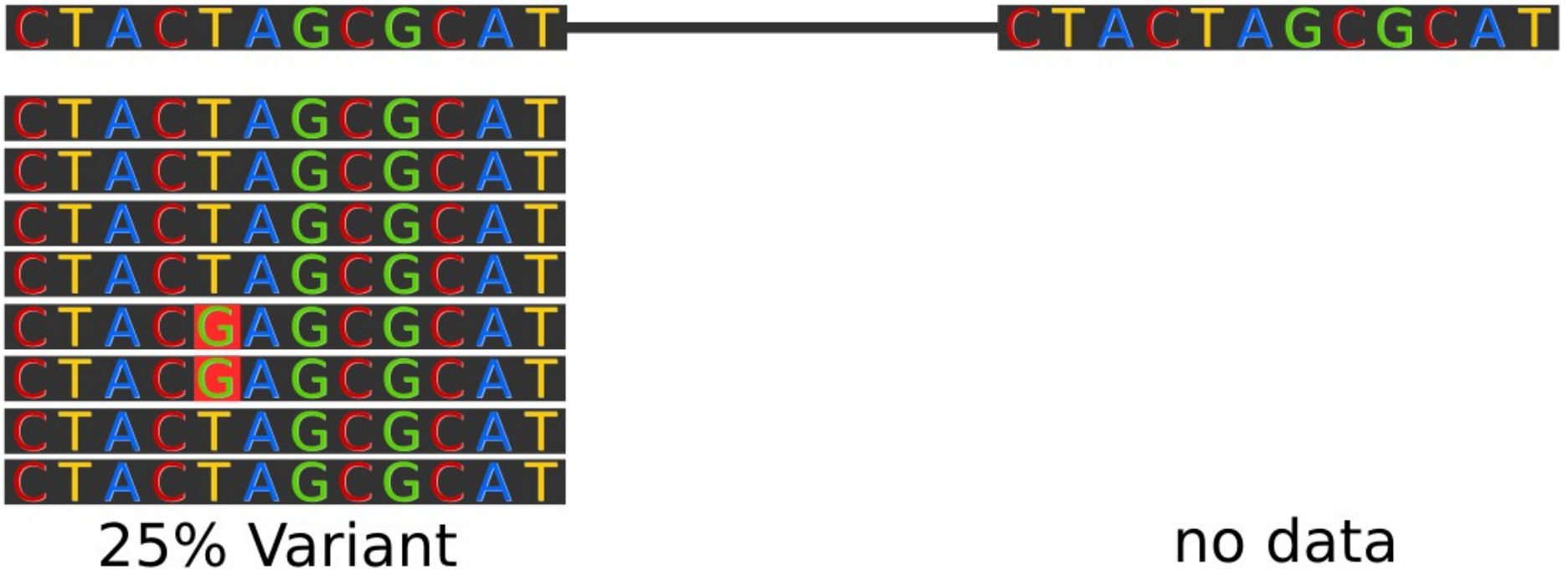
- To dustbin

deletion

deletion

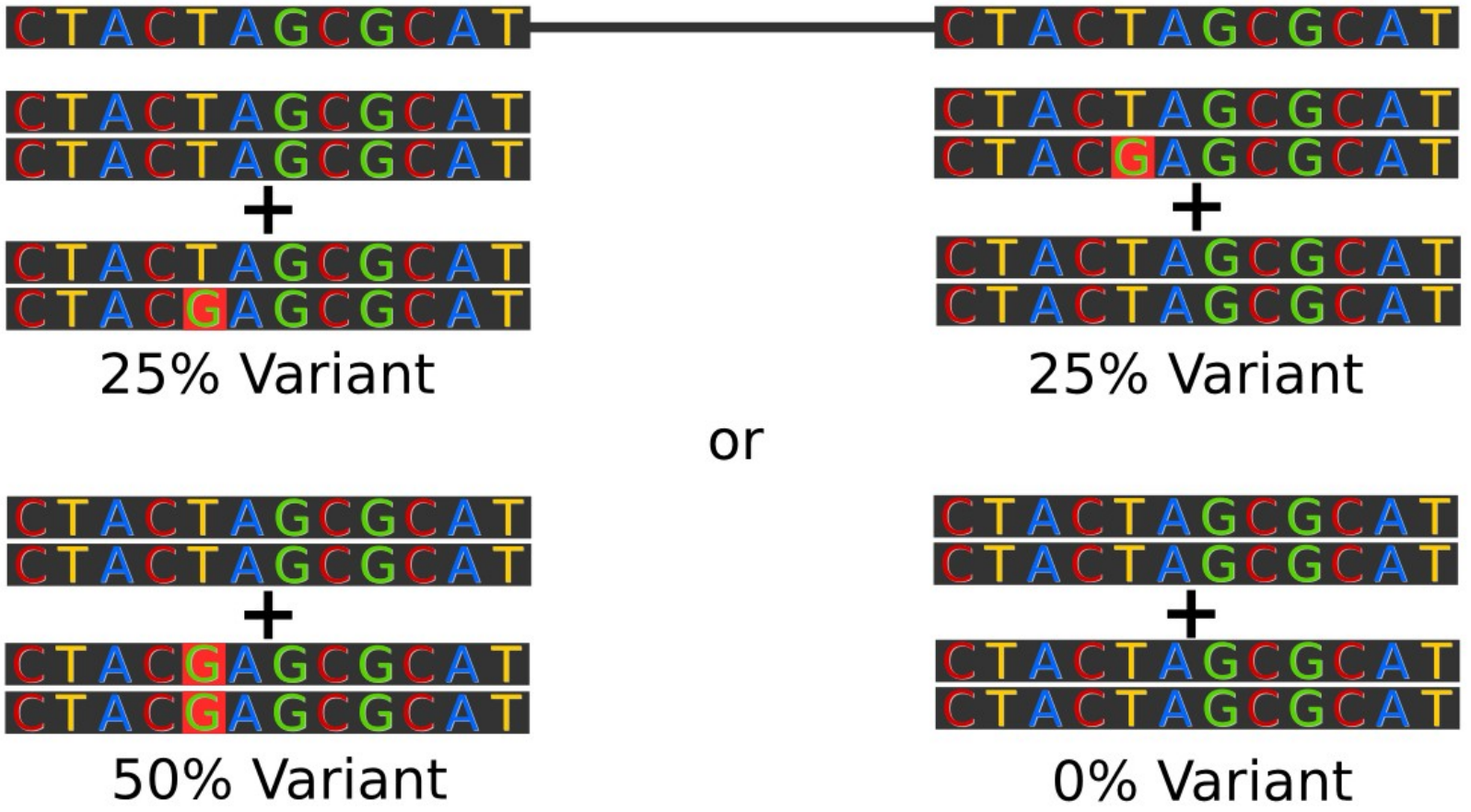
Alignment problems

- Map to first position



Alignment problems

- Map to random position



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

Reference based alignment

- Popular aligners
  - Bowtie 1 + 2 (http://bowtie-bio.sourceforge.net/)
  - BWA (http://bio-bwa.sourceforge.net/)
  - BLAST (http://blast.ncbi.nlm.nih.gov/)
- Different strengths for each
  - Read length
  - Paired end
  - Indels

A survey of sequence alignment algorithms for next-generation sequencing. Heng Li & Nils Homer, 2010

Assembly vs. Alignment

- Hybrid methods
  - Assemble contigs that are aligned back against the reference, many popular aligners can be used for this

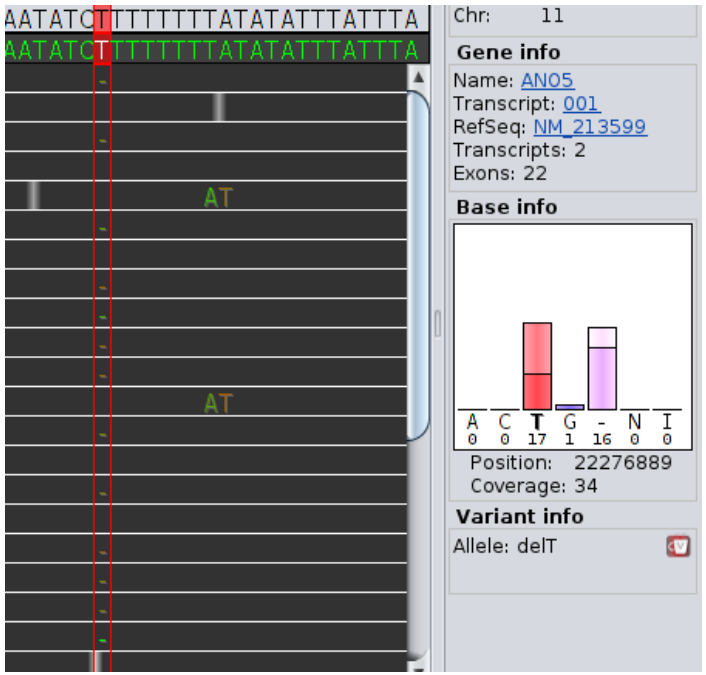
  - Reference aided assembly

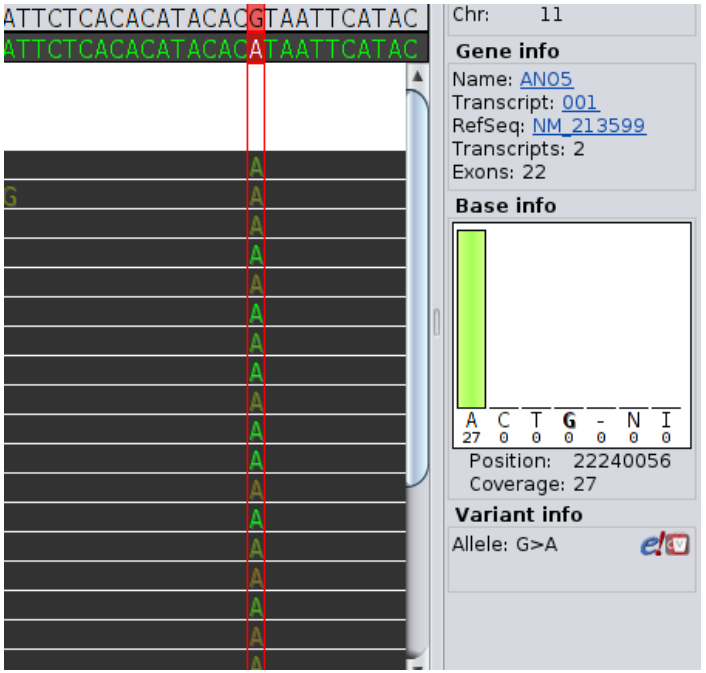
Variant calling

- Difference in underlying data (alignment vs assembly) require different strategies for variant calling
- Methods exist to combine both approaches
  - Reference based variant calling
  - Patient comparison of de novo assembly
  - Hybrid methods
    - Alignment of contigs against reference
    - Local de novo re-assembly

Variant calling

- Reference based variant calling
  - Compare aligned reads with reference





Variant calling

- Common reference based variant callers:
  - GATK
  - Samtools
  - FreeBayes

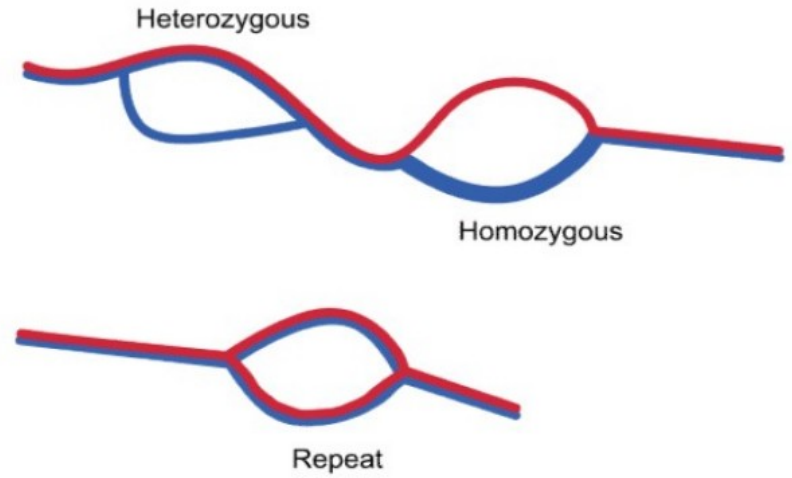
- Works very well for (in non repeat regions):
  - SNVs
  - Small indels

Variant calling

- De novo alignment
  - Either compare two patients
    - Useful for large structural variation detection
    - Can not be used to annotate variations with public databases
  - Or realign contigs against reference
    - Useful to annotate variants
    - Might loose information for the unaligned contigs

Variant calling

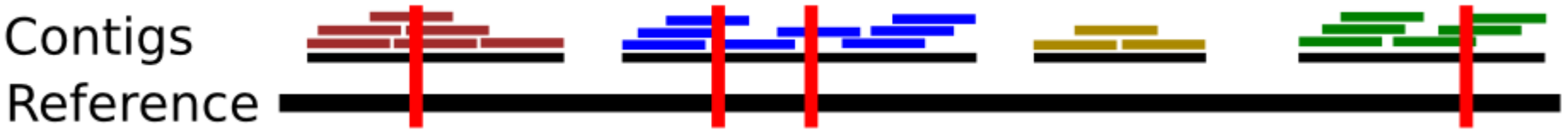
- Cortex
  - Colored de Bruijn graph based variant calling



- Works well for
  - Structural variations detection

Variant calling

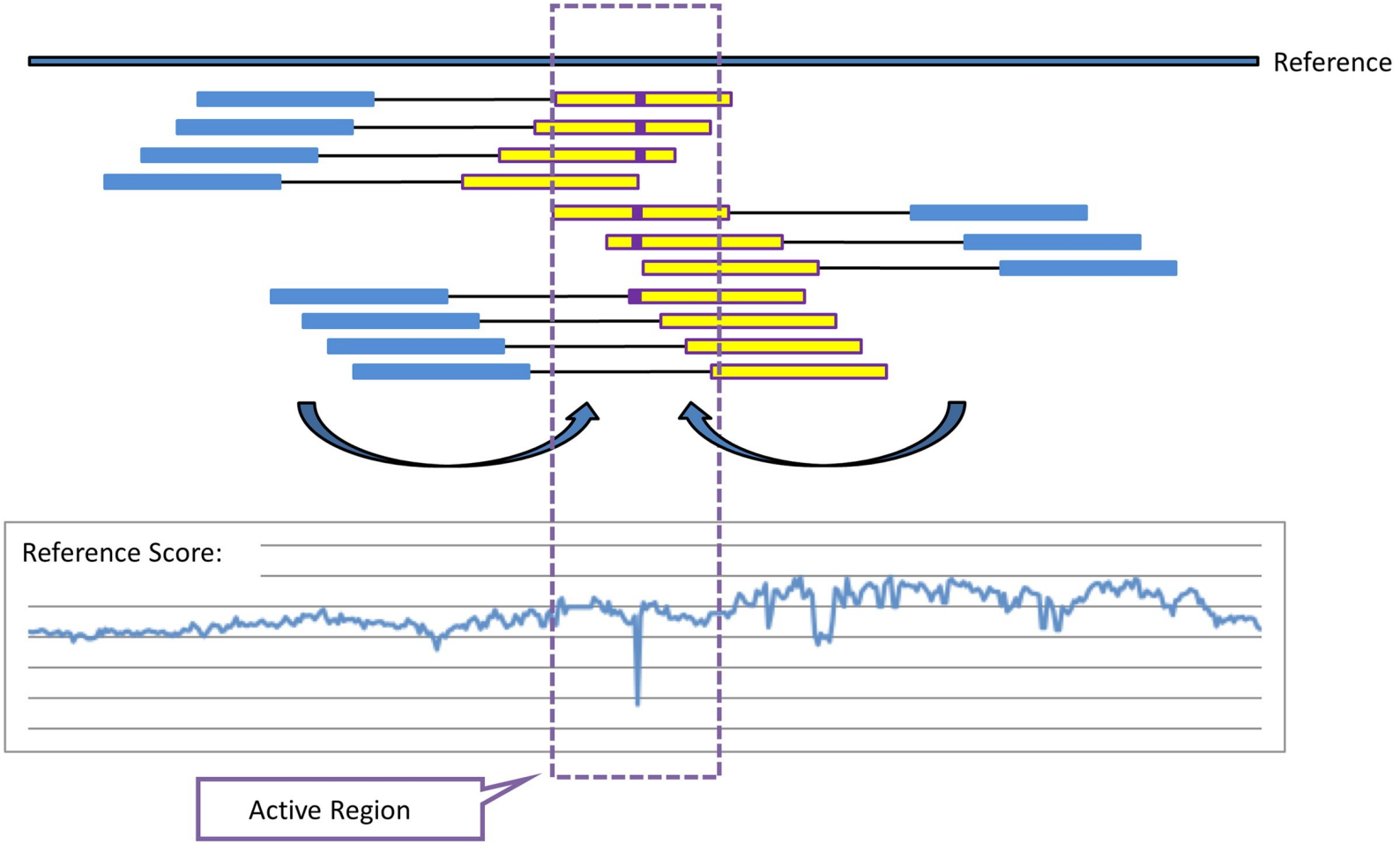
- Contig alignment against reference
  - Using aligners such as BWA
  - Uses standard reference alignment tools for variant detection
  - Helpful to „increase read size“ for better alignment
  - Variant detection is done using standard variant calling tools



Variant calling

- Local de novo assembly
  - Used by the Complete Genomics variant caller
- Every read around a variant is de novo assembled
- Contig is realigned back against the reference
- Final variant calling is done

Variant calling



Computational Techniques for Human Genome Resequencing Using Mated Gapped Reads, Paolo Carnevali et al., 2012

Variant calling

- Local de novo realignment allows for bigger features to be found than with traditional reference based variant calling
- Faster than complete assembly

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
  - Assembling tries 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

Other uses

- Transcriptomics, similar problematic to DNAseq
  - If small variations and gene expression analysis is done, alignment against reference is used
  - If unknown transcripts/genes are searched, de novo assembly is used
    - Used to detect transcripts with new introns, changed splice sites
    - Is able to handle RNA editing much better than alignment
    - Different underlying data (single strand, non uniform coverage, many small contigs)

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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Fachhochschule Westschweiz
University of Applied Sciences Western Switzerland

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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