CS-E5875 High-Throughput Bioinformatics
Genotype calling and de novo assembly

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Contents

Human genome

- DNA is a double-stranded molecule with each strand being a linear sequence of nucleotides
- A nucleotide consists of a phosphate group, sugar, and nucleoside
- A nucleoside is a nitrogenous base connected to a deoxyribose sugar
- There are four different nucleotides (depending on the nucleoside): adenine (A), cytosine (C), guanine (G), thymine (T)
- The nucleotides have a specific base pairing in double-stranded DNA:
  - Adenine pairs w/ thymine
  - Cytosine pairs w/ guanine
- Total length: about 3 billion nucleotides

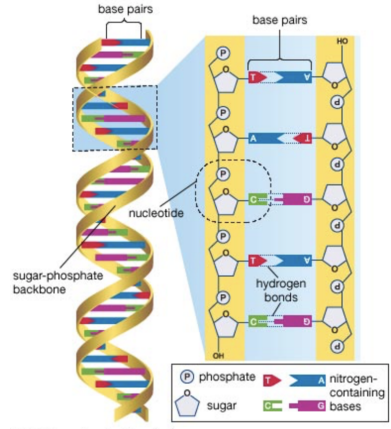

Figure from Wikipedia

Types of human genome variation

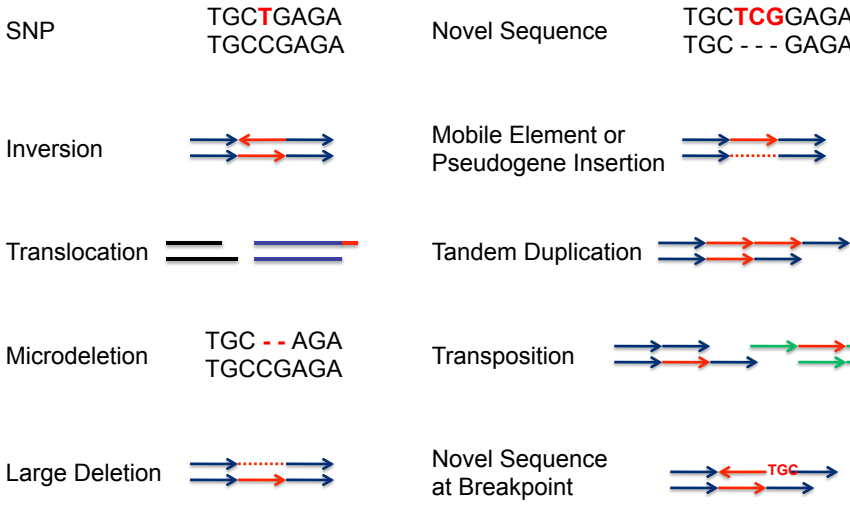

Figure from `https://web.stanford.edu/class/cs262/presentations/lecture4.pdf`

Single-nucleotide polymorphism

- Consider a specific nucleotide (chromosome and genomic coordinate) in human genome
- Most individuals have the same nucleotide at that position
- However, some individuals can have a different nucleotide at that position
- This nucleotide difference is called a genetic variant
- Different nucleotides at that variant position are called alleles
- There exist biallelic and multiallelic variants
  - Biallelic: a position in a genome can contain two different nucleotides
  - Multiallelic: a position in a genome can contain more than two different nucleotides
  - Much of the literature/published GWAS results focus on biallelic variants

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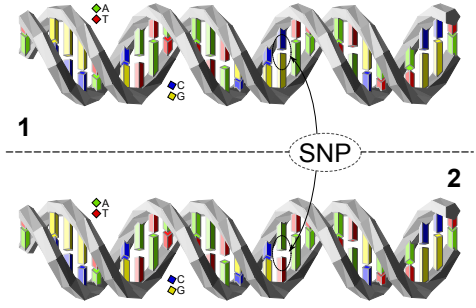
- Minor allele is defined to be the allele that occurs with a lower frequency
- Variants with a minor allele frequency (MAF) of at least 5% are typically called common single-nucleotide polymorphisms (SNPs)
- Variants with MAF between 0.5% and 5% are called as low-frequency variants

Single-nucleotide polymorphism

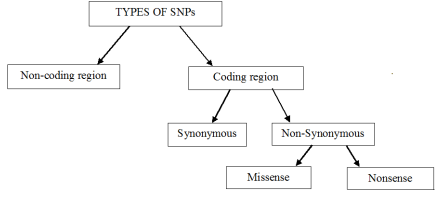
- An illustration of a SNP



Figures from https://en.wikipedia.org/wiki/Single-nucleotide_polymorphism

Types of single-nucleotide polymorphism

- Non-coding: SNP is located in a region of a genome that does not code for a protein
- Coding: SNP is located in a region of a genome that codes for a protein
- Synonymous: SNP does not change the amino acid sequence that is produced
- Nonsynonymous: SNP changes the amino acid sequence that is produced
- Missense: SNP causes a substitution of a different amino acid in the final amino acid sequence
- Nonsense: SNP causes a premature stop codon / truncated protein amino acid sequence / non-functional protein



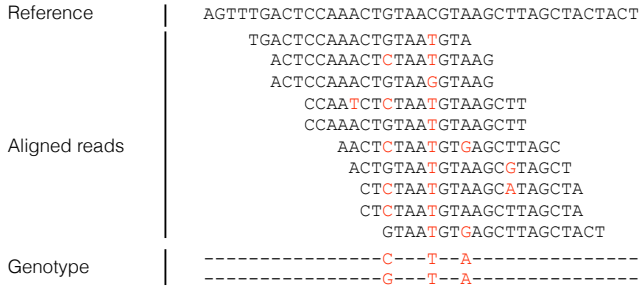
Figures from https://en.wikipedia.org/wiki/Single-nucleotide_polymorphism

Genotype

- The genotype of a diploid individual at a single genomic variant position is the combination of the two alleles in the two chromosome copies
- Denote the two alleles of a biallelic variant by A and B
- Note that both A and B can take values in {A,C,G,T}
  - The possible genotypes for the variant are then A/A, A/B and B/B
  - A/A: no mutation
  - A/B: heterozygous mutation
  - B/B: homozygous mutation
- For example: if the possible alleles of a biallelic SNP are A = G and B = T, then the possible genotypes are
  - A/A: G/G
  - A/B: G/T
  - B/B: T/T
- SNPs are the primary source of genetic differences between individuals

Genotype calling

- Assume we have measured short DNA sequencing reads from a large number of cells for several individuals
- Having aligned the short sequencing reads of all individuals to a reference genome
  - SNP calling identifies variable sites (using reads from all individuals)
  - Genotype calling determines the genotype for each individual separately at each site (using reads from a single individual separately)
  - Genotype calling is typically only done for positions in which a SNP variant has already been called



Genotype calling

- Challenges in SNP and genotype calling
  - A mismatch in an aligned read can be due to
    - A true SNP
    - An error while generating the sequencing library
    - Base calling error
    - Misalignment
    - Mistakes done earlier while building the reference sequence
  - Many NGS studies rely on low-coverage sequencing (<5x per site per individual, on average)
    - A high probability that only one of the two chromosomes of a diploid individual has been sampled at a specified site
- A probabilistic framework: so-called "genotype likelihoods" which incorporate errors that may have been introduced in base calling, alignment and assembly are coupled with prior information, such as allele frequencies and patterns of linkage disequilibrium (LD)

GATK: a simple Bayesian genotyper

- Genotyping with GATK tool (McKenna et al, 2010)
- GATK computes the posterior probability of each genotype, given the pileup of aligned reads that cover a given locus and expected heterozygosity of the sample
- Define:
  - G is the genotype
  - D represents the data (pileup of the aligned reads at a given position)
  - $P(G)$ is a prior probability of seeing this genotype (in a given population)

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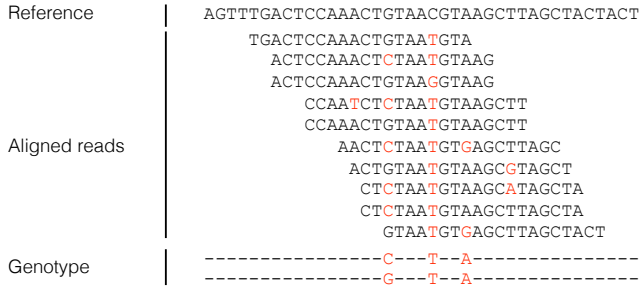
  - $P(G)$ is a prior probability of seeing this genotype (in a given population)
- The basic model is then (recall the Bayes' theorem)

$$P(G|D) = \frac{P(D|G)P(G)}{P(D)} \propto P(D|G)P(G),$$

GATK: a simple Bayesian genotyper

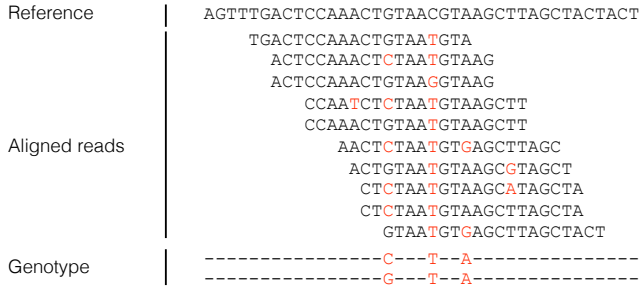


- The likelihood can be written as a product over the independent aligned reads

$$P(D|G) = \prod_{b_i \in \text{pileup}} P(b_i|G),$$

where b_i $(i = 1, \ldots, d)$ represents the base in the ith read covering the locus

GATK: a simple Bayesian genotyper

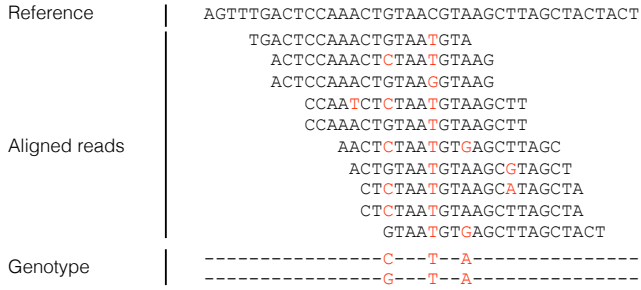


- For each position, decompose the genotype into its two alleles as $G = (A_1, A_2)$
- The probability of a base given the genotype is defined as

$$P(b_i|G) = P(b_i|(A_1, A_2)) = \frac{1}{2}P(b_i|A_1) + \frac{1}{2}P(b_i|A_2),$$

(because b_i can come from either of the chromosome copies with equal probability)

GATK: a simple Bayesian genotyper

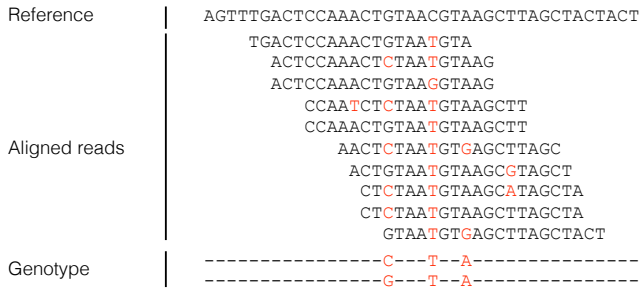


- Finally, the probability of seeing a base given an allele is

$$P(b_i|A) = \begin{cases} \frac{e_i}{3}, & \text{if } b_i \neq A \\ 1 - e_i, & \text{else} \end{cases},$$

where $e_i = 10^{-\frac{q_i}{10}}$ is the reversed phred scaled quality score at the base in the ith read

GATK: a simple Bayesian genotyper



- The maximum a posteriori (MAP) estimate of the genotype is then

$$\hat{G} = \arg\max_{G} P(G|D)$$

GATK: a simple Bayesian genotyper

- Consider a simplified example where the reference base at a given locus is A and the alternative is T and we have a single read with base b aligned to that position
- Assume that the possible genotypes are AA, AT and TT
- Applying the Bayes formula we get

$$
\begin{aligned}
P(AA|b) &= \frac{P(b|AA)P(AA)}{P(b|AA)P(AA) + P(b|AT)P(AT) + P(b|TT)P(TT)} \\
&= \frac{\left(\frac{1}{2}P(b|A) + \frac{1}{2}P(b|A)\right)P(AA)}{P(b|AA)P(AA) + P(b|AT)P(AT) + P(b|TT)P(TT)}
\end{aligned}
$$

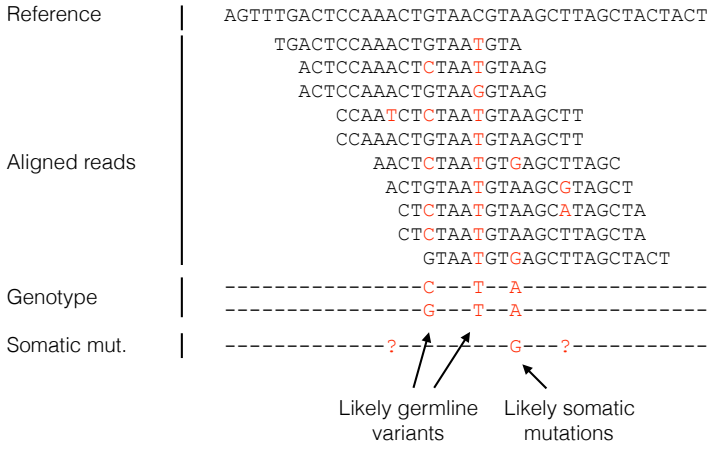
Contents

Germline vs. somatic mutations (quotes from Wikipedia)

- "A germline mutation is any detectable and heritable variation in the lineage of germ cells (cells that will develop into sex cells - namely sperm and ovum)."
- "Mutations in these cells can be transmitted to offspring if these cells are involved in the formation of a zygote"
- "Somatic mutations are changes to the genetics of a multicellular organism which are not passed on to its offspring through the germline."

Germline vs. somatic mutations

- Germline mutations that we have considered previously can be heterozygous or homozygous, i.e., appear with a frequency 50% or 100%
- Somatic mutations can be present with any frequency (across a population of cells)



Somatic mutation detection with Mutect

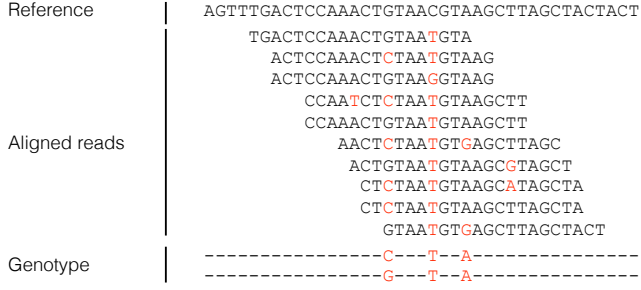
- Somatic mutation detection with Mutect (Cibulskis et al, 2013)
- Consider detecting a somatic mutation at a given position (chromosome and coordinate)
- Denote the reference allele as $r \in \{\mathrm{A}, \mathrm{C}, \mathrm{G}, \mathrm{T}\}$
- Assume d sequence reads overlap the position and denote
  - b_i is the base called in the ith read ($i \in \{1, \ldots, d\}$)
  - e_i is the probability of error of the base called in the ith read

$$e_i = 10^{-\frac{q_i}{10}}$$

where q_i is the associated Phred quality score

Somatic mutation detection with Mutect

- To detect a somatic mutation, try to explain the data using two models:
  1. Model M_0 in which there is no variant at the site and all non-reference bases are explained by sequencing noise
  2. Model M_f^m in which a variant allele $m \neq r$ truly exists at the site with an allele frequency f and reads are also subject to sequencing noise
- Note that M_0 is equivalent to M_f^m with $f = 0$ and $m = r$



Somatic mutation detection with Mutect

- The likelihood of the model M_f^m is given by

$$L(M_f^m) = P(\{b_i\}|\{e_i\}, r, m, f) = \prod_{i=1}^{d} P(b_i|e_i, r, m, f)$$

  assuming the sequencing errors are independent across reads

- If all substitution errors are equally likely and occur with probability $e_i/3$, then the likelihood is

$$P(b_i|e_i, r, m, f) = \begin{cases} fe_i/3 + (1-f)(1-e_i) & \text{if } b_i = r \\ f(1-e_i) + (1-f)e_i/3 & \text{if } b_i = m \\ e_i/3 & \text{otherwise} \end{cases}$$

Somatic mutation detection with Mutect

- Somatic mutation detection is performed by computing the likelihood ratio of the two models, M_0 and M_f^m

$$\text{LOD}_T(m, f) = \log_{10} \frac{L(M_f^m)P(m, f)}{L(M_0)(1 - P(m, f))}$$

  where $P(m, f)$ is a prior (e.g. expected probability of a mutated nucleotide m and its frequency for a given cancer type)

- The unknown frequency f and mutated nucleotide m can be estimated using the maximum likelihood method (or set to plug-in estimates) to obtain $\hat{f}$ and $\hat{m}$

Somatic mutation detection with Mutect

- Somatic mutation is called if the above LOD score exceeds a certain significance level
- Note that the above LOD score corresponds to a likelihood ratio statistic

Somatic mutation detection with Mutect

- The detected somatic mutations should be further filtered to avoid likely false positives
  - Check that the detected variant is not a heterozygous germline SNP, i.e., test

$$\mathrm{LOD}_N = \log_{10} \frac{L(M_0)P(m, f)}{L(M_{0.5}^m)P(\text{“germline”})},$$

where frequency has been set to $f = 0.5$ and terms have been reverted to avoid false positives

Somatic mutation detection with Mutect

- Filter other technical artifacts not accounted by the model

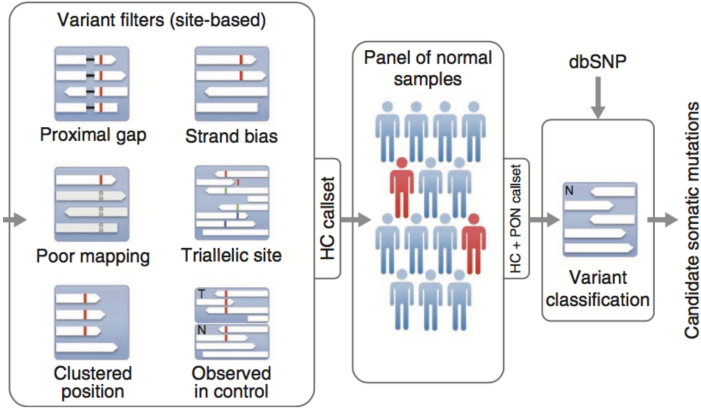


Figure from (Cibulskis et al, 2013)

Types of human genome variation

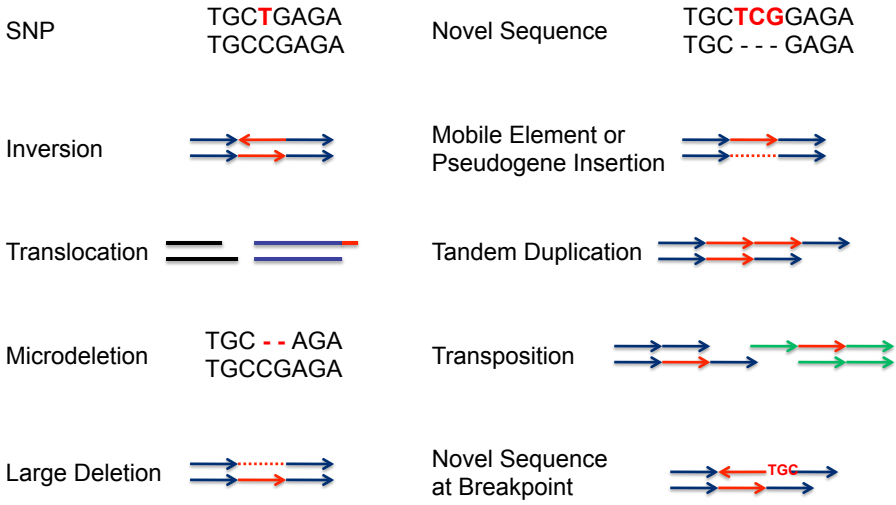


Figure from `https://web.stanford.edu/class/cs262/presentations/lecture4.pdf`

Contents

De novo genome assembly

- This section follows (Chaisson, et al, 2015)
- De novo: no reference genome available
- The goal of de novo genome assembly is to determine the sequence of a genome using only randomly sampled sequence fragments
  - Sequence fragments are typically less than one-millionth the size of a mammalian genome

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- De novo: no reference genome available
- The goal of de novo genome assembly is to determine the sequence of a genome using only randomly sampled sequence fragments
  - Sequence fragments are typically less than one-millionth the size of a mammalian genome
- Most current approaches involve some aspect of a whole-genome shotgun sequencing and assembly (WGSA) strategy
  - Random fragments from a genome are sequenced and computationally stitched together to generate sequence contigs and scaffolds
- Under ideal conditions (i.e., uniformly high sequence coverage across the whole genome and a genome devoid of repetitive sequences), an assembly may be determined with the simple approach of merging reads with maximal overlap

De novo genome assembly

- In practice such an approach does not work because:
  - Sequence coverage is almost never uniform
  - Genome contains repetitive sequences of varying length, and
  - Genome contains varying copy numbers (duplications)

Types of genome assembly gaps

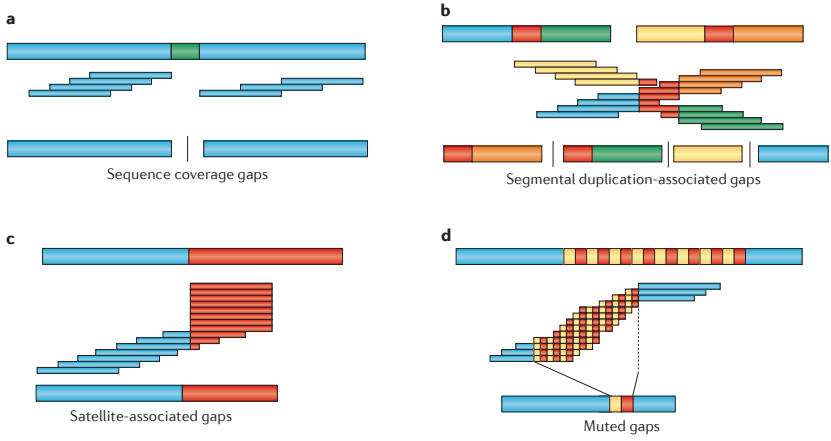

Figure from (Chaisson, et al, 2015)

Early de novo assembly methods

- The most-widely used mammalian genomes, human and mouse, were not assembled using WGSA
- Instead, human and mouse assemblies are relatively unique among mammalian genomes in that they were assembled almost entirely using clone-by-clone-based sequencing
  - Each genome/chromosome was divided into roughly 200-kb-long overlapping fragments that were cloned into bacterial artificial chromosomes (BACs) and individually assembled
  - These longer 200kb fragments were then connected

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  - Each genome/chromosome was divided into roughly 200-kb-long overlapping fragments that were cloned into bacterial artificial chromosomes (BACs) and individually assembled
  - These longer 200kb fragments were then connected
- When the result of a de novo assembly is a sequence per chromosome without gaps and with 99.99% base-pair accuracy, the assembly is considered complete; otherwise, it is considered a draft.
  - Even a recent build of the human genome (GRCh38) contains gaps

State-of-the-art assembly strategies

- Since 2013, de novo assembly of mammalian genomes has shifted from purely WGSA using HTS data to assembly with longer sequence reads generated either synthetically or by single-molecule sequencing (SMS) (e.g. PacBio, Nanopore)
- The main algorithmic approaches to de novo assembly are
  - Overlap-layout-consensus (OLC)
  - de Bruijn
  - (The string graph)

Overlap-layout-consensus (OLC)

- Contigs: Continuous (or 'contiguous') sequences produced in a de novo assembly, free of any gaps
- Basic steps of OLC algorithms:
  - Overlaps between all read pairs are first detected
  - Contigs are formed by iteratively merging overlapping reads until a read heuristically determined to be at the boundary of a repeat is reached

Overlap-layout-consensus (OLC)

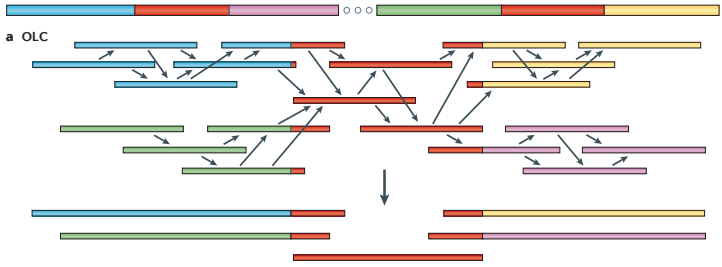

Figure from (Chaisson, et al, 2015)

- Some repeats can be resolved
- Imprecise read overlaps are allowed to account for sequencing errors

de Bruin algorithms

- Basic steps of de Bruin algorithms:
  - Start by replacing each read with the set of all-overlapping sequences of a shorter, fixed length (k typically between 31 and 200)
  - Contigs are formed by merging k-mers appearing adjacently in reads stopping at k-mers from repeat boundaries
- Requires highly accurate reads
- Initially discards some of the ability for reads to resolve repeats longer than k bases

de Bruin algorithms

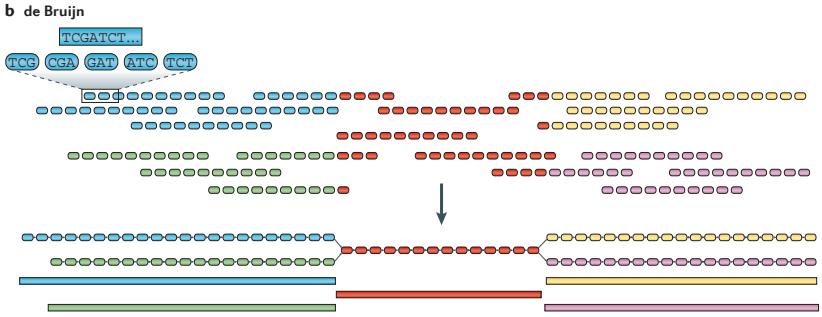

Figure from (Chaisson, et al, 2015)

Genome annotation de novo

- Full genome assembly methods can be developed further
- Things to do next:
- Gene finding:
  - Ab initio prediction methods: based on statistical signals within the DNA
    - E.g.: hidden Markov model-based prediction of genes: Genscan, Augustus, HMMgene
  - Align known genes of model species against the new genome
  - If RNA-seq available from the same species, align RNA-seq data to the newly discovered genome
- Gene annotation:
  - Function of the genes that can be aligned to new genome give some hint about the newly sequenced organism

References

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