Marine Genomics

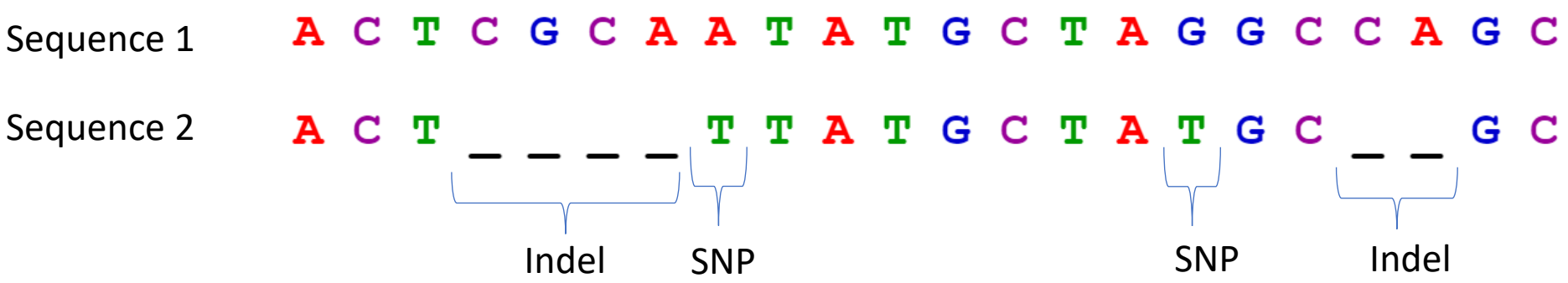
September 13th 2021

Mapping and calling variants

What is a genetic variant?

A region of the genome that differs from the reference (or another genome)

Signifies a mutation and can be a single base-pair, or larger insertion and/or deletion of several base-pairs.



What is a genotype?

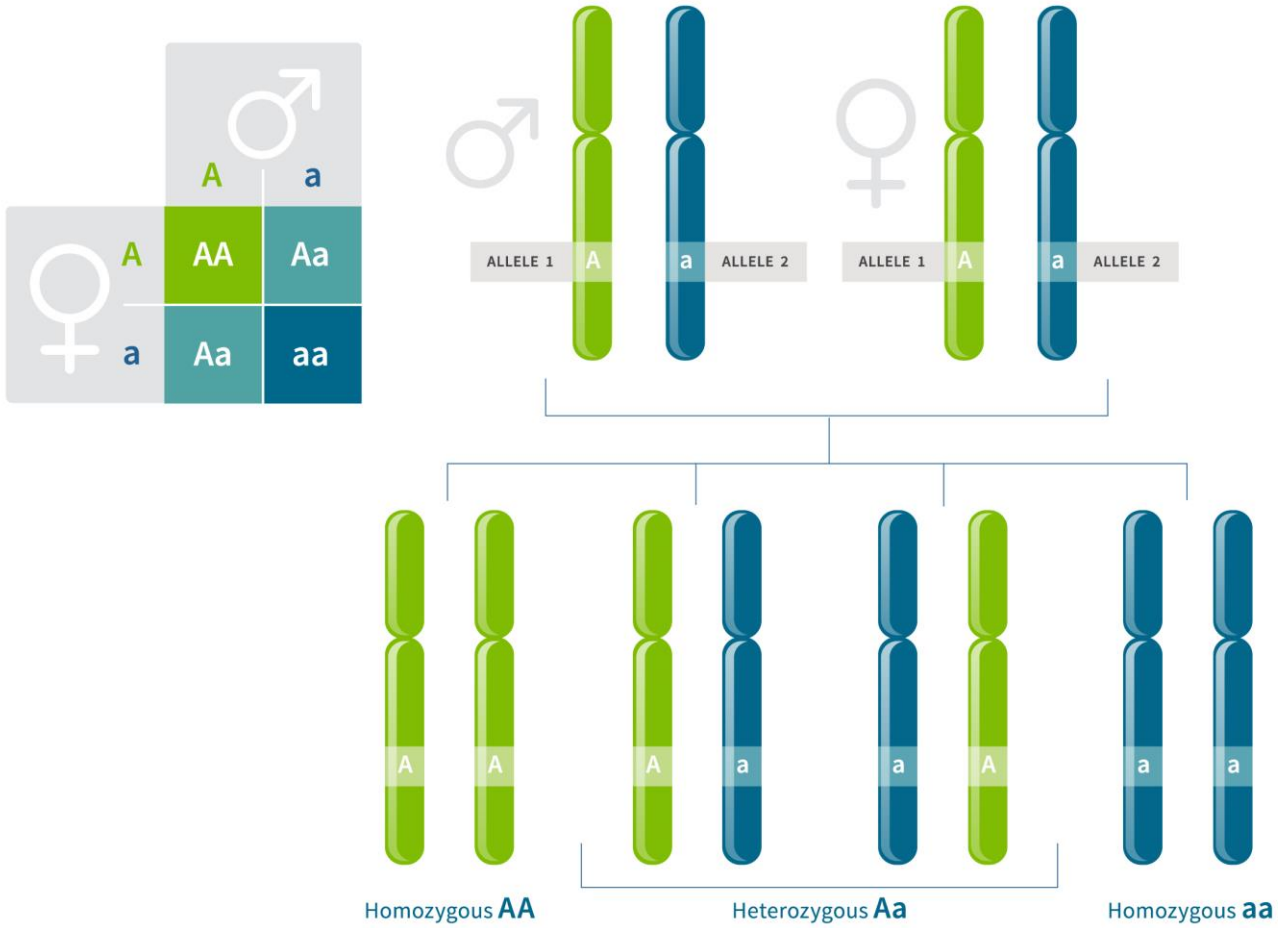
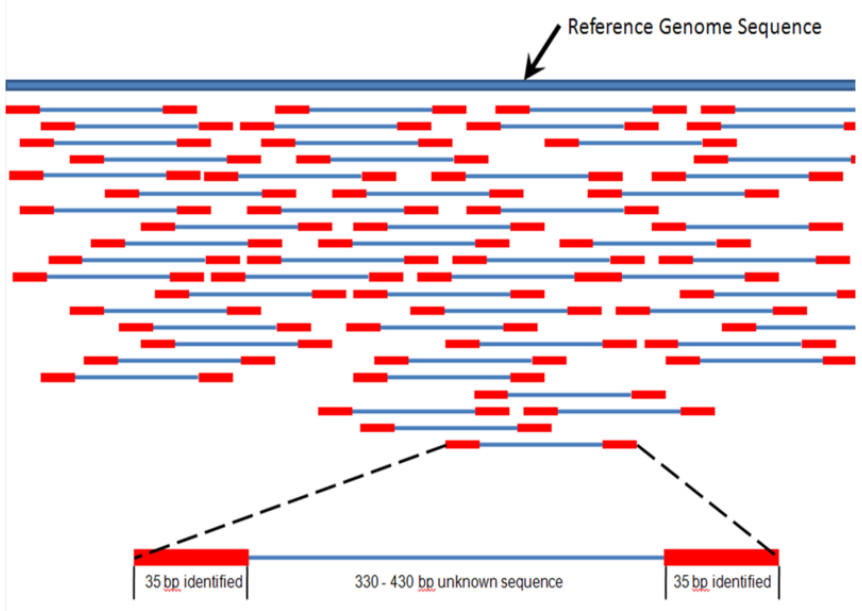


Image credit: https://www.ancestry.com/lp/genotype

How do we find a variant?

Map and align sequences from other individuals to a reference genome

- Does It matter what your reference genome is?
  - Is it the same or different species?
  - Is it from the same population?
- Short answer: Yes, it matters!



Genomes are continually being improved

- More genomes are being sequenced all the time
- Many marine organisms don't yet have a genome sequence available

Article | Open Access | Published: 07 April 2021

The structure, function and evolution of a complete human chromosome 8

Glennis A. Logsdon, Mitchell R. Vollger, [...] Evan E. Eichler

Nature (2021) | Cite this article

Abstract

The complete assembly of each human chromosome is essential for understanding human biology and evolution[1,2]. Here we use complementary long-read sequencing technologies to complete the linear assembly of human chromosome 8. Our assembly resolves the sequence of five previously long-standing gaps, including a 2.08-Mb centromeric α-satellite array, a 644-kb copy number polymorphism in the β-defensin gene cluster that is important for disease risk, and an 863-kb variable number tandem repeat at chromosome 8q21.2 that can function as a neocentromere. We show that the centromeric α-satellite array is generally methylated except for a 73-kb hypomethylated region of diverse higher-

Finding variants – some terminology

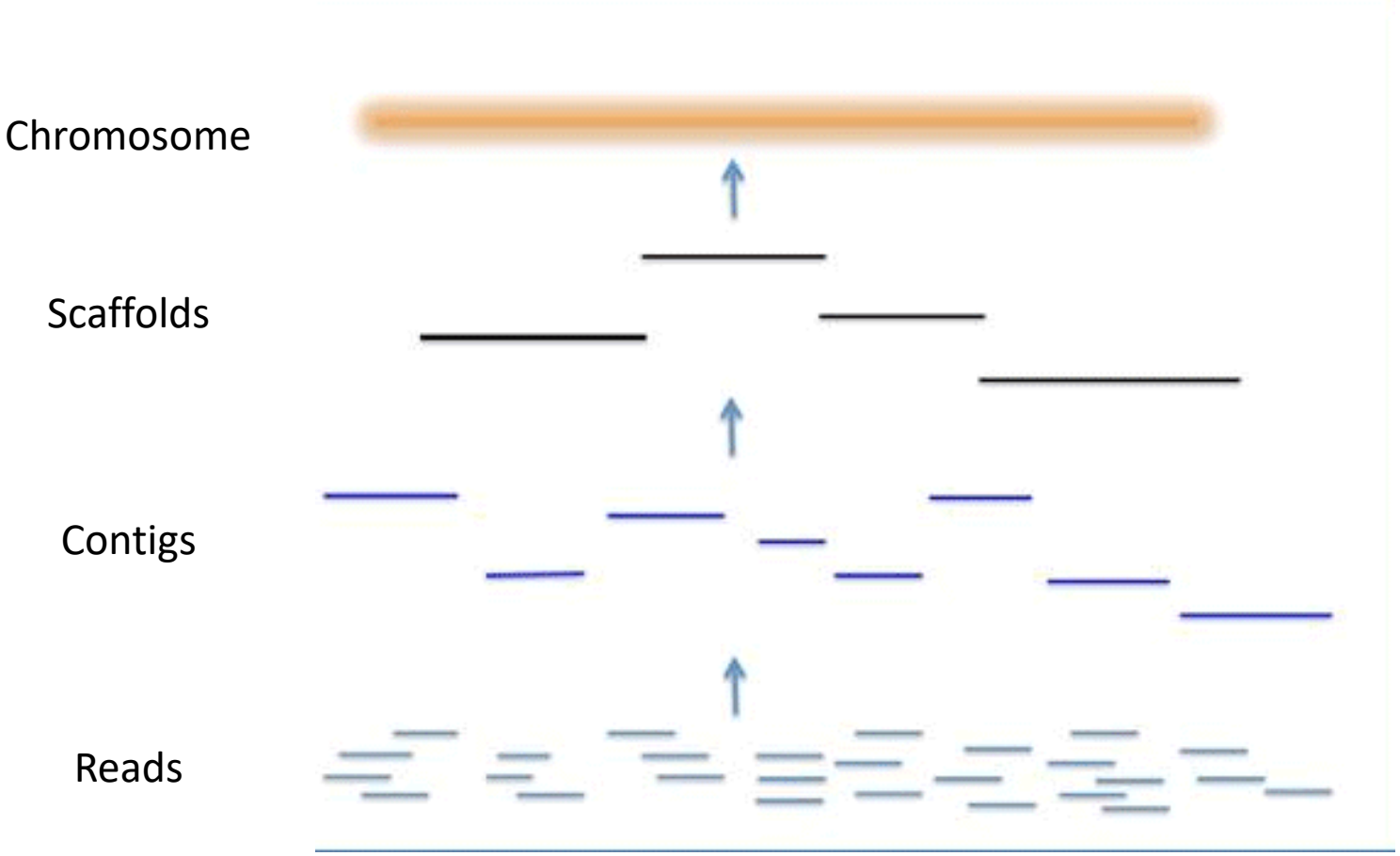


Figure modified from here https://www.ddbj.nig.ac.jp/ddbj/assembly-e.html

A reference genome is a collection of contigs

Finding variants – some terminology

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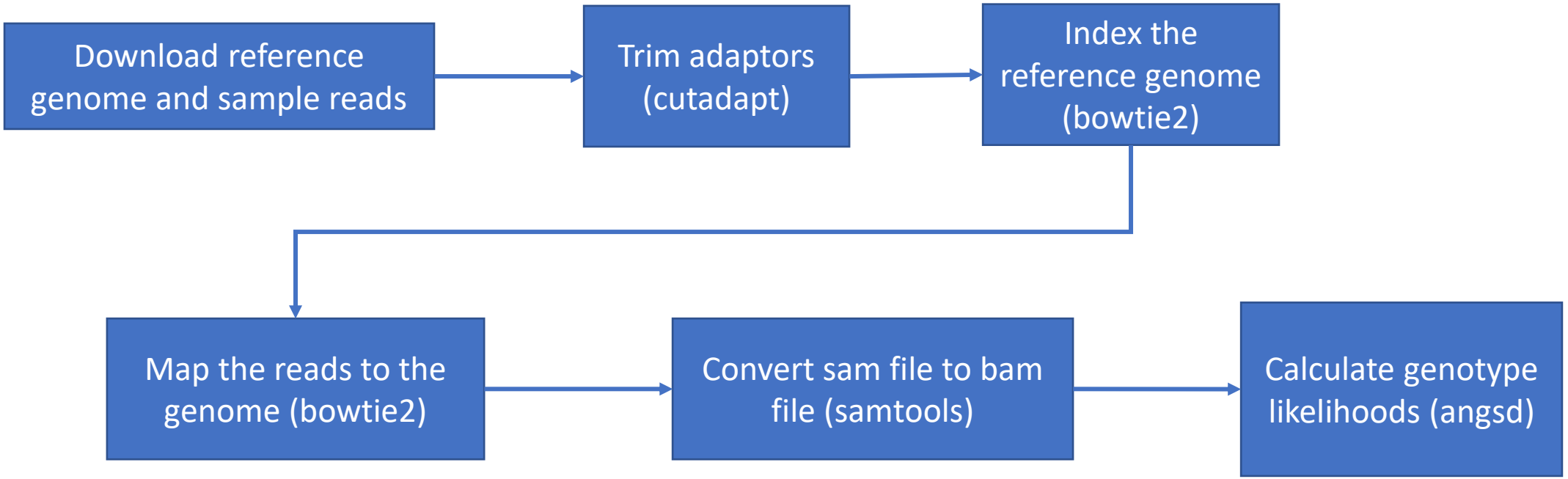
GAGATTCTCAAACTTTTGAGAGTAGTTCTCTTCCGAGAGAGGATCCTGCAAGCTTTCGATTCTCCTagtaattaaagaaaatgaaaaagttt
```

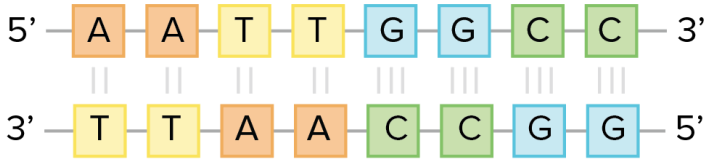
A reference genome is a collection of contigs

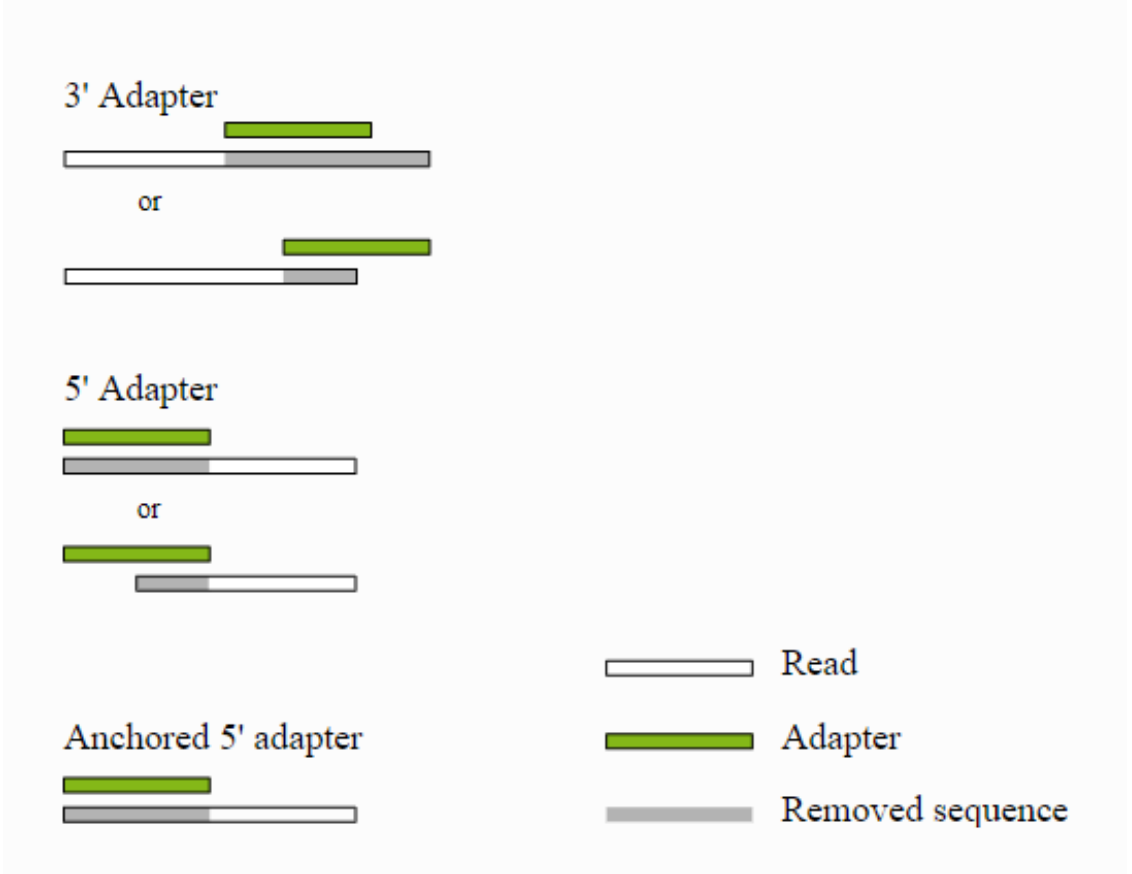
Typically, in fasta format

Finding variants - pipeline



Trimming adaptors from reads





Trimming adaptors from reads

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+
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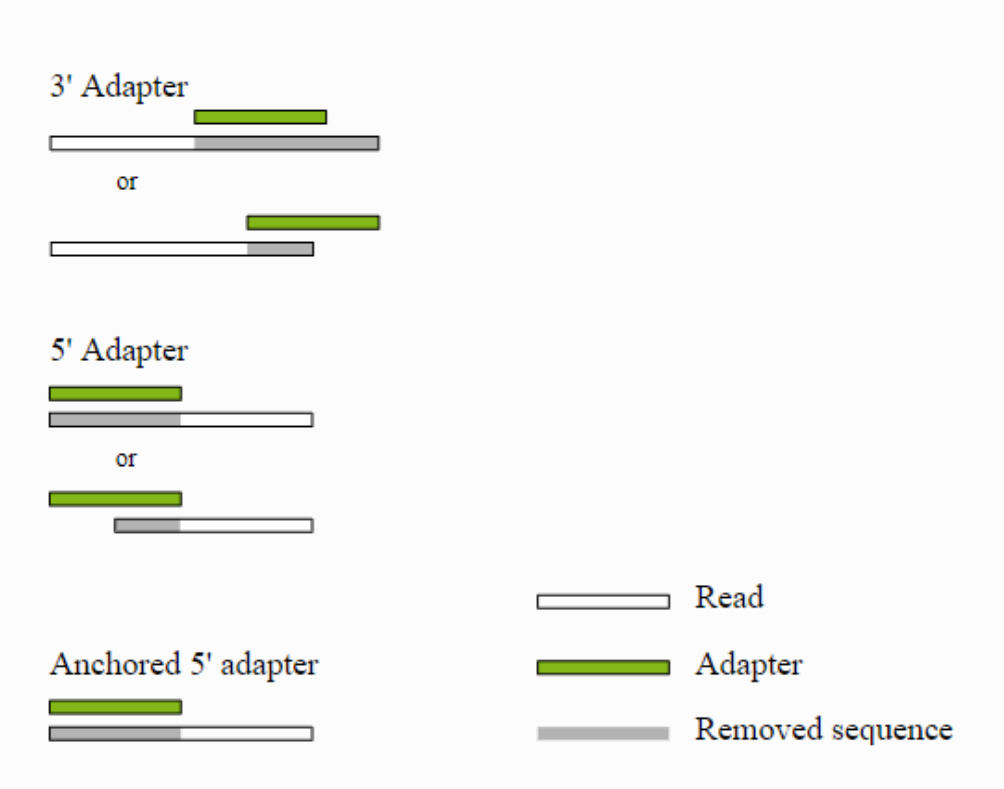

SAM and BAM file formats

Sequence Alignment Map, Binary Alignment Map

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Head of .sam file

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9489:;89:<5<;<;;:<;7=<<7;;2<<5.56;5;;:1:::=>>?B?7<><<<@;<;:3;8282;:;:::5        AS:i:-4 XN:i:0
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        XN:i:0  XM:i:2  XO:i:0  XG:i:0  NM:i:2  MD:Z:32A22T24   YT:Z:UU
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966357155588:89:957553222244407.254515666757:;;5:5966436,//4787878;8;:::        AS:i:-8 XS:i:-14
        XN:i:0  XM:i:2  XO:i:0  XG:i:0  NM:i:2  MD:Z:4A46T28    YT:Z:UU
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;:<99296;<;<:;4;5;;;;<A<<6<;998:0:;;;8883993:<3::6669::999999)96        YT:Z:UU
```

Tail of .sam file

SAM and BAM file formats

Sequence Alignment Map, Binary Alignment Map

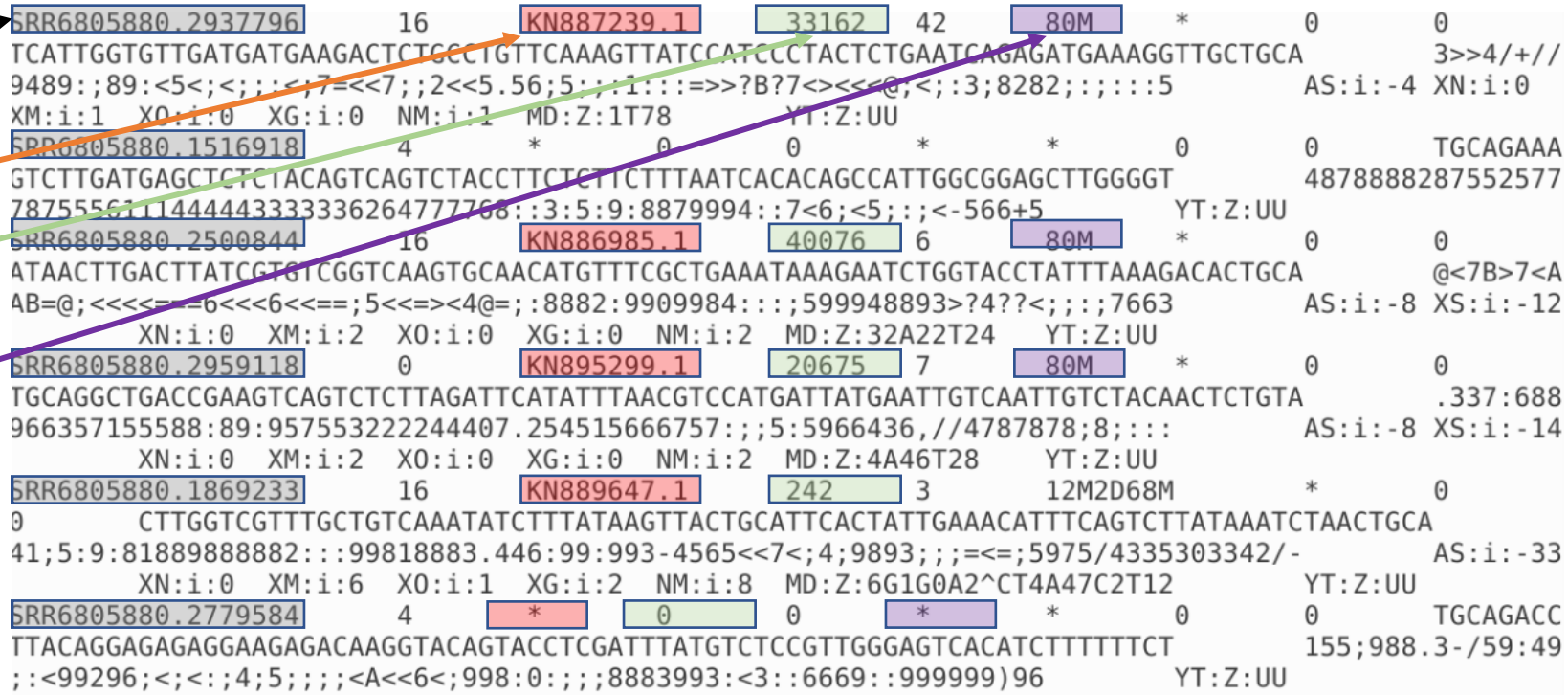
Name of read

Name of contig where read aligns

Position on contig where 5’ end starts

Alignment information “cigar string”

80M = contiguous match of 80bp



Tail of .sam file

“Calling” a genotype

Two main ways to estimate a genotype

Hard genotype calling versus genotype likelihoods

Depends on the type of data that you have. If you have reads with a high degree of coverage (many copies of the same read) you can do hard genotype calling. If you have variable coverage or low coverage you can do genotype likelihoods, to account for some uncertainty in the genotype.

Genotype likelihoods

In ANGSD http://www.popgen.dk/angsd/index.php/Genotype_Likelihoods

Accounts for some uncertainty in the genotype estimation

Theory

Genotype likelihoods are in this context the likelihood the data given a genotype. This is to be understood as we take all the information from our data for a specific position for a single individual, and we use this information to calculate the likelihood for our different genotypes. Since we assume diploid individuals it follows that we have 10 different genotypes.

0	1	2	3	4	5	6	7	8	9
AA	AC	AG	AT	CC	CG	CT	GG	GT	TT

And we write the genotype likelihood as

$$L(G = \{A_1, A_2\}|D) \propto Pr(D|G = A_1, A_2), \qquad A_1, A_2 \in \{A, C, G, T\}.$$