Introduction to variant calling

Bastiaan Star, Professor
Centre for Ecological and Evolutionary Synthesis (CEES)
Historical Ecology and Conservation Genomics
Department of Biosciences, University of Oslo (UiO)
Norway

@archaeogenomics

BIOS-IN 5K/9K
4th of Nov 2024

Centre for Ecological and Evolutionary Synthesis

UiO : University of Oslo

Evolutionary Biologist

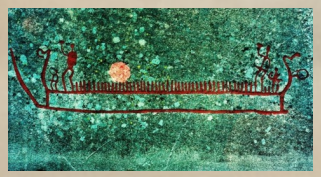
specialize in ancient DNA

Historical Ecology and Conservation Genomics
(10+ MSc, PhDs & Postdocs)

@archaeogenomics

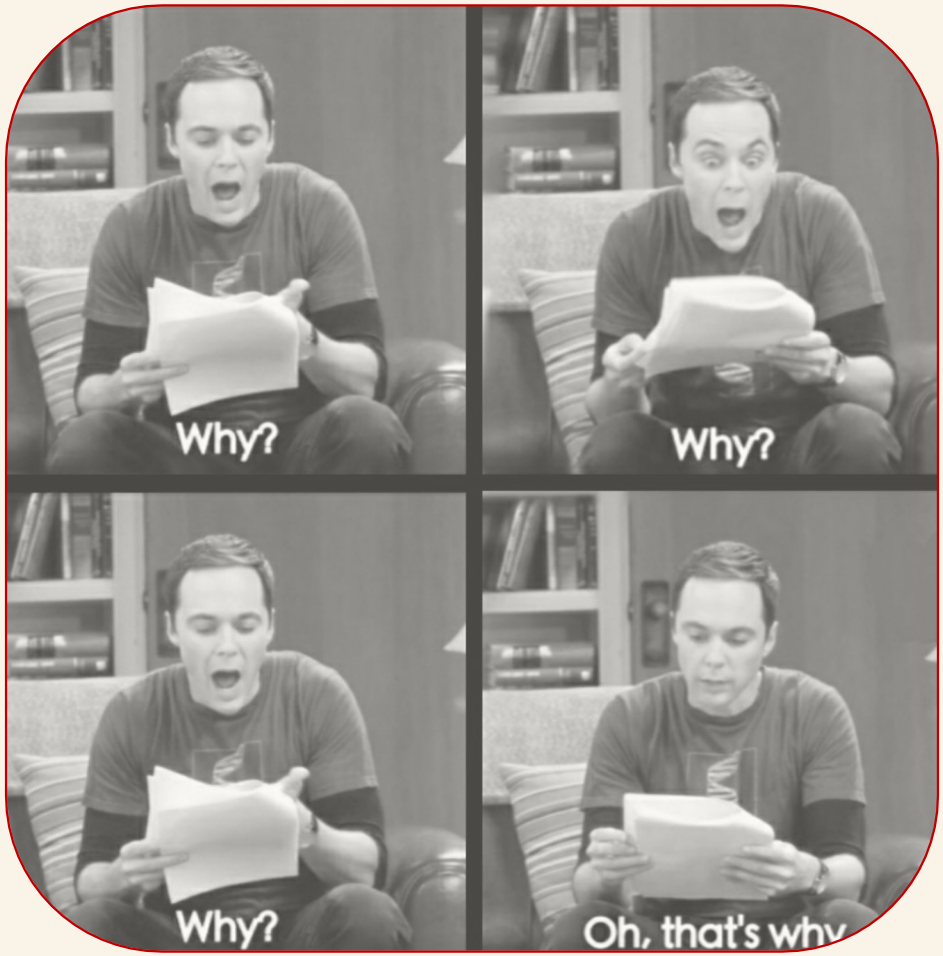
My background:

Historical Marine Ecology

Stone Age	Viking Age	Middle Ages	Modern Age
			
Subsistence	Early Trade	"Fish Horizon"	Globalisation
			

@archaeogenomics

As an evolutionary biologist - *Why* am I here?



Multidisciplinary research:
Archaeology
Biology
Ecology
Molecular methods/sequencing
Genomics
Bioinformatics

Today:

1) Introduction: variant calling, why do we want to do this, and what it is?

Today:

1) Introduction: variant calling, why do we want to do this, and what it is?
2) Variant calling pipelines/methods and limitations

Today:

1) Introduction: variant calling, why do we want to do this, and what it is?
2) Variant calling pipelines/methods and limitations
3) Practical session, going through (parts of) a SNP calling pipeline and interpret biological results

Introduction

What is genetic variation?
What does genetic variation do?
Why are we interested in genetic variation?

Please discuss between yourselfs (two or three) for a few minutes

Introduction

Genetic variation (genetic differences between individuals) is everywhere!

Genetic variation at different scales:

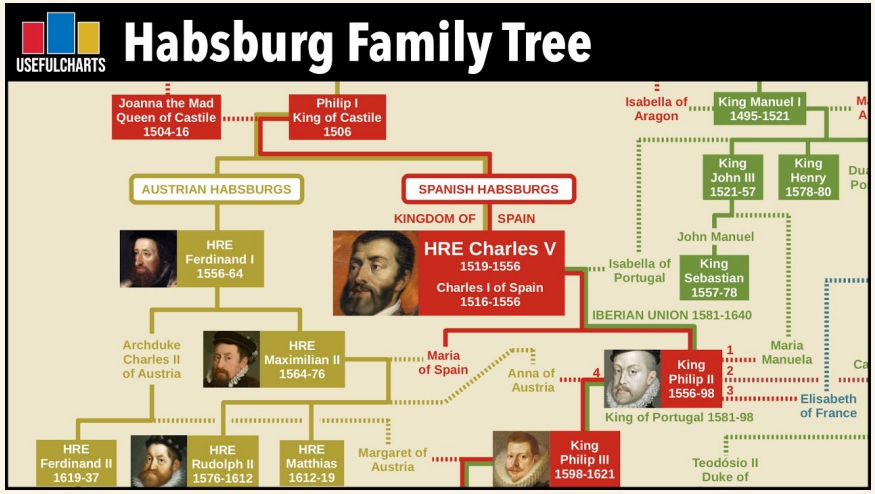
1) Biological differences (phenotypes) between species

Genetic variation at different scales :

1) Biological differences (phenotypes) between species
2) Biological differences within species

Genetic variation at different scales :

1) Biological differences (phenotypes) between species
2) Biological differences within species
3) Patterns of relatedness between individuals/ populations (23 and me)



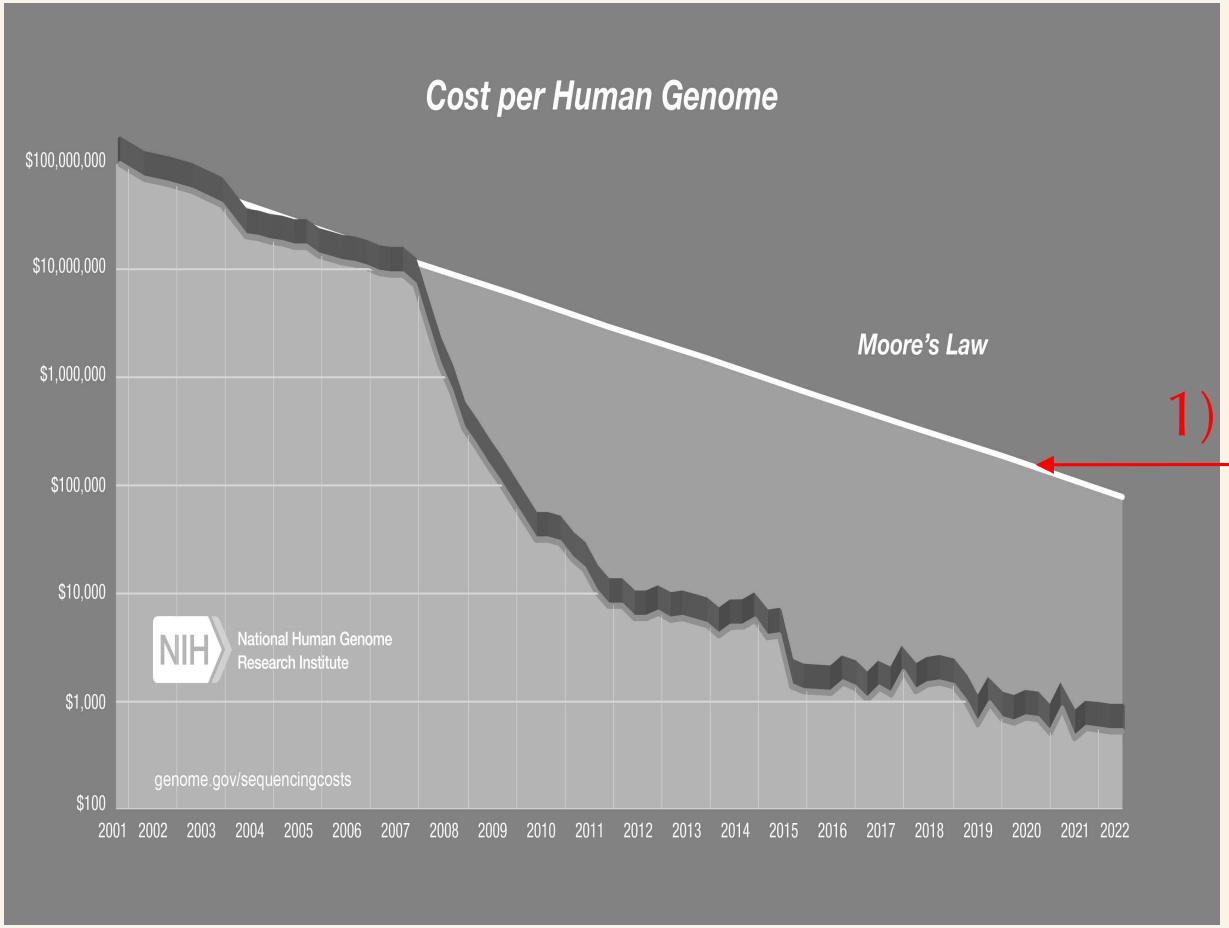
Genetic variation explains many observations within biology

Genetic variation explains many observations within biology

Knowing patterns of/quantifying genetic variation has enormous potential for a wide range of applications in society

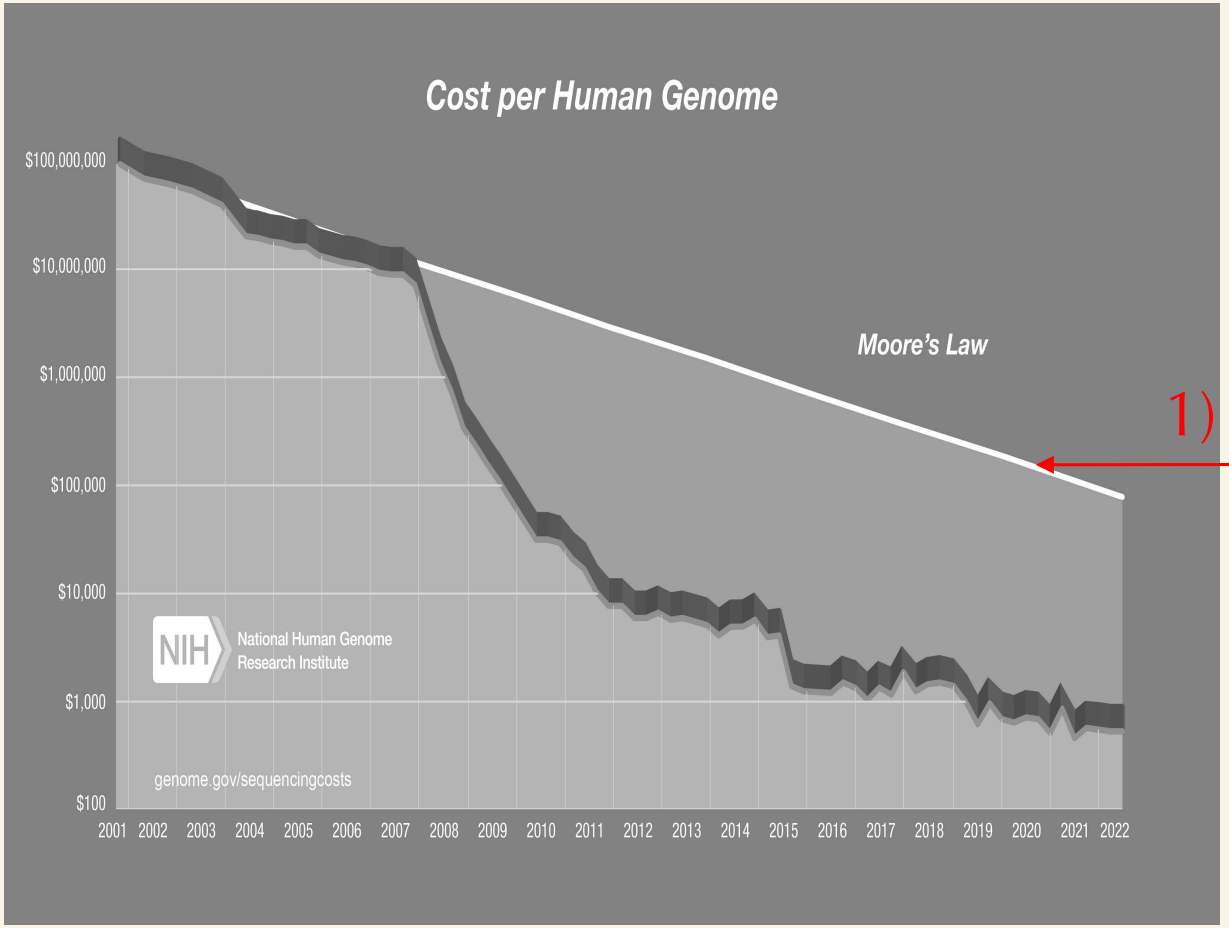
(e.g. personal medicine, forensic sciences, biodiversity assessments, crop improvement, animal breeding, conservation management, history & genealogy, etc etc)

Why are we here?



1) Moore's Law

Why are we here?

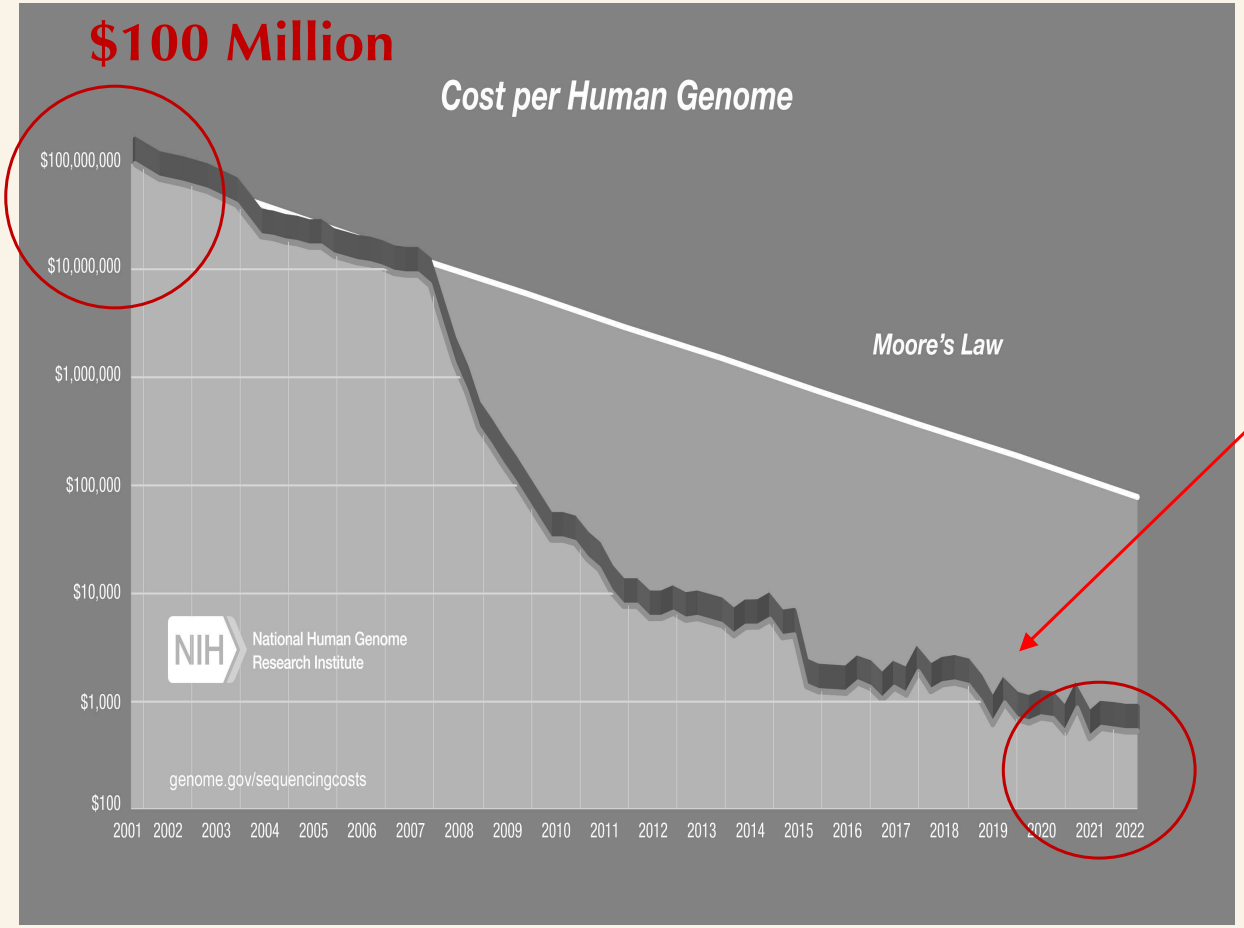


2000

1) Moore's Law

2022

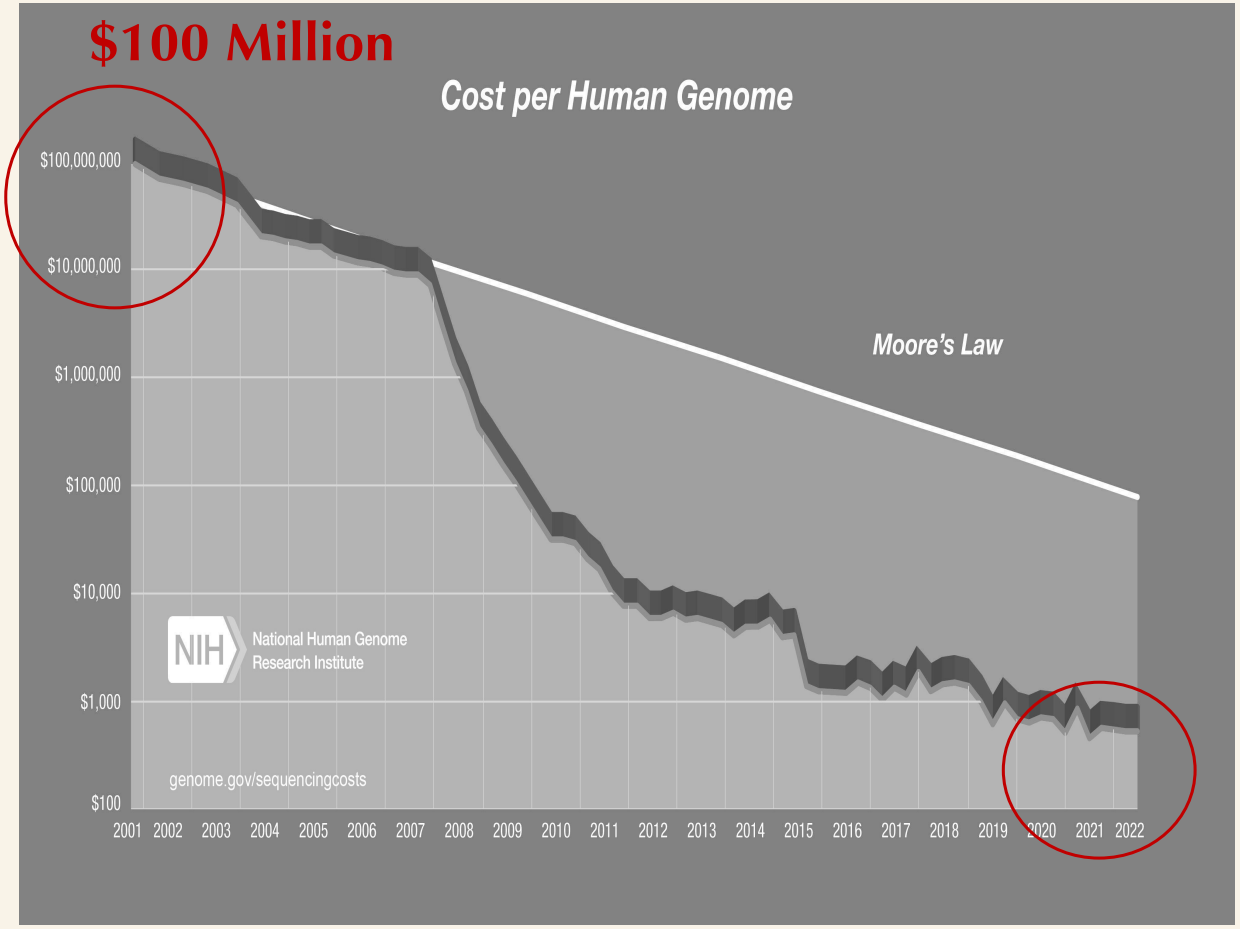
Why are we here?



2) **New** sequencing techniques

$1000

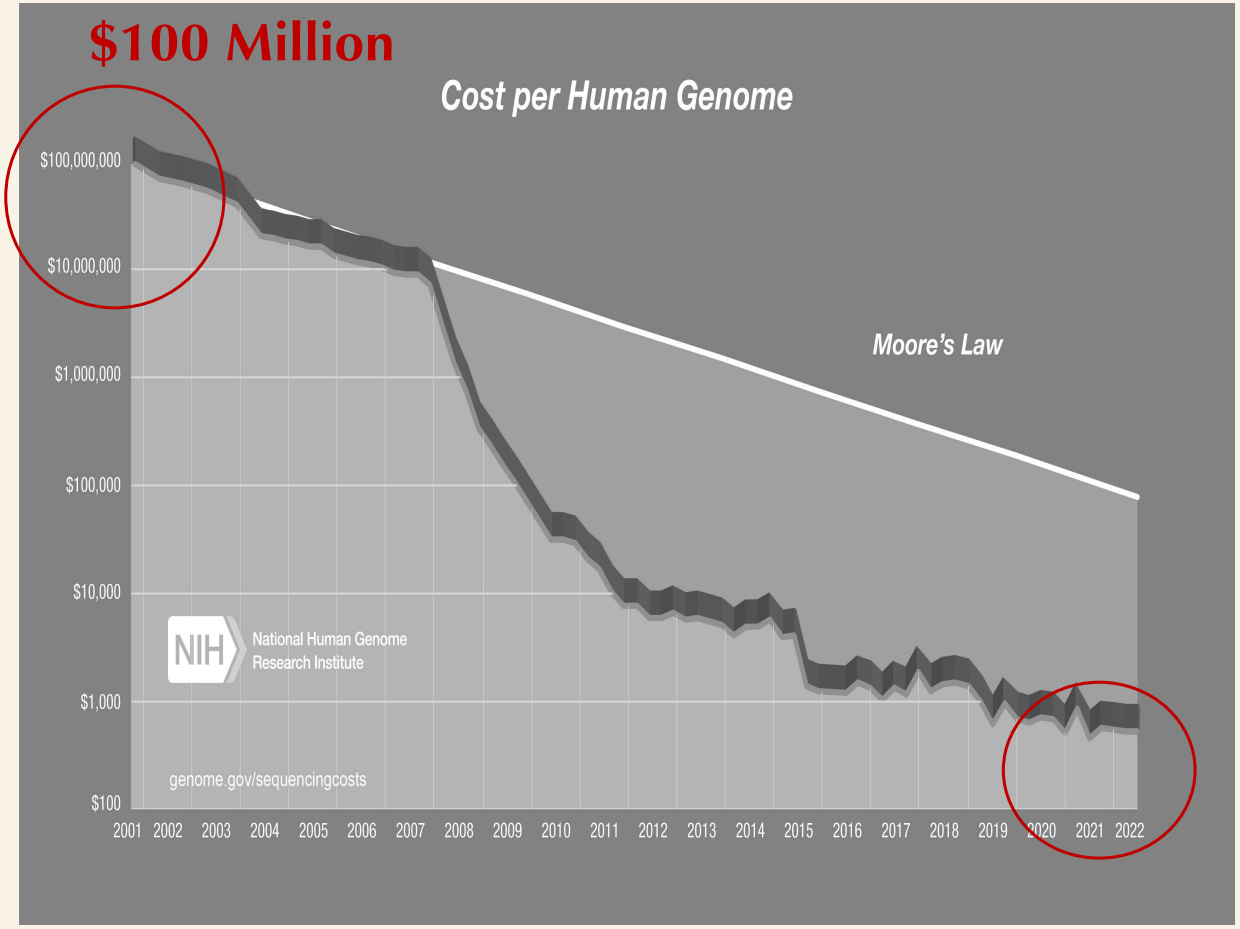
Why are we here?



Quick question!
What would this Maserati at
~1 million NOK
in 2000 cost now if similarly devaluated?

$1000

Why are we here? Phenomenal technical advances



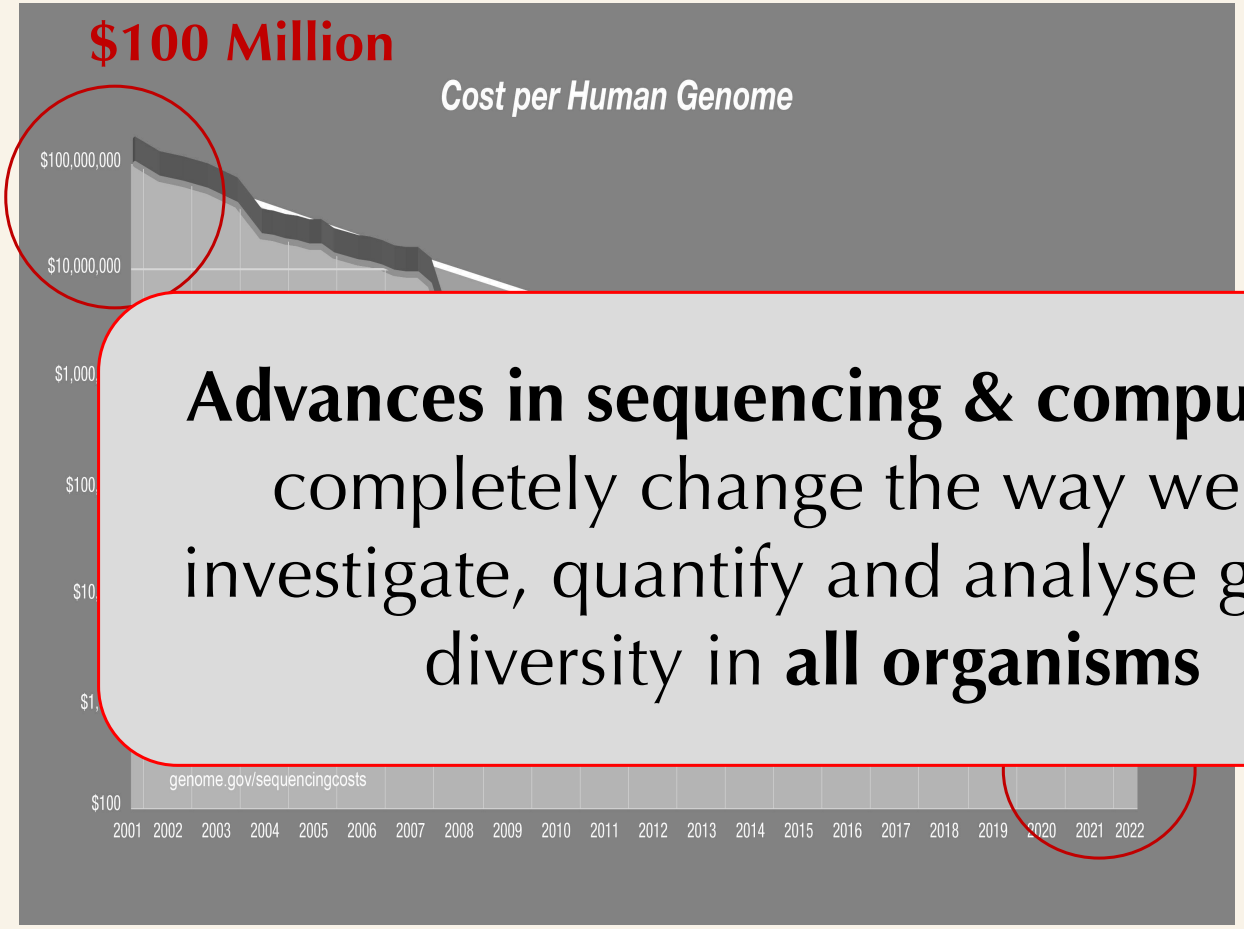
Quick question!
What would this Maserati at
~1 million NOK
in 2000 cost now if similarly devaluated?

10 NOK

$1000

Has fundamentally changed the way we do biology

Why are we here? Phenomenal technical advance



estion!
this
at
IOK
now
y
d?
K
$1000

Advances in sequencing & computation completely change the way we can investigate, quantify and analyse genetic diversity in **all organisms**

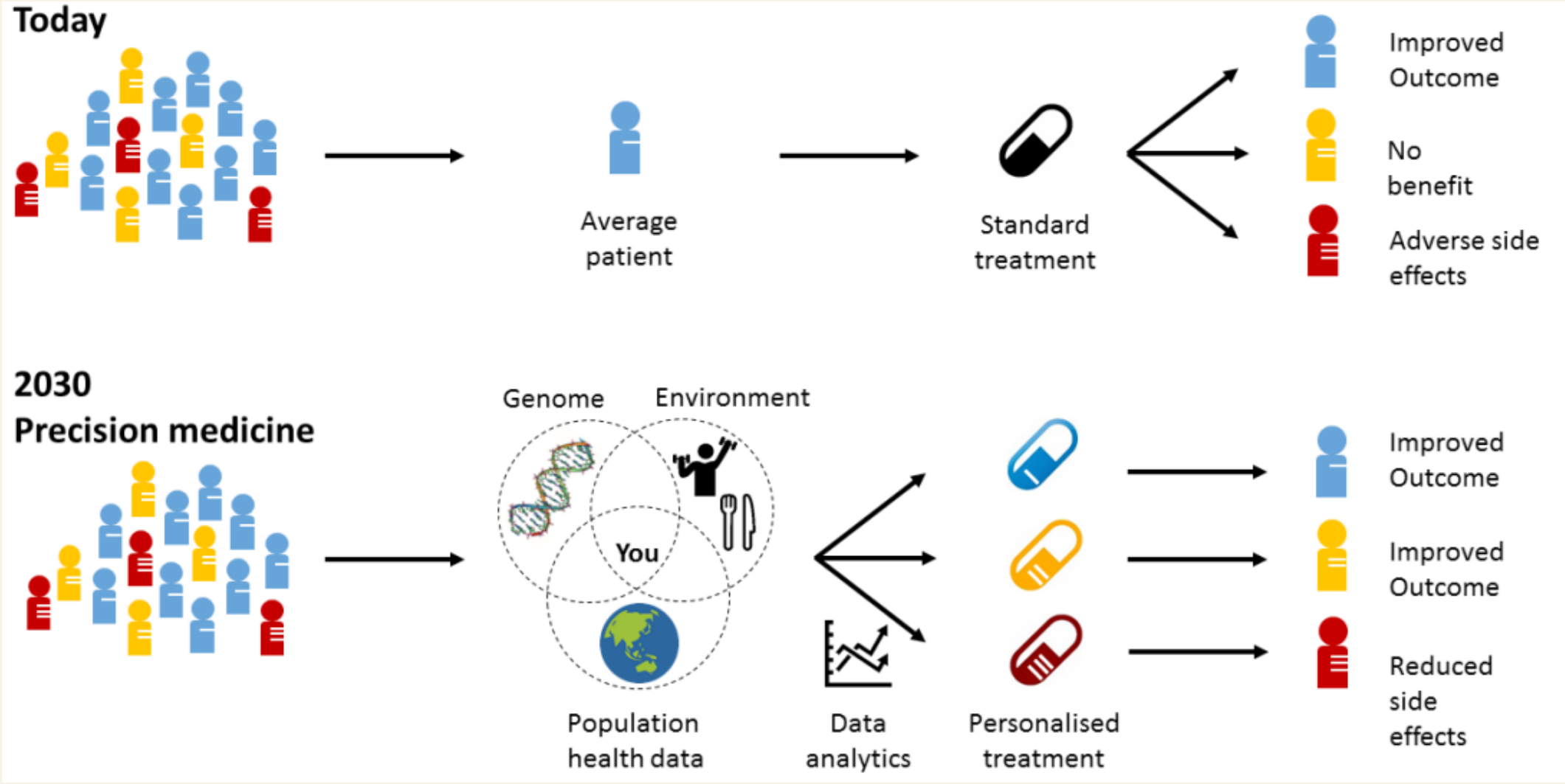
Has fundamentally changed the way we do biology

How has sequencing changed and is changing the world?

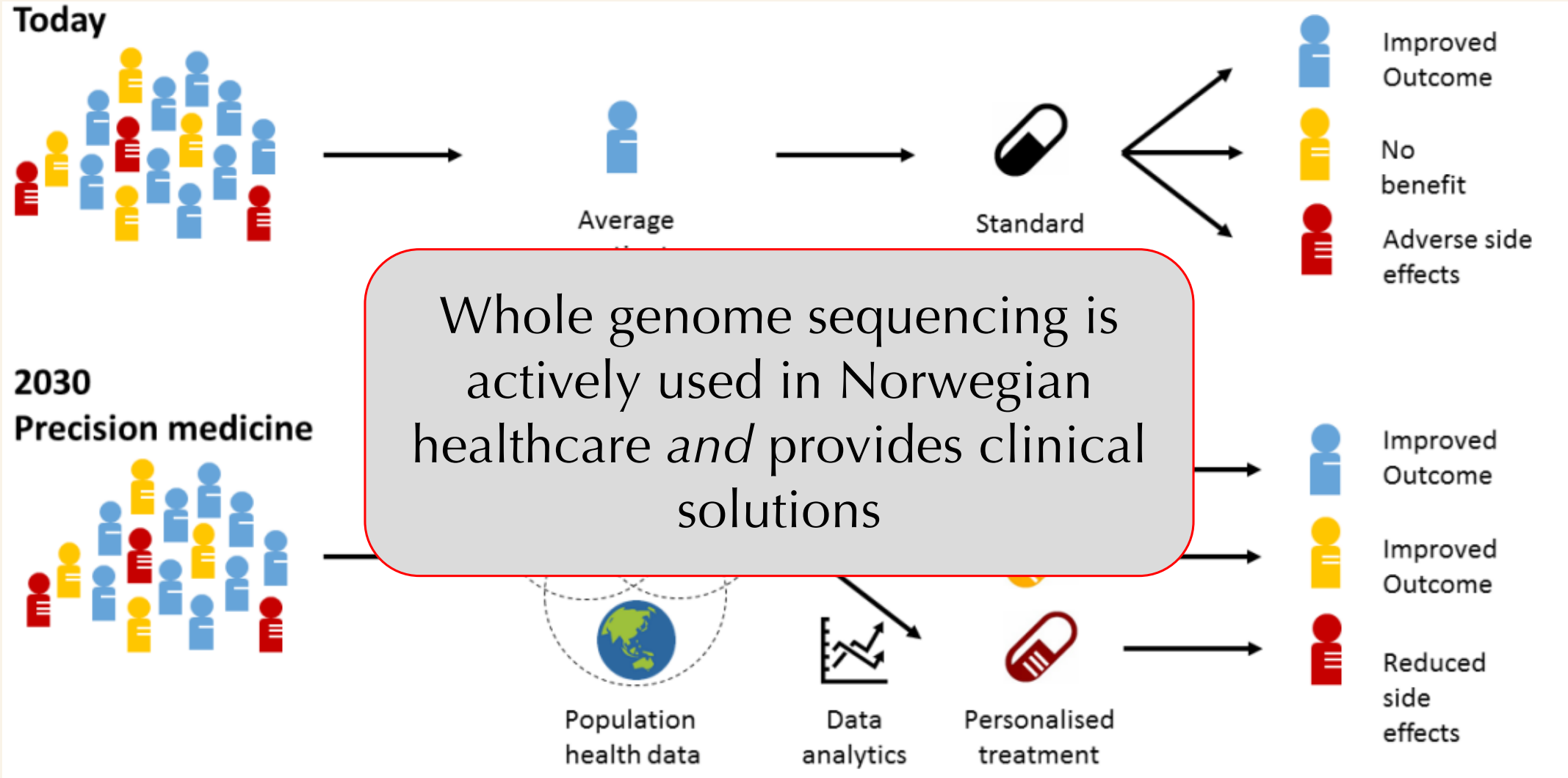
Changed healthcare

Sequencing (genome and exome) funded solely by *healthcare* systems

Dag Undlien (OUH)

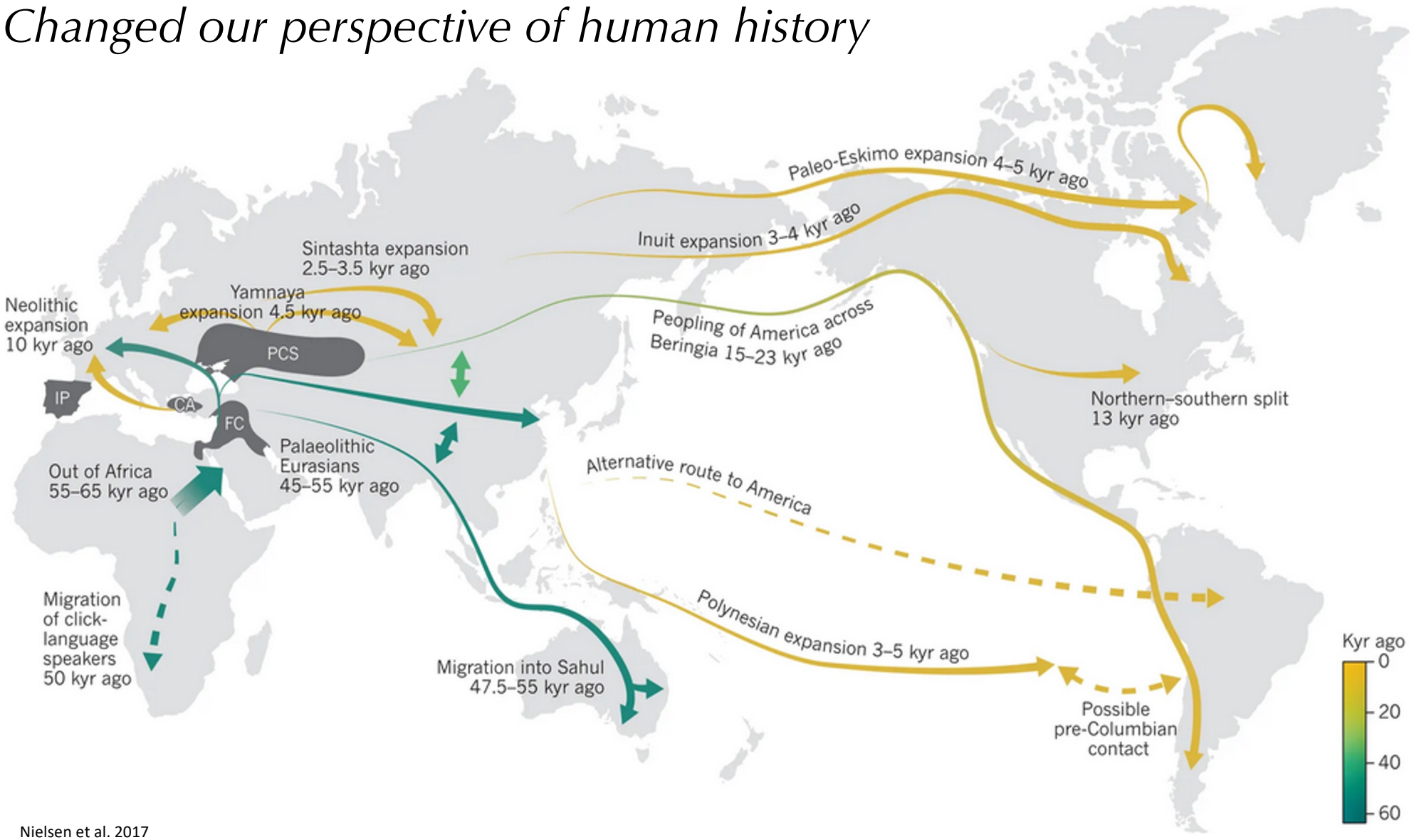


Dag Undlien (OUH)



Dag Undlien (OUH)

Changed our perspective of human history



Nielsen et al. 2017

Changed forensic capabilities

Using continuously expanding public genomic databases (e.g. 23 and me)…

The New York Times

Genealogists Turn to Cousins' DNA and Family Trees to Crack Five More Cold Cases

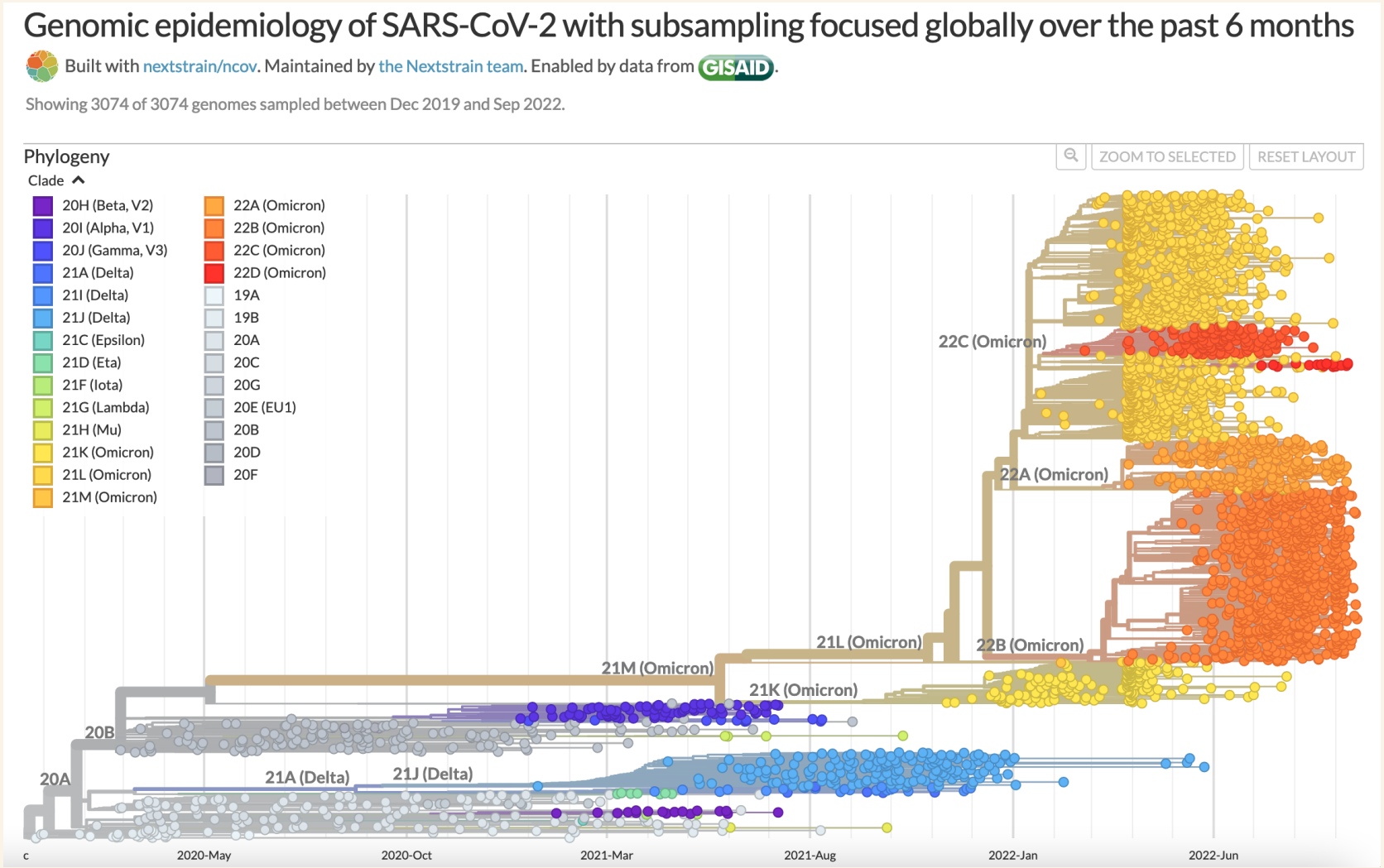
Police arrested a D.J. in Pennsylvania and a nurse in Washington State this week, the latest examples of the use of an open-source ancestry site since the break in the Golden State killer case.

Changed forensic capabilities

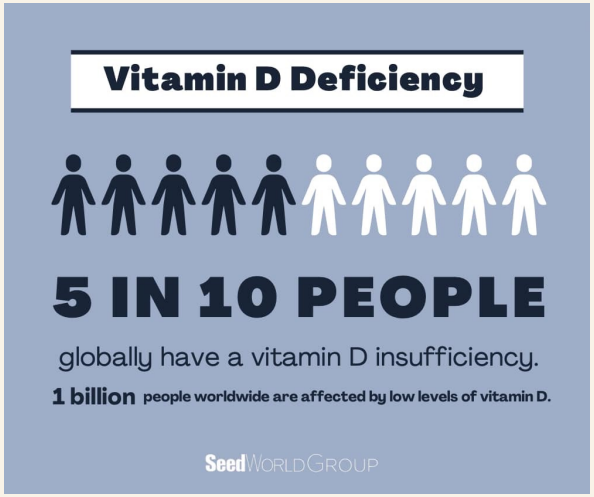
Or by the genetic testing of thousands of people!

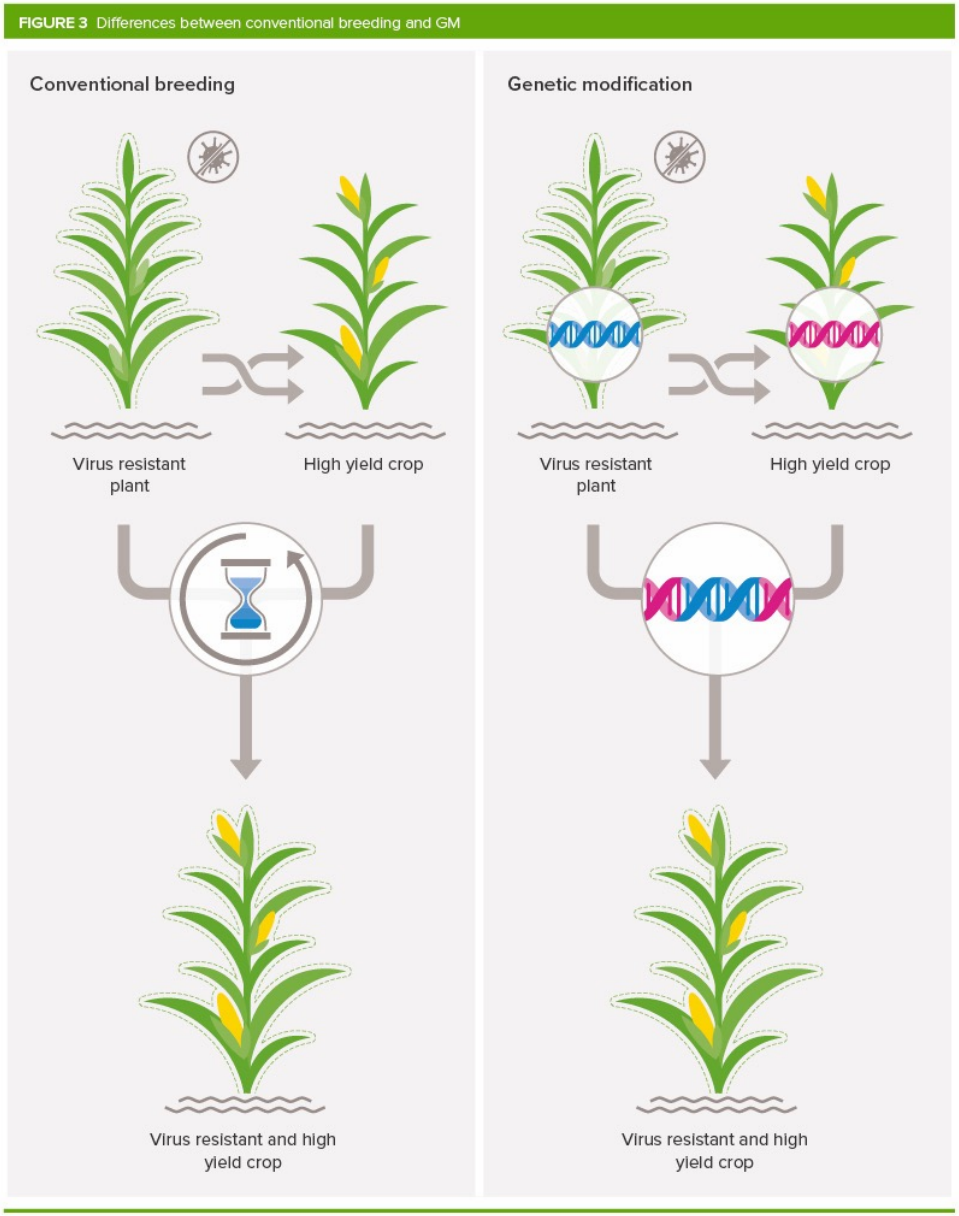
> As the *Times* reports, that law paved the way for a prosecutor in the Verstappen case to call for the voluntary DNA sampling of 21,500 Dutchmen, and the obligatory sampling of 1,500 men who were of "special interest" to investigators.
>
> The alleged killer, 55-year-old Jos Brech, was one of those 1,500 men who were mandated to provide a DNA sample. He never showed up. Dutch officials grew suspicious and took DNA samples from Brech's relatives. The results matched the DNA

Changed vaccine development and disease tracking

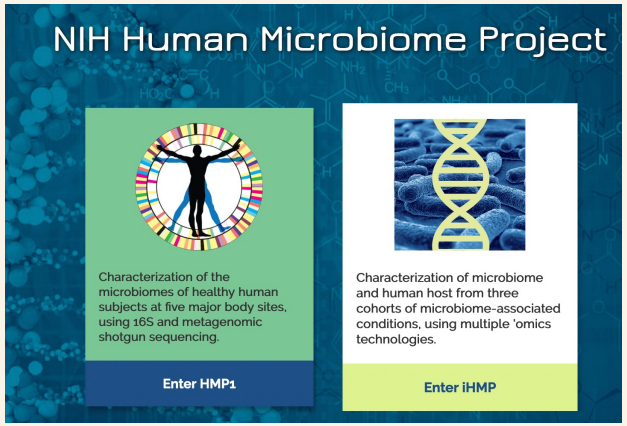


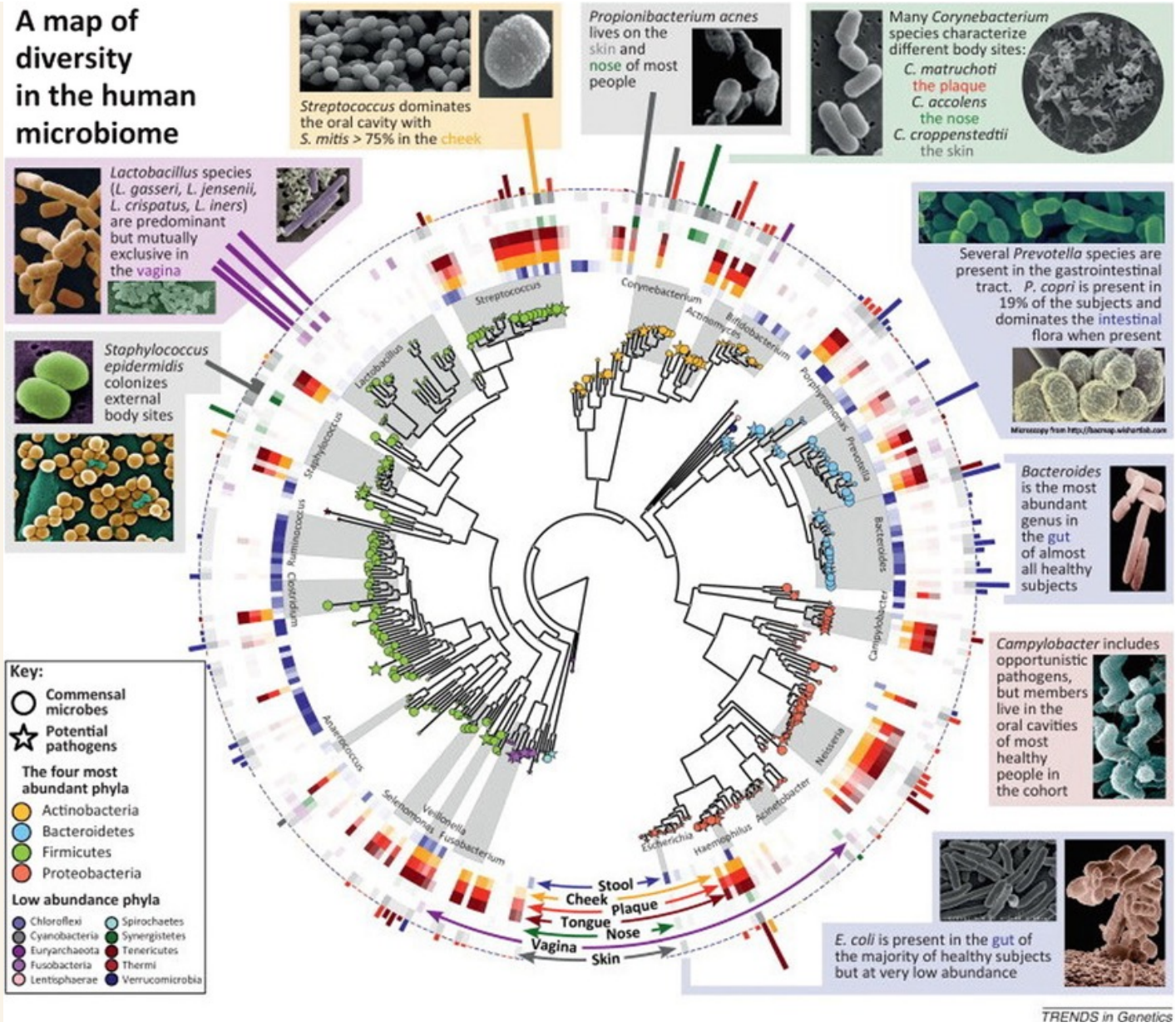
Changed improvement and selection of commercial crops



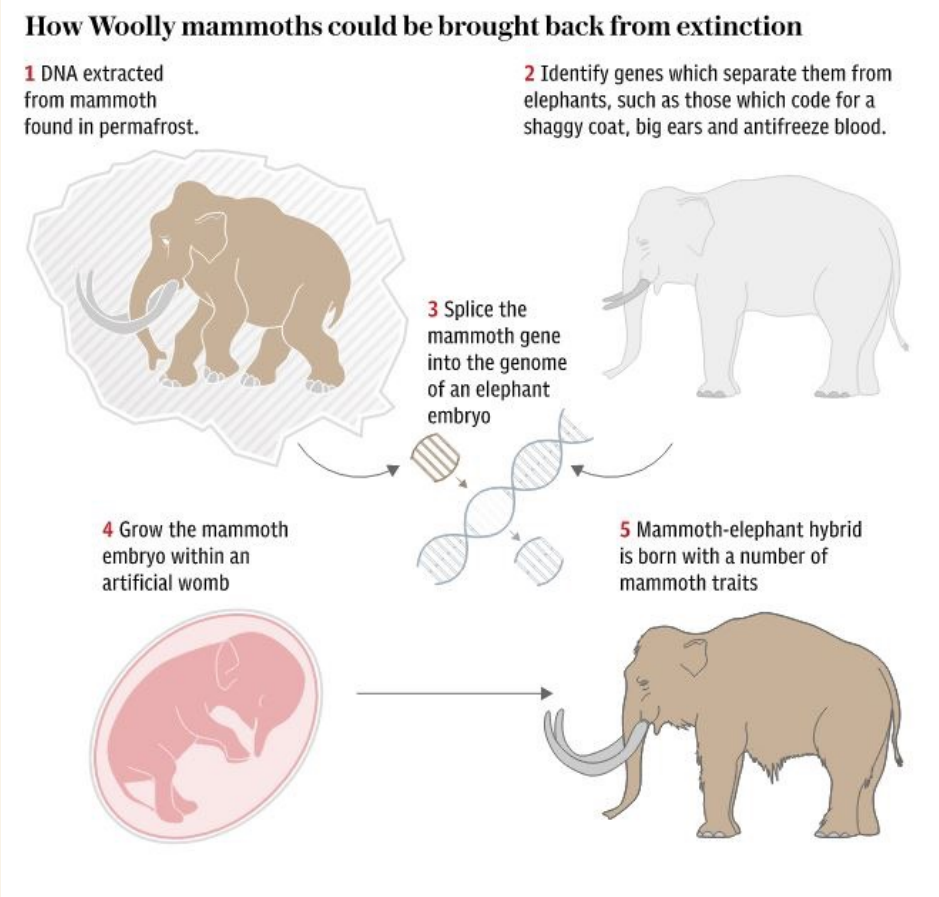


Changed our under-standing of the human microbiome

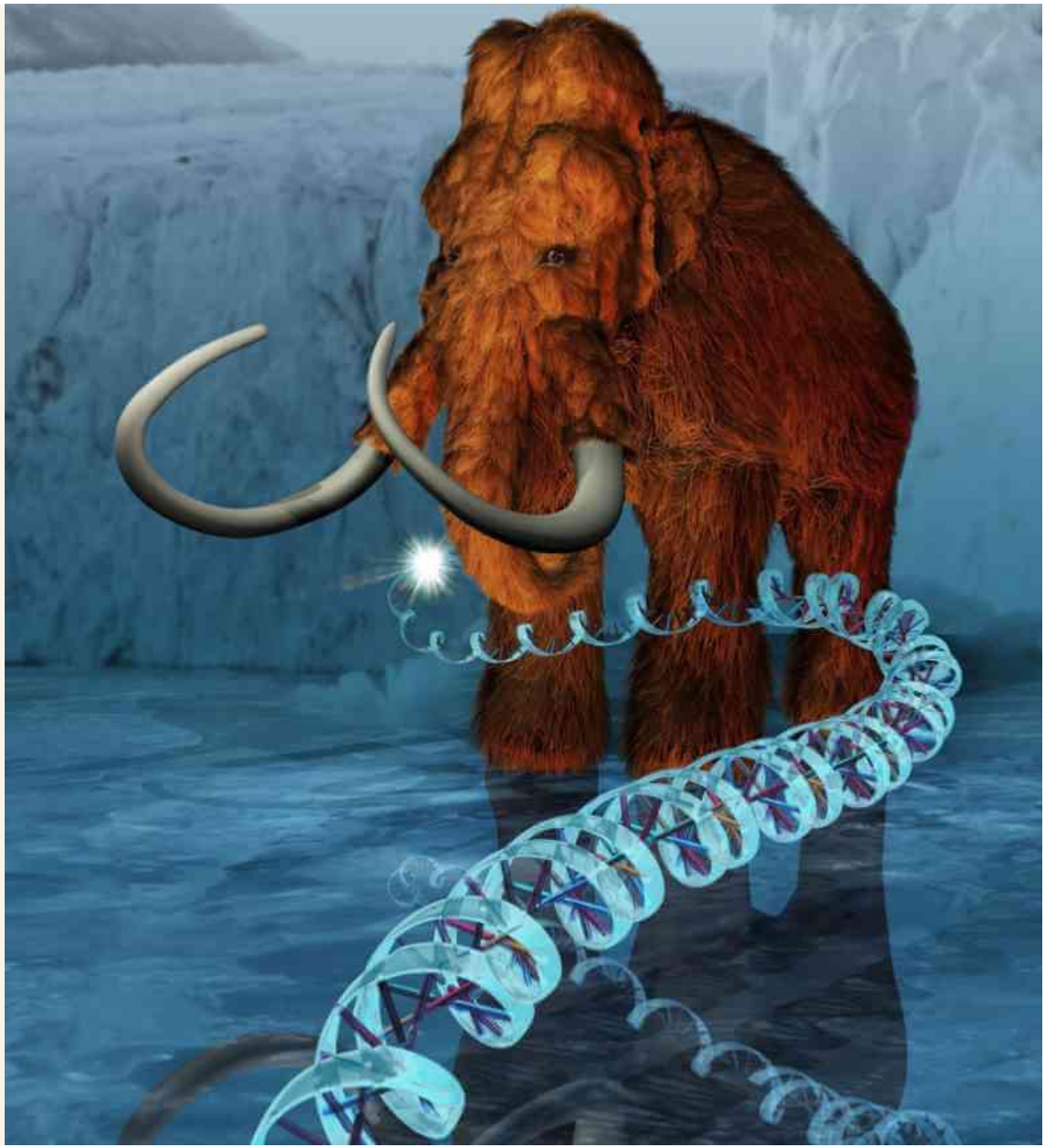




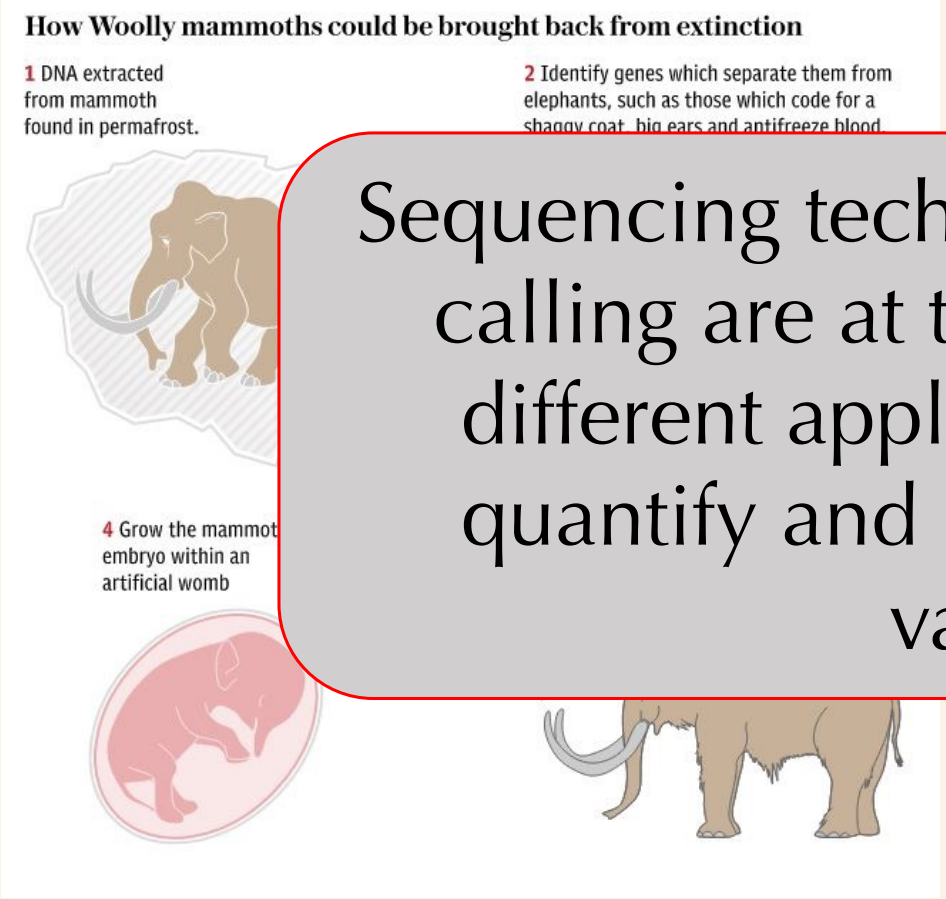
Changing our perspective of extinct species



https://instinctforfilm.com/feed/cloning-mammoths-global-warming/



Changing our perspective of extinct species



Sequencing technology and/or variant calling are at the basis of all these different applications in order to quantify and understand genetic variation

https://instinctforfilm.com/feed/cloning-mammoths-global-warming/

Available to high school students! 21.10 2022

NewScientist

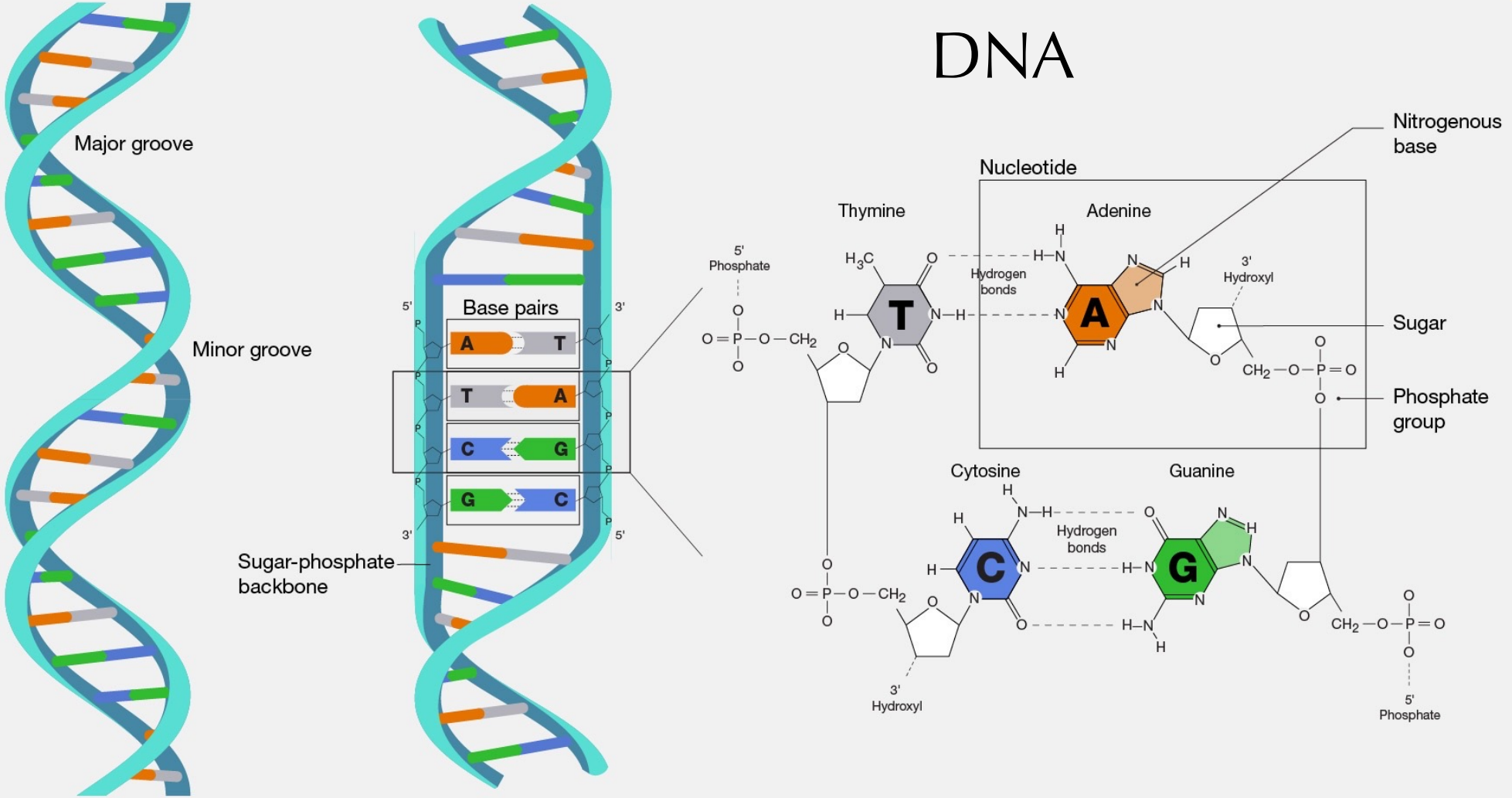
High school student is first to sequence the angelfish genome

17-year-old Indeever Madireddy sequenced the genome of his pet angelfish after it died – the first time this species has been sequenced

LIFE 21 October 2022

By Michael Le Page

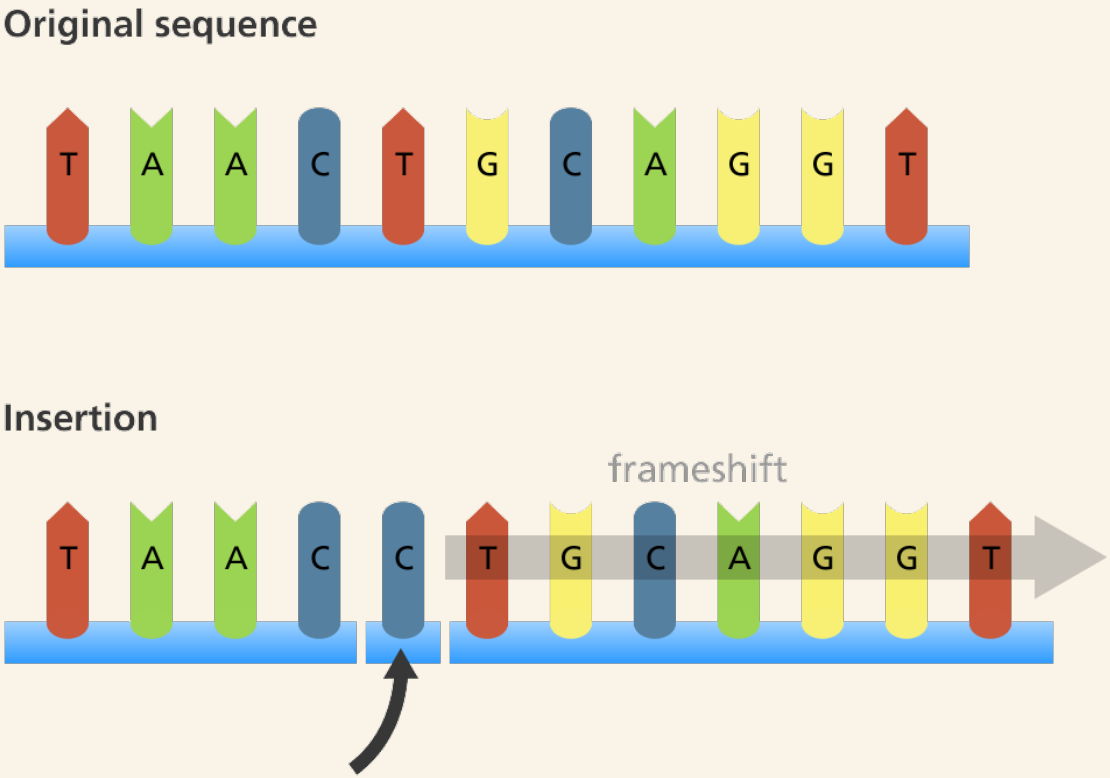
DNA


Major groove
Minor groove
Base pairs
A
T
T
A
C
G
G
C
5'
3'
Sugar-phosphate backbone
Nucleotide
Nitrogenous base
Thymine
Adenine
Cytosine
Guanine
Hydrogen bonds
Hydrogen bonds
5' Phosphate
3' Hydroxyl
Sugar
Phosphate group
3' Hydroxyl
5' Phosphate

What does genetic variation look like?

1) DNA (nucleotides) can be inserted or deleted (*indels*).

1) Insertion/Deletion (Indel)



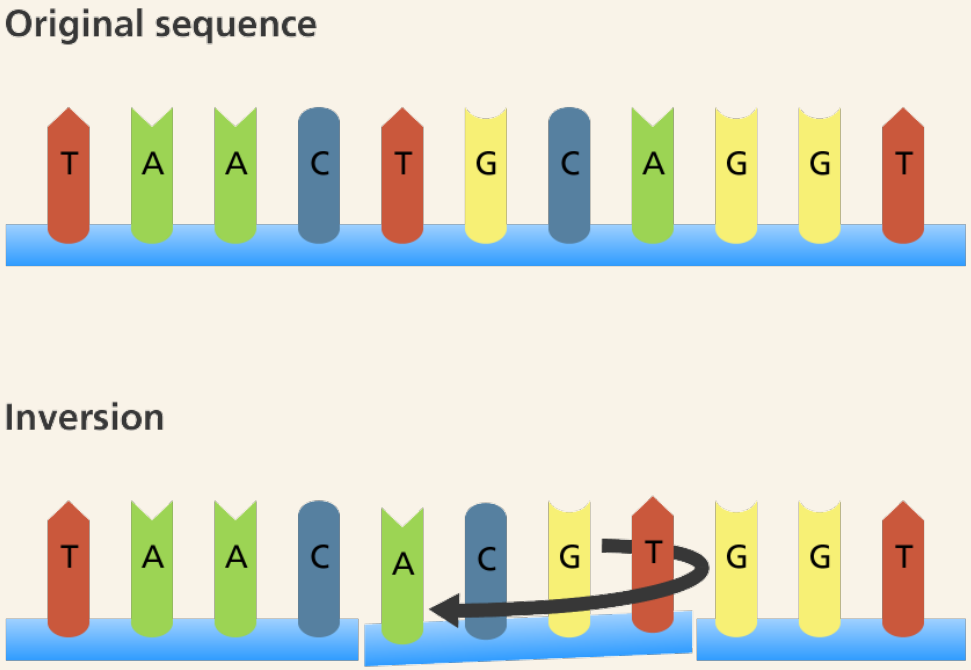
Can range from 1 base-pair (bp) to many bp

What does genetic variation look like?

1) DNA (nucleotides) can be inserted or deleted (*indels*).

2) DNA can be *structurally rearranged* (inversions/translocations)

2) Structural rearrangements (inversions/translocations)



Can be MILLIONS of bp long affecting the order of many genes simultaneously *(Supergenes)*

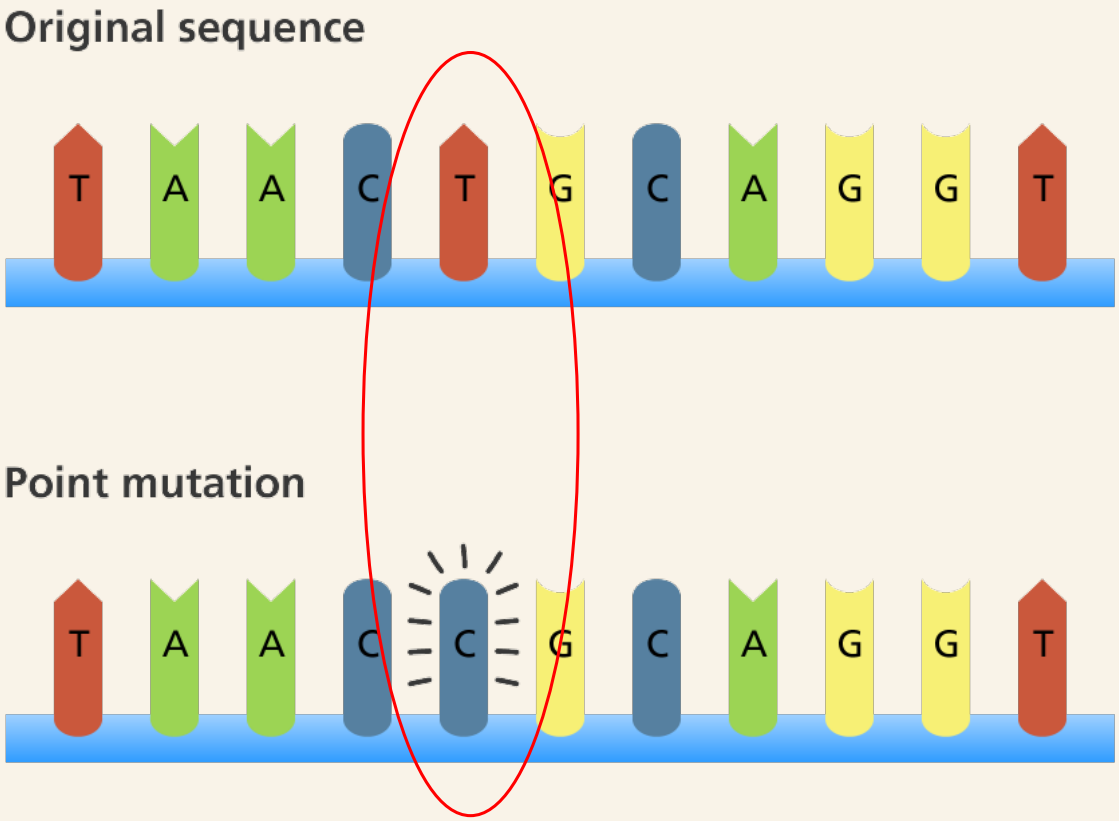
What does genetic variation look like?

1) DNA (nucleotides) can be inserted or deleted (*indels*).

2) DNA can be *structurally rearranged* (inversions/translocations)

3) DNA can be *altered* at a single base pair (Single Nucleotide Polymorphism or SNP)

3) Single Nucleotide Polymorphism (SNP)



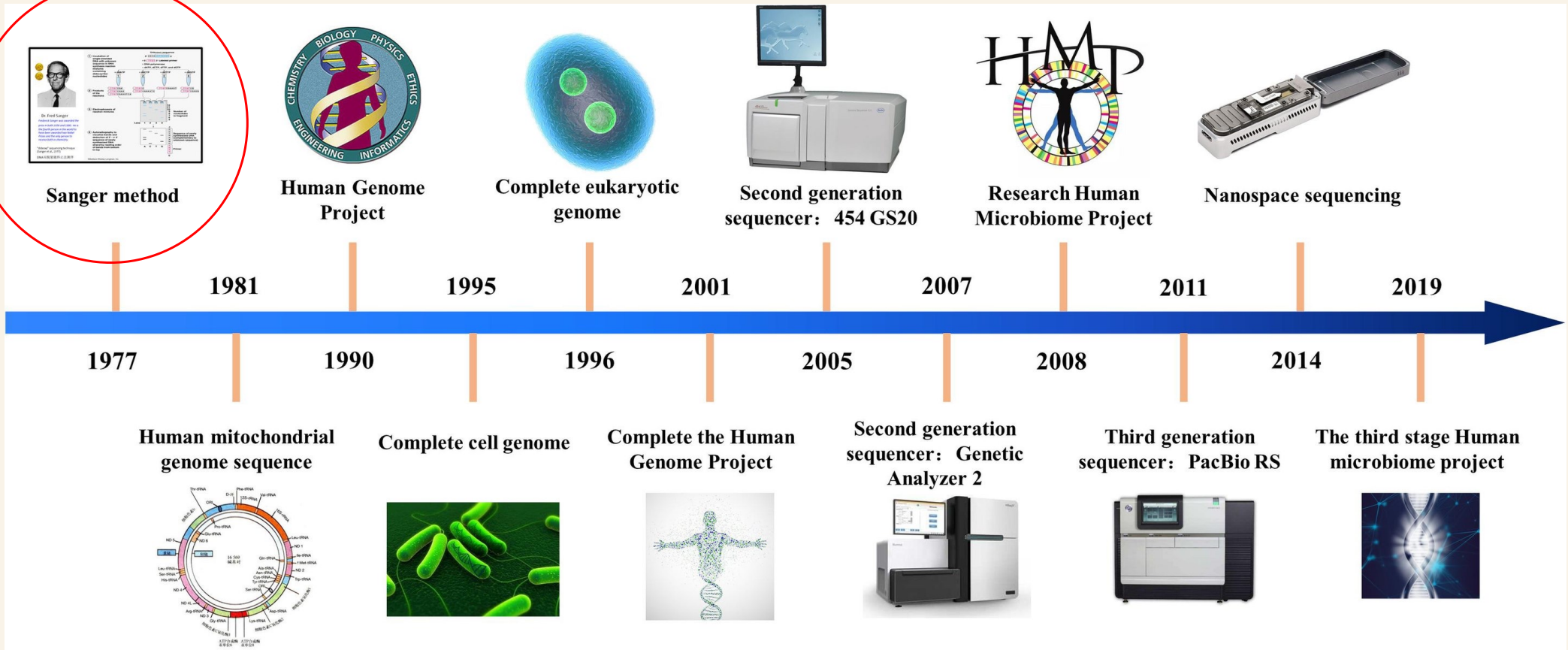
Extremely common

> 150 million known SNPs in humans (2015)

~100 SNPs unique in EVERY human

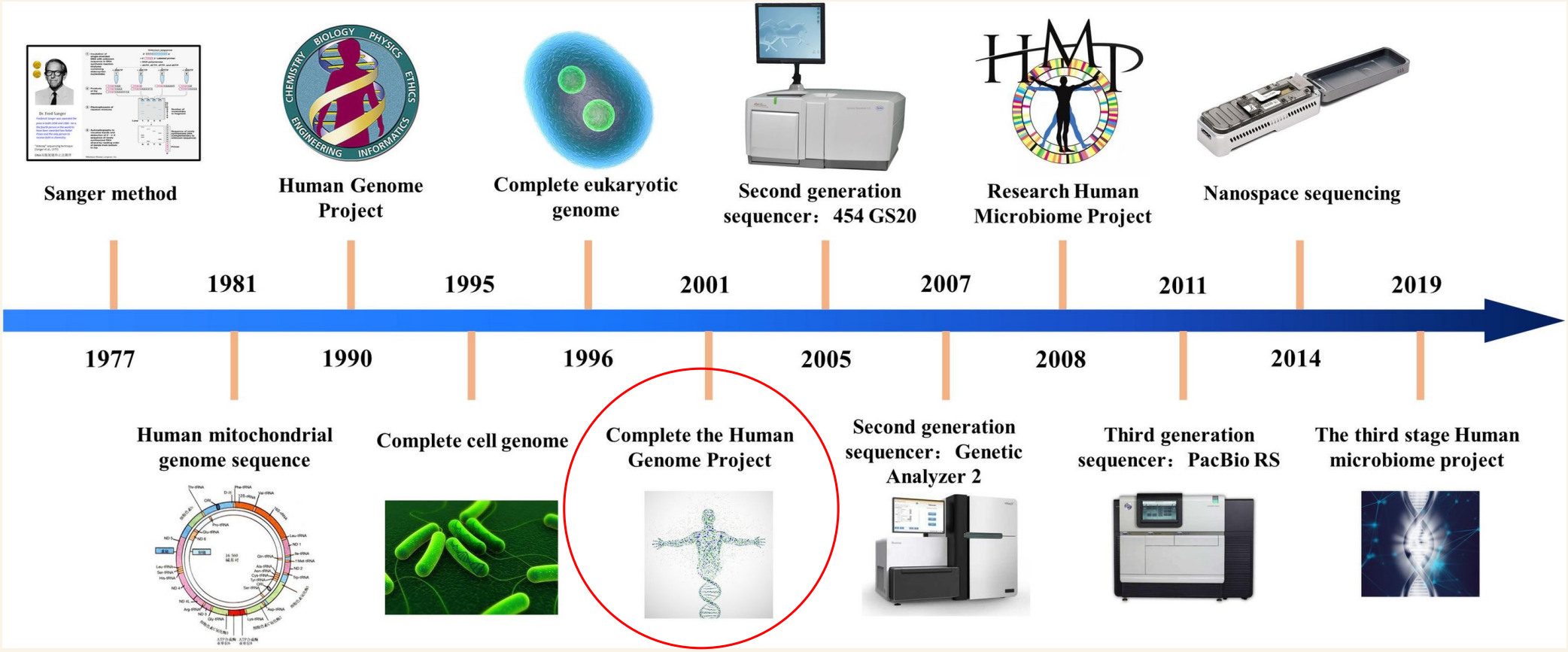
Putatively EVERY base in the human genome

How do we observe and quantify genetic variation?



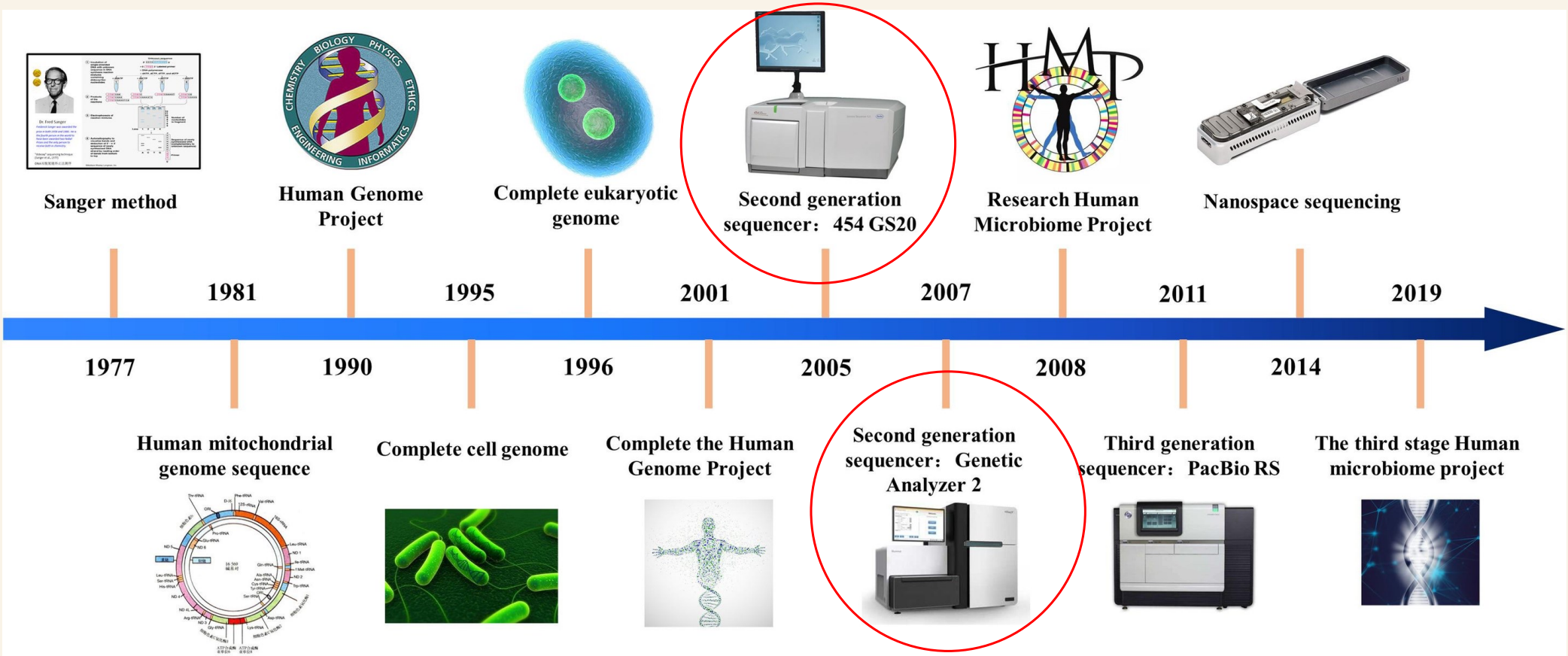
Sanger sequencing – leading sequencing technology for decades

How do we observe and quantify genetic variation?



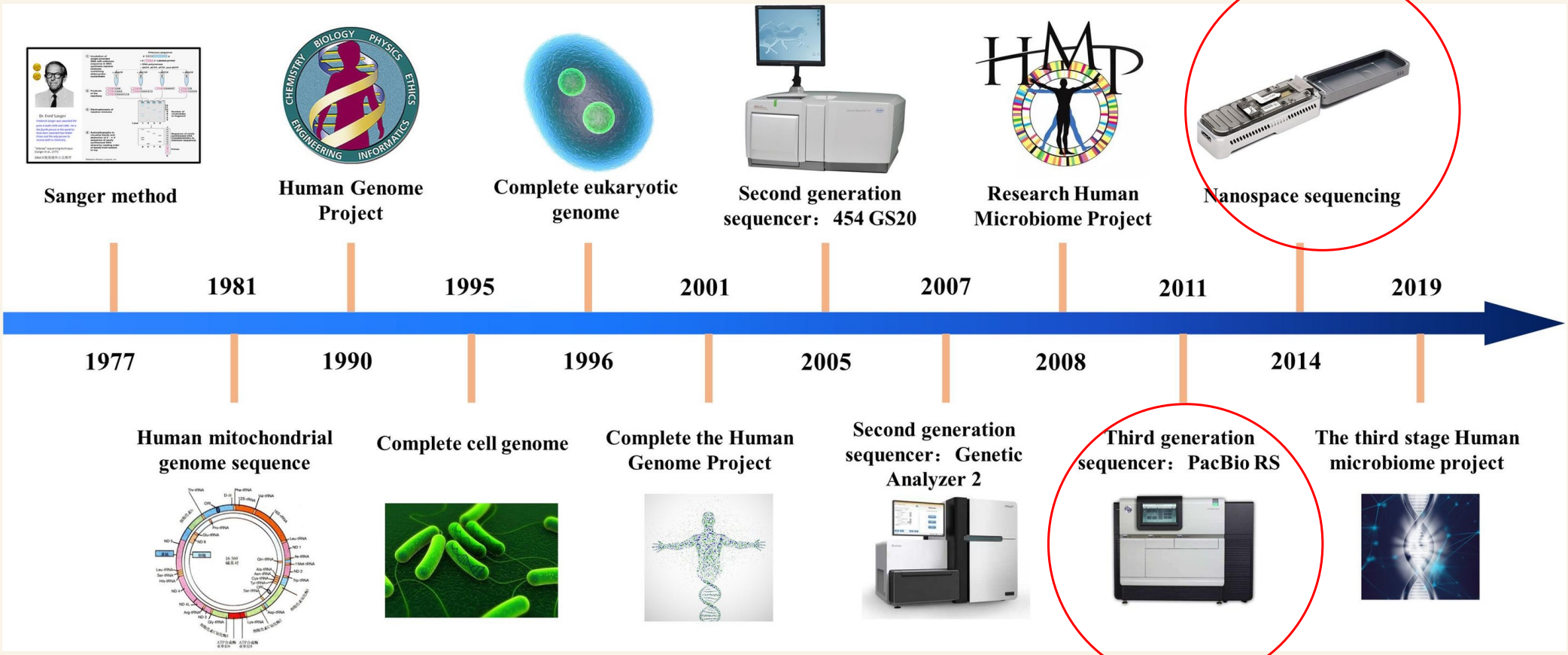
Human genome project: sparked a novel industry

How do we observe and quantify genetic variation?



“New” sequencing technologies (already outdated!)

How do we observe and quantify genetic variation?



Latest sequencing technologies that focus on long read sequencing

Two dominant technologies today

PacBio
Long read length (10k bp +)
More expensive
Specific applications

Two dominant technologies today

PacBio
Long read length (10k bp +)
More expensive
Specific applications

Illumina
Short read length (150-250 bp)
Cheap!
Workhorse of sequencing

Practical considerations: size matters!

PacBio
Long read length (10k bp +)
More expensive
Specific applications

Illumina
Short read length (150-250 bp)
Cheap!
Workhorse of sequencing

What variation can you assess with these different types of reads?

Type of variant	**Short reads**	**Long reads**
Indel	Only if small (~few bp)	Yes
Structural (inversion)	Difficult	Yes
SNP	Yes	Yes

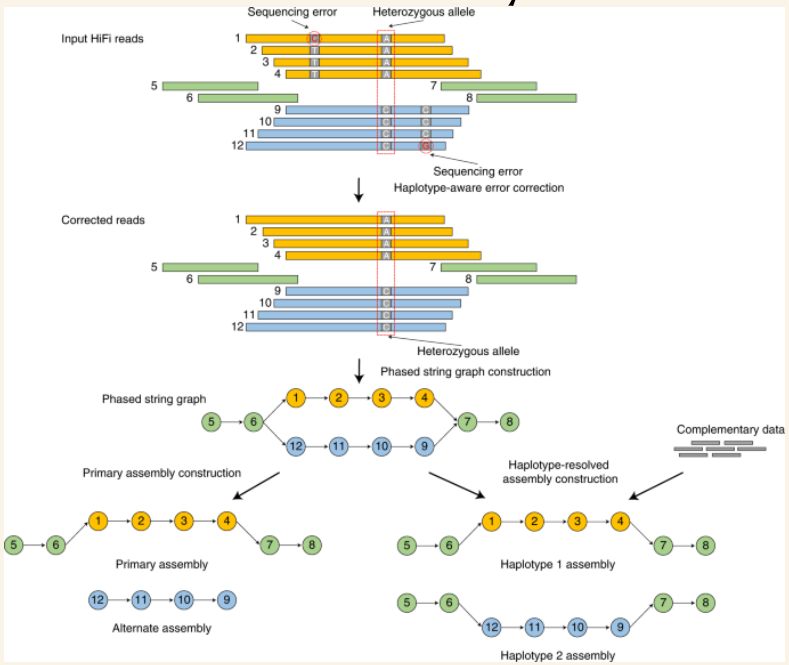
What variation can you assess with these different types of reads?

Type of variant	**Short reads**	**Long reads**
Indel	Only if small (~few bp)	Yes
Structural (inversion)	Difficult	Yes
SNP	Yes	Yes

Illumina ***re-sequencing*** domination means that SNPs are most reliably targeted and are most studied type of genetic variation

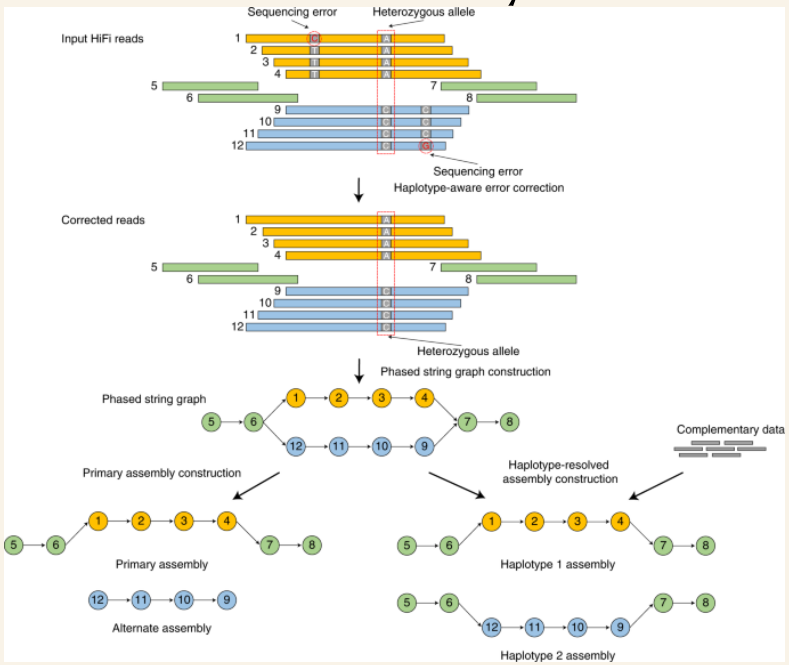
Yet there *are* different ways of assessing genetic variation

i.e. *de novo* haplotype aware assembly

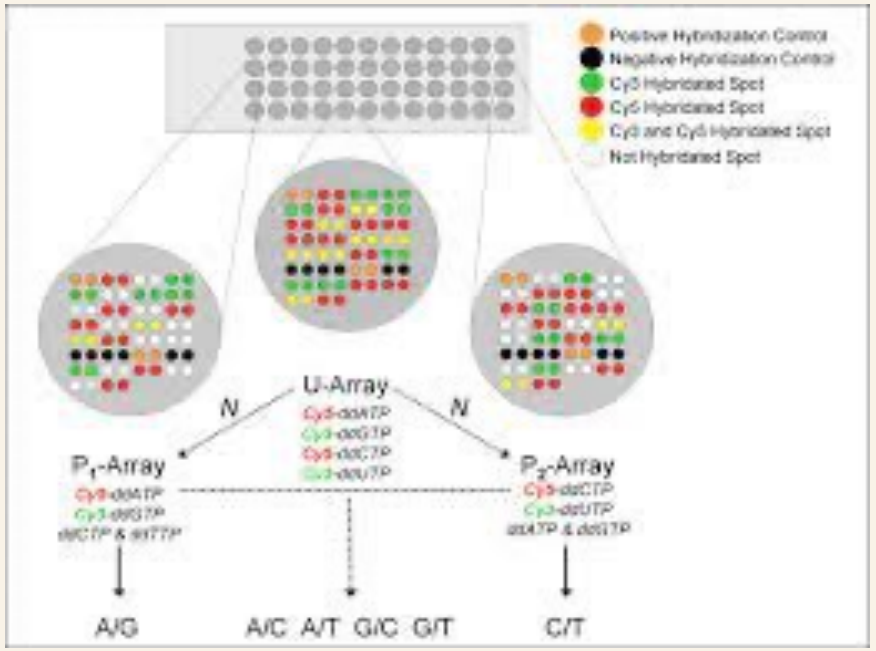


Yet there *are* different ways of assessing genetic variation

i.e. *de novo* haplotype aware assembly



SNP Chip/DNA micro array genotyping

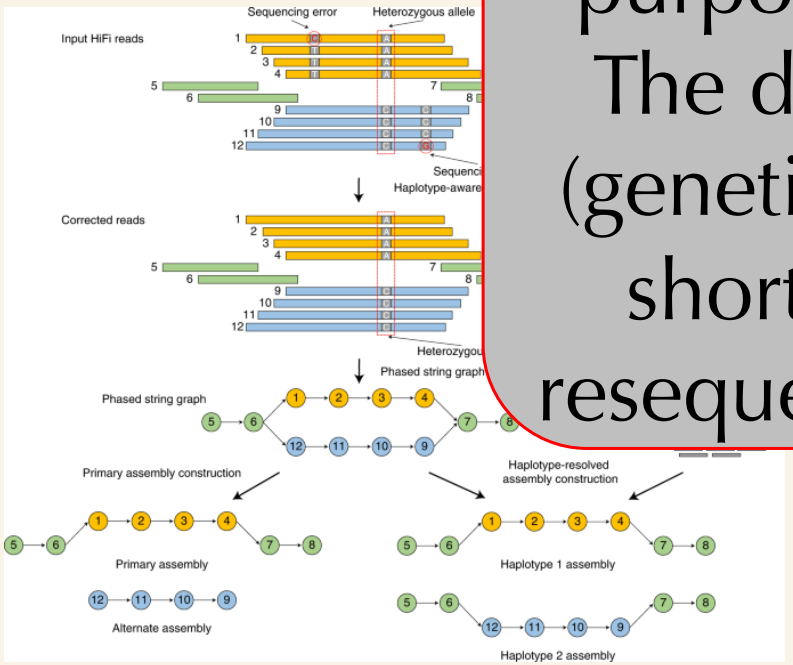


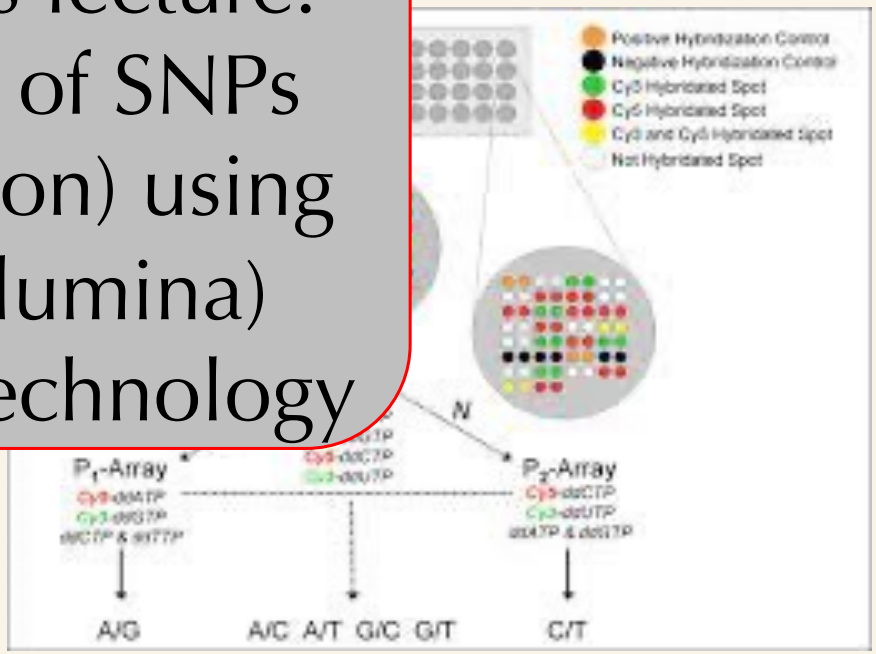
Yet there *are* different ways of assessing genetic variation

i.e. *de novo* haplotype a… assemb…

…P Chip …typing

Variant calling for purpose of this lecture: The detection of SNPs (genetic variation) using short read (Illumina) resequencing technology





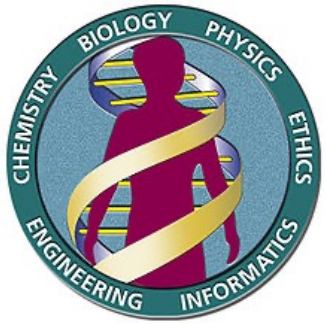
Questions?


WHO? WHERE?
WHEN? WHY? HOW?
WHAT? WHO?
HOW? WHERE?
WHO? WHERE?
WHAT?
WHY? WHAT?
WHEN?
WHERE? WHO?
HOW? WHAT?
WHO? WHERE?
WHY?
WHO? WHEN?
WHEN? WHAT?
WHO? WHY? HOW?
HOW? WHERE?
WHAT? WHY?
WHEN?
WHO? WHAT?
HOW?
WHAT? WHEN?
WHERE? WHY?
HOW? WHEN?
WHAT? WHO?
WHY? WHERE?
WHEN? HOW?
WHERE?

2) Variant calling pipelines/methods and limitations

2) Variant calling pipelines/methods and limitations

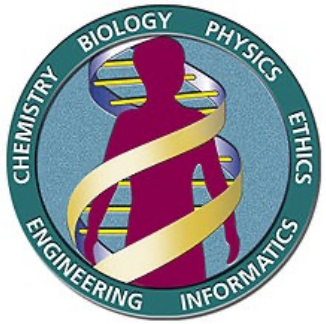
Variant calling **always** starts with a reference genome



Assembly of the first complex vertebrate genome
Human genome assembly project (2003)
Not easily repeated: it was massive task
Nowadays; much cheaper and faster

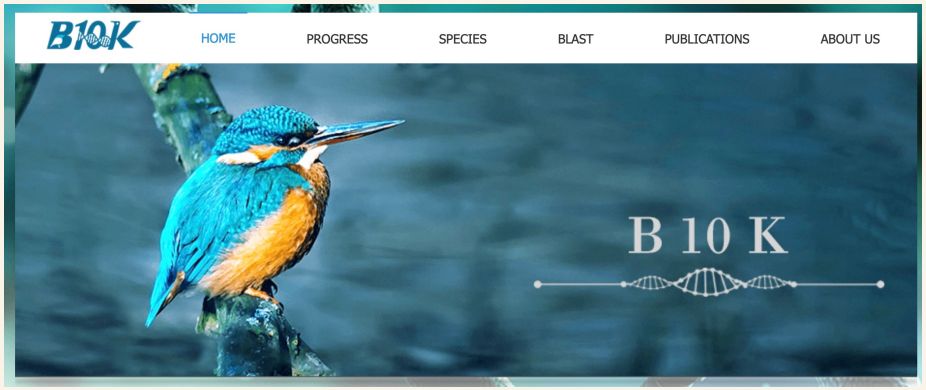
2) Variant calling pipelines/methods and limitations

Variant calling **always** starts with a reference genome



Assembly of the first complex vertebrate genome
Human genome assembly project (2003)
Not easily repeated: it was massive task
Nowadays; much cheaper and faster

Great push to provide reference genomes for many organisms!



B10 K:
10.000 bird genomes

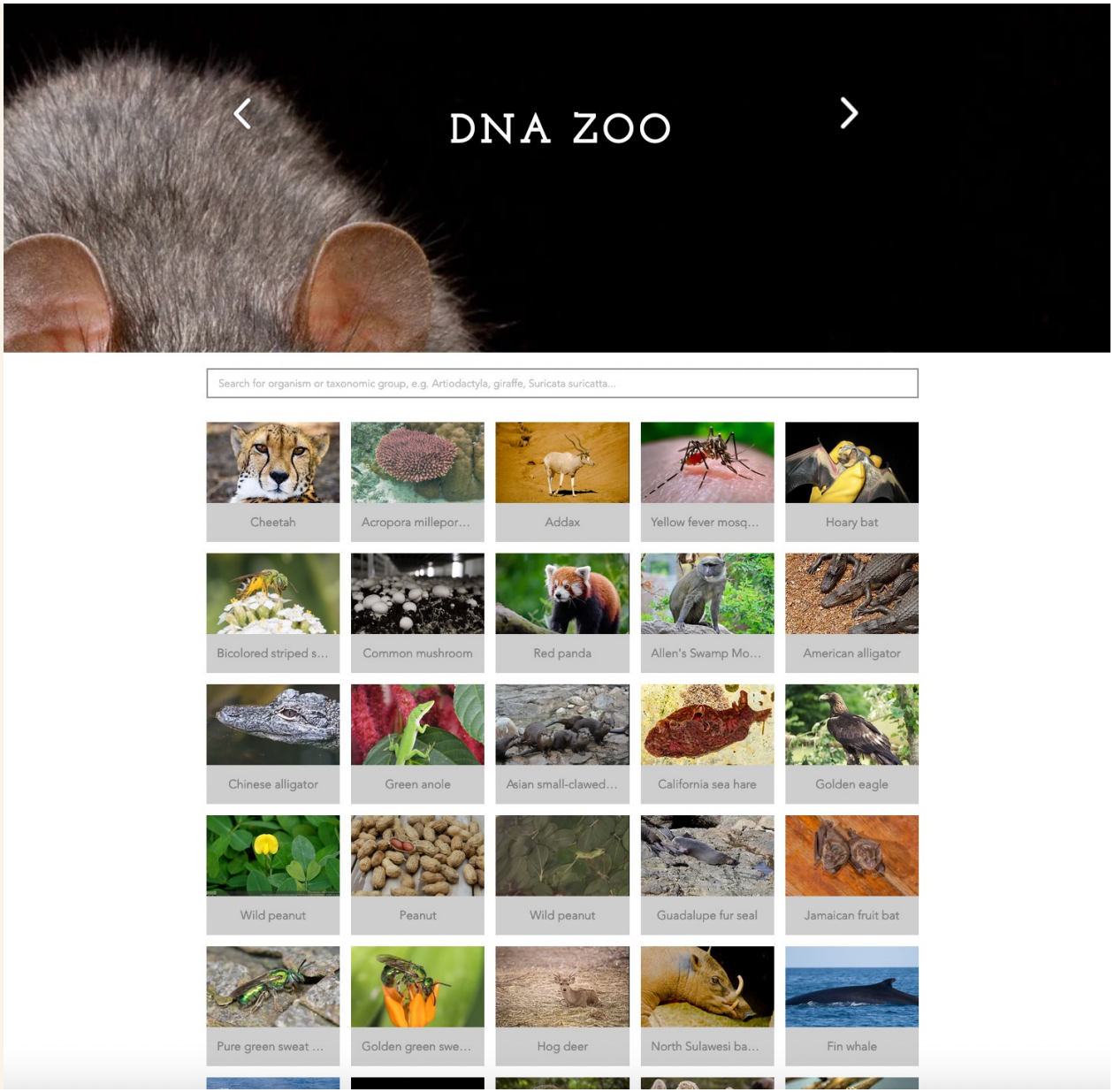
Deep evolutionary understanding of the entire living avian class

https://b10k.genomics.cn/

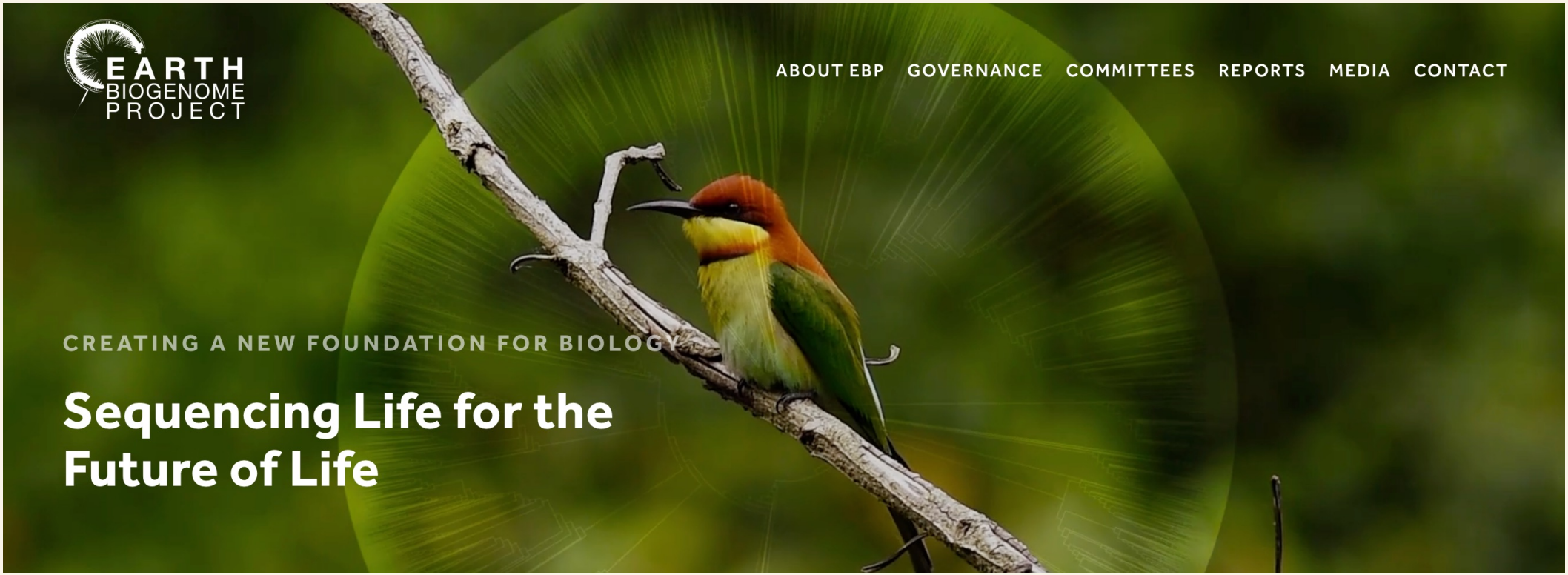
The *DNA Zoo*

facilitates conservation efforts by releasing high-quality genomics resources.

https://www.dnazoo.org



The most ambitious: Earth Biogenome Project



https://www.earthbiogenome.org/

The most ambitious: Earth Biogenome Project

EBP: moonshot for biology, aims to characterize the genomes of all of Earth's eukaryotic biodiversity over a period of ten years.

https://www.earthbiogenome.org/

The most ambitious: Earth Biogenome Project

EBP: moonshot for biology, aims to characterize the genomes of all of Earth's eukaryotic biodiversity over a period of ten years.

The vision: to create a new foundation for biology, with new solutions for preserving biodiversity and sustaining human societies.

https://www.earthbiogenome.org/

But what is a reference genome?

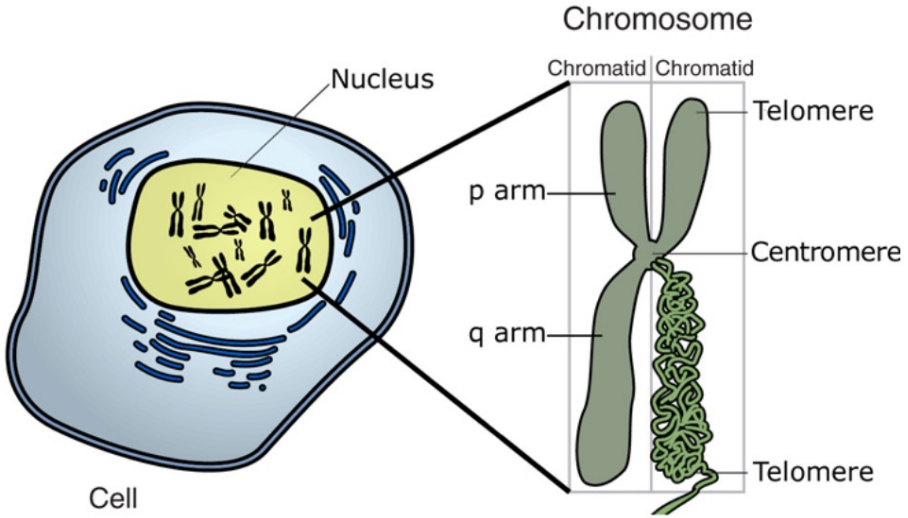
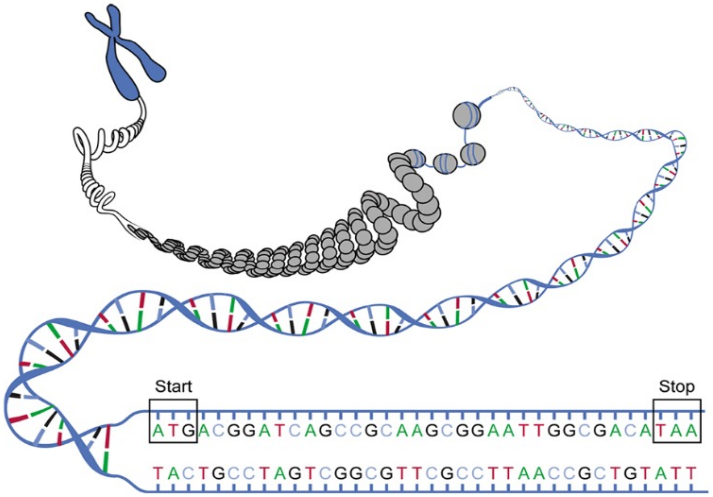
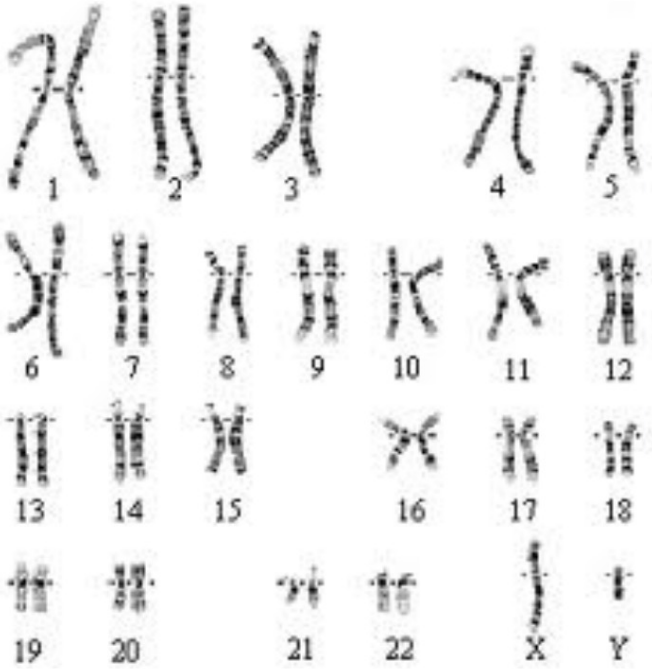


Image adapted from: National Human Genome Research Institute.





But what is a reference genome?

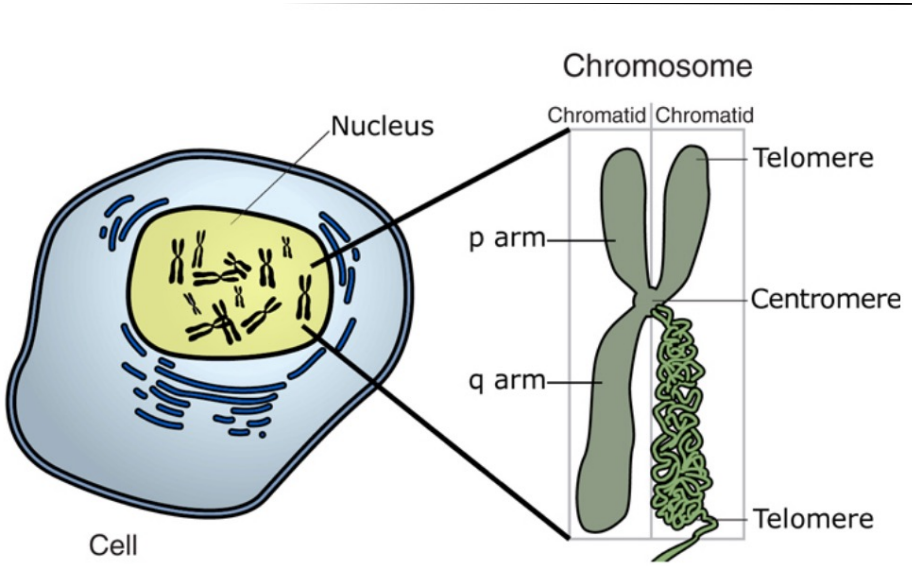
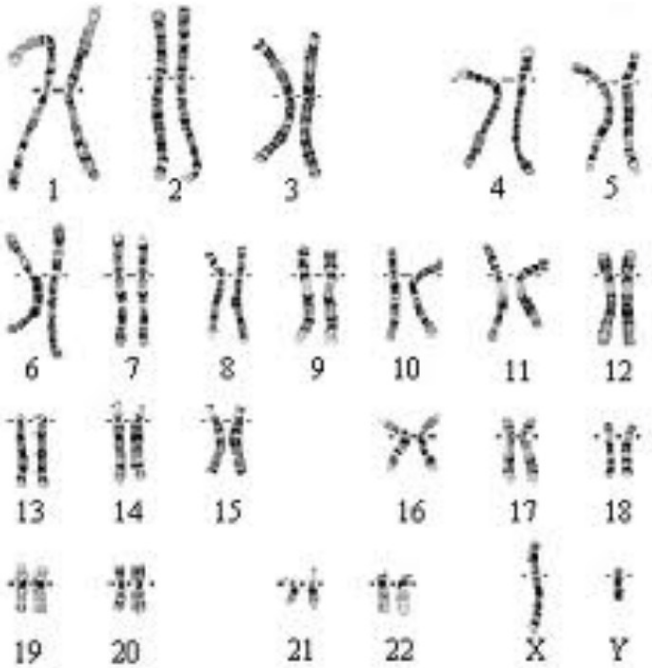
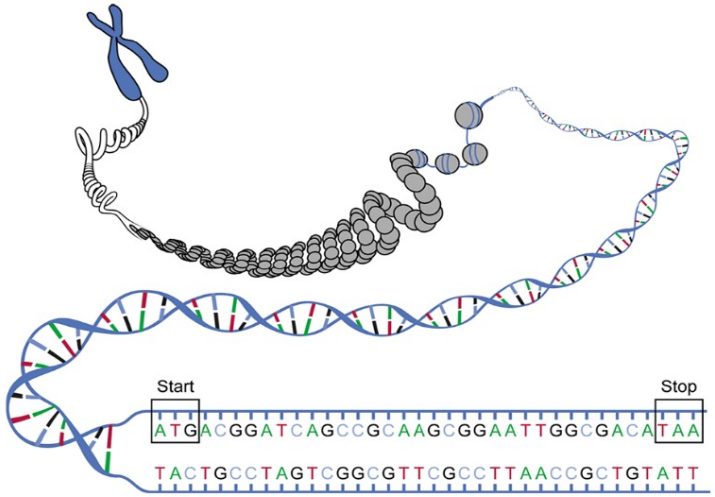


Image adapted from: National Human Genome Research Institute.





Digital representation / abstraction of a physical, biological phenomenon

A reference genome is …

Usually from a single individual

Result of a genome assembly process -> errors are introduced

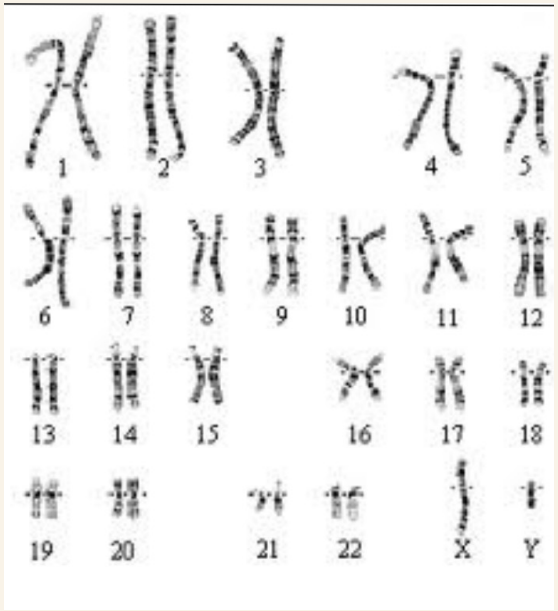
Of varying quality, that can vary from organism to organism

A reference genome is …

Usually from a single individual

Result of a genome assembly process -> errors are introduced

Of varying quality, that can vary from organism to organism



Sequencing and assembly

Digital version of the genome

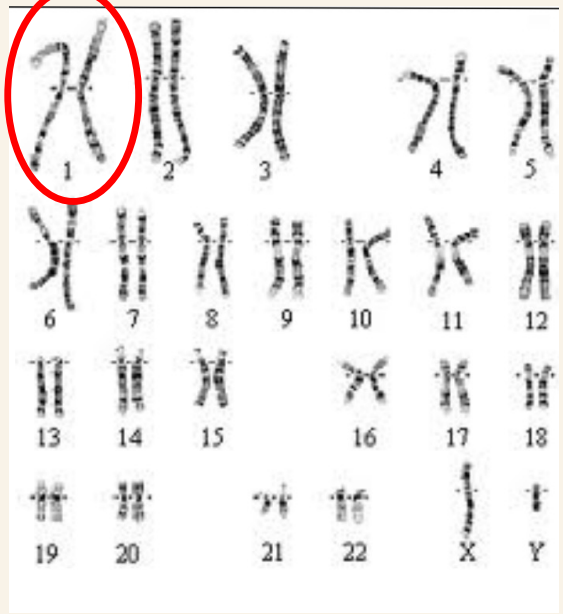
```
>Chr01
ACTACGTATATAGCATGATCATGCATGATACATGGCTAGT…
>Chr02
ATCATGCATGATACATGGCTAGTACTACGTATATAGCATG…
>Chr03
ATGATCATGCATGATAACTACGTATATAGCCATGGCTAGT…
>CHr04
CGTATATAGCATGATCATGACTACATGATACATGGCTAGT…
… …
```

A reference genome is …

Usually from a single individual

Result of a genome assembly process -> errors are introduced

Of varying quality, that can vary from organism to organism



Sequencing and assembly

Digital version of the genome

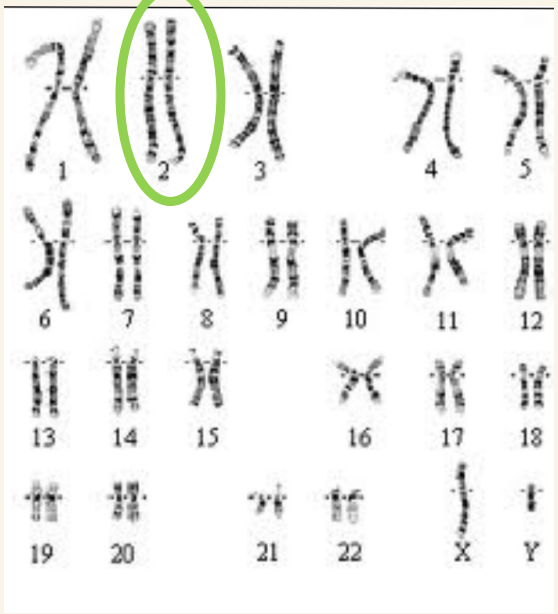
```
>Chr01
ACTACGTATATAGCATGATCATGCATGATACATGGCTAGT…
>Chr02
ATCATGCATGATACATGGCTAGTACTACGTATATAGCATG…
>Chr03
ATGATCATGCATGATAACTACGTATATAGCCATGGCTAGT…
>CHr04
CGTATATAGCATGATCATGACTACATGATACATGGCTAGT…
… …
```

A reference genome is …

Usually from a single individual

Result of a genome assembly process -> errors are introduced

Of varying quality, that can vary from organism to organism



Sequencing and assembly

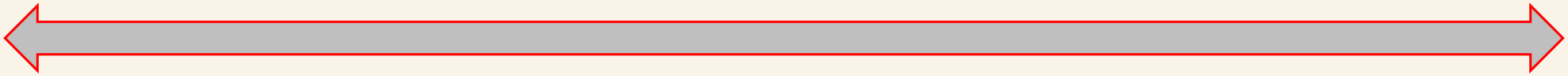
Digital version of the genome

```
>Chr01
ACTACGTATATAGCATGATCATGCATGATACATGGCTAGT…
>Chr02
ATCATGCATGATACATGGCTAGTACTACGTATATAGCATG…
>Chr03
ATGATCATGCATGATAACTACGTATATAGCCATGGCTAGT…
>CHr04
CGTATATAGCATGATCATGACTACATGATACATGGCTAGT…
… …
```

Quality scale of reference genomes

Poor

Good

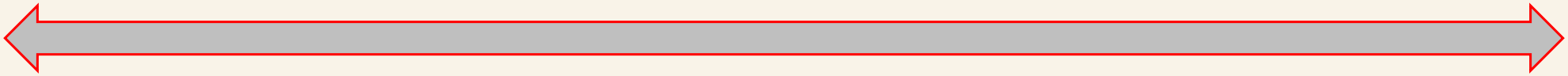


Chromosomes unclear
Thousands of loose fragments
Gaps (*nnnnn*) in sequences
Missing nucleotides

Quality scale of reference genomes

Poor

Good



Chromosomes unclear
Thousands of loose fragments
Gaps (*nnnnn*) in sequences
Missing nucleotides

Chromosomes resolved
Continuous sequences
No gaps
Most nucleotides covered, including centromeres and repetitive regions

A reference genome has a 2D coordinate system

```
>Chr01
ACTACGTATATAGCATGATCATGCATGATGATCATGCATGATACATGGCTAGT…
123456789……
```

Millions of nucleotides/bases

Note: some different coordinate systems exist (i.e. starting at 0 or 1)
or using the base or space as “location”

A reference genome has a 2D coordinate system

```
>Chr01
ACTACGTATATAGCATGATCATGCATGATGATCATGCATGATACATGGCTAGT…
123456789……
```

Note: some different coordinate systems exist (i.e. starting at 0 or 1)
or using the base or space as “location”

i.e.
```
A-C-T-A-C-G-T-A
1 2 3 4 5 6 7 8
  1 2 3 4 5 6 7
```

Such different systems are usually automatically recognized by different software

A reference genome has a 2D coordinate system

>Chr01

ACTACC…TAGT…

123456

This is all great, but where does the variant calling come in?

Note: sc

or using

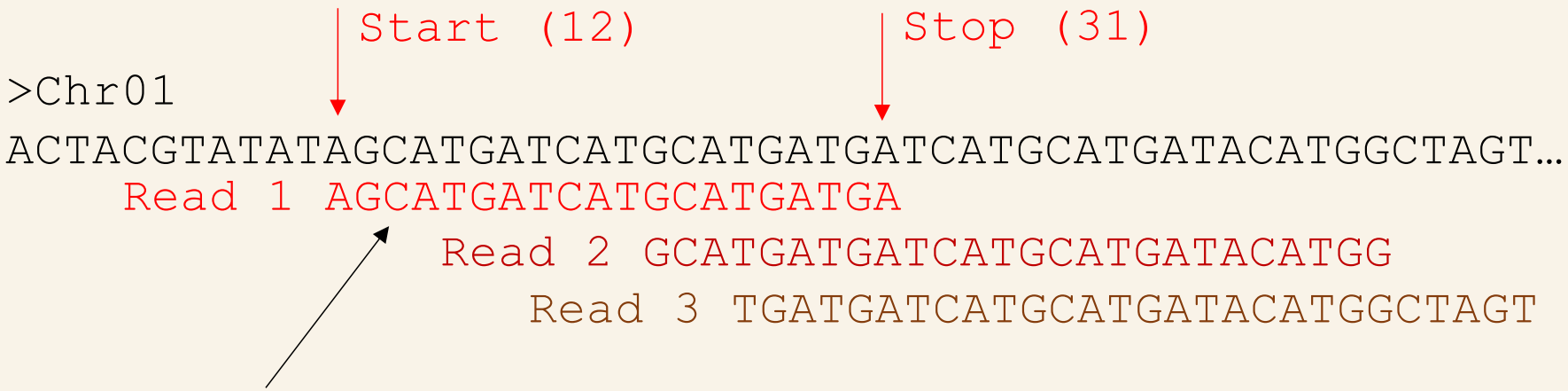
i.e. A-C-T A C G T A

1 2 3 4 5 6 7 8

1 2 3 4 5 6 7

Such different systems are usually automatically recognized by different software

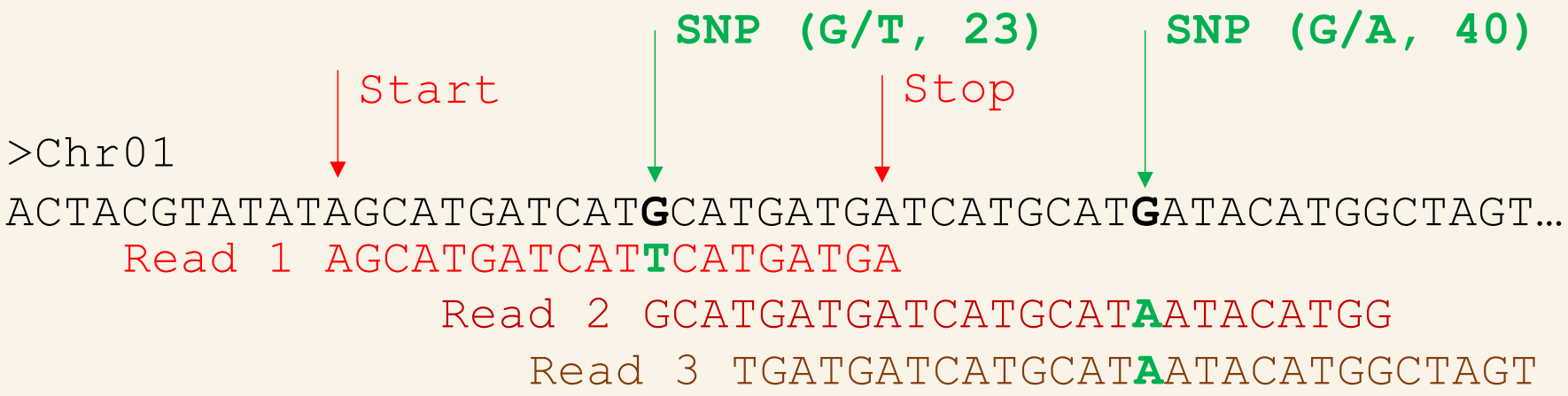
A multiple alignment towards a reference



Short read sequencing data is compared to the reference (looking for a ″match″)

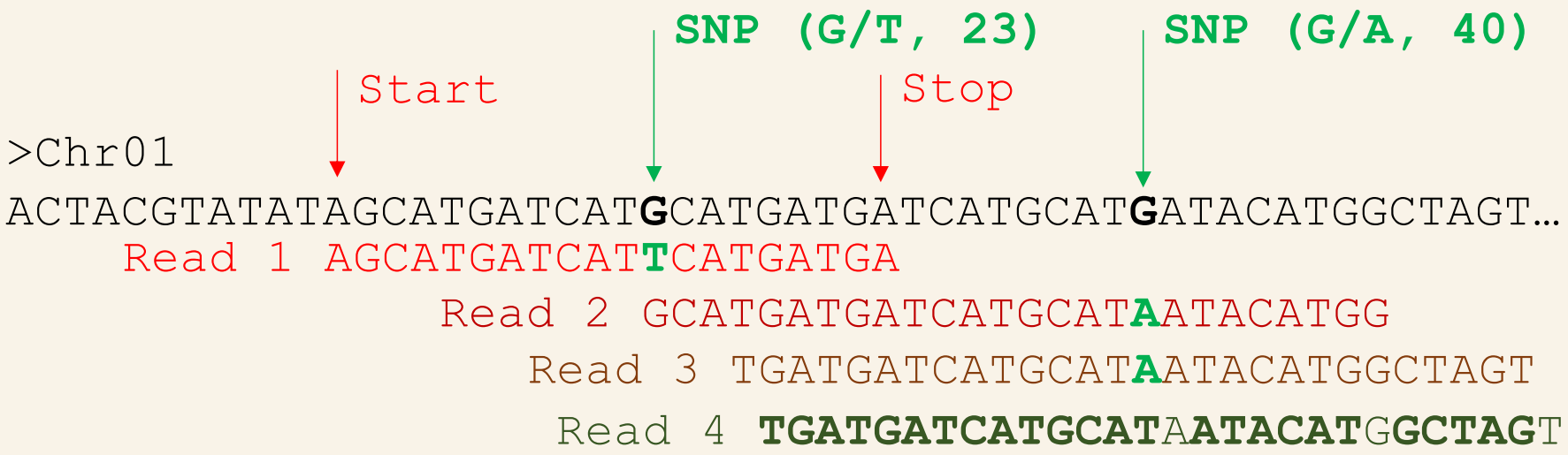
We first need such alignment before we can analyse variation

Read variation is analysed within this alignment context



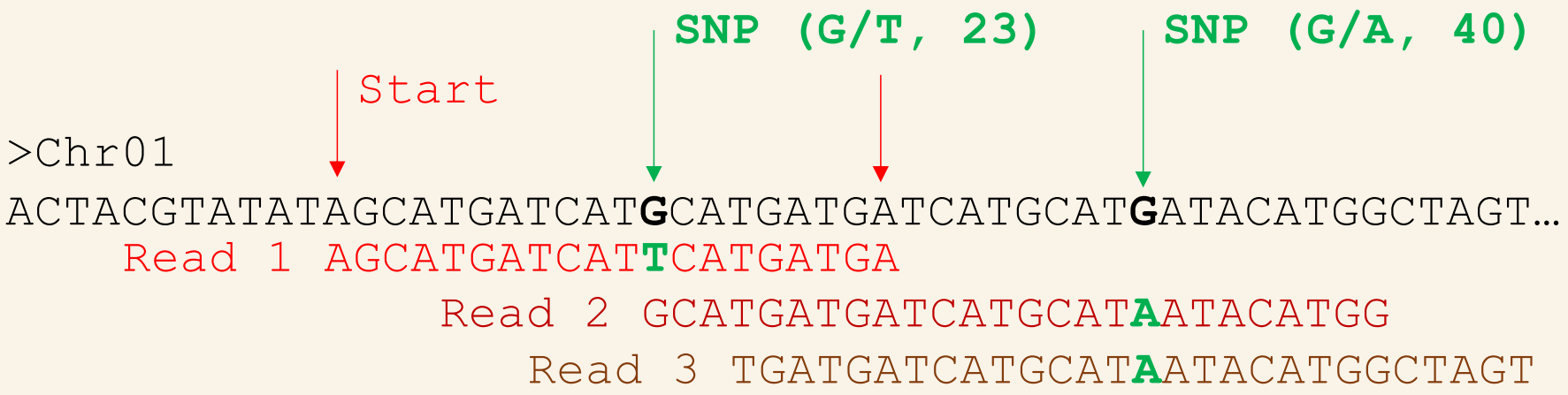
An accurate alignment is *essential* before we can trust any variant

Read variation is analysed within this alignment context



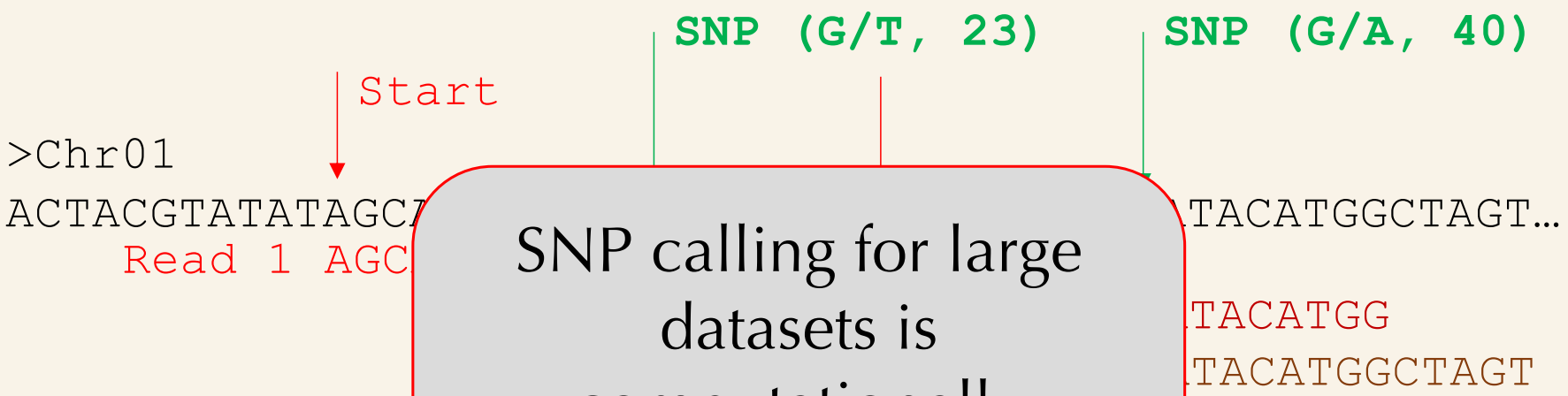
An accurate alignment is *essential* before we can trust any variant

Read variation is analysed within this alignment context



We usually analyse ***millions to billions*** of reads and compare these to reference genomes that consist of **billions** of nucleotides/bases (Human genome ~3Gb)

Read variation is analysed within this alignment context

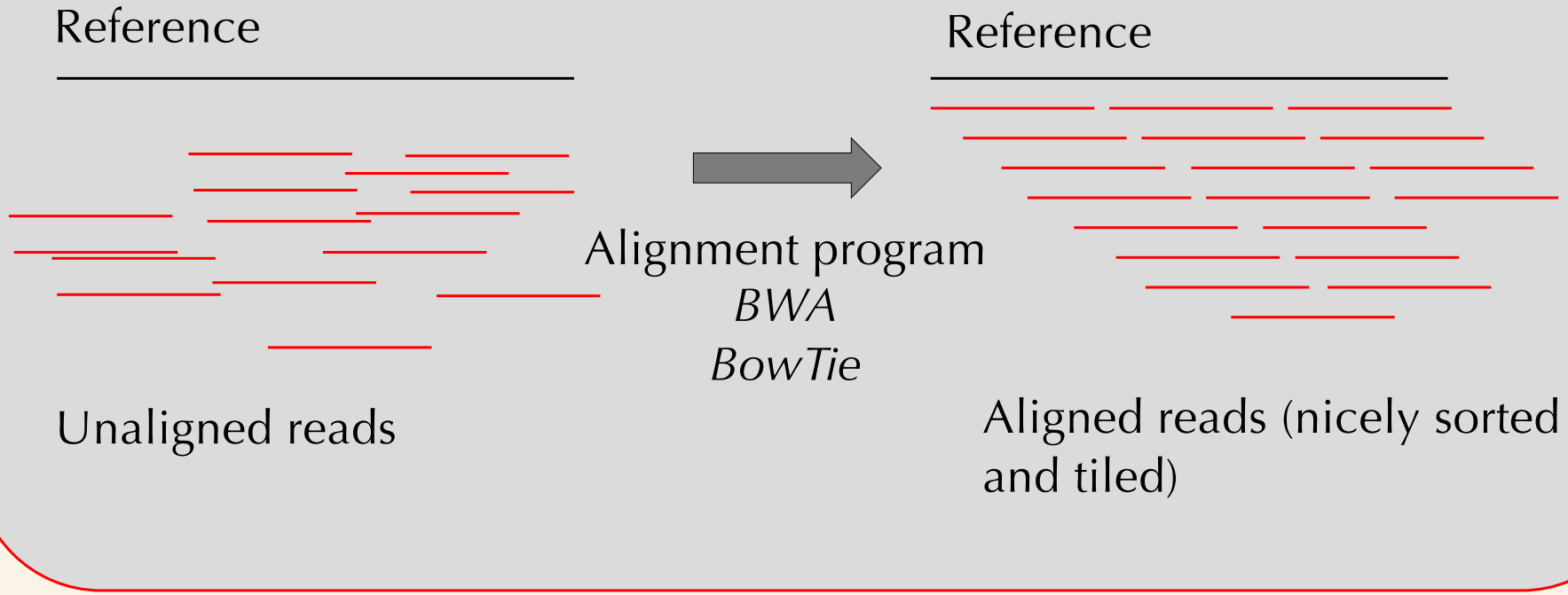


SNP calling for large datasets is computationally intensive -> work on remote HPC clusters

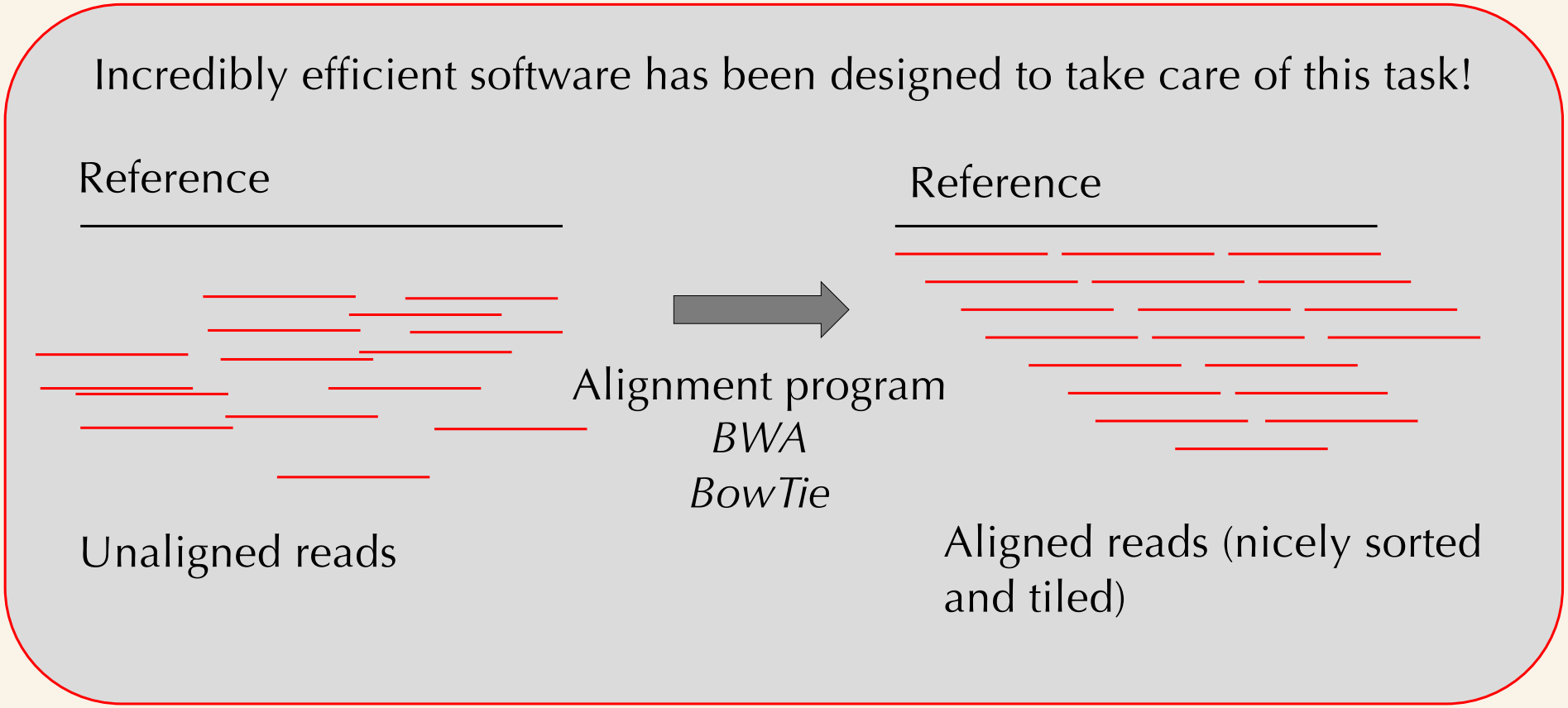
We usually of reads and compare th hat consist of **billions** of nucleotides/bases (Human genome ~3Gb)

Read variation is analysed within this alignment context

Incredibly efficient software has been designed to take care of this task!

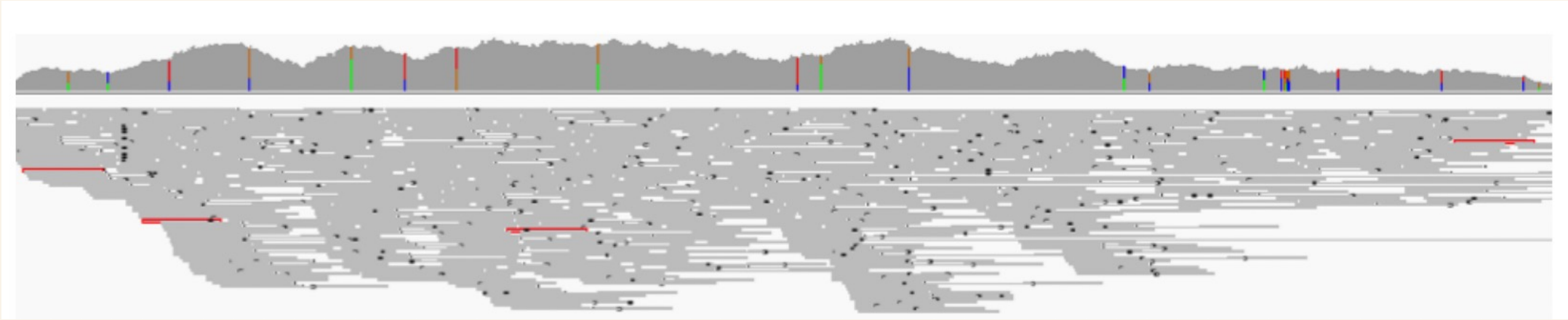


Read variation is analysed within this alignment context



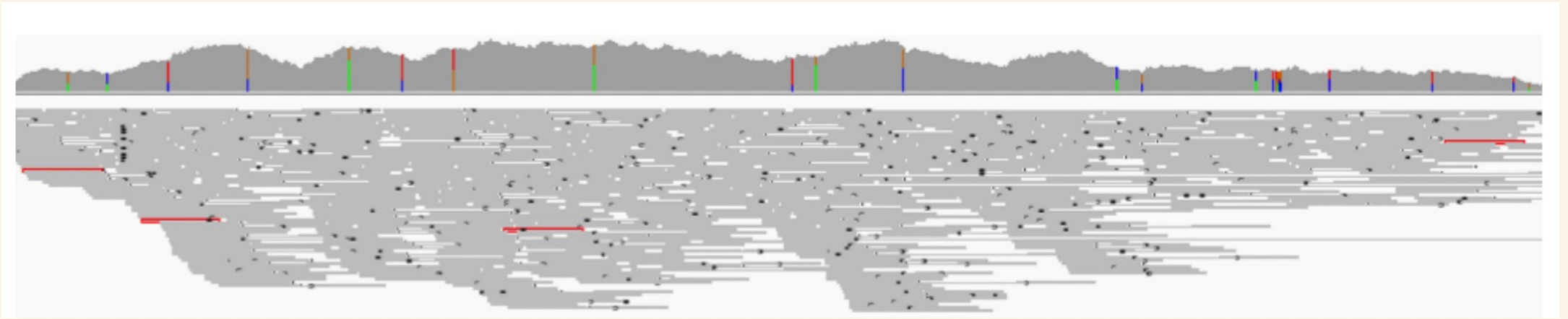
Standard program settings are usually sufficient

Visualisation of thousands of reads



Visualisation of thousands of reads

Genetic variation (SNPs) colours reflect which bases are variable (A-C, A-T, G-C, etc)



After aligning, we need another program to determine which bases are variable:

A SNP caller

SNP calling programs

Table 1. A brief summary of different tools.

caller	Bcftools	16GT	Freebayes	VarScan2	GATK
Code	C	Perl	C++	Java	Java
Model	HMM & MAQ	16-genotype probabilistic	Bayesian	heuristic algorithm	Bayesian
Sampling	Single & multiple	Single	Single	Single & multiple	Single & multiple
Variants	SNPs & indels	SNPs & indels	SNPs & indels&MNPs	SNPs & indels	SNPs & indels
Features	Sorting, indexing, etc.	easy to use, timesaving	straightforward	meet desired thresholds for read depth, base quality, variant allele frequency, and statistical significance	Realignment, per base recalibration, VQSR
Reference	Danecek et al., 2017 [15]	Luo et al., 2017 [19]	Garrison and Marth, 2012 [18]	Koboldt et al., 2012 [16]	Mckenna et al., 2010 [14]

https://doi.org/10.1371/journal.pone.0262574.t001 Liu J, Shen Q, Bao H (2022)

Many programs exist, and there is *continuous* development
For instance BCFtools/16GT are now recommended
Yet use of GATK is wide-spread (oldest, developed by Broad institute, good documentation)

What does a variant caller do?

Aims to provide statistical confidence in observing TRUE genetic variation

```
>Chr01
ACTACGTATATAGCATGATCATGCATGATGATCATGCATGATACATGGCTAGT…
     Read 1 AGCATGATCATTCATGATGA
```

Is this real or not?

Discuss between each other (few mins) and come with suggestions how to know this is real or not.

What does a variant caller do?

Aims to provide statistical confidence in observing TRUE genetic variation

```
>Chr01
ACTACGTATATAGCATGATCATGCATGATGATCATGCATGATACATGGCTAGT…
     Read 1 AGCATGATCATTCATGATGA
```

Is this real or not?

Sequencing data (as any type of data) comes with errors (wrong bases called) and/or uncertainty (low quality of bases) in the call

Solution? Generate LOTS more data!

What does a variant caller do?

With more data (read), more certainty is obtained: ***fold coverage***

```
>Chr01
ACTACGTATATAGCATGATCATGCATGATGATCATGCATGATACATGGCTAGT…
     Read 1 AGCATGATCATTCATGATGA
        Read 2 ATGATCATTCATGATGATCAT
          Read 3 GATCATTCATGATGATCATGCATGAT
            Read 4 TCATTCATGATGATCATGCAT
             Read 5 CATTCATGATGATCATGCATGATACATGG
```

5-fold coverage, all the same, we are pretty certain about this call (note: we usually strive for ~20 fold coverage)

What does a variant caller do?

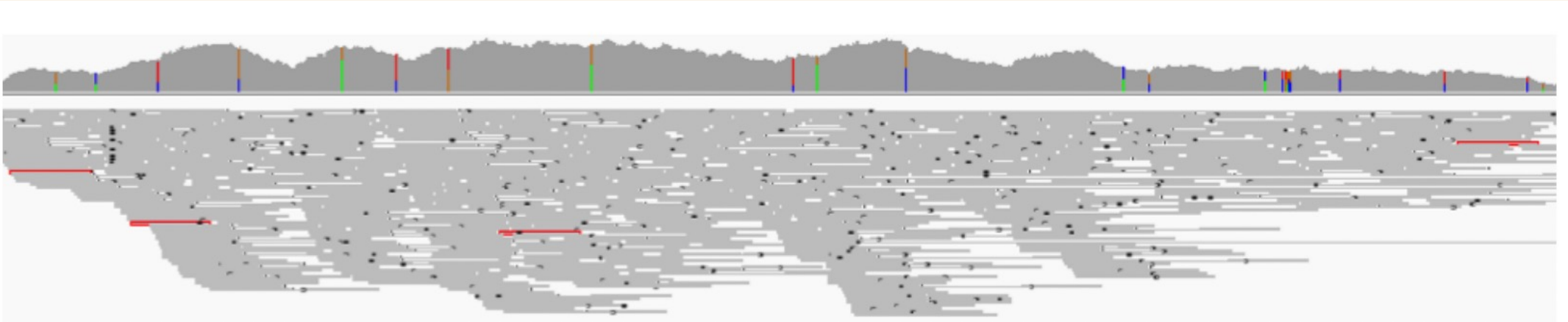
Another example

```
>Chr01
ACTACGTATATAGCATGATCATGCATGATGATCATGCATGATACATGGCTAGT…
     Read 1 AGCATGATCATTCATGATGA
        Read 2 ATGATCATTCATGATGATCAT
          Read 3 GATCATTCATGATGATCATGCATGAT
            Read 4 TCATACATGATGATCATGCAT
             Read 5 CATTCATGATGATCATGCATGATACATGG
```

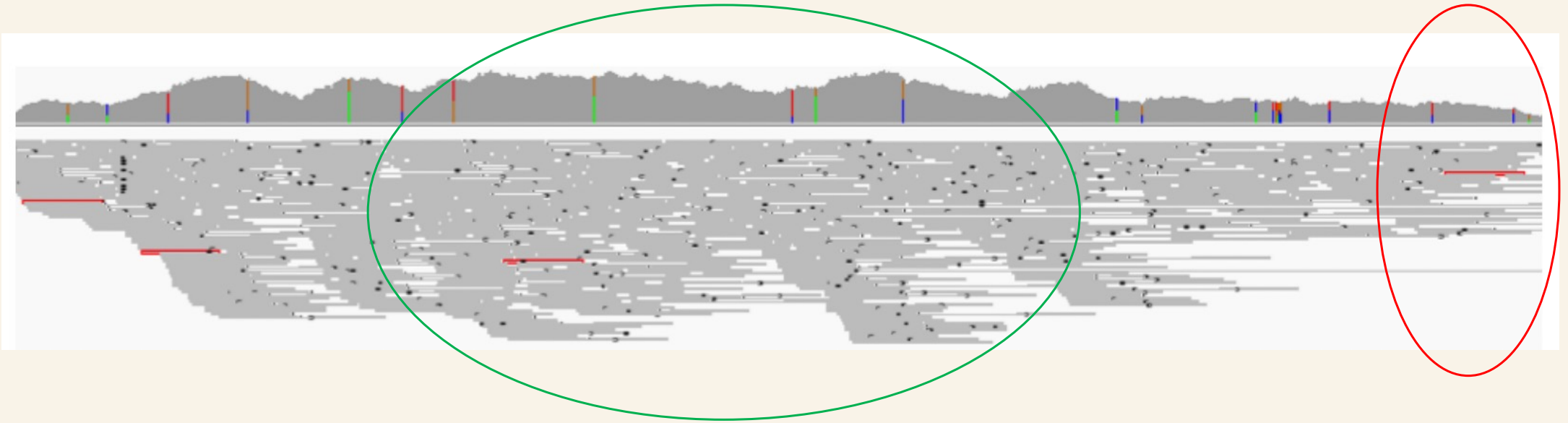
We cannot be so certain about the A, until we get more data

Coverage is the most important determinant for the quality of your data

Yet along a reference, you'll obtain variable coverage due to random processes, assembly quality, or genomic complexity



Yet along a reference, you'll obtain variable coverage due to random processes, assembly quality, or genomic complexity

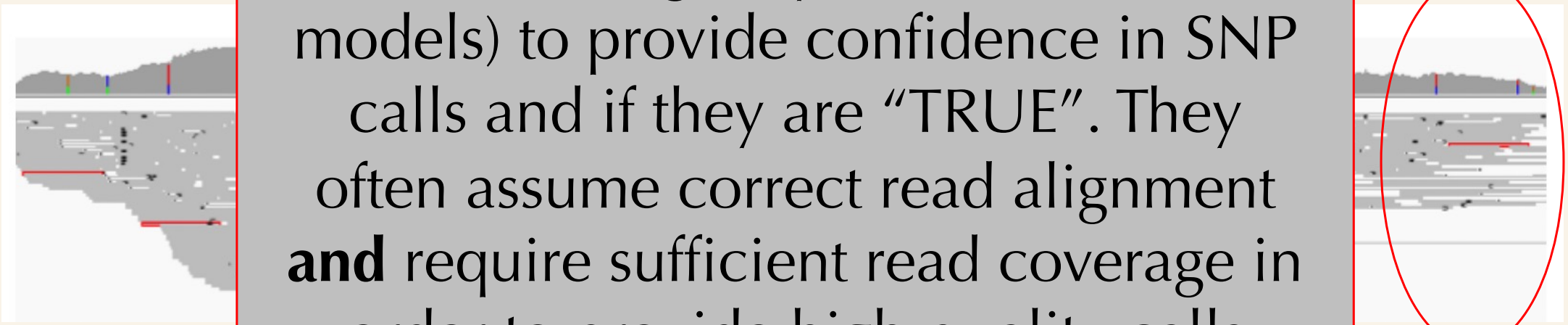


Higher coverage

Lower coverage

Yet along a reference, you'll obtain variable coverage due to random [illegible] plexity

SNP callers run complex statistical models (e.g. Bayesian or HMM models) to provide confidence in SNP calls and if they are "TRUE". They often assume correct read alignment **and** require sufficient read coverage in order to provide high-quality calls

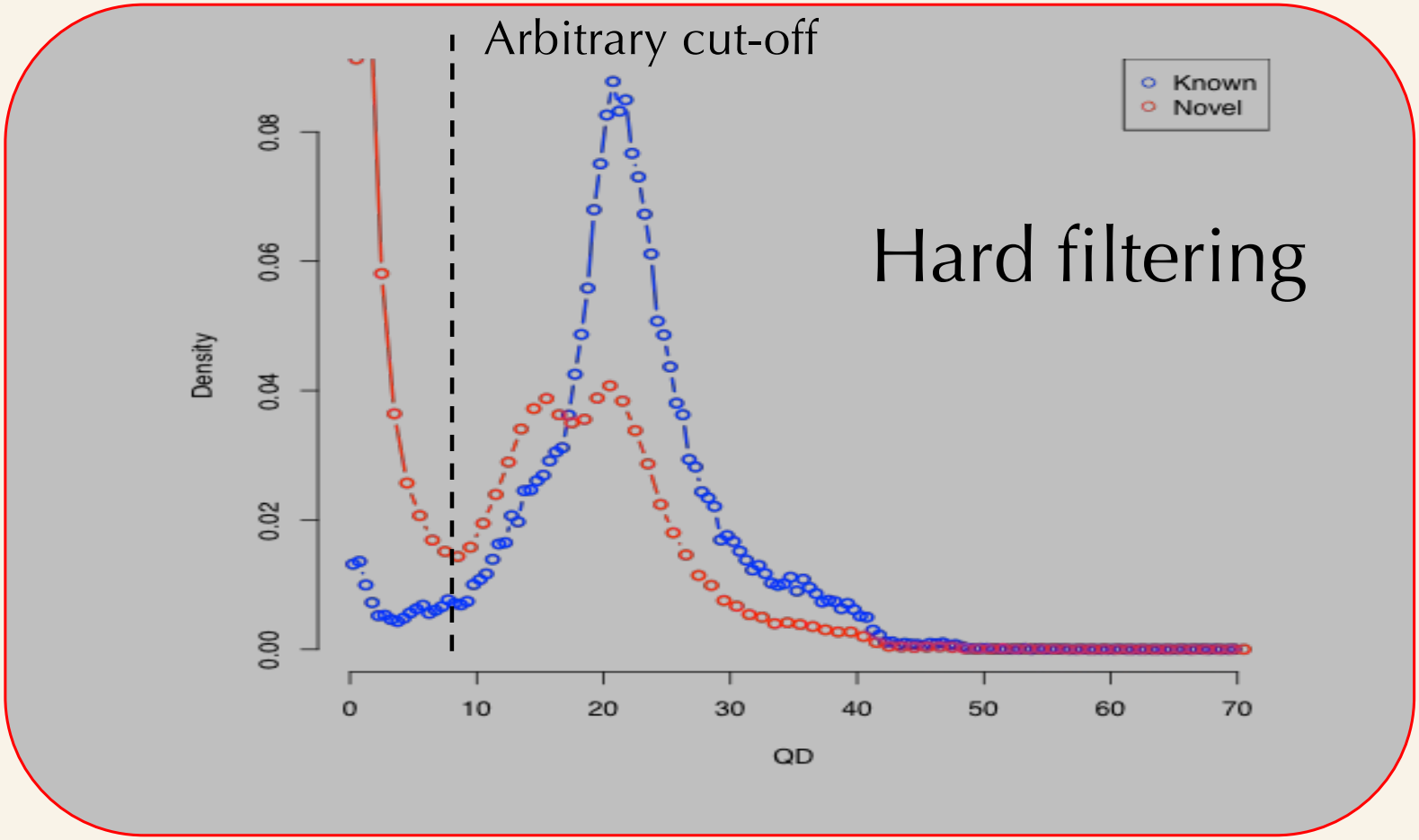


Higher coverage

Lower coverage

SNP callers will ALSO yield a large numbers of SNPs of which many will NOT be true (false positives)

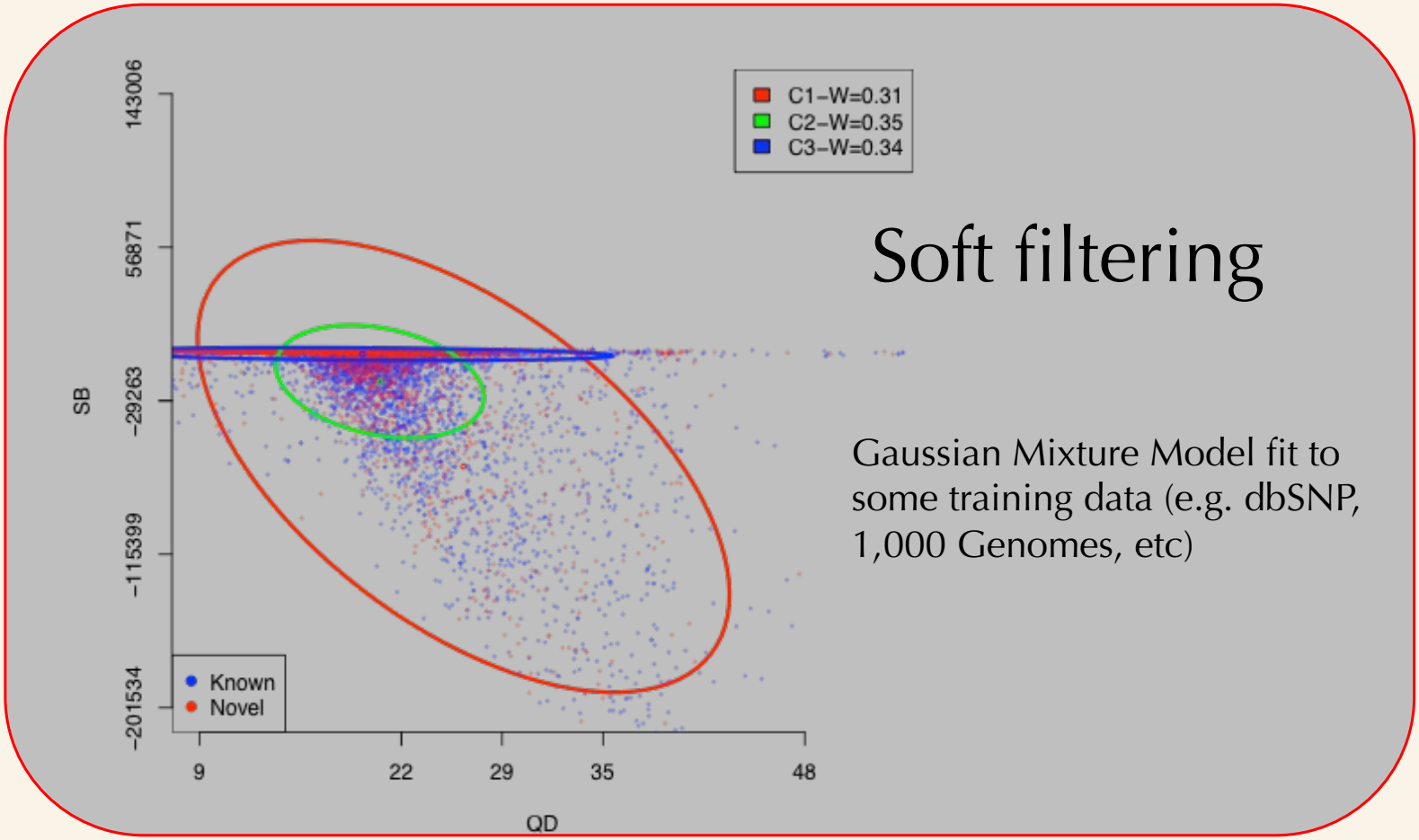
We need to ***filter*** our data to only retain the high quality part of the data



Mark de Pristo 2010

SNP callers will ALSO yield a large numbers of SNPs of which many will NOT be true (false positives)

We need to ***filter*** our data to only retain the high quality part of the data

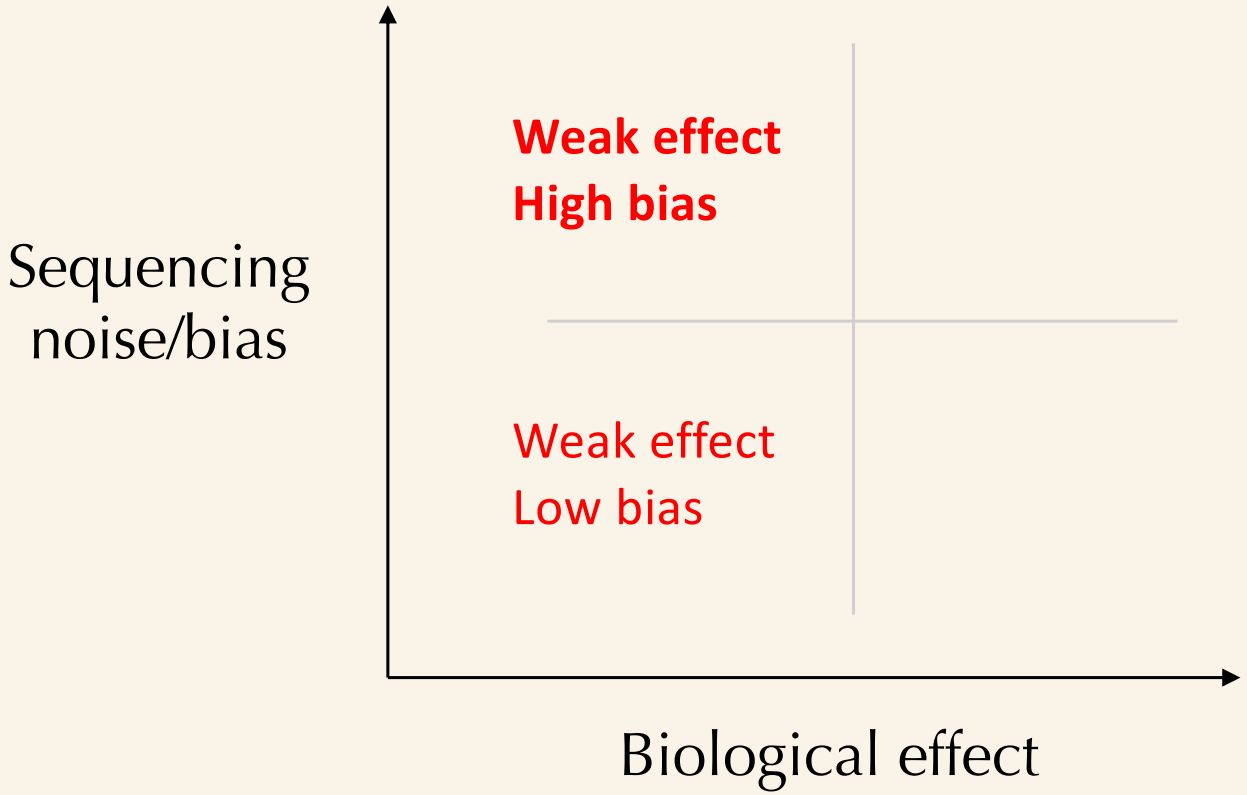


Soft filtering

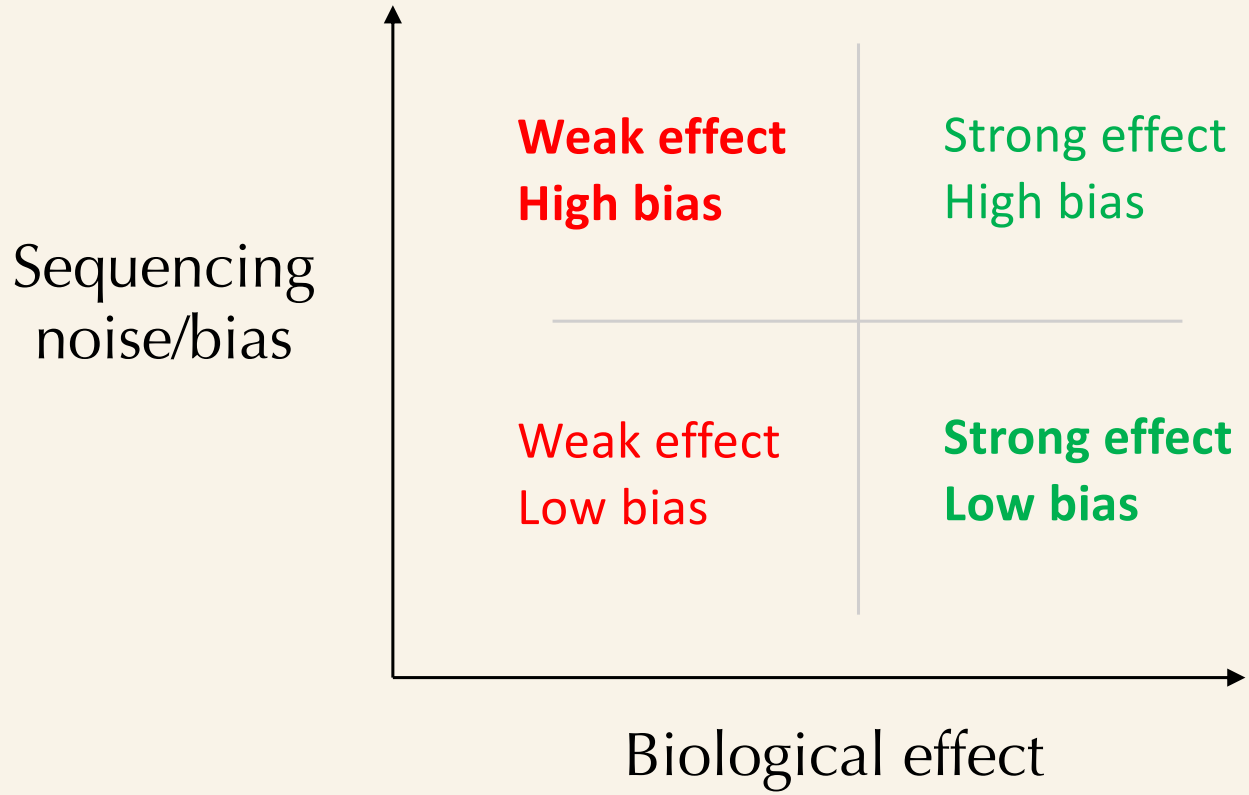
Gaussian Mixture Model fit to some training data (e.g. dbSNP, 1,000 Genomes, etc)

Mark de Pristo 2010

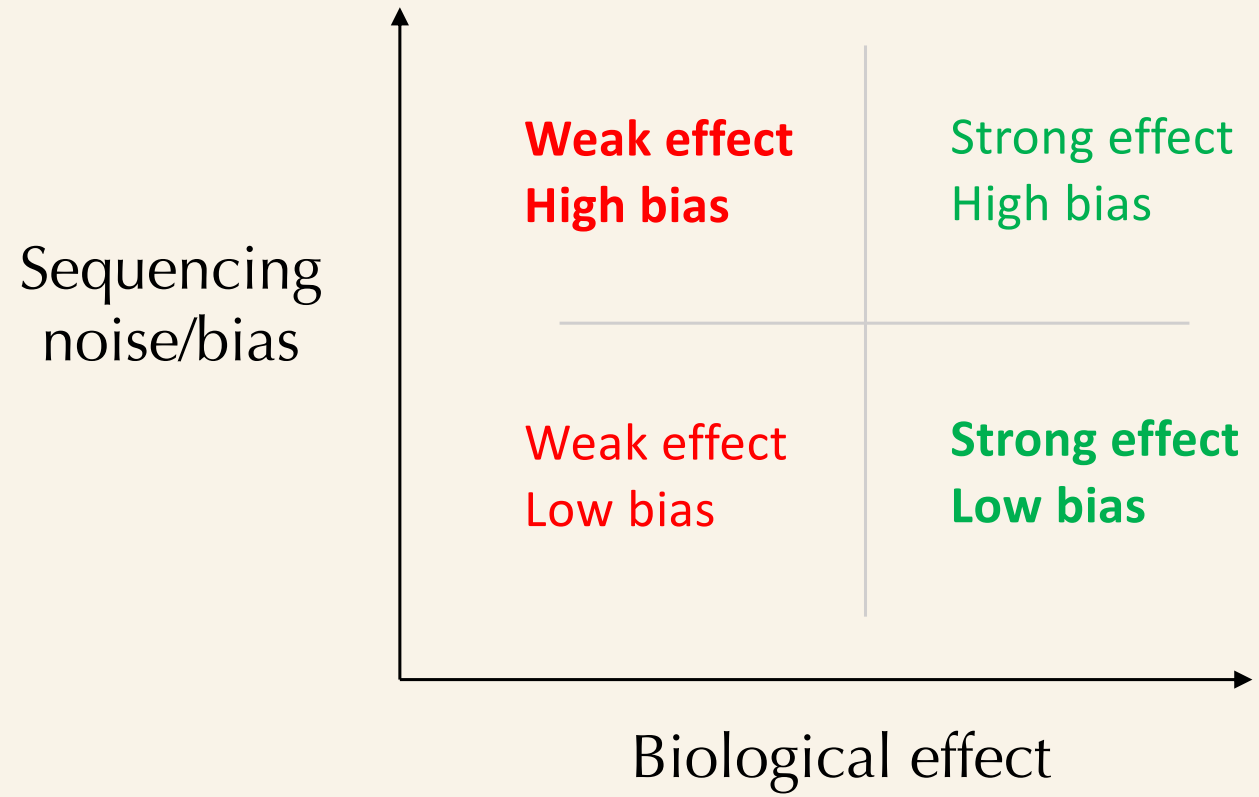
Yet there is no “fixed” approach to filtering your data



Yet there is no “fixed” approach to filtering your data



Yet there is no “fixed” approach to filtering your data



It is not always clear from the outset where you are! You need to explore your data and use preliminary analyses

Questions?


WHO? WHERE?
WHEN? WHY? HOW?
WHAT? WHO?
HOW? WHERE?
WHO? WHERE?
WHAT?
WHY? WHAT?
WHEN?
WHERE? WHO?
HOW? WHAT?
WHO? WHERE?
WHY?
WHO? WHEN?
WHEN? WHAT?
WHO? WHY? HOW?
HOW? WHERE?
WHAT? WHY?
WHEN?
WHO? WHAT?
HOW?
WHAT? WHEN?
WHERE? WHY?
HOW? WHEN?
WHAT? WHO?
WHY? WHERE?
WHEN? HOW?
WHERE?

After all this, what does a variant calling pipeline look like?

Reference

Reads

e.g. population data

After all this, what does a variant calling pipeline look like?

Reference

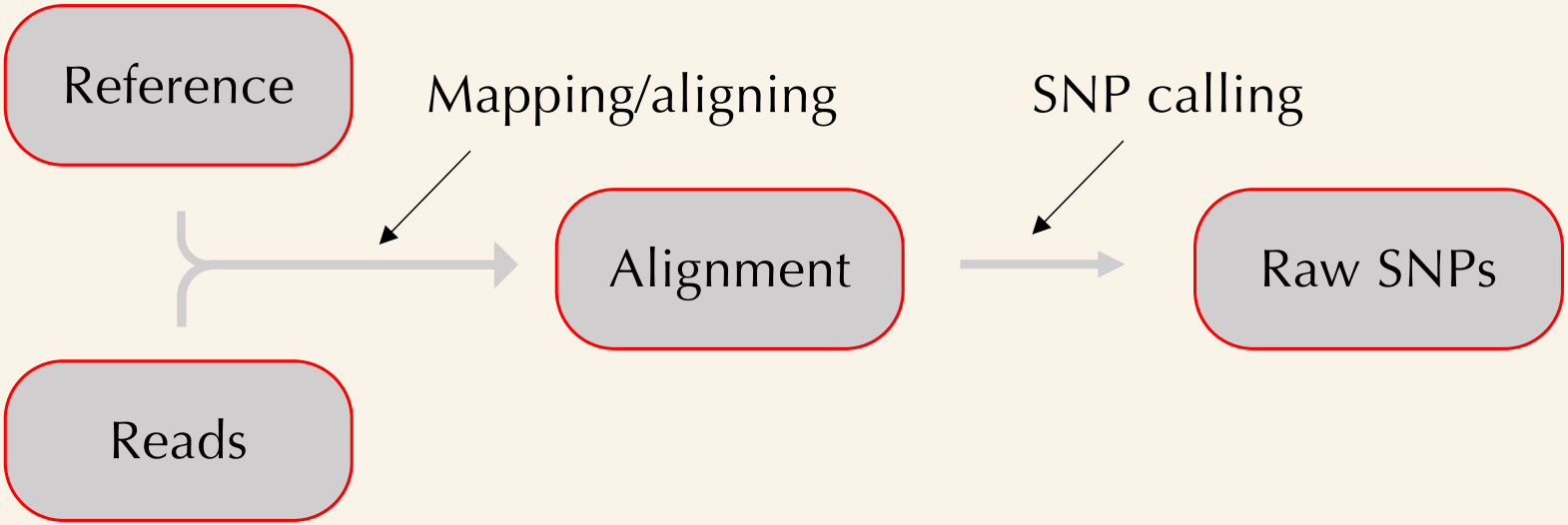
Mapping/aligning

Alignment

Reads

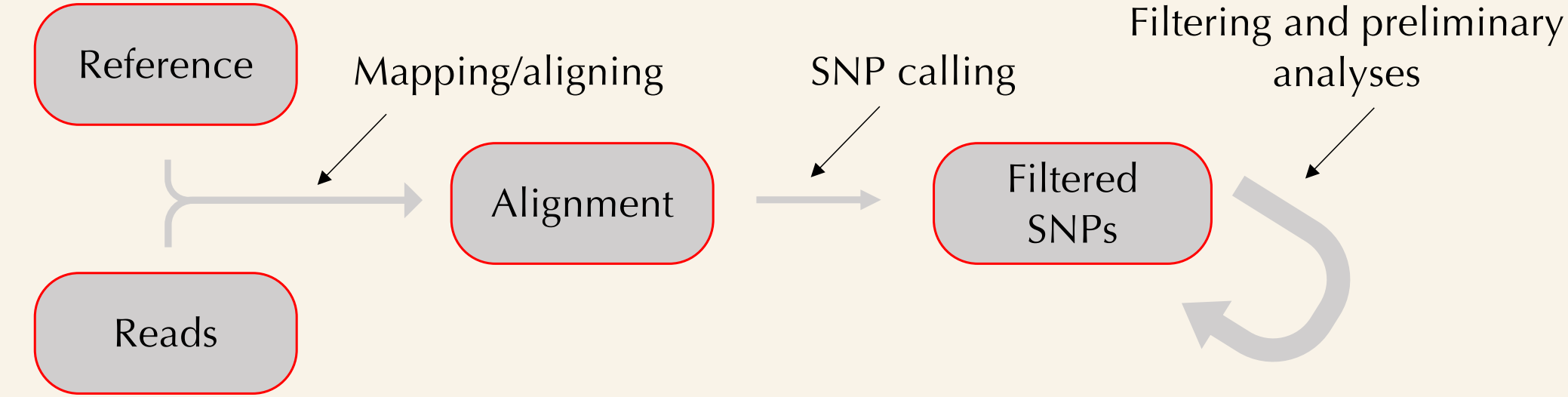
e.g.
population
data

After all this, what does a variant calling pipeline look like?



e.g.
population
data

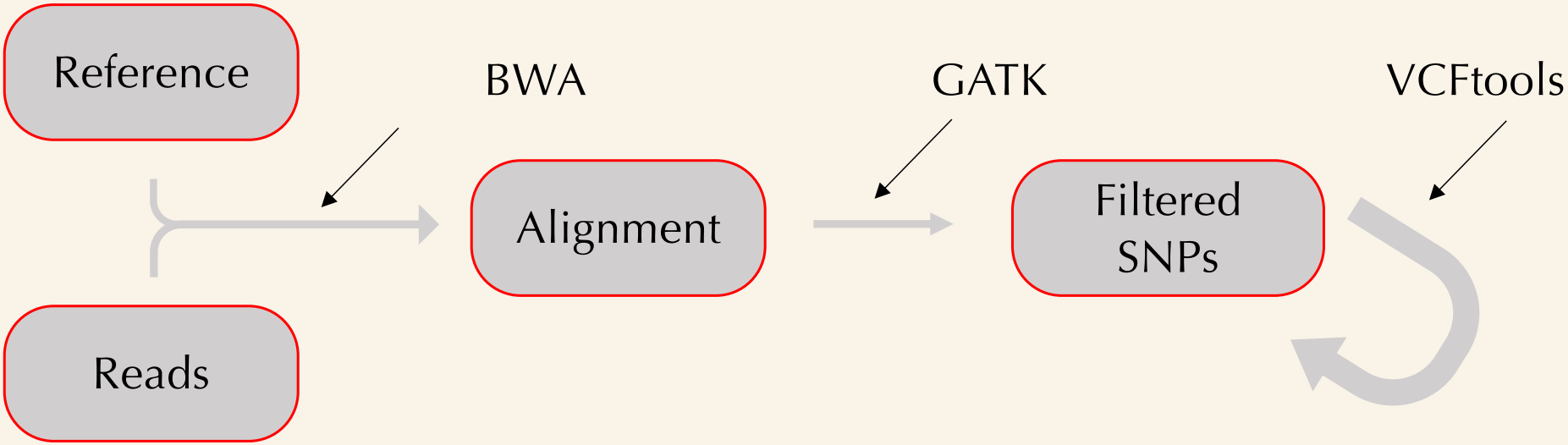
After all this, what does a variant calling pipeline look like?



e.g. population data

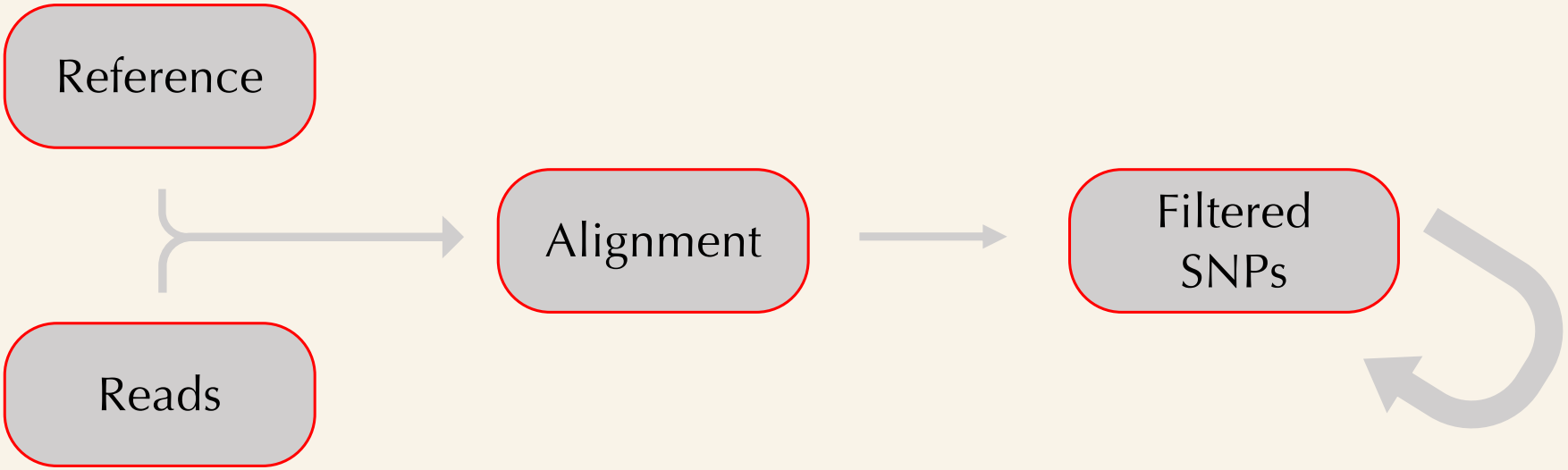
After all this, what does a variant calling pipeline look like?

A selection of programs that can be used

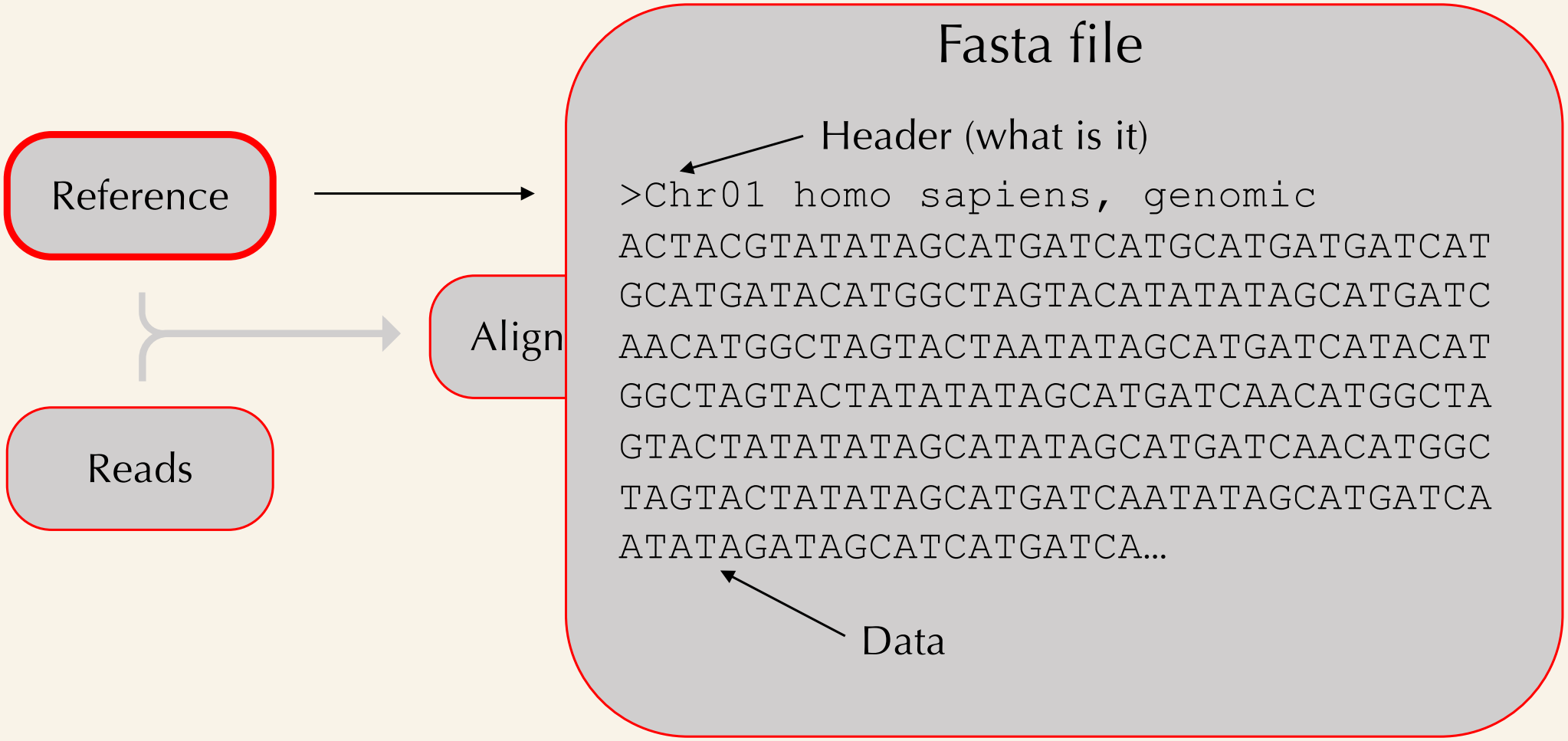


e.g. population data

Each of these steps requires specific files to work with!



Each of these steps requires specific files to work with!



Each of these steps requires specific files to work with!

Each of these steps requires specific files to work with!

Alignment

BAM file (binary alignment file)

Kind of data

"Header" with information about the file

```
@HD  VN:1.5  GO:none SO:coordinate
@SQ  SN:NC_004029.2  LN:16565
@RG  ID:L1i1_AGAACCG SM:WLR001       LB:L1i1_AGAACCG PU:Nistelberger-DNA1-2016-04-15
@RG  PL:ILLUMINA     PG:bwa
@PG  ID:bwa  PN:bwa  VN:0.7.17-r1188 CL:bwa samse Orosv1mt.fasta
@PG  ID:GATK IndelRealigner  VN:3.6-0-g89b7209  CL:knownAlleles=[] targetIntervals=WLR001/Wal_m
@PG  ID:samtools CL:samtools view -H WLR001.Wal_mt.realigned.bam
```

Reference

Sample name

Each of these steps requires specific files to work with!

Alignment

BAM file (binary alignment file)

Readname

Follow by data with information about each read aligment

Start of alignment

Matching bases

```
M_D00564:55:C9FG3ANXX:7  0       NC_004029.2     419     37      91M     TAAAAAGCTGCCGCTAATACAAAATATACTACGAAAGTGACT
M_D00564:55:C9FG3ANXX:7  0       NC_004029.2     474     37      58M     TTACACGACAGCTAAGACCCAAACTGGGATTAGATACCCCAC
M_D00564:55:C9FG3ANXX:7  0       NC_004029.2     515     37      56M     CTATGCTTAGCCATAAACACAAATAATTTGCACAACAAAATT
```

Reference name

Quality of alignment (37 is max)

CIGAR string (56 matching bases)

Each of these steps requires specific files to work with!

SNP data

↓

VCF file (Variant call format)

Again, a “Header” with lots of information about the file

```
##fileformat=VCFv4.2
##ALT=<ID=NON_REF,Description="Represents any possible alternative allele not already represented at this location
##FILTER=<ID=LowQual,Description="Low quality">
##FILTER=<ID=PASS,Description="All filters passed">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths for the ref and alt alleles in the order listed">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Approximate read depth (reads with MQ=255 or with bad mates are
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=MIN_DP,Number=1,Type=Integer,Description="Minimum DP observed within the GVCF block">
##FORMAT=<ID=PL,Number=G,Type=Integer,Description="Normalized, Phred-scaled likelihoods for genotypes as defined in
##FORMAT=<ID=RGQ,Number=1,Type=Integer,Description="Unconditional reference genotype confidence, encoded as a phred
##FORMAT=<ID=SB,Number=4,Type=Integer,Description="Per-sample component statistics which comprise the Fisher's Exac
##GATKCommandLine=<ID=GenomicsDBImport,CommandLine="GenomicsDBImport --genomicsdb-workspace-path Walrus_DB --varian
##GATKCommandLine=<ID=GenotypeGVCFs,CommandLine="GenotypeGVCFs --output Walrus_MT.vcf.gz --variant gendb://Walrus_D
##GATKCommandLine=<ID=HaplotypeCaller,CommandLine="HaplotypeCaller --sample-ploidy 1 --emit-ref-confidence GVCF --c
##INFO=<ID=AC,Number=A,Type=Integer,Description="Allele count in genotypes, for each ALT allele, in the same order
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency, for each ALT allele, in the same order as listed">
##INFO=<ID=AN,Number=1,Type=Integer,Description="Total number of alleles in called genotypes">
##INFO=<ID=BaseQRankSum,Number=1,Type=Float,Description="Z-score from Wilcoxon rank sum test of Alt Vs. Ref base qu
```

Each of these steps requires specific files to work with!

SNP data

↓

VCF file (Variant call format)

Followed by the data:

#CHROM	POS	ID	REF	ALT	QUAL		FILTER	INFO
NC_004029.2	131	.	T	C	356.22	.		AC=1;AF=0.022;AN=45;DP=143;FS=0.000;MLEAC=1;MLEAF=0
NC_004029.2	162	.	T	C	18479.23	.		AC=15;AF=0.333;AN=45;BaseQRankSum=0.00;DP=543;FS=0.
NC_004029.2	198	.	C	T	608.22	.		AC=1;AF=0.022;AN=45;DP=410;FS=0.000;MLEAC=1;MLEAF=0
NC_004029.2	387	.	G	A	547.22	.		AC=1;AF=0.022;AN=45;DP=408;FS=0.000;MLEAC=1;MLEAF=0
NC_004029.2	616	.	T	C	235.62	.		AC=1;AF=0.022;AN=45;DP=406;FS=0.000;MLEAC=1;MLEAF=0
NC_004029.2	741	.	C	T	819.22	.		AC=1;AF=0.022;AN=45;DP=412;FS=0.000;MLEAC=1;MLEAF=0
NC_004029.2	743	.	C	T	819	.		AC=1;AF=0.022;AN=45;DP=413;FS=0.000;MLEAC=1;MLEAF=0

↖

Reference name

Each of these steps requires specific files to work with!

SNP data

VCF file (Variant call format)

Followed by the data:

Geno**T**ype: **A**llele **D**epth: Read **D**e**P**th (DP): **G**enotype **Q**uality: **P**hred-scaled **L**ikelihood

FORMAT	WLR001	WLR002	WLR003	WLR004	WLR
GT:AD:DP:GQ:PL	0:0,0:0:0:0,0	0:2,0:2:90:0,90	0:0,0:0:0:0,0	0:0,0:0:0:0,0	
GT:AD:DP:GQ:PL	0:0,0:0:0:0,0	0:4,0:4:99:0,135	0:1,0:1:0:0,0	0:1,0:1:45:0,45	
GT:AD:DP:GQ:PL	0:0,0:0:0:0,0	0:5,0:5:46:0,46	0:0,0:0:0:0,0	0:2,0:2:90:0,90	
GT:AD:DP:GQ:PL	0:0,0:0:0:0,0	0:3,0:3:99:0,135	0:0,0:0:0:0,0	0:2,0:2:45:0,45	
GT:AD:DP:GQ:PL	0:0,0:0:0:0,0	0:0,0:0:0:0,0	0:0,0:0:0:0,0	0:0,0:0:0:0,0	
GT:AD:DP:GQ:PL	0:0,0:0:0:0,0	0:3,0:3:99:0,128	0:0,0:0:0:0,0	0:1,0:1:45:0,45	
GT:AD:DP:GQ:PL	0:0,0:0:0:0,0	0:3,0:3:99:0,128	0:0,0:0:0:0,0	0:1,0:1:45:0,45	
GT:AD:DP:GQ:PL	0:0,0:0:0:0,0	0:3,0:3:99:0,128	0:0,0:0:0:0,0	0:1,0:1:45:0,45	
GT:AD:DP:GQ:PL	0:0,0:0:0:0,0	0:3,0:3:99:0,135	0:0,0:0:0:0,0	0:1,0:1:42:0,42	
GT:AD:DP:GQ:PL	0:0,0:0:0:0,0	0:1,0:1:45:0,45	0:0,0:0:0:0,0	0:1,0:1:42:0,42	
GT:AD:DP:GQ:PL	0:1,0:1:45:0,45	0:1,0:1:45:0,45	0:0,0:0:0:0,0	0:3,0:3:99:0,119	
GT:AD:DP:GQ:PL	0:1,0:1:45:0,45	0:1,0:1:45:0,45	0:0,0:0:0:0,0	0:3,0:3:99:0,119	
GT:AD:DP:GQ:PL	0:1,0:1:0:0,0	0:1,0:1:0:0,0	0:0,0:0:0:0,0	0:0,0:0:0:0,0	
GT:AD:DP:GQ:PL	0:1,0:1:0:0,0	0:1,0:1:0:0,0	0:0,0:0:0:0,0	0:0,0:0:0:0,0	
GT:AD:DP:GQ:PL	0:0,0:0:0:0,0	0:1,0:1:45:0,45	0:0,0:0:0:0,0	0:0,0:0:0:0,0	
GT:AD:DP:GQ:PL	0:0,0:0:0:0,0	0:1,0:1:45:0,45	0:0,0:0:0:0,0	0:0,0:0:0:0,0	

After all this, what does a variant calling pipeline look like?

Fasta file

Reference

Mapping/aligning

SNP calling

Filtering and preliminary analyses

Alignment

Filtered SNPs

Reads

FastQ file

BAM file

VCF file

e.g. population data

Questions?


WHO? WHERE?
WHEN? WHY? HOW?
WHAT? WHO?
HOW? WHERE?
WHO? WHERE?
WHAT?
WHY? WHAT?
WHEN?
WHERE? WHO?
HOW? WHAT?
WHO? WHERE?
WHY?
WHO? WHEN?
WHEN? WHAT?
WHO? WHY? HOW?
HOW? WHERE?
WHAT? WHY?
WHEN?
WHO? WHAT?
HOW?
WHAT? WHEN?
WHERE? WHY?
HOW? WHEN?
WHAT? WHO?
WHY? WHERE?
WHEN? HOW?
WHERE?

Then it is *Quiz* time!

Today:

1) Introduction: variant calling, why do we want to do this, and what it is?
2) Variant calling pipelines/methods and pitfalls
3) Practical session, going through (parts of) a SNP calling pipeline and interpret biological results