CONFIDENTIAL

SevenBridges

Structural Variation

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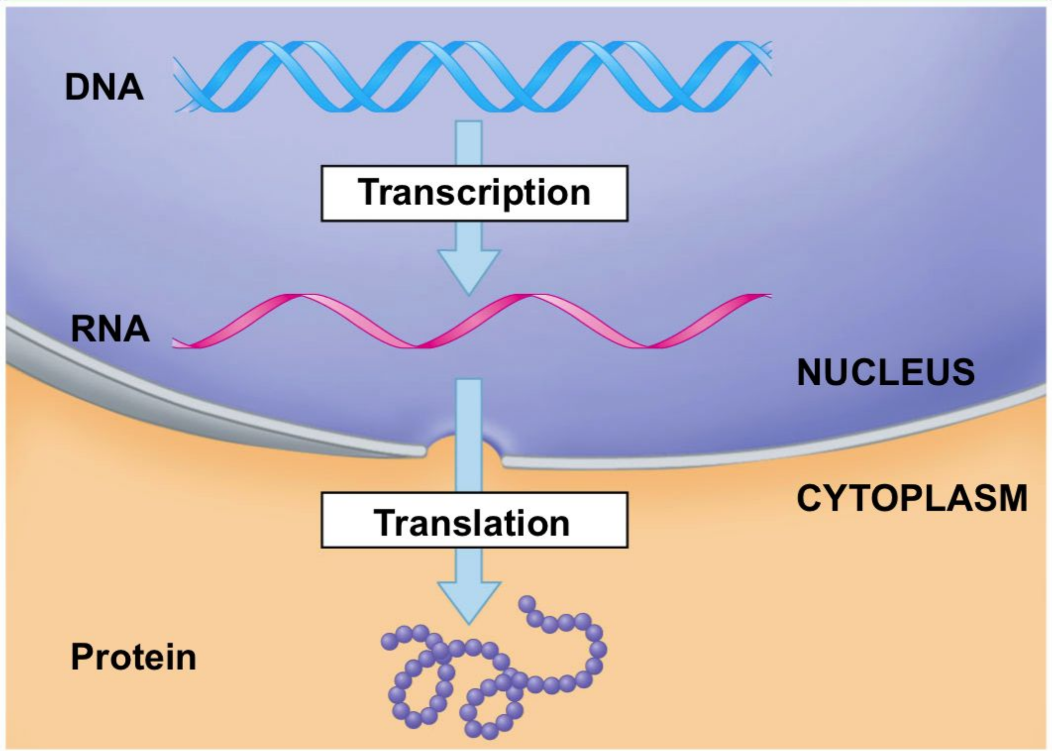
Genomic variation

Recap

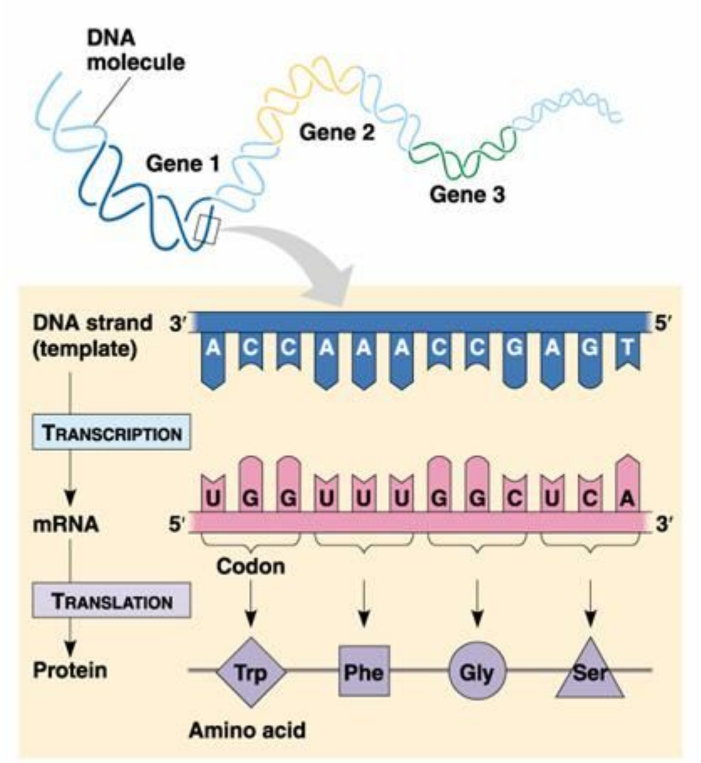
Genomic variation

- Represent **differences** between genomes which we are comparing
- Usually between a **sequenced genome** and a **reference genome**

Central dogma



Central dogma



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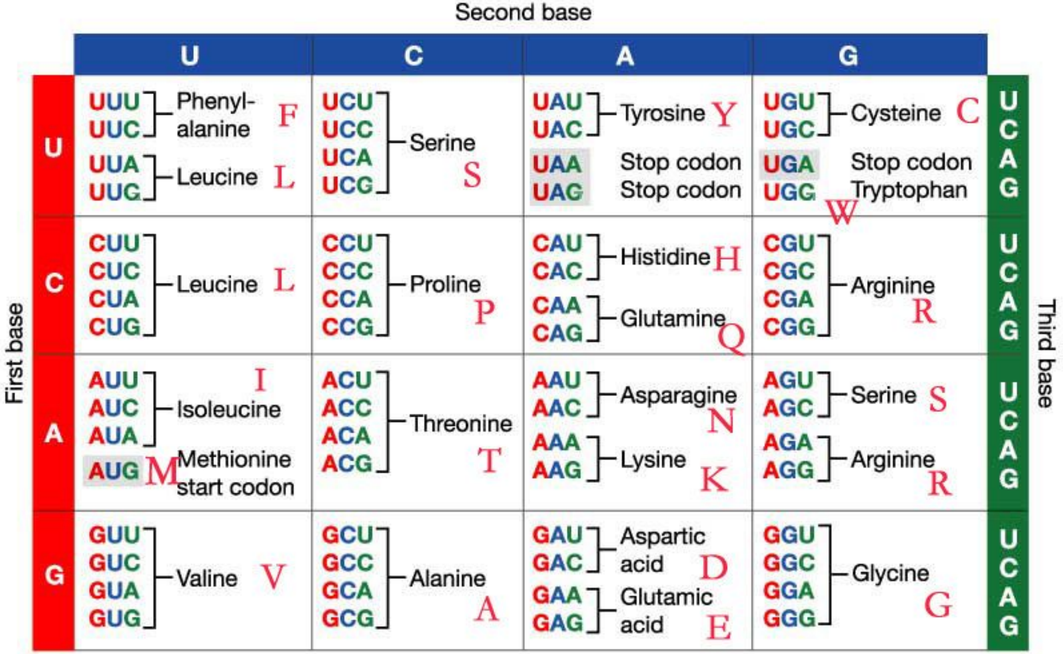


Second base

First base	U	C	A	G	Third base
U	UUU UUC Phenylalanine F UUA UUG Leucine L	UCU UCC UCA UCG Serine S	UAU UAC Tyrosine Y UAA Stop codon UAG Stop codon	UGU UGC Cysteine C UGA Stop codon UGG Tryptophan W	U C A G
C	CUU CUC CUA CUG Leucine L	CCU CCC CCA CCG Proline P	CAU CAC Histidine H CAA CAG Glutamine Q	CGU CGC CGA CGG Arginine R	U C A G
A	AUU AUC AUA Isoleucine I AUG M Methionine start codon	ACU ACC ACA ACG Threonine T	AAU AAC Asparagine N AAA AAG Lysine K	AGU AGC Serine S AGA AGG Arginine R	U C A G
G	GUU GUC GUA GUG Valine V	GCU GCC GCA GCG Alanine A	GAU GAC Aspartic acid D GAA GAG Glutamic acid E	GGU GGC GGA GGG Glycine G	U C A G

Genomic variants

- **Single Nucleotide Variants (SNV)**
  Length: 1bp
- **Small Insertions / Deletions (small INDELS)**
  Length: up to 50bp

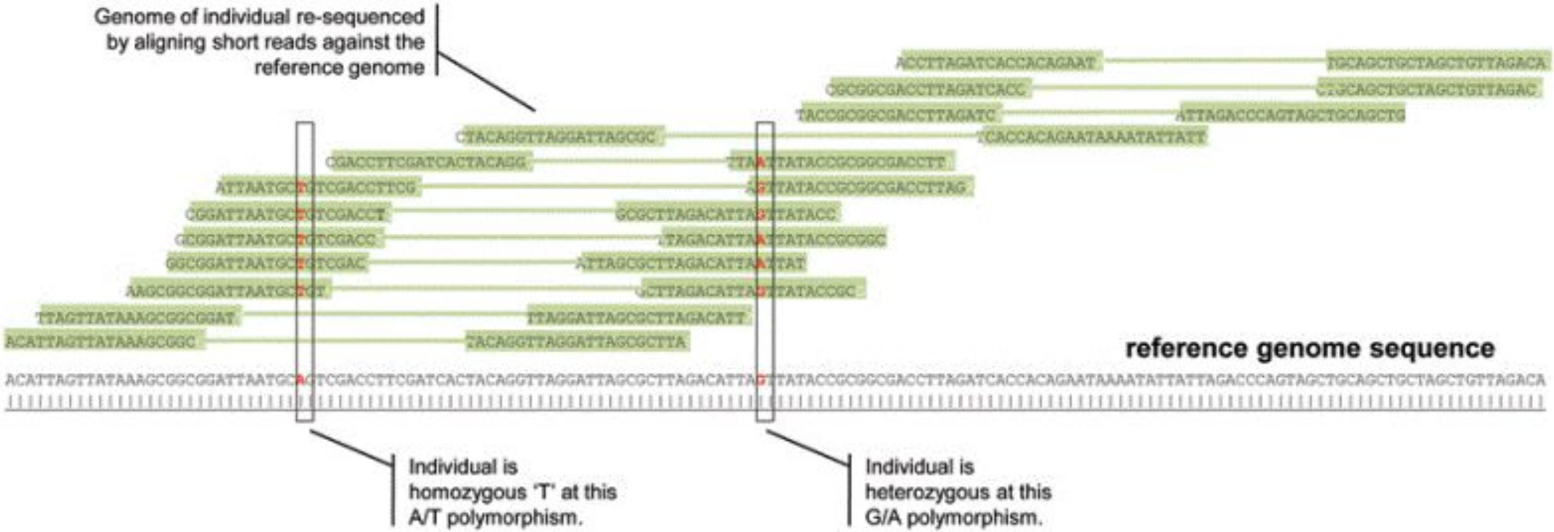
→ 25% developmental diseases

- **Structural Variations (SV)**
  Length: greater than 50bp

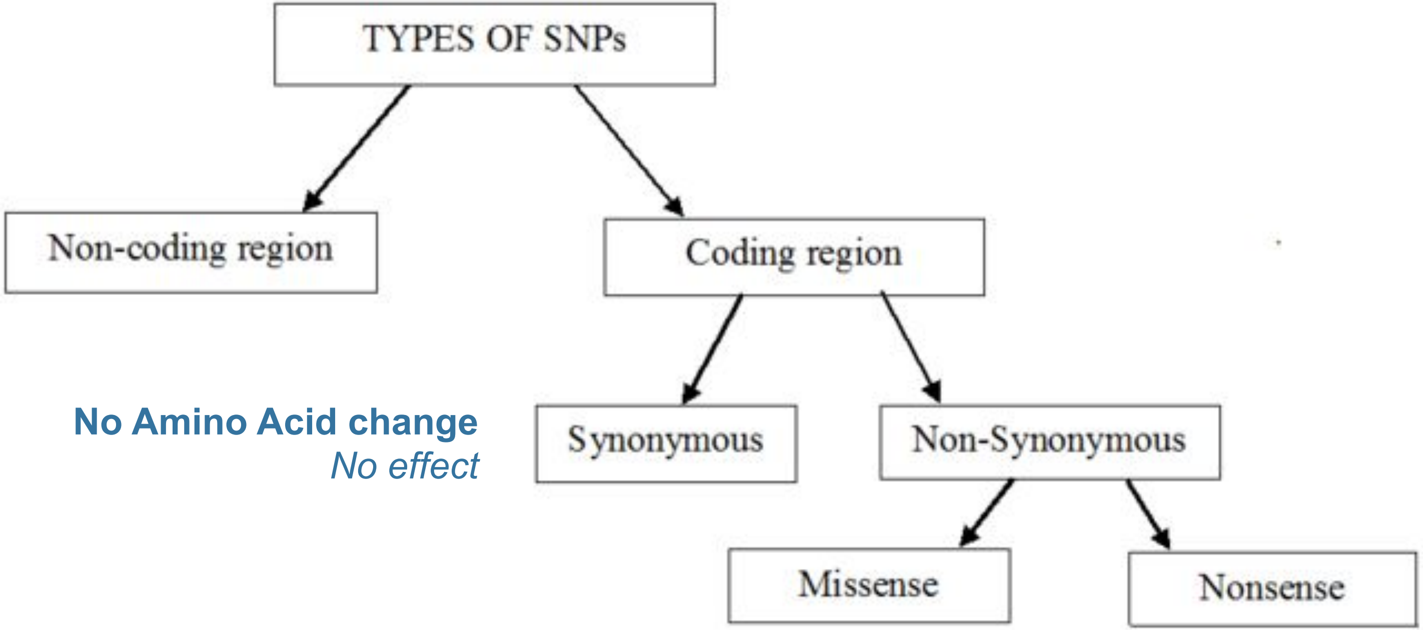
→ 20% developmental diseases

Genomic variants

- **Single Nucleotide Variants (SNV)**
  Length: 1bp



Single Nucleotide Variants (SNV)

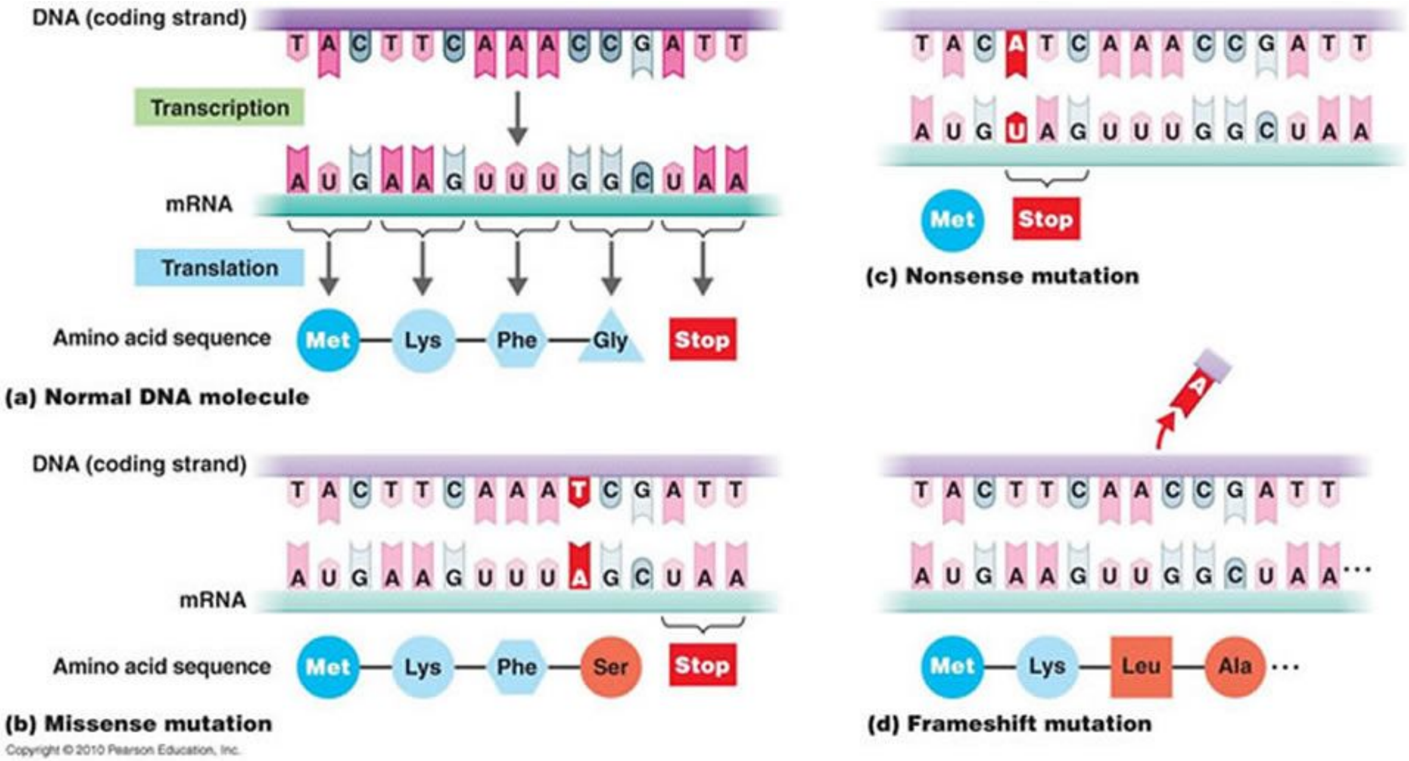


No Amino Acid change
No effect

Amino Acid change
Variable effect

Premature stop codon
Serious effect

Single Nucleotide Variants (SNV)


DNA (coding strand)
T A C T T C A A A C C G A T T
Transcription
A U G A A G U U U G G C U A A
mRNA
Translation
Amino acid sequence
Met
Lys
Phe
Gly
Stop
T A C A T C A A A C C G A T T
A U G U A G U U U G G C U A A
Met
Stop
A
DNA (coding strand)
T A C T T C A A A T C G A T T
A U G A A G U U U A G C U A A
mRNA
Amino acid sequence
Met
Lys
Phe
Ser
Stop
T A C T T C A A C C G A T T
A U G A A G U U G G C U A A...
Met
Lys
Leu
Ala
...

(a) Normal DNA molecule

(b) Missense mutation

(c) Nonsense mutation

(d) Frameshift mutation

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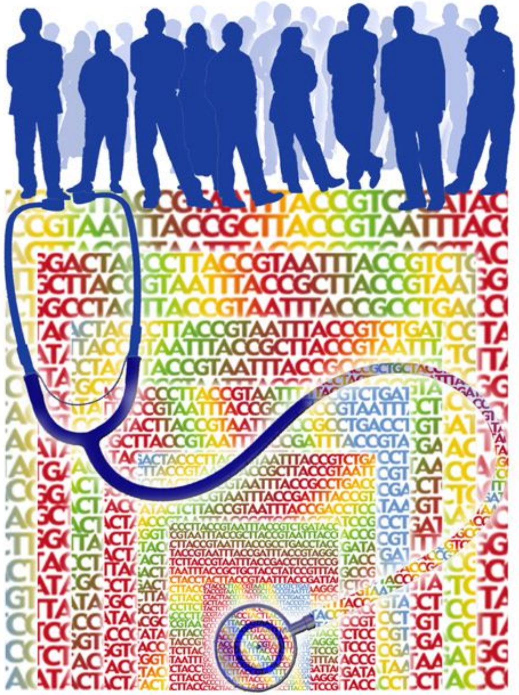
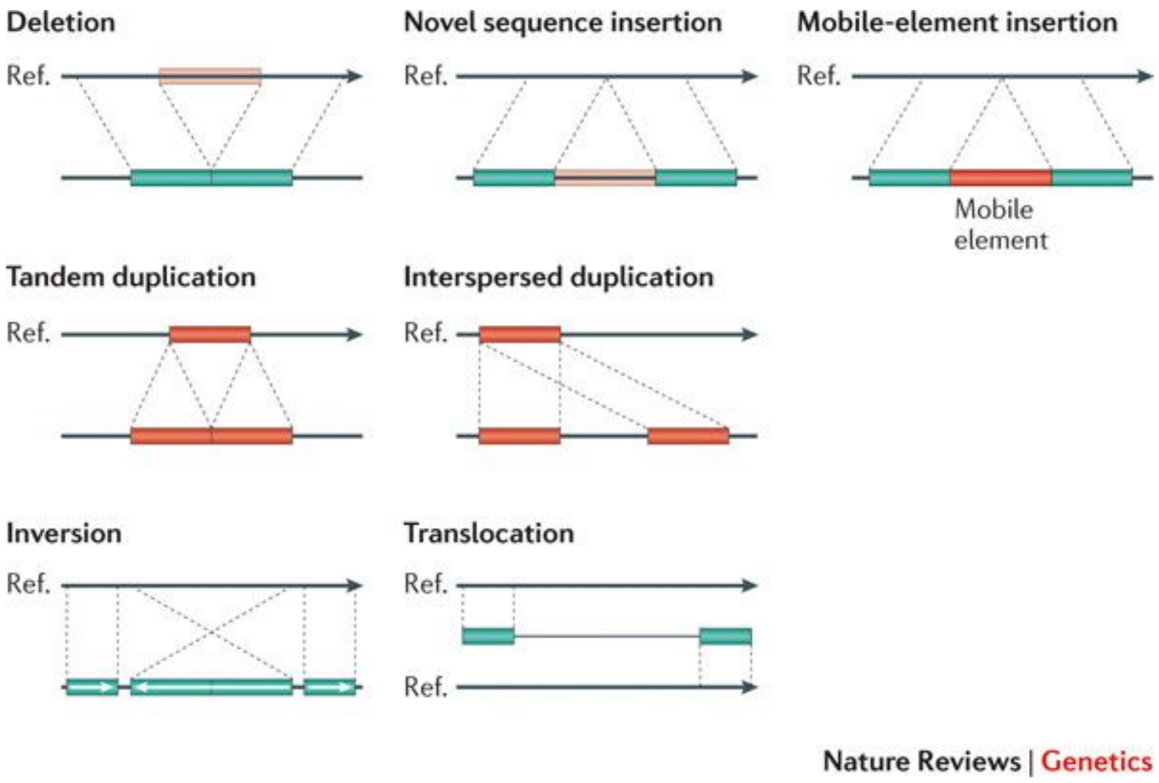
Structural variants

Structural variants (SV)

- Represent mutations in the genome > **50bp** in length
- Human genomes **differ more** as a consequence of **structural variation (SV)** than of a single-base-pair differences (SNV)
- Approximately **20000** SVs in each human genome



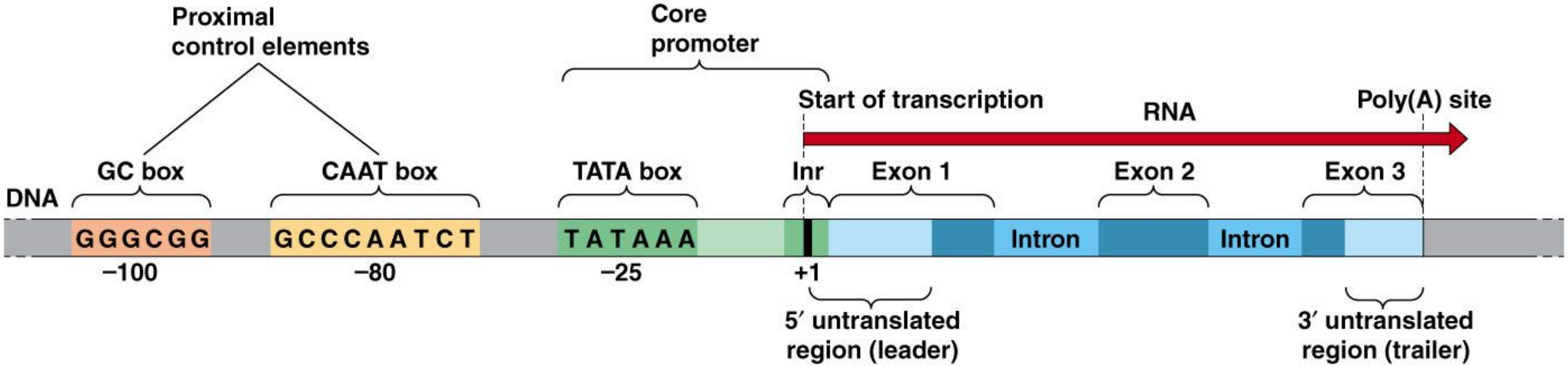
Structural variants (SV)



Effects of SV on the genome

- **Complete loss/gain** of a particular **region/gene**
- **Disruption of local interactions** in the genome
  - Increase/decrease expression of a gene
- **Disruption of global interactions** in the genome
  - Interaction with remote elements in the genome
  - Altering positions of chromosomes in the nucleus

Disruption of local interactions



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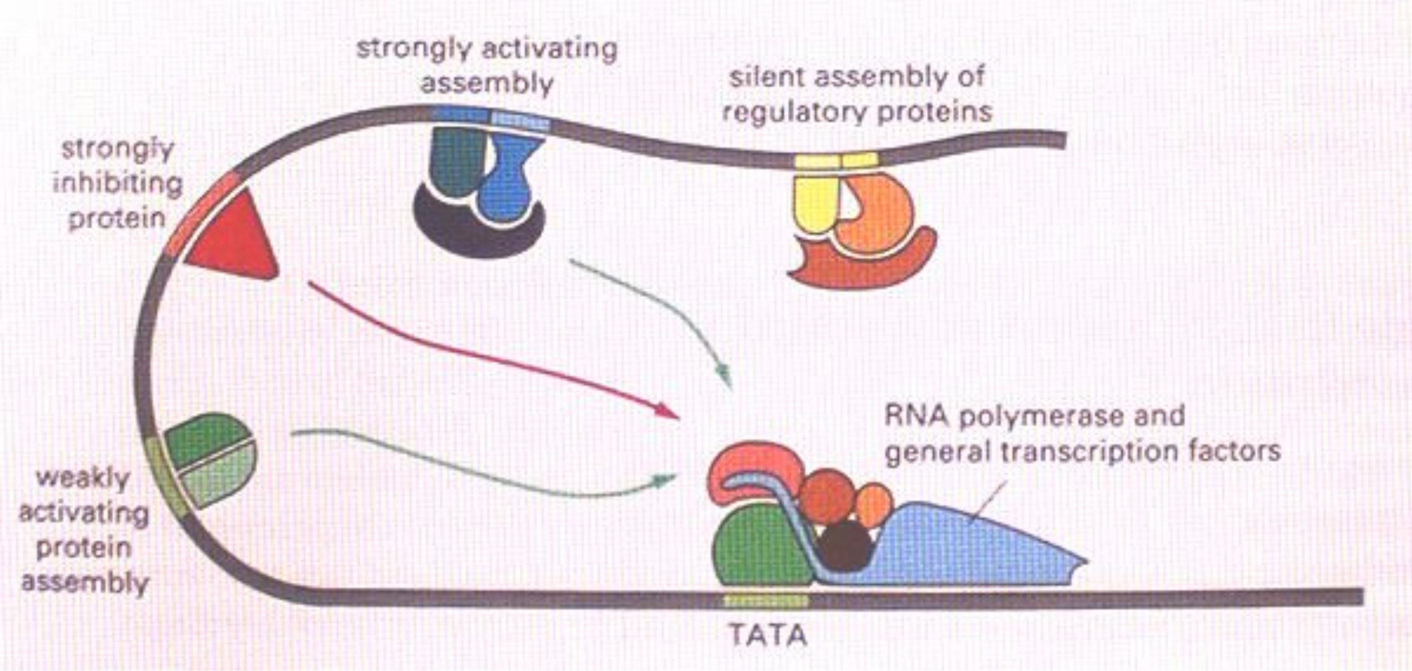
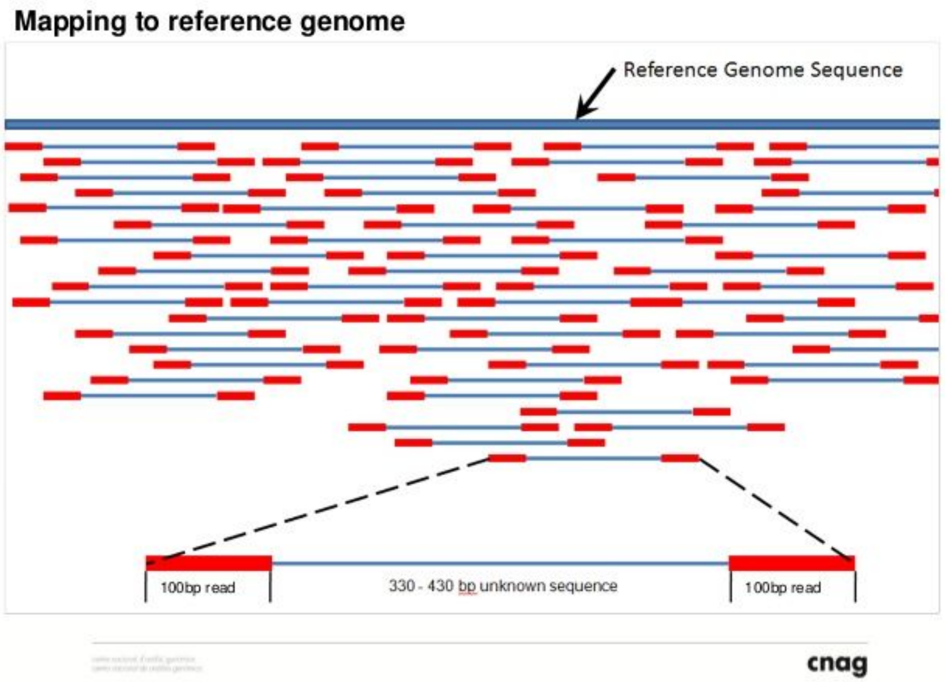
Disruption of global interactions


strongly activating assembly
silent assembly of regulatory proteins
strongly inhibiting protein
RNA polymerase and general transcription factors
weakly activating protein assembly
TATA

NGS short reads - recap

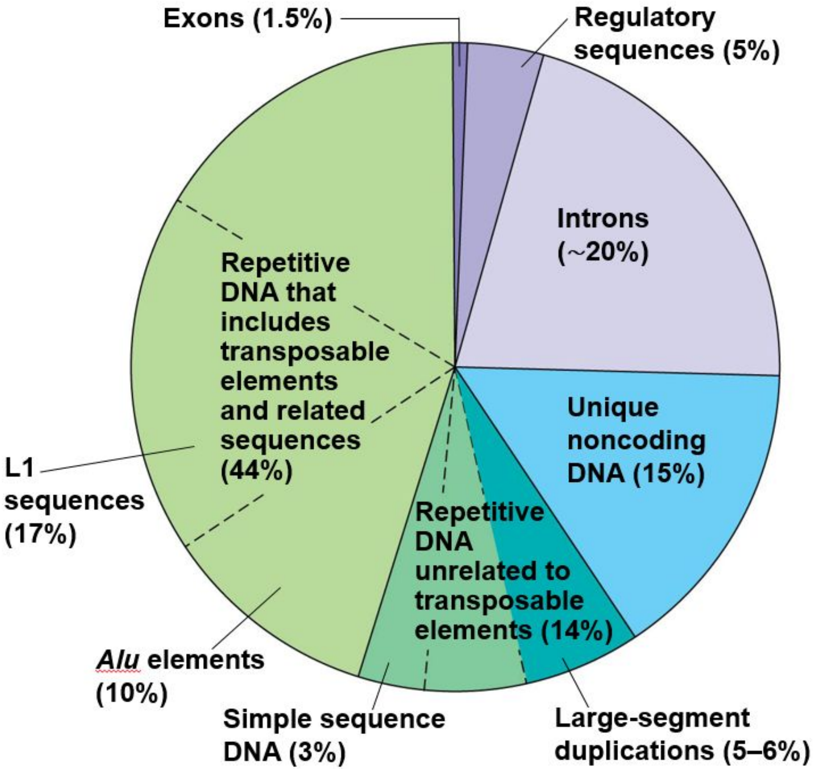
- Fragment size roughly **400-700bp**
- Paired-end (**PE**) reads **100-150bp** in length


Mapping to reference genome
Reference Genome Sequence
100bp read
330 - 430 bp unknown sequence
100bp read
cnag

Adapted from wikipedia

Genome structure

- **60% of the genome** is made of **repetitive sequences**
- Difficult to uniquely map a read to the correct position in the genome



SV detection - drawbacks

- Repetitive DNA
- Short reads (100-150bp)
- Short fragment size (distance between paired reads)

SV encoded in VCF

#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT NA00001

1 2827693 .
CCGTGGATGCGGGGACCCGCATCCCCTCTCCCTTCACAGCTGAGTGACCCACATCCCCTCTCCCCTCGCA C . PASS
SVTYPE=DEL;END=2827680;BKPTID=Pindel_LCS_D1099159;HOMLEN=1;HOMSEQ=C;**SVLEN=-66** GT:GQ 1/1:13.9

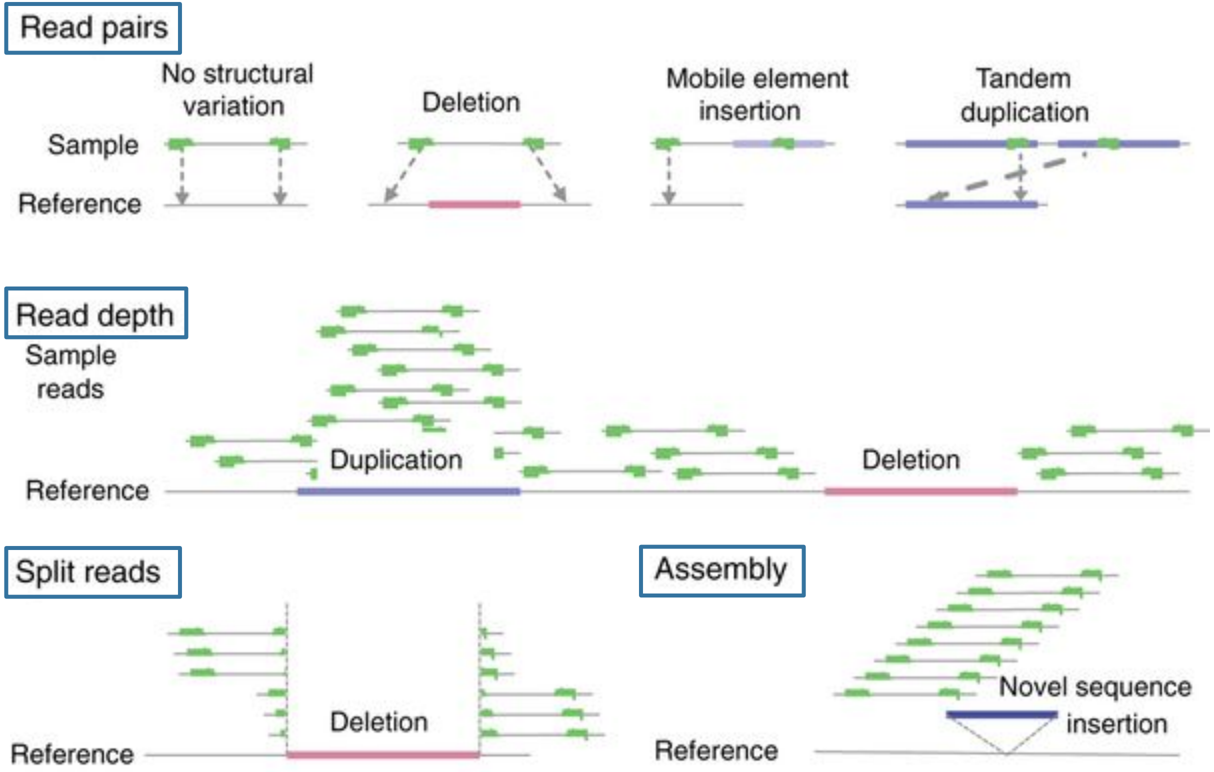
2 321682 . T <DEL> 6 PASS
IMPRECISE;**SVTYPE=DEL**;END=321887;**SVLEN=-105**;CIPOS=-56,20;CIEND=-10,62 GT:GQ 0/1:12

3 12665100 . A <DUP> 14 PASS
IMPRECISE;**SVTYPE=DUP**;END=12686200;**SVLEN=21100**;CIPOS=-500,500;CIEND=-500,500 GT:GQ:CN:CNQ ./.:0:3:16.2

SV classification

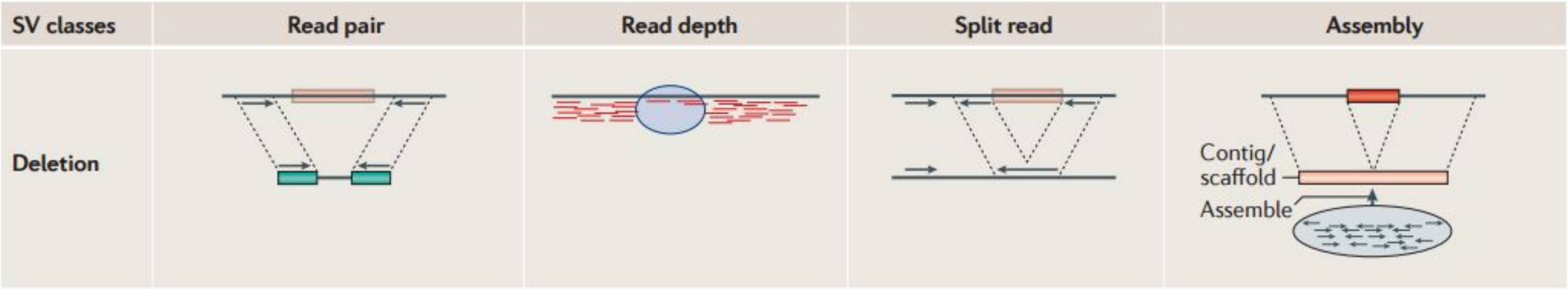
- **Balanced SVs -** *No change in length of the genome*
    - Inversions
    - Translocations
- **Unbalanced SVs** - *Alteration of genome length*
    - Insertions
    - **CNV** (copy number variation) - deletions, duplications

SV detection using short reads NGS



SV - Deletions

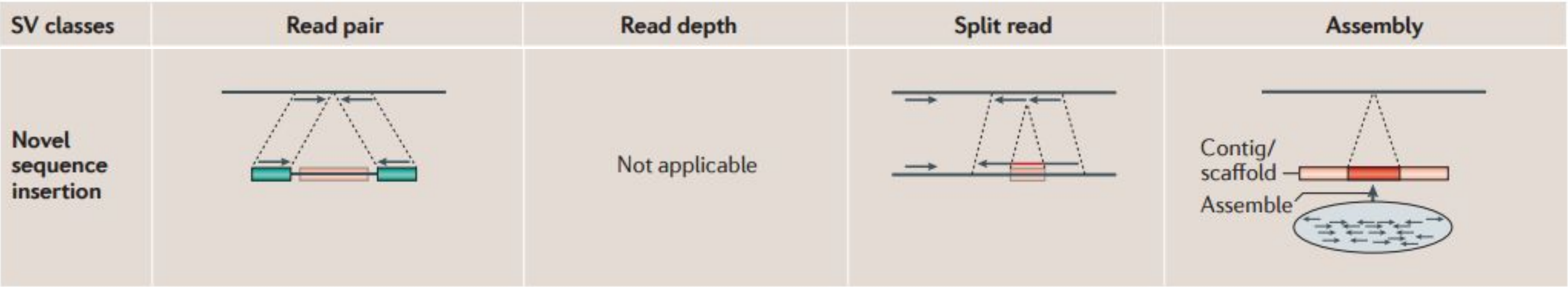
- **Read pair** - increased interpair mapping distance
- **Read depth** - fewer reads
- **Split read** - single read is “merged” from two segments surrounding deletion
- **Assembly -** assembled sequence shows “gap”



SV classes	Read pair	Read depth	Split read	Assembly
Deletion				

SV - Insertions

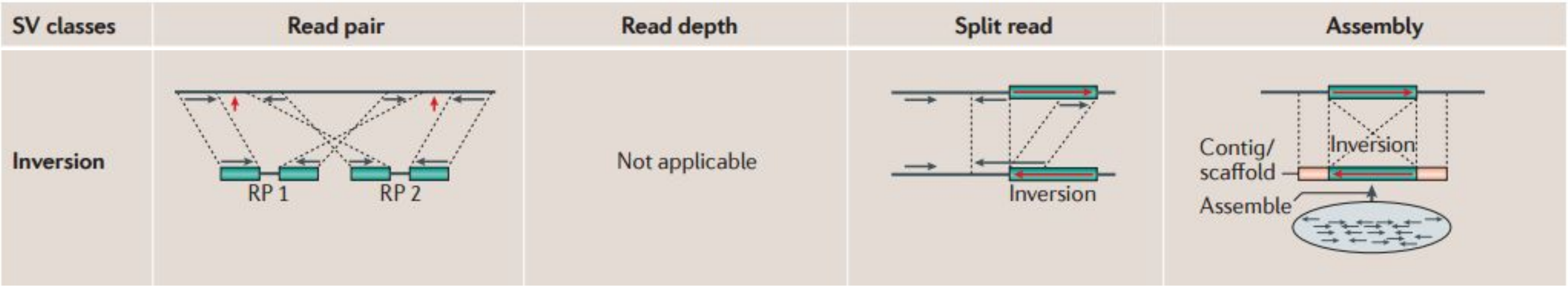
- **Read pair** - decreased interpair mapping distance
- **Read depth** - not applicable
- **Split read** - single read is split into two segments surrounding novel insertion sequence
- **Assembly -** assembled sequence contains novel sequence



SV classes	Read pair	Read depth	Split read	Assembly
Novel sequence insertion		Not applicable		

SV - Inversions

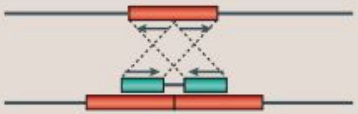
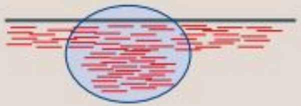
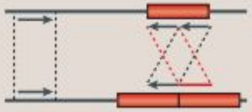
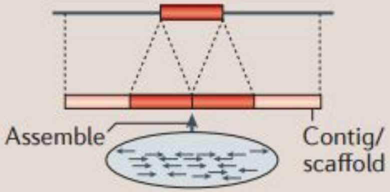
- **Read pair** - aberrant mapping and interpair distance
- **Read depth** - not applicable
- **Split read** - single read is split into two segments one of which is inverted
- **Assembly -** assembled sequence with inverted sequence



SV classes	Read pair	Read depth	Split read	Assembly
Inversion	RP 1 RP 2	Not applicable	Inversion	Contig/ scaffold Inversion Assemble

SV - Duplication

- **Read pair** - aberrant mapping and interpair distance
- **Read depth** - increased read depth
- **Split read** - single read is split into two segments one of which is inverted
- **Assembly -** assembled sequence with inverted sequence

SV classes	Read pair	Read depth	Split read	Assembly
Tandem duplication				

SV detection using long reads

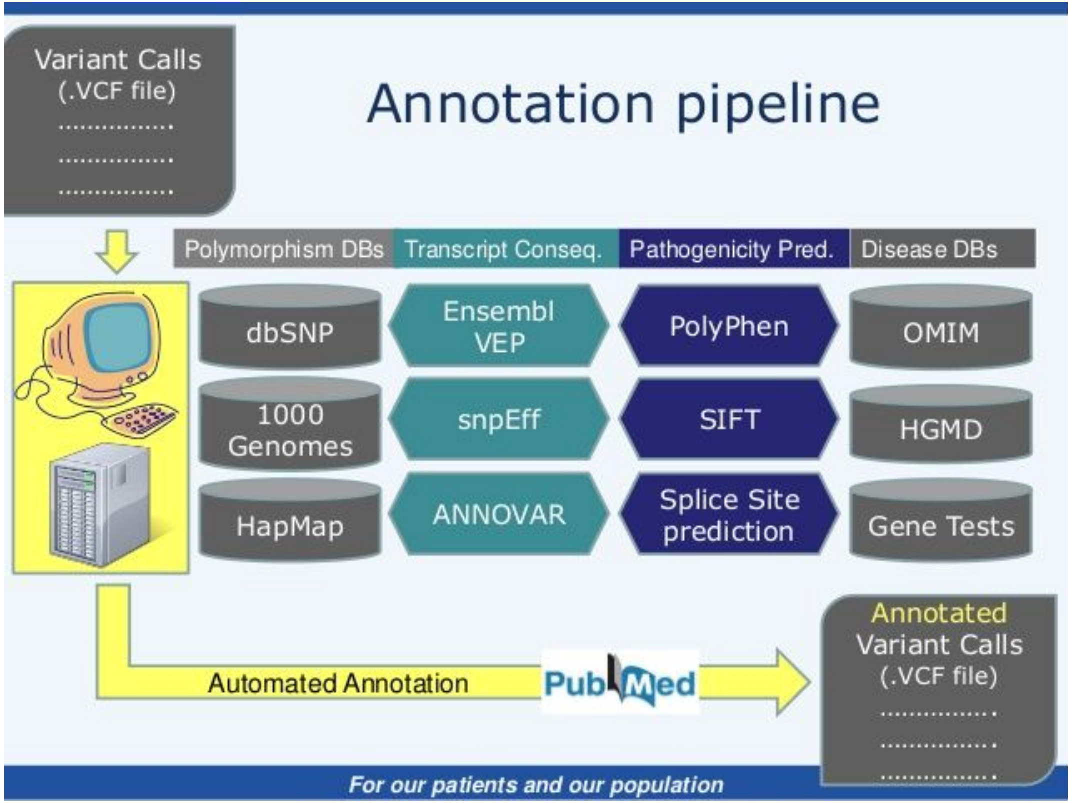
- **Pros**:
  - Ability for reads to span over entire variant
- **Cons**:
  - Higher error rate
  - Inability to detect inversions due to singe-end approach
- Still ineffective for extremely long variation

Variants annotation

Variants annotation

- Identify the **gene(s)** that **overlaps** with the **variant**
- Determine whether the variant is located in an **exon**
- If the variant is an **SNV**, determine whether the encoded amino acid is changed, if so annotate as missense
- If the variant is located right before or after an exon/intron boundary, annotate as splicing

Variants annotation pipeline



Variant Calls
(.VCF file)
…………….
…………….
…………….

Annotation pipeline

Polymorphism DBs	Transcript Conseq.	Pathogenicity Pred.	Disease DBs
dbSNP	Ensembl VEP	PolyPhen	OMIM
1000 Genomes	snpEff	SIFT	HGMD
HapMap	ANNOVAR	Splice Site prediction	Gene Tests

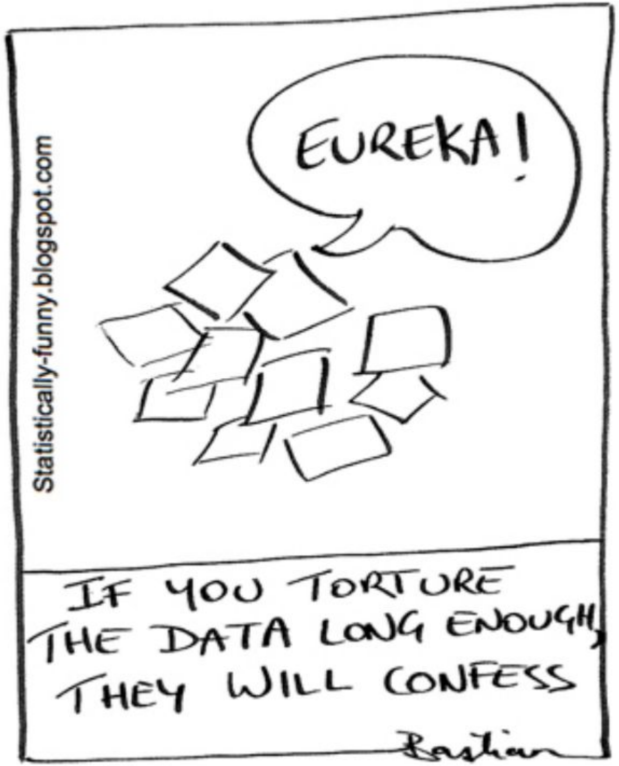
Automated Annotation

PubMed

Annotated
Variant Calls
(.VCF file)
…………….
…………….
…………….

For our patients and our population

Variant calling in short


Statistically-funny.blogspot.com
EUREKA!
IF YOU TORTURE THE DATA LONG ENOUGH, THEY WILL CONFESS
Bastian

Additional links

- Genome Sequencing and Structural Variation
- Encoding structural variants in VCF format
- Variant calling and annotation
- A geometric approach for classification and comparison of structural variants
- Structural variation in the human genome

SV - Deletions Exercise

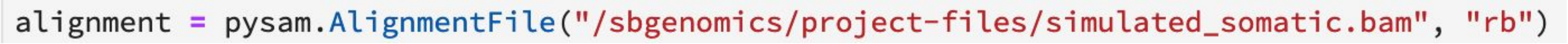
- Simplified deletion detection example based on **read depth** and **split reads**

- Find breakend candidates using split reads

- Detect SV type using read depth

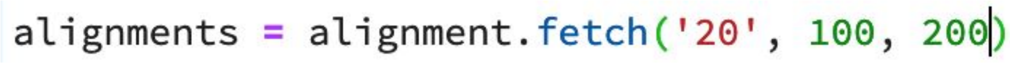
SV - Deletions Exercise

- Simplified deletion detection example based on **read depth** using pysam:
  - Load BAM file



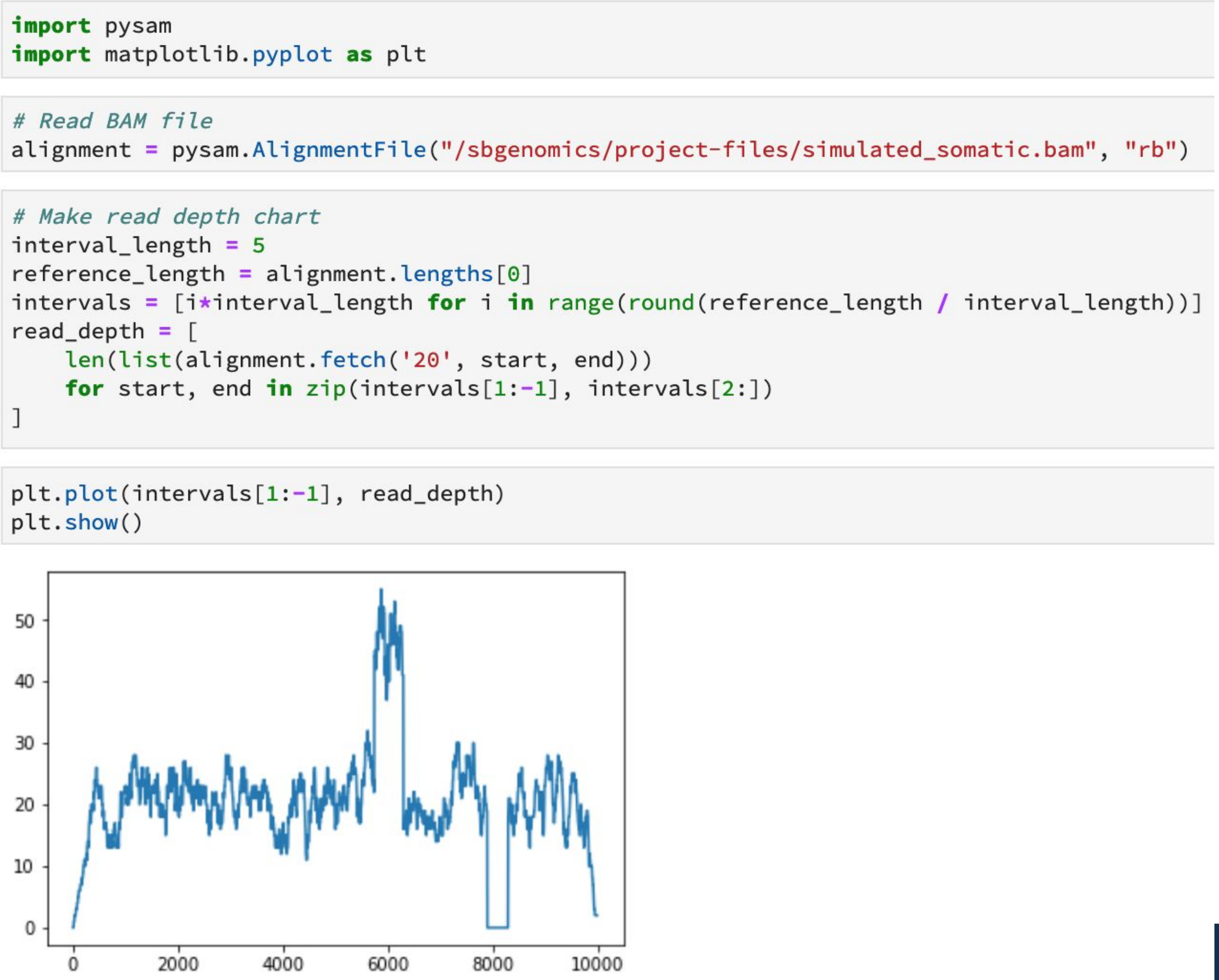
```
alignment = pysam.AlignmentFile("/sbgenomics/project-files/simulated_somatic.bam", "rb")
```

  - Plot read depth



```
alignments = alignment.fetch('20', 100, 200)
```

  - Find deletions



```
import pysam
import matplotlib.pyplot as plt
```

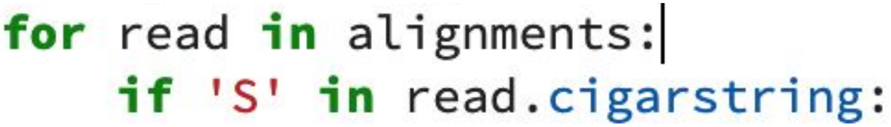
```
# Read BAM file
alignment = pysam.AlignmentFile("/sbgenomics/project-files/simulated_somatic.bam", "rb")
```

```
# Make read depth chart
interval_length = 5
reference_length = alignment.lengths[0]
intervals = [i*interval_length for i in range(round(reference_length / interval_length))]
read_depth = [
    len(list(alignment.fetch('20', start, end)))
    for start, end in zip(intervals[1:-1], intervals[2:])
]
```

```
plt.plot(intervals[1:-1], read_depth)
plt.show()
```

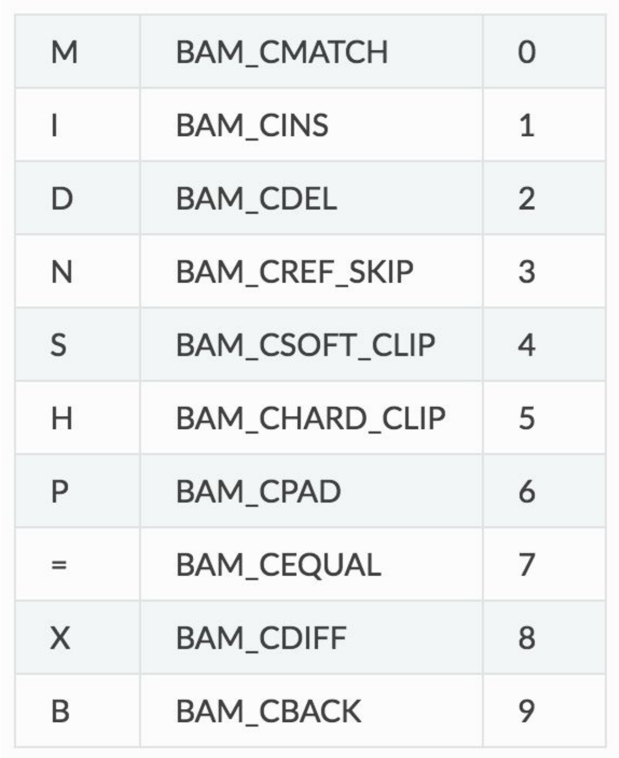
SV - Deletions Exercise

- Deletion detection based on split reads:
  - Locate soft clip locations
- CIGAR string



```
for read in alignments:
    if 'S' in read.cigarstring:
```

- 73M27S
  - U read-u imamo prvo 73 matcha



M	BAM_CMATCH	0
I	BAM_CINS	1
D	BAM_CDEL	2
N	BAM_CREF_SKIP	3
S	BAM_CSOFT_CLIP	4
H	BAM_CHARD_CLIP	5
P	BAM_CPAD	6
=	BAM_CEQUAL	7
X	BAM_CDIFF	8
B	BAM_CBACK	9