# Introduction to Genomics

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https://ngs-course.readthedocs.io/en/praha-february-2019/

# Genome

*The genome is the genetic material of an organism including both the genes and the non-coding sequences.*

# Bioinformatic perspective

- sequence
- physical map
- annotations
- versioned reference

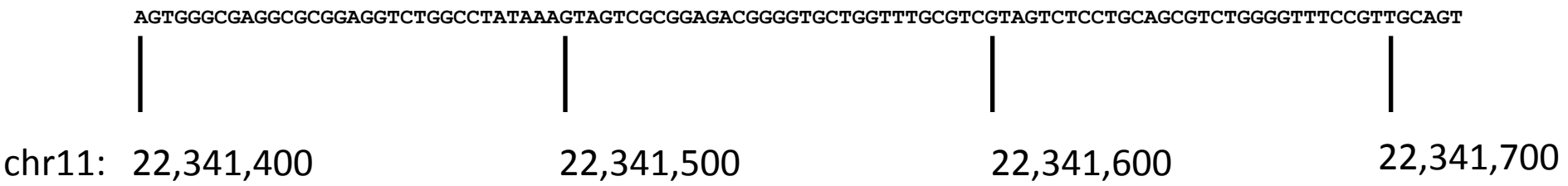
# Bioinformatic perspective

- sequence

```
AGTGGGCGAGGCGCGGAGGTCTGGCCTATAAAGTAGTCGCGGAGACGGGG
TGCTGGTTTGCGTCGTAGTCTCCTGCAGCGTCTGGGGTTTCCGTTGCAGT
CCTCGGAACCAGGACCTCGGCGTGGCCTAGCGAGTTATGGCGACGAAGGC
CGTGTGCGTGCTGAAGGGCGACGGCCCAGTGCAGGGCATCATCAATTTCG
AGCAGAAGGCAAGGGCTGGGACGGAGGCTTGTTTGCGAGGCCGCTCCCAC
CCGCTCGTCCCCCCGCGCACCTTTGCTAGGAGCGGGTCGCCCGCCAGGCC
TCGGGGCCGCCCTGGTCCAGCGCCCGGTCCCGGCCCGTGCCGCCCGGTCG
GTGCCTTCGCCCCCAGCGGTGCGGTGCCCAAGTGCTGAGTCACCGGGCGG
GCCCGGGCGCGGGGCGTGGGACCGAGGCCGCCGCGGGGCTGGGCCTGCGC
GTGGCGGGAGCGCGGGGAGGGATTGCCGCGGGCCGGGGAGGGGCGGGGGC
GGGCGTGCTGCCCTCTGTGGTCCTTGGGCCGCCGCCGCGGGTCTGTCGTG
GTGCCTGGAGCGGCTGTGCTCGTCCCTTGCTTGGCCGTGTTCTCGTTCCT
GAGGGTCCCGCGGACACCGAGTGGCGCAGTGCCAGGCCCAGCCCGGGGAT
GGCGACTGCGCCTGGGCCCGCCTGGTGTCTTCGCATCCCTCTCCGCTTTC
CGGCTTCAGCGCTCTAGGTCAGGGAGTCTTCGCTTTTGTACAGCTCTAAG
GCTAGGAATGGTTTTTATATTTTTAAAAGGCTTTGGAAAACAAAAATACG
CAACAGAGACCGTTTGTGTGACACTTTGCAGGGAAGTTTGCTGGCCTCTG
TTCTAGGTCATGATTGGGCTGCAAGGGCAGAGAAGGTAGCCTTGAACAGA
GGTCCTTTTCCTCCTCCTAAGCTCCGGGAGCCAGAGGTTTAACTGACCCT
```

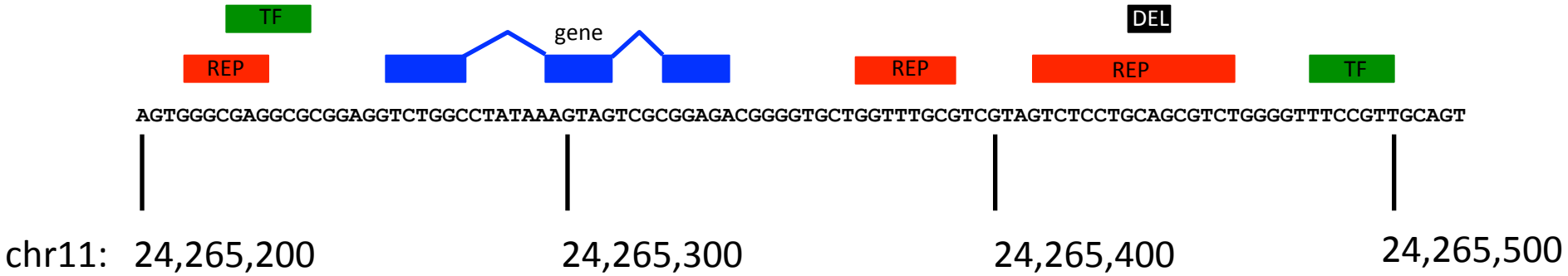
# Bioinformatic perspective

- physical map



# Bioinformatic perspective

- annotations



# Bioinformatic perspective

- versioned reference

# How to get a genome?

- get a sequence
- map the sequence
- annotate the sequence
- refine the sequence

# Get a sequence

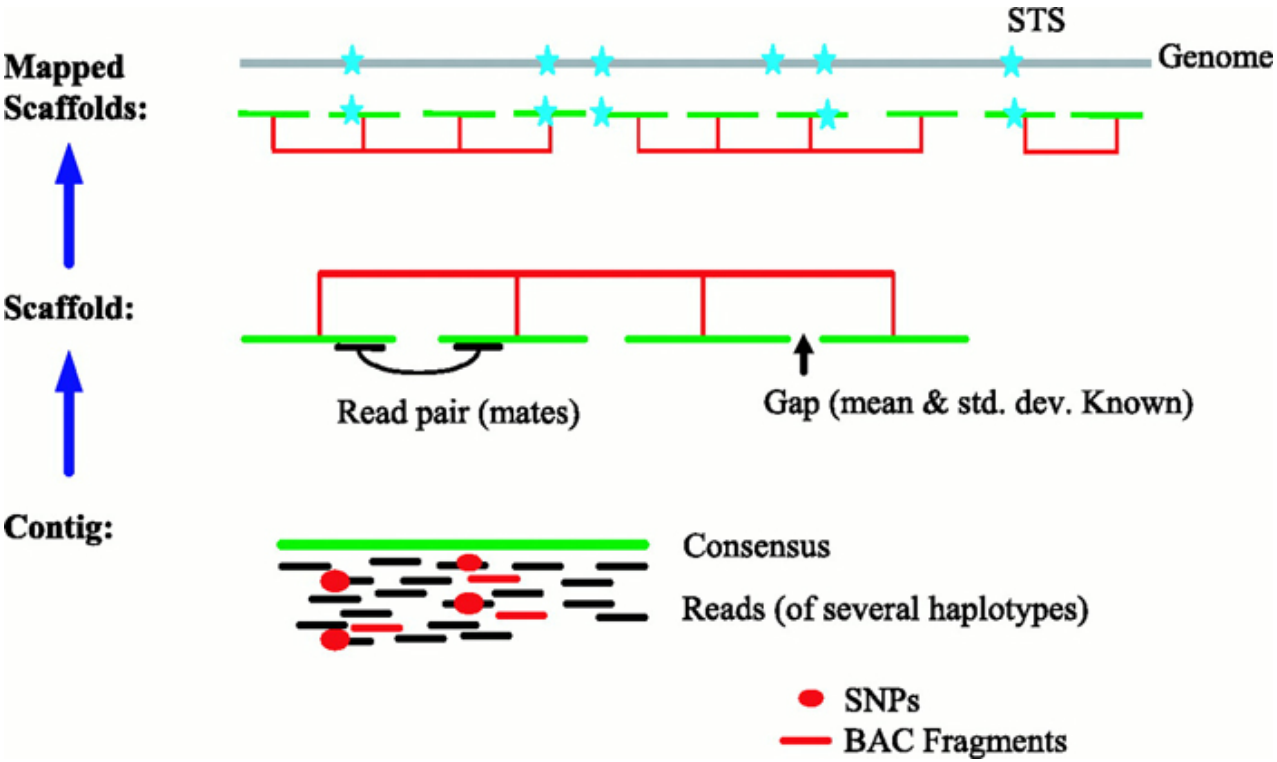
- Old ways (Sanger) or new ways (NGS)...

  *... all produce "reads"*

  *or "paired-end reads" ...*

# Get/Map the sequence

- *Reads* are *assembled* into continuous *contigs*
- *Paired-end reads* help to create a *scaffold* of contings
- Scaffolds are then mapped to *chromosomes*

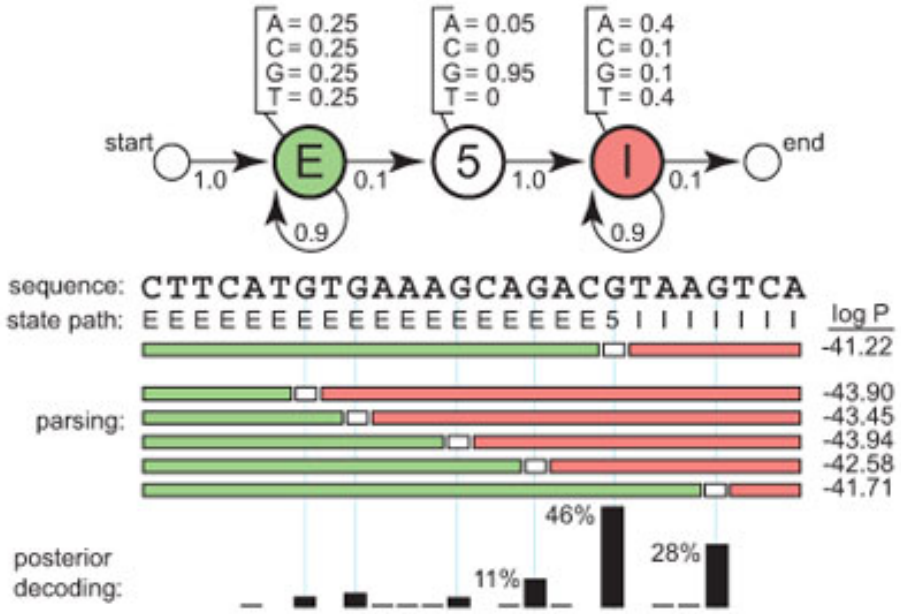


# Annotate the sequence

- Annotation approaches
  - sequence similarity
    - to known features
    - to homologous features in other organisms
  - feature prediction using models

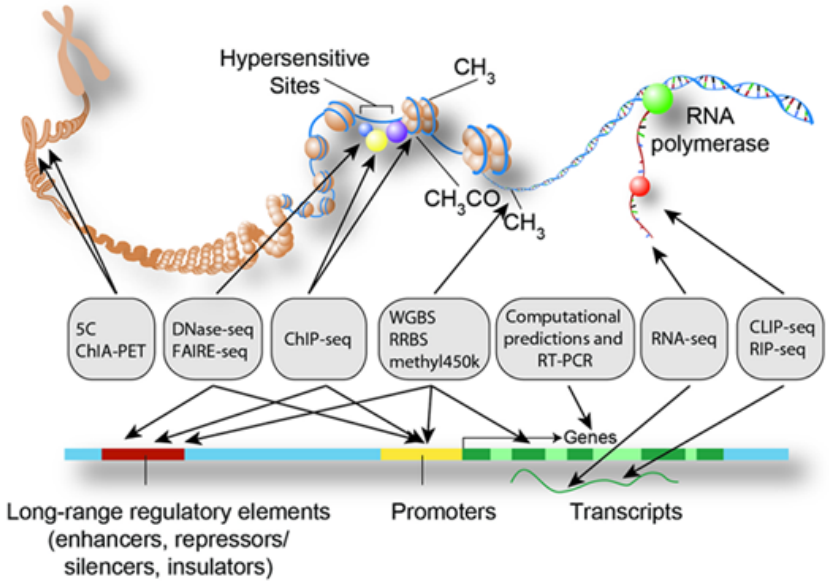
# Annotate the sequence

- Gene prediction
  - sequence similarity to ESTs, RNA-seq
  - homology – gene/protein families
  - using Hidden Markov Models to predict gene structure



# Annotate the sequence

- Other non-coding functional elements
  - TF binding sites, etc.
  - interspecies sequence conservation
  - ChIP-seq, DNAseI Hypersensitive Sites, etc.




# Annotate the sequence

- Other features
  - Variation data (SNPs, INDELS)
  - Structural variation data (CNVs)
  - Repeat data (RepeatMasker)
  - Epigenomic data (methylation, histone acetylation)
  - Functional data (Gene Ontology, KEGG, ...)
  - Gene Expression

# Where to find genomic data?



UCSC Genome Bioinformatics

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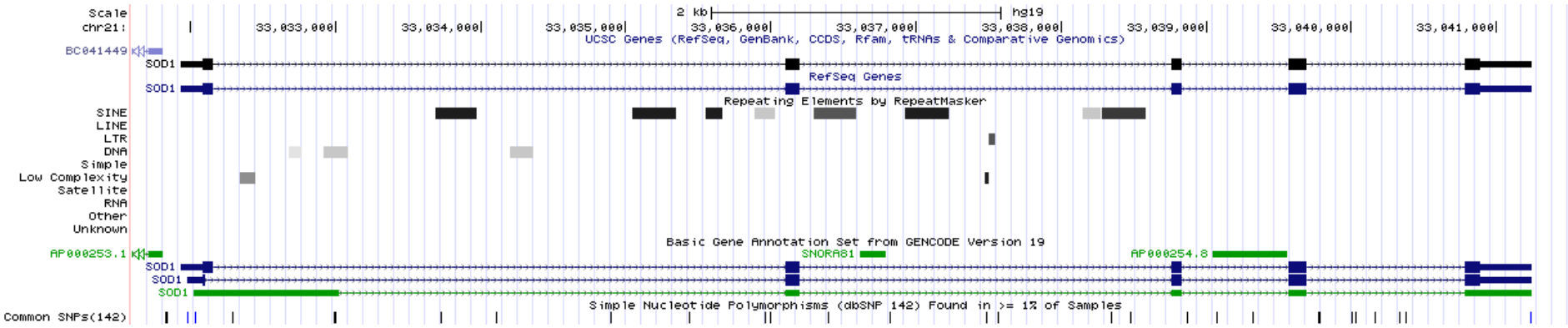
UCSC Genome Bioinformatics



# The way the genomic data are stored

- Regular text files of a specific format
  - easy to open and explore
  - easy to work with
  - .fasta, .fastq, .bed, .gff, .gtf, .vcf, ...
- Binaries
  - more efficient for large datasets
  - fast retrieval by specific tools
  - .2bit, .gz, .bcf

# Storing sequences: FASTA

```
>ID_seq|specific_info
AGTGGGCGAGGCGCGGAGGTCTGGCCTATAAAGTAGTCGCGGAGACGGGG
TGCTGGTTTGCGTCGTAGTCTCCTGCAGCGTCTGGGGTTTCCGTTGCAGT
CCTCGGAACCAGGACCTCGGCGTGGCCTAGCGAGTTATGGCGACGAAGGC
CGTGTGCGTGCTGAAGGGCGACGGCCCAGTGCAGGGCATCATCAATTTCG
AGCAGAAGGCAAGGGCTGGGACGGAGGCTTGTTTGCGAGGCCGCTCCCAC
CCGCTCGTCCCCCCGCGCACCTTTGCTAGGAGCGGGTCGCCCGCCAGGCC
TCGGGGCCGCCCTGGTCCAGCGCCCGGTCCCGGCCCGTGCCGCCCGGTCG
GTGCCTTCGCCCCCAGCGGTGCGGTGCCCAAGTGCTGAGTCACCGGGCGG
```

# Storing reads: FASTQ

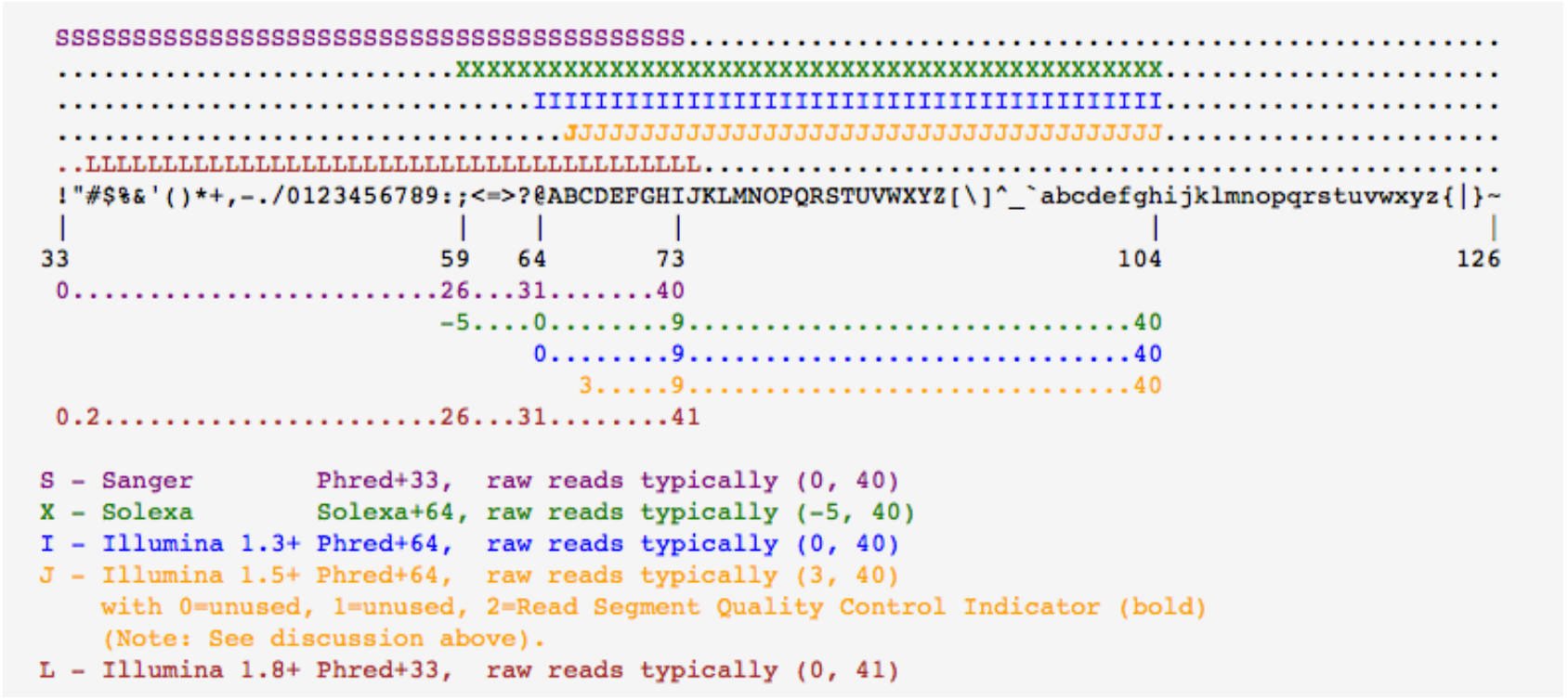
```
@ID_seq1
AGTGGGCGAGGCGCGGAGGTCTGGCCTATAAAGTAGTCGCGGAGACGGGG
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>
@ID_seq2
CCTCGGAACCAGGACCTCGGCGTGGCCTAGCGAGTTATGGCGACGAAGGC
+
')%'* (***+)*''))*%%++5C)(%%%(!((%).1***-+*5CF>>>>
```

# Storing reads: FASTQ

```
@ID_seq1
AGTGGGCGAGGCGCGGAGGTCTGGCCTATAAAGTAGTCGCGGAGACGGGG
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>
@ID_seq2
CCTCGGAACCAGGACCTCGGCGTGGCCTAGCGAGTTATGGCGACGAAGGC
+
')%'* (***+)*''))*%%++5C)(%%%(!((%).1***-+*5CF>>>>
```

ASCII

# FASTQ: ASCII to PHRED



# PHRED: quality scores

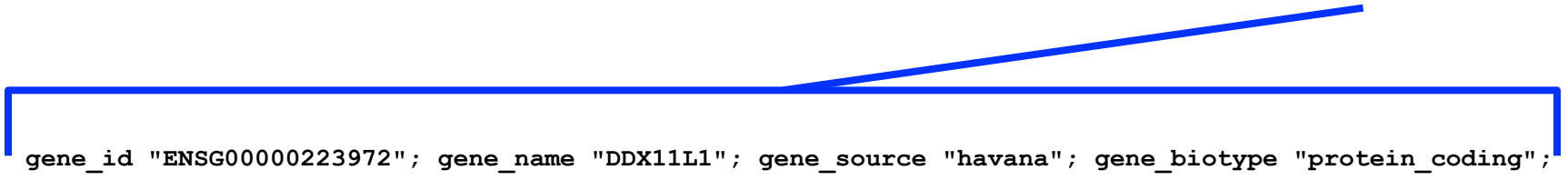
| Phred Quality Score | Probability of incorrect base call | Base call accuracy |
|---|---|---|
| 10 | 1 in 10 | 90% |
| 20 | 1 in 100 | 99% |
| 30 | 1 in 1000 | 99.9% |
| 40 | 1 in 10,000 | 99.99% |

$$\text{Phred} = -10 \log_{10} P$$

# Storing annotations: GFF/GTF

- GFF
  - General Feature Format (any kind of annotation/feature)
- GTF
  - Gene Transfer Format (specific form of GFF used to store gene annotation)
- 9 TAB separated fields
- actual content of individual fields depends on the database and type of data

| seqname | source | feature | start | end | score | strand | frame | attribute |
|---|---|---|---|---|---|---|---|---|
| 2 | protein_coding | CDS | 2419108 | 2419128 | . | + | 0 | gene_id "ENSG00000223972"; |
| X | protein_coding | CDS | 1186934 | 1440976 | . | - | 0 | gene_id "ENSG00000123546"; |




tag "value";

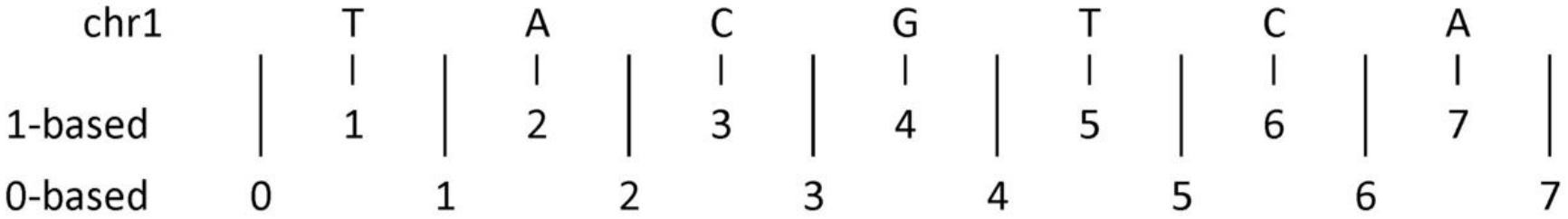
# Storing annotations: BED

- 3/4/6/12 columns
- used by UCSC Genome Browser to visualize various features

| chrom | chromStart | chromEnd | name | score | strand |
|---|---|---|---|---|---|
| 2 | 2419108 | 2419128 | ENSG00000223972 | . | + |
| X | 1186934 | 1440976 | ENSG00000123546 | . | - |

# Storing annotations: BED

- 0-based vs. 1-based coordinate system




| | 1-based | 0-based |
|---|---|---|
| Indicate a single nucleotide | chr1:4-4 G | chr1:3-4 G |
| Indicate a range of nucleotides | chr1:2-4 ACG | chr1:1-4 ACG |
| Indicate a single nucleotide variant | chr1:5-5 T/A | chr1:4-5 T/A |

# Storing variation data: VCF

- Variant Call Format

```
##fileformat=VCFv4.0
##fileDate=20110705
##reference=1000GenomesPilot-NCBI37
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=.,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
#CHROM POS     ID        REF  ALT     QUAL FILTER INFO                              FORMAT      Sample1
2      4370    rs6057    G    A       29   .      NS=2;DP=13;AF=0.5;DB;H2           GT:GQ:DP:HQ 0|0:48:1:52,51
2      7330    .         T    A       3    q10    NS=5;DP=12;AF=0.017               GT:GQ:DP:HQ 0|0:46:3:58,50
2      110696  rs6055    A    G,T     67   PASS   NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6:23,27
2      130237  .         T    .       47   .      NS=2;DP=16;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60
2      134567  microsat1 GTCT G,GTACT 50   PASS   NS=2;DP=9;AA=G                   GT:GQ:DP    0/1:35:4
```

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2      130237  .         T    .       47   .      NS=2;DP=16;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60
2      134567  microsat1 GTCT G,GTACT 50   PASS   NS=2;DP=9;AA=G                   GT:GQ:DP    0/1:35:4
```

**Header part**
**(description of abbreviations used in the data part)**

**Data part**

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2      4370    rs6057    G    A       29   .      NS=2;DP=13;AF=0.5;DB;H2           GT:GQ:DP:HQ 0|0:48:1:52,51
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2      130237  .         T    .       47   .      NS=2;DP=16;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60
2      134567  microsat1 GTCT G,GTACT 50   PASS   NS=2;DP=9;AA=G                   GT:GQ:DP    0/1:35:4
```

Table: Variants (rows) vs. Samples (columns)

Variation details (location, quality, type, etc.)

Samples + Genotypes

Data part

# Storing annotations: VCF

- Explore VCF file

```
< /data-shared/vcf_examples/luscinia_vars_flags.vcf.gz
zcat |  less -S
```