Genomics

Course: Work with genomic data in Unix

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http://ngs-course.readthedocs.org/en/praha-april-2015/

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

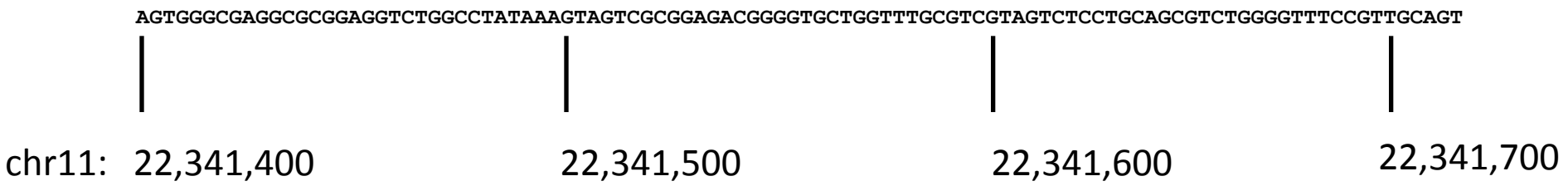
Bioinformatic perspective

- sequence

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

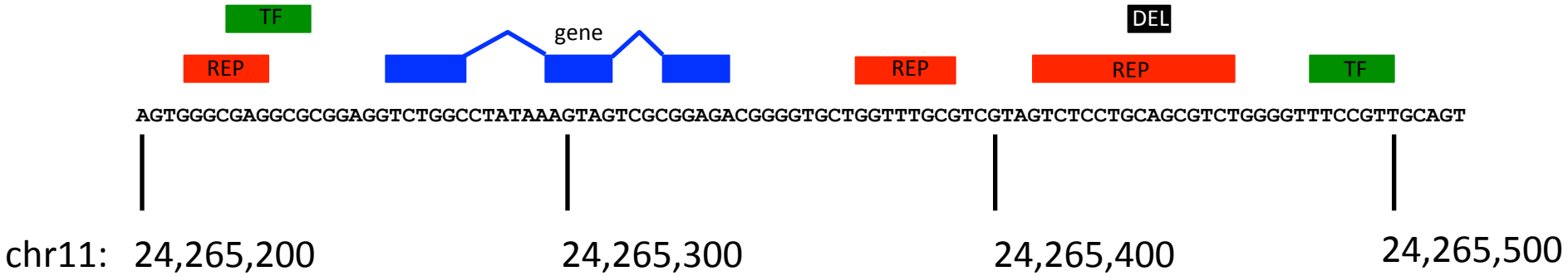
Bioinformatic perspective

- 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

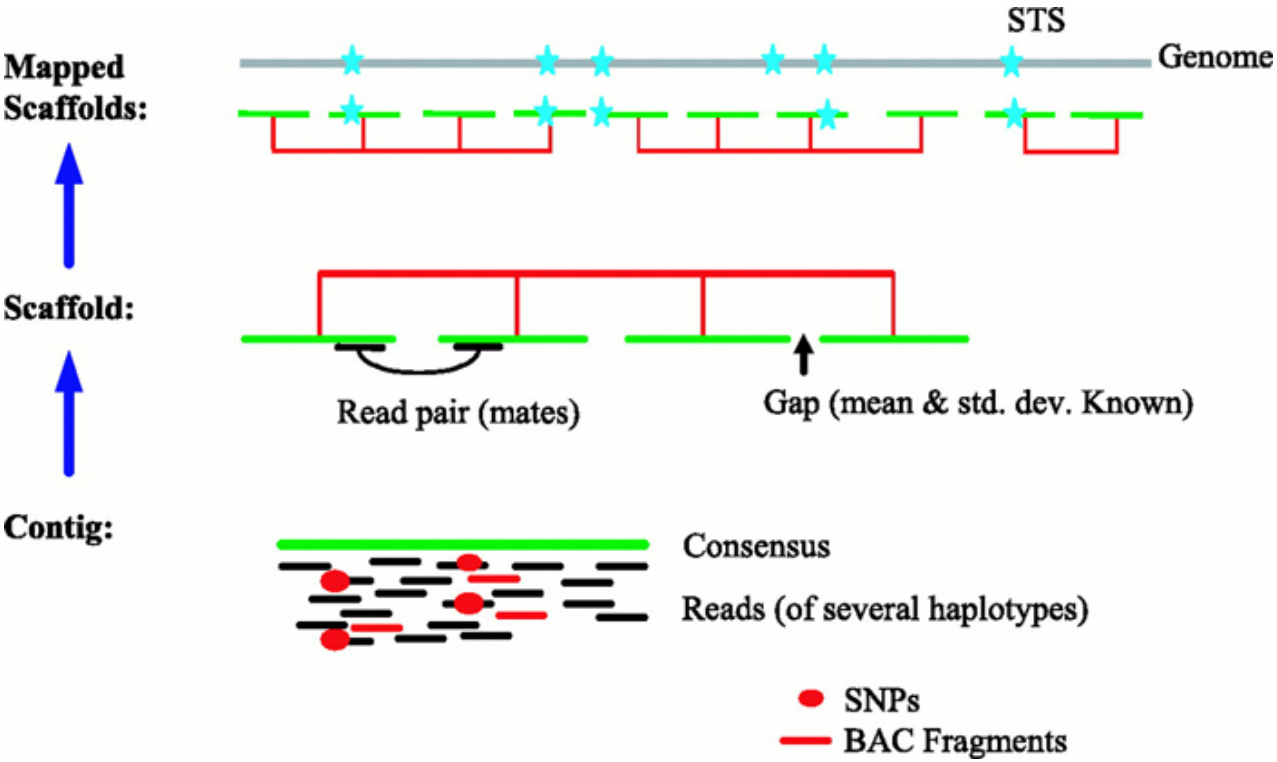
- Old ways (Sanger) or new ways (NGS)...

  ... all produce "reads"

  or "pairs of reads" ...

Map the sequence

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

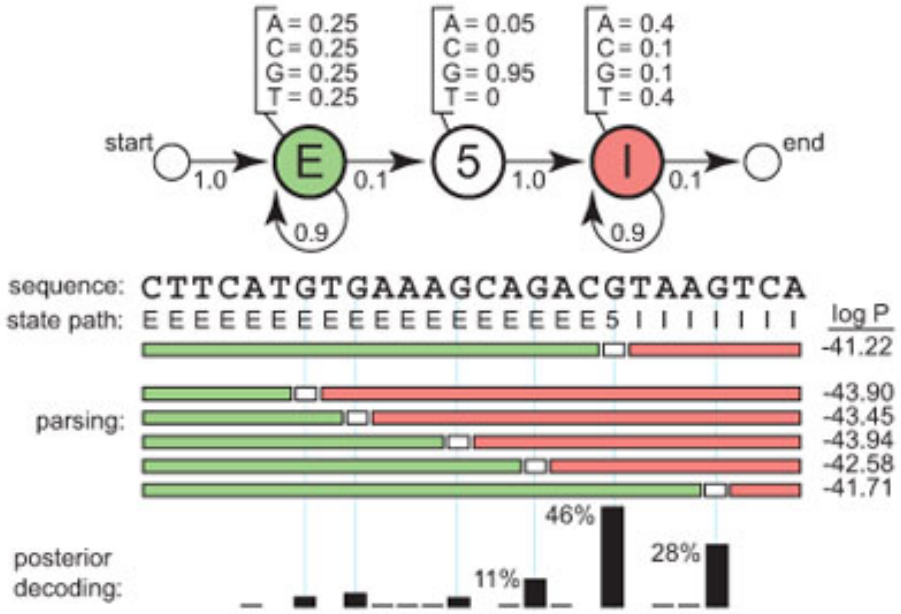


Annotate the sequence

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

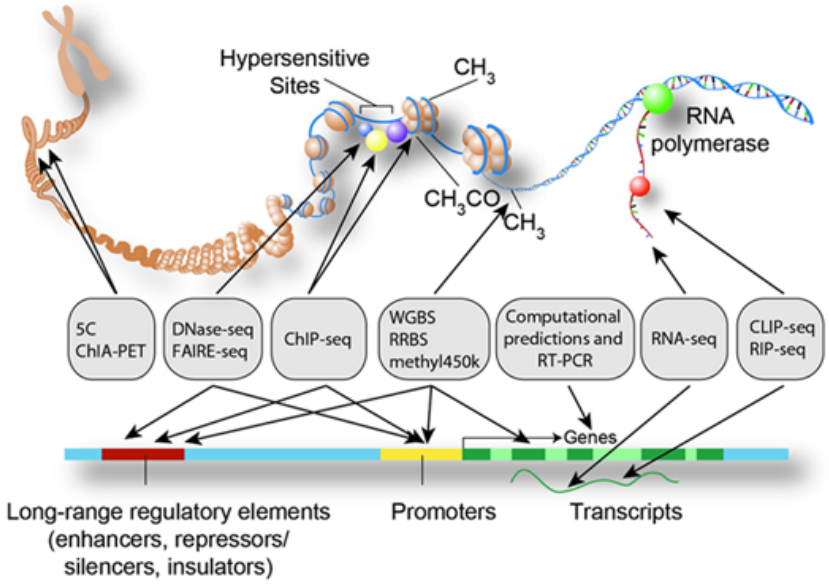
Annotate the sequence

- 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

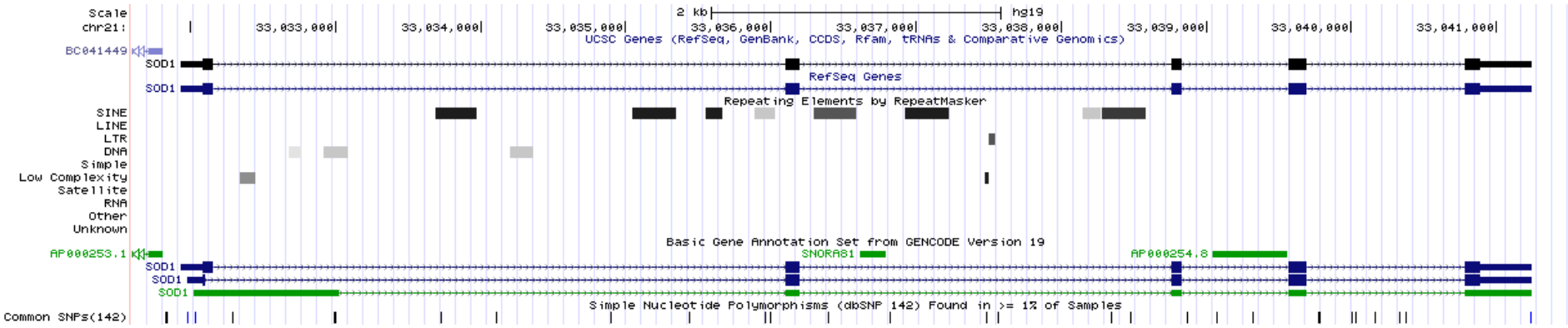
- Other features
  - Variation data (SNPs, INDELS)
  - Structural variation data (CNVs)
  - Repeat data (RepeatMasker)
  - Epigenomic data (methylation, histone acetylation)
  - Functional data (Gene Ontology, KEGG, ...)
  - Gene Expression

Where to find genomic data?

UCSC Genome Bioinformatics

Where to find genomic data?

UCSC Genome Bioinformatics



The way the genomic data are stored

- Regular text files of 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
```

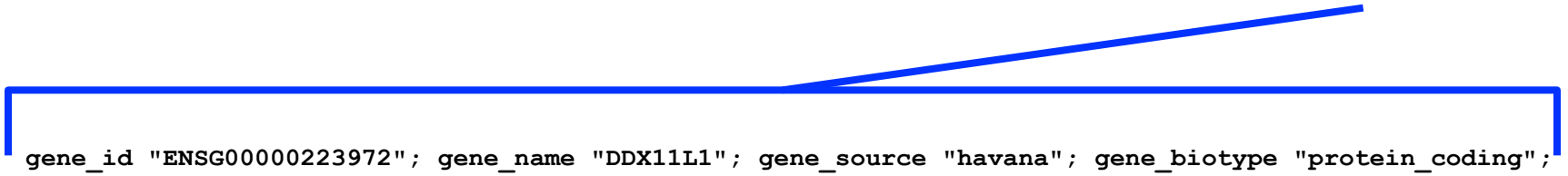
Storing reads: FASTQ

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

Storing 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: GTF

- Explore GTF file

```
cd
mkdir data

cp /data/mus_mda/05-fst2genes/Mus_musculus.NCBIM37.67.gtf.gz data/.

cd data
gunzip Mus_musculus.NCBIM37.67.gtf.gz

less -S Mus_musculus.NCBIM37.67.gtf
```

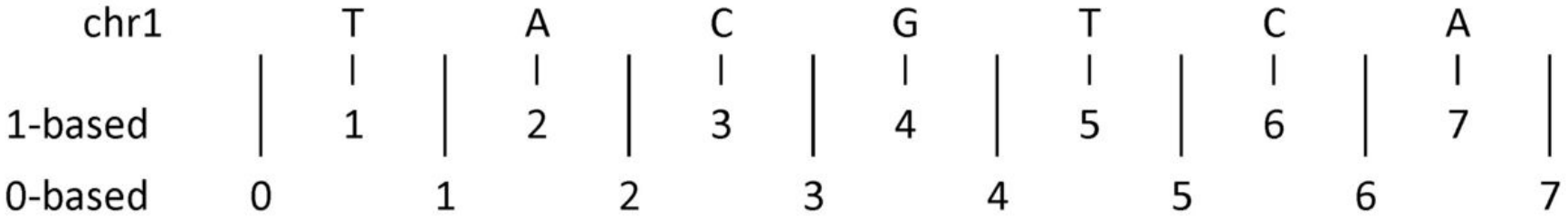
Storing annotations: BED

- 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

- Variation 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
```

Storing variation data: VCF

- Variation Call Format

```
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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
```

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

Data part

Storing variation data: VCF

- Variation Call Format

```
##fileformat=VCFv4.0
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##reference=1000GenomesPilot-NCBI37
##phasing=partial
##INFO=<ID=NS,Nu
##INFO=<ID=DP,Nu
##INFO=<ID=AF,Nu
##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">
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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
```

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

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

Samples + Genotypes

Data part

Storing annotations: VCF

- Explore VCF file

```
cd

cp /data/mus_mda/00-popdata/popdata_mda.vcf.gz data/.

cd data
gunzip popdata_mda.vcf.gz

less -S popdata_mda.vcf
```

Let's practise...