Variant calling
practical session

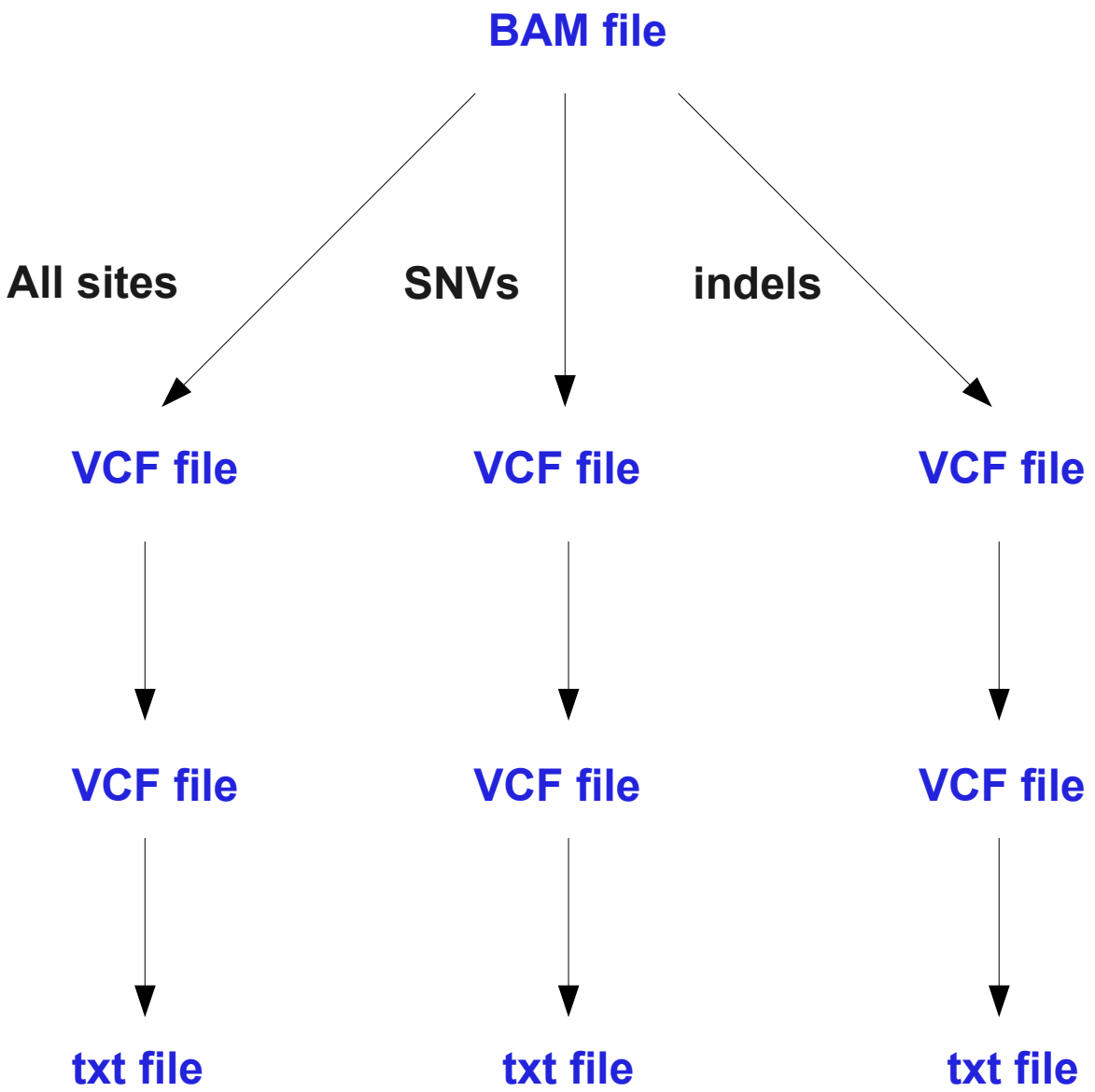
Jorge Jiménez
jjimeneza@cipf.es
BIER - CIBERER
Genomics Department
Centro de Investigacion Principe Felipe (CIPF)
(Valencia, Spain)

PRINCIPE FELIPE
CENTRO DE INVESTIGACION

ciberer
CENTRO DE INVESTIGACIÓN BIOMÉDICA EN RED DE ENFERMEDADES RARAS

Index

Scheme



Calling - GATK

Labeling - GATK

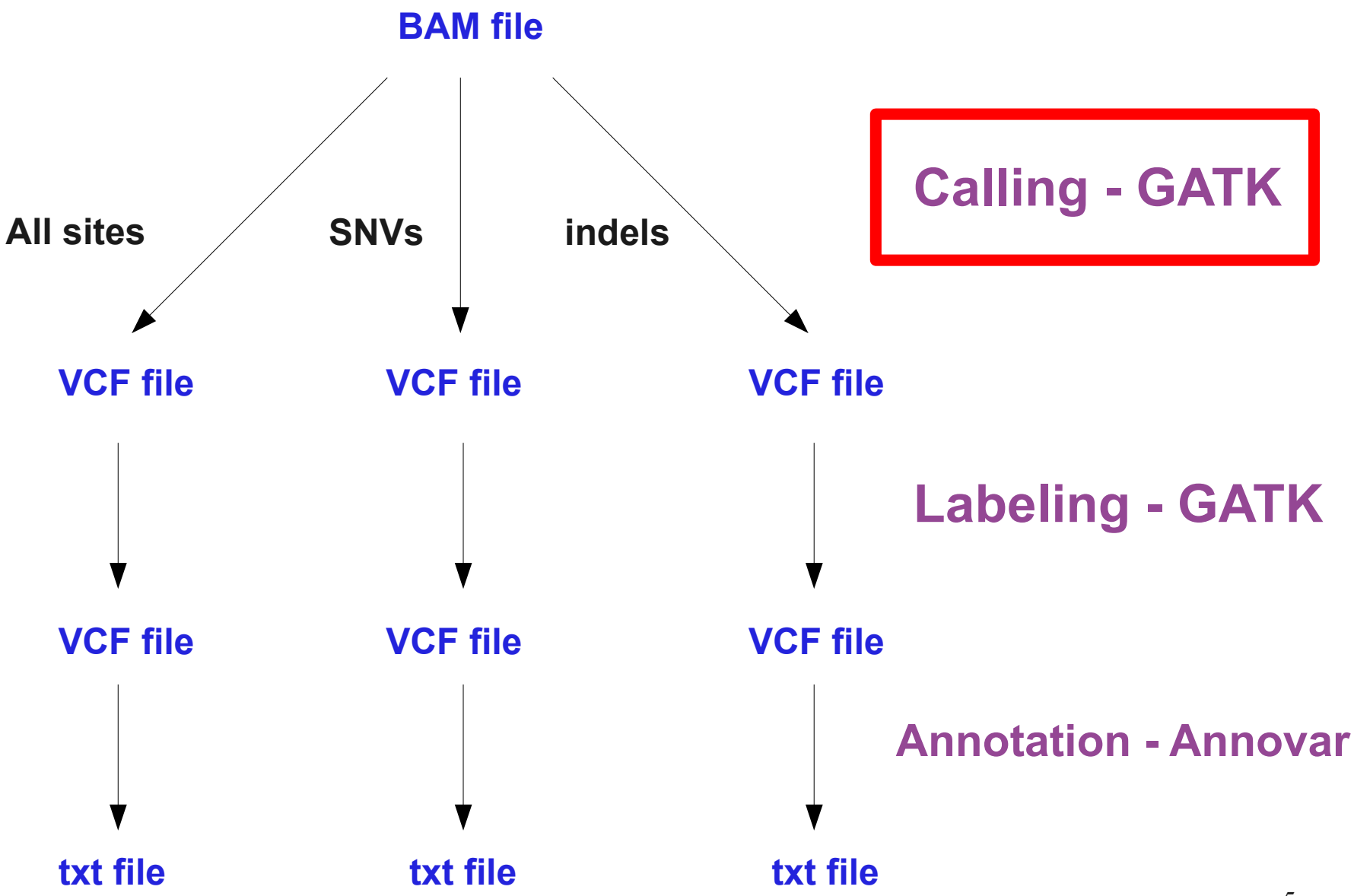
Annotation - Annovar

Working directory

Working directory

```
cd

cd mda12

ls

ls mapping

cd calling

ls
```

Scheme


BAM file
All sites
SNVs
indels
Calling - GATK
VCF file
VCF file
VCF file
Labeling - GATK
VCF file
VCF file
VCF file
Annotation - Annovar
txt file
txt file
txt file

Variant calling - GATK

GATK (http://www.broadinstitute.org/gsa/wiki/index.php/Home_Page)

We need:

- reference
- bed file of regions of capture
- BAM mapping file
- bases to print
- output file

Run program and see options

```
~/mda12/calling/software/GenomeAnalysisTK-1.4-15-gcd43f01/GenomeAnalysisTK.jar
```

It needs the parameter to do the calling:

UnifiedGenotyper

```
~/mda12/calling/software/GenomeAnalysisTK-1.4-15-gcd43f01/GenomeAnalysisTK.jar \
 -T UnifiedGenotyper
```

Reference and bed file

Checking the reference

```
head ~/mda12/resources/ref/human_g1k_v37.chr20.fasta

head -3000 ~/mda12/resources/ref/human_g1k_v37.chr20.fasta | tail
```

Checking the bed file

```
head ~/mda12/resources/ref/Exon_50mb_hg19_chr20.bed
```

```
20  68319  68439
20  76611  77091
20  123208 123358
20  125995 126237
20  126269 126389
20  138119 138269
20  139359 139719
20  168522 168762
20  170179 170299
20  207898 208018
```

SNV calling of all sites- GATK

SNV Calling of all sites

```
~/mda12/calling/software/GenomeAnalysisTK-1.4-15-gcd43f01/GenomeAnalysisTK.jar \
 -T UnifiedGenotyper \
 -R ~/mda12/resources/ref/human_g1k_v37.chr20.fasta \
 -L ~/mda12/resources/ref/Exon_50mb_hg19_chr20.bed \
 -I ~/mda12/resources/mapping/test_final.bam \
 -glm SNP \
 -out_mode EMIT_ALL_SITES \
 -o all_sites.vcf
```

Checking file

```
less all_sites.vcf
```

Counting lines

```
du -hs all_sites.vcf
wc -l all_sites.vcf
```

SNV calling only variants - GATK

Executing SNVs calling of variants

```
~/mda12/calling/software/GenomeAnalysisTK-1.4-15-gcd43f01/GenomeAnalysisTK.jar \
 -T UnifiedGenotyper \
 -R ~/mda12/resources/ref/human_g1k_v37.chr20.fasta \
 -L ~/mda12/resources/ref/Exon_50mb_hg19_chr20.bed \
 -I ~/mda12/resources/mapping/test_final.bam \
 -glm SNP \
 -o snvs.vcf
```

Checking file

```
less snvs.vcf
```

Counting lines

```
du -hs snvs.vcf
wc -l snvs.vcf
```

indel calling - GATK

Executing indels calling of variants

```
~/mda12/calling/software/GenomeAnalysisTK-1.4-15-gcd43f01/GenomeAnalysisTK.jar \
 -T UnifiedGenotyper \
 -R ~/mda12/resources/ref/human_g1k_v37.chr20.fasta \
 -L ~/mda12/resources/ref/Exon_50mb_hg19_chr20.bed \
 -I ~/mda12/resources/mapping/test_final.bam \
 -glm INDEL \
 -o indels.vcf
```

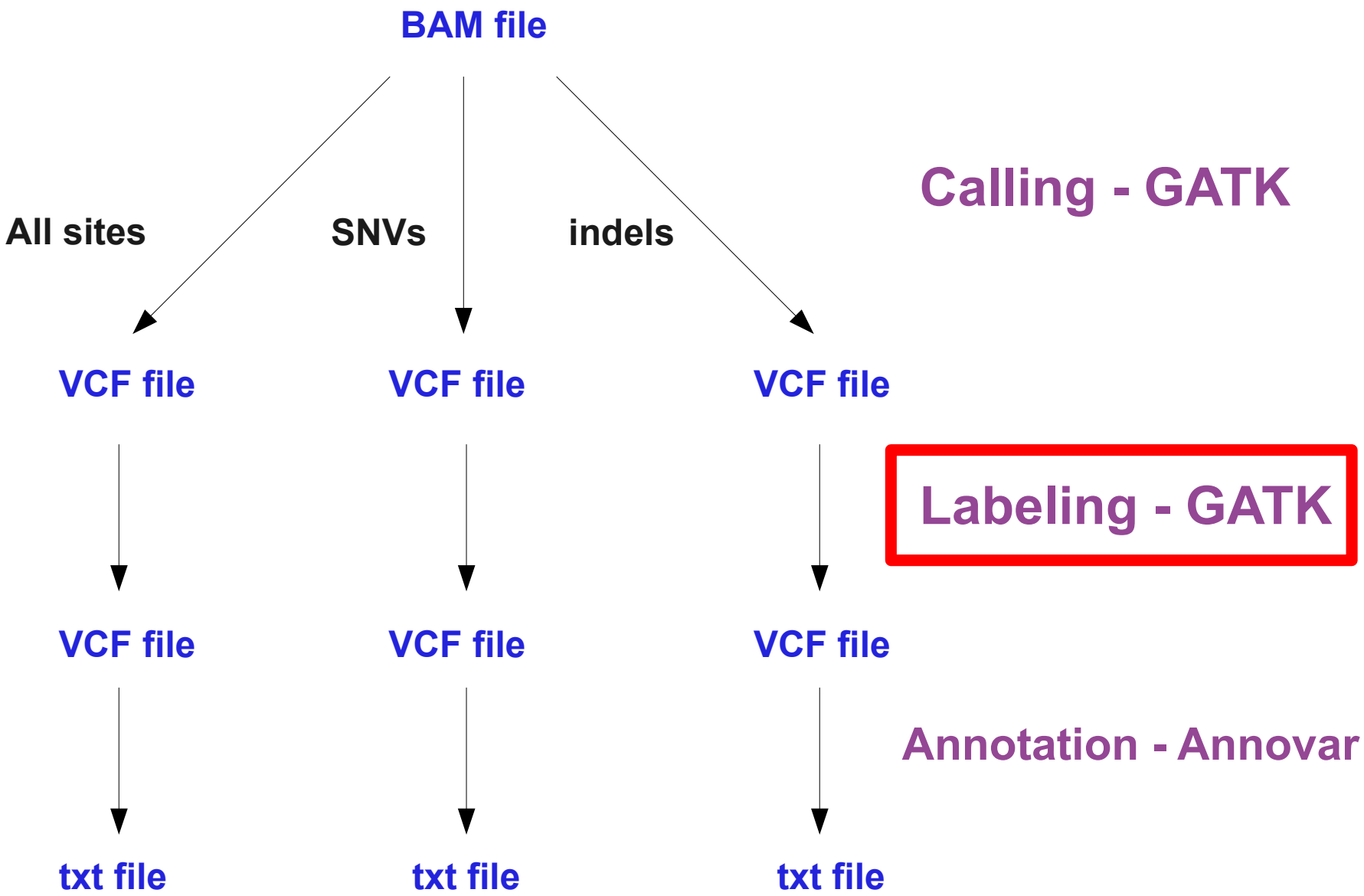
Checking file

```
less indels.vcf
```

Counting lines

```
du -hs indels.vcf
wc -l indels.vcf
```

Scheme


BAM file
All sites
SNVs
indels
Calling - GATK
VCF file
VCF file
VCF file
Labeling - GATK
VCF file
VCF file
VCF file
Annotation - Annovar
txt file
txt file
txt file

Labeling VCF files - GATK

Options for VariantFiltration:

- filter
- filter name
- reference
- input VCF file
- output VCF file

GATK parameter:

VariantFiltration

```
~/mda12/calling/software/GenomeAnalysisTK-1.4-15-gcd43f01/GenomeAnalysisTK.jar \
 -T VariantFiltration
```

Labeling all sites VCF file - GATK

Labeling all sites VCF file

```
~/mda12/calling/software/GenomeAnalysisTK-1.4-15-gcd43f01/GenomeAnalysisTK.jar \
 -T VariantFiltration \
 -filter "QD < 2.0 || MQ < 40.0 || FS > 60.0 || HaplotypeScore > 13.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0" \
 -filterName "STD_FILTER" \
 -R ~/mda12/resources/ref/human_g1k_v37.chr20.fasta \
 -V all_sites.vcf \
 -o all_sites_labeled.vcf
```

Checking files

```
wc -l all_sites_labeled.vcf
wc -l all_sites.vcf

grep PASS all_sites_labeled.vcf | wc -l
```

Labeling SNVs VCF file - GATK

Labeling SNVs VCF file

```
~/mda12/calling/software/GenomeAnalysisTK-1.4-15-gcd43f01/GenomeAnalysisTK.jar \
 -T VariantFiltration \
 -filter "QD < 2.0 || MQ < 40.0 || FS > 60.0 || HaplotypeScore > 13.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0" \
 -filterName "STD_FILTER" \
 -R ~/mda12/resources/ref/human_g1k_v37.chr20.fasta \
 -V snvs.vcf \
 -o snvs_labeled.vcf
```

Checking files

```
wc -l snvs_labeled.vcf
wc -l snvs.vcf

grep PASS snvs_labeled.vcf | wc -l
```

Labeling indels VCF file - GATK

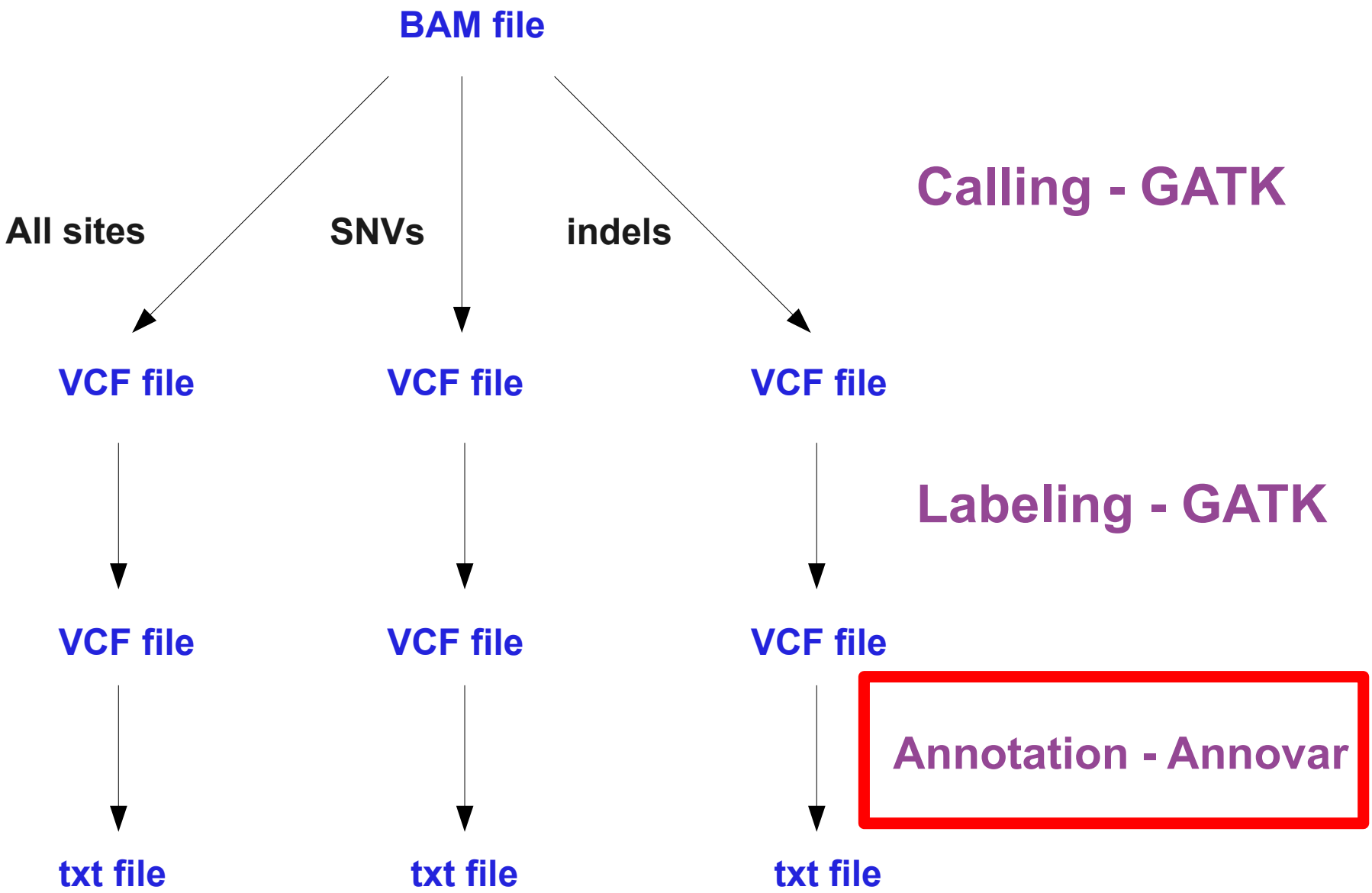
Labeling indels VCF file

```
~/mda12/calling/software/GenomeAnalysisTK-1.4-15-gcd43f01/GenomeAnalysisTK.jar \
 -T VariantFiltration \
 -filter "QD < 2.0 || ReadPosRankSum < -20.0 || FS > 200.0" \
 -filterName "STD_FILTER" \
 -R ~/mda12/resources/ref/human_g1k_v37.chr20.fasta \
 -V indels.vcf \
 -o indels_labeled.vcf
```

Checking files

```
wc -l indels.vcf
wc -l indels_labeled.vcf

grep PASS indels_labeled.vcf | wc -l
```

Scheme


BAM file
All sites
SNVs
indels
Calling - GATK
VCF file
VCF file
VCF file
Labeling - GATK
VCF file
VCF file
VCF file
Annotation - Annovar
txt file
txt file
txt file

Annotation

Software:

Annovar: http://www.openbioinformatics.org/annovar/

Steps:

1. Convert VCF file to annovar format file

2. Annotate variants: SNVs and indels

Output:

- only exonic variants.

- all variants.

Annotation of SNVs – Annovar (1)

Converting SNV VCF file to annovar format file

```
~/mda12/calling/software/annovar/convert2annovar.pl \
 -format vcf4 \
 -filter PASS snvs_labeled.vcf > \
 snvs_labeled.vcf.annovar
```

Checking files

```
wc -l snvs_labeled.vcf.annovar
grep PASS snvs_labeled.vcf | wc -l
```

```
head snvs_labeled.vcf.annovar
```

```
20   76962    76962    T   C   hom 8096.43  244 56.21    33.18
20   139362   139362   G   A   hom 2129.32  61  59.24    34.91
20   168728   168728   T   A   hom 6509173 58.52    37.62
20   209932   209932   G   T   het 308.17   72  51.77    4.28
20   210061   210061   G   A   het 1856.36  128 57.24    14.50
20   238507   238507   A   C   het 135.67   24  57.00    5.65
20   239688   239688   G   A   het 353.20   43  54.98    8.21
20   239697   239697   G   C   het 452.21   46  54.22    9.83
20   256573   256573   A   G   het 1113.27  81  57.09    13.74
20   256727   256727   T   A   het 533.82   45  58.97    11.86
```

Annotation of SNVs – Annovar (2)

Annotating

```
~/mda12/calling/software/annovar/annotate_variation.pl \
 --geneanno \
 --buildver hg19 \
 --dbtype gene \
 snvs_labeled.vcf.annovar \
 ~/mda12/calling/software/annovar/humandb/


ls -latr
```

Output

```
head snvs_labeled.vcf.annovar.exonic_variant_function


head snvs_labeled.vcf.annovar.variant_function


head snvs_labeled.vcf.annovar.log
```

Annotation of indels – Annovar (1)

Converting indels VCF file to annovar format file

```
~/mda12/calling/software/annovar/convert2annovar.pl \
 -format vcf4 \
 -filter PASS indels_labeled.vcf > \
 indels_labeled.vcf.annovar
```

Checking files

```
wc -l indels_labeled.vcf.annovar
grep PASS indels_labeled.vcf | wc -l
```

```
head indels_labeled.vcf.annovar
```

```
20  126156   126159   CAAA -    het 1926.74 113 55.75   17.05
20  126311   126312   CC   -    het 866.91  102 52.73   8.50
20  138179   138179   C    -    hom 1281.43 35  59.30   36.61
20  238436   238441   TGGTCT   -    het 402.53  20  54.78   20.13
20  746424   746432   TATCTGCCC    -    het 482.13  19  47.89   25.38
20  2618033  2618033  -    AAAA het 944.09  45  49.31   20.98
20  3740621  3740622  CA   -    hom 763.01  26  53.64   29.35
20  4880133  4880148  GCTCAATGCCTTCTGC -    hom 9459.12 139 45.11   68.05
20  10622081 10622081 A    -    het 1189.25 148 59.54   8.04
20  11790885 11790885 -    TT   hom 5752.58 110 60.45   52.30
```

Annotation of indels – Annovar (2)

Annotating

```
~/mda12/calling/software/annovar/annotate_variation.pl \
 --geneanno \
 --buildver hg19 \
 --dbtype gene \
 indels_labeled.vcf.annovar \
 ~/mda12/calling/software/annovar/humandb/
```

```
ls -latr
```

Output

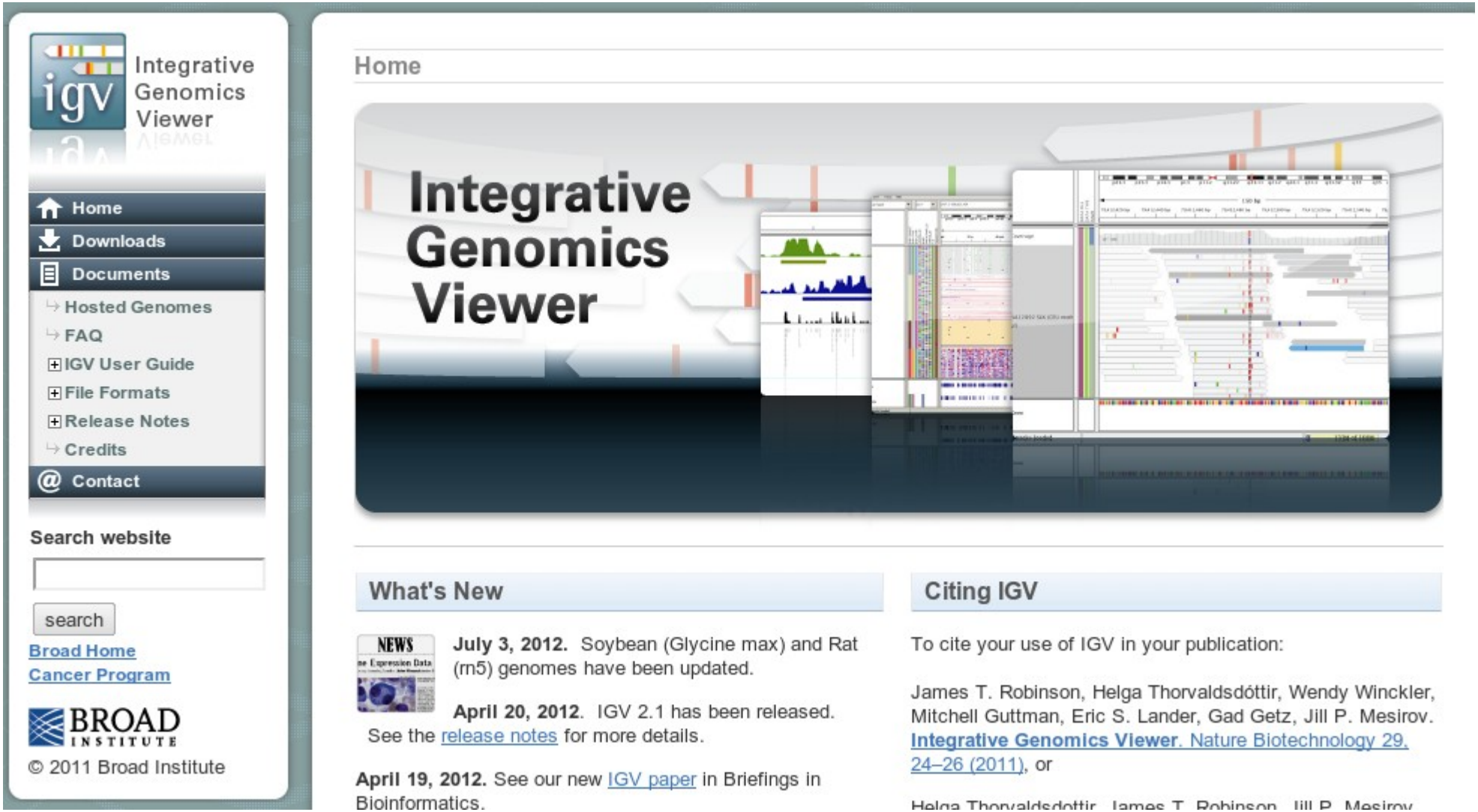
```
head indels_labeled.vcf.annovar.exonic_variant_function
```

```
head indels_labeled.vcf.annovar.variant_function
```

```
head indels_labeled.vcf.annovar.log
```

Visualization - IGV

IGV: http://www.broadinstitute.org/igv/



Questions?