umr BIOGER
Biologie et gestion des risques en agriculture

Variant Filtering

Ecole de bioinformatique –
Roscoff, novembre 2016

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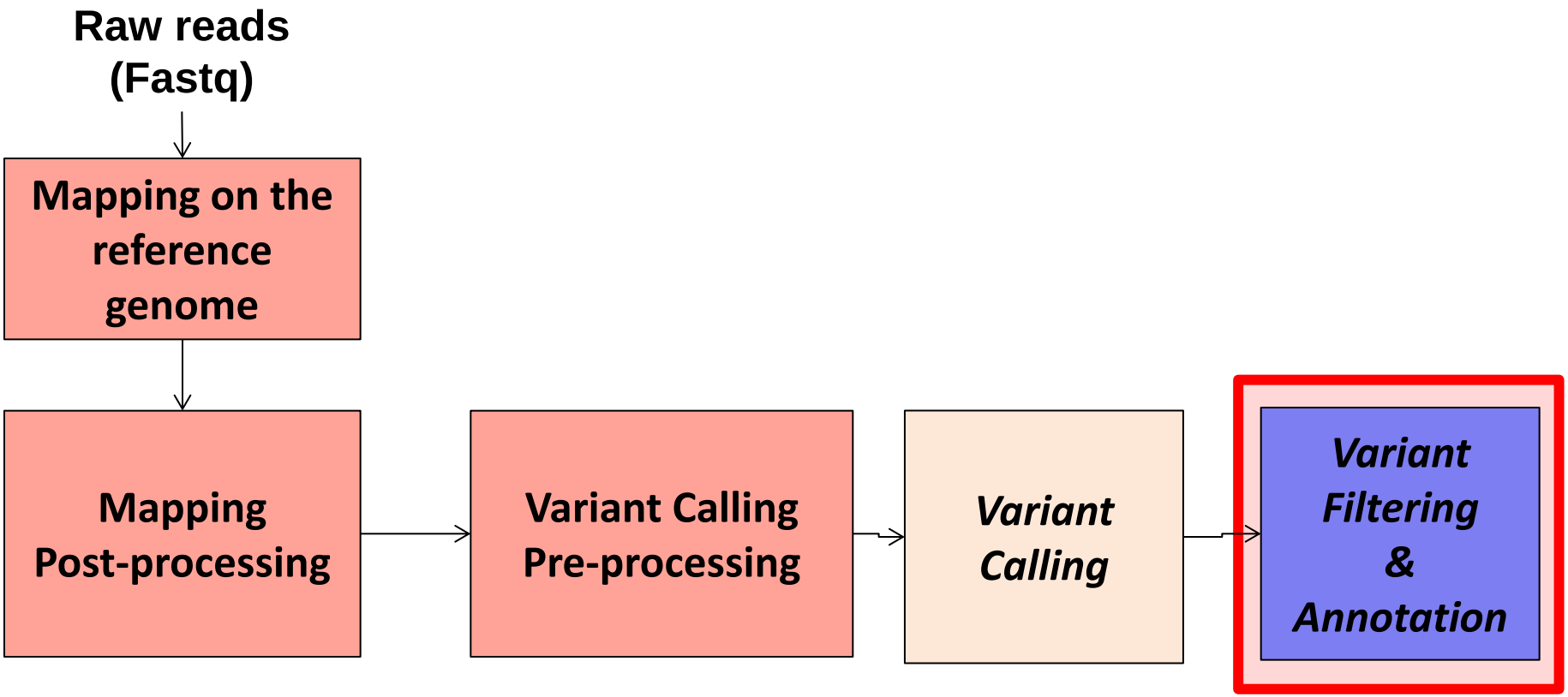
INRA
SCIENCE & IMPACT

Workflow


Raw reads
(Fastq)
Mapping on the reference genome
Mapping
Post-processing
Variant Calling
Pre-processing
Variant
Calling
Variant
Filtering
&
Annotation

Filters, why ?

Some use cases :

- Extract a subset of variants (localization, type)
- Combine variants from several analyses
- Compare obtained variants from several data types (RNA-Seq, Exome-Seq, Whole Genome)
- Identify new variants compare to a reference list
- Apply specific filter for Chip design
- …

Howto ?

Use specific tools to rewrite / annotate VCF File.

Reminder (VCF Format) :

```
##fileformat=VCFv4.1
##FORMAT=<ID=AD,Number=.,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 filtered)">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=PL,Number=G,Type=Integer,Description="Normalized, Phred-scaled likelihoods for genotypes as defined in the VCF specification">
##INFO=<ID=AC,Number=A,Type=Integer,Description="Allele count in genotypes, for each ALT allele, in the same order as listed">
##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 qualities">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP Membership">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Approximate read depth; some reads may have been filtered">
##INFO=<ID=DS,Number=0,Type=Flag,Description="Were any of the samples downsampled?">
##INFO=<ID=Dels,Number=1,Type=Float,Description="Fraction of Reads Containing Spanning Deletions">
##INFO=<ID=FS,Number=1,Type=Float,Description="Phred-scaled p-value using Fisher's exact test to detect strand bias">
##INFO=<ID=HaplotypeScore,Number=1,Type=Float,Description="Consistency of the site with at most two segregating haplotypes">
##FILTER=<ID=LowQual,Description="Low quality">
…
.
##contig=<ID=chr12,length=133851895>
##reference=file:///tmp/13905.1.galaxy.q/tmp-gatk-MPGS7G/gatk_input.fasta

#CHROM  POS     ID          REF  ALT  QUAL    FILTER  INFO                                                                                  FORMAT          Pickrell

Chr12   406292  rs2229351   G    A    994.77  .       AC=1;AF=0.500;AN=2;DB;DP=69;Dels=0.00;FS=4.853;MQ=37.00;MQ0=0;                        GT:AD:DP:GQ:PL  0/1:33,36:66:99:1023,0,994
Chr12   416046  rs35042439  C    CT   391.73  .       AC=1;AF=0.500;AN=2;DB;DP=46;FS=0.000;MQ=37.49;MQ0=0;QD=8.52;                          GT:AD:DP:GQ:PL  0/1:22,17:46:99:429,0,521
```

Tools

tool	suited to	link	Galaxy availability
VcfTools	-	https://vcftools.github.io/index.html	limited
BcfTools	Samtools	http://samtools.github.io/bcftools/	limited
vcflib	FreeBayes	https://github.com/ekg/vcflib	good
GATK	GATK	https://software.broadinstitute.org/gatk/	good

Methods

Remove variant entry (Hard Filtering) or add Filter info (Soft Filtering):

Before Filtering

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	Pickrell
.									
.									
Chr12	406292	rs2229351	G	A	994.77	.	AC=1;AF=0.500;AN=2;DB;DP=69;Dels=0.00;FS=4.853;**MQ=37.00**;MQ0=0;	GT:AD:DP:GQ:PL	0/1:33,36:66:99:1023,0,994
Chr12	416046	rs35042439	C	CT	391.73	.	AC=1;AF=0.500;AN=2;DB;DP=46;FS=0.000;**MQ=27.49**;MQ0=0;QD=8.52;	GT:AD:DP:GQ:PL	0/1:22,17:46:99:429,0,521

Filter : MQ < 30.0

After Filtering

##FILTER=<ID=LowQual,Description="Low quality, mapping < 30.0">

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	Pickrell
.									
.									
Chr12	406292	rs2229351	G	A	994.77	**PASS**	AC=1;AF=0.500;AN=2;DB;DP=69;Dels=0.00;FS=4.853;MQ=37.00	GT:AD:DP:GQ:PL	0/1:33,36:66:99:1023,0,994
Chr12	416046	rs35042439	C	CT	391.73	**LowQual**	AC=1;AF=0.500;AN=2;DB;DP=46;FS=0.000;MQ=27.49	GT:AD:DP:GQ:PL	0/1:22,17:46:99:429,0,521

Filter : **PASS** (Not Filtered), **.** (no data, filtering not performed), **LowQual** (Filtered)

By default, filtering is applied at variant level (INFO), genotype filtering (FORMAT) possible

Howto ?

- Understand VCF Format File
- Identify specific tags
- Fix Thresholds
- Find external ressources (dbSNP) to exclude / keep known Variants (other VCF File)
- Limit analysis to specific genomic locations (BED File)

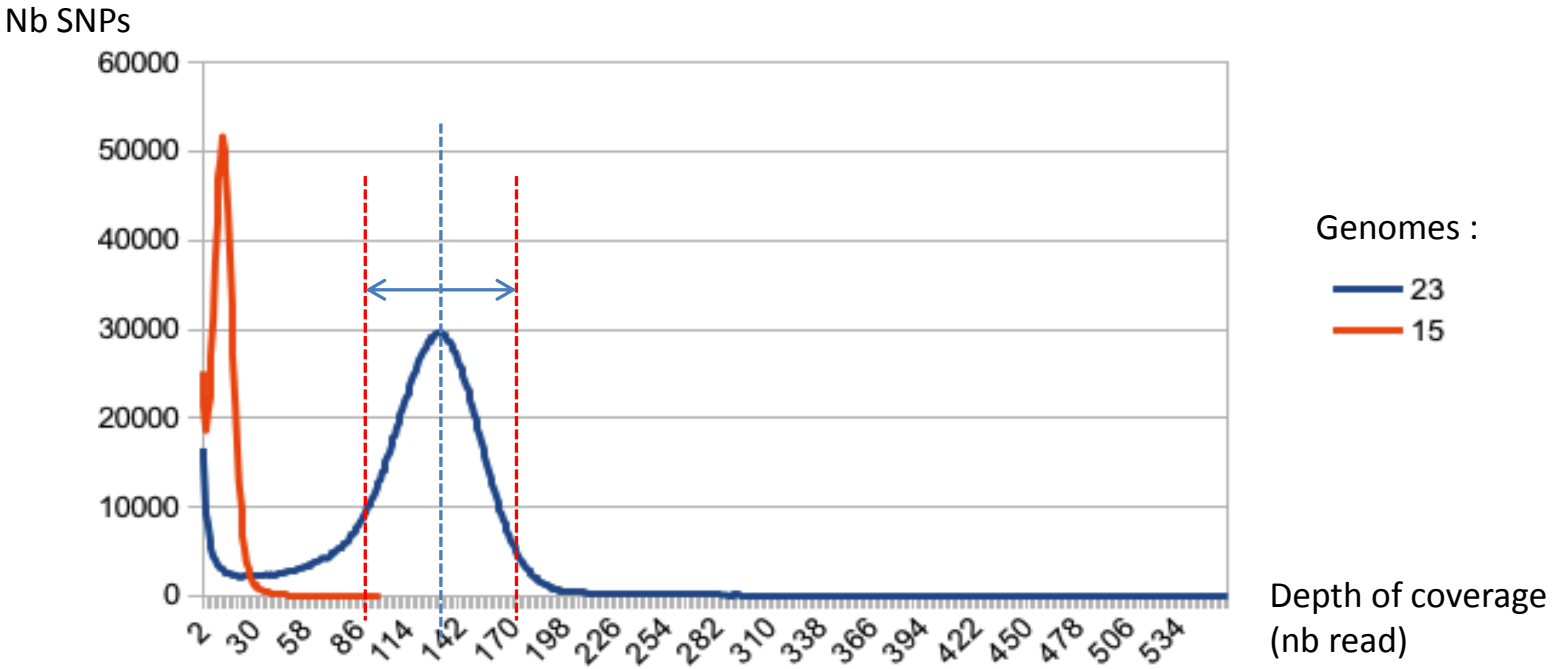
Criteria?

It depends of:

- Variant caller : methods, available info, VCF specific tags
- Data Type : DNA-Seq, Exome-Seq, RNA-Seq,
- Sequencing Technology : (depth, protocole)
- Reference genome: reliability of the reference sequence
- Studied species: Genome features (Transposable Elements, Tandem Repeats)
- Available ressources: reference variant sets

Criteria?

Depth (DP): Min / Max => d ± 4√d, d = average Read Depth



- Low DP : mapping errors, sequencing errors
- High DP : CNVs or Repeat Regions, mapping errors
- Reliable with High coverage > 40X
- DNA-Seq OK, Exome-Seq NOK

Li, H. (2014). Toward better understanding of artifacts in variant calling from high-coverage samples. *Bioinformatics*

Criteria?

Li, H. (2014). *Bioinformatics*

- Low Complexity regions (LC) : exclude variants located in LC regions
- Variant Quality (QU) : exclude variants with low quality
- Double Strand filter (DS) : exclude variants with number of reads (ALT allele) below a defined Threshold on reverse or forward strand
- Fisher Strand filter (FS) : reference / no-reference reads highly correlated with strand.
- Allele Balance (AB) : HET > 30%

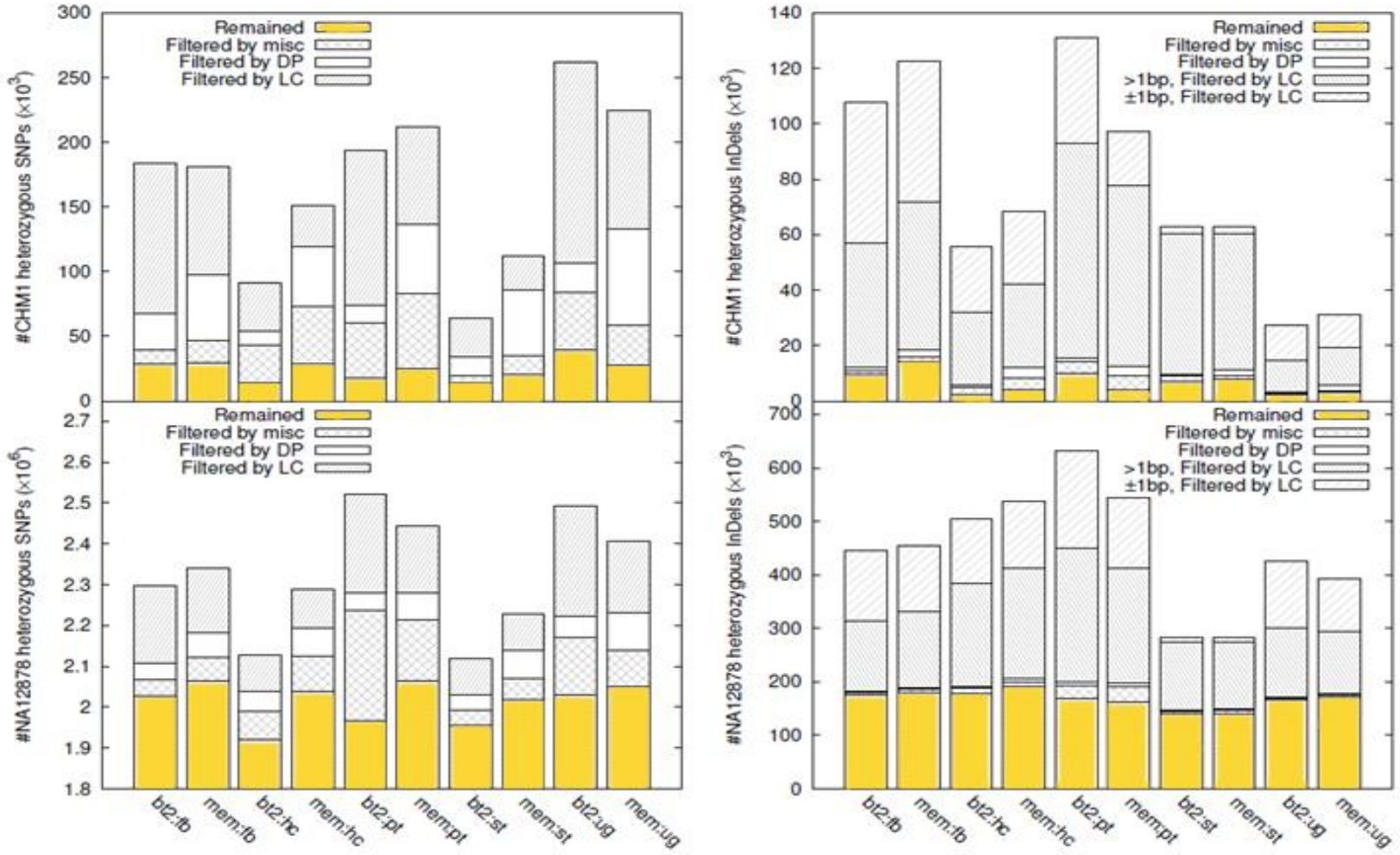
Meynert et al (2014). *BMC Bioinformatics*

Coverage uniformity vs coverage depth (whole genome vs exome-seq) -> critical for heterozygous sites

Criteria?

Haploid cells

Diploid cells



Li, H. (2014). *Bioinformatics*

misc = AB, DS, FS

genome VCF: gVCF and all sites file

"The key difference between a regular VCF and a gVCF is that the gVCF has records for all sites, whether there is a variant call there or not. The goal is to have every site represented in the file in order to do joint analysis of a cohort in subsequent steps. The records in a gVCF include an accurate estimation of how confident we are in the determination that the sites are homozygous-reference or not."

" The term GVCF is sometimes used simply to describe VCFs that contain a record for every position in the genome (or interval of interest) regardless of whether a variant was detected at that site or not."

Pros:

- Keep trace of rare variants
- Non-covered genome regions (structural variant)
- addition of new samples

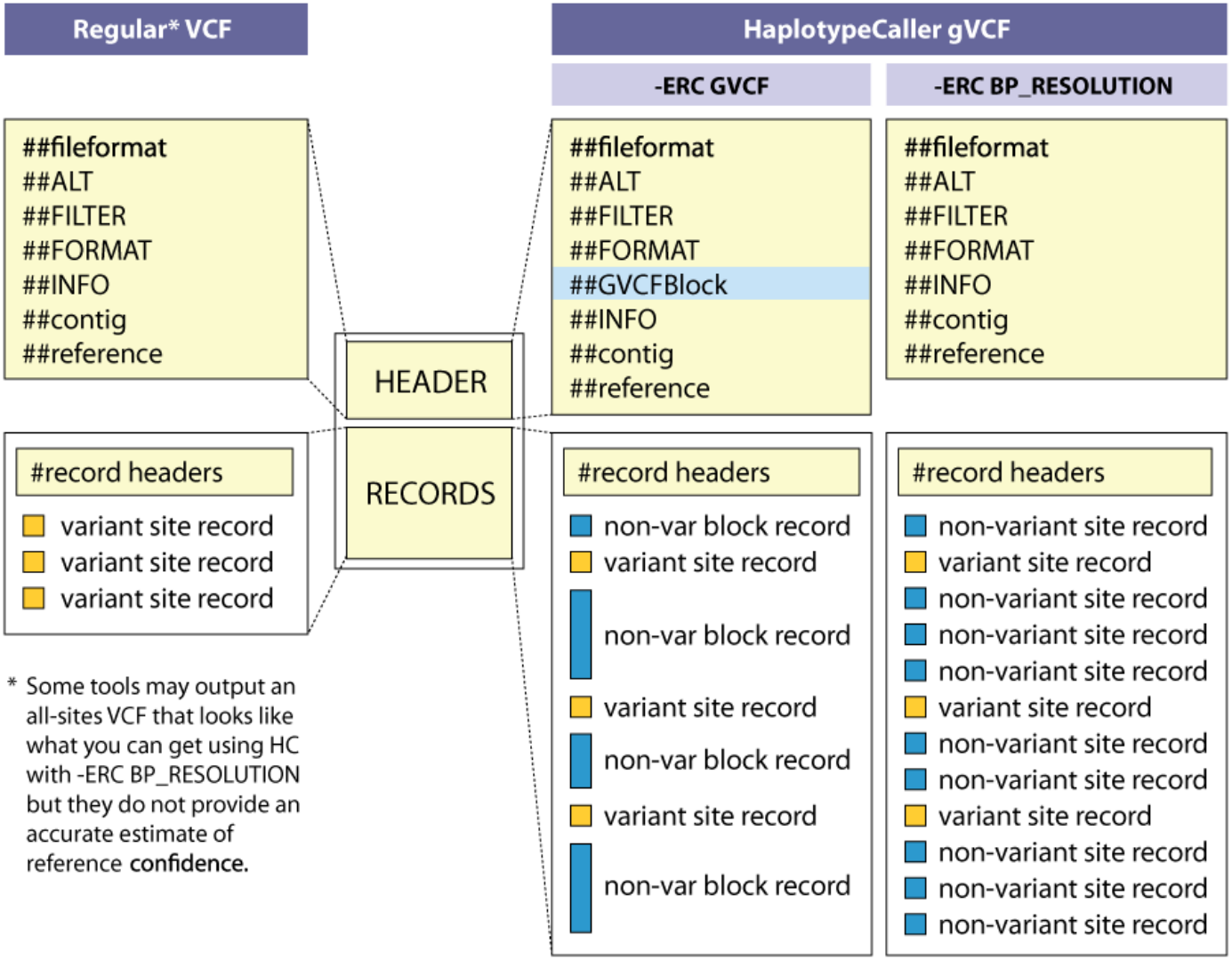
Cons:

- Computational time
- File size

https://software.broadinstitute.org/gatk/guide/article?id=4017

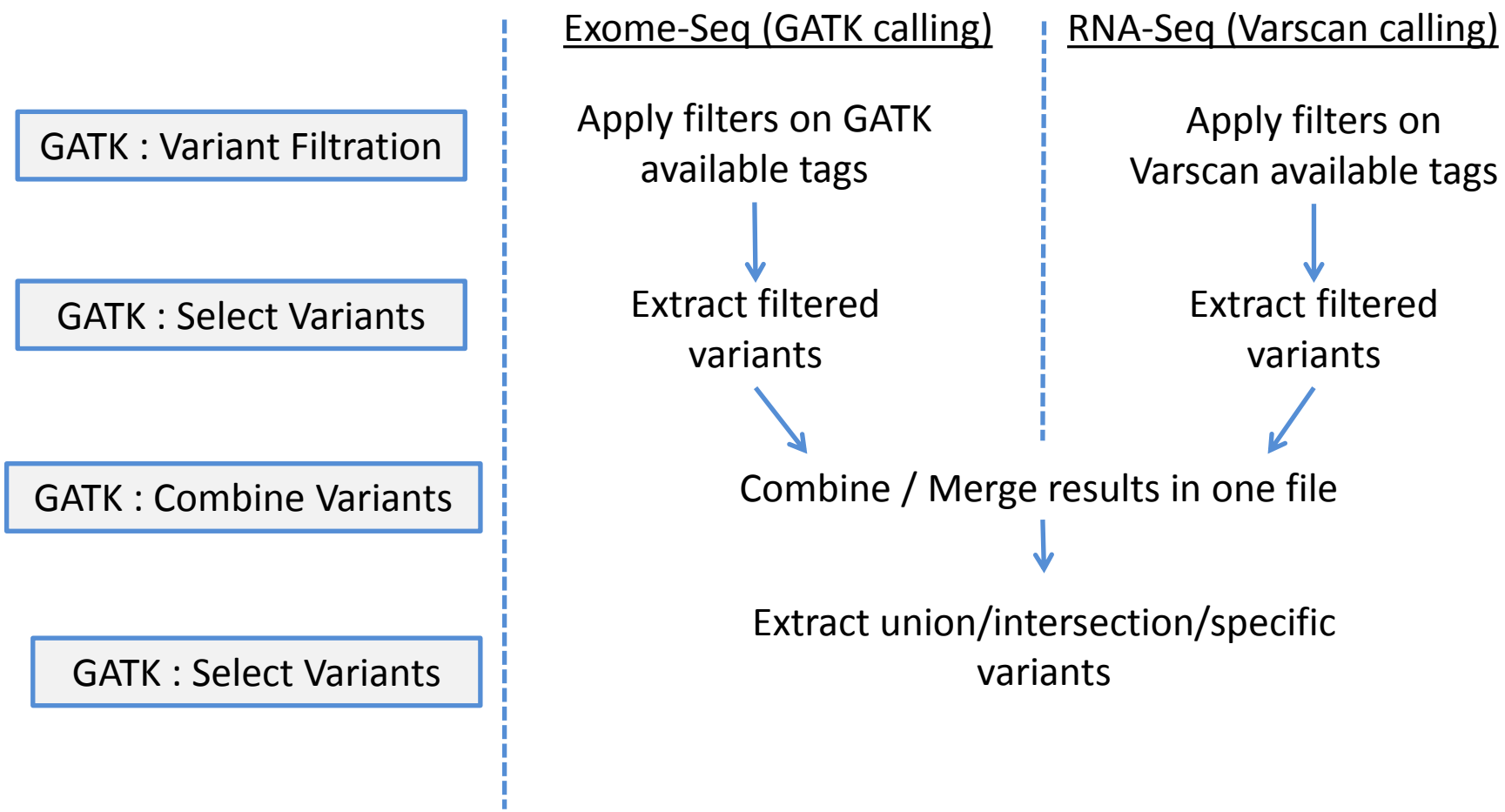
https://sites.google.com/site/gvcftools/home/about-gvcf/gvcf-conventions

genome VCF: gVCF and all sites file



https://software.broadinstitute.org/gatk/guide/article?id=4017

Tutorial


GATK : Variant Filtration
GATK : Select Variants
GATK : Combine Variants
GATK : Select Variants
Exome-Seq (GATK calling)
RNA-Seq (Varscan calling)
Apply filters on GATK available tags
Apply filters on Varscan available tags
Extract filtered variants
Extract filtered variants
Combine / Merge results in one file
Extract union/intersection/specific variants

GATK Tools

Variant Filtration:

-Modify FILTER column (Soft Filtering)
-Criteria on INFO Tags
-Criteria on FORMAT Tags
-Handle missing Values

Select Variants:

-Direct selection (exclude filtered variants = Hard Filtering)
-Criteria on INFO Tags
-Criteria on FILTER Tags
-No Criteria on FORMAT Tags
-Intersection / Union with other VCF Files
-Exclude / Include samples
-Selected genomic regions (BED File)

GATK Tools

JEXL = Java Expression Language

- Key, value
- Case-sensitive (Uppercase, Lowercase, MQ ≠ mq)
- Type-sensitive:

```
##FORMAT=<ID=AD,Number=.,Type=Integer,Description=".">
```

  - Integer = **2**
  - Float = **2.0**
  - String = **"two"**
- Operators:
  - Relational: ==, !=, <,>,<=, >=
  - Logical: && (AND) , || (OR)

http://gatkforums.broadinstitute.org/discussion/1255/what-are-jexl-expressions-and-how-can-i-use-them-with-the-gatk

Tutorial

Appendix: GATK, VCF Format

FORMAT Tags

GT	Genotype , 0/0, 0/1, 1/1
GQ	Genotype Quality (Highest value = 99)
AD / DP	Depth per Allele / Depth = global coverage
PL	Genotype Likelihoods , max 0 (Phred Score)

INFO Tags

AC,AF,AN	(AC) Alleles Count, and (AF) Allele Frequency for each ALT allele, (AN)Total number of allele
DB	If present, then the variant is in dbSNP.
DP	Coverage (reads that passed quality metrics)
DS	Were any of the samples downsampled because of too much coverage?
MQ and MQ0	Root Mean Square Mapping Quality and Mapping Quality Zero total count
BaseQualityRankSum	Test : quality of Reference reads vs ALT reads
MappingQualityRankSum	Test : Mapping quality of Reference reads vs ALT reads
ReadPosRankSum	Test: Distance of ALT reads from the end of the reads
HaplotypeScore	Consistency of the site with at most two segregating haplotype
QD	Variant Quality / depth of non-ref samples
FS	Test (Fisher) : Phred score p-value for strand bias
InbreedingCoeff	Inbreeding coefficient as estimated from the genotype likelihoods per-sample when compared against the Hardy-Weinberg expectation

http://gatkforums.broadinstitute.org/discussion/1268/how-should-i-interpret-vcf-files-produced-by-the-gatk

Appendix: GATK recommended filters

Use case : non-reference variant db, GATK recommended filters for recalibration

SNPs:

- QD < 2.0 (Variant Quality / depth of non-ref samples)
- MQ < 40.0 (Mapping Quality)
- FS > 60.0 (Phred score Fisher's test p-value for strand bias)
- HaplotypeScore > 13.0 (Consistency of the site with at most two segregating haplotype)
- MQRankSum < -12.5 (Mapping quality of Reference reads vs ALT reads)
- ReadPosRankSum < -8.0 (Distance of ALT reads from the end of the reads)

INDELs:

- QD < 2.0 (Variant Quality / depth of non-ref samples)
- ReadPosRankSum < -20.0 (Distance of ALT reads from the end of the reads)
- InbreedingCoeff < 0.8
- FS > 200.0 (Phred score Fisher's test p-value for strand bias)

http://gatkforums.broadinstitute.org/discussion/3225/how-can-i-filter-my-callset-if-i-cannot-use-vqsr-recalibrate-variants

Appendix: Phred Score

$$Q = -10 \log_{10} P \quad \text{and} \quad P = 10^{\frac{-Q}{10}}$$

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%
50	1 in 100,000	99.999%
60	1 in 1,000,000	99.9999%

Appendix: Filters

- Note that the InbreedingCoeff statistic is a population-level calculation that is only available with 10 or more samples. If you have fewer samples you will need to omit that particular filter statement.
- **For shallow-coverage (<10x): you cannot use filtering to reliably separate true positives from false positives. You must use the protocol involving variant quality score recalibration.**
- The maximum DP (depth) filter only applies to whole genome data, where the probability of a site having exactly N reads given an average coverage of M is a well-behaved function. First principles suggest this should be a binomial sampling but in practice it is more a Gaussian distribution. Regardless, the DP threshold should be set a 5 or 6 sigma from the mean coverage across all samples, so that the DP > X threshold eliminates sites with excessive coverage caused by alignment artifacts. Note that **for exomes, a straight DP filter shouldn't be used** because the relationship between misalignments and depth isn't clear for capture data.
- That said, all of the caveats about determining the right parameters, etc, are annoying and are largely eliminated by variant quality score recalibration.
- https://www.broadinstitute.org/gatk/guide/article?id=3225
- http://gatkforums.broadinstitute.org/discussion/2806/howto-apply-hard-filters-to-a-call-set