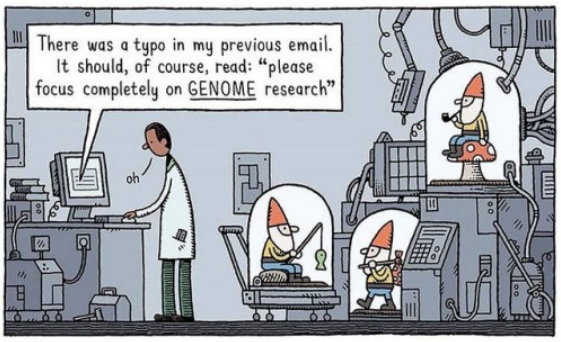
One SNP, two SNPs; many SNPs, few SNPs

How do I filter my data, and when do I stop?

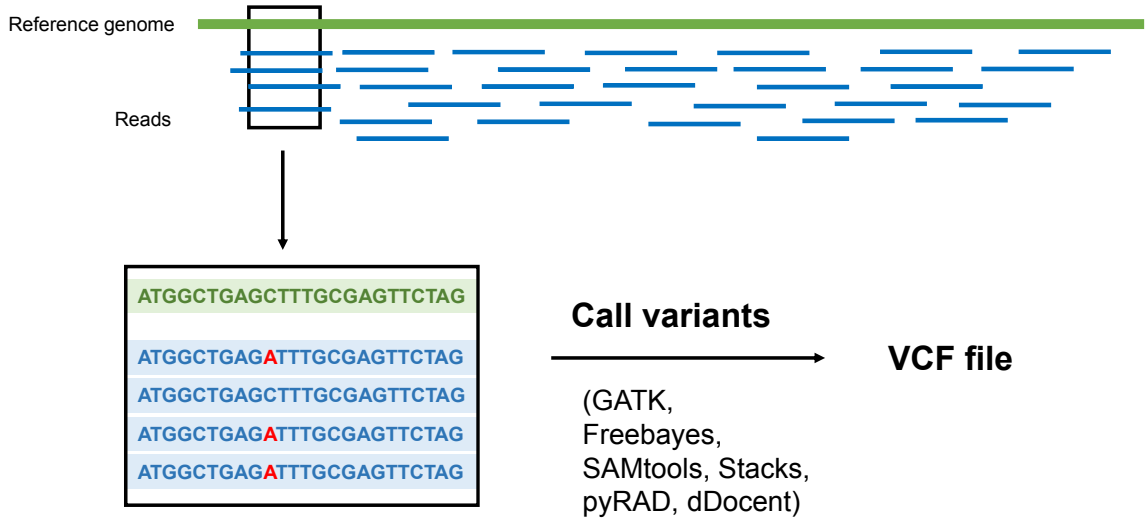
Maddie James
PhD candidate
Ortiz-Barrientos laboratory

Overview

- SNP calling
- What is a VCF file?
- Where do errors come from?
- Data filtering
- Recommendations



SNP calling


Reference genome
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ATGGCTGAGATTTGCGAGTTCTAG
ATGGCTGAGCTTTGCGAGTTCTAG
ATGGCTGAGATTTGCGAGTTCTAG
ATGGCTGAGATTTGCGAGTTCTAG
Call variants
(GATK,
Freebayes,
SAMtools, Stacks,
pyRAD, dDocent)
VCF file

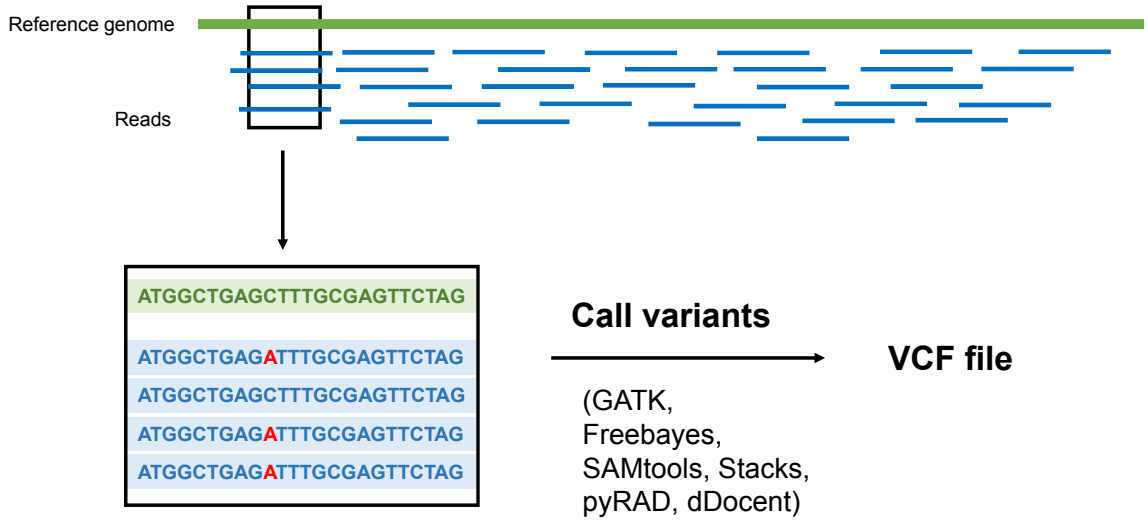
A note on calling SNPs

- Joint calling of SNPs
  - Uses information across all samples to call a SNP (good for samples with low coverage)
  - Assumes all samples are genetically similar
  - Typically, you joint call on “cohorts” of samples (aka populations, species)
  - SNPs from these cohorts are combined into one file
- The default settings of variant callers only output *variant* sites
  - This can be a problem when combining jointly-called cohorts
  - You can’t distinguish between sites that are invariant and sites with missing data
- When calling SNPs on cohorts, output variant and invariant sites (so you know what is missing vs what is invariant)

FreeBayes: --report-monomorphic

- Link: https://gatkforums.broadinstitute.org/gatk/discussion/3686/why-do-joint-calling-rather-than-single-sample-calling-retired

SNP calling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Call variants
(GATK,
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SAMtools, Stacks,
pyRAD, dDocent)
VCF file

What is a VCF file and what does it all mean?

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Information about the VCF file

List of contigs

Some extra detail:
https://samtools.github.io/hts-specs/VCFv4.2.pdf
https://gatkforums.broadinstitute.org/gatk/discussion/1268/what-is-a-vcf-and-how-should-i-interpret-it

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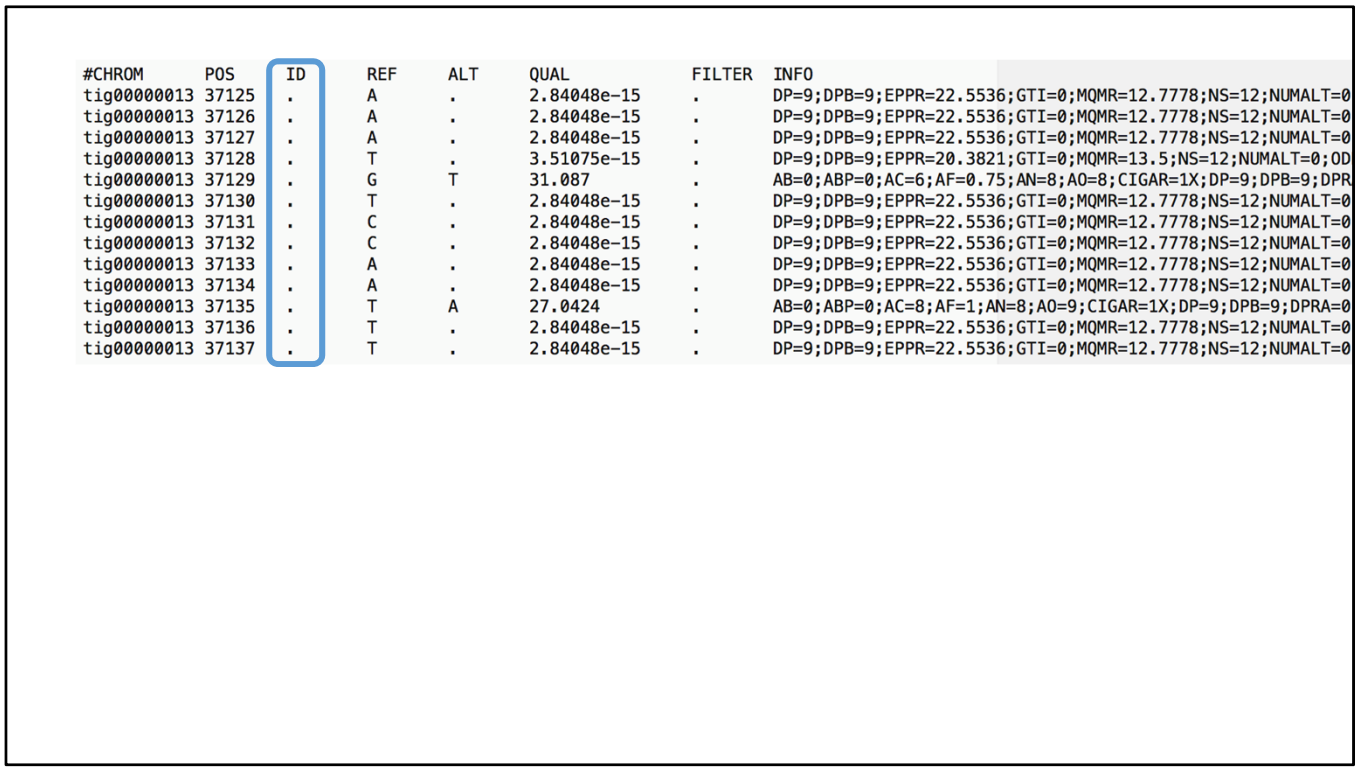

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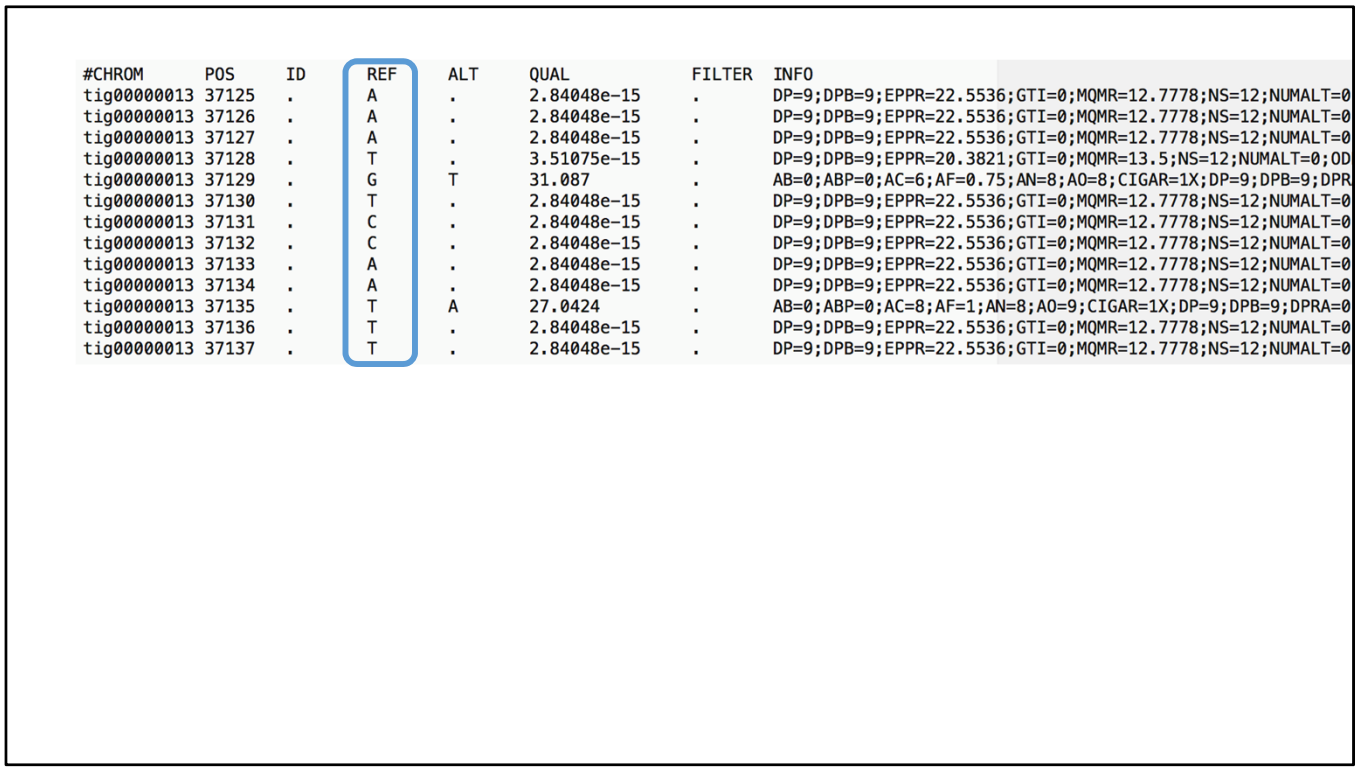

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#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO
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tig00000013	37137	.	T	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO
tig00000013	37125	.	A	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37126	.	A	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37127	.	A	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37128	.	T	.	3.51075e-15	.	DP=9;DPB=9;EPPR=20.3821;GTI=0;MQMR=13.5;NS=12;NUMALT=0;OD
tig00000013	37129	.	G	T	31.087	.	AB=0;ABP=0;AC=6;AF=0.75;AN=8;AO=8;CIGAR=1X;DP=9;DPB=9;DPR
tig00000013	37130	.	T	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37131	.	C	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37132	.	C	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37133	.	A	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37134	.	A	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37135	.	T	A	27.0424	.	AB=0;ABP=0;AC=8;AF=1;AN=8;AO=9;CIGAR=1X;DP=9;DPB=9;DPRA=0
tig00000013	37136	.	T	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37137	.	T	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO
tig00000013	37125	.	A	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37126	.	A	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37127	.	A	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37128	.	T	.	3.51075e-15	.	DP=9;DPB=9;EPPR=20.3821;GTI=0;MQMR=13.5;NS=12;NUMALT=0;OD
tig00000013	37129	.	G	T	31.087	.	AB=0;ABP=0;AC=6;AF=0.75;AN=8;AO=8;CIGAR=1X;DP=9;DPB=9;DPR
tig00000013	37130	.	T	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37131	.	C	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37132	.	C	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37133	.	A	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37134	.	A	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37135	.	T	A	27.0424	.	AB=0;ABP=0;AC=8;AF=1;AN=8;AO=9;CIGAR=1X;DP=9;DPB=9;DPRA=0
tig00000013	37136	.	T	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0
tig00000013	37137	.	T	.	2.84048e-15	.	DP=9;DPB=9;EPPR=22.5536;GTI=0;MQMR=12.7778;NS=12;NUMALT=0

FORMAT	H15-55	H15-67	H15-60	H15-69	H15-76	H15-58	H15-72
GT:GQ:DP:AD:RO:QR:AO:QA:GL	.	.	.	.	0/0:151.844:1:1:1:72:.:.:0	.	0/0:151.844:2:2:
GT:GQ:DP:AD:RO:QR:AO:QA:GL	.	.	.	.	0/0:151.844:1:1:1:72:.:.:0	.	0/0:151.844:2:2:
GT:GQ:DP:AD:RO:QR:AO:QA:GL	.	.	.	.	0/0:151.844:1:1:1:68:.:.:0	.	0/0:151.844:2:2:
GT:GQ:DP:AD:RO:QR:AO:QA:GL	.	.	.	.	0/0:150.924:1:1:1:72:.:.:0	.	0/0:150.924:2:2:
GT:GQ:DP:AD:RO:QR:AO:QA:GL	.	.	.	.	1/1:31.0715:1:0,1:0:0:1:68:-1.3,-0.30103,0	.	1/1:31.0715:2:0,
GT:GQ:DP:AD:RO:QR:AO:QA:GL	.	.	.	.	0/0:151.844:1:1:1:72:.:.:0	.	0/0:151.844:2:2:
GT:GQ:DP:AD:RO:QR:AO:QA:GL	.	.	.	.	0/0:151.844:1:1:1:72:.:.:0	.	0/0:151.844:2:2:
GT:GQ:DP:AD:RO:QR:AO:QA:GL	.	.	.	.	0/0:151.844:1:1:1:72:.:.:0	.	0/0:151.844:2:2:
GT:GQ:DP:AD:RO:QR:AO:QA:GL	.	.	.	.	0/0:151.844:1:1:1:72:.:.:0	.	0/0:151.844:2:2:
GT:GQ:DP:AD:RO:QR:AO:QA:GL	.	.	.	.	0/0:151.844:1:1:1:72:.:.:0	.	0/0:151.844:2:2:
GT:GQ:DP:AD:RO:QR:AO:QA:GL	.	.	.	.	1/1:26.7297:1:0,1:0:0:1:68:-1.3,-0.30103,0	.	1/1:26.8832:2:0,
GT:GQ:DP:AD:RO:QR:AO:QA:GL	.	.	.	.	0/0:151.844:1:1:1:68:.:.:0	.	0/0:151.844:2:2:
GT:GQ:DP:AD:RO:QR:AO:QA:GL	.	.	.	.	0/0:151.844:1:1:1:72:.:.:0	.	0/0:151.844:2:2:

We can use this information in our VCF file to filter our SNPs. We want distinguish between SNPs that are true variants vs SNPs that are errors.

Image sources:
https://www.yourgenome.org/facts/what-is-pcr-polymerase-chain-reaction
http://www.well.ox.ac.uk/ogc/sequencing-quality-monitoring-run/

See **dDocent** filtering: http://ddocent.com/filtering/

Link:
https://software.broadinstitute.org/gatk/documentation/article.php?id=4860

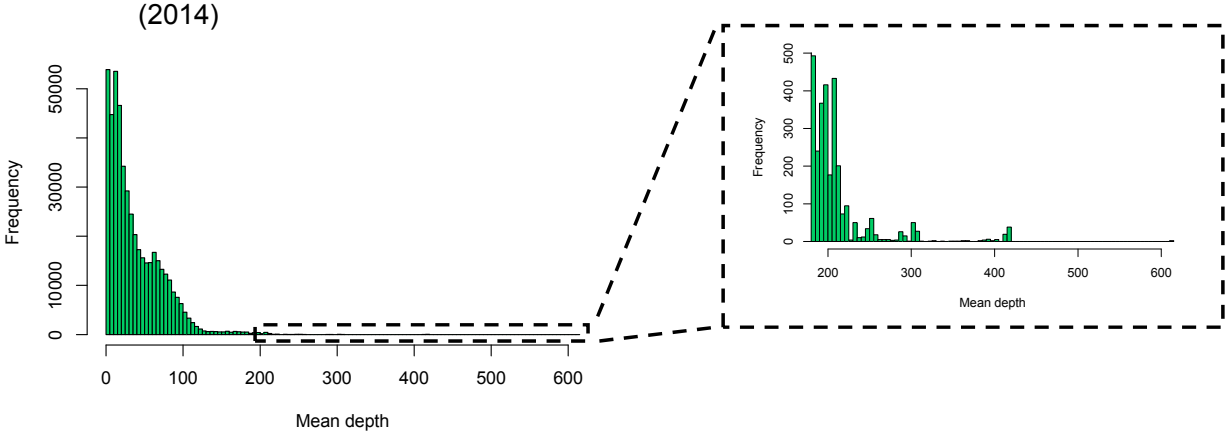
Minimum depth: Depends if you are joint calling or not. If you are calling SNPs for each sample by itself, you may want 10 reads as a minimum. If you are jointly calling (which uses information across all samples), you might be ok with 3 reads. But if you have really high coverage you might want to have 20 reads as a minimum.

Missing data per site: If you filter stringently here, and you have a bunch of low quality individuals (i.e. they are the ones contributing the most to the missing data), you will remove many sites. If we first have a relaxed missing data filter, and then remove low quality individuals, you will end up with more SNPs at the end.

Iterative filtering:
See O'Leary et al. (2018) "These aren't the loci you're looking for: Principles of effective SNP filtering for molecular ecologists"

Maximum mean depth

- We want to remove paralogues
  - Generally, the mean read depth per locus should be approximately normally distributed
  - 90th quantile; two times the mode (Willis et al. 2017); d+3*sqrt(d), d=mean depth; Li (2014)



Willis et al. (2017) "Haplotyping RAD loci: An efficient method to filter paralogs and account for physical linkage"

Li (2014) "Towards Better Understanding of Artifacts in Variant Calling from High-Coverage Samples"

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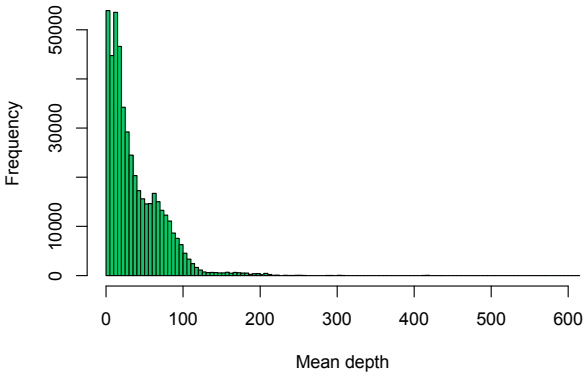


Create a list of mean depth per site:
vcftools --vcf input_file.vcf --site-mean-depth --out mean_depth

Results are stored in:
mean_depth.ldepth.mean

Filter for maximum mean depth:
vcftools --vcf input_file.vcf --max-meanDP 120 --recode --recode-INFO-all --out output_file.vcf

Minimum mean depth

- We want to be confident with out SNP calls
  - Commonly 20-30

```
vcftools --vcf input_file.vcf --min-meanDP 20 --recode --recode-INFO-all --out output_file
```

Depth and quality scores

See: http://ddocent.com/filtering/

- High coverage can lead to inflated quality scores
  - Removal of variants with high quality scores and high depth
  - Typical to remove these sites before the mean depth filtering

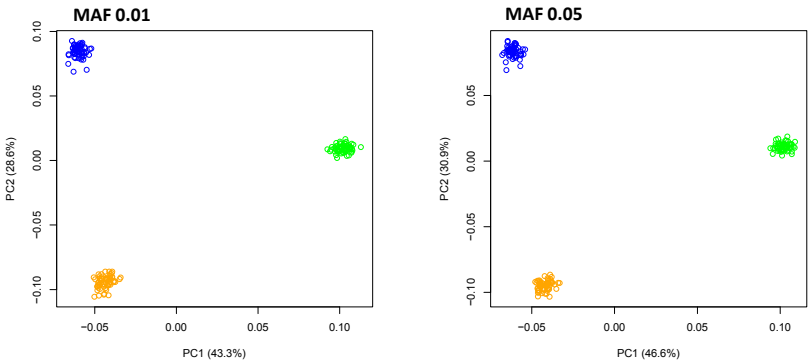
Mapping quality

- We want to filter out sites with reads that haven't mapped well to the reference genome
  - Typically 20-30

```
vcffilter -f "MQ > 30" input_file.vcf > output_file.vcf
(vcffilter is within vcflib)
```

Minor allele frequency

- Typically minor allele frequency of 0.01 or 0.05, or minor allele count of 1
  - Depends on what statistic you are measuring
  - Are you interested in rare variants? Singletons might be errors
  - Do some PCAs - does the structure change depending on the minor allele frequency?



~10,000 SNPs, three closely related populations, neutral loci

Minor allele frequency:
vcftools --vcf input_file.vcf --maf 0.01--recode --recode-INFO-all --out output_file

Minor allele count:
vcftools --vcf input_file.vcf --mac 1--recode --recode-INFO-all --out output_file

Are your results robust to differences in minor allele frequencies, or do you detect different structure depending on the minor allele frequency?

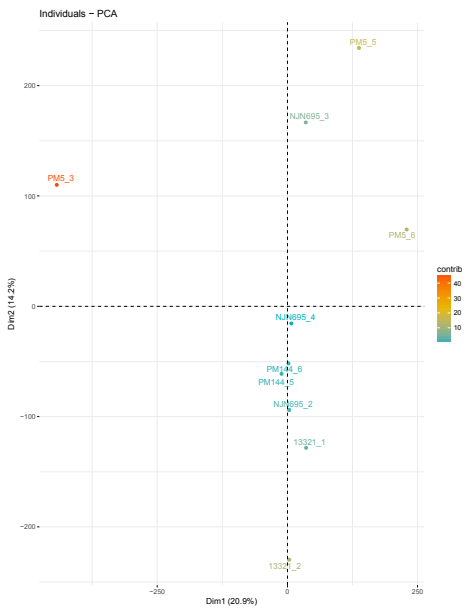
See Linck & Battey “Minor allele frequency thresholds strongly affect population structure inference with genomic datasets”

Missing data

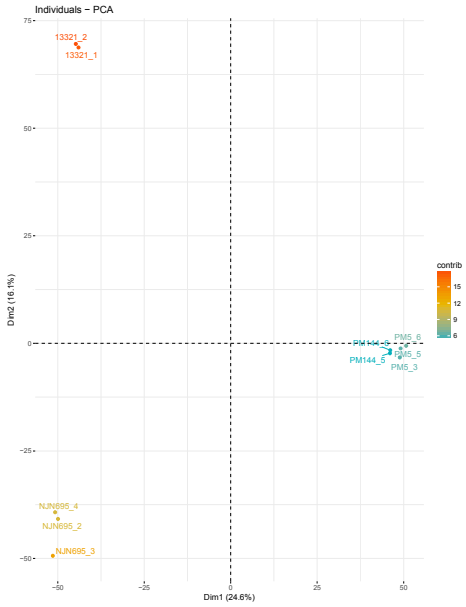
- Overall % of missing data per locus
  - Typically 0-20%
- Population specific missing data
  - Do you need each SNP to be sequenced in every population?
- Consider how each program deals with missing data!

Filter for overall 20% missing data:
vcftools --vcf input_file.vcf --max-missing 0.8 --recode --recode-INFO-all --out output_file

You might consider imputing missing data.



Missing data

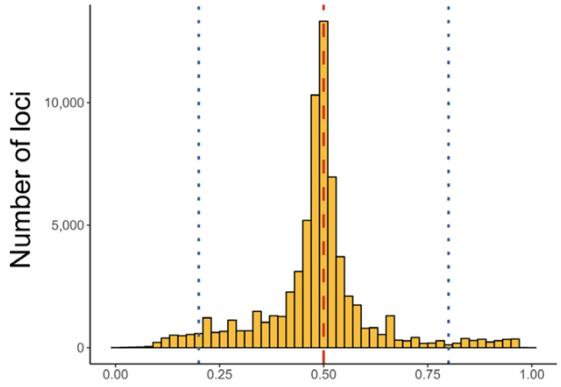


No missing data

PCAs by Alicia Toon

Allele balance

- For heterozygous sites, the ratio of the number of reads for the reference allele compared to the number of reads for the alternative allele

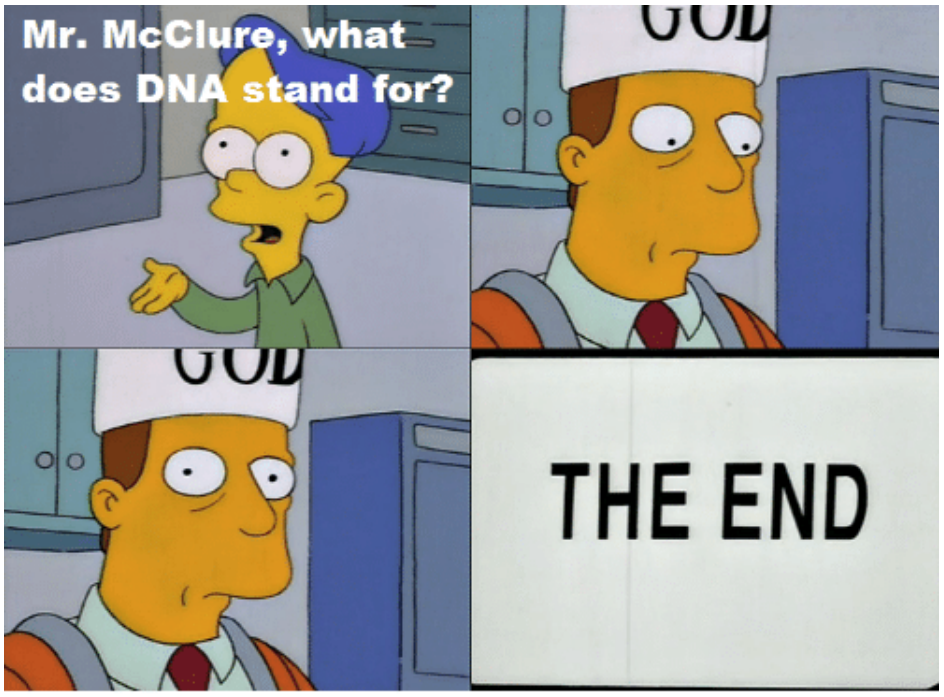


Source: O'Leary et al. (2018)

- We expect close to 0.5
- Typically remove sited with allele balance less than 0.25 and greater than 0.75

```
vcffilter -s -f "AB > 0.25 & AB < 0.75 | AB < 0.01" input_file.vcf > output_file.vcf
```

Allele balance not close to 0.5 could indicate false homozygotes (i.e. errors!)


Mr. McClure, what does DNA stand for?
GOD
GOD
THE END