Introduction to GWAS

Common Data Types and Formats

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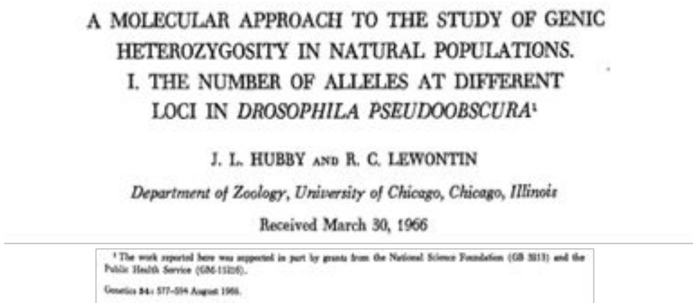
Genotyping and Sequencing

A very brief overview

The first steps – Biomarkers



A MOLECULAR APPROACH TO THE STUDY OF GENIC HETEROZYGOSITY IN NATURAL POPULATIONS.
I. THE NUMBER OF ALLELES AT DIFFERENT LOCI IN *DROSOPHILA PSEUDOOBSCURA*[1]

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Department of Zoology, University of Chicago, Chicago, Illinois

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Genetics **54**: 577–594 August 1966.

582 J. L. HUBBY AND R. C. LEWONTIN

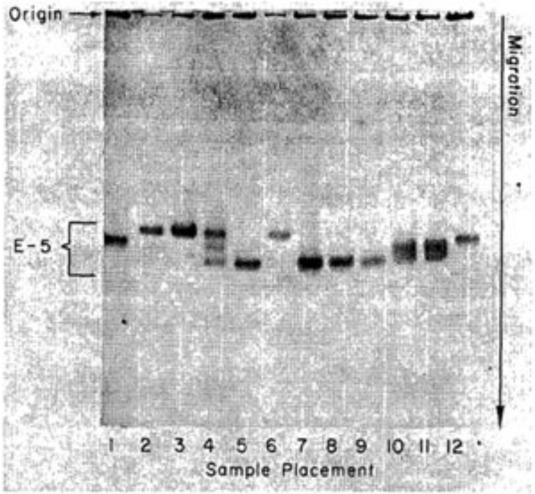
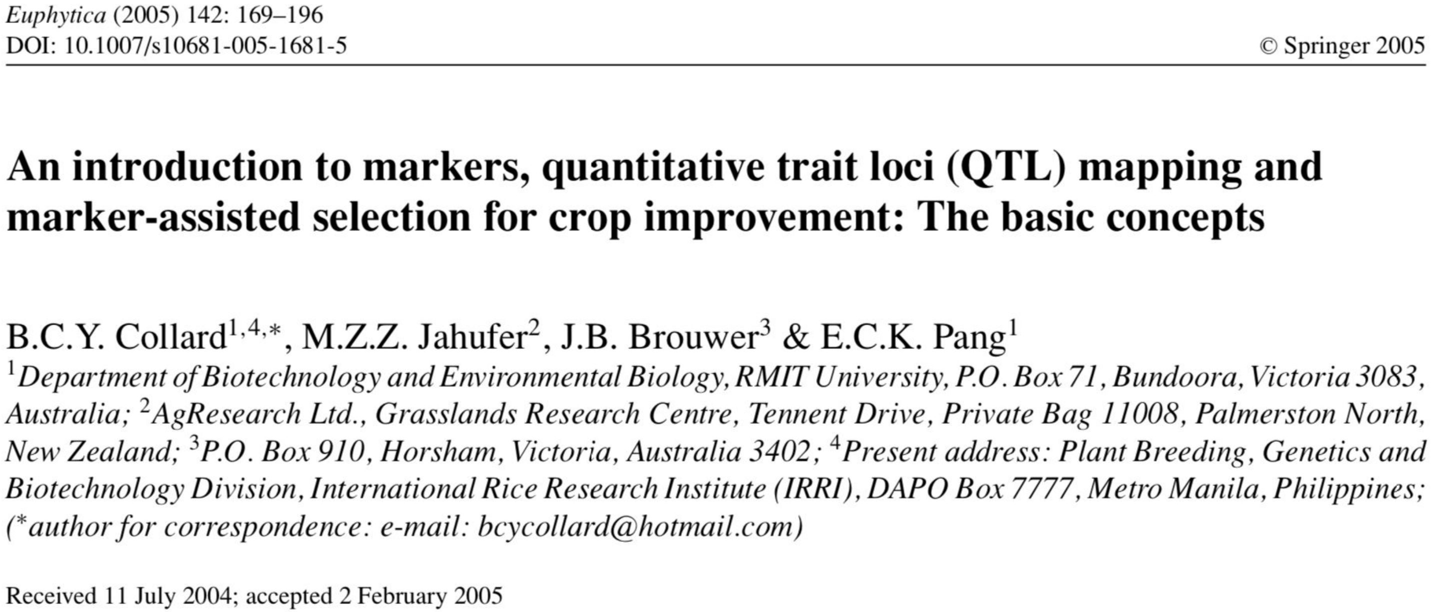


FIGURE 1.—Gel illustrating sample placement and typical results of strain analysis for Esterase-5. The first and the last samples were derived from the standard reference strain (E-$5^{1.00}$), while positions 2 through 6 were obtained from five individuals of one strain and positions 7 through 11 are from five individuals of a second strain. Positions 2, 3, and 6 contain Esterase-$5^{.95}$, position 5 contains Esterase-$5^{1.12}$, and position 4 contains Esterase-$5^{.95}$, Esterase-$5^{1.12}$, and a site of activity between them. Positions 7, 8, and 9 contain Esterase-$5^{1.12}$ and positions 10 and 11 contain Esterase-$5^{1.00}$ and Esterase-$5^{1.12}$. A site of activity midway between the latter two is barely discernible. In all the figures the direction of migration of the protein is down toward the anode.

From few to many markers – Molecular markers (DNA markers)

- arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA
- are usually located in non-coding regions of DNA
- are practically unlimited in number and are not affected by environmental factors and/or the developmental stage of the plant
- **RFLP, AFLP, RAPD, SSR (microsatellites), SNP**

From few to many markers – Molecular markers (DNA markers)



Euphytica (2005) 142: 169–196
DOI: 10.1007/s10681-005-1681-5

An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts

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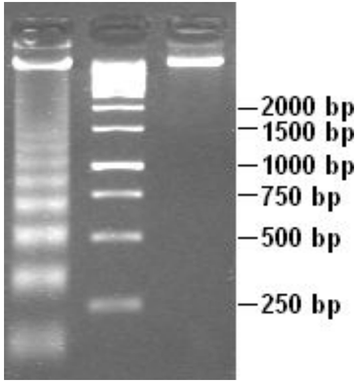
Genotyping Systems

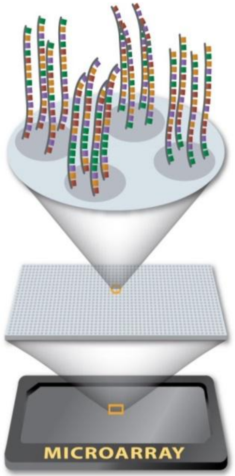
Marker gel

(a few markers)



SNP array (or GBS)

(100s -1,000,000s)

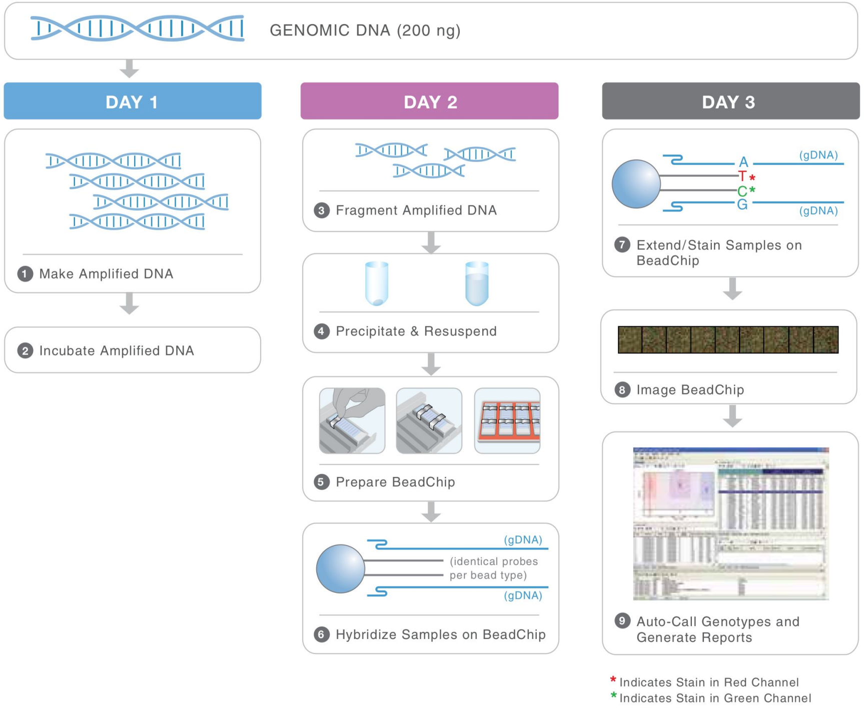


Genome sequencer

(1,000,000s +)

SNP array genotyping

Figure 1: Infinium Assay Protocol



GENOMIC DNA (200 ng)

DAY 1

1 Make Amplified DNA

2 Incubate Amplified DNA

DAY 2

3 Fragment Amplified DNA

4 Precipitate & Resuspend

5 Prepare BeadChip

6 Hybridize Samples on BeadChip

DAY 3

7 Extend/Stain Samples on BeadChip

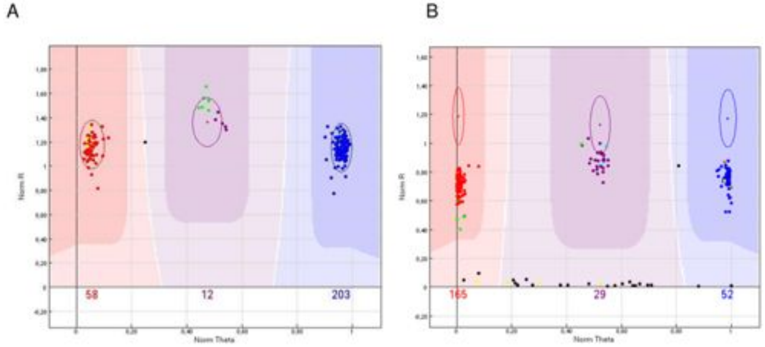
8 Image BeadChip

9 Auto-Call Genotypes and Generate Reports

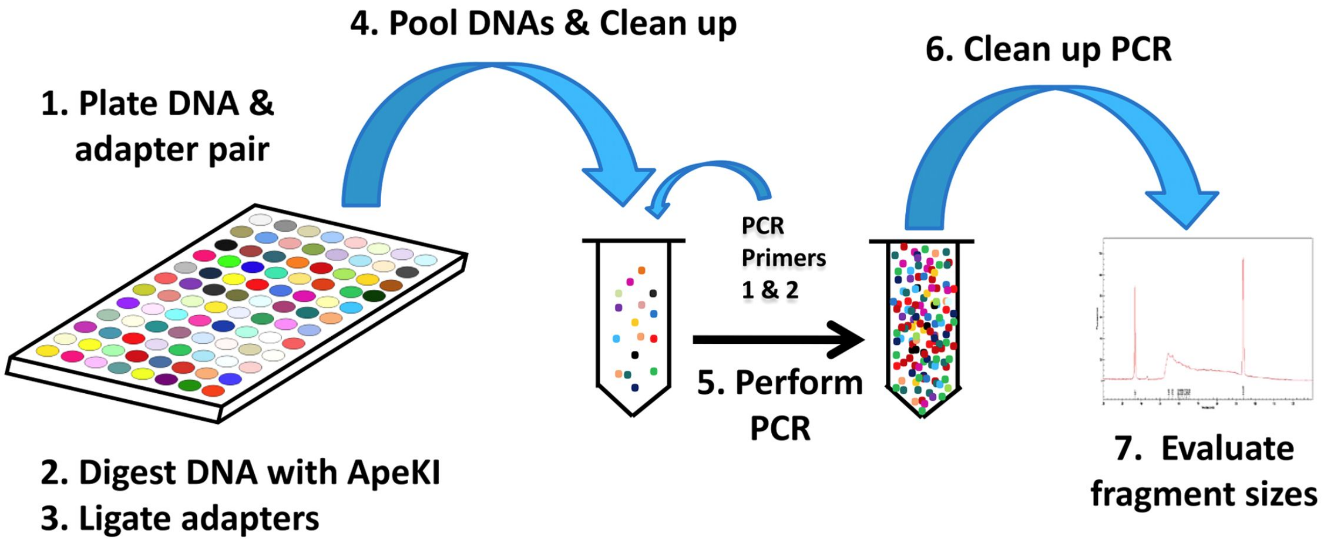
* Indicates Stain in Red Channel
* Indicates Stain in Green Channel

Genotype calling

3 genotypes: AA, AG, GG



Reduced representation sequencing – Genotyping-by-Sequencing (GBS)



1. Plate DNA & adapter pair

2. Digest DNA with ApeKI

3. Ligate adapters

4. Pool DNAs & Clean up

PCR Primers 1 & 2

5. Perform PCR

6. Clean up PCR

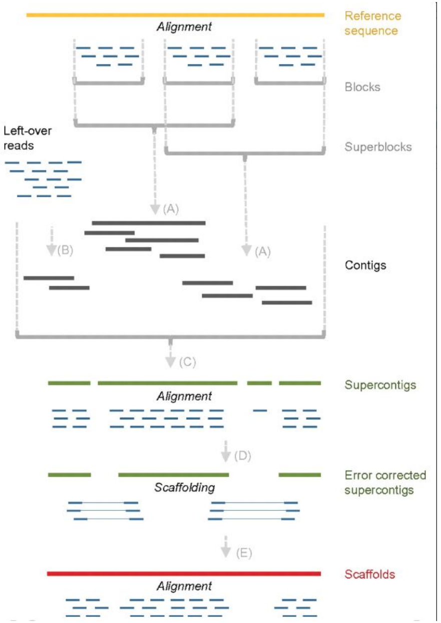
7. Evaluate fragment sizes

doi: https://doi.org/10.1371/journal.pone.0019379.g002

The Next Generation Sequencing Revolution

PHASE TWO : INTERPRETATION
I THINK I FOUND A CORNER PIECE
3 BILLION PIECES
GENOME
S.FELDMAN The Star-Ledger

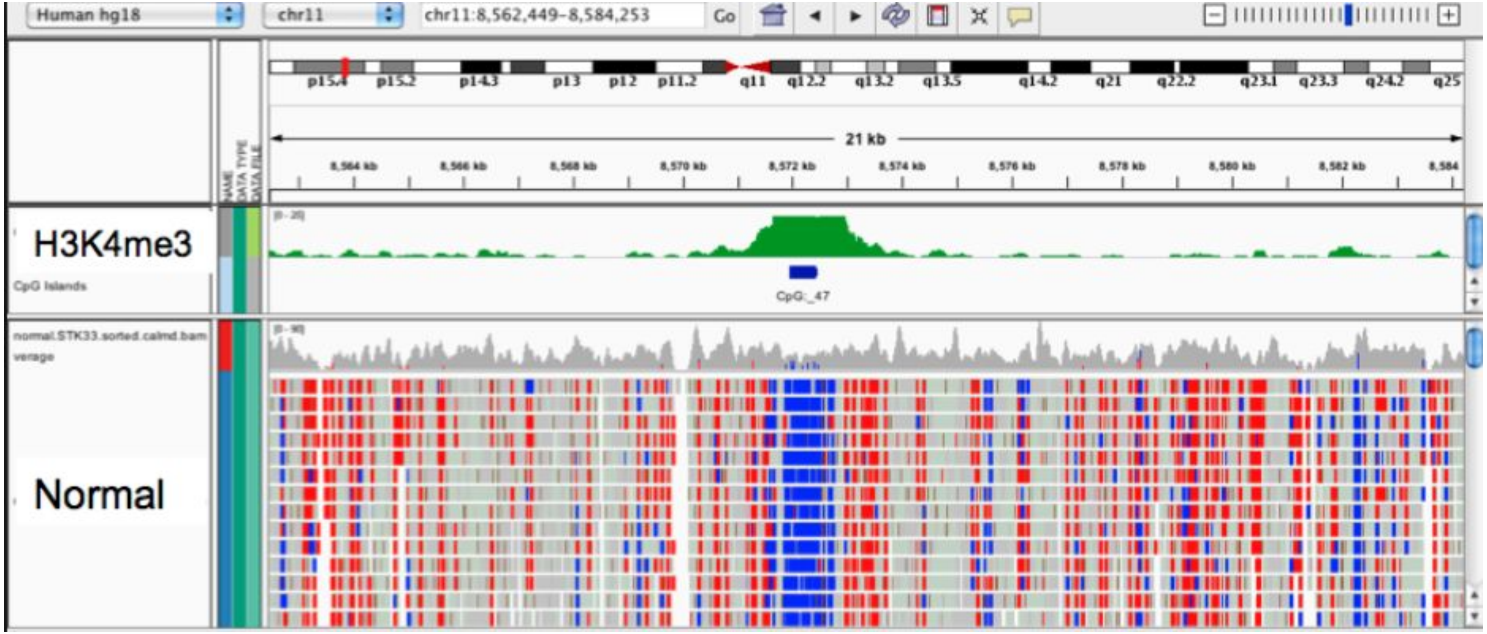
Hmmm...now the data is here….so what now?



Assembly!

The Next Generation Sequencing Revolution

Millions of polymorphisms in the genome sequences…



Genotyping formats

Two major genotyping formats (but there are many…)

Plink format ped/map files

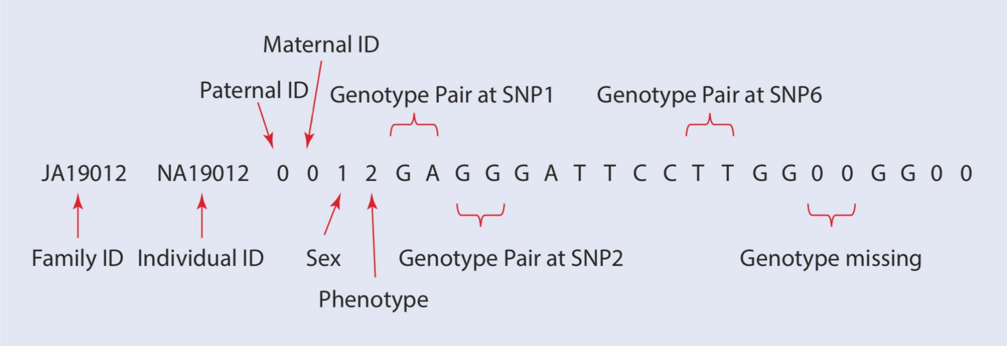
https://www.cog-genomics.org/plink2/

Variant calling format vcf files

https://www.internationalgenome.org/wiki/Analysis/vcf4.0/

https://vcftools.github.io/index.html

PLINK - the .ped file



Maternal ID

Paternal ID

Genotype Pair at SNP1

Genotype Pair at SNP6

JA19012 NA19012 0 0 1 2 G A G G G A T T C C T T G G 0 0 G G 0 0

Family ID

Individual ID

Sex

Phenotype

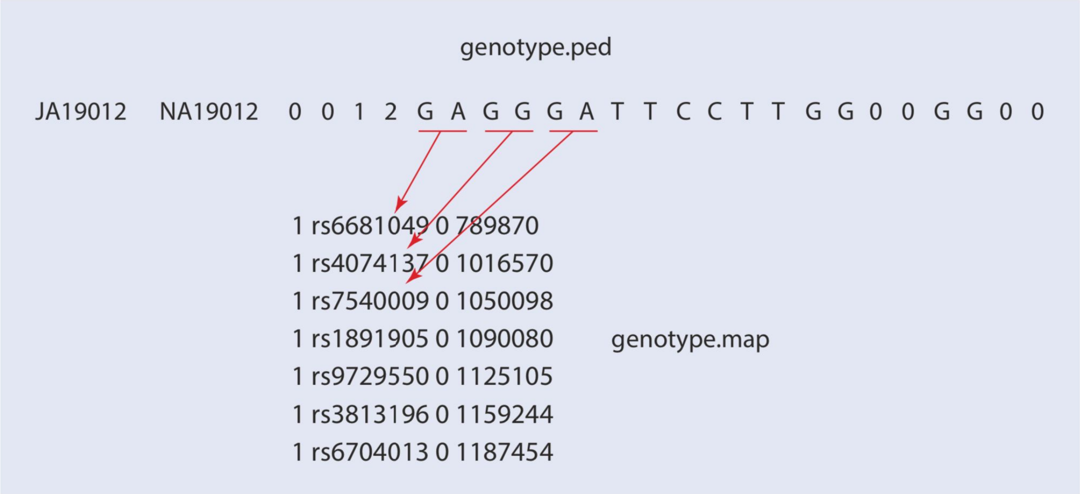
Genotype Pair at SNP2

Genotype missing

Column 1 = Family ID
Column 2 = Individual ID
Column 3 = Paternal ID (zero for missing)
Column 4 = Maternal ID (zero for missing)
Column 5 = Sex
Column 6 = Phenotype (1=unaffected, 2=affected, and 0=missing)
Column 7, 8 = genotype pair of the first SNP1 (zero for missing)
Column 9, 10 = genotype pair of the second SNP2 (zero for means missing)
…
Column 457393, 457394 = genotype pair of the last SNP228694

Kim J.H. (2019) GWAS Data Analysis. In: Genome Data Analysis. Learning Materials in Biosciences. Springer, Singapore

PLINK - the .map file



genotype.ped

```
JA19012   NA19012   0  0  1  2  G  A  G  G  G  A  T  T  C  C  T  T  G  G  0  0  G  G  0  0
```

```
1 rs6681049 0 789870
1 rs4074137 0 1016570
1 rs7540009 0 1050098
1 rs1891905 0 1090080
1 rs9729550 0 1125105
1 rs3813196 0 1159244
1 rs6704013 0 1187454
```

genotype.map

Column 1 = chromosome number
Column 2 = SNP ID
Column 3 = Genetic Distance (morgans)
Column 4 = physical base-pair position (bp)

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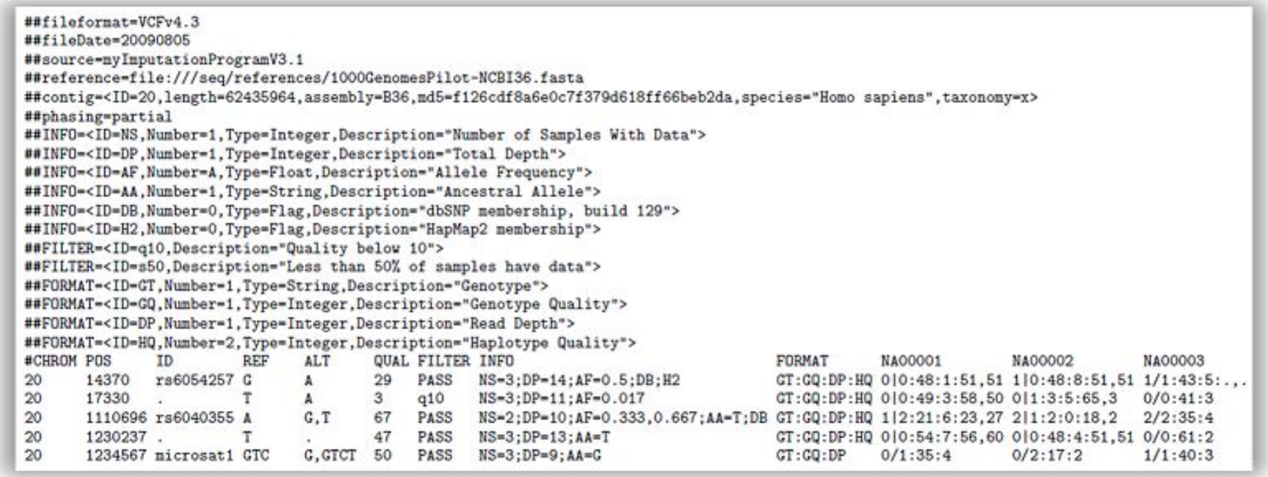
Variant calling format - the .vcf file

Data info



```
##fileformat=VCFv4.3
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens",taxonomy=x>
##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=A,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=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##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      NA00001        NA00002        NA00003
20     14370   rs6054257 G      A       29   PASS   NS=3;DP=14;AF=0.5;DB;H2           GT:GQ:DP:HQ 0|0:48:1:51,51 1|0:48:8:51,51 1/1:43:5:.,.
20     17330   .         T      A       3    q10    NS=3;DP=11;AF=0.017               GT:GQ:DP:HQ 0|0:49:3:58,50 0|1:3:5:65,3   0/0:41:3
20     1110696 rs6040355 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|1:2:0:18,2   2/2:35:4
20     1230237 .         T      .       47   PASS   NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 0/0:61:2
20     1234567 microsat1 GTC    G,GTCT  50   PASS   NS=3;DP=9;AA=G                    GT:GQ:DP    0/1:35:4       0/2:17:2       1/1:40:3
```

0/1 is not the same as **0|1** !! (unphased / phased)

CHROM → chromosome/contig

POS → position on chr/contig

ID → SNP name/ID

REF → reference genome allele

ALT → alternative allele (. / 1 or more)

QUAL → Phred scaled quality

-10log10 (call in Alt is wrong)

E.g. 1/10 chance of mistake → 10

FILTER □ quality filter (q10 → quality < 10)

INFO □ further information