Variant calling
in NGS experiments

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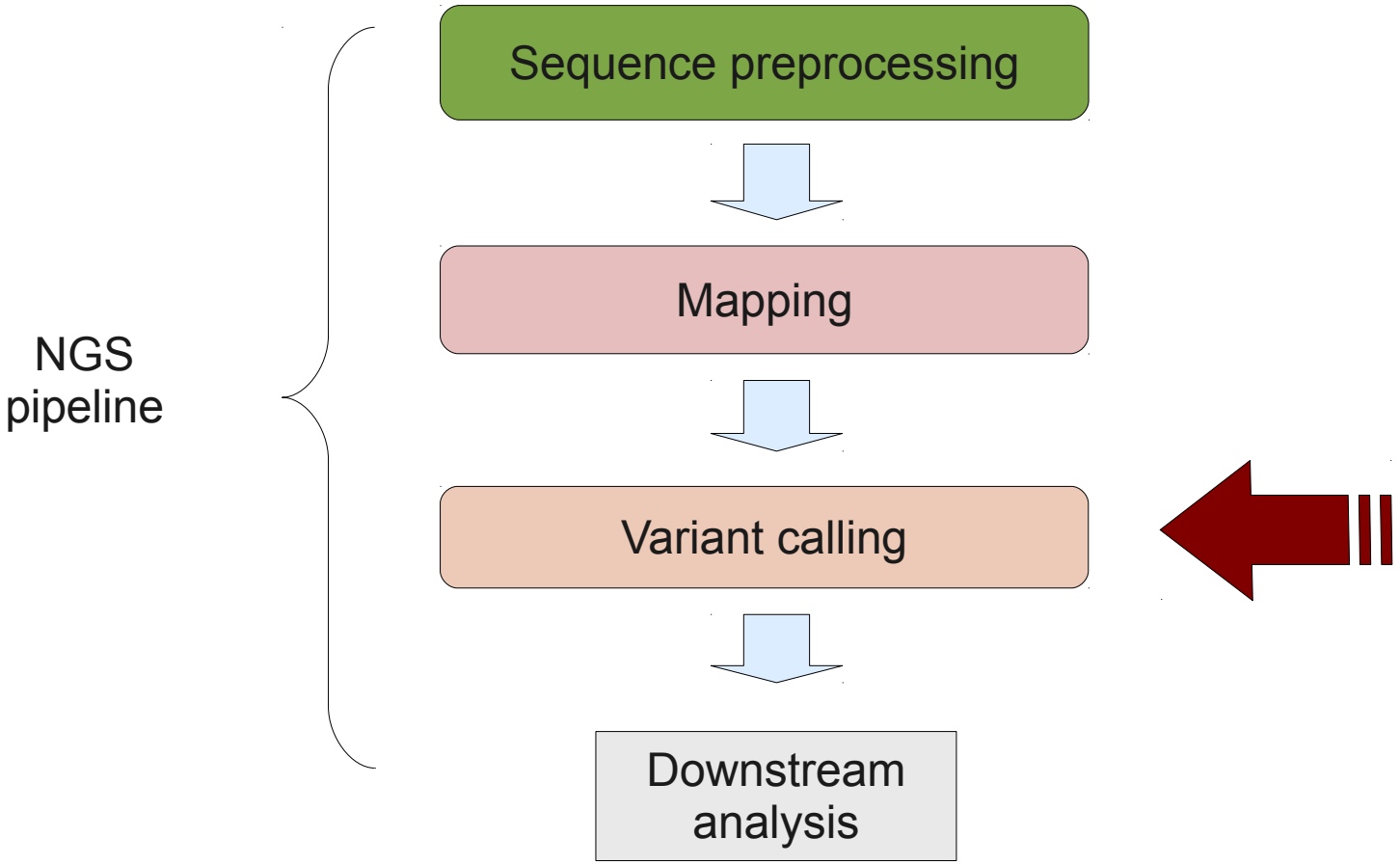
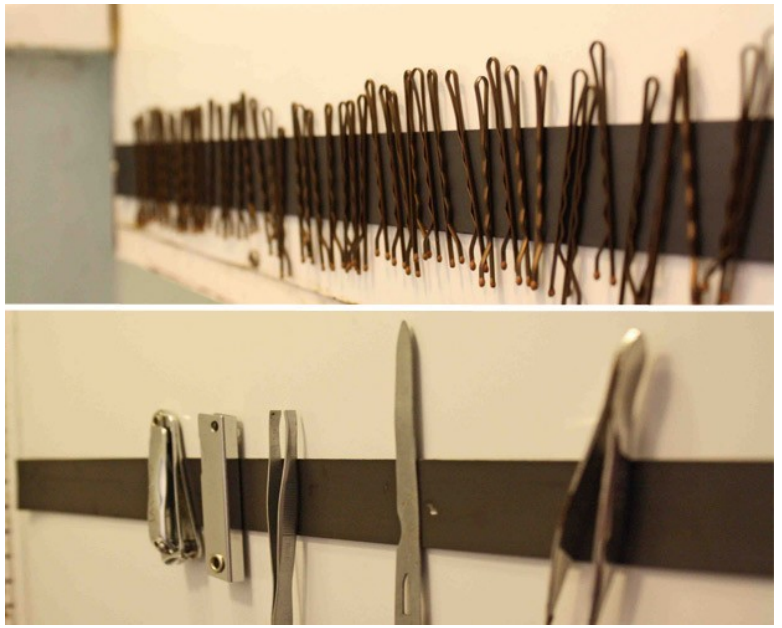
NGS Sequence preprocessing

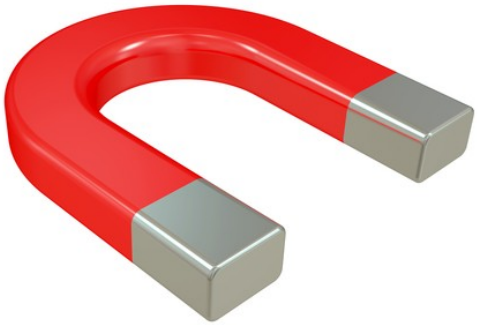
Where we are?



What is variant calling?

Finding A Needle In The Haystack?

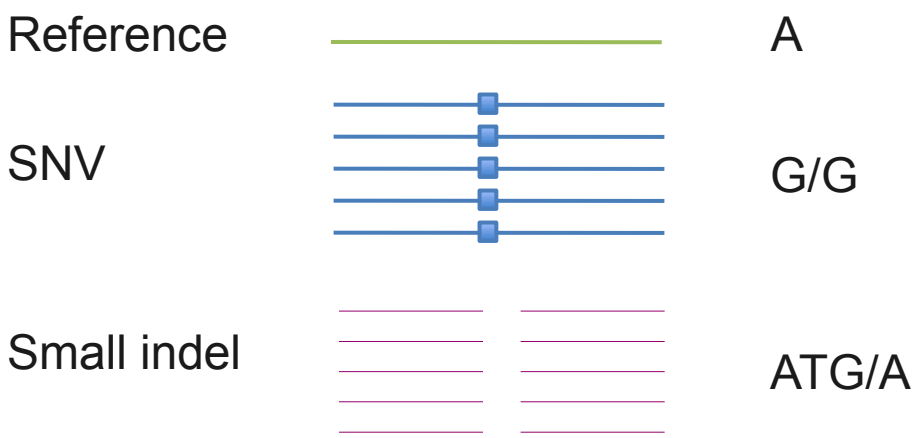


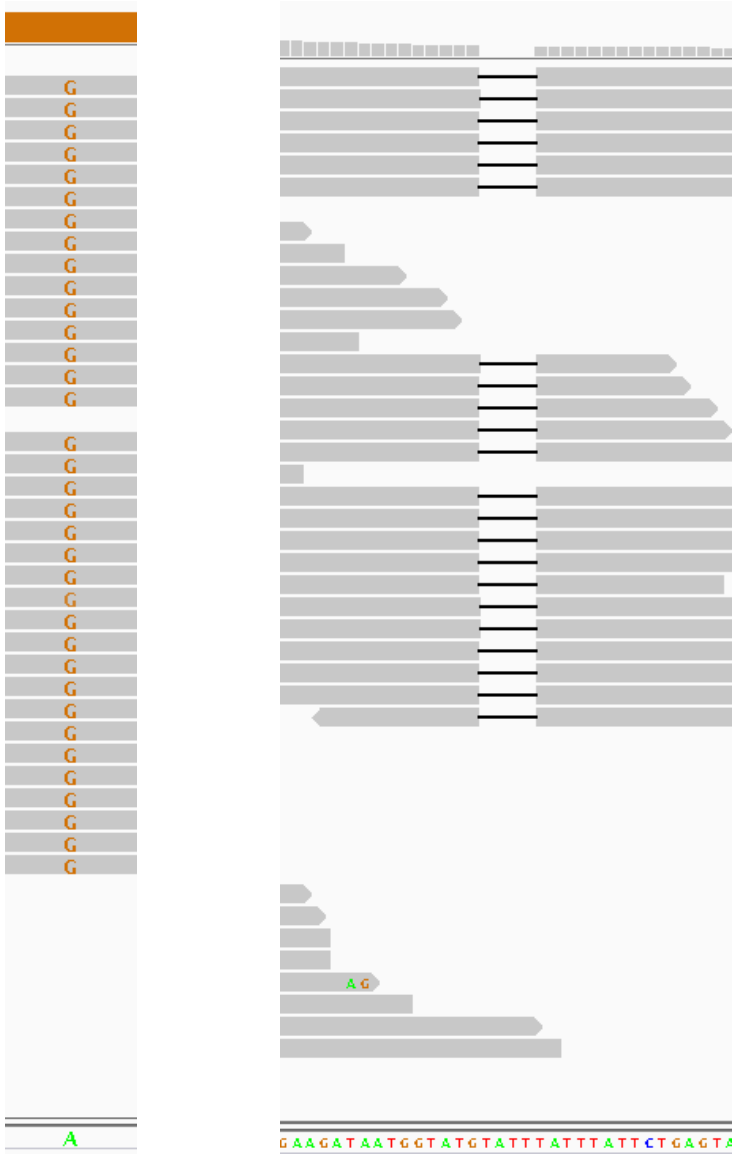


Variant types

SNV: Single nucleotide variant.

Indel: small insertion/deletion variant.





Genotype and variant calling – concepts

Phred Quality score:

$$Q_{\text{Phred}} = -10 \log_{10} P(\text{error}).$$

A score of 20 corresponds to 1% error rate in base calling

Variant calling: positions with at least one of the bases differs from reference.

Genotype calling: Process of determining the genotype of each variant.

Importance of base quality recalibration:

Obtaining well-calibrated quality scores is important, as SNP and genotype calling at a specific position in the genome depends on both the base calls and the per-base quality scores of the reads overlapping the position.

Nielsen R, Paul JS, Albrechtsen A, Song YS. Genotype and SNP calling from next-generation sequencing data. Nat Rev Genet. 2011 Jun;12(6):443-51. Review. PubMed PMID: 21587300.

Methods for calling

Early methods:

Counting the number of times each allele is observed.

Probabilistic methods:

They compute **genotype likelihood**.

Advantages:

- Provide statistical measures of uncertainty.

- Lead to higher accuracy of genotype calling.

- Provide a natural framework for incorporating information: AF, LD.

Calling algorithms

Software	Available from	Calling method	Prerequisites	Comments	Refs
SOAP2	http://soap.genomics.org.cn/index.html	Single-sample	High-quality variant database (for example, dbSNP)	Package for NGS data analysis, which includes a single individual genotype caller (SOAPsnp)	15
realSFS	http://128.32.118.212/thorfinn/realSFS/	Single-sample	Aligned reads	Software for SNP and genotype calling using single individuals and allele frequencies. Site frequency spectrum (SFS) estimation	-
Samtools	http://samtools.sourceforge.net/	Multi-sample	Aligned reads	Package for manipulation of NGS alignments, which includes a computation of genotype likelihoods (samtools) and SNP and genotype calling (bcftools)	53
GATK	http://www.broadinstitute.org/gsa/wiki/index.php/The_Genome_Analysis_Toolkit	Multi-sample	Aligned reads	Package for aligned NGS data analysis, which includes a SNP and genotype caller (Unifed Genotyper), SNP filtering (Variant Filtration) and SNP quality recalibration (Variant Recalibrator)	32,33
Beagle	http://faculty.washington.edu/browning/beagle/beagle.html	Multi-sample LD	Candidate SNPs, genotype likelihoods	Software for imputation, phasing and association that includes a mode for genotype calling	42
IMPUTE2	http://mathgen.stats.ox.ac.uk/impute/impute_v2.html	Multi-sample LD	Candidate SNPs, genotype likelihoods	Software for imputation and phasing, including a mode for genotype calling. Requires fine-scale linkage map	44
QCall	ftp://ftp.sanger.ac.uk/pub/rd/QCALL	Multi-sample LD	'Feasible' genealogies at a dense set of loci, genotype likelihoods	Software for SNP and genotype calling, including a method for generating candidate SNPs without LD information (NLDA) and a method for incorporating LD information (LDA). The 'feasible' genealogies can be generated using Margarita (http://www.sanger.ac.uk/resources/software/margarita)	54
MaCH	http://genome.sph.umich.edu/wiki/Thunder	Multi-sample LD	Genotype likelihoods	Software for SNP and genotype calling, including a method (GPT_Freq) for generating candidate SNPs without LD information and a method (thunder_glf_freq) for incorporating LD information	-

A more complete list is available from http://seqanswers.com/wiki/Software/list. LD, linkage disequilibrium; NGS, next-generation sequencing.

Nielsen R, Paul JS, Albrechtsen A, Song YS. Genotype and SNP calling from next-generation sequencing data. Nat Rev Genet. 2011 Jun;12(6):443-51. Review. PubMed PMID: 21587300.

Why GATK?

- Probabilistic method: Bayesian estimation of the most likely genotype.

- Calculates many parameters for each position of the genome.

- SNP and indel calling.

- Used in many NGS projects, including the 1000 Genomes Project, The Cancer Genome Atlas, etc.

- Base quality recalibration.

- Uses standard input and output files.

- Many tools for manage VCF files.

Indel calling

- Many available softwares like dindel, samtools, frebayes, ...

- Sequence aligners are often unable to perfectly map reads containing insertions or deletions.

- Indel-containing reads can be either less unmapped or arranged in gapless alignments.

- Mismatches in a particular read can interfere with the gap.

- Indel detection becomes difficult with so many missing reads.

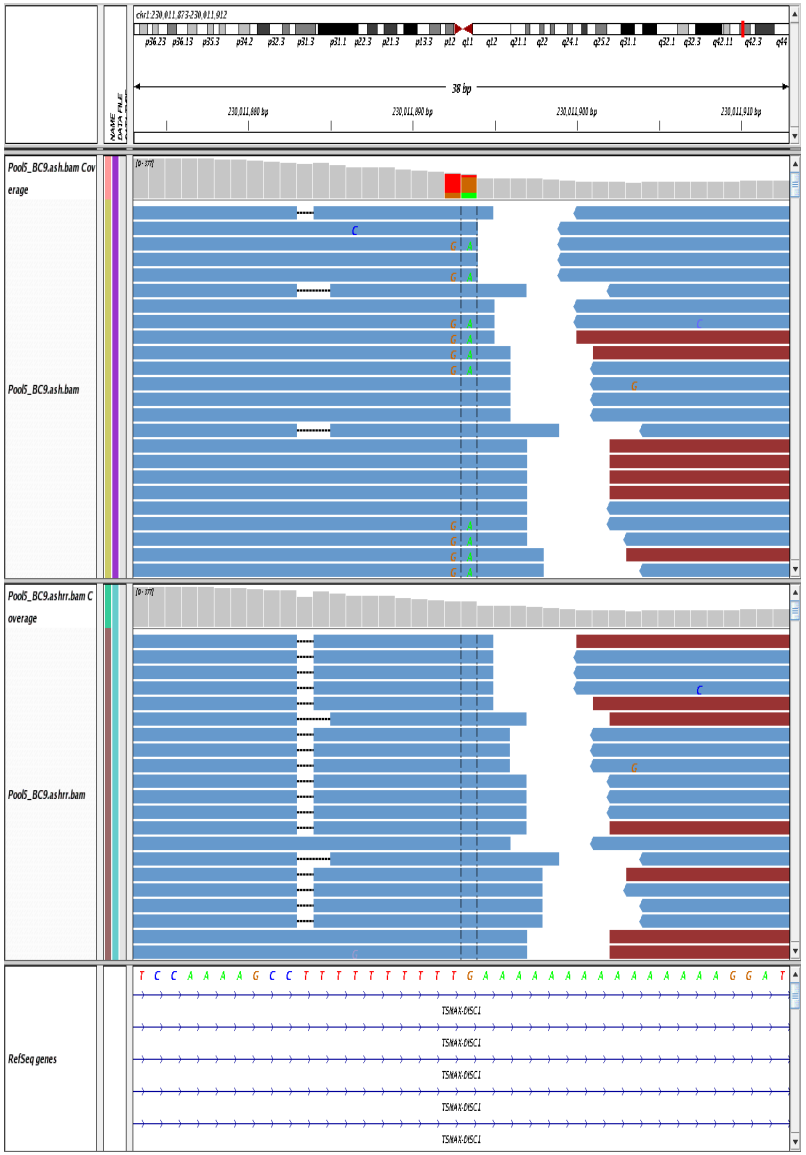
- Artifacts introduced by the gapless alignments cause the appearance of false positive SNPs (usually in clusters) → Local realignment

GATK

Local realignment

Local realignment of all reads at a specific location simultaneously to minimize mismatches to the reference genome

Reduces erroneous SNPs refines location of INDELS



DePristo MA, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet. 2011 May;43(5):491-8. PMID: 21478889

Calling all bases: missing values

We want to know the genotype of all the bases of the exome.

Calling:

- SNVs + all sites of capture kit

- indels

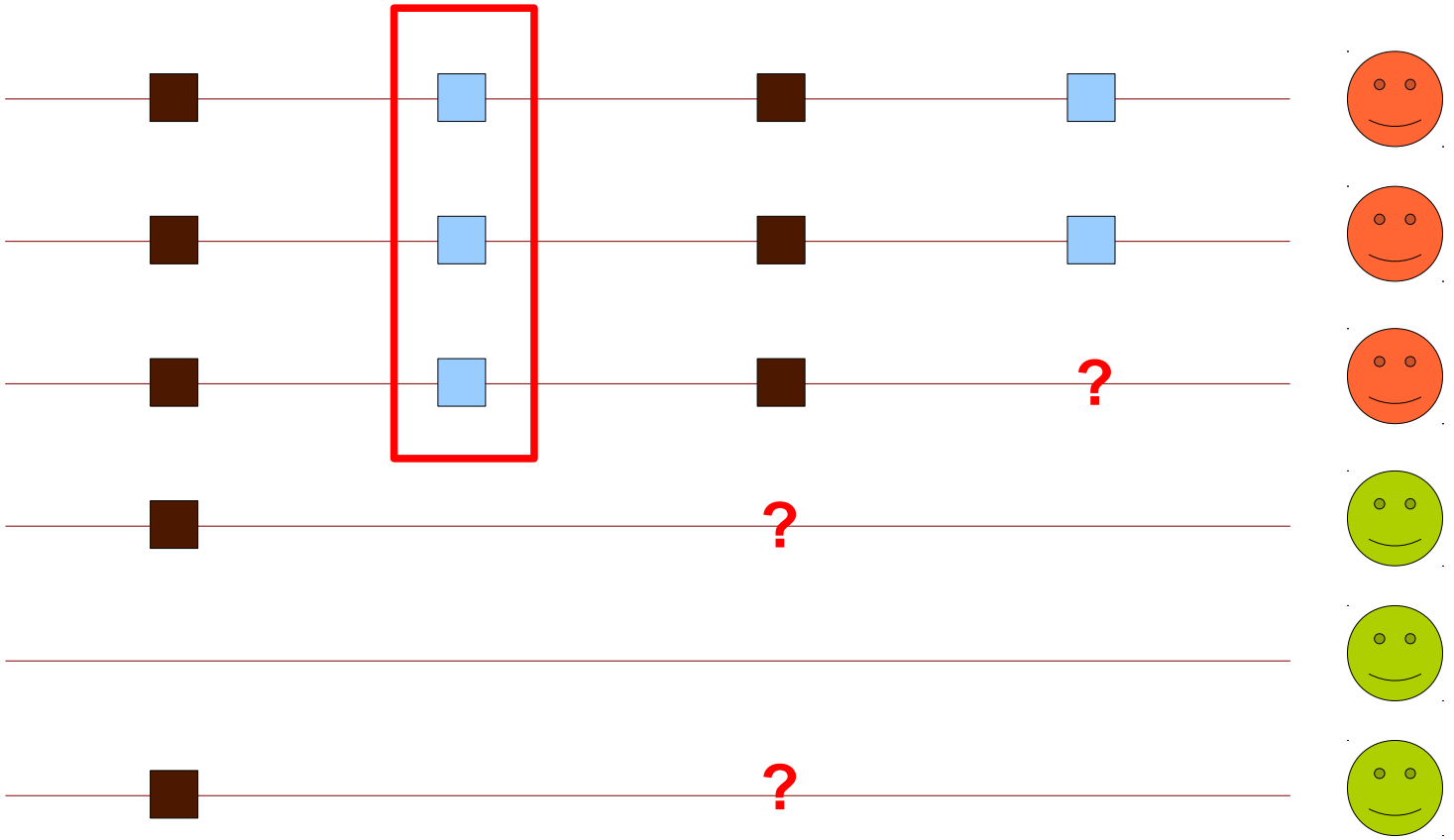
Two types of missing values:

No coverage: **./.** → Not sequenced base

Filtered: **-/-** → Low quality base

We do not know the genotype of these bases

Missing values



False positives and negatives

FALSE POSITIVES:

Error rate: 1/10,000 bases

False positives can be decreased by increasing the number of control and cases samples.

Genes: MUC4, MUC16, ORF, ...

FALSE NEGATIVES:

Variants not sequenced → missing values

Errors in variants

VCF format

```
##[HEADER LINES]
#CHROM  POS       ID          REF   ALT   QUAL      FILTER  INFO          FORMAT          NA12878
1       873762    .           T     G     5231.78   PASS    [ANNOTATIONS] GT:AD:DP:GQ:PL  0/1:173,141:282:99:255,0,255
1       877664    rs3828047   A     G     3931.66   PASS    [ANNOTATIONS] GT:AD:DP:GQ:PL  1/1:0,105:94:99:255,255,0
1       899282    rs28548431  C     T     71.77     PASS    [ANNOTATIONS] GT:AD:DP:GQ:PL  0/1:1,3:4:25.92:103,0,26
1       974165    rs9442391   T     C     29.84     LowQual [ANNOTATIONS] GT:AD:DP:GQ:PL  0/1:14,4:14:60.91:61,0,255
```

INFO fields:

```
AC=1;AF=0.50;AN=2;DP=315;Dels=0.00;HRun=2;HaplotypeScore=15.11;MQ=91.05;MQ0=15;QD=16.61;SB=-1533.02;VQSLOD=-1.5473
```

QD: QualByDepth. Variant confidence. Low scores are indicative of false positives calls and artifacts.

ReadPosRankSum: Mann-Whitney Rank Sum Test for the distance from the end of the read for reads with the alternate allele.

FS: Fisher's Exact Test to detect strand bias.

MQ: Root Mean Square of the mapping quality of the reads across all samples.

HaplotypeScore: Consistency of the site with two (and only two) segregating haplotypes.

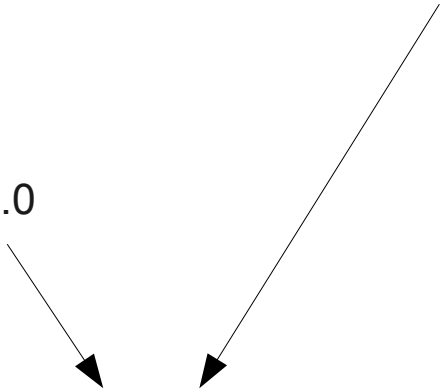
MQRankSum: Mann-Whitney Rank Sum Test for mapping qualities.

More info: http://www.broadinstitute.org/gsa/wiki/index.php/Understanding_the_Unified_Genotyper%27s_VCF_files

Filtering SNVs and indels

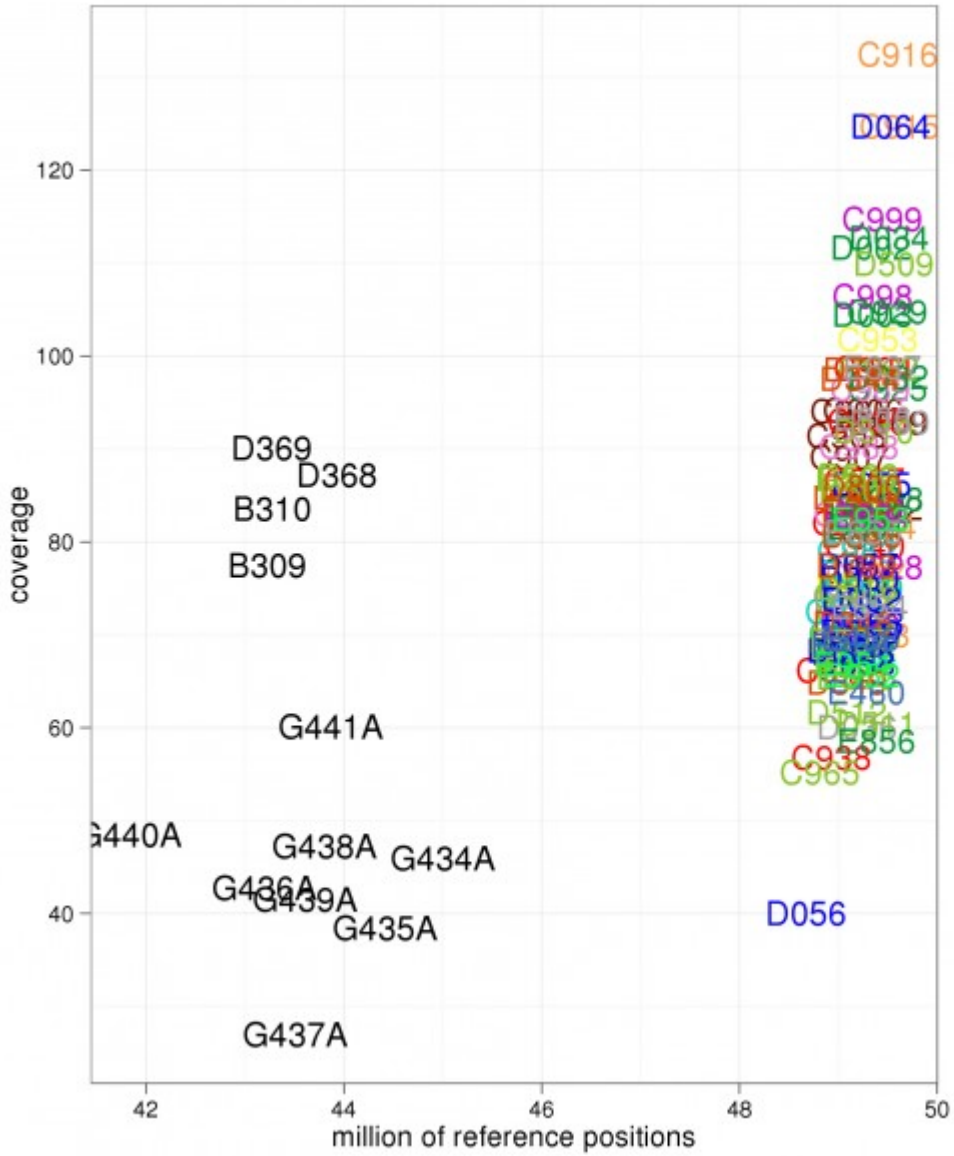
Filtering parameters for SNVs and indels are different.

SNVs	indels
QD < 2.0	QD < 2.0
MQ < 40.0	FS > 200.0
FS > 60.0	ReadPosRankSum < -20.0
HaplotypeScore > 13.0	
MQRankSum < -8.0	
ReadPosRankSum < -8.0	

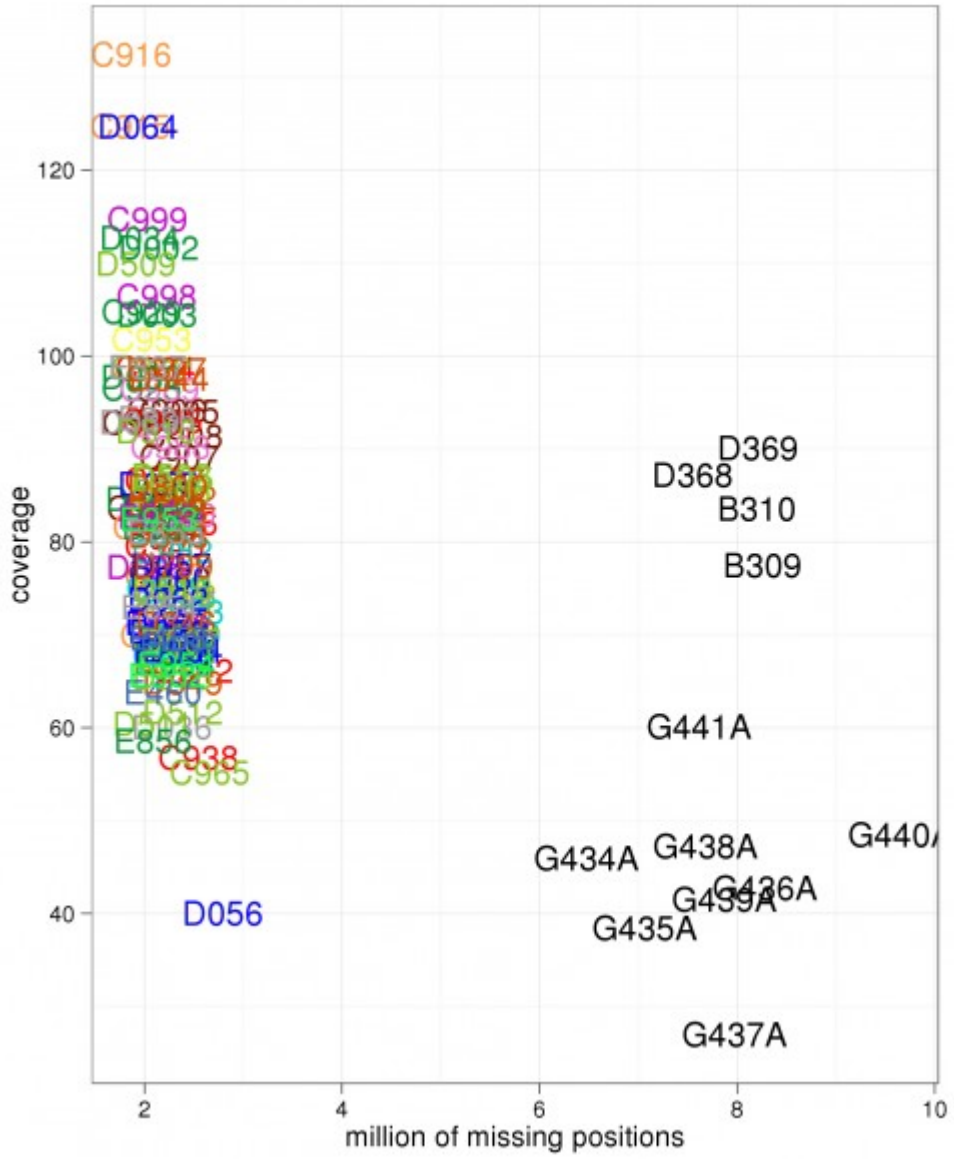


```
##[HEADER LINES]
#CHROM  POS       ID          REF   ALT   QUAL      FILTER      INFO          FORMAT          NA12878
1       873762    .           T     G     5231.78   PASS        [ANNOTATIONS] GT:AD:DP:GQ:PL  0/1:173,141:282:99:255,0,255
1       877664    rs3828047   A     G     3931.66   PASS        [ANNOTATIONS] GT:AD:DP:GQ:PL  1/1:0,105:94:99:255,255,0
1       899282    rs28548431  C     T     71.77     PASS        [ANNOTATIONS] GT:AD:DP:GQ:PL  0/1:1,3:4:25.92:103,0,26
1       974165    rs9442391   T     C     29.84     STD_FILTER  [ANNOTATIONS] GT:AD:DP:GQ:PL  0/1:14,4:14:60.91:61,0,255
```

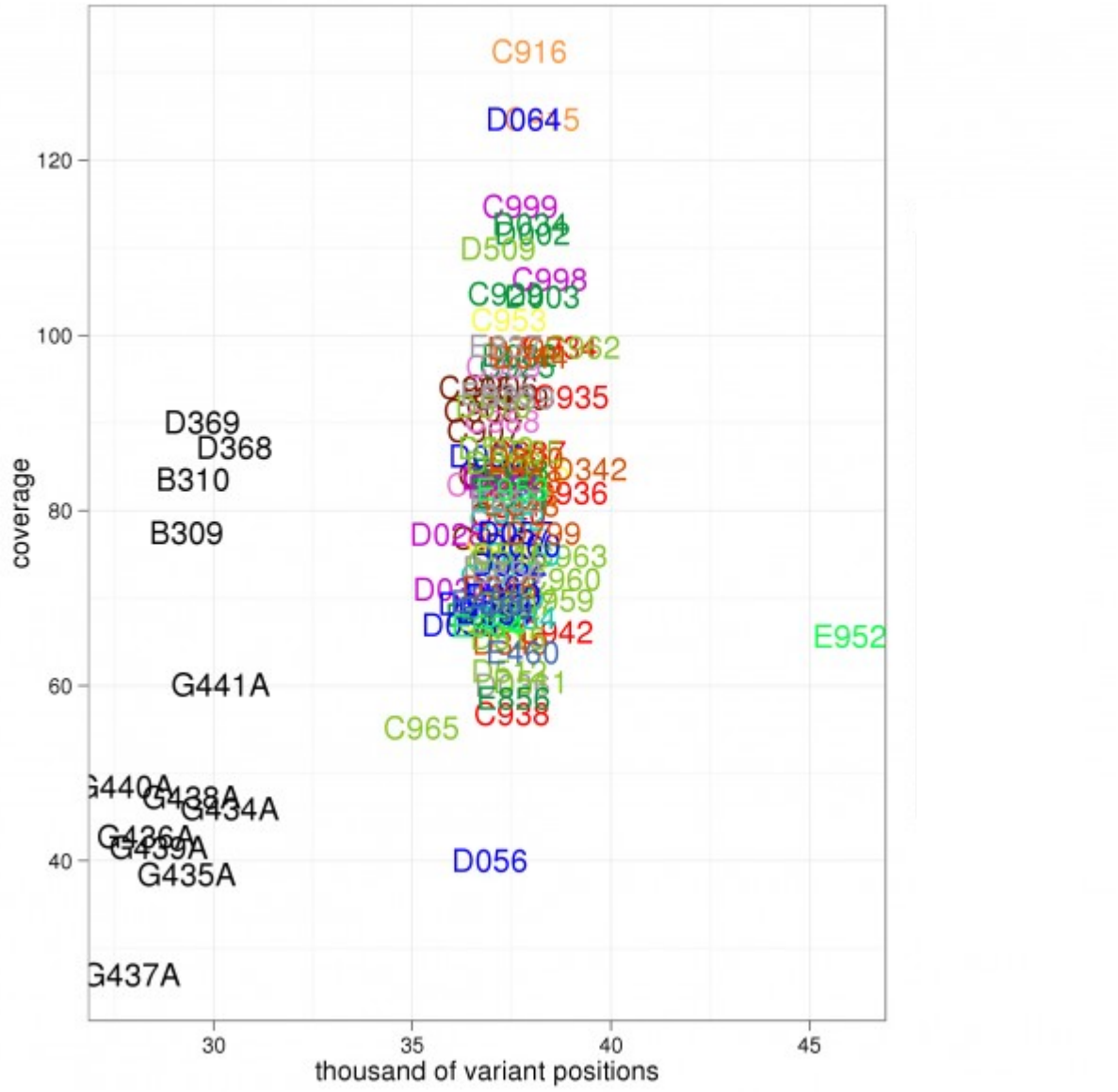
Some statistics: reference positions



Some statistics: missing positions



Some statistics: variant positions



Annotation

Definition:

An annotation is a note added by way of explanation or commentary.

Annotation of the variants:

- gene → RefSeq
- consequence type.
- dbSNP version 135.
- alelle frequency of alternative allele in 1000 genomes project.
- alelle frequency of alternative allele in NHLBI GO Exome Sequencing Project.
- PolyPhen.
- SIFT.
- conservation.
- disease associated to the variant.
- associated disease to the gene.
- GO terms.

Variant

Medina I, De Maria A, Bleda M, et al. "VARIANT: Command Line, Web service and Web interface for fast and accurate functional characterization of variants found by Next-Generation Sequencing." Nucleic Acids Res.. 2012;40(Web Server issue):W54-8.

- It can annotate single nucleotide variants (SNVs) and insertions/deletions.
- Very fast.
- web server.
- Input: VCF file.
- Annotation of all the transcripts.
- (...)

http://variant.bioinfo.cipf.es/

Annovar

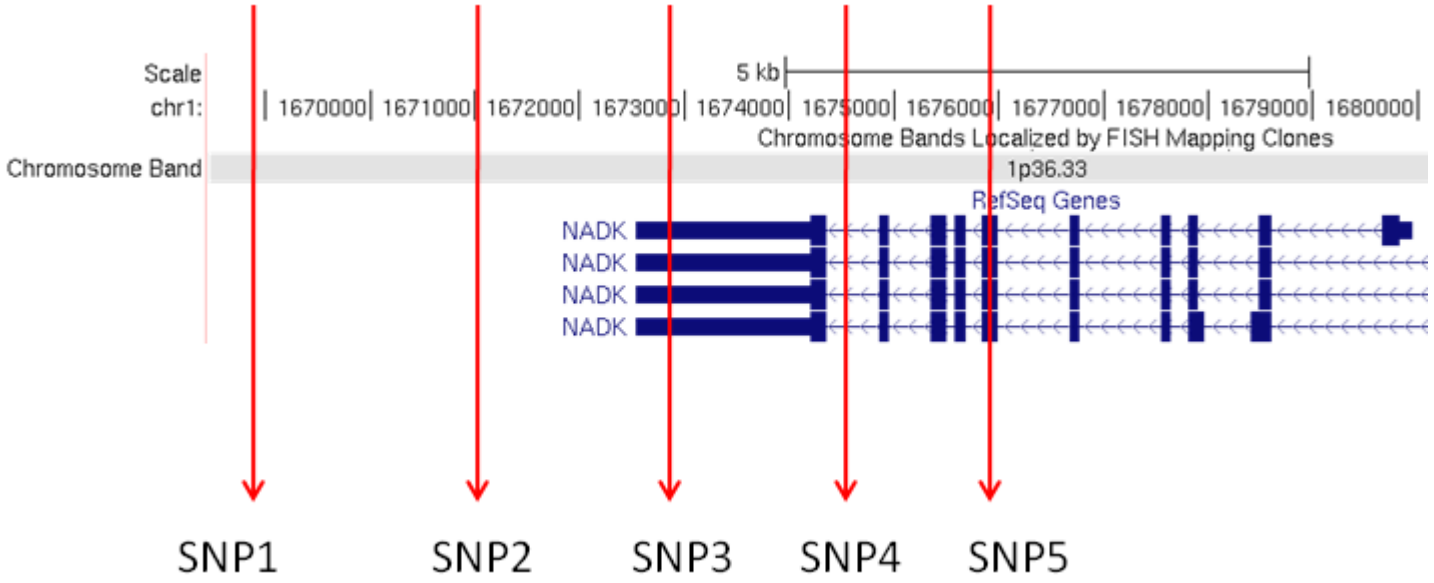
Wang K, Li M, Hakonarson H. ANNOVAR: Functional annotation of genetic variants from next-generation sequencing data. Nucleic Acids Res. 2010 Sep;38(16):e164.

- It can annotate single nucleotide variants (SNVs) and insertions/deletions.
- Gene-based, region-based and filter-based annotation of genetic variants.
- Versatile annotator: custom annotations.
- Only print the worst consequence.

frameshift substitution
stopgain
stoploss
nonframeshift
nonsynonymous SNV
synonymous SNV
unknown

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

Consequence type



frameshift substitution
stopgain
stoploss
nonframeshift
nonsynonymous SNV
synonymous SNV
unknown

exonic
splicing
ncRNA
UTR5
UTR3
intronic
upstream
downstream
intergenic

SIFT/PolyPhen

Assigns a “functional importance” score to SNV

SIFT:

Score < 0.5 → no benign variants

Score >= 0.5 → benign variants

http://sift.jcvi.org/

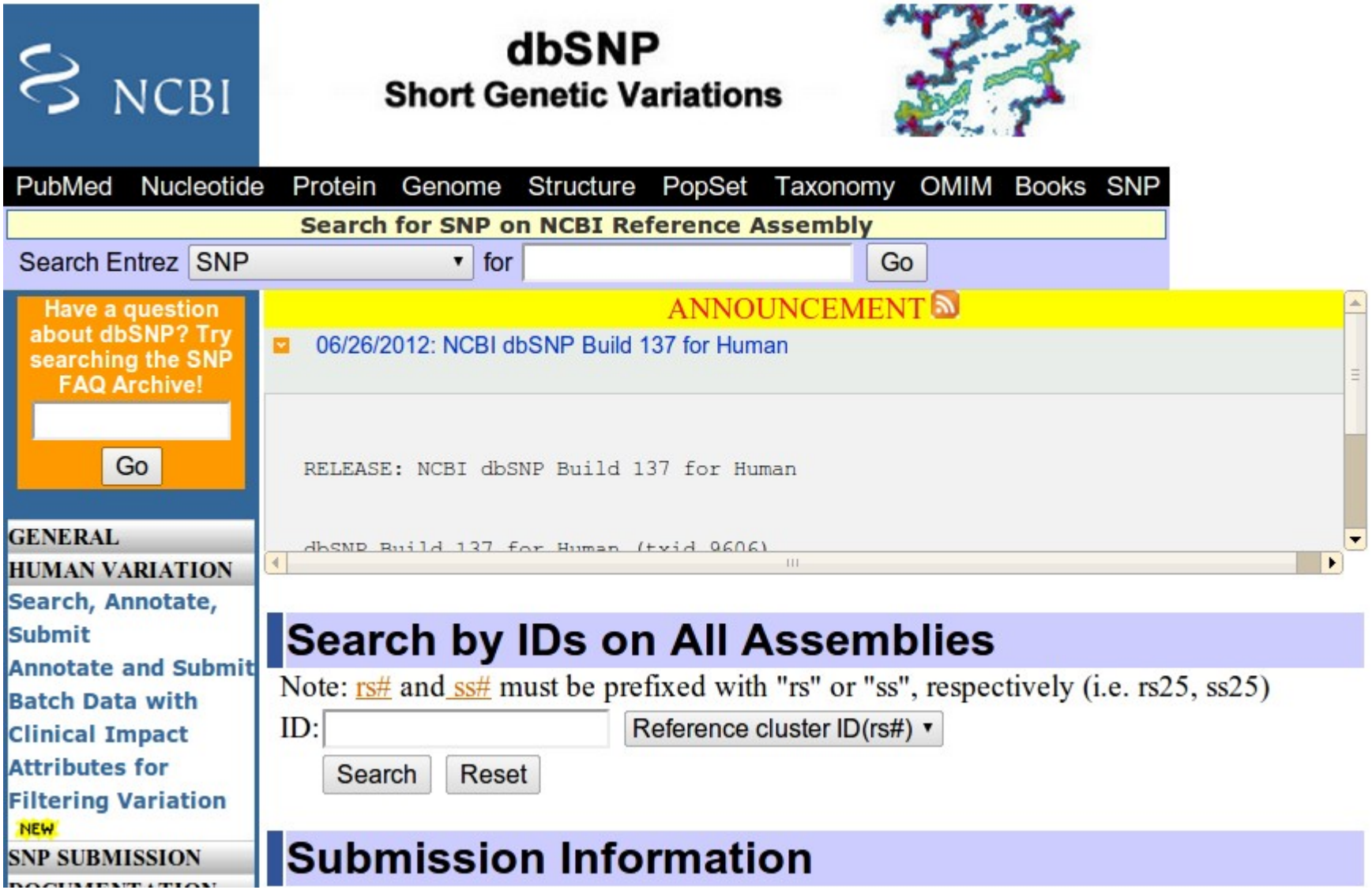
PolyPhen:

0 → benign variants

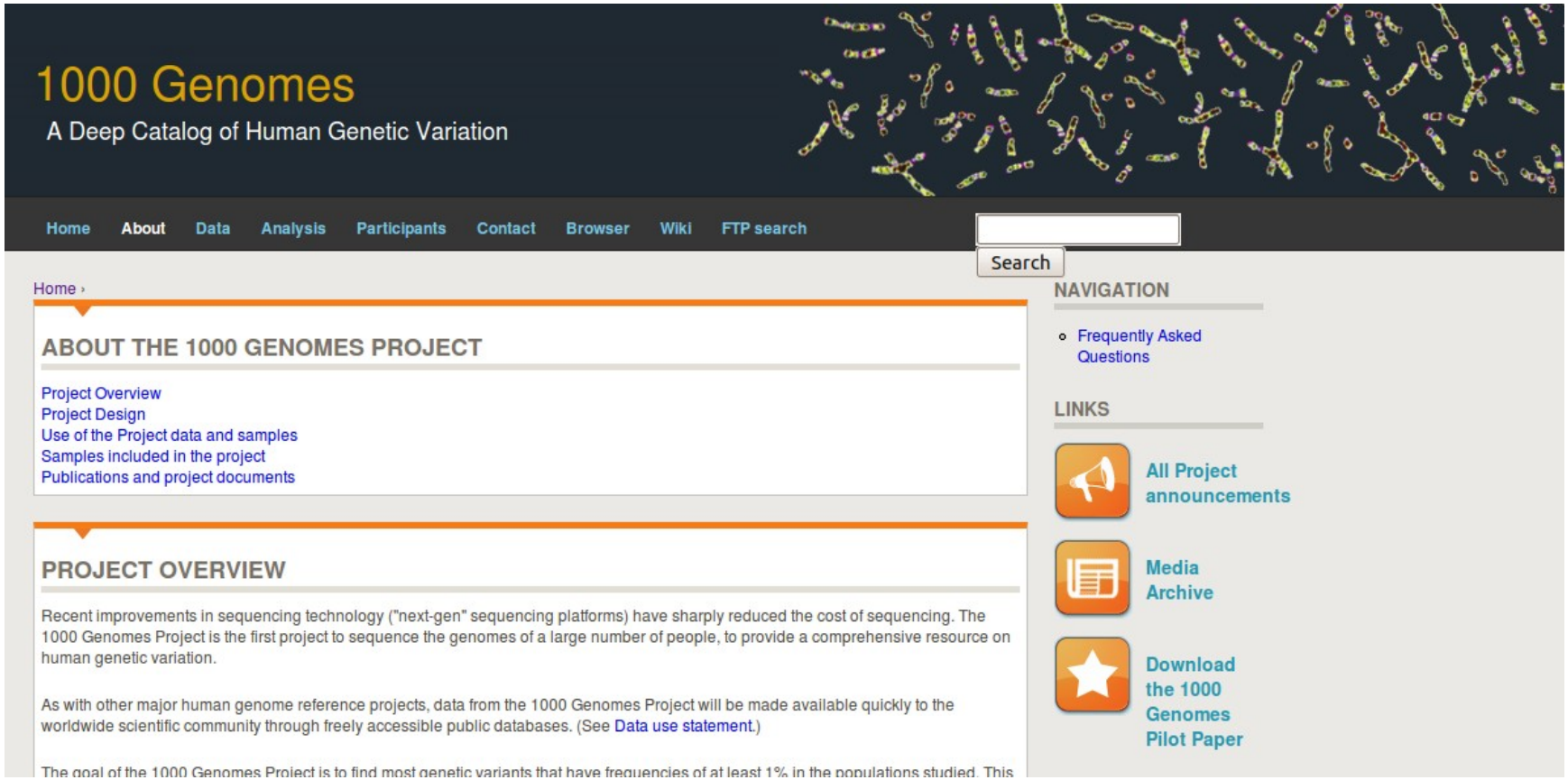
1 → no benign variants

http://genetics.bwh.harvard.edu/pph2/

dbSNP


NCBI
dbSNP
Short Genetic Variations
PubMed Nucleotide Protein Genome Structure PopSet Taxonomy OMIM Books SNP
Search for SNP on NCBI Reference Assembly
Search Entrez SNP for Go
Have a question about dbSNP? Try searching the SNP FAQ Archive!
Go
GENERAL
HUMAN VARIATION
Search, Annotate, Submit
Annotate and Submit Batch Data with Clinical Impact Attributes for Filtering Variation
NEW
SNP SUBMISSION
ANNOUNCEMENT
06/26/2012: NCBI dbSNP Build 137 for Human
RELEASE: NCBI dbSNP Build 137 for Human
dbSNP Build 137 for Human (txid 9606)
Search by IDs on All Assemblies
Note: rs# and ss# must be prefixed with "rs" or "ss", respectively (i.e. rs25, ss25)
ID:
Reference cluster ID(rs#)
Search
Reset
Submission Information

1000 genomes project



NHLBI Exome Sequencing Project (ESP)

The goal of the **NHLBI GO Exome Sequencing Project (ESP)** is to discover novel genes and mechanisms contributing to heart, lung and blood disorders by pioneering the application of next-generation sequencing of the protein coding regions of the human genome across diverse, richly-phenotyped populations and to share these datasets and findings with the scientific community to extend and enrich the diagnosis, management and treatment of heart, lung and blood disorders.

The groups participating and collaborating in the NHLBI GO ESP include:

- Seattle GO - University of Washington, Seattle, WA
- Broad GO - Broad Institute of MIT and Harvard, Cambridge, MA
- WHISP GO - Ohio State University Medical Center, Columbus, OH
- Lung GO - University of Washington, Seattle, WA
- WashU GO - Washington University, St. Louis, MO
- Heart GO - University of Virginia Health System, Charlottesville, VA
- ChargeS GO - University of Texas Health Sciences Center at Houston

The group includes some of the largest well-phenotyped populations in the United States, representing more than 200,000 individuals altogether from the:

- Women's Health Initiative (WHI)
- Framingham Heart Study (FHS)
- Jackson Heart Study (JHS)
- Multi-Ethnic Study of Atherosclerosis (MESA)
- Atherosclerosis Risk in Communities (ARIC)

TO DO

- Decrease number of false positives

Statistical analisys to detect errors

Statistical analysis to improve filters

- Improve indel calling

- Improve annotation

- (...)

Questions?