Variant Calling and Filtering for SNPs

Sequence Analysis Workshop
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Mary Kate Wing
University of Michigan
Center for Statistical Genetics

Goals of This Session

- Learn basics of Variant Call Format (VCF)
- Aligned sequences -> filtered snp calls
- Examine variants at particular genomic positions
- Evaluate quality of SNP calls

Session Design

- Same as yesterday

Variant Call Format (VCF)

- Describes variant positions
  - http://www.1000genomes.org/wiki/Analysis/Variant%20Call%20Format/vcf-variant-call-format-version-41
- Header
  - Each line starts with #
- Records
  - One for each variant position
  - Describes variant
  - Optional per sample genotype information

Variant Call Format: Header

```
##fileformat=VCFv4.1
##filedate=20140615
##source=glfMultiples
##minDepth=1
##maxDepth=10000000
##minMapQuality=0
##minPosterior=0.5000
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth at Site">
##INFO=<ID=MQ,Number=1,Type=Integer,Description="Root Mean Squared Mapping Quality">
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Coverage">
##INFO=<ID=AN,Number=1,Type=Integer,Description="Number of Alleles in Samples with Coverage">
##INFO=<ID=AC,Number=.,Type=Integer,Description="Alternate Allele Counts in Samples with Coverage">
##INFO=<ID=AF,Number=.,Type=Float,Description="Alternate Allele Frequencies">
##INFO=<ID=MQ30,Number=1,Type=Float,Description="Fraction of bases with mapQ<=30">
##FILTER=<ID=mq0,Description="Mapping Quality Below 0">
##FILTER=<ID=dp1,Description="Total Read Depth Below 1">
##FILTER=<ID=DP10000000,Description="Total Read Depth Above 10000000">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Most Likely Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Call Quality">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=PL,Number=.,Type=Integer,Description="Genotype Likelihoods for Genotypes in Phred Scale, fo
#CHROM  POS     ID      REF     ALT     QUAL    FILTER  INFO    FORMAT  HG00551 HG00553 HG00554 HG00637
```

Description of INFO, FILTER, & FORMAT fields

Description of the records fields

Order of per samples genotypes

Variant Call Format: Records

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	HG00551	HG00553
22	35999938		.	1) A	G	100	PASS	DP=127;MQ=59;NS=53;AN=10		
22	36000547		.	2) A	G	100	PASS	DP=485;MQ=59;NS=62;AN=12		
22	36000711		.	3) G	T	24	PASS	DP=376;MQ=59;NS=61;AN=12		
22	36707786		.	4) A	G,C	100	PASS	DP=373;MQ=59;NS=59;AN=11		
				A	B					

SNPs A : Reference B: Alternate

1) Alternate G 2) Alternate G

3) Alternate T 4) 2 Alternates bases: G & C

				A	B			
22	16123409	.	1)	G	GA	21	PASS	AC=1;AF=0.0
22	16136754	.	2)	TG	T	26	PASS	AC=2;AF=0.0
22	16139950	.	3)	G	GA	19	PASS	AC=88;AF=0.
22	16140022	.	4)	AAAGG	A	100	PASS	AC=40;AF=0.

INDELs A : Reference B: Alternate

1) Insertion of A 2) Deletion of G

3) Insertion of A 4) Deletion of AAGG

Variant Call Format: Records

This sample is Homozygous Alt for this variant

This sample is Heterozygous for this variant

```
GT:DP:GQ:PL    1/1:5:15:106,15,0      0/1:0:3:0,0,0    1/1:5:15:67,15,0
GT:DP:GQ:PL    1/1:7:21:101,21,0      1/1:1:4:4,3,0    1/1:5:15:43,15,0
GT:DP:GQ:PL    1/1:12:37:158,36,0     1/1:2:8:38,6,0   1/1:6:19:60,18,0
```

```
GT:DP:GQ:PL    1/1:7:15:73,21,0,73,21,73      2/2:1:5:23,23,23,3,3
```

This sample is Homozygous Alt1 for this variant

This sample is Homozygous Alt2 for this variant

Variant Call Format (VCF)

- It's a large file, how do I look at certain variants?
  - tabix
    - http://samtools.sourceforge.net/tabix.shtml
    - Generate tabix index (.tbi) file:
      - tabix -p vcf file.vcf.gz
    - View region:
      - tabix file.vcf.gz CHR:START-END

Why GotCloud snpcall?

Many of the same reasons as GotCloud align

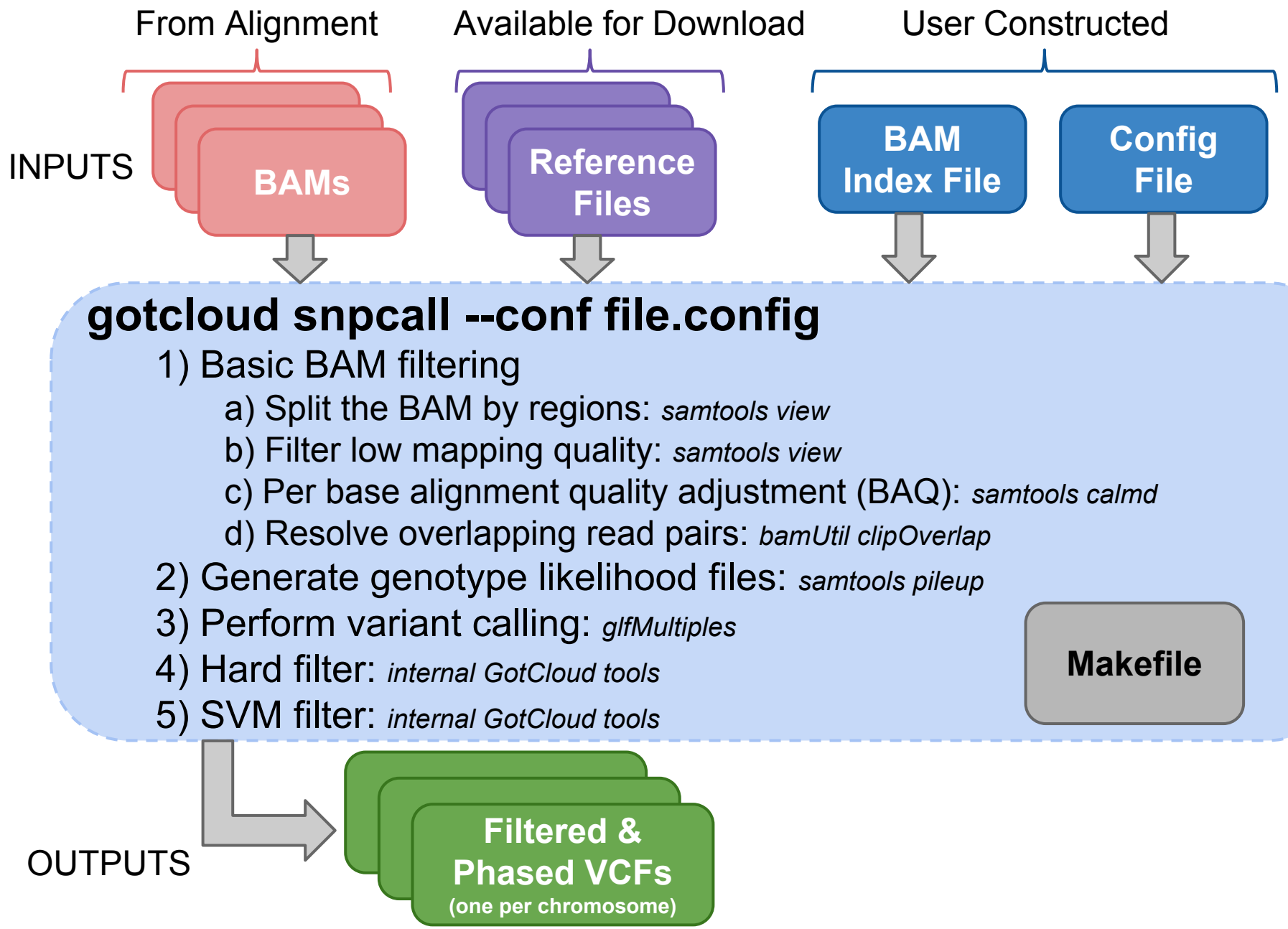
- All-in-one package for snp calling pipeline
  - You don't have to know the details of individual steps
  - Automates steps for you
- Robust parallelization
  - Automatically partitions chromosomes by regions
  - Takes advantage of clusters
    - Supports MOSIX, slurm, SGE, pbs (flux)
    - Can setup a cluster on Amazon
  - via GNU make
    - Reliable and fault-tolerant
    - Restart where it stopped upon unexpected crash

Why GotCloud snpcall?

- Easy to add new samples to your study
  - Just add them to your index
- Analyzes many samples together

- Each chromosome processed independently

GotCloud SnpCall Pipeline Overview



User Constructed Input: BAM Index File

- Points GotCloud to the BAMs
  - Alignment pipeline generates for you
  - For our tutorial: update it to include more BAMs
- Tab delimited

1) Sample name
one row per sample

HG00641	ALL
HG00640	ALL
HG00551	ALL
HG00553	ALL

3 .. N) BAM - typically only 1 BAM for sample,
but if more than one, separate with tabs

```
/home/mktrost/out/bams/HG00641.recal.bam
/home/mktrost/out/bams/HG00640.recal.bam
/home/mktrost/out/bams/HG00551.recal.bam
/home/mktrost/out/bams/HG00553.recal.bam
```

2) Population : alignment pipeline puts “ALL”
That is fine - it is not really necessary

User Constructed Input: GotCloud Configuration

- Same as Alignment Pipeline
- Overrides default settings
  - References files
  - Mapper to use
  - Path to Index file
  - Path to BAM Index File
  - Output directory
- Key = Value entries

User Constructed Input: GotCloud Configuration

```
IN_DIR = $(GOTCLOUD_ROOT)/../inputs

INDEX_FILE = $(IN_DIR)/align.index
FASTQ_PREFIX = $(IN_DIR)/fastq
BAM_PREFIX = $(IN_DIR)/

OUT_DIR = out
BAM_INDEX =  $(OUT_DIR)/bam.index

############
# References
REF_DIR = $(GOTCLOUD_ROOT)/../reference/chr22
AS = NCBI37  # Genome assembly identifier
REF = $(REF_DIR)/human.g1k.v37.chr22.fa
DBSNP_VCF =  $(REF_DIR)/dbsnp_135.b37.chr22.vcf.gz
HM3_VCF = $(REF_DIR)/hapmap_3.3.b37.sites.chr22.vcf.gz
INDEL_PREFIX = $(REF_DIR)/1kg.pilot_release.merged.indels.sites.hg19
OMNI_VCF = $(REF_DIR)/1000G_omni2.5.b37.sites.PASS.chr22.vcf.gz

MAP_TYPE = BWA_MEM

###############
CHRS = 22
```

Path to input files

For snpcall & indel -> path to rest of BAMs

Output Information

Use $(KEY) to refer to other KEYs
GOTCLOUD_ROOT is built in

#'s are comments

Path to chr22 reference files

For snpcall & indel -> chr22 only

Reference Files

- GotCloud snpcall uses:
  - Reference genome FASTA file
    - To identify differences (SNPs) between bases in sequence reads & the reference positions they mapped
- VCF files
  - indel - contains known insertions & deletions to help with filtering
  - omni - used as likely true positives for SVM filtering
  - hapmap - used as likely true positives for SVM filtering and for generating summary statistics
  - dbsnp - used for generating summary statistics

How good are the results?

${OUT}/vcfs/chr*/chr*.filtered.sites.vcf.summary

FILTER	#SNPs	#dbSNP	%dbSNP	%CpG Known	%CpG Novel	%Known Ts/Tv	%Novel Ts/Tv	%nCpG-K Ts/Tv	%nCpG-N Ts/Tv	%HM3 sens	%HM3 /SNP
INDEL5	56	50	89.3	10.0	0.0	1.78	1.00	1.50	1.00	0.005	1.786
INDEL5;SVM	9	9	100.0	0.0	NA	0.80	NA	0.80	NA	0.000	0.000
PASS	3870	3741	96.7	21.9	17.1	2.36	2.23	1.94	1.82	2.325	12.403
SVM	129	112	86.8	16.1	17.6	3.31	1.83	2.92	1.80	0.000	0.000

FILTER	#SNPs	#dbSNP	%dbSNP	%CpG Known	%CpG Novel	%Known Ts/Tv	%Novel Ts/Tv	%nCpG-K Ts/Tv	%nCpG-N Ts/Tv	%HM3 sens	%HM3 /SNP
INDEL5	65	59	90.8	8.5	0.0	1.57	1.00	1.35	1.00	0.005	1.538
PASS	3870	3741	96.7	21.9	17.1	2.36	2.23	1.94	1.82	2.325	12.403
SVM	138	121	87.7	14.9	17.6	2.90	1.83	2.55	1.80	0.000	0.000

PASS	3870	3741	96.7	21.9	17.1	2.36	2.23	1.94	1.82	2.325	12.403
FAIL	194	171	88.1	13.5	13.0	2.49	1.56	2.15	1.50	0.005	0.515
TOTAL	4064	3912	96.3	21.5	16.4	2.37	2.10	1.95	1.76	2.330	11.836

MultiAllele Ref/Alt	1
Repeated Positions	0
TOTAL SKIPPED	1

Genotype Refinement

- After snpcall, we run genotype refinement
  - improves the genotypes - higher quality
  - Beagle & thunder
- Outputs are VCFs
  - thunder breaks up by population

What will I need to configure in GotCloud for my own research?

- Exome/Targeted set in your configuration:

```
# Write loci file when performing pileup
WRITE_TARGET_LOCI = TRUE

# Directory to store target information
TARGET_DIR = target

# When all individuals has the same target
UNIFORM_TARGET_BED = path/to/file.bed

# When each individual has different targets
# Each line of file.txt contains [SM_ID] [TARGET_BED]
MULTIPLE_TARGET_MAP = path/to/file.txt

# Extend target by given # of bases
# Set this to what you want or to 0
OFFSET_OFF_TARGET = 50

# If a single chromosome is too small for SVM,
# set this to run SVM on all chromosomes combined
# Only for very small targetted projects
# Exome does not require this
#WGS_SVM = TRUE
```

What will I need to configure in GotCloud for my own research?

- Cluster support
  - Via configuration
    - BATCH_TYPE =
      - mosix, pbs, slurm, pbs, sge, slurmi, sgei
    - BATCH_OPTS =
      - Set to any options you would normally pass to your cluster
  - Via command line
    - --batchtype & --batchopts

Try it yourself

http://genome.sph.umich.edu/wiki/SeqShop:_Variant_Calling_and_Filtering_for_SNPs_Practical

- Interested in GotCloud?
  - http://genome.sph.umich.edu/wiki/GotCloud
  - Download:

    wget https://github.com/statgen/gotcloud/archive/master.tar.gz
  - Extract & Build:

    tar xvf master.tar.gz; cd gotcloud-master/src; make; cd ../..
  - Run:

    gotcloud-master/gotcloud
  - Join the mailing list: