MCCRD-SOP0011: Whole Exome Sequencing Data Analysis Pipeline and Specifications

Effective Date: 11/1/2017

Please check for revision status of the SOP at

https://pdmr.cancer.gov/sops/

PDMR NCI Patient-Derived Models Repository
An NCI Precision Oncology Initiative℠ Resource

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VERSION INFORMATION

1. Change History

Revision	Description
	Internal SOP used by MOCHA Laboratory
11/1/2017	Standardize SOP for posting to PDMR internal site for use by designated NCI intramural laboratories

1.0 PURPOSE/SCOPE

This Standing Operating Procedure (SOP) describes the pipeline and data analysis specifications for HiSeq PDX Exome Pipeline for Patient-Derived Models used/performed by the Molecular Characterization and Clinical Assay Development Laboratory (MoCha) at the Frederick National Laboratory for Cancer Research. **This SOP is for research purposes only and no clinical samples will be processed using this SOP. Any deviation from this SOP will be noted but will not be formally documented**.

2.0 REFERENCES

2.1 Li H., Handsaker B., Wysoker A., Fennell T., Ruan J., Homer N., Marth G., Abecasis G., Durbin R. and 1000 Genome Project Data Processing Subgroup (2009) The Sequence alignment/map (SAM) format and SAMtools. Bioinformatics, 25, 2078-9. PMID: 19505943

2.2 The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA, 2010 Genome Research 20:1297-303

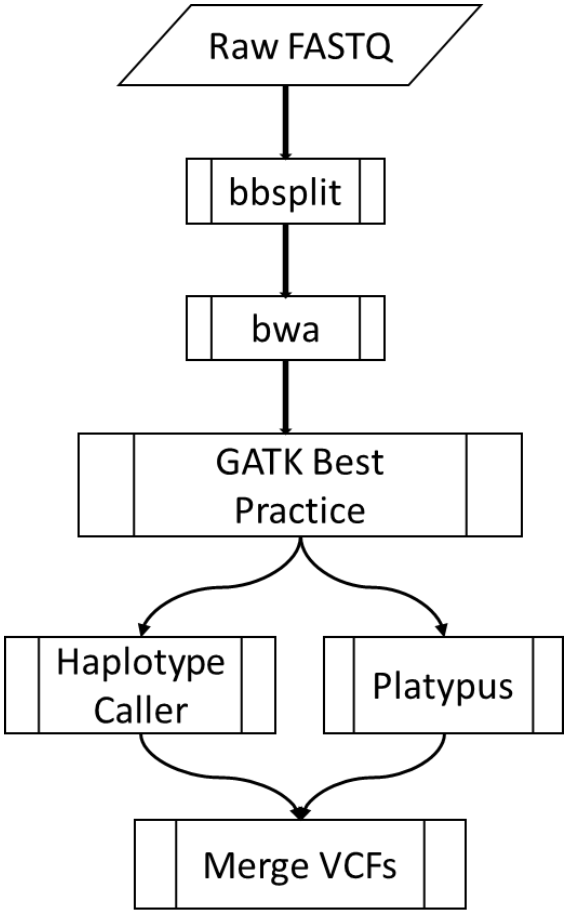
2.3 Justin M Zook, Brad Chapman, Jason Wang, David Mittelman, Oliver Hofmann, Winston Hide & Marc Salit: Integrating human sequence data sets provides a resource of benchmark SNP and indel genotype calls, Nature Biotechnology 32, 246–251 (2014) doi:10.1038/nbt.2835

2.4 Link to Sentieon tools: https://www.sentieon.com/

2.5 Link to GATK resource bundle. All files in brackets {} in the SOP are downloaded from https://software.broadinstitute.org/gatk/download/bundle

2.6 Link to Exome/Capture/RNASeq Pipeline on GitHub: https://github.com/FNL-MoCha/nextgenseq_pipeline

3.0 STANDARD VARIANT CALLING PIPELINE FOR PATIENT-DERIVED MODELS (PDM) EXOME



4.0 PROCEDURE

4.1 FASTQ files are generated with bcl2fastq (version: 2.17.1.14, Illumina). Adaptors are trimmed within this process using the default cutoff of the adapter-stringency option.

4.2 PDX Mouse reads are removed from the raw FASTQ files using bbsplit (bbtools v37.36).

4.2.1 bbtools bbsplit build=1 -Xmx10g path=<indexPath> in1=Sample.R1.fastq.gz in2=Sample.R2.fastq.gz basename=Sample_%_#.fastq.gz refstats=Sample_stat.txt

4.3 FASTQ files from step 3.2 are mapping against human genome reference hg19 using the BWA (v 0.7.10), resulting sam file is converted to sorted bam file using sentieon(sentieon-genomics/201711.01)

4.3.1 bwa mem -M -t <threads> -R '@RG\\tID:Sample\\tSM:Sample\\tLB:Sample\\tPL:Illumina' ucsc.hg19.fasta Sample.R1.fastq.gz Sample.R2.fastq.gz | sentieon util sort -o Sample.bwa.bam -t <threads> --sam2bam -i -

4.4 Custom Script is used to assess mapping quality, mean coverage and other QC statistics on the sample.

4.4.1 Available upon request

4.5 GATK Best Practice

4.5.1.1 Mark Duplicates.

- sentieon driver -t <threads> -i Sample.bwa.bam --algo LocusCollector --fun score_info Sample.markdup.txt.tmp
- sentieon driver -t <threads> -i Sample.bwa.bam --algo Dedup --score_info Sample.markdup.txt.tmp --metrics Sample.markdup.txt Sample.dd.bam

4.5.1.2 Local Realignment.

- sentieon driver -t <threads> -r ucsc.hg19.fasta -i Sample.dd.bam --algo Realigner -k {input.phase1} -k {input.mills} Sample.lr.bam

4.5.1.3 Base Quality Score Recalibration.

- sentieon driver -t <threads> -r ucsc.hg19.fasta -i Sample.lr.bam --algo QualCal -k {input.phase1} -k {input.mills} Sample.recalibration.matrix.txt
- sentieon driver -t <threads> -r ucsc.hg19.fasta -i Sample.lr.bam -q Sample.recalibration.matrix.txt --algo QualCal -k {input.phase1} -k {input.mills} Sample.recalibrationPost.matrix.txt --algo ReadWriter Sample.bwa.final.bam

4.6 Variant Calling

4.6.1.1 HaplotypeCaller

- sentieon driver -t <threads> -r ucsc.hg19.fasta -i Sample.bwa.final.bam --interval Sureselect.v5.padded.bed --algo Haplotyper -d {input.dbsnp} --min_base_qual 20 --min_map_qual 30 Sample.HC_DNASeq.raw.vcf
- merge 2 calls made on consecutive bases to one
    - Custom script available upon request

4.6.1.2 Platypus

- platypus callVariants –nCPU=<threads> --bufferSize=1000000 --maxReads=100000000 --bamFiles=Sample.bwa.final.bam --regions= Sureselect.v5.padded.bed --output=Sample.Platypus.raw.vcf --refFile=ucsc.hg19.fasta
- Select PASS Variants
- Remove 3 base-substitutions.

4.7 Merging VCFs from HaplotypeCaller and Platypus

4.7.1 Recode Tags in the vcf files

4.7.1.1 Custom perl script available upon request

- GATK Version: 3.4-0
- java -Xmx5g -XX:ParallelGCThreads=<threads> -jar GATK.jar -T CombineVariants -R ucsc.hg19.fasta -dcov 10000 --variant:HC Sample.HC_DNASeq.raw.vcf --variant:Platypus Sample.Platypus.raw.vcf -o Sample.vcf -genotypeMergeOptions PRIORITIZE -priority HC,Platypus -nt <threads>