MB-GATK-SGE pipeline

For GATK best practices: classic UG / v3.x HC / MuTect
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Classic Unified Genotyper workflow

BAM files merged using Picard threading used to off-load (de)compression/IO, shell script takes path/*.bam as input from command line

End of sample level workflow

Merge BAM files
Merge_BAM
MergeBAM.sh

Reads are realigned around indels, two stages:
i) Realignment Target Creation,
ii) Indel Realignment

2nd realignment of all reads
Realn_merged
Merged_RTC.sh
Merged_IDR.sh

Q scores for each base are recalibrated using machine learning. Two stages i) build model ii) apply it and "print" a new set of reads

Base Q Score Recal
BQSR_merged
Merged_BaseRecal.sh
Merged_PrintReads.sh

Variants called on all samples simultaneously, using Unified Genotyper, calls SNPs and indels separately owing to size of unified dataset

Unified Genotyper
UG_merged
UG_snps.sh
UG_indels.sh

Gaussian mixture model trained using 1000G, HapMap, dbSNP and Omni array data. Recalibrated variants are filtered at a desired truth level, SNPs and indels should not undergo VQSR together

Varient Q Score Recalibration
VQSR_UG
VQSR_snps_UG.sh
VQSE_indels_UG.sh
ApplyRecalibration_snps_UG.sh
ApplyRecalibration_indels_UG.sh

Common per-sample processing

Per sample level workflow

START
Raw Reads
FASTQ

Input is Raw .fastq.gz ideally reads should not be trimmed

Align Reads
BWA_MEM
BWA.sh
Submit_BWA.pl

BWA gives SAM output, -R used to encode read groups in SAM header

SAM to sorted index BAM
SamToSortedBam
SamToSortedBam.sh

SAM converted to sorted index BAM in one step using Picard: SortSam

Find PCR duplicates
MarkDuplicates
MarkDuplicates.sh

Duplicates marked not removed, using Picard: MarkDuplicates, this is more effective at finding duplicates than SAMtools

Sample level realignment
1stRealn
1st_RTC.sh
1st_IDR.sh

Reads are realigned around indels, two stages:
i) Realignment Target Creation,
ii) Indel Realignment

New Haplotype Caller workflow

Base Q Score Recal
BQSR_sample_lvl
BaseRecal.sh
PrintReads.sh

Q scores for each base are recalibrated using machine learning. Two stages i) build model ii) apply it and "print" a new set of reads

Haplotype Caller per sample
HC_sample_lvl
HC.sh

Variants called in new genomic VCF mode at sample level, this is quick, GATK 3.x uses AVX accelerated PairHMM on new CPUs

End of sample level workflow

Genotype and fuse gVFCs to a single VCF
GenotypeGVCFs
GenotypeGVCFs.sh

Individual gVCF files are fused here to make a single VCF, downstream analysis is as before

Variant Q Score Recalibration
VQSR_HC
VQSR_snps_HC.sh
VQSE_indels_HC.sh
ApplyRecalibration_snps_HC.sh
ApplyRecalibration_indels_HC.sh

Gaussian mixture model trained using 1000G, HapMap, dbSNP and Omni array data. Recalibrated variants are filtered at a desired truth level, SNPs and indels should not undergo VQSR together

MuTect 1.x somatic variant calling

Call tumor / normal pairs using MuTect
MuTect
MT.sh
Submit_MT.pl

MuTect subtracts the normal (germline) variants from the tumor (somatic) variants. It also reports if SNPs are novel i.e. not in COSMIC or dbSNP

Perl script submits MuTect jobs from a list of paired normal/tumour BAM files

MuTect handels heterogeneous and impure tumour samples

Recalibrated variant filtering

SelectRecaledVariants_snps.sh
SelectRecaledVariants_indels.sh

Select variants: passing recalibration, VQSlod >= 0, VQSlod >= 3
Filt_Recaled_VCF

Recalibrated variants can be filtered for those passing recalibration via the PASS flag. This can be further filtered via the VQSlod log odds ratio which is the likelihood of being a true variant versus being false under the trained Gaussian mixture model. VQSlod >= 0 tends to be a better subset with >= 3 being even better in terms of variant quality

Hard Filter variants if VQSR fails
Hard_filt
HardFilt_both_HC.sh
HardFilt_indels_UG.sh
HardFilt_snps_UG.sh

Should recalibration fail (owing to lack of bad variants) then hard (i.e. preset) filters can be applied to both the SNPs and indels