MB-GATK-SGE pipeline

For GATK best practices: classic UG / v3.5 HC / MuTect 1 & 2
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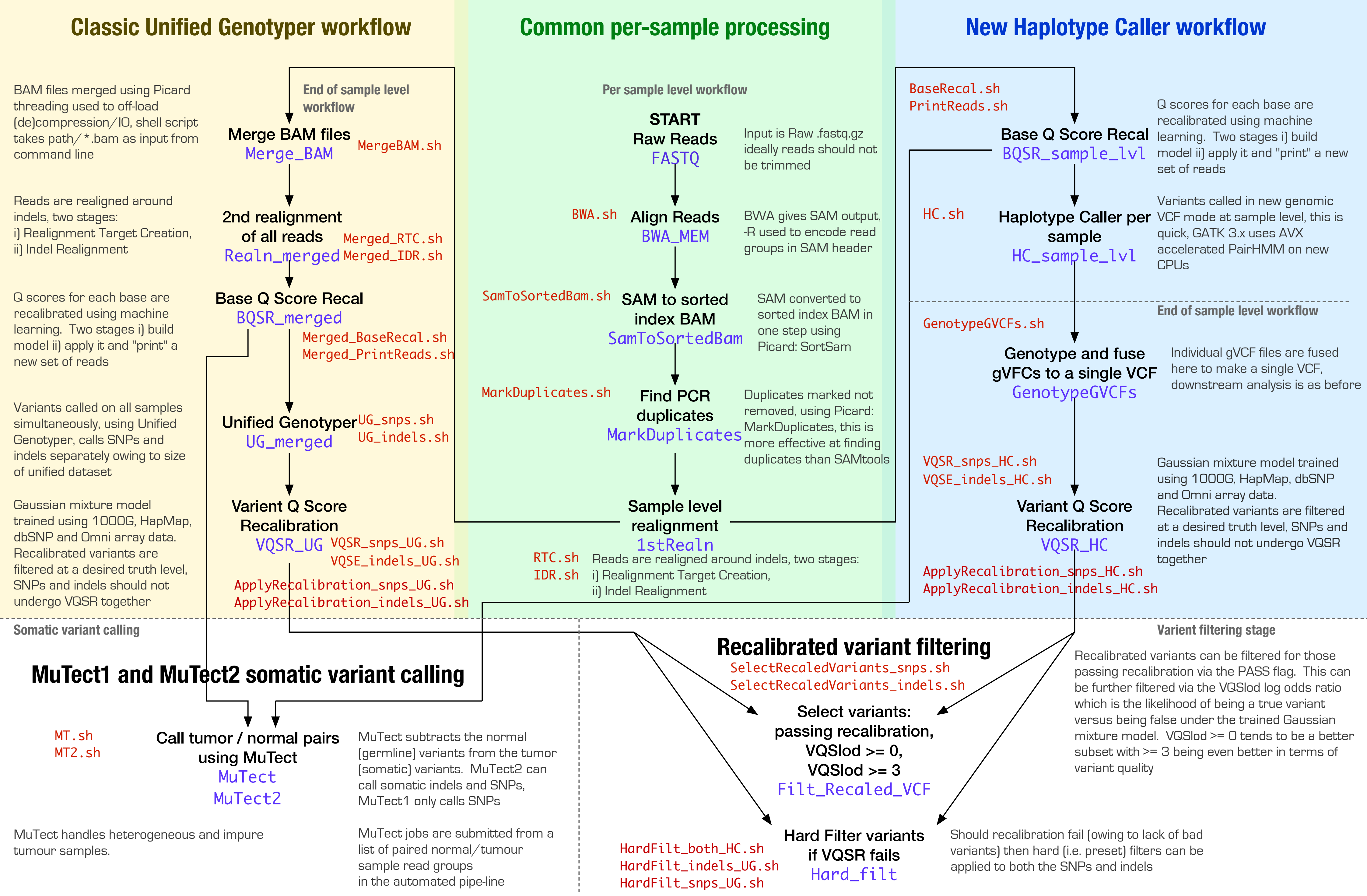

Classic Unified Genotyper workflow

End of sample level workflow

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

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

BWA.sh
Align Reads
BWA_MEM

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

SamToSortedBam.sh
SAM to sorted index BAM
SamToSortedBam

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

MarkDuplicates.sh
Find PCR duplicates
MarkDuplicates

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

Sample level realignment
1stRealn
RTC.sh
IDR.sh

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

New Haplotype Caller workflow

BaseRecal.sh
PrintReads.sh

Base Q Score Recal
BQSR_sample_lvl

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

HC.sh
Haplotype Caller per sample
HC_sample_lvl

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

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

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

VQSR_snps_HC.sh
VQSE_indels_HC.sh

Variant Q Score Recalibration
VQSR_HC
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

Somatic variant calling

MuTect1 and MuTect2 somatic variant calling

MT.sh
MT2.sh

Call tumor / normal pairs using MuTect
MuTect
MuTect2

MuTect subtracts the normal (germline) variants from the tumor (somatic) variants. MuTect2 can call somatic indels and SNPs, MuTect1 only calls SNPs

MuTect handles heterogeneous and impure tumour samples.

MuTect jobs are submitted from a list of paired normal/tumour sample read groups in the automated pipe-line

Varient filtering stage

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

HardFilt_both_HC.sh
HardFilt_indels_UG.sh
HardFilt_snps_UG.sh

Hard Filter variants if VQSR fails
Hard_filt

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