BaseRecal .sh

PrintReads.sh

VOSR\_snps\_HC.sh

HC.sh

## **MB-GATK-SGE** pipeline

### **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

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

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

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

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

End of sample level workflow Merge BAM files Merae BAM 2nd realignment of all reads Merged RTC.sh Realn merged\_IDR.sh Base Q Score Recal BQSR\_merged Merged\_BaseRecal.sh Meraed PrintReads.sh Unified Genotyper UG\_snps.sh UG\_indels.sh UG\_merged Varient Q Score Recalibration VOSR\_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 Input is Raw .fastq.gz **Raw Reads** ideally reads should not FAST0 be trimmed BWA.sh Alian Reads BWA gives SAM output. Submit\_BWA.pl -R used to encode read **BWA MFM** groups in SAM header SamToSortedBam.sh SAM to sorted SAM converted to sorted index BAM in index BAM one step using SamToSortedBam Picard: SortSam MarkDuplicates.sh Find PCR Duplicates marked not removed, using Picard: duplicates MarkDuplicates, this is MarkDuplicates more effective at finding duplicates than SAMtools Sample level realignment 1stRealn 1st RTC.sh Reads are realigned around indels, two stages: 1st\_IDR.sh i) Realignment Target Creation, ii) Indel Realignment

# **New Haplotype Caller workflow**

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

Variants called in new genomic Haplotype Caller per VCF mode at sample level, this is auick. GATK 3.x uses AVX sample accelerated PairHMM on new HC sample lvl **CPUs** 

End of sample level workflow GenotypeGVCFs.sh

Individual gVCF files are fused Genotype and fuse here to make a single VCF, aVFCs to a single VCF downstream analysis is as before GenotypeGVCFs

VOSE\_indels\_HC.sh Variant Q Score Recalibration

VOSR\_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

### MuTect 1.x somatic variant calling

MT.sh Submit\_MT.pl Call tumor / normal pairs using MuTect

MuTect

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

MuTect subtracts the normal

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

### Recalibrated variant filtering

SelectRecaledVariants\_snps.sh SelectRecaledVariants\_indels.sh

> Select variants: passing recalibration, VQSlod >= 0.VQSlod >= 3Filt\_Recaled\_VCF

Hard Filter variants HardFilt\_both\_HC.sh if VQSR fails HardFilt\_indels\_UG.sh Hard\_filt

HardFilt\_snps\_UG.sh

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

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

MuTect handels heterogeneous and impure tumour samples