ADBS Whole Genome Sequencing (WGS) analysis pipeline for Genomic-QC Report

Ravi More[1]

[1]ADBS, National Centre for Biological Sciences (NCBS), Bangalore 560065, Karnataka, India

dx.doi.org/10.17504/protocols.io.vuae6se

Ravi More

ABSTRACT

Whole Genome Sequencing (WGS) analysis pipeline devloped for generating Genomic-QC Report in Accelerator Program for Discovery in Brain Disorders Using Stem Cells (ADBS) program.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

Define paths and directories

1

COMMAND

```
SAMPLE_PATH="/path/to/sample"
SAMPLE_NAME="test_sample"
SOFTWARE_PATH="/path/to/software"
DATABASES_PATH="/path/to/databases"
TEMP_DIR="/path/to/temp"
```

Linux

Unzip the raw reads files from .gz to fastq format

2

COMMAND

```
gunzip $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME*.fastq.gz
```

Linux

QC check of R1 and R2 paired-end raw reads using FASTQC, Trimming poor quality reads using Prinseq-lite, and Adapter contimination removal using AfterQC

3 Software versions used:

FASTQC version 0.10.1
Prinseq-lite version 0.20.4
AfterQC version 0.9.6

COMMAND

```
$SOFTWARE_PATH/FastQC/fastqc $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_R1.fq

$SOFTWARE_PATH/FastQC/fastqc $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_R2.fq

cd $SAMPLE_PATH/$SAMPLE_NAME/

python $SOFTWARE_PATH/AfterQC-master/after.py -f -1 -t -1 -q 30 -1 $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_R1.fq -2 $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_R2.fq

$SOFTWARE_PATH/prinseq-lite-0.20.4/prinseq-lite.pl -fastq $SAMPLE_PATH/$SAMPLE_NAME/good/$SAMPLE_NAME\_R1.good.fq -fastq2 $SAMPLE_PATH/$SAMPLE_NAME/good/$SAMPLE_NAME\_R2.good.fq -out_good $SAMPLE_PA1

mv $SAMPLE_PATH/$SAMPLE_NAME/cleaned_1.fastq $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_cleaned_R1.fastq

mv $SAMPLE_PATH/$SAMPLE_NAME/cleaned_2.fastq $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_cleaned_R2.fastq

$SOFTWARE_PATH/FastQC/fastqc $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_cleaned_R1.fastq

$SOFTWARE_PATH/FastQC/fastqc $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_cleaned_R2.fastq

mkdir -p $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_4_FASTQC

mv $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_cleaned_R1_fastqc.zip $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_4_FASTQC/$SAMPLE_NAME\_cleaned_R1_fastqc.zip

mv $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_cleaned_R2_fastqc.zip $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_4_FASTQC/$SAMPLE_NAME\_cleaned_R2_fastqc.zip
```

Linux

Alignment of clened raw reads against Human Reference Genome hg19 GRCh37.p13 build using BWA and SAMTOOLS.

4 BWA version 0.5.9
Samtools version 1.3.1

COMMAND

```
# Align cleaned R1 reads with hg19
/softwares/bwa-0.5.9/bwa aln -t 30 $DATABASES_PATH/hg19_fa-chrMlast/hg19_chrM-last.fa $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_cleaned_R1.fastq > $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_R1.sai

# Align cleaned R2 reads with hg19
/softwares/bwa-0.5.9/bwa aln -t 30 $DATABASES_PATH/hg19_fa-chrMlast/hg19_chrM-last.fa $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_cleaned_R2.fastq > $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_R2.sai

#convert sai to sam by using cleaned fastq reads
/softwares/bwa-0.5.9/bwa sampe $DATABASES_PATH/hg19_fa-chrMlast/hg19_chrM-last.fa $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_R1.sai $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_R2.sai $SAMPLE_PATH/$

#convert sam to bam
/softwares/samtools1.3.1/bin/samtools view -bS $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME.sam > $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME.bam

#bam to sort file
/softwares/samtools1.3.1/bin/samtools sort $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME.bam -o $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_sorted.bam

#sort to flagstat
/softwares/samtools1.3.1/bin/samtools flagstat $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_sorted.bam > $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_sorted_flagstat.txt

#index the sorted bam
/softwares/samtools1.3.1/bin/samtools index $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_sorted.bam > $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_sortedbam.bai
```

Linux

Mark PCR duplicates and sorting BAM using PICARD Tools

5 Picard version 2.0.1
Samtools version 1.3.1

COMMAND

```
#Remove PCR duplicates
java -Djava.io.tmpdir=$TEMP_DIR/ -Xmx50g -jar $SOFTWARE_PATH/picard/build/libs/picard.jar AddOrReplaceReadGroups I="$SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_sorted.bam" O="$SAMPLE_PATH/$SAMPLE_NAME/$SA

java -Djava.io.tmpdir=$TEMP_DIR/ -Xmx50g -jar $SOFTWARE_PATH/picard/build/libs/picard.jar MarkDuplicates I="$SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_coordsort.bam" O="$SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_N

#Index the coordinate sorted bam file
/softwares/samtools1.3.1/bin/samtools index $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam
```

Linux

INDEL re-alignment using GATK tools

6 GATK version 3.6

COMMAND

```
java -Xmx8g -jar $SOFTWARE_PATH/GenomeAnalysisTK-3.6/GenomeAnalysisTK.jar -T RealignerTargetCreator -R $DATABASES_PATH/hg19_fa-chrMlast/hg19_chrM-last.fa -I $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUI
```

Linux

SNP and INDEL variant calling using Isaac Variant Caller tool and filter SNP and INDEL using rtg-tools

7 Isaac Variant Caller -- 1.0.7
rtg-tools version 3.7.1

COMMAND

```
$SOFTWARE_PATH/isaac_variant_caller-master/bin/configureWorkflow.pl --bam=$SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam --ref=$DATABASES_PATH/hg19_fa-chrMlast/hg19_chrM-last.fa --config=$SAMPLE_PA

cd $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/
make -j 16

gzip -dc $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/results/$SAMPLE_NAME\_RMDUP.genome.vcf.gz | $SOFTWARE_PATH/gvcftools-0.16/bin/extract_variants | awk '/^#/ || $7 == "PASS"' > $SAM

$SOFTWARE_PATH/rtg-tools-3.7.1/rtg vcffilter --snps-only -i $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/results/$SAMPLE_NAME\_RMDUP_all_passed_variants.vcf -o $SAMPLE_PATH/$SAMPLE

$SOFTWARE_PATH/rtg-tools-3.7.1/rtg vcffilter --non-snps-only -i $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/results/$SAMPLE_NAME\_RMDUP_all_passed_variants.vcf -o $SAMPLE_PATH/$SAM

cp $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/results/$SAMPLE_NAME\_snp_issac.vcf.gz $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/$SAMPLE_NAM

cp $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/results/$SAMPLE_NAME\_indel_issac.vcf.gz $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/$SAMPLE_NAM

gunzip $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/$SAMPLE_NAME\_snp.vcf.gz

gunzip $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/$SAMPLE_NAME\_indel.vcf.gz
```

Linux

Check the alignment QC of the bam file using Qualimap

8 Qualimap version 2.2.1

COMMAND

```
mkdir -p $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_5_ALIGNMENT_QC

$SOFTWARE_PATH/qualimap_v2.2.1/qualimap bamqc -bam $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam -gff $DATABASES_PATH/trueseq1.bed -outdir $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\
```

Linux

VCF QC of SNP and INDEL files using rtg-tools

9 rtg-tools version 3.7.1

COMMAND

```
mkdir -p $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/VCF_QC

$SOFTWARE_PATH/rtg-tools-3.7.1/rtg vcfstats $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/$SAMPLE_NAME\_snp.vcf > $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIAN

$SOFTWARE_PATH/rtg-tools-3.7.1/rtg vcfstats $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/$SAMPLE_NAME\_indel.vcf > $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIA

Linux
```

SNP AND INDEL variant annotation using ANNOVAR

10 ANNOVAR reference assembly 65 with reference hg19

COMMAND

```
mkdir -p $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/annotated_annovar

perl $SOFTWARE_PATH/annovar/convert2annovar.pl -format vcf4 $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/$SAMPLE_NAME\_snp.vcf > $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_N

perl $SOFTWARE_PATH/annovar/convert2annovar.pl -format vcf4 $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/$SAMPLE_NAME\_indel.vcf > $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_

#perl $SOFTWARE_PATH/annovar/table_annovar.pl $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/$SAMPLE_NAME\_snp.vcf.avinput $SOFTWARE_PATH/annovar/humandb/ -buildver hg19 -out $SA

perl $SOFTWARE_PATH/annovar/table_annovar.pl $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/$SAMPLE_NAME\_snp.vcf $SOFTWARE_PATH/annovar/humandb/ -buildver hg19 -out $SAMPLE_PA

perl $SOFTWARE_PATH/annovar/table_annovar.pl $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/$SAMPLE_NAME\_indel.vcf $SOFTWARE_PATH/annovar/humandb/ -buildver hg19 -out $SAMPLE_P

Linux
```

Mitochondria analysis

11 Extracting mitochondrial reads from BAM file and creatiing anather BAM file to input mtDNA-Server tool for Mitochondria analysis
Samtools version 1.3

COMMAND

```
mkdir -p $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_7_MITOCHONDRIA

/softwares/samtools1.3.1/bin/samtools view -b $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam chrM: -o $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_7_MITOCHONDRIA/$SAMPLE_NAME\_MT.bam

/softwares/samtools1.3.1/bin/samtools index $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_7_MITOCHONDRIA/$SAMPLE_NAME\_MT.bam

Linux
```

Blood Group Prediction

12 BOOGIE - Phenotype prediction from NGS data Version: 1.0

COMMAND

```
mkdir -p $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_10_blood_group

perl $SAMPLE_PATH/rename_phase2_blood_group_detection.pl $SAMPLE_NAME

perl $SAMPLE_PATH/rename_phase2_blood_group_summary.pl $SAMPLE_NAME

perl $SAMPLE_PATH/rename_phase2_blood_group_genes_extracter.pl $SAMPLE_NAME

chmod 755 $SAMPLE_PATH/$SAMPLE_NAME/*

perl $SAMPLE_PATH/$SAMPLE_NAME/phase2_blood_group_genes_extracter.pl

$SAMPLE_PATH/$SAMPLE_NAME/phase2_blood_group_detection.sh

perl $SAMPLE_PATH/$SAMPLE_NAME/phase2_blood_group_summary.pl

Linux
```

SNP-Chip rsID comparison with WGS rsID

13

COMMAND

```
mkdir -p $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_14_VIRTUAL_SNP
perl $SAMPLE_PATH/rename_phase2_1rsid_get.pl $SAMPLE_NAME
perl $SAMPLE_PATH/rename_phase2_2rsid_filter.pl $SAMPLE_NAME
perl $SAMPLE_PATH/rename_phase2_3rsid_venn.pl $SAMPLE_NAME
perl $SAMPLE_PATH/rename_phase2_4rsid_venn.pl $SAMPLE_NAME
perl $SAMPLE_PATH/rename_exonic_extract_common_indel.pl $SAMPLE_NAME
perl $SAMPLE_PATH/rename_exonic_extract_common_snp.pl $SAMPLE_NAME
perl $SAMPLE_PATH/rename_exonic_extract_rsid_indel.pl $SAMPLE_NAME
perl $SAMPLE_PATH/rename_exonic_extract_rsid_snp.pl $SAMPLE_NAME
perl $SAMPLE_PATH/rename_exonic_extract_unique_Illimina_snp.pl $SAMPLE_NAME
perl $SAMPLE_PATH/rename_exonic_extract_unique_indel_Illimina.pl $SAMPLE_NAME
perl $SAMPLE_PATH/rename_exonic_extract_unique_indel_sample.pl $SAMPLE_NAME
perl $SAMPLE_PATH/rename_exonic_extract_unique_sample_snp.pl $SAMPLE_NAME
perl $SAMPLE_PATH/rename_exonic_venn_snp_indel.pl $SAMPLE_NAME
chmod 755 $SAMPLE_PATH/$SAMPLE_NAME/*
perl $SAMPLE_PATH/$SAMPLE_NAME/phase2_1rsid_get.pl
perl $SAMPLE_PATH/$SAMPLE_NAME/phase2_2rsid_filter.pl
perl $SAMPLE_PATH/$SAMPLE_NAME/phase2_3rsid_venn.pl
perl $SAMPLE_PATH/$SAMPLE_NAME/phase2_4rsid_venn.pl
mkdir -p $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_14_VIRTUAL_SNP/exonic_rsid
perl $SAMPLE_PATH/$SAMPLE_NAME/exonic_extract_rsid_indel.pl
perl $SAMPLE_PATH/$SAMPLE_NAME/exonic_extract_rsid_snp.pl
perl $SAMPLE_PATH/$SAMPLE_NAME/exonic_extract_unique_indel_sample.pl
perl $SAMPLE_PATH/$SAMPLE_NAME/exonic_extract_unique_indel_Illimina.pl
perl $SAMPLE_PATH/$SAMPLE_NAME/exonic_extract_common_indel.pl
perl $SAMPLE_PATH/$SAMPLE_NAME/exonic_extract_unique_sample_snp.pl
perl $SAMPLE_PATH/$SAMPLE_NAME/exonic_extract_unique_Illimina_snp.pl
perl $SAMPLE_PATH/$SAMPLE_NAME/exonic_extract_common_snp.pl
```

Linux

Extract Damaging Varaints (SIFT, PolyPhen) from SNP file

14

COMMAND

```
mkdir -p $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/damaging
mkdir -p $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_13_VARIANT_CALLING/damaging/snp
perl $SAMPLE_PATH/$SAMPLE_NAME/phase2_damaging_1_get_snv_snp.pl
perl $SAMPLE_PATH/$SAMPLE_NAME/phase2_damaging_2_merge.pl
```

Linux

HLA Analysis using HLAVBSeq

15 Read data aligned to GRCh37/hg19 using **HLA-VBSeq Software to predict HLA types**
BWA version 0.5.9

COMMAND

```
erl $SAMPLE_PATH/rename_hla_calculation.pl $SAMPLE_NAME

$SOFTWARE_PATH/bwa.kit/bwa mem -t 8 -P -L 10000 -a $SOFTWARE_PATH/HLA/hla_all.fasta $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_cleaned_R1.fastq $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_cleaned_R2.fa

mkdir -p $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/

java -jar $SOFTWARE_PATH/HLA/HLAVBSeq.jar $SOFTWARE_PATH/HLA/hla_all.fasta $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_part.sam $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NA

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^A\*" | sort -k2 -n -r > $SAMPLE_PATH/$SAM

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^B\*" | sort -k2 -n -r > $SAMPLE_PATH/$SAM

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^C\*" | sort -k2 -n -r > $SAMPLE_PATH/$SAM

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DMA\*" | sort -k2 -n -r > $SAMPLE_PATH/$S

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DMB\*" | sort -k2 -n -r > $SAMPLE_PATH/$S

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DOA\*" | sort -k2 -n -r > $SAMPLE_PATH/$S

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DOB\*" | sort -k2 -n -r > $SAMPLE_PATH/$S

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DPA1\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DPB1\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DQA1\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DQB1\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DRA\*" | sort -k2 -n -r > $SAMPLE_PATH/$S

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DRB1\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DRB2\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DRB3\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DRB4\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DRB5\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DRB6\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DRB7\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DRB8\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^DRB9\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^E\*" | sort -k2 -n -r > $SAMPLE_PATH/$SAM

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^F\*" | sort -k2 -n -r > $SAMPLE_PATH/$SAM

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^G\*" | sort -k2 -n -r > $SAMPLE_PATH/$SAM

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^H\*" | sort -k2 -n -r > $SAMPLE_PATH/$SAM

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^J\*" | sort -k2 -n -r > $SAMPLE_PATH/$SAM

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^K\*" | sort -k2 -n -r > $SAMPLE_PATH/$SAM

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^L\*" | sort -k2 -n -r > $SAMPLE_PATH/$SAM

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^MICA\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^MICB\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^P\*" | sort -k2 -n -r > $SAMPLE_PATH/$SAM

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^TAP1\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^TAP2\*" | sort -k2 -n -r > $SAMPLE_PATH/$

perl $SOFTWARE_PATH/HLA/parse_result.pl $SOFTWARE_PATH/HLA/Allelelist.txt $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_11_HLA/$SAMPLE_NAME\_part_result | grep "^V\*" | sort -k2 -n -r > $SAMPLE_PATH/$SAM

perl $SAMPLE_PATH/$SAMPLE_NAME/hla_calculation.pl
```

Linux

Structual Variants (SV) Analysis using GASV

16 Geometric Analysis of Structural Variants (GASV) Version: 2.0

COMMAND

```
mkdir -p $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_15_SV

perl $SAMPLE_PATH/rename_SV_gasv.pl $SAMPLE_NAME

perl $SAMPLE_PATH/$SAMPLE_NAME/SV_gasv.sh

cp /home/odity/ravim/$SAMPLE_NAME\_RMDUP.bam.gasv.in.clusters $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_15_SV/$SAMPLE_NAME\_RMDUP.bam.gasv.in.clusters

mv $SAMPLE_PATH/$SAMPLE_NAME/*_null* $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_15_SV/

mv $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam.gasv.in $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_15_SV/

perl $SAMPLE_PATH/rename_SV_count_type.pl $SAMPLE_NAME

perl $SAMPLE_PATH/$SAMPLE_NAME/SV_count_type.pl
```

Linux

Gene Integration detection using string search

17 Samtools version 1.3

COMMAND

```
#cmyc gene end (GE) 65
#TGTTGCGGAAACGACGAGAACAGTTGAAACACAAACTTGAACAGCTACGGAACTCTTGTGCGTAA

#vector start (VS) 15
#GAATTCGCTAGCGAT

#cmyc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep TGTTGCGGAAACGACGAGAACAGTTGAAACACAAACTTGAACAGCTACGGAACTCTTGTGCGTAAGAATTCGCTAGCGAT

#rc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep ATCGCTAGCGAATTCTTACGCACAAGAGTTCCGTAGCTGTTCAAGTTTGTGTTTCAACTGTTCTCGTCGTTTCCGCAACA >

########################
#bmi
#gene end CTTCTTTTGCCAATAGACCTCGAAAATCATCAGTAAATGGGTCATCAGCAACTTCTTCTGGTTGA
#vec start GAATTCGCTAGCGAT

/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep CTTCTTTTGCCAATAGACCTCGAAAATCATCAGTAAATGGGTCATCAGCAACTTCTTCTGGTTGAGAATTCGCTAGCGAT >

#rc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep ATCGCTAGCGAATTCTCAACCAGAAGAAGTTGCTGATGACCCATTTACTGATGATTTTCGAGGTCTATTGGCAAAAGAAG

######################

#bclxl
#gene end
#GGTTCCTGACGGGCATGACTGTGGCCGGCGTGGTTCTGCTGGGCTCACTCTTCAGTCGGAAATGA

# vec start
#GAATTCGCTAGCGAT

/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep GGTTCCTGACGGGCATGACTGTGGCCGGCGTGGTTCTGCTGGGCTCACTCTTCAGTCGGAAATGAGAATTCGCTAGCGAT

#rc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep ATCGCTAGCGAATTCTCATTTCCGACTGAAGAGTGAGCCCAGCAGAACCACGCCGGCCACAGTCATGCCCGTCAGGAACC
######################

#KLF4
#gene end GTTTGTATTTTGCATACTCAAGGTGAGAATTAAGTTTTAAATAAACCTATAATATTTTATCTGAA
#vec start GAATTCGCTAGCGAT

/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep GTTTGTATTTTGCATACTCAAGGTGAGAATTAAGTTTTAAATAAACCTATAATATTTTATCTGAAGAATTCGCTAGCGAT

#rc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep ATCGCTAGCGAATTCTTCAGATAAAATATTATAGGTTTATTTAAAACTTAATTCTCACCTTGAGTATGCAAAATACAAAC
######################

#Lin28
#gene end TCCCTTCTCCTTTCCCTGGGAAAATACAATGAATAAATAAAGACTTATTGGTACGCAAACTGTCA
# vec start GAATTCGCTAGCGAT

/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep TCCCTTCTCCTTTCCCTGGGAAAATACAATGAATAAATAAAGACTTATTGGTACGCAAACTGTCAGAATTCGCTAGCGAT
######################
#rc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep ATCGCTAGCGAATTCTGACAGTTTGCGTACCAATAAGTCTTTATTTATTCATTGTATTTTCCCAGGGAAAGGAGAAGGGA
######################

#oct
#gene end AAAATGTTGTAGCCAACAAGACTGGGATTCCCACATGTGCCATTCCGGAGCCGGAAAAGCCCTCG
#vec start GAATTCGCTAGCGAT

/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep AAAATGTTGTAGCCAACAAGACTGGGATTCCCACATGTGCCATTCCGGAGCCGGAAAAGCCCTCGGAATTCGCTAGCGAT

#rc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep ATCGCTAGCGAATTCCGAGGGCTTTTCCGGCTCCGGAATGGCACATGTGGGAATCCCAGTCTTGTTGGCTACAACATTTT >
######################

#sox2
#gene end ACTTAAGTTTTTACTCCATTATGCACAGTTTGAGATAAATAAATTTTTGAAATATGGACACTGAA
#Vec start GAATTCGCTAGCGAT

/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep ACTTAAGTTTTTACTCCATTATGCACAGTTTGAGATAAATAAATTTTTGAAATATGGACACTGAAGAATTCGCTAGCGAT

#rc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep ATCGCTAGCGAATTCTTCAGTGTCCATATTTCAAAAATTTATTTATCTCAAACTGTGCATAATGGAGTAAAAACTTAAGT
```

```
# vector end 15 and gene start 65 in mapped region
##vector end 15 # TTGCGTACGGCCAGC

mkdir -p $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_16_GENE_INTEGRATION

#cmyc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep TTGCGTACGGCCAGCATGCCCCTCAACGTTAGCTTCACCAACAGGAACTATGACCTCGACTACGACTCGGTGCAGCCGTA >

#rc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep CGCTCTGCTGCTGCTGCTGGTAGAAGTTCTCCTCCTCGTCGCAGTAGAAATACGGCTGCACCGAGTCGTAGTCGAGGTCA >

#bmi
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep TTGCGTACGGCCAGCATGCATCGAACAACGAGAATCAAGATCACTGAGCTAAATCCCCACCTGATGTGTGTGCTTTGTGG

#rc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep AGAAGGAATGTAGACATTCTATTATGGTTGTGGCATCAATGAAGTACCCTCCACAAAGCACACACATCAGGTGGGGATTT

#bclxl
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep TTGCGTACGGCCAGCATGTCTCAGAGCAACCGGGAGCTGGTGGTTGACTTTCTCTCCTACAAGCTTTCCCAGAAAGGATA >

#rc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep CTGGGGCCTCAGTCCTGTTCTCTTCCACATCACTAAACTGACTCCAGCTGTATCCTTTCTGGGAAAGCTTGTAGGAGAGA >

#KLF4
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep TTGCGTACGGCCAGCAGTTTCCCGACCAGAGAGAACGAACGTGTCTGCGGGCGCGCGGGGAGCAGAGGCGGTGGCGGGCC

#rc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep GGGGCCAGAGGGGCGGGGGAGGGTCACTCGGCGGCTCCCGGTGCCGCCGCCGCCCGCCACCGCCTCTGCTCCCCGCGCGC >

#Lin28
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep TTGCGTACGGCCAGCGTGCGGGGGAAGATGTAGCAGCTTCTTCTCCGAACCAACCCTTTGCCTTCGGACTTCTCCGGGGC >

#rc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep GCCCCGGAGAAGTCCGAAGGCAAAGGGTTGGTTCGGAGAAGAAGCTGCTACATCTTCCCCCGCACGCTGGCCGTACGCAA

#oct
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep TTGCGTACGGCCAGCTTGCTTTTGCAGATGTACCTTCTTAAAGTTTTTTCTTAAAGTTTGGGAAATATTGAAATACGCTT >

#rc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep AAGCGTATTTCAATATTTCCCAAACTTTAAGAAAAAACTTTAAGAAGGTACATCTGCAAAAGCAAGCTGGCCGTACGCAA

#sox2
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep TTGCGTACGGCCAGCGGATGGTTGTCTATTAACTTGTTCAAAAAAGTATCAGGAGTTGTCAAGGCAGAGAAGAGAGTGTT

#rc
/softwares/samtools1.3/bin/samtools view $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_RMDUP.bam | grep AACACTCTCTTCTCTGCCTTGACAACTCCTGATACTTTTTTGAACAAGTTAATAGACAACCATCCGCTGGCCGTACGCAA >

Linux
```

Mycoplasma Contimination detection using BWA

18 BWA version 0.5.9
Samtools version 1.3.1

COMMAND

```
mkdir -p $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_18_Mycoplasma

#####Alaidlawii
/softwares/bwa-0.5.9/bwa aln -t 30 $SOFTWARE_PATH/Mycoplasma/Alaidlawii.fa $SAMPLE_PATH/$SAMPLE_NAME/$SAMPLE_NAME\_cleaned_R1.fastq > $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_18_Mycoplasma/

/softwares/bwa-0.5.9/bwa samse $SOFTWARE_PATH/Mycoplasma/Alaidlawii.fa $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_18_Mycoplasma/$SAMPLE_NAME\_R1_Alaidlawii.sai $SAMPLE_PATH/$SAMPLE_NAME/$SA

/softwares/samtools1.3.1/bin/samtools view -bS $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_18_Mycoplasma/$SAMPLE_NAME\_R1_Alaidlawii.sam > $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_18_My

/softwares/samtools1.3.1/bin/samtools sort $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_18_Mycoplasma/$SAMPLE_NAME\_R1_Alaidlawii.bam -o $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_18_Mycop

/softwares/samtools1.3.1/bin/samtools flagstat $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_18_Mycoplasma/$SAMPLE_NAME\_R1_Alaidlawii_sorted.bam > $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\

/softwares/samtools1.3.1/bin/samtools index $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_18_Mycoplasma/$SAMPLE_NAME\_R1_Alaidlawii_sorted.bam > $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_1

/softwares/samtools1.3.1/bin/samtools idxstats $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_18_Mycoplasma/$SAMPLE_NAME\_R1_Alaidlawii_sorted.bam

for BAM in $SAMPLE_PATH/$SAMPLE_NAME/Report_$SAMPLE_NAME\_18_Mycoplasma/*bam ; do

 CNT=`/softwares/samtools1.3.1/bin/samtools view -c -q20 $BAM`

 echo $BAM $CNT

done
```

Linux