Depth Analysis

Jacob Fuller

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# Alignments

I used Bowtie2 to align whole genome sequences of stickleback to the Glazer reference. I used Samtools to sort, index, and filter by whether MAPQ score ≥ 20

cd /lustre1/jcfuller/stick/genome/fastq/stickleback\_fastq/${sample}  
  
# Align  
export read1\_list=`ls -m \*\_1.fastq.gz | tr -d ' \n'`  
  
module load bowtie2/latest  
shopt -s nullglob  
set -- \*\_2.fastq.gz  
if [ "$#" -gt 0 ]  
 then  
 export read2\_list=`ls -m \*\_2.fastq.gz | tr -d ' \n'`  
 bowtie2 -p ${cores} --no-unal --very-sensitive -x /lustre1/jcfuller/stick/genome/bowtie/Glazer/Glazer \  
 -1 ${read1\_list} \  
 -2 ${read2\_list} \  
 --rg-id ${sample}\_${runNum} \  
 --rg SM:${sample} \  
 --rg PL:ILLUMINA \  
 --rg LB:${runNum} \  
 -S /lustre1/jcfuller/stick/genome/sam/${sample}.sam \  
 >& ${sample}\_${runNum}\_summary.txt  
  
 else  
 bowtie2 -p ${cores} --no-unal --very-sensitive -x /lustre1/jcfuller/stick/genome/bowtie/Glazer/Glazer \  
 -U ${read1\_list} \  
 --rg-id ${sample}\_${runNum} \  
 --rg SM:${sample} \  
 --rg PL:ILLUMINA \  
 --rg LB:${runNum} \  
 -S /lustre1/jcfuller/stick/genome/sam/${sample}.sam \  
 >& ${sample}\_${runNum}\_summary.txt  
fi  
  
# Sort, index  
module load samtools/latest  
cd /lustre1/jcfuller/stick/genome/sam  
samtools view -bh -@ $(expr ${cores} - 1) ${sample}.sam > ${sample}.bam  
samtools sort -o ${sample}\_sorted.bam -T ${sample}\_s -@ ${cores} ${sample}.bam  
mv ${sample}\_sorted.bam ${sample}.bam  
samtools index -b ${sample}.bam  
  
# Filter by whether MAPQ score ≥ 20  
if [ ${mapFilter} = true ]  
then  
 samtools view -bh -q 20 -@ $(expr ${cores} - 1) ${sample}.bam > ${sample}\_q.bam  
 samtools index -b ${sample}\_q.bam  
fi

# Acquiring Read Depth from Specific Chromosomes

You can use the Samtools "view" command with a specified range to view a section of the .bam file. I did this, and piped the result into the Bedtools "genomecov" command, with the -d option that produces the read depth at every basepair position. You must use bedtools v. 2.24.0 for this to work. V. 2.25.0 (on Sapelo) doesn't work as expected (unsure why). I'm only interested in the 3rd column of the bedtools results, so I pipe that into a output file.

#On UGA's Sapelo  
  
module load samtools/1.2  
#bedtools v. 2.25.0 causes weird issues  
module unload bedtools/2.25.0  
module load bedtools/2.24.0  
  
samtools view -b ${sample}.bam chrXXI | \  
bedtools genomecov \  
 -d \  
 -ibam stdin | \  
 awk '{print $3}' >> ${sample}\_XXI\_depth.txt