HS Rats Genotyping Pipeline

Pipeline Summary Report Design

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Pipeline Arguments

Line 1: home directory
Line 2: Flow cell directory
Line 3: Flow cell metadata Line 4: Sequencing data directory
Line 5: Reference genome
Line 6: Reference panels for STITCH
Line 7: Genetic map for BEAGLE
Line 8: Directory where you keep the code for the pipeline
Line 9: The general name of this run

previous_flow_cells_metadata

Paths to previous flow cells' metadata.

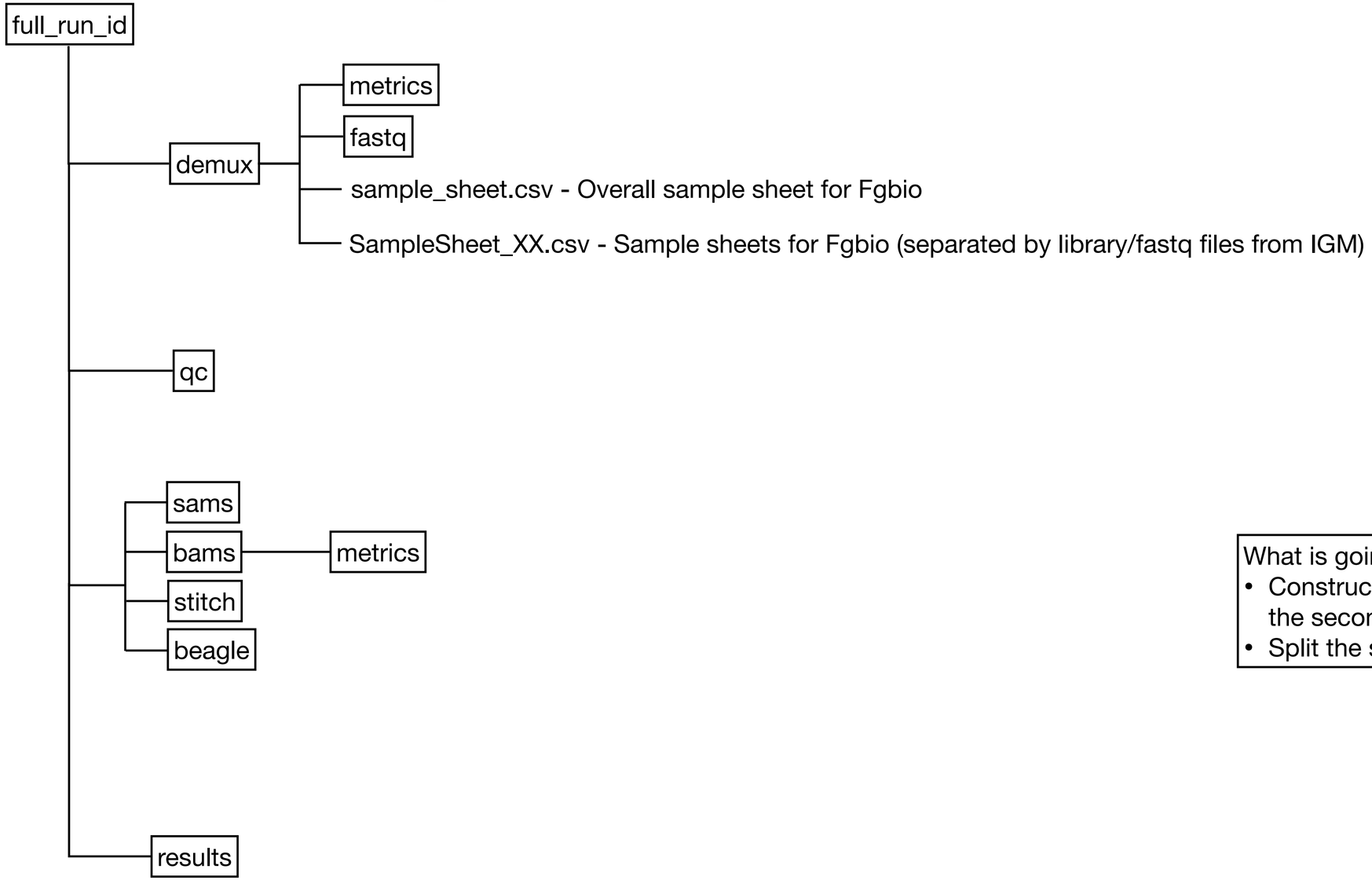
previous_flow_cells_bams

Paths to previous flow cells' BAM files.

pedigree_data

Paths to all flow cells' pedigree data.

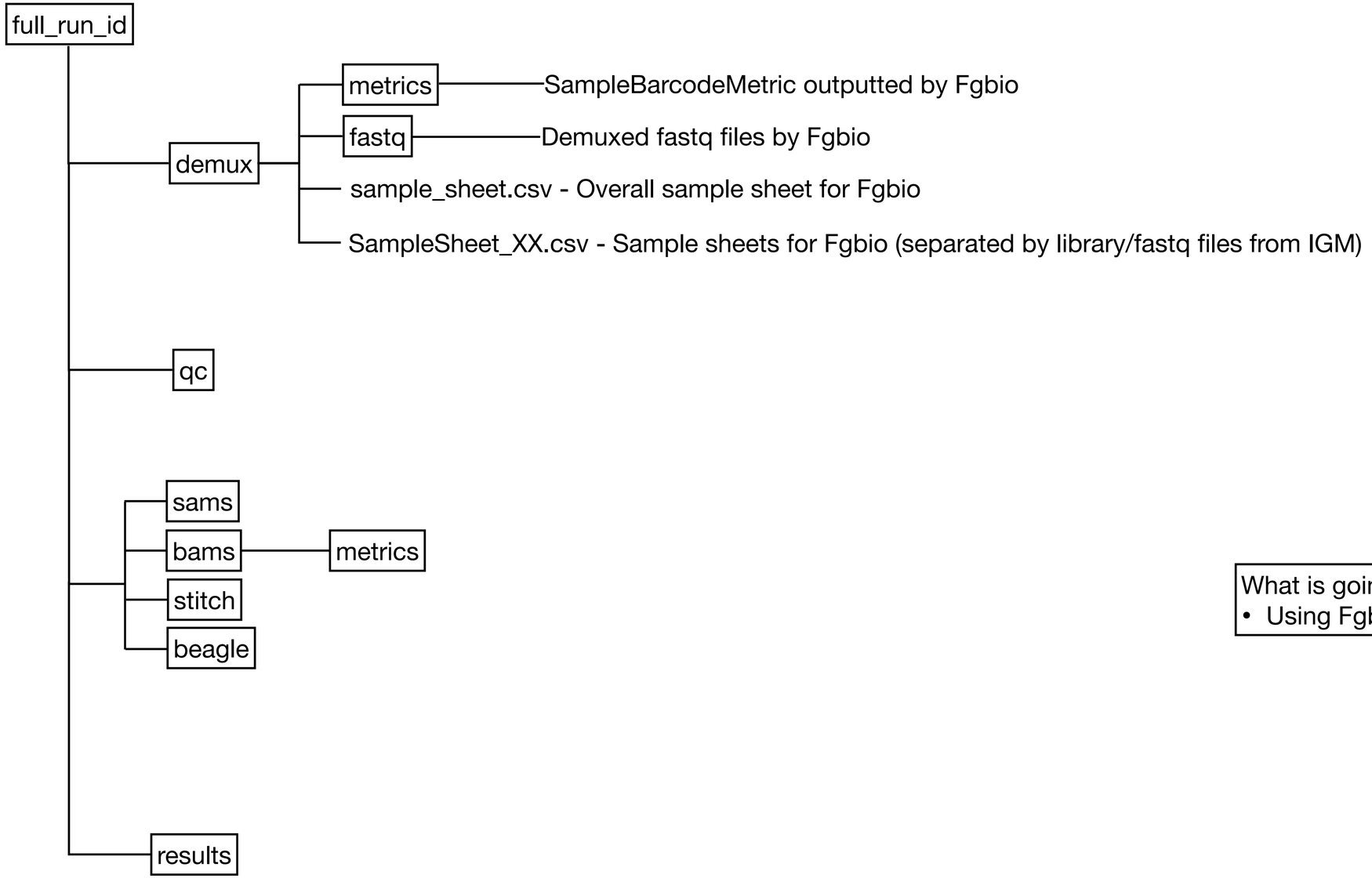
Step 1 - Preparation



What is going on:

- Construct the basic structure of the directory from the second line of the Pipeline Argument file
- Split the sample sheet for each library prep for Fgbio

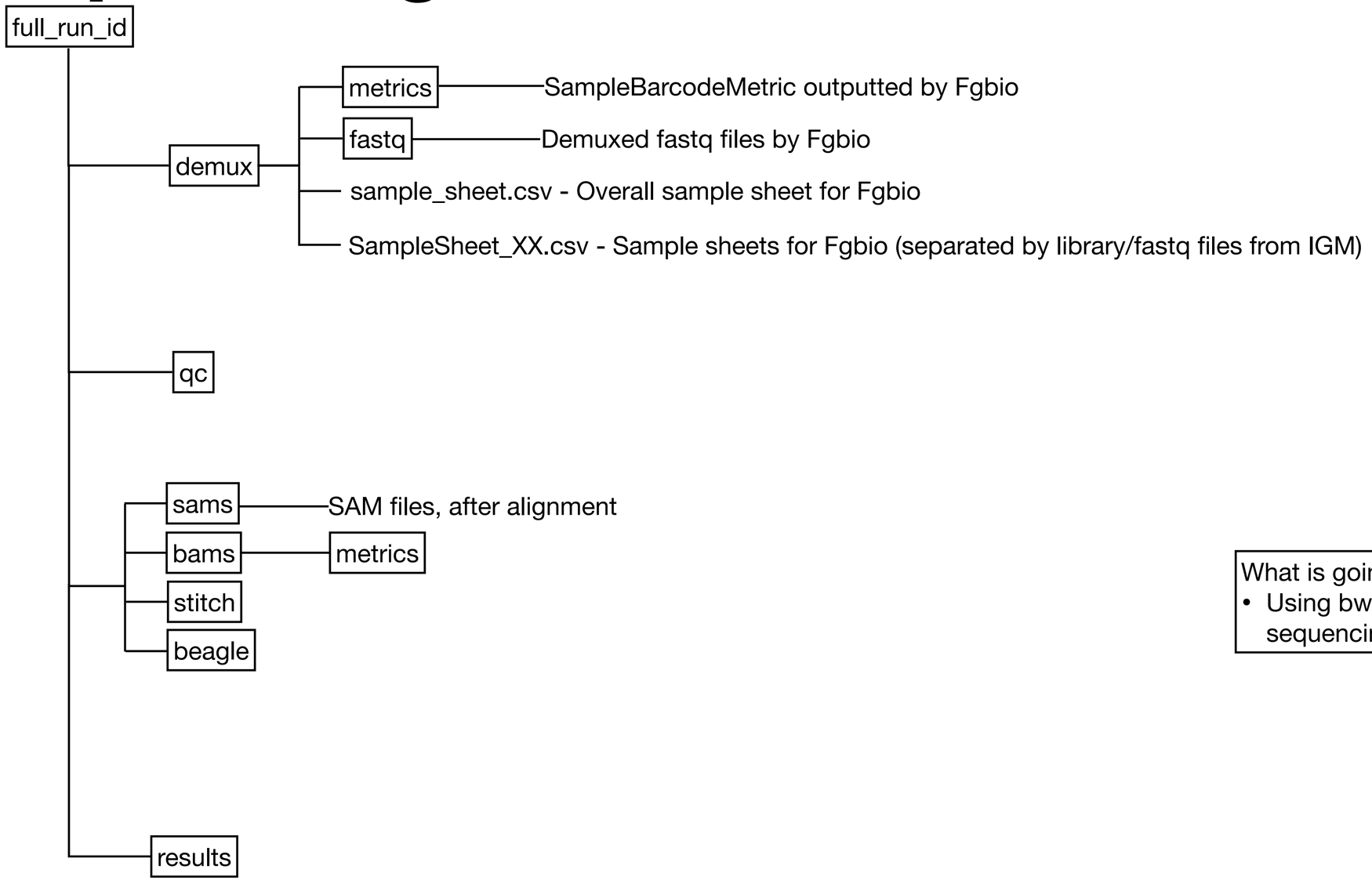
Step 2 - Demux



What is going on:

- Using Fgbio to demultiplex the fastq files

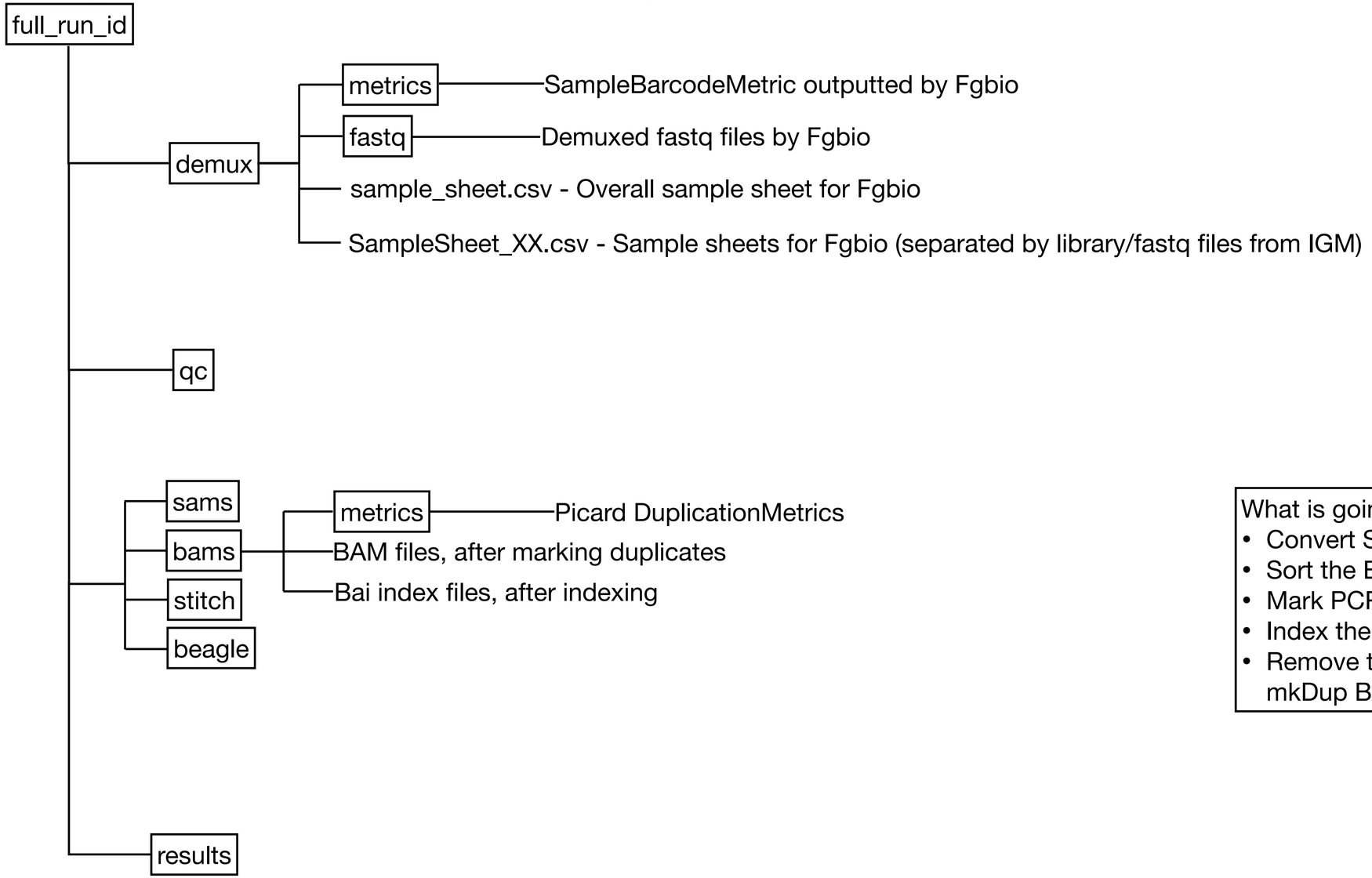
Step 3 - Alignment



What is going on:

- Using bwa mem to map the demultiplexed sequencing reads to reference genome

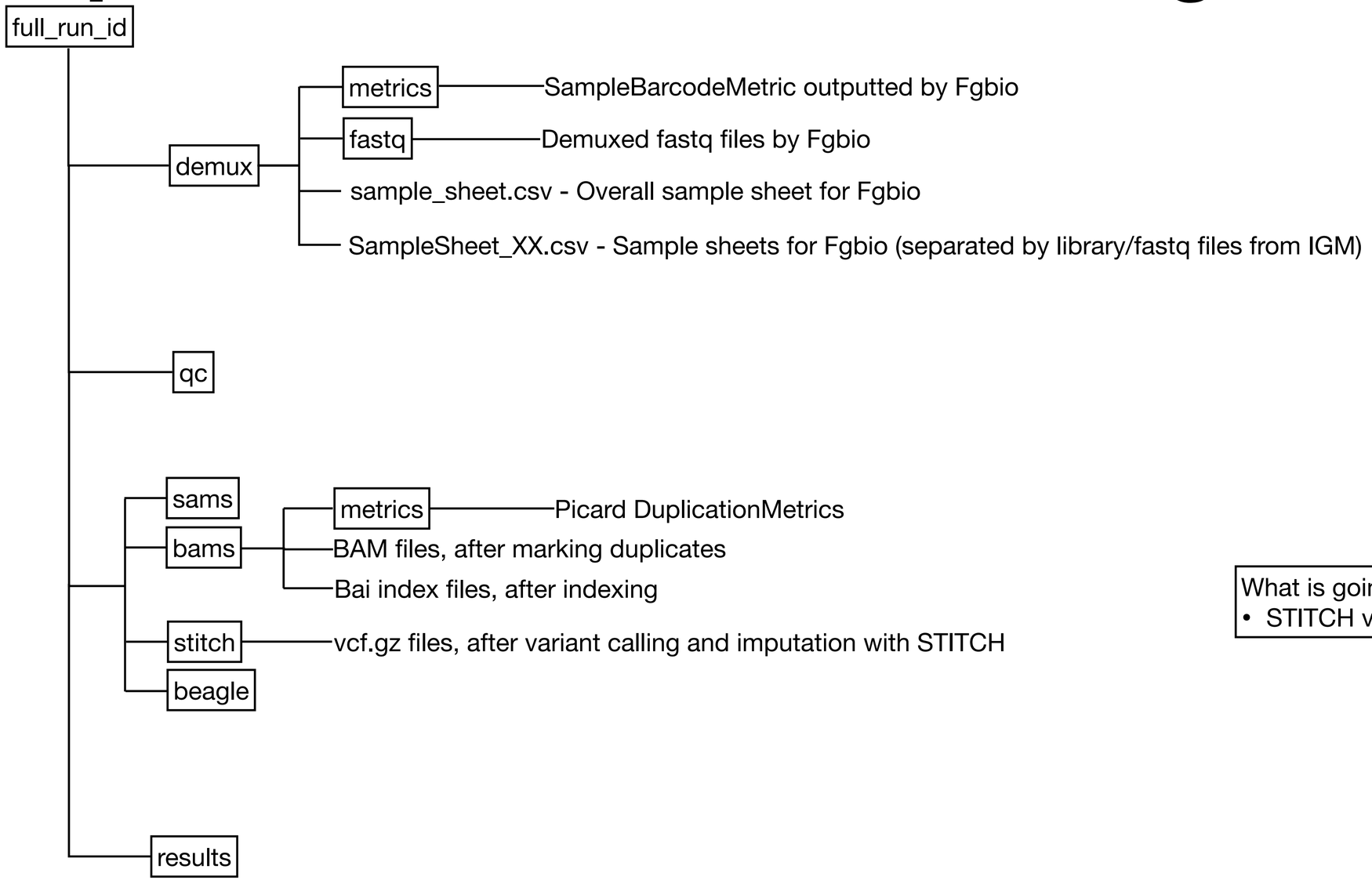
Step 4 - Mark Duplicates



What is going on:

- Convert SAM files to BAM files
- Sort the BAM files
- Mark PCR duplicates
- Index the marked duplicates BAM files
- Remove the SAM files, unsorted BAM files, and non-mkDup BAM files

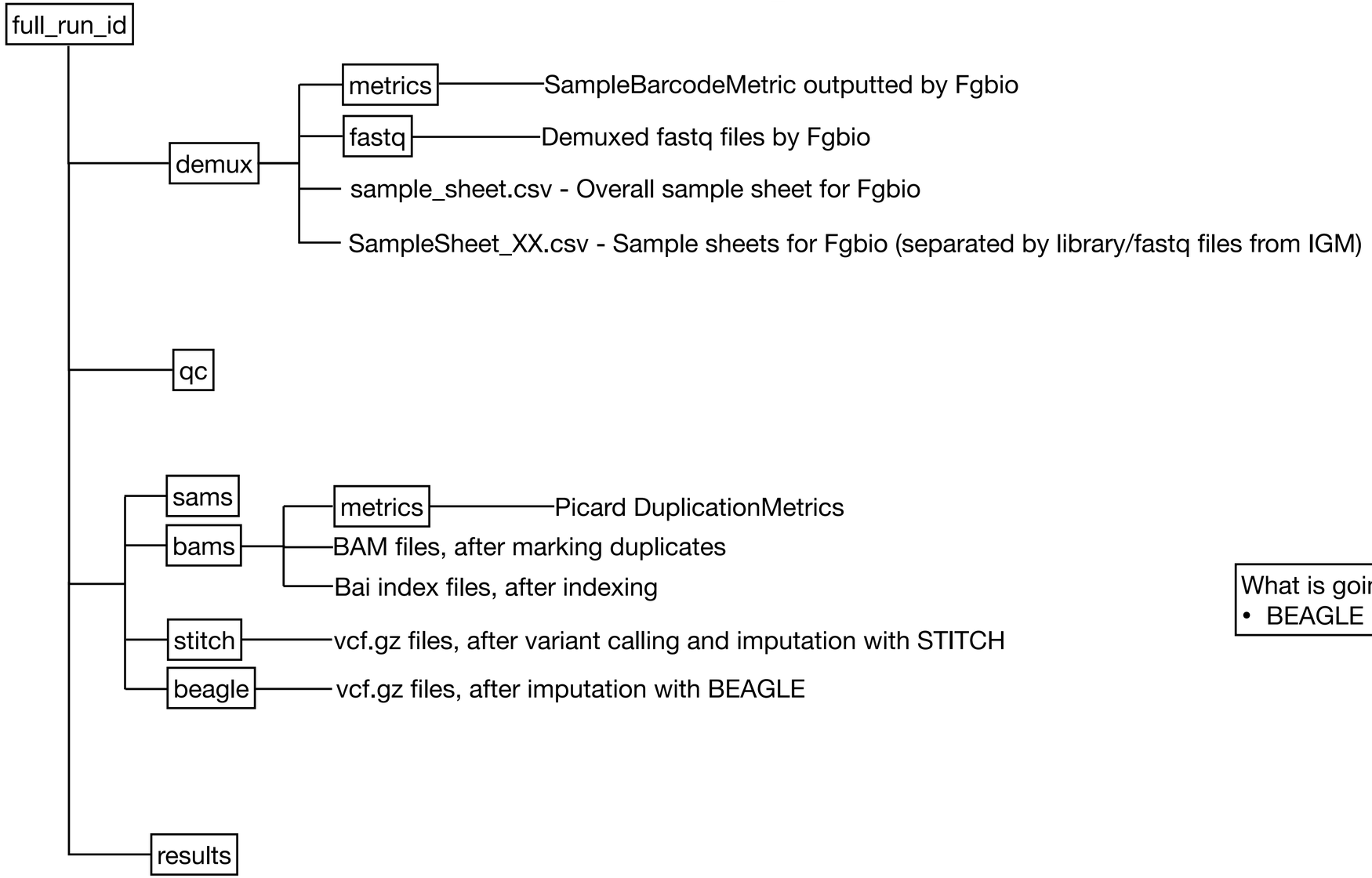
Step 5 - STITCH Variant Calling



What is going on:

- STITCH variant calling

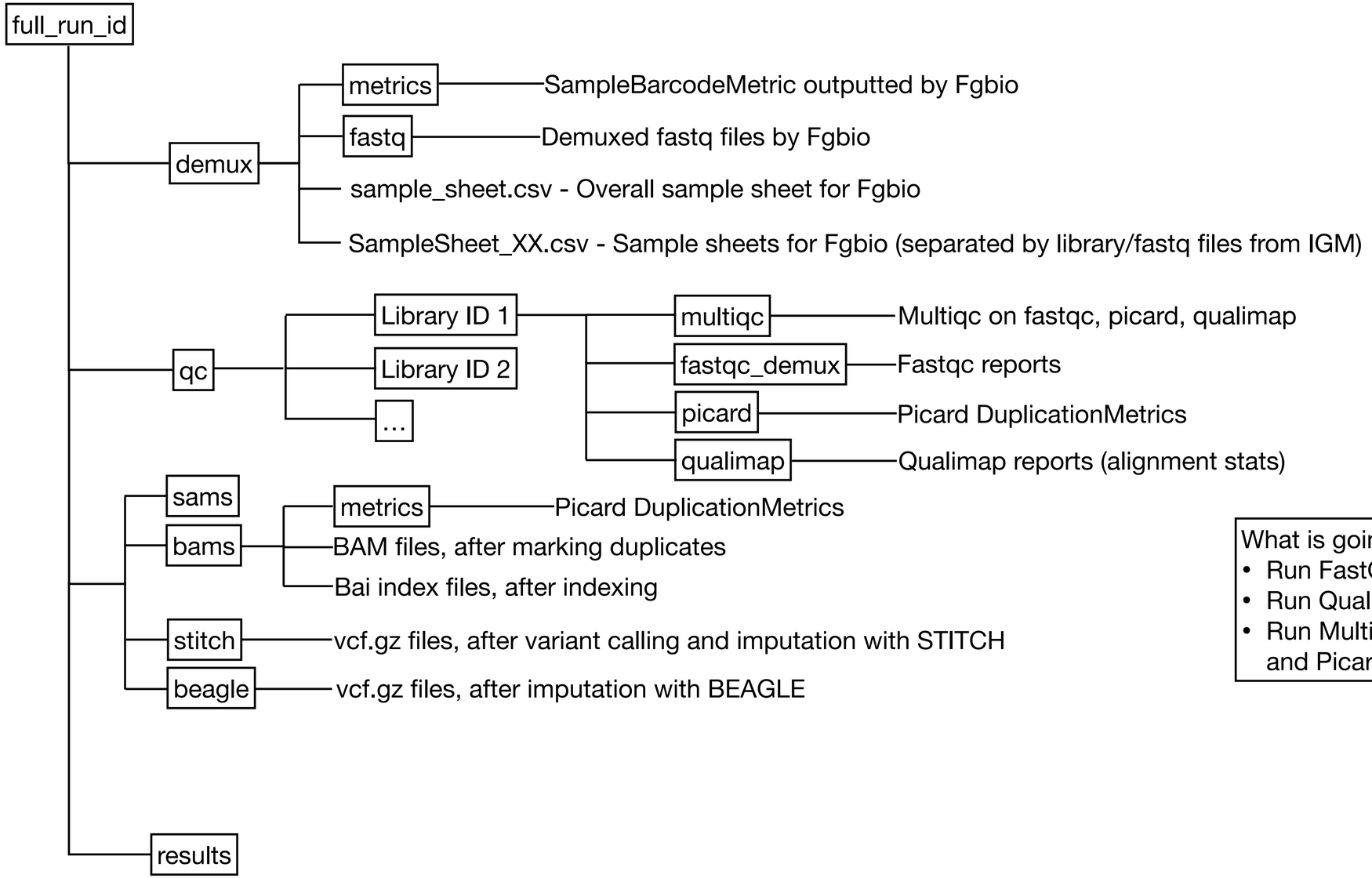
Step 6 - BEAGLE Imputation



What is going on:

- BEAGLE imputation

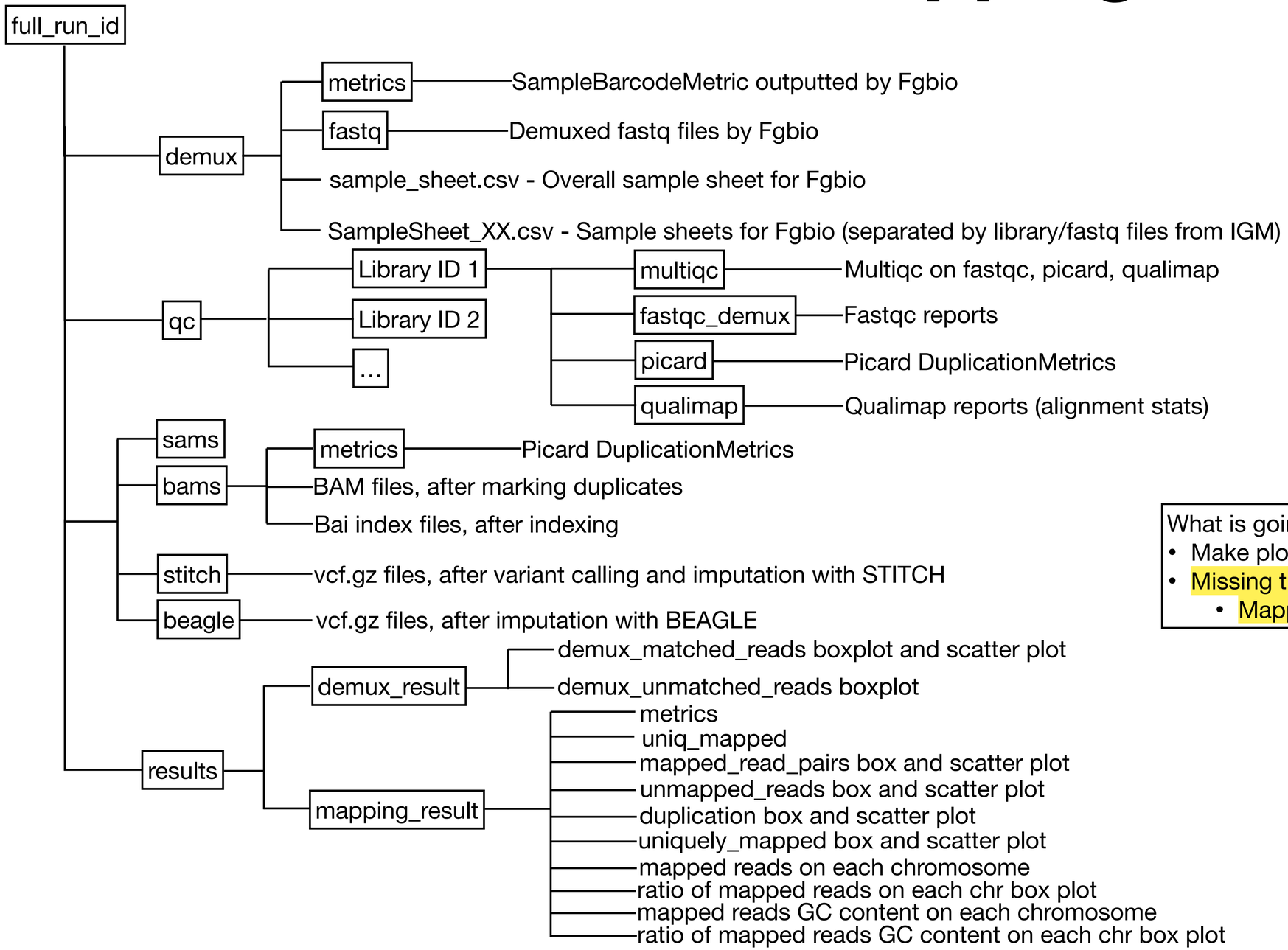
Result 1 - MultiQC



What is going on:

- Run FastQC on each library's fastq files
- Run Qualimap on each library's mapped bam files
- Run MultiQC on each library's FastQC, Qualimap, and Picard DuplicationMetrics results

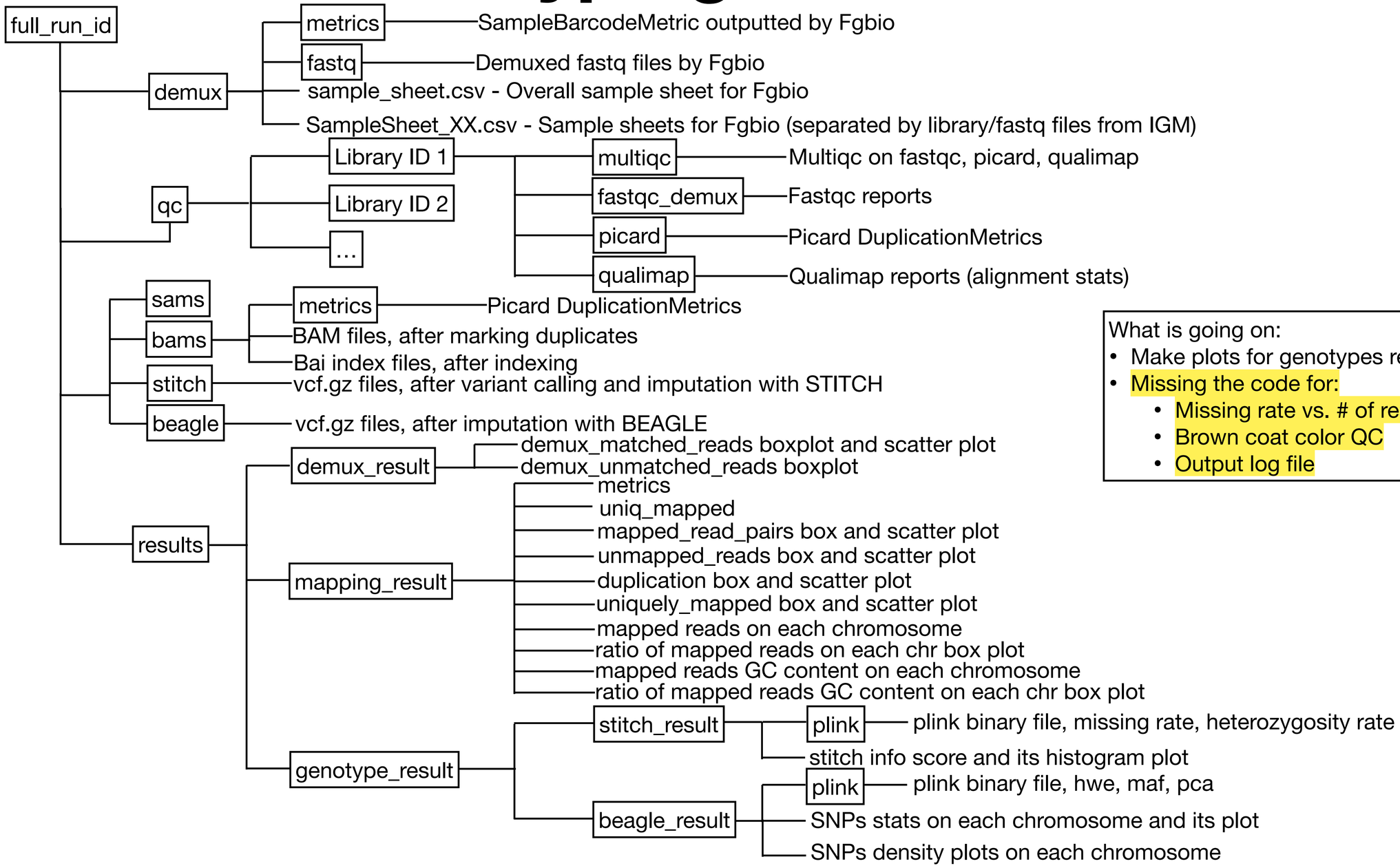
Result 2 - Demux and Mapping Results



What is going on:

- Make plots for demux result and mapping result
- Missing the code for:
  - Mapping quality histogram

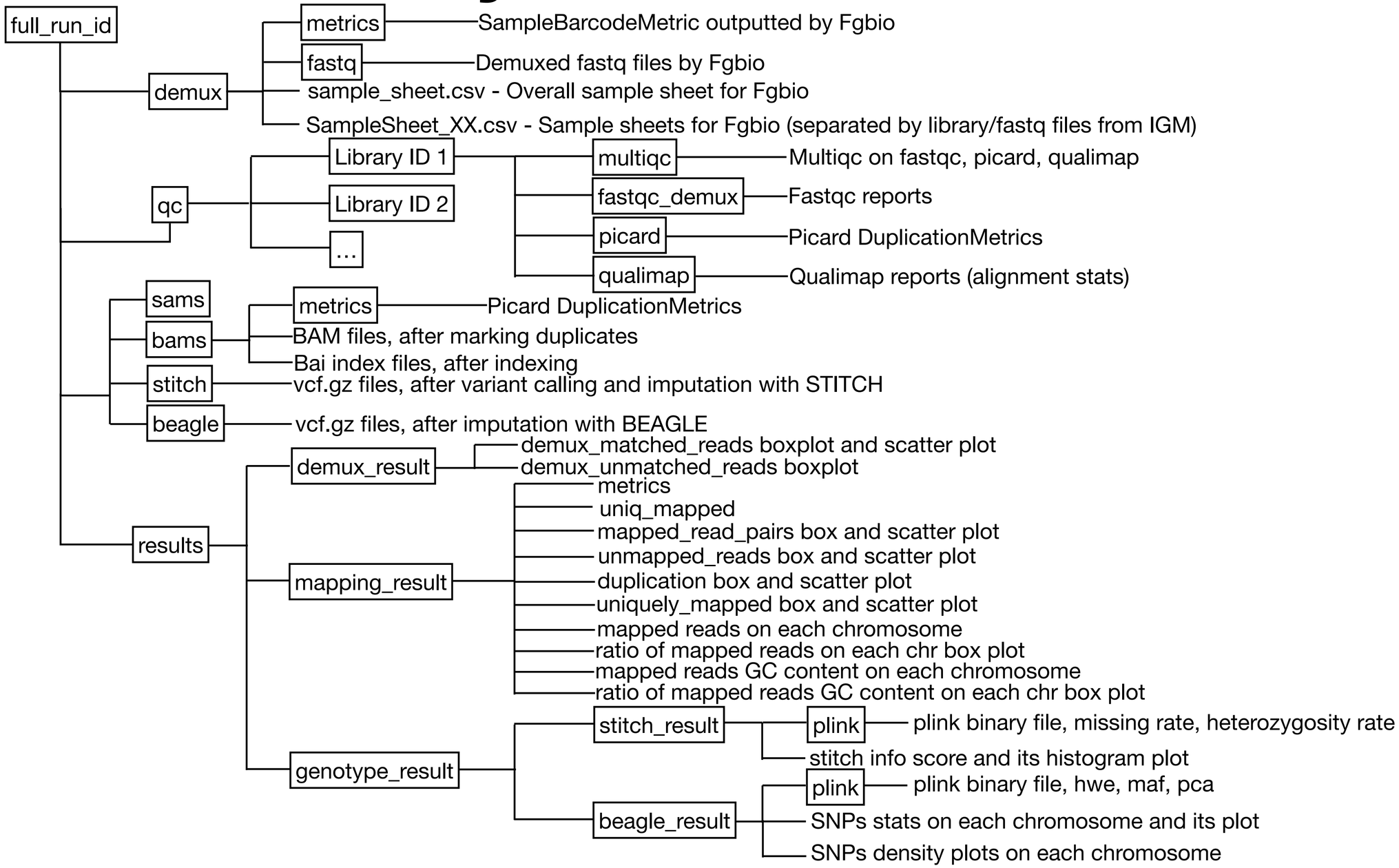
Result 3 - Genotyping Results



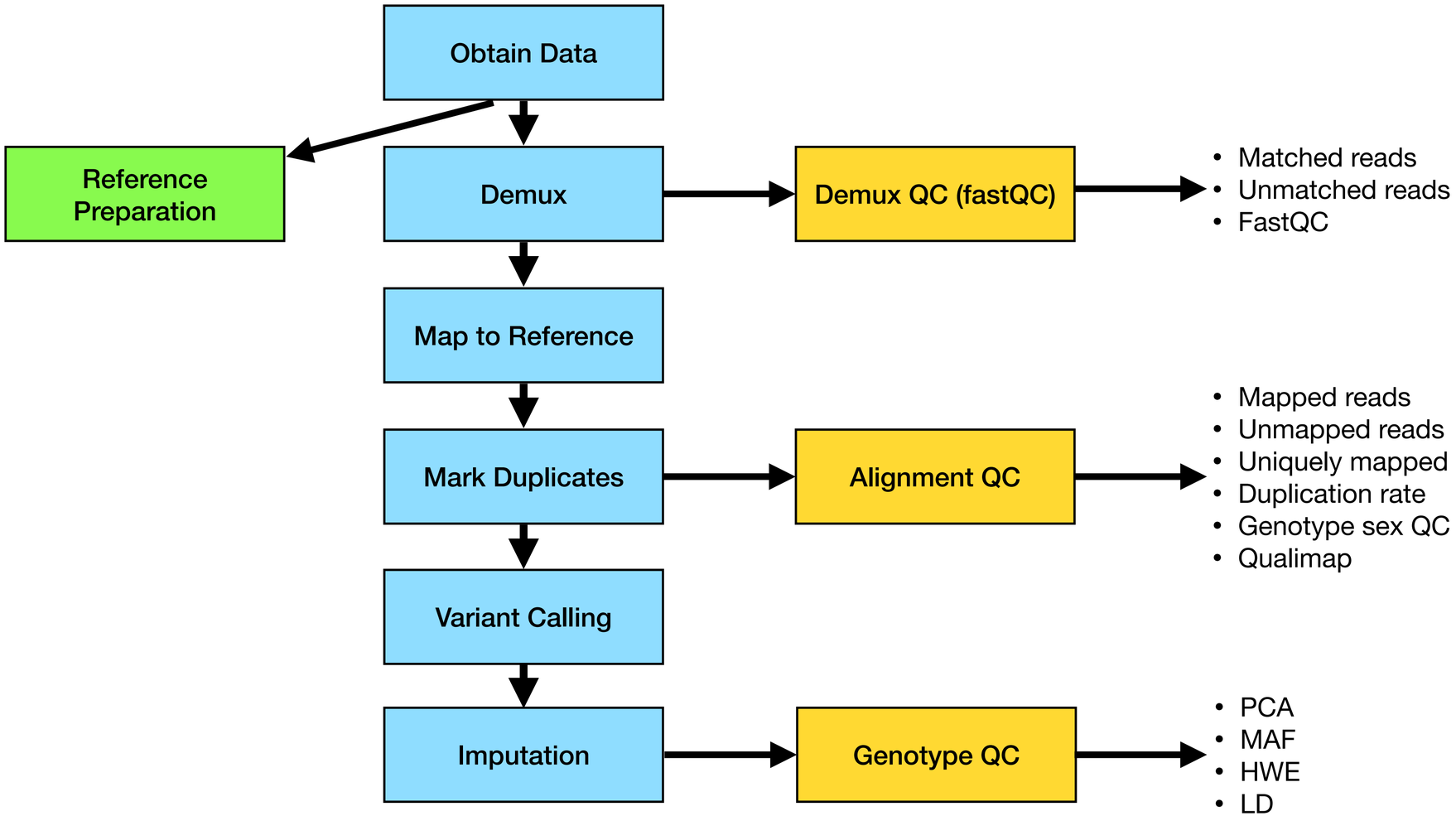
What is going on:

- Make plots for genotypes result
- Missing the code for:
  - Missing rate vs. # of reads
  - Brown coat color QC
  - Output log file

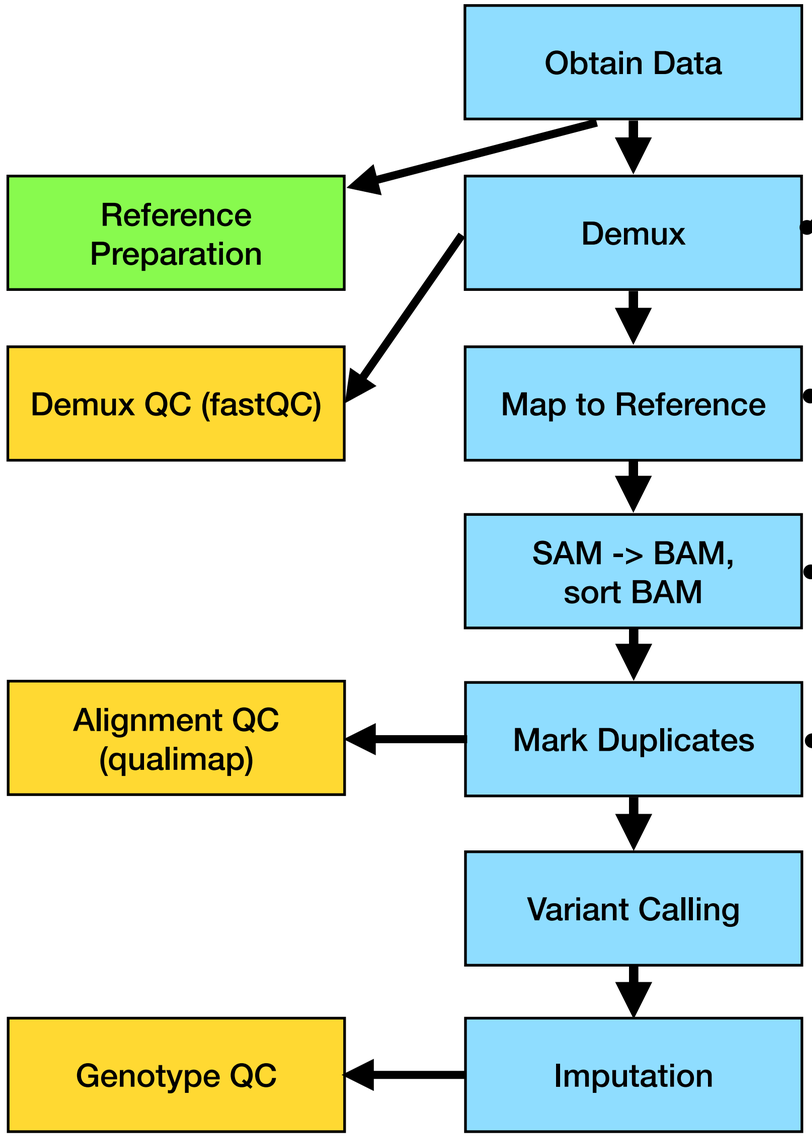
TSCC Directory Structure



Pipeline Flowchart



Pipeline Flowchart




```
java -Xmx40G -XX:+AggressiveOpts -XX:+AggressiveHeap \
    -jar /projects/ps-palmer/software/local/src/fgbio-1.2.0/fgbio-1.2.0.jar DemuxFastqs \
    --inputs ${pre_demux_fastq_R1} ${pre_demux_fastq_R2} \
    --metadata ${sample_sheet} \
    --read-structures 8B12M+T 8M+T \
    --output-type=Fastq \
    --threads $ncpu \
    --output ${out_path}/demux/fastq \
    --metrics ${out_path}/demux/metrics/${fastq_temp}demux_barcode_metrics.txt
```

```
/projects/ps-palmer/software/local/src/bwa-0.7.12/bwa mem -aM -t 2\
 -R "@RG\tID:${instrument_name}.${run_id}.${flowcell_id}.${flowcell_lane}\tLB:${library_id}
\tPL:ILLUMINA\tSM:${sample_id}\tPU:${flowcell_id}.${flowcell_lane}.${sample_barcode}" \
 ${reference_data} ${demux_data}/${f}_R1.fastq.gz \
 ${demux_data}/${f}_R2.fastq.gz > ${out_path}/sams/${f}.sam &
```

```
/projects/ps-palmer/software/local/src/samtools-1.10/samtools view -h -b \
  -t ${reference_data} -o ${out_path}/bams/${f}.bam ${mapped_data}/${f}.sam

/projects/ps-palmer/software/local/src/samtools-1.10/samtools sort -m 30G \
  -o ${out_path}/bams/${f}_sorted.bam ${out_path}/bams/${f}.bam
```

```
java -Xmx20G -XX:+AggressiveOpts -XX:+AggressiveHeap\
  -jar /projects/ps-palmer/software/local/src/picard-2.23.3/picard.jar MarkDuplicates \
  --INPUT ${out_path}/bams/${f}_sorted.bam \
  --REMOVE_DUPLICATES false \
  --ASSUME_SORTED true \
  --METRICS_FILE ${out_path}/bams/metrics/${f}_sorted_mkDup_metrics.txt \
  --OUTPUT ${out_path}/bams/${f}_sorted_mkDup.bam &

/projects/ps-palmer/software/local/src/samtools-1.10/samtools index \
  ${out_path}/bams/${f}_sorted_mkDup.bam ${out_path}/bams/${f}_sorted_mkDup.bai
```