```bash
#! /bin/bash


# Validate BAM File
java -Xmx4g -jar \${PICARD} \
  ValidateSamFile \
  INPUT=$bam \
  OUTPUT=${sample}.val_metrics \
  REFERENCE_SEQUENCE=${ref_fa} \
  MODE=SUMMARY \
  VALIDATE_INDEX=true \
  INDEX_VALIDATION_STRINGENCY=EXHAUSTIVE

# GC Bias Metrics
java -Xmx4g -jar \${PICARD} \
  CollectGcBiasMetrics \
  INPUT=$bam \
  OUTPUT=${sample}.gc_bias_metrics \
  REFERENCE_SEQUENCE=${ref_fa} \
  CHART_OUTPUT=${sample}.gc_bias_chart \
  SUMMARY_OUTPUT=${sample}.gc_bias_summary \
  IS_BISULFITE_SEQUENCED=false \
  METRIC_ACCUMULATION_LEVEL=ALL_READS \
  METRIC_ACCUMULATION_LEVEL=READ_GROUP \
  ALSO_IGNORE_DUPLICATES=true

# alignment summary
java -Xmx4g -jar \${PICARD} \
  CollectAlignmentSummaryMetrics \
  INPUT=$bam \
  OUTPUT=${sample}.alignment_summary \
  REFERENCE_SEQUENCE=${ref_fa} \
  MAX_INSERT_SIZE=100000 \
  EXPECTED_PAIR_ORIENTATIONS=${params.pair_orientation} \
  IS_BISULFITE_SEQUENCED=false \
  METRIC_ACCUMULATION_LEVEL=ALL_READS \
  METRIC_ACCUMULATION_LEVEL=READ_GROUP


#CollectRnaSeqMetrics
java -Xmx4g -jar \${PICARD} \
    CollectRnaSeqMetrics \
    INPUT=$bam \
    OUTPUT=${sample}.rna_seq_metrics \
    REF_FLAT=$ref_flat \
    CHART_OUTPUT=${sample}.rna_seq_metrics_chart \
    STRAND_SPECIFICITY=NONE \
    RIBOSOMAL_INTERVALS=$ref_rib \
    MINIMUM_LENGTH=500 \
    IGNORE_SEQUENCE=null \
    RRNA_FRAGMENT_PERCENTAGE=0.8 \
    METRIC_ACCUMULATION_LEVEL=ALL_READS \
    METRIC_ACCUMULATION_LEVEL=READ_GROUP \
    ASSUME_SORTED=true \
    STOP_AFTER=0


#CollectInsertSizeMetrics
java -Xmx4g -jar \${PICARD} \
```

```
CollectInsertSizeMetrics \
INPUT=$bam \
OUTPUT=${sample}.insert_size_metrics \
HISTOGRAM_FILE=${sample}.insert_size_histogram \
HISTOGRAM_WIDTH=null \
MINIMUM_PCT=0.05 \
METRIC_ACCUMULATION_LEVEL=ALL_READS \
METRIC_ACCUMULATION_LEVEL=READ_GROUP \
INCLUDE_DUPLICATES=false \
ASSUME_SORTED=true \
STOP_AFTER=0
```