

Sentieon DNA Pipeline for Variant Detection

Software-only solution, over 20× faster than GATK 3.3 with identical results

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Introduction

Recent advances in next generation sequencing (NGS) technologies have dramatically increased the rate of data output while significantly reducing costs. However, highly accurate analysis of NGS data is computationally intensive and creates a bottleneck in the overall sequencing workflow.

The current gold standard in variant calling is the Genome Analysis Toolkit (GATK)¹ Best Practice Workflow pipeline using HaplotypeCaller, which is regarded to have the highest accuracy for both single nucleotide polymorphisms (SNPs) and small insertions and deletions (indels).^{2,3} However, its slow computation speed often makes adoption challenging.

To address these challenges, the Sentieon DNA Software Package was developed to significantly decrease the analysis time and the computational resource requirements for variant detection without compromising accuracy. The result is a 20-to-50-fold increase in processing speed on the same hardware with results that are identical to the GATK pipeline, with differences within the numerical noise.

Sentieon DNA software package

The Sentieon DNA software is a package of tools used to perform ultrafast variant detection in genomic data obtained from NGS. It is designed to run on generic CPUs, without the need for specialized hardware (such as GPU, FPGAs, ASICs, etc.).

Sentieon DNA produces identical results to the GATK 3.3 pipeline with more than 20× speed improvement and includes all individual stages of the pipeline, namely: sample quality metrics calculation, duplicate read removal, indel realignment, base quality recalibration, and variant calling. The usage of Sentieon DNA is consistent with GATK and utilizes similar inputs, outputs, and parameters.

Sentieon DNA benchmarking methodology

A benchmarking comparison of Sentieon DNA and GATK 3.3 was performed using publically available genomic data from the 1000 Genomes Project (Appendix 1). The data was first mapped to the human reference genome hg19 using BWA⁴ 0.7.12 and SAMtools⁵ 1.2. The sorted.bam files were then used in two software scripts, which were created following the GATK Best Practice Workflows^{3,6} (see Appendix 2 for scripts). Each stage in GATK corresponds to a stage in Sentieon DNA, allowing for detailed, step-by-step evaluations of the two packages.

Six exome samples ranging from 3-347× coverage, and two full genome samples, with 6× and 14× coverage, were selected for the comparison of the two pipelines. The eight samples were analyzed individually using SAMtools 1.2/GATK 3.3 and Sentieon DNA 201505.02 on a 24 core, 2.4 GHz AMD Opteron 6234, 96GB memory server running Ubuntu 14.04.2 at the University of New Mexico.

Sentieon DNA is >20× faster than GATK 3.3

The runtime for the two pipelines using HaplotypeCaller variant calling was measured in core minutes. Exome runtime ranged from 108-2126 minutes for GATK 3.3 and 3-47 minutes for Sentieon DNA, while genome runtime was 2188 and 3978 minutes for GATK 3.3 and 66 and 198 minutes for Sentieon DNA (Appendix 3). Overall, Sentieon DNA provided a speed improvement over GATK 3.3 of 34-51× on the six exome samples and of 20-33× on the genome samples (Figure 1). For a comparison of UnifiedGenotyper variant calling, see Table 1 and Appendix 3.

Figure 1: Runtime Comparison

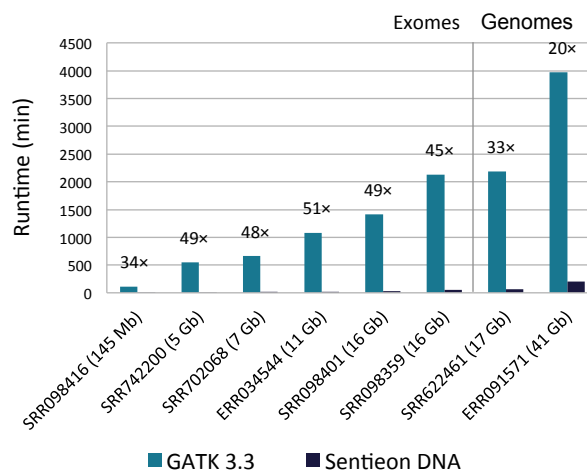


Figure 1. Runtime comparison of the pipelines using HaplotypeCaller variant calling in both software packages. Speed improvement of Sentieon DNA over GATK 3.3 is provided above each sample. Sentieon DNA runtime improvement ranges from 20–51× faster than GATK.

Table 1: Comparison of Sentieon DNA and GATK 3.3 Variant Calls

A. HaplotypeCaller

| Sample | Type | Sequencing Coverage | Sequenced Bases | Identical Variants | Differences Caused by Downsampling | Concordance: Sentieon vs. GATK without Downsampling | Differences: Missed INDEL | Differences: Added INDEL | Differences: Missed SNP | Differences: Added SNP |
|-----------|--------------|---------------------|-----------------|--------------------|------------------------------------|-----------------------------------------------------|---------------------------|--------------------------|-------------------------|------------------------|
| SRR098416 | Exome | 3x | 145M | 1420 | 0 | 100.000% | 0 | 0 | 0 | 0 |
| SRR742200 | Exome | 102x | 5G | 26454 | 8 | 99.985% | 2 | 0 | 1 | 1 |
| SRR702068 | Exome | 140x | 7G | 27296 | 32 | 100.000% | 0 | 0 | 0 | 0 |
| ERR034544 | Exome | 251x | 11G | 23995 | 14 | 99.983% | 0 | 0 | 3 | 1 |
| SRR098401 | Exome | 341x | 16G | 25067 | 18 | 100.000% | 0 | 0 | 0 | 0 |
| SRR098359 | Exome | 347x | 16G | 29104 | 28 | 99.993% | 0 | 0 | 0 | 2 |
| SRR622461 | Whole Genome | 6x | 17G | 1776194 | 1162 | 99.991% | 13 | 7 | 58 | 90 |
| ERR091571 | Whole Genome | 14x | 41G | 4317568 | 3159 | 99.992% | 27 | 23 | 110 | 188 |

B. UnifiedGenotyper

| Sample | Type | Sequencing Coverage | Sequenced Bases | Identical Variants | Differences Caused by Downsampling | Concordance: Sentieon vs. GATK without Downsampling | Differences: Missed INDEL | Differences: Added INDEL | Differences: Missed SNP | Differences: Added SNP |
|-----------|--------------|---------------------|-----------------|--------------------|------------------------------------|-----------------------------------------------------|---------------------------|--------------------------|-------------------------|------------------------|
| SRR098416 | Exome | 3x | 145M | 591 | 0 | 100.000% | 0 | 0 | 0 | 0 |
| SRR742200 | Exome | 102x | 5G | 33475 | 4 | 100.000% | 0 | 0 | 0 | 0 |
| SRR702068 | Exome | 140x | 7G | 34729 | 12 | 100.000% | 0 | 0 | 0 | 0 |
| ERR034544 | Exome | 251x | 11G | 29803 | 9 | 99.993% | 0 | 1 | 1 | 0 |
| SRR098401 | Exome | 341x | 16G | 31632 | 3 | 100.000% | 0 | 0 | 0 | 0 |
| SRR098359 | Exome | 347x | 16G | 36938 | 9 | 99.997% | 0 | 1 | 0 | 0 |
| SRR622461 | Whole Genome | 6x | 17G | 2352529 | 565 | 99.979% | 112 | 92 | 141 | 146 |
| ERR091571 | Whole Genome | 14x | 41G | 5341272 | 1180 | 99.981% | 247 | 193 | 330 | 243 |

Sentieon DNA produces identical results to GATK 3.3

The variant calling results of the two pipelines were analyzed for concordance using the program VarSeq™ from Golden Helix. Variants with quality-by-depth smaller than 2 and depth smaller than 5 were removed from the comparisons, as were variants called outside the exome capture area in the six exome samples.

In order to decrease runtime, GATK employs downsampling in areas of high coverage, which results in run-to-run variation in the variants called (Appendix 4). Sentieon DNA, however, does not downsample and produces consistent results between runs.

To identify the number of differing variant calls between the two pipelines that can be attributed to this downsampling, the GATK 3.3 pipeline was run an additional seven times for each sample. If all eight GATK 3.3 runs did not consistently call a variant, Sentieon DNA differences in these calls were attributed to downsampling by GATK.

The VarSeq™ analyses revealed over 99.8% concordance between the GATK 3.3 and Sentieon DNA variant calls (Figure 2). After removing the variation caused by GATK downsampling, the concordance between the two software packages increased to more than 99.99% (Table 1). In total, there were less than 1 in 10,000 true differences between the GATK 3.3 and Sentieon DNA analyses, which were caused by rounding differences between the two different software paths. This level of variance is 10x less than the numerical noise caused by run-to-run variation within GATK (Appendix 4).

Figure 2: Accuracy Comparison

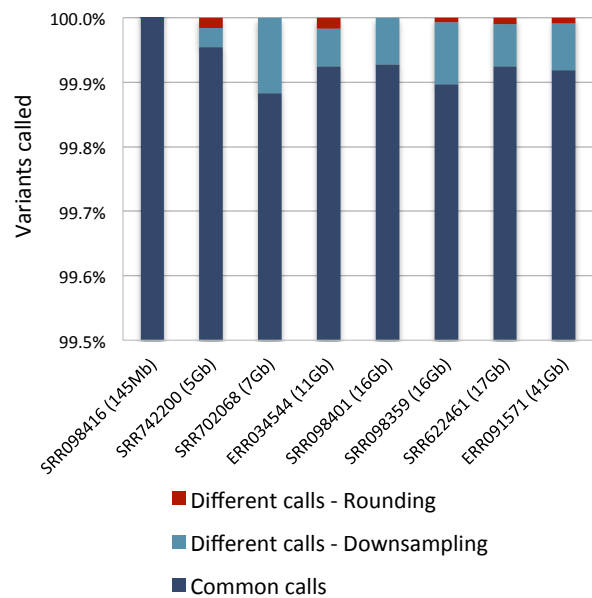
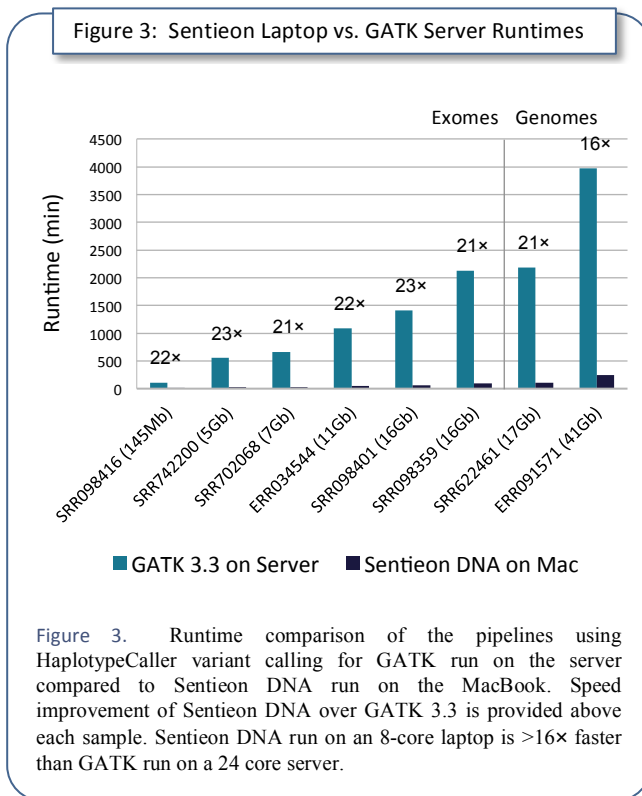


Figure 2. Concordance analysis for the variants called using the HaplotypeCaller in both software packages. Over 99.8% of the variant calls produced by GATK 3.3 and Sentieon DNA were identical. After removing the differences from GATK downsampling, the variant calls were over 99.99% concordant

Sentieon DNA run on a MacBook Pro Laptop

In addition to supporting all Linux distributions, Sentieon DNA is available for OS X versions 10.8 and above. A benchmarking comparison of Sentieon DNA for OS X was completed using the sorted.bam files from the six exome samples and the two full genome samples on a 2015 MacBook Pro laptop with a 2.8 GHz i7 processor with 8 Virtual Cores and 16GB Ram.

It was not feasible to re-analyze the samples using GATK on the laptop due to long processing times, so the MacBook Pro Sentieon DNA analyses were instead compared to the previous GATK results from the server (Figure 3, Appendix 3). Since Sentieon DNA produces consistent results with no run-to-run differences, the variants called using the MacBook Pro were identical to the results from the Linux server. Ultimately, Sentieon DNA run on the MacBook laptop outperformed GATK 3.3 on the server, providing a speed improvement of 16-26x.



Conclusions

The Sentieon DNA software package for variant detection produces identical SNP and indel variant identification to GATK 3.3 at >20x the speed. Transitioning pipelines from GATK to Sentieon DNA is easy due to consistent pipeline stages and similar user interface. Thus, Sentieon DNA enables drastically higher productivity, faster turn around time, and an order of magnitude increase in effective computing power of existing systems.

References

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Appendix 1: 1000 Genomes samples used in benchmarking

| Individual | Sample | Type | Technology | Sequenced Bases | Sequencing Coverage | Link |
|------------|-----------|--------------|----------------|-----------------|---------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| NA11930 | SRR098416 | Exome | Illumina HiSeq | 145M | 3x | ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data/NA11930/sequence_read/SRR098416* |
| NA12046 | SRR742200 | Exome | Illumina HiSeq | 5G | 102x | ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data/NA12046/sequence_read/SRR742200* |
| NA12155 | SRR702068 | Exome | Illumina HiSeq | 7G | 140x | ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data/NA12155/sequence_read/SRR702068* |
| NA11932 | ERR034544 | Exome | Illumina HiSeq | 11G | 251x | ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data/NA11932/sequence_read/ERR034544* |
| NA12878 | SRR098401 | Exome | Illumina HiSeq | 16G | 341x | ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data/NA12878/sequence_read/SRR098401* |
| NA12891 | SRR098359 | Exome | Illumina HiSeq | 16G | 347x | ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data/NA12891/sequence_read/SRR098359* |
| NA12878 | SRR622461 | Whole Genome | Illumina HiSeq | 17G | 6x | ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data/NA12878/sequence_read/SRR622461* |
| NA12878 | ERR091571 | Whole Genome | Illumina HiSeq | 41G | 14x | ftp://ftp-trace.ncbi.nih.gov/giab/ftp/technical/NA12878_data_other_projects/sequence_read/ERP001229/ILLUMINA/sequence_read/ERR091571* |

Appendix 2: Pipeline scripts

Read Alignment (used by both pipelines)

```
bwa mem -M -R "@RG\tID:$group\tSM:$sample\tPL:$pl" -t 24 $fasta $fastq_1 $fastq_2 | samtools view -Sb - >align.bam
samtools sort -@ 24 align.bam sorted
```

GATK 3.3

Sentieon DNA

| | | |
|------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sample metrics calculation | <pre>java -jar picard.jar CollectAlignmentSummaryMetrics INPUT=sorted.bam OUTPUT=aln_metrics.txt REFERENCE_SEQUENCE=\$fasta2 ADAPTER_SEQUENCE=null VALIDATION_STRINGENCY=SILENT java -jar picard.jar CollectGcBiasMetrics INPUT=sorted.bam OUTPUT=gc_metrics.txt SUMMARY_OUTPUT=gc_summary.txt CHART_OUTPUT=gcbias.pdf REFERENCE_SEQUENCE=\$fasta2 ASSUME_SORTED=true VALIDATION_STRINGENCY=SILENT java -jar picard.jar MeanQualityByCycle INPUT=sorted.bam OUTPUT=mq_metrics.txt CHART_OUTPUT=meanq_cycle.pdf REFERENCE_SEQUENCE=\$fasta2 VALIDATION_STRINGENCY=SILENT PF_READS_ONLY=true java -jar picard.jar QualityScoreDistribution INPUT=sorted.bam OUTPUT=qd_metrics.txt CHART_OUTPUT=qscore_dist.pdf REFERENCE_SEQUENCE=\$fasta2 VALIDATION_STRINGENCY=SILENT PF_READS_ONLY=true java -jar picard.jar CollectInsertSizeMetrics INPUT=sorted.bam OUTPUT=is_metrics.txt REFERENCE_SEQUENCE=\$fasta2</pre> | <pre>\$driver -r \$fasta -t 24 -i sorted.bam --algo MeanQualityByCycle mq_metrics.txt --algo QualDistribution qd_metrics.txt --algo GCBias --summary gc_summary.txt gc_metrics.txt --algo AlignmentStat aln_metrics.txt --algo InsertSizeMetricAlgo is_metrics.txt python \$dir/bin/plot.py metrics -o metrics-report.pdf gc=gc_metrics.txt qd=qd_metrics.txt mq=mq_metrics.txt isize=is_metrics.txt</pre> |
| Duplicate read removal | <pre>java -jar picard.jar MarkDuplicates M=dup_reads I=sorted.bam O=dedup.bam samtools index dedup.bam</pre> | <pre>\$driver -t 24 -i sorted.bam --algo LocusCollector --fun score_info score.txt \$driver -t 24 -i sorted.bam --algo Dedup --rmdup --score_info score.txt deduped.bam</pre> |
| Indel realignment | <pre>java -jar GenomeAnalysisTK.jar -T RealignerTargetCreator -R \$fasta2 -I dedup.bam -known \$dbsnp_Mill -o realigner.intervals java -jar GenomeAnalysisTK.jar -T IndelRealigner -R \$fasta2 -I dedup.bam -known \$dbsnp_Mill -targetIntervals realigner.intervals -o realigned.bam</pre> | <pre>\$driver -r \$fasta2 -t 24 -i deduped.bam --algo Realigner -k \$dbsnp_Mill realigned.bam</pre> |
| Base Quality Score Recalibration | <pre>java -jar GenomeAnalysisTK.jar -T BaseRecalibrator -nct 24 -R \$fasta2 -I realigned.bam -knownSites \$dbsnp -knownSites \$dbsnp_Mill -o recal.table java -jar GenomeAnalysisTK.jar -T PrintReads -nct 24 -R \$fasta2 -I realigned.bam -BQSR recal.table -o recal.bam java -jar GenomeAnalysisTK.jar -T BaseRecalibrator -nct 24 -R \$fasta2 -I realigned.bam -knownSites \$dbsnp -knownSites \$dbsnp_Mill -BQSR recal.table -o after_recal.table java -jar GenomeAnalysisTK.jar -T AnalyzeCovariates -R \$fasta2 -before recal.table -after after_recal.table -plots recal_plots.pdf</pre> | <pre>\$driver -r \$fasta2 -t 24 -i realigned.bam --algo QualCal -k \$dbsnp -k \$dbsnp_Mill recal_data.table \$driver -r \$fasta2 -t 24 -i realigned.bam -q recal_data.table --algo QualCal -k \$dbsnp -k \$dbsnp_Mill --pre recal_data.table --csv recal.csv recal_data.table.post python \$dir/bin/plot.py b24qsr -o recal_plots.pdf recal.csv</pre> |
| Variant calling – HaplotypeCaller | <pre>java -jar GenomeAnalysisTK.jar -T HaplotypeCaller -nct 24 -R \$fasta2 -I recal.bam -o HC.vcf</pre> | <pre>\$driver -r \$fasta2 -t 24 -i realigned.bam -q recal_data.table --algo Haplotype output-hc.vcf --algo ReadWriter recaled.bam</pre> |
| Variant calling – UnifiedGenotyper | <pre>java -jar GenomeAnalysisTK.jar -T UnifiedGenotyper -nt 24 -R \$fasta2 -I recal.bam -o UG.vcf -glm BOTH</pre> | <pre>\$driver -r \$fasta2 -t 24 -i realigned.bam -q recal_data.table --algo Genotyper output-ug.vcf</pre> |

Appendix 3: Runtime data per stage (in minutes)

| Sample Name | Type | Sequenced Bases | Sequencing Coverage | Stage | Sentieon Runtime on server | GATK Runtime on server | Sentieon Runtime on MacBook |
|-------------|--------------|-----------------|---------------------|------------------------------------|----------------------------|------------------------|-----------------------------|
| SRR098416 | Exome | 145M | 3× | Sample metrics calculation | 0.2 | 2.2 | 0.4 |
| | | | | Duplicate read removal | 0.3 | 1.8 | 1.0 |
| | | | | Indel realignment | 0.2 | 28.8 | 0.7 |
| | | | | Base Quality Score Recalibration | 2.1 | 21.0 | 1.1 |
| | | | | Variant calling – UnifiedGenotyper | 0.3 | 10.5 | 0.6 |
| | | | | Variant calling – HaplotypeCaller | 0.5 | 54.5 | 1.7 |
| SRR742200 | Exome | 5G | 102× | Sample metrics calculation | 0.5 | 14.6 | 1.3 |
| | | | | Duplicate read removal | 1.3 | 36.3 | 3.1 |
| | | | | Indel realignment | 1.5 | 69.2 | 3.1 |
| | | | | Base Quality Score Recalibration | 2.5 | 219.7 | 4.7 |
| | | | | Variant calling – UnifiedGenotyper | 0.7 | 15.2 | 1.5 |
| | | | | Variant calling – HaplotypeCaller | 5.4 | 213.1 | 12.3 |
| SRR702068 | Exome | 7G | 140× | Sample metrics calculation | 0.7 | 20.7 | 1.7 |
| | | | | Duplicate read removal | 1.9 | 55.5 | 4.3 |
| | | | | Indel realignment | 1.9 | 86.7 | 4.4 |
| | | | | Base Quality Score Recalibration | 3.9 | 297.6 | 8.0 |
| | | | | Variant calling – UnifiedGenotyper | 0.8 | 14.7 | 1.9 |
| | | | | Variant calling – HaplotypeCaller | 5.7 | 204.1 | 12.7 |
| ERR034544 | Exome | 11G | 251× | Sample metrics calculation | 0.9 | 31.7 | 2.6 |
| | | | | Duplicate read removal | 2.8 | 81.2 | 7.1 |
| | | | | Indel realignment | 3.1 | 123.0 | 7.3 |
| | | | | Base Quality Score Recalibration | 4.5 | 478.3 | 10.2 |
| | | | | Variant calling – UnifiedGenotyper | 1.2 | 18.1 | 2.9 |
| | | | | Variant calling – HaplotypeCaller | 9.9 | 370.5 | 23.4 |
| SRR098401 | Exome | 16G | 341× | Sample metrics calculation | 1.0 | 31.4 | 2.6 |
| | | | | Duplicate read removal | 2.8 | 80.9 | 6.6 |
| | | | | Indel realignment | 4.6 | 140.4 | 7.2 |
| | | | | Base Quality Score Recalibration | 4.6 | 406.9 | 10.1 |
| | | | | Variant calling – UnifiedGenotyper | 1.2 | 23.1 | 2.9 |
| | | | | Variant calling – HaplotypeCaller | 15.7 | 748.7 | 34.6 |
| SRR098359 | Exome | 16G | 347× | Sample metrics calculation | 1.6 | 56.4 | 4.3 |
| | | | | Duplicate read removal | 4.9 | 146.1 | 11.6 |
| | | | | Indel realignment | 7.9 | 284.5 | 15.9 |
| | | | | Base Quality Score Recalibration | 8.3 | 727.4 | 18.2 |
| | | | | Variant calling – UnifiedGenotyper | 1.8 | 27.5 | 4.5 |
| | | | | Variant calling – HaplotypeCaller | 24.6 | 912.0 | 49.6 |
| SRR622461 | Whole Genome | 17G | 6× | Sample metrics calculation | 1.4 | 46.6 | 3.6 |
| | | | | Duplicate read removal | 3.9 | 114.0 | 8.9 |
| | | | | Indel realignment | 6.9 | 202.5 | 10.9 |
| | | | | Base Quality Score Recalibration | 7.6 | 748.2 | 15.9 |
| | | | | Variant calling – UnifiedGenotyper | 2.0 | 31.5 | 4.4 |
| | | | | Variant calling – HaplotypeCaller | 46.6 | 1076.9 | 64.2 |
| ERR091571 | Whole Genome | 41G | 14× | Sample metrics calculation | 3.1 | 111.4 | 7.9 |
| | | | | Duplicate read removal | 11.0 | 296.9 | 25.7 |
| | | | | Indel realignment | 18.0 | 448.6 | 30.0 |
| | | | | Base Quality Score Recalibration | 15.8 | 1811.0 | 34.6 |
| | | | | Variant calling – UnifiedGenotyper | 5.2 | 47.8 | 11.7 |
| | | | | Variant calling – HaplotypeCaller | 149.7 | 1310.3 | 147.8 |

Appendix 4: Run to run differences in GATK due to downsampling

The run-to-run variation caused by re-running GATK 3.3 HaplotypeCaller 20 times is plotted below. Each run was compared to all other runs using the program VarSeq™ to determine the number of variants called in the run but not appearing in the other runs. The range of the variation in number of calls and the mean plus 1 standard deviation of the variation are shown. The difference between each run and the Sentieon DNA run is within the statistical variation due to the run-to-run differences.

