This document is a comprehensive walkthrough of the NGS_INTRO training environment and covers the deviation to the Welsh Genepark version supplied in the Docker container.

User input is coloured thusly and program output in this manner.

4204ad5da0ed login: guest

Password:

Welcome to Ubuntu 16.04 LTS (GNU/Linux 4.4.17-boot2docker x86 64)

* Documentation: https://help.ubuntu.com/

The programs included with the Ubuntu system are free software; the exact distribution terms for each program are described in the individual files in /usr/share/doc/*/copyright.

Ubuntu comes with ABSOLUTELY NO WARRANTY, to the extent permitted by applicable law.

Welcome to the IFR Dockerised version of the Welsh Genepark's Introduction to Command-line NGS Analysis course!

The original materials belong to Wesh Genepark and the original materials and software can be found at http://www.walesgenepark.cardiff.ac.uk/bioinformatics/training/

This was originally designed to be deployed onto Raspberry pi computers. Our version will run on any linux machine running Docker Host (or on Windows PC & Macs running Docker Toolbox (Kitematic)).

There are updated links to data files and we have provided an i86 version of the GATK (rather than the original ARM version).

*** NB ****

To run a command as administrator (user "root"), use "sudo <command>". See "man sudo_root" for details.

guest@4204ad5da0ed:~\$ /scripts/copy_course.sh
copying course material, tools & data from /home/guest to /coursehome
guest@4204ad5da0ed:~\$ ls /coursehome/
GenomeAnalysisTK.jar data docs
guest@4204ad5da0ed:~\$ cd /coursehome/

guest@4204ad5da0ed:/coursehome\$ cat data/Brca1Reads_1.1.fastq
data/Brca1Reads_1.2.fastq > data/reads.fq

guest@4204ad5da0ed:/coursehome\$ grep @chr data/reads.fq | wc -1
200000

guest@4204ad5da0ed:/coursehome\$ grep -c @chr data/reads.fq

200000
guest@4204ad5da0ed:/coursehome\$ mkdir fastqc

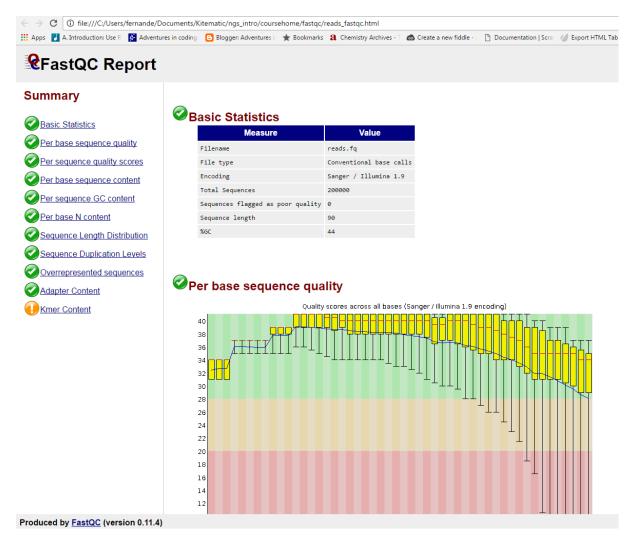
guest@14fd5fadf014:/coursehome\$ fastqc --extract -o fastqc data/reads.fq

Started analysis of reads.fq Approx 5% complete for reads.fq Approx 10% complete for reads.fq

```
Approx 15% complete for reads.fq
Approx 20% complete for reads.fq
Approx 25% complete for reads.fq
Approx 30% complete for reads.fq
Approx 35% complete for reads.fq
Approx 40% complete for reads.fq
Approx 45% complete for reads.fq
Approx 50% complete for reads.fq
Approx 55% complete for reads.fq
Approx 60% complete for reads.fq
Approx 65% complete for reads.fq
Approx 70% complete for reads.fq
Approx 75% complete for reads.fq
Approx 80% complete for reads.fq
Approx 85% complete for reads.fq
Approx 90% complete for reads.fq
Approx 95% complete for reads.fq
Approx 100% complete for reads.fq
Analysis complete for reads.fq
```

guest@14fd5fadf014:/coursehome\$ ls fastqc
reads_fastqc_reads_fastqc.html reads_fastqc.zip

You can view the HTML (web-page) output by clicking on the reads_fastqc.html file in fastqc directory from Windows side:



```
guest@14fd5fadf014:/coursehome$ ls -alFs fastqc/reads_fastqc
total 281
 4 drwxrwxrwx 1 guest staff 4096 Nov 17 2016 ./
 4 drwxrwxrwx 1 guest staff 4096 Nov 17 2016 ../
0 drwxrwxrwx 1 guest staff 0 Nov 17 2016 Icons/
4 drwxrwxrwx 1 guest staff 4096 Nov 17 2016 Images/
4 -rwxrwxrwx 1 guest staff 3839 Nov 17 2016 fastqc.fo*
 9 -rwxrwxrwx 1 guest staff 9197 Nov 17 2016 fastqc_data.txt*
255 -rwxrwxrwx 1 guest staff 261047 Nov 17 2016 fastqc report.html*
  1 -rwxrwxrwx 1 guest staff 406 Nov 17 2016 summary.txt*
guest@14fd5fadf014:/coursehome$ less fastqc/reads_fastqc/fastqc_data.txt
guest@14fd5fadf014:/coursehome$ gunzip --suffix=zip data/chr17.zip
guest@14fd5fadf014:/coursehome$ mv data/chr17 data/chr17.fa
guest@14fd5fadf014:/coursehome$ time bowtie-build data/chr17.fa data/hg19_chr17
  Output files: "data/hg19_chr17.*.ebwt"
  Line rate: 6 (line is 64 bytes)
  Lines per side: 1 (side is 64 bytes)
  Offset rate: 5 (one in 32)
  FTable chars: 10
  Strings: unpacked
  Max bucket size: default
  Max bucket size, sqrt multiplier: default
  Max bucket size, len divisor: 4
  Difference-cover sample period: 1024
  Endianness: little
  Actual local endianness: little
  Sanity checking: disabled
  Assertions: disabled
  Random seed: 0
  Sizeofs: void*:8, int:4, long:8, size_t:8
Input files DNA, FASTA:
  data/chr17.fa
Reading reference sizes
  Time reading reference sizes: 00:00:01
Calculating joined length
Writing header
Reserving space for joined string
Joining reference sequences
  Time to join reference sequences: 00:00:01
bmax according to bmaxDivN setting: 19448802
Using parameters --bmax 14586602 --dcv 1024
  Doing ahead-of-time memory usage test
  Passed! Constructing with these parameters: --bmax 14586602 --dcv 1024
Constructing suffix-array element generator
Building DifferenceCoverSample
  Building sPrime
  Building sPrimeOrder
  V-Sorting samples
  V-Sorting samples time: 00:00:01
  Allocating rank array
  Ranking v-sort output
  Ranking v-sort output time: 00:00:01
  Invoking Larsson-Sadakane on ranks
  Invoking Larsson-Sadakane on ranks time: 00:00:01
```

```
Sanity-checking and returning
Building samples
Reserving space for 12 sample suffixes
Generating random suffixes
QSorting 12 sample offsets, eliminating duplicates
QSorting sample offsets, eliminating duplicates time: 00:00:00
Multikey QSorting 12 samples
  (Using difference cover)
 Multikey QSorting samples time: 00:00:00
Calculating bucket sizes
  Binary sorting into buckets
  10%
  20%
  30%
  40%
  50%
  60%
  70%
  80%
  90%
  100%
  Binary sorting into buckets time: 00:00:03
Splitting and merging
 Splitting and merging time: 00:00:00
Split 1, merged 6; iterating...
  Binary sorting into buckets
  10%
  20%
  30%
  40%
  50%
  60%
  70%
  80%
  90%
  100%
                                  **[Lots of output omitted]**
Wrote 26421883 bytes to primary EBWT file: data/hg19_chr17.rev.1.ebwt
Wrote 9724408 bytes to secondary EBWT file: data/hg19_chr17.rev.2.ebwt
Re-opening _in1 and _in2 as input streams
Returning from Ebwt constructor
Headers:
    len: 77795210
    bwtLen: 77795211
    sz: 19448803
    bwtSz: 19448803
    lineRate: 6
    linesPerSide: 1
    offRate: 5
    offMask: 0xffffffe0
    isaRate: -1
    isaMask: 0xffffffff
    ftabChars: 10
    eftabLen: 20
    eftabSz: 80
    ftabLen: 1048577
    ftabSz: 4194308
    offsLen: 2431101
    offsSz: 9724404
```

```
isaLen: 0
    isaSz: 0
    lineSz: 64
    sideSz: 64
    sideBwtSz: 56
    sideBwtLen: 224
    numSidePairs: 173651
    numSides: 347302
    numLines: 347302
    ebwtTotLen: 22227328
    ebwtTotSz: 22227328
    reverse: 0
Total time for backward call to driver() for mirror index: 00:00:55
real
        1m55.467s
        1m49.170s
user
       0m3.610s
sys
uest@14fd5fadf014:/coursehome$ bowtie -S data/hg19 chr17 data/reads.fq
data/bowtie.sam
# reads processed: 200000
# reads with at least one reported alignment: 197212 (98.61%)
# reads that failed to align: 2788 (1.39%)
Reported 197212 alignments to 1 output stream(s)
Note the number of reads that fail to align. In a later session we will
investigate NGS trimmer programs and see their impact upon this area. This is
simulated data and so has a fairly good alignment.
guest@14fd5fadf014:/coursehome$ sed 's/\t255\t/\t60\t/g' data/bowtie.sam >
data/bowtie mapq60.sam
guest@14fd5fadf014:/coursehome$ samtools view -bS data/bowtie mapq60.sam >
data/bowtie.bam
[samopen] SAM header is present: 1 sequences.
guest@14fd5fadf014:/coursehome$ samtools sort data/bowtie.bam data/bowtie.sorted
guest@14fd5fadf014:/coursehome$ ls data
Brca1Reads_1.1.fastq bowtie.bam bowtie.sorted.bam bowties.sorted.bam
hg19_chr17.1.ebwt hg19_chr17.3.ebwt hg19_chr17.rev.1.ebwt reads.fq
Brca1Reads_1.2.fastq bowtie.sam bowtie_mapq60.sam chr17.fa
hg19_chr17.2.ebwt hg19_chr17.4.ebwt hg19_chr17.rev.2.ebwt
guest@14fd5fadf014:/coursehome$ samtools index data/bowtie.sorted.bam
guest@14fd5fadf014:/coursehome$ samtools tview data/bowtie.sorted.bam
guest@14fd5fadf014:/coursehome$ samtools tview data/bowtie.sorted.bam
data/chr17.fa
[fai_load] build FASTA index.
guest@14fd5fadf014:/coursehome$ picard-tools AddOrReplaceReadGroups
I=data/bowtie.sorted.bam O=data/bowtie_final.bam SORT_ORDER=coordinate RGPU=na
RGID=1 RGLB=input RGPL=Illumina RGSM=Company CREATE_INDEX=True
OpenJDK 64-Bit Server VM warning: ignoring option MaxPermSize=256m; support was
removed in 8.0
[Thu Nov 17 15:14:58 UTC 2016] net.sf.picard.sam.AddOrReplaceReadGroups
INPUT=data/bowtie.sorted.bam OUTPUT=data/bowtie final.bam SORT ORDER=coordinate
RGID=1 RGLB=input RGPL=Illumina RGPU=na RGSM=Company CREATE
              VERBOSITY=INFO QUIET=false VALIDATION STRINGENCY=STRICT
INDEX=true
COMPRESSION LEVEL=5 MAX RECORDS IN RAM=500000 CREATE MD5 FILE=false
```

```
[Thu Nov 17 15:14:58 UTC 2016] Executing as guest@14fd5fadf014 on Linux 4.4.17-
boot2docker amd64; OpenJDK 64-Bit Server VM 1.8.0_111-8u111-b14-2ubuntu0.16.04.2-
b14; Picard version: 1.113() JdkDeflater
                             AddOrReplaceReadGroups Created read group ID=1
       2016-11-17 15:14:58
PL=Illumina LB=input SM=Company
[Thu Nov 17 15:15:03 UTC 2016] net.sf.picard.sam.AddOrReplaceReadGroups done.
Elapsed time: 0.08 minutes.
Runtime.totalMemory()=32440320
guest@14fd5fadf014:/coursehome$ picard-tools CreateSequenceDictionary
R=data/chr17.fa O=data/chr17.dict
OpenJDK 64-Bit Server VM warning: ignoring option MaxPermSize=256m; support was
removed in 8.0
[Thu Nov 17 15:19:56 UTC 2016] net.sf.picard.sam.CreateSequenceDictionary
REFERENCE=data/chr17.fa OUTPUT=data/chr17.dict
TRUNCATE_NAMES_AT_WHITESPACE=true NUM_SEQUENCES=2147483647 VERBOSITY=INFO
QUIET=false V
ALIDATION STRINGENCY=STRICT COMPRESSION LEVEL=5 MAX RECORDS IN RAM=500000
CREATE INDEX=false CREATE MD5 FILE=false
[Thu Nov 17 15:19:56 UTC 2016] Executing as guest@14fd5fadf014 on Linux 4.4.17-
boot2docker amd64; OpenJDK 64-Bit Server VM 1.8.0_111-8u111-b14-2ubuntu0.16.04.2-
b14; Picard version: 1.113() JdkDeflater
[Thu Nov 17 15:19:56 UTC 2016] net.sf.picard.sam.CreateSequenceDictionary done.
Elapsed time: 0.02 minutes.
Runtime.totalMemory()=113770496
guest@14fd5fadf014:/coursehome$ java -jar GenomeAnalysisTK.jar \
> -R data/chr17.fa \
> -T UnifiedGenotyper \
> -I data/bowtie final.bam \
-o data/bowtie snps.vcf \
> -L chr17:41,196,311-41,277,499
INFO 15:44:27,781 HelpFormatter - ------
-----
INFO 15:44:27,783 HelpFormatter - The Genome Analysis Toolkit (GATK) v3.5-0-
g36282e4, Compiled 2015/11/25 04:03:56
INFO 15:44:27,784 HelpFormatter - Copyright (c) 2010 The Broad Institute
INFO 15:44:27,784 HelpFormatter - For support and documentation go to
http://www.broadinstitute.org/gatk
INFO 15:44:27,789 HelpFormatter - Program Args: -R data/chr17.fa -T
UnifiedGenotyper -I data/bowtie final.bam -o data/bowtie snps.vcf -L
chr17:41,196,311-41,277,499
INFO 15:44:27,815 HelpFormatter - Executing as guest@14fd5fadf014 on Linux
4.4.17-boot2docker amd64; OpenJDK 64-Bit Server VM 1.8.0_111-8
u111-b14-2ubuntu0.16.04.2-b14.
INFO 15:44:27,817 HelpFormatter - Date/Time: 2016/11/18 15:44:27
INFO 15:44:27,817 HelpFormatter - -------
.....
INFO 15:44:27,818 HelpFormatter - ------
INFO 15:44:27,972 GenomeAnalysisEngine - Strictness is SILENT
INFO 15:44:28,161 GenomeAnalysisEngine - Downsampling Settings: Method:
BY SAMPLE, Target Coverage: 250
INFO 15:44:28,176 SAMDataSource$SAMReaders - Initializing SAMRecords in serial
INFO 15:44:28,242 SAMDataSource$SAMReaders - Done initializing BAM readers: total
time 0.07
INFO 15:44:28,282 IntervalUtils - Processing 81189 bp from intervals
INFO 15:44:28,394 GenomeAnalysisEngine - Preparing for traversal over 1 BAM files
```

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15:44:28,461 GenomeAnalysisEngine - Done preparing for traversal
     15:44:28,462 ProgressMeter - [INITIALIZATION COMPLETE; STARTING PROCESSING]
INFO
INFO 15:44:28,462 ProgressMeter -
                                                  processed
                                                                   time
1M
                  total | remaining
INFO 15:44:28,464 ProgressMeter -
                                         Location |
                                                        sites | elapsed |
sites | completed | runtime |
                               runtime
INFO 15:44:28,564 StrandBiasTest - SAM/BAM data was found. Attempting to use read
data to calculate strand bias annotations values.
WARN 15:44:28,565 InbreedingCoeff - Annotation will not be calculated.
InbreedingCoeff requires at least 10 unrelated samples.
INFO 15:44:28,566 StrandBiasTest - SAM/BAM data was found. Attempting to use read
data to calculate strand bias annotations values.
INFO 15:44:37,642 ProgressMeter -
                                                      81189.0
                                                                  9.0 s
       100.0%
                 9.0 s
                             0.0 s
INFO 15:44:37,643 ProgressMeter - Total runtime 9.18 secs, 0.15 min, 0.00 hours
INFO 15:44:37,643 MicroScheduler - 0 reads were filtered out during the traversal
out of approximately 127221 total reads (0.00%)
INFO 15:44:37,644 MicroScheduler - -> 0 reads (0.00% of total) failing
BadCigarFilter
INFO 15:44:37,650 MicroScheduler - -> 0 reads (0.00% of total) failing
BadMateFilter
INFO 15:44:37,651 MicroScheduler - -> 0 reads (0.00% of total) failing
DuplicateReadFilter
INFO 15:44:37,656 MicroScheduler -
                                     -> 0 reads (0.00% of total) failing
FailsVendorQualityCheckFilter
INFO 15:44:37,657 MicroScheduler -
                                     -> 0 reads (0.00% of total) failing
MalformedReadFilter
INFO 15:44:37,657 MicroScheduler -
                                     -> 0 reads (0.00% of total) failing
MappingQualityUnavailableFilter
INFO 15:44:37,657 MicroScheduler - -> 0 reads (0.00% of total) failing
NotPrimaryAlignmentFilter
INFO 15:44:37,657 MicroScheduler - -> 0 reads (0.00% of total) failing
UnmappedReadFilter
INFO 15:44:39,147 GATKRunReport - Uploaded run statistics report to AWS S3
guest@14fd5fadf014:/coursehome$ grep -v "^#" data/bowtie_snps.vcf | wc -1
guest@14fd5fadf014:/coursehome$ bwa index -a bwtsw data/chr17.fa
[bwa_index] Pack FASTA... 0.92 sec
[bwa index] Construct BWT for the packed sequence...
[BWTIncCreate] textLength=162390420, availableWord=23426280
[BWTIncConstructFromPacked] 10 iterations done. 38642372 characters processed.
[BWTIncConstructFromPacked] 20 iterations done. 71387764 characters processed.
[BWTIncConstructFromPacked] 30 iterations done. 100487892 characters processed.
[BWTIncConstructFromPacked] 40 iterations done. 126348132 characters processed.
[BWTIncConstructFromPacked] 50 iterations done. 149328740 characters processed.
[bwt_gen] Finished constructing BWT in 57 iterations.
[bwa_index] 49.63 seconds elapse.
[bwa_index] Update BWT... 0.64 sec
[bwa_index] Pack forward-only FASTA... 0.62 sec
[bwa index] Construct SA from BWT and Occ... 20.26 sec
[main] Version: 0.7.12-r1039
[main] CMD: bwa index -a bwtsw data/chr17.fa
[main] Real time: 73.621 sec; CPU: 72.060 sec
```

```
guest@14fd5fadf014:/coursehome$ bwa aln data/chr17.fa data/reads.fq >
data/bwa.sai
[bwa_aln] 17bp reads: max_diff = 2
[bwa_aln] 38bp reads: max_diff = 3
[bwa_aln] 64bp reads: max_diff = 4
[bwa_aln] 93bp reads: max_diff = 5
[bwa_aln] 124bp reads: max_diff = 6
[bwa aln] 157bp reads: max diff = 7
[bwa aln] 190bp reads: max diff = 8
[bwa_aln] 225bp reads: max_diff = 9
[bwa aln core] calculate SA coordinate... 39.04 sec
[bwa_aln_core] write to the disk... 0.08 sec
[bwa aln core] 200000 sequences have been processed.
[main] Version: 0.7.12-r1039
[main] CMD: bwa aln data/chr17.fa data/reads.fq
[main] Real time: 40.028 sec; CPU: 39.610 sec
guest@14fd5fadf014:/coursehome$ bwa samse -f data/bwa.sam data/chr17.fa
data/bwa.sai data/reads.fq -r "@RG\tID:1\tSM:1"
[bwa aln core] convert to sequence coordinate... 1.14 sec
[bwa_aln_core] refine gapped alignments... 0.20 sec
[bwa_aln_core] print alignments... 0.88 sec
[bwa_aln_core] 200000 sequences have been processed.
[main] Version: 0.7.12-r1039
[main] CMD: bwa samse -f data/bwa.sam -r @RG\tID:1\tSM:1 data/chr17.fa
data/bwa.sai data/reads.fq
[main] Real time: 3.577 sec; CPU: 2.600 sec
guest@14fd5fadf014:/coursehome$ samtools view -bS data/bwa.sam > data/bwa.bam
[samopen] SAM header is present: 1 sequences.
guest@14fd5fadf014:/coursehome$ samtools sort data/bwa.bam data/bwa.sorted
guest@14fd5fadf014:/coursehome$ Is data/bwa*
data/bwa.bam data/bwa.sai data/bwa.sam data/bwa.sorted.bam
guest@14fd5fadf014:/coursehome$ samtools index data/bwa.sorted.bam
guest@14fd5fadf014:/coursehome$ java -jar GenomeAnalysisTK.jar -R data/chr17.fa -T
UnifiedGenotyper -I data/bwa.sorted.bam -o data/bwa snp
s.vcf -L chr17:41,196,311-41,277,499
INFO 16:07:11,214 HelpFormatter - -------
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INFO 16:07:11,216 HelpFormatter - The Genome Analysis Toolkit (GATK) v3.5-0-
g36282e4, Compiled 2015/11/25 04:03:56
INFO 16:07:11,217 HelpFormatter - Copyright (c) 2010 The Broad Institute
INFO 16:07:11,218 HelpFormatter - For support and documentation go to
http://www.broadinstitute.org/gatk
INFO 16:07:11,225 HelpFormatter - Program Args: -R data/chr17.fa -T
UnifiedGenotyper -I data/bwa.sorted.bam -o data/bwa_snps.vcf -L chr17
:41,196,311-41,277,499
                                **[Ooutput omitted]**
INFO 16:07:20,251 MicroScheduler - -> 0 reads (0.00% of total) failing
BadCigarFilter
INFO 16:07:20,251 MicroScheduler - -> 0 reads (0.00% of total) failing
BadMateFilter
INFO 16:07:20,252 MicroScheduler - -> 0 reads (0.00% of total) failing
DuplicateReadFilter
INFO 16:07:20,252 MicroScheduler - -> 0 reads (0.00% of total) failing
FailsVendorQualityCheckFilter
```

```
MalformedReadFilter
INFO 16:07:20,253 MicroScheduler -
                                       -> 0 reads (0.00% of total) failing
MappingQualityUnavailableFilter
INFO 16:07:20,253 MicroScheduler -
                                       -> 0 reads (0.00% of total) failing
NotPrimaryAlignmentFilter
INFO 16:07:20,256 MicroScheduler - -> 0 reads (0.00% of total) failing
UnmappedReadFilter
INFO 16:07:26,793 GATKRunReport - Uploaded run statistics report to AWS S3
guest@14fd5fadf014:/coursehome$ grep -v "^#" data/bwa snps.vcf | wc -1
21
guest@14fd5fadf014:/coursehome$ grep -v "^#" data/bwa_snps.vcf | cut -f 1,2 | tr
"\t" ":" | sort > data/bwa_snps.txt
guest@14fd5fadf014:/coursehome$ grep -v "^#" data/bowtie_snps.vcf | cut -f 1,2 |
tr "\t" ":" | sort > data/bowtie_snps.txt
guest@14fd5fadf014:/coursehome$ sdiff data/bowtie_snps.txt data/bwa_snps.txt
chr17:41200031
chr17:41200036
chr17:41200040
chr17:41201130
                                                                 chr17:41201130
chr17:41201198
                                                                 chr17:41201198
chr17:41209153
                                                                 chr17:41209153
chr17:41215396
                                                                 chr17:41215396
chr17:41215756
                                                                 chr17:41215756
chr17:41216205
                                                                 chr17:41216205
chr17:41223265
                                                                 chr17:41223265
chr17:41226398
chr17:41226499
                                                                 chr17:41226499
                                                                 chr17:41228280
chr17:41228280
chr17:41228282
chr17:41228587
                                                                 chr17:41228587
chr17:41229773
                                                                 chr17:41229773
chr17:41237250
chr17:41237251
chr17:41237252
chr17:41237254
chr17:41244405
                                                                 chr17:41244405
chr17:41244436
                                                                 chr17:41244436
chr17:41244530
chr17:41244582
                                                                 chr17:41244582
chr17:41244779
chr17:41244780
chr17:41244781
                                                                 chr17:41244964
chr17:41244964
chr17:41245034
chr17:41245096
chr17:41245098
                                                                 chr17:41245900
chr17:41245900
chr17:41246000
chr17:41252687
                                                                 chr17:41252687
chr17:41252697
                                                                 chr17:41252697
chr17:41260789
chr17:41263566
                                                                 chr17:41263566
chr17:41266038
chr17:41271293
                                                                 chr17:41271293
chr17:41271294
chr17:41271305
```

-> 0 reads (0.00% of total) failing

INFO 16:07:20,253 MicroScheduler -

chr17:41276032

chr17:41276032