

## [Record] 180609 (Sat) snakemake --cores 12 map

**Notebook:** A01工作任务  
**Created:** 6/9/2018 6:42 PM **Updated:** 6/10/2018 9:29 AM  
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**Tags:** @2018 Duke Data+

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fix the path of picard in map.smk first

run the command

```
(env-py3) clintko@phl-biostats-ub1:/data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe$ snakemake --cores 12 map
Building DAG of jobs...
Using shell: /bin/bash
Provided cores: 12
Rules claiming more threads will be scaled down.
Job counts:
   count  jobs
     2    MergeBamAlignment
     2    STAR_align
     2    TagReadWithGeneExon
     2    bam_hist
     2    bead_errors_metrics
     1    map
     1    multiqc_star
     2    plot_knee_plot
     1    plot_yield
     2    sort_sam
    17
```

This message is strange.

```
rule STAR_align:
  input: data/Surface_naph_filtered.fastq.gz, /data/clintko/DropSeq_Lung/GRCm38_v2/STAR_INDEX/SA_49/SA
  output: data/Surface_naph/Aligned.out.bam
  log: data/Surface_naph/Log.final.out
  jobid: 15
  wildcards: sample=Surface_naph
  threads: 12

/data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/.snakemake/scripts/bpij4ovc.wrapper.py:18: SyntaxWarning: assertion is always true,
erhaps remove parentheses?
  assert(fq1 is not None, "input-> fq1 is a required input parameter")
/data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/.snakemake/scripts/bpij4ovc.wrapper.py:23: SyntaxWarning: assertion is always true,
erhaps remove parentheses?
  assert(len(fq1) == len(fq2), "input-> equal number of files required for fq1 and fq2")
```

after 5~10 min (I think), I have seen it had already move to the next step

```
/data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/.snakemake/scripts/bpij4ovc.wrapper.py:18: SyntaxWarning: assertion is always true,
erhaps remove parentheses?
  assert(fq1 is not None, "input-> fq1 is a required input parameter")
/data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/.snakemake/scripts/bpij4ovc.wrapper.py:23: SyntaxWarning: assertion is always true,
erhaps remove parentheses?
  assert(len(fq1) == len(fq2), "input-> equal number of files required for fq1 and fq2")
Finished job 15.
1 of 17 steps (6%) done

rule sort_sam:
  input: data/Surface_naph/Aligned.out.bam
  output: data/Surface_naph/Aligned.sorted.bam
  jobid: 37
  wildcards: sample=Surface_naph

[Sat Jun 09 18:57:51 EDT 2018] picard.sam.SortSam INPUT=data/Surface_naph/Aligned.out.bam OUTPUT=data/Surface_naph/Aligned.sorted.bam SORT_ORDER=
=queryname TMP_DIR=[/data/clintko/DropSeq_Lung/tmp] VERBOSITY=INFO QUIET=false VALIDATION_STRINGENCY=STRICT COMPRESSION_LEVEL=5 MAX_RECORDS_
N_RAM=500000 CREATE_INDEX=false CREATE_MD5_FILE=false GA4GH_CLIENT_SECRETS=client_secrets.json
[Sat Jun 09 18:57:51 EDT 2018] Executing as clintko@phl-biostats-ub1 on Linux 4.13.0-43-generic amd64; OpenJDK 64-Bit Server VM 1.8.0_171-b11-0ubuntu0.17.10.1-b11; Picard version: 2.10.3-SNAPSHOT
INFO 2018-06-09 18:59:33 SortSam Read 10,000,000 records. Elapsed time: 00:01:42s. Time for last 10,000,000: 102s. Last read position: 7:109,520,567
```

```
[Sat Jun 09 18:57:51 EDT 2018] picard.sam.SortSam INPUT=data/Surface_naph/Aligned.out.bam OUTPUT=data/Surface_naph/Aligned.sorted.bam SORT_ORDER=
=queryname TMP_DIR=[/data/clintko/DropSeq_Lung/tmp] VERBOSITY=INFO QUIET=false VALIDATION_STRINGENCY=STRICT COMPRESSION_LEVEL=5 MAX_RECORDS_IN
N_RAM=500000 CREATE_INDEX=false CREATE_MD5_FILE=false GA4GH_CLIENT_SECRETS=client_secrets.json
[Sat Jun 09 18:57:51 EDT 2018] Executing as clintko@ph1-biostats-ub1 on Linux 4.13.0-43-generic amd64; OpenJDK 64-Bit Server VM 1.8.0_171-8u171-
b11-0ubuntu0.17.10.1-b11; Picard version: 2.10.3-SNAPSHOT
INFO 2018-06-09 18:59:33 SortSam Read 10,000,000 records. Elapsed time: 00:01:42s. Time for last 10,000,000: 102s. Last read position: 7:109,520,567
INFO 2018-06-09 19:01:17 SortSam Read 20,000,000 records. Elapsed time: 00:03:26s. Time for last 10,000,000: 104s. Last read position: 1:24,615,981
INFO 2018-06-09 19:03:02 SortSam Read 30,000,000 records. Elapsed time: 00:05:10s. Time for last 10,000,000: 104s. Last read position: 2:84,641,967
INFO 2018-06-09 19:04:46 SortSam Read 40,000,000 records. Elapsed time: 00:06:55s. Time for last 10,000,000: 104s. Last read position: 1:36,366,119
INFO 2018-06-09 19:06:30 SortSam Read 50,000,000 records. Elapsed time: 00:08:38s. Time for last 10,000,000: 103s. Last read position: 11:48,805,613
INFO 2018-06-09 19:08:13 SortSam Read 60,000,000 records. Elapsed time: 00:10:22s. Time for last 10,000,000: 103s. Last read position: 2:32,963,569
INFO 2018-06-09 19:09:57 SortSam Read 70,000,000 records. Elapsed time: 00:12:06s. Time for last 10,000,000: 103s. Last read position: 17:33,824,342
INFO 2018-06-09 19:11:30 SortSam Finished reading inputs, merging and writing to output now.
INFO 2018-06-09 19:13:01 SortSam Wrote 10,000,000 records from a sorting collection. Elapsed time: 00:15:10s. Time for last 10,000,000: 89s. Last read position: 7:45,122,453
INFO 2018-06-09 19:15:00 SortSam Wrote 20,000,000 records from a sorting collection. Elapsed time: 00:17:08s. Time for last 10,000,000: 118s. Last read position: 7:90,441,976
INFO 2018-06-09 19:16:58 SortSam Wrote 30,000,000 records from a sorting collection. Elapsed time: 00:19:06s. Time for last 10,000,000: 118s. Last read position: 9:25,101,348
INFO 2018-06-09 19:18:43 SortSam Wrote 40,000,000 records from a sorting collection. Elapsed time: 00:20:52s. Time for last 10,000,000: 105s. Last read position: 4:117,967,849
INFO 2018-06-09 19:20:39 SortSam Wrote 50,000,000 records from a sorting collection. Elapsed time: 00:22:48s. Time for last 10,000,000: 116s. Last read position: 3:37,715,039
INFO 2018-06-09 19:22:35 SortSam Wrote 60,000,000 records from a sorting collection. Elapsed time: 00:24:44s. Time for last 10,000,000: 116s. Last read position: MT:6,755
INFO 2018-06-09 19:24:17 SortSam Wrote 70,000,000 records from a sorting collection. Elapsed time: 00:26:26s. Time for last 10,000,000: 101s. Last read position: 14:49,713,271
[Sat Jun 09 19:26:04 EDT 2018] picard.sam.SortSam done. Elapsed time: 28.23 minutes.
Runtime.totalMemory()=442893568
Removing temporary output file data/Surface_naph/Aligned.out.bam.
Finished job 37.
2 of 17 steps (12%) done
```

```
rule MergeBamAlignment:
    input: data/Surface_naph_trimmed_unmapped.bam, data/Surface_naph/Aligned.sorted.bam, /data/clintko/DropSeq_Lung/GRCm38_v2/GRCm38.dict
    output: data/Surface_naph/Aligned.merged.bam
    jobid: 30
    wilddcards: sample=Surface_naph

[Sat Jun 09 19:26:06 EDT 2018] picard.sam.MergeBamAlignment UNMAPPED BAM=data/Surface_naph_trimmed_unmapped.bam ALIGNED BAM=[data/Surface_naph/Aligned.sorted.bam] OUTPUT=data/Surface_naph/Aligned.merged.bam REFERENCE_SEQUENCE=/data/clintko/DropSeq_Lung/GRCm38_v2/GRCm38.fasta PAIRED_RUN=false INCLUDE_SECONDARY_ALIGNMENTS=false CLIP_ADAPTERS=true IS_BISULFITE_SEQUENCE=false ALIGNED_READS_ONLY=false MAX_INSERTIONS_OR_DELETIONS=7 ATTRIBUTES_TO_REVERSE=[Q0, U2] ATTRIBUTES_TO_REVERSE_COMPLEMENT=[E2, S0] READ1_TRIM=0 READ2_TRIM=0 ALIGNER_PROPER_PAIR_FLAGS=false SORT_ORDER=coordinate PRIMARY_ALIGNMENT_STRATEGY=BestMapq CLIP_OVERLAPPING_READS=true ADD_MATE_CIGAR=true UNMAP_CONTAMINANT_READS=false MIN_UNCLIPPED_BASES=32 MATCHING_DICTIONARY_TAGS=[M5, LN] UNMAPPED_READ_STRATEGY=DO_NOT_CHANGE VERBOSITY=INFO QUIET=false VALIDATION_STRINGENCY=STRICT COMPRESSION_LEVEL=5 MAX_RECORDS_IN_RAM=500000 CREATE_INDEX=false CREATE_MD5_FILE=false GA4GH_CLIENT_SECRETS=client_secrets.json
[Sat Jun 09 19:26:06 EDT 2018] Executing as clintko@ph1-biostats-ub1 on Linux 4.13.0-43-generic amd64; OpenJDK 64-Bit Server VM 1.8.0_171-8u171-
b11-0ubuntu0.17.10.1-b11; Picard version: 2.10.3-SNAPSHOT
INFO 2018-06-09 19:26:06 SamAlignmentMerger Processing SAM file(s): [data/Surface_naph/Aligned.sorted.bam]
INFO 2018-06-09 19:26:25 AbstractAlignmentMerger Merged 1,000,000 records. Elapsed time: 00:00:18s. Time for last 1,000,000: 18s. Last read position: 11:96,953,694
INFO 2018-06-09 19:26:43 AbstractAlignmentMerger Merged 2,000,000 records. Elapsed time: 00:00:37s. Time for last 1,000,000: 18s. Last read position: 9:62,026,464
INFO 2018-06-09 19:27:06 AbstractAlignmentMerger Merged 3,000,000 records. Elapsed time: 00:01:00s. Time for last 1,000,000: 23s. Last read position: 7:27,133,572
INFO 2018-06-09 19:27:23 AbstractAlignmentMerger Merged 4,000,000 records. Elapsed time: 00:01:17s. Time for last 1,000,000: 17s. Last read position: 11:100,203,296
INFO 2018-06-09 19:27:46 AbstractAlignmentMerger Merged 5,000,000 records. Elapsed time: 00:01:39s. Time for last 1,000,000: 22s. Last read position: 6:66,930,244
INFO 2018-06-09 19:28:02 AbstractAlignmentMerger Merged 6,000,000 records. Elapsed time: 00:01:56s. Time for last 1,000,000: 16s. Last read position: 19:9,085,226
INFO 2018-06-09 19:28:19 AbstractAlignmentMerger Merged 7,000,000 records. Elapsed time: 00:02:13s. Time for last 1,000,000: 16s. Last read position: 2:37,836,448
INFO 2018-06-09 19:28:43 AbstractAlignmentMerger Merged 8,000,000 records. Elapsed time: 00:02:36s. Time for last 1,000,000: 23s. Last read position: 13:21,833,297
INFO 2018-06-09 19:28:59 AbstractAlignmentMerger Merged 9,000,000 records. Elapsed time: 00:02:53s. Time for last 1,000,000: 16s. Last read position: 10:120,224,719
INFO 2018-06-09 19:29:17 AbstractAlignmentMerger Merged 10,000,000 records. Elapsed time: 00:03:10s. Time for last 1,000,000: 17s. Last read position: 16:5,888,865
```

```

Time for last 10,000,000: 124s. Last read position: 10:63,022,740
INFO 2018-06-09 19:51:20 AbstractAlignmentMerger Written in coordinate order to output 40,000,000 records. Elapsed time: 00:08:09s.
Time for last 10,000,000: 124s. Last read position: 14:51,105,741
INFO 2018-06-09 19:53:25 AbstractAlignmentMerger Written in coordinate order to output 50,000,000 records. Elapsed time: 00:10:13s.
Time for last 10,000,000: 124s. Last read position: 19:10,502,675
INFO 2018-06-09 19:55:29 AbstractAlignmentMerger Written in coordinate order to output 60,000,000 records. Elapsed time: 00:12:17s.
Time for last 10,000,000: 124s. Last read position: */*
INFO 2018-06-09 19:56:17 AbstractAlignmentMerger Wrote 78586987 alignment records and 10204928 unmapped reads.
[Sat Jun 09 19:56:17 EDT 2018] picard.sam.MergeBamAlignment done. Elapsed time: 30.19 minutes.
Runtime.totalMemory()=2622488576
Removing temporary output file data/Surface_naph.Aligned.sorted.bam.
Finished job 30.
3 of 17 steps (18%) done

rule TagReadWithGeneExon:
  input: data/Surface_naph.Aligned.merged.bam, /data/clintko/DropSeq_Lung/GRCm38_v2/GRCm38.91.refFlat
  output: data/Surface_naph_gene_exon_tagged.bam
  jobid: 20
  wildcards: sample=Surface_naph

+ java -Xmx16g -Djava.io.tmpdir=/data/clintko/DropSeq_Lung/tmp -jar /data/clintko/DropSeq_Lung/Drop-seq tools-1.13/jar/dropseq.jar TagReadWithGeneExon INPUT=data/Surface_naph.Aligned.merged.bam OUTPUT=data/Surface_naph_gene_exon_tagged.bam ANNOTATIONS_FILE=/data/clintko/DropSeq_Lung/GRCm38_v2/GRCm38.91.refFlat TAG-GE CREATE_INDEX=true
[Sat Jun 09 19:56:18 EDT 2018] org.broadinstitute.dropseqrna.metrics.TagReadWithGeneExon INPUT=data/Surface_naph.Aligned.merged.bam OUTPUT=data/Surface_naph_gene_exon_tagged.bam TAG-GE ANNOTATIONS_FILE=/data/clintko/DropSeq_Lung/GRCm38_v2/GRCm38.91.refFlat CREATE_INDEX=true STRAND_TAG=GS FUNCTION TAG-XF USE_STRAND INFO=true ALLOW_MULTIPLE_GENE_READS=false VERBOSITY=INFO QUIET=false VALIDATION_STRINGENCY=STRICT COMPRESSION_LEVEL=5 MAX_RECORDS_IN_RAM=500000 CREATE_MD5_FILE=false GA4GH_CLIENT_SECRETS=client_secrets.json
[Sat Jun 09 19:56:18 EDT 2018] Executing as clintko@phl-biostats-ub1 on Linux 4.13.0-43-generic amd64; OpenJDK 64-Bit Server VM 1.8.0_171-8u171-b11-0ubuntu0.17.10.1-b11; Picard version: 1.13(7bed8f4_1513008033)
INFO 2018-06-09 19:56:29 TagReadWithGeneExon Processed 1,000,000 records. Elapsed time: 00:00:10s. Time for last 1,000,000: 9s. Last read position: 1:24,614,113
INFO 2018-06-09 19:56:39 TagReadWithGeneExon Processed 2,000,000 records. Elapsed time: 00:00:20s. Time for last 1,000,000: 10s. Last read position: 1:55,080,243
INFO 2018-06-09 19:56:50 TagReadWithGeneExon Processed 3,000,000 records. Elapsed time: 00:00:32s. Time for last 1,000,000: 11s. Last read position: 1:133,157,943
INFO 2018-06-09 19:57:14 TagReadWithGeneExon Processed 4,000,000 records. Elapsed time: 00:00:56s. Time for last 1,000,000: 12s. Last read position: 2:5,882,854
INFO 2018-06-09 19:57:33 TagReadWithGeneExon Processed 5,000,000 records. Elapsed time: 00:01:14s. Time for last 1,000,000: 13s. Last read position: 2:61,800,213
INFO 2018-06-09 19:57:44 TagReadWithGeneExon Processed 6,000,000 records. Elapsed time: 00:01:26s. Time for last 1,000,000: 14s. Last read position: 2:152,736,743
INFO 2018-06-09 19:58:04 TagReadWithGeneExon Processed 7,000,000 records. Elapsed time: 00:01:45s. Time for last 1,000,000: 15s. Last read position: 2:157,104,875
INFO 2018-06-09 19:58:14 TagReadWithGeneExon Processed 8,000,000 records. Elapsed time: 00:01:56s. Time for last 1,000,000: 16s. Last read position: */*
INFO 2018-06-09 20:10:55 TagReadWithGeneExon Processed 60,000,000 records. Elapsed time: 00:14:37s. Time for last 1,000,000: 17s. Last read position: */*
INFO 2018-06-09 20:11:02 TagReadWithGeneExon Processed 61,000,000 records. Elapsed time: 00:14:43s. Time for last 1,000,000: 18s. Last read position: */*
INFO 2018-06-09 20:11:08 TagReadWithGeneExon Processed 62,000,000 records. Elapsed time: 00:14:50s. Time for last 1,000,000: 19s. Last read position: */*
INFO 2018-06-09 20:11:28 TagReadWithGeneExon Processed 63,000,000 records. Elapsed time: 00:15:10s. Time for last 1,000,000: 20s. Last read position: */*
INFO 2018-06-09 20:11:36 TagReadWithGeneExon TOTAL READS [53400989] CORRECT_STRAND [51736108] WRONG_STRAND [677113] AMBIGUOUS_STRAND [1561360]
FIXED [1561360] AMBIGUOUS REJECTED READS [987768]
[Sat Jun 09 20:11:36 EDT 2018] org.broadinstitute.dropseqrna.metrics.TagReadWithGeneExon done. Elapsed time: 15.30 minutes.
Runtime.totalMemory()=1485307904
Removing temporary output file data/Surface_naph.Aligned.merged.bam.
Finished job 20.
4 of 17 steps (24%) done

rule bead_errors_metrics:
  input: data/Surface_naph_gene_exon_tagged.bam
  output: data/Surface_naph_final.bam
  jobid: 7
  wildcards: sample=Surface_naph

+ java -Xmx16g -Djava.io.tmpdir=/data/clintko/DropSeq_Lung/tmp -jar /data/clintko/DropSeq_Lung/Drop-seq tools-1.13/jar/dropseq.jar DetectBeadSynthesisErrors INPUT=data/Surface_naph_gene_exon_tagged.bam OUTPUT=data/Surface_naph_final.bam OUTPUT_STATS=logs/Surface_naph_synthesis_stats.txt SUMMARY=logs/Surface_naph_synthesis_stats_summary.txt NUM_BARCODES=4000 PRIMER_SEQUENCE=AATGATACGGCGACCACCGAGATCTACACGCTGTCCGCGGAAGCAGTGGTATCAACGAGATAC
[Sat Jun 09 20:11:38 EDT 2018] org.broadinstitute.dropseqrna.beadsynthesis.DetectBeadSynthesisErrors OUTPUT_STATS=logs/Surface_naph_synthesis_stats.txt INPUT=[data/Surface_naph_gene_exon_tagged.bam] SUMMARY=logs/Surface_naph_synthesis_stats_summary.txt OUTPUT=data/Surface_naph_final.bam PRIMER_SEQUENCE=AATGATACGGCGACCACCGAGATCTACACGCTGTCCGCGGAAGCAGTGGTATCAACGAGATAC NUM_BARCODES=4000 EDIT_DISTANCE=0 CELL_BARCODE_TAG=XC MOLECULAR_BARCODE_TAG=XM GENE_EXON_TAG=GE STRAND_TAG=GS READ MQ=10 MIN UMIS PER CELL=25 MAX_NUM_ERRORS=1 VERBOSITY=INFO QUIET=false VALIDATION_STRINGENCY=STRICT COMPRESSION_LEVEL=5 MAX_RECORDS_IN_RAM=500000 CREATE_INDEX=false CREATE_MD5_FILE=false GA4GH_CLIENT_SECRETS=client_secrets.json
[Sat Jun 09 20:11:38 EDT 2018] Executing as clintko@phl-biostats-ub1 on Linux 4.13.0-43-generic amd64; OpenJDK 64-Bit Server VM 1.8.0_171-8u171-b11-0ubuntu0.17.10.1-b11; Picard version: 1.13(7bed8f4_1513008033)
INFO 2018-06-09 20:11:38 AbstractDetectBeadSynthesisErrors Gathering barcodes for the top [4000] cells
WARNING: BAM index file /data/clintko/DropSeq_Lung/Drop-seq tools-1.13/dropSeqPipe/data/Surface_naph_gene_exon_tagged.bai is older than BAM /data/clintko/DropSeq_Lung/Drop-seq tools-1.13/dropSeqPipe/data/Surface_naph_gene_exon_tagged.bam
INFO 2018-06-09 20:11:56 BAMTagHistogram Processed 10,000,000 records. Elapsed time: 00:00:18s. Time for last 10,000,000: 18s. Last read position: 3:135,465,874
INFO 2018-06-09 20:12:16 BAMTagHistogram Processed 20,000,000 records. Elapsed time: 00:00:37s. Time for last 10,000,000: 19s. Last read position: 7:45,122,513
INFO 2018-06-09 20:12:35 BAMTagHistogram Processed 30,000,000 records. Elapsed time: 00:00:56s. Time for last 10,000,000: 19s. Last read position: 10:63,022,740
INFO 2018-06-09 20:12:54 BAMTagHistogram Processed 40,000,000 records. Elapsed time: 00:01:15s. Time for last 10,000,000: 19s. Last read position: 1:161,241,131
WARNING: BAM index file /data/clintko/DropSeq_Lung/Drop-seq tools-1.13/dropSeqPipe/data/Surface_naph_gene_exon_tagged.bai is older than BAM /data/clintko/DropSeq_Lung/Drop-seq tools-1.13/dropSeqPipe/data/Surface_naph_gene_exon_tagged.bam
INFO 2018-06-09 20:13:47 UMIIterator Processed 1,000,000 records. Elapsed time: 00:00:13s. Time for last 1,000,000: 13s. Last read position: 1:161,241,131

```

there are some strange warning

Besides those weird warnings, it seems that everything is ok

```

000: 6s. Last read position: X:106,019,664
INFO 2018-06-09 20:32:16 DetectBeadSynthesisErrors Processed 53,000,000 records. Elapsed time: 00:09:28s. Time for last 1,000
000: 18s. Last read position: MT:11,265
INFO 2018-06-09 20:32:22 DetectBeadSynthesisErrors Processed 54,000,000 records. Elapsed time: 00:09:34s. Time for last 1,000
000: 6s. Last read position: /*
INFO 2018-06-09 20:32:29 DetectBeadSynthesisErrors Processed 55,000,000 records. Elapsed time: 00:09:41s. Time for last 1,000
000: 6s. Last read position: /*
INFO 2018-06-09 20:32:48 DetectBeadSynthesisErrors Processed 56,000,000 records. Elapsed time: 00:10:00s. Time for last 1,000
000: 19s. Last read position: /*
INFO 2018-06-09 20:32:54 DetectBeadSynthesisErrors Processed 57,000,000 records. Elapsed time: 00:10:07s. Time for last 1,000
000: 6s. Last read position: /*
INFO 2018-06-09 20:33:01 DetectBeadSynthesisErrors Processed 58,000,000 records. Elapsed time: 00:10:13s. Time for last 1,000
000: 6s. Last read position: /*
INFO 2018-06-09 20:33:21 DetectBeadSynthesisErrors Processed 59,000,000 records. Elapsed time: 00:10:33s. Time for last 1,000
000: 19s. Last read position: /*
INFO 2018-06-09 20:33:27 DetectBeadSynthesisErrors Processed 60,000,000 records. Elapsed time: 00:10:39s. Time for last 1,000
000: 6s. Last read position: /*
INFO 2018-06-09 20:33:47 DetectBeadSynthesisErrors Processed 61,000,000 records. Elapsed time: 00:10:59s. Time for last 1,000
000: 19s. Last read position: /*
INFO 2018-06-09 20:33:53 DetectBeadSynthesisErrors Processed 62,000,000 records. Elapsed time: 00:11:06s. Time for last 1,000
000: 6s. Last read position: /*
INFO 2018-06-09 20:34:00 DetectBeadSynthesisErrors Processed 63,000,000 records. Elapsed time: 00:11:12s. Time for last 1,000
000: 6s. Last read position: /*
[Sat Jun 09 20:34:17 EDT 2018] org.broadinstitute.dropseqrna.beadsynthesis.DetectBeadSynthesisErrors done. Elapsed time: 22.66 minutes.
Runtime.totalMemory()=7057965056
Removing temporary output file data/Surface_naph_gene_exon_tagged.bam.
Finished job 7.
5 of 17 steps (29%) done

```

I wonder what this means? it appears at the start of mapping as well

```

rule STAR_align:
  input: data/Gland_homeo_filtered.fastq.gz, /data/clintko/DropSeq_Lung/GRCm38_v2/STAR_INDEX/SA_49/SA
  output: data/Gland_homeo/Aligned.out.bam
  log: data/Gland_homeo/Log.final.out
  jobid: 10
  wildcards: sample=Gland_homeo
  threads: 12

/data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/.snakemake/scripts/271sfnz.wrapper.py:18: SyntaxWarning: assertion is always true,
erhaps remove parentheses?
  assert(fq1 is not None, "input-> fq1 is a required input parameter")
/data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/.snakemake/scripts/271sfnz.wrapper.py:23: SyntaxWarning: assertion is always true,
erhaps remove parentheses?
  assert(len(fq1) == len(fq2), "input-> equal number of files required for fq1 and fq2")
Finished job 10.
6 of 17 steps (35%) done

```

```

rule bam_hist:
  input: data/Surface_naph_final.bam
  output: logs/Surface_naph_hist_out_cell.txt
  jobid: 4
  wildcards: sample=Surface_naph

rule sort_sam:
  input: data/Gland_homeo/Aligned.out.bam
  output: data/Gland_homeo/Aligned.sorted.bam
  jobid: 35
  wildcards: sample=Gland_homeo

```

```

localrule plot_yield:
  input: logs/Surface_naph_CELL_barcode.txt, logs/Gland_homeo_CELL_barcode.txt, logs/Surface_naph_UMI_barcode.txt, logs/Gland_homeo_UMI_barcode.txt, logs/Surface_naph_reads_left.txt, logs/Gland_homeo_reads_left.txt, data/Surface_naph/Log.final.out, data/Gland_homeo/Log.final.out, logs/Surface_naph_reads_left_trim.txt, logs/Gland_homeo_reads_left_trim.txt
  output: plots/yield.pdf
  jobid: 3

+ java -Xmx16g -Djava.io.tmpdir=/data/clintko/DropSeq_Lung/tmp -jar /data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/jar/dropseq.jar BAMTagHistogram TAG=XC I=data/Surface_naph_final.bam READ_QUALITY=10 O=logs/Surface_naph_hist_out_cell.txt

```

error occur when requiring multiqc



```

localrule multiqc_star:
    input: data/Surface_naph/Log.final.out, data/Gland_homeo/Log.final.out
    output: reports/star.html
    jobid: 5

[Sat Jun 09 20:42:48 EDT 2018] org.broadinstitute.dropseqrna.metrics.BAMTagHistogram INPUT=data/Surface_naph_final.bam OUTPUT=logs/Surface_naph
hist_out_cell.txt TAG=XC READ_QUALITY=10 FILTER_PCR_DUPLICATES=false VERBOSITY=INFO QUIET=false VALIDATION_STRINGENCY=STRICT COMPRESSION_LEV
L=5 MAX_RECORDS_IN_RAM=500000 CREATE_INDEX=false CREATE_MD5_FILE=false GA4GH_CLIENT_SECRETS=client_secrets.json
[Sat Jun 09 20:42:48 EDT 2018] Executing as clintko@phl-biostats-ub1 on Linux 4.13.0-43-generic amd64; OpenJDK 64-Bit Server VM 1.8.0_171-8u171
b11-0ubuntu0.17.10.1-b11; Picard version: 1.13(7bed8f4 1513008033)
[Sat Jun 09 20:42:48 EDT 2018] picard.sam.SortSam INPUT=data/Gland_homeo/Aligned.out.bam OUTPUT=data/Gland_homeo/Aligned.sorted.bam SORT_ORDER=
ueryname TMP_DIR=[/data/clintko/DropSeq_Lung/tmp] VERBOSITY=INFO QUIET=false VALIDATION_STRINGENCY=STRICT COMPRESSION_LEVEL=5 MAX_RECORDS_IN
RAM=500000 CREATE_INDEX=false CREATE_MD5_FILE=false GA4GH_CLIENT_SECRETS=client_secrets.json
[Sat Jun 09 20:42:48 EDT 2018] Executing as clintko@phl-biostats-ub1 on Linux 4.13.0-43-generic amd64; OpenJDK 64-Bit Server VM 1.8.0_171-8u171
b11-0ubuntu0.17.10.1-b11; Picard version: 2.10.3-SNAPSHOT
/bin/bash: multiqc: command not found
Traceback (most recent call last):
  File "/data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/.snakemake/scripts/_u1b9sn0.wrapper.py", line 24, in <module>
    "multiqc"
  File "/local_data/env-py3/lib/python3.6/site-packages/snakemake/shell.py", line 130, in __new__
    raise sp.CalledProcessError(retcode, cmd)
subprocess.CalledProcessError: Command 'set -euo pipefail; multiqc -m star --force -o reports -n star.html data/Gland_homeo data/Surface_naph
' returned non-zero exit status 127.
Error in rule multiqc_star:
  jobid: 5
  output: reports/star.html

RuleException:
CalledProcessError in line 62 of /data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/rules/map.smk:
Command 'set -euo pipefail; /local_data/env-py3/bin/python3 /data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/.snakemake/scripts/_u1
9sn0.wrapper.py' returned non-zero exit status 1.
  File "/data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/rules/map.smk", line 62, in __rule_multiqc_star
  File "/usr/lib/python3.6/concurrent/futures/thread.py", line 56, in run
Loading required package: viridisLite
Warning: Ignoring unknown parameters: binwidth, bins, pad
Warning: Ignoring unknown parameters: binwidth, bins, pad
pdf
2
Finished job 3.
7 of 17 steps (41%) done

```

This is the first error that appear so far

```

subprocess.CalledProcessError: Command 'set -euo pipefail; multiqc -m star --force -o reports -n star.html data/Gland_homeo data/Surface_naph
' returned non-zero exit status 127.
Error in rule multiqc_star:
  jobid: 5
  output: reports/star.html

RuleException:
CalledProcessError in line 62 of /data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/rules/map.smk:
Command 'set -euo pipefail; /local_data/env-py3/bin/python3 /data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/.snakemake/scripts/_u1
9sn0.wrapper.py' returned non-zero exit status 1.
  File "/data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/rules/map.smk", line 62, in __rule_multiqc_star
  File "/usr/lib/python3.6/concurrent/futures/thread.py", line 56, in run

```

```

subprocess.CalledProcessError: Command 'set -euo pipefail; multiqc -m star --force -o reports -n star.html data/Gland_homeo data/Surface_naph
' returned non-zero exit status 127.
Error in rule multiqc_star:
  jobid: 5
  output: reports/star.html

RuleException:
CalledProcessError in line 62 of /data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/rules/map.smk:
Command 'set -euo pipefail; /local_data/env-py3/bin/python3 /data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/.snakemake/scripts/_u1
9sn0.wrapper.py' returned non-zero exit status 1.
  File "/data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/rules/map.smk", line 62, in __rule_multiqc_star
  File "/usr/lib/python3.6/concurrent/futures/thread.py", line 56, in run
Loading required package: viridisLite
Warning: Ignoring unknown parameters: binwidth, bins, pad
Warning: Ignoring unknown parameters: binwidth, bins, pad
pdf
2
Finished job 3.
7 of 17 steps (41%) done
INFO 2018-06-09 20:43:07 BAMTagHistogram Processed 10,000,000 records. Elapsed time: 00:00:18s. Time for last 10,000,000: 18s. L
st read position: 3:135,466,484
INFO 2018-06-09 20:43:26 BAMTagHistogram Processed 20,000,000 records. Elapsed time: 00:00:37s. Time for last 10,000,000: 18s. L
st read position: 7:45,122,520
INFO 2018-06-09 20:43:44 BAMTagHistogram Processed 30,000,000 records. Elapsed time: 00:00:55s. Time for last 10,000,000: 18s. L
st read position: 10:67,014,984
INFO 2018-06-09 20:44:03 BAMTagHistogram Processed 40,000,000 records. Elapsed time: 00:01:14s. Time for last 10,000,000: 18s. L
st read position: 14:52,088,502
INFO 2018-06-09 20:44:21 BAMTagHistogram Processed 50,000,000 records. Elapsed time: 00:01:32s. Time for last 10,000,000: 18s. L
st read position: 19:11,482,001
INFO 2018-06-09 20:44:26 SortSam Read 10,000,000 records. Elapsed time: 00:01:38s. Time for last 10,000,000: 98s. Last read posi
ion: 17:23,900,655
INFO 2018-06-09 20:44:36 BAMTagHistogram Processed 60,000,000 records. Elapsed time: 00:01:47s. Time for last 10,000,000: 15s. L
st read position: */*
[Sat Jun 09 20:44:41 EDT 2018] org.broadinstitute.dropseqrna.metrics.BAMTagHistogram done. Elapsed time: 1.89 minutes.
Runtime.totalMemory()=2700083200
Finished job 4.
8 of 17 steps (47%) done
INFO 2018-06-09 20:46:06 SortSam Read 20,000,000 records. Elapsed time: 00:03:17s. Time for last 10,000,000: 99s. Last read posi
ion: 17:23,919,328
INFO 2018-06-09 20:47:46 SortSam Read 30,000,000 records. Elapsed time: 00:04:57s. Time for last 10,000,000: 99s. Last read posi
ion: 17:23,882,564
INFO 2018-06-09 20:49:26 SortSam Read 40,000,000 records. Elapsed time: 00:06:38s. Time for last 10,000,000: 100s. Last read posi
ion: 4:137,395,582
INFO 2018-06-09 20:51:06 SortSam Read 50,000,000 records. Elapsed time: 00:08:17s. Time for last 10,000,000: 99s. Last read posi

```

it turns out to run the pipeline, at least 20 GB memory is required. Below is the screenshot of command "top"

```

60243 clintko 20 0 20.310g 6.069g 20508 S 100.0 4.8 10:47.10 java
1647 root -51 0 0 0 0 S 6.0 0.0 308:47.26 irq/75-nvidia
1670 root -51 0 0 0 0 S 5.0 0.0 237:33.18 irq/90-nvidia
60444 clintko 20 0 46736 4272 3296 R 0.7 0.0 0:00.07 top
2392 gdm 20 0 614704 24388 18740 S 0.3 0.0 1:44.14 gsd-color
42192 wxiaoa 20 0 1954088 236140 132516 S 0.3 0.2 1:11.52 Web Content
60400 root 20 0 0 0 0 S 0.3 0.0 0:00.15 kworker/12:1
1 root 20 0 220628 8236 5572 S 0.0 0.0 0:06.36 systemd

```

This is strange. Why does it stop? The message mention the process stopped b/c of one error. The only error occurred is caused by multiqc, which help generating html file. I don't think this will cause problems during the preprocessing.

```

Finished job 4.
8 of 17 steps (47%) done
INFO 2018-06-09 20:46:06 SortSam Read 20,000,000 records. Elapsed time: 00:03:17s. Time for last 10,000,000: 99s. Last read posi
ion: 17:23,919,328
INFO 2018-06-09 20:47:46 SortSam Read 30,000,000 records. Elapsed time: 00:04:57s. Time for last 10,000,000: 99s. Last read posi
ion: 17:23,882,564
INFO 2018-06-09 20:49:26 SortSam Read 40,000,000 records. Elapsed time: 00:06:38s. Time for last 10,000,000: 100s. Last read posi
ion: 4:137,395,582
INFO 2018-06-09 20:51:06 SortSam Read 50,000,000 records. Elapsed time: 00:08:17s. Time for last 10,000,000: 99s. Last read posi
ion: 9:25,101,145
INFO 2018-06-09 20:52:45 SortSam Read 60,000,000 records. Elapsed time: 00:09:57s. Time for last 10,000,000: 99s. Last read posi
ion: 19:33,976,621
INFO 2018-06-09 20:54:25 SortSam Read 70,000,000 records. Elapsed time: 00:11:36s. Time for last 10,000,000: 99s. Last read posi
ion: 17:23,919,170
INFO 2018-06-09 20:56:05 SortSam Read 80,000,000 records. Elapsed time: 00:13:16s. Time for last 10,000,000: 100s. Last read posi
ion: 17:23,882,650
INFO 2018-06-09 20:56:19 SortSam Finished reading inputs, merging and writing to output now.
INFO 2018-06-09 20:57:47 SortSam Wrote 10,000,000 records from a sorting collection. Elapsed time: 00:14:58s. Time for last 10,000,
00: 86s. Last read position: 17:23,882,548
INFO 2018-06-09 20:59:42 SortSam Wrote 20,000,000 records from a sorting collection. Elapsed time: 00:16:53s. Time for last 10,000,
00: 115s. Last read position: 7:90,441,976
INFO 2018-06-09 21:01:26 SortSam Wrote 30,000,000 records from a sorting collection. Elapsed time: 00:18:37s. Time for last 10,000,
00: 103s. Last read position: 11:120,729,738
INFO 2018-06-09 21:03:01 SortSam Wrote 40,000,000 records from a sorting collection. Elapsed time: 00:20:12s. Time for last 10,000,
00: 95s. Last read position: 7:90,441,976
INFO 2018-06-09 21:04:54 SortSam Wrote 50,000,000 records from a sorting collection. Elapsed time: 00:22:06s. Time for last 10,000,
00: 113s. Last read position: 17:23,882,582
INFO 2018-06-09 21:06:34 SortSam Wrote 60,000,000 records from a sorting collection. Elapsed time: 00:23:45s. Time for last 10,000,
00: 99s. Last read position: 19:33,976,491
INFO 2018-06-09 21:08:26 SortSam Wrote 70,000,000 records from a sorting collection. Elapsed time: 00:25:38s. Time for last 10,000,
00: 112s. Last read position: 5:146,835,574
INFO 2018-06-09 21:10:04 SortSam Wrote 80,000,000 records from a sorting collection. Elapsed time: 00:27:16s. Time for last 10,000,
00: 98s. Last read position: 17:23,882,548
[Sat Jun 09 21:10:23 EDT 2018] picard.sam.SortSam done. Elapsed time: 27.58 minutes.
Runtime.totalMemory()=4827119616
Removing temporary output file data/Gland_homeo/Aligned.out.bam.
Finished job 35.
9 of 17 steps (53%) done
Shutting down, this might take some time.
Exiting because a job execution failed. Look above for error message
Complete log: /data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/.snakemake/log/2018-06-09T184909.666092.snakemake.log
(env-py3) clintko@phl-biostats-ub1: /data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe$

```

**I rerun the snakemake mapping. Notice that the job counts are different than the first time I executed. I wondered why?**

```

(env-py3) clintko@phl-biostats-ub1: /data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe$ snakemake --cores 12 map
Building DAG of jobs...
Using shell: /bin/bash
Provided cores: 12
Rules claiming more threads will be scaled down.
Job counts:
  count  jobs
   1     MergeBamAlignment
   1     TagReadWithGeneExon
   1     bam_hist
   1     bead_errors_metrics
   1     map
   1     multiqc_star
   2     plot_knee_plot
   8
localrule multiqc_star:
  input: data/Surface_naph/Log.final.out, data/Gland_homeo/Log.final.out
  output: reports/star.html
  jobid: 6
localrule plot_knee_plot:
  input: logs/Surface_naph_hist_out_cell.txt
  output: plots/Surface_naph_knee_plot.pdf
  jobid: 8
  wildcards: sample=Surface_naph
rule MergeBamAlignment:
  input: data/Gland_homeo.trimmed_unmapped.bam, data/Gland_homeo.Aligned.sorted.bam, /data/clintko/DropSeq_Lung/GRCm38_v2/GRCm38.dict
  output: data/Gland_homeo.Aligned.merged.bam
  jobid: 23
  wildcards: sample=Gland_homeo

```

it encounter the error very quick

```

rule MergeBamAlignment:
    input: data/Gland_homeo_trimmed_unmapped.bam, data/Gland_homeo.Aligned.sorted.bam, /data/clintko/DropSeq_Lung/GRCm38_v2/GRCm38.dict
    output: data/Gland_homeo.Aligned.merged.bam
    jobid: 23
    wildcards: sample=Gland_homeo

[Sat Jun 09 21:19:36 EDT 2018] picard.sam.MergeBamAlignment UNMAPPED BAM=data/Gland_homeo_trimmed_unmapped.bam ALIGNED BAM=[data/Gland_homeo.Aligned.sorted.bam] OUTPUT=data/Gland_homeo.Aligned.merged.bam REFERENCE_SEQUENCE=/data/clintko/DropSeq_Lung/GRCm38_v2/GRCm38.fasta PAIRED_RUN=false INCLUDE_SECONDARY_ALIGNMENTS=false CLIP_ADAPTERS=true IS_BISULFITE_SEQUENCE=false ALIGNED_READS_ONLY=false MAX_INSERTIONS_OR_DELETIONS=1 ATTRIBUTES_TO_REVERSE=[OQ, U2] ATTRIBUTES_TO_REVERSE_COMPLEMENT=[E2, SQ] READ1_TRIM=0 READ2_TRIM=0 ALIGNER_PROPER_PAIR_FLAGS=false SORT_ORDER=coordinate PRIMARY_ALIGNMENT_STRATEGY=BestMapq CLIP_OVERLAPPING_READS=true ADD_MATE_CIGAR=true UNMAP_CONTAMINANT_READS=false MIN_UNCLIPPED_BASES=32 MATCHING_DICTIONARY_TAGS=[M5, LN] UNMAPPED_READ_STRATEGY=DO_NOT_CHANGE VERBOSITY=INFO QUIET=false VALIDATION_STRINGENCY=STRICT COMPRESSION_LEVEL=5 MAX_RECORDS_IN_RAM=500000 CREATE_INDEX=false CREATE_MD5_FILE=false GA4GH_CLIENT_SECRETS=client_secrets.json
[Sat Jun 09 21:19:36 EDT 2018] Executing as clintko@phl-biostats-ub1 on Linux 4.13.0-43-generic amd64; OpenJDK 64-Bit Server VM 1.8.0_171-b11-0ubuntu0.17.10.1-b11; Picard version: 2.10.3-SNAPSHOT
/bin/bash: multiqc: command not found
Traceback (most recent call last):
  File "/data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/.snakemake/scripts/ln8qpr6b.wrapper.py", line 24, in <module>
    "multiqc"
  File "/local_data/env-py3/lib/python3.6/site-packages/snakemake/shell.py", line 130, in __new__
    raise sp.CallProcessError(retcode, cmd)
subprocess.CallProcessError: Command 'set -euo pipefail; multiqc -m star --force -o reports -n star.html data/Surface_naph data/Gland_homeo' returned non-zero exit status 127.
Error in rule multiqc_star:
  jobid: 6
  output: reports/star.html

RuleException:
CalledProcessError in line 62 of /data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/rules/map.smk:
Command 'set -euo pipefail; /local_data/env-py3/bin/python3 /data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/.snakemake/scripts/ln8qpr6b.wrapper.py' returned non-zero exit status 1.
  File "/data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/rules/map.smk", line 62, in __rule_multiqc_star
  File "/usr/lib/python3.6/concurrent/futures/thread.py", line 56, in run
INFO 2018-06-09 21:19:36 SamAlignmentMerger Processing SAM file(s): [data/Gland_homeo.Aligned.sorted.bam]
Warning message:
Removed 195968 rows containing missing values (geom_point).
Finished job 8.
1 of 8 steps (12%) done

```

It reaches to the end quickly (I mean it only reach to the 2 of 8 steps

```

INFO 2018-06-09 21:37:32 AbstractAlignmentMerger Merged 58,000,000 records. Elapsed time: 00:17:55s. Time for last 1,000,000: 16s
Last read position: 17:23,919,325
INFO 2018-06-09 21:39:37 AbstractAlignmentMerger Written in coordinate order to output 10,000,000 records. Elapsed time: 00:01:59s.
Time for last 10,000,000: 118s. Last read position: 5:115,345,746
INFO 2018-06-09 21:41:44 AbstractAlignmentMerger Written in coordinate order to output 20,000,000 records. Elapsed time: 00:04:05s.
Time for last 10,000,000: 126s. Last read position: 9:89,699,346
INFO 2018-06-09 21:43:47 AbstractAlignmentMerger Written in coordinate order to output 30,000,000 records. Elapsed time: 00:06:09s.
Time for last 10,000,000: 123s. Last read position: 12:55,490,460
INFO 2018-06-09 21:46:02 AbstractAlignmentMerger Written in coordinate order to output 40,000,000 records. Elapsed time: 00:08:24s.
Time for last 10,000,000: 135s. Last read position: 17:23,882,624
INFO 2018-06-09 21:47:50 AbstractAlignmentMerger Written in coordinate order to output 50,000,000 records. Elapsed time: 00:10:11s.
Time for last 10,000,000: 107s. Last read position: 17:31,141,180
INFO 2018-06-09 21:50:08 AbstractAlignmentMerger Written in coordinate order to output 60,000,000 records. Elapsed time: 00:12:29s.
Time for last 10,000,000: 138s. Last read position: */*
INFO 2018-06-09 21:51:53 AbstractAlignmentMerger Wrote 81100041 alignment records and 10914531 unmapped reads.
[Sat Jun 09 21:51:53 EDT 2018] picard.sam.MergeBamAlignment done. Elapsed time: 32.29 minutes.
Runtime.totalMemory()=835190784
Removing temporary output file data/Gland_homeo.Aligned.sorted.bam.
Finished job 23.
2 of 8 steps (25%) done
Shutting down, this might take some time.
Exiting because a job execution failed. Look above for error message
Complete log: /data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe/.snakemake/log/2018-06-09T211933.817711.snakemake.log
(env-py3) clintko@phl-biostats-ub1:/data/clintko/DropSeq_Lung/Drop-seq_tools-1.13/dropSeqPipe$

```