Demultiplexing Summary

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Demultiplexing Script

The runtime for my script was about 14 hours. This is likely attributed to Python's sluggish rate of gzipping files. It is also a probably a consequence of checking the index lines for each filtering step.

```
#!/usr/bin/env python3
# module load python3/3.6.1
import argparse
import gzip
def get_arguments():
    parser = argparse.ArgumentParser(description="Allows users to know how to navigate this program")
    parser.add_argument("-r1", "--read1", help="Forward Read File", required=True, type=str)
    parser.add_argument("-i1", "--index1", help="Forward Index File", required=True, type=str)
    parser.add_argument("-i2", "--index2", help="Reverse Index File", required=True, type=str)
    parser.add_argument("-r2", "--read2", help="Reverse Read File", required=True, type=str)
    parser.add_argument("-qs", "--quality_score", help="Cutoff for quality score", required=True, type=int)
    parser.add_argument("-I", "--Index_library", help="Input index library. Indexes must be in the 5th column", required=True, t
    return parser.parse_args()
################################
###### Argparse Variables ######
################################
args = get_arguments()
r1 = args.read1
i1 = args.index1
i2 = args.index2
r2 = args.read2
qscore = args.quality_score
indexes = args.Index_library
################################
#### Higher order functions ####
################################
def reverse_complement(seq):
   """Returns the reverse complement of a DNA sequence"""
   complement = {'A': 'T', 'C': 'G', 'G': 'C', 'T': 'A', 'N':'N'}
   return ''.join([complement[base] for base in seq[::-1]])
def convert_phred(letter):
    """Converts a single character into a phred score"""
    return(ord(letter)-33)
################################
### Isolate Indexes from file ###
################################
index_list = []
with open(indexes) as i:
    for col in i:
        col = col.strip("\n").split("\t")
        index_list.append(col[4])
index_list = index_list[1:] # remove the header line
################################
# Open and Create Output Files #
################################
ctr_dict = {}
F_index_dict = {}
R_index_dict = {}
for index in index_list:
    Forward = gzip.open(index + "_forward.gz","wt")
    Reverse = gzip.open(index + "_reverse.gz","wt")
    F_index_dict[index] = Forward
    R_index_dict[index] = Reverse
    ctr_dict[index] = 0
bad_ctr = 0
LQ_Fz = gzip.open("bad_reads_forward.gz", "wt")
```

```
LQ_Rz = gzip.open("bad_reads_reverse.gz", "wt")
################################
### Bad Read Filter Counters ###
################################
undetermined = 0
low_qscore = 0
false_read = 0
index_hopping = 0
################################
# Open Input Files, Sort Reads #
################################
total_count = 0
with gzip.open(r1, "rt") as r1, gzip.open(i1, "rt") as i1, gzip.open(i2, "rt") as i2, gzip.open(r2, "rt") as r2:
    while True: # keep looping until the break

        r_header1 = r1.readline()
        r_seq1 = r1.readline()
        r_plus1 = r1.readline().strip()
        r_qscore1 = r1.readline()

        # set condition for when the loop finally ends so that there isn't an error
        if r_header1 == '':
            break

        total_count += 1

        i_header1 = i1.readline()
        i_seq1= i1.readline()
        i_plus1 = i1.readline()
        i_qscore1 = i1.readline()

        i_header2 = i2.readline()
        i_seq2 = i2.readline()
        i_plus2 = i2.readline()
        i_qscore2 = i2.readline()

        r_header2 = r2.readline()
        r_seq2 = r2.readline()
        r_plus2 = r2.readline().strip()
        r_qscore2 = r2.readline()

        isBad = False

        # filter out indexes with undetermined nucleotides
        if "N" in i_seq1 or "N" in i_seq2:
            isBad = True
            undetermined += 1
            r_plus1 += "Undetermined | "
            r_plus2 += "Undetermined | "

        # calculate the mean qscore of the index sequence
        converted_NT = []
        for NT in i_qscore1:
            converted_NT.append(convert_phred(NT))
        converted_NT_mean = sum(converted_NT) / len(converted_NT)

        # filter out indexes with a low mean qscore
        if converted_NT_mean < qscore:
            isBad = True
            low_qscore += 1
            r_plus1 += "Low Quality | "
            r_plus2 += "Low Quality | "

        # filter out indexes that do not appear in the index library
        if i_seq1.strip() not in index_list and reverse_complement(i_seq2.strip()) not in index_list:
            isBad = True
            false_read += 1
```

```
            r_plus1 += "Index not recognized | "
            r_plus2 += "Index not recognized | "

        # filter and track occurrences of index hopping
        else:
            if reverse_complement(i_seq1.strip()) != i_seq2.strip():
                isBad = True
                index_hopping += 1
                r_plus1 += "Index hopping | "
                r_plus2 += "Index hopping | "

        # build final bad output files
        if isBad:
            LQ_Fz.write(r_header1.strip()+':'+i_seq1.strip()+'\n')
            LQ_Fz.write(r_seq1)
            LQ_Fz.write(r_plus1 + '\n')
            LQ_Fz.write(r_qscore1)

            LQ_Rz.write(r_header2.strip()+':'+i_seq2.strip()+'\n')
            LQ_Rz.write(r_seq2)
            LQ_Rz.write(r_plus2 + '\n')
            LQ_Rz.write(r_qscore2)

            bad_ctr += 1

        # once all the bad reads have been filtered out, write good reads to their respective files
        if isBad == False:

            F_index_dict[i_seq1.strip()].write(r_header1.strip()+':'+i_seq1.strip()+'\n')
            F_index_dict[i_seq1.strip()].write(r_seq1)
            F_index_dict[i_seq1.strip()].write(r_plus1 + '\n')
            F_index_dict[i_seq1.strip()].write(r_qscore1)

            R_index_dict[i_seq1.strip()].write(r_header2.strip()+':'+i_seq2.strip()+'\n')
            R_index_dict[i_seq1.strip()].write(r_seq2)
            R_index_dict[i_seq1.strip()].write(r_plus2 + '\n')
            R_index_dict[i_seq1.strip()].write(r_qscore2)

            ctr_dict[i_seq1.strip()] += 1
################################
###### Close Output Files ######
################################

for index in index_list:
    F_index_dict[index].close()
    R_index_dict[index].close()
LQ_Fz.close()
LQ_Rz.close()
################################
## Relevant Output Statements ##
################################
print("INDEX", "\t", "RAW COUNT", "\t", "PROPORTION (%)")
for k in ctr_dict:
    print(k, "\t", ctr_dict[k], "\t", (ctr_dict[k]/total_count) * 100)

bad_proportion = (bad_ctr / total_count) * 100
print("Bad reads compose {}% of the entire file.".format(bad_proportion))
print("There are {} instances of indexes with undetermined NTs in their sequences.".format(undetermined))
print("There are {} instances of indexes with a mean phred quality score below {}.".format(low_qscore, qscore))
print("There are {} instances of indexes that are not included in the list of indexes.".format(false_read))
print("There are {} instances of index hoppping.".format(index_hopping))
```

Sbatch script submitted to Talapas

```
#!/usr/bin/env bash
```

```
#SBATCH --partition=long        ### Partition (like a queue in PBS)
#SBATCH --job-name=Demult          ### Job Name
#SBATCH --time=1-8:00:00       ### Wall clock time limit in Days-HH:MM:SS
#SBATCH --nodes=1               ### Number of nodes needed for the job
#SBATCH --ntasks-per-node=28     ### Number of tasks to be launched per Node
#SBATCH --mail-type=ALL
#SBATCH --mail-user=mrezzoni@uoregon.edu

R1="/projects/bgmp/shared/2017_sequencing/1294_S1_L008_R1_001.fastq.gz"
I1="/projects/bgmp/shared/2017_sequencing/1294_S1_L008_R2_001.fastq.gz"
I2="/projects/bgmp/shared/2017_sequencing/1294_S1_L008_R3_001.fastq.gz"
R2="/projects/bgmp/shared/2017_sequencing/1294_S1_L008_R4_001.fastq.gz"
I_table="/projects/bgmp/mrezzoni/Bi624/HW2/indexes.txt"

./demultiplexing_script.py -r1 $R1 -i1 $I1 -i2 $I2 -r2 $R2 -qs 30 -I $I_table
```

Test files

```
############## Altered Index List ##############

# sample    group   treatment   index   index sequence
# 1 2A  control B1  AAAAAAAA
# 2 2B  control A5  CGATCGAT
# 3 2B  control C1  GATCAAGG
# 4 2C  mbnl    B9  AACAGCGA
# 6 2D  mbnl    C9  TAGCCATG
# 7 2E  fox C3  CGGTAATC
# 8 2F  fox B3  CTCTGGAT
# 10    2G  both    C4  TACCGGAT
# 11    2H  both    A11 CTAGCTCA
# 14    3B  control C7  CACTTCAC
# 15    3C  mbnl    B2  GCTACTCT
# 16    3D  mbnl    A1  ACGATCAG
# 17    3E  fox B7  TATGGCAC
# 19    3F  fox A3  TGTTCCGT
# 21    3G  both    B4  GTCCTAAG
# 22    3H  both    A12 TCGACAAG
# 23    4A  control C10 TCTTCGAC
# 24    4A  control A2  ATCATGCG
# 27    4C  mbnl    C2  ATCGTGGT
# 28    4D  mbnl    A10 TCGAGAGT
# 29    4E  fox B8  TCGGATTC
# 31    4F  fox A7  GATCTTGC
# 32    4G  both    B10 AGAGTCCA
# 34    4H  both    A8  AGGATAGC


############## Test Read 1 ##############

# @K00337:83:HJKJNBBXX:8:1101:1265:1191 1:N:0:1
# GNCTGGCATTCCCAGAGACATCAGTACCCAGTTGGTTCAGACAGTTCCTCTATTGGTTGACAAGGTCTTCATTTCTAGTGATATCAACACGGTGTCTACAA
# +
# A#A-<FJJJ<JJJJJJJJJJJJJJJFJJJJFFJJFJJJAJJJJ-AJJJJJJJFFJJJJJJFFA-7<AJJJFFAJJJJJF<F--JJJJJJF-A-F7JJJJ
# @K00337:83:HJKJNBBXX:8:1101:1286:1191 1:N:0:1
# CNACCTGTCCCCAGCTCACAGGACAGCACACCAAAGGCGGCAACCCACACCCAGTTTTACAGCCACACAGTGCCTTGTTTTACTTGAGGACCCCCCCACTCC
# +
# A#AAFJJJJJJJJJFJJJJJJJJJJJJJJJJJJJJJJFJJJJJJJJJJJJJJAJJJJJJJJJJJJJJFJJJJJFFFFJJJJJJJJJJJJJJJJ77F
# @K00337:83:HJKJNBBXX:8:1101:1347:1191 1:N:0:1
# GNGGTCTTCTACCTTTCTCTTCTTTTTTGGAGGAGTAGAATGTTGAGAGTCAGCAGTAGCCTCATCATCACTAGATGGCATTTCTTCTGAGCAAAACAGGT
# +
# A#AFFFJFJJFJJJJFJJJJJJJJAJJFJJJJJFJFJ7<FAFJJFJFJJFJFJJJFJAAJJJFJJJJJJJJJJJJJJAJJJFAJJJJJFFJJJAJJJ<F-
# @K00337:83:HJKJNBBXX:8:1101:1367:1191 1:N:0:1
# GNGCTCTTCCCCACACCATTGGGACCCACGATGCAAATCCGGGAGTCCATGTCGATGCCGAAATCTAGATTCTTAAAGAGTGGCTTCTGCCCCTCGTAGCC
# +
# A#<AAFJFJJJJFJJFJJ7JFJJJFJFAJJ<FF<<JJ<JJ<F<JJFAJJFFFJJJJJJA--77FJ--<<-AA<<AFJJJJJJFJJJFFFJ-<7--7-FFFA
# @K00337:83:HJKJNBBXX:8:1101:1407:1191 1:N:0:1
```

```
# TNCTAAAATAAATAAAATCCCTAGGTTTAAATTAATTAATGGGTGTGGTATTGGTAGGGGAACTCATAGACTTAATGCTAGTGTGAGTGATAGGGTAGGTG
# +
# A#A-FJJJJJJJJJJJJJJJJJJJJJJJJJJJJJFJJFJFJFJJJJJJJJJJFFJJJJJJFJJJJJJJJJJJJJJJJJFJ<FJJAFJJJF<J7FJJJF


############### Test Index 1 ###############

# @K00337:83:HJKJNBBXX:8:1101:1265:1191 2:N:0:1
# AAAAAAAA
# +
# #AA<FJJJ
# @K00337:83:HJKJNBBXX:8:1101:1286:1191 2:N:0:1
# AAAAAAAA
# +
# #AAAFJJJ
# @K00337:83:HJKJNBBXX:8:1101:1347:1191 2:N:0:1
# AAAAAAAA
# +
# #AAFFJAJ
# @K00337:83:HJKJNBBXX:8:1101:1367:1191 2:N:0:1
# AAAAAAAN
# +
# #AAAFJJ<
# @K00337:83:HJKJNBBXX:8:1101:1407:1191 2:N:0:1
# AAAAAAAA
# +
# #AAFFJJF


############### Test Index 2 ###############

# @K00337:83:HJKJNBBXX:8:1101:1265:1191 2:N:0:1
# TTTTTTTT
# +
# #AA<FJJJ
# @K00337:83:HJKJNBBXX:8:1101:1286:1191 2:N:0:1
# TTTTTTTT
# +
# #AAAFJJJ
# @K00337:83:HJKJNBBXX:8:1101:1347:1191 2:N:0:1
# TTTTTTTT
# +
# #AAFFJAJ
# @K00337:83:HJKJNBBXX:8:1101:1367:1191 2:N:0:1
# TTTTTTTT
# +
# #AAAFJJ<
# @K00337:83:HJKJNBBXX:8:1101:1407:1191 2:N:0:1
# TTTTTTTT
# +
# #AAFFJJF


############### Test Read 2 ###############

# @K00337:83:HJKJNBBXX:8:1101:1265:1191 4:N:0:1
# NTTTTGATTTACCTTTCAGCCAATGAGAAGGCCGTTCATGCAGACTTTTTTAATGATTTTGAAGACCTTTTTGATGATGATGATGTCCAGTGAGGCCTCCC
# +
# #AAFAFJJ-----F---7-<FA-F<AFFA-JJJ77<FJFJFJJJJJJJJJJAFJFFAJJJJJJJJFJF7-AFFJJ7F7JFJJFJ7FFF--A<A7<-A-7--
# @K00337:83:HJKJNBBXX:8:1101:1286:1191 4:N:0:1
# NTGTGTAGACAAAAGTTTTCATGAGTCTGTAAGCTGTCTATTGTCTCCTGAAAAGAAACCAGAAGTTTTCCCCTAAATGTGTTTAGAATGCTTATTCTAAT
# +
# #A-AFFJJFJJJJJJJJJJJJJJJ<JAJFJJJJF<JFJJJAJJJJJJJJJJJJJJJJJJFJJJAJJFJJJFJJJF<JJA-JJJ-<AFAF--FF<JAFJF
# @K00337:83:HJKJNBBXX:8:1101:1347:1191 4:N:0:1
# NAAATGCCATCTAGTGATGATGAGGCTACTGCTGACTCTCAACATTCTACTCCTCCAAAAAAGAAGAGAAAGATTCCAACCCCCAGAACCGATGACCGGCA
# +
# #AAFFJJJJJJJJJJJJJJJJJJJJJJJJJFJJJJJJJJFJJJJJJJJJJJFJJFJJJFJFJAJAF<7AJF<J--7AA7<FJ----7A-F-77-77------
# @K00337:83:HJKJNBBXX:8:1101:1367:1191 4:N:0:1
```

```
# NAGAAATTCAAGGTGCTTAAGGTTGGGGGCAATTCAACAAAGCAATAGGAAAAGCAAATAAAGGAAGGCCTGACTCGAAAACATCAGAATCATGTATGGAA
# +
# #A-7AF----<7---77--7<<<F-7---77F--77-<--7--<-----77<--<-<<<<<-7-7-7-F<<A-7-F-A-7-7------7-----7--77-7
# @K00337:83:HJKJNBBXX:8:1101:1407:1191 4:N:0:1
# NTAAGACTTAAAACCTTGTTCCCAGAGGTTCAAATCCTCTCCCTAATAGTGTTCTTTATTAATATCCTAACACTCCTCGTCCCCATTCTAATCGCCATACC
# +
# #AAFFJJJJJJJJJJJJJJJJJJFJJJF7FFJJFF7AJJFJJJJJJJJJJFJJJJJJJJJJJJFJJJJJJJJJFJJJJJJJFFFJFF7AFJF7FFJAJ-F
```

Output from test files

```
# INDEX      RAW COUNT   PROPORTION (%)
# AAAAAAAA   1   20.0
# CGATCGAT   0   0.0
# GATCAAGG   0   0.0
# AACAGCGA   0   0.0
# TAGCCATG   0   0.0
# CGGTAATC   0   0.0
# CTCTGGAT   0   0.0
# TACCGGAT   0   0.0
# CTAGCTCA   0   0.0
# CACTTCAC   0   0.0
# GCTACTCT   0   0.0
# ACGATCAG   0   0.0
# TATGGCAC   0   0.0
# TGTTCCGT   0   0.0
# GTCCTAAG   0   0.0
# TCGACAAG   0   0.0
# TCTTCGAC   0   0.0
# ATCATGCG   0   0.0
# ATCGTGGT   0   0.0
# TCGAGAGT   0   0.0
# TCGGATTC   0   0.0
# GATCTTGC   0   0.0
# AGAGTCCA   0   0.0
# AGGATAGC   0   0.0
# Bad reads compose 80.0% of the entire file.
# There are 1 instances of indexes with undetermined NTs in their sequences.
# There are 4 instances of indexes with a mean phred quality score below 30.
# There are 0 instances of indexes that are not included in the list of indexes.
# There are 1 instances of index hoppping.
```

Output from assigned samples

```
# INDEX      RAW COUNT   PROPORTION (%)
# GTAGCGTA   6423487     1.768353678388878
# CGATCGAT   4370218     1.203099045060928
# GATCAAGG   4877454     1.3427385658401032
# AACAGCGA   7247905     1.995311809203185 4
# TAGCCATG   8048298     2.2156559782980567
# CGGTAATC   3693609     1.0168319888683928
# CTCTGGAT   28060203    7.7248328192130895
# TACCGGAT   61542468    16.942332048765696
# CTAGCTCA   13196603    3.632958462792515
# CACTTCAC   3197050     0.880131792512877 7
# GCTACTCT   5162621     1.4212436073238208
# ACGATCAG   6186925     1.7032293490538877
# TATGGCAC   8622836     2.3738234013307786
# TGTTCCGT   10905086    3.0021153528055797
# GTCCTAAG   6461033     1.7786899034343697
# TCGACAAG   2947217     0.811354023595009
# TCTTCGAC   34754032    9.56761029111521
# ATCATGCG   7264829     1.999970901321384
# ATCGTGGT   4814723     1.3254690369068285
# TCGAGAGT   5608742     1.544058475845626
```

```
# TCGGATTC   2840779     0.7820521772893567
# GATCTTGC   2773588     0.7635548327777811
# AGAGTCCA   7709563     2.122403935716036
# AGGATAGC   6771157     1.8640654815520914
# Bad reads compose 30.218113040988513% of the entire file.
# There are 4205183 instances of indexes with undetermined NTs in their sequences.
# There are 99116240 instances of indexes with a mean phred quality score below 30.
# There are 7867981 instances of indexes that are not included in the list of indexes.
# There are 23623721 instances of index hoppping.
```

Files created after script completed

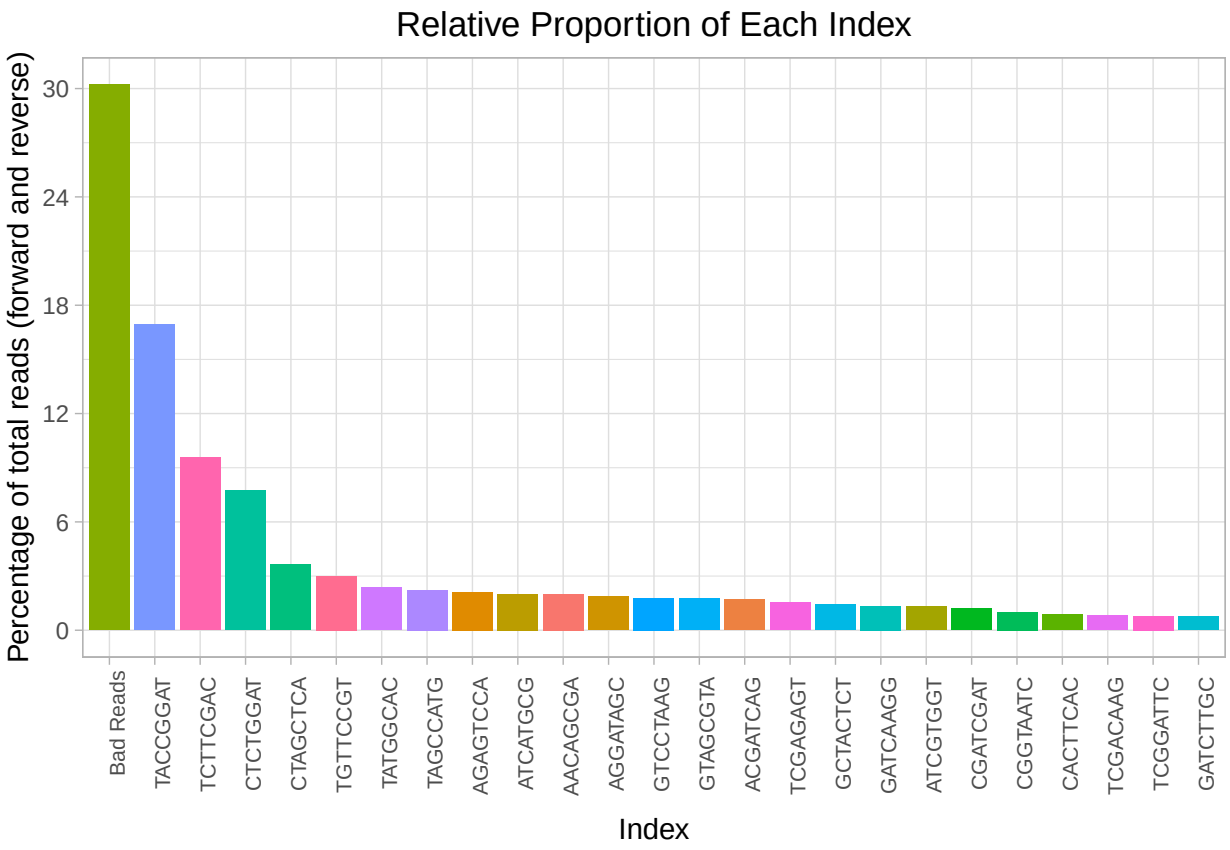
```
[mrezzoni@talapas-ln1 zipped_output]$ ls
AACAGCGA_forward.gz  ATCATGCG_forward.gz   CGATCGAT_forward.gz  GATCAAGG_forward.gz  GTCCTAAG_forward.gz  TCGACAAG_forward.gz  TGTTCCGT_forward.gz
AACAGCGA_reverse.gz  ATCATGCG_reverse.gz   CGATCGAT_reverse.gz  GATCAAGG_reverse.gz  GTCCTAAG_reverse.gz  TCGACAAG_reverse.gz  TGTTCCGT_reverse.gz
ACGATCAG_forward.gz  ATCGTGGT_forward.gz   CGGTAATC_forward.gz  GATCTTGC_forward.gz  TACCGGAT_forward.gz  TCGAGAGT_forward.gz
ACGATCAG_reverse.gz  ATCGTGGT_reverse.gz   CGGTAATC_reverse.gz  GATCTTGC_reverse.gz  TACCGGAT_reverse.gz  TCGAGAGT_reverse.gz
AGAGTCCA_forward.gz  bad_reads_forward.gz  CTAGCTCA_forward.gz  GCTACTCT_forward.gz  TAGCCATG_forward.gz  TCGGATTC_forward.gz
AGAGTCCA_reverse.gz  bad_reads_reverse.gz  CTAGCTCA_reverse.gz  GCTACTCT_reverse.gz  TAGCCATG_reverse.gz  TCGGATTC_reverse.gz
AGGATAGC_forward.gz  CACTTCAC_forward.gz   CTCTGGAT_forward.gz  GTAGCGTA_forward.gz  TATGGCAC_forward.gz  TCTTCGAC_forward.gz
AGGATAGC_reverse.gz  CACTTCAC_reverse.gz   CTCTGGAT_reverse.gz  GTAGCGTA_reverse.gz  TATGGCAC_reverse.gz  TCTTCGAC_reverse.gz
[mrezzoni@talapas-ln1 zipped_output]$
```

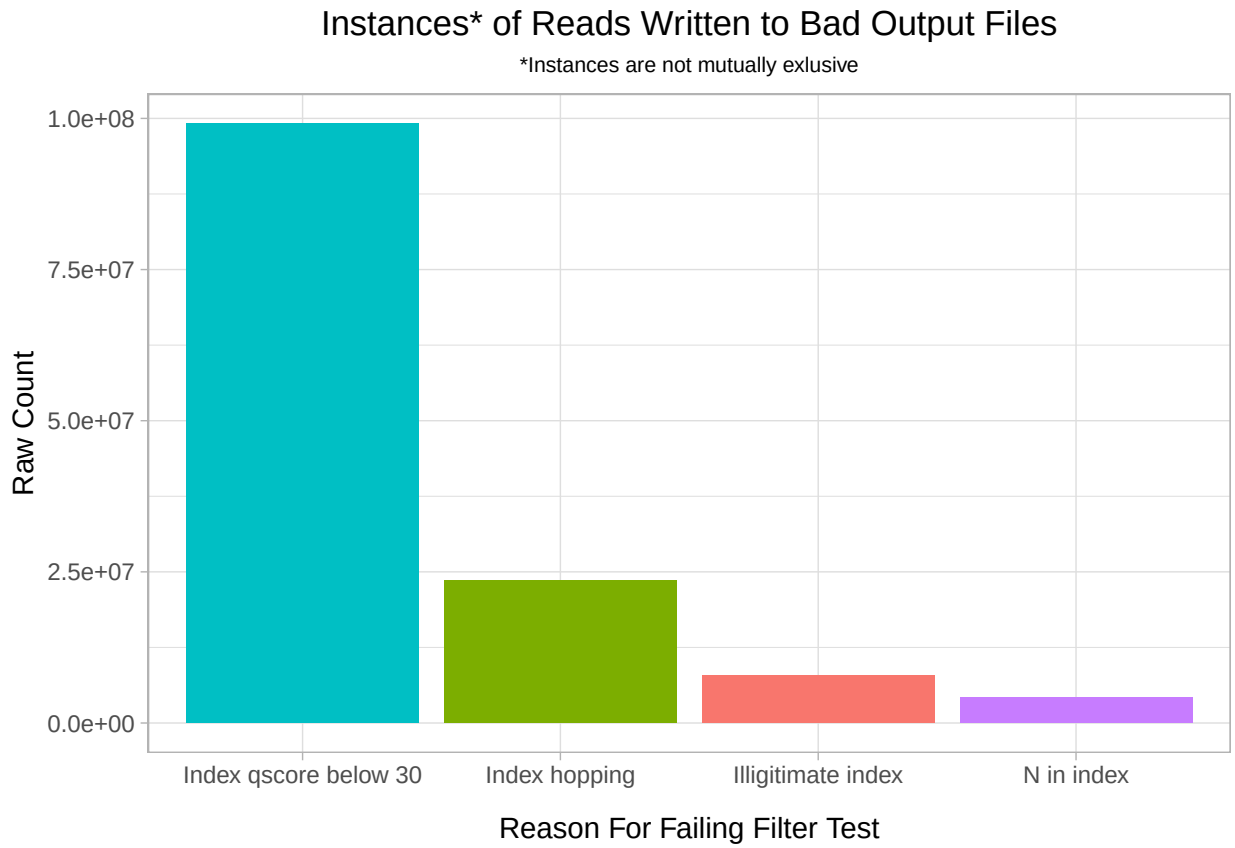
Head -8 output for AACAGCGA and bad reads

```
############### AACAGCGA Forward ###############

# @K00337:83:HJKJNBBXX:8:1101:1479:1701 1:N:0:1:AACAGCGA
# CCTTTGCTTAATCTGCTCTTGTCTTTCAGCACTAAGCTGTTCTTTTTCTTCTTTCATGGCTGAAATTTTTGTTTTCAGTTCTCTAACCTGGCGCTCAATAT
# +
# AAAAAJJFJJJJJJJJJJJFJJJJFJJJJJJFJJJJJFFJJJJJJJJJJJJJJFJJJJJJJAFJJJJJJJJJJJJFF<JJJJJJJJJFJFJJJJFJJJ
# @K00337:83:HJKJNBBXX:8:1101:1966:1701 1:N:0:1:AACAGCGA
# ATGTTCAGTTGTCTCTGGACTTACAAGTTCCTCAGTACACTGCTCCAGAGCCTCCCTCTCTTCATCCATGTCCTGACTGGTCTCCTTGCTGGCTTCTGAGC
# +
# AAFAFJJJJJJJJJJJJJFJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ

############### AACAGCGA Reverse ###############

# @K00337:83:HJKJNBBXX:8:1101:1479:1701 4:N:0:1:TCGCTGTT
# CAACGAGACGCGCGCTAAGCTCGACGAGCTTTCTGCTAAGCGAGAAACGAGTGGAGAGAAATCCAGACAACTAAGGGATGCCCAGCAGGATGCAAGAGACA
# +
# A<AFFJJJJJJJJJJJJJJAJJJJJJJJJJFJJJJJJJJJJJJJJJJJJJJJJJJJJJJFJJJJJJJJJ7JJJJJJJJJJJJJJJJJJFJJJJJJJJJ
# @K00337:83:HJKJNBBXX:8:1101:1966:1701 4:N:0:1:TCGCTGTT
# CTTGTGAGAACTGAACCACGAGAGCAGCTCCTTGATCAGCTGCAAAAGAAACAACCACCAATGATGCTAAACAGCTCAGAAGCCAGCAAGGAGACCAGTCA
# +
# AAFFFJJJAJJJJJJJJJJJJJJJJJJJJJFJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ


############### Bad Forward ###############

# @K00337:83:HJKJNBBXX:8:1101:1265:1191 1:N:0:1:NCTTCGAC
# GNCTGGCATTCCCAGAGACATCAGTACCCAGTTGGTTCAGACAGTTCCTCTATTGGTTGACAAGGTCTTCATTTCTAGTGATATCAACACGGTGTCTACAA
# +Undetermined | Low Quality | Index not recognized |
# A#A-<FJJJ<JJJJJJJJJJJJJJJJFJJJJFFJJFJJJAJJJJ-AJJJJJJJFFJJJJJJFFA-7<AJJJFFAJJJJJF<F--JJJJJJF-A-F7JJJJ
# @K00337:83:HJKJNBBXX:8:1101:1286:1191 1:N:0:1:NACAGCGA
# CNACCTGTCCCCAGCTCACAGGACAGCACACCAAAGGCGGCAACCCACACCCAGTTTACAGCCACACAGTGCCTTGTTTTACTTGAGGACCCCCCCACTCC
# +Undetermined | Low Quality | Index not recognized |
# A#AAFJJJJJJJJJFJJJJJJJJJJJJJJJJJJJJJJJFJJJJJJJJJJJJJJAJJJJJJJJJJJJJJFJJJJJFFFFJJJJJJJJJJJJJJJJ77F

############### Bad Reverse ###############

# @K00337:83:HJKJNBBXX:8:1101:1265:1191 4:N:0:1:NTCGAAGA
# NTTTTGATTTACCTTTCAGCCAATGAGAAGGCCGTTCATGCAGACTTTTTTAATGATTTTGAAGACCTTTTTGATGATGATGATGTCCAGTGAGGCCTCCC
# +Undetermined | Low Quality | Index not recognized |
# #AAFAFJJ-----F---7-<FA-F<AFFA-JJJ77<FJFJFJJJJJJJJJJAFJFFAJJJJJJJJFJF7-AFFJJ7F7JFJJFJ7FFF--A<A7<-A-7--
# @K00337:83:HJKJNBBXX:8:1101:1286:1191 4:N:0:1:NCGCTGTT
# NTGTGTAGACAAAAGTTTTCATGAGTCTGTAAGCTGTCTATTGTCTCCTGAAAAGAAACCAGAAGTTTTCCCCTAAATGTGTTTAGAATGCTTATTCTAAT
```

```
# +Undetermined | Low Quality | Index not recognized |
# #A-AFFJJFJJJJJJJJJJJJJJJJ<JAJFJJJJF<JFJJJAJJJJJJJJJJJJJJJJJJFJJJAJJFJJJFJJJF<JJA-JJJ-<AFAF--FF<JAFJF
```

Output plots




Instances* of Reads Written to Bad Output Files
*Instances are not mutually exlusive
1.0e+08
7.5e+07
5.0e+07
2.5e+07
0.0e+00
Raw Count
Index qscore below 30
Index hopping
Illigitimate index
N in index
Reason For Failing Filter Test