# Demultiplex part 2

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# Demultiplexing code:

Error correct and quality score check of reads commented out. Unit test designed with these in mind.

```
#!/usr/bin/env python3
#SBATCH --partition=long
                             ### Partition (like a queue in PBS)
#SBATCH --job-name=RRPS7
                                  ### Job Name
#SBATCH --time=1-20:01:00
                              ### Wall clock Oime 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-user=rarichardson92@qmail.com
#SBATCH --mail-type=BEGIN, END, FAIL
# Don't forget to load modules in bash, easybuild, prl, python/3.6.0 before running co
de!
import argparse
def getarguments():
    parser=argparse.ArgumentParser(description = "Demultiplexes gzipped index and rea
d outputs. requires fastq files and a file with tab seperated indexes. Outputs files b
ased on read index. Requires carefully choosen indexes for single base error correctio
n.")
    parser.add_argument("-r1", help = "Defines name and path of read1 file to use in p
rogram. Required, must be a string.", required = True, type = str)
    parser.add argument("-r2", help = "Defines name and path of read2 file to use in p
rogram. Required, must be a string.", required = True, type = str)
    parser.add argument("-i1", help = "Defines name and path of index1 file to use in
program. Required, must be a string.", required = True, type = str)
    parser.add_argument("-i2", help = "Defines name and path of index2 file to use in
program. Required, must be a string.", required = True, type = str)
    parser.add argument("-a","--allindex", help = "Defines name and path of tab sepera
ted file with all indexes to use in program. Required, must be a string.", required =
True, type = str)
    parser.add_argument("-n", help = "Number of records in files (must all be equal, w
ill only process up to that number). Required, must be an integer.", required = True,
type = int)
    parser.add_argument("-qi", help = "Mean quality score cutoff for indexes. Optiona
1. Defaults to 30, must be an integer.", required = False, type = int, default=30)
    #parser.add argument("-qr", help = "Mean quality score cutoff for reads. Optiona
L. Defaults to 30, must be an integer.", required = False, type = int, default=30)
    parser.add_argument("-m", help = "Index mismatch tolerance. Optional. Defaults to
1, must be an integer.", required = False, type = int, default=1)
    return parser.parse_args()
args=getarguments()
index1=str(args.i1)
index2=str(args.i2)
read1=str(args.r1)
read2=str(args.r2)
allindex=str(args.allindex)
number=int(args.n)
indexq=int(args.qi)
#readq=int(args.qr)
mis=int(args.m)
#Assumes input gzip files
```

```
#Initializes user defined variables
#set for number of records in fastq file
import gzip
def reverse(index):
    """Generates the complimentary sequence of input"""
    index=index.strip('\n')
    comp=""
    #holds reverse sequence
    for i in reversed(range(len(index))):
        if index[i] == "A":
            comp=comp+"T"
        elif index[i] == "T":
            comp=comp+"A"
        elif index[i] == "C":
            comp=comp+"G"
        elif index[i] == "G":
            comp=comp+"C"
        elif index[i] == "N":
            comp=comp+"N"
    return(comp)
def makedict(allindex):
    """Generates a dictionary with indexes as keys and the reverse compliments as valu
   indexdict={}
    #holds dictionary
   with open(allindex, "r") as ai:
        indexstring=ai.readline()
        indexstring=indexstring.strip('\n')
        list=indexstring.split('\t')
        for index in list:
            indexdict[index]=reverse(index)
    return(indexdict)
def convert phred(letter):
    """Converts a single character into a phred score. Will not tolerate letters outsi
de of Allchar,
    which are the letters used for +33 quality scores."""
   Allchar="!\"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJK"
    if letter in Allchar:
        return Allchar.find(letter)
    else:
        raise ValueError("Invalid entry for +33 phred scores.")
def checkqual(line):
    """Checks mean quality of input line."""
    line=line.strip('\n')
    line_qscores=0
    #holds total quality scores for a line
    letternum=0
    #counts letters in a line
    for letter in line:
```

```
line_qscores+=convert_phred(letter)
        letternum += 1
    return (line qscores/letternum)
#def errorcorrect(readindex,indexdict, mis):
     """Corrects errors of base pairs in index reads. Prevents correction where the mi
smatch is ambiguous to which index it should be corrected to. Returns forward index o
r 0 for noncorrection/no match. Assumes reverse compliments are more than mismatch bas
e difference."""
#
     onecorrect=0
#
     passingdict={}
     Doubleflag=False
#
#
     Least=mis+1
#
     #hold index of corrected index or 0 for multiples and no matches
     readindex=readindex.strip('\n')
#
     length=len(readindex)
    #determines length of index, assumes equal lengths for index input and index in di
ctionary
     for index in indexdict:
#
#
         mismatch=0
#
         for i in range(length-1):
             if index[i]!=readindex[i]:
#
                 mismatch+=1
#
#
         if mismatch<least:</pre>
             passingdict[index]=mismatch
#
         readindex=reverse(readindex)
        #Reverse compliments read index, repeats above process
         for index in indexdict:
             mismatch=0
             for i in range(length-1):
                 if index[i]!=readindex[i]:
#
#
                     mismatch+=1
             if mismatch<least:</pre>
                 if index not in passingdict:
#
#
                      passingdict[index]=mismatch
                 elif mismatch<passingdict[index]:</pre>
#
                      passingdict[index]=mismatch
         for item in passingdict:
#
             if passingdict[item]==least:
#
                 Doubleflag=True
             if passingdict[item]<least:</pre>
#
#
                 least=passingdict[item]
#
                 Doubleflag=False
                 onecorrect=item
#
         if Doubleflag==True:
#
#
             onecorrect=0
#
     return(onecorrect)
indexdict={}
indexdict=makedict(allindex)
indexset=set(indexdict.values())
```

```
#makes index:reversecompliment dictionary
indexhop={}
indexgood={}
for index in indexdict:
    indexhop[index]=0
    indexgood[index]=0
#makes dictionaries for good and bad index tallies; index hopping categorized by index
# index:###
r1dict={}
r2dict={}
RN=0
#record number
with gzip.open(read1, "rt") as r1:
    with gzip.open(read2, "rt") as r2:
        with gzip.open(index1, "rt") as i1:
            with gzip.open(index2, "rt") as i2:
                with open("unknown.r1", "wt") as u1:
                    with open("unknown.r2", "wt") as u2:
                        for index in indexdict:
                            r1dict[index]=open(index+".r1.fastq", "wt")
                            r2dict[index]=open(index+".r2.fastq", "wt")
                        while(RN < number):</pre>
                            r1header=r1.readline()
                            r1seq=r1.readline()
                            r1plus=r1.readline()
                            r1qual=r1.readline()
                            r2header=r2.readline()
                            r2seq=r2.readline()
                            r2plus=r2.readline()
                            r2qual=r2.readline()
                            i1header=i1.readline()
                            i1seq=i1.readline()
                            i1plus=i1.readline()
                            i1qual=i1.readline()
                            i2header=i2.readline()
                            i2seq=i2.readline()
                            i2plus=i2.readline()
                            i2qual=i2.readline()
                            #opens all files and saves one record as variables, increm
ents RN by 1
                            indexcheck=0
                            #Used as a variable to check indexes with dictionary
                            checknumi1=checkqual(i1qual)
                            checknumi2=checkqual(i2qual)
                            #checknumr1=checkqual(r1qual)
                            #checknumr2=checkqual(r2qual)
                            #Checks quality for all sequence lines
```

```
if checknumi1>=indexq and checknumi2>=indexq:
                            #and checknumr1>=readq and checknumr2>=readq:
                                #Quality check - passing to continue
                                if i1seq.strip('\n') not in indexdict and i1seq.strip
('\n') not in indexset:
                                    #Check if NOT in dictionary
                                    indexcheck=0
                                        #errorcorrect(i1seq, indexdict, mis)
                                    #Attempts error correction, if successful, forwar
d index assigned for comparison (otherwise indexcheck==0)
                                else:
                                    if i1seq.strip('\n') in indexdict:
                                        indexcheck=i1seq.strip('\n')
                                    else:
                                        indexcheck=reverse(i1seq.strip('\n'))
                                        #For above if, else: if in dictionary keys or
values, assigns forward index as indexcheck
                                if indexcheck!=0:
                                    #Where not no match (AKA match found)
                                    if indexcheck!=i2seq.strip('\n') and reverse(index
check)!=i2seq.strip('\n'):
                                    #and indexcheck!=errorcorrect(i2seq.strip('\n'), i
ndexdict, mis):
                                        #check if saved index or reverse is not equal
to index 2, as well as corrected index2
                                        u1.write(r1header)
                                        u1.write(r1seq)
                                        u1.write(r1plus)
                                        u1.write(r1qual)
                                        u2.write(r2header)
                                        u2.write(r2seq)
                                        u2.write(r2plus)
                                        u2.write(r2qual)
                                        #Writes to unknown files
                                        if i2seq.strip('\n') in indexdict or i2seq.str
ip('\n') in indexset:
                                        #or errorcorrect(i2seq.strip('\n'), indexdic
t, mis) in indexdict:
                                            indexhop[indexcheck]+=1
                                            #Discriminates high QC sequence errors fro
m index hopping
                                    else:
                                        #index1 == index2
                                        r1dict[indexcheck].write(r1header)
                                        r1dict[indexcheck].write(r1seq)
                                        r1dict[indexcheck].write(r1plus)
                                        r1dict[indexcheck].write(r1qual)
                                        r2dict[indexcheck].write(r2header)
                                        r2dict[indexcheck].write(r2seq)
```

```
r2dict[indexcheck].write(r2plus)
                                        r2dict[indexcheck].write(r2qual)
                                        indexgood[indexcheck]+=1
                                else:
                                #No index could be found that matches the read (indexc
heck==0)
                                    u1.write(r1header)
                                    u1.write(r1seq)
                                    u1.write(r1plus)
                                    u1.write(r1qual)
                                    u2.write(r2header)
                                    u2.write(r2seq)
                                    u2.write(r2plus)
                                    u2.write(r2qual)
                                    #Writes to unknown files
                            else:
                                #Failed Quality check, discard lines
                                u1.write(r1header)
                                u1.write(r1seq)
                                u1.write(r1plus)
                                u1.write(r1qual)
                                u2.write(r2header)
                                u2.write(r2seq)
                                u2.write(r2plus)
                                u2.write(r2qual)
                                #Writes to unknown files
for index in indexdict:
    r1dict[index].close()
    r2dict[index].close()
goodsum=0
hopsum=0
print("")
print("Parameters:")
print("Demultiplexing run on the following files:", read1, read2, index1, index2)
print("Number of records set to "+str(number))
print("Index mismatch tolerance set to "+str(mis))
#Can probably be changed to be manipulated later on
print("Index quality score mean cutoff set to "+str(indexq))
print("Index file used for demultiplexing:", allindex)
print("Full set of input indexes as follows:")
print(indexdict.keys())
print("")
print("Please refer arguments in the \"--help\" menu to adjust parameters.")
print("")
print("Number of good reads per index, percentage of all reads:")
for index in indexgood:
    if indexgood[index] > 0:
        print(index, indexgood[index], str((indexgood[index]/number)*100)+"%")
        goodsum+=indexgood[index]
```

```
print("Index hopping per index (sorted by index 1), percentage of all reads:")
for index in indexhop:
    if indexhop[index] > 0:
        print(index, indexhop[index], str((indexhop[index]/number)*100)+"%")
        hopsum+=indexhop[index]
print("")
print("Total number of read sets: "+str(number))
print("Total percentage of high quality read sets: "+str(((goodsum+hopsum)/number)*10
0)+"%")
print("Total number of high quality read sets without index hopping: "+str(goodsum))
print("Total number of high quality read sets with index hopping: "+str(hopsum))
if goodsum+hopsum != 0:
    print("Percentage of high quality read sets with index hopping: "+str((hopsum/(goo
dsum+hopsum))*100)+"%")
else:
    print("Percentage of high quality read sets with index hopping: 0")
```

## Example input:

```
./demultipart2.2.py -r1 unittestread1.gz -r2 unittestread2.gz -i1 unittestindex1.gz -i2 unittestindex2.gz -a Indexall.txt -n 10 -qi 35
```

### Example output:

```
Parameters:
Demultiplexing run on the following files: unittestread1.gz unittestread2.gz unittesti
ndex1.gz unittestindex2.gz
Number of records set to 10
Index mismatch tolerance set to 1
Index quality score mean cutoff set to 35
Index file used for demultiplexing: Indexall.txt
Full set of input indexes as follows:
dict_keys(['ACGATCAG', 'TCGACAAG', 'TCTTCGAC', 'ATCATGCG', 'AACAGCGA', 'TACCGGAT', 'CG
GTAATC', 'TCGGATTC', 'GCTACTCT', 'CTCTGGAT', 'GATCTTGC', 'CTAGCTCA', 'TAGCCATG', 'TGTT
CCGT', 'GTCCTAAG', 'AGAGTCCA', 'TCGAGAGT', 'CGATCGAT', 'TATGGCAC', 'GTAGCGTA', 'AGGATA
GC', 'ATCGTGGT', 'CACTTCAC', 'GATCAAGG'])
Please refer arguments in the "--help" menu to adjust parameters.
Number of good reads per index, percentage of all reads:
AACAGCGA 1 10.0%
GTAGCGTA 1 10.0%
GATCAAGG 1 10.0%
Index hopping per index (sorted by index 1), percentage of all reads:
Total number of read sets: 10
Total percentage of high quality read sets: 30.0%
Total number of high quality read sets without index hopping: 3
Total number of high quality read sets with index hopping: 0
Percentage of high quality read sets with index hopping: 0.0%
```

## Demultiplexing file tests:

Unfortunately, even after implementing some optimization techniques discussed in class on 16Oct2018, program is still slower than ideal. Several different runs were attempted to acheive completion. The following is the line count results of a 6 hour updated python script where index quality is set to 36:

Additional updates will be added as completion occurs.

```
[rrichard@n053 part2]$ wc -1 *.fastq
  13643040 AACAGCGA.r1.fastq
  13643040 AACAGCGA.r2.fastq
  13576592 ACGATCAG.r1.fastq
  13576592 ACGATCAG.r2.fastq
  17276064 AGAGTCCA.r1.fastq
  17276064 AGAGTCCA.r2.fastq
  13939846 AGGATAGC.r1.fastq
  13939846 AGGATAGC.r2.fastq
  15492828 ATCATGCG.r1.fastq
  15492828 ATCATGCG.r2.fastq
  10338620 ATCGTGGT.r1.fastq
  10338620 ATCGTGGT.r2.fastq
   5153076 CACTTCAC.r1.fastq
    5153076 CACTTCAC.r2.fastq
   9612288 CGATCGAT.r1.fastq
   9612288 CGATCGAT.r2.fastq
   5978864 CGGTAATC.r1.fastq
    5978864 CGGTAATC.r2.fastq
  29400858 CTAGCTCA.r1.fastq
   29400858 CTAGCTCA.r2.fastq
  55015834 CTCTGGAT.r1.fastq
  55015834 CTCTGGAT.r2.fastq
   9876424 GATCAAGG.r1.fastq
   9876424 GATCAAGG.r2.fastq
   6143816 GATCTTGC.r1.fastq
   6143816 GATCTTGC.r2.fastq
   9314886 GCTACTCT.r1.fastq
   9314886 GCTACTCT.r2.fastq
  12585190 GTAGCGTA.r1.fastq
  12585190 GTAGCGTA.r2.fastq
  13906714 GTCCTAAG.r1.fastq
  13939734 GTCCTAAG.r2.fastq
  96877770 TACCGGAT.r1.fastq
  96910790 TACCGGAT.r2.fastq
  16648678 TAGCCATG.r1.fastq
  16648678 TAGCCATG.r2.fastq
  15558300 TATGGCAC.r1.fastq
  15558300 TATGGCAC.r2.fastq
   5549346 TCGACAAG.r1.fastq
   5549346 TCGACAAG.r2.fastq
  14501110 TCGAGAGT.r1.fastq
  14501110 TCGAGAGT.r2.fastq
   6308934 TCGGATTC.r1.fastq
   6308934 TCGGATTC.r2.fastq
  64399920 TCTTCGAC.r1.fastq
  64432940 TCTTCGAC.r2.fastq
  26031424 TGTTCCGT.r1.fastq
```

```
26031424 TGTTCCGT.r2.fastq
  974359904 total
[rrichard@n053 part2]$ zcat unknown.r1.gz | wc -1
gzip: unknown.r1.gz: unexpected end of file
414694824
#Equivelent assumed for unknown.r2.gz
[rrichard@n053 part2]$ squeue -u rrichard
            JOBID PARTITION
                              NAME
                                        USER ST
                                                      TIME NODES NODELIST(REASON)
          2445257
                       long
                               RRPS7 rrichard R 1-08:32:39
                                                                1 n056
                                                                1 n068
          2446664
                       long RRPS7 rrichard R
                                                   6:00:46
                              RRPS7 rrichard R 5:52:23
RRPS7 rrichard R 5:53:25
                       long
                                                                1 n063
          2446668
          2446666
                       long RRPS7 rrichard R
                                                                1 n020
```

bash rrichard R

7:14

1 n053

short

2447099

Head of files:

[rrichard@n053 part2]\$ head TGTTCCGT.r1.fastq

@K00337:83:HJKJNBBXX:8:1101:4401:1701 1:N:0:1

TCAGCTTTCAGTTTGTCTAAGACCCAGGCGTACTTGAAGGAGCCCTTTCCCATCTCAGCAGCCTCCTTCTCAAACTTTTCGATGGT TCGCTTGTCGATTCC

+

@K00337:83:HJKJNBBXX:8:1101:6187:1701 1:N:0:1

CCTTGGGGTTCGGGCTGATGTACCAGTTCTTCTGAGGCACAGACGGCTGAGTAGGGAACACACAGGTCTGACCTGTCTCCATGTTG
CAGTAGACCTTGATG

+

@K00337:83:HJKJNBBXX:8:1101:6350:1701 1:N:0:1

GCCTCCTTGGTCTTCTTGTAACCTTCAACCTTATCTTCAACCACCAGCGGCAGCTCAAGAACCTCCTCAATACGATGACCTTTAGA CATCACCAAAGCTGG

[rrichard@n053 part2]\$ head TGTTCCGT.r2.fastq

@K00337:83:HJKJNBBXX:8:1101:4401:1701 4:N:0:1

CAAAAATGGGAAAGGAAAAGACTCACATCAACATCGTCGTAATCGGACACGTAGATTCCGGCAAGTCCACCACAACCGGCCACCTG
ATCTACAAATGTGGT

+

@K00337:83:HJKJNBBXX:8:1101:6187:1701 4:N:0:1

+

@K00337:83:HJKJNBBXX:8:1101:6350:1701 4:N:0:1

CAACCCAAAAACGATATGCCATCTGTTCTGCCCTGGCTGCCTCGGCCTTACCAGCTTTGGTGATGTCTAAAGGTCATCGTATTGAG
GAGGTTCTTGAGCTG

```
[rrichard@n053 part2]$ zcat unknown.r1.gz | head
@K00337:83:HJKJNBBXX:8:1101:1265:1191 1:N:0:1
GNCTGGCATTCCCAGAGACATCAGTACCCAGTTGGTTCAGACAGTTCCTCTATTGGTTGACAAGGTCTTCATTTCTAGTGATATCA
ACACGGTGTCTACAA
JJJJJF-A-F7JJJJ
@K00337:83:HJKJNBBXX:8:1101:1286:1191 1:N:0:1
{\tt CNACCTGTCCCCAGCTCACAGGACAGCACACCAAAGGCGGCAACCCACACCCAGTTTTACAGCCACACAGTGCCTTGTTTTACTTG}
AGGACCCCCACTCC
JJJJJJJJJJJJ77F
@K00337:83:HJKJNBBXX:8:1101:1347:1191 1:N:0:1
{\tt GNGGTCTTCTACCTTTCTTTTTTGGAGGAGTAGAATGTTGAGAGTCAGCAGTAGCCTCATCATCACTAGATGGCATTTCTT}
CTGAGCAAAACAGGT
[rrichard@n053 part2]$ zcat unknown.r2.gz | head
@K00337:83:HJKJNBBXX:8:1101:1265:1191 4:N:0:1
NTTTTGATTTACCTTTCAGCCAATGAGAAGGCCGTTCATGCAGACTTTTTTAATGATTTTTGAAGACCTTTTTTGATGATGATGATGATGT
CCAGTGAGGCCTCCC
FF--A<A7<-A-7--
@K00337:83:HJKJNBBXX:8:1101:1286:1191 4:N:0:1
NTGTGTAGACAAAAGTTTTCATGAGTCTGTAAGCTGTCTATTGTCTCCTGAAAAGAAACCAGAAGTTTTCCCCTAAATGTGTTTAG
AATGCTTATTCTAAT
-<AFAF--FF<JAFJF
@K00337:83:HJKJNBBXX:8:1101:1347:1191 4:N:0:1
NAAATGCCATCTAGTGATGATGAGGCTACTGCTGACTCTCAACATTCTACTCCTCCAAAAAAAGAAGAAGAAGATTCCAACCCCCAG
AACCGATGACCGGCA
```

ONE test completed with the original, unmodified script (commented out areas included). At quality scores for reads and indexes set to 38, the process took ~20 hours with output as follows:

#### Parameters:

Demultiplexing run on the following files: 1294\_S1\_L008\_R1\_001.fastq.gz 1294\_S1\_L008\_R 4 001.fastq.gz 1294 S1 L008 R2 001.fastq.gz 1294 S1 L008 R3 001.fastq.gz

Number of records set to 363246735

Index mismatch tolerance set to 0

Index quality score mean cutoff set to 38 and read quality score cutoff set to 38

Index file used for demultiplexing: Indexall.txt

Full set of input indexes as follows:

dict\_keys(['ACGATCAG', 'TCGACAAG', 'TCTTCGAC', 'ATCATGCG', 'AACAGCGA', 'TACCGGAT', 'CG
GTAATC', 'TCGGATTC', 'GCTACTCT', 'CTCTGGAT', 'GATCTTGC', 'CTAGCTCA', 'TAGCCATG', 'TGTT
CCGT', 'GTCCTAAG', 'AGAGTCCA', 'TCGAGAGT', 'CGATCGAT', 'TATGGCAC', 'GTAGCGTA', 'AGGATA
GC', 'ATCGTGGT', 'CACTTCAC', 'GATCAAGG'])

Please refer arguments in the "--help" menu to adjust parameters.

Number of good reads per index, percentage of all reads:

Index hopping per index (sorted by index 1), percentage of all reads:

Total number of read sets: 363246735

Total percentage of high quality read sets: 0.0%

Total number of high quality read sets without index hopping: 0

Total number of high quality read sets with index hopping: 0

Percentage of high quality read sets with index hopping: 0.0%

[rrichard@n053 justincase2]\$ zcat unknown.r1.run1.gz | wc -1

1452986940

[rrichard@n053 justincase2]\$ zcat unknown.r2.run1.gz | wc -1

1452986940

[rrichard@talapas-ln1 justincase2]\$ zcat unknown.r1.run1.gz |head @K00337:83:HJKJNBBXX:8:1101:1265:1191 1:N:0:1 GNCTGGCATTCCCAGAGACATCAGTACCCAGTTGGTTCAGACAGTTCCTCTATTGGTTGACAAGGTCTTCATTTCTAGTGATATCA ACACGGTGTCTACAA JJJJJF-A-F7JJJJ @K00337:83:HJKJNBBXX:8:1101:1286:1191 1:N:0:1 CNACCTGTCCCCAGCTCACAGGACAGCACCAAAGGCGGCAACCCACACCCAGTTTTACAGCCACACAGTGCCTTGTTTTACTTG AGGACCCCCACTCC JJJJJJJJJJJJ77F @K00337:83:HJKJNBBXX:8:1101:1347:1191 1:N:0:1  ${\tt GNGGTCTTCTACCTTTCTTTTTTGGAGGAGTAGAATGTTGAGAGTCAGCAGTAGCCTCATCATCACTAGATGGCATTTCTT}$ CTGAGCAAAACAGGT [rrichard@talapas-ln1 justincase2]\$ zcat unknown.r2.run1.gz |head @K00337:83:HJKJNBBXX:8:1101:1265:1191 4:N:0:1 CCAGTGAGGCCTCCC FF--A<A7<-A-7--@K00337:83:HJKJNBBXX:8:1101:1286:1191 4:N:0:1 NTGTGTAGACAAAAGTTTTCATGAGTCTGTAAGCTGTCTATTGTCTCCTGAAAAGAAACCAGAAGTTTTCCCCTAAATGTGTTTAG AATGCTTATTCTAAT -<AFAF--FF<JAFJF @K00337:83:HJKJNBBXX:8:1101:1347:1191 4:N:0:1 NAAATGCCATCTAGTGATGATGAGGCTACTGCTGACTCTCAACATTCTACTCCTCCAAAAAAAGAAGAAGAATTCCAACCCCCAG **AACCGATGACCGGCA**