

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 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-user=rarichardson92@gmail.com
#SBATCH --mail-type=BEGIN,END,FAIL
# Don't forget to load modules in bash, easybuild, prl, python/3.6.0 before running code!
import argparse
def getarguments():
    parser=argparse.ArgumentParser(description = "Demultiplexes gzipped index and read outputs. requires fastq files and a file with tab separated indexes. Outputs files based on read index. Requires carefully chosen indexes for single base error correction.")
    parser.add_argument("-r1", help = "Defines name and path of read1 file to use in program. Required, must be a string.", required = True, type = str)
    parser.add_argument("-r2", help = "Defines name and path of read2 file to use in program. 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 separated 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, will 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. Optional. Defaults to 30, must be an integer.", required = False, type = int, default=30)
    #parser.add_argument("-qr", help = "Mean quality score cutoff for reads. Optional. 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

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#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 values"""
    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 outside 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:

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        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 mismatch is ambiguous to which index it should be corrected to. Returns forward index or 0 for noncorrection/no match. Assumes reverse compliments are more than mismatch base 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 dictionary
#    for index in indexdict:
#        mismatch=0
#        for i in range(length-1):
#            if index[i]!=readindex[i]:
#                mismatch+=1
#        if mismatch<least:
#            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:
#            if index not in passingdict:
#                passingdict[index]=mismatch
#            elif mismatch<passingdict[index]:
#                passingdict[index]=mismatch
#    for item in passingdict:
#        if passingdict[item]==least:
#            Doubleflag=True
#        if passingdict[item]<least:
#            least=passingdict[item]
#            Doubleflag=False
#            onecorrect=item
#    if Doubleflag==True:
#        onecorrect=0
#    return(onecorrect)
indexdict={}
indexdict=makedict(allindex)
indexset=set(indexdict.values())

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#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
1
# 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):
                            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()
                            RN+=1
                        #opens all files and saves one record as variables, increments 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

```



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        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]

```

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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)*100)+"%")
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/(goodsum+hopsum))*100)+"%")
else:
    print("Percentage of high quality read sets with index hopping: 0")

```

Example input:

```

./demultipart2.2.py -r1 unitttestread1.gz -r2 unitttestread2.gz -i1 unitttestindex1.gz -i2 unitttestindex2.gz -a Indexall.txt -n 10 -qi 35

```


Example output:

```
Parameters:
Demultiplexing run on the following files: unittestread1.gz unittestread2.gz unittestindex1.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', 'CGGTAATC', 'TCGGATTG', 'GCTACTCT', 'CTCTGGAT', 'GATCTTGC', 'CTAGCTCA', 'TAGCCATG', 'TGTTCCGT', 'GTCCTAAG', 'AGAGTCCA', 'TCGAGAGT', 'CGATCGAT', 'TATGGCAC', 'GTAGCGTA', 'AGGATAGC', '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 achieve 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 -l *.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 TGTCCGT.r1.fastq
```

```
26031424 TGTTCCT.r2.fastq
974359904 total
```

```
[rrichard@n053 part2]$ zcat unknown.r1.gz | wc -l
```

```
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	ODELIST(REASON)
2445257	long	RRPS7	rrichard	R	1-08:32:39	1	n056
2446664	long	RRPS7	rrichard	R	6:00:46	1	n068
2446668	long	RRPS7	rrichard	R	5:52:23	1	n063
2446666	long	RRPS7	rrichard	R	5:53:25	1	n020
2447099	short	bash	rrichard	R	7:14	1	n053

Head of files:

[illegible]

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_R4_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', 'TCGGATTG', '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 -l
```

```
1452986940
```

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

```
1452986940
```

[illegible]