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# pipeline to generate read counts and phenotype scores directly from gzipped sequencing data
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import os
import sys
import gzip
import multiprocessing
import fnmatch
import glob
import argparse
```

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### Sequence File to Trimmed Fasta Functions ###
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def parallelSeqFileToCountsParallel(fastqGzFileNameList, fastaFileNameList,
countFileNameList, processPool, libraryFasta, startIndex=None, stopIndex=None,
test=False):
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```
    if len(fastqGzFileNameList) != len(fastaFileNameList):
        raise ValueError('In and out file lists must be the same length')
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```
    arglist = zip(fastqGzFileNameList, fastaFileNameList, countFileNameList,
[libraryFasta]*len(fastaFileNameList),
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[startIndex]*len(fastaFileNameList), [stopIndex]*len(fastaFileNameList),
[test]*len(fastaFileNameList))
```

```
    readsPerFile = processPool.map(seqFileToCountsWrapper, arglist)
```

```
    return zip(countFileNameList, readsPerFile)
```

```
def seqFileToCountsWrapper(arg):
    return seqFileToCounts(*arg)
```

```
def seqFileToCounts(infileName, fastaFileName, countFileName, libraryFasta,
startIndex=None, stopIndex=None, test=False):
```

```
    printNow('Processing %s' % infileName)
```

```
    fileType = None
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```
    for fileTup in acceptedFileTypes:
        if fnmatch.fnmatch(infileName, fileTup[0]):
            fileType = fileTup[1]
            break
```

```
    if fileType == 'fqgz':
        linesPerRead = 4
        infile = gzip.open(infileName, mode='rt')
    elif fileType == 'fq':
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        linesPerRead = 4
        infile = open(infileName)
    elif fileType == 'fa':
        linesPerRead = 2
        infile = open(infileName)
    else:
        raise ValueError('Sequencing file type not recognized!')

    seqToIdDict, idsToReadcountDict, expectedReadLength =
parseLibraryFasta(libraryFasta)

    curRead = 0
    numAligning = 0

    with open(fastaFileName, 'w') as unalignedFile:
        for i, fastqLine in enumerate(infile):
            if i % linesPerRead != 1:
                continue

            else:
                seq = fastqLine.strip()[startIndex:stopIndex]

                if i == 1 and len(seq) != expectedReadLength:
                    raise ValueError('Trimmed read length does not match expected
reference read length')

                if seq in seqToIdDict:
                    for seqId in seqToIdDict[seq]:
                        idsToReadcountDict[seqId] += 1

                    numAligning += 1

                else:
                    unalignedFile.write('>%d\n%s\n' % (i, seq))

                curRead += 1

                #allow test runs using only the first N reads from the fastq file
                if test and curRead >= testLines:
                    break

    with open(countFileName, 'w') as countFile:
        for countTup in (sorted(zip(idsToReadcountDict.keys(),
idsToReadcountDict.values()))):
            countFile.write('%s\t%d\n' % countTup)

    printNow('Done processing %s' % infileName)

    return curRead, numAligning, numAligning * 100.0 / curRead

```

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### Map File to Counts File Functions ###

def parseLibraryFasta(libraryFasta):
    seqToIds, idsToReadcounts, readLengths = dict(), dict(), []

    curSeqId = ''
    curSeq = ''

    with open(libraryFasta) as infile:
        for line in infile:
            if line[0] == '>':
                if curSeqId != '' and curSeq != '':
                    if curSeq not in seqToIds:
                        seqToIds[curSeq] = []
                    seqToIds[curSeq].append(curSeqId)

                    idsToReadcounts[curSeqId] = 0

                    readLengths.append(len(curSeq))

                curSeqId = line.strip()[1:]
                curSeq = ''

            else:
                curSeq += line.strip().upper()

    #at the end, add the final item that was not covered in the loop
    if curSeqId != '' and curSeq != '':
        if curSeq not in seqToIds:
            seqToIds[curSeq] = []
        seqToIds[curSeq].append(curSeqId)

        idsToReadcounts[curSeqId] = 0

        readLengths.append(len(curSeq))

    if len(seqToIds) == 0 or len(idsToReadcounts) == 0 or readLengths[0] == 0:
        raise ValueError('library fasta could not be parsed or contains no
sequences')
    elif max(readLengths) != min(readLengths):
        print(min(readLengths), max(readLengths))
        raise ValueError('library reference sequences are of inconsistent lengths')

    return seqToIds, idsToReadcounts, readLengths[0]

### Utility Functions ###
def parseSeqFileNames(fileNameList):

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infileList = []
outfileBaseList = []

for inputFileNames in fileNameList:
    #iterate through entered
    #filenames for sequence files
    for filename in glob.glob(inputFileName):
        #generate all possible
        #files given wildcards
        for fileType in list(zip(*acceptedFileTypes))[0]:
            #iterate through
            #allowed filetypes
            if fnmatch.fnmatch(filename,fileType):
                infileList.append(filename)

outfileBaseList.append(os.path.split(filename)[-1].split('.')[0])

return infileList, outfileBaseList

def makeDirectory(path):
    try:
        os.makedirs(path)
    except OSError:
        #printNow(path + ' already exists')
        pass

def printNow(printInput):
    print(printInput)
    sys.stdout.flush()

### Global variables ###
acceptedFileTypes = [('*.fastq.gz', 'fqgz'),
                    ('*.fastq', 'fq'),
                    ('*.fq', 'fq'),
                    ('*.fa', 'fa'),
                    ('*.fasta', 'fa'),
                    ('*.fna', 'fa')]

testLines = 10000

if __name__ == '__main__':
    parser = argparse.ArgumentParser(description='Process raw sequencing data from
screens to counts files in parallel')
    parser.add_argument('Library_Fasta', help='Fasta file of expected library
reads.')
    parser.add_argument('Out_File_Path', help='Directory where output files should
be written.')
    parser.add_argument('Seq_File_Names', nargs='+', help='Name(s) of sequencing
file(s). Unix wildcards can be used to select multiple files at once. The script
will search for all *.fastq.gz, *.fastq, and *.fa(/fasta/fna) files with the given
wildcard name.')

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parser.add_argument('-p', '--processors', type=int, default = 1)
parser.add_argument('--trim_start', type=int)
parser.add_argument('--trim_end', type=int)
parser.add_argument('--test', action='store_true', default=False, help='Run the
entire script on only the first %d reads of each file. Be sure to delete or move
all test files before re-running script as they will not be overwritten.' %
testLines)

args = parser.parse_args()
#printNow(args)

###catch input mistakes###
numProcessors = max(args.processors, 1)

infileList, outfileBaseList = parseSeqFileNames(args.Seq_File_Names)
if len(infileList) == 0:
    sys.exit('Input error: no sequencing files found')

try:
    seqToIdDict, idsToReadcountDict, expectedReadLength =
parseLibraryFasta(args.Library_Fasta)

    printNow('Library file loaded successfully:\n\t%.2E elements (%.2E unique
sequences)\t%dbp reads expected' \
            % (len(idsToReadcountDict), len(seqToIdDict), expectedReadLength))

except IOError:
    sys.exit('Input error: library fasta file not found')

except ValueError as err:
    sys.exit('Input error: ' + err.args[0])

trimmedFastaPath = os.path.join(args.Out_File_Path, 'unaligned_reads')
makeDirectory(trimmedFastaPath)
countFilePath = os.path.join(args.Out_File_Path, 'count_files')
makeDirectory(countFilePath)

fastaFileNameList = [outfileName + '_unaligned.fa' for outfileName in
outfileBaseList]
fastaFilePathList = [os.path.join(trimmedFastaPath, fastaFileName) for
fastaFileName in fastaFileNameList]
countFilePathList = [os.path.join(countFilePath, outfileName + '_' +
os.path.split(args.Library_Fasta)[-1] + '.counts') for outfileName in
outfileBaseList]

pool = multiprocessing.Pool(min(len(infileList), numProcessors))

try:

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        resultList = parallelSeqFileToCountsParallel(infileList, fastaFilePathList,
countFilePathList, pool, args.Library_Fasta, args.trim_start, args.trim_end,
args.test)
    except ValueError as err:
        sys.exit('Error while processing sequencing files: ' + ' '.join(err.args))

    for filename, result in resultList:
        print(filename + ':\n\t%.2E reads\t%.2E aligning (%.2f%%)' % result)

    pool.close()
    pool.join()

    printNow('Done processing all sequencing files')
```