##Modify script: <https://github.counsyl.com/bioinformatics/oligo_design/blob/master/barcode_design.py>

##New name: barcode\_design\_rk\_2.py

python barcode\_design\_rk\_2.py -l 8 -e 2 -i ExistingBarcodes.txt -NB newbarcodes -n 100

##Combine ExistingBarcodes.txt and newbarcodes to make a list of barcodes wc: 671 + 100 = 771 :ExistingBarcodes\_barcodes.txt

# Run ExistingBarcodes\_barcodes.txt through a test script : barcode\_design\_rk\_test.py

## This is to test the full list and make sure they are all compatible

python barcode\_design\_rk\_test.py -l 8 -e 2 -i ExistingBarcodes\_barcodes.txt -o newbarcodes\_test = 772 (not sure where the extra line is coming from…)

## another test to make sure the test script is doing what it should ...

##make a file ExisitngBarcodes\_test.txt which is ExistingBarcodes\_test.txt with part of the list duplicated. = 803 barcodes. The barcode\_design\_rk\_test.py should remove the duplicated barcodes.

python barcode\_design\_rk\_test.py -l 8 -e 2 -i ExistingBarcodes\_test.txt -o newbarcodes\_test2.txt

# Newbarcodes\_test2.txt : wc = 772 !

python barcode\_design\_rk\_2.py -l 8 -e 2 -i ExistingBarcodes.txt -NB BrandNewBarcodes -n 1000

# Combine ExistingBarcodes.txt and BrandNewBarcodes.txt to make new file : ExistingBarcodes\_BrandNewBarcodes.txt

# We then tested ExistingBarcodes\_BrandNewBarcodes.txt

using illumina bcl2fastq to demux the barcode list.

<https://docs.google.com/document/d/1J4CbYuaZzp40WPAMNzuyXB0nqGDO5v3gZhLGhbaLpgo/edit>

# Bcl2fastq was not happy. This list is no good. I did some troubleshooting…

# ExistingBarcodes.txt is a good list of barcodes according to Bcl2fastq but the BrandNewBarcodes.txt is not. My hypothesis is that we need to use a distance of 3 instead of 2.

# Make a new list of ~800 barcodes with distance of 3 and this is now a good list according to Bcl2fastq

# Okay, now make a list of new barcodes with the Existing list

python barcode\_design\_rk\_2.py -l 8 -e 3 -i ExistingBarcodes.txt -NB BrandNewBarcodes\_dist3 -n 250

#and combine them into one file : ExistingBarcodes\_BrandNewBarcodes\_dist3.txt

# Test to make sure this list is all good… and it is! But I only generated 250 more barcodes

bcl2fastq --runfolder-dir rawdata/HNNMWBBXX\_180830\_K00413\_0063\_A/ --output-dir demux\_newbarcodes/ --sample-sheet example\_dragon\_samplesheet\_ExistingBarcodes\_BrandNewBarcodes\_dist3.csv