Notes why and how to add the ABL L298L data:

11.15.2023 Haider Inam

Summary: Our ABL SM Library was made on REFSEQ ABL background for almost everything. But for 16 residues from 290-305 of the kinase, the library has a synonymous SNP on position 298. This SNP causes our in-house variant caller to think that there are two mutants on each read for these positions (one L298L and the other one being Twist's SNV/MNV). These are automatically discarded by our variant caller. I could have fixed the variant caller to account for this discrepancy, but the easier approach is to just do two separate variant calling pipelines for the L298L and the non-L298L regions.

* No L298L means a reference was used that had the normal CTG at residue 298 instead of a CTA.
* L298L means a CTA at residue 298 instead of the normal CTG.

To combat the L298L problem:

* We align our reads on a non-L298L reference and then an L298L reference. This is why you will see an L298L folder in each directory on the AWS instance
  + The two types of datasets originate from the same consensus family calls, i.e. the dunovo consensus family files.
  + The dunovo consensus calls were aligned first to a reference without L298L and then to a reference with L298L
  + To automatically do the L298L alignments: do the exact same 7 step consensus calling pipeline, except that in step 4 (the align ABL part), do an L298L step as well.
    - For example, when you typically run bash 4. align-abl-parallel, in this case, also run 4.1.align-abl-parallel-l298l.
    - This script will make an L298L folder within your sample and put all the L298L alignments in this sample.
    - You can continue the rest of the 3 steps as usual (plotting barcodes, calculating coverages, and making a for-export directory.
    - This extra piece of code will modify your for-export directory such that it has an L298L folder with the L298L alignments in it. You can now use the variant caller to call both the L298L alignments and the non-L298L alignments.
* For the reads aligned to the L298L reference, we extract the mutants called between ABL residues 290-305 and merge them with the non-L298L-called variants
  + The add\_l298l.R function (in the code directory) takes in a non-L298L variant caller outputs and L298L variant caller outputs, and merges them.
* To automatically add L298L for all the samples in a directory, you can use the following R code (this code is also present in the ABL\_SM\_CRISPR\_Cut\_Analysis.Rmd file):

source("code/variantcaller/add\_l298l.R")

for(i in c(1:18)){

sample=paste("sample",i,sep = "")

# sample="sample1"

input\_df\_nol298l=read.csv(paste("data/Consensus\_Data/novogene\_lane18/",sample,"/nol298l/duplex/variant\_caller\_outputs/variants\_unique\_ann.csv",sep=""))

input\_df\_l298l=read.csv(paste("data/Consensus\_Data/novogene\_lane18/",sample,"/l298l/duplex/variant\_caller\_outputs/variants\_unique\_ann.csv",sep=""))

output\_df=add\_l298l(input\_df\_nol298l,input\_df\_l298l)

write.csv(output\_df,

paste("data/Consensus\_Data/novogene\_lane18/",sample,"/duplex/variant\_caller\_outputs/variants\_unique\_ann.csv",sep = ""))

}