

# Supplementary Information

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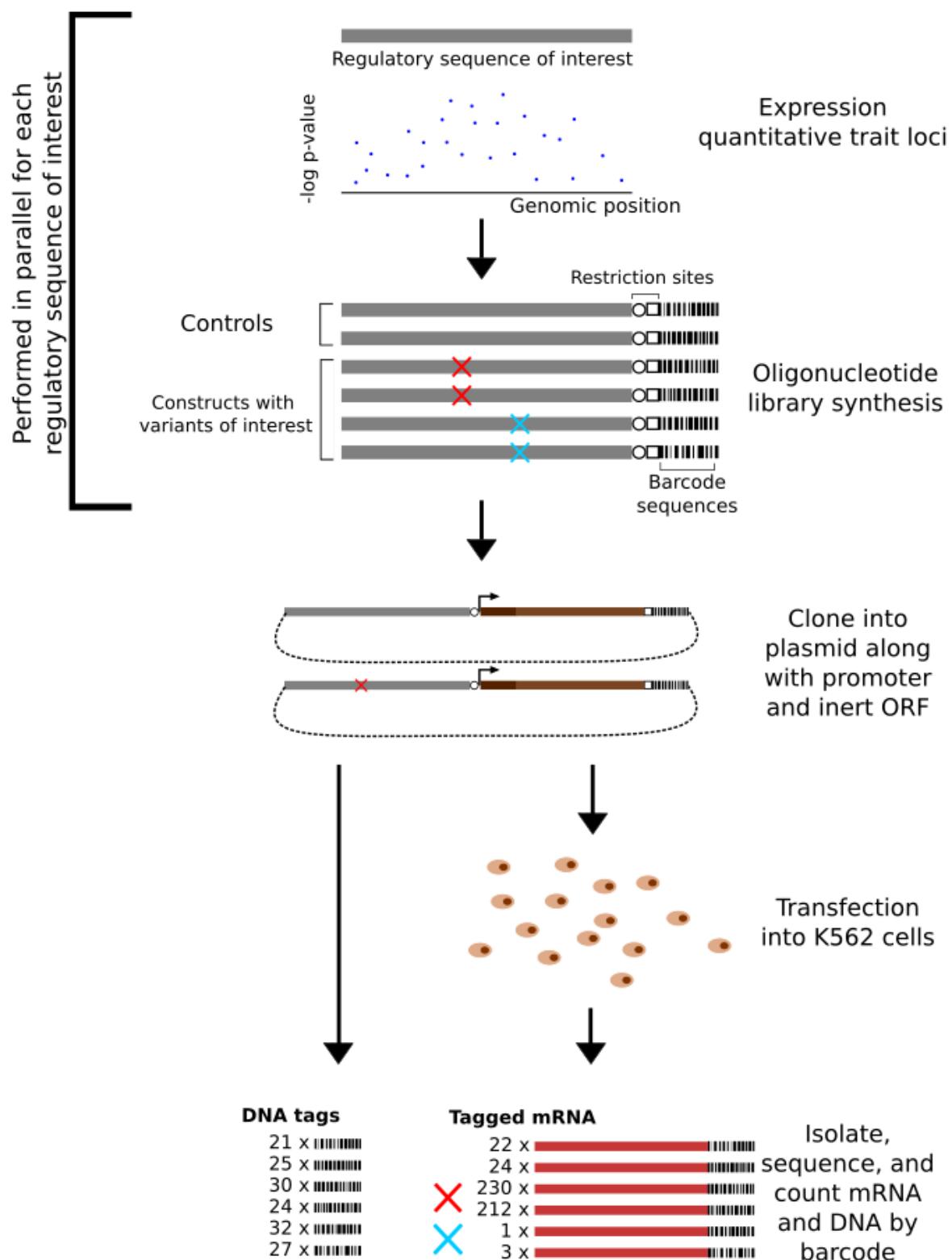
*6/21/2017*

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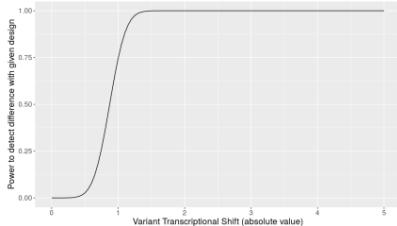
## S1 - MPRA diagrams

### S1.1 MPRA experimental diagram



## S1.2 MPRA Design Tools Workflow

Interactively design experiment based on desired statistical power



Generate MPRA sequences

ID	type	allele	barcode	sequence
rs701269	ref	C	GAAACGTTCTG	ACTGGCCAGTGTAAAT...
rs701269	ref	C	GATGCCGTCAC	ACTGGCCAGTGTAAAT...
rs701269	ref	C	GGACGCTATCTG	ACTGGCCAGTGTAAAT...
rs701269	ref	C	CTCGACTTTAGT	ACTGGCCAGTGTAAAT...
rs701269	ref	C	ATAGGTTGGACC	ACTGGCCAGTGTAAAT...
rs701269	mut	A	ACGGCGTGTAGC	ACTGGCCAGTGTAAAT...
rs701269	mut	A	GACTACGTACTT	ACTGGCCAGTGTAAAT...
rs701269	mut	A	CCGGTCGGAGGC	ACTGGCCAGTGTAAAT...
rs701269	mut	A	ATCTAACATGTGT	ACTGGCCAGTGTAAAT...
rs701269	mut	A	AACAAACCGCAAT	ACTGGCCAGTGTAAAT...

## S1.3 MPRA Design Tools Sequence Element Layout

Forward PCR primer **TG** Genomic context with appropriate allele **KpnI site** **XbaI site** **Barcode** **GGC** Reverse PCR primer

When using enzymes other than KpnI and XbaI through use of the R package, the corresponding segments of the sequence are replaced by the arguments `enzyme1` and `enzyme2` respectively.

## S2 - MPRA Activity Variance

Below is an analysis and visualization the variance of MPRA activity measurements. We do this by computing the activity of each barcode for an allele, then taking the standard deviation of those activities. Repeating this for all alleles, we can see what the distribution of standard deviations are across alleles.

### S2.1 - MPRA Activity Variance in Tewhey et al., Cell 2016

The study's data was acquired through direct correspondence with the authors. We compute the activity of each barcode in each transfection. Then, for every allele in every transfection, we compute the standard deviation of the activities. We then produce a histogram of the standard deviations in each transfection.

```
library(tidyverse)
library(magrittr)
library(parallel)
library(knitr)
library(nortest)

dir = '/mnt/bigData2/andrew/MPRA/Tewhey/indivTags/'

files = list.files(dir,
                  pattern = '.expanded$')

getFileDepth = function(file){
  # function to get the total number of reads in a sample.
  # Used to normalize counts by sample depth.

  read_tsv(paste0(dir, file),
            col_names = c('allele', 'barcode', 'count')) %>%
    .$count %>%
    sum
}
```

```
#apply the above function to the sample files
fileDepths = data_frame(src = files,
                        fileDepth = mclapply(src,
                                             getFileDepth,
                                             mc.cores = 19) %>% unlist)
```

A few example DNA barcode counts by sample file:

```
dnaCounts = map(1:5, ~read_tsv(paste0(dir, files[.x])),
                col_names = c('allele', 'barcode', 'count')) %>%
  mutate(src = files[.x])) %>%
  reduce(bind_rows)

dnaCounts %>% # show a random sample of the counts
  sample_n(5) %>%
  kable

dnaCounts %<>%
  left_join(fileDepths, by = 'src') %>%
  mutate(depthAdjCount = 1e6*count/fileDepth)

depthAdjDNAmeanCount = dnaCounts %>%
  group_by(barcode) %>%
  summarise(allele = allele[1],
            bcMean = mean(depthAdjCount)) %>%
  ungroup
#grouping together the huge number of barcodes takes a while,
#so this is saved and loaded

save(dnaCounts,
      file = '~/designMPRA/outputs/tewheyDNACounts.RData')
save(depthAdjDNAmeanCount,
      file = '~/designMPRA/outputs/tewheyDepthAdjDNACounts.RData')
```

Allele	Barcode	Count	Transfection File Name
rs116303217_altA	CATGCTCGTTAGGGTCCGCC	7	Geuv_90K_ctrl.r4.tag.ct.indiv.expanded
rs55906525_RC_B	GTCACGCTTAGCCAATGAGA	20	Geuv_90K_ctrl.r4.tag.ct.indiv.expanded
rs13228599_RC_B	ACGAATCACAAACAAATGTAT	19	Geuv_90K_ctrl.r5.tag.ct.indiv.expanded
rs9358930_A	AGAACAAACGTTGTCCACCT	22	Geuv_90K_ctrl.r3.tag.ct.indiv.expanded
rs17721766_A	CCATCGTACTAGGAAGTCGA	7	Geuv_90K_ctrl.r5.tag.ct.indiv.expanded

So to get the DNA normalization factor we simply take the mean of the DNA counts across the DNA transfections. For example:

```
depthAdjDNAmeanCount %>%
  head %>%
  set_names(c('Barcode', 'Allele', 'Mean Depth Adjusted DNA Count')) %>%
  kable
```

Barcode	Allele	Mean Depth Adjusted DNA Count
AAAAAAAAAAAAACCAAGCGG	rs116221068_B	0.0835755
AAAAAAAAAAAAACGTACTTC	rs9977746_B	0.0245955
AAAAAAAAAAAATGGCTCAC	rs6051692_RC_A	0.0228665

Barcode	Allele	Mean Depth Adjusted DNA Count
AAAAAAAAAAAAATTCCACGT	rs2915876_RC_A	0.0628202
AAAAAAAAAAACAAAAGTCC	rs2234161_B	0.2756719
AAAAAAAAAACAGTGTTC	rs59955136_A	0.2367092

So for example the first barcode for allele rs116221068\_B was counted 0.0835755 times on average across the plasmid sequencing runs (after adjusting for depth). The depth adjustment is performed as follows:

$$10^6 * \frac{\text{count}}{\text{sum of barcode counts in sequencing run}}$$

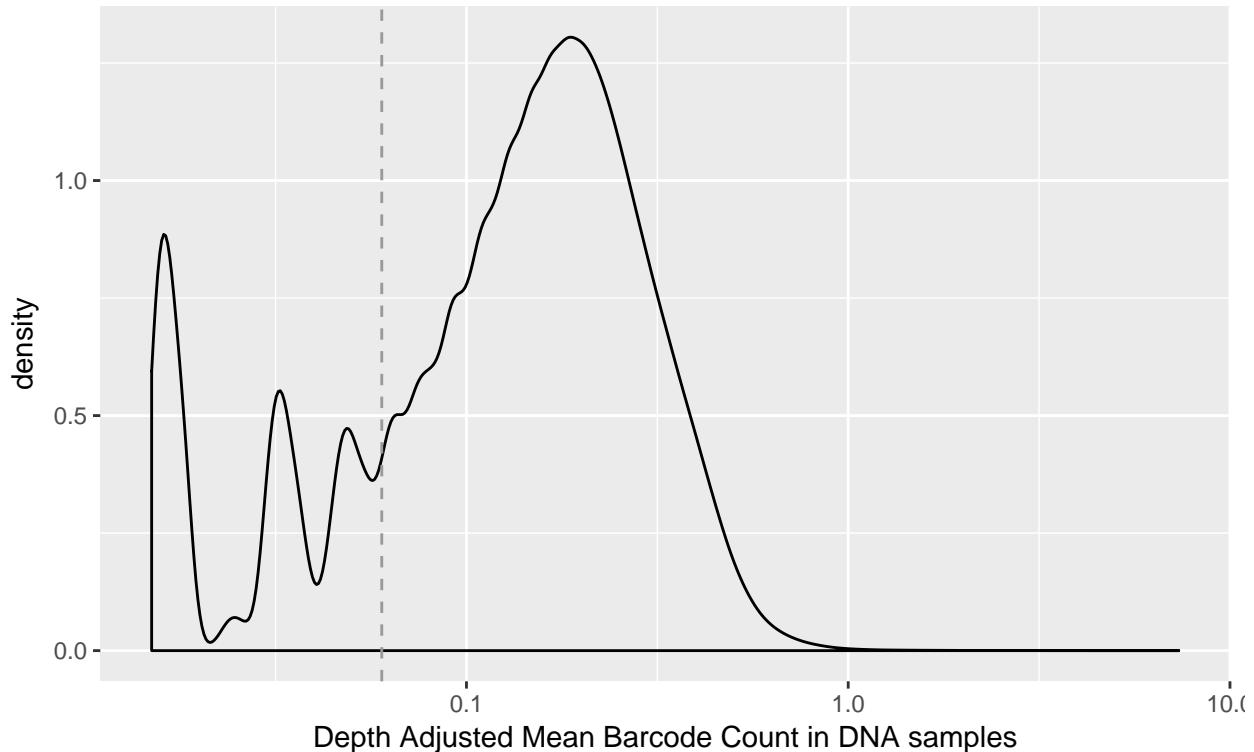
This normalizes each observed count according to how deeply the replicate was sequenced.

Tewhey et al., Cell 2016 had five plasmid replicates, so the estimates of the mean DNA counts from this study will likely be more precise than the counterparts in Ulirsch et al., Cell 2016 which had only two plasmid replicates. This will in turn make the downstream activity measurements more stable, thus the activity standard deviations in this paper will likely be lower.

We cut out barcodes that were not well represented in the DNA samples. Visual inspection of a (log-scale) density plot of the mean depth-normalized count shows that .06 would be a reasonable cutoff (shown as a dotted vertical line). This cuts out 4221460 out of 19611641 barcodes. The first few modes represents failed barcodes with very low counts while the large mode represents well-performing barcodes (for further example see Ulirsch et al., Cell 2016 Figure 1B):

```
depthAdjDNAmeanCount %>%
  ggplot(aes(bcMean)) +
  geom_density(adjust = 2) +
  scale_x_log10() +
  geom_vline(xintercept = .06,
             lty = 2,
             color = 'grey60') +
  xlab('Depth Adjusted Mean Barcode Count in DNA samples') +
  ggtitle('Density plot of depth-adjusted DNA count mean\nacross DNA replicates in Tewhey et al., 2016')
```

Density plot of depth-adjusted DNA count mean  
across DNA replicates in Tewhey et al., 2016



Then we compute the activity levels of each barcode by taking the depth adjusted count from an RNA sequencing run, dividing through the depth adjusted mean count from the DNA runs, then taking the log:

```

library(parallel)
library(nortest) # for lillie.test()

# For a given data file
computeTransfectionStatistics = function(fileNum){
  # Get the file's depth normalization factor
  depthNum = fileDepths %>% filter(src == files[fileNum]) %>% .$fileDepth

  # Read in the counts
  rnaCounts = read_tsv(paste0(dir, files[fileNum]),
                       col_names = c('allele', 'barcode', 'count')) %>%
    mutate(depthAdjCount = 1e6*count/depthNum) #normalize them for depth

  # Then compute statistics for the file
  alleleStatistics = depthAdjDNAmeanCount %>%
    filter(bcMean > .06) %>% # this is where we introduce the cutoff
    left_join(rnaCounts, by = c('allele', 'barcode')) %>% #join onto DNA counts
    mutate(activity = log(depthAdjCount/bcMean)) %>% #compute activity
    group_by(allele) %>% #for each allele
    summarise(alleleMean = mean(activity, na.rm = TRUE), #compute statistics
              alleleSD = sd(activity, na.rm = TRUE),
              numBarcodes = sum(!is.na(count)),
              lillieforsP = ifelse(numBarcodes > 4,
                                  lillie.test(na.omit(activity))$p.value,

```

```

    NA)) %>%
  filter(numBarcodes > 1) %>% # take only alleles which had > 1 barcode
  mutate(file = files[fileNum]) # and add on a file identifier
# The filter removes alleles that only had zero or one barcodes show up in
# the RNA, for which a standard deviation is not meaningful

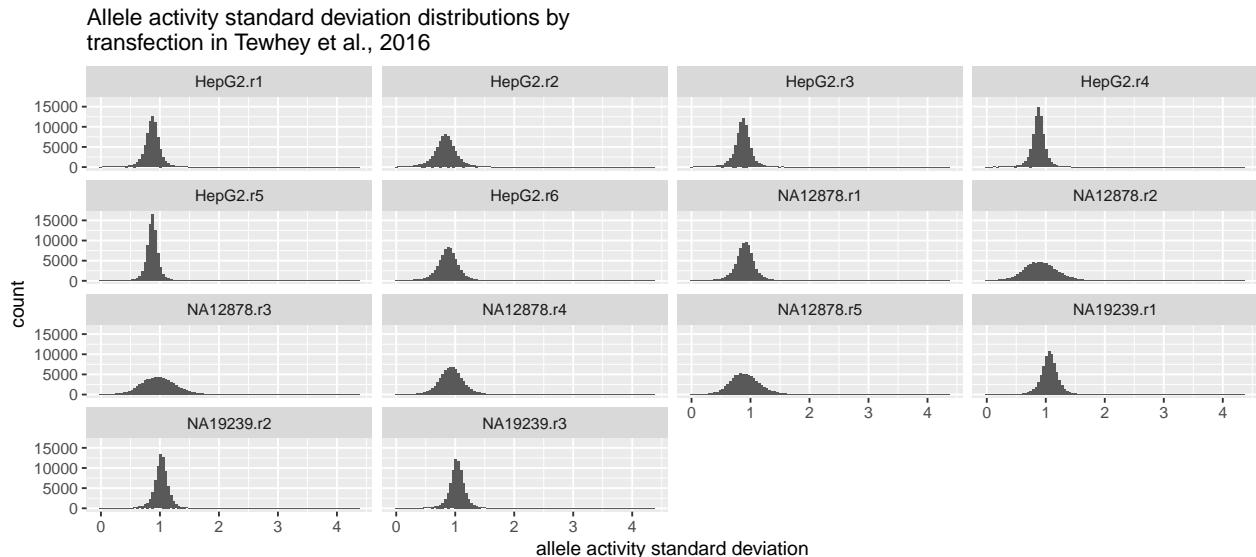
  return(alleleStatistics)
}

#apply the above function to the RNA data files
transfectionStatistics = mclapply(6:19,
                                computeTransfectionStatistics,
                                mc.cores = 14)

# Join the results together and clean up the file names
allTransfectionsStats = transfactionStatistics %>%
  reduce(bind_rows) %>%
  mutate(file = gsub('Geuv_90K_', '', file) %>% gsub('.tag.ct.indiv.expanded', '', .))
save(allTransfectionsStats,
     file = '~/designMPRA/outputs/tewheyAllTransfectionsStats.RData')

allTransfectionsStats %>%
  ggplot(aes(alleleSD)) +
  geom_histogram(bins = 100) +
  facet_wrap('file') +
  xlab('allele activity standard deviation') +
  ggtitle('Allele activity standard deviation distributions by\ntransfection in Tewhey et al., 2016')

```



There is variability in the distribution of allele activity standard deviations by transfection, but they are commonly above 1 (the mean of every allele in every transfection in this study is .926). Because activity is a log quantity, a standard deviation of 1 corresponds to an average variability of  $\exp(1) \approx 2.7$ -fold more mRNA molecules out per DNA molecule in within barcode replicates of the same allele.

We can look at the activity distribution of the two alleles for a single SNP tested. We'll take rs1674999 as an example. A random sample of the raw counts:

```

# counts = map(1:19, ~read_tsv(paste0(dir, files[.x])),
#               col_names = c('allele', 'barcode', 'count')) %>%
#               mutate(src = files[.x])) %>%
#   reduce(bind_rows)
#
# rs1674999counts = counts %>% filter(grepl('rs1674999', allele))

load('~/designMPRA/outputs/tewheyrs1674999counts.RData')

rs1674999counts %>%
  set_names(c('Allele', 'Barcode', 'Count', 'Transfection File Name')) %>%
  sample_n(5) %>%
  kable

```

Allele	Barcode	Count	Transfection File Name
rs1674999_A	TGACCTTTAGTATTGATCT	6	Geuv_90K_ctrl.r1.tag.ct.indiv.expanded
rs1674999_B	GATGAAGACATGTAGCCATA	7	Geuv_90K_ctrl.r4.tag.ct.indiv.expanded
rs1674999_B	TGTGTCGCCCATGGTCGTGC	3	Geuv_90K_NA19239.r2.tag.ct.indiv.expanded
rs1674999_B	TCGCGGTTGTACTAGGTGAC	34	Geuv_90K_NA12878.r5.tag.ct.indiv.expanded
rs1674999_A	AAAAGGGAAAGGGATAGTG	1	Geuv_90K_HepG2.r1.tag.ct.indiv.expanded

And a plot of the activity levels of all barcodes in all samples:

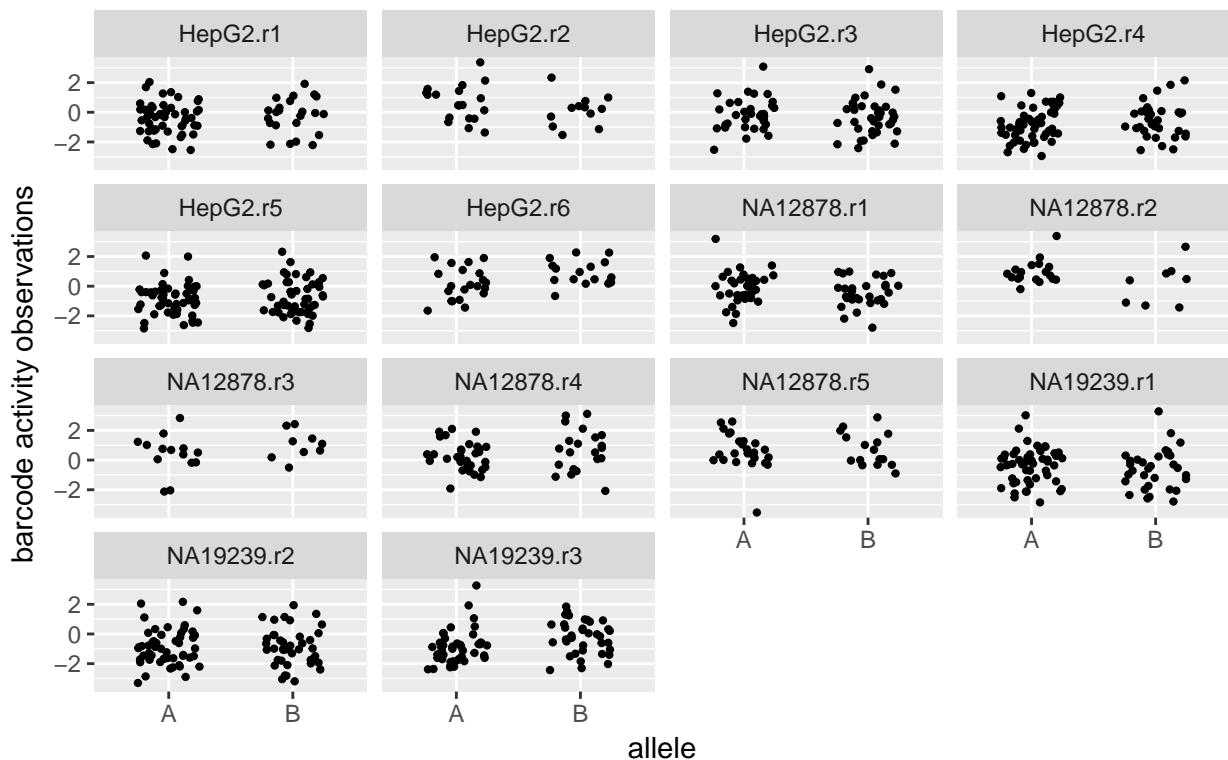
```

assureRNAandDNA = function(barcodeDat){
  # function for only including barcodes with DNA & RNA measurements
  any(grepl('ctrl',
    barcodeDat$src)) & any(grepl('HepG2|NA[0-9]{5}',
    barcodeDat$src))
}

rs1674999counts %>%
  group_by(barcode) %>%
  nest %>%
  filter(map_lgl(data, assureRNAandDNA)) %>%
  unnest %>%
  left_join(fileDepths, by = 'src') %>%
  mutate(depthAdjCount = count*1e6/fileDepth) %>% #adjust count for sample depth
  group_by(barcode) %>% nest %>%
  mutate(ctrlMean = map_dbl(data, # take mean count of control samples
                            ~filter(.x, grepl('ctrl', .x$src))$depthAdjCount %>% mean)) %>%
  unnest %>%
  filter(!grepl('ctrl', src)) %>%
  mutate(activity = log(depthAdjCount / ctrlMean)) %>% #compute activity
  mutate(replicate = gsub('Geuv_90K_', '', src) %>% gsub('.tag.ct.indiv.expanded', '', .),
         allele = gsub('rs1674999_', '', allele)) %>%
  ggplot(aes(allele, activity)) +
  geom_jitter(height = 0, width = .25, size = .8) +
  facet_wrap('replicate') +
  ylab('barcode activity observations') +
  ggtitle('Activity measurements of rs1674999\nin Tewhey et al., 2016 by sample')

```

## Activity measurements of rs1674999 in Tewhey et al., 2016 by sample



This SNP doesn't seem to have a large transcriptional shift between the alleles (both alleles have roughly the same average activity), but one can see that this level of activity variance (close to 1.0 in most transfections, which is fairly common within the experiment as can be seen by the histograms of standard deviations above) is large. Detecting a low magnitude transcriptional shift (for example a 33% increase in mRNA per DNA corresponding to a TS of  $\log(1.33) = .285$ ) at 90% power at the significance level required to overcome multiple corrections would require aggregating an even larger number of data points than shown here. A power calculation with `pwr::pwr.t.test` shows this:

```
library(pwr)

pwr.t.test(d = .285,
            sig.level = .05/39479, # bonferroni correction for the number of pairs tested in the paper
            power = .9)

##
##      Two-sample t test power calculation
##
##              n = 930.0622
##              d = 0.285
##      sig.level = 1.266496e-06
##              power = 0.9
##      alternative = two.sided
##
## NOTE: n is number in *each* group
```

## S2.2 - MPRA Activity Variance in Ulirsch et al., Cell 2016

Repeating the analysis with a different study. This data was acquired from the authors' website. The code differs slightly due to differences in how the data was provided by the authors, but the analysis is the same.

A few example barcode counts:

```
dir = "/mnt/labhome/andrew/MPRA/paper_data/"

UMPRA = read_delim(file = paste0(dir, "Raw/", "RBC_MPRA_minP_raw.txt"),
                    delim = "\t",
                    col_names = T,
                    col_types = cols(chr = "c"))

UMPRA %>% # show a few example counts
  select(chr, pos, ref, alt, byallele, K562_minP_DNA1:K562_GATA1_minP_RNA4) %>%
  gather(key = src, value = count, K562_minP_DNA1:K562_GATA1_minP_RNA4) %>%
  sample_n(5) %>%
  set_names(c('Chrom', 'Position', 'Reference',
             'Alternate', 'Allele ID', 'Transfection', 'Count')) %>%
kable
```

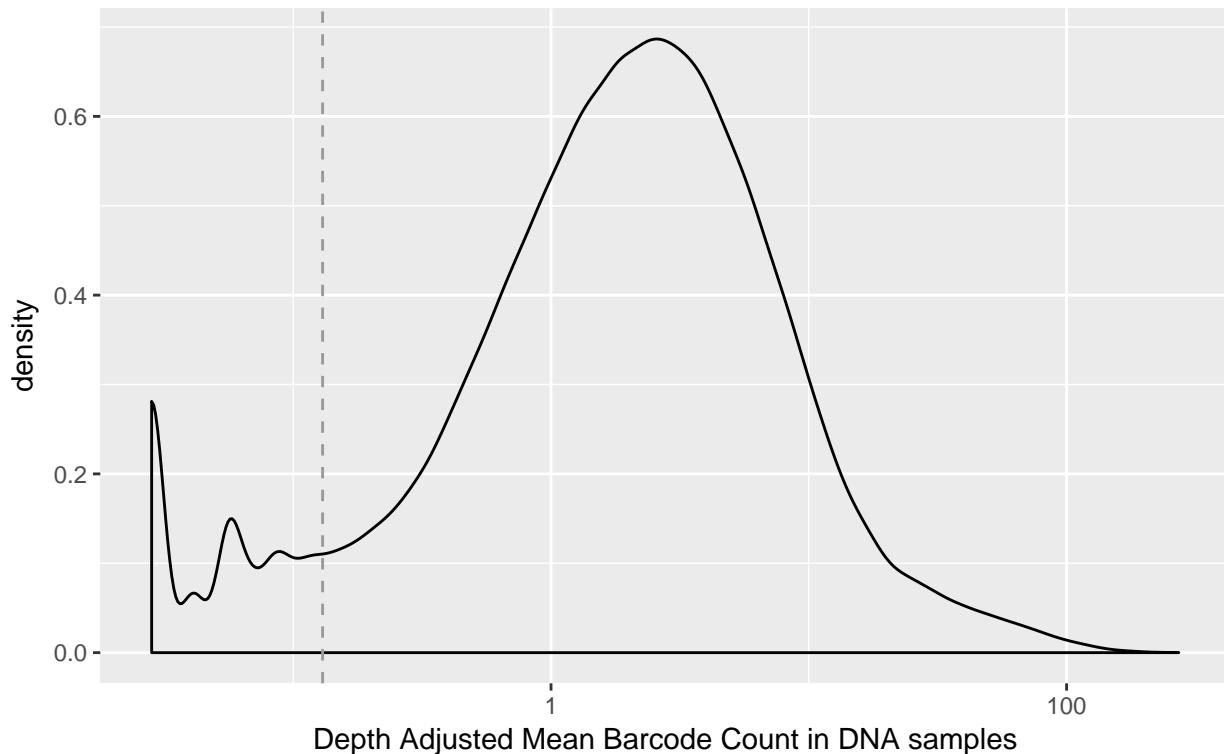
Chrom	Position	Reference	Alternate	Allele ID	Transfection	Count
12	2474661	C	T	12 2474661 1/2 Mut	K562_GATA1_minP_RNA3	251
17	42294462	A	G	17 42294462 1/2 Ref	K562_CTRL_minP_RNA6	1
1	158628014	T	G	1 158628014 1/2 Ref	K562_CTRL_minP_RNA3	38
6	32612079	T	C	6 32612079 1/3 Ref	K562_GATA1_minP_RNA3	3
1	203650945	C	T	1 203650945 1/3 Ref	K562_CTRL_minP_RNA1	1

Here we'll use a mean depth-normalized DNA count of .13:

```
depthNormalize = function(sampleCounts){
  sampleCounts*1e6/sum(sampleCounts)
}

UMPRA %>%
  mutate_at(vars(contains('K562')), depthNormalize) %>%
  mutate(dnaMean = (K562_minP_DNA1 + K562_minP_DNA2)/2) %>%
  ggplot(aes(dnaMean)) +
  geom_density() +
  scale_x_log10() +
  geom_vline(xintercept = .13,
             lty = 2,
             color = 'grey60') +
  ggtitle('Density plot of depth-adjusted DNA count mean\nacross DNA replicates in Ulirsch et al., 2016')
  xlab('Depth Adjusted Mean Barcode Count in DNA samples')
```

Density plot of depth-adjusted DNA count mean  
across DNA replicates in Ulirsch et al., 2016

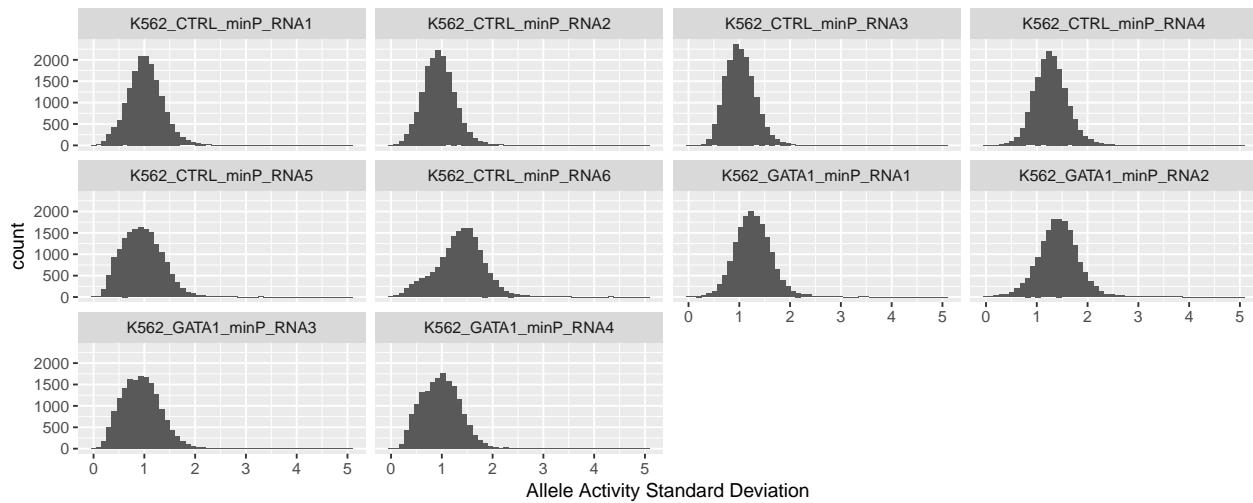


And here are the distributions of allele activity standard deviations by sample:

```
activitySDs = UMPRA %>%
  mutate_at(vars(contains('K562')), depthNormalize) %>%
  mutate(dnaMean = (K562_minP_DNA1 + K562_minP_DNA2)/2) %>%
  filter(dnaMean > .13) %>%
  select(-K562_minP_DNA1, -K562_minP_DNA2) %>%
  gather(sample, depthAdjCount, K562_CTRL_minP_RNA1:K562_GATA1_minP_RNA4) %>%
  mutate(activity = log(depthAdjCount / dnaMean)) %>%
  group_by(byallele, sample) %>%
  summarise(alleleSD = sd(activity[is.finite(activity)]), na.rm = TRUE)) %>%
  ungroup
# the use of is.finite() removes barcodes with 0 RNA counts (-Inf activity)

activitySDs %>%
  ggplot(aes(alleleSD)) +
  geom_histogram(bins = 50) +
  facet_wrap('sample') +
  xlab('Allele Activity Standard Deviation') +
  ggtitle('Allele activity standard deviation distributions\nby transfection in Ulirsch et al., 2016')
```

Allele activity standard deviation distributions  
by transfection in Ulirsch et al., 2016



Again, this plot shows that for a randomly chosen single allele, the standard deviation of its activity measurements are commonly around or above 1. The mean activity standard deviation across all samples in this study was NA.

If we combine both studies, we can see the typical range of activity standard deviations. The third output exponentiates the standard deviations, showing the corresponding standard fold-change in mRNA out to DNA in.

```
bothStudies = c(allTransfectionsStats$alleleSD, #Tewhey et al.
                activitySDs$alleleSD) #Ulirsch et al.

# Mean activity standard deviation in both studies:
bothStudies %>%
  mean(na.rm = TRUE)

## [1] 0.9523771

# Exponentiated
bothStudies %>%
  mean(na.rm = TRUE) %>%
  exp

## [1] 2.591864

# Central 95% interval of activity standard deviations in both studies:
bothStudies %>%
  na.omit %>%
  quantile(c(.025, .975))

##      2.5%    97.5%
## 0.4531956 1.5874324

# Exponentiated
bothStudies %>%
  na.omit %>%
  quantile(c(.025, .975)) %>%
  exp

##      2.5%    97.5%
## 1.573332 4.891174
```

### S3 - Activity Normality Assumption

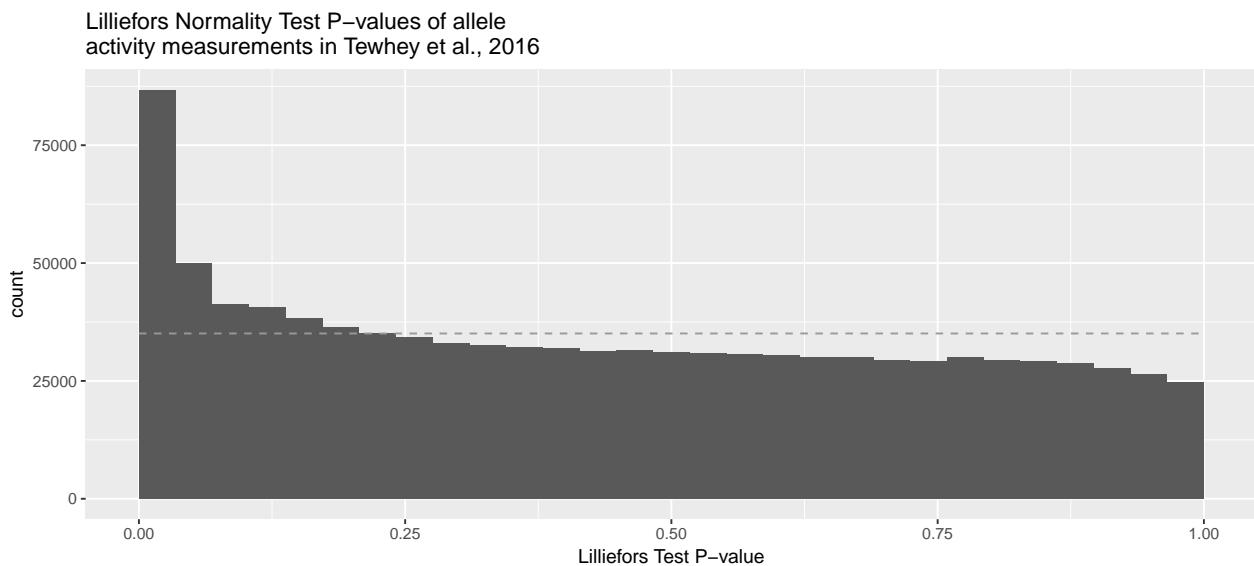
The “Power” tab of the application uses a t-test to estimate the power to detect functional variants with given activity variance across a range of transcriptional shifts.

In the earlier section S2.1 describing the activity variance in MPRA assays, we also calculated the p-value of a Lilliefors test for each allele. A Lilliefors test is a modified Kolmogorov-Smirnov test that tests if the data come from a normal distribution with unspecified mean and variance. A low p-value from a Lilliefors test suggests that the data come from a non-normal distribution.

Looking at the distribution of these p-values will tell us how commonly the normality assumption of our t-test holds. If all samples come from normal distributions, the p-values should follow a  $Unif(0, 1)$  distribution (shown here scaled to this number of allele observations as a grey horizontal line).

```
segment = data_frame(x1 = 0,
                      x2 = 1,
                      y1 = nrow(allTransfectionsStats) / 30,
                      y2 = nrow(allTransfectionsStats) / 30)

allTransfectionsStats %>%
  na.omit() %>% #we returned NA for alleles with <4 observations
  ggplot(aes(lillieforsP)) +
  geom_histogram(breaks = seq(0,1,length.out = 30)) +
  xlab('Lilliefors Test P-value') +
  geom_segment(aes(x = x1,
                   xend = x2,
                   y = y1,
                   yend = y2),
               data = segment,
               lty = 2,
               color = 'grey60') +
  ggtitle('Lilliefors Normality Test P-values of allele\activity measurements in Tewhey et al., 2016')
```



While a subset of the variants from Tewhey et al. clearly skew from the  $Unif(0, 1)$  distribution one would expect from normally distributed samples, the fraction of alleles that defy the expected pattern is relatively small. This suggests that a t-test should usually provide a reasonable approximation of the true power.

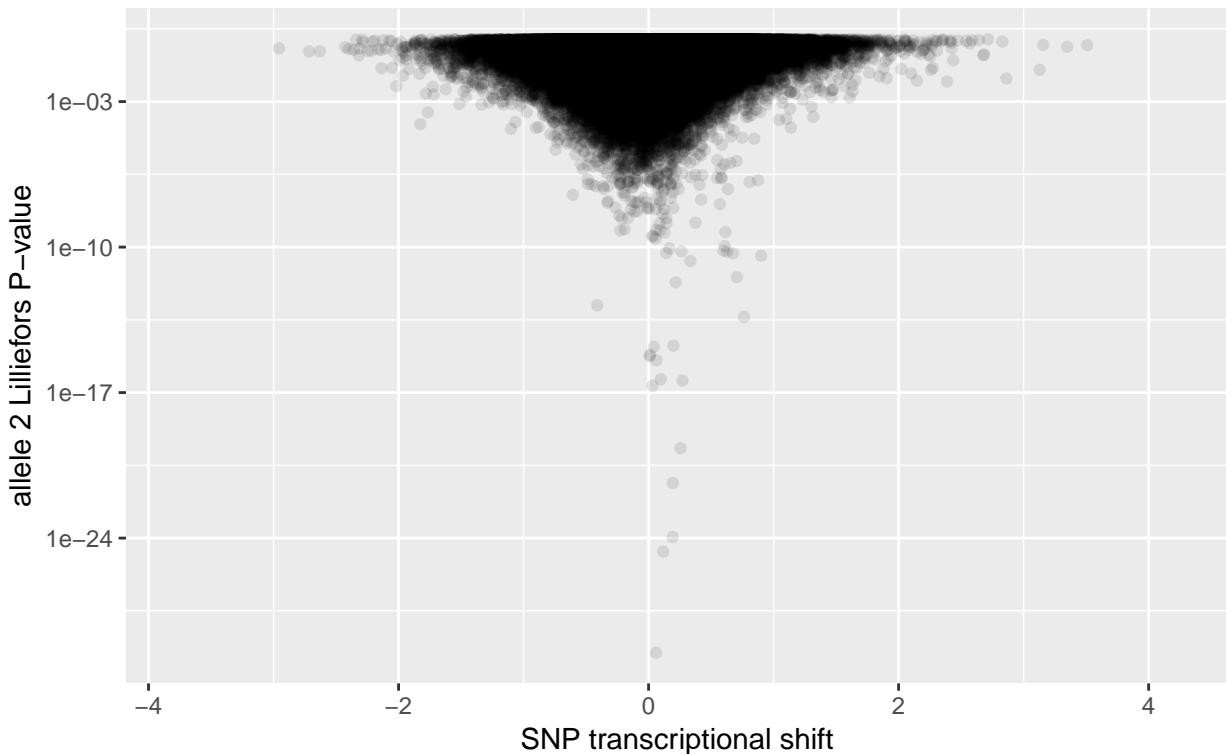
There is no indication that SNPs with large transcriptional shifts (i.e. those one aims to detect with the assay)

systematically defy the normality assumption. The following plot shows this with the observed transcriptional shift on the x-axis and p-value of a Lilliefors test on the activity measurements of the second allele on the y-axis (the plot for the first allele looks very similar). If the most important SNPs systematically defied the normality assumptions, the SNPs on the left and right sides would show the lowest p-values, which is not observed:

```
shiftNonNormality = allTransflectionsStats %>%
  separate(allele,
    into = c('SNP', 'allele'),
    sep = '_(?=[AB]$)|_(?=alt[AB]$)') %>%
  separate(allele,
    into = c('alt', 'allele'),
    sep = '(?=[AB]$)') %>%
  unite(SNP, SNP:alt) %>%
  mutate(SNP = gsub('_$', ' ', SNP)) %>%
  unite(SNPfile, SNP, file) %>%
  group_by(SNPfile) %>%
  summarise(numAlleles = n(),
    TS = ifelse(numAlleles == 2, alleleMean[2] - alleleMean[1], NA),
    allele1lilliefors = lillieforsP[1],
    allele2lilliefors = lillieforsP[2])

shiftNonNormality %>%
  ggplot(aes(TS, allele2lilliefors)) +
  geom_point(alpha = .1) +
  scale_y_log10() +
  xlab('SNP transcriptional shift') +
  ylab('allele 2 Lilliefors P-value') +
  ggtitle('SNP transcriptional shift vs. allele 2 Lilliefors\nNormality Test P-value in Tewhey et al., 2012')
```

## SNP transcriptional shift vs. allele 2 Lilliefors Normality Test P-value in Tewhey et al., 2016



Furthermore, t-tests are generally considered robust against minor violations of the normality assumption (Lehmann 1986).

All of this together suggests that having a t-test underlie our power calculations would be reasonable. Researchers won't know the activity variance their experimental setups will achieve nor the true transcriptional shifts of their variants *a priori* in any case. The "Power" tab of the application is meant to use a few assumptions in order to provide approximate power estimates that researchers can use as rough guidelines for their experiments.

### S4 - Power calculations

The power calculations are done with `pwr.t.test` from the R `pwr` package using the following R code:

```
tibble::data_frame(meanDiff = seq(0,5, by = .05),
                  pwr = pwr.t.test(n = input$nbarcode*input$nBlock,
                                    d = meanDiff / input$sigma,
                                    sig.level = input$alpha / input$nsnp)$power)
```

This returns the power to detect a transcriptional shift across a range from zero to five using user inputs (denoted `input$inputName`) on:

- \* the number of barcodes per allele
- \* the number of transfection replicates (blocks)
- \* the number of variants being tested (used for correcting the significance level to account for multiple testing)
- \* the desired significance level
- \* the assumed variance of activity measurements

This data frame is then plotted.

## S5 - Barcode design

We generated the set of all possible DNA 12-mers then screened these according to the design parameters in Melnikov et al., Nature Biotechnology 2012 intended to assure that the barcodes are inert. These involve the following parameters:

- \* each nucleotide occurs at least once
- \* no runs of single nucleotides greater than length 3
- \* do not contain restriction sites for KpnI/XbaI + do not start with TCT (this creates a restriction site with XbaI with the preceding sequence)
- \* they do not match any human miR seed sequences

This was done with the `generate12mers.R` script in the package (copied below; not run in the generation of this document).

```
library(tcR)
library(Biostrings)
nucruns = vector(mode = 'character', length = 4) %>% DNAStringSet
ni = 1
for (i in 4) {
  for (j in c('A', 'G', 'T', 'C')) {
    nucruns[ni] = rep(j, i) %>% paste(collapse = '') %>% DNAStringSet
    ni = ni + 1
  }
}

twelvemers = generate.kmers(12) %>% DNAStringSet
cat(paste0('done generating 12mers at ', Sys.time()))

#tmp = twelvemers[sample.int(length(twelvemers), size = 10)]

#Each nucleotide occurs at least one
missingone = apply(alphabetFrequency(twelvemers)[,1:4], 1, function(x){any(x == 0)})
twelvemers = twelvemers[!missingone]
cat(paste0('done removing twelvemers missing a nucleotide at ', Sys.time()))

#Cut out those with nucleotide runs of 4 or more in a row
hasnucruns = vcountPDict(nucruns, twelvemers) %>% colSums
hasnucruns = hasnucruns > 0
twelvemers = twelvemers[!hasnucruns]
cat(paste0('done removing 12mers with runs of 4 or more at ', Sys.time()))

#Cut out those that start with TCT (creates an alternative digestion site for XbaI)
tctStart = subseq(twelvemers, 1, 3) == DNAString('TCT')
twelvemers = twelvemers[!tctStart]
cat(paste0('done removing 12mers starting with TCT at ', Sys.time()))

#Cut out those that match the miRNA seed sequences
#For now let's just use the human ones since there are fewer and it won't take as long
#isolated species names with cat mature.fa | grep '>' | cut -f 3,4 -d \ > mirBaseSpecies.txt
species = read.table '~/plateletMPRA/mirBaseSpecies.txt' %>%
  as.tbl %>%
  transmute(name = paste(V1 %>% as.character, V2 %>% as.character)) #%%>%
  #filter(!duplicated(name))
human = grepl('Homo sapiens', species$name)
```

```

allSeeds = readRNAStringSet('~/plateletMPRA/mature.fa')

seedSeqs = allSeeds %>% subseq(2,7) %>% DNAStringSet #>% unique
humanSeedSeqs = seedSeqs[human] %>% unique
seedSeqs = allSeeds %>% subseq(2,7) %>% DNAStringSet %>% unique

haveSeedlist = vwhichPDict(humanSeedSeqs, twelvemers) #this takes ~40 minutes. All seeds takes ~1h45m
save(list = c('twelvemers', 'haveSeedlist', 'humanSeedSeqs'),
      file = '~/designMPRA/outputs/haveHumanRNAiSeeds.RData')
haveSeed = sapply(haveSeedlist, function(x){length(x) > 0})

twelvemers = twelvemers[!haveSeed]
cat(paste0('done removing those with mirSeeds at ', Sys.time()))

print(length(twelvemers))
save(twelvemers, file = '~/designMPRA/outputs/inertTwelveMers.RData')

```

## S6 - References

1. Melnikov, A., Murugan, A., Zhang, X., Tesileanu, T., Wang, L., Rogov, P., ... Mik-kelsen, T. S. (2012). Systematic dissection and optimization of inducible enhancers in human cells using a massively parallel reporter assay. *Nature Bio-technology*, 30(3), 271–7.
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3. Ulirsch, J. C., Nandakumar, S. K., Wang, L., Giani, F. C., Zhang, X., Rogov, P., ... Sankaran, V. G. (2016). Systematic Functional Dissection of Common Genetic Variation Affecting Red Blood Cell Traits. *Cell*, 165(6), 1530–1545.
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