Enzmatic Error Correction Figures

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R Stuff

Knitr Options

```
knitr::opts_chunk$set(fig.width = 17.5, fig.height = 10.94, dpi=300)
knitr::opts_chunk$set(fig.path = "./paper/")
knitr::opts_chunk$set(dev='pdf')
knitr::opts_chunk$set(warning=FALSE)

# see http://stackoverflow.com/q/36230790 to scroll output
# needs to be really big to prevent wrapping from happening before the scroll bar comes up
options(width = 240)
```

Initialization

```
# plotting utils
library(scales)
library(grid)
library(gridExtra)

# data.table backend
library(dtplyr)
library(data.table)

# tidyverse!
library(stringr)
library(broom)
library(magrittr)
library(tidyverse)
```

Style Choices

```
theme_pub <- function(base_size = 13, base_family = "") {
  require(grid)
  # based on https://github.com/noamross/noamtools/blob/master/R/theme_nr.R
  # start with theme_bw and modify from there!
  theme_bw(base_size = base_size, base_family = base_family) +# %+replace%
    theme(
      # grid lines
      panel.grid.major.x = element_line(colour="#ECECEC", size=0.5, linetype=1),
      panel.grid.minor.x = element_blank(),
      panel.grid.minor.y = element_blank(),
      panel.grid.major.y = element_line(colour="#ECECEC", size=0.5, linetype=1),
      panel.background   = element_blank(),

      # axis options
      axis.ticks.y   = element_blank(),
      axis.title.x   = element_text(size=rel(2.25), vjust=0.25),
      axis.title.y   = element_text(size=rel(2.25), vjust=0.35),
      axis.text      = element_text(color="black", size=rel(1.5)),

      # legend options
      legend.title    = element_blank(),
      legend.key      = element_rect(fill="white"),
      legend.key.size = unit(1, "cm"),
      legend.text     = element_text(size=rel(2)),

      # facet options
      strip.text = element_text(size=rel(2)),

      # title options
      plot.title = element_text(size=rel(3), vjust=0.25, hjust=0.5)
      )
  }

# set the theme and brewer color
theme_set(theme_pub())
cbbPalette <- c("#000000", "#E69F00", "#56B4E9", "#009E73", "#F0E442", "#0072B2", "#D55E00", "#CC79A7")
```

Helper Functions

```
DistribUncert2 <- function(df) {
  # Takes a df with uncertain types and manually distributes them, returning a
  # df of counts ready for plotting. Since we cannot place the insertion or
  # deletion precisely, we will assign fractional counts to each position based
  # on the nature of the difference. For example, a deletion in a 'AAA' repeat
  # could be at any of the A's, so we will assign a 1/3 count to each position
  #
  # Args:
  #   df - a data frame that must have the following:
  #     ColNames: Pos, Diff, Type
  #     Type: Must contain 'UI' and 'UD'
  # Returns:
  #   df - data frame with colnames Pos, Type, Count
  require(dplyr, magrittr, stringr)
  uncert <- df %>%
    filter(Type %in% c('UI', 'UD')) %>%
    mutate(
      To=str_sub(Diff, 2, 2),
      From=str_sub(Diff, 1, 1),
      Diff=str_sub(Diff, -1),
      Type=str_sub(Type, -1),
      FracCount=1/as.numeric(From)
    ) %>%
    select(c(-From, -To))

  # filter the canonical types and summarize their counts as well
  canon <- df %>%
    filter(!(Type %in% c('UI', 'UD'))) %>%
    mutate(FracCount=1)

  return(bind_rows(uncert, canon))
}

LabelMaker <- function(graph, label){
  # Takes a ggplot and adds a label to the top-left corner.
  # Must be used in conjunction with grid.arrange to plot
  # See: https://stackoverflow.com/a/29863172
  # Args:
  #   graph - a ggplot
  #   label - text string to add as label
  # Returns:
  #   gtable with plot and label
  require(ggplot2, grid, gridExtra)
  myplot <- arrangeGrob(
    graph,
    top = textGrob(
      label,
      x = unit(0, 'npc'),
      y = unit(1, 'npc'),
      just = c('left', 'top'),
      gp = gpar(fontsize=32)
    )
  )

  return(myplot)
}
```

Data Loading

We can get about at 10x speed-up by using pure `data.table`'s, however, some of `dplyr`'s functionality does not seem to behave quite right (especially joins). This is a known issue, and is being worked on.

```
ref.seq <- "GCTGCCGATTTCCATAAGATGCCTCCACGTCTCCGAAGAACTACATGGTGAATGTGTGAAGGCATTTTGAACCAATCCTCGAGCAGTGTTG
refCounts <- data.table(
  Char = c('A', 'T', 'G', 'C', 'N'),
  Count = c(str_count(ref.seq, 'A'),
            str_count(ref.seq, 'T'),
            str_count(ref.seq, 'G'),
            str_count(ref.seq, 'C'),
            str_count(ref.seq, 'N'))
  )

# set working directory here for local dev
```

```r
# setwd('/FOO/BAR/BAZ')

# constants for all samples
readCounts <- fread('./output/read-counts.txt', header=T)

# requisite information for all treatments
charCounts <- fread('zcat ./output/char-counts.txt.gz', header=T)
allSamps <- fread('zcat ./output/errs-all-samples.csv.gz', header=T)

# the zcat trick above may not work on your machine...
# charCounts <- fread('./output/char-counts.txt', header=T)
# allSamps <- fread('./output/errs-all-samples.csv', header=T)

# subset variables for easy running
nonDoped <- allSamps %>% filter(Sample == '1_nonDoped')
doped <- allSamps %>% filter(Sample == '1_DopedTemp')
```

Main Figures

Figure 2 - Error Analysis for a Standard Oligo Assembly

```r
#-------------------------------------------------------------------------------
# Panel 1
# Plot the position of all types of errors

# We need to make sure that any 0's are actually caught for plotting
# nonDoped %>%
#   DistribAndNorm(., 1) %>%
#   complete(Type, Pos, fill=list('Norm'=0))

positions <- nonDoped %>%
  DistribUncert2() %>%
  count(Pos, Type, wt=FracCount) %>%
  filter(Type != 'S') %>%
  ungroup() %>%
  mutate(
    Norm = n / subset(readCounts, Sample == '1_nonDoped')$Reads,
    Type = Type %>%
      factor(levels = c('M', 'I', 'D', 'P')) %>%
      recode(D = 'Single Base Deletions', I = 'Single Base Insertions',
             P = 'Multiple Base Deletions', M = 'Mismatches')
  ) %>%
  ggplot(aes(x=Pos, y=Norm)) +
  geom_point(size=3) +
  facet_wrap(~ Type, ncol=2) +
  stat_smooth(se=F) +
  labs(x = 'Position',
       y = 'Error Rate',
       title = 'Error Rate vs. Position for Error Sub-types') +
  scale_y_log10() +
  annotation_logticks(sides='l')

#-------------------------------------------------------------------------------
# Panel 1b
# Percentage of Error Subtypes

sub_type <- nonDoped %>%
    DistribUncert2() %>%
  count(Type, wt=FracCount) %>%
  mutate(
    Norm = n / sum(n) * 100,
    Type = Type %>%
      factor(levels = c('M', 'D', 'P', 'I', 'S')) %>%
      recode(M = 'MM', D = 'Del.', P = 'M. Del.', I = 'Ins.', S = 'M. Ins.')
    ) %T>%
  {arrange(., -Norm) %>% print()} %>%
  ggplot(aes(x=Type, y=Norm)) +
  geom_bar(stat='identity') +
  theme(plot.title = element_text(size=rel(2))) +
  labs(
    y = 'Percent',
    x = 'Error Type',
    title = 'Percentage of Error Sub-types'
  )
```

```
## Source: local data table [5 x 3]
##
## # tbl_dt [5 × 3]
##      Type      n       Norm
##    <fctr>  <dbl>      <dbl>
## 1      MM 155254 75.0682971
## 2    Del.  29313 14.1733997
## 3 M. Del.  16946  8.1937172
## 4    Ins.   4897  2.3677938
## 5 M. Ins.    407  0.1967923
```

```
#-------------------------------------------------------------------------------
# Panel 1c
# plot the distribution of total mismatches per position
mm_freq <- nonDoped %>%
  filter(Type == 'M') %>%
  mutate(
    To = str_sub(Diff, 2, 2),
    From = str_sub(Diff, 1, 1)
  ) %>%
  count(Pos, From) %>%
  ungroup() %>%
  # left_join(rename(refCounts, From=Char), by='From') %>%
  mutate(Norm = n / subset(readCounts, Sample == '1_nonDoped')$Reads) %T>%
  { # pairwise wilcox test and print median values for paper
    group_by(., From) %>%
      summarise(med=median(Norm)) %>%
      arrange(-med) %>%
      print; # <- ; critical for . to be interpreted correctly
    with(., pairwise.wilcox.test(n, From)) %>% print
  } %>%
  ggplot(aes(x = From, y = Norm)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(position=position_jitter(w = 0.5), size=0.75, alpha=0.8) +
  # stat_summary(fun.y = median, fun.ymin = median, fun.ymax = median, geom = 'crossbar', width = 0.5)
  labs(
    y = 'Error Rate',
    title = 'Mismatches per Position'
  ) +
  theme(
    axis.text.x = element_text(size=28),
    axis.title.x = element_blank(),
    plot.title = element_text(size=rel(2.25))
  ) +
  scale_y_continuous(labels = scientific_format())
```

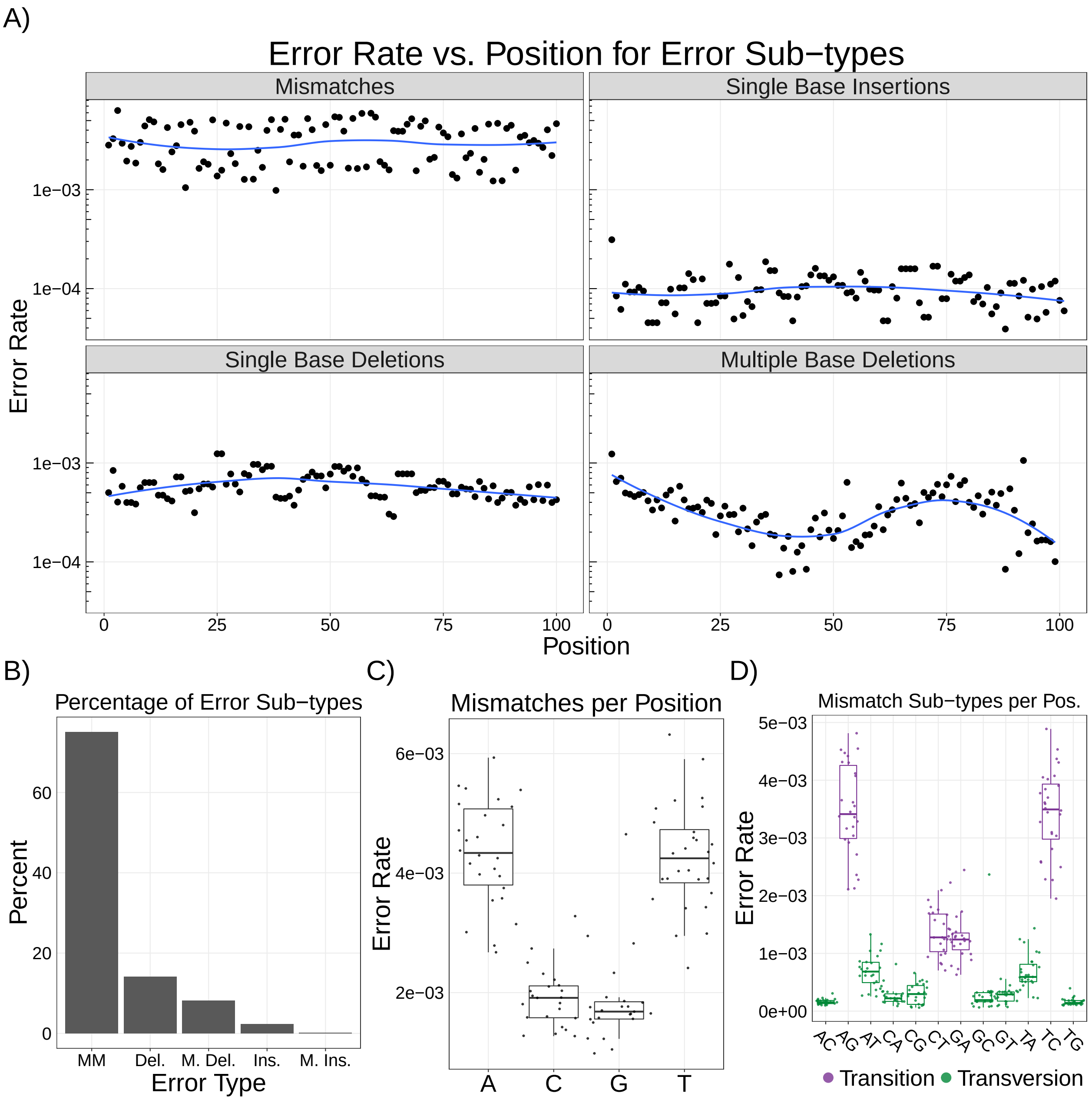
```
## Source: local data table [4 x 2]
##
## # tbl_dt [4 × 2]
##    From         med
##   <chr>       <dbl>
## 1     A 0.004337145
## 2     T 0.004247793
## 3     C 0.001912329
## 4     G 0.001682274
##
##  Pairwise comparisons using Wilcoxon rank sum test
##
## data:  n and From
##
##   A       C       G
## C 3.3e-12 -       -
## G 7.2e-08 0.47    -
## T 0.58    1.2e-12 5.7e-08
##
## P value adjustment method: holm
```

```
#-------------------------------------------------------------------------------
# Panel 1d
# what bases are most likely mutated to
# We will normallize by the total count in each "from" group
mm_type <- nonDoped %>%
  filter(Type == 'M') %>%
  count(Pos, Diff) %>%
  ungroup() %>%
  mutate(
    Char=str_sub(Diff, 1, 1),
    Class=Diff %>%
      recode(AT='Transversion', AG='Transition', AC='Transversion',
             TA='Transversion', TG='Transversion', TC='Transition',
             GA='Transition', GT='Transversion', GC='Transversion',
             CA='Transversion', CT='Transition', CG='Transversion')
  ) %>%
  # left_join(refCounts, by='Char') %>%
  mutate(Norm = n / subset(readCounts, Sample == '1_nonDoped')$Reads) %T>%#(Count * subset(readCounts,
  {# significance testing and printing for paper
    group_by(., Diff) %>%
      summarise(med=median(Norm)) %>%
      arrange(-med) %>%
      print; # <- ; critical for . to be interpreted correctly
      with(., pairwise.wilcox.test(Norm, Diff)) %>% print
  } %>%
  ggplot(aes(x = Diff, y = Norm, color=Class)) +
  geom_boxplot(outlier.shape = NA, show.legend = FALSE) +
  geom_jitter(position=position_jitter(w = 0.5), size=0.75, alpha=0.8) +
  # stat_summary(fun.y = median, fun.ymin = median, fun.ymax = median, geom = 'crossbar', width = 0.5,
  labs(
    y = 'Error Rate',
    title = 'Mismatch Sub-types per Pos.'
    ) +
  theme(
    legend.position='bottom',
    legend.key.size=unit(0.75, "cm"),
    axis.title.x=element_blank(),
    axis.text.x = element_text(angle = 315, vjust=0.5),
    plot.title = element_text(size=rel(1.75))
  ) +
  scale_y_continuous(labels = scientific_format()) +
  scale_color_manual(values = c('#7b3294', '#008837')) +
  guides(colour = guide_legend(override.aes = list(size=5)))
```

```
## Source: local data table [12 x 2]
##
## # tbl_dt [12 × 2]
##     Diff          med
##    <chr>        <dbl>
## 1     TC 0.0034939539
## 2     AG 0.0034128186
## 3     CT 0.0012776245
## 4     GA 0.0012406515
## 5     AT 0.0006850286
## 6     TA 0.0005925959
## 7     CG 0.0002957845
## 8     GT 0.0002896223
## 9     CA 0.0002218383
## 10    GC 0.0001910275
## 11    AC 0.0001540544
## 12    TG 0.0001427571
##
##  Pairwise comparisons using Wilcoxon rank sum test
##
## data:  Norm and Diff
##
##    AC      AG      AT      CA      CG      CT      GA      GC      GT      TA      TC
## AG 2.9e-08 -       -       -       -       -       -       -       -       -       -      
## AT 4.0e-08 2.5e-13 -       -       -       -       -       -       -       -       -      
## CA 0.0134  2.0e-12 8.1e-06 -       -       -       -       -       -       -       -      
## CG 0.2814  2.0e-12 1.7e-05 1.0000  -       -       -       -       -       -       -      
## CT 8.4e-08 2.0e-12 1.1e-06 5.1e-11 1.3e-11 -       -       -       -       -       -      
## GA 8.4e-08 5.3e-11 2.8e-05 8.7e-11 2.6e-11 1.0000  -       -       -       -       -      
## GC 0.4843  1.3e-07 1.3e-05 1.0000  1.0000  3.2e-06 3.1e-06 -       -       -       -      
## GT 0.0248  8.4e-08 8.7e-06 1.0000  1.0000  2.3e-07 2.3e-07 1.0000  -       -       -      
## TA 2.0e-08 6.9e-14 1.0000  4.0e-07 5.3e-05 5.4e-07 3.1e-06 8.1e-06 4.5e-06 -       -      
## TC 1.5e-08 1.0000  6.9e-14 6.3e-13 6.3e-13 1.2e-12 6.8e-12 6.4e-08 4.9e-08 1.7e-14 -      
## TG 1.0000  1.5e-08 2.7e-08 0.0077  0.2857  4.9e-08 4.9e-08 0.4373  0.0220  1.4e-08 7.0e-09
## 
## P value adjustment method: holm
```

```r
#-------------------------------------------------------------------------------
# plot everything!
grid.arrange(
  LabelMaker(positions, 'A)'),
  arrangeGrob(
    LabelMaker(sub_type, 'B)'),
    LabelMaker(mm_freq, 'C)'),
    LabelMaker(mm_type, 'D)'),
    nrow=1),
  ncol=1,
  heights = c(1, 0.67)
  )
```

```
## `geom_smooth()` using method = 'loess'
```



```
# pairwise test of medians for each group
nonDoped %>%
  DistribUncert2() %>%
  count(Type, Pos, wt=FracCount) %>%
  mutate(Norm = n / subset(readCounts, Sample == '1_nonDoped')$Reads) %T>%
  with(., pairwise.wilcox.test(Norm, Type) %>% print) %>%
  group_by(Type) %>%
  summarise(med=median(Norm), mean=mean(Norm))
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test
##
## data:  Norm and Type
```

```
##
##   D      I      M      P
## I <2e-16 -      -      -
## M <2e-16 <2e-16 -      -
## P <2e-16 <2e-16 <2e-16 -
## S <2e-16 <2e-16 <2e-16 <2e-16
##
## P value adjustment method: holm
```

```
## Source: local data table [5 x 3]
##
## # tbl_dt [5 × 3]
##    Type          med         mean
##   <chr>        <dbl>        <dbl>
## 1     D 5.638391e-04 6.021062e-04
## 2     I 9.654076e-05 9.959134e-05
## 3     M 3.079034e-03 3.189008e-03
## 4     P 3.348116e-04 3.515968e-04
## 5     S 6.162176e-06 8.277247e-06
```

```
# insertions at position 1
nonDoped %>%
  DistribUncert2() %>%
  filter(Type == 'I', Pos == 1) %>%
  count(Diff)
```

```
## Source: local data table [4 x 2]
##
## # tbl_dt [4 × 2]
##    Diff     n
##   <chr> <int>
## 1     T    57
## 2     C    16
## 3     G    78
## 4     A     1
```

```
# differences in medians in annealing regions and outside
nonDoped %>%
    DistribUncert2() %>%
    count(Type, Pos, wt=FracCount) %>%
    mutate(Norm = n / subset(readCounts, Sample == '1_nonDoped')$Reads) %>%
    filter(Type == 'P') %>%
  mutate(Region = if_else(Pos >= 36 & Pos <=64, 'Anneal', 'No')) %T>%
  {wilcox.test(Norm ~ Region, data=.) %>% print} %>%
  group_by(Region) %>%
  summarise(med=median(Norm), IQR=IQR(Norm))
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Norm by Region
## W = 371, p-value = 7.507e-07
## alternative hypothesis: true location shift is not equal to 0
## Source: local data table [2 x 3]
##
## # tbl_dt [2 × 3]
##   Region          med          IQR
##    <chr>        <dbl>        <dbl>
## 1     No 0.0003892441 0.0002018113
## 2 Anneal 0.0001889734 0.0001314598
```

Figure 3 - Error Correction and Percent Perfects

```
# reduce the all of the reads down to errors/read (this may take a while...)
# join with the length of every read and fill in perfects with a 0 count
# errRate calculation from Furhman paper
errRates <- allSamps %>%
  count(Sample, Name) %>%
  left_join(charCounts, ., by = c('Sample', 'Name')) %>%
  replace_na(list(n=0)) %>%
  group_by(Sample) %>%
  summarise(
    errRate = mean(n * (1000/Len)),
    sem = sd(n*(1000/Len)) / sqrt(n())
  ) %>%
  left_join(readCounts, by='Sample') %>%
  mutate(
    PercentPerf = (Reads - Errs) / Reads * 100,
    Treatment = str_sub(Sample, 1, 1),
    Sample = str_sub(Sample, 3),
    # Pretty printing for figures
    Sample = Sample %>%
      recode(nonDoped='Standard Oligo', DopedTemp='Doped Oligo',
             MutS_1900nM='MutS (1900nM)', MutS_950nM='MutS (950nM)',
             T7EndoIFurhmann='T7 EndoI (Fuhrmann)', T4EndoVII='T4 EndoVII',
             T7EndoI='T7 EndoI (0U T7 Lig.)',
             `T7EndoI-e3T7Ligase`='T7 EndoI (1e3U T7 Lig.)',
             `T7EndoI-e4T7Ligase`='T7 EndoI (1e4U T7 Lig.)',
             `ErrASE-nonDoped` = 'Standard Oligo (ErrASE)')
  )
```

```
# manual ordering for nice plots
plt.order <- c('Standard Oligo (ErrASE)', 'Standard Oligo', 'MutS (1900nM)',
           'MutS (950nM)', 'ErrASE', 'T7 EndoI (Fuhrmann)',
           'T4 EndoVII', 'Surveyor', 'T7 EndoI (0U T7 Lig.)',
           'T7 EndoI (1e3U T7 Lig.)', 'T7 EndoI (1e4U T7 Lig.)',
           'EndoV', 'Doped Oligo')

errRates %>%
  # add in 0's for doped and nonDoped
  select(c(-Errs, -Reads, -sem)) %>%
  bind_rows(
    data.table(Sample=c('Doped Oligo', 'Standard Oligo'),
               errRate=c(0.0, 0.0),
               PercentPerf=c(0.0, 0.0),
               Treatment=c('2', '2'))
  ) %>%
  gather(Metric, Value, PercentPerf, errRate) %>%
  mutate(
    Metric=if_else(Metric == 'PercentPerf',
                   "Percent Perfect Reads",
                   "Error Frequency (per kb)") %>%
      factor(levels=c("Percent Perfect Reads",
                      "Error Frequency (per kb)")),
    Sample = factor(Sample, levels = plt.order)
  ) %>%
  ggplot(aes(x=Sample, y=Value, fill=Treatment)) +
  geom_bar(stat='identity', position='dodge') +
  facet_wrap(~ Metric, nrow=2) +
  theme(
    axis.text.x=element_text(angle=315, hjust=0.15, vjust=0.90, size=rel(1.0)),
    axis.title.x=element_blank(),
    axis.title.y=element_blank(),
    legend.title=element_text(size=rel(2.25)),
    legend.position="bottom"
    ) +
  scale_y_continuous(breaks=seq(0,60,10)) +
  scale_fill_manual(name="Treatment Round:",
                      values=c("#ca0020", "#0571b0"))
```

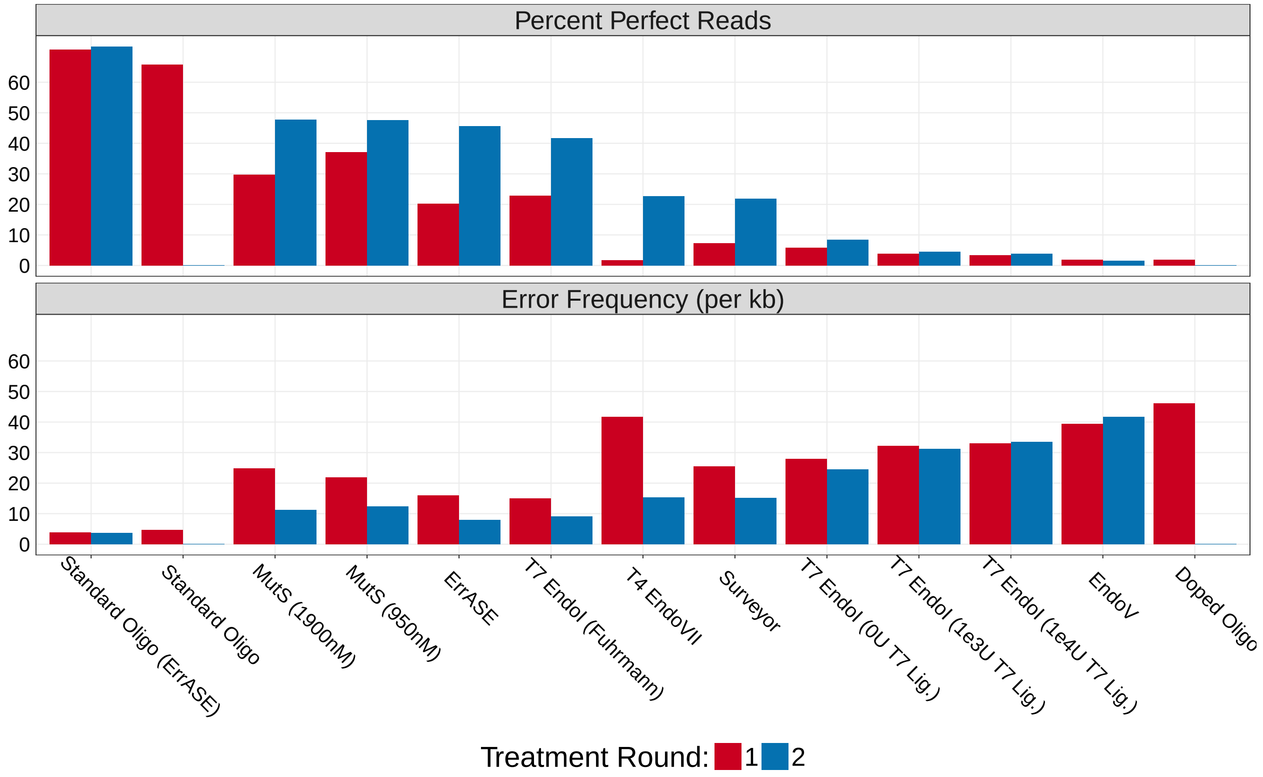
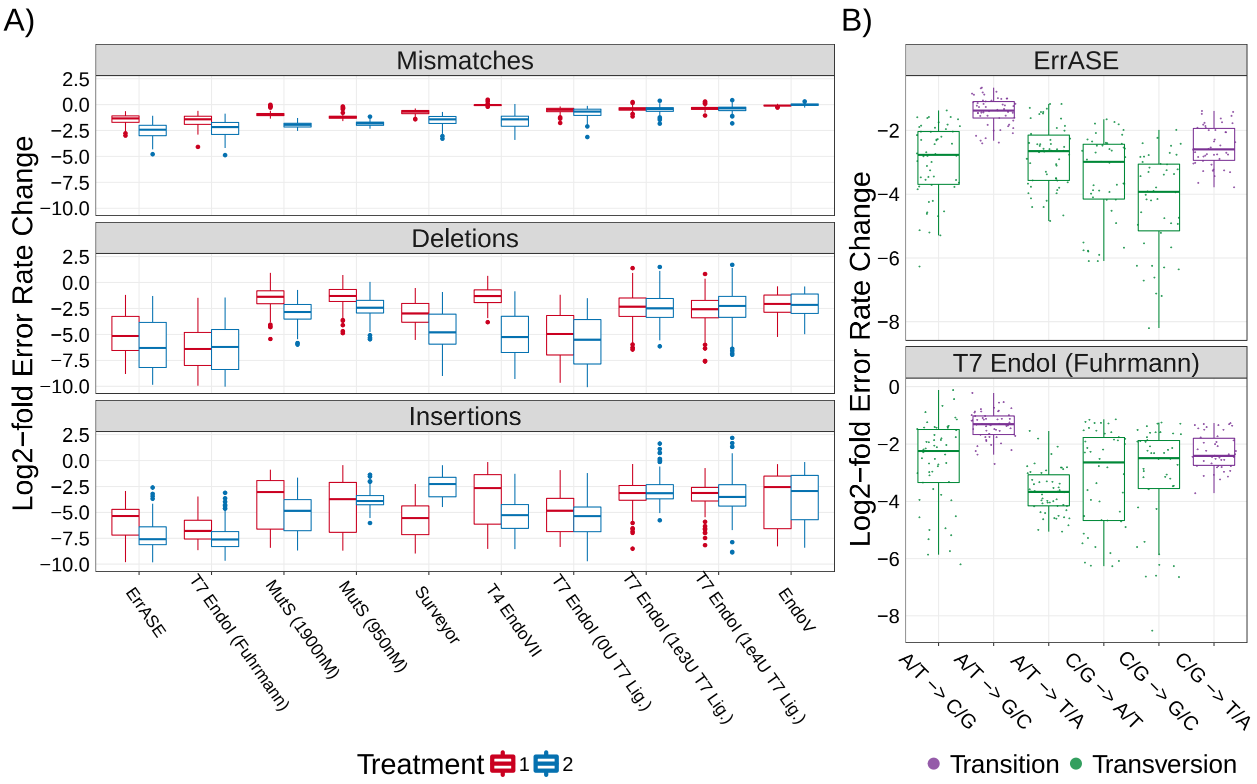


Figure 4 - Enzyme Preferences

```r
enzPref <- allSamps %>%
  DistribUncert2() %>%
  count(Sample, Type, Pos, wt=FracCount) %>%
  ungroup() %>%
  left_join(readCounts, by = 'Sample') %>%
  mutate(
    Treatment=str_sub(Sample, 1, 1),
    Sample=str_sub(Sample, 3),
    Norm=n / Reads
  ) %>%
  # Grab the normalized data from DopedTemp for easy divide
  left_join(.,
            filter(., Sample == 'DopedTemp') %>%
              transmute(Type=Type, Pos=Pos, Dope_Norm=Norm),
            by = c('Type', 'Pos')
  ) %>%
  mutate(
    Rel_Norm = Norm / Dope_Norm,
    Fold_2 = log2(Rel_Norm),
    Fold = 2^-Fold_2,
    # pretty print for figures
    Sample = Sample %>%
      recode(nonDoped='Standard Oligo', DopedTemp='Doped Oligo',
             MutS_1900nM='MutS (1900nM)', MutS_950nM='MutS (950nM)',
             T7EndoIFurhmann='T7 EndoI (Fuhrmann)', T4EndoVII='T4 EndoVII',
             T7EndoI='T7 EndoI (0U T7 Lig.)',
             `T7EndoI-e3T7Ligase`='T7 EndoI (1e3U T7 Lig.)',
             `T7EndoI-e4T7Ligase`='T7 EndoI (1e4U T7 Lig.)',
             `ErrASE-nonDoped` = 'Standard Oligo (ErrASE)')
  )

#-------------------------------------------------------------------------------
# specific call-outs for indels and mismatches
enzPref.idm <- allSamps %>%
  DistribUncert2() %>%
  filter(Type %in% c('I', 'M', 'D')) %>%
  count(Sample, Type, Pos, Diff, wt=FracCount) %>%
  ungroup() %>%
  left_join(readCounts, by = 'Sample') %>%
  mutate(
    Treatment=str_sub(Sample, 1, 1),
    Sample=str_sub(Sample, 3),
    Norm=n / Reads
    ) %>%
  # Grab the normalized data from DopedTemp for easy divide
  left_join(.,
            filter(., Sample == 'DopedTemp') %>%
              transmute(Diff=Diff, Type=Type, Pos=Pos, Dope_Norm=Norm),
            by = c('Type', 'Pos', 'Diff')
            ) %>%
  mutate(
    Rel_Norm = Norm / Dope_Norm,
    Fold_2 = log2(Rel_Norm),
    Fold = 2^-Fold_2,
    Class= Diff %>%
      recode(AT='Transversion', AG='Transition', AC='Transversion',
             TA='Transversion', TG='Transversion', TC='Transition',
             GA='Transition', GT='Transversion', GC='Transversion',
             CA='Transversion', CT='Transition', CG='Transversion'),
    Sample = Sample %>%
      recode(nonDoped='Standard Oligo', DopedTemp='Doped Oligo',
             MutS_1900nM='MutS (1900nM)', MutS_950nM='MutS (950nM)',
             T7EndoIFurhmann='T7 EndoI (Fuhrmann)', T4EndoVII='T4 EndoVII',
             T7EndoI='T7 EndoI (0U T7 Lig.)',
             `T7EndoI-e3T7Ligase`='T7 EndoI (1e3U T7 Lig.)',
             `T7EndoI-e4T7Ligase`='T7 EndoI (1e4U T7 Lig.)',
             `ErrASE-nonDoped` = 'Standard Oligo (ErrASE)')

  )
```

```
# order by error frequency
plt.order <- c('Standard Oligo (ErrASE)', 'Standard Oligo',
               'ErrASE', 'T7 EndoI (Fuhrmann)', 'MutS (1900nM)',
               'MutS (950nM)', 'Surveyor', 'T4 EndoVII',
               'T7 EndoI (0U T7 Lig.)', 'T7 EndoI (1e3U T7 Lig.)',
               'T7 EndoI (1e4U T7 Lig.)', 'EndoV', 'Doped Oligo')

#------------------------------------------------------------------------------
# Panel 1
# Plot the positional distribution across enzymes for indels and mm's
pan1 <- enzPref %>%
  filter(!Sample %in% c('Doped Oligo', 'Standard Oligo', 'Standard Oligo (ErrASE)')) %>%
  mutate(
    Type = case_when(Type == 'D' | Type == 'P' ~ 'Deletions',
                     Type == 'I' | Type == 'S' ~ 'Insertions',
                     TRUE ~ 'Mismatches'),
    # factor madness for proper ordering
    Type = factor(Type, levels = c('Mismatches', 'Deletions', 'Insertions')),
    Sample = factor(Sample, levels = plt.order)
  ) %>%
  ggplot(aes(x=Sample, y=Fold_2, color=Treatment)) +
  geom_boxplot() +
  facet_wrap(~ Type, ncol = 1) +
  theme(
    legend.position='bottom',
    legend.text=element_text(size=rel(1.5)),
    legend.title=element_text(size=rel(2.25)),
    axis.text.x=element_text(angle=305, hjust=0.15, vjust=0.90, size=rel(0.85)),
    axis.title.x=element_blank()
  ) +
  guides(colour = guide_legend(override.aes = list(size=2))) +
  scale_color_manual(
    name = 'Treatment',
    values=c("#ca0020", "#0571b0")
  ) +
  labs(y='Log2-fold Error Rate Change')

#-------------------------------------------------------------------------------
# Panel 2
# Specific call outs for ErrASE/T7 Endo
pan2 <- enzPref.idm %>%
  filter(
    Type == 'M',
    Treatment == '2',
    Sample %in% c('ErrASE', 'T7 EndoI (Fuhrmann)')
  ) %>%
  mutate(Sym = Diff %>% recode(AC = 'A/T -> C/G', TG='A/T -> C/G',
                               AG = 'A/T -> G/C', TC='A/T -> G/C',
                               AT = 'A/T -> T/A', TA='A/T -> T/A',
                               CA = 'C/G -> A/T', GT='C/G -> A/T',
                               CG = 'C/G -> G/C', GC='C/G -> G/C',
                               CT = 'C/G -> T/A', GA='C/G -> T/A')
  ) %>%
  ggplot(aes(x=Sym, y=Fold_2, color=Class)) +
  geom_boxplot(
    outlier.shape = NA,
    show.legend = FALSE
  ) +
  geom_point(
    position=position_jitter(),
    size = 0.25,
    alpha = 0.8
  ) +
  facet_wrap(~ Sample, ncol=1, scales='free_y') +
  theme(
    legend.position = 'bottom',
    axis.title.x=element_blank(),
    axis.text.x = element_text(angle = 315, vjust=0.5)
  ) +
  guides(colour = guide_legend(override.aes = list(size=5))) +
  scale_color_manual(values = c('#7b3294', '#008837')) +
  labs(y='Log2-fold Error Rate Change')

grid.arrange(
  LabelMaker(pan1, 'A)'),
  LabelMaker(pan2, 'B)'),
  ncol = 2,
  widths = c(0.67, 0.33)
)
```



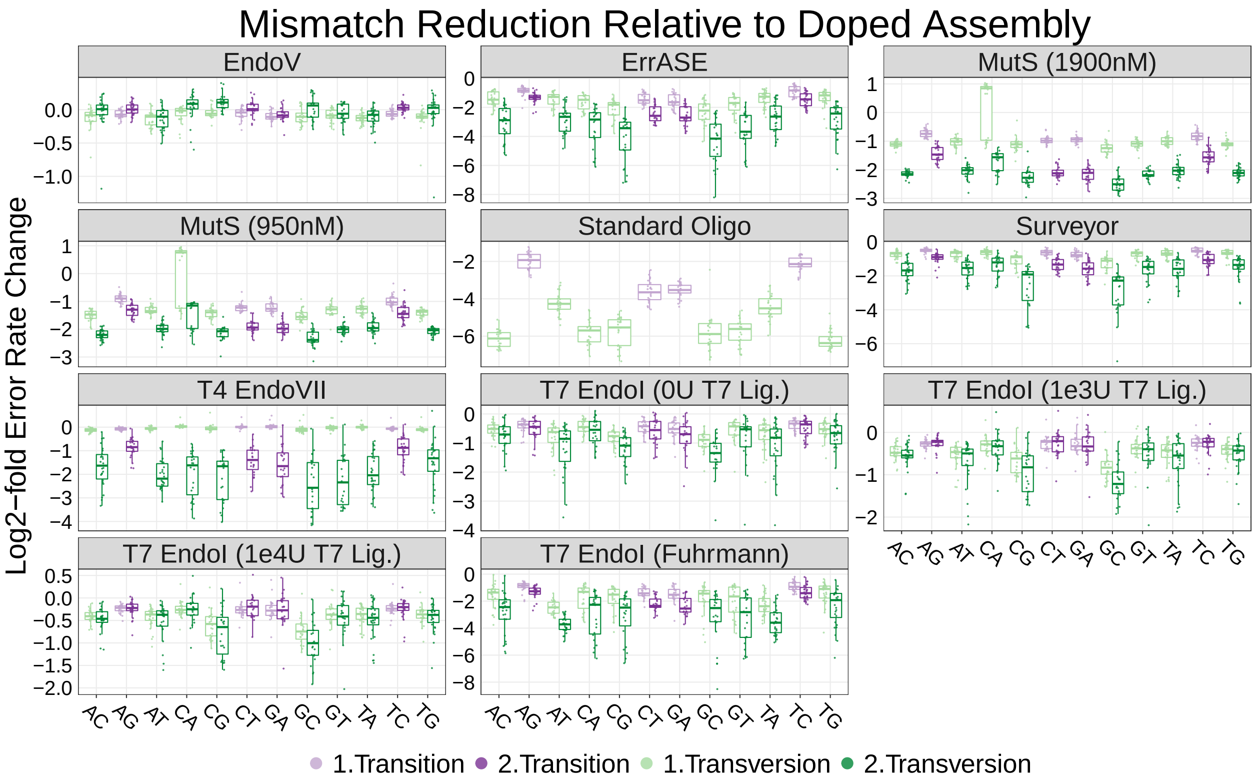
```
# table version of plot
enzPref %>%
  filter(!Sample %in% c('Doped Oligo', 'Standard Oligo', 'Standard Oligo ErrASE')) %>%
  mutate(
    Type = case_when(Type == 'D' | Type == 'P' ~ 'Deletions',
                     Type == 'I' | Type == 'S' ~ 'Insertions',
                     TRUE ~ 'Mismatches')
  ) %>%
  group_by(Sample, Treatment, Type) %>%
  summarise(
    Mean=mean(Fold),
    Median=median(Fold)
  ) %>%
  ungroup() %>%
  arrange(Sample, Treatment, Type)
```

```
## Source: local data table [66 x 5]
##
## # tbl_dt [66 × 5]
##    Sample Treatment        Type      Mean    Median
##     <chr>     <chr>       <chr>     <dbl>     <dbl>
## 1   EndoV         1   Deletions  5.565447  4.152186
## 2   EndoV         1 Insertions  58.663793  5.906338
## 3   EndoV         1 Mismatches   1.064883  1.064428
## 4   EndoV         2   Deletions  5.845421  4.404096
## 5   EndoV         2 Insertions  38.613960  7.666019
## 6   EndoV         2 Mismatches   1.010861  1.008456
## 7  ErrASE         1   Deletions 68.864735 36.052656
## 8  ErrASE         1 Insertions 114.955902 40.655895
## 9  ErrASE         1 Mismatches   2.880278  2.510900
## 10 ErrASE         2  Deletions 188.193013 78.790890
## # ... with 56 more rows
```

Figure 4 - Supplement

Sup - Mismatch Preferences

```
enzPref.idm %>%
  filter(
    Type == 'M',
    !Sample %in% c('Doped Oligo', 'Standard Oligo (ErrASE)')
  ) %>%
  ggplot(
    aes(x=Diff,
        y=Fold_2,
        color=interaction(Treatment, Class))
  ) +
  geom_boxplot(
    outlier.shape = NA,
    show.legend = FALSE
  ) +
  geom_point(
    position=position_jitterdodge(),
    size = 0.25,
    alpha = 0.8
  ) +
  facet_wrap(~ Sample, ncol=3, scales='free_y') +
  theme(
    legend.position = 'bottom',
    axis.title.x=element_blank(),
    axis.text.x = element_text(angle = 315, vjust=0.5)
  ) +
  guides(colour = guide_legend(override.aes = list(size=5))) +
  scale_color_manual(values = c('#c2a5cf','#7b3294', '#a6dba0','#008837')) +
  labs(
    title='Mismatch Reduction Relative to Doped Assembly',
    y='Log2-fold Error Rate Change'
  )
```



There appears to be some real differences here, let's do some testing.

```
# first run anova to make sure there are diffs in medians
enzPref.idm %>%
  filter(
    Type %in% c('D', 'I', 'M'),
    !Sample %in% c('Doped Oligo', 'Standard Oligo', 'Standard Oligo (ErrASE)')
  ) %>%
  mutate(Diff = factor(Diff)) %>%
  group_by(Sample, Treatment, Type) %>%
  do(tidy(kruskal.test(Fold_2 ~ Diff, data=.))) %>%
  ungroup() %>%
  select(Sample, Treatment, Type, statistic, p.value)
```

```
## Source: local data table [60 x 5]
##
## # tbl_dt [60 × 5]
##                      Sample Treatment  Type statistic    p.value
##                       <chr>     <chr> <chr>     <dbl>      <dbl>
## 1                     EndoV         1     D  4.130051 0.24775910
## 2                    ErrASE         1     D  7.111247 0.06843521
## 3              MutS (1900nM)        1     D  3.926210 0.26954313
## 4               MutS (950nM)        1     D  9.139608 0.02749117
## 5                  Surveyor         1     D  6.943758 0.07371217
## 6                T4 EndoVII         1     D  3.826506 0.28081807
## 7     T7 EndoI (0U T7 Lig.)         1     D  2.789177 0.42528460
## 8   T7 EndoI (1e3U T7 Lig.)         1     D  4.480190 0.21406410
## 9   T7 EndoI (1e4U T7 Lig.)         1     D  3.789947 0.28505758
## 10      T7 EndoI (Fuhrmann)         1     D  1.974915 0.57762954
## # ... with 50 more rows
```

```
# transition vs transversions
enzPref.idm %>%
  filter(
    Type == 'M',
    !Sample %in% c('Doped Oligo', 'Standard Oligo', 'Standard Oligo (ErrASE)')
  ) %>%
  group_by(Sample, Treatment) %>%
  do(tidy(wilcox.test(Fold_2 ~ Class, data=.))) %>%
  ungroup() %>%
  select(Sample, Treatment, statistic, p.value)
```

```
## Source: local data table [20 x 4]
##
## # tbl_dt [20 × 4]
##                      Sample Treatment statistic      p.value
##                       <chr>     <chr>     <dbl>        <dbl>
## 1                     EndoV         1     11650 1.986610e-02
## 2                    ErrASE         1     14421 4.343993e-10
## 3             MutS (1900nM)         1     15318 6.023112e-14
## 4              MutS (950nM)         1     15836 1.737675e-16
## 5                  Surveyor         1     14181 3.584885e-09
## 6                T4 EndoVII         1     11555 2.818224e-02
## 7     T7 EndoI (0U T7 Lig.)         1     13773 1.002606e-07
## 8   T7 EndoI (1e3U T7 Lig.)         1     15384 2.943936e-14
## 9   T7 EndoI (1e4U T7 Lig.)         1     15151 3.547354e-13
## 10      T7 EndoI (Fuhrmann)         1     15033 1.201505e-12
## 11                    EndoV         2     10348 6.236937e-01
## 12                   ErrASE         2     16455 8.023391e-20
## 13            MutS (1900nM)         2     14586 9.538181e-11
## 14             MutS (950nM)         2     15691 9.418908e-16
## 15                 Surveyor         2     14587 9.449412e-11
## 16               T4 EndoVII         2     15043 1.084638e-12
## 17    T7 EndoI (0U T7 Lig.)         2     13539 5.856954e-07
## 18  T7 EndoI (1e3U T7 Lig.)         2     15360 3.823047e-14
## 19  T7 EndoI (1e4U T7 Lig.)         2     14644 5.527648e-11
## 20      T7 EndoI (Fuhrmann)         2     15721 6.661996e-16
```

```
# median values for paper
enzPref.idm %>%
  filter(Treatment == '2') %>%
  group_by(Sample, Type, Diff) %>%
  summarise(med = median(Fold)) %>%
  spread(Sample, med)
```

```
## Source: local data table [20 x 13]
## Groups:
##
## # grouped_dt [20 × 13]
##      Type  Diff     EndoV     ErrASE `MutS (1900nM)` `MutS (950nM)` `Standard Oligo (ErrASE)`  Surveye
## *   <chr> <chr>     <dbl>      <dbl>           <dbl>          <dbl>                     <dbl>     <dbl
## 1       D     A 1.9407476  27.268578        5.612455       4.402694                 15.995512  6.6450
## 2      D     C 1.9375507  19.428743        5.216762       3.677288                 30.117109  4.65869
## 3      D     G 3.1062003  64.141297        7.276764       5.240921                 70.168749 13.39179
## 4      D     T 2.3074515  25.498670        6.174086       5.880878                 46.239703 11.52934
## 5      I     A 8.2996398  77.855939       14.969087      21.842308                 69.359554  8.78198
## 6      I     C 9.1831863  87.715424       16.608982      20.562173                 73.058730 13.76810
## 7      I     G 7.0873865 108.114360       30.762161      21.748965                117.911242 12.84161
## 8      I     T 8.2498679  83.781344       27.466216      19.714083                 75.965226  8.75280
## 9      M    AC 0.9949576   7.387999        4.444978       4.610612                 56.765547  3.20429
## 10     M    AG 0.9975086   2.463517        2.767391       2.459252                  3.638588  1.85592
## 11     M    AT 1.0751901   6.289659        4.047035       3.971451                 16.447679  2.89752
## 12     M    CA 0.9410410   7.160285        2.954518       2.219500                 45.379807  2.30738
## 13     M    CG 0.9283197  10.818895        4.857978       4.210227                 77.405262  3.77559
## 14     M    CT 0.9944130   5.989511        4.358451       3.839514                 12.789122  2.51748
## 15     M    GA 1.0612757   6.510108        4.312037       3.941436                 12.406390  2.98030
## 16     M    GC 0.9575938  17.784310        5.686389       5.229828                 97.387928  4.85570
## 17     M    GT 1.0426257  12.786200        4.560414       3.995814                 46.507634  2.78411
## 18     M    TA 1.0551532   6.222358        4.084956       3.885780                 18.062219  3.00594
## 19     M    TC 0.9800512   2.731725        2.966423       2.729911                  4.165589  2.09230
## 20     M    TG 0.9808719   5.373149        4.325695       4.099834                 56.282996  2.55891
```

```r
# ErrASE cg/gc, ag/tc
errase.pref <- enzPref.idm %>%
  filter(
    Sample == 'ErrASE',
    Type == 'M'
  ) %>%
  mutate(
    GC = if_else(Diff == 'GC' | Diff == 'CG', 'GC', 'No'),
    AG = if_else(Diff == 'AG' | Diff == 'TC', 'AG', 'No')
  ) %>%
  select(Pos, Diff, Treatment, Fold, GC, AG) %>%
  gather(Test, Val, GC, AG)

# p.val invariant of fold vs fold_2
errase.pref %>%
  group_by(Treatment, Test) %>%
  do(tidy(wilcox.test(Fold ~ Val, data=.))) %>%
  ungroup() %>%
  select(Test, Treatment, statistic, p.value)
```

```
## # A tibble: 4 × 4
##    Test Treatment statistic      p.value
##   <chr>     <chr>     <dbl>        <dbl>
## 1    AG         1      1704 1.192852e-17
## 2    GC         1      9647 2.100637e-12
## 3    AG         2       779 3.113673e-24
## 4    GC         2      9489 1.630541e-11
```

```r
errase.pref %>%
  group_by(Test, Treatment, Val) %>%
  summarise(med = median(Fold))
```

```
## Source: local data frame [8 x 4]
## Groups: Test, Treatment [?]
##
##    Test Treatment   Val       med
##   <chr>     <chr> <chr>     <dbl>
## 1    AG         1    AG  1.776546
## 2    AG         1    No  2.922308
## 3    AG         2    AG  2.600594
## 4    AG         2    No  7.147050
## 5    GC         1    GC  3.949500
## 6    GC         1    No  2.422313
## 7    GC         2    GC 15.203511
## 8    GC         2    No  5.401971
```

```r
# T7 ta/at, cg/gc(ligase), ag/tc
t7.pref <- enzPref.idm %>%
  filter(
    str_detect(Sample, 'T7'),
    Type == 'M'
  ) %>%
  mutate(
    AT = if_else(Diff == 'AT' | Diff == 'TA', 'AT', 'No'),
    GC = if_else(Diff == 'GC' | Diff == 'CG', 'GC', 'No'),
    AG = if_else(Diff == 'AG' | Diff == 'TC', 'AG', 'No')
  ) %>%
  select(Sample, Pos, Diff, Treatment, Fold, AT, GC, AG) %>%
  gather(Test, Val, AT, GC, AG)

t7.pref %>%
  group_by(Sample, Treatment, Test) %>%
  do(tidy(wilcox.test(Fold ~ Val, data=.))) %>%
  ungroup() %>%
  select(Sample, Test, Treatment, statistic, p.value)
```

```
## # A tibble: 24 × 5
##                     Sample  Test Treatment statistic      p.value
##                      <chr> <chr>     <chr>     <dbl>        <dbl>
## 1    T7 EndoI (0U T7 Lig.)    AG         1      3750 5.466407e-07
## 2    T7 EndoI (0U T7 Lig.)    AT         1      8189 7.381498e-03
## 3    T7 EndoI (0U T7 Lig.)    GC         1      9446 2.807120e-11
## 4    T7 EndoI (0U T7 Lig.)    AG         2      3869 1.562894e-06
## 5    T7 EndoI (0U T7 Lig.)    AT         2      8248 5.413589e-03
## 6    T7 EndoI (0U T7 Lig.)    GC         2      9150 9.989281e-10
## 7  T7 EndoI (1e3U T7 Lig.)    AG         1      3424 2.490561e-08
## 8  T7 EndoI (1e3U T7 Lig.)    AT         1      8157 8.698338e-03
## 9  T7 EndoI (1e3U T7 Lig.)    GC         1      9664 1.676603e-12
## 10 T7 EndoI (1e3U T7 Lig.)    AG         2      3532 7.171005e-08
## # ... with 14 more rows
```

```r
t7.pref %>%
  group_by(Sample, Test, Treatment, Val) %>%
  summarise(med = median(Fold))
```

```
## Source: local data frame [48 x 5]
## Groups: Sample, Test, Treatment [?]
##
##                    Sample  Test Treatment   Val      med
##                     <chr> <chr>     <chr> <chr>    <dbl>
## 1  T7 EndoI (OU T7 Lig.)    AG         1    AG 1.267042
## 2  T7 EndoI (OU T7 Lig.)    AG         1    No 1.486359
## 3  T7 EndoI (OU T7 Lig.)    AG         2    AG 1.345917
## 4  T7 EndoI (OU T7 Lig.)    AG         2    No 1.716004
## 5  T7 EndoI (OU T7 Lig.)    AT         1    AT 1.522887
## 6  T7 EndoI (OU T7 Lig.)    AT         1    No 1.419058
## 7  T7 EndoI (OU T7 Lig.)    AT         2    AT 1.789891
## 8  T7 EndoI (OU T7 Lig.)    AT         2    No 1.605018
## 9  T7 EndoI (OU T7 Lig.)    GC         1    GC 1.752622
## 10 T7 EndoI (OU T7 Lig.)    GC         1    No 1.396459
## # ... with 38 more rows
```

```
# muts ag/tc cg/gc
muts.pref <- enzPref.idm %>%
  filter(
    str_detect(Sample, 'MutS'),
    Type == 'M'
  ) %>%
  mutate(
    GC = if_else(Diff == 'GC' | Diff == 'CG', 'GC', 'No'),
    AG = if_else(Diff == 'AG' | Diff == 'TC', 'AG', 'No')
  ) %>%
  select(Sample, Pos, Diff, Treatment, Fold, GC, AG) %>%
  gather(Test, Val, GC, AG)

muts.pref %>%
  group_by(Sample, Treatment, Test) %>%
  do(tidy(wilcox.test(Fold ~ Val, data=.))) %>%
  ungroup() %>%
  select(Sample, Test, Treatment, statistic, p.value)
```

```
## # A tibble: 8 × 5
##          Sample  Test Treatment statistic      p.value
##           <chr> <chr>     <chr>     <dbl>        <dbl>
## 1 MutS (1900nM)    AG         1      2150 7.196440e-15
## 2 MutS (1900nM)    GC         1      9248 3.162290e-10
## 3 MutS (1900nM)    AG         2       935 4.796316e-23
## 4 MutS (1900nM)    GC         2      9825 1.888872e-13
## 5  MutS (950nM)    AG         1      1533 8.763013e-19
## 6  MutS (950nM)    GC         1      8941 1.043401e-08
## 7  MutS (950nM)    AG         2      1005 1.598121e-22
## 8  MutS (950nM)    GC         2      9371 7.134109e-11
```

```
muts.pref %>%
  group_by(Sample, Test, Treatment, Val) %>%
  summarise(med = median(Fold))
```

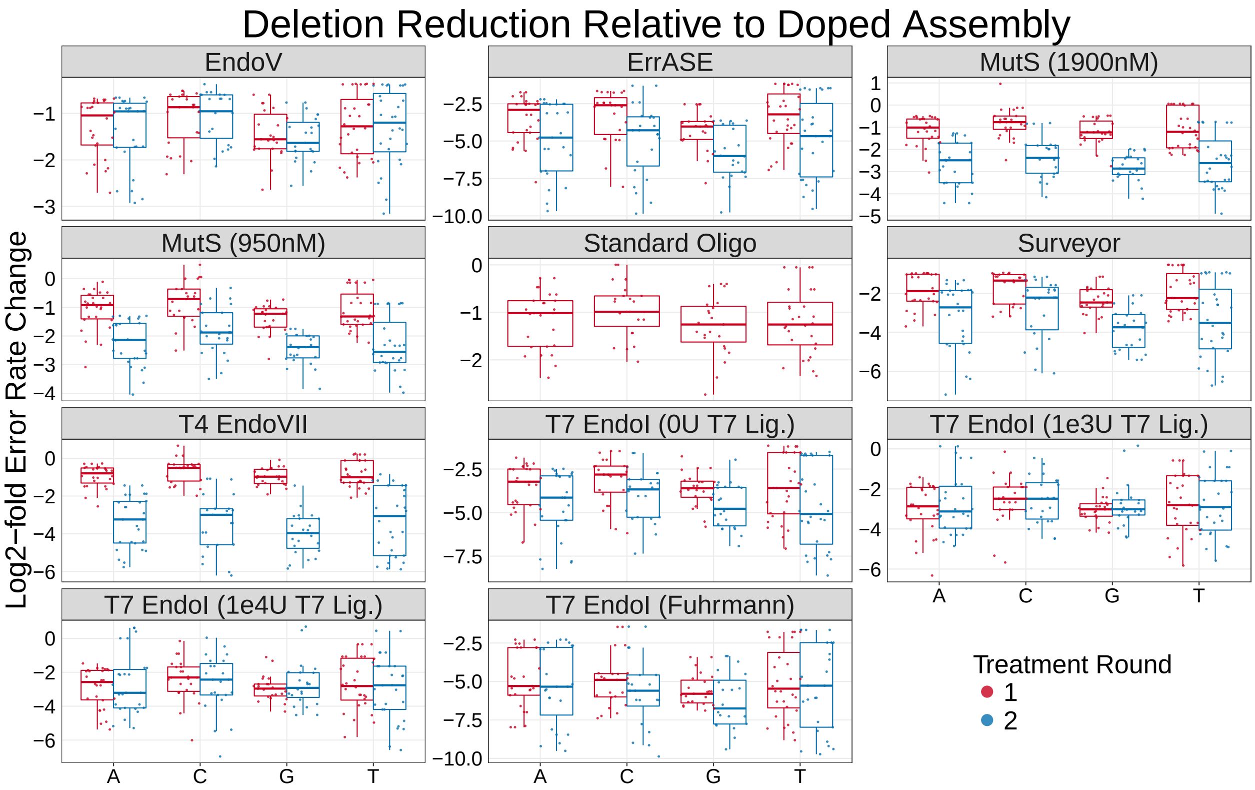
```
## Source: local data frame [16 x 5]
## Groups: Sample, Test, Treatment [?]
```

```
##
##            Sample  Test Treatment   Val      med
##             <chr> <chr>     <chr> <chr>    <dbl>
## 1  MutS (1900nM)    AG         1    AG 1.744946
## 2  MutS (1900nM)    AG         1    No 2.074040
## 3  MutS (1900nM)    AG         2    AG 2.805817
## 4  MutS (1900nM)    AG         2    No 4.357599
## 5  MutS (1900nM)    GC         1    GC 2.296331
## 6  MutS (1900nM)    GC         1    No 1.962474
## 7  MutS (1900nM)    GC         2    GC 5.130657
## 8  MutS (1900nM)    GC         2    No 4.092990
## 9   MutS (950nM)    AG         1    AG 1.958103
## 10  MutS (950nM)    AG         1    No 2.527475
## 11  MutS (950nM)    AG         2    AG 2.610612
## 12  MutS (950nM)    AG         2    No 4.070371
## 13  MutS (950nM)    GC         1    GC 2.758041
## 14  MutS (950nM)    GC         1    No 2.373760
## 15  MutS (950nM)    GC         2    GC 4.656740
## 16  MutS (950nM)    GC         2    No 3.798405
```

Sup - Deletion Preferences

Enzyme specificties for single base deletions

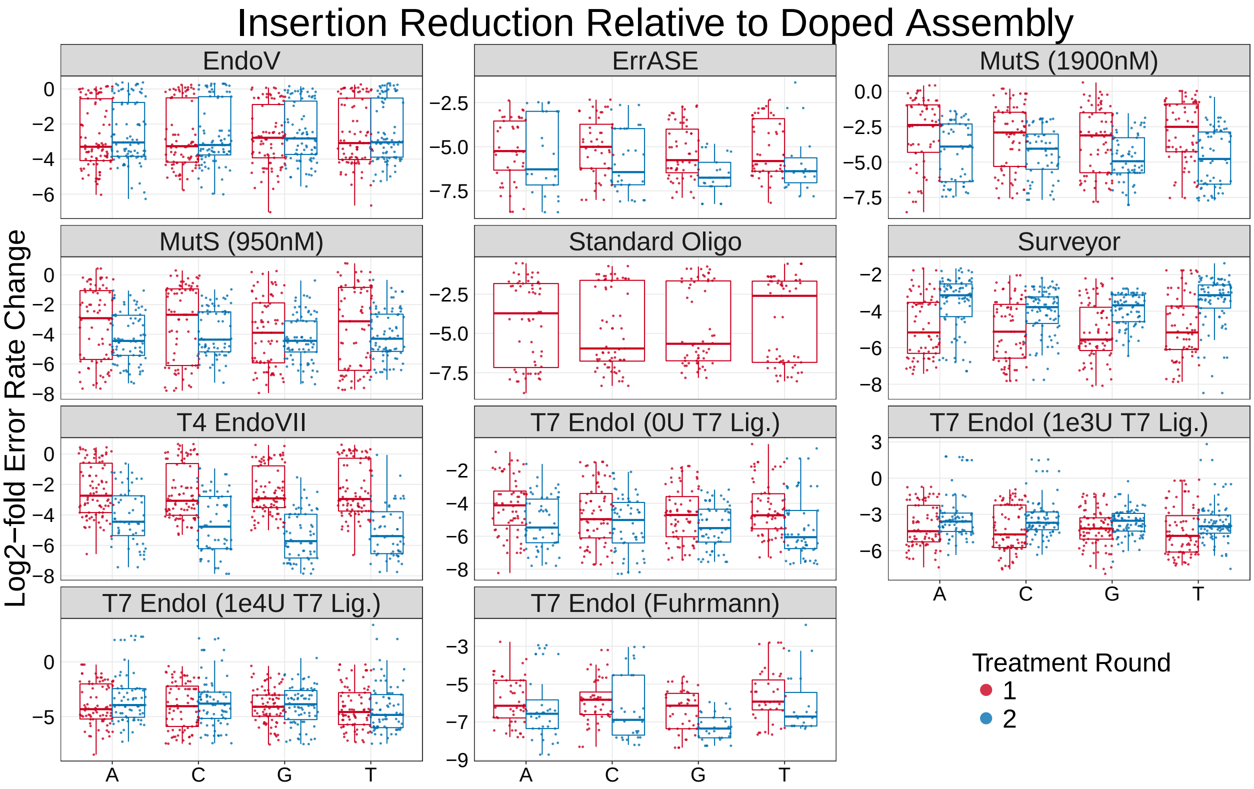
```
enzPref.idm %>%
  filter(
    Type == 'D',
    !Sample %in% c('Doped Oligo', 'Standard Oligo (ErrASE)')
  ) %>%  ggplot(aes(x=Diff, y=Fold_2, color=Treatment)) +
  geom_boxplot(
    outlier.shape = NA,
    show.legend = FALSE
  ) +
  geom_point(
    position=position_jitterdodge(),
    size = 0.5,
    alpha = 0.8
  ) +
  facet_wrap(~ Sample, ncol=3, scales='free_y') +
  theme(
    axis.title.x=element_blank(),
    legend.title=element_text(size=rel(2)),
    legend.position=c(0.85, 0.10)
    ) +
  guides(colour = guide_legend(override.aes = list(size=5))) +
  scale_color_manual(
    name = 'Treatment Round',
    values = c('#ca0020', '#0571b0')
  ) +
  labs(
    title='Deletion Reduction Relative to Doped Assembly',
       y='Log2-fold Error Rate Change'
  )
```



Sup - Insertion Preferences

Enzyme specificties for single base insertions

```
enzPref.idm %>%
  filter(
    Type == 'I',
    !Sample %in% c('Doped Oligo', 'Standard Oligo (ErrASE)')
  ) %>%  ggplot(aes(x=Diff, y=Fold_2, color=Treatment)) +
  geom_boxplot(
    outlier.shape = NA,
    show.legend = FALSE
  ) +
  geom_point(
    position=position_jitterdodge(),
    size = 0.5,
    alpha = 0.8
  ) +
  facet_wrap(~ Sample, ncol=3, scales='free_y') +
  theme(
    axis.title.x=element_blank(),
    legend.title=element_text(size=rel(2)),
    legend.position=c(0.85, 0.10)
    ) +
  guides(colour = guide_legend(override.aes = list(size=5))) +
  scale_color_manual(
    name = 'Treatment Round',
    values = c('#ca0020', '#0571b0')
  ) +
  labs(
    title='Insertion Reduction Relative to Doped Assembly',
       y='Log2-fold Error Rate Change'
    )
```



Extra Supplement

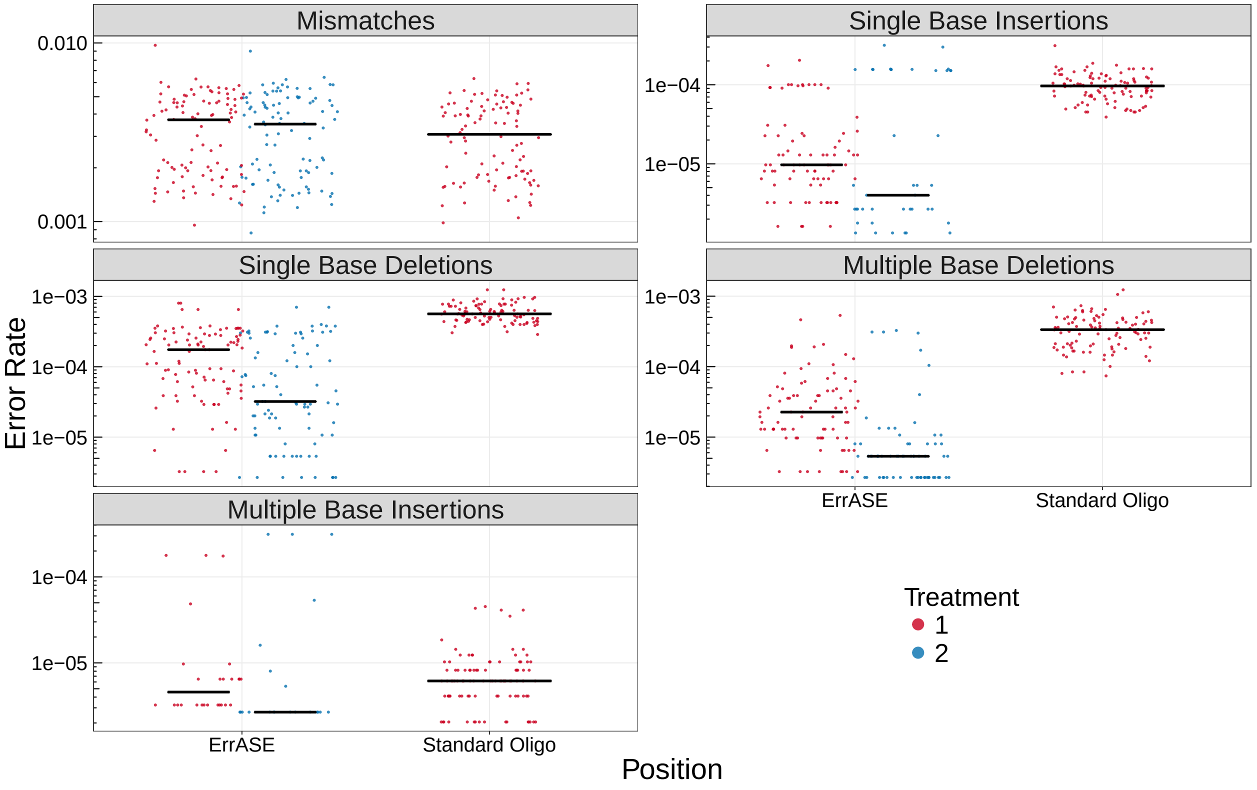
Doped Oligo

Non-Doped w/ ErrASE

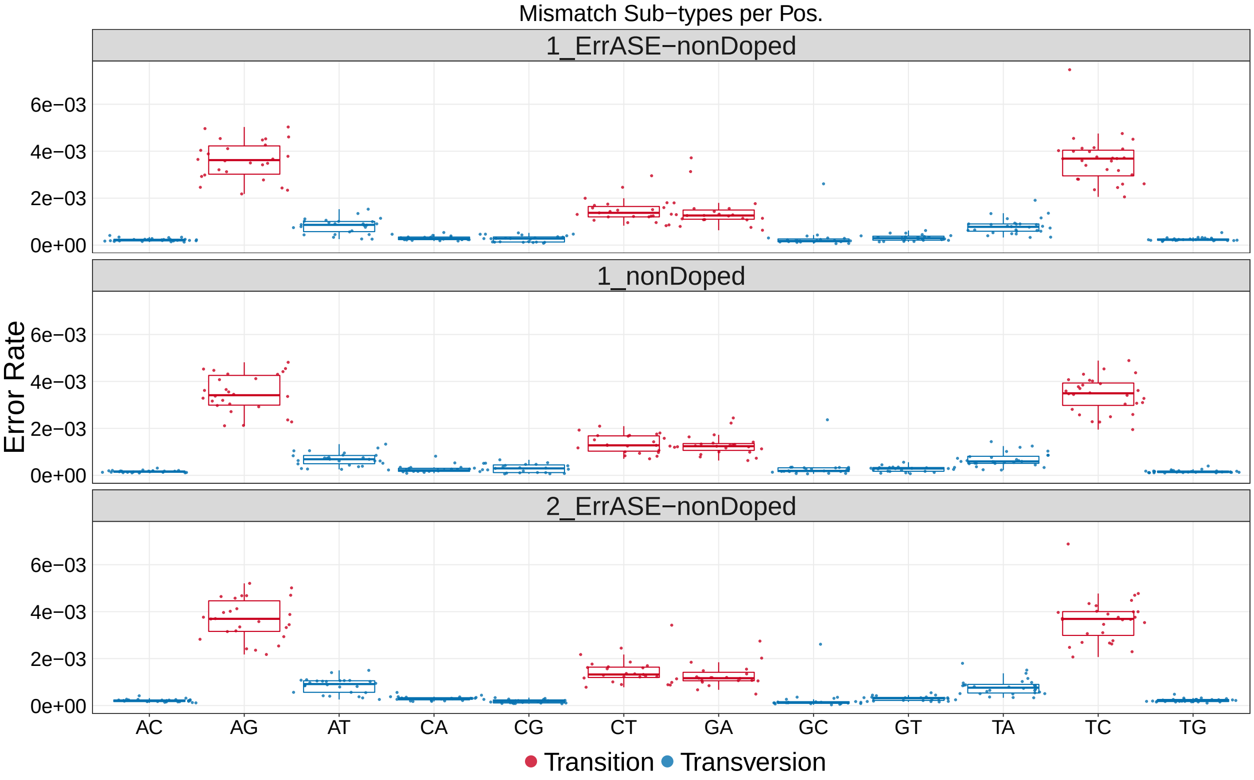
Can we figure out what the noise floor is for our method? Is there a difference between the standard oligo and its error corrected counterpart?

```
allSamps %>%
  filter(Sample %in% c('1_nonDoped', '1_ErrASE-nonDoped', '2_ErrASE-nonDoped')) %>%
  DistribUncert2() %>%
  count(Sample, Type, Pos, wt=FracCount) %>%
  ungroup() %>%
  left_join(readCounts, by='Sample') %>%
  mutate(
    Norm = n / Reads,
    Treatment = str_sub(Sample, 1, 1),
    Sample = str_sub(Sample, 3),
    Sample = Sample %>%
      recode(`ErrASE-nonDoped`='ErrASE',
             nonDoped='Standard Oligo'),
    Type = Type %>%
      factor(levels = c('M', 'I', 'D', 'P', 'S')) %>%
      recode(D = 'Single Base Deletions', I = 'Single Base Insertions',
             P = 'Multiple Base Deletions', S = 'Multiple Base Insertions',
             M = 'Mismatches')
  ) %T>%
  {
    group_by(., Type) %>%
      do(with(.,
              tidy(pairwise.wilcox.test(Norm, interaction(Sample, Treatment))))) %>%
      print
  } %>%
  ggplot(aes(x=Sample, y=Norm, color=Treatment, group=Treatment)) +
  facet_wrap(~ Type, ncol=2, scales='free_y') +
  geom_point(
    size=0.75,
    alpha=0.8,
    position=position_jitterdodge()
  ) +
  stat_summary(
    fun.y = median, fun.ymin = median, fun.ymax = median,
    geom = 'crossbar',
    width = 0.5,
    color='black',
    position=position_dodge(width=0.7)
  ) +
  scale_y_log10() +
  annotation_logticks(sides='l') +
  theme(
    legend.title=element_text(size=rel(2)),
    legend.position=c(0.75, 0.15)
  ) +
  guides(colour = guide_legend(override.aes = list(size=5))) +
  scale_color_manual(
    name = 'Treatment',
    values=c("#ca0020", "#0571b0")
  ) +
  labs(x = 'Position', y = 'Error Rate')
```

```
## Source: local data table [15 x 4]
## Groups: Type
##
## # grouped_dt [15 × 4]
##                         Type           group1           group2      p.value
##                       <fctr>           <fctr>            <chr>        <dbl>
## 1     Single Base Deletions Standard Oligo.1         ErrASE.1 2.806476e-28
## 2     Single Base Deletions         ErrASE.2         ErrASE.1 1.693221e-04
## 3     Single Base Deletions         ErrASE.2 Standard Oligo.1 1.782420e-29
## 4    Single Base Insertions Standard Oligo.1         ErrASE.1 4.223533e-20
## 5    Single Base Insertions         ErrASE.2         ErrASE.1 1.344410e-01
## 6    Single Base Insertions         ErrASE.2 Standard Oligo.1 8.154933e-04
## 7                Mismatches Standard Oligo.1         ErrASE.1 7.007785e-01
## 8                Mismatches         ErrASE.2         ErrASE.1 9.019551e-01
## 9                Mismatches         ErrASE.2 Standard Oligo.1 9.019551e-01
## 10  Multiple Base Deletions Standard Oligo.1         ErrASE.1 5.450814e-27
## 11  Multiple Base Deletions         ErrASE.2         ErrASE.1 2.052926e-11
## 12  Multiple Base Deletions         ErrASE.2 Standard Oligo.1 1.928125e-24
## 13 Multiple Base Insertions Standard Oligo.1         ErrASE.1 4.973757e-01
## 14 Multiple Base Insertions         ErrASE.2         ErrASE.1 3.150188e-02
## 15 Multiple Base Insertions         ErrASE.2 Standard Oligo.1 1.969505e-01
```



```
allSamps %>%
  filter(Sample %in% c('1_nonDoped', '1_ErrASE-nonDoped', '2_ErrASE-nonDoped')) %>%
  filter(Type == 'M') %>%
  count(Sample, Pos, Diff) %>%
  ungroup() %>%
  left_join(readCounts, by = 'Sample') %>%
  mutate(
    Norm = n / Reads,
    Class= Diff %>%
      recode(AT='Transversion', AG='Transition', AC='Transversion',
             TA='Transversion', TG='Transversion', TC='Transition',
             GA='Transition', GT='Transversion', GC='Transversion',
             CA='Transversion', CT='Transition', CG='Transversion')
  ) %>%
  ggplot(aes(x = Diff, y = Norm, color=Class)) +
  geom_boxplot(outlier.shape = NA, show.legend = FALSE) +
  geom_jitter(position=position_jitter(w = 0.5), size=0.75, alpha=0.8) +
  facet_wrap(~ Sample, ncol = 1) +
  labs(
    y = 'Error Rate',
    title = 'Mismatch Sub-types per Pos.'
  ) +
  theme(
    legend.position='bottom',
    legend.key.size=unit(0.75, "cm"),
    axis.title.x=element_blank(),
    plot.title = element_text(size=rel(1.75))
  ) +
  scale_y_continuous(labels = scientific_format()) +
  scale_color_manual(values=c("#ca0020", "#0571b0")) +
  guides(colour = guide_legend(override.aes = list(size=5)))
```



Doped Analysis

Here we will run the same sorts of analysis as Figure 2 on the Doped oligo.

```
positions_dope <- doped %>%
  DistribUncert2() %>%
  count(Pos, Type, wt=FracCount) %>%
  filter(Type != 'S') %>%
  ungroup() %>%
  mutate(
    Norm = n / subset(readCounts, Sample == '1_nonDoped')$Reads,
    Type = Type %>%
      factor(levels = c('M', 'I', 'D', 'P')) %>%
      recode(D = 'Single Base Deletions', I = 'Single Base Insertions',
             P = 'Multiple Base Deletions', M = 'Mismatches')
  ) %>%
  ggplot(aes(x=Pos, y=Norm)) +
  geom_point(size=3) +
  facet_wrap(~ Type, ncol=2) +
  stat_smooth(se=F) +
  labs(x = 'Position',
       y = 'Error Rate',
       title = 'Error Rate vs. Position for Error Sub-types') +
  scale_y_log10(labels = scientific_format()) +
  annotation_logticks(sides='l')

#-------------------------------------------------------------------------------
# Panel 1b
# Percentage of Error Subtypes

sub_type_dope <- doped %>%
  DistribUncert2() %>%
  count(Type, wt=FracCount) %>%
  mutate(
    Norm = n / sum(n) * 100,
    Type = Type %>%
      factor(levels = c('M', 'D', 'P', 'I', 'S'))  %>%
      recode(M = 'MM', D = 'Del.', P = 'M. Del.', I = 'Ins.', S = 'M. Ins.')
  ) %T>%
  {arrange(., -Norm) %>% print()} %>%
  ggplot(aes(x=Type, y=Norm)) +
  geom_bar(stat='identity') +
  theme(plot.title = element_text(size=rel(2))) +
  labs(
    y = 'Percent',
    x = 'Error Type',
    title = 'Percentage of Error Sub-types'
  )
```

```
## Source: local data table [5 x 3]
##
## # tbl_dt [5 × 3]
##      Type       n      Norm
##    <fctr>   <dbl>     <dbl>
## 1      MM 1620841 90.867916
## 2    Del.   54805  3.072489
## 3 M. Del.   48431  2.715149
## 4    Ins.   33058  1.853304
## 5 M. Ins.   26598  1.491142
```

```
#-------------------------------------------------------------------------------
# Panel c
# plot the distribution of total mismatches per position
mm_freq_dope <- doped %>%
  filter(Type == 'M') %>%
  mutate(
    To = str_sub(Diff, 2, 2),
    From = str_sub(Diff, 1, 1)
  ) %>%
  count(Pos, From) %>%
  ungroup() %>%
  mutate(Norm = n / subset(readCounts, Sample == '1_nonDoped')$Reads) %T>%
  { # pairwise wilcox test and print median values for paper
    group_by(., From) %>%
      summarise(med=median(Norm)) %>%
      arrange(-med) %>%
      print; # <- ; critical for . to be interpreted correctly
    with(., pairwise.wilcox.test(n, From)) %>% print
  } %>%
  ggplot(aes(x = From, y = Norm)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(position=position_jitter(w = 0.5), size=0.75, alpha=0.8) +
  # stat_summary(fun.y = median, fun.ymin = median, fun.ymax = median, geom = 'crossbar', width = 0.5)
  labs(y = 'Error Rate per Base',
       title = 'Mismatches per Pos.') +
  theme(
    axis.text.x = element_text(size=28),
    axis.title.x = element_blank(),
    plot.title = element_text(size=rel(2.25))
  ) +
  scale_y_continuous(labels = scientific_format())
```

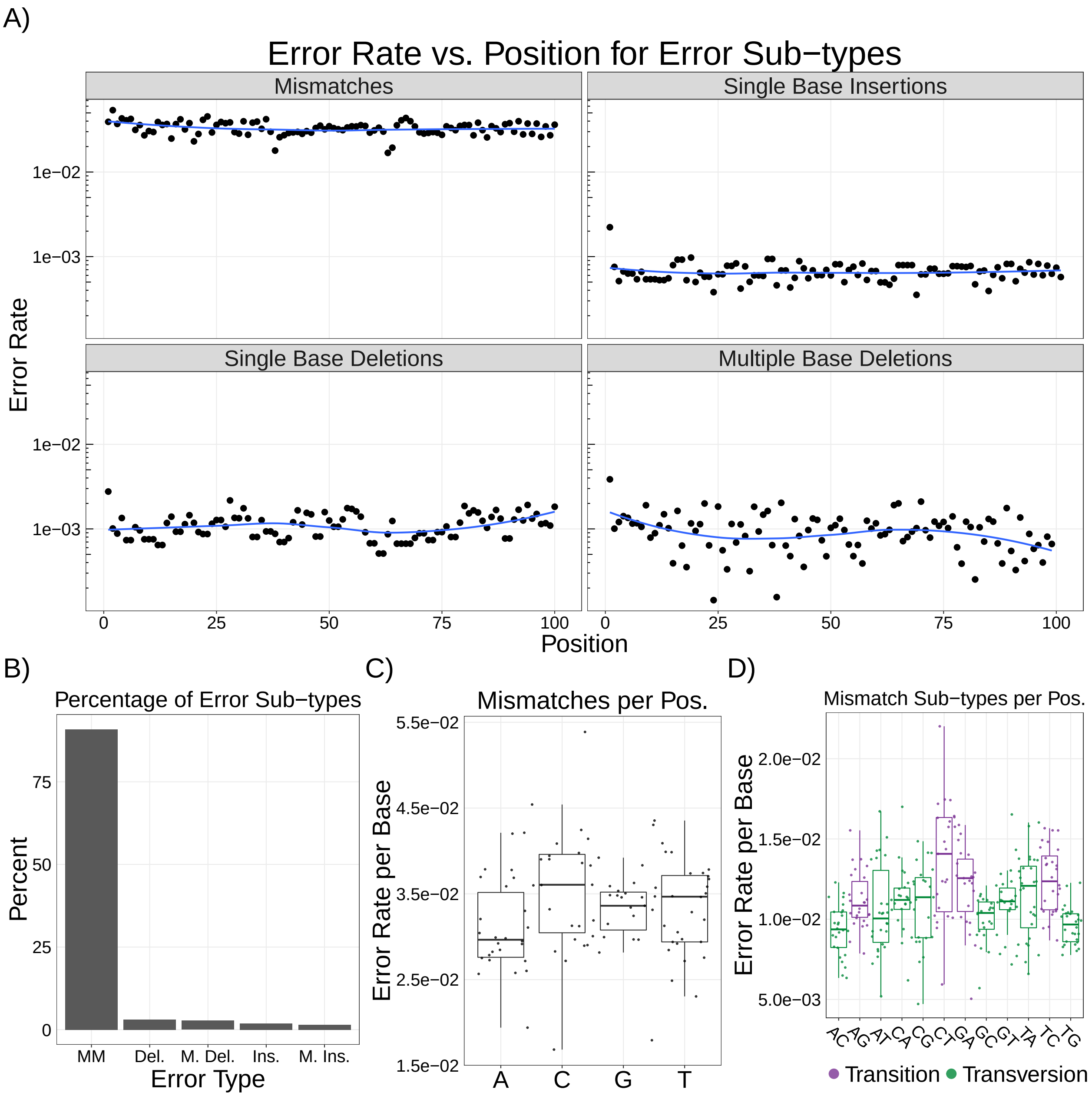
```
## Source: local data table [4 x 2]
##
## # tbl_dt [4 × 2]
##    From        med
##   <chr>      <dbl>
## 1     C 0.03606105
## 2     T 0.03467046
## 3     G 0.03361262
## 4     A 0.02965034
##
##  Pairwise comparisons using Wilcoxon rank sum test
##
## data:  n and From
##
##   A     C     G
## C 0.049 -     -
## G 0.206 0.489 -
## T 0.409 0.489 0.918
##
## P value adjustment method: holm
```

```
#--------------------------------------------------------------------------------
# Panel d
# what bases are most likely mutated to
# We will normallize by the total count in each "from" group
mm_type_dope <- doped %>%
  filter(Type == 'M') %>%
  count(Pos, Diff) %>%
  ungroup() %>%
  mutate(
    Char=str_sub(Diff, 1, 1),
    Class= Diff %>%
      recode(AT='Transversion', AG='Transition', AC='Transversion',
             TA='Transversion', TG='Transversion', TC='Transition',
             GA='Transition', GT='Transversion', GC='Transversion',
             CA='Transversion', CT='Transition', CG='Transversion')

  ) %>%
  mutate(Norm = n / subset(readCounts, Sample == '1_nonDoped')$Reads) %T>%
  {# significance testing and printing for paper
    group_by(., Diff) %>%
      summarise(med=median(Norm)) %>%
      arrange(-med) %>%
      print; # <- ; critical for . to be interpreted correctly
      with(., pairwise.wilcox.test(Norm, Diff)) %>% print
  } %>%
  ggplot(aes(x = Diff, y = Norm, color=Class)) +
  geom_boxplot(outlier.shape = NA, show.legend = FALSE) +
  geom_jitter(position=position_jitter(w = 0.5), size=0.75, alpha=0.8) +
  # stat_summary(fun.y = median, fun.ymin = median, fun.ymax = median, geom = 'crossbar', width = 0.5,
  labs(
    y = 'Error Rate per Base',
    title = 'Mismatch Sub-types per Pos.'
    ) +
  theme(
    legend.position='bottom',
    legend.key.size=unit(0.75, "cm"),
    axis.title.x=element_blank(),
    axis.text.x = element_text(angle = 315, vjust=0.5),
    plot.title = element_text(size=rel(1.75))
  ) +
  scale_y_continuous(labels = scientific_format()) +
  scale_color_manual(values = c('#7b3294', '#008837')) +
  guides(colour = guide_legend(override.aes = list(size=5)))
```

```
## Source: local data table [12 x 2]
##
## # tbl_dt [12 × 2]
##     Diff         med
##    <chr>       <dbl>
## 1     CT 0.014072356
## 2     GA 0.012556461
## 3     TC 0.012362352
## 4     TA 0.012078892
## 5     CG 0.011369215
## 6     CA 0.011200782
## 7     GT 0.011112458
## 8     AG 0.010847484
## 9     GC 0.010389429
## 10    AT 0.010046401
## 11    TG 0.009670508
## 12    AC 0.009366508
##
##  Pairwise comparisons using Wilcoxon rank sum test
##
## data:  Norm and Diff
##
##    AC      AG      AT      CA      CG      CT      GA      GC      GT      TA      TC
## AG 0.03251 -       -       -       -       -       -       -       -       -       -
## AT 1.00000 1.00000 -       -       -       -       -       -       -       -       -
## CA 0.02235 1.00000 1.00000 -       -       -       -       -       -       -       -
## CG 0.87035 1.00000 1.00000 1.00000 -       -       -       -       -       -       -
## CT 0.00022 0.50669 0.07019 0.81013 0.40479 -       -       -       -       -       -
## GA 0.00295 1.00000 1.00000 1.00000 1.00000 1.00000 -       -       -       -       -
## GC 1.00000 1.00000 1.00000 0.65909 1.00000 0.02070 0.02620 -       -       -       -
## GT 0.09648 1.00000 1.00000 1.00000 1.00000 0.81013 1.00000 1.00000 -       -       -
## TA 0.08809 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 -       -
## TC 1.7e-05 0.75909 0.20620 1.00000 1.00000 1.00000 1.00000 0.00730 1.00000 1.00000 -
## TG 1.00000 0.02293 1.00000 0.00730 1.00000 0.00025 0.00325 1.00000 0.07019 0.17116 3.4e-06
##
## P value adjustment method: holm
```

```
#-------------------------------------------------------------------------------
# plot everything!
grid.arrange(
  LabelMaker(positions_dope, 'A)'),
  arrangeGrob(
    LabelMaker(sub_type_dope, 'B)'),
    LabelMaker(mm_freq_dope, 'C)'),
    LabelMaker(mm_type_dope, 'D)'),
    nrow=1),
  ncol=1,
  heights = c(1, 0.67)
  )
```

```
## `geom_smooth()` using method = 'loess'
```



```
#----------------------------------------------------------------------------
# Values for text

# differences in medians in annealing regions and outside
doped %>%
  DistribUncert2() %>%
  count(Type, Pos, wt=FracCount) %>%
  mutate(Norm = n / subset(readCounts, Sample == '1_nonDoped')$Reads) %>%
  filter(Type == 'P') %>%
  mutate(Region = if_else(Pos >= 36 & Pos <=64, 'Anneal', 'No')) %T>%
  {wilcox.test(Norm ~ Region, data=.) %>% print} %>%
  group_by(Region) %>%
  summarise(med=median(Norm), IQR=IQR(Norm))
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Norm by Region
## W = 956.5, p-value = 0.6556
## alternative hypothesis: true location shift is not equal to 0
```

```
## Source: local data table [2 x 3]
##
## # tbl_dt [2 × 3]
##   Region          med          IQR
##    <chr>        <dbl>        <dbl>
## 1 Anneal 0.0009695157 0.0006059473
## 2     No 0.0010126509 0.0005694878
```

Here we will calculate the per-base error rate

```
doped %>%
  DistribUncert2() %>%
  count(Sample, Name, Type, wt=FracCount) %>%
  left_join(
            filter(charCounts, Sample == '1_DopedTemp'),
            by=c('Sample', 'Name')
  ) %>%
  group_by(Type) %>%
  summarise(m=sum(n/Len)/filter(readCounts, Sample == '1_DopedTemp')$Reads)
```

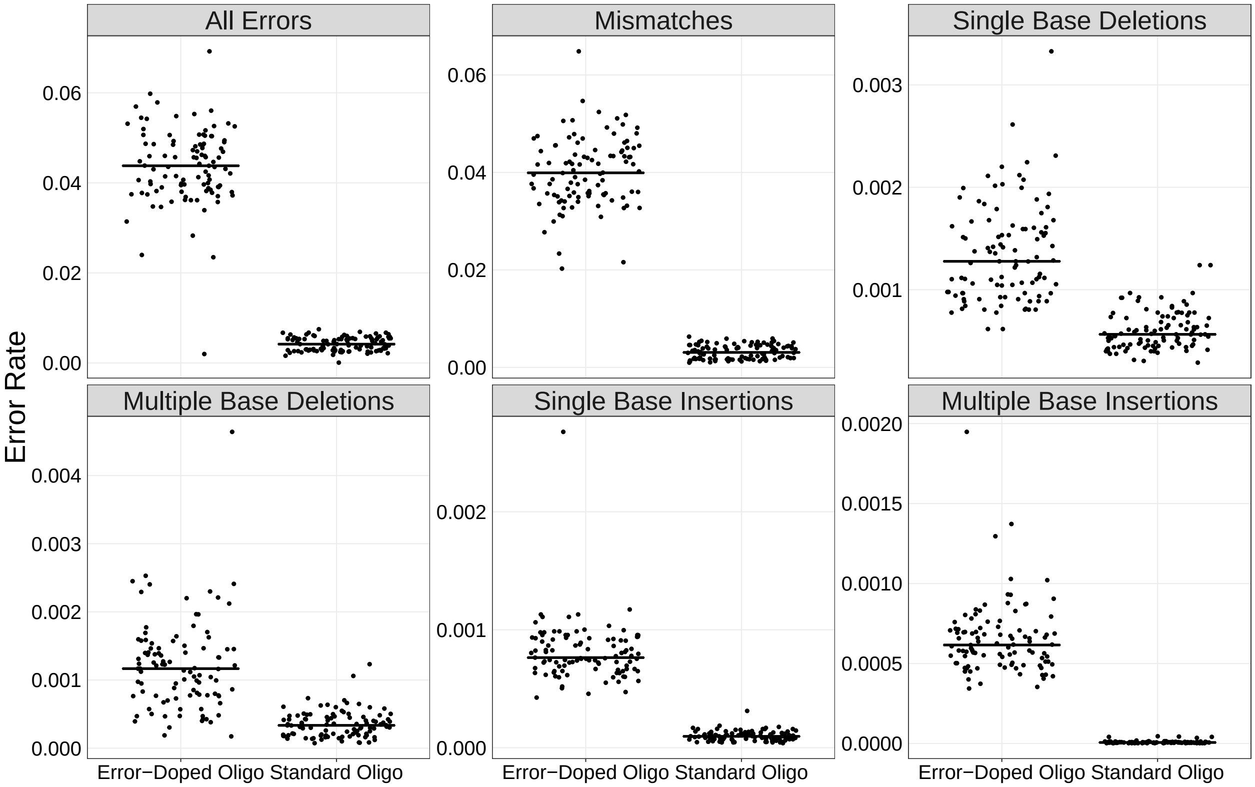
```
## Source: local data table [5 x 2]
##
## # tbl_dt [5 × 2]
##    Type            m
##   <chr>        <dbl>
## 1     M 0.0405901054
## 2     D 0.0015592068
## 3     I 0.0008250557
## 4     P 0.0015810213
## 5     S 0.0005879444
```

Doped vs Non-Doped Error Rates

Here we compare the doped oligo to the non-doped. We see that all error rates are significantly higher in the doped sample.

```
allSamps %>%
  filter(Sample %in% c('1_DopedTemp', '1_nonDoped')) %>%
  DistribUncert2() %>%
  count(Sample, Type, Pos, wt=FracCount) %>%
  ungroup() %>%
  left_join(readCounts, by='Sample') %>%
  select(., -Errs) %>%
  # count all errors regardless of type
  bind_rows(.,
            count(., Sample, Reads, Pos, wt=n) %>% mutate(Type = 'A') %>% rename(n=nn)) %>%
  mutate(
    Norm = n / Reads,
    Sample = if_else(Sample == '1_DopedTemp',
                     'Error-Doped Oligo',
                     'Standard Oligo'),
    Type = Type %>%
      factor(levels = c('A', 'M', 'D', 'P', 'I', 'S')) %>%
      recode(D = 'Single Base Deletions', I = 'Single Base Insertions',
             P = 'Multiple Base Deletions', S = 'Multiple Base Insertions',
             M = 'Mismatches', A = 'All Errors')
  ) %T>%
  { # significance test and summarise for paper
    group_by(., Sample, Type) %>%
      summarise(med=median(Norm), mean=mean(Norm)) %>%
      arrange(Sample) %>%
      print();
    group_by(., Type) %>%
      summarise(p.val=wilcox.test(Norm ~ Sample, data=.)$p.value) %>%
        print()
    } %>%
  ggplot(aes(x=Sample, y=Norm)) +
  geom_jitter(position=position_jitter(w = 0.35)) +
  stat_summary(fun.y = median, fun.ymin = median, fun.ymax = median, geom = 'crossbar', width = 0.75) +
  facet_wrap(~ Type, scales='free_y', ncol=3) +
  labs(y = 'Error Rate') +
  theme(axis.title.x=element_blank())
```

```
## Source: local data table [12 x 4]
## Groups: Sample
##
## # grouped_dt [12 × 4]
##               Sample                     Type          med         mean
##                <chr>                   <fctr>        <dbl>        <dbl>
## 1  Error-Doped Oligo    Single Base Deletions 1.276961e-03 1.354963e-03
## 2  Error-Doped Oligo   Single Base Insertions 7.639514e-04 8.092122e-04
## 3  Error-Doped Oligo               Mismatches 3.992449e-02 4.007261e-02
## 4  Error-Doped Oligo  Multiple Base Deletions 1.166942e-03 1.209471e-03
## 5  Error-Doped Oligo Multiple Base Insertions 6.156113e-04 6.510807e-04
## 6  Error-Doped Oligo               All Errors 4.379741e-02 4.366322e-02
## 7     Standard Oligo    Single Base Deletions 5.638391e-04 6.021062e-04
## 8     Standard Oligo   Single Base Insertions 9.654076e-05 9.959134e-05
## 9     Standard Oligo               Mismatches 3.079034e-03 3.189008e-03
## 10    Standard Oligo  Multiple Base Deletions 3.348116e-04 3.515968e-04
## 11    Standard Oligo Multiple Base Insertions 6.162176e-06 8.277247e-06
## 12    Standard Oligo               All Errors 4.175901e-03 4.206082e-03
## Source: local data table [6 x 2]
##
## # tbl_dt [6 × 2]
##                       Type        p.val
##                     <fctr>        <dbl>
## 1    Single Base Deletions 6.545164e-30
## 2   Single Base Insertions 1.194815e-34
## 3                Mismatches 2.561855e-34
## 4  Multiple Base Deletions 2.309269e-26
## 5 Multiple Base Insertions 8.611414e-35
## 6                All Errors 2.161343e-33
```

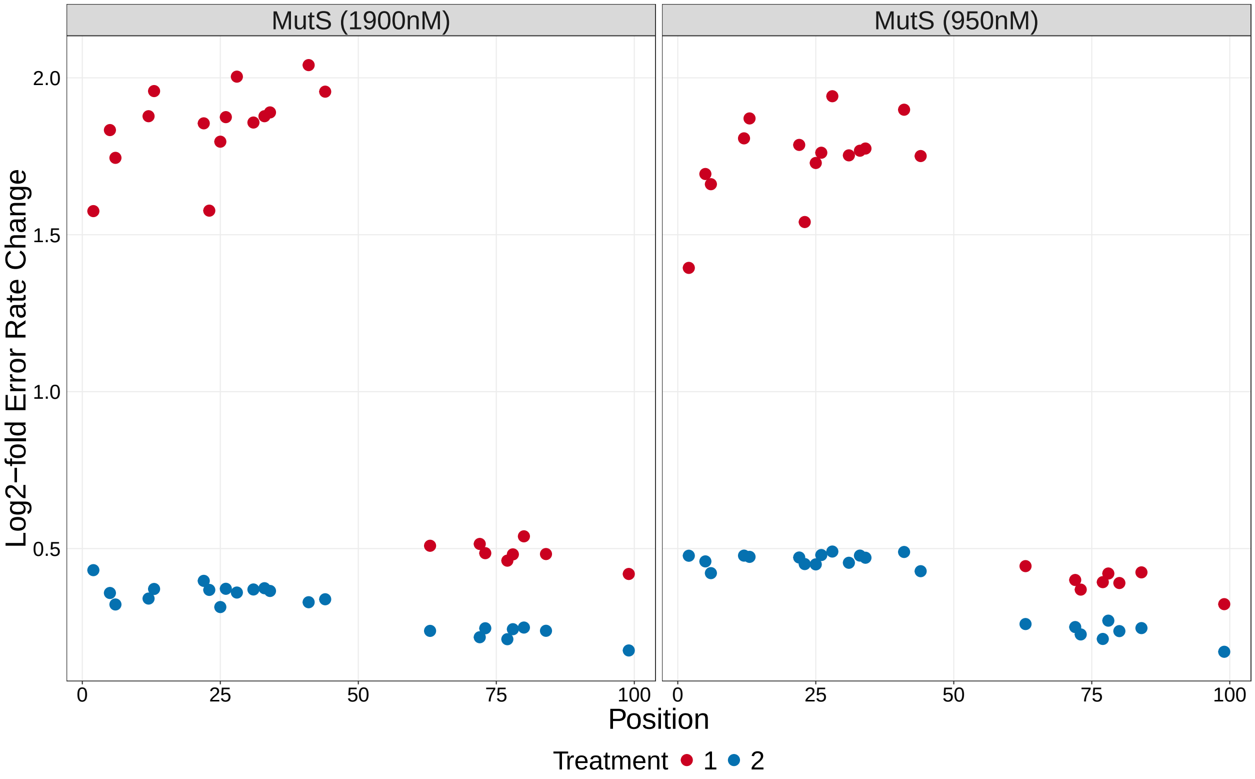


Misc

MutS Mismatch Preferences

There's some really strange bi-modal correction of CA mismatches with MutS across both samples. Not sure what it could be…

```
enzPref.idm %>%
  filter(
    str_detect(Sample, 'MutS'),
    Type == 'M',
    Diff == 'CA'
    ) %>%
  ggplot(aes(x=Pos, y=Rel_Norm, color=Treatment)) +
  geom_point(size=5) +
  facet_wrap(~ Sample, ncol=2) +
  guides(colour = guide_legend(override.aes = list(size=5))) +
  theme(
    legend.title = element_text(size=rel(2)),
    legend.position = 'bottom'
  ) +
  scale_color_manual(
    name = 'Treatment',
    values=c("#ca0020", "#0571b0")
  ) +
  labs(x = 'Position', y = 'Log2-fold Error Rate Change')
```

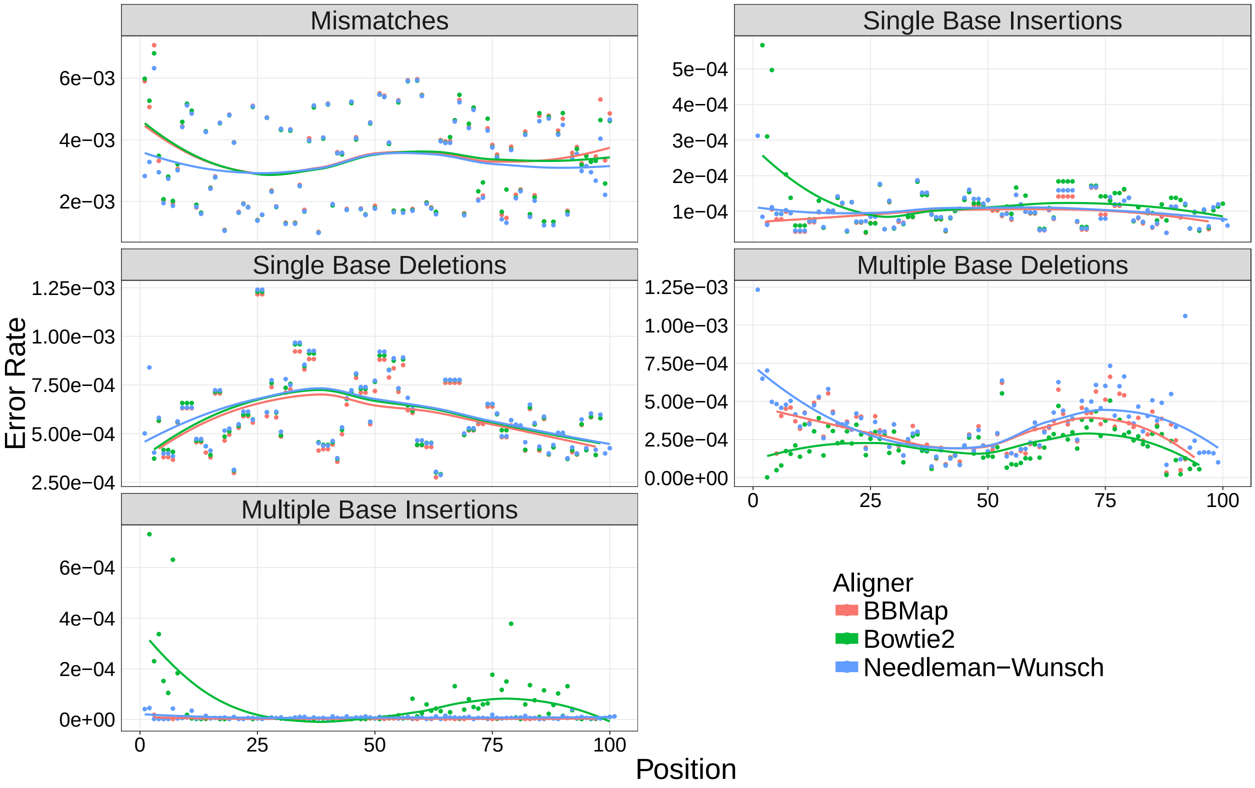


Aligner Comparision

Here we will compare BBMap, Bowtie2, and our NW aligner

```
bind_rows(
  list(bbmap=fread('./pipeline/1_nonDoped.bbmap.csv', header=T),
       bowtie=fread('./pipeline/1_nonDoped.bowtie.csv', header=T),
       nw=select(nonDoped, -Sample)),
  .id = 'Aligner'
) %>%
  DistribUncert2() %>%
  count(Aligner, Pos, Type, wt=FracCount) %>%
  ungroup() %>%
  mutate(
    Norm=n / subset(readCounts, Sample == '1_nonDoped')$Reads,
    Type = Type %>%
      factor(levels = c('M', 'I', 'D', 'P', 'S')) %>%
      recode(D = 'Single Base Deletions', I = 'Single Base Insertions',
             P = 'Multiple Base Deletions', S = 'Multiple Base Insertions',
             M = 'Mismatches')) %>%
  ggplot(aes(x=Pos, y=Norm, color=Aligner)) +
  facet_wrap(~ Type, ncol=2, scales='free_y') +
  geom_point() +
  stat_smooth(se=F) +
  theme(
    legend.title=element_text(size=rel(2)),
    legend.position=c(0.75, 0.15)
  ) +
  guides(colour = guide_legend(override.aes = list(size=5))) +
  scale_color_discrete(name = 'Aligner',
                       labels = c('BBMap', 'Bowtie2', 'Needleman-Wunsch')) +
  labs(x = 'Position', y = 'Error Rate') +
  scale_y_continuous(labels = scientific_format())
```

```
## `geom_smooth()` using method = 'loess'
```



Classic Table

```
single <- allSamps %>%
    filter(!Type %in% c('S', 'P')) %>%
      DistribUncert2() %>%
      group_by(Sample, Type, Diff) %>%
      summarise(n=sum(FracCount)) %>%
      ungroup()

# just count the number of multiple counts
```

```r
multiple <- allSamps %>%
  filter(Type %in% c('S', 'P')) %>%
  group_by(Sample, Type) %>%
  summarise(n=n(), Diff='N/A') %>%
  ungroup()

# grab transitions/transversions
trans <- single %>%
  filter(Type == 'M') %>%
  mutate(
    Type= Diff %>%
      recode(AT='Transversion', AG='Transition', AC='Transversion',
             TA='Transversion', TG='Transversion', TC='Transition',
             GA='Transition', GT='Transversion', GC='Transversion',
             CA='Transversion', CT='Transition', CG='Transversion')
  ) %>%
  group_by(Sample, Type) %>%
  summarise(Diff='N/A', n=sum(n)) %>%
  ungroup()

bind_rows(single, multiple, trans) %>%
  mutate(
    Type = Type %>%
      recode(D = 'Single Base Deletions', I = 'Single Base Insertions',
             P = 'Multiple Base Deletions', S = 'Multiple Base Insertions',
             M = 'Mismatches')
  ) %>%
  spread(Sample, n) %>%
  # manual selection maddness to aid downstream processing
  select(
    Type, Diff,
    `1_nonDoped`, matches('ErrASE-'), `1_DopedTemp`,
    matches('_ErrASE'), contains("1900"), contains("950"),
    contains("Survey"), contains("Furhmann"), `1_T7EndoI`,
    `2_T7EndoI`, contains("e3T7"), contains("e4T7"),
    contains("T4"), contains("EndoV")
  ) %>%
  write.csv('Table_1.csv', quote=FALSE, row.names=FALSE)
```