Polymerase fidelity estimates

Load required libraries, load and merge MAGERI results.

```
library(plyr); library(ggplot2); library(reshape2); library(gplots); library(knitr); library(RColorBrew
```

```
##
## Attaching package: 'gplots'

## The following object is masked from 'package:stats':
##
##     lowess

##
## Attaching package: 'dplyr'

## The following objects are masked from 'package:plyr':
##
##     arrange, count, desc, failwith, id, mutate, rename, summarise,
##     summarize

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```
load_variant_table <- function(path, project, sample) {
  fname <- paste(path, paste(project, sample, "variant.caller.txt", sep ="."), sep = "/")
  df.1 <- read.table(fname, header=T, sep="\t")
  df.1$project <- project
  df.1$sample <- sample
  df.1
}

# mutation signatures
sign.rep <- data.frame(mutation.signature = c("A>C","A>G","A>T","C>A","C>G","C>T","G>A",
                                              "G>C","G>T","T>A","T>C","T>G"),
                       mutation.signature.rep = c("A>C,T>G","A>G,T>C","A>T,T>A","C>A,G>T",
                                                  "C>G,G>C","C>T,G>A","C>T,G>A","C>G,G>C",
                                                  "C>A,G>T","A>T,T>A","A>G,T>C","A>C,T>G"))

path <- "data/"
df.meta <- read.table(paste(path, "metadata.txt", sep="/"), header=T, sep = "\t")

# load and concatenate all samples
df.0 <- data.frame()

for (i in 1:nrow(df.meta)) {
  df.0 <- rbind(df.0, load_variant_table(path, df.meta$project[i], df.meta$sample[i]))
}

# append metadata
df.0 <- merge(df.0, df.meta, all.x=T, all.y=F)
```

```r
# split mutation signature
df.0$mut.split <- sapply(df.0$mutation, function(x) strsplit(as.character(x),"[S:>]"))
df.0$mutation.pos <- as.integer(sapply(df.0$mut.split, function(x) x[2]))
df.0$mutation.from <- sapply(df.0$mut.split, function(x) x[3])
df.0$mutation.to <- sapply(df.0$mut.split, function(x) x[4])
df.0$mut.split  <- NULL

# mutation signature groups
df.0$mutation.signature <- paste(df.0$mutation.from, df.0$mutation.to, sep =">")
df.0 <- merge(df.0, sign.rep, all.x=T, all.y=F)


# exclude bases that are not in reference
df.0 <- subset(df.0, !(mutation.pos %in% 22:25))

# shift back mutations
df.0$mutation.pos <- ifelse(df.0$mutation.pos > 25, df.0$mutation.pos - 4,
                            df.0$mutation.pos)

df.0$mutation <- with(df.0, paste("S", mutation.pos, ":", mutation.from, ">",
                                  mutation.to, sep=""))

df <- subset(df.0, project %in% c("polerr2016-1", "polerr2016-2"))
df.linpcr <- subset(df.0, project %in% c("polerr73", "polerr82"))

template <- paste("TAGCGTGAAGACGACAGAACCATGGGATCCATTATCGGCGGCGAATTTACCACCATTGAAAACCAGCCGTGGTTT",
                  "GCGGCGATTTATCGTCGTCATCGTGGCGGCAGCGTGACCTATGTGTGCGGCGGCAGCCTGATTAGCCCGTGCTGG",
                  sep="") # 4 index bases removed
```

Estimating error rates

In this section we compute linear PCR error rate from Proj73/82 and error rate in conventional PCR from Polerr2016 project. Note that the linear PCR error rate is substantially higher than rate per cycle of conentional PCR. For some cases, e.g. Phusion it accounts for ~1/2 of errors observed in Polerr2016.

```r
# Compute linear PCR error rate

df.er.linpcr <- ddply(df.linpcr, .(project, name), summarize, mismatches = sum(count.major),
                      umi.count = mean(coverage))
df.er.linpcr <- ddply(df.er.linpcr, .(name), summarize, mismatches = sum(mismatches),
                      umi.count = round(sum(umi.count)))

df.er.linpcr <- data.frame(name = df.er.linpcr$name,
                           linpcr.er = df.er.linpcr$mismatches/df.er.linpcr$umi.count /
                             nchar(template))

# Compute uncrorrected error rate

df.er <- ddply(df, .(project, name, cycles), summarize,
               mismatches = sum(count.major), umi.count = round(mean(coverage)))

df.er <- merge(df.er, df.er.linpcr, by = "name", all.x = T)
df.er$err.rate <- with(df.er, mismatches / umi.count / nchar(template) / mean(cycles))
df.er$delta <- with(df.er,
                    sqrt(mismatches / umi.count * (1 - mismatches / umi.count) / umi.count) /
                      nchar(template) / mean(cycles))
df.er$err.lb <- df.er$err.rate - 1.96 * df.er$delta
df.er$err.ub <- df.er$err.rate + 1.96 * df.er$delta

# Error rates corrected for linear PCR errors

df.er$mismatches.corr <- with(df.er, mismatches - linpcr.er * umi.count * nchar(template))
df.er$err.rate.corr <- with(df.er, mismatches.corr / umi.count / nchar(template) / mean(cycles))
df.er$delta.corr <- with(df.er, sqrt(mismatches.corr / umi.count *
                                       (1 - mismatches.corr / umi.count) / umi.count) /
                 nchar(template) / mean(cycles))
df.er$err.lb.corr <- df.er$err.rate.corr - 1.96 * df.er$delta.corr
df.er$err.ub.corr <- df.er$err.rate.corr + 1.96 * df.er$delta.corr

df.er$cycles <- NULL

kable(df.er)
```

name	project	mismatches	umi.count	linpcr.er	err.rate	delta	err.lb	err.ub	mismatc
Encyclo	polerr2016-1	24557	185560	0.0001279	4.41e-05	3e-07	4.36e-05	4.46e-05	209
Encyclo	polerr2016-2	14211	101516	0.0001279	4.67e-05	4e-07	4.60e-05	4.74e-05	122
Kappa HF	polerr2016-2	2519	57052	0.0000817	1.47e-05	3e-07	1.42e-05	1.53e-05	18
Kappa HF	polerr2016-1	339	7876	0.0000817	1.43e-05	8e-07	1.29e-05	1.58e-05	2
SD-HS	polerr2016-2	10362	58518	0.0002689	5.90e-05	5e-07	5.80e-05	6.01e-05	80
SD-HS	polerr2016-1	6714	33076	0.0002689	6.77e-05	7e-07	6.62e-05	6.91e-05	53
SNP-detect	polerr2016-2	848	32310	0.0000504	8.70e-06	3e-07	8.20e-06	9.30e-06	6
SNP-detect	polerr2016-1	457	13870	0.0000504	1.10e-05	5e-07	1.00e-05	1.20e-05	3
Taq-HS	polerr2016-1	1875	15137	0.0001836	4.13e-05	9e-07	3.95e-05	4.30e-05	14
Taq-HS	polerr2016-2	3113	24082	0.0001836	4.31e-05	7e-07	4.17e-05	4.45e-05	24
Tersus	polerr2016-2	5504	154226	0.0000598	1.19e-05	2e-07	1.16e-05	1.22e-05	41
Tersus	polerr2016-1	2550	46927	0.0000598	1.81e-05	3e-07	1.74e-05	1.88e-05	21
Tersus-SNP-buffer	polerr2016-2	1299	30683	0.0000510	1.41e-05	4e-07	1.34e-05	1.49e-05	10
Tersus-SNP-buffer	polerr2016-1	6282	130891	0.0000510	1.60e-05	2e-07	1.56e-05	1.64e-05	52
TruSeq	polerr2016-2	362	16705	0.0000410	7.20e-06	4e-07	6.50e-06	8.00e-06	2
TruSeq	polerr2016-1	312	14164	0.0000410	7.30e-06	4e-07	6.50e-06	8.10e-06	2
Velox	polerr2016-1	16802	132733	0.0001292	4.22e-05	3e-07	4.16e-05	4.28e-05	142
Velox	polerr2016-2	6298	44331	0.0001292	4.74e-05	6e-07	4.63e-05	4.84e-05	54

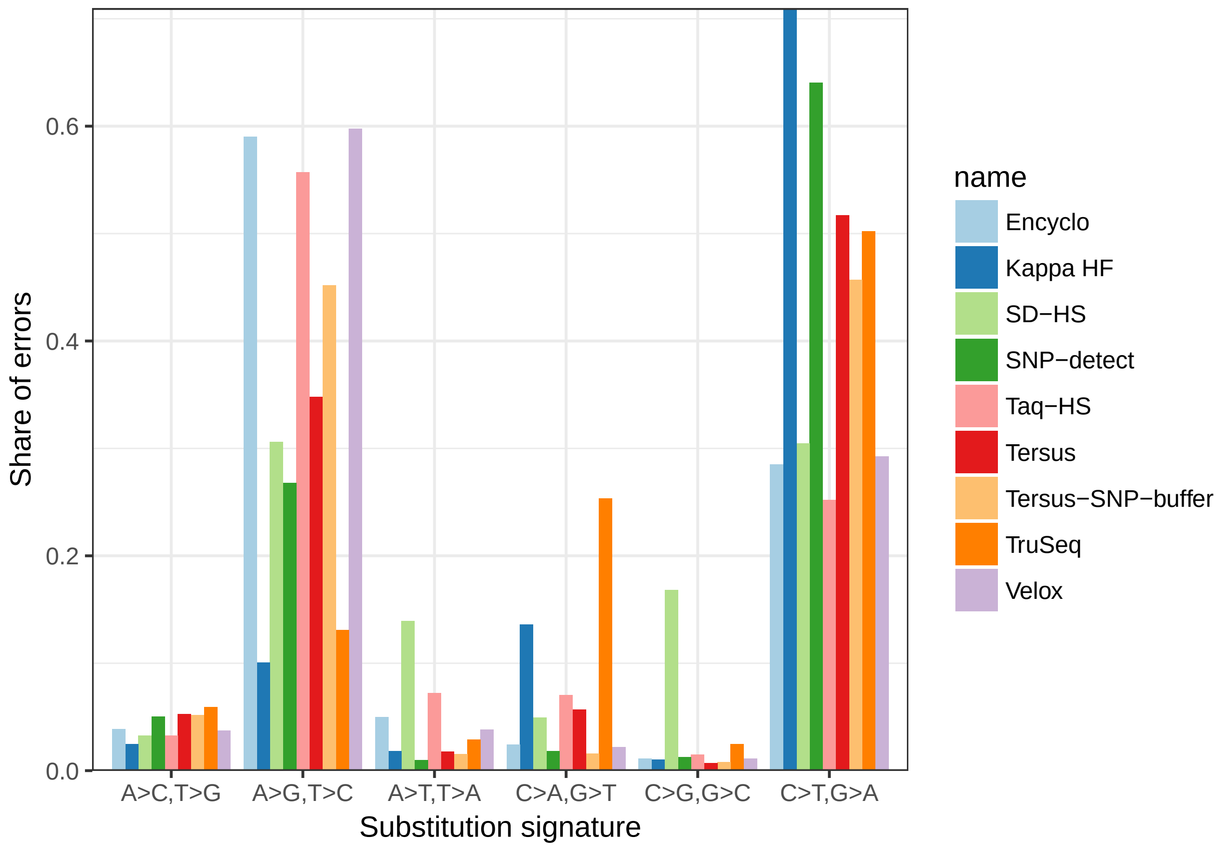
```
write.table(df.er, file="er.txt", quote=F, sep="\t", row.names = F)
```

Patterns and recurrent errors

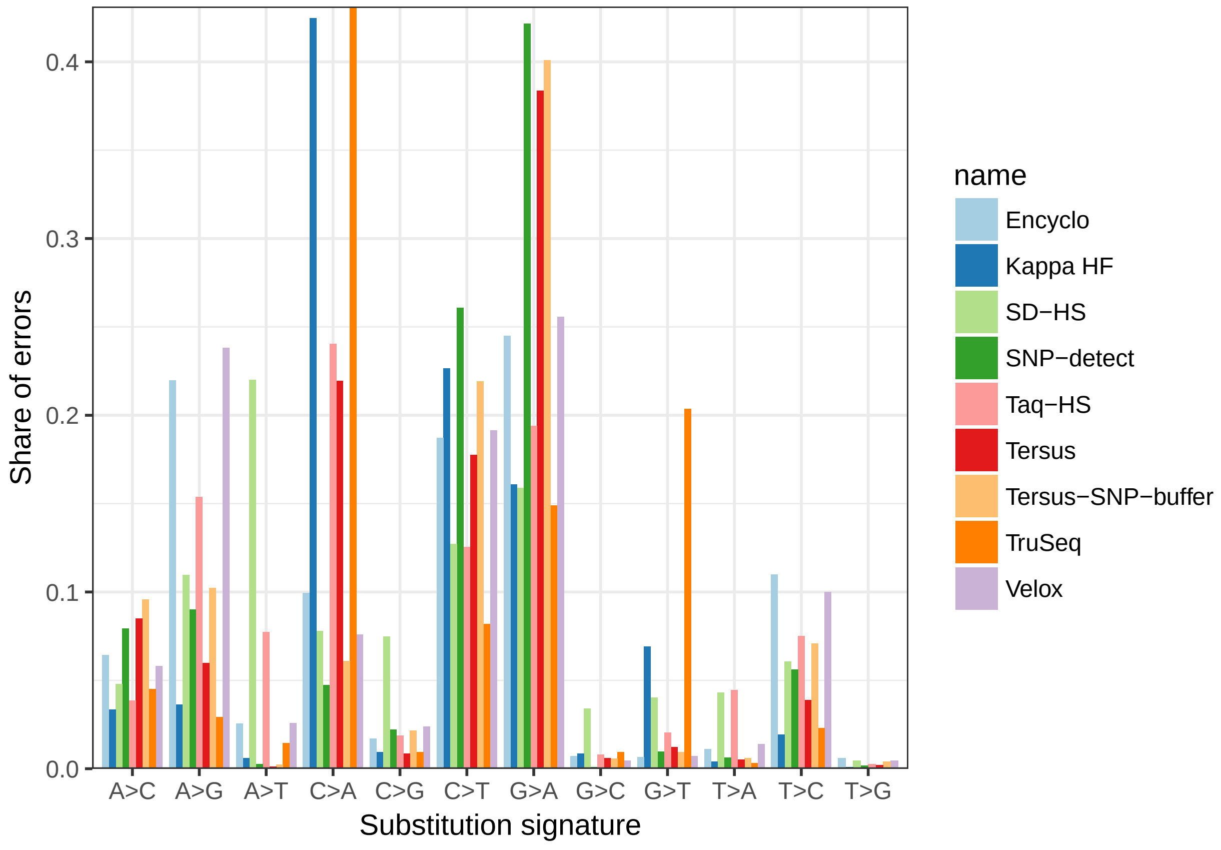
Substitution signature preferences:

```
base.freqs <- ddply(data.frame(base = strsplit(template, "")[[1]]), .(base), summarize,
                    count=length(base))
bases <- base.freqs$base
base.freqs <- base.freqs$count
```

```r
names(base.freqs) <- bases

df.pattern <- ddply(df, .(name, mutation.signature.rep, mutation.from), summarize,
                    count.sum = sum(count.major))
df.pattern <- ddply(df.pattern, .(mutation.from), transform, count.sum = count.sum /
                      base.freqs[mutation.from])
df.pattern <- ddply(df.pattern, .(name), transform, freq = count.sum / sum(count.sum))

ggplot(df.pattern, aes(x = mutation.signature.rep, weight = freq,
               fill = name)) + geom_bar(position = position_dodge()) +
  xlab("Substitution signature") + scale_y_continuous("Share of errors", expand=c(0,0)) +
  scale_fill_brewer(palette = "Paired") + theme_bw()
```



```r
write.table(dcast(df.pattern, name ~ mutation.signature.rep, value.var = "freq",
                  fun.aggregate = sum),
            file="pattern.txt", quote=F, sep="\t", row.names = F)

# same for linpcr

df.pattern.linpcr <- ddply(df.linpcr, .(name, mutation.signature, mutation.from), summarize,
                           count.sum = sum(count.major))
df.pattern <- ddply(df.pattern, .(mutation.from), transform,
                    count.sum = count.sum / base.freqs[mutation.from])
df.pattern.linpcr <- ddply(df.pattern.linpcr, .(name), transform,
                           freq = count.sum / sum(count.sum))

ggplot(df.pattern.linpcr, aes(x = mutation.signature, weight = freq,
               fill = name)) + geom_bar(position = position_dodge()) +
  xlab("Substitution signature") + scale_y_continuous("Share of errors", expand=c(0,0)) +
  scale_fill_brewer(palette = "Paired") + theme_bw()
```



```
write.table(dcast(df.pattern.linpcr, name ~ mutation.signature, value.var = "freq",
                  fun.aggregate = sum),
            file="pattern.lpcr.txt", quote=F, sep="\t", row.names = F)
```

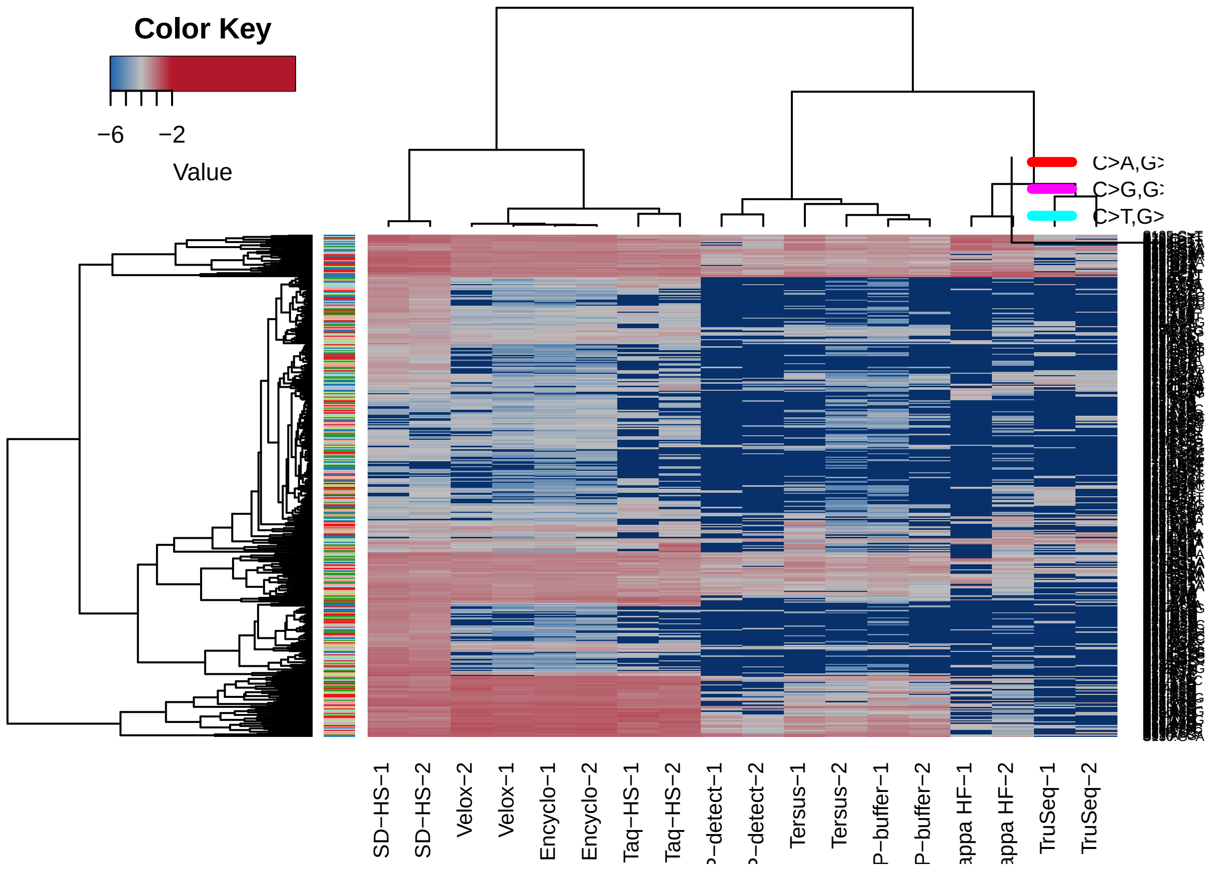
Clustering of polymerase error profiles:

```
df$freq <- df$count.major / df$coverage
df$name_proj <- paste(df$name, ifelse(df$project == "polerr2016-1", "1", "2"), sep="-")
mat.profile <- dcast(df, name_proj ~ mutation, value.var = "freq")
rownames(mat.profile) <- mat.profile[,1]
mat.profile[,1] <- NULL
mat.profile <- t(as.matrix(mat.profile))
mat.profile[is.na(mat.profile)] <- 0

df.aux <- unique(data.frame(mutation = df$mutation, signature = df$mutation.signature.rep))
df.aux$mutation <- as.character(df.aux$mutation)

df.color <- merge(data.frame(mutation = rownames(mat.profile)), df.aux)

df.color.legend <- data.frame(colors = brewer.pal(6, "Paired"),
                              signature = levels(df.color$signature))

df.color <- merge(df.color, df.color.legend)
```

```r
rowcolor <- as.character(df.color$colors)
names(rowcolor) <- df.color$mutation

rowDend <- hclust(dist(mat.profile))
colDend <- hclust(as.dist((1-cor(mat.profile))/2), method = "ward")
```

```
## The "ward" method has been renamed to "ward.D"; note new "ward.D2"
```

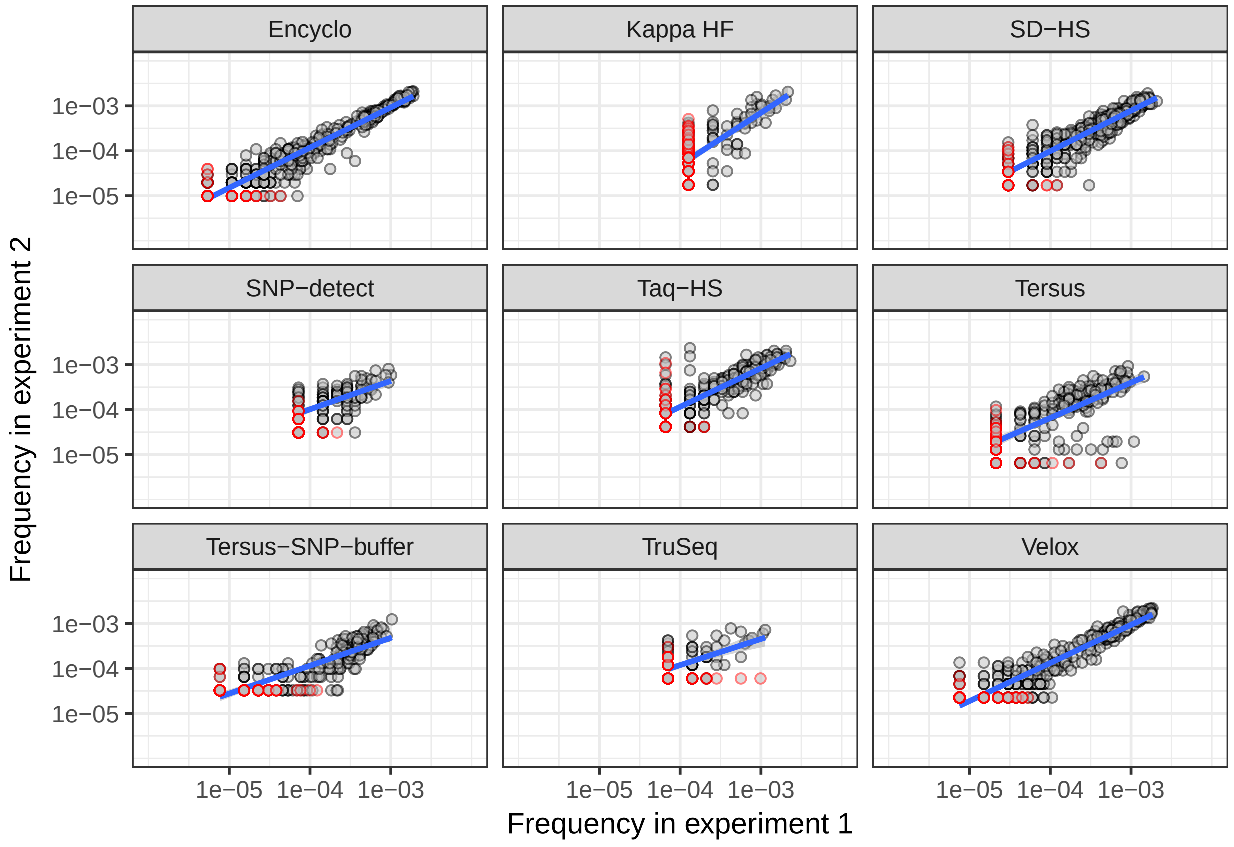
```r
heatmap.2(log10(mat.profile), col=c("#08306b", colorpanel(99, "#2166ac", "grey", "#b2182b")),
          dendrogram = "both", RowSideColors = rowcolor, breaks = seq(-6,-2,length.out = 101),
          Rowv = as.dendrogram(rowDend),
          Colv = as.dendrogram(colDend),
          density.info = "none", trace="none")

legend(y=1.2, x=.85, xpd=TRUE,
    legend = df.color.legend$signature,
    col = df.color.legend$colors,
    lty= 1,
    lwd = 5,
    cex=.7
    )
```



Recurrent errors

```r
df.1x <- ddply(subset(df, project == "polerr2016-1"), .(name, mutation), summarize,
               freq = count.major / coverage, coverage = coverage)
```

```
df.1y <- ddply(subset(df, project == "polerr2016-2"), .(name, mutation), summarize,
               freq = count.major / coverage, coverage = coverage)

df.1 <- merge(df.1x, df.1y, by=c("name","mutation"), all = T)
mask1 <- is.na(df.1$freq.x)
mask2 <- is.na(df.1$freq.y)
df.1$miss <- mask1 | mask2

df.1 <- ddply(df.1, .(name), transform,
              freq.x = ifelse(is.na(freq.x), 1/mean(coverage.x, na.rm = T), freq.x),
              freq.y = ifelse(is.na(freq.y), 1/mean(coverage.y, na.rm = T), freq.y))

ggplot() +
  geom_point(data=subset(df.1, !miss), aes(x=freq.x, y=freq.y), shape=21, fill="grey", alpha=0.5) +
  geom_smooth(data=subset(df.1, !miss & name != "Phusion"), aes(x=freq.x, y=freq.y), method="lm") +
  geom_point(data=subset(df.1, miss), aes(x=freq.x, y=freq.y), shape=21, color="red", fill="grey",
             alpha=0.5) +
  facet_wrap(~name) +
  scale_x_log10("Frequency in experiment 1", limits=c(1e-6,1e-2), breaks = c(1e-5, 1e-4, 1e-3)) +
  scale_y_log10("Frequency in experiment 2", limits=c(1e-6,1e-2), breaks = c(1e-5, 1e-4, 1e-3)) +
  theme_bw()
```

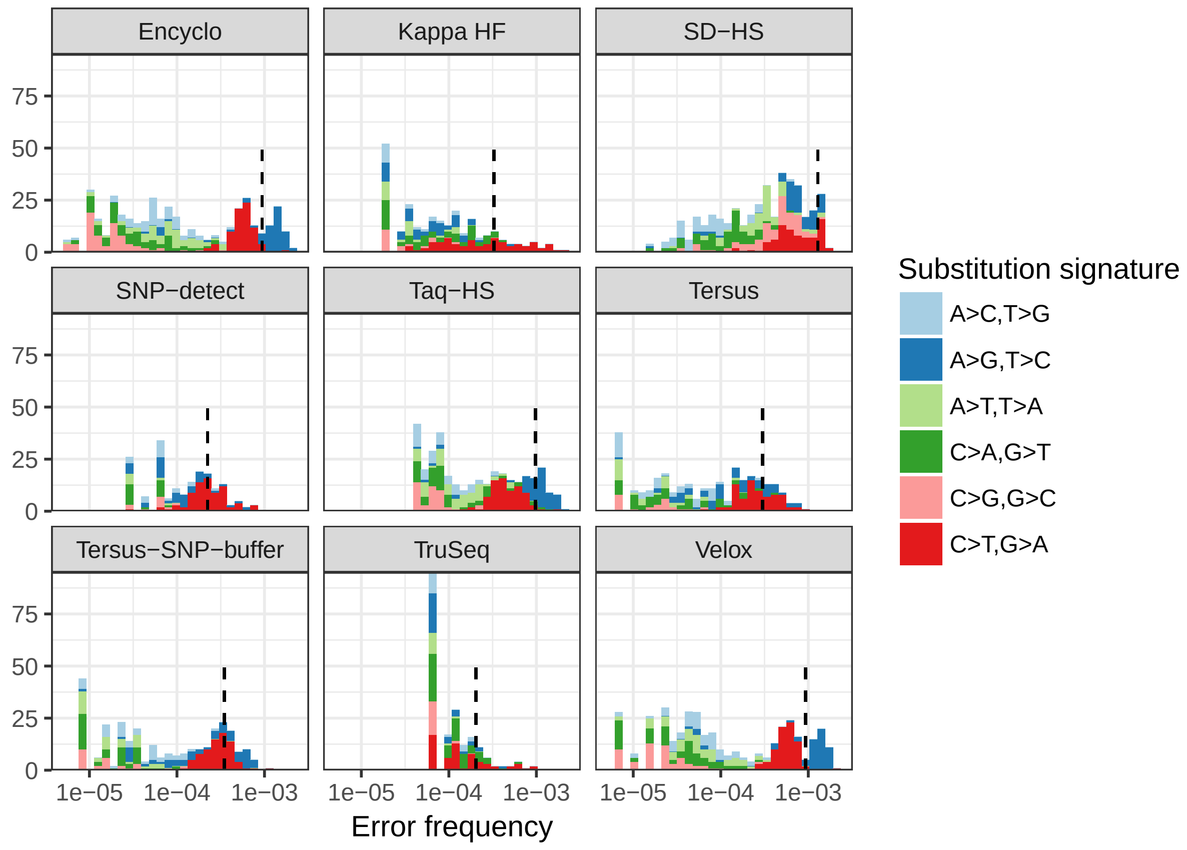


Indibidual mutations

Frequency distribution of individual mutations, grouped by their pattern.

```
df.1 <- subset(df, project %in% c("polerr2016-1", "polerr2016-2"))
df.1 <- ddply(df.1, .(name, mutation.pos, mutation.signature.rep), summarize, count.major=sum(count.maj
              coverage=sum(coverage))
df.mean.err <- ddply(df.1, .(name), summarize, mean.err.rate = sum(count.major) / mean(coverage) /
                       nchar(template))
# ^ this one is the gloal mean

df.1 <- merge(df.1, df.mean.err, all.x=T, all.y=F)

ggplot(df.1) +
  geom_histogram(aes(x = count.major / coverage, fill = mutation.signature.rep)) +
  geom_linerange(aes(x = mean.err.rate, ymin = 0, ymax=50), linetype = "dashed", color="black") +
  scale_fill_brewer("Substitution signature", palette = "Paired") +
  scale_x_log10("Error frequency") +
  scale_y_continuous("", expand=c(0,0)) + facet_wrap(~name) + theme_bw()
```

```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```



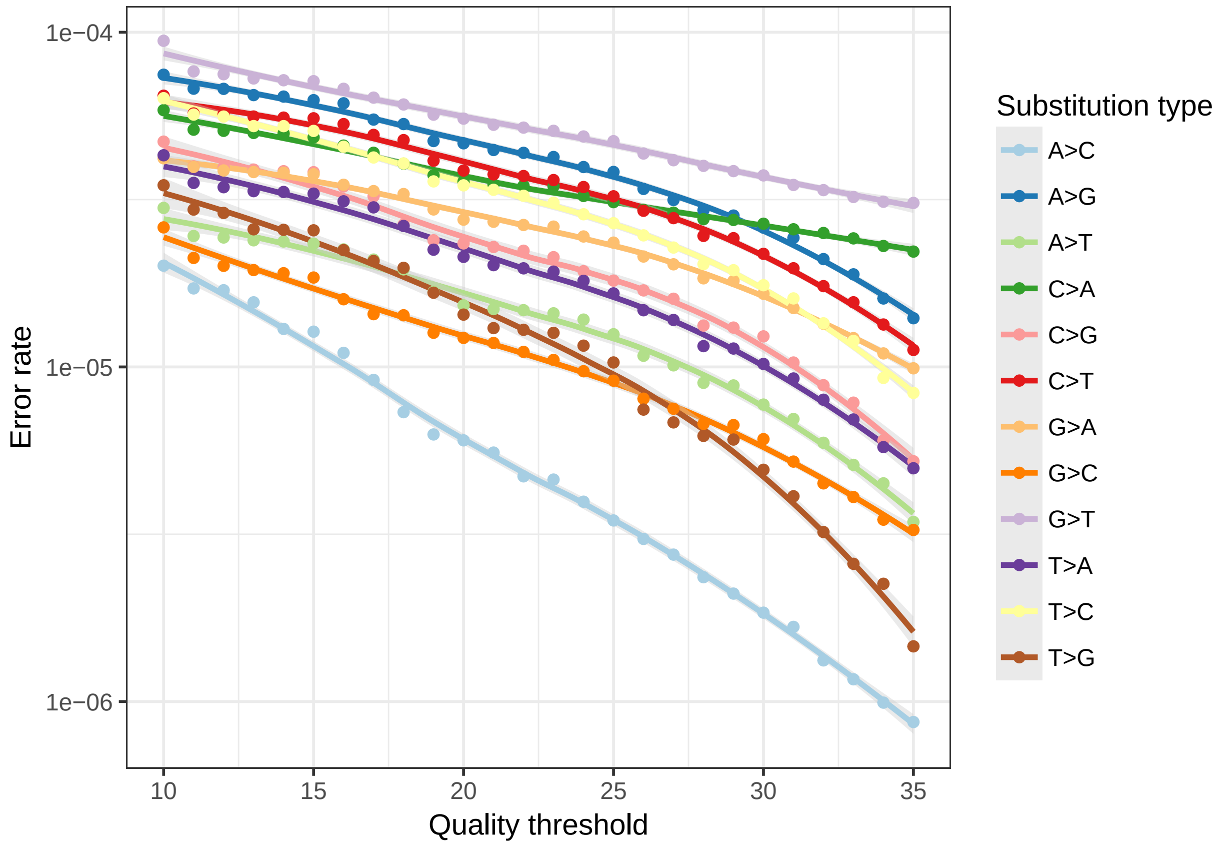
```
  #facet_wrap(~name, scales = "free_y") + theme_bw()
```

Sequence quality and error patterns

Error frequency for different substitution type and quality threshold.

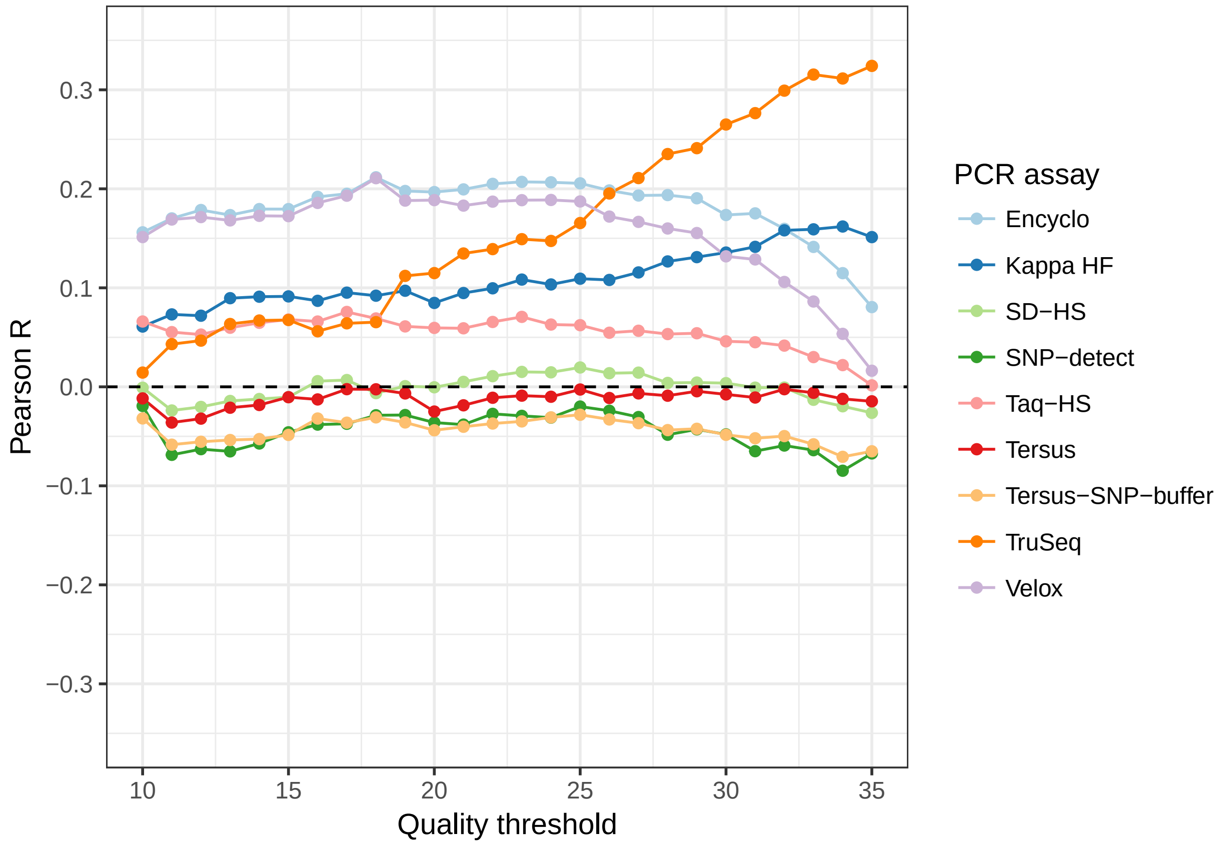
```
df.qq <- read.table("data/mmqc.txt", header=T, sep="\t", stringsAsFactors = F)
df.qq$mutation.signature <- as.factor(paste(df.qq$from, df.qq$to, sep = ">"))
df.qq <- merge(df.qq, sign.rep, all.x=TRUE)

df.qq.cov <- ddply(subset(df.qq, from == to), .(from, qual), summarize, total=sum(count))

df.qq.1 <- merge(df.qq, df.qq.cov)

df.qq.1 <- ddply(subset(df.qq.1, from != to), .(mutation.signature, qual), summarize,
                 count = sum(count), freq = count / total[1])

ggplot(df.qq.1, aes(x=qual, y=freq, color=mutation.signature)) +
  geom_smooth(fill="grey80") + geom_point() +
  scale_color_brewer("Substitution type", palette = "Paired") +
  scale_y_log10("Error rate") +
  scale_x_continuous("Quality threshold") +
  theme_bw()
```

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



Correlation across polymerase types.

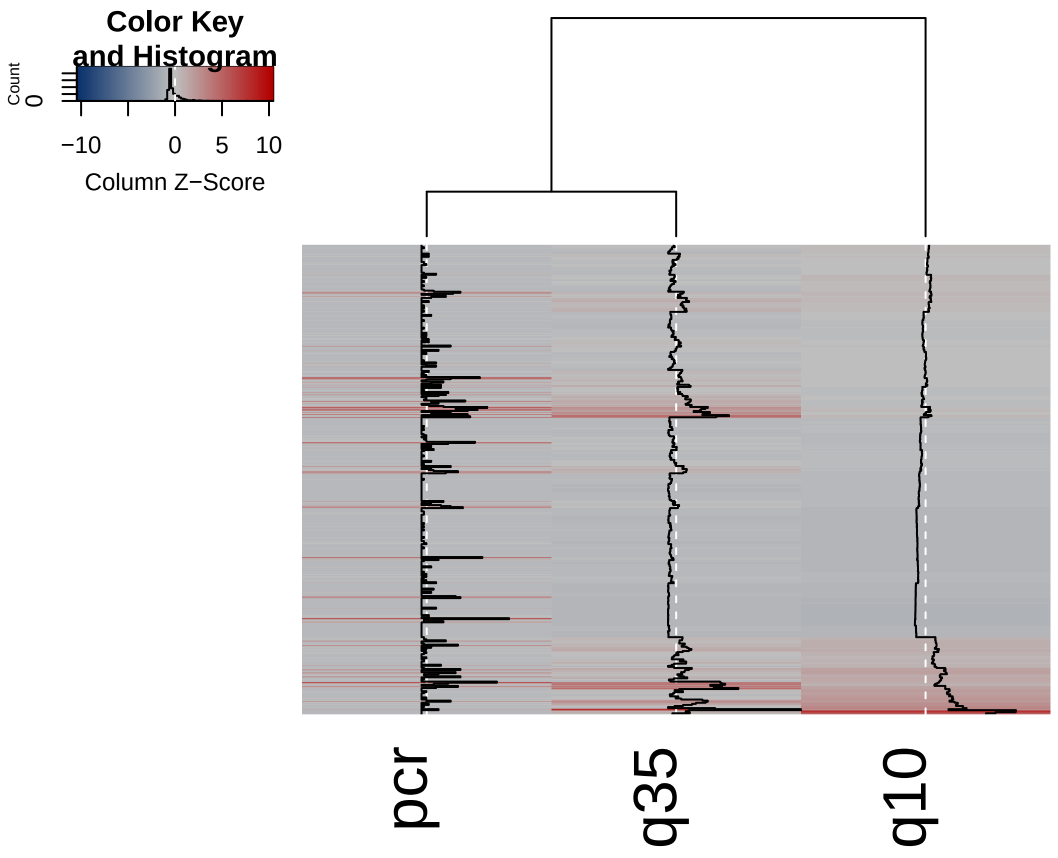
```
df.linpcr.2 <- ddply(df.linpcr, .(name, mutation.pos, mutation.from, mutation.to), summarize,
                     count = sum(count.major))

colnames(df.linpcr.2) <- c("name", "pos", "from", "to", "count.pcr")

df.qq.2 <- merge(df.qq, df.linpcr.2)
df.qq.corr <- ddply(df.qq.2, .(qual, name), summarize, r = cor(count, count.pcr))

ggplot(df.qq.corr, aes(x=qual, color=name, y = r)) +
  geom_line() + geom_point() + geom_hline(yintercept = 0, linetype = "dashed") +
  scale_color_brewer("PCR assay", palette = "Paired") +
  scale_y_continuous("Pearson R", limits = c(-0.35, 0.35),
                     breaks = c(-0.3, -0.2, -0.1, 0, 0.1, 0.2, 0.3)) +
  xlab("Quality threshold") +
  theme_bw()
```



Clustering TruSeq, Q10 and Q35 error profiles.

```
df.qq.trs <- subset(df.linpcr.2, name=="TruSeq")
df.qq.trs <- data.frame(pos = df.qq.trs$pos, to = df.qq.trs$to, count.trs = df.qq.trs$count.pcr)
df.qq.q10 <- subset(df.qq, qual==10 & from != to)
df.qq.q35 <- subset(df.qq, qual==35 & from != to)
df.qq.clust <- merge(df.qq.trs, df.qq.q10, by = c("pos", "to"), all.y=T)
df.qq.clust <- merge(df.qq.clust, df.qq.q35, by = c("pos", "to"))
df.qq.clust <- data.frame(subst = paste(df.qq.clust$pos, df.qq.clust$to),
                          pcr=df.qq.clust$count.trs,
                          q10=df.qq.clust$count.x,
                          q35=df.qq.clust$count.y)
rownames(df.qq.clust) <- df.qq.clust$subst
df.qq.clust$subst <- NULL

mat.qq.clust <- as.matrix(df.qq.clust)
mat.qq.clust[is.na(mat.qq.clust)] <- 0
```

```r
heatmap.2(mat.qq.clust, dendrogram = "column",  labRow = FALSE, scale="col",
          col=colorpanel(100, "#08306b", "grey", "#b30000"), tracecol="black", linecol = "white")
```



Sequence context

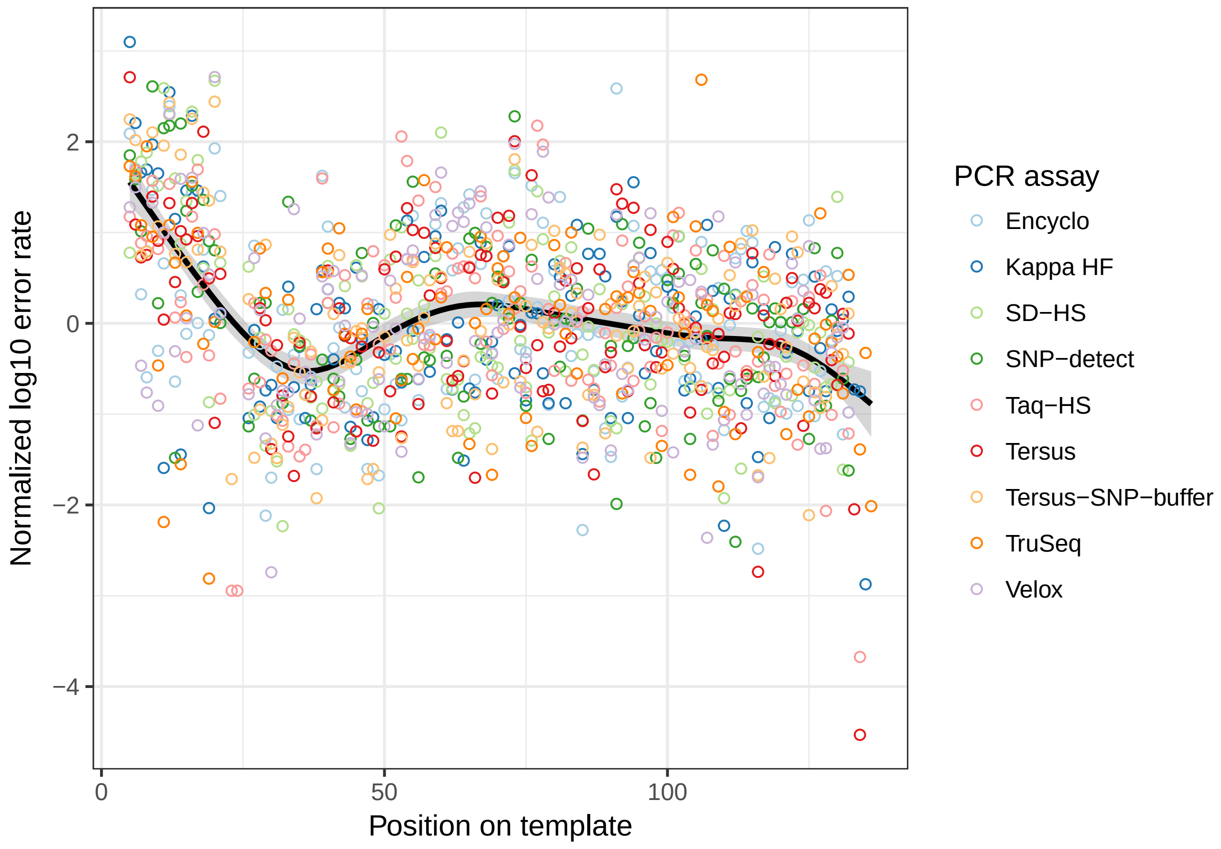
Load data

```r
df.kmer = read.table("data/kmer_errors.txt", header = T, sep = "\t")
```

Normalize error rate for each polymerase and base

```r
df.kmer.posnorm = df.kmer %>%
  dplyr::group_by(name, mut.from, mut.pos) %>%
  dplyr::summarize(freq = log10(sum(count) / sum(coverage)), gc = ifelse(mut.from %in% c("G", "C"), 1, 

df.kmer.posnorm = df.kmer.posnorm %>%
  dplyr::group_by(name, mut.from) %>%
  dplyr::mutate(freq.norm = scale(freq)[,1])

ggplot(df.kmer.posnorm, aes(x = mut.pos, y = freq.norm)) +
  #geom_line(aes(group = name)) +
  geom_smooth(color = "black") +
  geom_point(aes(color = name), shape = 21) +
  xlab("Position on template") + ylab("Normalized log10 error rate") +
  #facet_grid(.~mut.from) +
  scale_color_brewer("PCR assay", palette = "Paired") +
  theme_bw()
```

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



```
cor.test(df.kmer.posnorm$mut.pos, df.kmer.posnorm$freq.norm, method = "spearman")
```

```
## Warning in cor.test.default(df.kmer.posnorm$mut.pos, df.kmer.posnorm
## $freq.norm, : Cannot compute exact p-value with ties

##
##  Spearman's rank correlation rho
##
## data:  df.kmer.posnorm$mut.pos and df.kmer.posnorm$freq.norm
## S = 248560000, p-value = 7.186e-12
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##        rho
## -0.2072036
```

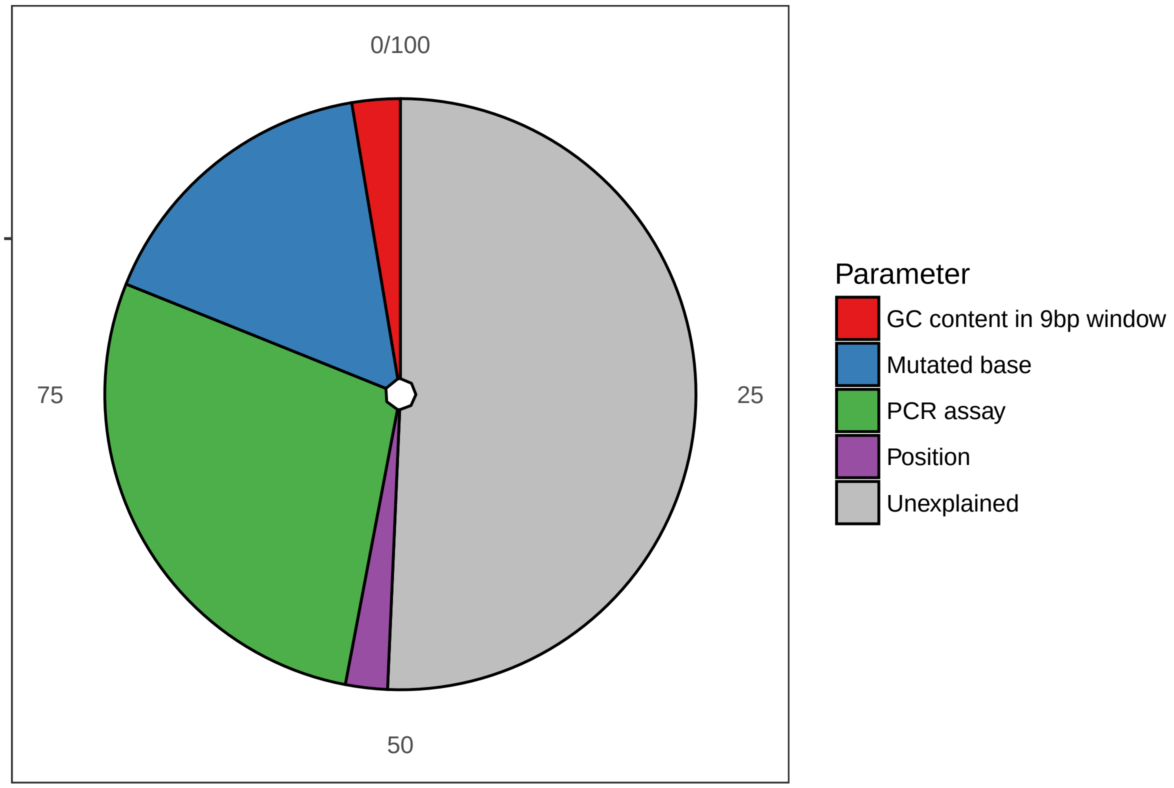
K-mer model

```
df.kmer.pos = df.kmer %>%
  dplyr::group_by(name, mut.pos, mut.from, region.gc) %>%
  dplyr::summarize(freq.pos = sum(count) / sum(coverage))

fit = aov(log10(freq.pos) ~ mut.pos + region.gc + name + mut.from, df.kmer.pos)
af <- anova(fit)
afss <- af$"Sum Sq"
afss <- cbind(af,PctExp=afss/sum(afss)*100)
```

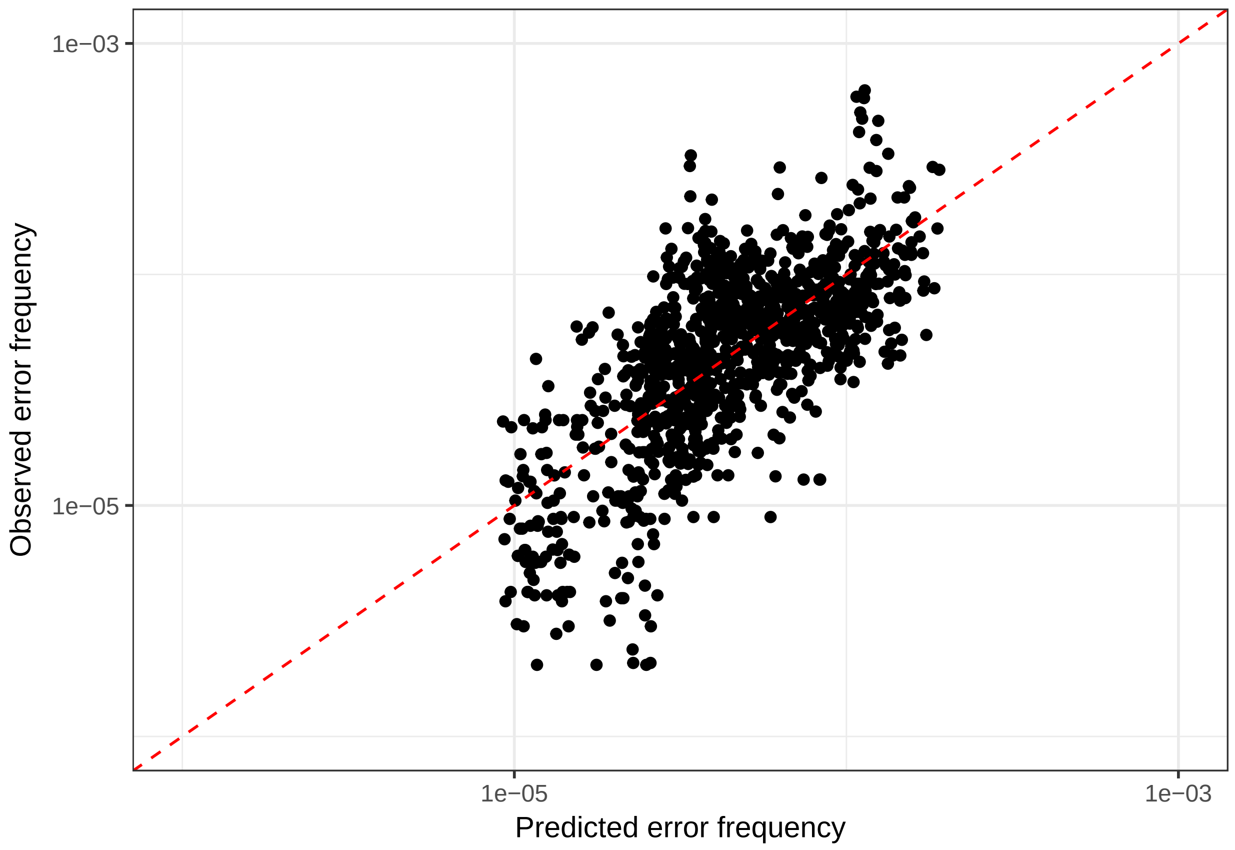
```r
print(afss)
```

```
##              Df    Sum Sq    Mean Sq   F value       Pr(>F)    PctExp
## mut.pos       1  3.999454 3.99945388  47.90793 7.726697e-12  2.293204
## region.gc     1  4.592552 4.59255224  55.01243 2.450133e-13  2.633274
## name          8 48.995320 6.12441497  73.36203 4.535112e-96 28.092900
## mut.from      3 28.409779 9.46992632 113.43663 1.071380e-63 16.289578
## Residuals 1059 88.407528 0.08348209        NA           NA 50.691043
```

```r
afss$param <- c("Position", "GC content in 9bp window", "PCR assay", "Mutated base", "Unexplained")

ggplot(afss, aes(x = "", y = PctExp, fill = param)) +
  geom_bar(stat = "identity", color = "black") +
  coord_polar(theta = "y") + xlab("") + ylab("") +
  scale_fill_manual("Parameter", values = c("#e41a1c", "#377eb8", "#4daf4a", "#984ea3", "grey")) +
  theme_bw() + theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())
```



```r
df.kmer.pos$freq.mdl = predict(fit, df.kmer.pos, type="response")

ggplot(df.kmer.pos, aes(x = 10^freq.mdl, y = freq.pos)) +
  geom_point() +
  geom_abline(slope = 1, intercept = 0, color = "red", linetype = "dashed") +
  scale_x_log10("Predicted error frequency", limits = c(1e-6, 1e-3)) +
  scale_y_log10("Observed error frequency", limits = c(1e-6, 1e-3)) +
  theme_bw()
```

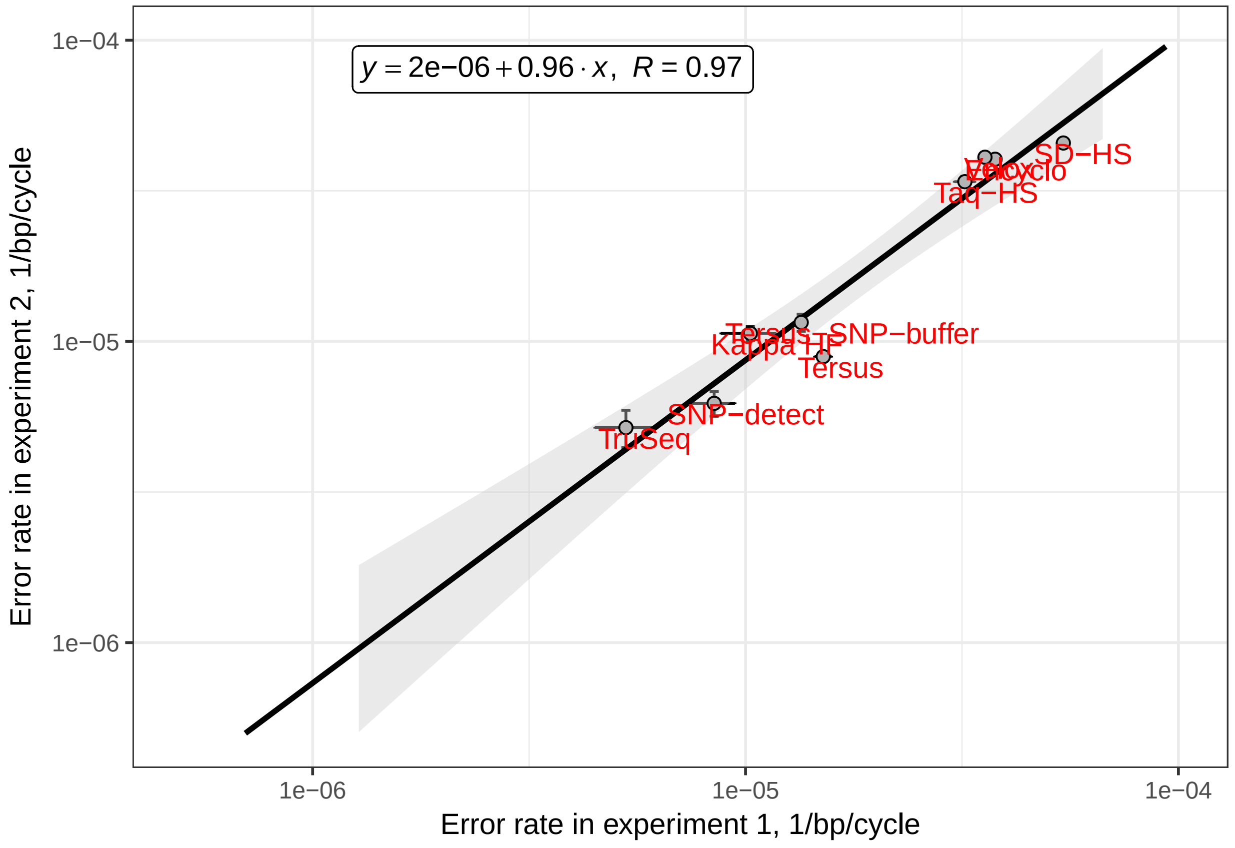


Supplementary data

Error rate consistency between two independent experimental replicas from Polerr2016 dataset.

```
df.er.cast <- dcast(df.er, name ~ project,
                    value.var = "err.rate.corr")
df.er.cast2 <- dcast(df.er, name ~ project,
                    value.var = "delta")

df.er.cast <- merge(df.er.cast, df.er.cast2, by = "name")
colnames(df.er.cast) <- c("name", "replica1.x", "replica2.x", "replica1.y", "replica2.y")

m <- lm(replica1.x ~ replica2.x, df.er.cast);
eq <- substitute(italic(y) == a + b %.% italic(x)*","~~italic(R)~"="~r,
     list(a = format(coef(m)[1], digits = 2),
          b = format(coef(m)[2], digits = 2),
          r = format(sqrt(summary(m)$r.squared), digits = 2)))
lbl<-as.character(as.expression(eq))

ggplot(df.er.cast, aes(x=replica1.x, y=replica2.x)) +
  geom_errorbarh(aes(xmax=replica1.x+1.96*replica1.y,xmin=replica1.x-1.96*replica1.y)) +
  geom_errorbar(aes(ymax=replica2.x+1.96*replica2.y,ymin=replica2.x-1.96*replica2.y)) +
  geom_smooth(method = "lm", color="black", fill="grey80", fullrange = T) +
  geom_point(size=2, shape=21, fill="grey70") +
  geom_text(aes(label=name), vjust=1, hjust = .3, color="red") +
  geom_label(aes(x = 1e-6, y = 8e-5, label = lbl), hjust=-0.1, parse = TRUE)+
  scale_x_log10(name="Error rate in experiment 1, 1/bp/cycle", limits=c(5e-7,1e-4)) +
  scale_y_log10(name="Error rate in experiment 2, 1/bp/cycle", limits=c(5e-7,1e-4)) +
  theme_bw()
```

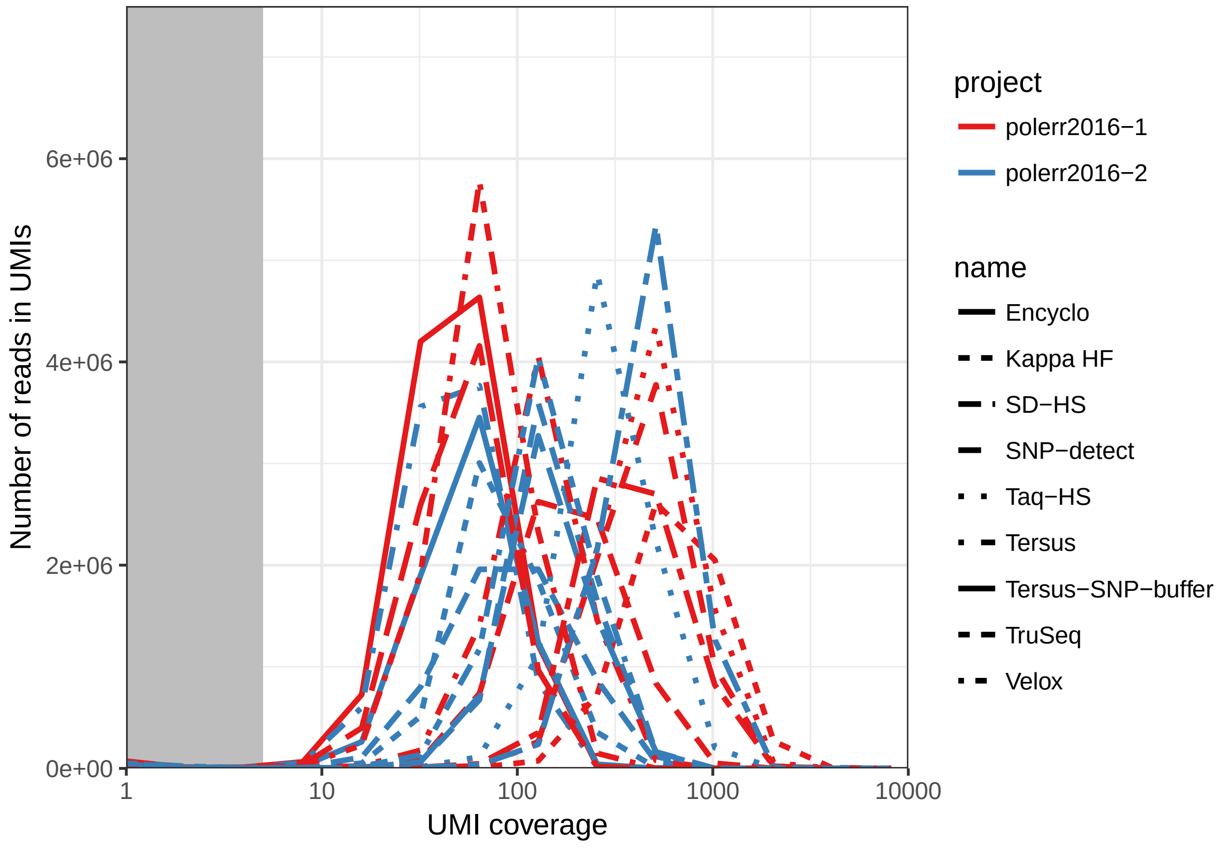
```
## Warning: Removed 6 rows containing missing values (geom_smooth).
```



UMI coverage histogram

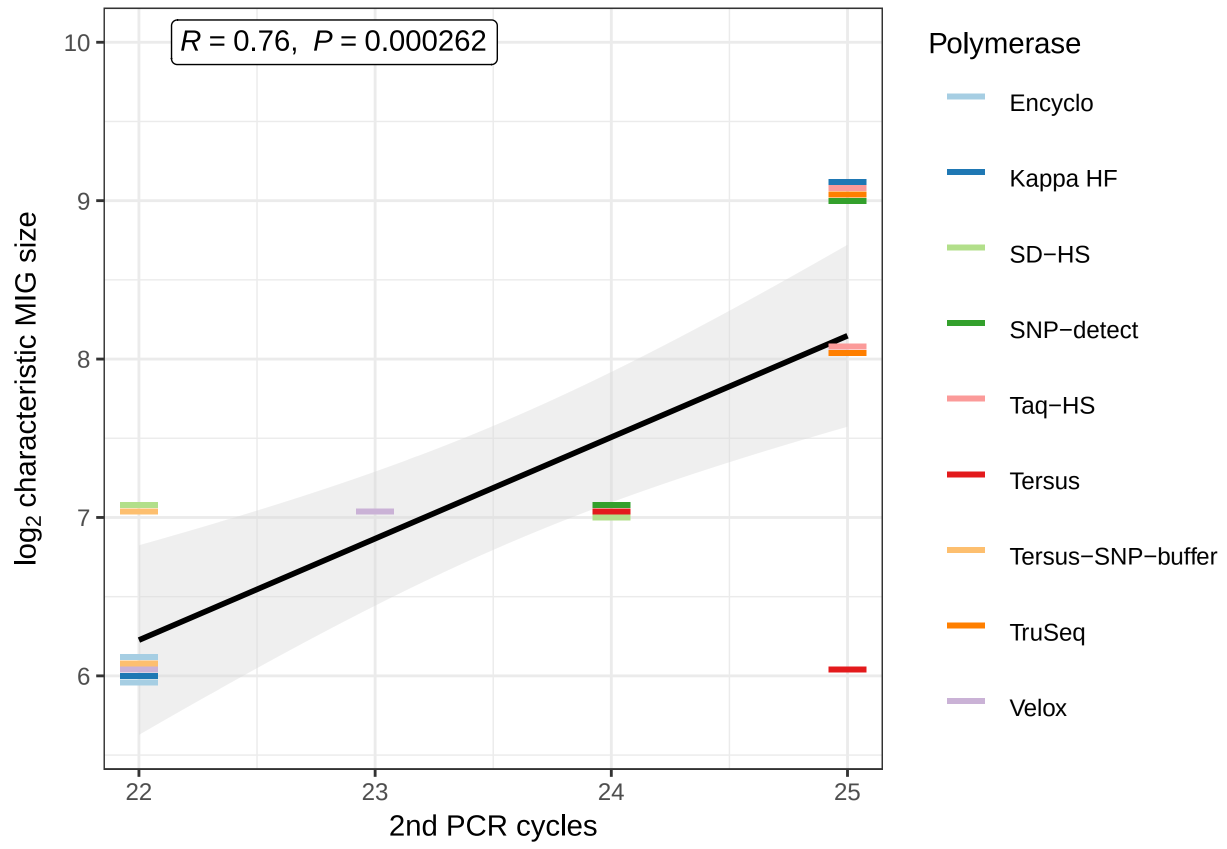
```
df.meta <- subset(df.meta, project %in% c("polerr2016-1", "polerr2016-2"))

df.h <- data.frame(mig.size.bin = integer(), read.count = integer(), name=character(),
                   project=character())

for (proj in unique(df.meta$project)) {
  for (sample in unique(subset(df.meta, project == proj)$sample)) {
    mask <- which(df.meta$sample == sample & df.meta$project == proj)
    name <- df.meta$name[mask][1]
    cycles_2 <- df.meta$cycles_2[mask][1]
    df.hh <- read.table(paste(path, paste(proj, sample, "umi.histogram.txt", sep="."), sep="/"),
                        header=T, sep="\t")
    df.h <- rbind(df.h, data.frame(mig.size.bin = df.hh$mig.size.bin, read.count = df.hh$read.count,
                                   name=name, project=proj, cycles_2=cycles_2))
  }
}

ggplot(df.h) +
  geom_rect(aes(xmin=1, xmax=5, ymin=0, ymax=Inf), fill="grey") +
  geom_line(aes(x=mig.size.bin, y=read.count, color=project, linetype=name,
                group = interaction(project, name)), size=1) +
  scale_x_log10("UMI coverage", limits = c(1,1e4), expand=c(0,0)) +
  scale_y_continuous("Number of reads in UMIs", expand=c(0,0), limits=c(0,7.5e6)) +
  scale_color_brewer(palette = "Set1") +
  theme_bw()
```

```
## Warning: Removed 108 rows containing missing values (geom_path).
```



Coverage and PCR cycles

```
library(ggbeeswarm)
df.o <- ddply(df.h, .(project, name, cycles_2), summarize,
              peak = log2(mig.size.bin[which(read.count == max(read.count))]))

m <- lm(peak ~ cycles_2, df.o)
eq <- substitute(italic(R)~"="~r*","~~italic(P)~"="~p,
     list(
     r = format(sqrt(summary(m)$r.squared), digits = 2),
     p = format(summary(m)$coefficient[[8]], digits = 3)
     ))
lbl<-as.character(as.expression(eq))

ggplot()+
geom_label(aes(x = 22, y = 10, label = lbl), hjust=-0.1, parse = TRUE)+
stat_smooth(data=df.o, aes(cycles_2, peak), method=lm, color="black", fill="grey85") +
geom_beeswarm(data=df.o, aes(x=cycles_2, y=peak, group = interaction(name,cycles_2), color=name), group
            shape=45, size = 10) +
scale_x_continuous(name="2nd PCR cycles", breaks=10:30) +
scale_y_continuous(expression('log'[2]~'characteristic MIG size'), breaks=2:20) +
scale_color_brewer(name  ="Polymerase", palette = "Paired") +
theme_bw()
```


R = 0.76, P = 0.000262
log2 characteristic MIG size
2nd PCR cycles
Polymerase
Encyclo
Kappa HF
SD−HS
SNP−detect
Taq−HS
Tersus
Tersus−SNP−buffer
TruSeq
Velox