Polymerase fidelity estimates

Load required libraries, load and merge MAGERI results.

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

```
## Warning: package 'ggplot2' was built under R version 3.2.4
```

```
## Warning: package 'gplots' was built under R version 3.2.4
```

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

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

```
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>
                       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>

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)

# 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 <- "TAGCGTGAAGACGACAGAACCATGGGATCCATTATCGGCGGCGAATTTACCACCATTGAAAACCAGCCGTGGTTTGCGGCGATTTATCGT
```

Estimating the 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.

```
# Compute linear PCR error rate

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

df.er.linpcr <- data.frame(name = df.er.linpcr$name, linpcr.er = df.er.linpcr$mismatches/df.er.linpcr$u

# 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$delta.corr <- with(df.er, sqrt(mismatches.corr / umi.count * (1 - mismatches.corr / umi.count) /
                 nchar(template) / mean(cycles))
df.er$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)
```

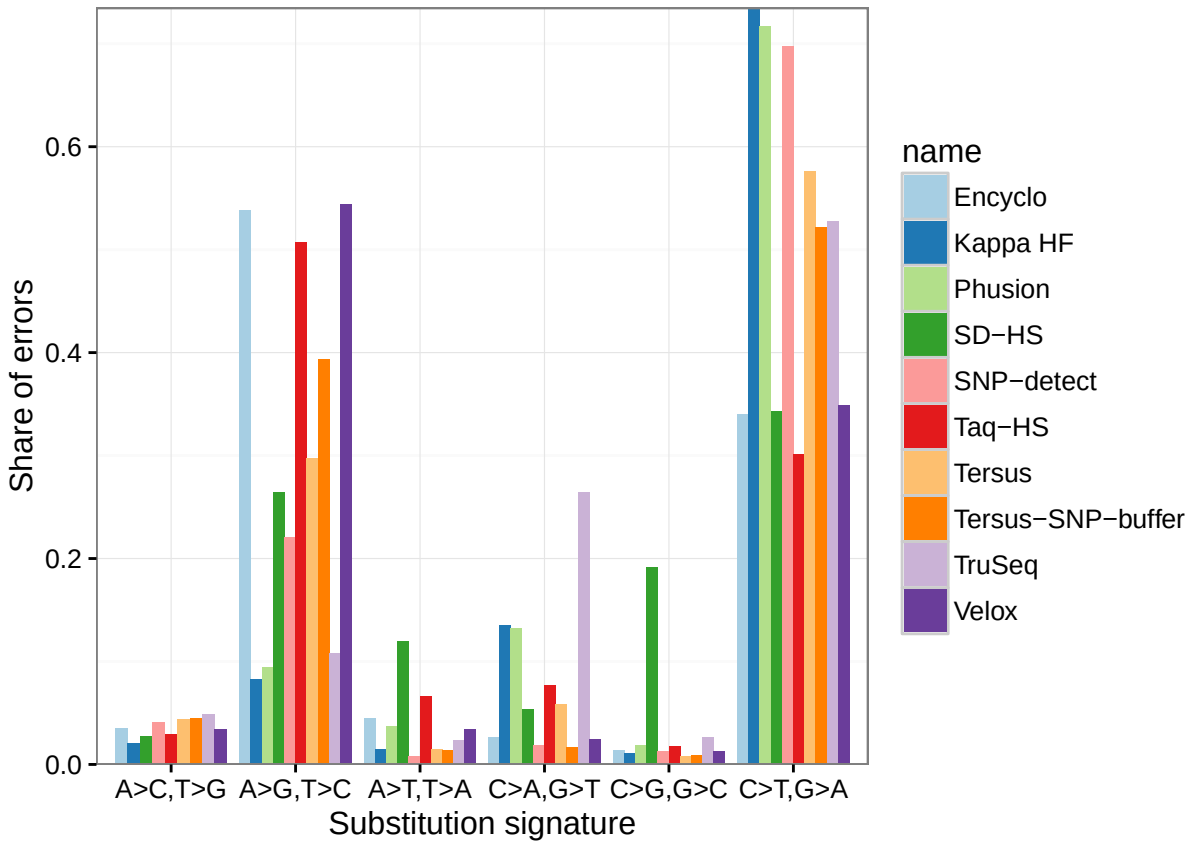
name	project	mismatches	umi.count	linpcr.er	err.rate	delta	err.lb	err.ub	misma
Encyclo	polerr2016-1	24557	185560	0.0001279	4.30e-05	3.0e-07	4.25e-05	4.35e-05	20
Encyclo	polerr2016-2	14211	101516	0.0001279	4.55e-05	4.0e-07	4.48e-05	4.62e-05	12
Kappa HF	polerr2016-1	339	7876	0.0000817	1.40e-05	7.0e-07	1.25e-05	1.55e-05	
Kappa HF	polerr2016-2	2519	57052	0.0000817	1.44e-05	3.0e-07	1.38e-05	1.49e-05	1
Phusion	polerr2016-2	23	1351	0.0000499	5.50e-06	1.1e-06	3.30e-06	7.80e-06	
Phusion	polerr2016-1	30	1348	0.0000499	7.20e-06	1.3e-06	4.70e-06	9.80e-06	
SD-HS	polerr2016-2	10362	58518	0.0002689	5.76e-05	5.0e-07	5.66e-05	5.86e-05	8
SD-HS	polerr2016-1	6714	33076	0.0002689	6.60e-05	7.0e-07	6.46e-05	6.74e-05	5
SNP-detect	polerr2016-1	457	13870	0.0000504	1.07e-05	5.0e-07	9.70e-06	1.17e-05	
SNP-detect	polerr2016-2	848	32310	0.0000504	8.50e-06	3.0e-07	8.00e-06	9.10e-06	
Taq-HS	polerr2016-1	1875	15137	0.0001836	4.03e-05	9.0e-07	3.86e-05	4.20e-05	1
Taq-HS	polerr2016-2	3113	24082	0.0001836	4.20e-05	7.0e-07	4.07e-05	4.34e-05	2
Tersus	polerr2016-2	5504	154226	0.0000598	1.16e-05	2.0e-07	1.13e-05	1.19e-05	4
Tersus	polerr2016-1	2550	46927	0.0000598	1.77e-05	3.0e-07	1.70e-05	1.83e-05	2
Tersus-SNP-buffer	polerr2016-2	1299	30683	0.0000510	1.38e-05	4.0e-07	1.30e-05	1.45e-05	1
Tersus-SNP-buffer	polerr2016-1	6282	130891	0.0000510	1.56e-05	2.0e-07	1.52e-05	1.60e-05	5
TruSeq	polerr2016-1	312	14164	0.0000410	7.20e-06	4.0e-07	6.40e-06	7.90e-06	
TruSeq	polerr2016-2	362	16705	0.0000410	7.00e-06	4.0e-07	6.30e-06	7.80e-06	
Velox	polerr2016-1	16802	132733	0.0001292	4.12e-05	3.0e-07	4.06e-05	4.17e-05	14
Velox	polerr2016-2	6298	44331	0.0001292	4.62e-05	5.0e-07	4.51e-05	4.73e-05	5

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

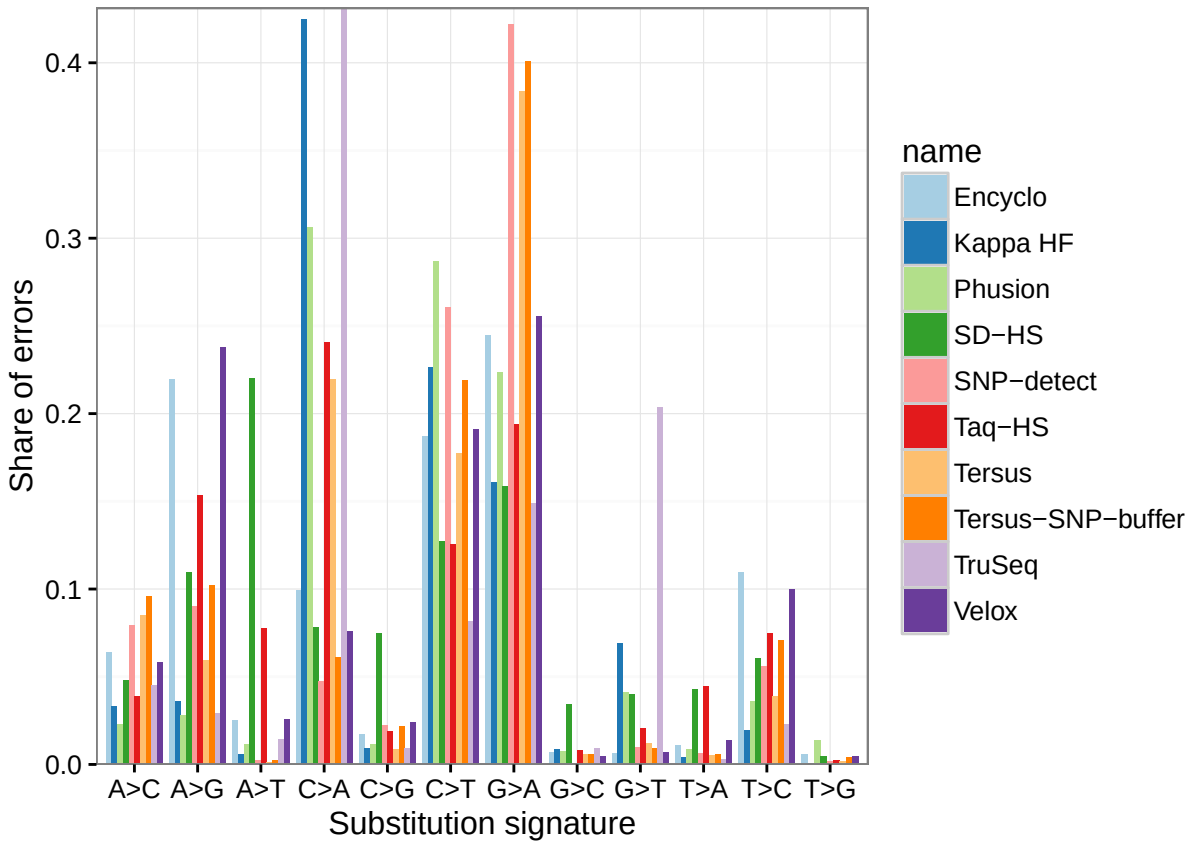
Patterns and recurrent errors

Substitution signature preferences:

```
df.pattern <- ddply(df, .(name, mutation.signature.rep), summarize, count.sum = sum(count.major))
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()
```




```
# same for linpcr

df.pattern.linpcr <- ddply(df.linpcr, .(name, mutation.signature), summarize, count.sum = sum(count.maj
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()
```

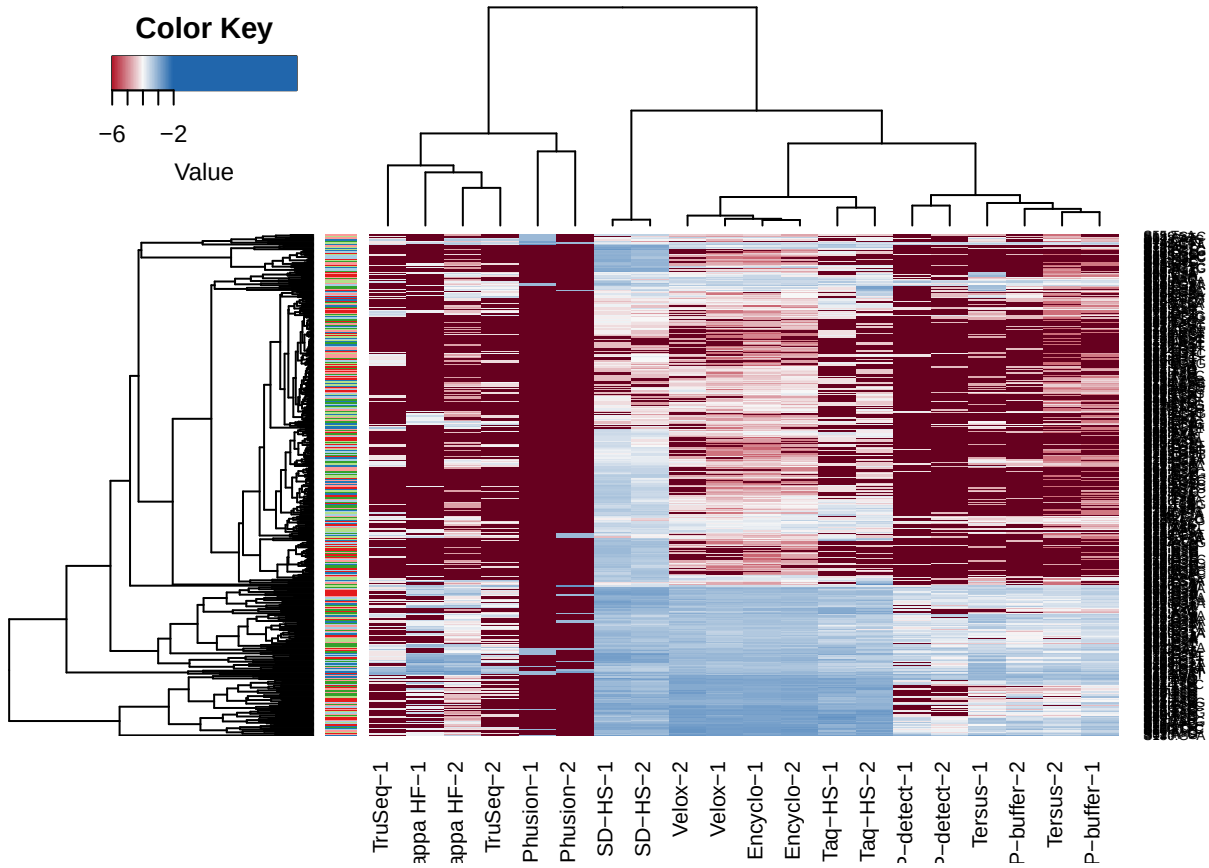


Clustering of polymerase error profiles:

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

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

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

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

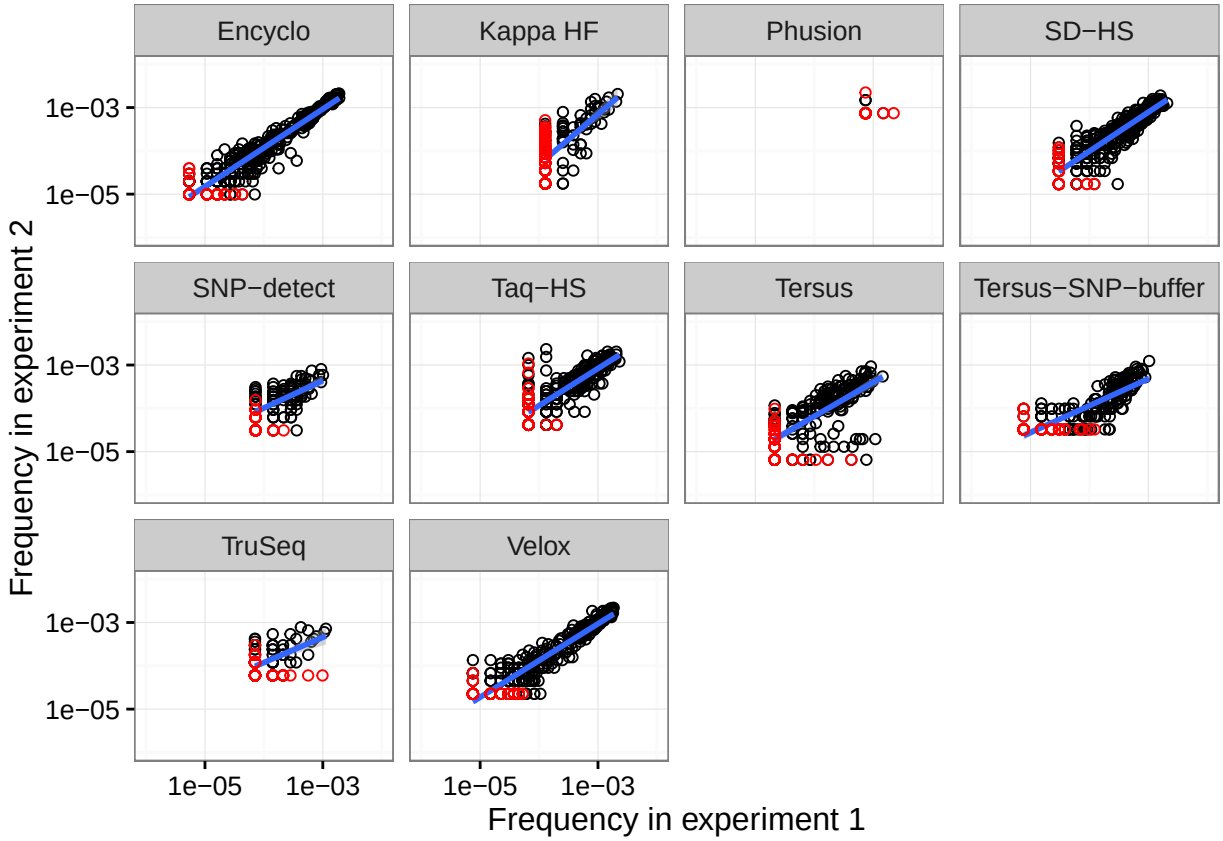
```
heatmap.2(log10(mat.profile), col=c("#67001f", colorpanel(99, "#b2182b", "#f7f7f7", "#2166ac")),
          dendrogram = "both", RowSideColors = rowcolor, breaks = seq(-6,-2,length.out = 101),
          Rowv = as.dendrogram(rowDend),
          Colv = as.dendrogram(colDend),
          density.info = "none", trace="none")
```



Recurrent errors

```
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) +
  geom_smooth(data=subset(df.1, !miss & name != "Phusion"), aes(x=freq.x, y=freq.y), method="lm") +
```

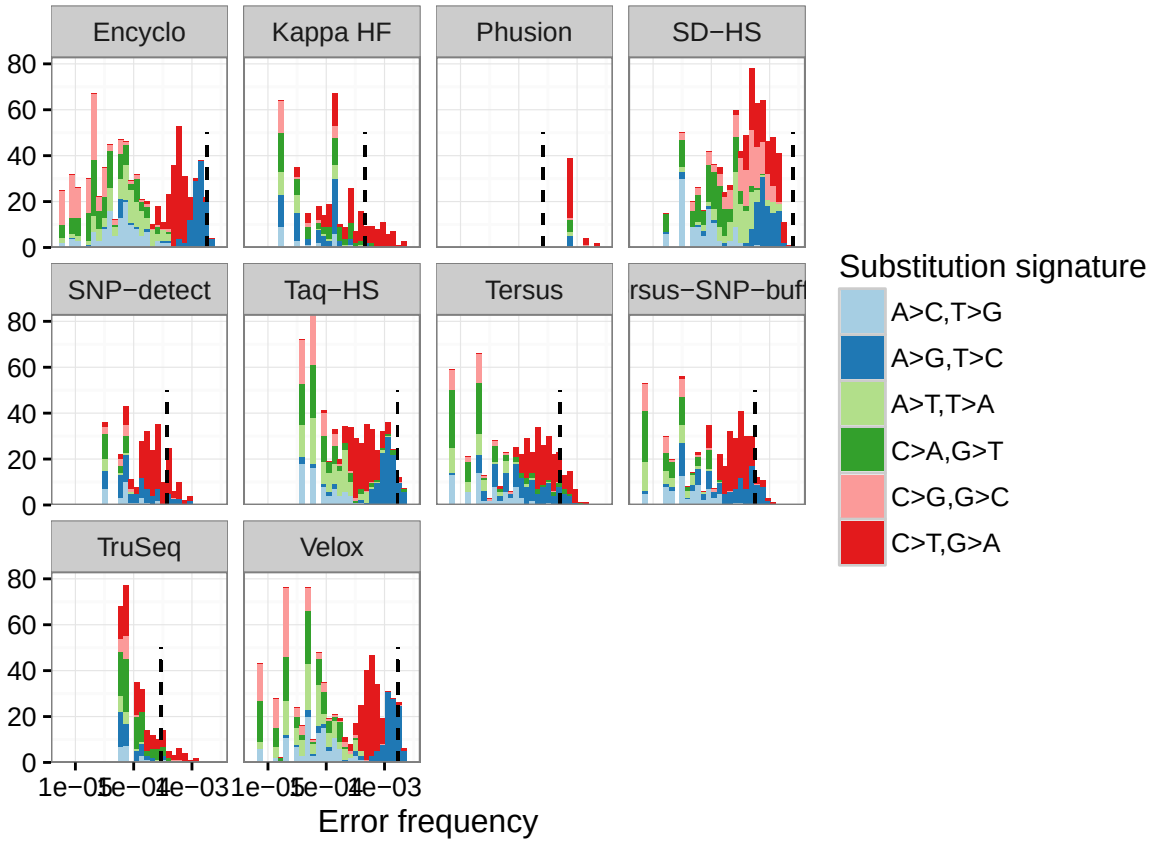
```
  geom_point(data=subset(df.1, miss), aes(x=freq.x, y=freq.y), shape=21, color="red") +
  facet_wrap(~name) +
  scale_x_log10("Frequency in experiment 1", limits=c(1e-6,1e-2)) +
  scale_y_log10("Frequency in experiment 2", limits=c(1e-6,1e-2)) +
  theme_bw()
```



Frequency distribution of individual mutations, grouped by their pattern.

```
df.mean.err <- ddply(df, .(name), summarize, mean.err.rate = sum(count.major) / mean(coverage) / nchar(
# ^ this one is the gloal mean

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

ggplot(df) +
  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()
```

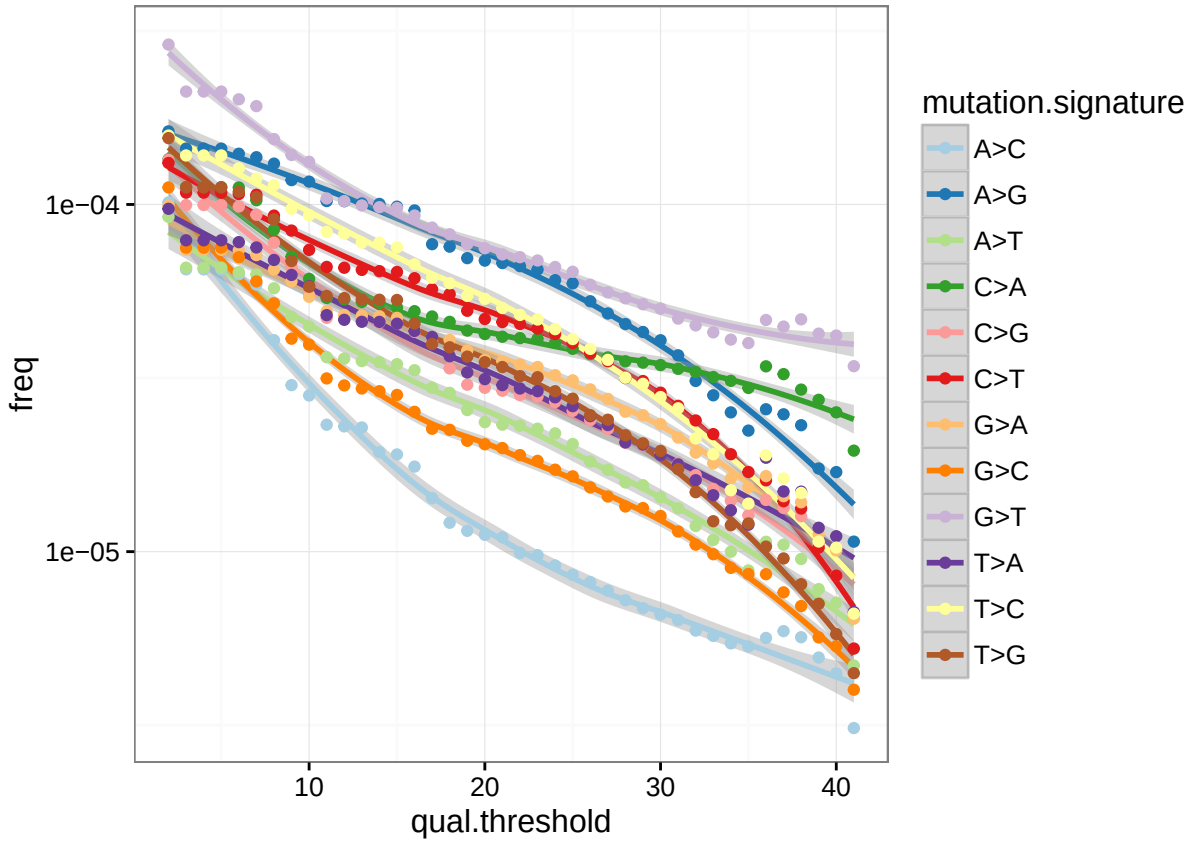
```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```



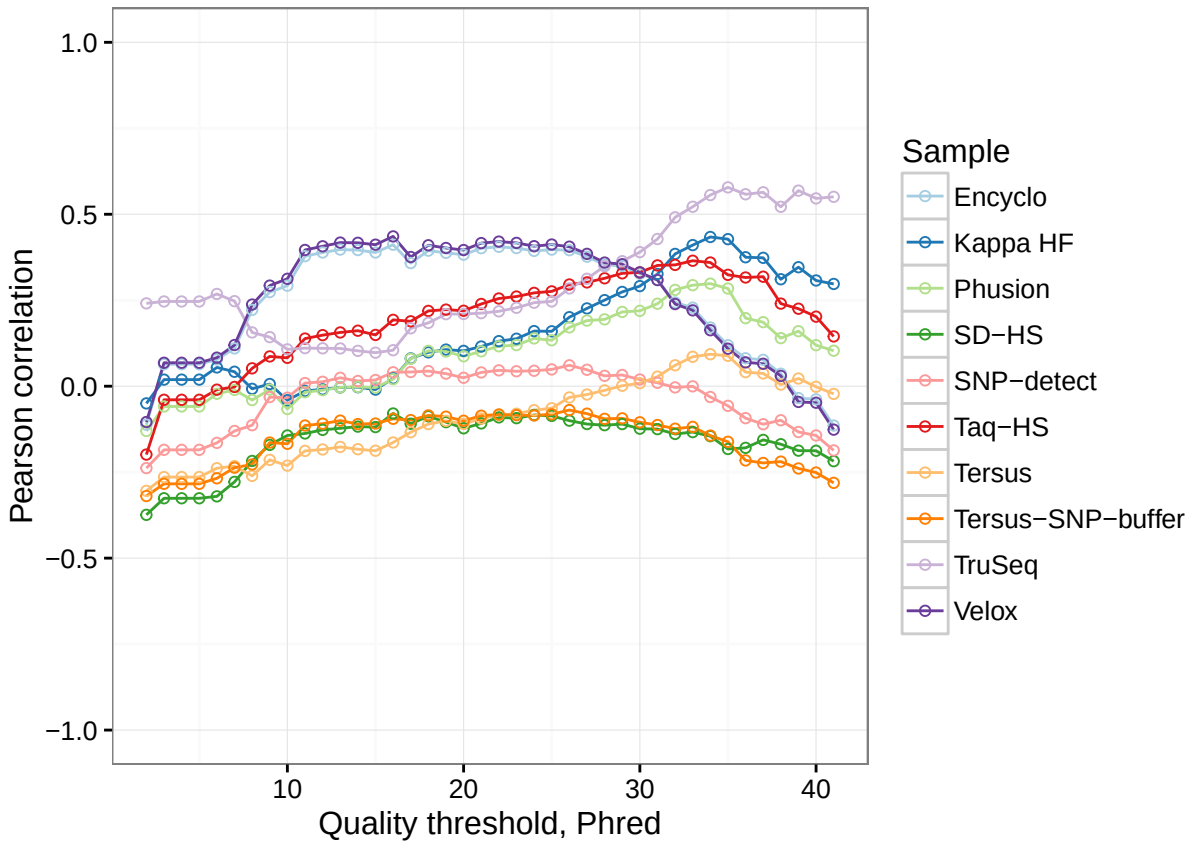

```
  #facet_wrap(~name, scales = "free_y") + theme_bw()
```

Sequence quality and error patterns

```
df.q <- read.table("data/mmqc.txt", header=T,sep="\t")
df.q <- melt(df.q, id.vars = "qual.threshold")
df.q$variable <- as.factor(gsub(".", ">", df.q$variable, fixed = T))
colnames(df.q) <- c("qual.threshold", "mutation.signature", "freq")

# Error profiles for different quality threhsolds

ggplot(df.q, aes(x=qual.threshold, color=mutation.signature, y=freq)) +
  geom_smooth() + geom_point() + scale_y_log10() +
  scale_color_brewer(palette = "Paired") + theme_bw()
```




```
# Correlation between error profiles

df.linpcrT <- ddply(df.linpcr, .(mutation.signature, name), summarize, freq = sum(count.major) / sum(co
df.q_truseq <- merge(df.q, df.linpcrT, by = c("mutation.signature"), all.x=T)
df.q_truseq$freq.y[is.na(df.q_truseq$freq.y)] <- 0

df.q_truseq <- ddply(df.q_truseq, .(qual.threshold, name), summarize, R = cor(freq.x, freq.y))

ggplot(df.q_truseq, aes(x=qual.threshold, y=R, color=name)) +
  geom_line() + geom_point(shape=21) + scale_color_brewer("Sample", palette = "Paired") +
  scale_y_continuous("Pearson correlation", limits=c(-1,1)) + xlab("Quality threshold, Phred") +
  theme_bw()
```



Supplementary data

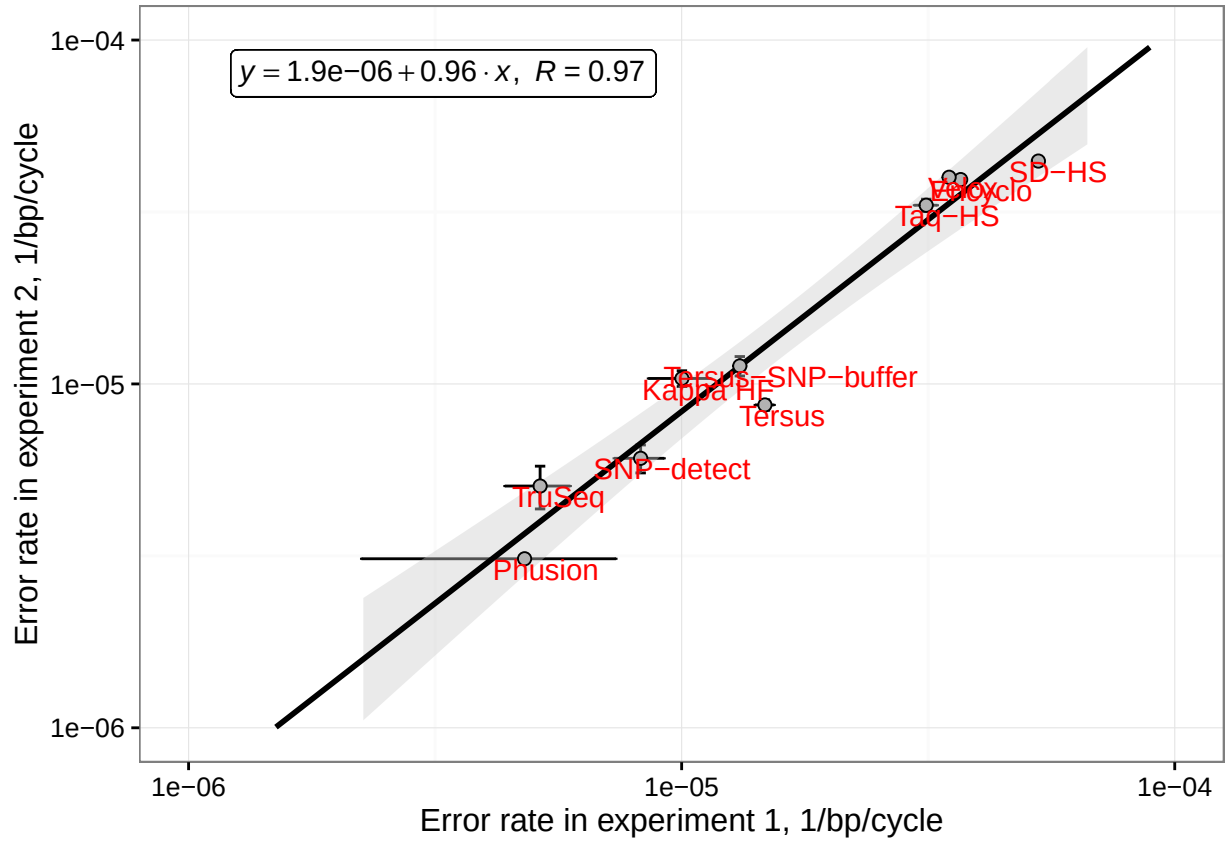
Error rate consistency between two independent experimental replicas from Polerr2016 dataset.

```
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(1e-6,1e-4)) +
  scale_y_log10(name="Error rate in experiment 2, 1/bp/cycle", limits=c(1e-6,1e-4)) +
  theme_bw()
```

```
## Warning: Removed 1 rows containing missing values (geom_errorbar).
```

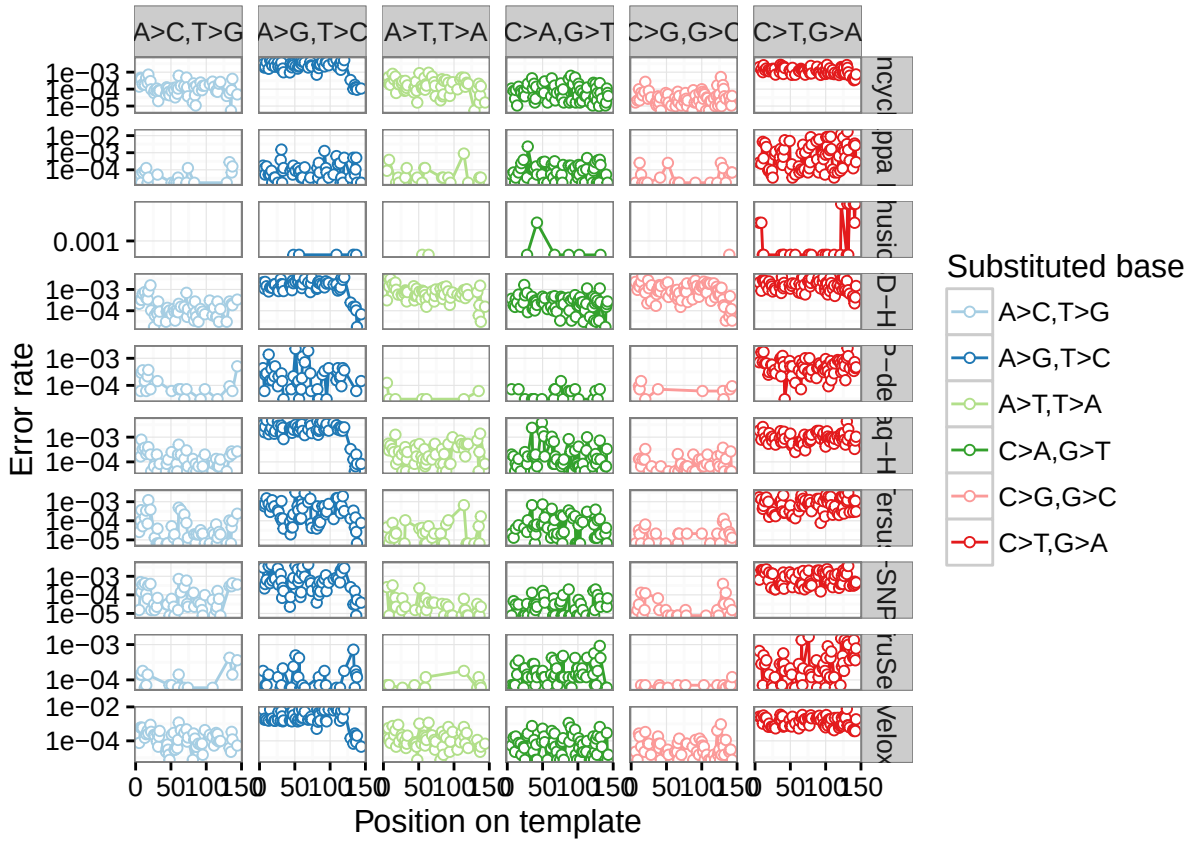
```
## Warning: Removed 9 rows containing missing values (geom_smooth).
```



Error distribution by position

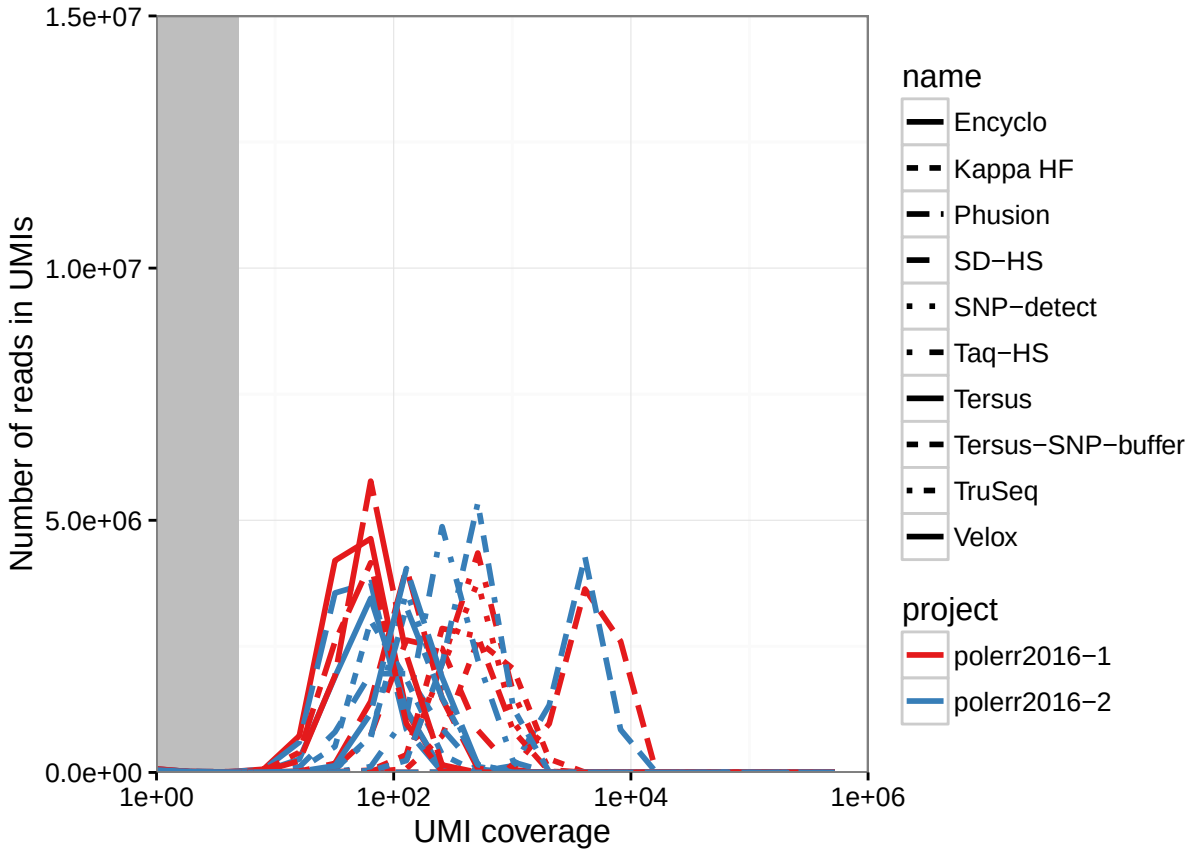
```
df.pos <- ddply(df, .(name, mutation.pos, mutation.from, mutation.signature.rep),
                summarize, count = sum(count.major), freq = sum(count.major) / coverage[1])

ggplot(df.pos, aes(x = mutation.pos, y = freq, color = mutation.signature.rep)) +
  geom_line() + geom_point(shape=21, fill="white") +
  scale_color_brewer("Substituted base", palette = "Paired") +
  xlab("Position on template") + scale_y_log10("Error rate") +
  facet_grid(name~mutation.signature.rep, scales="free_y") +
  theme_bw()
```

```
## geom_path: Each group consists of only one observation. Do you need to
## adjust the group aesthetic?
```



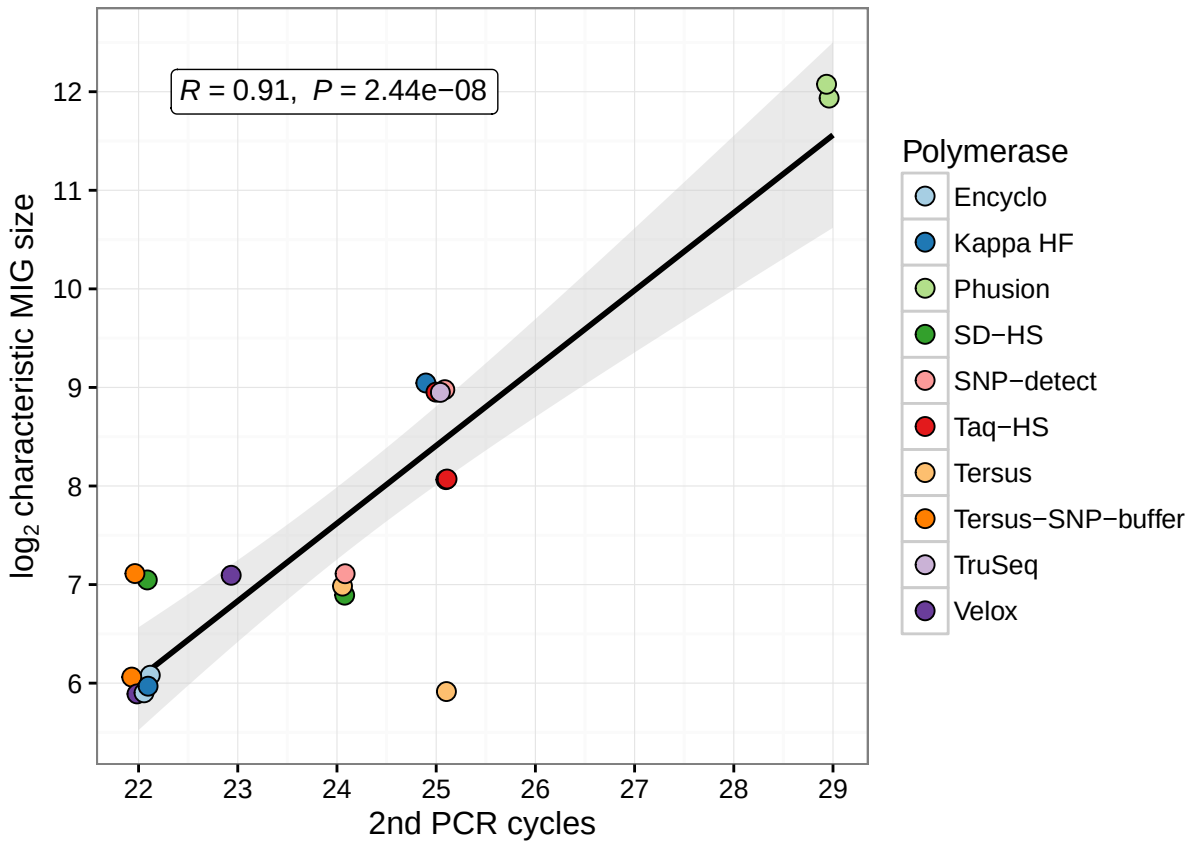
UMI coverage histogram

```
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=characte

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(1,1e6), expand=c(0,0)) +
  scale_y_continuous("Number of reads in UMIs", expand=c(0,0), limits=c(0,1.5e7)) +
  scale_color_brewer(palette = "Set1") +
  theme_bw()
```



Coverage and PCR cycles

```
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 = 12, label = lbl), hjust=-0.1, parse = TRUE)+
stat_smooth(data=df.o, aes(cycles_2, peak), method=lm, color="black", fill="grey80") +
geom_jitter(data=df.o, aes(cycles_2, peak, fill=name), size=3, width=0.3, height=0.3, color="black", sha
scale_x_continuous(name="2nd PCR cycles", breaks=10:30) +
scale_y_continuous(expression('log'[2]~'characteristic MIG size'), breaks=2:20) +
scale_fill_brewer(name  ="Polymerase", palette = "Paired") +
theme_bw()
```


R = 0.91, P = 2.44e−08
log2 characteristic MIG size
2nd PCR cycles
12
11
10
9
8
7
6
22
23
24
25
26
27
28
29
Polymerase
Encyclo
Kappa HF
Phusion
SD−HS
SNP−detect
Taq−HS
Tersus
Tersus−SNP−buffer
TruSeq
Velox