Benchmark of error rate inference from UMI-tagged data and PCR error model

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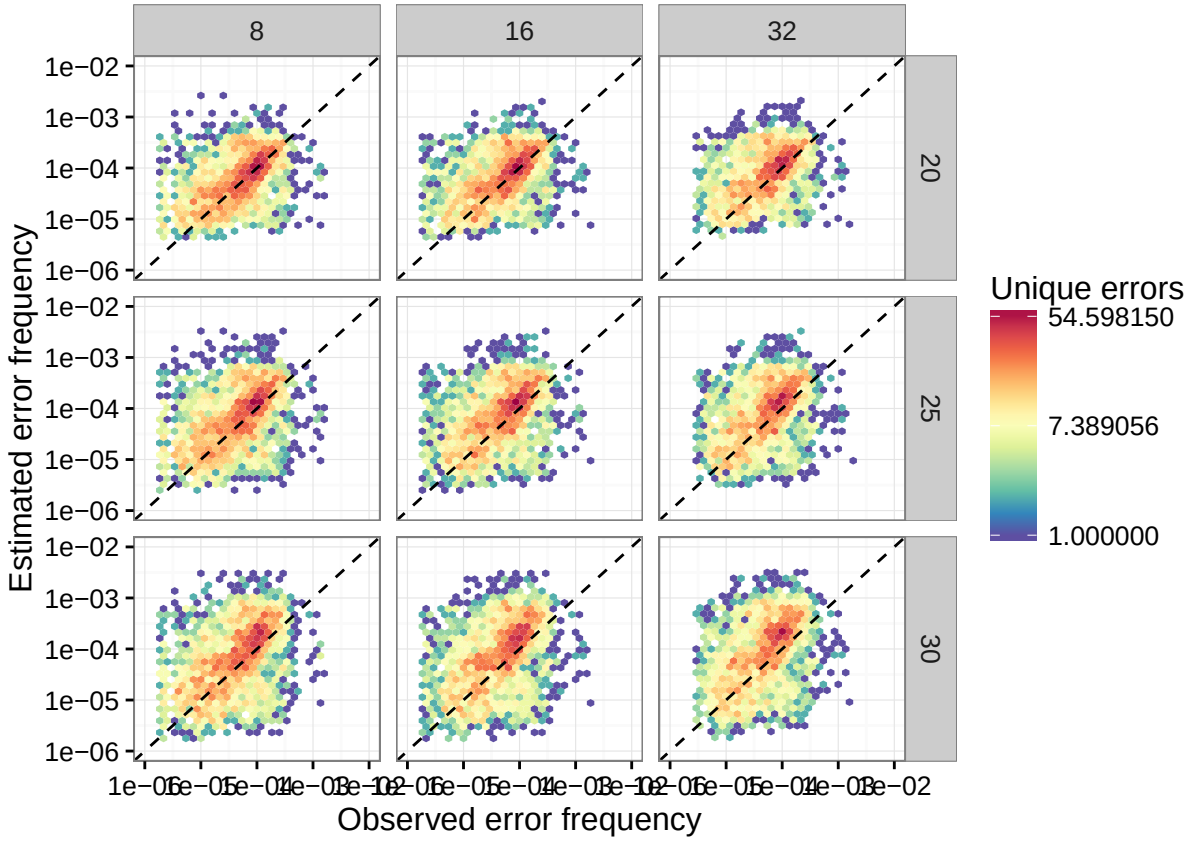
Load data from 2 independent experiments with 10 different PCR assays.

```
library(plyr)
library(ggplot2)
library(RColorBrewer)

df <- data.frame()

for (q in c(20, 25, 30)) {
  for (m in c(8, 16, 32)) {
    for (proj in c("73", "82")) {
      for (sample in c("encyclo", "kappa-hf-taq", "phusion", "sd-hs", "snp-detect",
                       "taq-hs", "tersus", "tersus-snp-buff", "truseq", "velox")) {
        .df <- read.table(
            paste("data",
              paste(paste(q, m, paste("polerr", proj, sep=""), sep="_"), sample, "variant.caller.txt", s
                    sep ="/"),
            header=T, sep="\t", stringsAsFactors = F)
        .df$q <- q
        .df$m <- m
        .df$proj <- proj
        .df$sample <- sample
        df <- rbind(df, .df)
      }
    }
  }
}

df <- subset(df, count > 0 &
               !grepl("D", mutation) & !grepl("I", mutation) & global.est == 0 &
               coverage > 0)

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

Model estimates for error rate:

```
rf <- colorRampPalette(rev(brewer.pal(11, 'Spectral')))
r <- rf(32)

ggplot(df, aes(x=freq, y=error.rate)) +
  stat_binhex() +
  geom_abline(intercept = 0, slope=1, linetype="dashed") +
```

```
  scale_x_log10("Observed error frequency", limits=c(1e-6, 1e-2),
                breaks=c(1e-6, 1e-5, 1e-4, 1e-3, 1e-2)) +
  scale_y_log10("Estimated error frequency", limits=c(1e-6, 1e-2),
                breaks=c(1e-6, 1e-5, 1e-4, 1e-3, 1e-2)) +
  facet_grid(q~m) +
  scale_fill_gradientn("Unique errors", colors=r, trans="log") +
  theme_bw()
```




```
a <- aov(log10(freq) ~ I(log10(error.rate)) + q + m + proj, df)
summary(a)
```

```
##                          Df Sum Sq Mean Sq  F value   Pr(>F)
## I(log10(error.rate))      1   1435  1434.5 5769.797  < 2e-16 ***
## q                         1      9     9.4   37.988 7.21e-10 ***
## m                         1      6     6.3   25.189 5.23e-07 ***
## proj                      1      0     0.2    0.776    0.378
## Residuals             30074   7477     0.2
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
for (mm in unique(df$m)) {
  for (qq in unique(df$q)) {
    print(paste("m =", mm , "q =", qq, with(subset(df, q == qq & m == mm), cor(freq, error.rate, method
  }
}
```

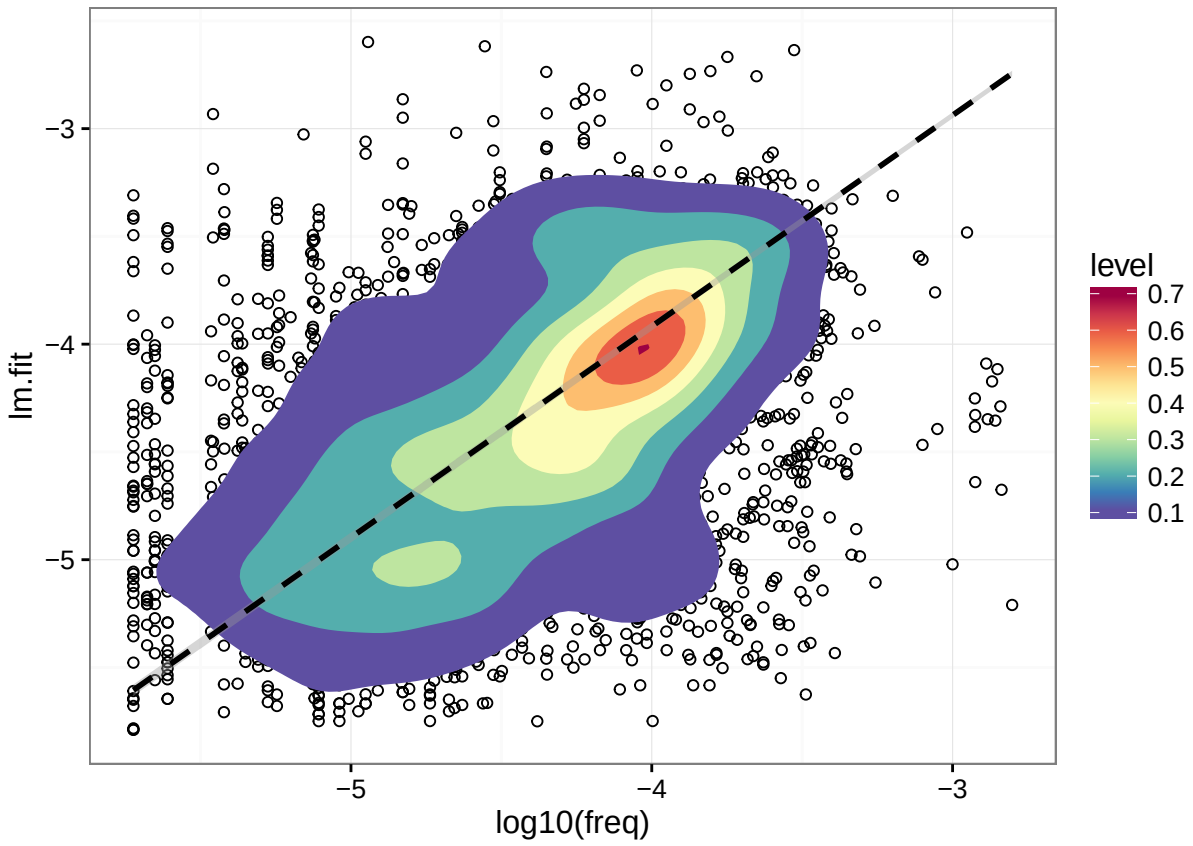
```
## [1] "m = 8 q = 20 0.439187907278551"
## [1] "m = 8 q = 25 0.440171986104134"
```

```
## [1] "m = 8 q = 30 0.437514996064882"
## [1] "m = 16 q = 20 0.419952825360284"
## [1] "m = 16 q = 25 0.416393783658744"
## [1] "m = 16 q = 30 0.420338819999655"
## [1] "m = 32 q = 20 0.38017513895925"
## [1] "m = 32 q = 25 0.399270957195644"
## [1] "m = 32 q = 30 0.40434020987666"
```

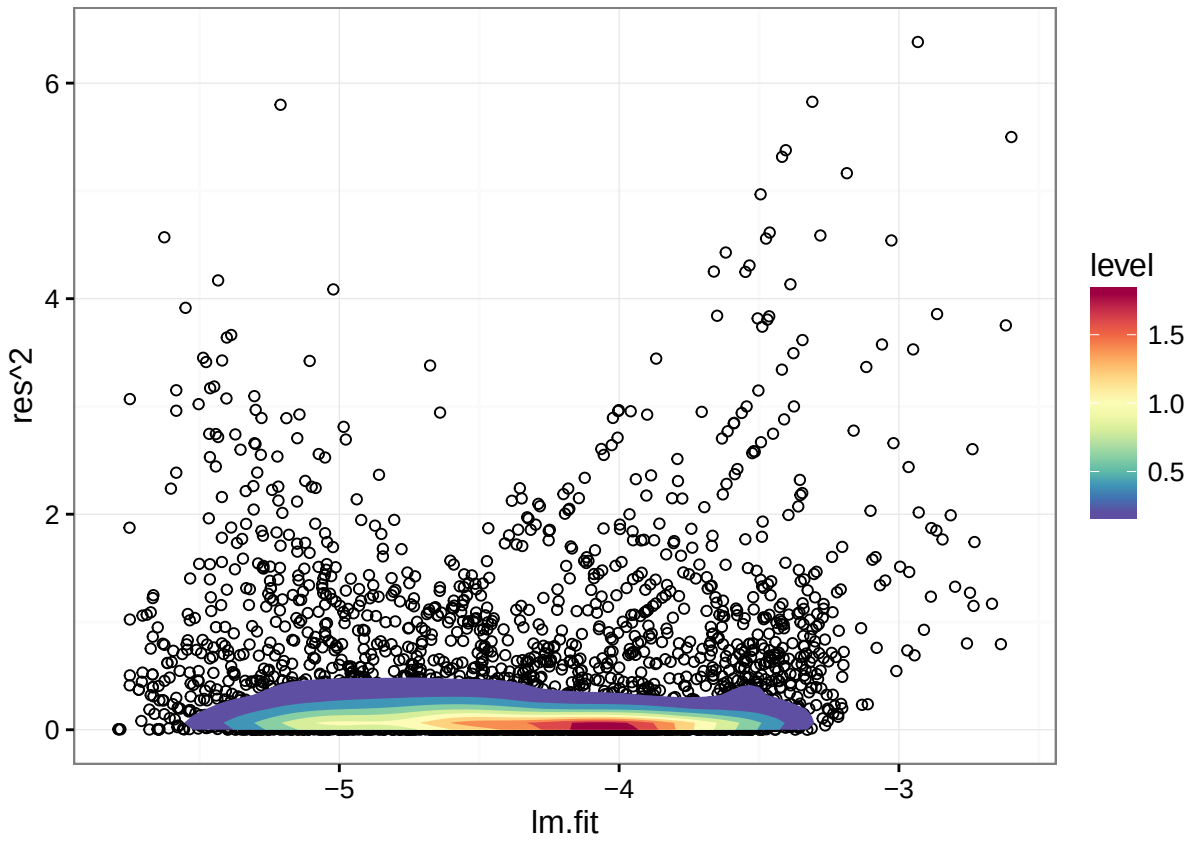
```
df.1 <- subset(df, q == 25 & m == 8)

fit <- lm(log10(freq) ~ I(log10(error.rate)) - 1, data = df.1)
summary(fit)
```

```
##
## Call:
## lm(formula = log10(freq) ~ I(log10(error.rate)) - 1, data = df.1)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -2.52623 -0.39962 -0.04251  0.30744  2.40817
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## I(log10(error.rate)) 1.034687   0.002479   417.4   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.6409 on 3666 degrees of freedom
## Multiple R-squared:  0.9794, Adjusted R-squared:  0.9794
## F-statistic: 1.742e+05 on 1 and 3666 DF,  p-value: < 2.2e-16
```

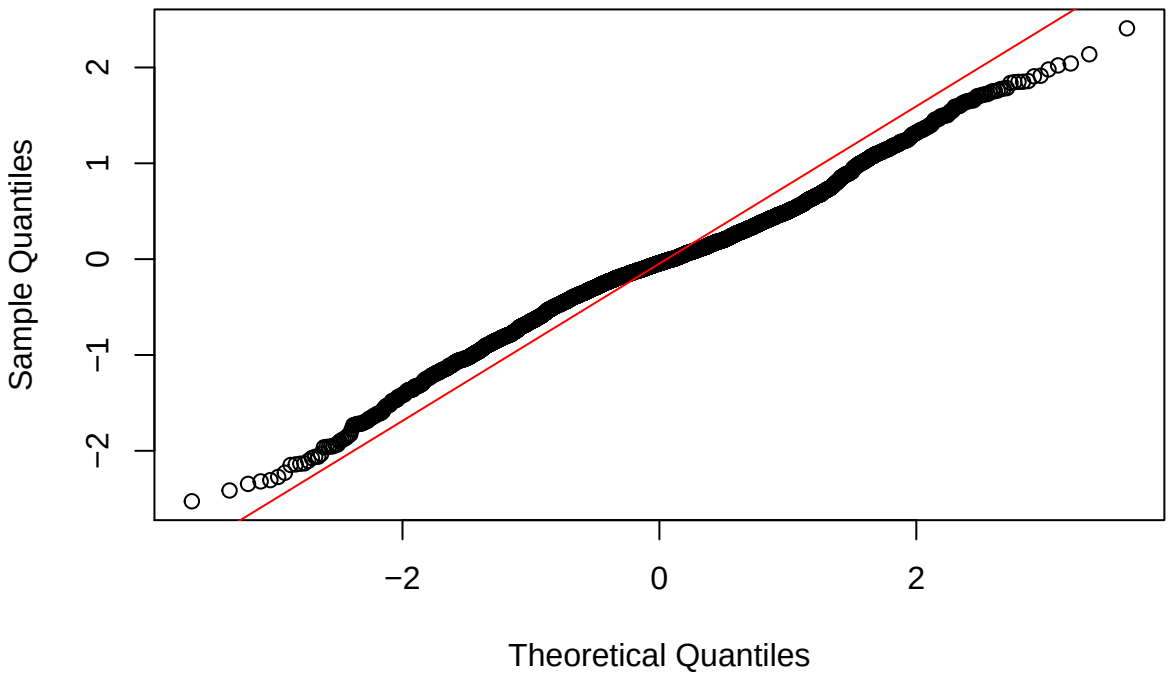
```
df.1$lm.fit <- fitted.values(fit)
df.1$res <- residuals(fit)

ggplot(df.1, aes(x=log10(freq), y=lm.fit)) +
  geom_point(shape=21) +
  stat_density2d(aes(fill=..level..), geom="polygon") +
  geom_smooth(method="lm", formula=y ~ x - 1, linetype="dashed", color="black") +
  scale_fill_gradientn(colors=r) + theme_bw()
```




```
# No correlation between residual sd and MBEM error rate

ggplot(df.1, aes(x=lm.fit, y=res^2)) +
  geom_point(shape=21) +
  stat_density2d(aes(fill=..level..), geom="polygon") +
  scale_fill_gradientn(colors=r) + theme_bw()
```




```
# Residuals are distributed normally

mdl.res.sd <- sd(df.1$res)

qqnorm(df.1$res)
qqline(df.1$res, distribution = function(p) qnorm(p, mean = 0, sd = mdl.res.sd),
       col = 2)
```




```
shapiro.test(df.1$res)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  df.1$res
## W = 0.98917, p-value = 3.907e-16
```

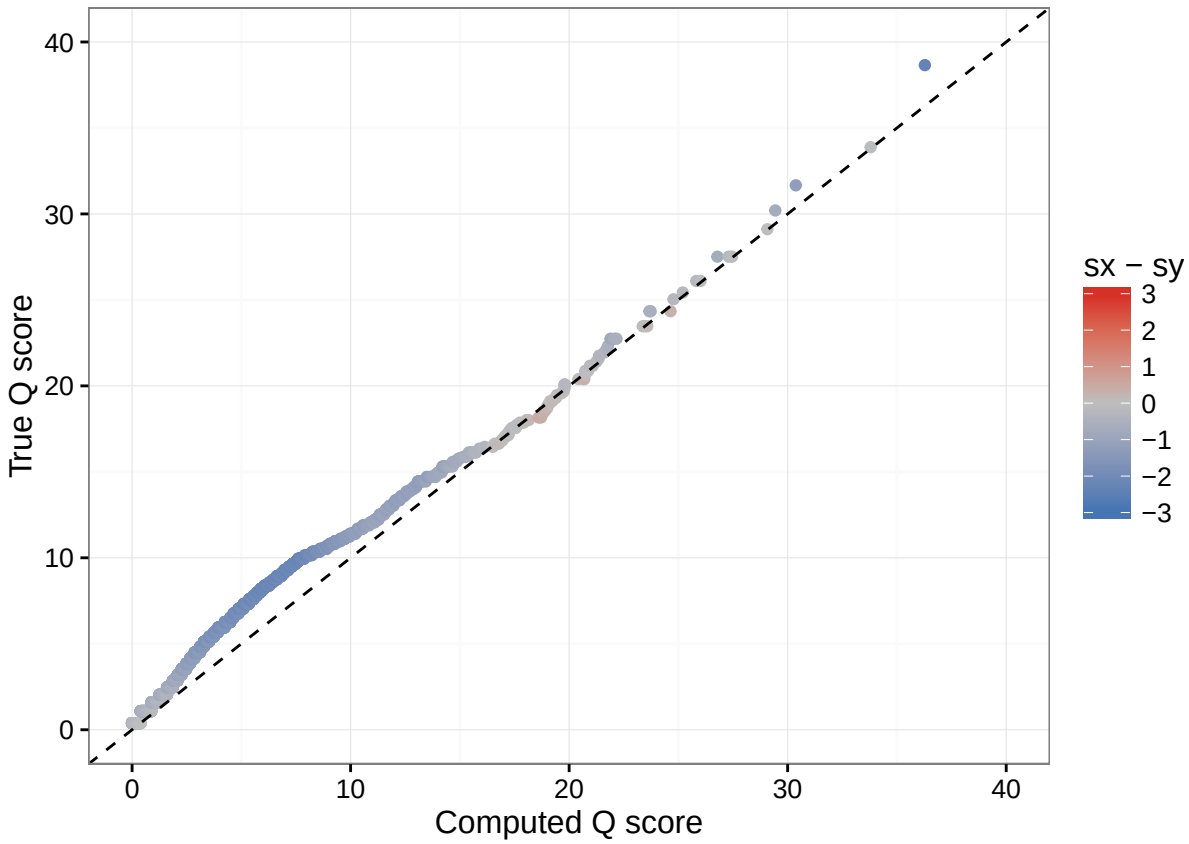
Computing error rate statistics:

```
#
df.1 <- subset(df, q == 25 & m == 8)

#lambda.m <- 5.2505409 + 0.3545431 * round(df.1$error.rate * df.1$coverage)
#lambda.var <- 35.089371 + 3.886613 * round(df.1$error.rate * df.1$coverage)

#df.1$alpha <- 1 / (exp(mdl.res.sd^2) - 1)
#df.1$beta <- df.1$alpha / exp(mdl.res.sd^2/2) / df.1$error.rate / df.1$coverage

#df.1$phi <- df.1$minor.count.local * df.1$read.fraction.in.minors
#n <- 20
#lambda <- 0.8
#df.1$alpha <- df.1$phi * (1 + lambda^2) / (1 + lambda)^2 / (df.1$phi / n / lambda + 1)
#df.1$beta <- (1 + lambda^2) / (1 + lambda)^2 / (df.1$phi / n / lambda + 1)

er.m <- df.1$error.rate * exp(mdl.res.sd^2 / 2)
er.v <- df.1$error.rate ^ 2 * exp(mdl.res.sd^2) * (exp(mdl.res.sd^2) - 1)

df.1$alpha <- er.m * (er.m * (1 - er.m) / er.v - 1)
df.1$beta <- (1 - er.m) * (er.m * (1 - er.m) / er.v - 1)
```

```
# source("https://bioconductor.org/biocLite.R")
# biocLite("Biobase")
# install.packages("TailRank", repos="http://R-Forge.R-project.org")
library(TailRank)
```

```
## Loading required package: oompaBase
```

```
df.1$pval <- with(df.1, mapply(function(x, y, a, b, z)
                ifelse(x <= 5, 1.0 - pbb(x, N = y, u = a, v = b) + 0.5 * dbb(x, N = y, u = a, v = b),
                       1.0 - pnorm(log10(x / y), mean = log10(z), sd = mdl.res.sd)),
                count, coverage, alpha, beta, error.rate))
#df.1$pval <- with(df.1,
#                 1.0 - pnbinom(count, prob = beta / (1 + beta), size = alpha) +
#                 0.5 * dnbinom(count, prob = beta / (1 + beta), size = alpha))

#df.1$pval <- with(df.1,
#                 1.0 - pbinom(count, prob = error.rate, size = coverage) +
#                   0.5 * dbinom(count, prob = error.rate, size = coverage))

df.count.summary <- ddply(df.1, .(count), summarize, weight = length(count))

df.1$pval.true <- with(df.count.summary,
                     sapply(df.1$count,
                       function(x)
                         sum(ifelse(x > count, 0, ifelse(x == count, 0.5 * weight, weight)))) / sum(wei

sx <- sort(-10*log10(df.1$pval))
sy <- sort(-10*log10(df.1$pval.true))
lenx <- length(sx)
leny <- length(sy)
if (leny < lenx)sx <- approx(1L:lenx, sx, n = leny)$y
if (leny > lenx)sy <- approx(1L:leny, sy, n = lenx)$y

ggplot(data.frame(q=sx, q_true=sy), aes(x=q, y=q_true, color=sx-sy)) +
  geom_point() +
  geom_abline(intercept = 0, slope = 1, color = "black", linetype="dashed") +
  scale_x_continuous("Computed Q score", limits=c(0, 40)) +
  scale_y_continuous("True Q score", limits=c(0, 40)) +
  scale_color_gradient2(low = "#4575b4", mid="grey", high="#d73027", midpoint=0, limits=c(-3,3)) +
  theme_bw()
```


True Q score
Computed Q score
0
10
20
30
40
sx − sy
3
2
1
0
−1
−2
−3