

# Sequencing: GLM for gene expression

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```
suppressPackageStartupMessages(library(SummarizedExperiment))
se <- readRDS("data/seParathyroid.rds")
```

```
## extract data from one gene
y <- assays(se)$counts[5,]

## extract covariates for each sample
treatment <- colData(se)$treatment
table(treatment)
```

```
## treatment
## Control    DPN    OHT
##         7     8     8
```

```
time <- colData(se)$time
table(time)
```

```
## time
## 24h 48h
## 11 12
```

```
patient <- colData(se)$patient
table(patient)
```

```
## patient
## 1 2 3 4
## 6 6 6 5
```

```
table(patient, treatment, time)
```

```
## , , time = 24h
##
##      treatment
## patient Control DPN OHT
##      1      1   1   1
##      2      1   1   1
##      3      1   1   1
##      4      0   1   1
##
## , , time = 48h
##
##      treatment
## patient Control DPN OHT
##      1      1   1   1
##      2      1   1   1
##      3      1   1   1
##      4      1   1   1
```

```
boxplot((y/colSums(assays(se)$counts)) ~ interaction(treatment, time))
```

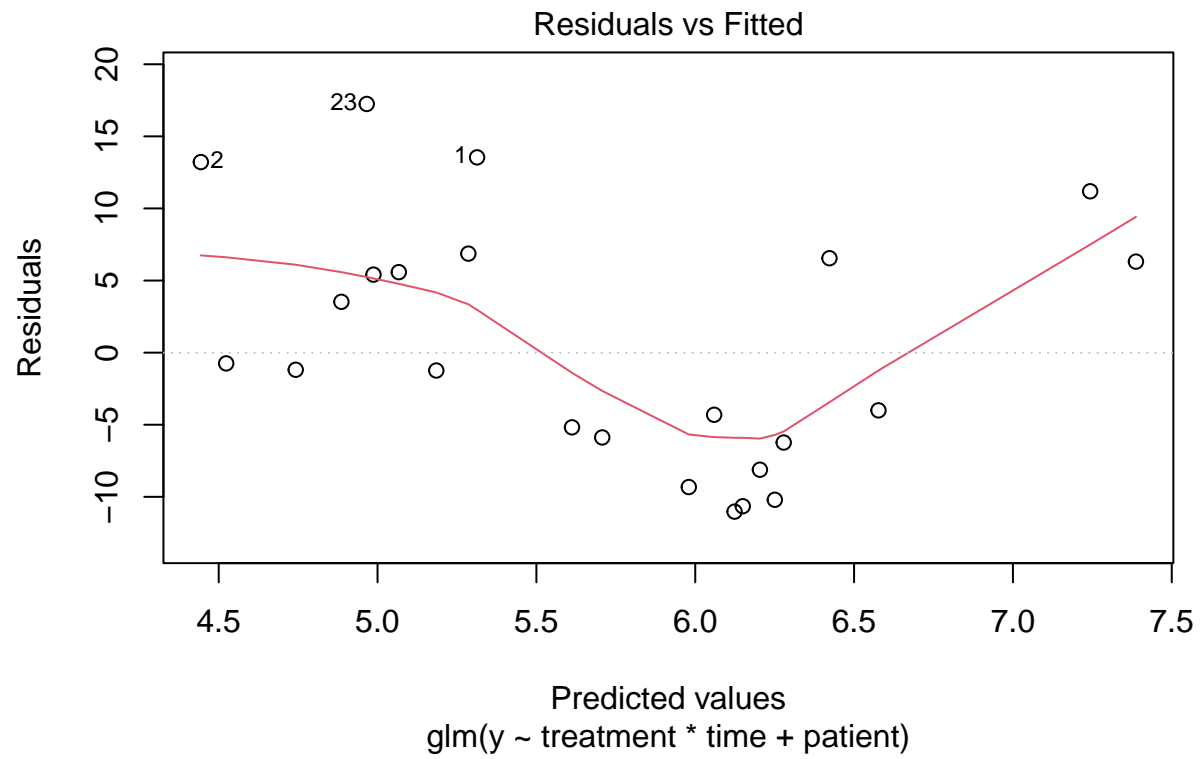


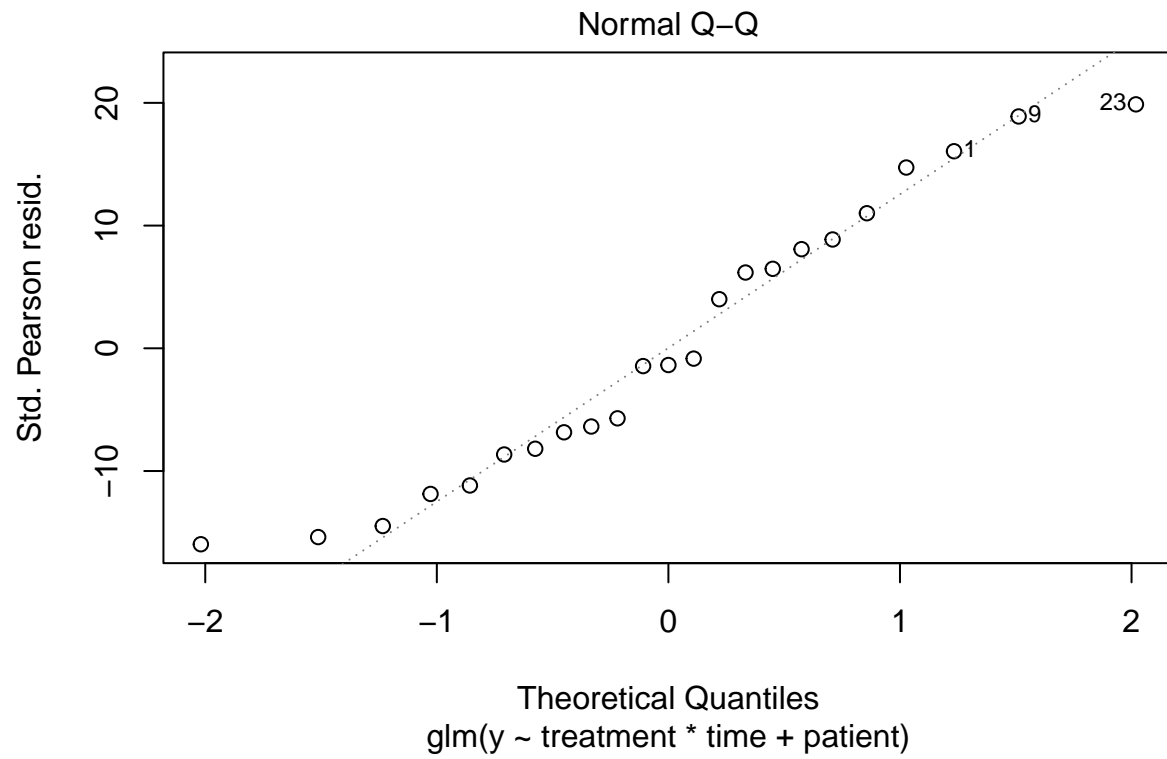
```
## dotplot for each treatment, matching patient samples
df <- data.frame(y=y,
                 treatment=treatment,
                 time=time,
                 patient=patient)
ggplot(df, aes(x=time, y=y)) +
  geom_point() +
  geom_line(aes(group = patient)) +
  facet_grid(.~treatment) +
  theme_classic()
```



## 1 Poisson GLM

```
m <- glm(y ~ treatment*time + patient,
         family = "poisson")
plot(m) # Extra-Poisson variation?
```









## 1.1 Check overdispersion

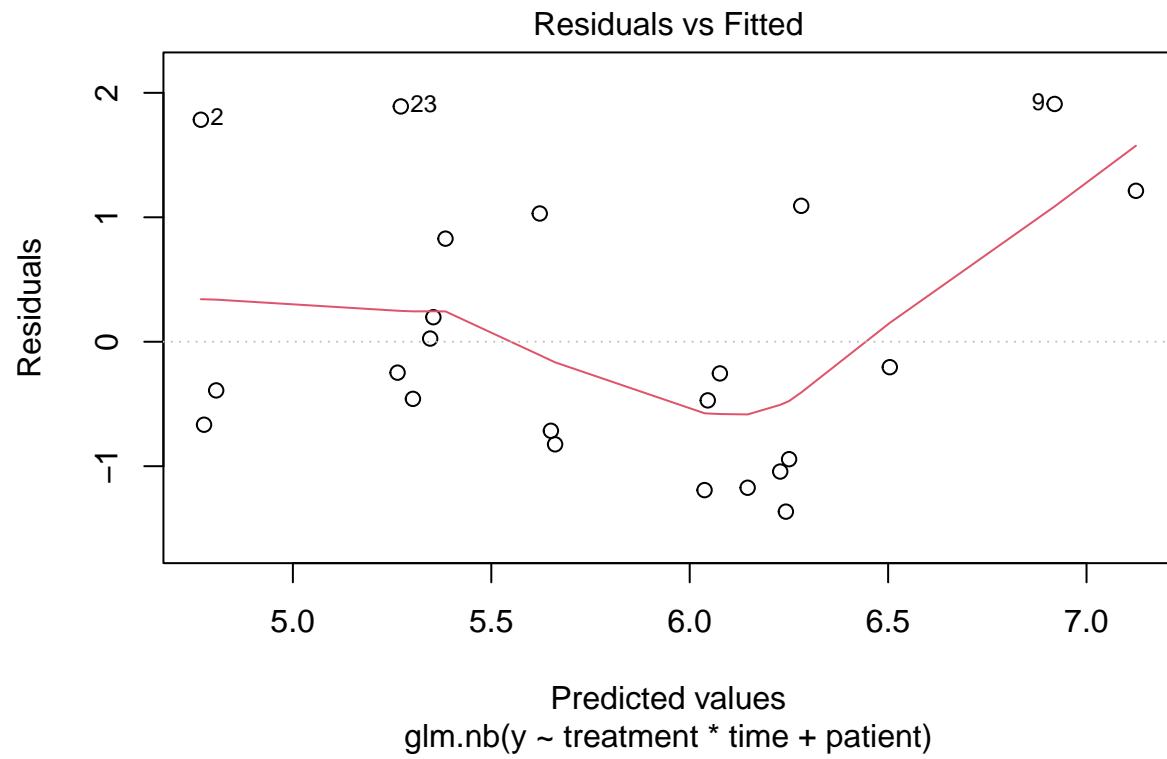
```
ePearson <- resid(m, type="pearson")
n <- length(y)
p <- length(coef(m))
sum(ePearson^2) / (n-p) # huge overdispersion.
```

```
## [1] 115.2631
```

## 2 Negative binomial model

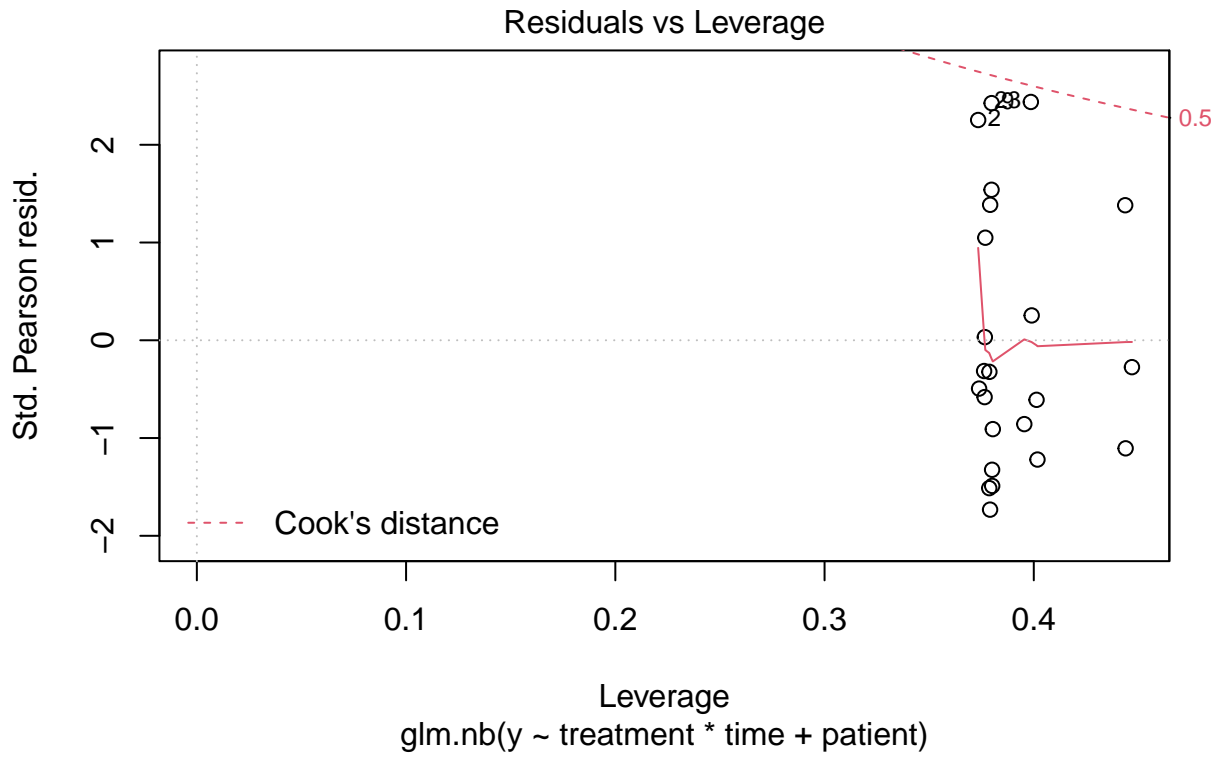
```
library(MASS)
mNB <- glm.nb(y ~ treatment*time + patient)
plot(mNB)
```











```
summary(mNB)
```

```
##
## Call:
## glm.nb(formula = y ~ treatment * time + patient, init.theta = 5.797944761,
##   link = log)
##
## Deviance Residuals:
##   Min       1Q   Median       3Q      Max
## -1.7692  -0.8726  -0.2641   0.8303   1.5579
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)    5.622089   0.279514  20.114 < 2e-16 ***
## treatmentDPN     0.415319   0.324036   1.282 0.199945
## treatmentOHT     0.620294   0.323896   1.915 0.055479 .
## time48h        -0.854023   0.325959  -2.620 0.008792 **
## patient2         0.882139   0.241916   3.646 0.000266 ***
## patient3         0.038663   0.242737   0.159 0.873450
## patient4         0.008082   0.258521   0.031 0.975059
## treatmentDPN:time48h 0.162526  0.440457   0.369 0.712132
## treatmentOHT:time48h -0.124510  0.440453  -0.283 0.777417
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

```
## (Dispersion parameter for Negative Binomial(5.7979) family taken to be 1)
##
##      Null deviance: 90.709  on 22  degrees of freedom
## Residual deviance: 23.560  on 14  degrees of freedom
## AIC: 308.63
##
## Number of Fisher Scoring iterations: 1
##
##
##              Theta:  5.80
##             Std. Err.:  1.69
##
## 2 x log-likelihood: -288.632
```

## 2.1 Statistical inference

We will test seven different contrasts.

```
L <- matrix(0, nrow = length(coef(mNB)), ncol = 7)
rownames(L) <- names(coef(mNB))
colnames(L) <- c("DPNvsCON24", "DPNvsCON48",
                 "OHTvsCON24", "OHTvsCON48",
                 "DPNvsCONInt", "OHTvsCONInt",
                 "OHTvsDPNInt")
# DPN vs control at 24h
L[2,"DPNvsCON24"] <- 1
# DPN vs control at 48h
L[c(2,8),"DPNvsCON48"] <- 1
# OHT vs control at 24h
L[3,"OHTvsCON24"] <- 1
# OHT vs control at 48h
L[c(3,9),"OHTvsCON48"] <- 1
# DPN control interaction
L[8,"DPNvsCONInt"] <- 1
# OHT control interaction
L[9,"OHTvsCONInt"] <- 1
# OHT DPN interaction
L[c(9,8),"OHTvsDPNInt"] <- c(1, -1)

L
```

	DPNvsCON24	DPNvsCON48	OHTvsCON24	OHTvsCON48	DPNvsCONInt
## (Intercept)	0	0	0	0	0
## treatmentDPN	1	1	0	0	0
## treatmentOHT	0	0	1	1	0
## time48h	0	0	0	0	0
## patient2	0	0	0	0	0
## patient3	0	0	0	0	0
## patient4	0	0	0	0	0
## treatmentDPN:time48h	0	1	0	0	1
## treatmentOHT:time48h	0	0	0	1	0
	OHTvsCONInt	OHTvsDPNInt			
## (Intercept)	0	0			

```
## treatmentDPN          0          0
## treatmentOHT          0          0
## time48h               0          0
## patient2              0          0
## patient3              0          0
## patient4              0          0
## treatmentDPN:time48h   0         -1
## treatmentOHT:time48h   1          1
```

### 2.1.1 Wald test

```
beta <- matrix(coef(mNB), ncol = 1)
waldStats <- c()
for(ll in 1:ncol(L)){
  curL <- L[,ll,drop=FALSE]
  curWald <- t(curL) %*% beta %*% solve(t(curL) %*% vcov(mNB) %*% curL) %*% t(beta) %*% curL
  waldStats[ll] <- curWald
}

waldStats
```

```
## [1] 1.64277658 3.75083592 3.66762230 2.75849551 0.13615681 0.07991173 0.46952521
```

```
pvalues <- 1-pchisq(waldStats, df=1)
pvalues
```

```
## [1] 0.19994494 0.05278111 0.05547928 0.09673917 0.71213195 0.77741707 0.49320567
```

### 2.1.2 Likelihood ratio test

Implementing these contrasts using a likelihood ratio test is possible, but is not trivial. It would require a reparameterization of our model using the contrasts of interest. In this reparameterization, one variable may correspond to one contrast. We may then compare a full to an alternative model, dropping this variable, using a likelihood ratio test. While it is important to know that this is possible, we will not implement the reparameterization ourselves as it is considered outside the scope of this course.

## 3 Residuals

### 3.1 Deviance residuals

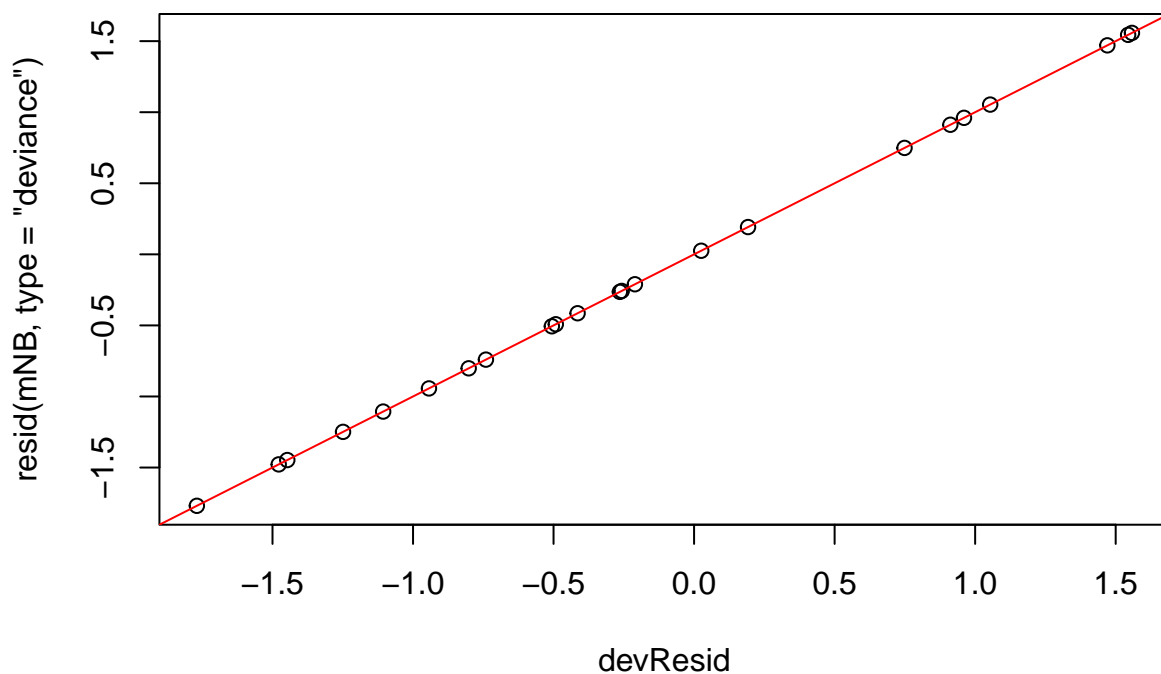
```
## residual deviance
sum(2*(dnbinom(x=y, mu=y, size=mNB$theta, log=TRUE) - dnbinom(x=y, mu=fitted(mNB), size=mNB$theta, log=
## [1] 23.5601
```

```
## deviance residual
devResid <- sign(y-fitted(mNB)) * sqrt(2*(dnbinom(x=y, mu=y, size=mNB$theta, log=TRUE) -
      dnbinom(x=y, mu=fitted(mNB), size=mNB$theta, log=TRUE)))

range(devResid - resid(mNB, type="deviance"))
```

```
## [1] -8.070651e-10  9.068435e-10
```

```
plot(devResid, resid(mNB, type="deviance")) ; abline(0,1, col="red")
```

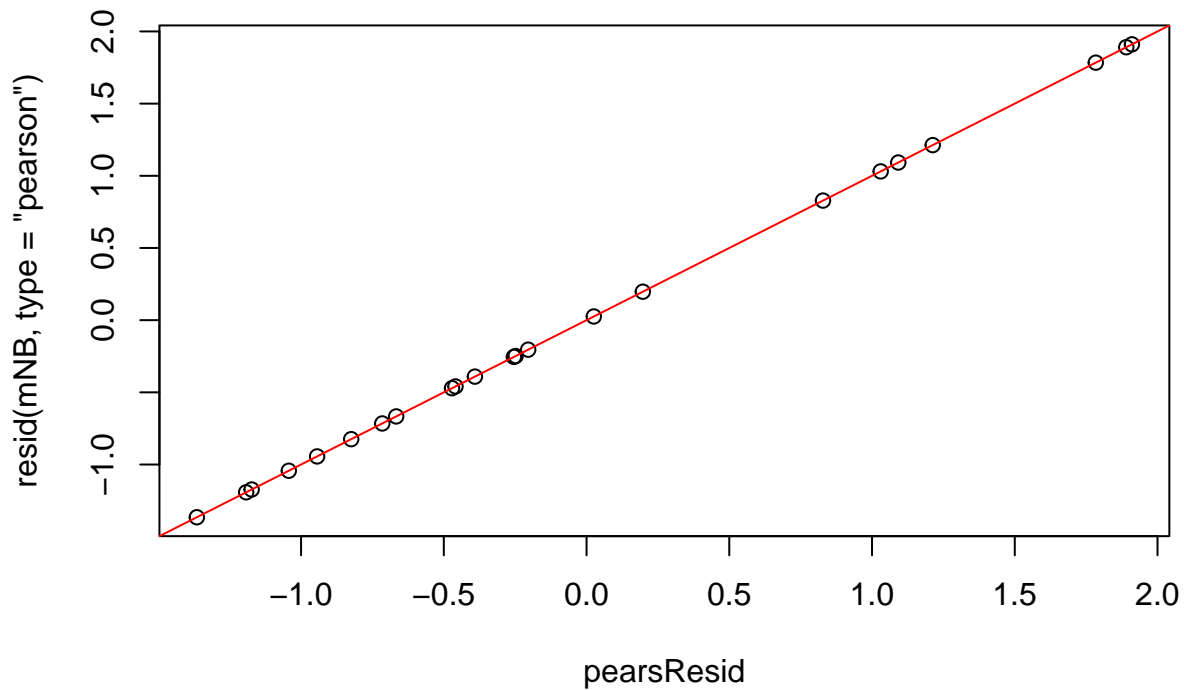


### 3.2 Pearson residuals

```
pearsResid <- (y - fitted(mNB)) / sqrt(fitted(mNB) + 1/mNB$theta * fitted(mNB)^2)
range(pearsResid - resid(mNB, type="pearson"))
```

```
## [1] -9.888568e-10  7.026788e-10
```

```
plot(x=pearsResid, y=resid(mNB, type="pearson")) ; abline(0,1, col="red")
```



### 3.3 Goodness-of-fit

```
X2 <- sum(pearsResid^2)
1-pchisq(X2, df=length(y) - length(coef(mNB)))
```

```
## [1] 0.04729423
```

## 4 Re-analysis upon basic normalization

A very simple normalization would use an offset to account for sequencing depth. Verify if our hypothesis test results remain upon using this basic normalization.

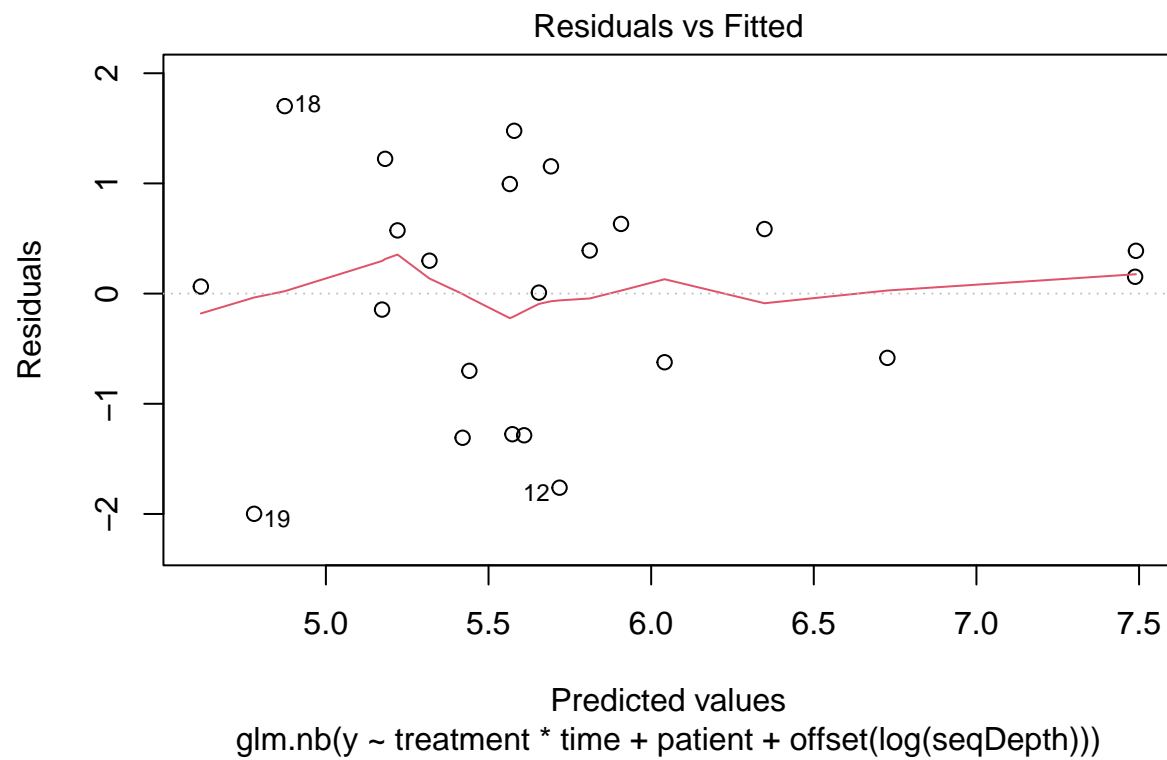
```
seqDepth <- colSums(assays(se)$counts)
```

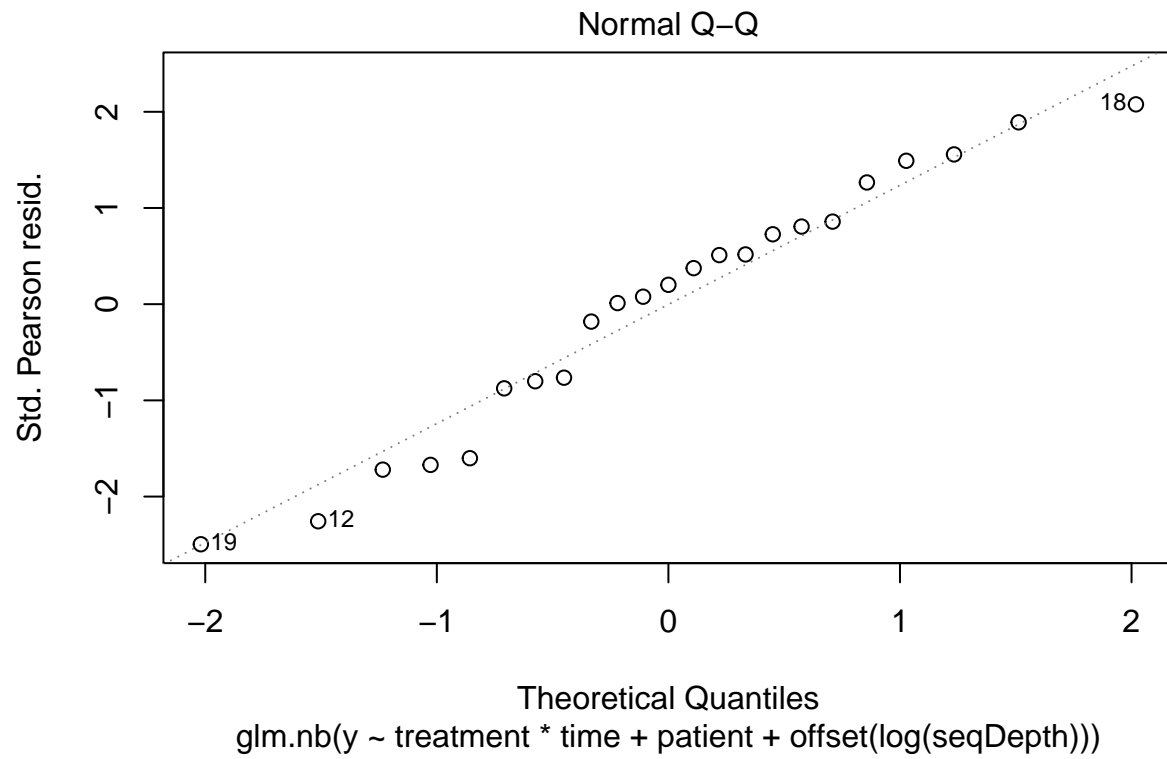
### 4.1 Statistical inference

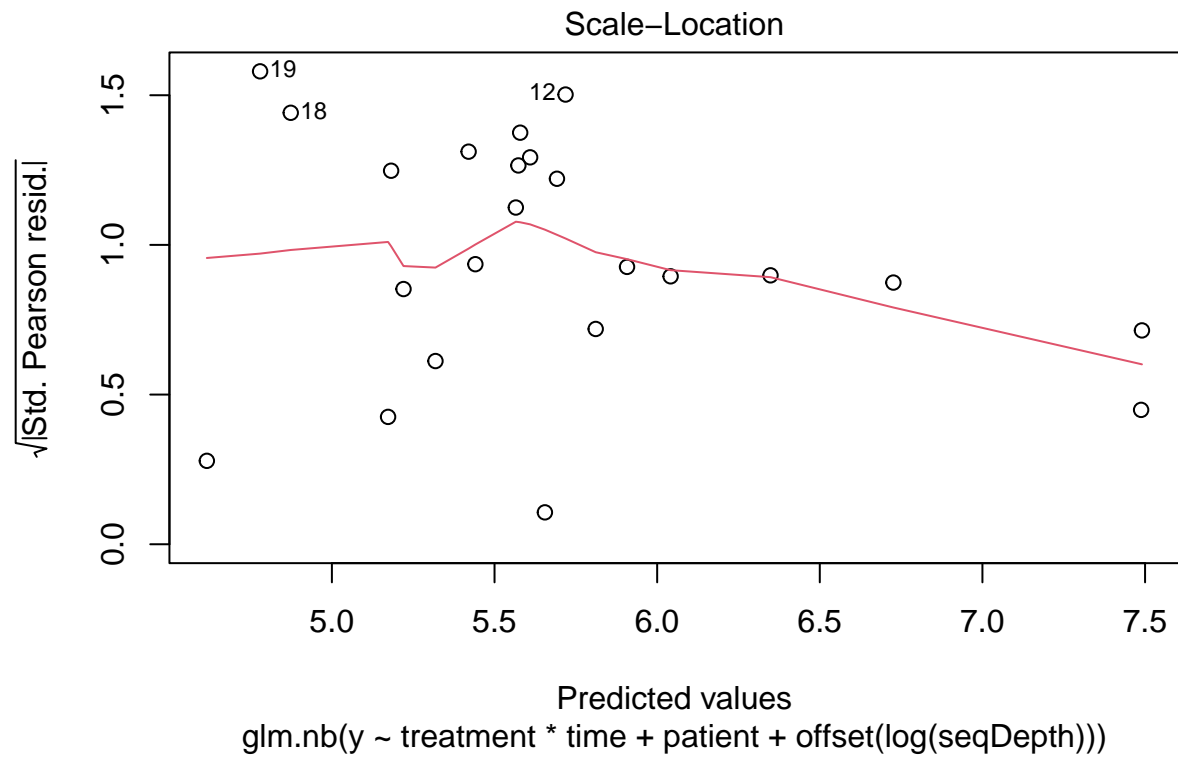
## 5 Negative binomial model, corrected for sequencing depth

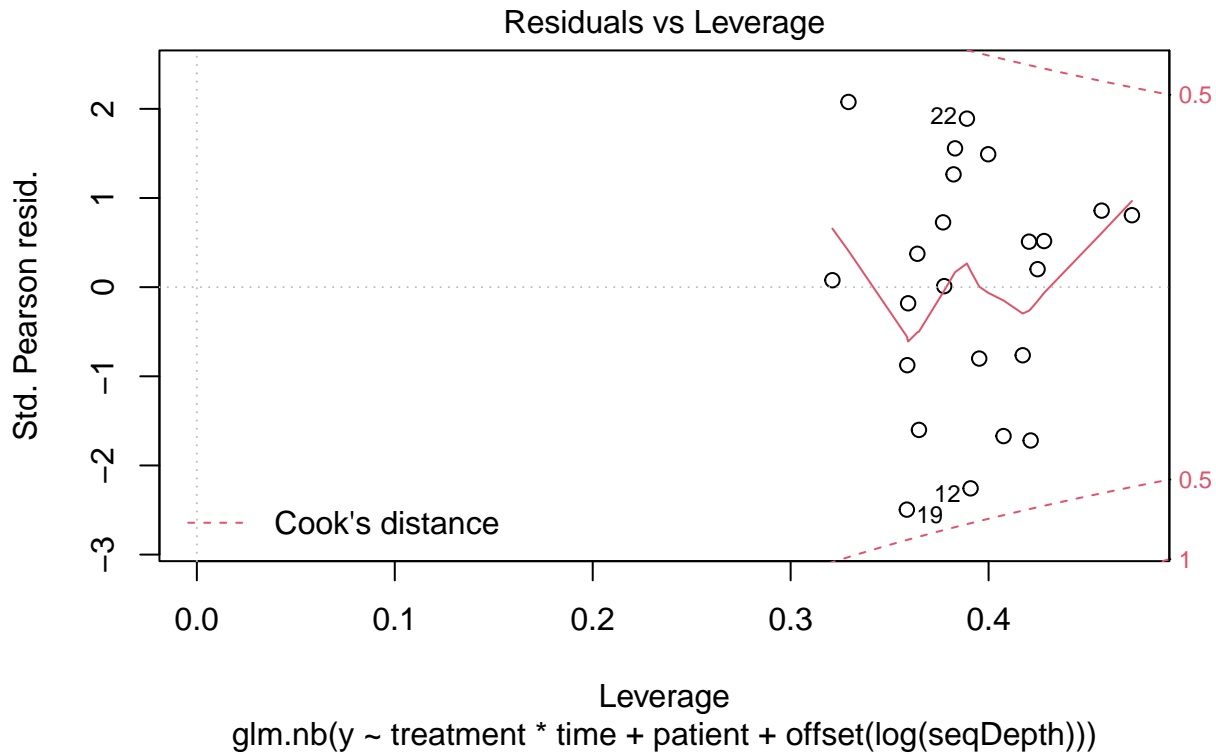


```
library(MASS)
mNBOffset <- glm.nb(y ~ treatment*time + patient +
  offset(log(seqDepth)))
plot(mNBOffset)
```









```
summary(mNBOffset)
```

```
##
## Call:
## glm.nb(formula = y ~ treatment * time + patient + offset(log(seqDepth)),
##       init.theta = 82.98036979, link = log)
##
## Deviance Residuals:
##      Min       1Q   Median       3Q      Max
## -2.1746  -0.6788   0.1520   0.5961   1.6033
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)    -10.11672    0.08217  -123.120  < 2e-16 ***
## treatmentDPN      0.09023    0.09418   0.958 0.338022
## treatmentOHT      0.13403    0.09362   1.432 0.152224
## time48h         -0.89874    0.10008  -8.980  < 2e-16 ***
## patient2         0.49680    0.07174   6.925 4.37e-12 ***
## patient3        -0.27195    0.07419  -3.666 0.000247 ***
## patient4        -0.06219    0.07888  -0.788 0.430428
## treatmentDPN:time48h  0.15540    0.13277   1.170 0.241820
## treatmentOHT:time48h  0.17758    0.13292   1.336 0.181560
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

```
## (Dispersion parameter for Negative Binomial(82.9804) family taken to be 1)
##
##      Null deviance: 422.284  on 22  degrees of freedom
## Residual deviance:  24.637  on 14  degrees of freedom
## AIC: 253.94
##
## Number of Fisher Scoring iterations: 1
##
##
##              Theta:  83.0
##             Std. Err.:  34.2
##
## 2 x log-likelihood: -233.939
```

## 5.1 Wald tests

```
betaOffset <- matrix(coef(mNBOffset), ncol = 1)
waldStatsOffset <- c()
for(ll in 1:ncol(L)){
  curL <- L[,ll,drop=FALSE]
  curWald <- t(curL) %*% betaOffset %*% solve(t(curL) %*% vcov(mNBOffset) %*% curL) %*% t(betaOffset) %
  waldStatsOffset[ll] <- curWald
}

waldStatsOffset

## [1]  0.91792044  6.84868664  2.04982086 10.79379449  1.36995308  1.78481023
## [7]  0.03194304

pvaluesOffset <- 1-pchisq(waldStatsOffset, df=1)
pvaluesOffset

## [1] 0.338021590 0.008870646 0.152224100 0.001018409 0.241819946 0.181559766
## [7] 0.858152733
```