

RNAScope-McKO-new-probe

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```
#analysis of MOR McKO validation using new RNAScope probe
library(tibble)
library(dplyr)

##
## Attaching package: 'dplyr'

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

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

library(stringr)
library(ggplot2)
library(ggpubr)
library(ggthemes)

#creating the data frame from values logged on google sheet
genotype <- c("WT", "WT", "WT", "WT", "McKO", "McKO", "McKO", "McKO")
sex <- c("F", "F", "M", "M", "F", "F", "M", "M")
number <- c(46, 47, 6, 77, 95, 98, 58, 92)
positive <- c(13, 17, 7, 13, 0, 1, 2, 4)
total <- c(16, 17, 7, 14, 1, 9, 6, 19)

data <- tibble(genotype, sex, number, positive, total)

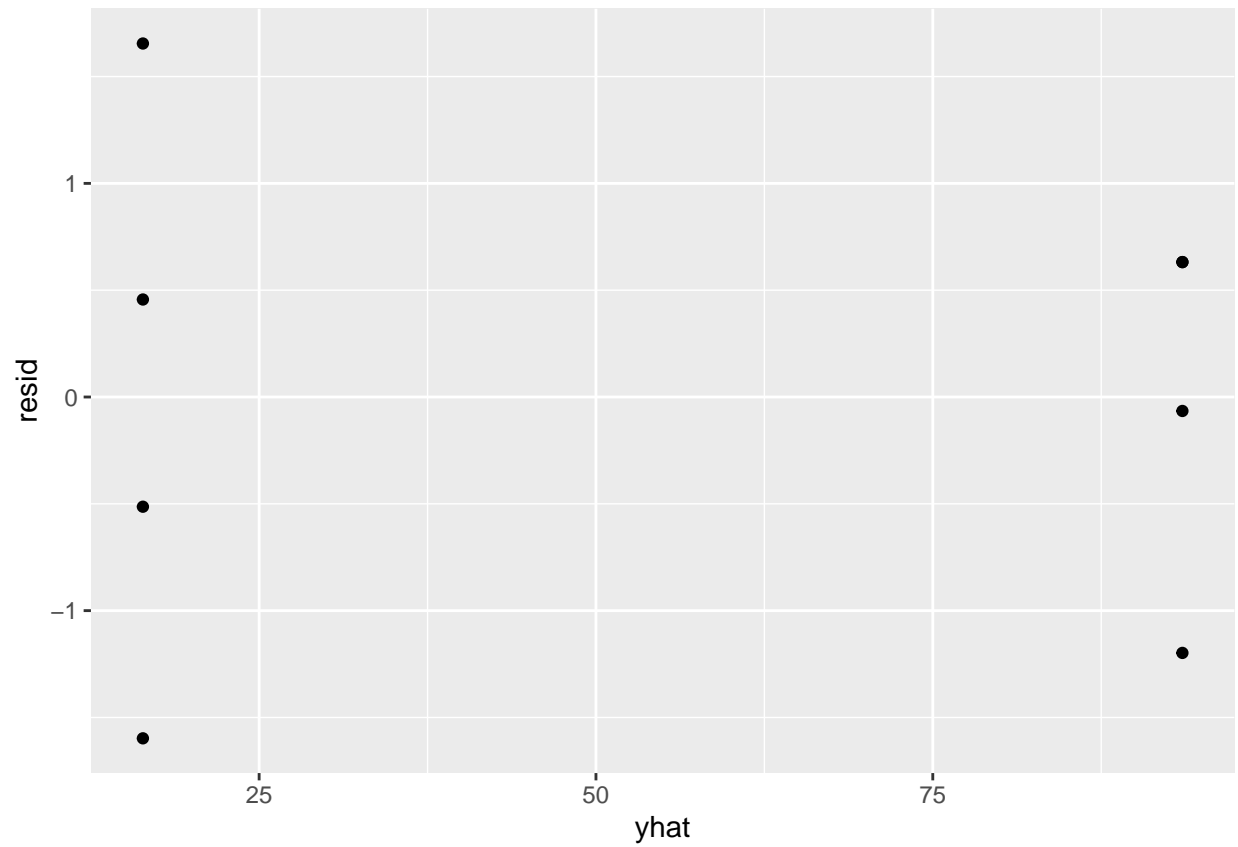
data <- mutate(data,
               number = str_c(number),
               percent = positive/total*100)

#assessing normality
shapiro.test(data$percent)

##
## Shapiro-Wilk normality test
##
## data:  data$percent
## W = 0.84703, p-value = 0.08888

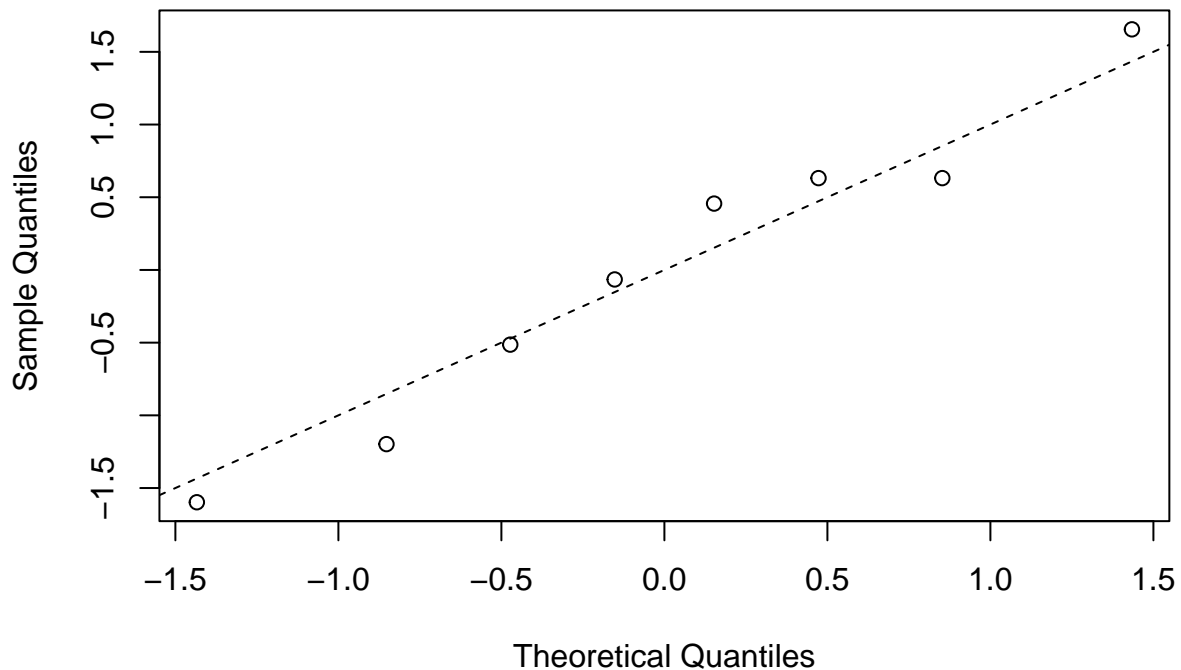
#creating linear model and checking assumptions
morlm <- lm(percent ~ genotype, data)
```

```
resid <- rstandard(morlm)
yhat <- morlm$fitted.values
ggplot(data, aes(x=yhat, y=resid)) +
  geom_point()
```



```
qqnorm(resid)
abline(0, 1, lty = 2)
```

Normal Q-Q Plot



```
#t-test to assess whether genotype predicts percent of positive MOR+ ipRGCs
#it does - statistically significant decrease in McKO versus WT
t.test(percent ~ genotype, data)
```

```
##
## Welch Two Sample t-test
##
## data: percent by genotype
## t = -9.2199, df = 5.0241, p-value = 0.0002457
## alternative hypothesis: true difference in means between group McKO and group WT is not equal to 0
## 95 percent confidence interval:
## -98.63223 -55.67280
## sample estimates:
## mean in group McKO mean in group WT
## 16.37427 93.52679
```

```
#checking for a sex difference, there is none
t.test(percent ~ sex, data)
```

```
##
## Welch Two Sample t-test
##
## data: percent by sex
## t = -0.42736, df = 5.7499, p-value = 0.6847
## alternative hypothesis: true difference in means between group F and group M is not equal to 0
## 95 percent confidence interval:
## -93.11462 65.67363
```

```

## sample estimates:
## mean in group F mean in group M
##      48.09028      61.81078

#checking the power
wts_only <- data %>% filter(genotype == "WT")
sd(wts_only$percent)

## [1] 8.850102

#sd is 8.85
mckos_only <- data %>% filter(genotype == "McKO")
#calculating delta (change in means between groups)
mean(wts_only$percent) - mean(mckos_only$percent)

## [1] 77.15252

#delta is 77.15
power.t.test(n=4, delta=77.15, sd=8.85, type = "one.sample", alternative="one.sided")

##
##      One-sample t test power calculation
##
##              n = 4
##            delta = 77.15
##              sd = 8.85
##          sig.level = 0.05
##              power = 1
##      alternative = one.sided

#power is 1

range(wts_only$percent)

## [1] 81.25 100.00

range(mckos_only$percent)

## [1] 0.00000 33.33333

#same t.test as above but using ggpubr to put p-value into geom_bracket
stat.test <- compare_means(percent ~ genotype, data, method = "t.test")

mcko_plot <- ggplot(data, aes(genotype, percent)) +
  geom_point(alpha=0.75) +
  stat_summary(fun = 'mean', geom="bar", aes(alpha=0.5)) +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1, alpha=0.5) +
  theme_few() +
  theme(legend.position = "none") +
  labs(y="% ipRGCs with MOR mRNA",
       title = "RNAscope labeling of MOR mRNA") +
  geom_bracket(
    aes(xmin = "WT", xmax = "McKO", label = signif(p, 2)),
    data = stat.test, y.position = 110) +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1)))

ggsave(filename="../figures/RNAscope_McKO_new_probe.png",

```

```
plot=mcko_plot, height=3, width=3)
```