

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)
```

```
#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
```

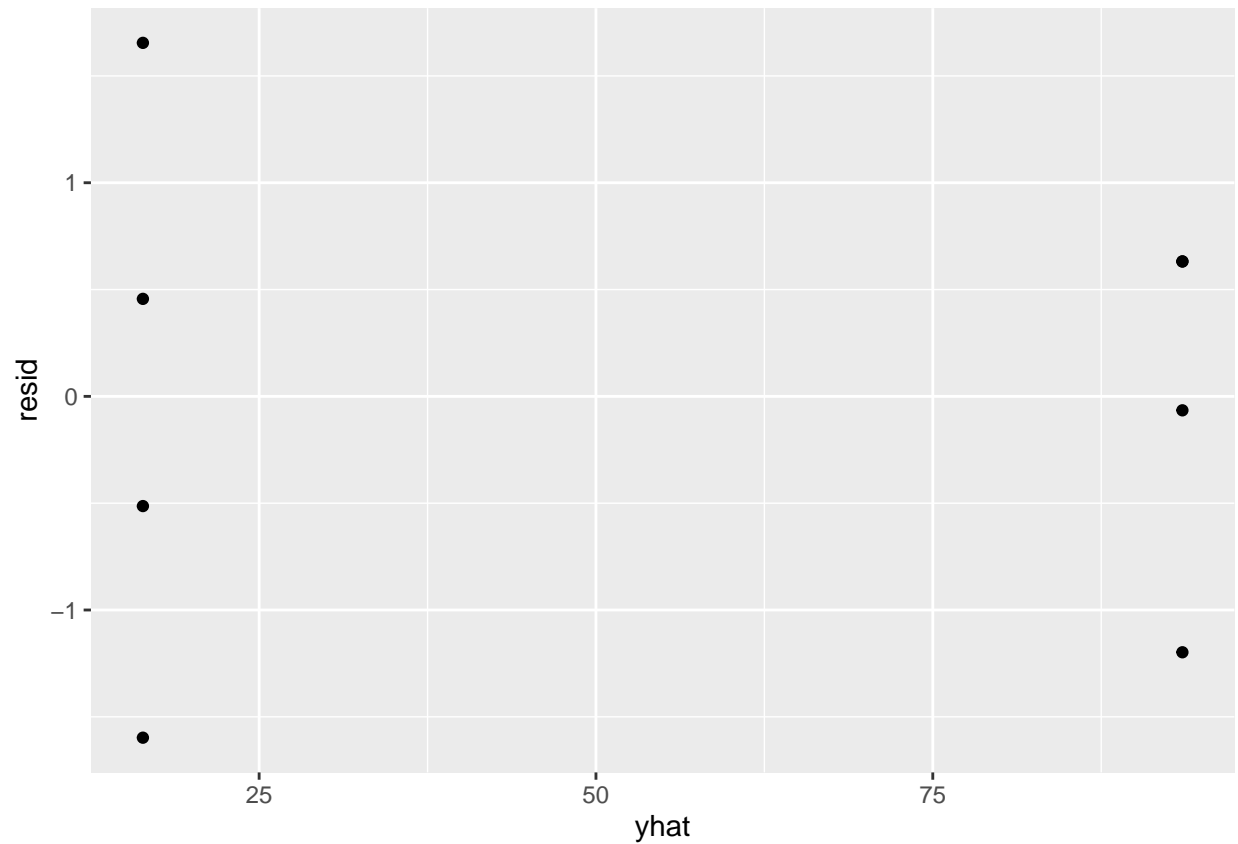
```
##
```

```
## data: data$percent
```

```
## W = 0.84703, p-value = 0.08888
```

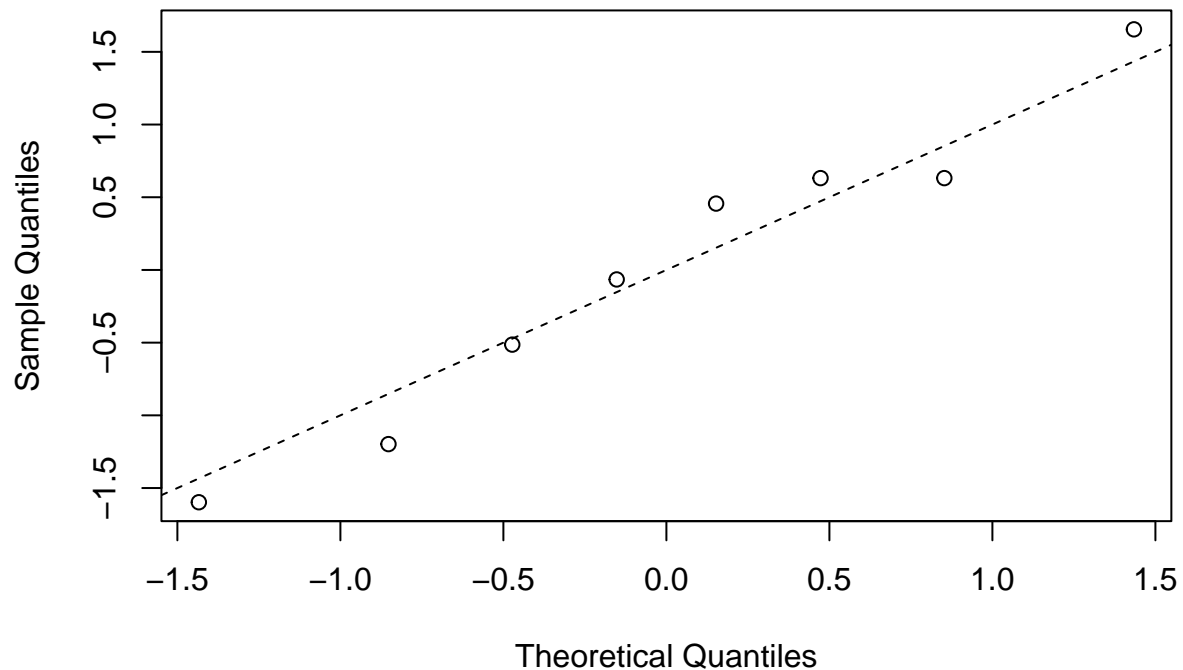
```
#creating linear model and checking assumptions
morlm <- lm(percent ~ genotype, data)

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 == "McK0")
#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
```