

Pycno Run 5

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Set Up

Load Libraries

Values Set

```
IPC_name = "Moa"  
Target_name = "Pycno"
```

LoD + LoQ Analysis - Pycno Plate Run 5

Load Data

```
sample_set_up <- read_excel(here("qPCR_raw_data","2025-12-23_161523_Pycno_5_v3.xls"), sheet="Sample Set")  
amplification_data<- read_excel(here("qPCR_raw_data","2025-12-23_161523_Pycno_5_v3.xls"), sheet="Amplification")  
results_data<- read_excel(here("qPCR_raw_data","2025-12-23_161523_Pycno_5_v3.xls"), sheet="Results", sheet_index=2)
```

Merge Tables

```
amplification_data %>%  
  left_join(sample_set_up) -> combined_qPCR_data
```

Now we are ready to start analyzing the data.

First thing we want to do is check to see how our internal positive control is working.

Moa Map

```

columns = seq(from =1, to=24, by=1)
columns_t= as.tibble(columns) %>% dplyr::rename("Column"=value)
rows = capitalize(letters[1:16])
rows_t= as.tibble(rows) %>% dplyr::rename("Row"=value)

cross_join(columns_t, rows_t) -> full_plate

combined_qPCR_data %>%
  filter(., `Target Name`==IPC_name) %>%
  mutate(., Row = str_sub(`Well Position`,start = 1L, end = 1L),
        Column= as.numeric(str_sub(`Well Position`,start = 2L, end = -1L ))) -> moa_samples

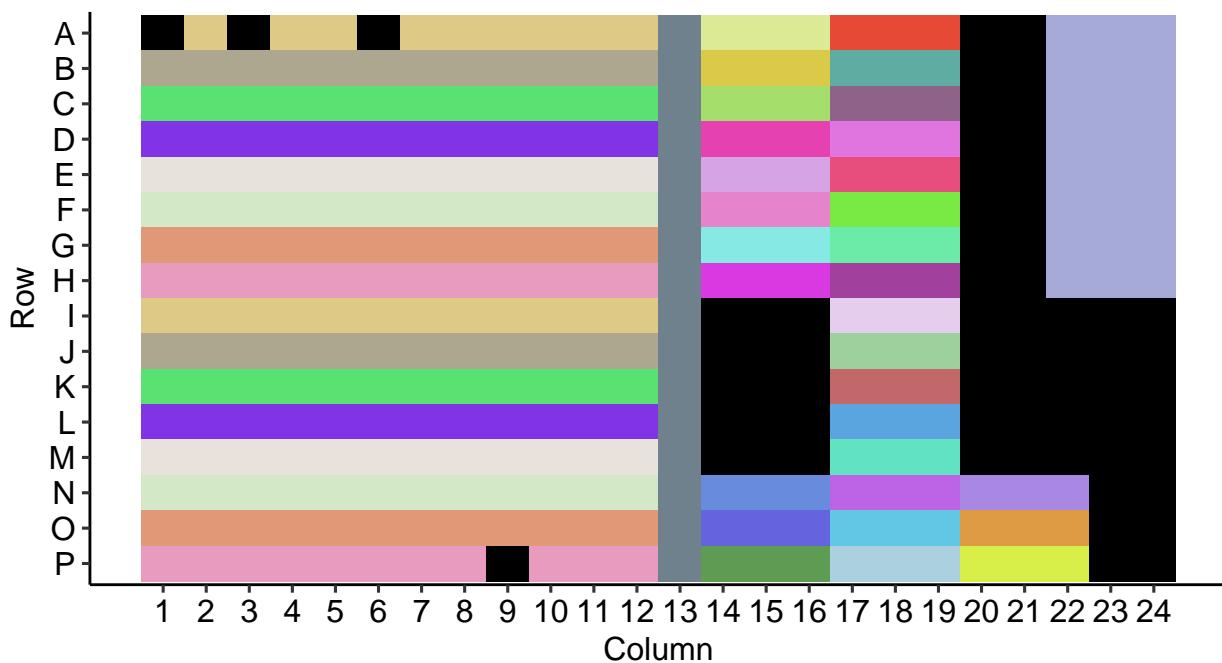
full_plate %>%
  left_join(moa_samples) -> moa_plate

palette <- distinctColorPalette(length(moa_plate$`Sample Name` %>% unique()))

moa_plate %>%
  mutate(Row = factor(Row, levels = rows)) %>%
  ggplot(aes(x=Column, y=fct_rev(Row), fill=`Sample Name` )) +geom_tile() +scale_x_continuous(breaks=co

```

Moa-IPC Standards & Samples



Pycno Map

```

combined_qPCR_data %>%
  filter(., `Target Name` == Target_name) %>%
  mutate(., Row = str_sub(`Well Position`, start = 1L, end = 1L),
        Column= as.numeric(str_sub(`Well Position`, start = 2L, end = -1L ))) -> pycno_samples

full_plate %>%
  left_join(pycno_samples) -> pycno_plate
  
```

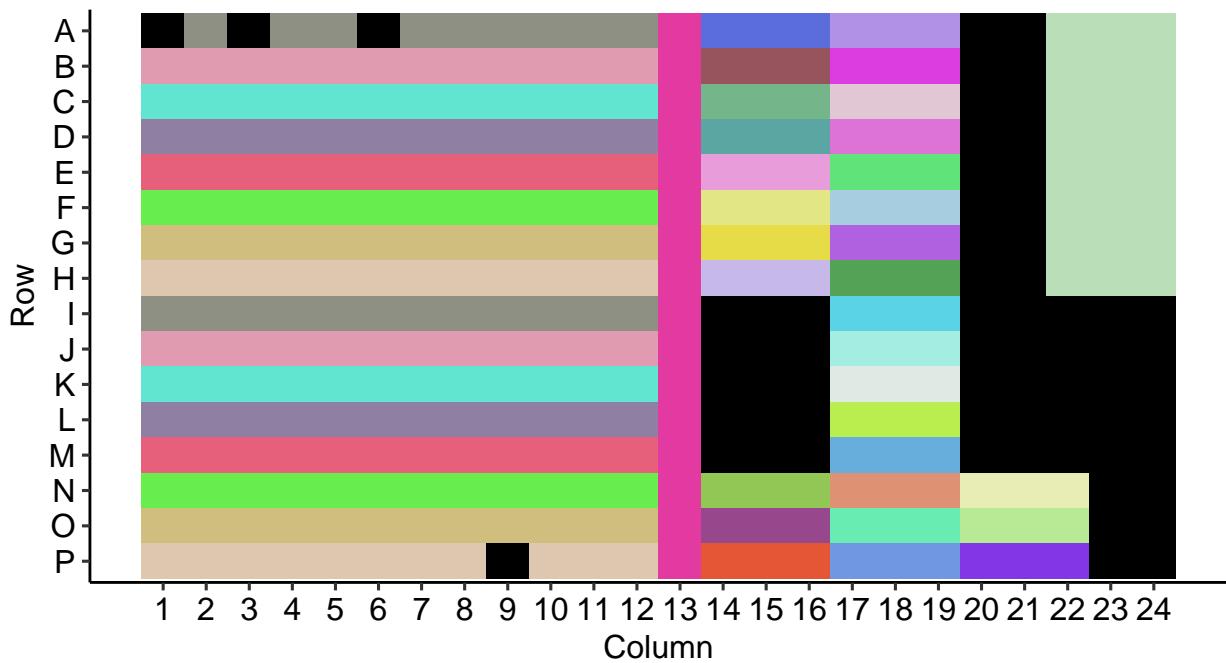
```

palette <- distinctColorPalette(length(pycno_plate$`Sample Name` %>% unique()))

pycno_plate %>%
  mutate(Row = factor(Row, levels = rows)) %>%
  ggplot(aes(x=Column, y=fct_rev(Row), fill=`Sample Name` )) +geom_tile() +scale_x_continuous(breaks=co)

```

Pycno Standards & Samples



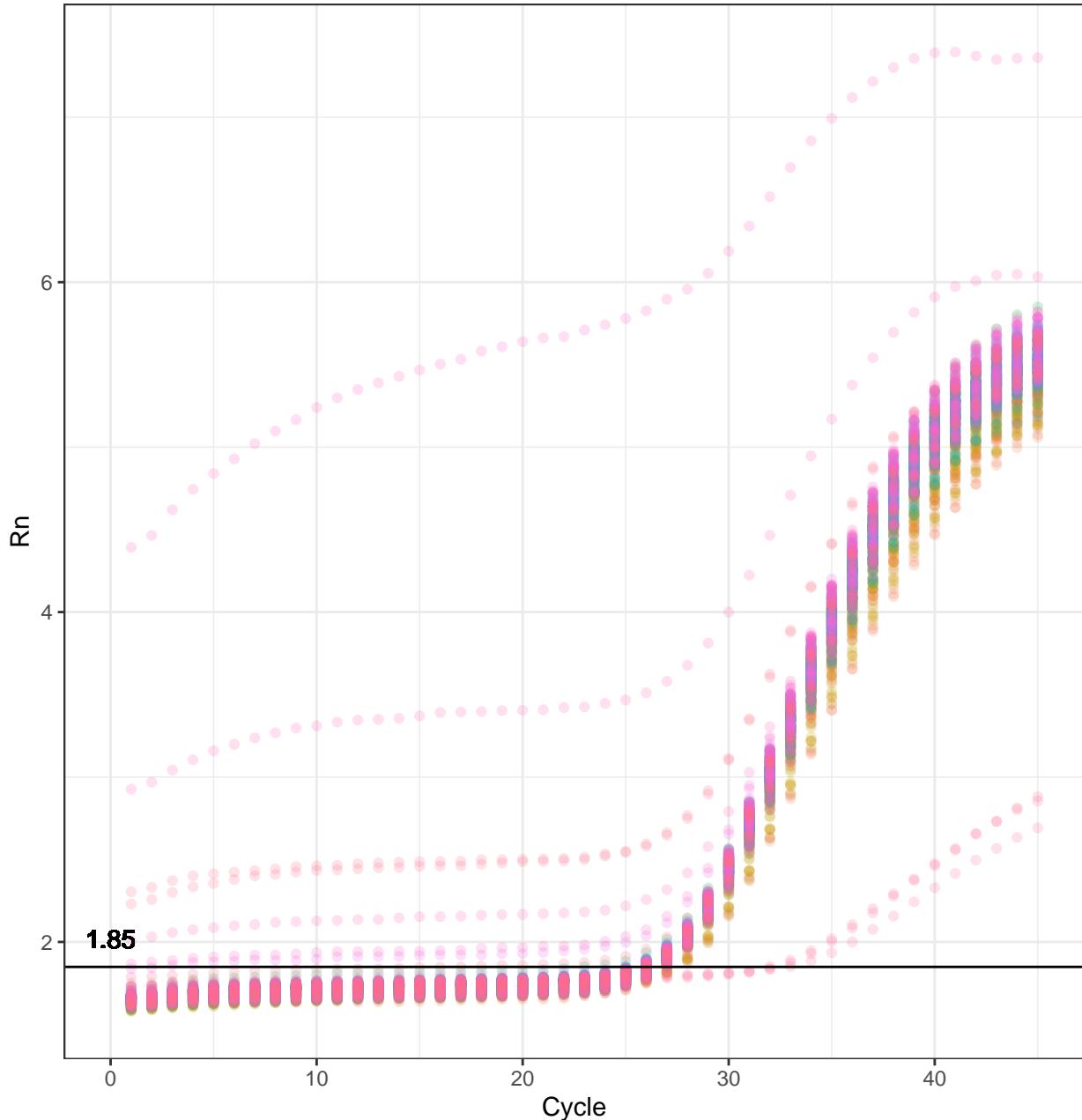
Moa Rn

```
combined_qPCR_data %>%
  filter(., str_detect(`Target Name`, IPC_name)) -> moa_standard

moa_standard$Rn %>% max() -> max_Rn_moa
h_moa= max_Rn_moa*0.25

moa_standard %>%
  # filter(., `Well Position`=="A24") %>% A24 did not perform well
  ggplot(., aes(y=Rn, x=Cycle, color=`Well Position`)) +
    geom_point(alpha=0.2) + geom_hline(yintercept =h_moa) +
    geom_text(aes(0,h_moa,label = round(h_moa,2), vjust = -1),color="black") +theme_bw() +ggtitle("Moa Rn")
```

Moa Rn



There is quite a bit of spread in our IPC. Will remove any samples outside normal distribution. #####
Remove Samples with Anomalous Outlier IPC values

```
moa_standard %>%
  filter(., Cycle == 40) -> moa_standard_c40

moa_standard_c40 %>%
  dplyr::summarise(mean_Rn = ci(Rn, confidence = 0.99)[1],
                  lower_ci_mpg = ci(Rn, confidence = 0.99)[2],
                  upper_ci_mpg = ci(Rn, confidence = 0.99)[3],
                  sd_Rn = ci (Rn, confidence = 0.99)[4]) -> CI0.95_moa
```

```

Sum = groupwiseMean(Rn ~ Cycle, data=moa_standard_c40, conf = 0.99)

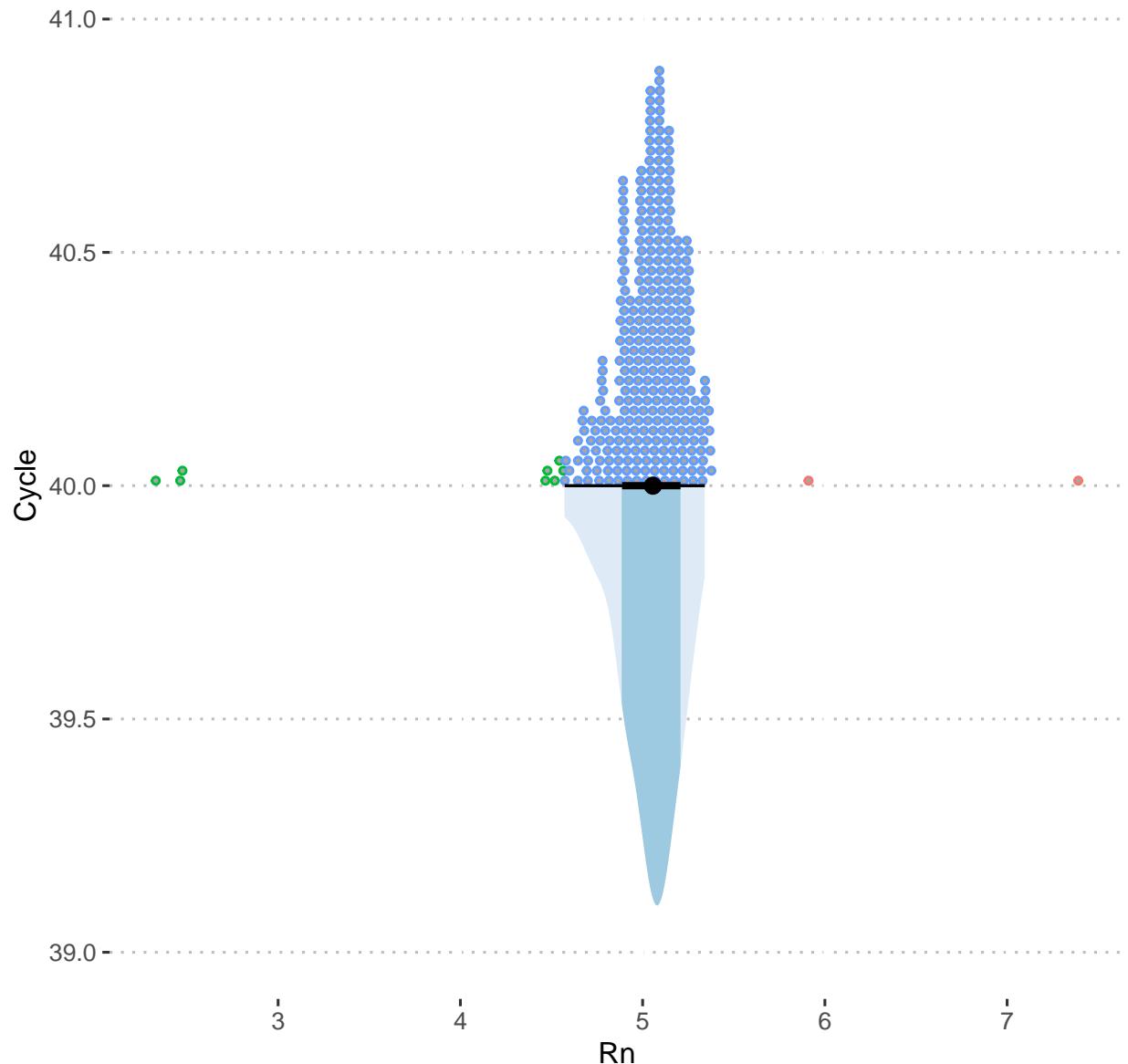
moa_standard_c40 %>%
summarise(
  Q1 = quantile(Rn, 0.25, na.rm = TRUE),
  Q3 = quantile(Rn, 0.75, na.rm = TRUE),
  IQR = Q3 - Q1,
  lower_bound = Q1 - 1.5 * IQR,
  upper_bound = Q3 + 1.5 * IQR
) -> moa_iqr

moa_standard_c40 %>%
  mutate(. , type=case_when(Rn > moa_iqr$upper_bound ~ "Outlier High",
                           Rn < moa_iqr$lower_bound ~ "Outlier Low",
                           TRUE~"To Keep")) -> moa_standard_c40

moa_standard_c40 %>%
  ggplot(., aes(x= Rn, y=Cycle)) +  geom_dots(layout='weave', side = "top", aes(color=type)) + stat_sla

```

level 0.95 0.66 type Outlier High Outlier Low To Keep



```
moa_standard_c40 %>%
  filter(., type != "To Keep") -> moa_outliers_to_drop
```

```
combined_qPCR_data %>%
  filter(., `Well Position` != moa_outliers_to_drop$`Well Position`) -> combined_qPCR_data_cleaned

results_data %>%
  filter(., `Well Position` != moa_outliers_to_drop$`Well Position`) -> results_data_cleaned
```

Filter Data

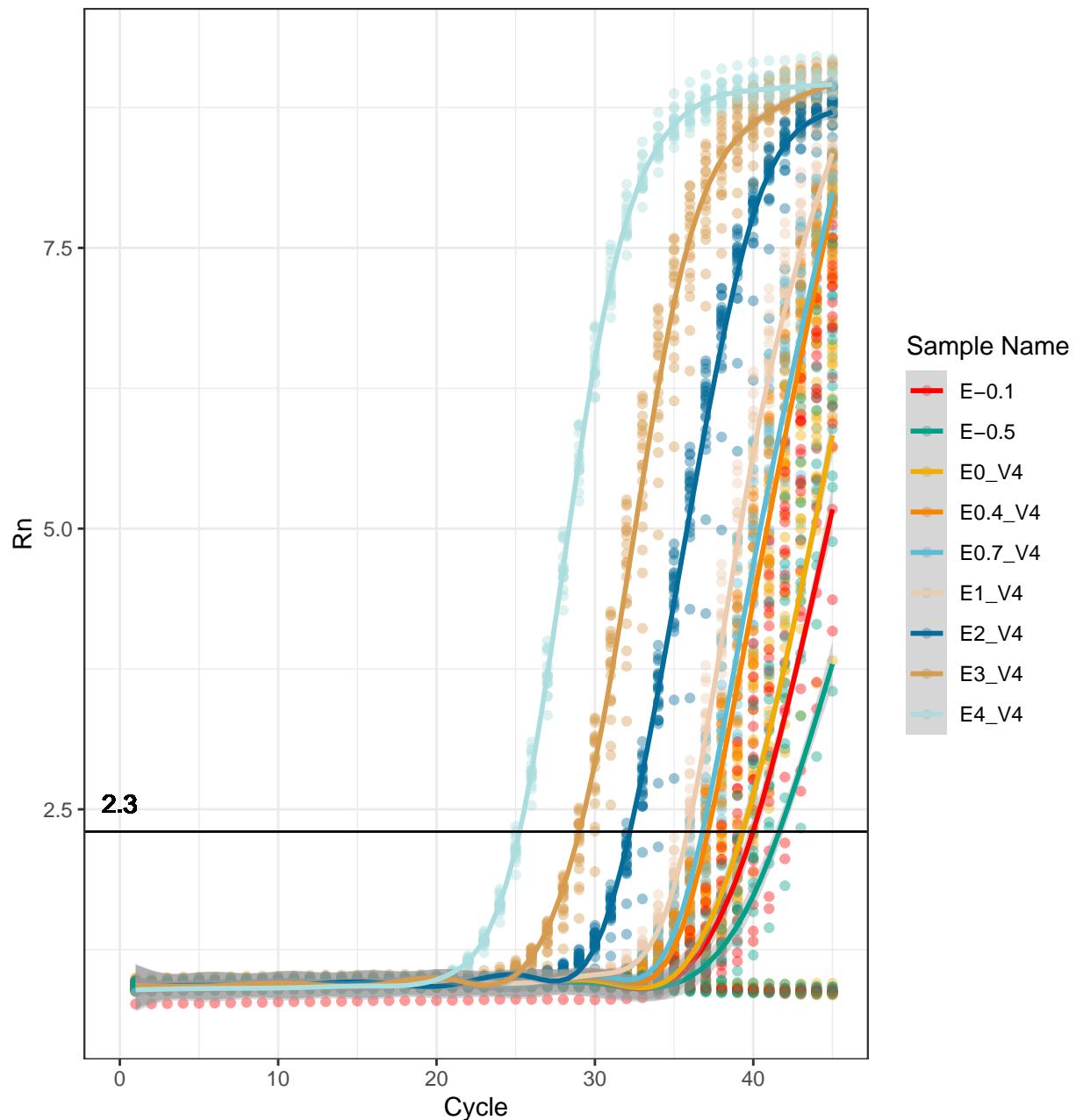
Pycno Rn

```
combined_qPCR_data_cleaned %>%
  filter(., str_detect(`Target Name`, Target_name)) %>%
    filter(., Task=="STANDARD")-> pycno_standard

pycno_standard$Rn %>% max() -> max_Rn
h= max_Rn*0.25

pycno_standard %>%
  ggplot(., aes(y=Rn, x=Cycle, color=`Sample Name`)) +geom_point(alpha=0.4) +geom_smooth() + geom_hline(y=
  geom_text(aes(0,h,label = round(h,2), vjust = -1),color="black") +theme_bw() + ggtile("Pycno Rn") +s
```

Pycno Rn



Looks great. We expect higher concentration standards to surpass the threshold (here set to 25% of the maximum Rn fluorescence value) at fewer cycles and we see that in the data.

Pycno Ct versus Quantity

```
pycno_standard %>%
  mutate(., Threshold = if_else(Rn > h, "Above", "Below")) -> pycno_standard
```

```
pycno_standard %>%
```

```

group_by(`Well Position`, `Sample Name`) %>%
  filter(., rank(Threshold, ties.method="first")==1) %>%
  filter(., Threshold == "Above") -> pycno_standard_above

pycno_standard %>%
  ungroup() %>%
  filter(., !`Well Position` %in% pycno_standard_above$`Well Position`) %>%
  group_by(`Well Position`, `Sample Name`) %>%
  filter(., rank(Threshold, ties.method="last")==1) -> pycno_standard_below

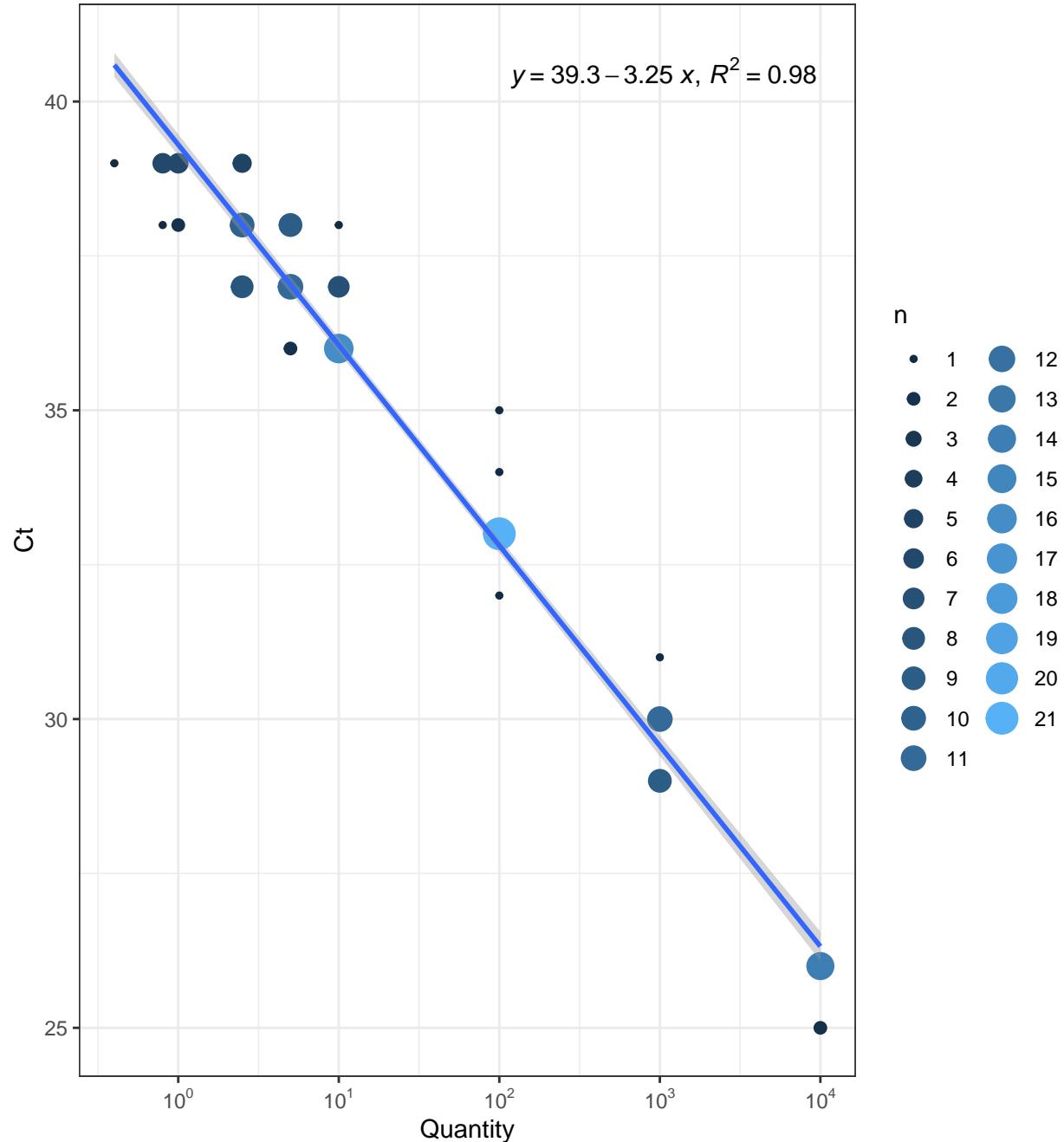
bind_rows(pycno_standard_above, pycno_standard_below) -> pycno_standard_threshold

set_breaks = function(limits) {
  seq(limits[1], limits[2], by = 1)
}

pycno_standard_threshold %>%
  filter(., Cycle < 40 ) %>%
  ggplot(., aes(x=`Quantity`, y = Cycle)) +geom_count(aes(color = ..n.., size = ..n..)) +
  scale_x_log10("Quantity",
    breaks = trans_breaks("log10", function(x) 10^x),
    labels = trans_format("log10", math_format(10^.x))) +
  guides(color = 'legend') +
  scale_size_continuous(breaks = set_breaks)+
  scale_color_continuous(breaks = set_breaks)+
  stat_poly_line() +
  stat_poly_eq(use_label(c("eq", "R2")),label.x = "right",
  label.y = "top") +theme_bw() +ylab("Ct") -> run5_ct_versus_quant

run5_ct_versus_quant

```



```
# View the structure of the generated plot object
# The computed data is stored within the plot object
grob_data_run5 <- ggplot_build(run5_ct_versus_quant)$data[[3]]

# The result is a list; unlist it to get a character vector
coef_char_run5 <- unlist(grob_data_run5$coefs)

standard_curve_coefs_run5 <- enframe(coef_char_run5, name = "Metric", value = "Value") %>%
  mutate( Metric = recode(Metric,
    "(Intercept)" = "Intercept",
    "n" = "n",
    "log(Quantity)" = "log(Quantity)",
    "log(Quantity)^2" = "log(Quantity)^2",
    "log(Quantity)*n" = "log(Quantity)*n",
    "n*log(Quantity)" = "n*log(Quantity)"))

```

```

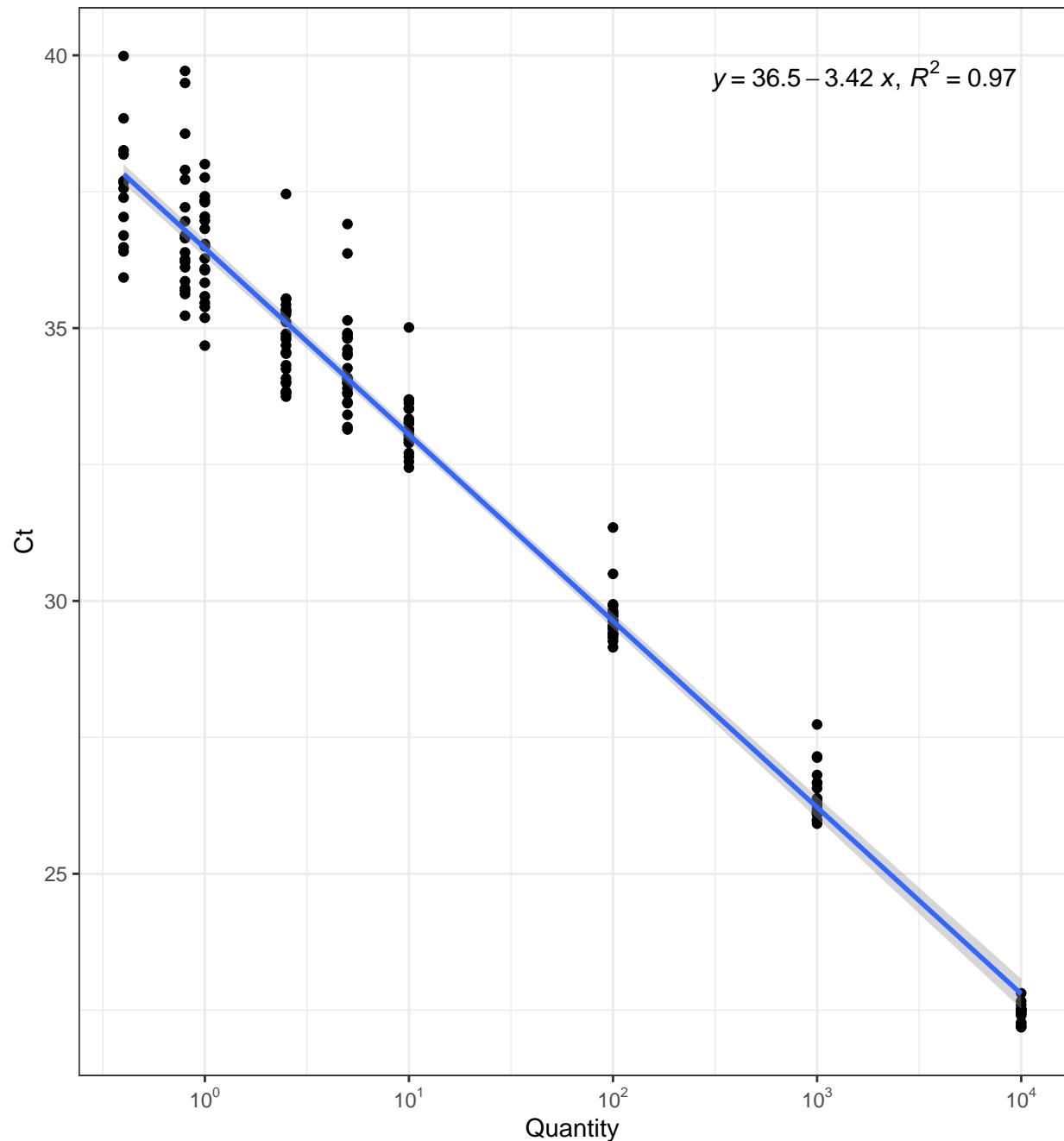
"x" = "Slope"))

# Extract the R^2 value from the 'r.squared' column
r_squared_value_run5 <- grob_data_run5$r.squared

results_data_cleaned %>%
  filter(., `Target Name`==Target_name) %>%
  filter(., Task=="STANDARD") %>%
  mutate(., CT=as.numeric(CT)) -> pycno_results_standard

pycno_results_standard %>%
  ggplot(., aes(x=`Quantity`, y = CT)) +geom_point() +
  scale_x_log10("Quantity",
    breaks = trans_breaks("log10", function(x) 10^x),
    labels = trans_format("log10", math_format(10^.x))) +
  guides(color = 'legend') +
  scale_size_continuous(breaks = set_breaks)+
  scale_color_continuous(breaks = set_breaks)+
  stat_poly_line() +
  stat_poly_eq(use_label(c("eq", "R2")),label.x = "right",
  label.y = "top") +theme_bw() +ylab("Ct")

```



Klymus et al. 2020 LoD + LoQ

LoD

LOD was defined as the lowest concentration at which 95% of the technical replicates exhibited positive amplification.

```
pycno_standard_threshold %>%
  group_by(`Sample Name`, Threshold, Quantity) %>%
```

```
count()
```

Raw data with R fit

```
## # A tibble: 12 x 4
## # Groups:   Sample Name, Threshold, Quantity [12]
##   `Sample Name` Threshold  Quantity     n
##   <chr>        <chr>       <dbl> <int>
## 1 E-0.1        Above      0.800    18
## 2 E-0.1        Below      0.800     6
## 3 E-0.5        Above      0.400    13
## 4 E-0.5        Below      0.400    10
## 5 E0.4_V4      Above      2.5      24
## 6 E0.7_V4      Above      5        24
## 7 E0_V4        Above      1        20
## 8 E0_V4        Below      1        4
## 9 E1_V4        Above      10       24
## 10 E2_V4       Above     100      24
## 11 E3_V4       Above    1000     21
## 12 E4_V4       Above   10000     16
```

```
pycno_standard_threshold %>%
  group_by(`Sample Name`, Threshold, Quantity) %>%
  count() %>%
  pivot_wider(names_from = Threshold, values_from = n, values_fill=0) %>%
  mutate(., Perc = Above/(Above+Below)) %>%
  arrange(desc(Quantity)) %>% kable(digits=2)
```

Sample Name	Quantity	Above	Below	Perc
E4_V4	10000.0	16	0	1.00
E3_V4	1000.0	21	0	1.00
E2_V4	100.0	24	0	1.00
E1_V4	10.0	24	0	1.00
E0.7_V4	5.0	24	0	1.00
E0.4_V4	2.5	24	0	1.00
E0_V4	1.0	20	4	0.83
E-0.1	0.8	18	6	0.75
E-0.5	0.4	13	10	0.57

```
# LoD is 2.5 copies/ $\mu$ L
```

Here we see that 95% of the technical replicates exhibited positive amplification between 1 and 2.5 copies per μ L.

```
pycno_results_standard %>%
  mutate(., detection = case_when(is.na(CT) ~ "Non-detection",
                                  TRUE ~ "Detection")) %>%
  group_by(`Sample Name`, detection, Quantity) %>%
  count()
```

QuantStudio 5 fit

```
## # A tibble: 12 x 4
## # Groups:   Sample Name, detection, Quantity [12]
##   `Sample Name` detection     Quantity     n
##   <chr>        <chr>          <dbl>    <int>
## 1 E-0.1        Detection     0.800     18
## 2 E-0.1        Non-detection 0.800      6
## 3 E-0.5        Detection     0.400     13
## 4 E-0.5        Non-detection 0.400     10
## 5 E0.4_V4      Detection     2.5       24
## 6 E0.7_V4      Detection     5         24
## 7 E0_V4        Detection     1         20
## 8 E0_V4        Non-detection 1         4
## 9 E1_V4        Detection     10        24
## 10 E2_V4       Detection    100        24
## 11 E3_V4       Detection   1000       21
## 12 E4_V4       Detection  10000      16

pycno_results_standard %>%
  mutate(., detection = case_when(is.na(CT) ~ "Non-detection",
                                   TRUE ~ "Detection")) %>%
  group_by(`Sample Name`, detection, Quantity) %>%
  count() %>%
  pivot_wider(names_from = detection, values_from = n, values_fill=0) %>%
  mutate(., Perc = Detection/(Detection+`Non-detection`)) %>%
  arrange(desc(Quantity)) %>% kable(digits=2)
```

Sample Name	Quantity	Detection	Non-detection	Perc
E4_V4	10000.0	16	0	1.00
E3_V4	1000.0	21	0	1.00
E2_V4	100.0	24	0	1.00
E1_V4	10.0	24	0	1.00
E0.7_V4	5.0	24	0	1.00
E0.4_V4	2.5	24	0	1.00
E0_V4	1.0	20	4	0.83
E-0.1	0.8	18	6	0.75
E-0.5	0.4	13	10	0.57

```
# LoD is 2.5 copies/µL
```

Using the QuantStudio5 estimated quantities, we can also see that our limit of quantification here appears to be between 1 and 2.5 copies.

LoQ

LOQ was determined at the lowest concentration at which the relative standard deviation of back-calculated concentrations was <35%

```

pycno_results_standard %>%
  mutate(., detection = case_when(is.na(CT) ~ "Non-detection",
                                 TRUE ~ "Detection")) %>% group_by(`Well Position`, `Sample Name`) %>%
  mutate(., Observed_Quantity= if_else(detection=="Detection",10^((CT -`Y-Intercept`)/Slope), 0 )) %>%
  mutate(., Concentration_L = Observed_Quantity*100/2 * 1) %>%
  group_by(`Sample Name`,Quantity) %>%
  dplyr::summarise(., mean_conc = round(mean(Concentration_L),2), sd_conc = round(sd(Concentration_L),2))

```

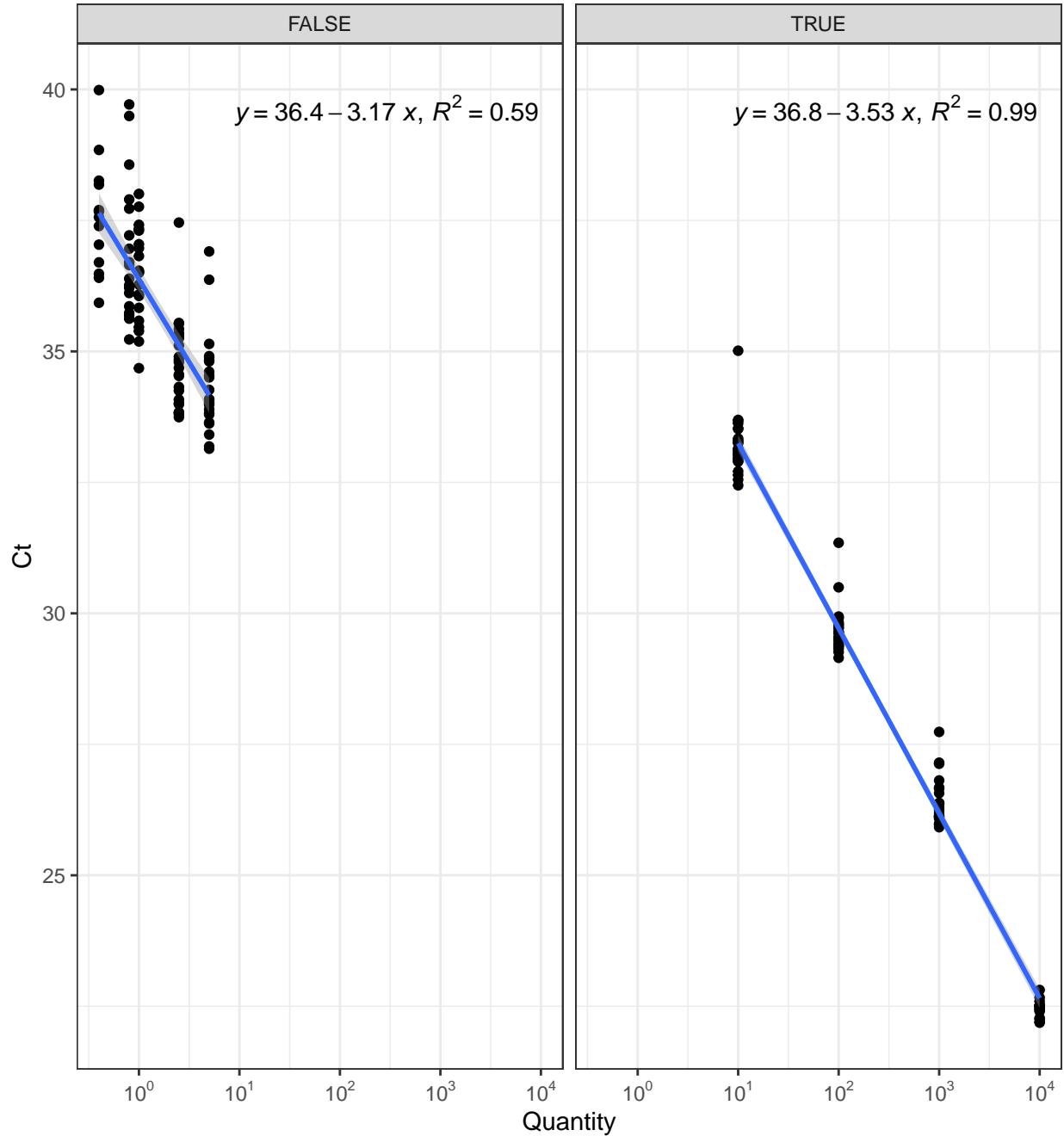
Sample Name	Quantity	mean_conc	sd_conc	n()	perc_sd_conc
E-0.5	0.4	16.94	20.79	23	122.73
E-0.1	0.8	37.25	34.69	24	93.13
E0_V4	1.0	50.59	41.82	24	82.66
E0.4_V4	2.5	177.66	81.01	24	45.60
E0.7_V4	5.0	238.35	111.48	24	46.77
E1_V4	10.0	474.70	135.31	24	28.50
E2_V4	100.0	5014.39	1147.06	24	22.88
E3_V4	1000.0	45094.94	11707.33	21	25.96
E4_V4	10000.0	634593.60	70796.37	16	11.16

The lowest concentration at which the relative standard deviation of back-calculated concentrations was <35% was between 5 and 10 copies per μL .

```

pycno_results_standard %>%
  ggplot(., aes(x=`Quantity`, y = CT)) +geom_point() +
  scale_x_log10("Quantity",
    breaks = trans_breaks("log10", function(x) 10^x),
    labels = trans_format("log10", math_format(10^.x))) +
  guides(color = 'legend') +
  scale_size_continuous(breaks = set_breaks)+
  scale_color_continuous(breaks = set_breaks)+
  stat_poly_line() +
  stat_poly_eq(use_label(c("eq", "R2")),label.x = "right",
  label.y = "top") + facet_wrap(~Quantity>9) +theme_bw() +ylab("Ct")

```



```
# Cycle = 41.7-3.52* log10(Quantity)
# (Cycle -41.7)/-3.52 = log10(Quantity)
```

Lesperance eLowQuant LoD and LoQ

Instructions

This file performs the computations in the publication, ‘A Statistical Model for Calibration and Computation of Detection and Quantification Limits for Low Copy Number Environmental DNA samples’ by Lesperance,

- Create a folder call Outputs in your working directory.
- Put your data file in your working directory and put the name of the file in the chunk labeled ‘READIN’ below.
- Data set csv file requirements. Columns: Target, Lab, Cq, SQ
- For nondetects, set Cq to be empty or NA value
- For negative controls, set Sq to be empty or 0 or NA value
- Include negative controls in the csv file!
- DO NOT DUPLICATE Target names over different Labs!!
- This code uses observations with nonempty data and where phat < 1 (num detect < num technical replicates)
- Only the SQ’s up to the first one with phat==1 are used.
- Allows for variable numbers of SQ levels per Target.
- Assumes SQs == NA are zero, i.e. are negative controls.
- The code uses the R function optim. A convergence code 0 indicates successful completion.
- Ignore warnings if results are sensible.
- EXECUTE EACH CHUNK LOOKING AT THE OUTPUT. IN PARTICULAR, LOOK AT THE GRAPHS IN THE CHUNK CALLED ‘PlotPois’ TO DETERMINE IF THE MODEL IS APPROPRIATE. IT IS NOT APPROPRIATE IF ‘lm Rsq’ is small, i.e. near zero!
- You can send results to files by setting the sink.indicator and Manusink to TRUE and running the code in RStudio. This currently does not work for all results files when knitting.
- If you wish to knit to pdf AND you do NOT have a version of Latex installed on your computer, then run the following in your RStudio console:
`install.packages("tinytex"); tinytex::install_tinytex()`

Process/Summarize samples by Lab; Compute the Poisson estimates of SQ

Hindson et al “High-Throughput Droplet Digital PCR System for Absolute Quantitation of DNA Copy Number”, Anal. Chem., 2011, 83 (22), pp 8604–8610 use a Poisson approximation for quantitation. Before that, Dube et al. 2008, “Mathematical analysis of copy number variation in a DNA sample using digital PCR on a nanofluidic device”, PloS One, Vol 3, Issue 8, e2876, model the number of molecules in each chamber as a Poisson process, giving the relationship between p and λ .

```
## [1] 10  6  
  
## dim, before negative controls added
```

Target	SQ	detect	n	Cqmean	Lab
Pycno:10	Min. : 0.00	Min. : 0.0	Min. :16.00	Min. :22.46	NOAA PMEL OME:10
NA	1st Qu.: 0.85	1st Qu.:16.5	1st Qu.:23.25	1st Qu.:29.68	NA
NA	Median : 3.75	Median :20.5	Median :24.00	Median :34.35	NA
NA	Mean : 1111.97	Mean :18.4	Mean :22.80	Mean :32.42	NA
NA	3rd Qu.: 77.50	3rd Qu.:24.0	3rd Qu.:24.00	3rd Qu.:36.44	NA
NA	Max. :10000.00	Max. :24.0	Max. :24.00	Max. :37.55	NA
NA	NA	NA	NA	NA's :1	NA

```
##
##
## Table: NOAA PMEL OME
##
## Target      SQ   detect     n    phat
## -----      --   -----  ---  -----
## Pycno      0.0      0    24  0.000
## Pycno      0.4     13    23  0.565
## Pycno      0.8     18    24  0.750
## Pycno      1.0     20    24  0.833
## Pycno      2.5     24    24  1.000
## Pycno      5.0     24    24  1.000
## Pycno     10.0     24    24  1.000
## Pycno    100.0     24    24  1.000
## Pycno  1000.0     21    21  1.000
## Pycno 10000.0     16    16  1.000
```

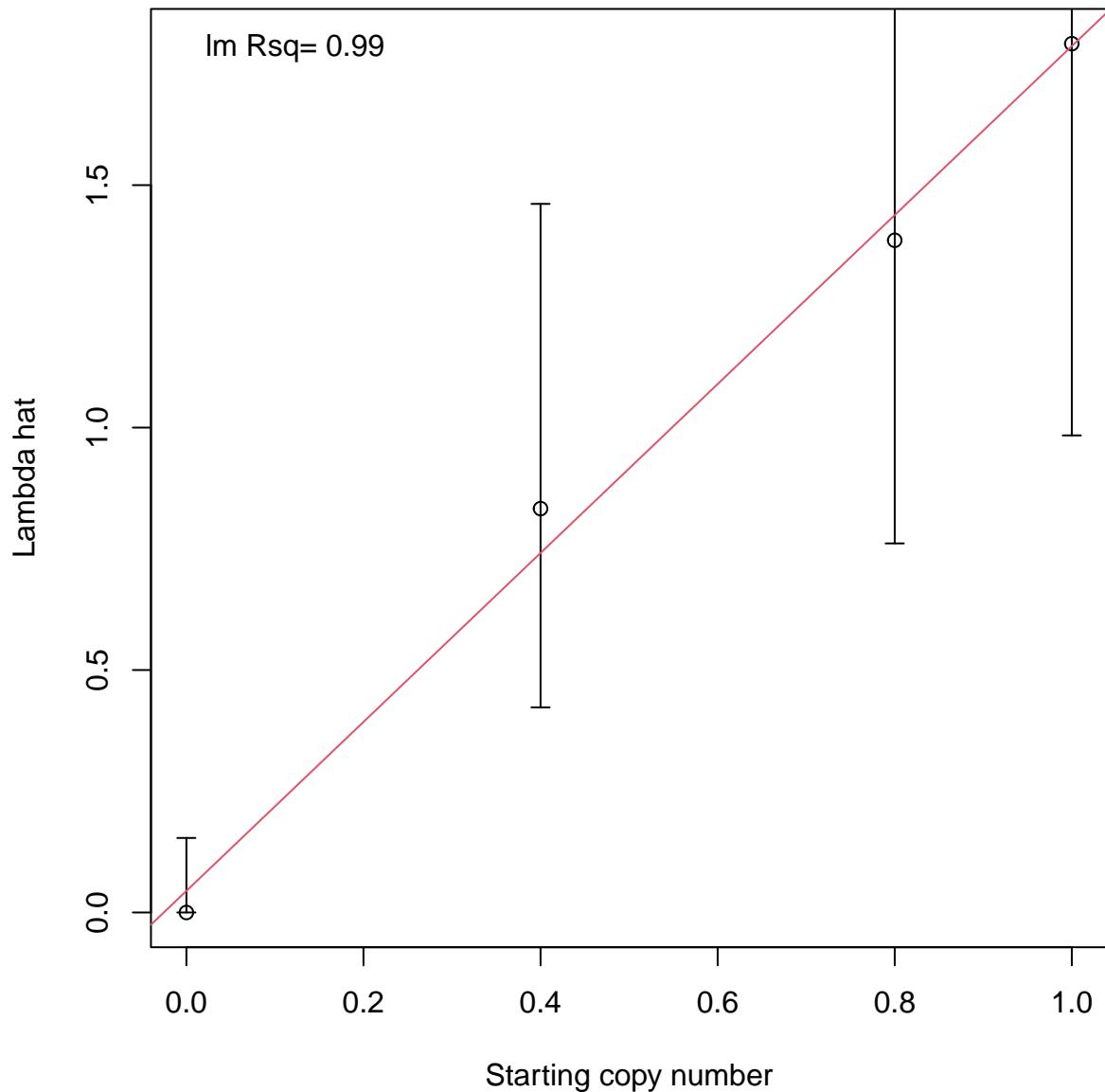
Plot the Poisson estimates (and CI) of SQ for levels that had non-detects

Only the first levels of SQ that had non-detects are analyzed. Red line is least squares linear regression line.

LOOK AT THE GRAPHS IN THE CHUNK CALLED ‘PlotPois’ TO DETERMINE IF THE MODEL IS APPROPRIATE. IT IS NOT APPROPRIATE IF ‘lm Rsq’ is small, i.e. near zero!

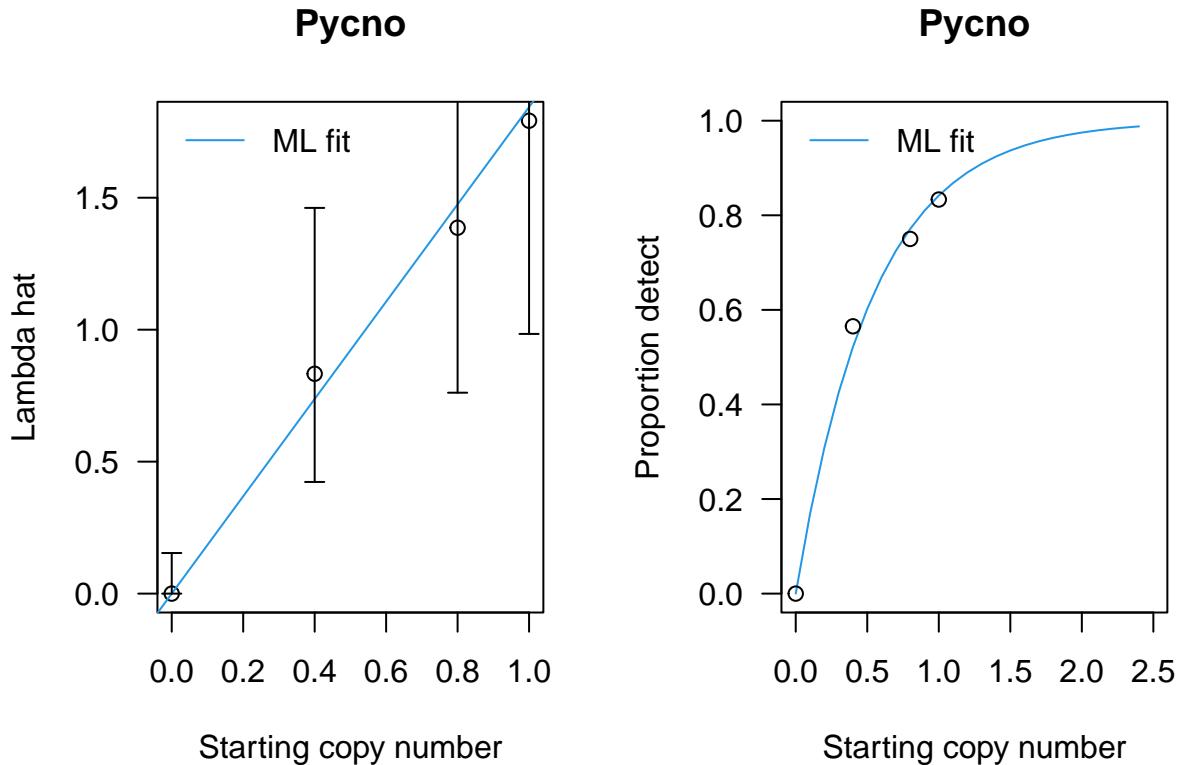
ML ??will error if all phats==1

NOAA PMEL OME, Pycno



Both the intercept and no intercept models are fit to the data. The ‘best’ of the two models is the one with the largest Likelihood Ratio test p-value. The ‘best’ model will be identified in the chunk called *Manuscript*.

Estimate Poisson models - no intercept model



```
##
##
##
##
##   Pycno
## Convergence= 0
##      Estimate Std. Err Z value Pr(>z)
## beta     1.844    0.282    6.53 6.5e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.2479441 , df=  3 , p-value= 0.9695018
```

Estimate predicted Sq given number detects and technical replicates - no intercept (not shown)

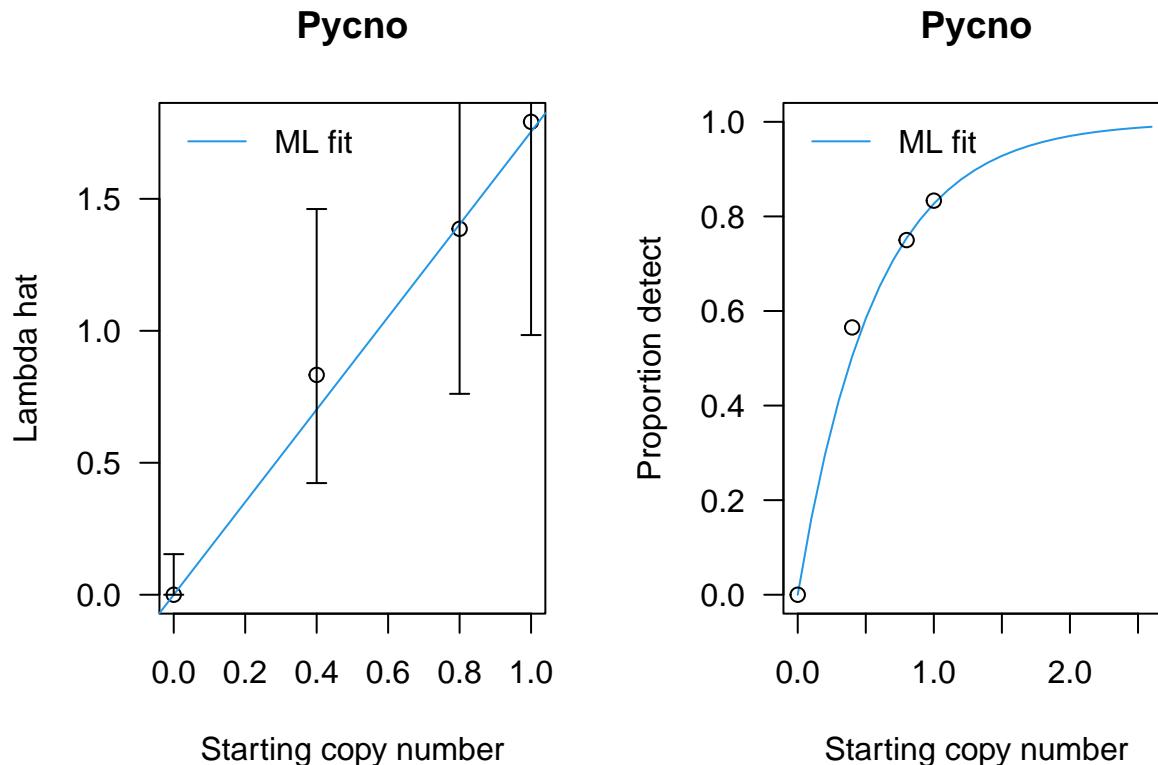
The estimated SQ is easily obtained for given new values of nn0=number of replicates, nd0=number detected and the estimated slope betaS as: Shat <- -(log((nn0 - nd0)/nn0)) / betaS[1] The standard errors are obtained from the Hessian matrix (or via the function CalibS0Or.ddLLik()) .

Estimate predicted Sq given consecutive numbers of detects given number of technical replicates - no intercept

The estimated SQ is easily obtained for given new values of nn0=number of replicates, nd0=number detected and the estimated slope betaS as: Shat <- -(log((nn0 - nd0)/nn0)) / betaS[1] The standard errors are obtained from the Hessian matrix (or via the function CalibS0Or.ddLLik()) .

```
##  
## Pycno  
## ML estimate of SQ for numbers of detects and 8 replicates  
##  
##  
##  NumDetects      SQ0     SE_SQ0  
## -----  
##      0      0.000      NaN  
##      1      0.072      0.073  
##      2      0.156      0.113  
##      3      0.255      0.154  
##      4      0.376      0.200  
##      5      0.532      0.261  
##      6      0.752      0.352  
##      7      1.128      0.536
```

Estimate Poisson models - intercept model



```
##
##
##
##
##   Pycno
## Convergence= 0
##      Estimate Std. Err Z value Pr(>z)
## alpha 1.49e-08 4.22e-01    0.00 1.000
## beta  1.75e+00 6.94e-01    2.53 0.011 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.352375 , df=  2 , p-value= 0.8384608
```

Estimate predicted Sq given number detects and technical replicates - intercept model (not shown)

Estimate predicted Sq given consecutive numbers of detects given number of technical replicates - intercept model

The estimated SQ is easily obtained for given new values of nn0=number of replicates, nd0=number detected and the estimated slope betaS as: Shat <- -(log((nn0 - nd0)/nn0) + betaS[1]) / betaS[2] The standard errors are obtained from the Hessian matrix (or via the function CalibS0.ddLLik()).

```
##  
## Pycno  
## ML estimate of SQ for numbers of detects and 8 replicates  
##  
##  
##  NumDetects      SQ0     SE_SQ0  
## -----  
##    0    0.000    0.000  
##    1    0.056    0.478  
##    2    0.146    0.240  
##    3    0.250    0.221  
##    4    0.376    0.228  
##    5    0.537    0.266  
##    6    0.769    0.358  
##    7    1.165    0.577
```

Determine Lc, Ld, Lq (LOB, LOD, LOQ) - no intercept model

Follows Lavagnini and Magno 2007, Mass Spectrometry Reviews *The notation in the paper was changed from the Lavagnini 2007 paper and is shown in brackets here.*

- Lc (*LOB Limit of blank*) = critical level is the assay signal above which a response is reliably attributed to the presence of analyte
- Ld (*Ld = expected number detects out of NN replicates at concentration LOD*) = signal corresponding to an analyte concentration x_d (*=LOD Limit of Detection*) level which may be a priori expected to be recognized
- Lq = quantification limit is a signal with a precision which satisfies an expected value ($=\gamma_Q$)

Lc corresponds to a critical response level or a false positive rate, i.e. critical number of detects given NN replicates, above which we would reject the null hypothesis that the concentration/copy number is zero at $\alpha = \alpha_{Lc}$ ($=\gamma_{FP}$). It is the critical response level corresponding to the false positive rate of α_{Lc} . Essentially, the test is positive if the $Y \sim \text{Binomial}(m, p) > Lc$. The False Positive Rate is $P(Y > Lc | S=0)$.

Ld is computed to correspond to the false negative rate, $\beta = \beta_{Ld}$ ($=\gamma_{FN}$) here. It is computed so that the probability of observing a new (unknown concentration) response less than or equal to Lc is less than or equal to β_{Ld} . The probability of observing Lc or less detects if the concentration is x_d (*=LOD Limit of Detection*) or more is less than or equal to β_{Ld} . The values of Lc depend on the number of replicates, NN, so x_d does as well. Ld is the expected number of detects at values x_d (*=LOD Limit of Detection*) and NN replicates. False negative rate Ld computation: $P(Y \leq Lc | p_{xd}) \leq \beta_{Ld}$, ($=\gamma_{FN}$) and solve for x_d (*=LOD Limit of Detection*).

Lq is less well defined. The literature suggests using $Lq = \beta_{Lq} + 10 \text{ s.e.}(\beta_{Lq})$, but this uses the normality assumption. Other literature suggests using the “analyte concentration x_q (*=LOQ Limit of Quantification*) for which the experimental relative standard deviation of the responses reaches a fixed level ($=\gamma_Q$), for example, the level 0.1.” Lavagnini and Magno 2007. I interpret the term “relative standard deviation” to mean the coefficient of variation, $CV = sd/mean$.

In the exercise below, we use the fits from the ML models to estimate the Lc, Ld and Lq, for various values of NN replicates for a new observation, i.e. a new (unknown concentration) response number of detects. Both the intercept and no intercept models are considered.

Determine Lc, Ld, Lq (LOB, LOD and LOQ) - intercept model

Estimates, Lc, Ld, Lq (LOB, LOD, LOQ) and confidence limits for a given number of technical reps NN[NNi]

Chooses the model (intercept versus no intercept) with the best LLR test fit, i.e. the largest p-value for the LLR test. A table of values for all assays is printed.

```
## Limits for best choice model for N= 8
```

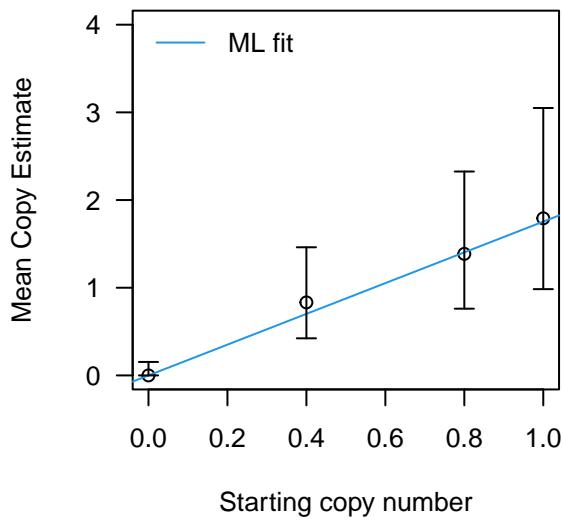
```
## InterModel      alpha      aSE      beta      bSE      Lc      SdLow
##      0.00      0.00      0.00     1.84      0.28      0.00      0.16
##      Sd      SdUp      SqLow      Sq      SqUp
##      0.20      0.29      0.59     0.77     1.10
```

Tables and Graphs for the manuscript

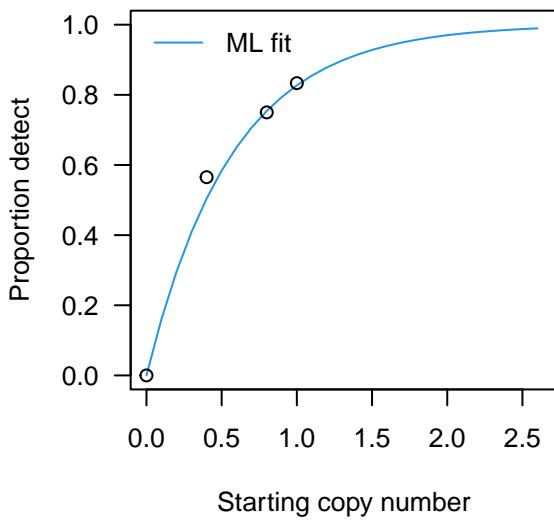
Revised for general use to use all eligible targets.

```
##  
##  
##      alpha   aSE    beta   bSE   LOB   LODL   LOD   LODU   LOQL   LOQ   LOQU  
## -----  -----  -----  -----  -----  -----  -----  -----  -----  -----  -----  
## Pycno     0     0.4    1.8    0.7     0     0     0.2     1     0.2     0.8     3.6  
  
##  
##  
##      alpha   aSE    beta   bSE   LOB   LODL   LOD   LODU   LOQL   LOQ   LOQU  
## -----  -----  -----  -----  -----  -----  -----  -----  -----  -----  -----  
## Pycno     0     0     1.8    0.3     0     0.2     0.2     0.3     0.6     0.8     1.1  
  
##  
##  
##      alpha   aSE    beta   bSE   LOB   LODL   LOD   LODU   LOQL   LOQ   LOQU  
## -----  -----  -----  -----  -----  -----  -----  -----  -----  -----  -----  
## Pycno     0     0     1.8    0.3     0     0.2     0.2     0.3     0.6     0.8     1.1  
  
##  
##  
## Table: Pycno  
##  
##      S num.detect   n p.tilde lambda.tilde  
## -----  -----  -----  -----  
##  0.0       0   24    0.000    0.000  
##  0.4      13   23    0.565    0.833  
##  0.8      18   24    0.750    1.386  
##  1.0      20   24    0.833    1.792  
  
##  
##  Pycno  
## Convergence= 0  
##      Estimate Std.Err Z value Pr(>z)  
## alpha  1.49e-08 4.22e-01    0.00  1.000  
## beta   1.75e+00 6.94e-01    2.53  0.011 *  
## ---  
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1  
## LLR test stat= 0.352375 , df=  2 , p-value= 0.8384608  
##  
##  
##  Pycno  
## Convergence= 0  
##      Estimate Std.Err Z value Pr(>z)  
## beta    1.844   0.282    6.53 6.5e-11 ***  
## ---  
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1  
## LLR test stat= 0.2479441 , df=  3 , p-value= 0.9695018
```

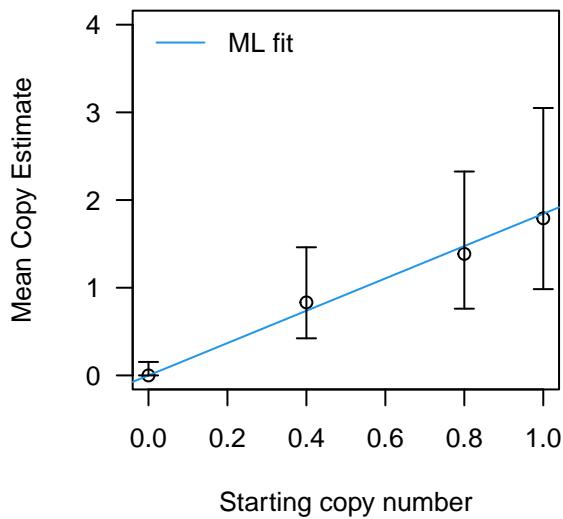
Pycno



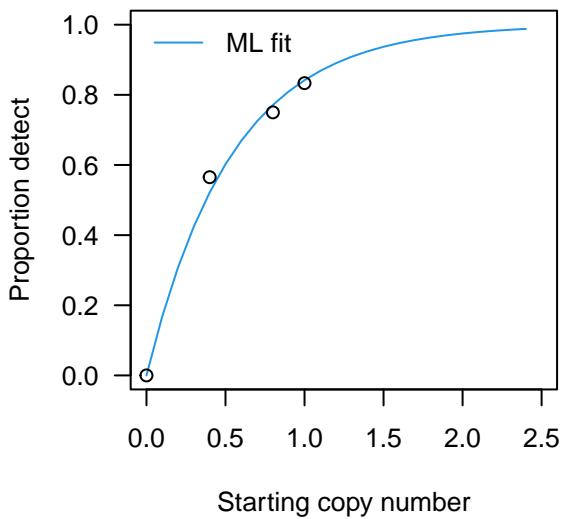
Intercept Pycno



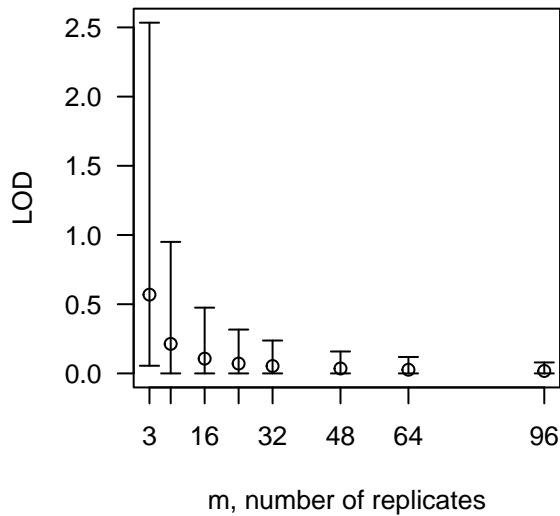
Pycno



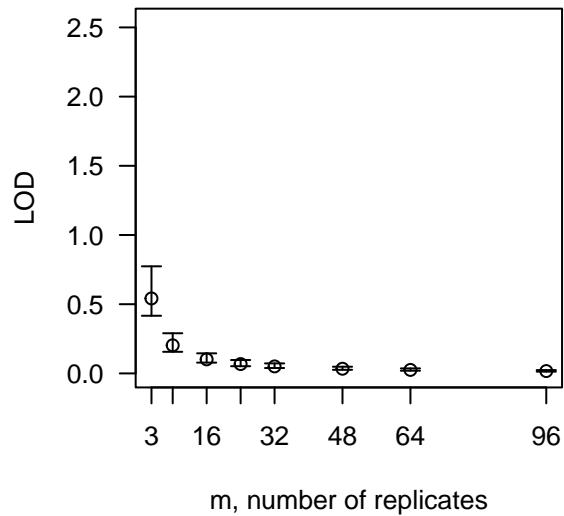
No intercept Pycno



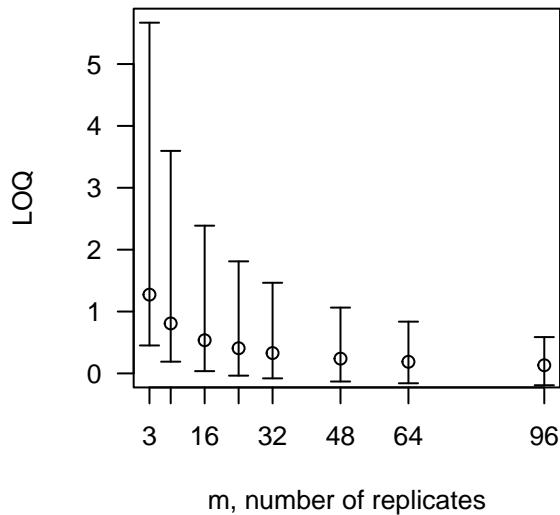
Limits detect – intercept Pycno



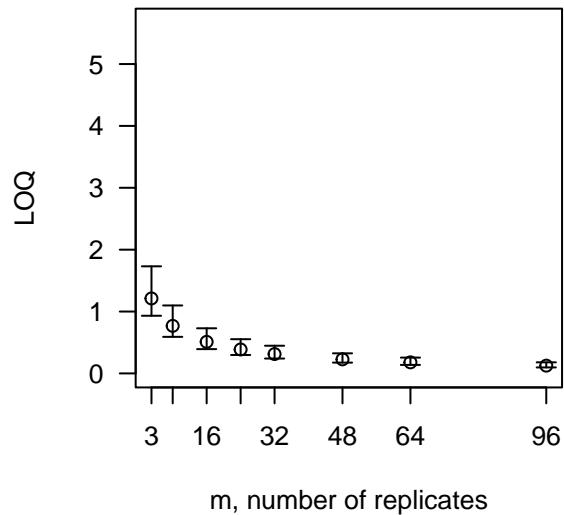
Limits detect – no intercept Pycno



Limits quant – intercept Pycno



Limits quant – no intercept Pycno



FHL Analysis Transplanted Individual Detection

Load Data

```
sample_set_up_run1 <- read_excel(here("qPCR_raw_data", "2025-05-23_132340_Pycno_test_1.xlsx"), sheet="Sam  
amplification_data_run1 <- read_excel(here("qPCR_raw_data", "2025-05-23_132340_Pycno_test_1.xlsx"), sheet="Amplification")
```

Merge Tables

```
amplification_data_run1 %>%
  left_join(sample_set_up_run1) %>%
  mutate(., `Sample Name` = if_else(is.na(`Sample Name`), str_c(`Target Name`, "_", Task, "_", Quantity), `Sample Name`))
  mutate(., `Sample Name` = if_else(is.na(`Sample Name`), "Negative Control", `Sample Name`)) -> combined
```

Now we are ready to start analyzing the data.

First thing we want to do is check to see how our internal positive control is working.

Moa Map

```
columns = seq(from = 1, to=24, by=1)
columns_t= as.tibble(columns) %>% dplyr::rename("Column"=value)
rows = capitalize(letters[1:16])
rows_t= as.tibble(rows) %>% dplyr::rename("Row"=value)

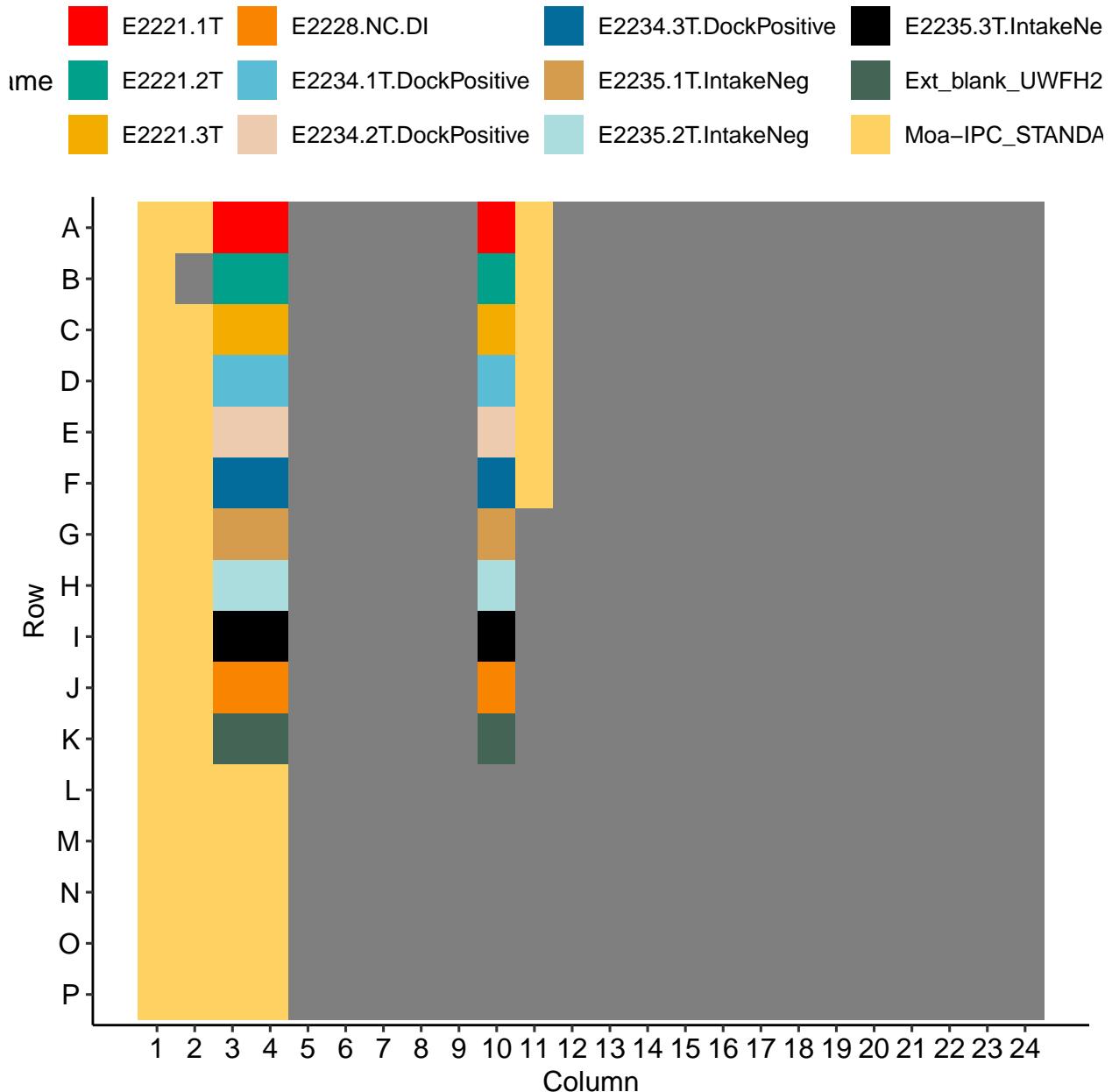
cross_join(columns_t, rows_t) -> full_plate

combined_qPCR_data_run1 %>%
  filter(., `Target Name`=="Moa-IPC") %>%
  mutate(., Row = str_sub(`Well Position`,start = 1L, end = 1L),
         Column= as.numeric(str_sub(`Well Position`,start = 2L, end = -1L))) -> moa_samples_run1

full_plate %>%
  left_join(moa_samples_run1) -> moa_plate_run1

moa_plate_run1 %>%
  mutate(Row = factor(Row, levels = rows)) %>%
  ggplot(aes(x=Column, y=fct_rev(Row), fill=`Sample Name` )) +geom_tile() +scale_x_continuous(breaks=co
```

Moa-IPC Standards & Samples



Pycno Map

```
combined_qPCR_data_run1 %>%
  filter(`Target Name` == "Pycno") %>%
  mutate(`Row` = str_sub(`Well Position`, start = 1L, end = 1L),
        `Column` = as.numeric(str_sub(`Well Position`, start = 2L, end = -1L))) -> pycno_samples_run1

full_plate %>%
  left_join(pycno_samples_run1) -> pycno_plate_run1
```

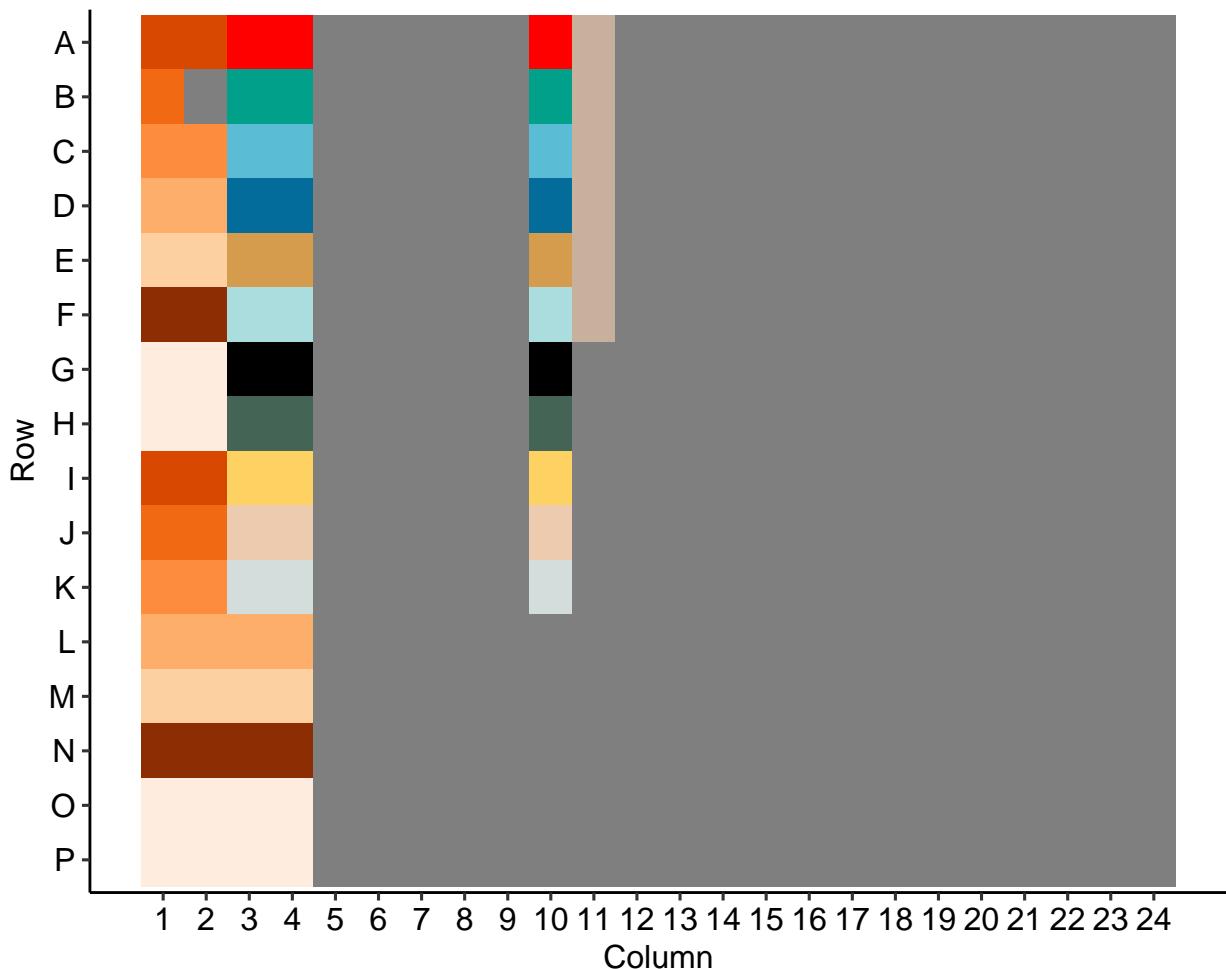
```

pycno_plate_run1 %>%
  mutate(Row = factor(Row, levels = rows)) %>%
  ggplot(aes(x=Column, y=fct_rev(Row), fill=`Sample Name` )) +geom_tile() +scale_x_continuous(breaks=co

```

Pycno Standards & Samples

E2221.1T		E2234.1T.DockPositive		E2235.2T.IntakeNeg		Pycno_STANDARD_1	
E2221.2T		E2234.2T.DockPositive		E2235.3T.IntakeNeg		Pycno_STANDARD_10	
E2221.3T		E2234.3T.DockPositive		Ext_blank_UWFH25		Pycno_STANDARD_100	
E2228.NC.DI		E2235.1T.IntakeNeg		Negative Control		Pycno_STANDARD_1000	



Moa Rn

```

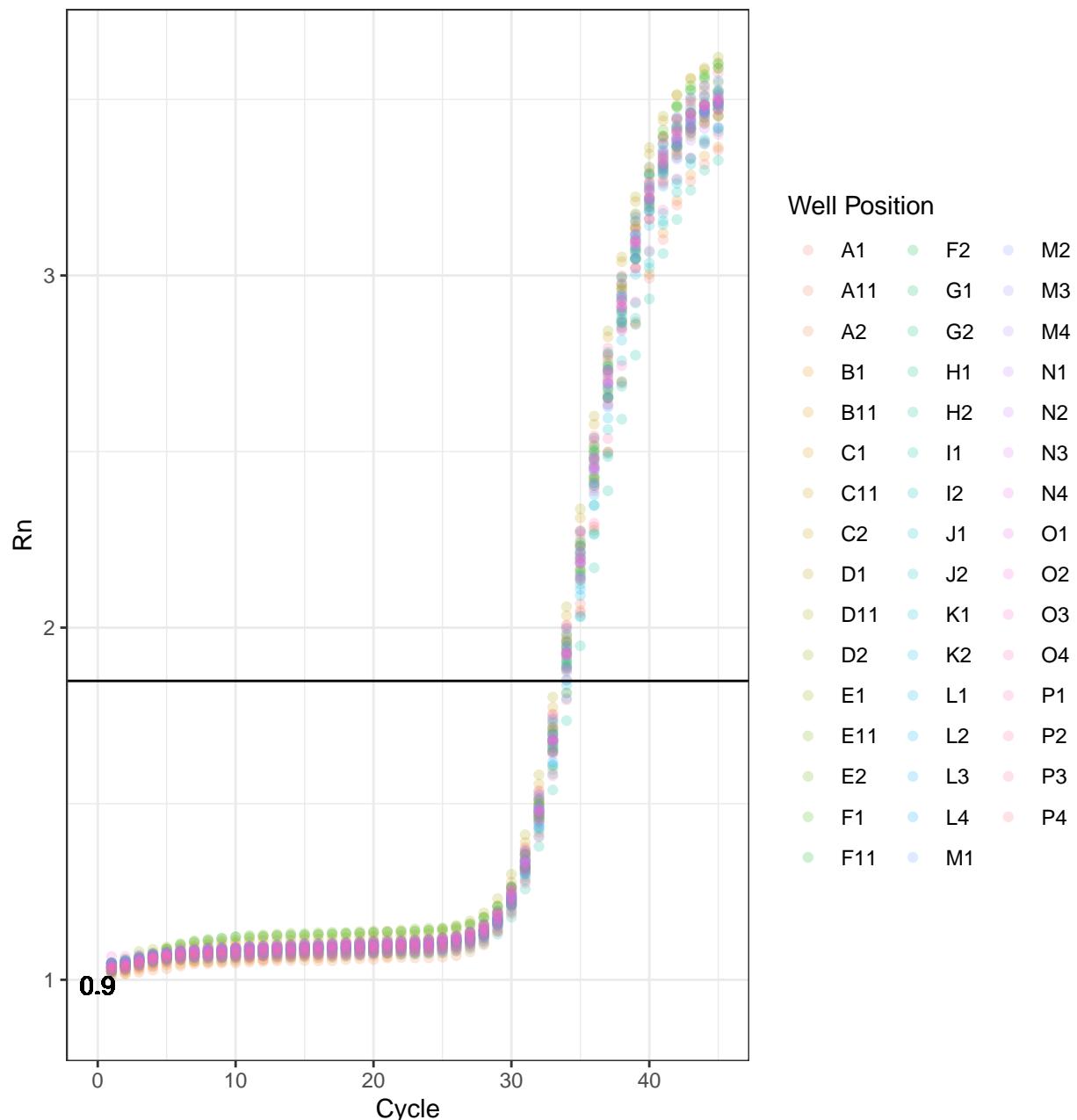
combined_qPCR_data_run1 %>%
filter(., str_detect(`Sample Name`,"Moa-IPC_STANDARD")) -> moa_standard_run1

moa_standard_run1$Rn %>% max() -> max_Rn_moa_run1
h_moa_run1= max_Rn_moa_run1*0.25

moa_standard_run1 %>%
ggplot(., aes(y=Rn, x=Cycle, color=`Well Position`)) +
  geom_point(alpha=0.2) + geom_hline(yintercept =h_moa) +
  geom_text(aes(0,h_moa_run1,label = round(h_moa_run1,2), vjust = -1),color="black") +theme_bw() +ggti

```

Moa Rn



There is quite a bit of spread in our IPC. Will remove any samples outside normal distribution. ##### Remove Samples with Anomalous Outlier IPC values

```
moa_standard_run1 %>%
  filter(., Cycle == 40) -> moa_standard_c40_run1

moa_standard_c40_run1 %>%
  dplyr::summarise(mean_Rn = ci(Rn, confidence = 0.99)[1],
                   lower_ci_mpg = ci(Rn, confidence = 0.99)[2],
                   upper_ci_mpg = ci(Rn, confidence = 0.99)[3],
                   sd_Rn = ci(Rn, confidence = 0.99)[4]) -> CI0.95_moa_run1

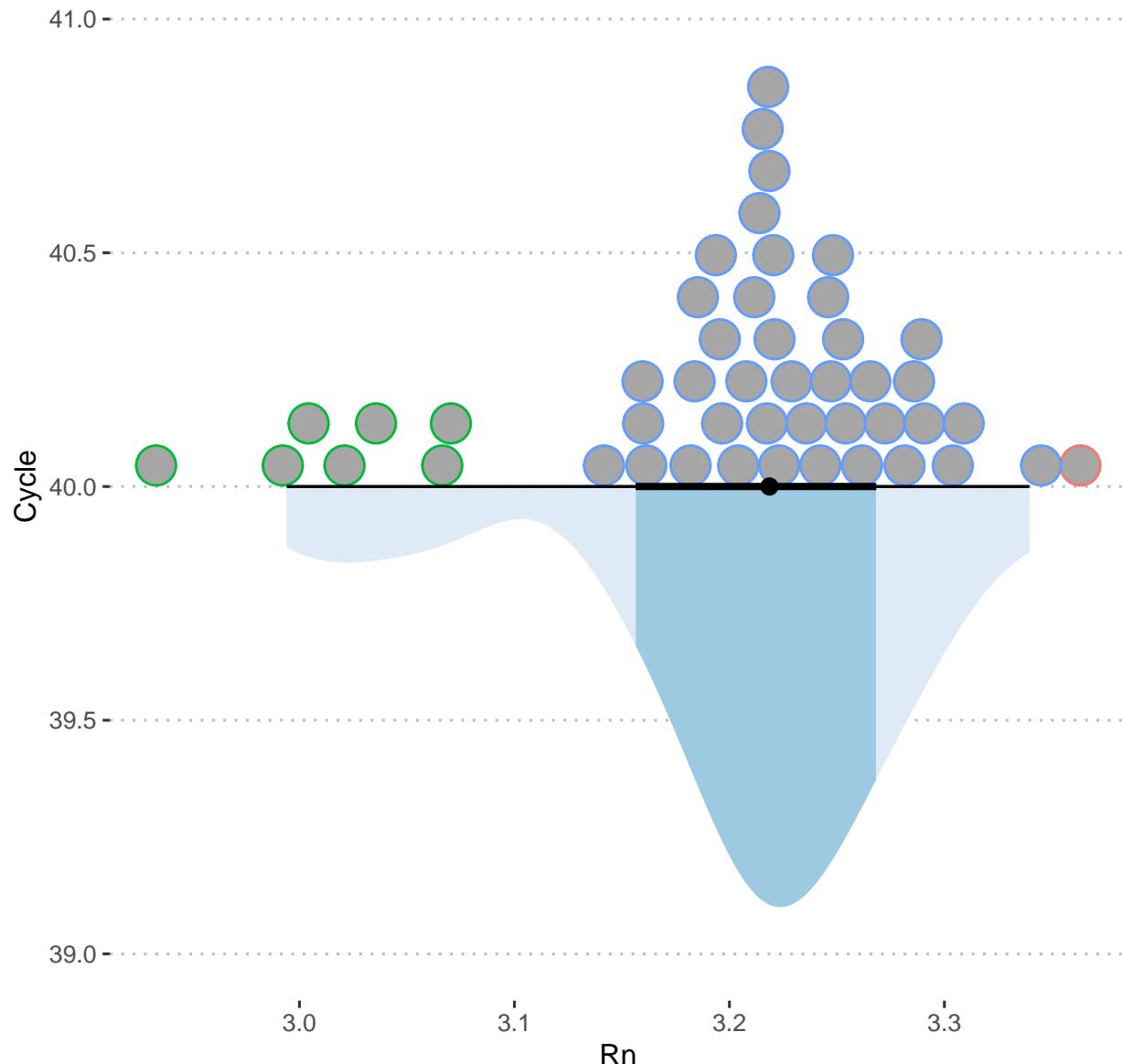
Sum_run1 = groupwiseMean(Rn ~ Cycle, data=moa_standard_c40_run1, conf = 0.99)

moa_standard_c40_run1 %>%
  summarise(
    Q1 = quantile(Rn, 0.25, na.rm = TRUE),
    Q3 = quantile(Rn, 0.75, na.rm = TRUE),
    IQR = Q3 - Q1,
    lower_bound = Q1 - 1.5 * IQR,
    upper_bound = Q3 + 1.5 * IQR
  ) -> moa_iqr_run1

moa_standard_c40_run1 %>%
  mutate(., type=case_when(Rn > moa_iqr_run1$upper_bound ~ "Outlier High",
                          Rn < moa_iqr_run1$lower_bound ~ "Outlier Low",
                          TRUE ~ "To Keep")) -> moa_standard_c40_run1

moa_standard_c40_run1 %>%
  ggplot(., aes(x= Rn, y=Cycle)) + geom_dots(layout='weave', side = "top", aes(color=type)) + stat_sla
```

level 0.95 0.66 type Outlier High Outlier Low To Keep



```
moa_standard_c40_run1 %>%
  filter(., type!="To Keep") -> moa_outliers_to_drop_run1
```

```
combined_qPCR_data_run1 %>%
  filter(., `Well Position` != moa_outliers_to_drop_run1$`Well Position`) -> combined_qPCR_data_cleaned
```

Filter Data

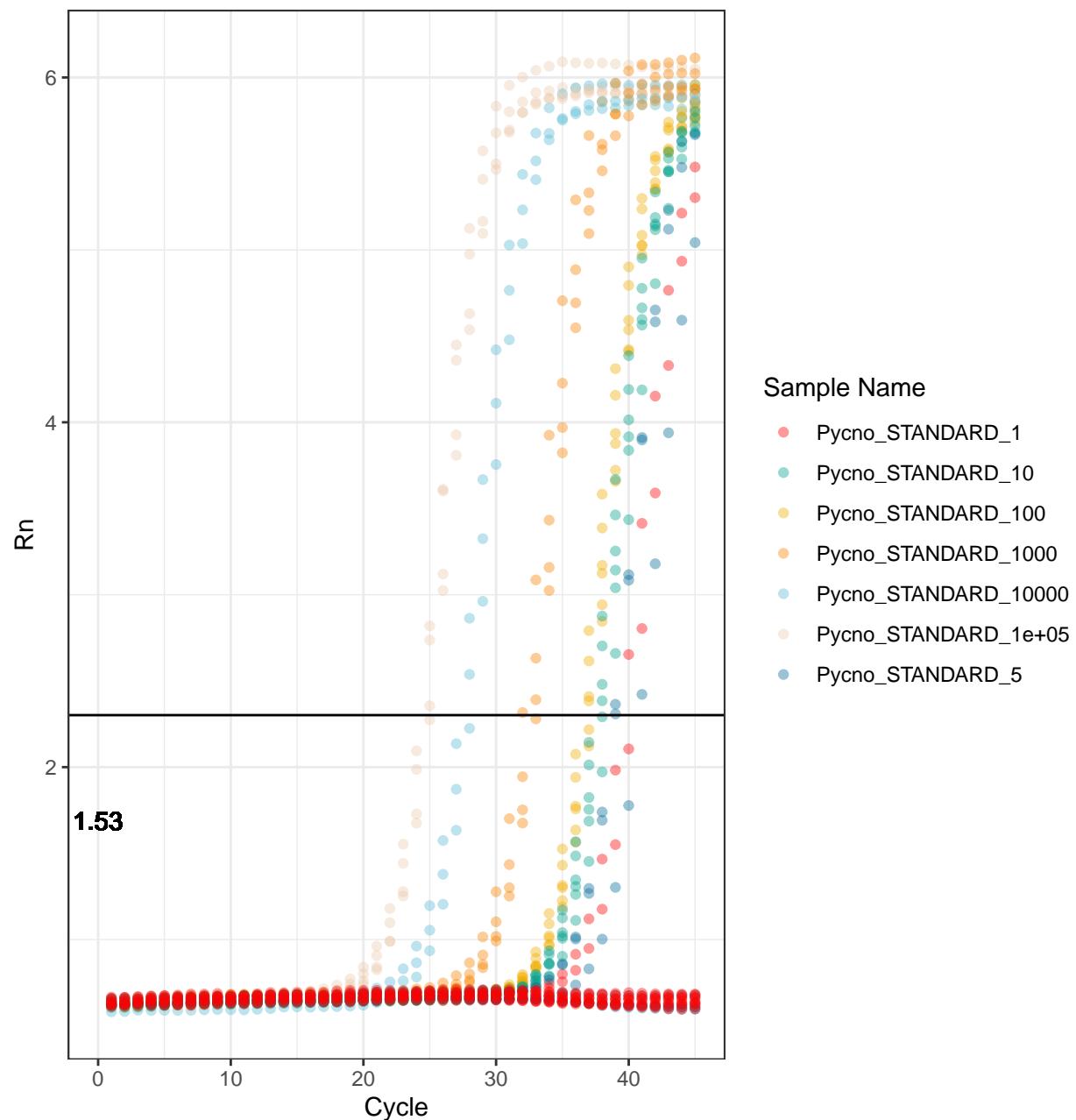
Pycno Rn

```
combined_qPCR_data_run1 %>%
filter(., str_detect(`Sample Name`,"Pycno_STANDARD")) -> pycno_standard_run1

pycno_standard_run1$Rn %>% max() -> max_Rn_run1
h_run1= max_Rn_run1*0.25

pycno_standard_run1 %>%
ggplot(., aes(y=Rn, x=Cycle, color=`Sample Name`)) +geom_point(alpha=0.4) + geom_hline(yintercept =h) +
  geom_text(aes(0,h_run1,label = round(h_run1,2), vjust = -1),color="black") +theme_bw() + ggtitle("Pycno Rn")
```

Pycno Rn



```
pycno_standard_run1 %>%
  mutate(., Threshold = if_else(Rn > h, "Above", "Below")) -> pycno_standard_run1

pycno_standard_run1 %>%
  group_by(`Well Position`, `Sample Name`) %>%
  filter(., rank(Threshold, ties.method="first") == 1) %>%
  filter(., Threshold == "Above") -> pycno_standard_above_run1
```

```

pycno_standard_run1 %>%
  ungroup() %>%
  filter(., !`Well Position` %in% pycno_standard_above$`Well Position`) %>%
  group_by(`Well Position`, `Sample Name`) %>%
  filter(., rank(Threshold, ties.method="last") == 1) -> pycno_standard_below_run1

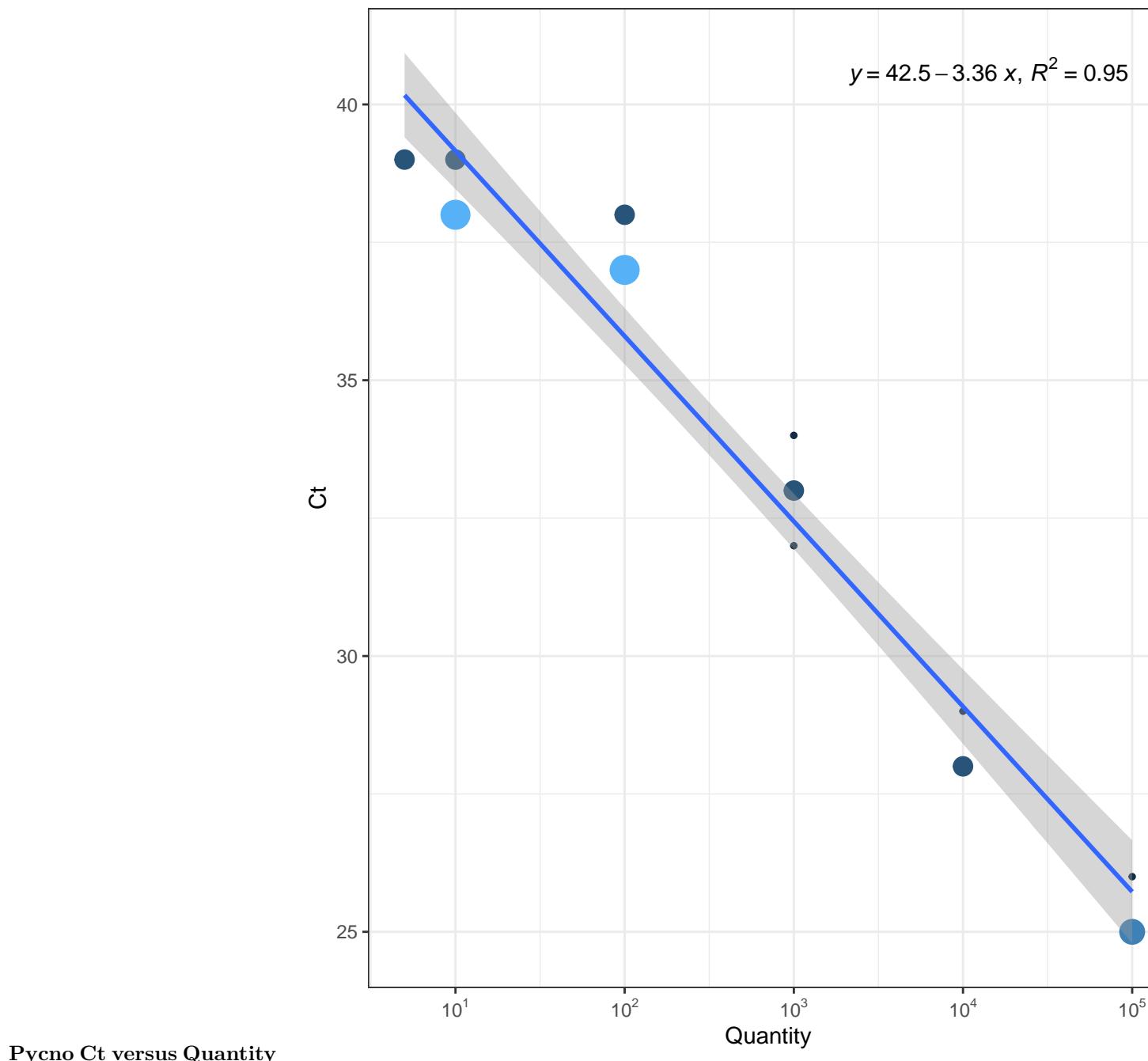
bind_rows(pycno_standard_above_run1, pycno_standard_below_run1) -> pycno_standard_threshold_run1

set_breaks = function(limits) {
  seq(limits[1], limits[2], by = 1)
}

pycno_standard_threshold_run1 %>%
  filter(., Cycle < 40 ) %>%
  ggplot(., aes(x=`Quantity`, y = Cycle)) +geom_count(aes(color = ..n.., size = ..n..)) +
  scale_x_log10("Quantity",
    breaks = trans_breaks("log10", function(x) 10^x),
    labels = trans_format("log10", math_format(10^.x))) +
  guides(color = 'legend') +
  scale_size_continuous(breaks = set_breaks)+
  scale_color_continuous(breaks = set_breaks)+
  stat_poly_line() +
  stat_poly_eq(use_label(c("eq", "R2")),label.x = "right",
  label.y = "top") +theme_bw() +ylab("Ct") -> run1_ct_versus_quant

run1_ct_versus_quant

```



```
# View the structure of the generated plot object
# The computed data is stored within the plot object
grob_data_run1 <- ggplot_build(run1_ct_vs_quant)$data[[3]]

# The result is a list; unlist it to get a character vector
coef_char_run1 <- unlist(grob_data_run1$coefs)

standard_curve_coefs_run1 <- enframe(coef_char_run1, name = "Metric", value = "Value") %>%
  mutate( Metric = recode(Metric,
    "(Intercept)" = "Intercept",
    "log(Quantity)" = "Slope"))
```

```

        "x" = "Slope"))

# Extract the R^2 value from the 'r.squared' column
r_squared_value_run1 <- grob_data_run1$r.squared

```

Merge Sample Metadata

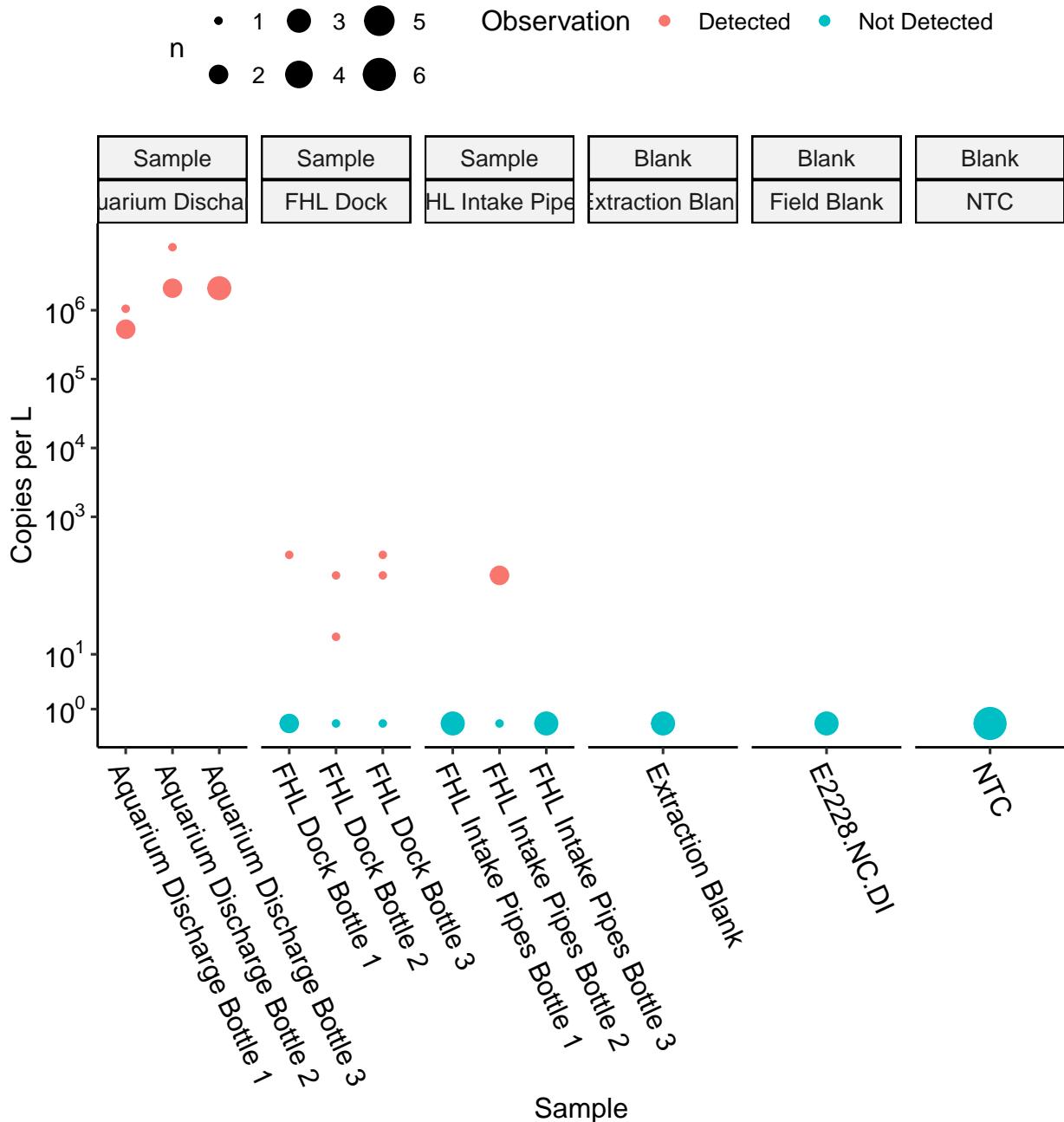
```

sample_metadata<- read_excel(here("qPCR_raw_data","ElectronicArchive_CruiseSampleMetadata_20250516_FHL.."))

combined_qPCR_data_run1 %>%
  filter(., `Target Name`=="Pycno") %>%
  filter(., !str_detect(`Task`,"STANDARD")) %>%
  mutate(., Threshold = if_else(Rn > h, "Above","Below")) %>%
  group_by(`Well Position`, `Sample Name`) %>%
  filter(., rank(Threshold, ties.method="first")==1) %>%
  mutate(., Quantity= if_else(Threshold=="Above",10^((Cycle -standard_curve_coefs_run1$Value[[1]])/standard_curve_coefs_run1$Value[[2]]),if_else(is.na(Quantity), 0, Quantity))) %>%
  mutate(., Concentration = Quantity*100/2 * 1) %>%
  mutate(., Observation = if_else(Quantity ==0, "Not Detected", "Detected")) %>%
  mutate(., `Sample Name` = if_else(str_detect(`Sample Name`,"Negative Control"), "NTC", `Sample Name`)) %>%
  ungroup() %>%
  left_join(sample_metadata, by=c("Sample Name"="Field_Name")) %>%
  mutate(., `Sample Name` = if_else(`Sample Name`=="Ext_blank_UWFH25","Extraction Blank",`Sample Name`)) %>%
  mutate(., Negative_control = if_else(is.na(Negative_control), TRUE, Negative_control)) %>%
  group_by(`Sample Name`) %>%
  mutate(Technical_Replicate = row_number(`Sample Name`)) %>%
  mutate(., Station = if_else(is.na(Station) & Negative_control==TRUE | Task=="NTC", `Sample Name`, Station)) %>%
  mutate(., Name_plotting = str_c(Station," Bottle ", Biological_Replicate," PCR ",Technical_Replicate)) %>%
  mutate(., Name_plotting = if_else(is.na(Name_plotting),str_c(`Sample Name`, " PCR ",Technical_Replicate)) %>%
    mutate(., Name_plotting_b = str_c(Station," Bottle ", Biological_Replicate)) %>%
  mutate(., Name_plotting_b = if_else(is.na(Name_plotting_b),`Sample Name`,Name_plotting_b)) %>%
  mutate(., Type = if_else(is.na(Station),"Extraction Blank",Station )) %>%
  mutate(., Type = if_else(str_detect(Type,".NC"),"Field Blank",Type )) %>%
  mutate(., Sample_Type = if_else(Negative_control==FALSE,"Sample","Blank")) -> pycno_sample_data_4_plotting

pycno_sample_data_4_plotting_run1 %>%
  mutate(across(Sample_Type, ~factor(., levels=c("Sample","Blank")))) %>%
  ggplot(., aes(x=Name_plotting_b, y=Concentration, color=Observation)) + geom_count() +
  scale_y_continuous(
    trans = scales::pseudo_log_trans(base = 10),
    breaks = c(1,10,1000,10000,100000,1000000),
    labels = scales::trans_format("log10", scales::math_format(10^.x))
  ) + theme_pubr() + theme(axis.text.x = element_text(angle = -65, vjust = 1, hjust=0)) + facet_wrap(~

```



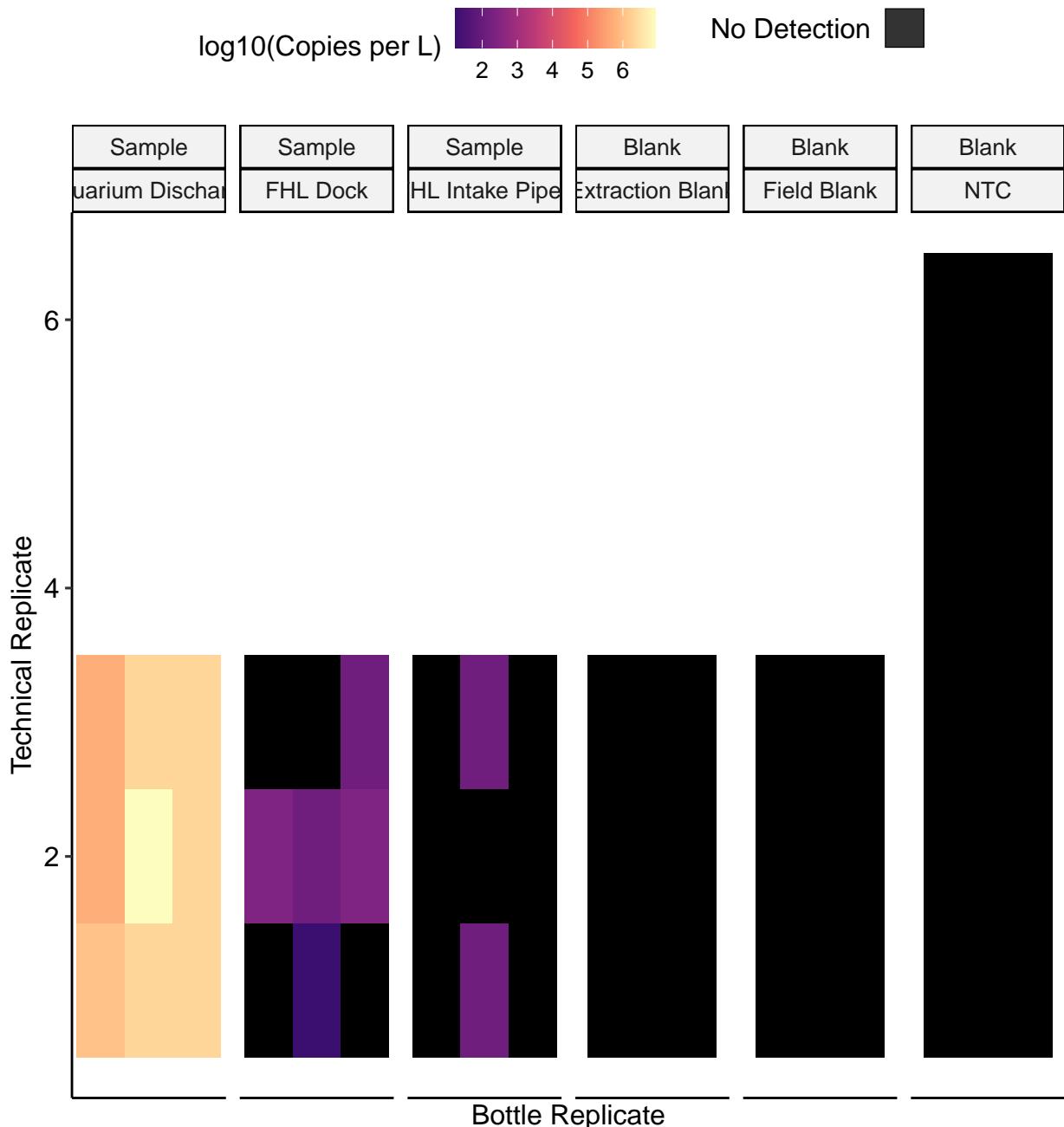
```
# pycno_sample_data_4_plotting_run1 %>%
#   filter(., str_detect(Name_plotting_b, "Aquarium")) %>%
#   group_by(`Target Name`) %>%
#   dplyr::summarise(mean(Concentration))
```

```
pycno_sample_data_4_plotting_run1 %>%
  mutate(across(Sample_Type, ~factor(., levels=c("Sample", "Blank")))) %>%
  group_by(Name_plotting_b) %>%
  mutate(., Replicate = row_number()) %>%
  ungroup() %>%
```

```

mutate(., Concentration=if_else(Concentration==0,NA,Concentration) ) %>%
ggplot(., aes(x=Name_plotting_b, y=Replicate, fill=log10(Concentration), color="")) + geom_tile() + theme(
  guides(colour=guide_legend("No Detection", override.aes=list(colour="black")))) +
  labs(fill = "log10(Copies per L)")

```



Here we see ~2 million copies per L of *Pycnopodia* target DNA in the Friday Harbor Aquarium discharge water. The discharge is from a flow through system with 39 individual stars of at least 40 cm in diameter.

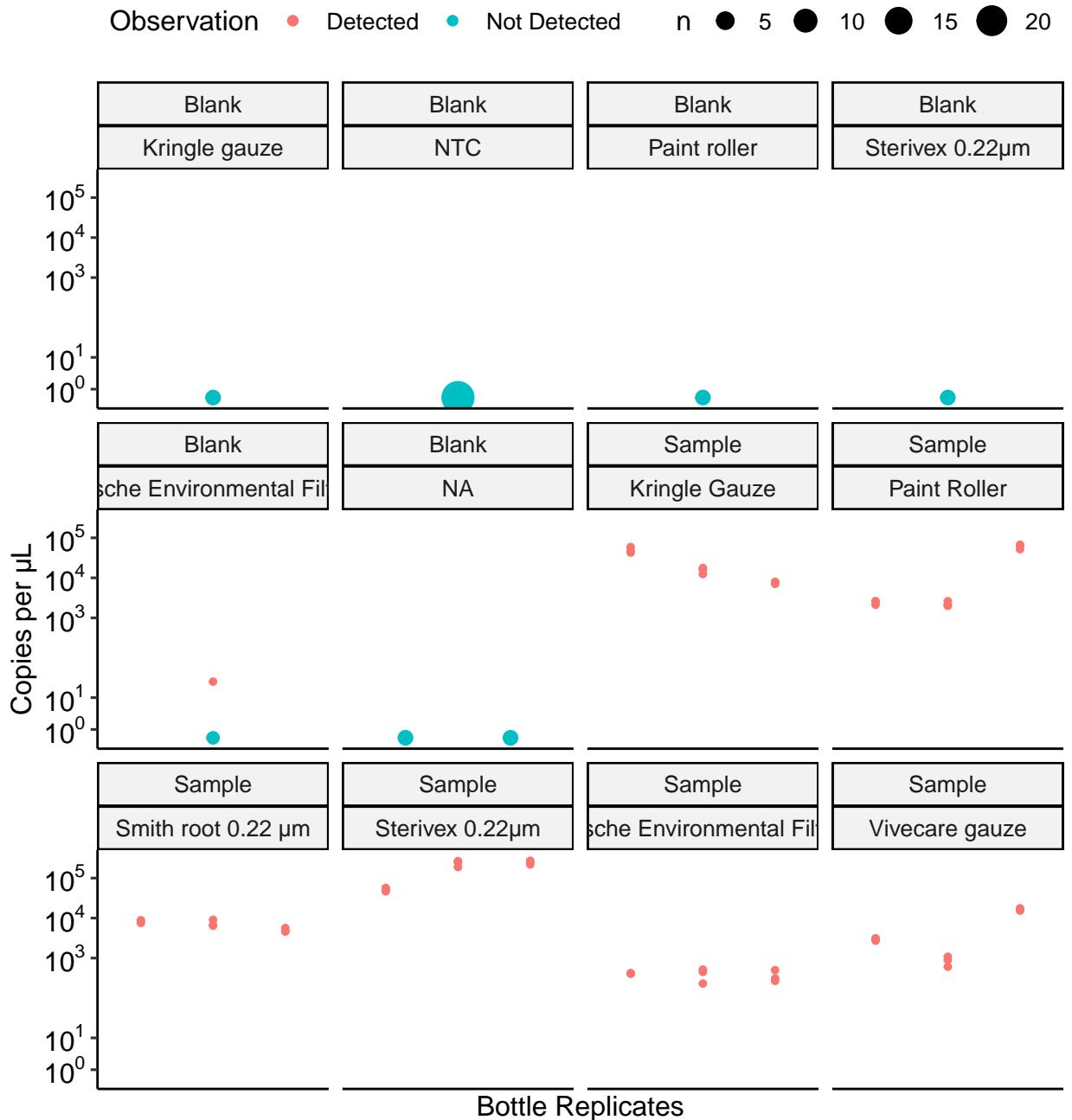
We were able to detect the 10 transplanted stars from at least one technical replicate from each of the three surface eDNA samples taken ~5m above the individuals. We only detected a single individual located at 10m depth from one of three surface samples taken at the Friday Harbor Lab intake pipes.

These results demonstrate that the eDNA qPCR assay can detect Pycnopodia from both mesocosms and the field.

FHL Analysis Passive Versus Active Filtration

```
results_data_cleaned %>%
  filter(., `Target Name`=="Pycno") %>%
  filter(., !str_detect(`Task`,"STANDARD")) %>%
  mutate(., `Sample Name`=str_replace(`Sample Name`,".", "."))
  left_join(sample_metadata, by=c("Sample Name"="Sample_Name")) %>%
  group_by(`Sample Name`) %>%
  mutate(., Negative_control = if_else(is.na(Negative_control), TRUE, Negative_control)) %>%
  mutate(Technical_Replicate = row_number(`Sample Name`)) %>%
  mutate(., Station = if_else(is.na(Station) & Negative_control=="TRUE" | Task=="NTC", `Sample Name`, Sample_Name))
  mutate(., Name_plotting = str_c(Station, " ", Field_collection_method, " ", Biological_Replicate, " ", Technical_Replicate))
  mutate(., Name_plotting = if_else(is.na(Name_plotting),str_c(`Sample Name`, " ", Technical_Replicate),Name_plotting))
  mutate(., Name_plotting_b = str_c(Station, " ", Field_collection_method, " ", Biological_Replicate)) %>%
  mutate(., Name_plotting_b = if_else(is.na(Name_plotting_b),`Sample Name`,Name_plotting_b)) %>%
  mutate(., Quantity=if_else(is.na(Quantity), 0, Quantity)) %>%
  mutate(., Concentration_L = Quantity*100/2 * 1) %>%
  mutate(., Observation = if_else(Quantity ==0, "Not Detected", "Detected")) %>%
  mutate(., Type = if_else(is.na(Station),"Extraction Blank",Station )) %>%
  mutate(., Type = if_else(str_detect(Type, ".NC"),"Field Blank",Type )) %>%  mutate(., Sample_Type = if_else(Type=="Extraction Blank", "Extraction Blank", "Field Blank"))
  mutate(., Field_collection_method = if_else(Task=="NTC","NTC",Field_collection_method)) %>%
  mutate(., Field_collection_method = if_else(Type=="Extraction Blank","Extraction Blank",Field_collection_method))

pycno_sample_data_4_plotting %>%
  filter(., !str_detect(Name_plotting_b, "FHL")) %>%
  ggplot(., aes(x=Name_plotting_b, y=Concentration_L, color=Observation)) + geom_count() +
  scale_y_continuous(
    trans = scales::pseudo_log_trans(base = 10),
    breaks = c(1,10,1000,10000,100000,1000000,10000000,100000000),
    labels = scales::trans_format("log10", scales::math_format(10^.x)))
  ) +theme_pubr() + theme(axis.text.x = element_text(angle = -65, vjust = 1, hjust=-0.05)) + facet_wrap(~
```



We had minor contamination in the passive filter. It's 2 orders of magnitude less than any of the samples so not a major concern. Currently not adjusting the values here, but could subtract this value from all observed positive results.

```
pycno_sample_data_4_plotting %>%
  filter(., !str_detect(Name_plotting_b, "FHL")) %>%
  filter(., Sample_Type!="Blank") %>%
  mutate(., Method= case_when(str_detect(Field_collection_method, "Sterivex")~"Active Filtration", str_detect(Field_collection_method, "Vivecare gauze")~"Passive Filtration", TRUE~"Passive Filtration" )) %> pycno_sample_data_4_plotting_fm
```

Kruskal Wallace test

```

# Perform one-way ANOVA
kw_test_results <- kruskal.test(Concentration_L ~ Field_collection_method, pycno_sample_data_4_plotting_fm)

# Perform post-hoc Tukey HSD test
pairwise.wilcox.test(pycno_sample_data_4_plotting_fm$Concentration_L, pycno_sample_data_4_plotting_fm$Field_collection_method,
                      p.adjust.method = "bonferroni") -> pairwise_results

full_p_matrix <- fullPTable(pairwise_results$p.value)
multcompLetters(full_p_matrix) -> cld

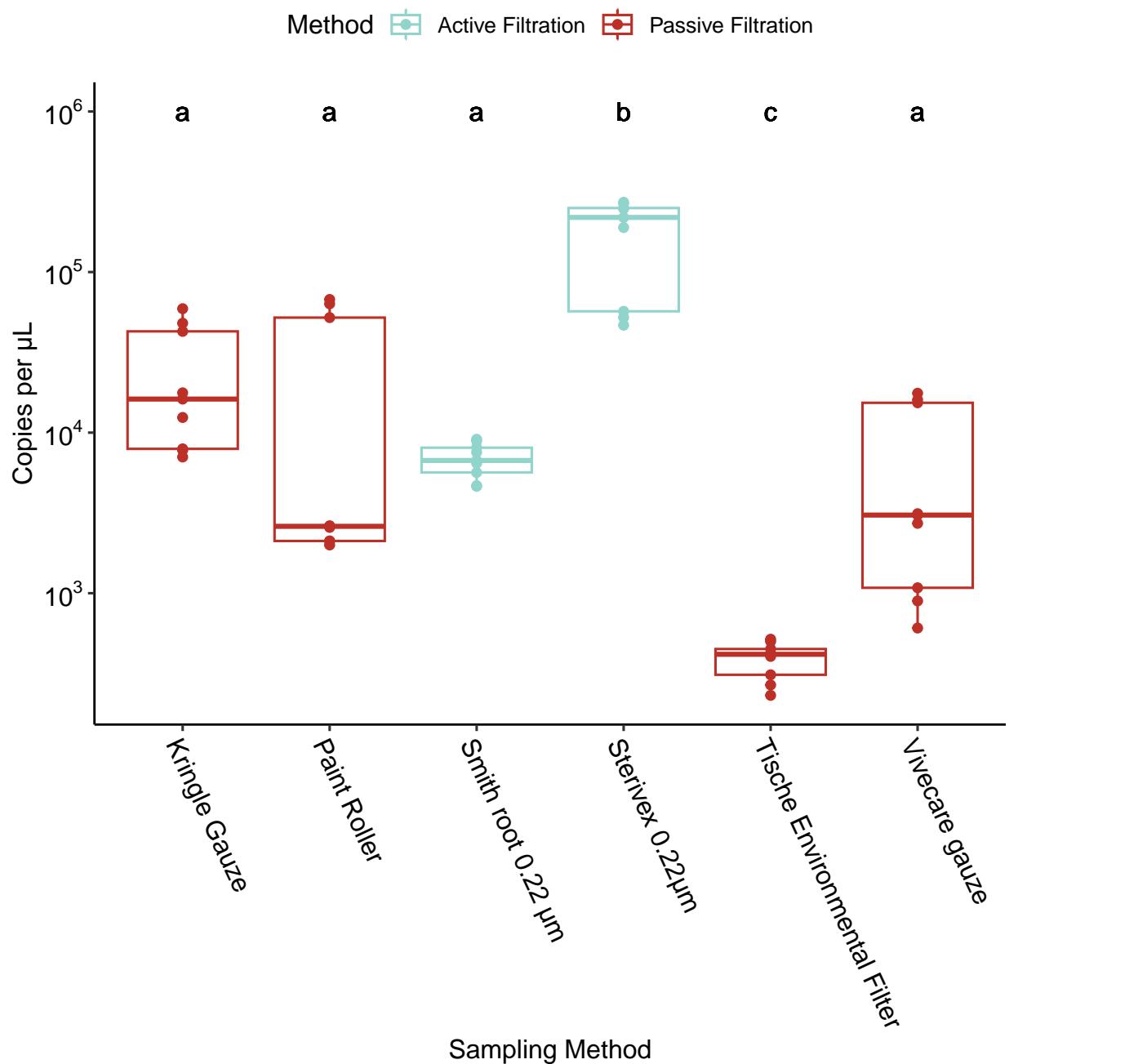
cld_tibble_optionA <- as_tibble(cld$Letters, rownames = "group") %>%
  dplyr::select(Field_collection_method=group, Letters=value)

pairwise_results

## 
##  Pairwise comparisons using Wilcoxon rank sum exact test
##
## data: pycno_sample_data_4_plotting_fm$Concentration_L and pycno_sample_data_4_plotting_fm$Field_collection_method
## 
##          Kringle Gauze Paint Roller Smith root 0.22 µm
## Paint Roller           1.00000   -      - 
## Smith root 0.22 µm    0.08453   1.00000   - 
## Sterivex 0.22µm       0.00740   0.02777   0.00062 
## Tische Environmental Filter 0.00062   0.00062   0.00062 
## Vivecare gauze        0.21288   1.00000   1.00000 
## 
##          Sterivex 0.22µm Tische Environmental Filter
## Paint Roller           -          - 
## Smith root 0.22 µm    -          - 
## Sterivex 0.22µm       -          - 
## Tische Environmental Filter 0.00062   - 
## Vivecare gauze        0.00062   0.00062 
## 
## P value adjustment method: bonferroni

pycno_sample_data_4_plotting_fm %>%
  left_join(cld_tibble_optionA) %>%
  ggplot(., aes(x=Field_collection_method, y=Concentration_L)) +
  geom_boxplot(aes(color=Method)) +
  geom_point(aes(color=Method)) +
  scale_y_continuous(
    trans = scales::pseudo_log_trans(base = 10),
    breaks = c(1,10,1000,10000,100000,1000000,10000000,100000000),
    labels = scales::trans_format("log10", scales::math_format(10^.x)))
  ) +theme_pubr() +theme(axis.text.x = element_text(angle = -65, vjust = 1, hjust=0)) + ylab("Copies")

```



```
pycno_sample_data_4_plotting %>
  filter(., !str_detect(Name_plotting_b, "FHL")) %>
  filter(., Sample_Type!="Blank") %>
  mutate(., Method= case_when(str_detect(Field_collection_method, "Sterivex")~"Active Filtration", str_detect(Field_collection_method, "Tische Environmental Filter")~"Passive Filtration" )) %>
  group_by(Field_collection_method, Method) %>
  dplyr::summarize(Max=max(Concentration_L), Min=min(Concentration_L), Mean=mean(Concentration_L), median=median(Concentration_L), IQR = IQR(Concentration_L)) %>% left_join(cld_tibble_optionA) %>% kable(digits=2)
```

Field_collection_method	Method		Max	Min	Mean	median	IQR	Letters
Kringle Gauze	Passive Filtration		59142.98	7050.73	24327.36	16182.52	34791.46	a
Paint Roller	Passive Filtration		67489.01	1996.79	21888.52	2608.77	49932.24	a
Smith root 0.22 μm	Active Filtration		9090.73	4630.68	6851.24	6709.28	2415.34	a
Sterivex 0.22 μm	Active Filtration		272054.76	46670.11	177984.78	218896.17	193436.08	b
Tische Environmental Filter	Passive Filtration		516.85	231.09	390.68	416.38	138.75	c
Vivec care gauze	Passive Filtration		17594.03	606.86	6715.40	3066.18	14260.65	a

Here we see that Sterivex 0.22 μm filters captured nearly an order of magnitude more Pycnopodia DNA than either Smith Root active filters or any of the passive filters deployed here. This is notable given that Sterivex have a smaller surface area (10cm^2) than the Smith Root 47mm filter (17.3 cm^2) and suggests that differences in the housing or sample integrity are driving these apparent differences. These results highlight that passive filters can be used for field applications to detect Pycnopodia, albeit at a significant disadvantage in that even the best performing materials captured less than a 10th of the DNA captured by active sterivex filtration methods.