

# Probe Efficiency and Single Spore Analysis

2025-08-04

```
#Load necessary libraries  
library(readxl)
```

```
## Warning: package 'readxl' was built under R version 4.3.3
```

```
library(ggplot2)
```

```
## Warning: package 'ggplot2' was built under R version 4.3.3
```

```
library(dplyr)
```

```
## Warning: package 'dplyr' was built under R version 4.3.3
```

```
##
```

```
## 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(cowplot)
```

```
## Warning: package 'cowplot' was built under R version 4.3.3
```

```
library(rstatix)
```

```
## Warning: package 'rstatix' was built under R version 4.3.3
```

```
##
```

```
## Attaching package: 'rstatix'
```

```
## The following object is masked from 'package:stats':
```

```
##
```

```
##   filter
```

Load in the dataset (probe efficiency tests)

*#Read each Excel file into a separate dataframe*

```
df_197198 <- read_excel(file.path(
  "C:/Users/Joyalea/Documents/UBCO/Thesis",
  "Final_Files",
  "RQ1_AMFAbundance",
  "197198_SyntheticDNA.xlsx"
))
df_240448 <- read_excel(file.path(
  "C:/Users/Joyalea/Documents/UBCO/Thesis",
  "Final_Files",
  "RQ1_AMFAbundance",
  "240448_SyntheticDNA.xlsx"
))
df_240720 <- read_excel(file.path(
  "C:/Users/Joyalea/Documents/UBCO/Thesis",
  "Final_Files",
  "RQ1_AMFAbundance",
  "240720_SyntheticDNA.xlsx"
))
summary(df_197198)
```

```
##      Well      ExptType      Experiment      Sample
## Length:21      Length:21      Mode:logical      Length:21
## Class :character      Class :character      NA's:21      Class :character
## Mode  :character      Mode  :character                      Mode  :character
##
##
##      TargetType      Target      Status      Concentration
## Length:21      Length:21      Length:21      Min.   : 0.000
## Class :character      Class :character      Class :character      1st Qu.: 0.330
## Mode  :character      Mode  :character      Mode  :character      Median : 4.000
##                                          Mean   : 6.538
##                                          3rd Qu.:12.300
##                                          Max.   :18.300
##      CopiesPer20uLWell      Positives      Negatives      AcceptedDroplets
## Min.   : 0.0      Min.   : 0.00      Min.   :15685      Min.   :15850
## 1st Qu.: 6.6      1st Qu.: 5.00      1st Qu.:17755      1st Qu.:17800
## Median : 80.0      Median : 63.00      Median :17992      Median :18140
## Mean   :130.8      Mean   : 99.52      Mean   :18028      Mean   :18127
## 3rd Qu.:246.0      3rd Qu.:186.00      3rd Qu.:18638      3rd Qu.:18725
## Max.   :366.0      Max.   :279.00      Max.   :18975      Max.   :19217
```

```
summary(df_240448)
```

```
##      Well      ExptType      Experiment      Sample
## Length:21      Length:21      Mode:logical      Length:21
## Class :character      Class :character      NA's:21      Class :character
## Mode  :character      Mode  :character                      Mode  :character
##
##
##
```

```
## TargetType      Target      Status      Concentration
## Length:21      Length:21      Length:21      Min.   : 0.000
## Class :character Class :character Class :character 1st Qu.: 0.120
## Mode  :character Mode  :character Mode  :character Median : 1.200
##                                         Mean  : 5.807
##                                         3rd Qu.: 7.400
##                                         Max.   :21.000
## CopiesPer20uLWell
## Min.   : 0.0
## 1st Qu.: 2.4
## Median : 24.0
## Mean   :116.1
## 3rd Qu.:148.0
## Max.   :420.0
```

```
summary(df_240720)
```

```
## Well      ExptType      Experiment      Sample
## Length:21 Length:21      Mode:logical Length:21
## Class :character Class :character NA's:21      Class :character
## Mode  :character Mode  :character      Mode  :character
##
##
## TargetType      Target      Status      Concentration
## Length:21      Length:21      Length:21      Min.   : 0.00
## Class :character Class :character Class :character 1st Qu.: 0.46
## Mode  :character Mode  :character Mode  :character Median : 5.10
##                                         Mean  :16.40
##                                         3rd Qu.: 7.40
##                                         Max.   :71.30
## CopiesPer20uLWell
## Min.   : 0.0
## 1st Qu.: 9.2
## Median :102.0
## Mean   : 328.0
## 3rd Qu.:148.0
## Max.   :1426.0
```

```
#Combine the datasets
df_197198$Primer <- "197198"
df_240448$Primer <- "240448"
df_240720$Primer <- "240720"

#Combine all
df_all <- dplyr::bind_rows(df_197198, df_240448, df_240720)

#Add a new column: Copies_24 = Concentration * 24 [total reaction volume]
df_all <- df_all %>%
  mutate(Copies_24 = Concentration * 24)

summary(df_all)
```

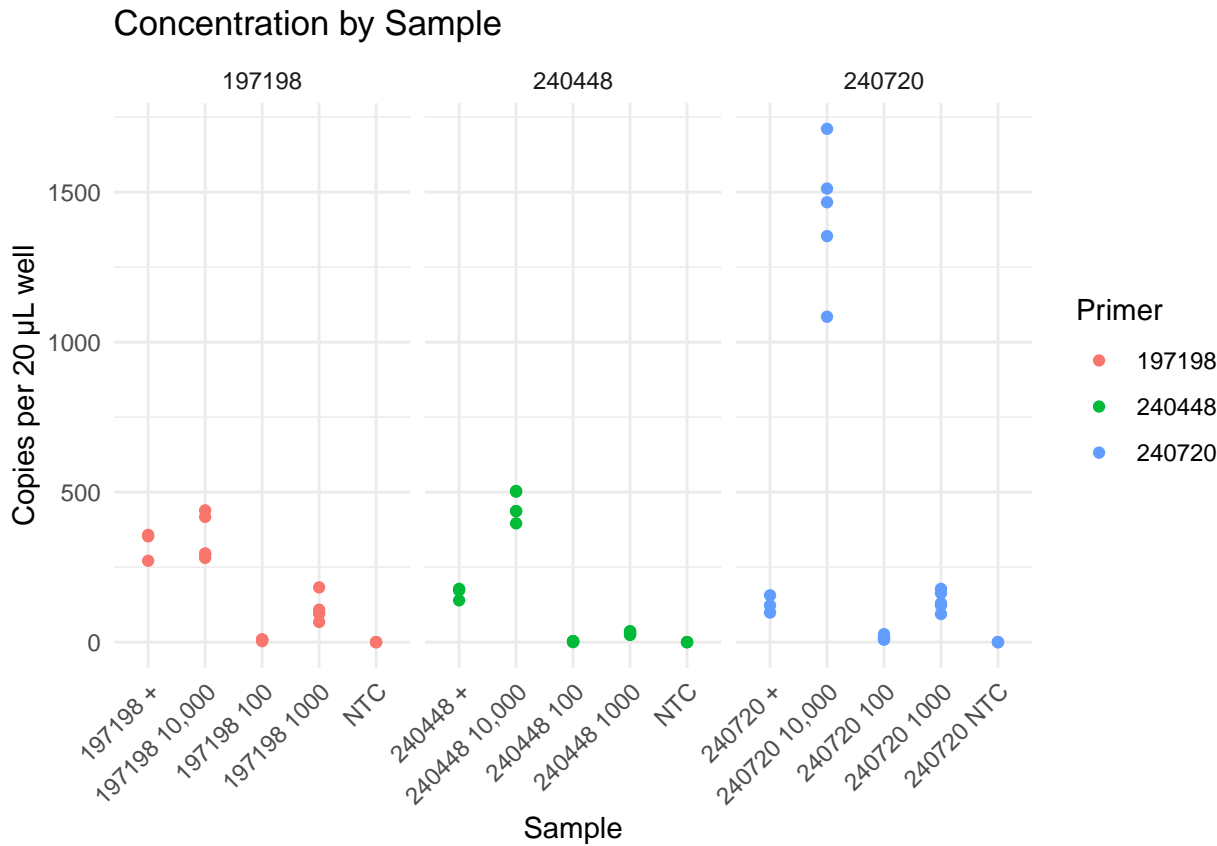
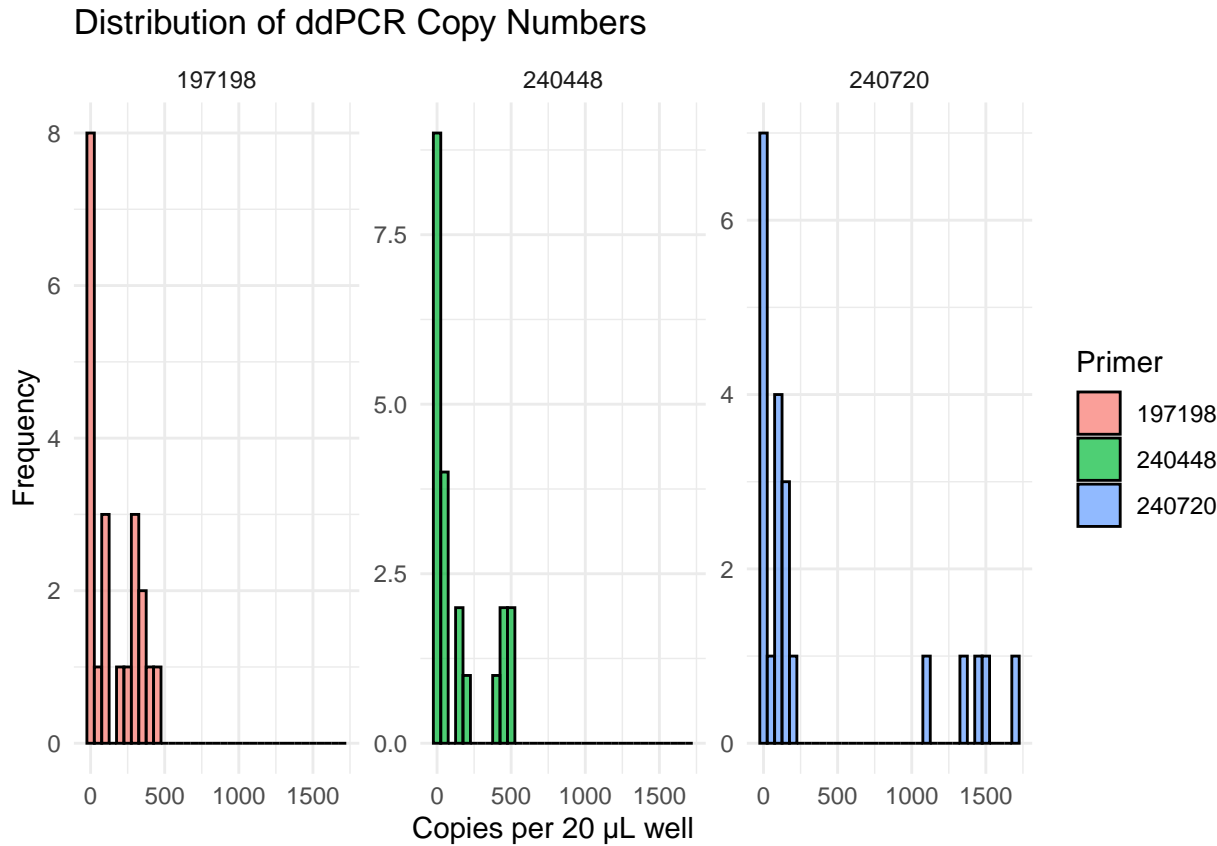
```
## Well      ExptType      Experiment      Sample
```

```

## Length:63      Length:63      Mode:logical     Length:63
## Class :character Class :character NA's:63           Class :character
## Mode  :character Mode  :character           Mode  :character
##
##
##
## TargetType      Target      Status      Concentration
## Length:63      Length:63      Length:63      Min.   : 0.000
## Class :character Class :character Class :character 1st Qu.: 0.325
## Mode  :character Mode  :character Mode  :character Median : 4.000
##                                           Mean  : 9.582
##                                           3rd Qu.:12.000
##                                           Max.   :71.300
##
## CopiesPer20uLWell Positives      Negatives      AcceptedDroplets
## Min.   : 0.0      Min.   : 0.00      Min.   :15685      Min.   :15850
## 1st Qu.: 6.5      1st Qu.: 5.00      1st Qu.:17755      1st Qu.:17800
## Median : 80.0      Median : 63.00      Median :17992      Median :18140
## Mean   : 191.6      Mean   : 99.52      Mean   :18028      Mean   :18127
## 3rd Qu.: 240.0      3rd Qu.:186.00      3rd Qu.:18638      3rd Qu.:18725
## Max.   :1426.0      Max.   :279.00      Max.   :18975      Max.   :19217
##                                           NA's    :42      NA's    :42
## Primer          Copies_24
## Length:63      Min.   : 0.0
## Class :character 1st Qu.: 7.8
## Mode  :character Median : 96.0
##                                           Mean   : 230.0
##                                           3rd Qu.: 288.0
##                                           Max.   :1711.2
##
##

```

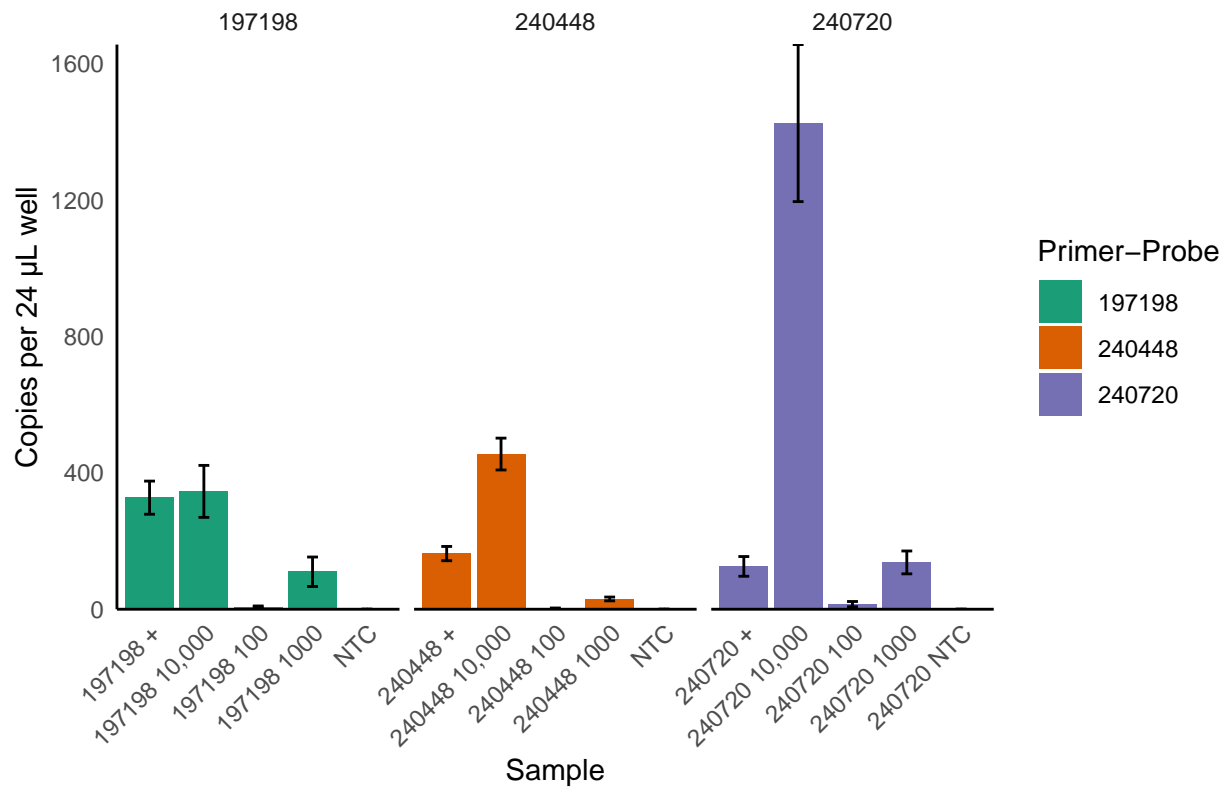
Visualise probe efficiencies



```
## 'summarise()' has grouped output by 'Primer'. You can override using the
## '.groups' argument.
```

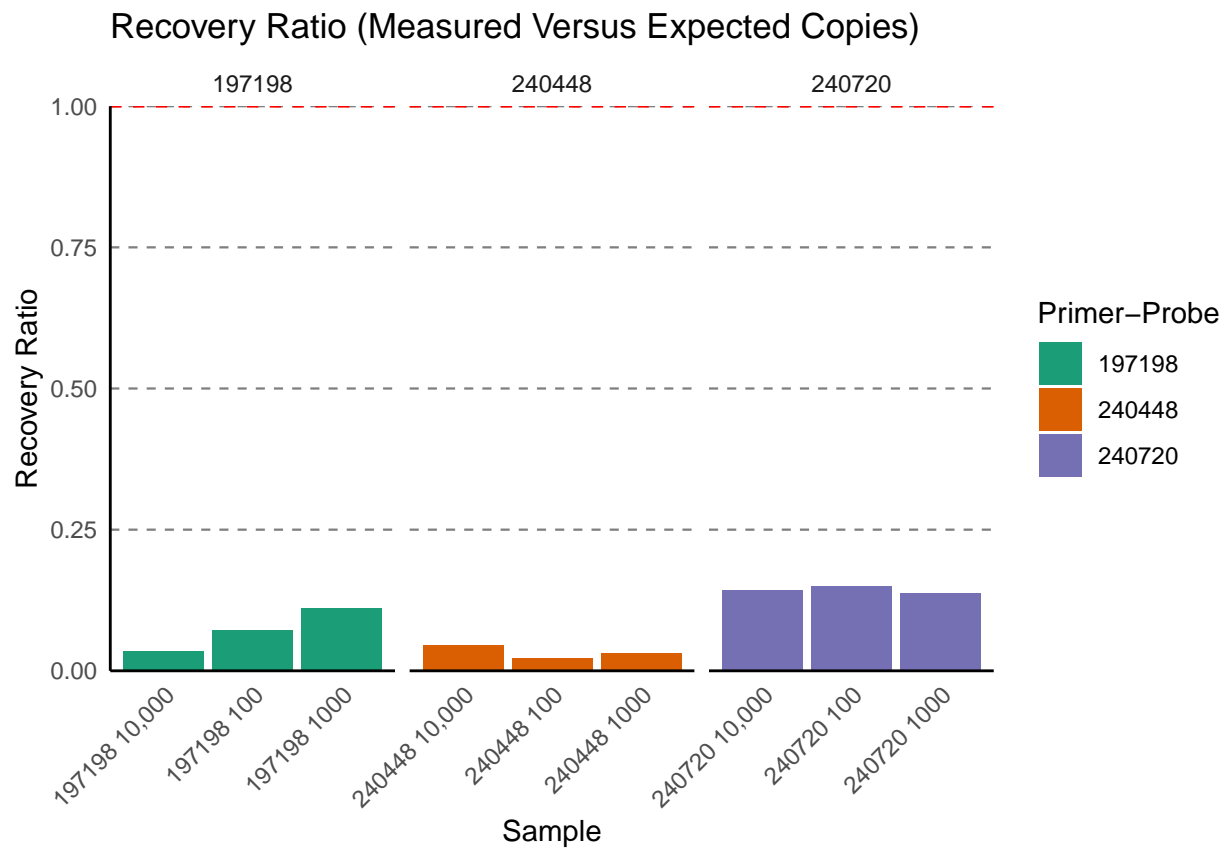
```
## # A tibble: 15 x 6
##   Primer Sample      mean_copies sd_copies      n cv_percent
##   <chr>  <chr>          <dbl>      <dbl> <int>    <dbl>
## 1 197198 197198 +          327.        48.6      3     14.8
## 2 197198 197198 10,000      346.        76.2      5     22.0
## 3 197198 197198 100         7.2         2.35      5     32.6
## 4 197198 197198 1000       110.        43.2      5     39.3
## 5 197198 NTC              0           0        3      NaN
## 6 240448 240448 +          163.        20.9      3     12.8
## 7 240448 240448 10,000     455.        46.7      5     10.3
## 8 240448 240448 100        2.16         1.42      5     65.7
## 9 240448 240448 1000       30.2         5.59      5     18.5
## 10 240448 NTC              0           0        3      NaN
## 11 240720 240720 +          126.        28.9      3     23.0
## 12 240720 240720 10,000    1426.       230.      5     16.1
## 13 240720 240720 100        15.0         7.67      5     51.1
## 14 240720 240720 1000      137.        33.5      5     24.4
## 15 240720 240720 NTC              0           0        3      NaN
```

Mean ddPCR Copy Number with SD



```
## # A tibble: 15 x 8
##   Primer Sample      mean_copies sd_copies      n cv_percent expected_copies
##   <chr>  <chr>          <dbl>      <dbl> <int>    <dbl>          <dbl>
```

```
## 1 197198 197198 + 327. 48.6 3 14.8 NA
## 2 197198 197198 10,000 346. 76.2 5 22.0 10000
## 3 197198 197198 100 7.2 2.35 5 32.6 100
## 4 197198 197198 1000 110. 43.2 5 39.3 1000
## 5 197198 NTC 0 0 3 NaN 0
## 6 240448 240448 + 163. 20.9 3 12.8 NA
## 7 240448 240448 10,000 455. 46.7 5 10.3 10000
## 8 240448 240448 100 2.16 1.42 5 65.7 100
## 9 240448 240448 1000 30.2 5.59 5 18.5 1000
## 10 240448 NTC 0 0 3 NaN 0
## 11 240720 240720 + 126. 28.9 3 23.0 NA
## 12 240720 240720 10,000 1426. 230. 5 16.1 10000
## 13 240720 240720 100 15.0 7.67 5 51.1 100
## 14 240720 240720 1000 137. 33.5 5 24.4 1000
## 15 240720 240720 NTC 0 0 3 NaN 0
## # i 1 more variable: recovery_ratio <dbl>
```



## Single spore extractions

```
#Upload the dataset
df_spore <-
  read.csv(file.path(
    "C:/Users/Joyalea/Documents/UBCO/Thesis",
```

```

"Final_Files",
"RQ1_AMFAbundance",
"SingleSpore_AllIsolates.csv"))
head(df_spore)

```

##	Well	ExptType	Experiment	Sample	TargetType	Target	
## 1	A01	Absolute Quantification	NA	197198 1	Ch1Positive Control	FAM	
## 2	B01	Absolute Quantification	NA	197198 2	Ch1Positive Control	FAM	
## 3	C01	Absolute Quantification	NA	197198 3	Ch1Positive Control	FAM	
## 4	D01	Absolute Quantification	NA	197198 4	Ch1Positive Control	FAM	
## 5	E01	Absolute Quantification	NA	197198 5	Ch1Positive Control	FAM	
## 6	F01	Absolute Quantification	NA	197198 6	Ch1Positive Control	FAM	
##	Status	Concentration	Supermix	CopiesPer20uLWell			
## 1	Manual	26.2	ddPCR Supermix for Probes (no dUTP)	524			
## 2	Manual	14.8	ddPCR Supermix for Probes (no dUTP)	296			
## 3	Manual	29.4	ddPCR Supermix for Probes (no dUTP)	588			
## 4	Manual	35.7	ddPCR Supermix for Probes (no dUTP)	714			
## 5	Manual	22.8	ddPCR Supermix for Probes (no dUTP)	456			
## 6	Manual	19.9	ddPCR Supermix for Probes (no dUTP)	398			
##	TotalConfMax	TotalConfMin	PoissonConfMax	PoissonConfMin	Positives	Negatives	
## 1	NA	NA	29.1	24.7	323	14347	
## 2	NA	NA	16.7	13.7	214	16958	
## 3	NA	NA	32.3	28.0	413	16298	
## 4	NA	NA	38.7	34.1	525	17053	
## 5	NA	NA	25.3	21.5	324	16551	
## 6	NA	NA	22.3	18.7	274	16042	
##	Ch1.Ch2.	Ch1.Ch2..1	Ch1.Ch2..2	Ch1.Ch2..3	Linkage	AcceptedDroplets	CNV
## 1	NA	NA	NA	NA	NA	14670	NA
## 2	NA	NA	NA	NA	NA	17172	NA
## 3	NA	NA	NA	NA	NA	16711	NA
## 4	NA	NA	NA	NA	NA	17578	NA
## 5	NA	NA	NA	NA	NA	16875	NA
## 6	NA	NA	NA	NA	NA	16316	NA
##	TotalCNVMax	TotalCNVMin	PoissonCNVMax	PoissonCNVMin	ReferenceCopies		
## 1	NA	NA	NA	NA	NA		
## 2	NA	NA	NA	NA	NA		
## 3	NA	NA	NA	NA	NA		
## 4	NA	NA	NA	NA	NA		
## 5	NA	NA	NA	NA	NA		
## 6	NA	NA	NA	NA	NA		
##	UnknownCopies	Ratio	TotalRatioMax	TotalRatioMin	PoissonRatioMax		
## 1	NA	NA	NA	NA	NA		
## 2	NA	NA	NA	NA	NA		
## 3	NA	NA	NA	NA	NA		
## 4	NA	NA	NA	NA	NA		
## 5	NA	NA	NA	NA	NA		
## 6	NA	NA	NA	NA	NA		
##	PoissonRatioMin	FractionalAbundance	TotalFractionalAbundanceMax				
## 1	NA		NA				
## 2	NA		NA				
## 3	NA		NA				
## 4	NA		NA				
## 5	NA		NA				



## 6	NA	NA	NA
##	TotalFractionalAbundanceMin	PoissonFractionalAbundanceMax	
## 1	NA	NA	
## 2	NA	NA	
## 3	NA	NA	
## 4	NA	NA	
## 5	NA	NA	
## 6	NA	NA	
##	PoissonFractionalAbundanceMin	ReferenceAssayNumber	TargetAssayNumber
## 1	NA	1	1
## 2	NA	1	1
## 3	NA	1	1
## 4	NA	1	1
## 5	NA	1	1
## 6	NA	1	1
##	Threshold	MeanAmplitudeofPositives	MeanAmplitudeofNegatives
## 1	7783	9381.3	1234.6
## 2	7783	8583.8	1185.9
## 3	7783	8517.8	1203.8
## 4	7783	8428.8	1181.1
## 5	7783	8398.2	1224.1
## 6	7783	8788.1	1220.8
##	MeanAmplitudeTotal	ExperimentComments	MergedWells TotalConfMax68
## 1	1414.0	NA	NA NA
## 2	1278.1	NA	NA NA
## 3	1384.6	NA	NA NA
## 4	1397.6	NA	NA NA
## 5	1361.9	NA	NA NA
## 6	1347.9	NA	NA NA
##	TotalConfMin68	PoissonConfMax68	PoissonConfMin68 TotalCNVMax68 TotalCNVMin68
## 1	NA	28	0 NA NA
## 2	NA	16	0 NA NA
## 3	NA	31	0 NA NA
## 4	NA	37	0 NA NA
## 5	NA	24	0 NA NA
## 6	NA	21	0 NA NA
##	PoissonCNVMax68	PoissonCNVMin68	TotalRatioMax68 TotalRatioMin68
## 1	NA	NA	NA NA
## 2	NA	NA	NA NA
## 3	NA	NA	NA NA
## 4	NA	NA	NA NA
## 5	NA	NA	NA NA
## 6	NA	NA	NA NA
##	PoissonRatioMax68	PoissonRatioMin68	TotalFractionalAbundanceMax68
## 1	NA	NA	NA
## 2	NA	NA	NA
## 3	NA	NA	NA
## 4	NA	NA	NA
## 5	NA	NA	NA
## 6	NA	NA	NA
##	TotalFractionalAbundanceMin68	PoissonFractionalAbundanceMax68	
## 1	NA	NA	
## 2	NA	NA	
## 3	NA	NA	

```
## 4 NA NA
## 5 NA NA
## 6 NA NA
## PoissonFractionalAbundanceMin68
## 1 NA
## 2 NA
## 3 NA
## 4 NA
## 5 NA
## 6 NA
```

```
#Add Primer column
df_spore <- df_spore %>%
  mutate(
    Primer = case_when(
      grepl("197198", Sample) ~ "197198",
      grepl("240448", Sample) ~ "240448",
      grepl("240720", Sample) ~ "240720",
      TRUE ~ NA_character_
    ),
    Copies_24 = Concentration * 24 # New column
  )

#Convert primer to a factor
df_spore$Primer<-as.factor(df_spore$Primer)

#Calculate summary stats of the primers (MEAN)
summary_stats_spore <- df_spore %>%
  group_by(Primer) %>%
  summarise(
    mean_copies = mean(Copies_24, na.rm = TRUE),
    sd_copies = sd(Copies_24, na.rm = TRUE),
    n = n(),
    cv_percent = (sd_copies / mean_copies) * 100
  ) %>%
  ungroup()

summary_stats_spore
```

```
## # A tibble: 3 x 5
##   Primer mean_copies sd_copies      n cv_percent
##   <fct>      <dbl>    <dbl> <int>    <dbl>
## 1 197198      468.     371.    13      79.3
## 2 240448      616.     438.    13      71.1
## 3 240720      208.     150.    13      71.8
```

```
#Calculate summary stats of the primers
summary_stats_spore_sample <- df_spore %>%
  group_by(Primer, Sample) %>%
  summarise(
    mean_copies = mean(Copies_24, na.rm = TRUE),
    sd_copies = sd(Copies_24, na.rm = TRUE),
    n = n(),
```

```

    cv_percent = (sd_copies / mean_copies) * 100
  ) %>%
  ungroup()

```

## 'summarise()' has grouped output by 'Primer'. You can override using the  
## '.groups' argument.

```

#Calculate summary stats of the primers
summary_stats_spore_sample_median <- df_spore %>%
  group_by(Primer) %>%
  summarise(
    median_copies = median(Copies_24, na.rm = TRUE),
    sd_copies = sd(Copies_24, na.rm = TRUE),
    n = n(),
  ) %>%
  ungroup()
summary_stats_spore_sample_median

```

```

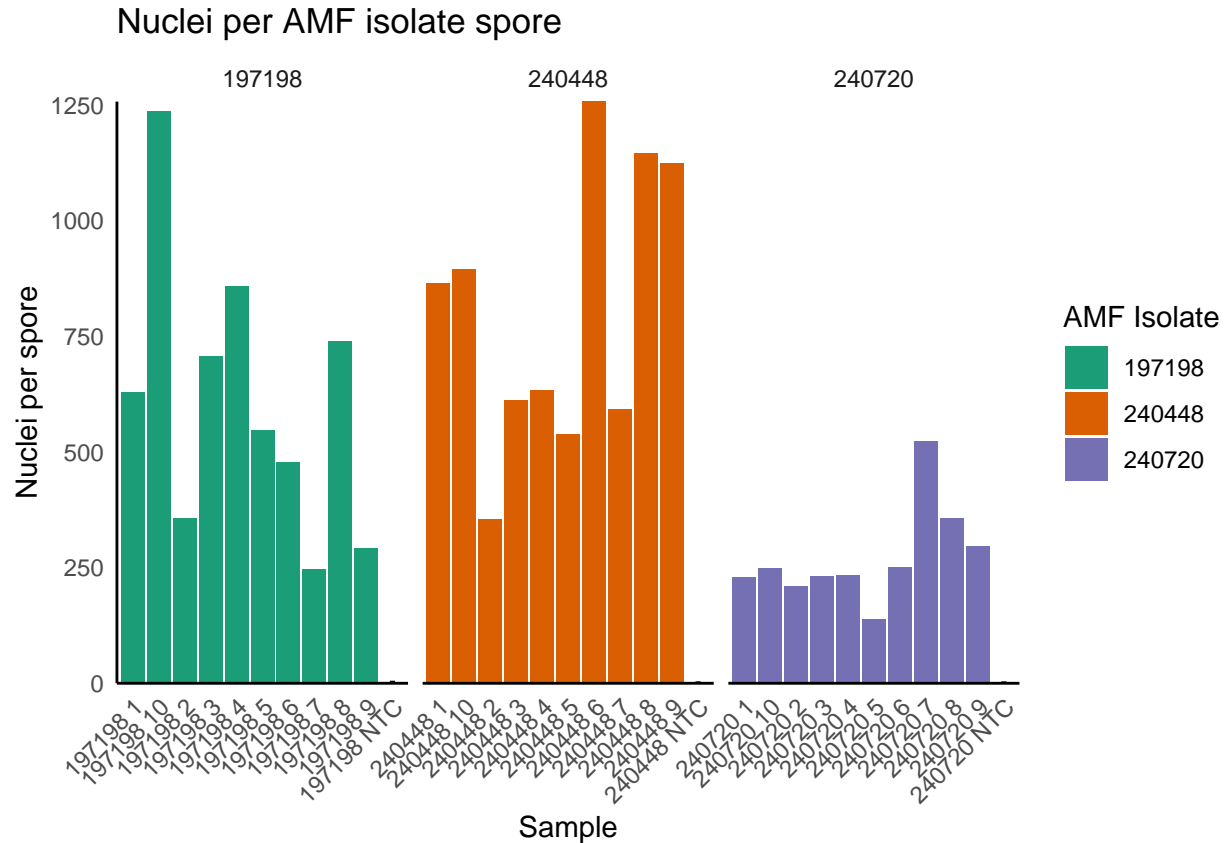
## # A tibble: 3 x 4
##   Primer median_copies sd_copies      n
##   <fct>         <dbl>    <dbl> <int>
## 1 197198         478.     371.    13
## 2 240448         612     438.    13
## 3 240720         230.     150.    13

```

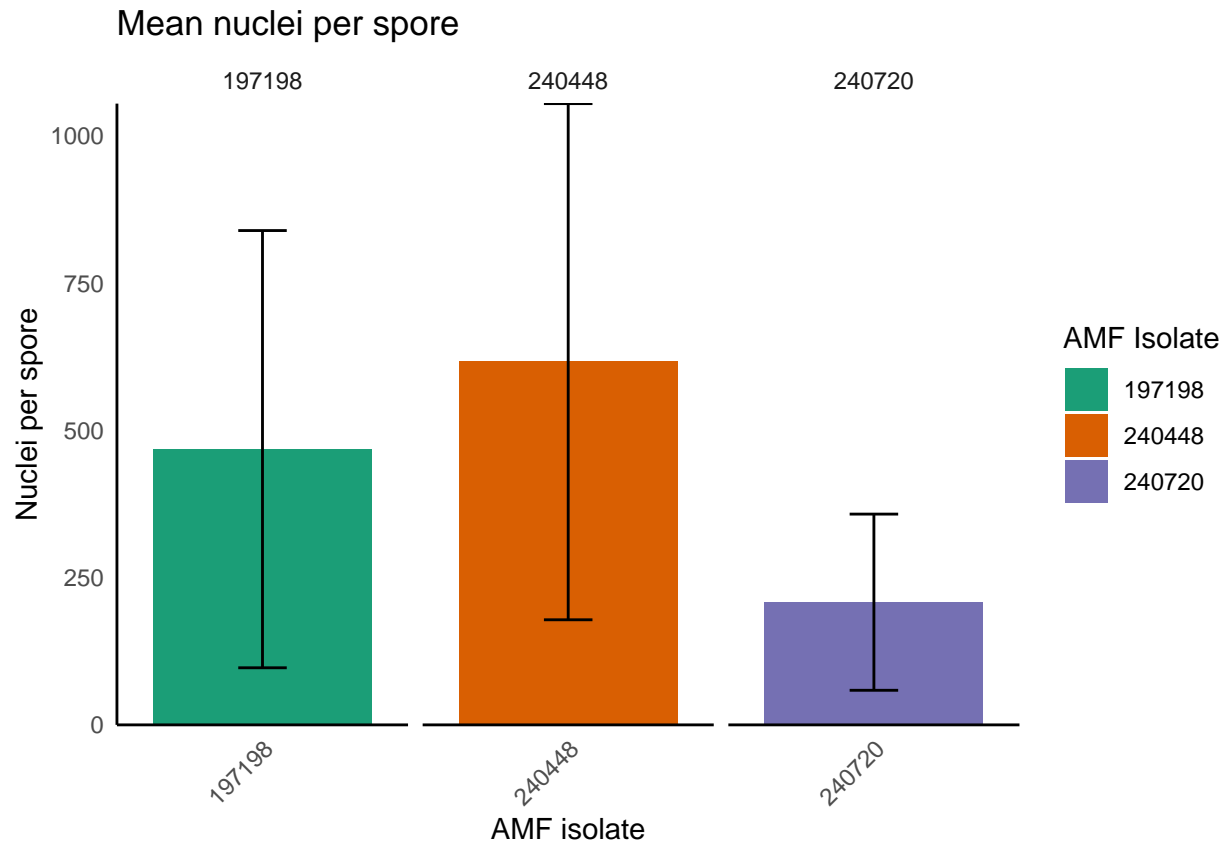
```

#Plot the mean + SD
spores_plot<-ggplot(summary_stats_spore_sample, aes(x = Sample, y = mean_copies, fill = Primer)) +
  geom_col(position = position_dodge(width = 0.9)) +
  geom_errorbar(aes(ymin = mean_copies - sd_copies,
                    ymax = mean_copies + sd_copies),
                width = 0.2,
                position = position_dodge(width = 0.9)) +
  facet_wrap(~ Primer, scales = "free_x") +
  labs(
    title = "Nuclei per AMF isolate spore",
    x = "Sample",
    y = "Nuclei per spore"
  ) +
  scale_fill_brewer(palette = "Dark2", name = "AMF Isolate") +
  scale_y_continuous(expand = c(0, 0)) +
  theme_minimal() +
  theme(
    axis.text.x = element_text(angle = 45, hjust = 1),
    panel.grid.major = element_blank(), # remove major grid lines
    panel.grid.minor = element_blank(), # remove minor grid lines
    panel.border = element_blank(),
    axis.line = element_line(color = "black") # retain x and y axis lines
  )
print(spores_plot)

```



```
mean_spore<-ggplot(summary_stats_spore, aes(x = Primer, y = mean_copies, fill = Primer)) +
  geom_col(position = position_dodge(width = 0.9)) +
  geom_errorbar(aes(ymin = mean_copies - sd_copies,
                    ymax = mean_copies + sd_copies),
               width = 0.2,
               position = position_dodge(width = 0.9)) +
  facet_wrap(~ Primer, scales = "free_x") +
  labs(
    title = "Mean nuclei per spore",
    x = "AMF isolate",
    y = "Nuclei per spore"
  ) +
  scale_fill_brewer(palette = "Dark2", name = "AMF Isolate") +
  scale_y_continuous(expand = c(0, 0)) +
  theme_minimal() +
  theme(
    axis.text.x = element_text(angle = 45, hjust = 1),
    panel.grid.major = element_blank(),
    panel.grid.minor = element_blank(),
    panel.border = element_blank(),
    axis.line = element_line(color = "black")
  )
print(mean_spore)
```



```
#Combine plots vertically
combined_plot_spore <- plot_grid(
  spores_plot, mean_spore,
  labels = c("A", "B"),
  ncol = 1,
  align = "v"
)

#Compare whether nuclear abundance significantly differs
#Do spores of three AMF isolates differ in nuclear abundance?
#Test assumptions of normality
#Shapiro test
by(df_spore$Copies_24, df_spore$Primer, shapiro.test) #pass
```

```
## df_spore$Primer: 197198
##
##  Shapiro-Wilk normality test
##
## data:  dd[, ]
## W = 0.94739, p-value = 0.5593
##
## -----
## df_spore$Primer: 240448
##
##  Shapiro-Wilk normality test
##
```

```
## data: dd[x, ]
## W = 0.92066, p-value = 0.2561
##
## -----
## df_spore$Primer: 240720
##
## Shapiro-Wilk normality test
##
## data: dd[x, ]
## W = 0.90729, p-value = 0.1684
```

```
#Levene test
car::leveneTest(Copies_24 ~ Primer, data = df_spore) #fail
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 2  5.1344 0.01092 *
##      36
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#assumption of homogeneity of variance violated, use a Kruskal Wallis
```

```
#Copies per 24uL [reaction volume used] (copies/spore) ~ primer-probe
kruskal.test(Copies_24 ~ Primer, data = df_spore) #Significant
```

```
##
## Kruskal-Wallis rank sum test
##
## data: Copies_24 by Primer
## Kruskal-Wallis chi-squared = 7.569, df = 2, p-value = 0.02272
```

```
#Post-hoc Dunn
FSA::dunnTest(Copies_24 ~ Primer, data = df_spore, method = "bh")
```

```
## Registered S3 methods overwritten by 'FSA':
##      method      from
## confint.boot car
## hist.boot     car
## Dunn (1964) Kruskal-Wallis multiple comparison
## p-values adjusted with the Benjamini-Hochberg method.
```

```
##      Comparison      Z      P.unadj      P.adj
## 1 197198 - 240448 -0.6909998 0.489565695 0.48956570
## 2 197198 - 240720  1.9607118 0.049912651 0.07486898
## 3 240448 - 240720  2.6517116 0.008008492 0.02402548
```

```
#Significant difference between DAOM 240448 and DAOM 240720
```

```
# Calculate y_star just above the max of mean + SD
y_star <- max(summary_stats_spore$mean_copies + summary_stats_spore$sd_copies, na.rm = TRUE) + 10
```

```

#Add signfiicance star to the plot
mean_spore <- ggplot(summary_stats_spore, aes(x = Primer, y = mean_copies, fill = Primer)) +
  geom_col(position = position_dodge(width = 0.9)) +
  geom_errorbar(aes(ymin = mean_copies - sd_copies,
                    ymax = mean_copies + sd_copies),
                width = 0.2,
                position = position_dodge(width = 0.9)) +
  labs(
    title = "Mean nuclei per spore",
    x = "AMF Isolate",
    y = "Nuclei per spore"
  ) +
  scale_fill_brewer(palette = "Dark2", name = "AMF Isolate") +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1)))+
  theme_minimal() +
  theme(
    axis.text.x = element_text(angle = 45, hjust = 1),
    panel.grid.major = element_blank(),
    panel.grid.minor = element_blank(),
    panel.border = element_blank(),
    axis.line = element_line(color = "black")
  ) +
  # Bracket and star (outside theme!)
  geom_segment(aes(x = 2, xend = 3, y = y_star, yend = y_star)) +
  geom_segment(aes(x = 2, xend = 2, y = y_star, yend = y_star - 10)) +
  geom_segment(aes(x = 3, xend = 3, y = y_star, yend = y_star - 10)) +
  geom_text(aes(x = 2.5, y = y_star + 3), label = "*", size = 6)

print(mean_spore)

```

