Homework 12: Regression

XDASI Fall 2021

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SARS-CoV-2 Diagnostics

Background

This question is about the detection and quantification of viral loads in actual COVID-19 tests.

The data you will work with is from the very first experiment we performed as part of a pilot study in the spring of 2020 to test the sensitivity of an assay system that we developed at NYUAD using the Fluidigm microfluidic platform. We showed that our assay is quantitative and that we could detect very low titers of virus that were previously classified as negative samples in the diagnostic lab. The results of this study have been published:

Xie et al., *Processes* 2020 (PDF) - Supplement (PDF)

As for many real-world datasets, in this homework you will see that most of your effort will be devoted to checking and cleaning the data before you get to the fun part! Unfortunately, that's just the way the cookie crumbles. ;-)

The assays

This dataset contains results of qRT-PCR assays for SARS-CoV-2 nasopharyngeal swabs from a clinical laboratory in Abu Dhabi.

The samples were previously classified as "**Negative**" or "**Positive**" for presence of SARS-CoV-2 viral particles using a standard CDC-approved diagnostic protocol in a clinical laboratory ("PHD Diagnostics"). These protocols typically involve and RNA extraction step followed by reverse transcription and qPCR amplification. Samples were called "Positive" by PHD if one or more of the viral genes could be detected with PCR amplification within 32 PCR cycles.

The samples were then tested using the NYUAD assay system, which includes an RNA extraction step followed pre-amplification by standard RT-PCR, and then additional cycles of qPCR using the Fluidigm microfluidic assay system. The two-step amplification contributes to the sensitivity of our assay. In addition, very small volumes of reagents are used in the microfluidic system, which both enables numerous replicate assays and lowers the cost of reagents.

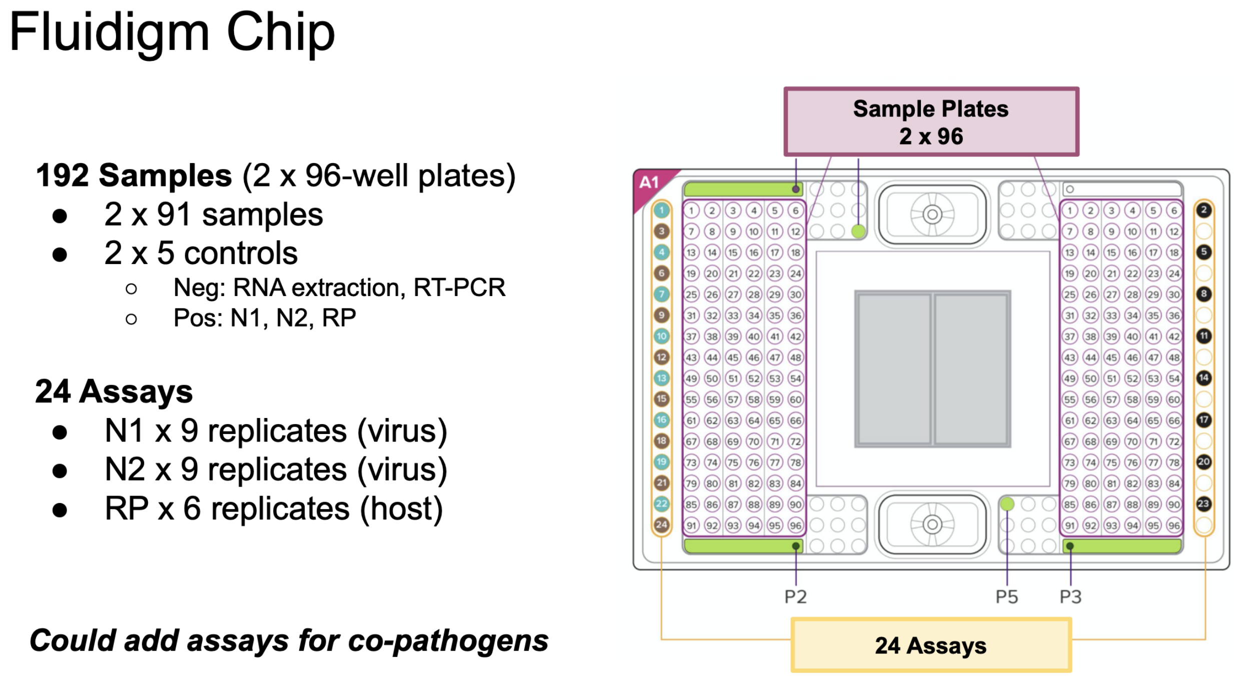
The dataset

- **Samples**: The Fluidigm system is able to assay two 96-well plates of samples at once.
  - We assayed extracted RNA from 182 clinical samples: 91 "Positives" and 91 "Negatives", based on the PHD assay. For each class, 45 were run on one plate and 46 on the other plate.
- **Controls**: We reserved 5 wells on each plate for controls:
  - `RX`: 1 negative control for the RNA extraction (extraction performed using buffer only)
  - `NT`: 1 negative control for RT-PCR (reactions run using amplification mix only)
  - `CoV`: a dilution series of 3 positive controls containing plasmid DNA encoding the N gene, at 3 different concentrations, for quantification of viral load:
    - 50 copies/ul
    - 500 copies/ul
    - 5000 copies/ul
- **Assays**: For each sample / control, we performed 24 different assays at the same time. These were:
  - 9 replicate assays for amplification of the viral N gene using the N1 probe set
  - 9 replicate assays for amplification of the viral N gene using the N2 probe set
  - 6 replicate assays for amplification of the human RP gene

Note that in order to detect viral RNA, we used **two different probe sets for the SARS-CoV-2 N gene, N1 and N2. We expect the N1 and N2 assays to be positive for the dilution series controls and for clinical samples that contain viral particles, and negative for the other controls.**

The **human RP gene** is a control for the quality of the clinical samples and should be present in all clinical samples if the swab was ok. **We expect the RP assays to be positive for all samples and negative for all controls.**

The layout for the Fluidigm chip is illustrated below:



Fluidigm Chip

192 Samples (2 x 96-well plates)

- 2 x 91 samples
- 2 x 5 controls
  - Neg: RNA extraction, RT-PCR
  - Pos: N1, N2, RP

24 Assays

- N1 x 9 replicates (virus)
- N2 x 9 replicates (virus)
- RP x 6 replicates (host)

Could add assays for co-pathogens

The data files

1) Fluidigm output

The raw data file from the Fluidigm system has a lot of extra metadata and some of the column data are not that well labeled. To provide an easier starting point for this analysis, the file has been massaged slightly and supplemented with additional labels. Otherwise the file is pretty much the way it comes off the machine.

The columns are as follows:

- **ID**: Sample well + Assay well combination on Fluidigm chip (e.g. `S138-A01`)
- **Sample**: Sample well
- **Assay**: Assay well
- **SampleName**: A sample barcode or control name
  - **Negative Controls**: These should all be negative!
    - `RX QC`: Quality control for RNA extraction
    - `NT QC`: Quality control for reverse transcription and preamplification
  - **Standard Controls**: Plasmid DNA with SARS-CoV-2 N gene at known concentrations
    - `CoV 50`: 50 copies / ul
    - `CoV 500`: 500 copies / ul
    - `CoV 5000`: 5000 copies / ul
  - **Samples**: A barcoded clinical sample (e.g. `9900039147` or `04MI200640957A`)
- **Type**: `Unknown` (**you can ignore this**)
- **rConc**: Known concentration of viral RNA (copies / ul)
  - ***NOTE: This column contains valid numerical data for standards only! For any row with a value of "1", the concentration has not yet been determined!!! (This is the way the data are emitted from the system; I'm not sure if this was a dummy value set by the lab staff, or if the machine defaults to this if no other value is entered.)***
- **AssayName**: the tests that were performed (for each control or sample well, there are a total of 24 assays)
  - `N1`: SARS-CoV-2 N gene, probe set 1 (9 replicates)
  - `N2`: SARS-CoV-2 N gene, probe set 2 (9 replicates)
  - `RP`: Human RP gene (6 replicates)
- **Value**: Number of PCR cycles at which a signal was detected (numerical)
  - ***NOTE: This is the C_t value, or "Cycles to threshold". A value of 999 means that NOTHING WAS DETECTED.***
- **Quality**: Quality score for assay (**you can ignore this**)
- **Call**: `Pass` (good assay) or `Flag` (bad assay or Value = 999)
- **Threshold**: Quality cutoff for `Pass` (**you can ignore this**)
- **Plate**: `Plate1` or `Plate2`

2) PHD diagnostic results

The diagnostic results contains two columns for each sample tested:

- **Sample_barcode**: a barcode ID, matches **SampleName** in the Fluidigm file
- **Class**: `Negative` or `Positive`
  - ***This is the status of each clinical sample according to PHD diagnostic lab***

The overall goal

We would like to answer two basic questions:

1. Is our assay more sensitive than the one used by the clinical diagnostic lab? 2. Can we correlate the viral load we measure in our lab with the status of samples according to the clinical diagnosis?

To do this, we will use ***linear regression*** and compare the status of each sample based on our own assay vs. the status based on the clinical lab.

We will need to do some quality control on the data before we start, however, to get rid of data that are not reliable (e.g. due to pipetting errors, contamination, etc.)

Q1: Prepare the data

Below you will organize the data to make it easier for you to work with it. Feel free to delete any columns you don't need to use, or to add columns that you think will help you keep track of everything.

A. Load the dataset

Read in the Fluidigm data file and take a look at it.

```
# Import massaged Fluidigm data file
fluid.all = read.csv("data/Fluidigm_1691207281.csv", stringsAsFactors = TRUE)
head(fluid.all)
```

```
##   X       ID Sample Assay SampleName    Type rConc AssayName    Value   Quality
## 1 1 S138-A01   S138   A01     CoV 50 Unknown    50        N1 15.71392 0.9927153
## 2 2 S138-A02   S138   A02     CoV 50 Unknown    50        N1 15.60800 0.9787948
## 3 3 S138-A03   S138   A03     CoV 50 Unknown    50        N1 15.67827 0.9881885
## 4 4 S138-A04   S138   A04     CoV 50 Unknown    50        N1 15.70482 0.9923112
## 5 5 S138-A05   S138   A05     CoV 50 Unknown    50        N1 15.50040 0.9725491
## 6 6 S138-A06   S138   A06     CoV 50 Unknown    50        N1 15.69728 0.9924170
##   Call  Threshold  Plate
## 1 Pass 0.03341885 Plate1
## 2 Pass 0.03341885 Plate1
## 3 Pass 0.03341885 Plate1
## 4 Pass 0.03341885 Plate1
## 5 Pass 0.03341885 Plate1
## 6 Pass 0.03341885 Plate1
```

B. Subset the negative and standard controls

- Make separate data frames for the negative controls and standard controls (refer to the above key for details).
    - Remember that there are two sets of negative controls: "RX QC" and "NT QC"
    - The standard controls are: "CoV 50", "CoV 500", "CoV 5000"

Note: To simplify your life, you may wish to remove columns that you are not interested in anymore. You should keep the sample ID and/or SampleName, AssayName, Value, Call, and Plate columns. You will also need to keep rConc for the Standards.

Hint: You can do this any way you like. One convenient method is to use the `dplyr` paradigm `filter %>% select %>% arrange`.

```
# ============================================================================ #
# negative controls ("RX QC" and "NT QC")
neg.ctls = fluid.all %>% filter(SampleName %in% c("RX QC", "NT QC")) %>%
  select(ID,SampleName,AssayName,Value,Call,Plate) %>%
  arrange(Plate,ID)

# ============================================================================ #
# standards ("CoV 50", "CoV 500", "CoV 5000")
std.ctls = fluid.all %>% filter(!rConc == 1) %>%  # shortcut
  select(ID,SampleName,rConc,AssayName,Value,Call,Plate) %>%
  arrange(Plate,ID)
```

C. Sanity check Controls

It's always a good idea to check whether your controls look ok. Use whatever method you like to inspect the data and answer the following questions. Looking at plots and using those to make a decision about whether they look ok would be fine here.

Please note:

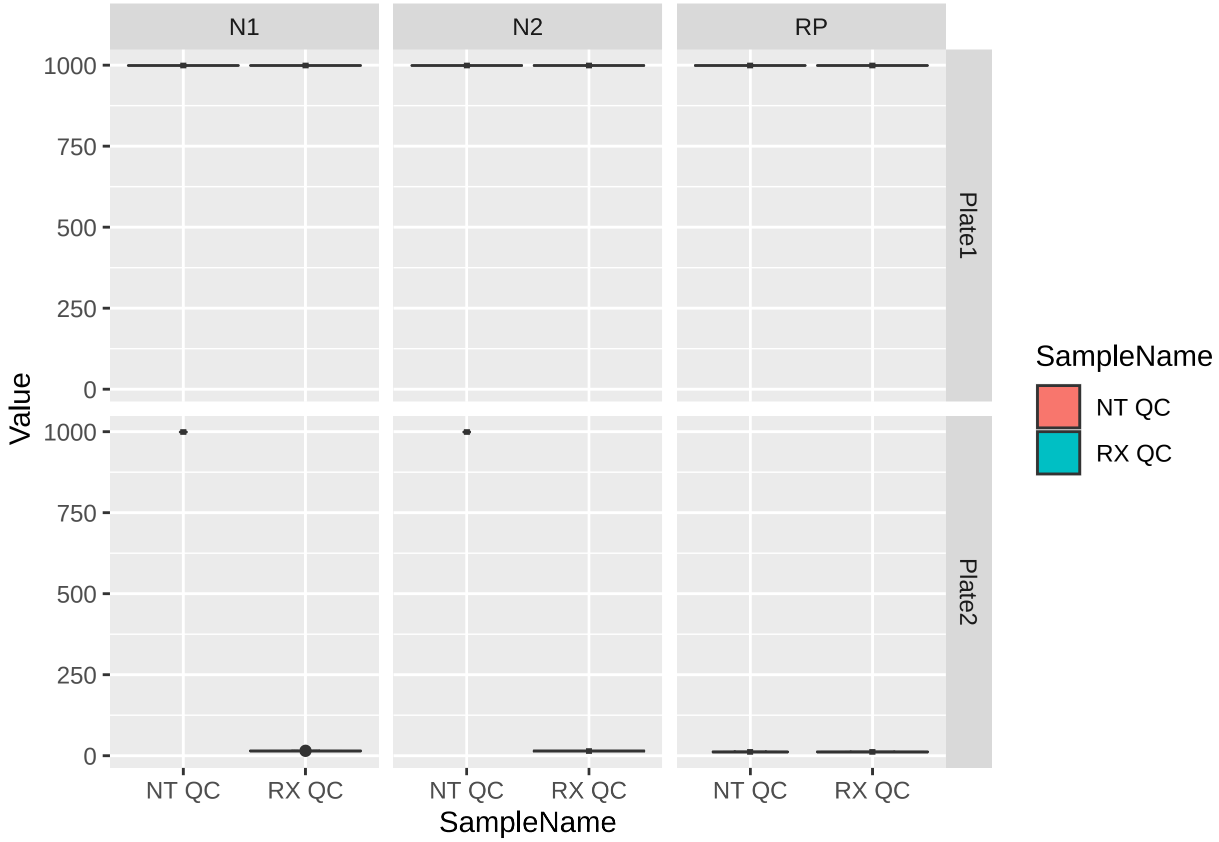
- The negative controls should not amplify anything, so the PCR Value for those should be 999. This automatically raises a value of "Flag" in the "Call" column.
- The positive controls should amplify the viral N gene using both sets of primers (N1 and N2), but not the human RP gene.

Negative controls First, use `ggplot` to take a look at the negative controls.

- Make a boxplot or violin plot using `SampleName` and `Value`.
- Use `facet_grid(Plate ~ AssayName)` to show the data for each assay separated by plate.

Once you get the syntax working, you can use the same template to plot the standard controls below.

```
# take a look at the data ...
# View(neg.ctls)

# plot Ct for all negative controls
ggplot(neg.ctls, aes(x=SampleName, y=Value, fill=SampleName)) +
  geom_violin() +
  geom_boxplot(width=0.05, fill="white") +
  facet_grid(Plate ~ AssayName)
```



Do all of the **negative** controls all look ok? Why or why not? If not, what could explain the results?

Hint: Check the description of the data file above to remind yourself what the Ct value for the negative controls should be.

```
# Your answer here

No -- there should be no N1, N2, or RP detected for the QC wells, so
we would expect to see Value = 999 and Call = "Flag".

However, some of them amplify something on Plate 2.
This is probably due to cross-contamination or errors during plate loading.

RX controls (RNA extraction):
all three probes show some signal, so it's possible that one of the
positive clinical samples got loaded in the wrong place (prior to RNA extraction).

NT controls (RT and pre-amplifcation):
N1 and N2 are negative, but RP amplified -- possible contamination
from a negative lab sample, or one of the lab people?
```

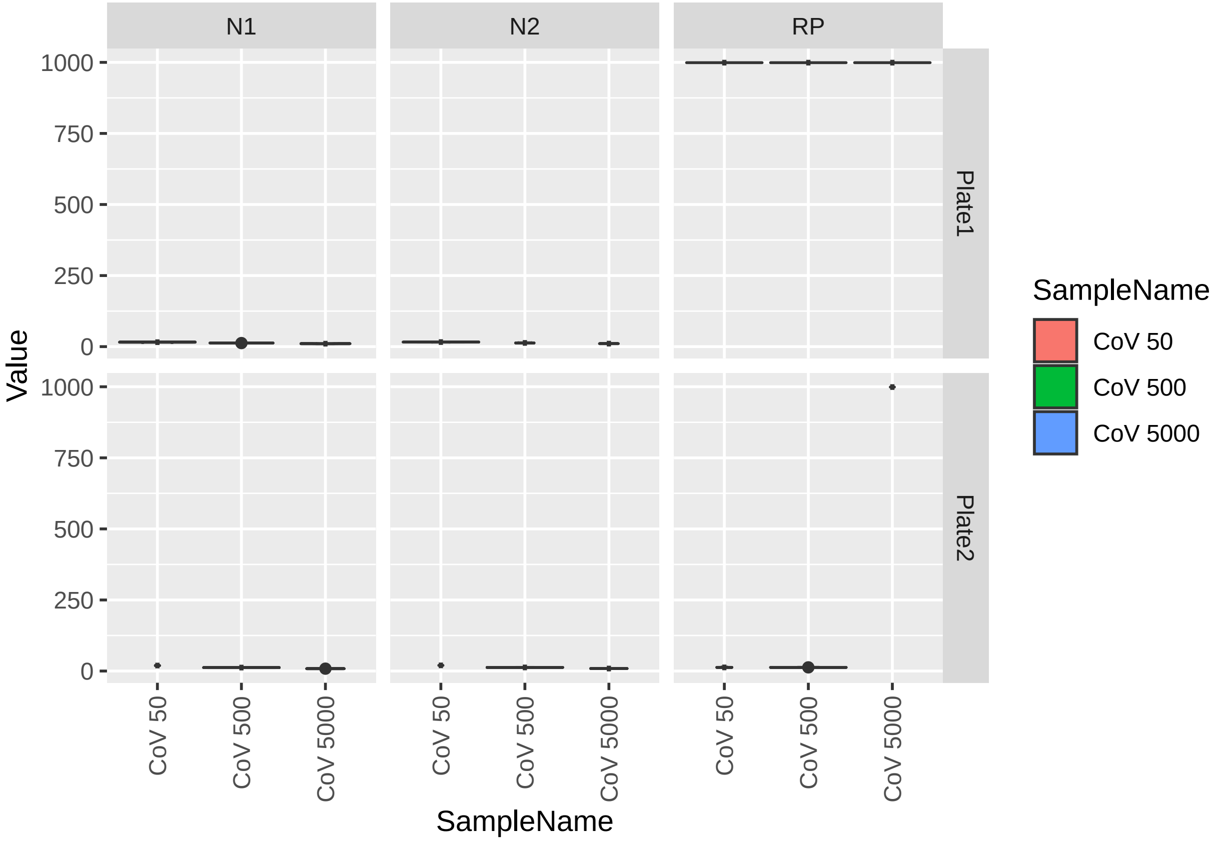
Do you think you proceed with the analysis using the data on one of the two plates? Explain.

```
# your answer here

Yes, Plate 1 looks ok
```

Standard controls Now take a look at the standards.

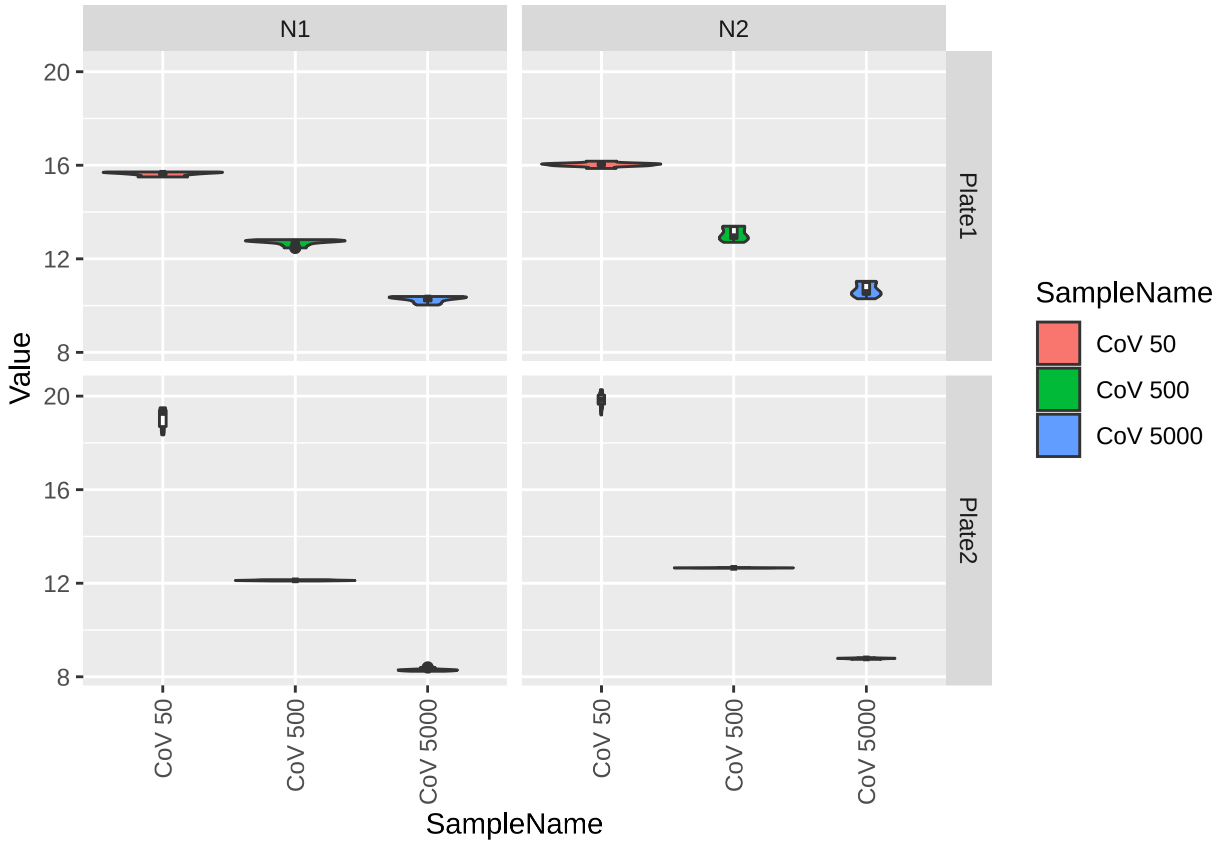
Note that the Value for all of the positive controls for the N gene should be some number below 100 and for the human RP gene should be 999 (since no human RNA should be amplified using the N gene plasmid).

```
# take a look at the data ...
# View(std.ctls)

# ggplot for all standard controls
ggplot(std.ctls, aes(x=SampleName, y=Value, fill=SampleName)) +
  geom_violin() +
  geom_boxplot(width=0.05, fill="white") +
  facet_grid(Plate ~ AssayName) +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1))
```



After looking at all 3 assays, make a new data frame that contains only the N1 and N2 assays for the Standards (remove the RP data from the dataset) and replot the subsetted data. Note that the Ct values for the standards should be under 20, and the number of cycles should decrease with increasing concentration of the control plasmid (since it takes fewer cycles to amplify a larger amount of starting material).

```
# look more closely at just the N1 and N2 results
std.ctls.n1.n2 = std.ctls[std.ctls$AssayName != "RP",]
#std.ctls.n1.n2 = std.ctls %>% filter(AssayName %in% c("N1","N2"))

ggplot(std.ctls.n1.n2, aes(x=SampleName, y=Value, fill=SampleName)) +
  geom_violin() +
  geom_boxplot(width=0.05, fill="white") +
  facet_grid(Plate ~ AssayName) +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1))
```



Do the **standard** controls all look ok? Why or why not?

```
# Your answer here

No -- there should be no RP detected, but some of the RP also amplify on Plate 2.
Again, this reflects some kind of contamination somewhere along the line.
```

Based on the standard controls, do you think you can proceed with the analysis for at least one of the plates? Explain.

```
# your answer here

Yes, Plate 1 looks ok
```

D. Prepare Sample data

Subset Fluidigm data and add Clinical results

- Extract the sample data from the Fluidigm file (again, `filter %>% select %>% arrange` could be useful here.)
- Load the PHD result data and merge the two files using the sample barcode IDs.

  - To join the tables, you can use the inner_join() command.

```
# ============================================================================ #
# samples

# filter fluid.all to keep just the samples and none of the controls
samples = fluid.all %>%
  filter(! SampleName %in% c("RX QC", "NT QC", "Cov 50", "CoV 500", "CoV 500"))  %>%
  select(ID,SampleName,rConc,AssayName,Value,Call,Plate) %>%
  arrange(Plate,ID)

# Import PHD results from .csv file
phd.calls = read.csv("data/PHD_results.csv", stringsAsFactors = FALSE)

# Add a column to the Fluidigm file containing the PHD results
# use the inner_join() function to do this
samples = inner_join(samples, phd.calls, by.x = c("SampleName"), by.y = c("Sample_barcode"), row.names =
```

```
## Joining, by = "SampleName"
```

```
# clean up
rm(phd.calls)
```

Make a placeholder for NYUAD results As we go along we will classify the samples as "Inconclusive", "Negative", or "Positive", according to the NYUAD results. So let's add a placeholder column that we will fill up as we go along.

- Add a new column to samples called "NYUAD_Class" and fill it with NA.
- Rename "Class" to "PHD_Class" to distinguish our results from the clinical results.

```
# Add a column to sample for NYUAD classes and rename Class to PHD_Class
samples = samples %>%  rename(PHD_Class = Class) %>% mutate(NYUAD_Class = NA)
head(samples)
```

```
##         ID SampleName rConc AssayName Value Call  Plate PHD_Class NYUAD_Class
## 1 S001-A01 9900039147     1        N1   999 Pass Plate1  Negative          NA
## 2 S001-A02 9900039147     1        N1   999 Pass Plate1  Negative          NA
## 3 S001-A03 9900039147     1        N1   999 Pass Plate1  Negative          NA
## 4 S001-A04 9900039147     1        N1   999 Pass Plate1  Negative          NA
## 5 S001-A05 9900039147     1        N1   999 Pass Plate1  Negative          NA
## 6 S001-A06 9900039147     1        N1   999 Pass Plate1  Negative          NA
```

Q2: Quality Control for Standard Dilution Series

A. Standard curves

In order to quantify viral load, we want to make sure that the dilution series for our standards look suitable for quantification of the viral load in our samples.

First, use ggplot to plot just the N1 and N2 control data with a simple linear regression line of Ct against the concentration (recall we subsetted these above as std.ctls.n1.n2).

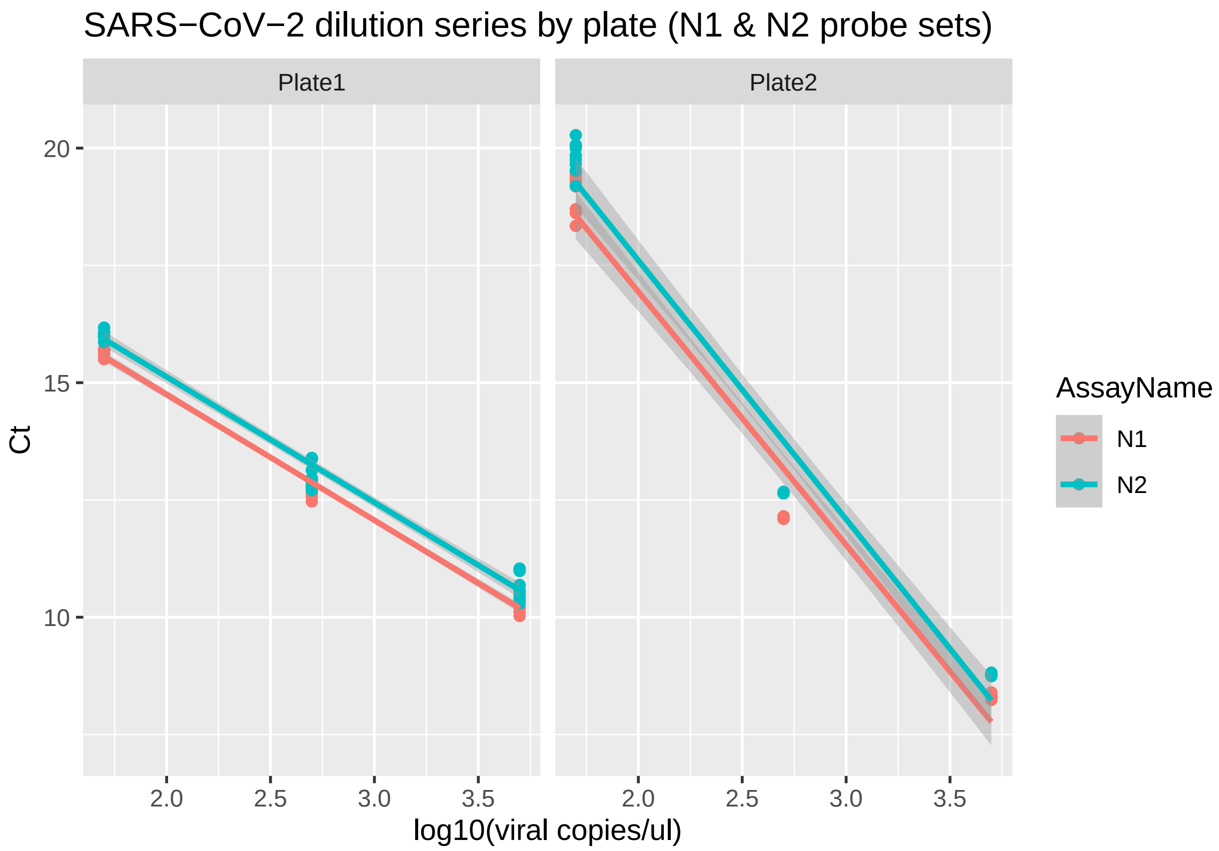
Hints:

- Map `color` to **AssayName** and facet the plot by **Plate** to visualize the results for N1 and for N2 separately on each plate (you can use `facet_wrap(~Plate)`).
- Note that Ct units are in PCR cycles, so you will need to do the aesthetic mapping using a transformation of the data.
  - Since you have a 10-fold dilution series (50, 500, 5000 copies/ul), you can use a log transformation with base 10 (`log10()`) on the x-axis instead of using `rConc` directly.
- Plot the individual data points, and then add the regression lines using `geom_smooth(method="lm")`.

Optional: if you want to show the original concentrations instead of the log10 values on the graph, you can use this function inside the ggplot command (I think there may be easier ways to do this, but this works): `scale_x_continuous(label = function(x){return(round(10^x,1))})`

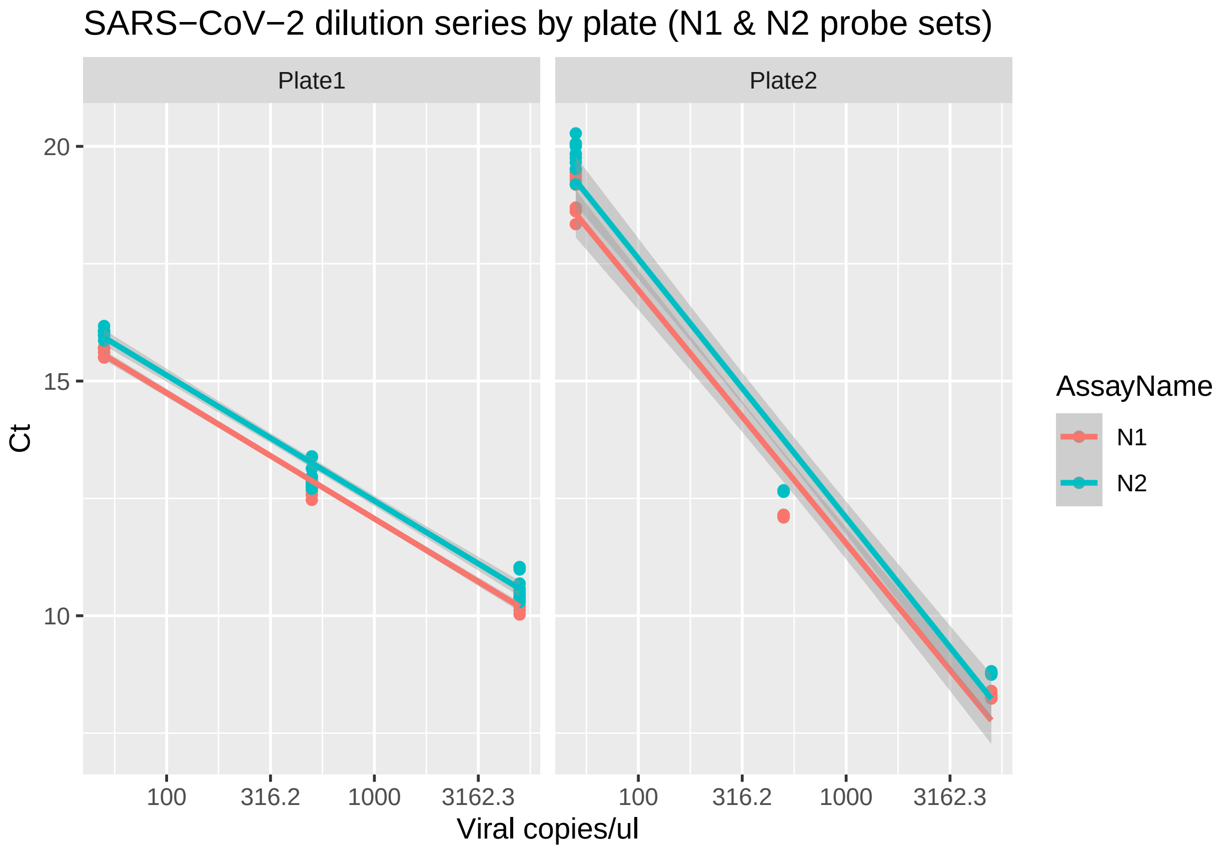
```
# subset N1 and N2 assays from standard control set
#std.ctls.n1.n2 = std.ctls %>% filter(AssayName %in% c("N1","N2"))

# side-by-side graphs for N1 and N2 on Plate 1 and Plate 2
ggplot(std.ctls.n1.n2, aes(x=log10(rConc), y=Value, color=AssayName)) +
  geom_point() +
  geom_smooth(method="lm") +
  ggtitle("SARS-CoV-2 dilution series by plate (N1 & N2 probe sets)") +
  xlab("log10(viral copies/ul)") + ylab("Ct") +
  facet_wrap(~Plate)
```

```
## `geom_smooth()` using formula 'y ~ x'
```



```
# with original concentrations
ggplot(std.ctls.n1.n2, aes(x=log10(rConc), y=Value, color=AssayName)) +
  geom_point() +
  geom_smooth(method="lm") +
  ggtitle("SARS-CoV-2 dilution series by plate (N1 & N2 probe sets)") +
  scale_x_continuous(label = function(x){return(round(10^x,1))}) +
  xlab("Viral copies/ul") + ylab("Ct") +
  facet_wrap(~Plate)
```

```
## `geom_smooth()` using formula 'y ~ x'
```



How do the standard regression lines look? Does everything look consistent between the N1 and N2 assays? How about between Plates?

```
# Your answer here

No -- the N1 and N2 assays look similar, but the lines from the two plates are really different.
```

B. Test for interaction between rConc and Plate

Something looks fishy here. It seems that when this experiment was performed, something was not quite right.

- Make a linear model for the standards that includes an interaction term between the (log-transformed) concentration and the plate and examine the results using `summary()`

```
# lm for standards with Plate interaction term
# you may do this for N1, N2, or both combined
summary(lm(Value ~ log10(rConc) * Plate, data = filter(std.ctls, AssayName == "N1")))
```

```
##
## Call:
## lm(formula = Value ~ log10(rConc) * Plate, data = filter(std.ctls,
##     AssayName == "N1"))
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.07253 -0.15805  0.08585  0.47959  0.93936
##
## Coefficients:
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)                 20.1092     0.3852   52.20   <2e-16 ***
## log10(rConc)                -2.6807     0.1366  -19.62   <2e-16 ***
## PlatePlate2                  7.6200     0.5448   13.99   <2e-16 ***
## log10(rConc):PlatePlate2    -2.7147     0.1932  -14.05   <2e-16 ***
## ---
## Signif. codes:  0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1
##
## Residual standard error: 0.5796 on 50 degrees of freedom
## Multiple R-squared:  0.975,  Adjusted R-squared:  0.9735
## F-statistic: 649.5 on 3 and 50 DF,  p-value: < 2.2e-16
```

```
summary(lm(Value ~ log10(rConc) * Plate, data = filter(std.ctls, AssayName == "N2")))
```

```
##
## Call:
## lm(formula = Value ~ log10(rConc) * Plate, data = filter(std.ctls,
##     AssayName == "N2"))
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1.1103 -0.2844  0.1153  0.5078  1.0081
##
## Coefficients:
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)                 20.4752     0.4083   50.15   <2e-16 ***
## log10(rConc)                -2.6758     0.1448  -18.48   <2e-16 ***
## PlatePlate2                  8.1692     0.5774   14.15   <2e-16 ***
## log10(rConc):PlatePlate2    -2.8426     0.2048  -13.88   <2e-16 ***
## ---
## Signif. codes:  0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1
##
## Residual standard error: 0.6143 on 50 degrees of freedom
## Multiple R-squared:  0.973,  Adjusted R-squared:  0.9714
## F-statistic:   601 on 3 and 50 DF,  p-value: < 2.2e-16
```

```
summary(lm(Value ~ log10(rConc) * Plate, data = std.ctls.n1.n2))
```

```
##
## Call:
## lm(formula = Value ~ log10(rConc) * Plate, data = std.ctls.n1.n2)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.36419 -0.30069  0.01769  0.33912  1.36122
##
## Coefficients:
##                           Estimate Std. Error t value Pr(>|t|)
## (Intercept)                20.2922     0.2999   67.67   <2e-16 ***
## log10(rConc)               -2.6783     0.1063  -25.19   <2e-16 ***
## PlatePlate2                 7.8946     0.4241   18.62   <2e-16 ***
## log10(rConc):PlatePlate2  -2.7787     0.1504  -18.48   <2e-16 ***
## ---
## Signif. codes:  0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1
##
## Residual standard error: 0.638 on 104 degrees of freedom
## Multiple R-squared:  0.9692, Adjusted R-squared:  0.9684
## F-statistic:  1093 on 3 and 104 DF,  p-value: < 2.2e-16
```

What is the difference in slopes between the slopes for the standards on Plate 1 vs. Plate 2? Is the interaction term significant?

```
# Your answer here

Yes, the difference in slopes is -2.7 or -2.8 (depending on whether one uses N1,
N2, or both in the model).

The P-values for the interaction terms are very small.
```

C. R-squared and Efficiency

If our standard dilutions are ok, we would like the amount of variation explained by the model to be high. We will use a cutoff of $R^2 > 0.975$ as a QC measure.

Also, for a good PCR amplification, the amount of material should double in each cycle. We can check to see if our standards follow this pattern by computing the **efficiency of amplification**, which is given by this equation:

$$Conc = (1 + Eff)^{C_t}$$

In practice, it is computed this way:

$$Eff = 10^{-1/slope} - 1$$

where the slope is derived from a linear regression of Cycles to Threshold (Ct) values plotted against the log_{10} values of the template amounts.

A slope of -3.32 indicates an amplification efficiency of 100%, and **efficiency between 90% and 110% is generally considered to be good.** This corresponds to a slope between approximately -3.1 and -3.6.

Below, you will try to figure out which dilution series looks ok and which one does not.

- First, subset the dilution series by AssayName (N1 or N2) and Plate, and generate linear models for each combination separately (e.g. N1 on Plate 1, N2 on Plate 1, etc.)
    - **Hint**: You can use `data = filter(std.controls, ... )` inside the lm, instead of creating separate data frame for each combination of plate and assay, but do whatever you feel most comfortable with.

```
# make 4 separate linear models for N1 and N2 on Plate 1 or Plate 2
lm.n1.p1 = lm(Value ~ log10(rConc),
              data = filter(std.ctls, Plate == "Plate1" & AssayName == "N1"))
lm.n2.p1 = lm(Value ~ log10(rConc),
              data = filter(std.ctls, Plate == "Plate1" & AssayName == "N2"))
lm.n1.p2 = lm(Value ~ log10(rConc),
              data = filter(std.ctls, Plate == "Plate2" & AssayName == "N1"))
lm.n2.p2 = lm(Value ~ log10(rConc),
              data = filter(std.ctls, Plate == "Plate2" & AssayName == "N2"))

# look at the summaries of the models
summary(lm.n1.p1)
```

```
##
## Call:
## lm(formula = Value ~ log10(rConc), data = filter(std.ctls, Plate ==
##     "Plate1" & AssayName == "N1"))
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.40570 -0.10434  0.00655  0.15202  0.19658
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  20.10916    0.10929  184.00   <2e-16 ***
## log10(rConc) -2.68073    0.03876  -69.16   <2e-16 ***
## ---
## Signif. codes:  0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1
##
## Residual standard error: 0.1644 on 25 degrees of freedom
## Multiple R-squared:  0.9948, Adjusted R-squared:  0.9946
## F-statistic:  4784 on 1 and 25 DF,  p-value: < 2.2e-16
```

```
summary(lm.n2.p1)
```

```
##
## Call:
## lm(formula = Value ~ log10(rConc), data = filter(std.ctls, Plate ==
##     "Plate1" & AssayName == "N2"))
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.54804 -0.13911  0.05744  0.14061  0.46089
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  20.47516    0.17758  115.30   <2e-16 ***
## log10(rConc) -2.67578    0.06298  -42.49   <2e-16 ***
## ---
## Signif. codes:  0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1
##
## Residual standard error: 0.2672 on 25 degrees of freedom
## Multiple R-squared:  0.9863, Adjusted R-squared:  0.9858
## F-statistic:  1805 on 1 and 25 DF,  p-value: < 2.2e-16
```

```
summary(lm.n1.p2)
```

```
##
## Call:
## lm(formula = Value ~ log10(rConc), data = filter(std.ctls, Plate ==
##     "Plate2" & AssayName == "N1"))
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1.0725 -1.0247  0.4845  0.5871  0.9394
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   27.7292     0.5337   51.96   <2e-16 ***
## log10(rConc)  -5.3955     0.1893  -28.51   <2e-16 ***
## ---
## Signif. codes:  0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1
##
## Residual standard error: 0.803 on 25 degrees of freedom
## Multiple R-squared:  0.9702, Adjusted R-squared:  0.969
## F-statistic: 812.7 on 1 and 25 DF,  p-value: < 2.2e-16
```

```
summary(lm.n2.p2)
```

```
##
## Call:
## lm(formula = Value ~ log10(rConc), data = filter(std.ctls, Plate ==
##     "Plate2" & AssayName == "N2"))
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1.1103 -1.0903  0.5138  0.5625  1.0081
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   28.6444     0.5494   52.14   <2e-16 ***
## log10(rConc)  -5.5184     0.1948  -28.32   <2e-16 ***
## ---
## Signif. codes:  0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1
##
## Residual standard error: 0.8267 on 25 degrees of freedom
## Multiple R-squared:  0.9698, Adjusted R-squared:  0.9686
## F-statistic: 802.1 on 1 and 25 DF,  p-value: < 2.2e-16
```

To evaluate the models, we will do the following:

- Extract the R-squared value from each lm summary (accessible as `summary(your.model)$r.squared`).
- Examine the slope and efficiency from each model (slope is available as one of the model coefficients).

Below this has been done for you and the results put into a tabular format to make them easier to compare. ***Note that I called the data frame containing just the standard controls `std.ctls`. If you used a different name, just substitute the name of your data frame below.***

```
# Look at the slope/efficiency and R^2
# Here is an outline for a data frame containing these data, if you want to use it
# (but you don't have to)
std.ctl.qc = data.frame(Model = c("N1-P1","N2-P1","N1-P2","N2-P2"),
                        Rsquared = c(summary(lm.n1.p1)$r.squared,
                                     summary(lm.n2.p1)$r.squared,
                                     summary(lm.n1.p2)$r.squared,
                                     summary(lm.n2.p2)$r.squared),
                        Slope = c(summary(lm.n1.p1)$coef[2],
                                  summary(lm.n2.p1)$coef[2],
                                  summary(lm.n1.p2)$coef[2],
                                  summary(lm.n2.p2)$coef[2]),
                        Eff = c(10^{-1/summary(lm.n1.p1)$coef[2]} - 1,
                                10^{-1/summary(lm.n2.p1)$coef[2]} - 1,
                                10^{-1/summary(lm.n1.p2)$coef[2]} - 1,
                                10^{-1/summary(lm.n2.p2)$coef[2]} - 1))
std.ctl.qc
```

```
##   Model  Rsquared     Slope       Eff
## 1 N1-P1 0.9948010 -2.680727 1.3606583
## 2 N2-P1 0.9863413 -2.675779 1.3644110
## 3 N1-P2 0.9701547 -5.395458 0.5322904
## 4 N2-P2 0.9697755 -5.518417 0.5177890
```

These fits don't look perfect, but one plate looks a lot better than the other. So, you will need to choose one of these to use for quantifying your viral samples.

Which plate's dilution series should you choose to quantify viral titers in the clinical samples, and why?

```
# Your answer here

We will use the standards from Plate 1:
- R-squared is above 0.975
- The slope/efficiency is not in the ideal range, but it's better than for Plate 2.
```

NOTE: We indeed verified later that there were some problems with a bunch of wells in one quadrant on one of the plates that were probably due to loading errors / contamination.

Q3: QC and clean data for clinical samples

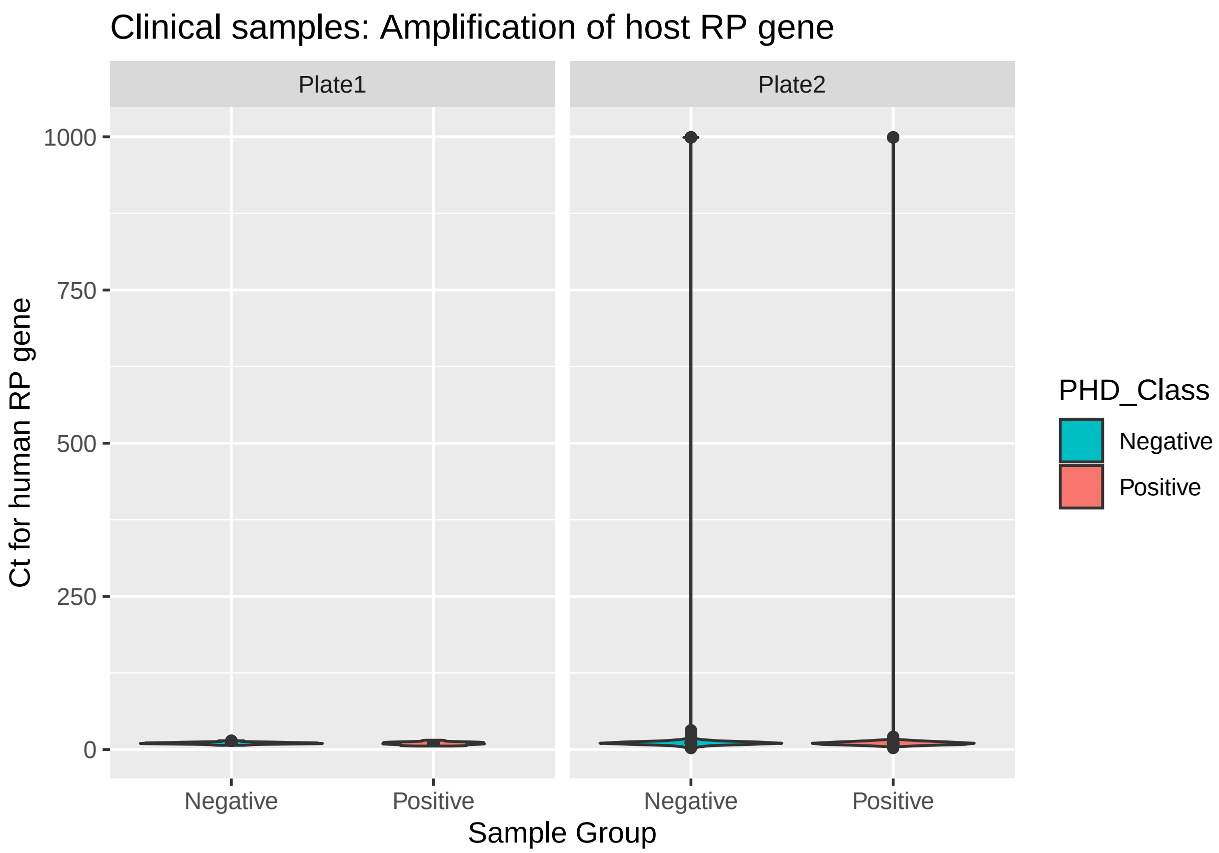
Now we need to check on the N1/N2/RP Assays for the clinical samples.

A. Host RP gene

Distribution of Ct values

- Make a violin plot of the clinical sample data for **just the host RP gene** (again, you can use `data = filter(...)` rather than make a separate data frame first, but do whatever you feel most comfortable with).
- Separate the data by PHD diagnosis ("PHD_Class"), and facet by Plate.

```
# distribution of Ct values for RP genes in clinical samples
ggplot(filter(samples, AssayName == "RP"),
       aes(x=PHD_Class, y=Value, fill=PHD_Class)) +
  geom_violin() +
  geom_boxplot(width=0.05, fill="white") +
  facet_wrap(~ Plate, nrow=1) +
  scale_fill_manual(values=c("#00BFC4","#F8766D")) +
  labs(title="Clinical samples: Amplification of host RP gene",
       x="Sample Group",
       y = "Ct for human RP gene")
```



What should we expect to see for the RP assays in the clinical samples? Does everything look ok here?

```
# Your answer here

If a sample is good, then the human control gene should be detectable at least.
If not, then we will have to take another sample because a negative result will
```

```
be uninformative.

Some of the samples on Plate 2 look invalid (Ct = 999).
```

Filter samples for RP quality Since some of the samples don't look great, we will filter them before proceeding to the quantification.

- Filter the data and keep only those samples for which at least 4 RP assays:
  - were called as "Pass" (QC on quality), and
  - have a Ct "Value"< 16 (meaning a decent amount of RP gene was detected).
- Also flag the assays that were removed by labeling them as "Inconclusive" in the `samples` dataframe (set `NYUAD_Class` to "Inconclusive").

Note: Since this is tedious, I have filled in this part for you.

```
# ============================================================================ #
# subset valid samples
valid.sample.ids = samples %>%
  group_by(SampleName) %>%
  filter(AssayName == "RP" & Call == "Pass") %>%
  summarize( TotalPass = sum(Value < 16) ) %>%
  filter(TotalPass > 3)

valid.samples = merge(samples, valid.sample.ids, by = "SampleName") %>%
  arrange(ID) %>% select(-TotalPass)

# ============================================================================ #
# number of valid samples
nrow(valid.sample.ids)
## [1] 171

# ============================================================================ #
# Flag inconclusive samples
samples$NYUAD_Class[ ! samples$SampleName %in% valid.samples$SampleName ] = "Inconclusive"
```

You should now have a filtered list with a full set of assays (N1, N2, and RP) for samples that you deem to be of "good quality".

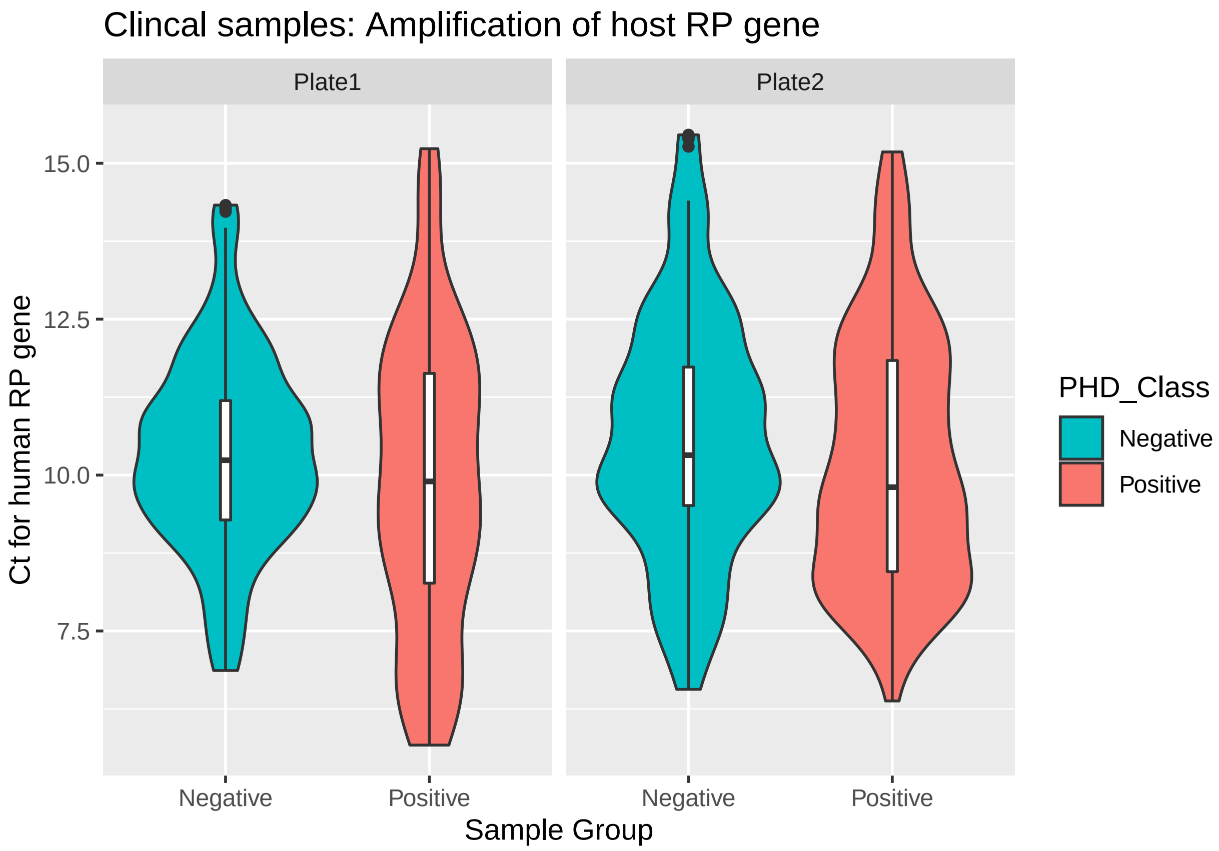
How many samples are left?

```
# Your answer here

171 samples are left.
```

Make a new violin plot of the Ct values for the RP gene assays for the remaining samples.

- Filter the RP data from `valid.samples`
- Make a plot with the data separated by "PHD_class" (i.e. use this for the aesthetic mapping instead of "AssayName" to create a separate violin for the "Negative" and "Positive" results according to the diagonstic lab)
- Facet by "Plate"

```
# subset the rp data from the valid samples (we will also need this below)
samples.rp = filter(valid.samples, AssayName == "RP")

# violin plot of Ct values for valid assays, filtered for RP gene, by PHD diagnosis
ggplot(samples.rp,
       aes(x=PHD_Class, y=Value, fill=PHD_Class)) +
  geom_violin() +
  geom_boxplot(width=0.05, fill="white") +
  facet_wrap(~as.factor(Plate), nrow=1) +
  scale_fill_manual(values=c("#00BFC4","#F8766D")) +
  labs(title="Clincal samples: Amplification of host RP gene",
       x="Sample Group",
       y = "Ct for human RP gene")
```

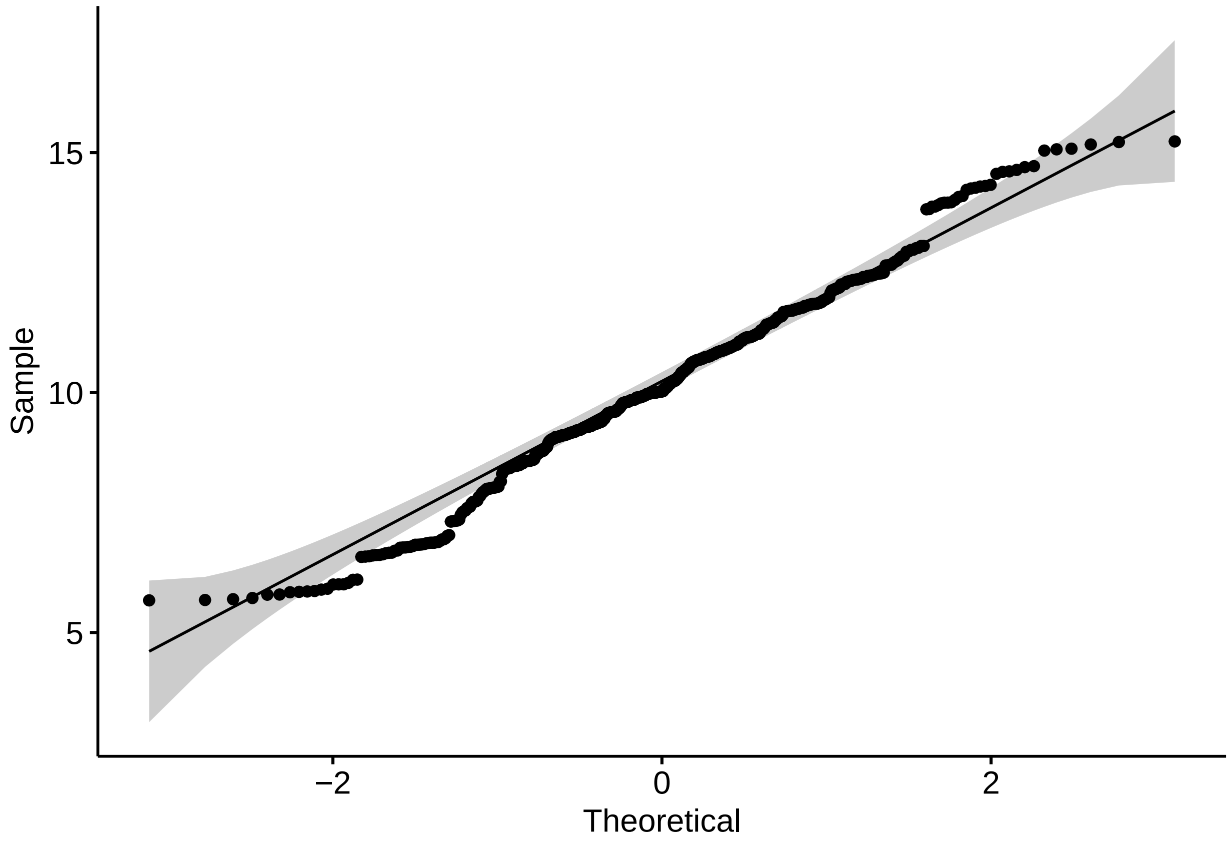


Compare RP distributions If we want to use standards from only one of the plates for quantification, we should probably make sure that the samples amplified similarly on both plates, even though it looks like something went wrong with the standards on one of the plates. (In other words, since we only have one good set of controls, we want to make sure we can use the standards to quantify the clinical samples on both plates).

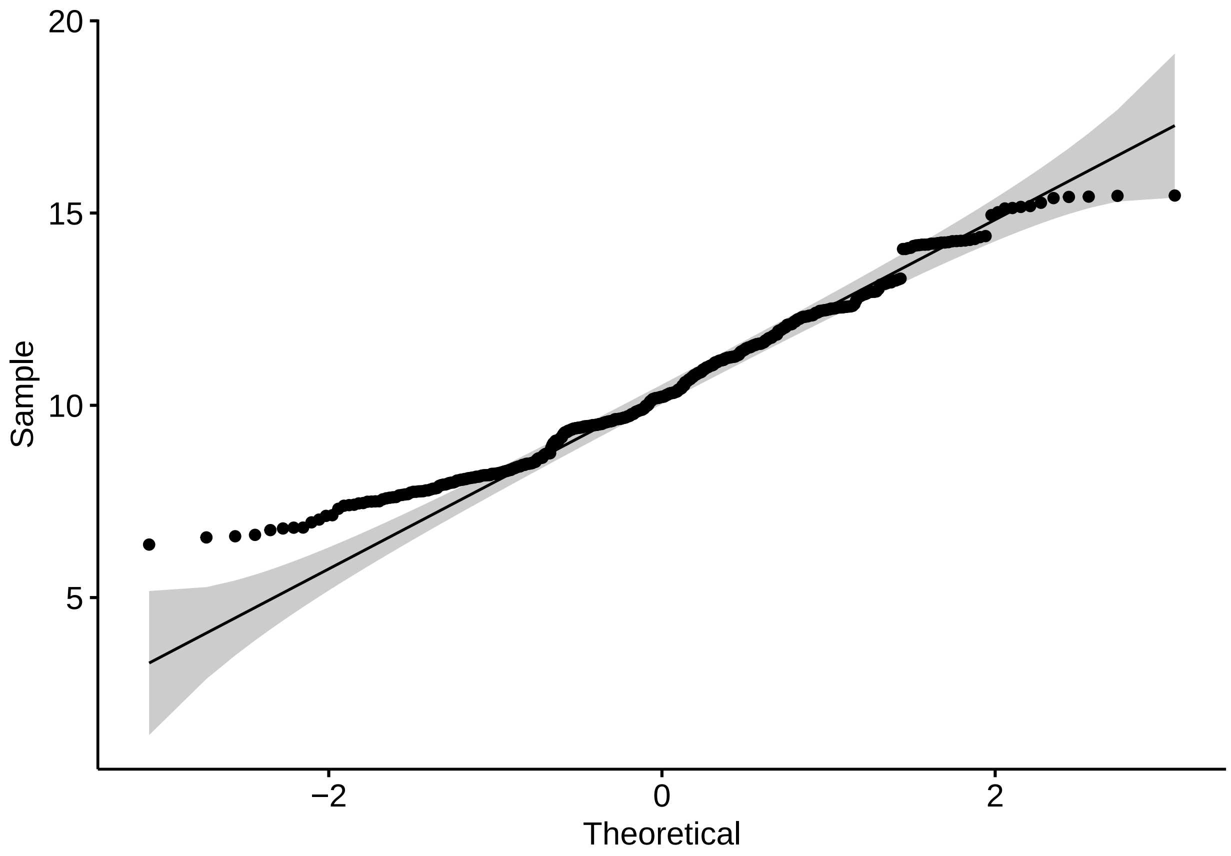
- First, get just the Ct data for Plate1 and Plate2 separately.
- Make QQ plots for the Ct values on both plates to see if the data look sort of normal.
  - Note that the `ggpubr` package contains a nice command called `ggqqplot()` for making QQ plots, if you want to try it out.

- Next, perform a test for normality to see if the distributions on both plates look approximately normal.
- Finally, identify and use an appropriate test to see whether these samples likely came from the same distribution.

```
## Plate 1 and Plate 2 Ct values
rp.p1 = samples.rp %>% filter(Plate == "Plate1") %>% select(Value)
rp.p2 = samples.rp %>% filter(Plate == "Plate2") %>% select(Value)

## QQ plots

# Plate 1
# qqnorm(rp.p1$Value)
# qqline(rp.p1$Value)
ggqqplot(rp.p1$Value) # part of the ggpubr package
```



```
# Plate 2
# qqnorm(rp.p2$Value)
# qqline(rp.p2$Value)
ggqqplot(rp.p2$Value) # part of the ggpubr package
```



```
## Test for normality
shapiro.test(rp.p1$Value)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  rp.p1$Value
## W = 0.98953, p-value = 0.0006189
```

```
shapiro.test(rp.p2$Value)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  rp.p2$Value
## W = 0.97711, p-value = 7.569e-07
```

```
## Test for difference in sample distributions
#t.test(rp.p1$Value,rp.p2$Value) # not valid since data don't look normal

# Test for equality of non-normal distributions
# Wilcoxon rank sum test gives a little more power than the KS test
wilcox.test(rp.p1$Value,rp.p2$Value)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  rp.p1$Value and rp.p2$Value
## W = 122634, p-value = 0.07593
## alternative hypothesis: true location shift is not equal to 0
```

```
ks.test(rp.p1$Value,rp.p2$Value)
```

```
##
##  Two-sample Kolmogorov-Smirnov test
##
## data:  rp.p1$Value and rp.p2$Value
## D = 0.082807, p-value = 0.06021
## alternative hypothesis: two-sided
```

Based on these results, and using a p-value cutoff of 0.05, does it seem ok to use the standards from only one of the plates to quantify the viral load in all the samples?

```
# Your answer here

The samples do not look normally distributed, but both the KS and Wilcoxon rank sum test
give p-values > 0.05 (barely), so the samples from both plates seem to come from
the same distribution.

Therefore it should be ok to use the standards from plate 1 to quanify the samples
on both plates (we don not have a good alternative choice anyway...)
```

B. Viral N gene

Filter samples for viral quantification We won't be able to quantify viral load in any samples that did not amplify N1 or N2, so we will want to filter those out.

- Starting with the "valid" samples from above, filter the data so that you only keep results for the samples that passed QC ("Pass) and showed a valid Ct value (Value < 999) at NYUAD.
- Keep any samples that amplified **either** N1 or N2 (sometimes, for unknown reasons, only one or the other amplified), AND that had at least 4 good replicates in total.

NOTE: Data cleaning is tedious. We have done this for you below.

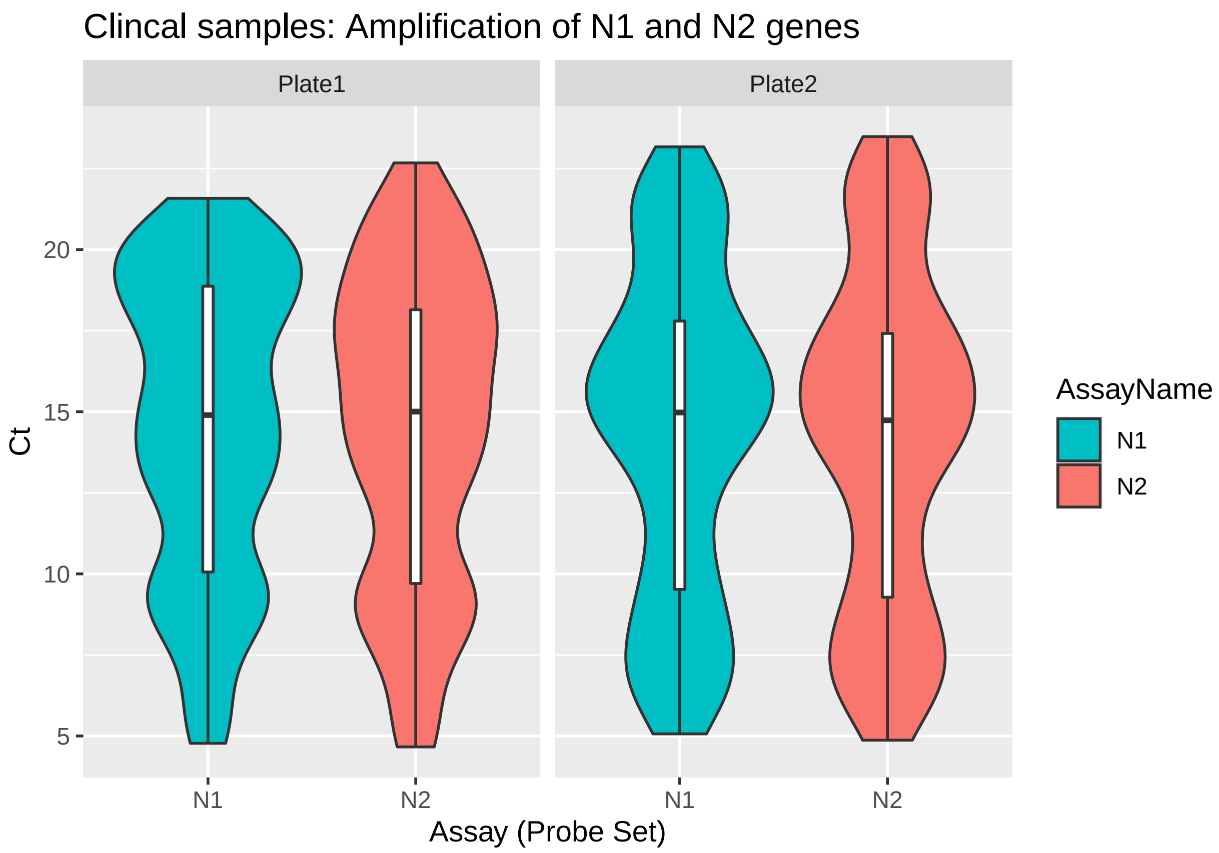
```
# =========================================================================== #
# "positive" samples at NYUAD
nyuad.pos.sample.ids = valid.samples %>%
  group_by(SampleName) %>%
  filter(Call == "Pass" & Value < 999 &
           AssayName %in% c("N1","N2")) %>%
  summarize( TotalPass = sum(Value < 999) ) %>%
  filter(TotalPass > 3)

nyuad.pos.samples = merge(valid.samples, nyuad.pos.sample.ids, by = "SampleName") %>%
  filter(Call == "Pass" & Value < 999 & AssayName %in% c("N1","N2")) %>%
  select(ID,SampleName,PHD_Class,AssayName,Value,Plate) %>%
  arrange(Plate, ID, AssayName)

# ============================================================================ #
nrow(nyuad.pos.sample.ids)
## [1] 102

# ============================================================================ #
# Add NYUAD_Class labels to sample table
samples$NYUAD_Class[ samples$SampleName %in% nyuad.pos.samples$SampleName ] = "Positive"
samples$NYUAD_Class[which(is.na(samples$NYUAD_Class))] = "Negative"
```

How many samples are left?

```
# Your answer here

102 samples are left.
```

Make a new violin plot with the cleaned data, showing the Ct values for the N1 and N2 genes faceted by plate.

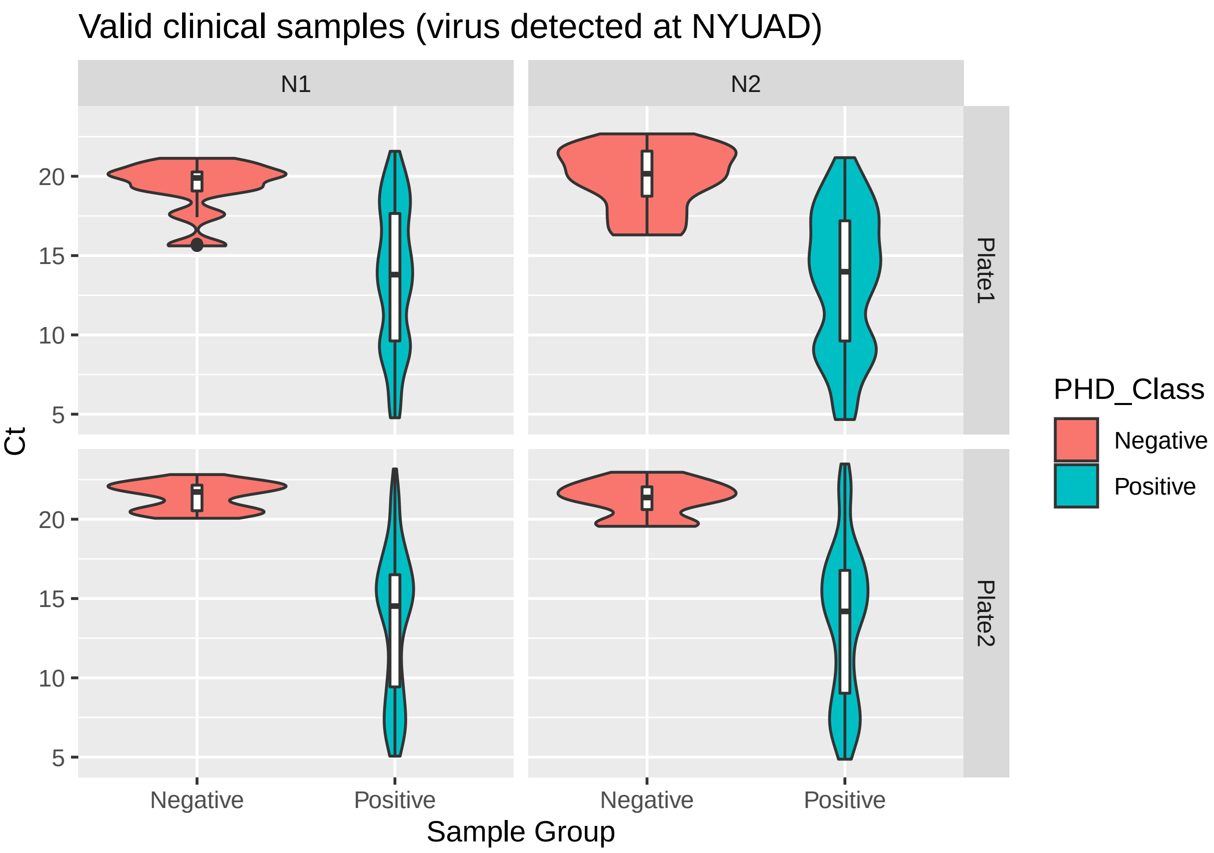
```
# violin plot of N1 and N2 Ct distributions in cleaned data, by assay
ggplot(nyuad.pos.samples, aes(x=AssayName, y=Value, fill=AssayName)) +
  geom_violin() +
  geom_boxplot(width=0.05, fill="white") +
  facet_wrap(~as.factor(Plate), nrow=1) +
  scale_fill_manual(values=c("#00BFC4","#F8766D")) +
  labs(title="Clincal samples: Amplification of N1 and N2 genes",
       x="Assay (Probe Set)",
       y = "Ct")
```



We can also plot the N1 and N2 assays based on whether they gave a positive or negative result in the clinical diagnostic assays performed at PHD. Modify your ggplot above to do this:

- Instead of using "AssayName" as the aesthetic mapping, use "PHD_class".
- Instead of using `facet_wrap()`, use `facet_grid(Plate ~ AssayName)`.

```
# by PHD diagnosis
ggplot(nyuad.pos.samples, aes(x=PHD_Class, y=Value, fill=PHD_Class)) +
  geom_violin() +
  geom_boxplot(width=0.05, fill="white") +
  facet_grid(Plate ~ AssayName) +
  labs(title = "Valid clinical samples (virus detected at NYUAD)",
       x = "Sample Group",
       y = "Ct")
```



Do you notice anything interesting bout the Ct values for the samples that PHD called “Negative” vs. “Positive”?

```
# your answer here

Yes, the negative ones generally took a lot longer to amplify.
```

Check for consistency among assay replicates We also want to discard samples that showed high variability between replicates, since these wouldn’t give us much confidence in our estimate of viral load; those samples would need to be re-analyzed again later.

Below you will compare the variation in the original N1/N2 data vs. the variation in your cleaned up data. To help out with this, I already filtered the data for you below using the following steps:

- Filter the original `samples` data frame and keep just the N1 and/or N2 assays with Ct Value < 999 (these are “good” samples)
- Compute the **mean** and **st.andard deviation** across the replicates for each assay (N1 and N2), and save this summary data in a new data frame (there will now be one row per sample.)
  - Hint: you can use the very convenient `dplyr` paradigm `filter %>% group_by %>% summarise` for this.)
- Do the same for the filtered “Positives” dataset (`nyuad.pos.samples`).

```
# sample variation (all clinical samples with Ct < 999)
sample.clean.var = samples %>%
  filter(AssayName %in% c("N1","N2") & Value < 999) %>%
  group_by(SampleName,AssayName,PHD_Class,Plate) %>%
  summarise(Ct.mean = mean(Value), Ct.sd = sd(Value), n = length(Value))
## `summarise()` has grouped output by 'SampleName', 'AssayName', 'PHD_Class'. You can override using t

# sample variation (all "NYUAD Positive" samples)
nyuad_pos.clean.var = nyuad.pos.samples %>%
  group_by(SampleName,AssayName,PHD_Class,Plate) %>%
  summarise(Ct.mean = mean(Value), Ct.sd = sd(Value), n = length(Value))
## `summarise()` has grouped output by 'SampleName', 'AssayName', 'PHD_Class'. You can override using t
```

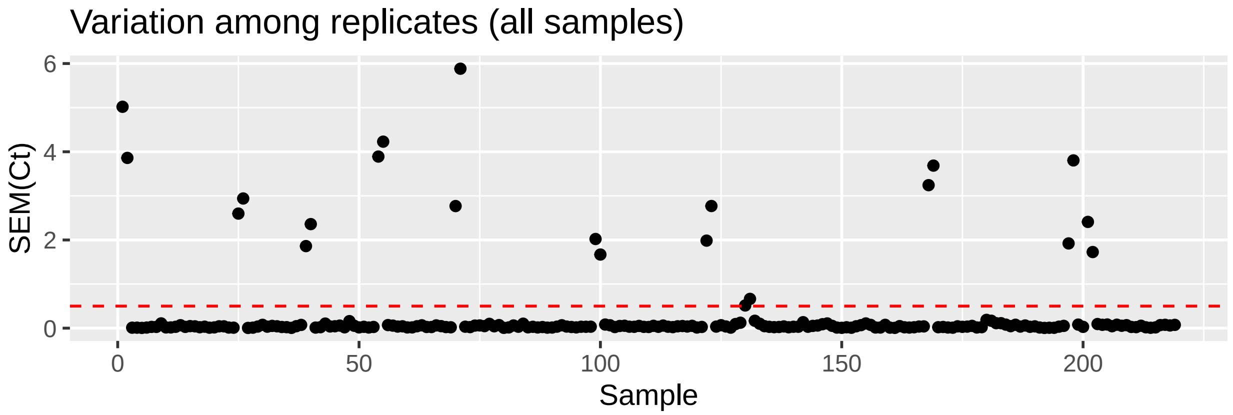
Your task is to draw some graphs to visually compare the variation in Ct values in these two datasets.

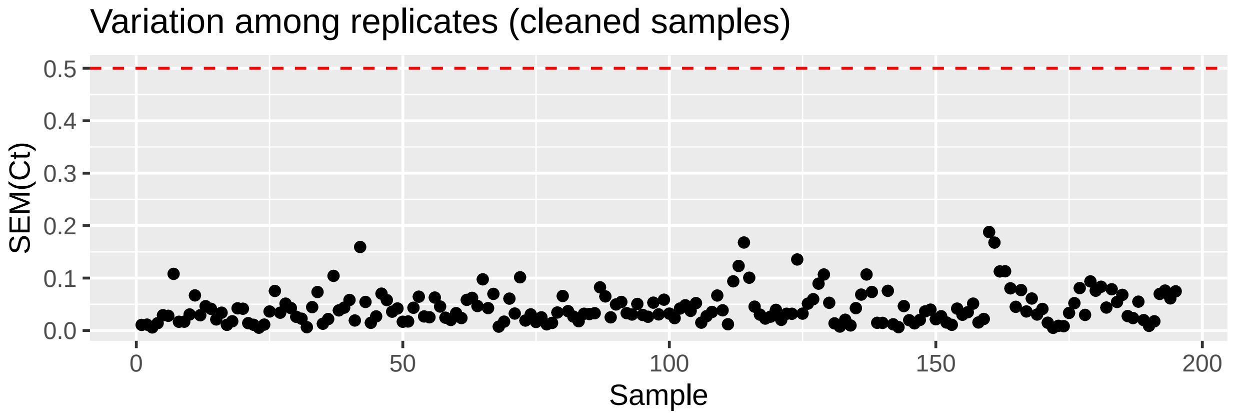
- First, for each of the two datasets we just made (cleaned sample data and NYUAD "Positive" samples), add a column containing the SEM for the Ct values.

```
## Add SEM(Ct) column to each dataset
sample.clean.var = sample.clean.var %>% mutate(Ct.se = Ct.sd/sqrt(n))
nyuad_pos.clean.var = nyuad_pos.clean.var %>% mutate(Ct.se = Ct.sd/sqrt(n))
```

Then, for each dataset, make a plot of the SEM(Ct) on the y-axis vs. the samples on the x-axis. + Include a horizontal line at SEM(Ct) = 0.5 on each of the graphs. + Hint: for the x-axis aesthetic mapping, you can use the expression `seq(SampleName)` in order to plot the samples along the x-axis.

```
## Plot variation as SEM(Ct) vs. sample index

# All samples with N1/N2 detected
p1 = ggplot(data=sample.clean.var, aes(x=seq(SampleName), y=Ct.se)) +
  geom_point() +
  geom_hline(yintercept=0.5, linetype="dashed", color = "red") +
  xlab("Sample") + ylab("SEM(Ct)") +
  ggtitle("Variation among replicates (all samples)")

# Cleaned data
p2 = ggplot(data=nyuad_pos.clean.var, aes(x=seq(Ct.se), y=Ct.se)) +
  geom_point() +
  geom_hline(yintercept=0.5, linetype="dashed", color = "red") +
  xlab("Sample") + ylab("SEM(Ct)") +
  ggtitle("Variation among replicates (cleaned samples)")

ggarrange(p1,p2,nrow=2)
```





If everything went ok with your filtering, you should find that some of the samples in the original dataset had high variation (s.e. > 0.5), and that the QC steps you performed above also removed those samples from your final "Positives" dataset.

So luckily, you don't need to do any more filtering for this step!!!

Q4: Compare Results and Estimate Viral Load

Finally! Now we've gotten to th fun part!!!

A. Compare sample classification from PHD and NYUAD

Now that you have a clean set of samples with reliable data, let's see how we did compared to PHD.

- First, make a 3-column summary table containing the sample names, PHD class, and NYUAD class for the samples in the original `sample` dataset.
  - **Hint**: you need to collapse all the replicates first in order to answer this question properly. You can use `group_by %>% summarize` to make a data frame with just one row per sample that you can then use to make your table.
- Next, make a contingency table comparing NYUAD's Positive / Negative / Inconclusive calls vs. PHD's Negative / Positive calls (this has been done for you below.)

```
# get summary table of classifications for each sample (one row per sample)
sample.classes = samples %>% group_by(SampleName, PHD_Class, NYUAD_Class) %>% summarise
```

```
## ‘summarise()‘ has grouped output by ’SampleName’, ’PHD_Class’. You can override using the ‘.groups‘ a
```

```
head(sample.classes)
```

```
## # A tibble: 6 x 3
## # Groups:   SampleName, PHD_Class [6]
##   SampleName     PHD_Class NYUAD_Class
##   <chr>          <chr>     <chr>
## 1 04MI200640957A Negative  Negative
## 2 04MI200647725A Negative  Inconclusive
## 3 04MI200650984A Negative  Negative
## 4 92MI200630550  Positive  Positive
## 5 92MI200642724  Positive  Positive
## 6 92MI200643329  Positive  Positive
```

```
# make a contingency table
with(sample.classes, table(PHD_Class, NYUAD_Class, dnn = c("PHD","NYUAD")))
```

```
##           NYUAD
## PHD         Inconclusive Negative Positive
##   Negative             7       67       17
##   Positive             4        2       85
```

How many samples that were called “Positive” by PHD did NYUAD fail to detect?

```
# Your answer here

Two were negative and 4 were inconclusive.
```

How many samples that were called “Negative” by PHD did NYUAD detect as positive?

```
# Your answer here

NYUAD detected virus in 17 samples that PHD called as Negative. NYUAD also had
inconclusive results for 7 of the negative samples from PHD.
```

NOTE*: In our analysis we actually included an additional class for “Inconsistent” samples, which were the ones where we only detected virus using one of the two probesets (N1 or N2). We had 9 samples in this class, so we officially reported 8 fewer Positives that were reported as Negative by PHD, and only one Negative that was reported as Positive by PHD.*

B. Linear model for prediction

Use the “good” standard dilution series you identified above, create a linear model for viral load based on the Ct values for the standards.

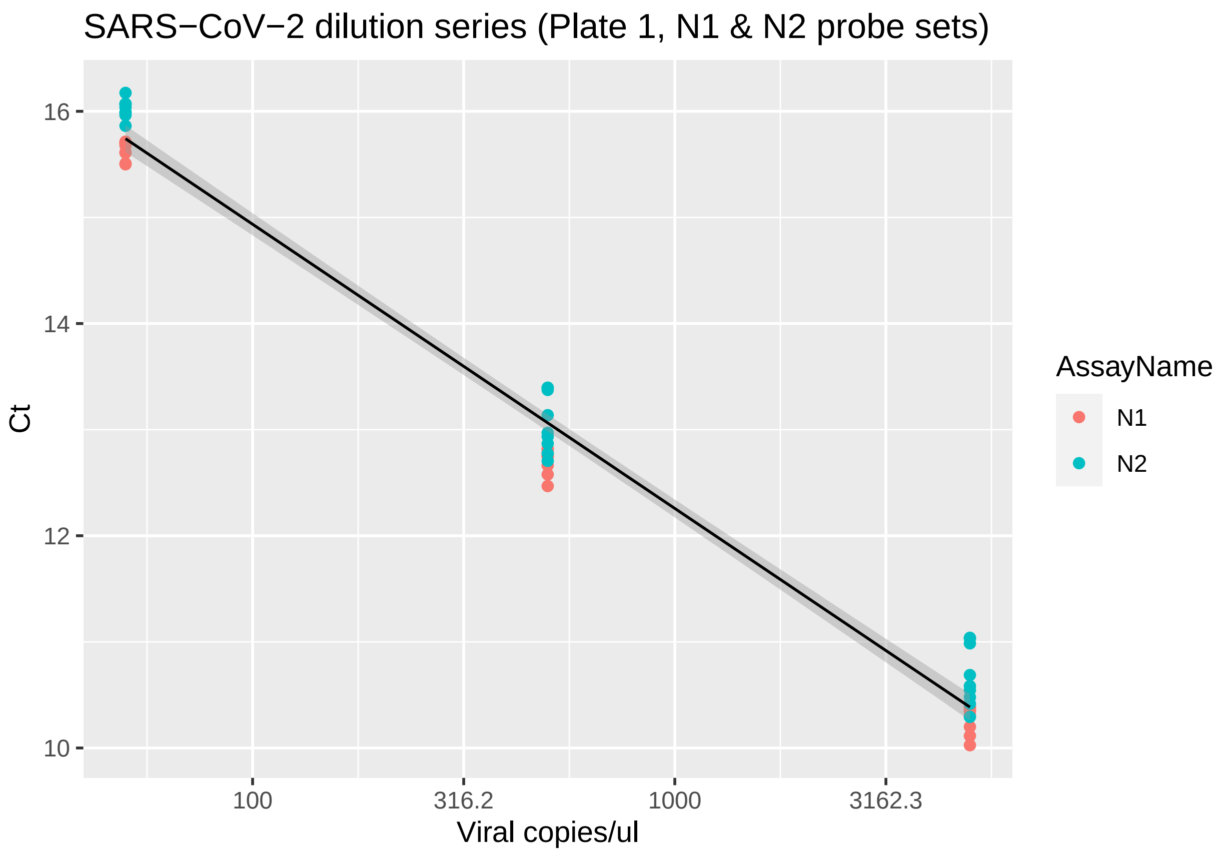
Note: Even though the N1 and N2 didn’t amplify **exactly** the same, they were pretty close, so just combine them as replicates for the purpose of making the model.

```r
# filter out N1 and N2 controls from Plate 1
std.ctls.p1 = std.ctls.n1.n2 %>% filter(Plate == "Plate1")

# linear model for rConc based on combined N1 and N2 Ct from "good" plate
lm.std.rConc = lm(log10(rConc) ~ Value, std.ctls.p1)
```

Plot the linear model with a 95% CI (remember that you can use `geom_smooth` for this).

```r
## ggplot of linear model for Ct vs. conc, with regression line and CI bands

# simple plot
# ggplot(std.ctls.p1, aes(x=log10(rConc), y=Value)) +
#   geom_point(aes(color=AssayName)) +
#   geom_smooth(method="lm", color="black", size=0.5) +
#   ggtitle("SARS-CoV-2 dilution series (Plate 1, N1 & N2 probe sets)") +
#   xlab("log10(viral copies/ul)") + ylab("Ct")

# with original concentrations
ggplot(std.ctls.p1, aes(x=log10(rConc), y=Value)) +
  geom_point(aes(color=AssayName)) +
  geom_smooth(method="lm", color="black", size=0.5) +
  ggtitle("SARS-CoV-2 dilution series (Plate 1, N1 & N2 probe sets)") +
  scale_x_continuous(label = function(x){return(round(10^x,1))}) +
  xlab("Viral copies/ul") + ylab("Ct")
```

```
## `geom_smooth()` using formula 'y ~ x'
```



C. Predict viral loads for samples

First, use the model you just made to make predictions of the viral load for each of the samples in your “NYUAD Positives” dataset. Make sure to use “**prediction**” intervals instead of “confidence” intervals for the prediction (recall that CI bands are narrower than PI bands).

NOTE: Since you will report one estimate of viral load for each sample, you will need to decide whether to take the mean of the replicates for each sample and then predict on that, or whether you want to predict on individual measurements and take the mean afterward. **You must do one of these things for your final data report.** *For simplicity, I have made a placeholder in the code for taking the means first, but you can do it either way.*

```
# combine sample measurements for N1 and N2 for each sample to a grand mean
# - you want to end up with one row per sample
# - you can use `group_by()` and `summarise()` to do this
nyuad.pos.sample.means = nyuad.pos.samples %>%
  group_by(SampleName, PHD_Class, Plate) %>%
  summarize(Value = mean(Value))
```

```
## ‘summarise()‘ has grouped output by ’SampleName’, ’PHD_Class’. You can override using the ‘.groups‘ a
```

```
# use lm for prediction on controls to get CIs for them
pred.sample.rConc = predict.lm(lm.std.rConc,
                               newdata = nyuad.pos.sample.means,
                               interval = "predict")
```

```
# make a data frame out of the predictions and take a look at the result
df.pred.sample.rConc = as.data.frame(pred.sample.rConc)
head(df.pred.sample.rConc)
```

```
##          fit       lwr         upr
## 1  5.0518672  4.817522  5.28621241
## 2  2.0013554  1.781697  2.22101373
## 3  2.2988448  2.080170  2.51751925
## 4  1.9397190  1.719791  2.15964706
## 5  4.1644728  3.939877  4.38906843
## 6 -0.3049185 -0.548891 -0.06094594
```

Next, make a new data frame that combines the prediction results with your sample data, appended as three new columns: the fit, the lower prediction interval bound, and the upper prediction interval bound.

```
# make a data frame adding 3 columns to the sample means data frame
# with fit, lwr, and upr
df.pred.sample.rConc = cbind(nyuad.pos.sample.means,
                             conc.fit = df.pred.sample.rConc$fit,
                             conc.lwr = df.pred.sample.rConc$lwr,
                             conc.upr = df.pred.sample.rConc$upr)
head(df.pred.sample.rConc)
```

```
## # A tibble: 6 x 7
## # Groups:   SampleName, PHD_Class [6]
##   SampleName    PHD_Class Plate  Value conc.fit conc.lwr conc.upr
##   <chr>         <chr>     <fct>  <dbl>    <dbl>    <dbl>    <dbl>
## 1 92MI200630550 Positive  Plate2  6.65    5.05     4.82    5.29
## 2 92MI200642724 Positive  Plate2 15.0     2.00     1.78    2.22
## 3 92MI200643329 Positive  Plate2 14.2     2.30     2.08    2.52
## 4 9900039096    Positive  Plate2 15.1     1.94     1.72    2.16
## 5 9900039097    Positive  Plate1  9.07    4.16     3.94    4.39
## 6 9900039101    Negative  Plate2 21.2    -0.305   -0.549  -0.0609
```

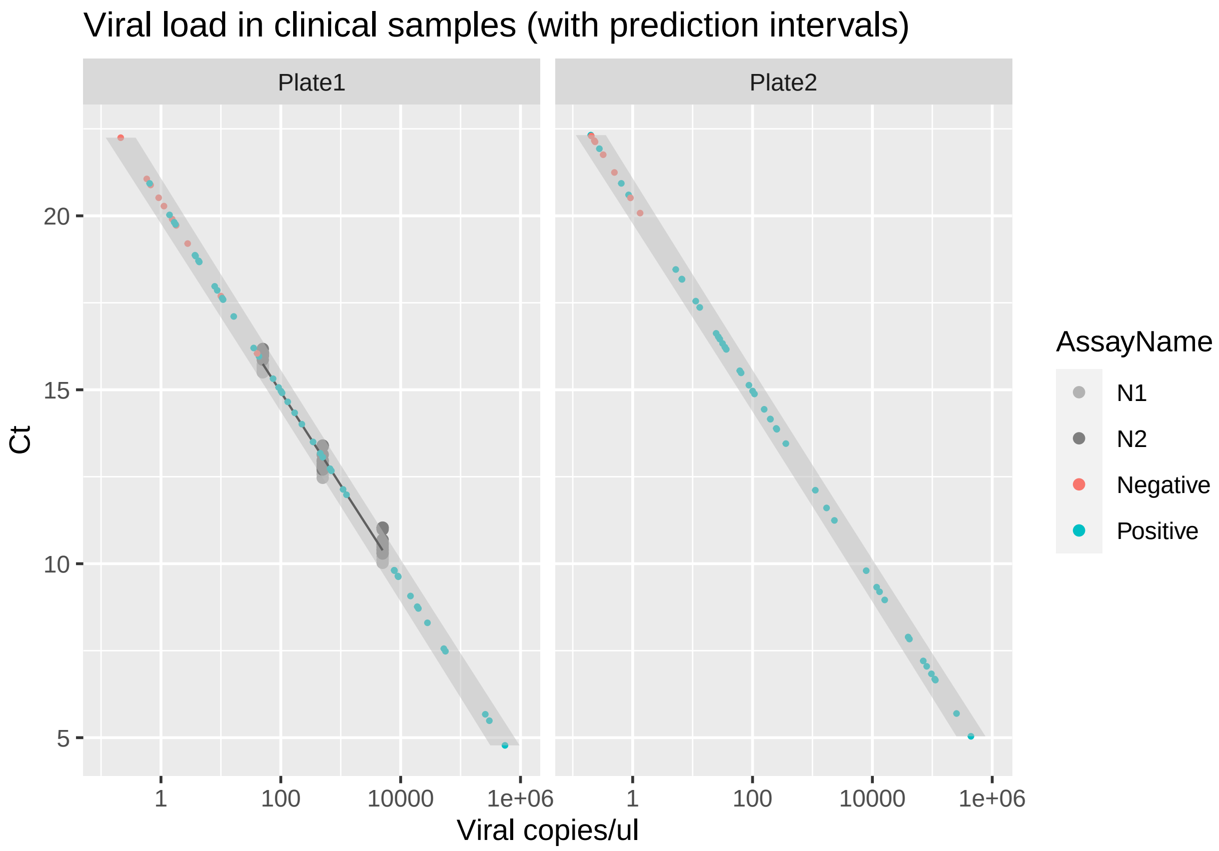
Finally, to see how your predictions look, plot them along with the control data.

- Create a ggplot object showing:
  - The control data points for the standards
  - The regression line from your controls
  - Points for your predictions, mapping `color` to the PHD diagnosis
  - Prediction Intervals (PI's) for the new predictions (use the new columns you just added)

```
# ============================================================================ #
# base plot: data points for N1/N2 standards from Plate 1
p0 = ggplot(std.ctls.p1, aes(x=log10(rConc), y=Value,
                             color=AssayName)) +       # map color to assay names
  geom_point() +
  scale_color_manual(values=c("gray70","gray50",       # for N1/N2 controls
                              "#F8766D","#00BFC4")) +  # for Neg/Pos classes
  facet_wrap(~Plate)  # wrap by Plate
```

```
# add standard controls regression line
p.fit = p0 +
  geom_smooth(method="lm", color="black", size=0.4, se = FALSE)

# add data points for sample predictions
# (points are the fitted values, map color to PHD classification)
p.fit = p.fit +
  geom_point(data = df.pred.sample.rConc, # the data frame containing predicted values
             mapping = aes(x = conc.fit, y = Value, color = PHD_Class), size=0.5)

# add PI for predictions (min and max are the lower and upper PI bounds)
p.fit = p.fit +
  geom_ribbon(data = df.pred.sample.rConc, # the data frame containing predicted values
              mapping = aes(y = Value, xmin = conc.lwr, xmax = conc.upr),
              fill = "gray", alpha = 0.5, inherit.aes = FALSE)

# convert back to copies / ul and add some labels
p.fit = p.fit +
  scale_x_continuous(label = function(x){return(round(10^x,1))}) +
  xlab("Viral copies/ul") + ylab("Ct") +
  ggtitle("Viral load in clinical samples (with prediction intervals)")

p.fit
```

```
## `geom_smooth()` using formula 'y ~ x'
```



Note that if you wanted to get specific confidence intervals for individual samples, you'd need to do the prediction on each point individually and then calculate the statistics for the variation afterward.

What do you conclude about the sensitivity of the NYUAD Fluidigm system?

```
# Your answer here

The assay is very sensitive and can detect viral particles down to less than one
copy per microliter.
```

How does the viral load estimated by NYUAD relate to the original diagnoses of the PHD clinical samples as "Negative" or "Positive"? Do you see a trend?

```
# Your answer here

Most of the samples detected by NYU that had a "Negative" clinical diagnosis
also carried very low viral titers, whereas "Positive" samples carried a much
wider range that approached close to 1M particles / ul. This makes sense that if
our assays is more sensitive, we would be likely to detect virus in samples with
low titers that could be missed by the standard diagnostic test.
```

D. Export data file with predicted viral loads for clinicas samples

Finally, you are ready to prepare a report to send back to the clinic!

- First, back-transform the predicted viral loads into proper units of "copies/ul" for reporting, and add this as a new column to `df.pred.sample.rConc`.
  - Call the new column `Copies_per_ul`.
  - Since these numbers span a huge range and will be ugly, use `signif()` to trim the output to two significant digits.
- Next, prepare a data table containing just the "SampleName", "PHD_Class", "NYUAD_Class", and "Copies_per_ul".
  - You can use `left_join()` to combine `sample.classes` with **selected columns** from `df.pred.sample.rConc`. This command will join the data frames based on common columns.
  - Make sure to specify `sample.classes` as the first argument.
- Finally, write your viral load estimates to a file (please remember to substitute your own name for the placeholder `YOURNAMEHERE`).
  - When you submit your work, upload this output file along with your .Rmd file.

```
# back-calculate actual viral loads and add as a new column
df.pred.sample.rConc = df.pred.sample.rConc %>%
  mutate(Copies_per_ul = signif(10^conc.fit,2))

# make a file for the final data report
data.report = left_join(sample.classes,
                        select(df.pred.sample.rConc,SampleName,Copies_per_ul))
```

```
## Adding missing grouping variables: ‘PHD_Class‘
```

```
## Joining, by = c("SampleName", "PHD_Class")
```

```r
head(data.report)
```

```
## # A tibble: 6 x 4
## # Groups:   SampleName, PHD_Class [6]
##   SampleName     PHD_Class NYUAD_Class  Copies_per_ul
##   <chr>          <chr>     <chr>                <dbl>
## 1 04MI200640957A Negative  Negative                NA
## 2 04MI200647725A Negative  Inconclusive            NA
## 3 04MI200650984A Negative  Negative                NA
## 4 92MI200630550  Positive  Positive            110000
## 5 92MI200642724  Positive  Positive               100
## 6 92MI200643329  Positive  Positive               200
```

```r
# export data file
# write.csv(data.report, "data/PHD_samples.viral_load.YOURNAMEHERE.csv")
write.csv(data.report, "data/PHD_samples.viral_load.ANSWER_KEY.csv")
```

Congratulations! You've just completed your first COVID PCR testing analysis!