Hydrolytic Extracellular Enzyme Activity Calculations

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Contents

1 Setup

1.1 Plate Templates

Start by filling out your template files and metadata file.

You need one completed **template file** for each plate, in CSV format. There are some templates in the `empty-templates` sub-directory for hydrolytic and oxidative enzyme assays regularly performed in the Gutknecht lab. **Alternatively** you can use a script that HB wrote to automate the creation of `plater` templates (see `plater-prep` project here: https://github.com/havablair/plater-prep)

Name your template files with the ID of your plate. This will be added as its own column by plater so that you can identify which plate the data is coming from.

Files used with this version of the script should contain the following four blocks of information:

–"template" = standard, blank, and sample IDs. Empty wells (no sample, standard, or check) may be coded as ".", "0" (zero), "NA", or left blank.

–"bad_wells" = identifies any wells that have known problems (ex. pipetting errors). Code these as "bad". Leave all other wells empty in this part of the template.

– "conc_uM" = identifies the concentration of the substrate or MUB used in the well. The concentration should be in umol/L (uM). Examples: sub_300 = 300 uM enzyme substrate. mub_1.25 = 1.25 uM MUB standard.

–"data" = raw fluorescence data from the plate reader

1.2 Metadata

You also need one completed **metadata file** (data for all plates you are analyzing with this script can be included in the same file). Find "template_metadata.csv" in the `empty-templates` sub-directory. Make sure that the plate_id column **exactly matches** the names of your filled templates.

Use 24-hour clock time (https://en.wikipedia.org/wiki/24-hour_clock) to specify time in the metadata sheet.

1.3 File organization

The plate templates you want to process must all be in the **filled-templates subfolder**, and that folder must not contain any other files. R will attempt to read in all files in the designated folder.

Your **metadata file** should be in the main project folder (eea-hydrolytic-template)

Now you are ready to read in your files. If you get an error, check whether you have any extra CSV files in your filled-templates folder. Also check to make sure the files are saved as CSV, not XLSX. (However, script can easily be adjusted to read XLSX).

1.4 Read in plate templates & metadata

```r
# read in completed template files from subfolder
file.names <- dir("./filled-templates/")
file.paths <- paste0("./filled-templates/", file.names)
plates <- read_plates(file.paths)
colnames(plates) <- c("plate", "wells", "id", "fluor", "bad_wells",
    "conc_uM")
unique(plates$plate)  # To check that all plates read in correctly
```

```
##  [1] "plate_b"   "test_2006" "test_2008" "test_2014" "test_2016" "test_2022"
##  [7] "test_2024" "test_2050" "test_2051" "test_2082" "test_2083" "test_2090"
## [13] "test_2092"
```

```
# read in plate metadata - fill in the name of your metadata
# file
plate_metadata <- read.csv("./eea_metadata_test.csv", stringsAsFactors = FALSE)

colnames(plate_metadata) <- c("plate", "ph_buffer", "moist_soil_mass_g",
    "vol_buffer_ml", "substrates", "tin", "tin_moist", "tin_dry",
    "time_soil_added", "time_naoh_added", "time_plate_read")
```

2 Metadata calculations

2.1 Incubation time, soil oven dry equivalent

```
# parse the time columns so we can do math on them
plate_metadata <- plate_metadata %>% mutate(time_soil_added = parse_hm(time_soil_added),
    time_naoh_added = parse_hm(time_naoh_added), time_plate_read = parse_hm(time_plate_read))

# calculate incubation time and convert from seconds to hours
plate_metadata <- plate_metadata %>% mutate(inc_time_hr = as.numeric(((time_plate_read -
    time_soil_added)/60/60)))

# calculate soil moisture content and dry soil equivalent
plate_metadata <- plate_metadata %>% mutate(mc_soil_moist = tin_moist -
    tin, mc_soil_dry = tin_dry - tin, soil_water_content = (mc_soil_moist -
    mc_soil_dry)/mc_soil_dry, soil_ov_dry_eq_g = moist_soil_mass_g -
    (moist_soil_mass_g * soil_water_content))
```

3 Initial Quality control

3.1 Remove empty wells and known bad wells

```
# Remove empty wells
no_missing <- subset(plates, !is.na(id) & id != "empty")

# Remove bad wells
no_bad <- subset(no_missing, is.na(bad_wells))

# Create a dataframe with the details about which wells were
# removed as 'bad'.  Can write this to CSV now, or wait until
# after later QC checks to compile a list of samples that
# need to be redone.
bad_list <- subset(no_missing, !is.na(bad_wells))

# print a message about how many wells were removed
wells_removed <- nrow(plates) - nrow(no_bad)
```

```
glue("Removed {wells_removed} wells that were missing or bad")
```

```
## Removed 193 wells that were missing or bad
```

```
# filter out plate b here - it will be used for the emission
# coefficient calculation
plate_b <- no_bad %>% filter(plate == "plate_b")

# clean_data contains all of the plate a data
clean_data <- no_bad %>% filter(plate != "plate_b")

# nest data so we can keep our calculations organized nicely
# by plate
clean_nested <- clean_data %>% group_by(plate) %>% nest()
```

4 Standards calculations

4.1 B Plate: MUB standard calculations (for emission coefficient calculation)

Boone, R.D., Grigal, D.F., Sollins, P., Ahrens, R.J., & Armstrong, D.E. (1999). "Soil Sampling, Preparation, Archiving, and Quality Control" in Eds. Robertson, G. P., Coleman, D. C., Sollins, P., & Bledsoe, C. S. Standard soil methods for long-term ecological research. Oxford University Press on Demand. p 15-16

CV = (standard deviation / mean) * 100

Sometimes called relative standard deviation (RSD) in QA/QC literature according to Boone et al. 1999.

"Laboratory replicates with RSD [CV] values </= 10% indicate that analyses are sufficiently precise" Boone et al., 1999 p.16

```
# exract MUB concentrations from the conc_uM column and drop
# the empty 'bad_wells' column
plate_b <- plate_b %>% mutate(conc_uM = as.numeric(str_replace(conc_uM,
    "mub_", ""))) %>% select(-bad_wells)

# calculate the mean fluorescence across technical replicates
# for each MUB concentration
plate_b_means <- plate_b %>% group_by(id) %>% filter(str_detect(id,
    "MUB")) %>% summarise(mub_std_fluor = mean(fluor), n = n(),
    sd = sd(fluor), cv = sd(fluor)/mean(fluor) * 100)

# calculate the plate blank (buffer only)
plate_b_blank <- plate_b %>% filter(str_detect(id, "Buf")) %>%
    summarise(fluor_blank = mean(fluor), n = n())

fluor_blank <- plate_b_blank$fluor_blank

# subtract the fluorescence of the plate blank (id = Buf)
# from the mean fluorescence of the standards
plate_b_means <- plate_b_means %>% mutate(plate_blank = fluor_blank,
    corr_fluor = mub_std_fluor - plate_blank, mub_conc_uM = as.numeric(str_replace(id,
        "MUB", "")))
```

```
plate_b_means
```

```
## # A tibble: 4 x 8
##   id        mub_std_fluor     n    sd    cv plate_blank corr_fluor mub_conc_uM
##   <chr>             <dbl> <int> <dbl> <dbl>       <dbl>      <dbl>       <dbl>
## 1 MUB0.16           1486.     4  111.  7.45        66.1      1420.       0.16
## 2 MUB0.625          5891.     4  466.  7.91        66.1      5825.       0.625
## 3 MUB1.25          11928.     4  845.  7.08        66.1     11862.       1.25
## 4 MUB2.5           22628      4  721.  3.19        66.1     22562.       2.5
```

4.2 A Plates: Calculate plate blank and homogenate blank

```
# function to calculate plate blanks (Buffer only)
get_plate_blank <- function(data) {
    buf <- data %>% filter(id == "Buf")
    mean(buf$fluor)
}

# apply function to nested df
with_a_blanks <- clean_nested %>% mutate(plate_blank = map_dbl(data,
    get_plate_blank))

# function to calculate the fluor for HOMogenate BLanks
# (hombl = buffer + homogenate)
get_hombl <- function(data) {
    buf_soil <- data %>% filter(str_detect(id, "Hombl"))
    mean(buf_soil$fluor)
}

# apply function to nested df
calc_hombl <- with_a_blanks %>% mutate(hombl = map_dbl(data,
    get_hombl))

head(calc_hombl)
```

```
## # A tibble: 6 x 4
## # Groups:   plate [6]
##   plate     data              plate_blank hombl
##   <chr>     <list>                  <dbl> <dbl>
## 1 test_2006 <tibble [80 x 5]>        61.2  113.
## 2 test_2008 <tibble [80 x 5]>        60.8  116.
## 3 test_2014 <tibble [80 x 5]>        68.6  131.
## 4 test_2016 <tibble [80 x 5]>        59.2  125
## 5 test_2022 <tibble [80 x 5]>        64.4  121.
## 6 test_2024 <tibble [80 x 5]>        58.6  128.
```

4.3 A Plates: Calculate mean fluor of the MUB standard + soil wells (for quench coeff calculation)

```
# function to average the MUB+soil (quench) standard wells to
# get mean fluorescence for each MUB concentration

quench_std_fun <- function(data, hombl) {
    data %>% select(wells, id, fluor, conc_uM) %>% filter(str_detect(id,
        "quench")) %>% mutate(conc_uM = as.numeric(str_replace(conc_uM,
        "mub_", ""))) %>% group_by(conc_uM) %>% summarise(quench_fluor = mean(fluor),
        n = n(), sd = sd(fluor), cv = sd(fluor)/mean(fluor) *
            100, hombl = hombl, corr_fluor = quench_fluor - hombl)  #also corrects for hom blank here
}

# apply function to nested df
quench_nested <- calc_hombl %>% mutate(quench_std_values = map2(data,
    hombl, quench_std_fun))

head(quench_nested)
```

```
## # A tibble: 6 x 5
## # Groups:   plate [6]
##   plate     data               plate_blank hombl quench_std_values
##   <chr>     <list>                   <dbl> <dbl> <list>
## 1 test_2006 <tibble [80 x 5]>         61.2  113. <tibble [4 x 7]>
## 2 test_2008 <tibble [80 x 5]>         60.8  116. <tibble [4 x 7]>
## 3 test_2014 <tibble [80 x 5]>         68.6  131. <tibble [4 x 7]>
## 4 test_2016 <tibble [80 x 5]>         59.2  125  <tibble [4 x 7]>
## 5 test_2022 <tibble [80 x 5]>         64.4  121. <tibble [4 x 7]>
## 6 test_2024 <tibble [80 x 5]>         58.6  128. <tibble [4 x 7]>
```

5 Linear models

5.1 Linear model functions

```
# function to run a linear model x = MUB concentration, y =
# mean fluorescence

lm_mod_ftn <- function(df) {
    lm(corr_fluor ~ conc_uM, data = df)
}

# functions to extract linear model details calculated above
# into a nicer format for putting in our graphs

b_fun <- function(mod) {
    coefficients(mod)[[1]]
}
```

```r
slope_fun <- function(mod) {
    coefficients(mod)[[2]]
}

r_sq_fun <- function(mod) {
    summary(mod)[["r.squared"]]
}

# leaving this max fluorescence function out for now because
# I'm not sure we need it.  max_fluor_fun <- function(data){
# max(data$corr_std_mean) }
```

5.2 A Plates: Linear model calcs for MUB+soil standards (quench)

```r
# calculate linear model for quench (A plates)

quench_lm_calcs <- quench_nested %>% mutate(quench_lm = map(quench_std_values,
    lm_mod_ftn))

# Extract linear models details from the homogenate control
# linear model
quench_lm_details <- quench_lm_calcs %>% mutate(intcpt_quench = map_dbl(quench_lm,
    b_fun), slope_quench = map_dbl(quench_lm, slope_fun), r_squared_quench = map_dbl(quench_lm,
    r_sq_fun))

# nest the linear model details in a dataframe
nest_quench_stats <- quench_lm_details %>% nest(lm_stats_quench = c(intcpt_quench,
    slope_quench, r_squared_quench))
```

5.2.1 R^2 values for MUB+Soil standards (A Plate)

```
## [1] "The following plates have R^2 < 0.99 for MUB+soil standards, check graphs and raw data for outl

## [1] "MUB+soil standards are located in columns 7 and 8 of A Plates"
```

5.3 B Plate: Linear model calculations for MUB standards (emission)

```r
# fix name of mub concentration column so it works with the
# lm function
plate_b_rename <- plate_b_means %>% rename(conc_uM = mub_conc_uM)

# apply linear model ftn to plate b data
lm_plate_b <- lm_mod_ftn(plate_b_rename)

# save linear model details
intcpt_emis <- b_fun(lm_plate_b)
slope_emis <- slope_fun(lm_plate_b)
rsq_emis <- r_sq_fun(lm_plate_b)
```

```
# add linear model details to plate b dataframe
lm_stats_emis <- plate_b_rename %>% mutate(intcpt_emis = intcpt_emis,
    slope_emis = slope_emis, rsq_emis = rsq_emis)
```

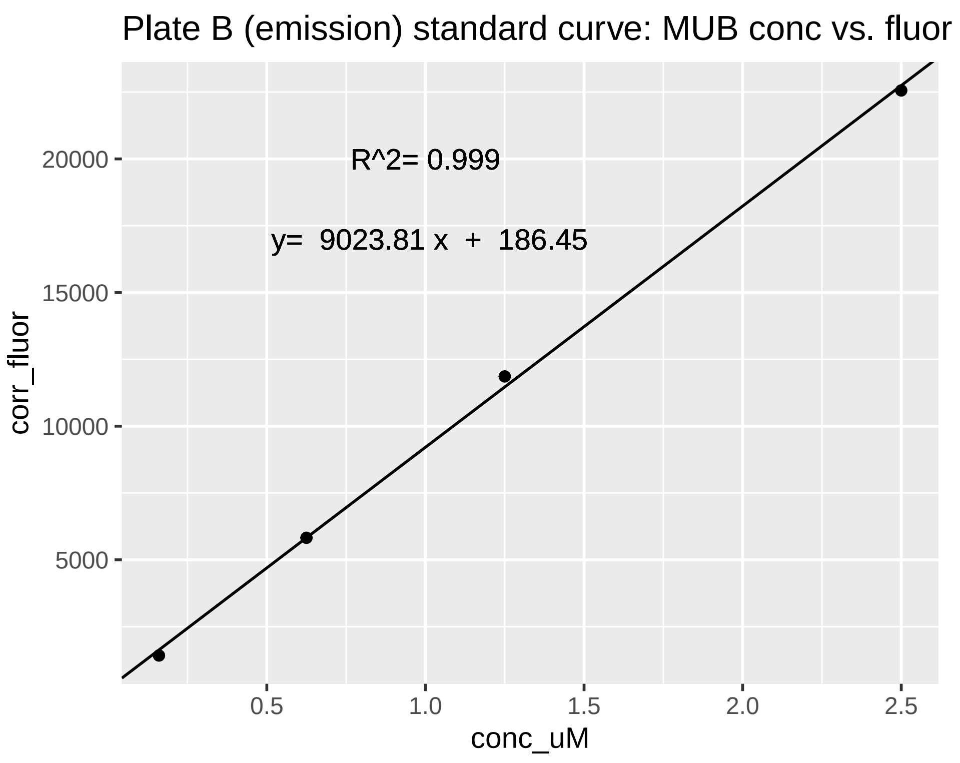
5.3.1 R^2 values for MUB standard curve (B Plate)

```
## The R^2 value for the linear model of the MUB standard curve is 0.9991, if this value is <0.99, revi
```

6 Standard curve graphs

6.1 B Plate: Plot MUB standard curve (emission)

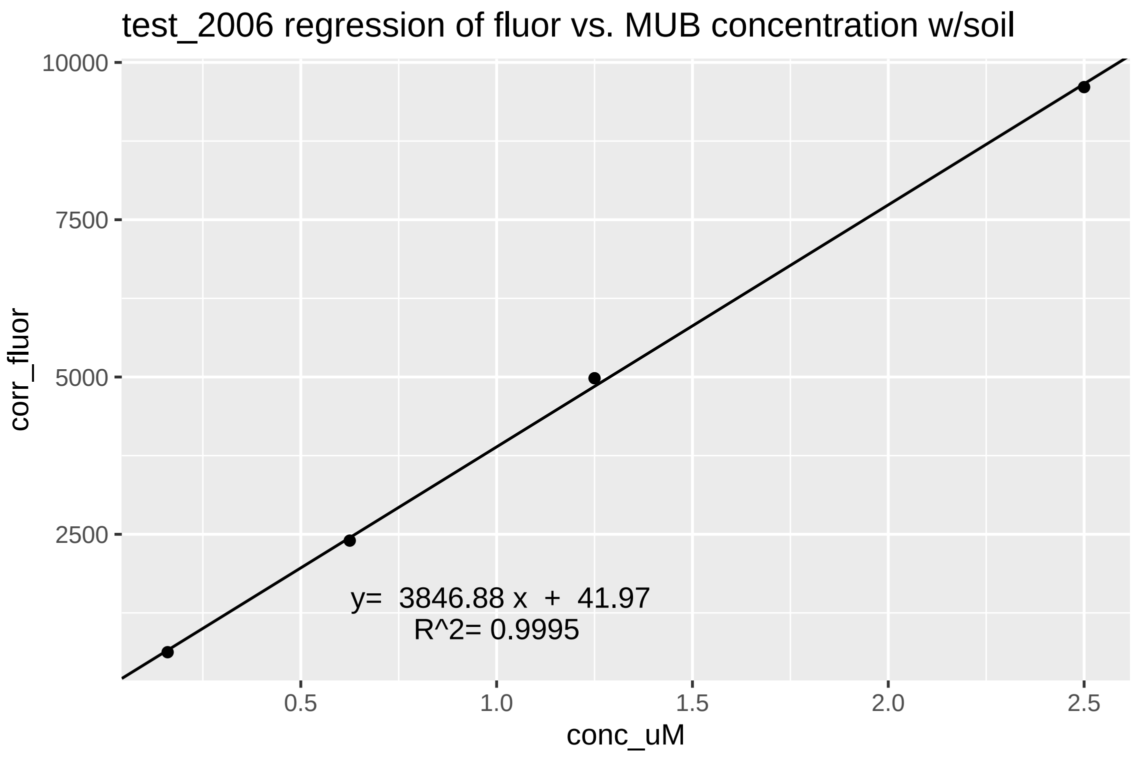
```
## consider writing this into a function so that multiple B
## plates (from multiple runs) could be processed together.

b_plot <- ggplot(lm_stats_emis) + geom_point(aes(x = conc_uM,
    y = corr_fluor)) + geom_abline(aes(slope = slope_emis, intercept = intcpt_emis)) +
    geom_text(data = lm_stats_emis, aes(x = 1, y = 20000, label = paste("R^2=",
        round(rsq_emis, digits = 3))), inherit.aes = FALSE) +
    geom_text(aes(x = 1, y = 17000, label = paste(" y= ", round(slope_emis,
        digits = 2), "x", " + ", round(intcpt_emis, digits = 2))),
        inherit.aes = FALSE) + labs(title = glue("Plate B (emission) standard curve: MUB conc vs. fluor

b_plot
```



6.2 A Plate: plot homogenate + MUB standard curves (quench)

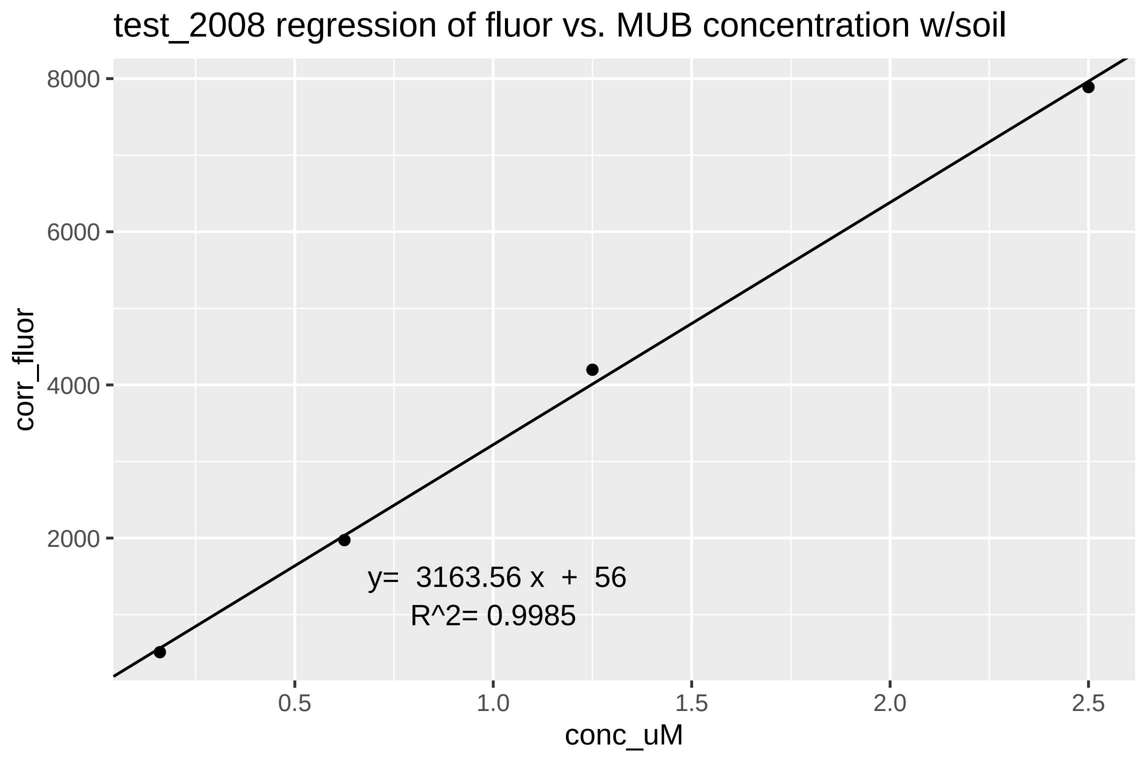
```
# plotting function
plot_homog_fun <- function(quench_std_values, lm_stats_quench,
    plate) {

    g <- ggplot() + geom_point(data = quench_std_values, aes(x = conc_uM,
        y = corr_fluor)) + geom_abline(data = lm_stats_quench,
        aes(slope = slope_quench, intercept = intcpt_quench)) +
        geom_text(data = lm_stats_quench, aes(x = 1, y = 1000,
            label = paste("R^2=", round(r_squared_quench, digits = 4))),
            inherit.aes = FALSE) + geom_text(data = lm_stats_quench,
        aes(x = 1, y = 1500, label = paste(" y= ", round(slope_quench,
            digits = 2), "x", " + ", round(intcpt_quench, digits = 2))),
        inherit.aes = FALSE) + labs(title = glue("{plate} regression of fluor vs. MUB concentration w/s
    return(g)
}

# run the plotting function for each plate and store the
# plots in our nested dataframe
homog_plots <- nest_quench_stats %>% mutate(plot_homog_control = pmap(list(quench_std_values,
    lm_stats_quench, plate), plot_homog_fun))

homog_plots$plot_homog_control
```

```
## [[1]]
```

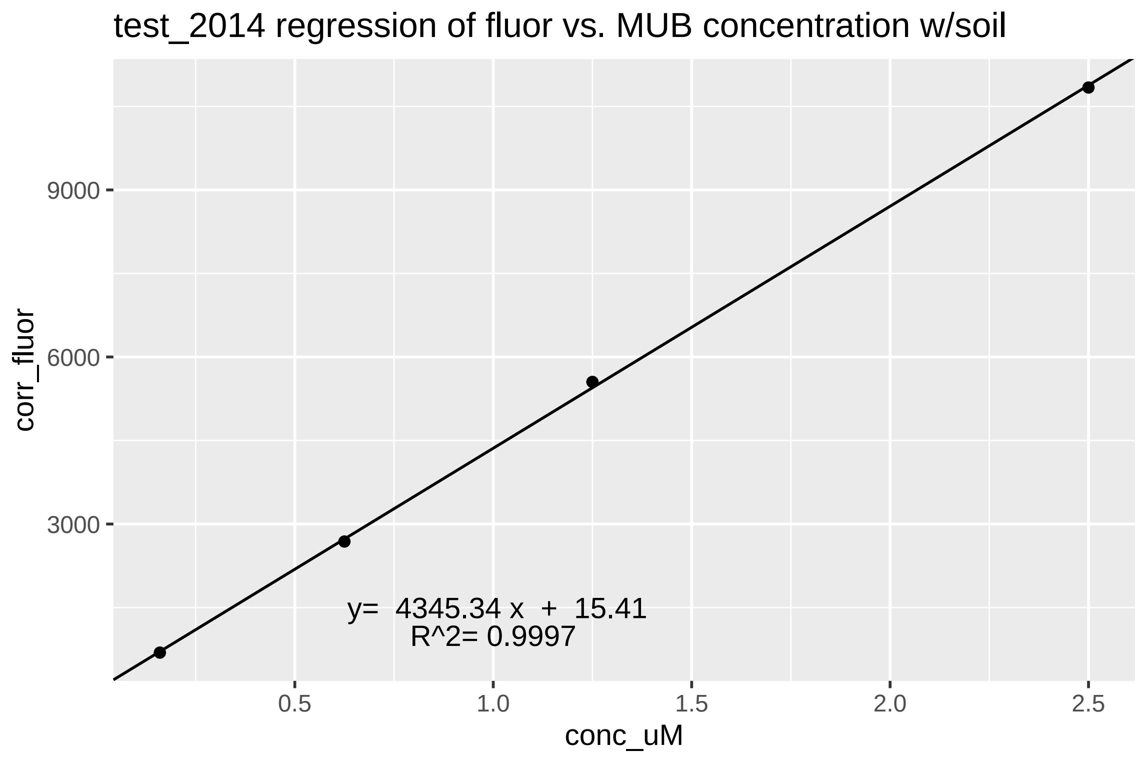


```
##
```

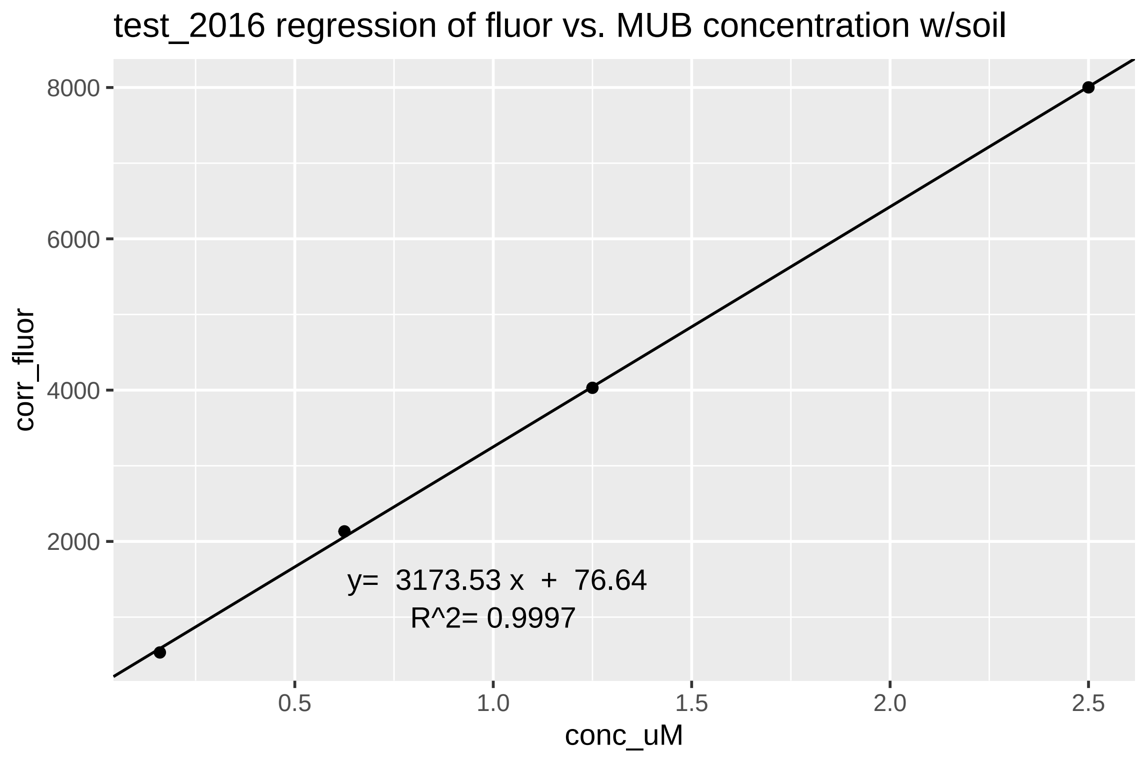
```
## [[2]]
```


test_2008 regression of fluor vs. MUB concentration w/soil
corr_fluor
conc_uM
y= 3163.56 x + 56
R^2= 0.9985
8000
6000
4000
2000
0.5
1.0
1.5
2.0
2.5

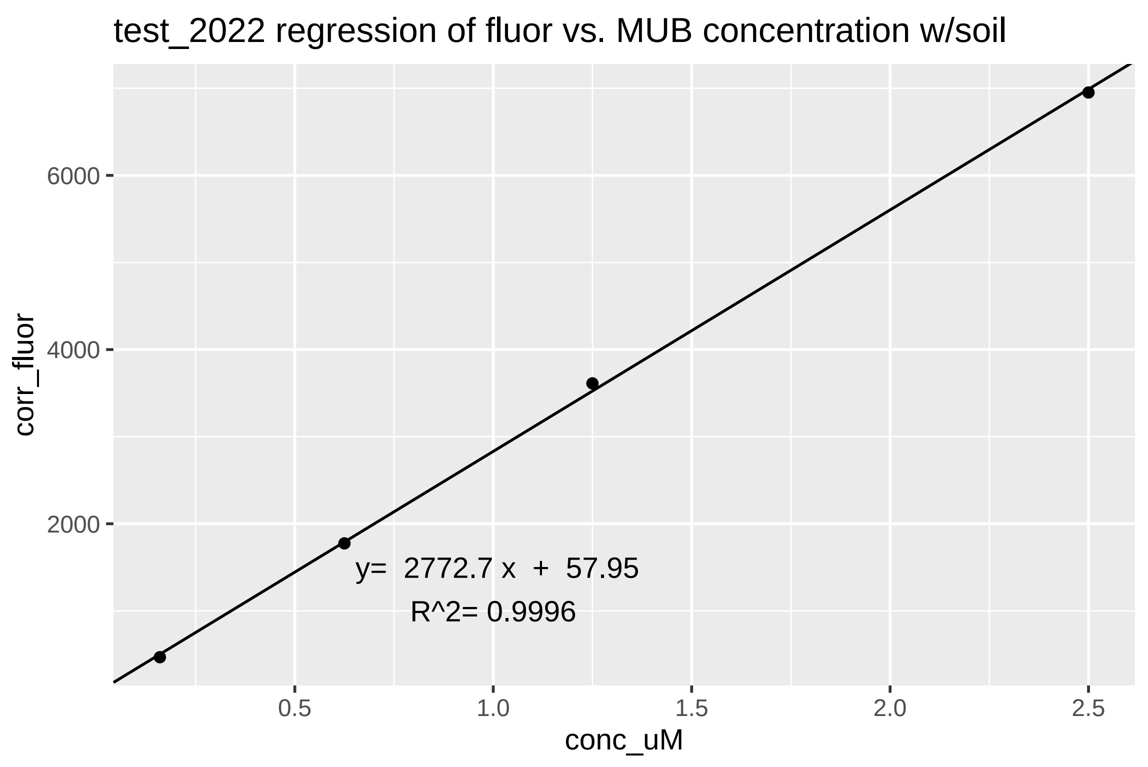
```
##
## [[3]]
```


test_2014 regression of fluor vs. MUB concentration w/soil
corr_fluor
conc_uM
y= 4345.34 x + 15.41
R^2= 0.9997
9000
6000
3000
0.5
1.0
1.5
2.0
2.5

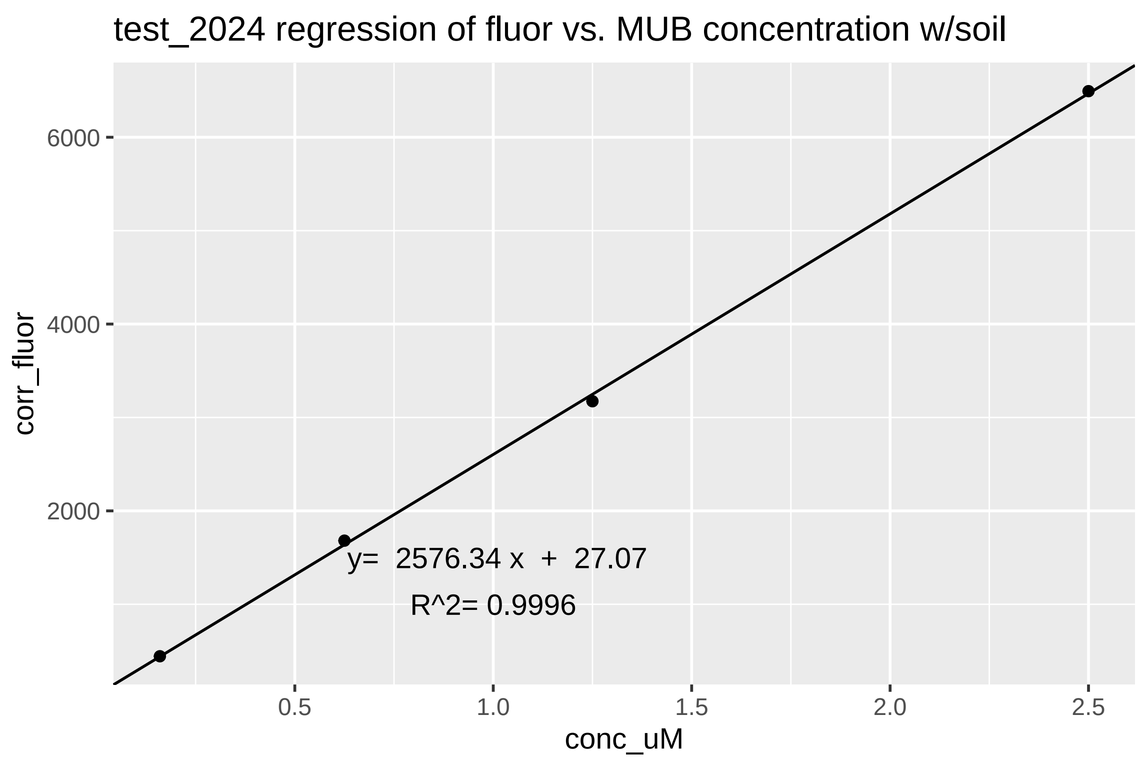
```
##
## [[4]]
```


test_2016 regression of fluor vs. MUB concentration w/soil
8000
6000
4000
2000
corr_fluor
0.5
1.0
1.5
2.0
2.5
conc_uM
y= 3173.53 x + 76.64
R^2= 0.9997

```
##
## [[5]]
```

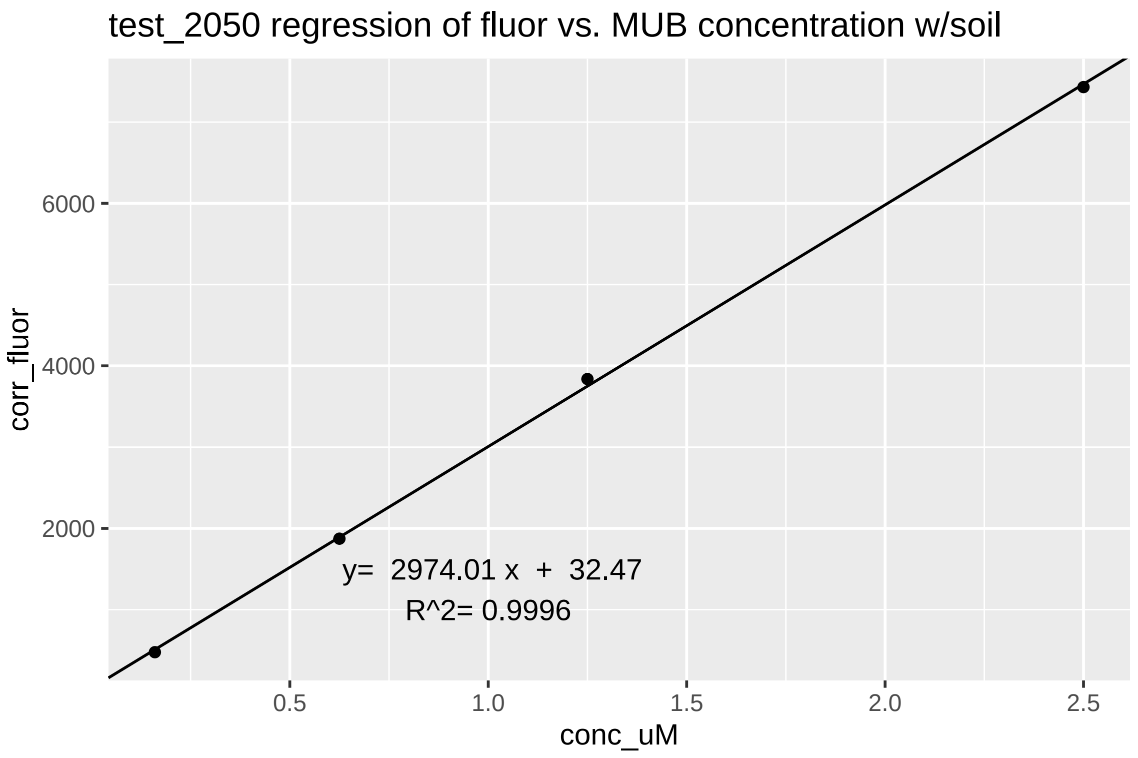

test_2022 regression of fluor vs. MUB concentration w/soil
y= 2772.7 x + 57.95
R^2= 0.9996
corr_fluor
conc_uM
6000
4000
2000
0.5
1.0
1.5
2.0
2.5

```
##
## [[6]]
```

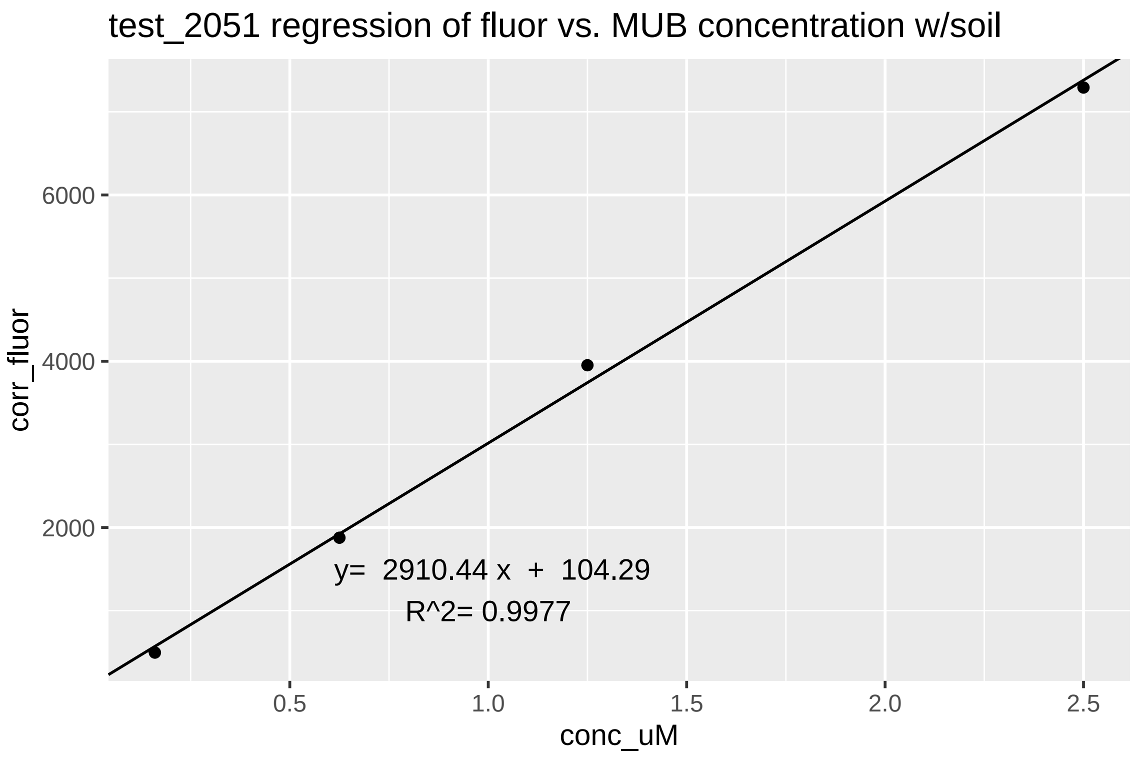

test_2024 regression of fluor vs. MUB concentration w/soil
y= 2576.34 x + 27.07
R^2= 0.9996
corr_fluor
conc_uM
6000
4000
2000
0.5
1.0
1.5
2.0
2.5

```
##
```

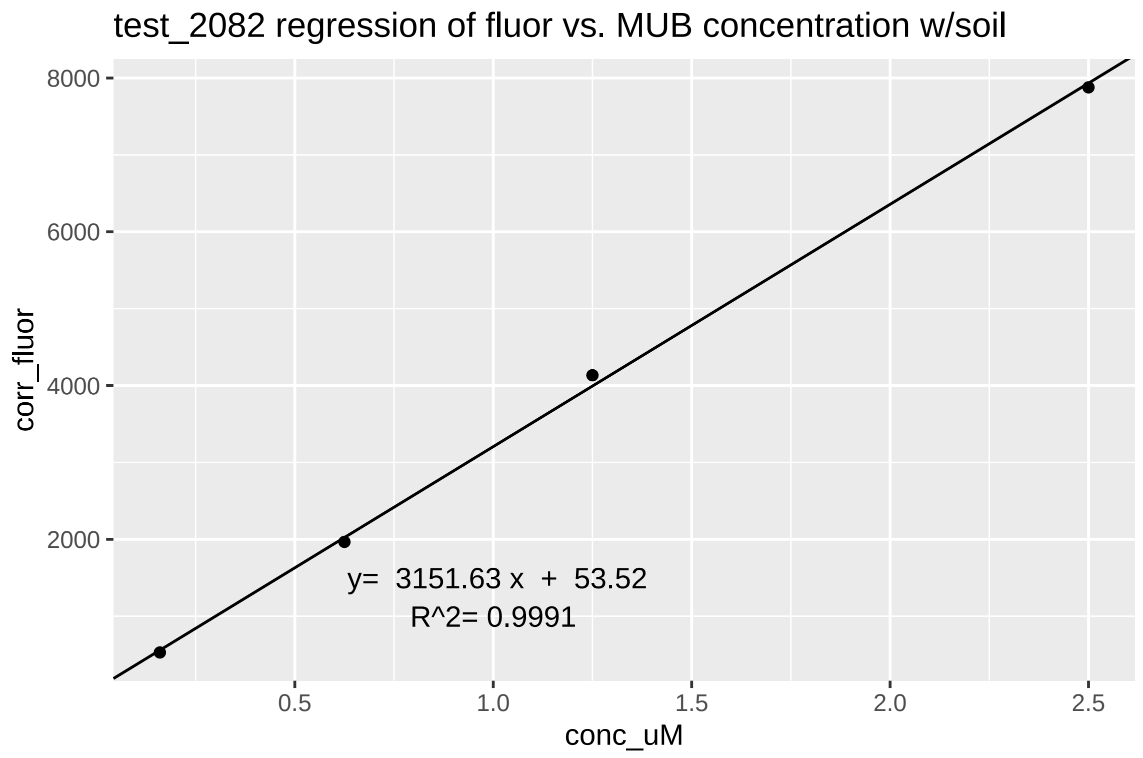
```
## [[7]]
```


test_2050 regression of fluor vs. MUB concentration w/soil
y= 2974.01 x + 32.47
R^2= 0.9996
corr_fluor
conc_uM
6000
4000
2000
0.5
1.0
1.5
2.0
2.5

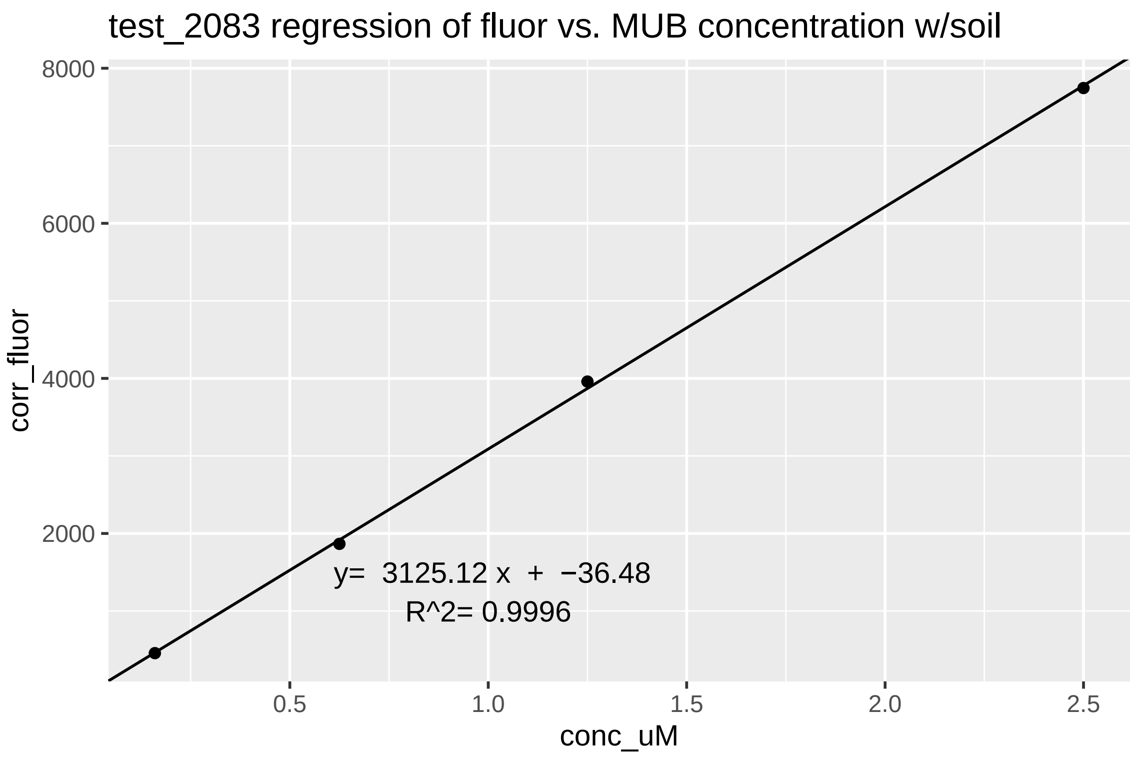
```
##
## [[8]]
```


test_2051 regression of fluor vs. MUB concentration w/soil
y= 2910.44 x + 104.29
R^2= 0.9977
corr_fluor
conc_uM
6000
4000
2000
0.5
1.0
1.5
2.0
2.5

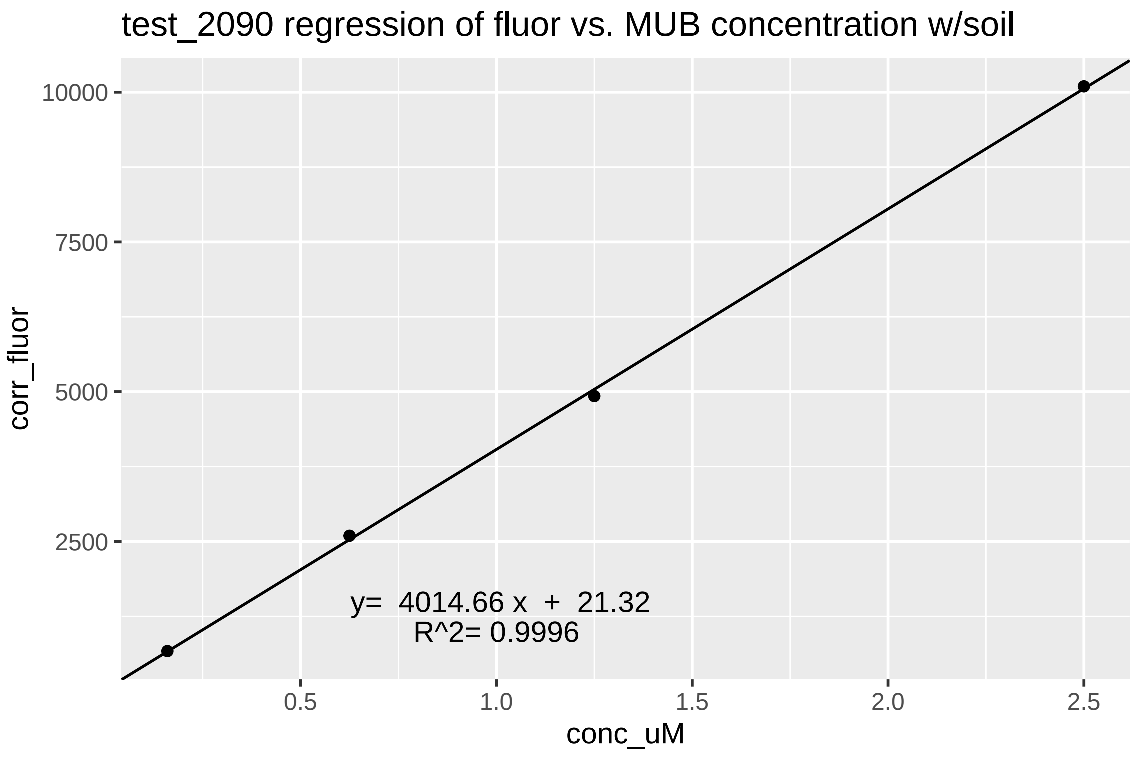
```
##
## [[9]]
```


test_2082 regression of fluor vs. MUB concentration w/soil
8000
6000
4000
2000
corr_fluor
0.5
1.0
1.5
2.0
2.5
conc_uM
y= 3151.63 x + 53.52
R^2= 0.9991

```
##
## [[10]]
```

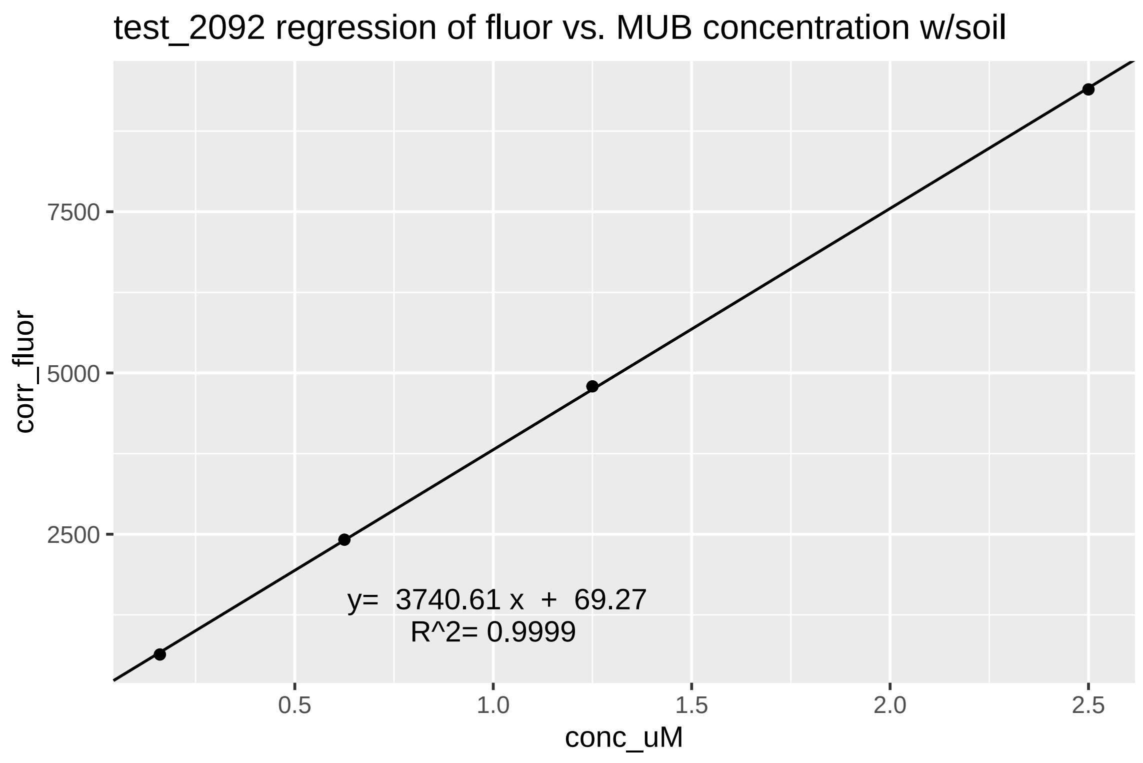

test_2083 regression of fluor vs. MUB concentration w/soil
y= 3125.12 x + −36.48
R^2= 0.9996
corr_fluor
conc_uM
8000
6000
4000
2000
0.5
1.0
1.5
2.0
2.5

```
##
## [[11]]
```


test_2090 regression of fluor vs. MUB concentration w/soil
y= 4014.66 x + 21.32
R^2= 0.9996
corr_fluor
conc_uM
10000
7500
5000
2500
0.5
1.0
1.5
2.0
2.5

```
##
```

```
## [[12]]
```



7 Coefficient calculations

7.1 Calculate emission coefficient

The emission coefficient is the slope (m) from plate b (fluorescence vs MUB conc NO SOIL) divided by the assay volume

Pay attention to units! **slope (m) units** from the standard curves = fluor/uM = fluor/ (umol/L) = fluor L / umol = fluor mL / nmol

assay volume units are 250uL = 0.00025 L = 0.250 mL

emission coefficient units are fluorescence/nmol.

```r
assay_vol_ml <- 0.25

# just grabbing the first element of slope_emis because the
# slope is the same for all 4 rows in this df ( we only have
# 1 B plate). In future consider writing a function that will
# return this for multiple B plates

slope_emis <- lm_stats_emis$slope_emis[1]

# calculate slope of the emission curve and the emisson
# coefficient
emis_calc <- homog_plots %>% mutate(slope_emis = slope_emis[1],
    emis_coeff = slope_emis/assay_vol_ml)
```

7.2 Calculate the quench coefficient

The quench coefficient is calculated as the slope of the quench (MUB + soil) standard curve divided by the slope of the emission (MUB) standard curve.

Add more information about what the quench coefficient actually is. . .

```
coeff_calcs <- emis_calc %>% mutate(quench_coeff = map2_dbl(lm_stats_quench,
    slope_emis, ~as.numeric(.x[["slope_quench"]])/.y))
```

8 Assay well calculations

8.1 A Plates: Mean of technical reps for assay & substrate control wells

```
# Calculate the mean fluor for the assay wells & substrate
# control wells (note using the un-nested dataframe here)

assay_sub_blnks <- clean_data %>% filter(str_detect(id, "assay") |
    str_detect(id, "sub-blank")) %>% group_by(plate, id) %>%
    summarise(fluor_mean = mean(fluor), n = n(), sd = sd(fluor,
        na.rm = T), cv = sd(fluor, na.rm = T)/mean(fluor) * 100)

# format dataframe 'wide' so that substrate controls are in
# columns adjacent to assay wells for each enzyme
wide <- assay_sub_blnks %>% pivot_wider(id_cols = c(plate, id),
    names_from = id, values_from = fluor_mean, names_prefix = "mean_fluor_",
    names_sep = "_")

# grab relevant coeffs from nested dataframe
coeffs <- coeff_calcs %>% select(plate, plate_blank, hombl, emis_coeff,
    quench_coeff)

# join coeffs to assay/substrate well calculations
wide_all <- left_join(wide, coeffs, by = "plate")
```

8.2 Calculate net fluorescence

Net fluorescence = ((assay-homogenate control) / quench coefficient) - (substrate control-plate blank)

```
# net fluorescence calculation for each enzyme
net_fluor_calcs <- wide_all %>%
mutate(BG_net_fluor = (((`mean_fluor_BG-assay` - hombl)/quench_coeff) -
    (`mean_fluor_BG-sub-blank` - plate_blank)), Cello_net_fluor = (((`mean_fluor_Cello-assay` -
    hombl)/quench_coeff) - (`mean_fluor_Cello-sub-blank` - plate_blank)),
    NAG_net_fluor = (((`mean_fluor_NAG-assay` - hombl)/quench_coeff) -
        (`mean_fluor_NAG-sub-blank` - plate_blank)), P_net_fluor = (((`mean_fluor_P-assay` -
        hombl)/quench_coeff) - (`mean_fluor_P-sub-blank` - plate_blank)))
```

8.3 Calculate enzyme activity

Activity(nmol/g^-1 h^-1) = [Net fluor x buffer vol (mL)] / [emis coeff x homogenate vol(mL) x time(h) x ode soil mass mass(g)]

Activity units are nmol / g^-1 hr^-1

```
# buffer vol is the volume of buffer used to make the soil
# slurries. In the regular Gutknecht lab protocol, this is 50
# mL
buffer_vol_ml <- 50

# vol of homogenate added to the assay wells in mL
homogenate_vol_ml <- 0.2

# bring in plate metadata (incubation time, ov dry soil eq,
# etc)
calcs_with_meta <- left_join(net_fluor_calcs, plate_metadata,
    by = "plate")

# activity calculation for each enzyme
activity_calcs <- calcs_with_meta %>% mutate(BG_activity = (BG_net_fluor *
    buffer_vol_ml)/(emis_coeff * homogenate_vol_ml * inc_time_hr *
    soil_ov_dry_eq_g), Cello_activity = (Cello_net_fluor * buffer_vol_ml)/(emis_coeff *
    homogenate_vol_ml * inc_time_hr * soil_ov_dry_eq_g), NAG_activity = (NAG_net_fluor *
    buffer_vol_ml)/(emis_coeff * homogenate_vol_ml * inc_time_hr *
    soil_ov_dry_eq_g), P_activity = (P_net_fluor * buffer_vol_ml)/(emis_coeff *
    homogenate_vol_ml * inc_time_hr * soil_ov_dry_eq_g))
```

9 Save data to file

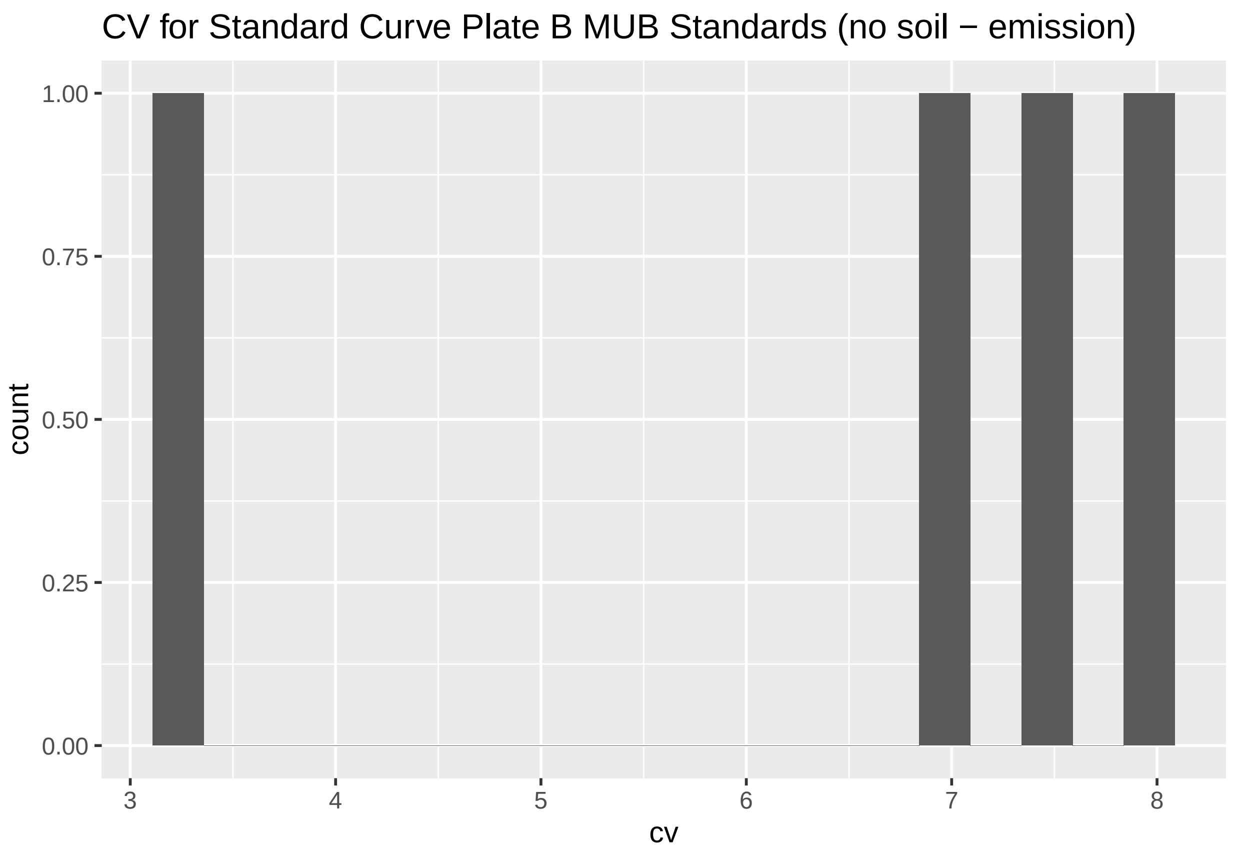
```
# select plate name and final activity calculations
final_activity <- activity_calcs %>%
  select(plate, BG_activity, Cello_activity, NAG_activity, P_activity)

# save a copy of the final data in the results sub-directory
write.csv(final_activity, "./results/hydrolytic_mgtest_2020-07-03.csv", row.names = FALSE)

# save a list of the "bad" wells you removed

write.csv(bad_list, "./results/bad_hydrolytic_mgtest_2020-07-03.csv", row.names = FALSE)
```

10 Visualizing variability

10.1 MUB Standards (Plate B - Emission)

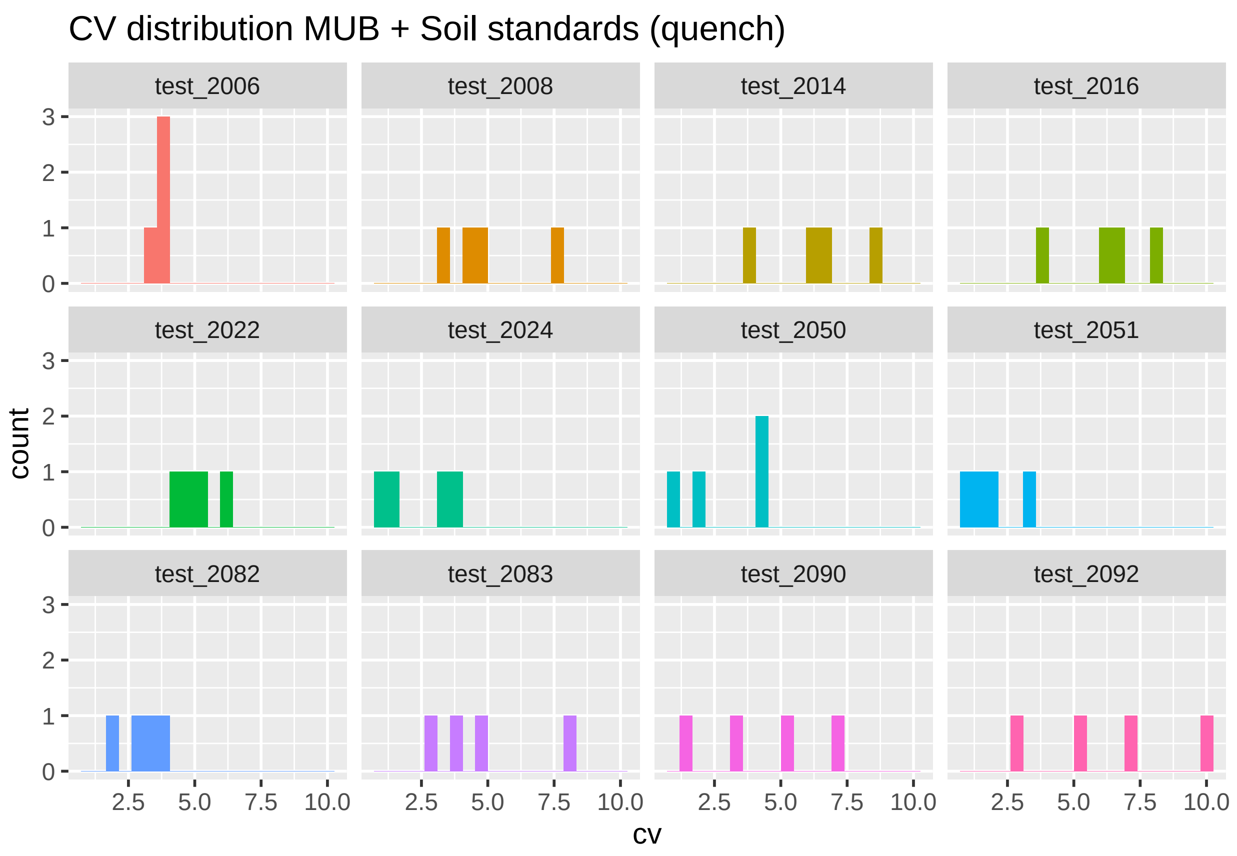
```
# to visualize variability on plate B (emission)
plate_b_means %>%
```

```r
ggplot() +
geom_histogram(aes(cv), bins = 20) +
labs(title = "CV for Standard Curve Plate B MUB Standards (no soil - emission)")
```



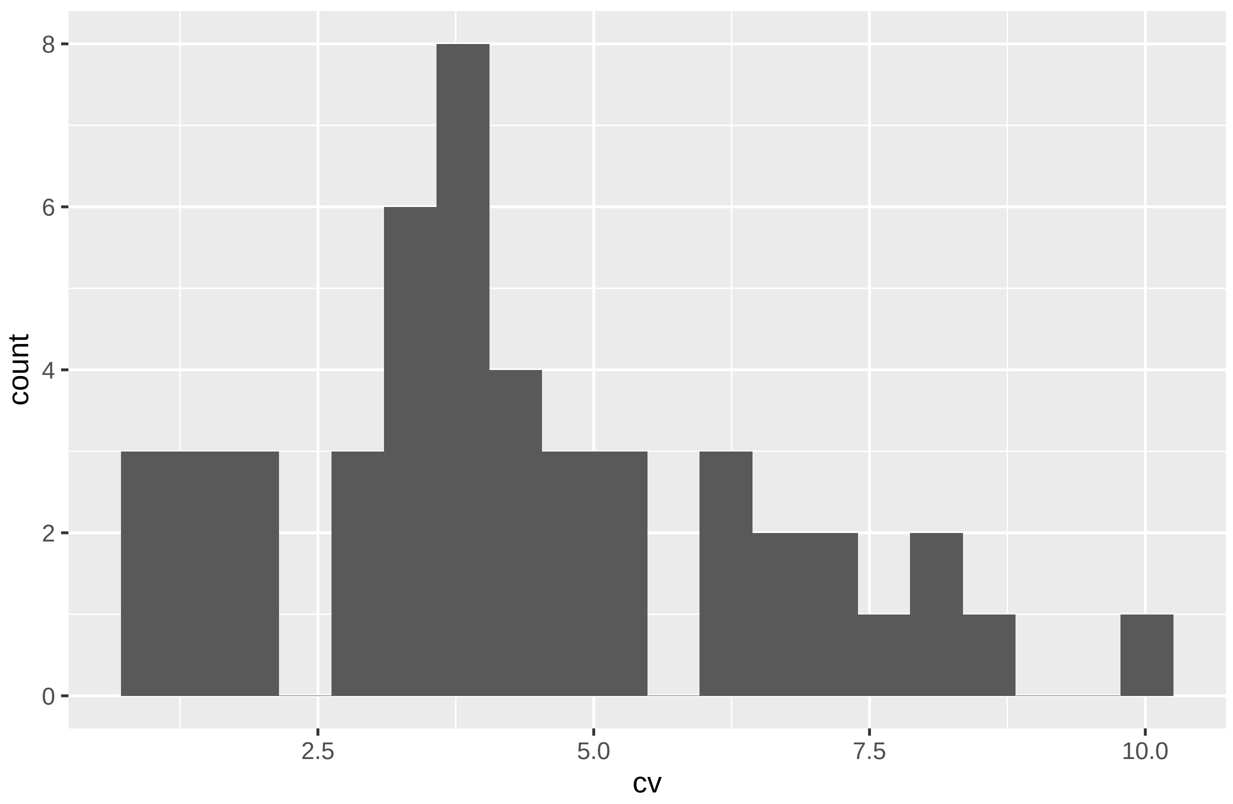
10.2 MUB + Soil Standards (Plate A- Quench)

```
# quench std data
quench_var <- quench_nested %>%
  select(-hombl, -data, -plate_blank) %>%
  unnest(quench_std_values)

# visualize quench data by plate
(quench_var_plot <- quench_var %>%
  ggplot() +
  geom_histogram(aes(cv, fill = plate), bins = 20) +
  labs(title = "CV distribution MUB + Soil standards (quench)") +
  facet_wrap(vars(plate)) +
  theme(legend.position = "none"))
```



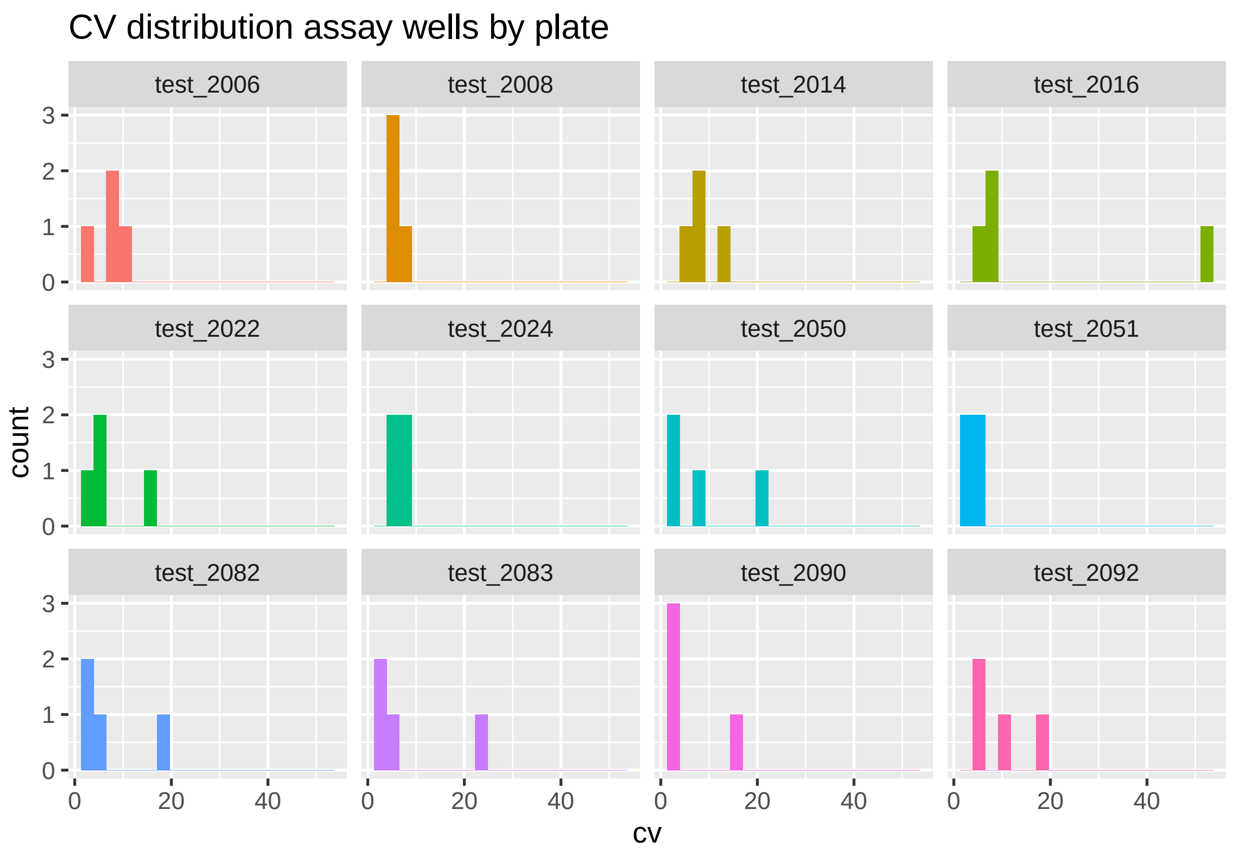
```
# visualize quench data overall
(quench_var_plot <- quench_var %>%
  ggplot() +
  geom_histogram(aes(cv), bins = 20) +
  labs(title = "CV distribution MUB + soil standards (quench)") +
  theme(legend.position = "none"))
```

CV distribution MUB + soil standards (quench)

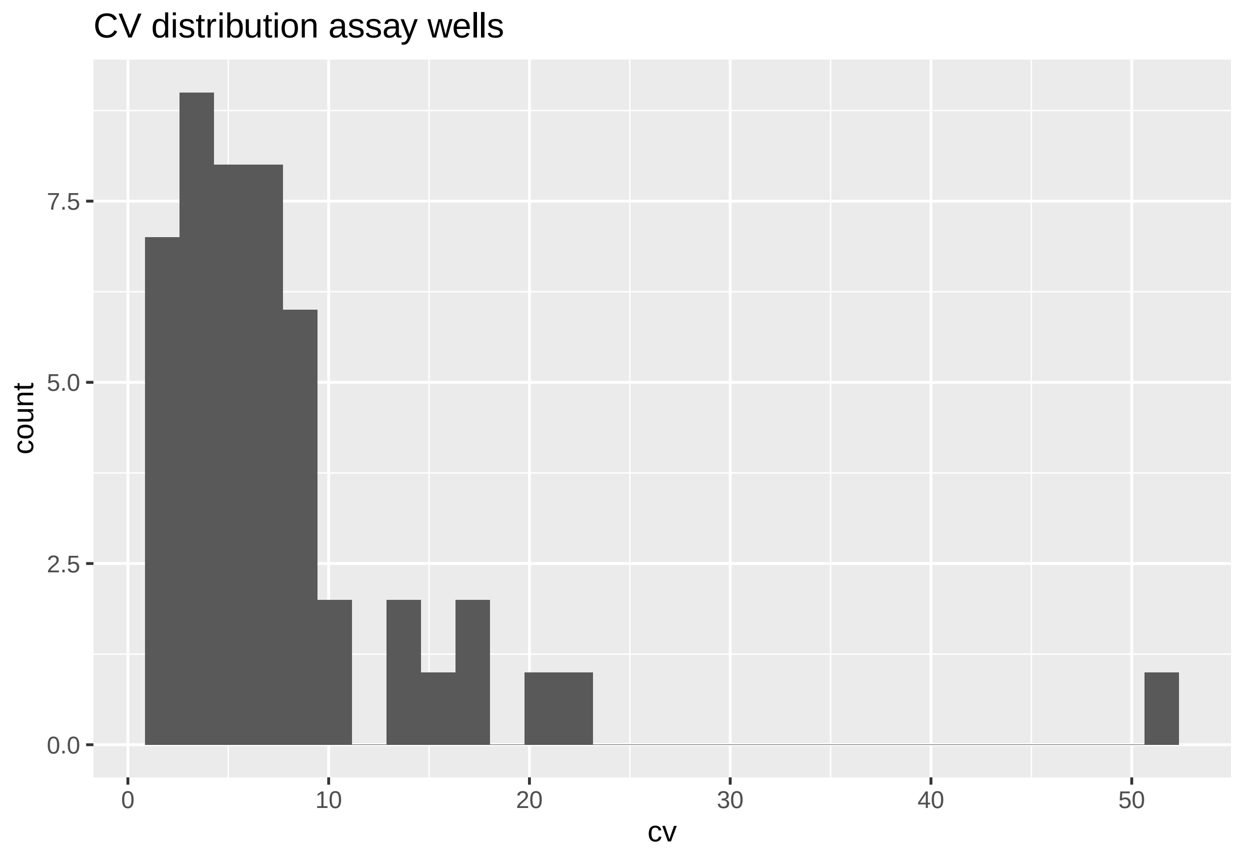


10.3 Assay Wells (Plate A)

```
# to visualize the assay well variability by plate
(assay_var_plot <- assay_sub_blnks %>%
  filter(str_detect(id,"assay")) %>%
  ggplot() +
  geom_histogram(aes(cv, fill = plate), bins = 20) +
  labs(title = "CV distribution assay wells by plate") +
    facet_wrap(vars(plate)) +
    theme(legend.position = "none"))
```

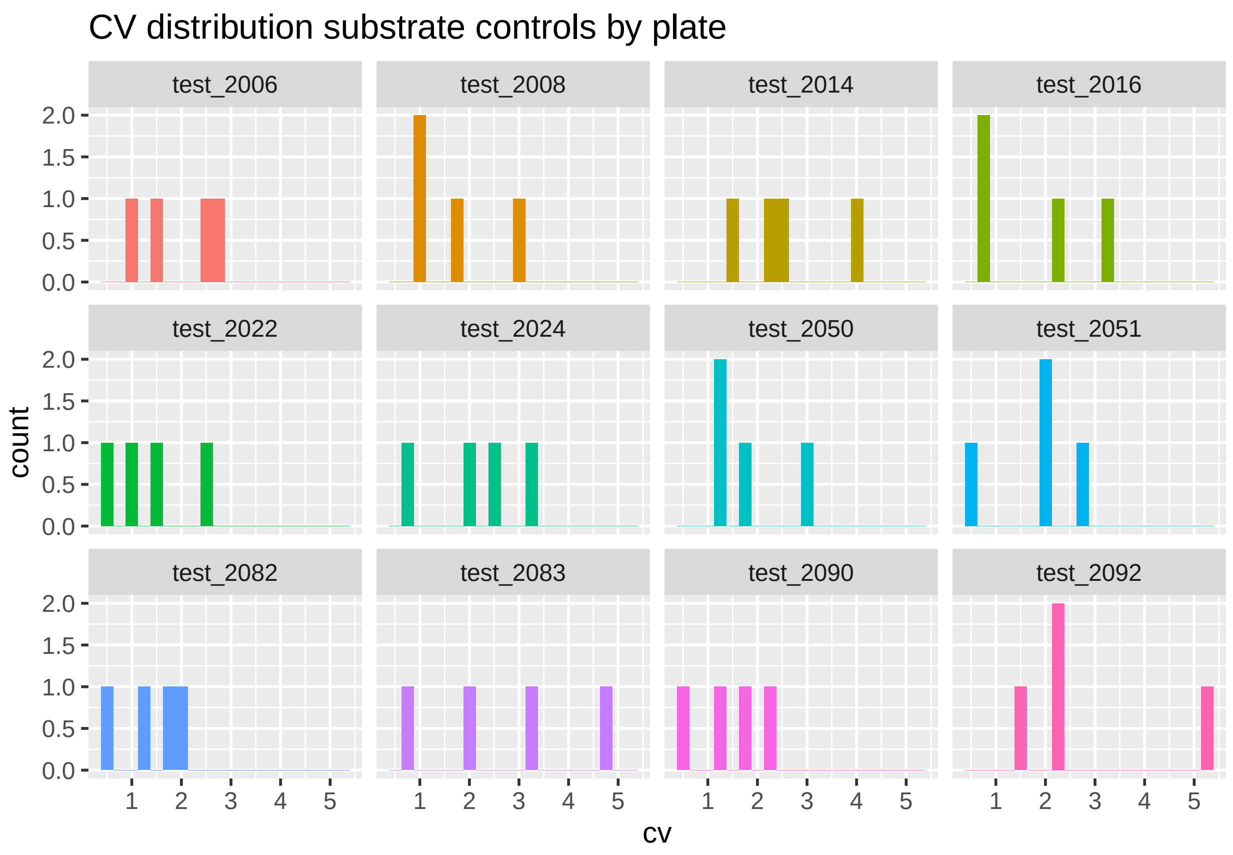


```
# assay well variabiilty overall
(assay_var_plot <- assay_sub_blnks %>%
  filter(str_detect(id,"assay")) %>%
  ggplot() +
  geom_histogram(aes(cv), bins = 30) +
  labs(title = "CV distribution assay wells") +
    theme(legend.position = "none"))
```

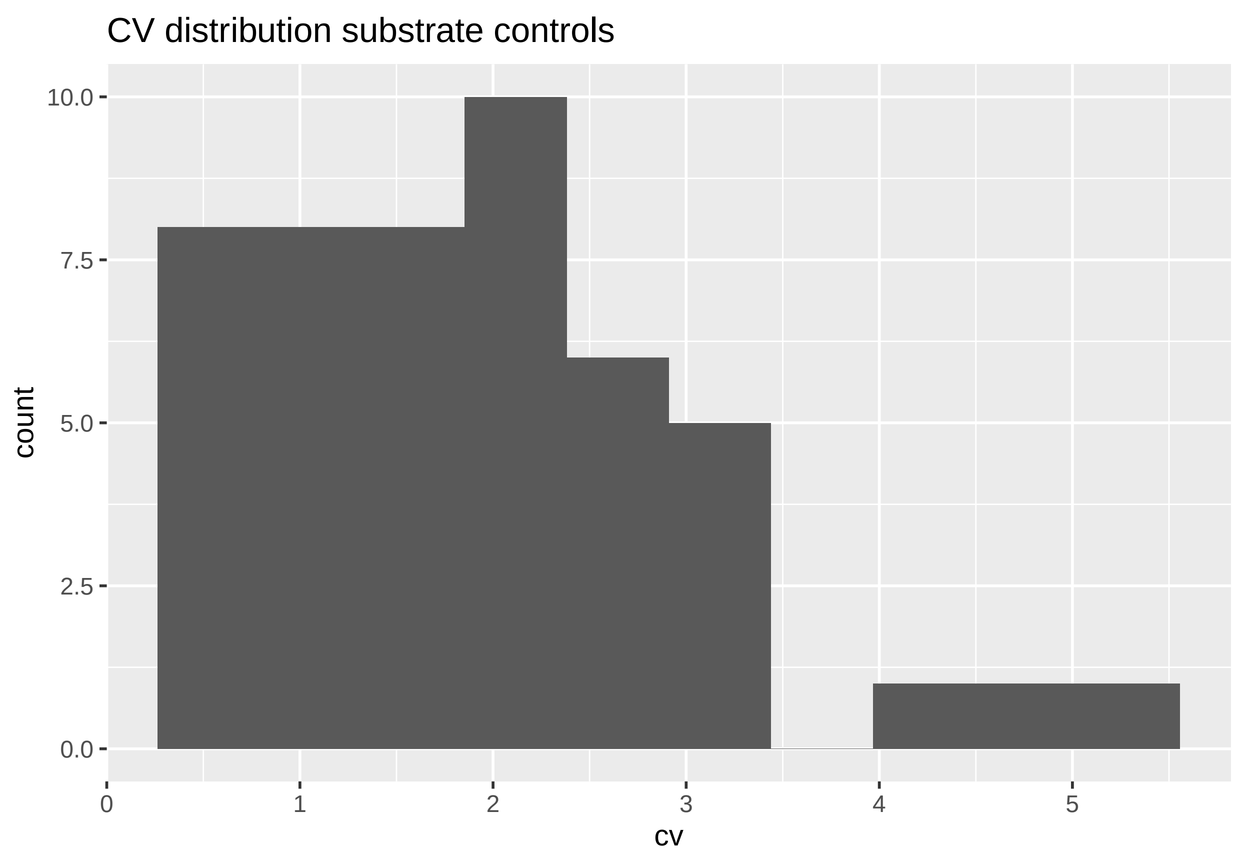

CV distribution assay wells
count
cv
0.0
2.5
5.0
7.5
0
10
20
30
40
50

10.4 Substrate blank wells (Plate A)

```
# visualize the substrate blank variability by plate
(subs_var_plot <- assay_sub_blnks %>%
  filter(str_detect(id,"blank")) %>%
  ggplot() +
  geom_histogram(aes(cv, fill = plate), bins = 20) +
  labs(title = "CV distribution substrate controls by plate") +
    facet_wrap(vars(plate)) +
    theme(legend.position = "none"))
```



```
# visualize substrate blank variability overall
(subs_var_plot <- assay_sub_blnks %>%
  filter(str_detect(id,"blank")) %>%
  ggplot() +
  geom_histogram(aes(cv), bins = 10) +
  labs(title = "CV distribution substrate controls") +
    theme(legend.position = "none"))
```



CV distribution substrate controls
count
cv
10.0
7.5
5.0
2.5
0.0
0
1
2
3
4
5