Luminex Xponent: Plate- level Quality Control

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Contents

Aim

This code aims to do a plate- level quality control analysis for Luminex studies through bead counts, Background MFI, and Standard curve analysis.

R Markdown

This is an R Markdown document, areas that between the `{r Plate QC-}` frames that are require manual entries as described in the **bolded text**. When entries are completed, press the *knit* button at the top of the page.

Dependencies

- R version 4.3.1 (2023-06-16)
  - `tidyverse` (v. 2.0.0)
  - `here` (v. 1.0.1)

Required Inputs

1. Confirm working directory:

This is the project folder where the project is found. All folders and files added to the code will be within this folder. If needed you can set the working directory using the `setwd()` function.

Working directory: /Users/sahal/Documents/R Projects/Luminex

2. Choose file (input required):

Chose the raw plate file from working directory by adding what folder within (in the quotes). In the example below, the plate csv is in the `Project/data/raw/luminex/` folder.

3. Set minimum bead count:

Set the **minimum bead count** as `min_beadcount`. In this example, the standard we have set here is `50`. All bead counts <50 will be identified.

4 Define the `file_path`:

For example, here "Project/data/qc" folder is where the *PlateName_beadqc_df.csv will be saved*

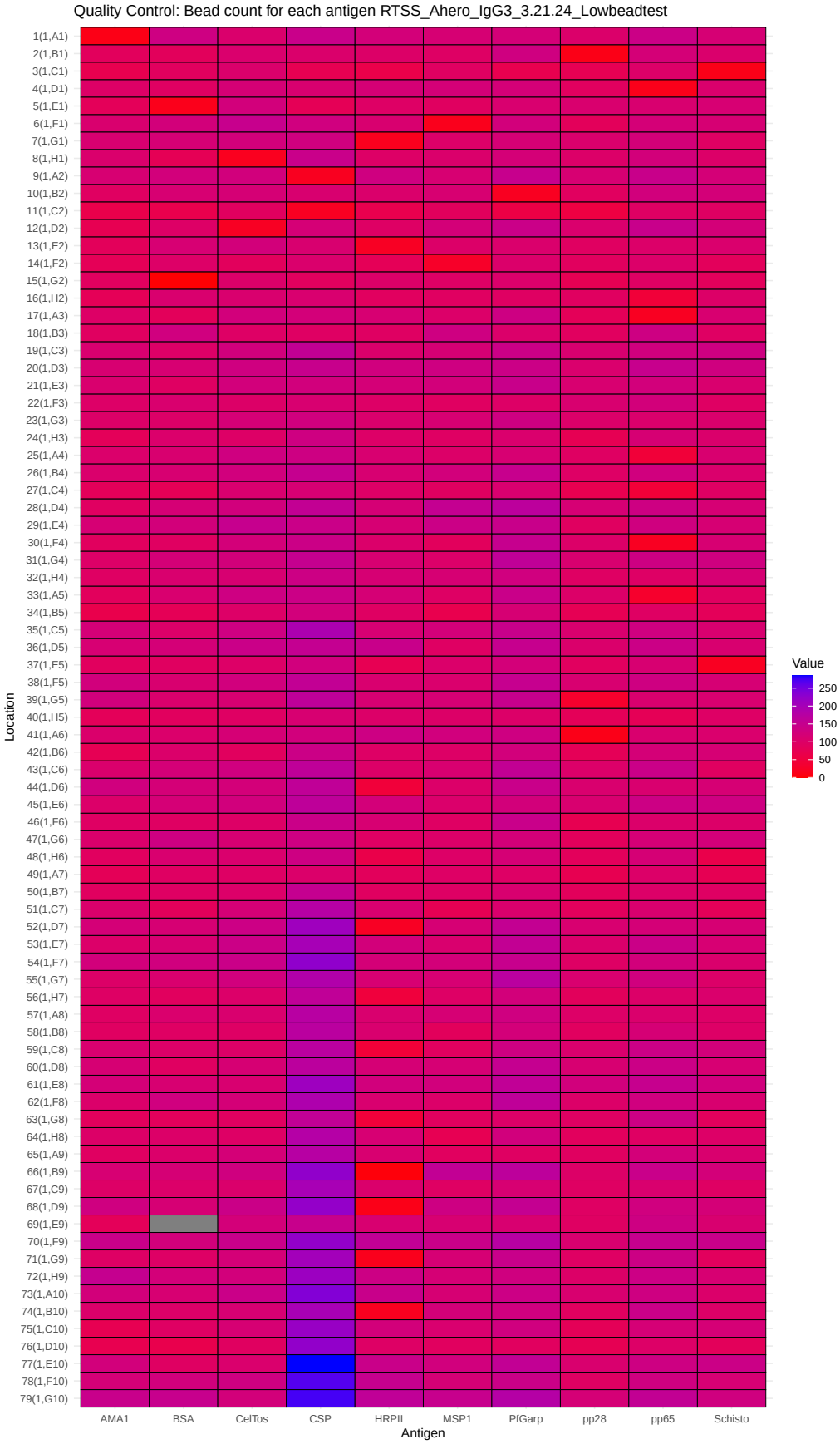
```
#Input here is in YAML
   # Read parameters from YAML header
    input_file <- params$input_file
    min_beadcount <- params$min_beadcount
    min_Rsquared <- params$min_Rsquared
    file_path <- params$file_path
```

Quality Control

Bead count per Antigen

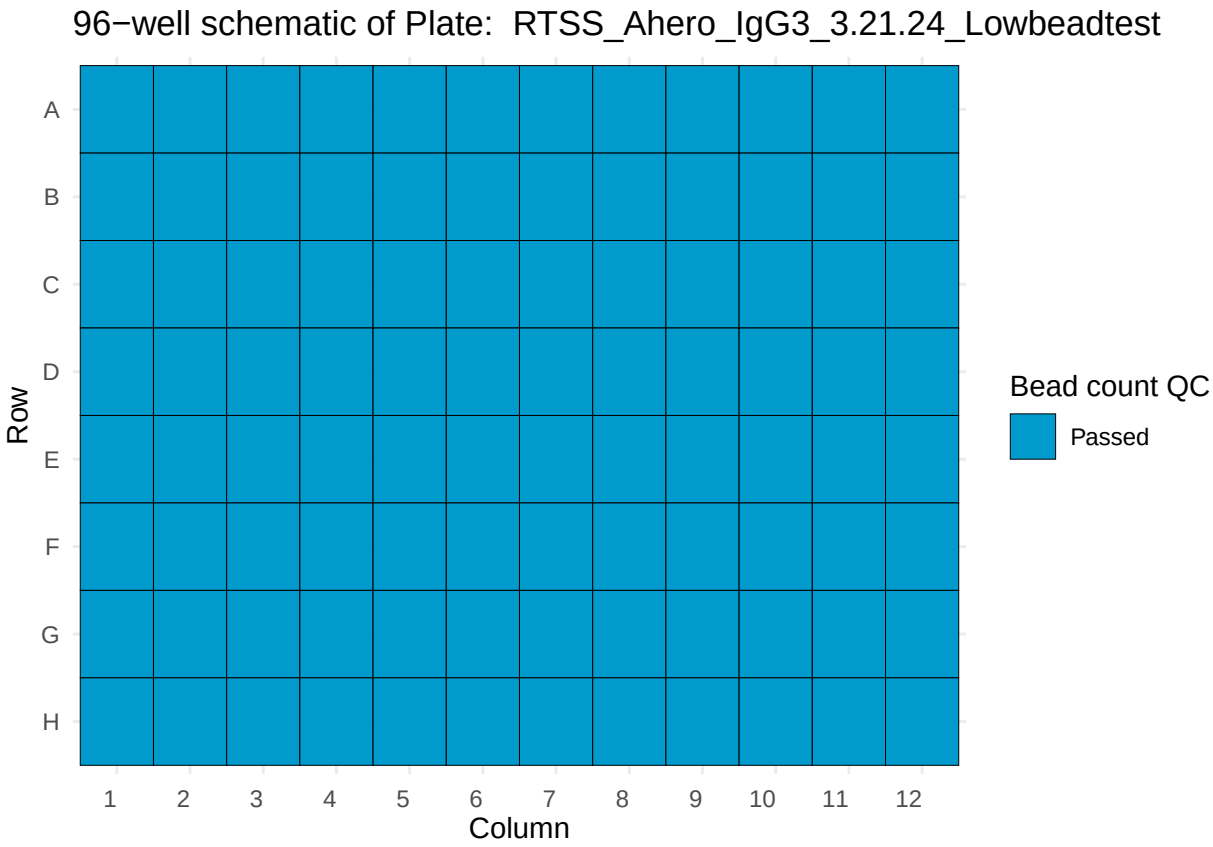
This code assists in check if low bead counts are associated with specific antigens. Note the plot below is not oriented as a 96-well plate given it displays multiple antigens per well.

```
## The bead QC report has been exported to data/qc/RTSS_Ahero_IgG3_3.21.24_Lowbeadtest_beadqc.png
```


Quality Control: Bead count for each antigen RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
Location
1(1,A1)
2(1,B1)
3(1,C1)
4(1,D1)
5(1,E1)
6(1,F1)
7(1,G1)
8(1,H1)
9(1,A2)
10(1,B2)
11(1,C2)
12(1,D2)
13(1,E2)
14(1,F2)
15(1,G2)
16(1,H2)
17(1,A3)
18(1,B3)
19(1,C3)
20(1,D3)
21(1,E3)
22(1,F3)
23(1,G3)
24(1,H3)
25(1,A4)
26(1,B4)
27(1,C4)
28(1,D4)
29(1,E4)
30(1,F4)
31(1,G4)
32(1,H4)
33(1,A5)
34(1,B5)
35(1,C5)
36(1,D5)
37(1,E5)
38(1,F5)
39(1,G5)
40(1,H5)
41(1,A6)
42(1,B6)
43(1,C6)
44(1,D6)
45(1,E6)
46(1,F6)
47(1,G6)
48(1,H6)
49(1,A7)
50(1,B7)
51(1,C7)
52(1,D7)
53(1,E7)
54(1,F7)
55(1,G7)
56(1,H7)
57(1,A8)
58(1,B8)
59(1,C8)
60(1,D8)
61(1,E8)
62(1,F8)
63(1,G8)
64(1,H8)
65(1,A9)
66(1,B9)
67(1,C9)
68(1,D9)
69(1,E9)
70(1,F9)
71(1,G9)
72(1,H9)
73(1,A10)
74(1,B10)
75(1,C10)
76(1,D10)
77(1,E10)
78(1,F10)
79(1,G10)
AMA1
BSA
CelTos
CSP
HRPII
MSP1
PfGarp
pp28
pp65
Schisto
Antigen
Value
250
200
150
100
50
0

Bead Count: per well

This code results in a 96 plate- well schematic indicating wells with low wells. A :ist of wells, sample IDs, and analytes that are less than the minimum bead count set. This file is exported to `file_path` (chosen above) as *PlateName_beadqc_low_df.csv*. Values in this list will be `Na` in the Median MFI dataframe. `file_path`: data/qc (this can be edited in the **Bead Count** section)




```
## [1] Location Sample   Antigen  Plate
## <0 rows> (or 0-length row.names)
```

```
## The bead QC list has been exported to data/qc//RTSS_Ahero_IgG3_3.21.24_Lowbeadtest_beadqc_low_df.csv
##  This file will be added to in data/qc/qc_compile.csv
```

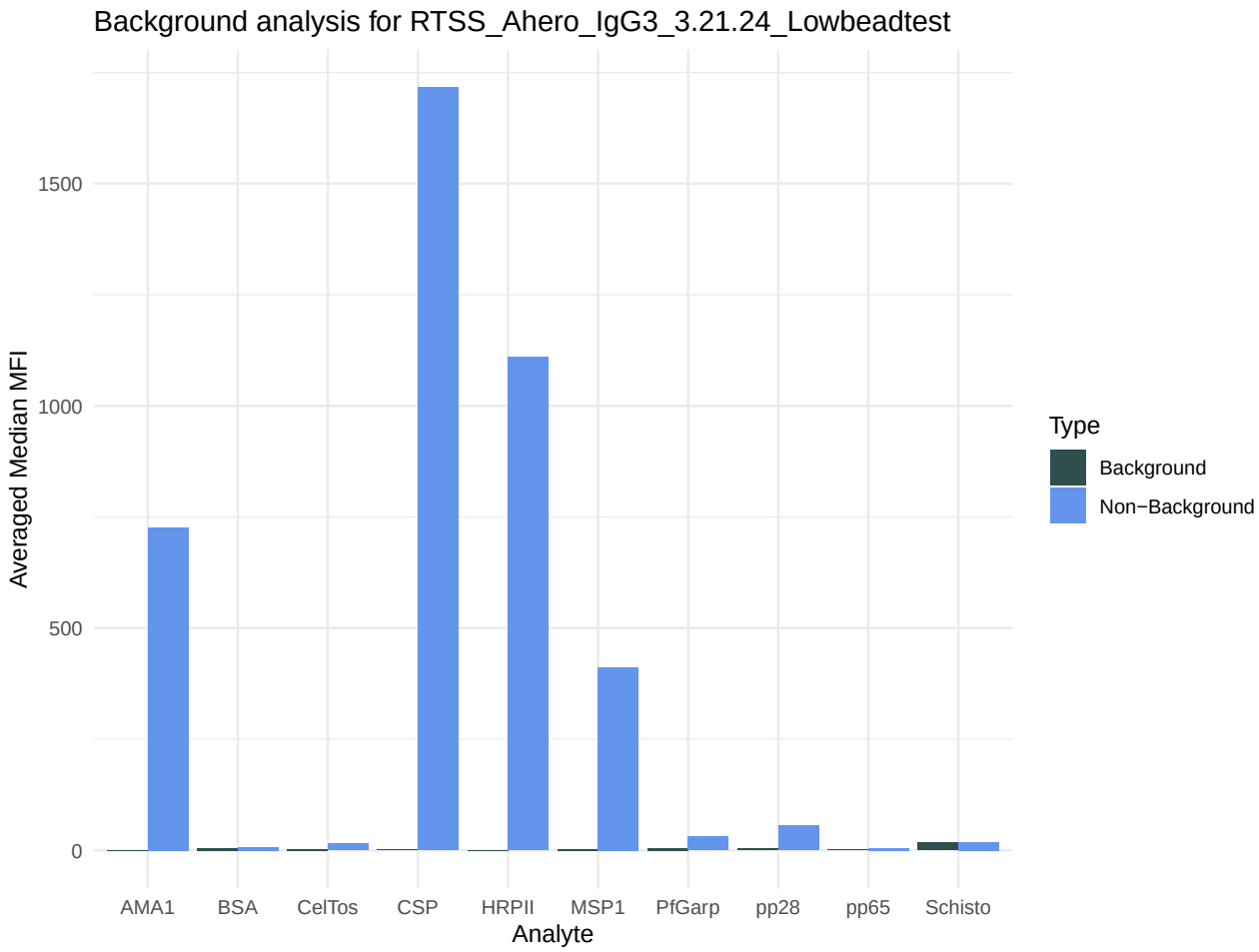
QC of median MFI data

This extracts the median MFI data, with all well-analyte combinations <50 beads/well replaced with *NA*. It completes the BSA MFI subtraction as well

note: negative values are kept

Background MFI

This code averages the median MFI values for all background and non-background samples. This requires all Background samples to have "Background" within the characters in the `Sample` column



Standard curve

This code will attempt to create standard curves. This requires the samples have "Standard" within the characters in the `Sample` column. This will extraction `Dilution_Factor` as the number within the name (x -1).

For example: *Standard 2* will have a `Dilution_factor` of -2

This code will run for all `analyte`

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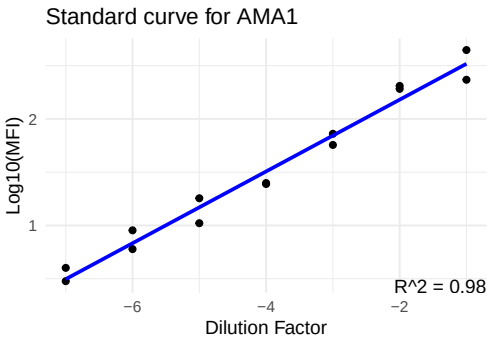
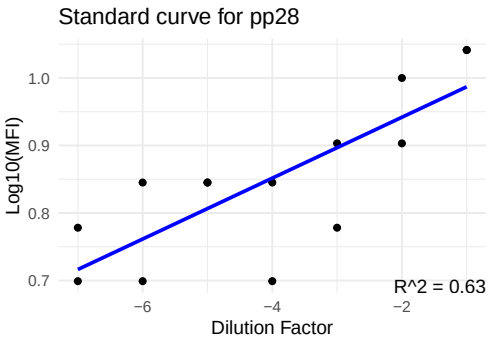
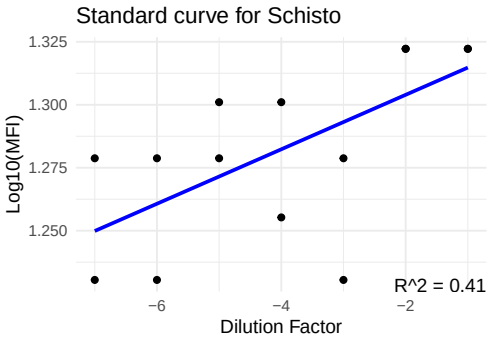
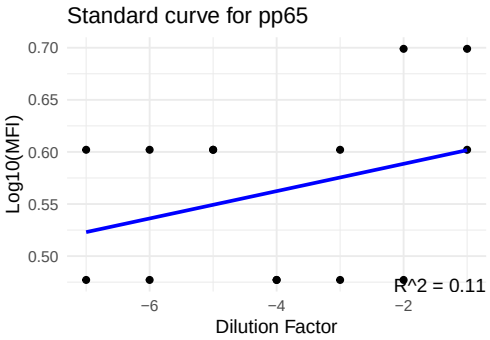
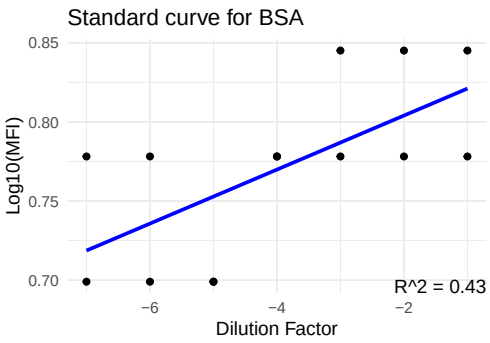
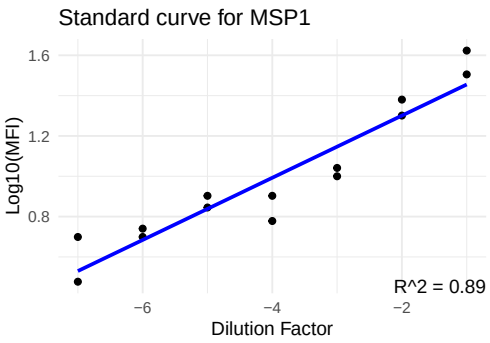


Standard curve for AMA1
Log10(MFI)
Dilution Factor
R^2 = 0.98

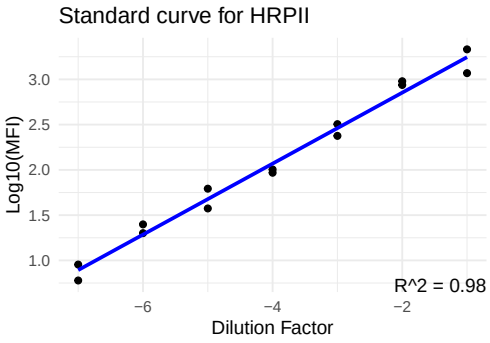

Standard curve for pp28
Log10(MFI)
Dilution Factor
R^2 = 0.63

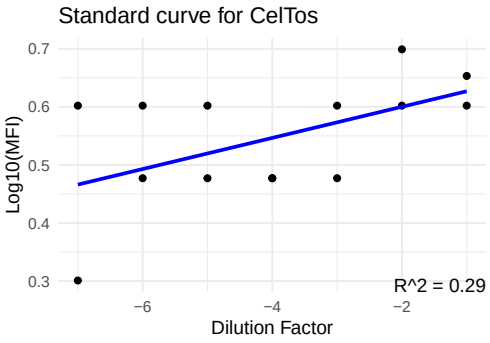

Standard curve for Schisto
Log10(MFI)
Dilution Factor
R^2 = 0.41

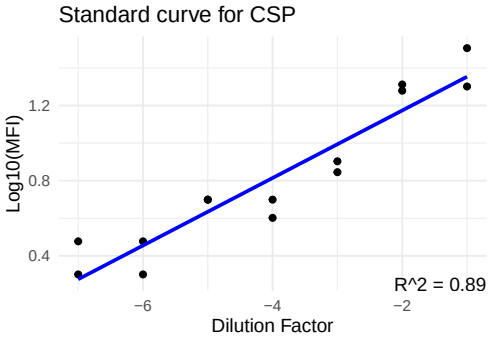

Standard curve for pp65
Log10(MFI)
Dilution Factor
R^2 = 0.11

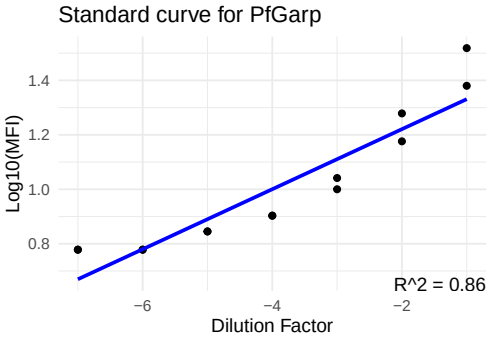

Standard curve for BSA
Log10(MFI)
Dilution Factor
R^2 = 0.43


Standard curve for MSP1
Log10(MFI)
Dilution Factor
R^2 = 0.89


Standard curve for HRPII
Log10(MFI)
Dilution Factor
R^2 = 0.98


Standard curve for CelTos
Log10(MFI)
Dilution Factor
R^2 = 0.29


Standard curve for CSP
Log10(MFI)
Dilution Factor
R^2 = 0.89


Standard curve for PfGarp
Log10(MFI)
Dilution Factor
R^2 = 0.86

```
## TableGrob (5 x 2) "arrange": 10 grobs
##     z     cells    name           grob
## 1   1 (1-1,1-1) arrange gtable[layout]
## 2   2 (1-1,2-2) arrange gtable[layout]
## 3   3 (2-2,1-1) arrange gtable[layout]
## 4   4 (2-2,2-2) arrange gtable[layout]
## 5   5 (3-3,1-1) arrange gtable[layout]
## 6   6 (3-3,2-2) arrange gtable[layout]
## 7   7 (4-4,1-1) arrange gtable[layout]
## 8   8 (4-4,2-2) arrange gtable[layout]
## 9   9 (5-5,1-1) arrange gtable[layout]
## 10 10 (5-5,2-2) arrange gtable[layout]
```

QC- Plate standard curve

The minimum R squared value set for this study is 0.9. In order to pass quality control for appropriate standard curve response, at least one analyte requires a minimum Rsquared > than that value.

```
##    Analyte R_squared min_Rsq
## 1     AMA1 0.9756595    TRUE
## 2     pp28 0.6322933   FALSE
## 3  Schisto 0.4113194   FALSE
## 4     pp65 0.1065765   FALSE
## 5      BSA 0.4344079   FALSE
## 6     MSP1 0.8911352   FALSE
## 7    HRPII 0.9840924    TRUE
## 8   CelTos 0.2923485   FALSE
## 9      CSP 0.8920984   FALSE
## 10  PfGarp 0.8648147   FALSE
```

```
## [1] "This plate has passed plate-level quality control for standard curve linearity."
## [1] "At least one analyte meets the criteria for the preset minimum R-squared (see min_Rsquared in Y
## [1] "Median MFI from this plate will be included in the compiled study dataframe"
```