Luminex Xponent: Plate-level Quality Control

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Contents

Aim

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

Instructions

1. **Modify YAML Parameters:**
   - Open this document in RStudio.
   - Edit the YAML parameters at the top of the document:
     - `input_file`: Path to your input CSV file.
     - `min_beadcount`: Set the minimum bead count threshold.
     - `min_Rsquared`: Set the minimum R-squared value for standard curve QC.
     - `file_path`: Specify the output directory for plots and PDFs.
     - '
2. **Run the Analysis:**
   - After modifying the YAML parameters, click the *knit* button at the top of the page to generate the report.

Code Explanation

Dependencies

- R version 4.3.1 (2023-06-16)
  - `tidyverse` (v. 2.0.0)
  - `here` (v. 1.0.1)
  - `gridExtra` (v. 2.3)

This code chunk will ensure these packages are installed and loaded.

Import and Data Extraction

1. **Raw data import:** Import raw data CSV file into R.

```
# No input required
  #YAML extraction
    # 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
```

```
    background_col <- params$background

# Raw data import
    lines <- readLines(input_file)
```

Warning in readLines(input_file): incomplete final line found on
'data/raw/luminex/RTSS_Ahero_IgG3_3.21.24_Lowbeadtest.csv'

2. **Extract dataframes:** find median MFI and bead count data. This includes a check to ensure the median MFI and beadcount dataframes align.

## [1] "Column names are consistent between the median MFI and bead count data frames."

3. **Identify low bead count wells:** this searches the beadcount data frame for well-antigen data with low bead count, as set in the YAML.This will export a csv file of the samples which will need to be rerun.

The bead QC list has been exported to data/qc/RTSS_Ahero_IgG3_3.21.24_Lowbeadtest_beadqc_low_df.csv

Visualization of bead count data

This code generates a well schematic identifying wells identified as having low bead counts. as well as a heatmap of antigen/well locations and the quantity of beads, to assist in determining the cause of low bead counts (low antigen concentration in mastermix, vortexing between wells, etc.)

96–well schematic of Plate: RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
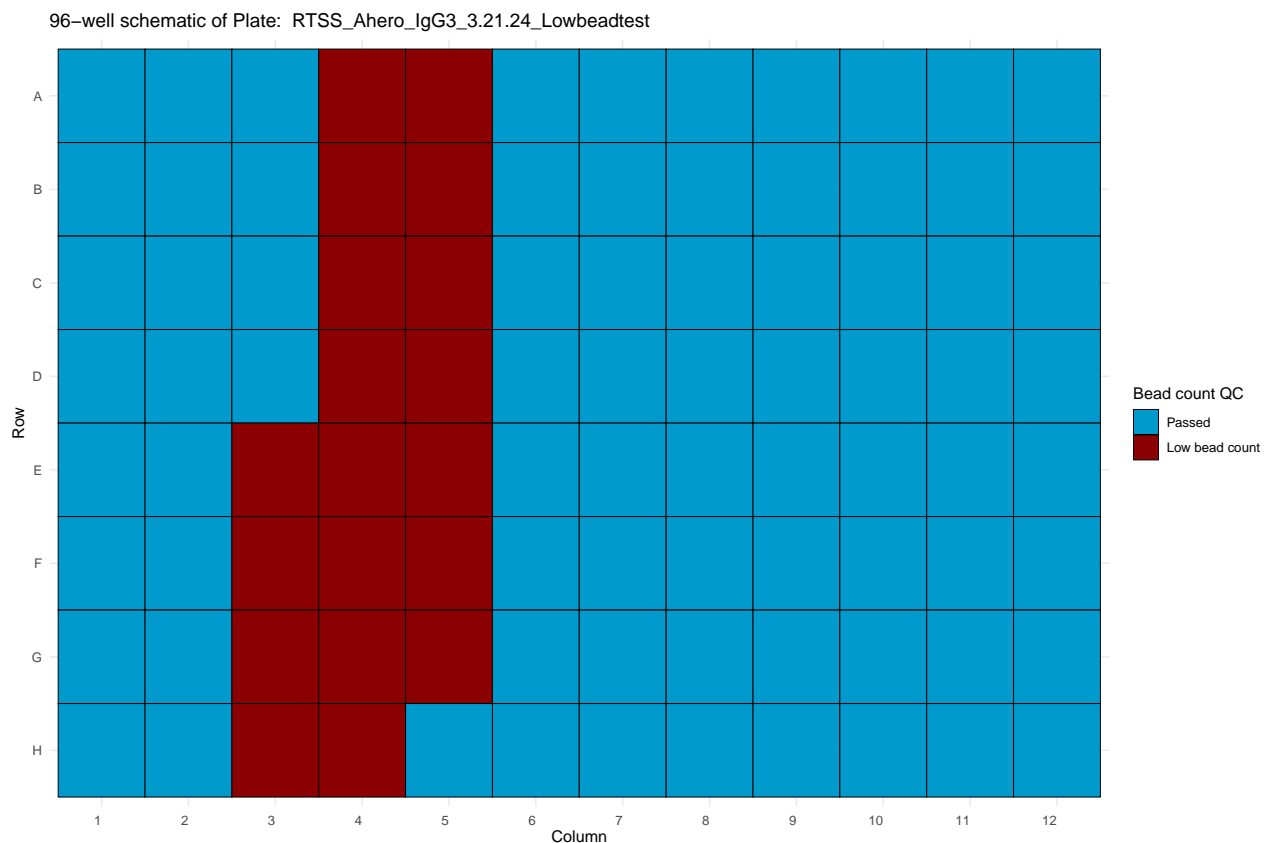


```
## # A tibble: 19 x 4
##    Location Sample     Antigen Plate
##    <chr>    <chr>      <chr>   <chr>
##  1 21(1,E3) RAM-002    AMA1    RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
##  2 22(1,F3) RAC-002-T01 pp28   RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
```

```
##  3 23(1,G3) RAC-002-T07 Schisto RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
##  4 24(1,H3) RAC-002-T08 pp65    RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
##  5 25(1,A4) RAM-005     BSA     RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
##  6 26(1,B4) RAC-005-T01 MSP1    RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
##  7 27(1,C4) RAC-005-T08 HRPII   RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
##  8 28(1,D4) RAM-007     CelTos  RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
##  9 29(1,E4) RAC-007-T01 CSP     RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 10 30(1,F4) RAC-007-T07 PfGarp  RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 11 31(1,G4) RAC-007-T08 CSP     RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 12 32(1,H4) RAM-008     CelTos  RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 13 33(1,A5) RAC-008-T01 HRPII   RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 14 34(1,B5) RAC-008-T07 MSP1    RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 15 35(1,C5) RAC-008-T08 BSA     RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 16 36(1,D5) RAM-009     pp65    RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 17 37(1,E5) RAC-009-T01 Schisto RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 18 38(1,F5) RAC-009-T07 pp28    RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
## 19 39(1,G5) RAC-009-T08 AMA1    RTSS_Ahero_IgG3_3.21.24_Lowbeadtest
```

The bead QC list has been exported to data/qc/RTSS_Ahero_IgG3_3.21.24_Lowbeadtest_beadqc_low_df.csv

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
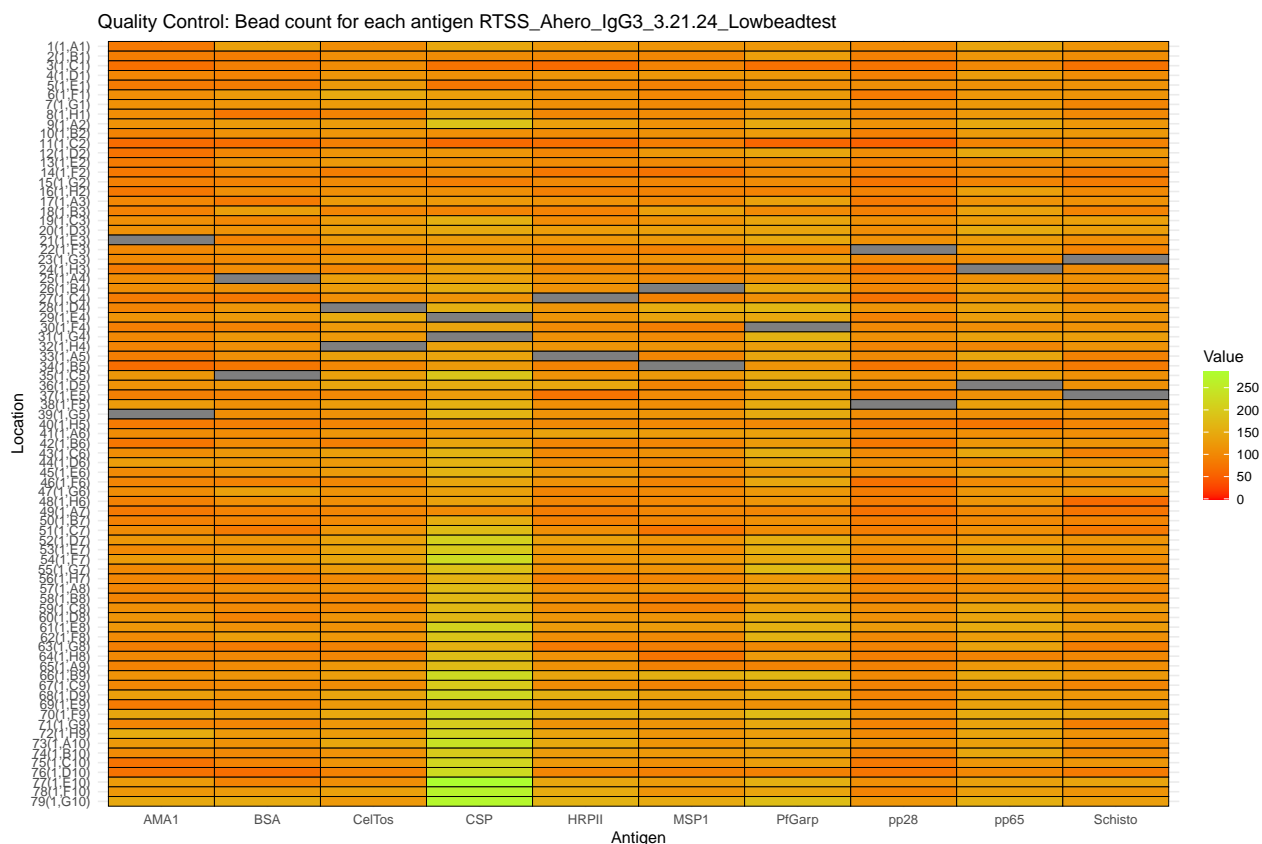
4. **Modify Median MFI data:** All plate locations with a low beadcount with be switched to a `NA` value for standard curve evaluation

Median MFI data has been modified for low bead count wells.

5. **Evaluate standard curves:** Make sure the background and standard analytes are defined in the YAML params

This first chunk conducts background MFI subtraction, based on the analyte defined in the YAML.

```
## Background MFI subtraction completed.
```

This code chunk

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

Standard curve for AMA1

Standard curve for CelTos

Standard curve for HRPII
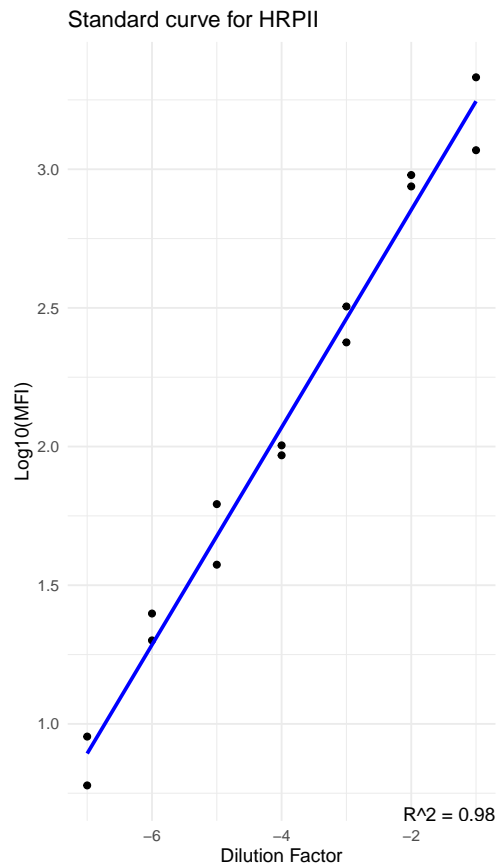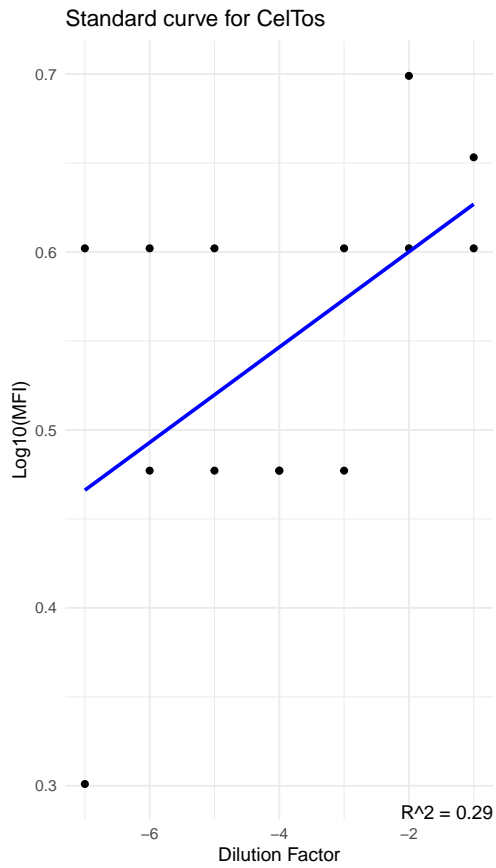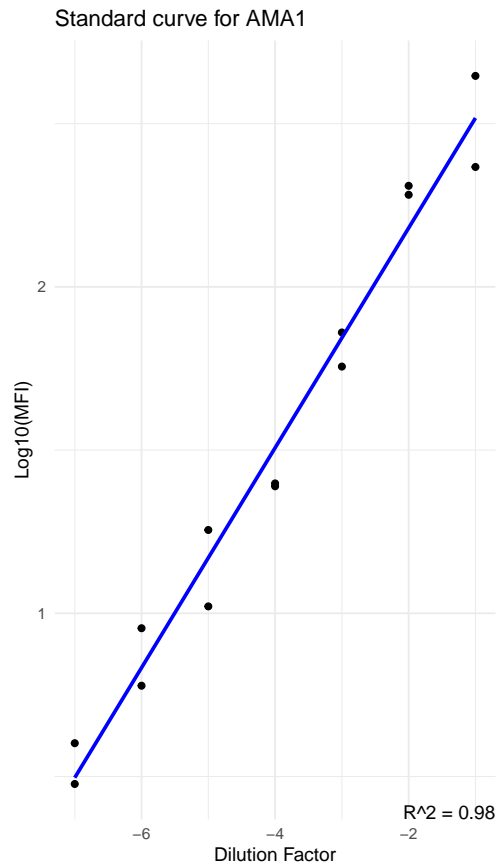
```
## TableGrob (2 x 2) "arrange": 3 grobs
##   z    cells    name             grob
## 1 1 (1-1,1-1) arrange gtable[layout]
## 2 2 (1-1,2-2) arrange gtable[layout]
## 3 3 (2-2,1-1) arrange gtable[layout]

##   Analyte R_squared
## 1    AMA1 0.9756595
## 2  CelTos 0.2923485
## 3   HRPII 0.9840924

##   Analyte R_squared min_Rsq
## 1    AMA1 0.9756595    TRUE
## 2  CelTos 0.2923485   FALSE
## 3   HRPII 0.9840924    TRUE

## [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 YA
## [1] "Median MFI from this plate will be included in the compiled study dataframe."
```

Create quality controlled Median MFI data

```
##   Analyte R_squared min_Rsq
## 1    AMA1 0.9756595    TRUE
## 2  CelTos 0.2923485   FALSE
## 3   HRPII 0.9840924    TRUE

## [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 YA
## [1] "Median MFI from this plate will be included in the compiled study dataframe."

## Plate passed QC. All NA values have been replaced with 'Failed QC (bead)'.

## The cleaned median MFI data has been exported to data/clean/luminex/RTSS_Ahero_IgG3_3.21.24_Lowbeadto
```

Developer notes

–>