Luminex Xponent: Multiple Plates (QC, Aggregation, Median MFI standardization

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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_folder`: Path to your input folder containing CSV files.
     - `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)
  - `stringr` (v. 1.5.1)

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

Project- level Quality Control

Blurb for Explanation

This is designed to perform plate-level quality control analysis for Luminex assays. The process involves the following steps:

1. **Setup and Initialization**: Load necessary libraries and ensure all required packages are installed.

2. **Reading Input Files**: Read the directory path from the parameters and list all CSV files in the specified folder.

3. **Processing Each File**: For each CSV file:

   - **Data Extraction**: Extract median MFI and bead count data.
   - **Data Alignment**: Ensure the data frames are properly aligned and contain no missing values.
   - **Bead Count Quality Control**: Identify and mark wells with low bead counts, generating a list of these wells and creating visual plots.
   - **Background MFI Subtraction**: Subtract background MFI values from the median MFI data.
   - **Standard Curve Analysis**: Evaluate standard curves for specified analytes, calculate R-squared values, and create plots for visualization.
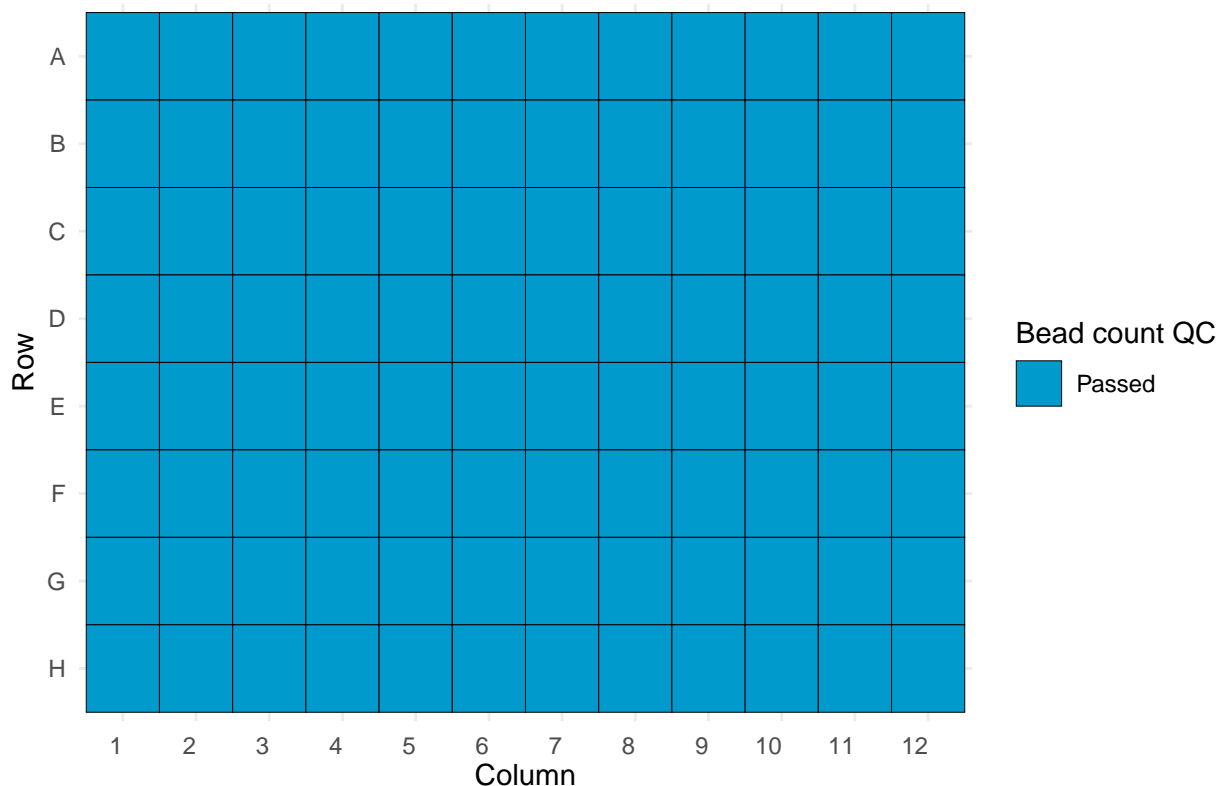   - **Final Data Cleaning**: Modify the median MFI data based on the QC results and export cleaned data.

4. **Creating Summary Files**:

   - **Standard QC Results**: Compile and save the results of the standard curve QC for each plate.
   - **Bead QC Data**: Combine and save the bead QC data from all plates into a single CSV file.
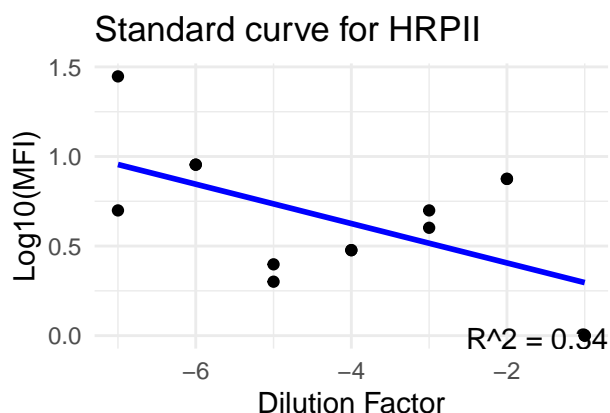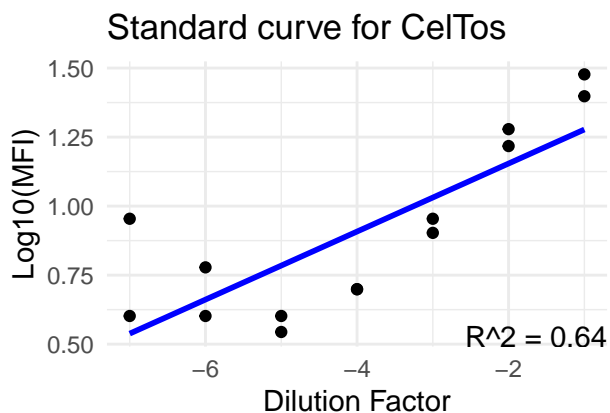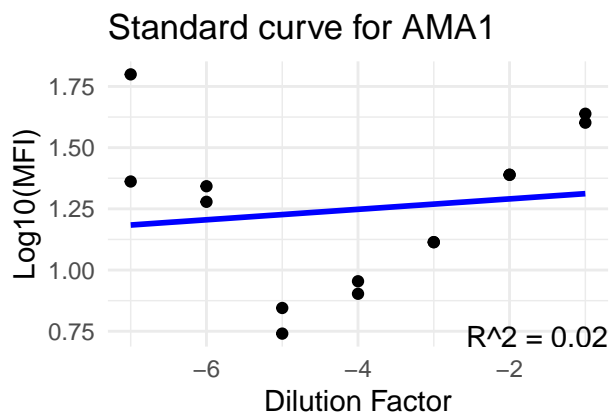
This document helps ensure that the data from Luminex assays meet the necessary quality standards, making it easier to identify and address any issues with bead counts or standard curve linearity. The final cleaned data and QC results are saved for further analysis and record-keeping.

```
## [1] "Column names are consistent between the median MFI and bead count data frames."
## The bead QC list has been exported to data/qc/RTSS_Ahero_IgA1.2_3.21.24_AM_beadqc_low_df.csv
```

96−well schematic of Plate: RTSS_Ahero_IgA1.2_3.21.24_AM


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

Standard curve for AMA1

Log10(MFI)

Dilution Factor

R^2 = 0.02

Standard curve for CelTos

Log10(MFI)

Dilution Factor

R^2 = 0.64

Standard curve for HRPII

Log10(MFI)

Dilution Factor

R^2 = 0.34

```
## 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]
## [1] "This plate has failed plate-level QC for standard curves."
## [1] "No analytes met the preset minimum R-squared (see min_Rsquared in YAML)."
## [1] "All MFI values from this plate will display as 'Failed Plate QC'."
## Plate failed QC. All median MFI data set to 'Failed QC (standards)'.
## The cleaned median MFI data has been exported to data/clean/luminex/RTSS_Ahero_IgA1.2_3.21.24_AM_clea
## [1] "Column names are consistent between the median MFI and bead count data frames."
## The bead QC list has been exported to data/qc/RTSS_Ahero_IgG1_3.20.24_MK_beadqc_low_df.csv
```
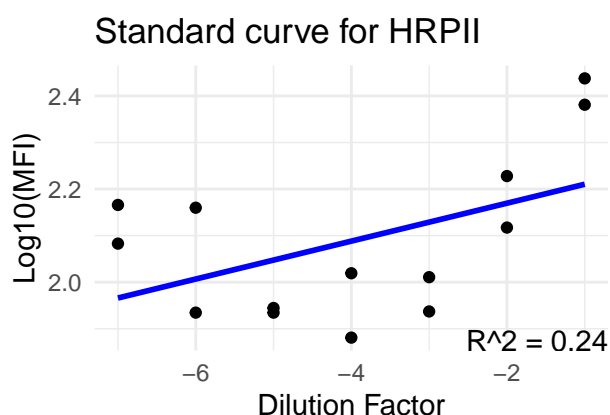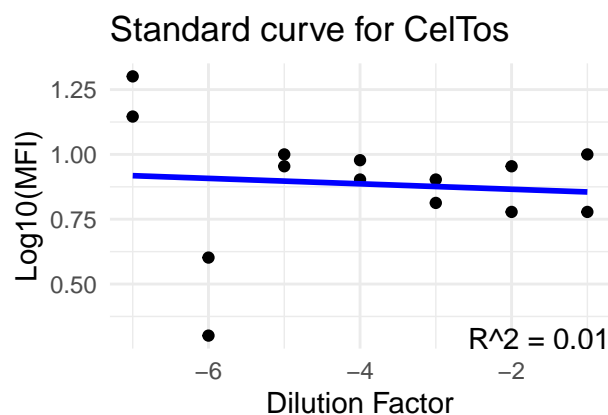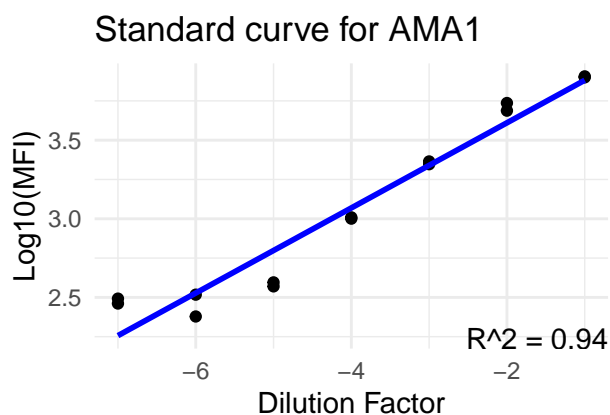
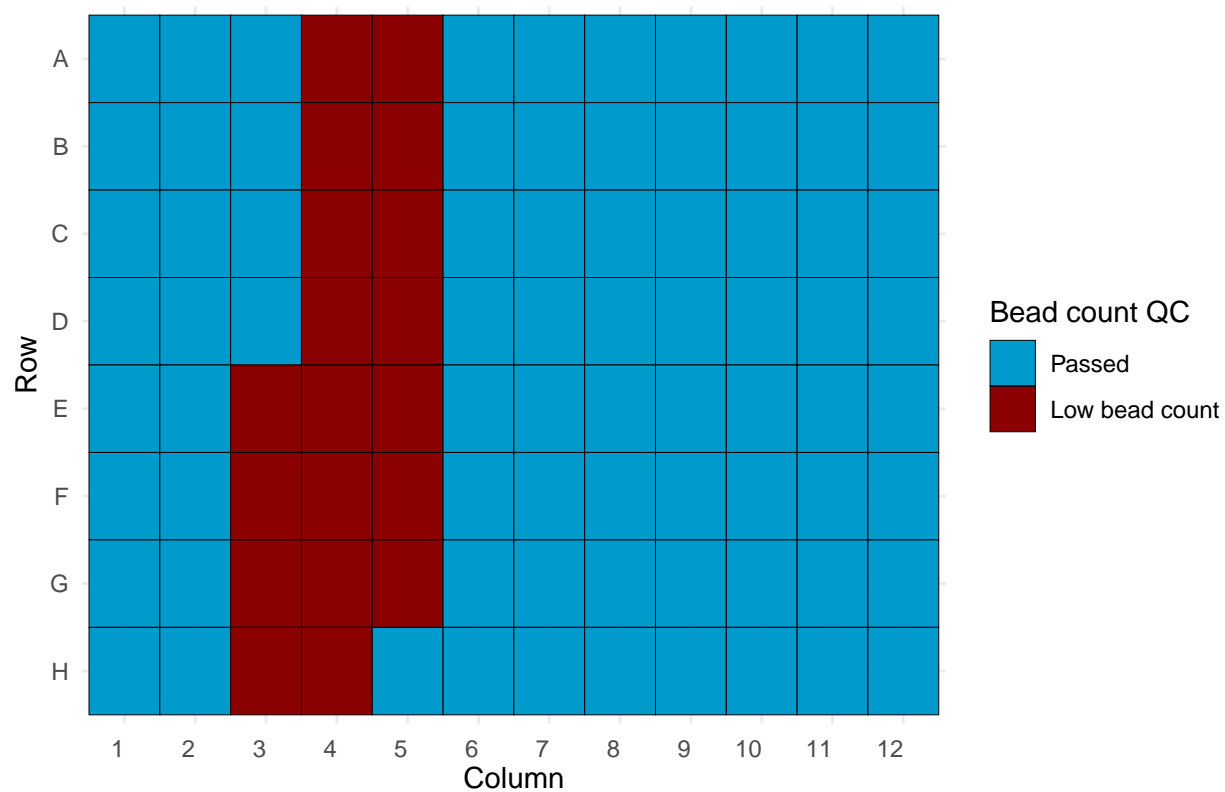## 96−well schematic of Plate: RTSS_Ahero_IgG1_3.20.24_MK

Bead count QC

Passed

Background MFI subtraction completed.

Standard curve for AMA1

Standard curve for CelTos

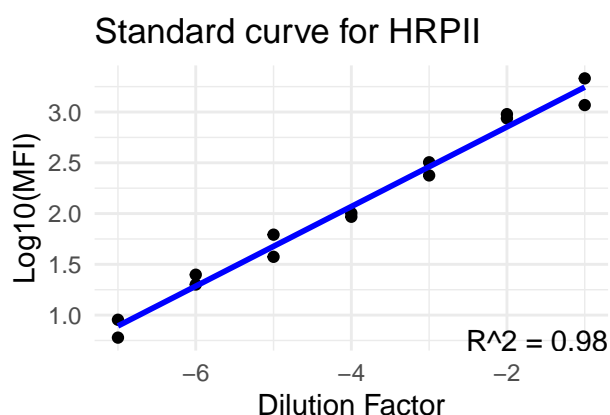

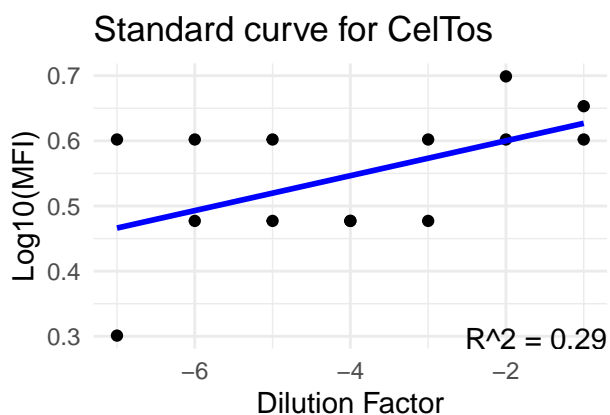## Standard curve for HRPII
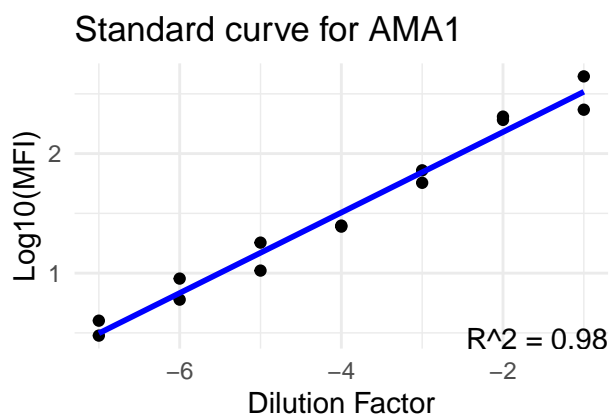


```
## TableGrob (2 x 2) "arrange": 3 grobs
##   z     cells    name           grob
## 1 1 (1-1,1-1) arrange gtable[layout]
## 2 2 (1-1,2-2) arrange gtable[layout]
## 3 3 (2-2,1-1) arrange gtable[layout]
## [1] "This plate has passed plate-level quality control for standard curve linearity."
## [1] "At 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_IgG1_3.20.24_MK_clean
## [1] "Column names are consistent between the median MFI and bead count data frames."
## The bead QC list has been exported to data/qc/RTSS_Ahero_IgG3_3.21.24_Lowbeadtest_beadqc_low_df.csv
```

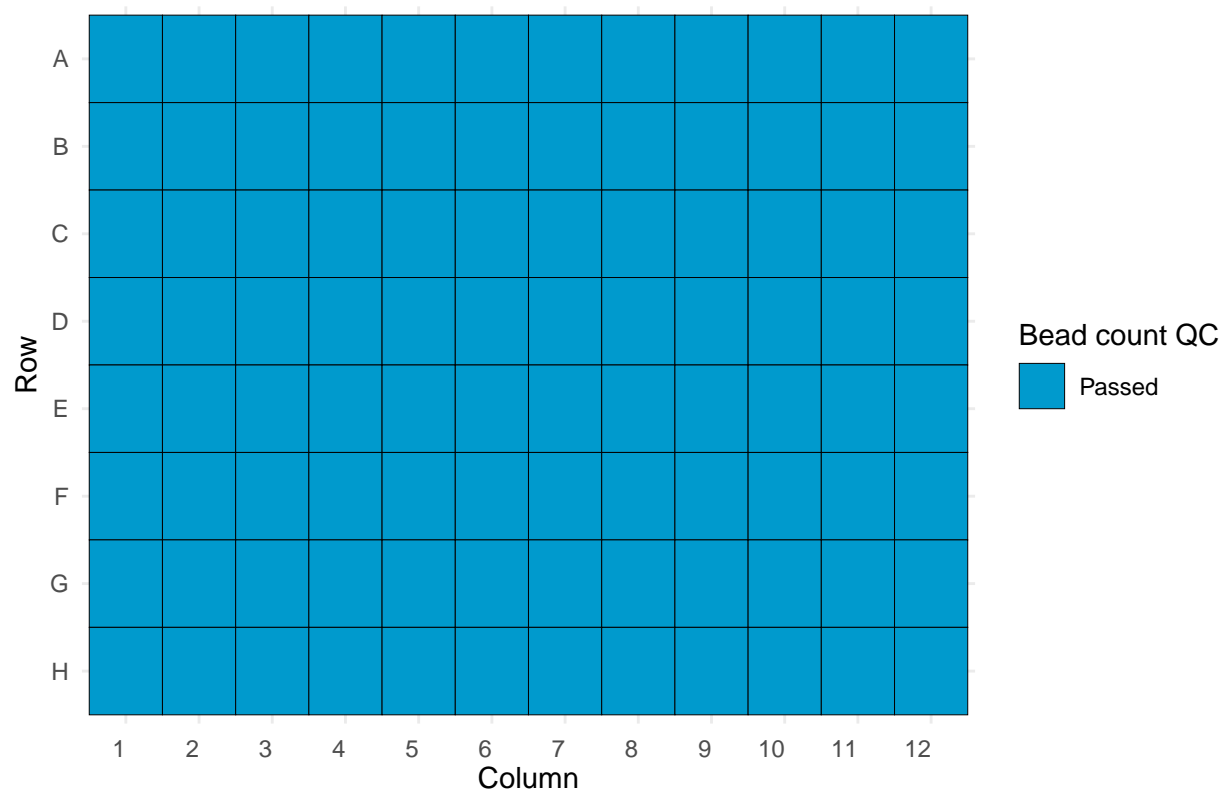## 96−well schematic of Plate: RTSS_Ahero_IgG3_3.21.24_Lowbeadtest

Background MFI subtraction completed.

Standard curve for AMA1

R^2 = 0.98

Standard curve for CelTos

R^2 = 0.29

Standard curve for HRPII

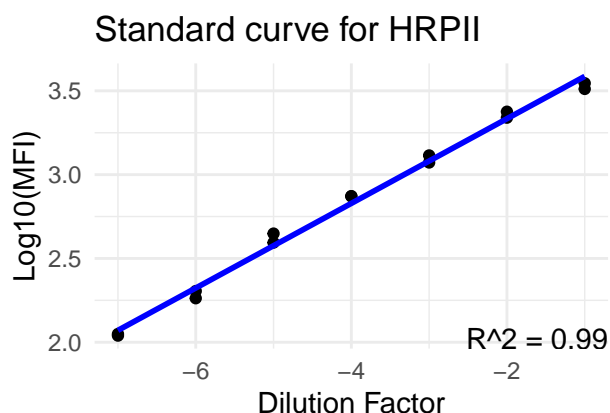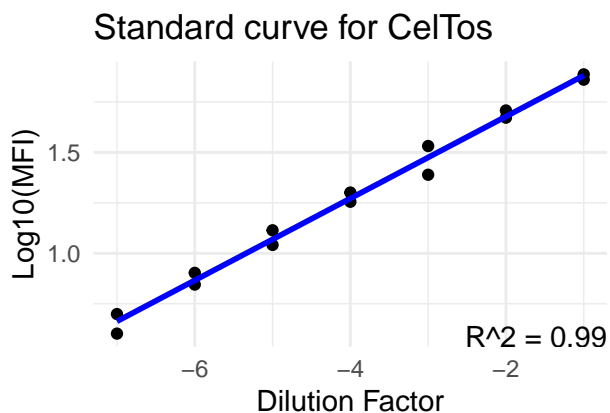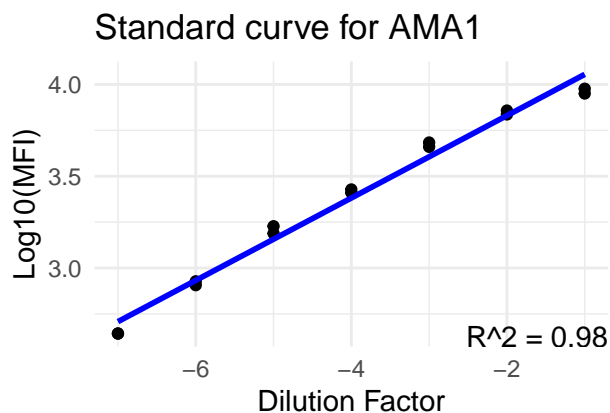R^2 = 0.98

```
## 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]
## [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."
## 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
## [1] "Column names are consistent between the median MFI and bead count data frames."
## The bead QC list has been exported to data/qc/RTSS_Ahero_TotalIgG_3.20.24_AM_beadqc_low_df.csv
```

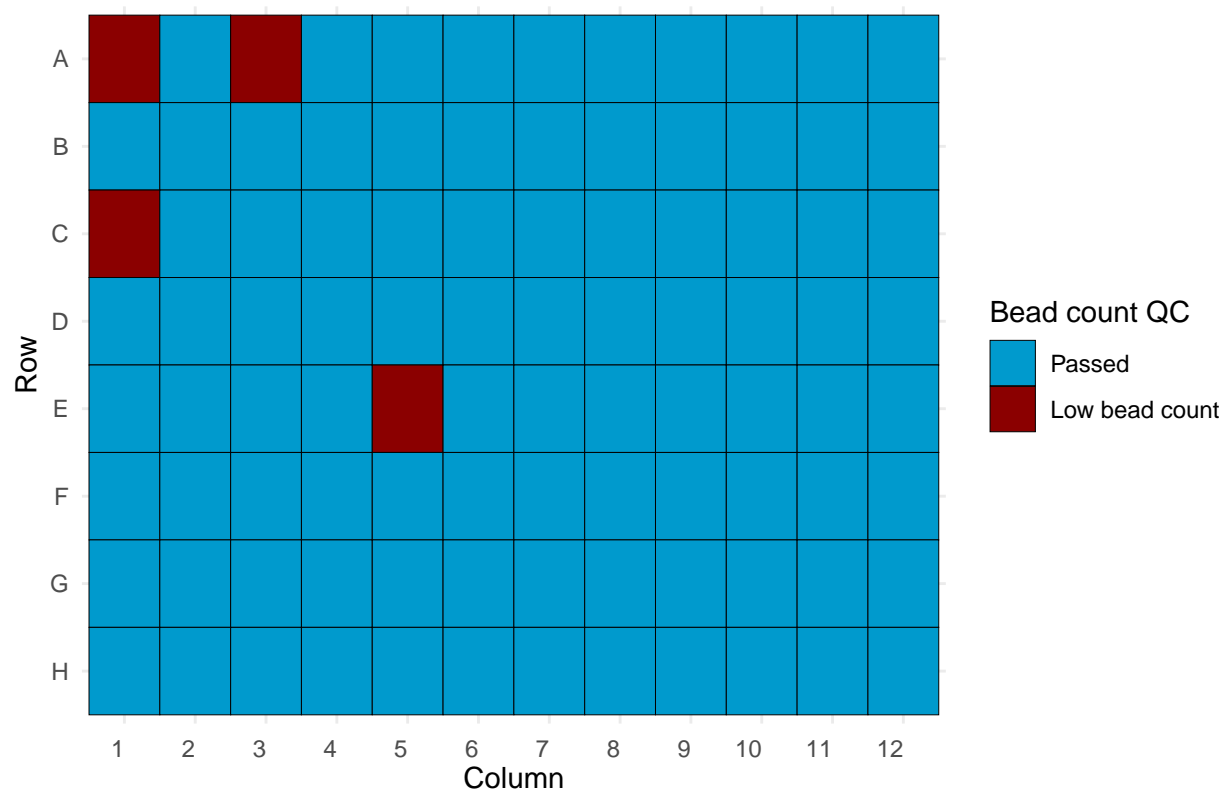## 96−well schematic of Plate: RTSS_Ahero_TotalIgG_3.20.24_AM

Bead count QC

Passed

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

Standard curve for AMA1

Standard curve for CelTos
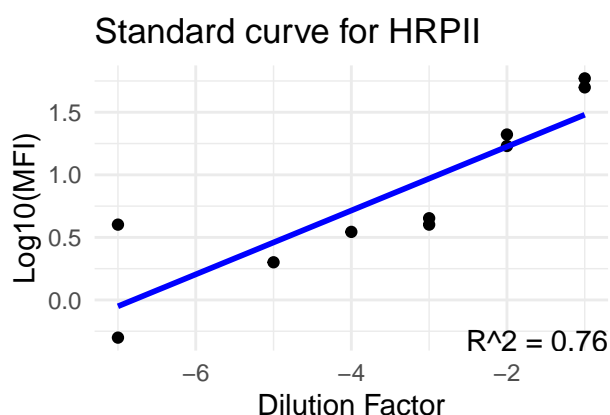

Standard curve for HRPII
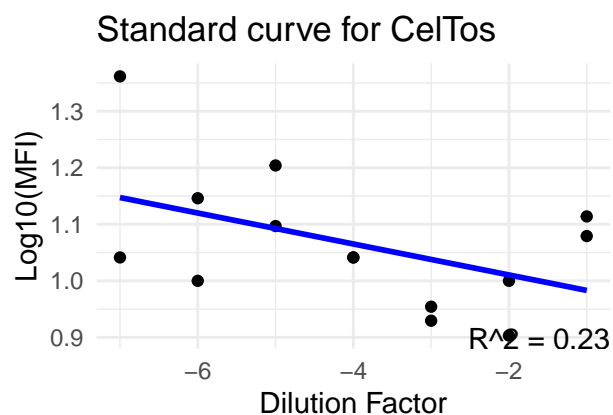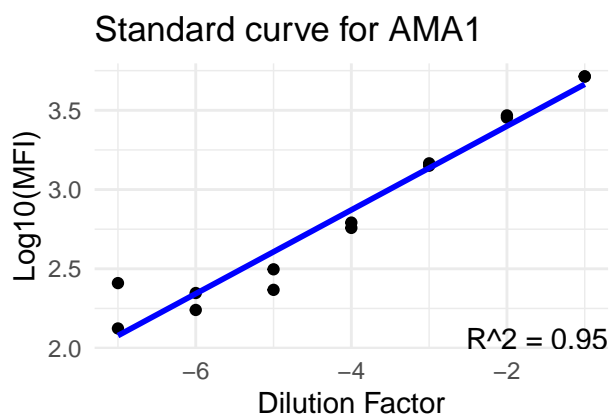


```
## TableGrob (2 x 2) "arrange": 3 grobs
##   z     cells    name            grob
## 1 1 (1-1,1-1) arrange gtable[layout]
## 2 2 (1-1,2-2) arrange gtable[layout]
## 3 3 (2-2,1-1) arrange gtable[layout]
## [1] "This plate has passed plate-level quality control for standard curve linearity."
## [1] "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."
## 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_TotalIgG_3.20.24_AM_cl
## [1] "Column names are consistent between the median MFI and bead count data frames."
## The bead QC list has been exported to data/qc/RTSS_Chul.(SET1)_IgG1_3.23.24_AM_beadqc_low_df.csv
```

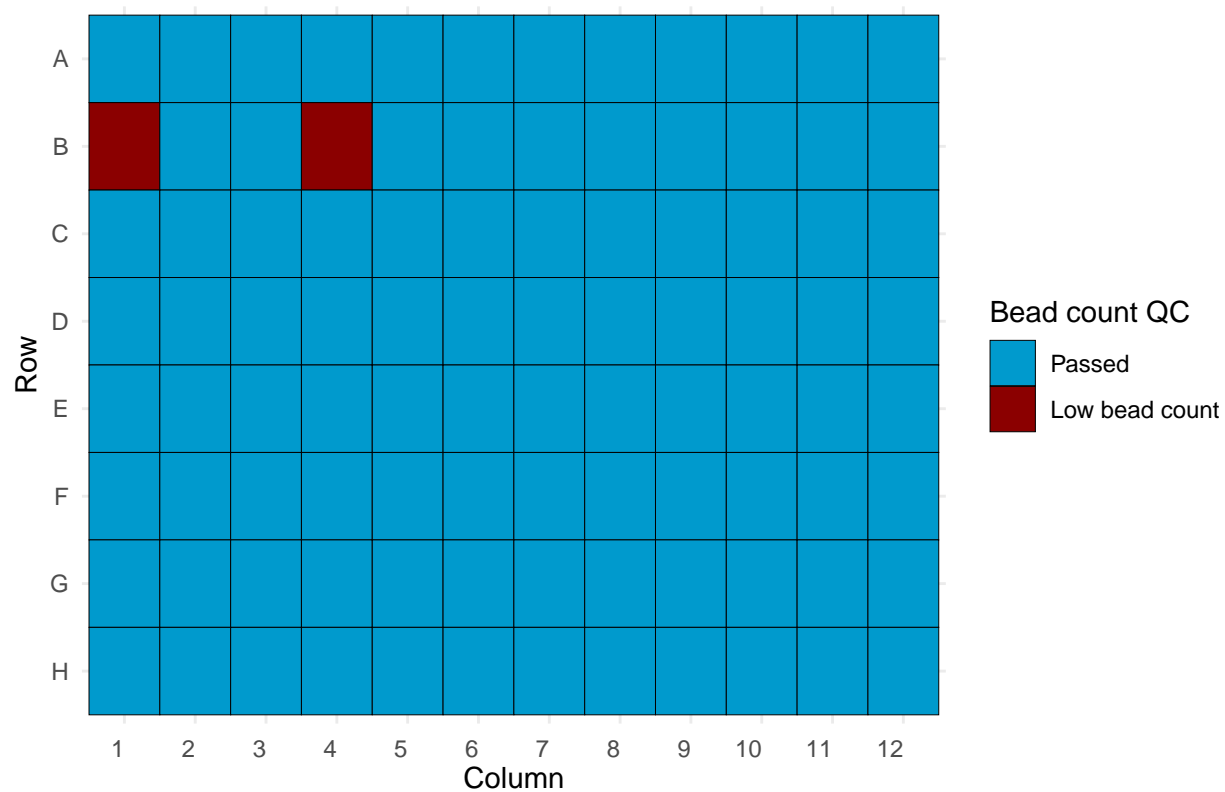## 96–well schematic of Plate: RTSS_Chul.(SET1)_IgG1_3.23.24_AM

Background MFI subtraction completed.

Standard curve for AMA1

Standard curve for CelTos

Standard curve for HRPII
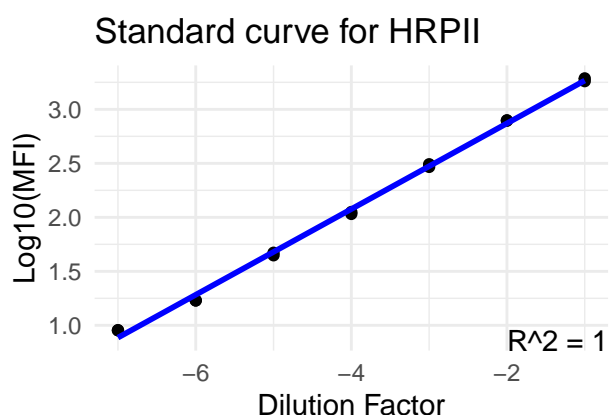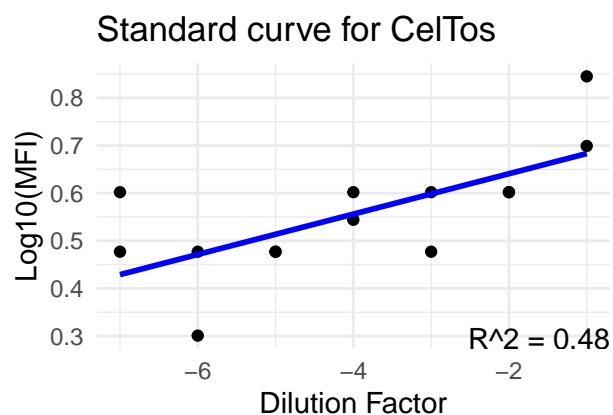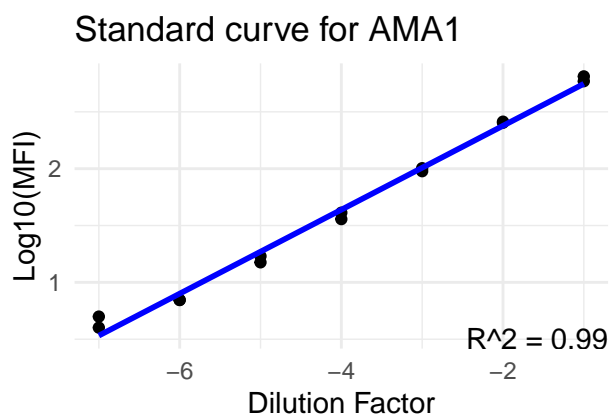


```
## TableGrob (2 x 2) "arrange": 3 grobs
##   z      cells     name              grob
## 1 1 (1-1,1-1) arrange gtable[layout]
## 2 2 (1-1,2-2) arrange gtable[layout]
## 3 3 (2-2,1-1) arrange gtable[layout]
## [1] "This plate has passed plate-level quality control for standard curve linearity."
## [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."
## 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_Chul.(SET1)_IgG1_3.23.24_AM
## [1] "Column names are consistent between the median MFI and bead count data frames."
## The bead QC list has been exported to data/qc/RTSS_Chul.(SET1)_IgG3_3.23.24_AK_beadqc_low_df.csv
```

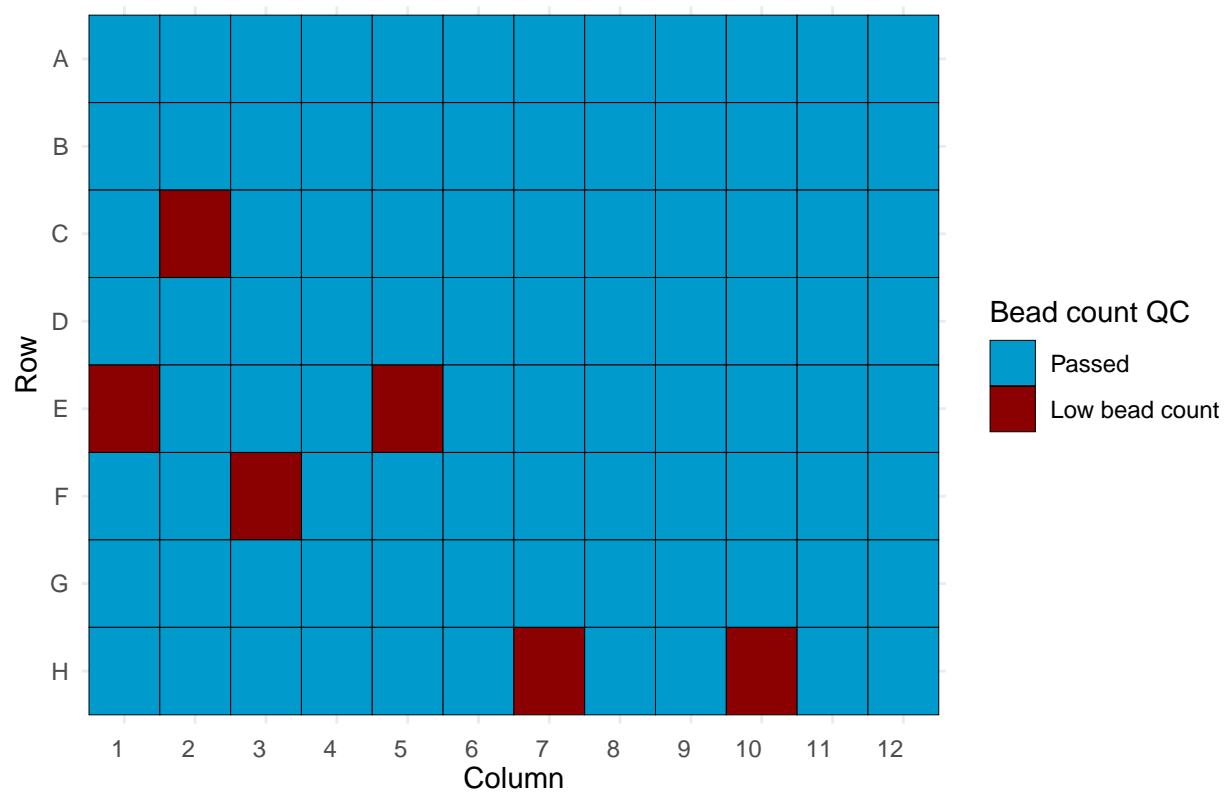## 96−well schematic of Plate: RTSS_Chul.(SET1)_IgG3_3.23.24_AK

Background MFI subtraction completed.

Standard curve for AMA1

Log10(MFI)

R^2 = 0.99

Dilution Factor

Standard curve for CelTos

Log10(MFI)

R^2 = 0.48

Dilution Factor

Standard curve for HRPII

Log10(MFI)

R^2 = 1

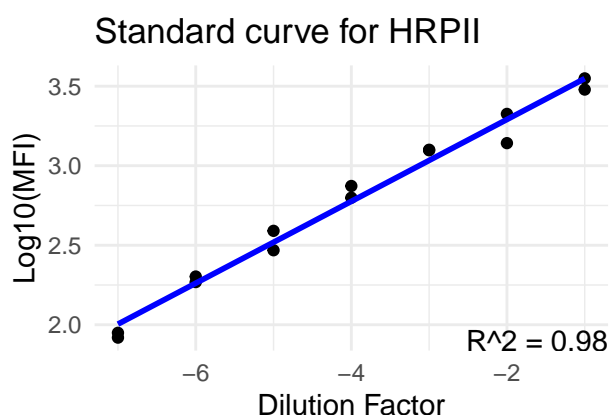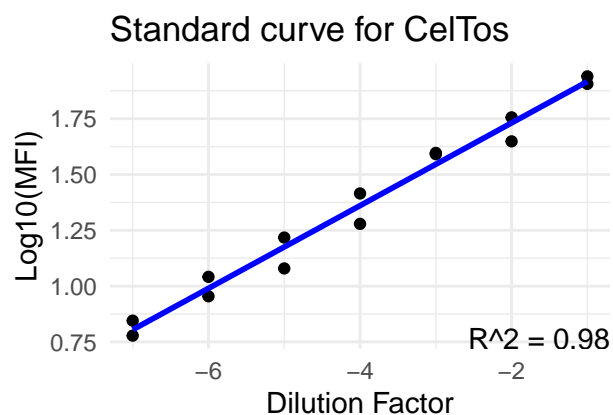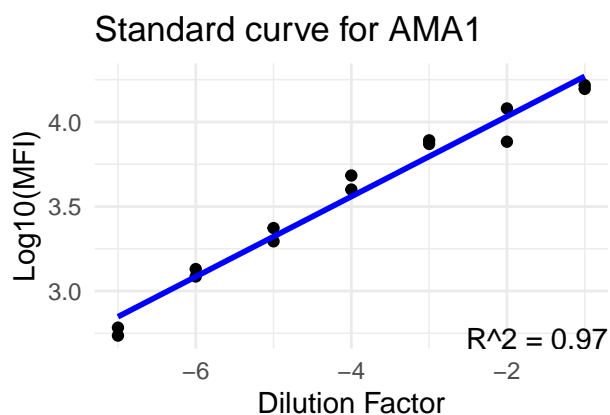Dilution Factor

```
## 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]
## [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_Chul.(SET1)_IgG3_3.23.24_AK
## [1] "Column names are consistent between the median MFI and bead count data frames."
## The bead QC list has been exported to data/qc/RTSS_Chul.(SET1)_TotalIgG_3.23.24_JN_beadqc_low_df.csv
```

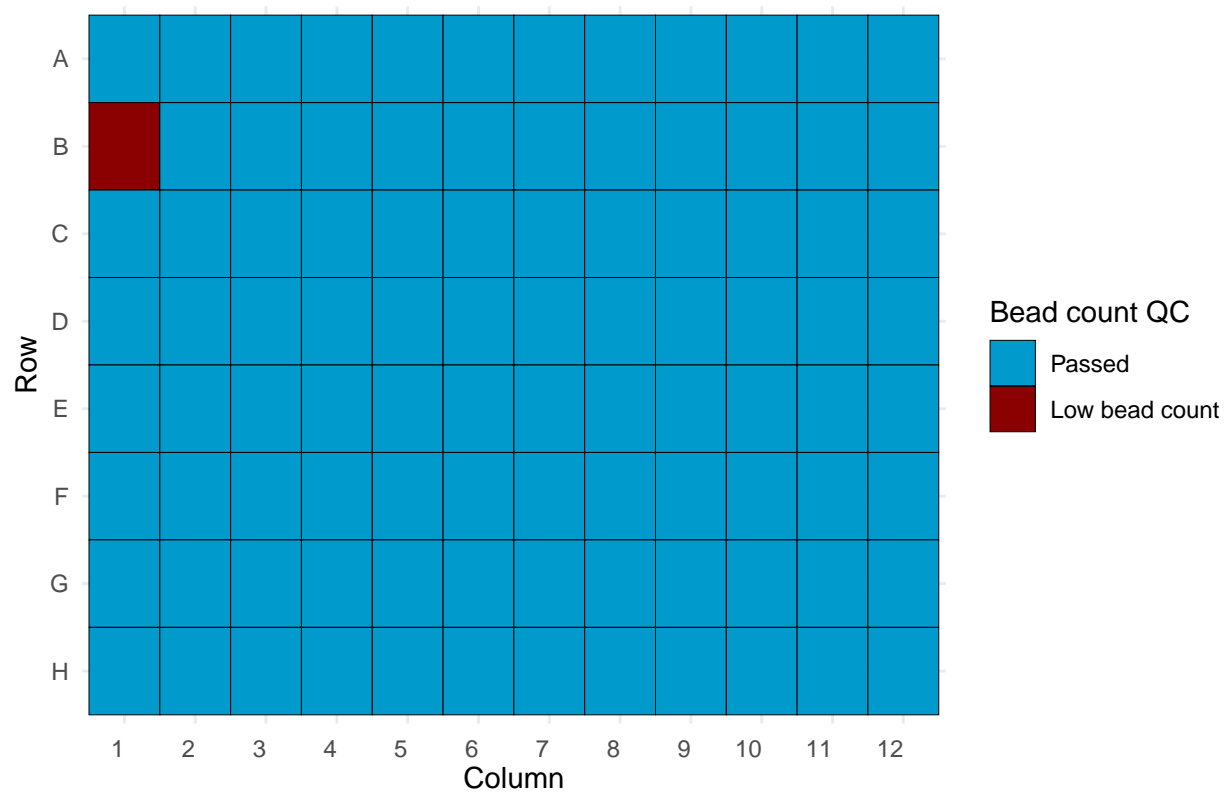## 96–well schematic of Plate: RTSS_Chul.(SET1)_TotalIgG_3.23.24_JN

Background MFI subtraction completed.

Standard curve for AMA1

Standard curve for CelTos

Standard curve for HRPII
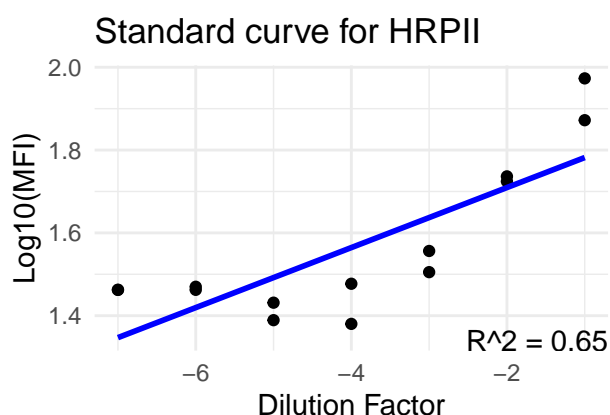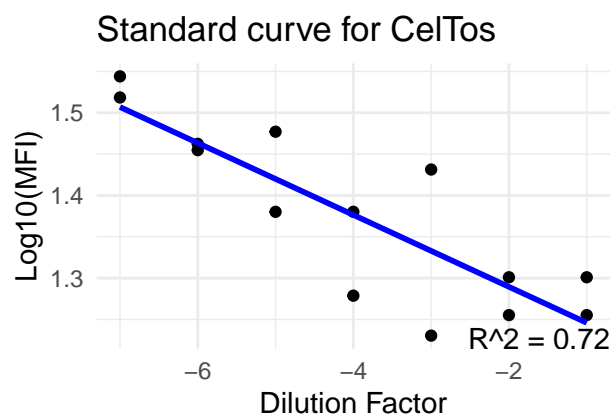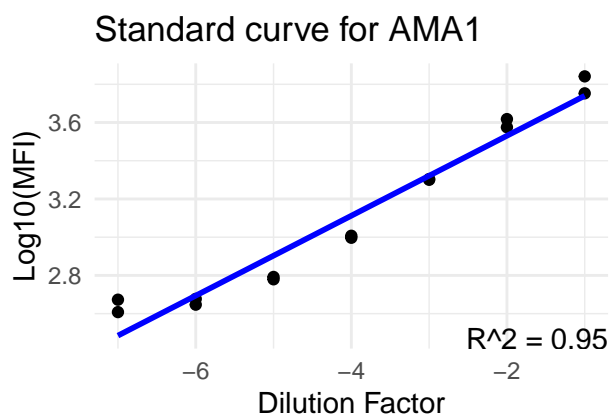


```
## TableGrob (2 x 2) "arrange": 3 grobs
##   z     cells   name            grob
## 1 1 (1-1,1-1) arrange gtable[layout]
## 2 2 (1-1,2-2) arrange gtable[layout]
## 3 3 (2-2,1-1) arrange gtable[layout]
## [1] "This plate has passed plate-level quality control for standard curve linearity."
## [1] "At 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."
## 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_Chul.(SET1)_TotalIgG_3.23.2
## [1] "Column names are consistent between the median MFI and bead count data frames."
## The bead QC list has been exported to data/qc/RTSS_Chul.(SET2)_IgG1_3.25.24_MK_beadqc_low_df.csv
```

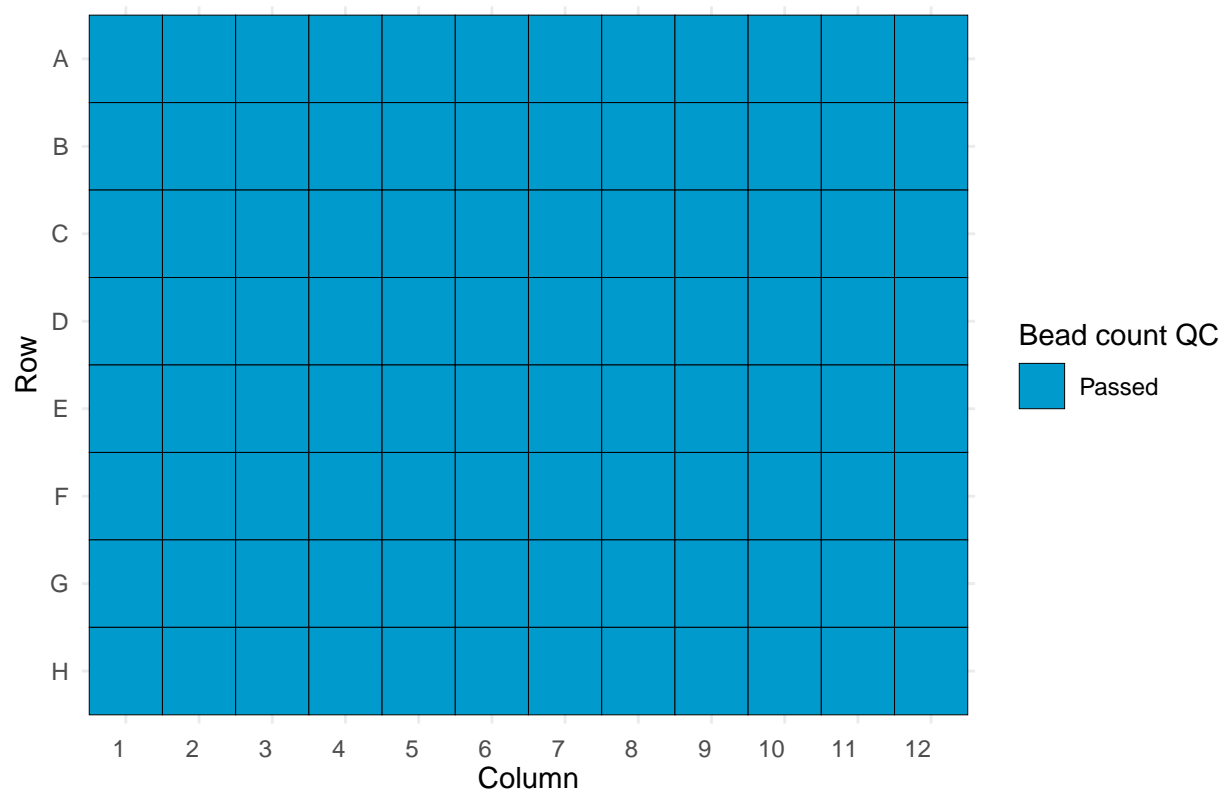## 96−well schematic of Plate: RTSS_Chul.(SET2)_IgG1_3.25.24_MK

Background MFI subtraction completed.

Standard curve for AMA1

$R^2 = 0.95$

Standard curve for CelTos

$R^2 = 0.72$

Standard curve for HRPII
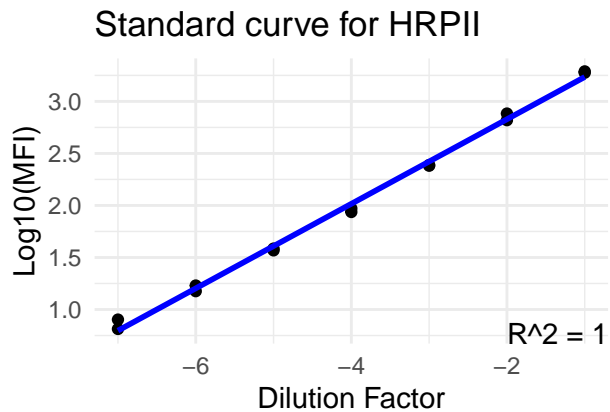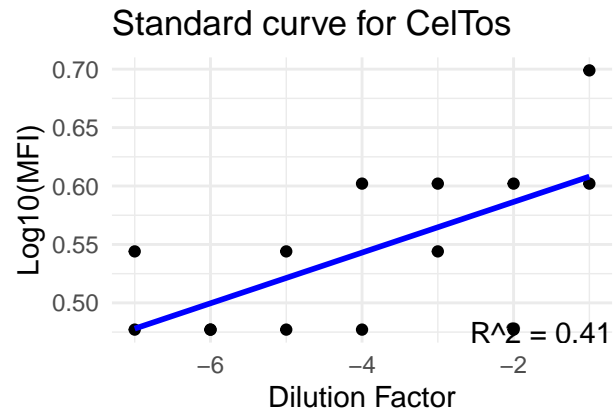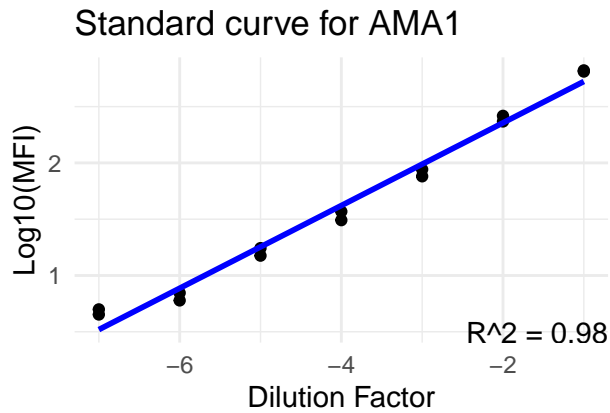

$R^2 = 0.65$

```
## 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]
## [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."
## 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_Chul.(SET2)_IgG1_3.25.24_MK_
## [1] "Column names are consistent between the median MFI and bead count data frames."
## The bead QC list has been exported to data/qc/RTSS_Chul.(SET2)_IgG3_3.26.24_JN_beadqc_low_df.csv
```

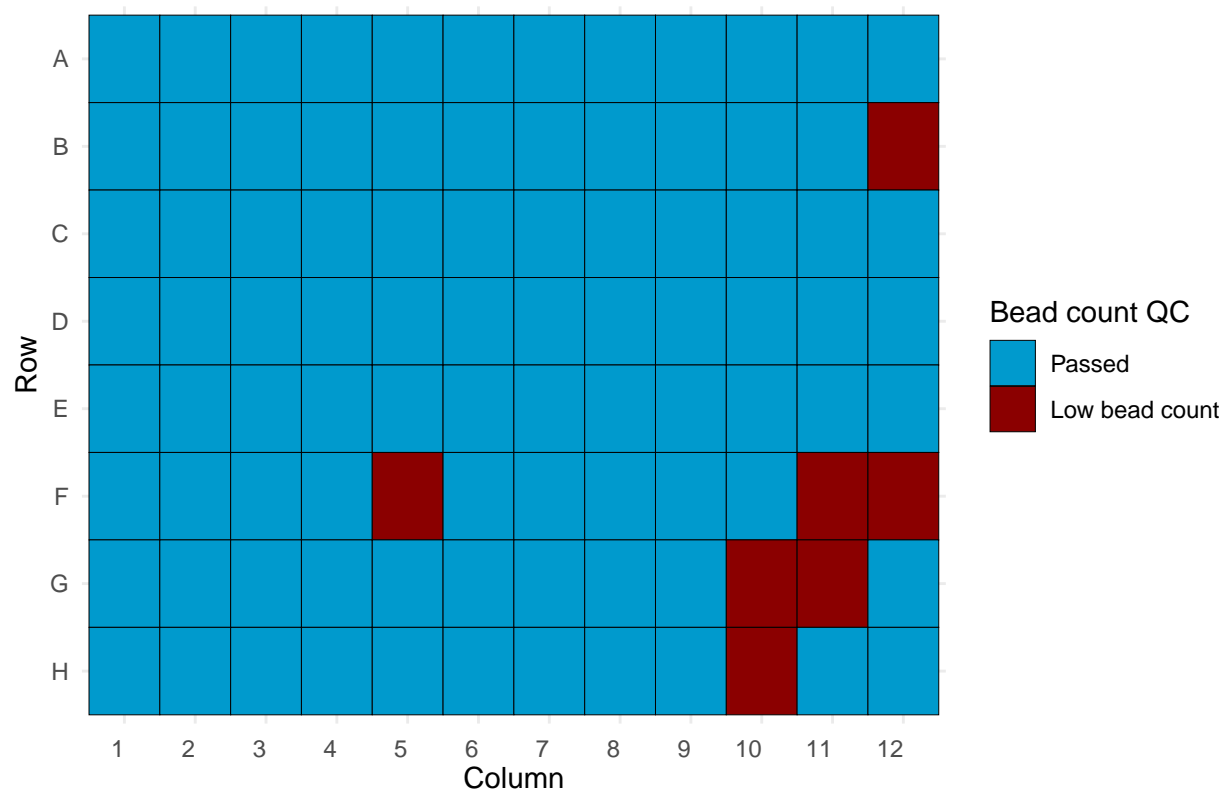## 96–well schematic of Plate: RTSS_Chul.(SET2)_IgG3_3.26.24_JN

Bead count QC

Passed

Background MFI subtraction completed.

Standard curve for AMA1

R^2 = 0.98

Standard curve for CelTos

R^2 = 0.41

Standard curve for HRPII
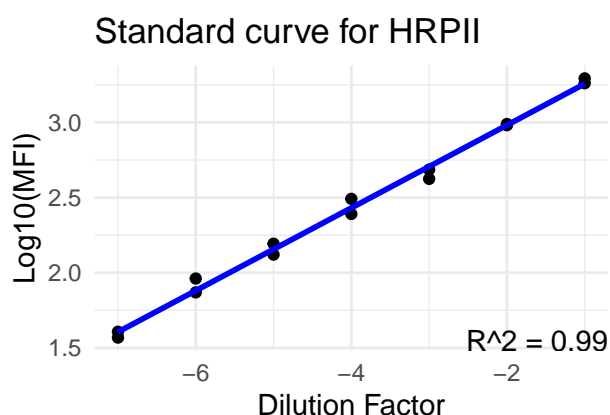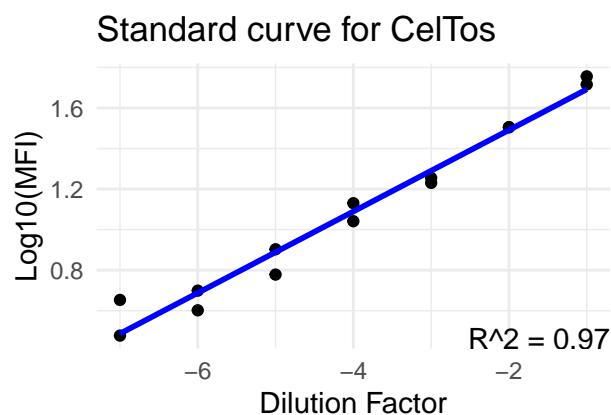

R^2 = 1

```
## 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]
## [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."
## 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_Chul.(SET2)_IgG3_3.26.24_JN
## [1] "Column names are consistent between the median MFI and bead count data frames."
## The bead QC list has been exported to data/qc/RTSS_Chul.(SET2)_TotalIgG_3.25.24_AM_beadqc_low_df.csv
```

96−well schematic of Plate: RTSS_Chul.(SET2)_TotalIgG_3.25.24_AM

Background MFI subtraction completed.

Standard curve for AMA1

R^2 = 0.99

Standard curve for CelTos

R^2 = 0.97

Standard curve for HRPII

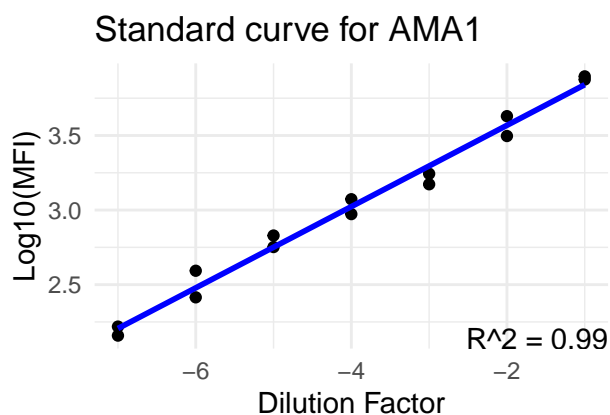R^2 = 0.99

```
## 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]
## [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."
## 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_Chul.(SET2)_TotalIgG_3.25.2
```

```
## [[1]]
## NULL
##
## [[2]]
## NULL
##
## [[3]]
## NULL
##
## [[4]]
## NULL
##
## [[5]]
## NULL
##
## [[6]]
## NULL
```

```
## 
## [[7]]
## NULL
## 
## [[8]]
## NULL
## 
## [[9]]
## NULL
## 
## [[10]]
## NULL
```

Standard QC results have been exported to data/qc/RTSS_all_plates_standardsqc.csv

Compiled bead QC data has been exported to data/qc/RTSS_all_plates_beadqc.csv