

## User Manual - MRMPlusGUI

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### Introduction

MRMPlusGUI as a freely available, open-source tool in the platform independent Java programming language to compute analytical measures as recommended recently by the Clinical Proteomics Tumor Analysis Consortium Assay Development Working Group for "Tier 2" assays. While it helps to validate peptides and associated transitions as stable, quantifiable, and reproducible representatives of proteins of interest, it streamlines the assay development analytical workflow - thus guarding against analytical errors that quite often are propagated.

For assay experiments design and generating MRMPlusGUI input files, please see the Clinical Proteomics Tumor Analysis Consortium (CPTAC), Assay Development Working Group (ADWG), guidance document. This may be found here - <https://assays.cancer.gov/guidance-document/>

### Running MRMPlusGUI

#### HOW TO RUN MRMPlusGUI

Please see <https://bitbucket.org/paiyetan/mrmplusgui/overview> and <https://bitbucket.org/paiyetan/mrmplusgui/wiki>.

PS: Current implementation of MRMPlus computes analytical measures for "Response Curve" and "Mini-Validation of Repeatability"

#### SYSTEM REQUIREMENT

Java 6 or above. You may download the latest version from <http://www.java.com/en/download/index.jsp>

#### HOW TO INSTALL

- Download the program folder
- Unzip MRMPlusGUI.xxxx.xx.xx.zip folder.
- Place MRMPlusGUI.xxxx.xx.xx folder in directory of choice.

#### RUNNING THE PROGRAM

- Navigate to Program directory/folder.
- Modify the "MRMPlus.config" file options accordingly (see below).
- (For Windows OS) Double click the "MRMPlusGUI.bat", OR
- (For Unix-like OS) Run the command on the terminal (assuming you're in the MRMPlusGUI.xxxx.xx.xx directory).

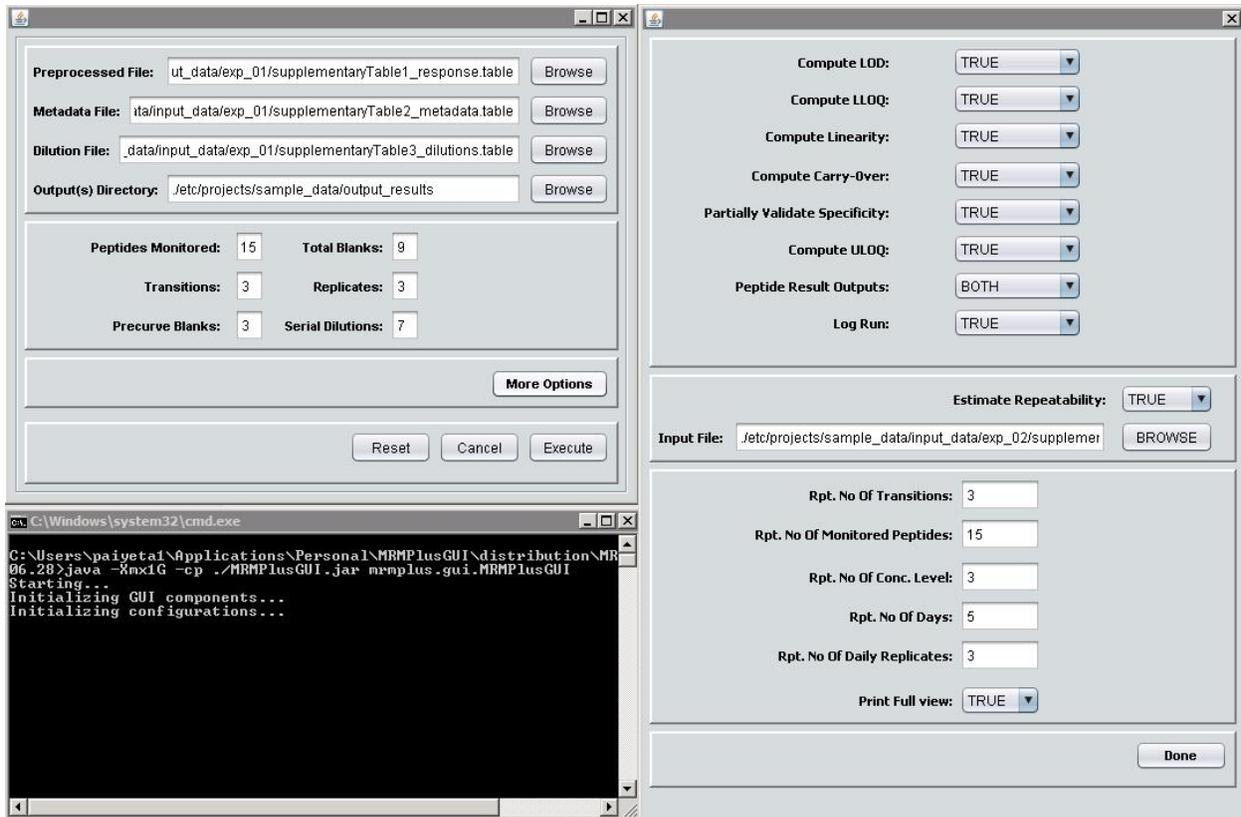
```
java -Xmx1G -cp ./MRMPlusGUI.jar mrmplus.gui.MRMPlusGUI
```

### MRMPlusGUI input files

Inputs to MRMPlusGUI include:

1. Skyline-derived output files in tab-delimited format for  
(a) Response Curve Experiment, and optionally

- (b) Repeatability Experiment
2. User defined experiment metadata, and
  3. a dilution data file



Current implementation of MRMPPlus computes analytical measures for “Response Curve” and “Mini-Validation of Repeatability”. For specific information on installing and running MRMPPlusGUI, please see <https://bitbucket.org/paiyetan/mrmpplusgui/wiki>.

The Skyline-derived file for “Response Curve” experiments is anticipated to include the following defined data columns:

- PeptideSequence
- ReplicateName
- PrecursorCharge
- ProductCharge
- FragmentIon
- light PrecursorMz
- light ProductMz
- light RetentionTime
- light Area
- heavy PrecursorMz
- heavy ProductMz
- heavy RetentionTime

- heavy Area

The specific order of the columns is not necessary as MRMPPlus can automatically locate the respective column index.

The metadata file in a tab-delimited format describes the response curve experiment. Its expected columns are:

- RunOrder
- ReplicateName
- AnalyteType
- Replicate
- CalibrationPoint

The 'RunOrder' column indicates the order in which the experiment and data acquisition for the particular assay (experimental replicate and calibration point) was performed. The 'ReplicateName' maps the row information in the metadata file to the respectively associated analyte in the Skyline derived files. The 'AnalyteType' column differentiates blank dilution from true dilution experiments. The 'Replicate' column describes to what replicate an experiment belongs. The 'CalibrationPoint' column indicates the specific calibration point for the experiment. The dilution file maps calibration points described in the experiment metadata with the associated concentration of spiked-in (or heavy stable isotope-labeled) peptides.

(Optionally) To compute measures for 'Mini-Validation of Repeatability', MRMPPlus requires a tab-delimited Skyline derived file with column names:

- PeptideSequence
- ReplicateName
- PrecursorCharge
- ProductCharge
- FragmentIon
- light PrecursorMz
- light ProductMz
- light RetentionTime
- light Area
- heavy PrecursorMz
- heavy ProductMz
- heavy RetentionTime and
- heavy Area

Other inputs to MRMPPlus include user-defined program parameters inputted via the graphical user interface (GUI) or through the 'MRMPPlus.config' file modification(s).

### **MRMPPlusGUI outputs**

Measures computed include, LOD, LLOQ, linearity, carry-over, partial validation of specificity, ULOQ, inter- and intra-assay coefficient of variation, and total assay coefficient of variation to assess analytical performance of multiplexed peptide assays.

The result table columns in the current implementation of MRMPPlus for 'Response Curve' experiment analysis are as follows:

- Peptide
- Transition
- LOD.average
- LOD.stDeviation
- LOD.value
- LOD.usedSpiked
- LOD.zeroFlagged
- LOD.concPointUsed
- LOD.undetectable
- LLOQ.CaliPoint
- LLOQ.Coefficient
- LLOQ.MeanValue
- LLOQ.StDeviation
- Curve.Slope
- Curve.Intercept
- Curve.Rsquared
- Curve.Predicted
- Curve.Observed
- Curve.Diff(%)
- Curve.SlopeStdErr
- Curve.SlopeStdErr(%)
- CarryOver.maxPeakArea
- CarryOver.postMaxPeakArea
- CarryOver.carryOver(%)
- PVSpec.hasValuesOverLLOQ
- PVSpec.pointOfMaxDev (Cali\_Point)
- PVSpec.pointOfMaxDev (Mean)
- PVSpec.maxDeviation (%)
- ULOQ.CaliPoint
- ULOQ.Coefficient
- ULOQ.MeanValue and
- ULOQ.StDeviation

LOD.average, and LOD.stDeviation describe measures of central tendencies, mean and standard deviation respectively, at the limit of detection – LOD. LOD.concPointUsed indicates the calibration point at which an LOD was computable. LOD.value reports the estimated LOD. LOD.usedSpiked reports a Boolean value which indicates that a spiked in experiment, typically of the lowest calibration point and or lowest concentration was used to compute the LOD. LOD.zeroFlagged similarly is a Boolean value which indicates whether any of the values used to compute LOD are zero. LLOQ.Coefficient, LLOQ.MeanValue, and LLOQ.StDeviation describe the coefficient of variation, mean and standard deviation at the lower limit of quantification – LLOQ. LLOQ.CaliPoint indicates at what calibration point the LLOQ was determined.

Curve.Slope, Curve.Intercept, Curve.Diff, and Curve.SlopeStdErr indicate the analytical response curve properties – slope of the curve, the curve’s intercept on the y-axis, the percentage difference between an observed peptide-transition mid-point concentration and expected concentration from a linear regression modeled response curve (a measure of linearity), and the standard error of the estimate of the slope.

The CarryOver.carryOver property estimates the percent of highest (CarryOver.maxPeakArea) concentration sample found in the immediate blank experiment run (CarryOver.postMaxPeakArea). The 'PVSpec.hasValuesOverLLOQ', 'PVSpec.pointOfMaxDev (Cali\_Point)', 'PVSpec.pointOfMaxDev (Mean)', and 'PVSpec.maxDeviation' properties describe the specificity of a given transition to the assayed peptide. According to ADWG specification, no transition ratio (ratios of peak areas of different transitions of same peptide) should deviate >30% from the mean in all samples above LLOQ.

The Boolean property 'PVSpec.hasValuesOverLLOQ' indicates whether the specific transition has quantifiable values above the LLOQ. 'PVSpec.pointOfMaxDev (Cali\_Point)' reports the calibration point of observed maximum deviation from the mean. It is reasoned that if the point of maximally observed deviation is less than ADWG-specified 30%, it can be assumed that the transition is partially validated as specific to its associated peptide. 'PVSpec.pointOfMaxDev (Mean)', and 'PVSpec.maxDeviation' report the mean and standard deviation at the identified point of maximum deviation. 'ULOQ.CaliPoint', 'ULOQ.Coefficient', 'ULOQ.MeanValue', and 'ULOQ.StDeviation' describe the upper limit of quantification’s calibration point, coefficient of variation, mean value, and standard deviation respectively.

The result table columns in the current implementation of MRMPPlus for ‘Mini-Validation of Repeatability’ experiment analysis are;

- Peptide
- Transition
- intraAssayCV.Me
- interAssayCV.Me
- totalCV.Me
- intraAssayCV.Hi
- interAssayCV.Hi
- totalCV.Hi
- intraAssayCV.Lo
- interAssayCV.Lo
- totalCV.Lo
- ValidatedLLOQ.concLevel
- PVSpec.maxDeviation
- PVSpec.meanAtMaxDev
- PVSpec.dayAtMaxDev
- PVSpec.concLevelAtMaxDev

The 'intraAssayCV.Me', 'interAssayCV.Me', and 'totalCV.Me' properties report the intra-assay, inter-assay, and total coefficient of variations respectively at the repeatability experiments analytes determined medium concentration. The 'intraAssayCV.Hi', 'interAssayCV.Hi', and 'totalCV.Hi' properties, report the intra-assay, inter-assay, and total coefficient of variations

respectively at the high concentration while the 'intraAssayCV.Lo', 'interAssayCV.Lo', and 'totalCV.Lo' properties report the coefficients at the low concentration. 'ValidatedLLOQ.concLevel' indicates the lowest of three concentrations (medium, high, and low) at which the total estimated variability is < 20%. The reported 'PVSpec.maxDeviation', 'PVSpec.meanAtMaxDev', 'PVSpec.dayAtMaxDev', and 'PVSpec.concLevelAtMaxDev' properties are as described for the Response Curve experiment analysis result.

### THE MRMPlus.config FILE

The ".config" file contains the program associated defaults and user-modifiable options. The currently implemented options include.

- **logRun**, a logical specifying whether to log intermediary computation outputs. By default, MRMPlusGUI logs its intermediary results. These are logged in the "./logs" directory.
- **peptidesResultsOutputted**, possible values are "BOTH", "SUMMED", OR "TRANSITIONS". Option specifies what levels, peptide performance metrics are to be computed and outputted. For "TRANSITION", performance metrics are estimated at each respective transition of a peptide. However, at the summed level, summed values for monitored transitions per peptide are utilized to compute performance metrics. "BOTH" specifies that performance metrics are to be computed at both "transition" and "summed" level. MRMPlusGUI defaults to "BOTH"
- **outputDirectory**, specifies the directory where computed results are to be outputted.

### Response Curve Experiment options

- **header**, a logical indicating whether input response file has a header (column attribute) line. By default, MRMPlusGUI expects a header.
- **preprocessedFile**, specifies the file path to the Skyline preprocessed response curve experiment data.
- **metadataFile**, specifies the file path to the user defined response curve experiment metadata information.
- **dilutionFile**, specifies the absolute path to the user defined response curve experiment dilution information.
- **peptidesMonitored**, specifies the total number of peptides monitored in the experiment
- **noOftransitions**, specifies the number of transitions monitored per peptide in the experiment
- **preCurveBlanks**, specifies the number blank experiments ran before true experiments
- **totalBlanks**, specifies the total number of blank experiments ran throughout the experiment
- **replicates**, specifies the number of replicates per concentration level (dilution)
- **serialDilutions**, specifies the number of concentration levels (dilutions)
- **computeLOD**, a logical specifying to compute limit of detection
- **computeLLOQ**, a logical specifying to compute lower limit of quantification
- **computeLinearity**, a logical specifying to estimate the linear character of the curve
- **computeCarryOver**, a logical specifying to estimate analytes' carryover

- **computePartialValidationOfSpecificity**, a logical specifying to partially validate the specificity of peptide transitions
- **computeULOQ**, a logical specifying to compute upper limit of quantification

### Repeatability Experiment options

- **computeMiniValidationOfRepeatability**, a logical specifying whether to compute metrics for repeatability experiment, defaults to FALSE
- **preprocessedRepeatabilityData**, required option if **computeMiniValidationOfRepeatability** is set to TRUE. The file path to the Skyline preprocessed repeatability experiment data.
- **repeatabilityNoOftransitions**, the number of transitions monitored per peptide
- **repeatabilityNoOfPeptidesMonitored**, the total number of peptides monitored
- **repeatabilityNoOfConcentrationLevels**, the number of concentration levels considered for repeatability metric estimation
- **repeatabilityOverNoOfDays**, the number of days over which repeat experiments were performed
- **repeatabilityNoOfReplicatesPerDay**, the number of replicates per experiment per day
- **repeatabilityPrintFullView**, logical specifying if all computed metrics are to be printed