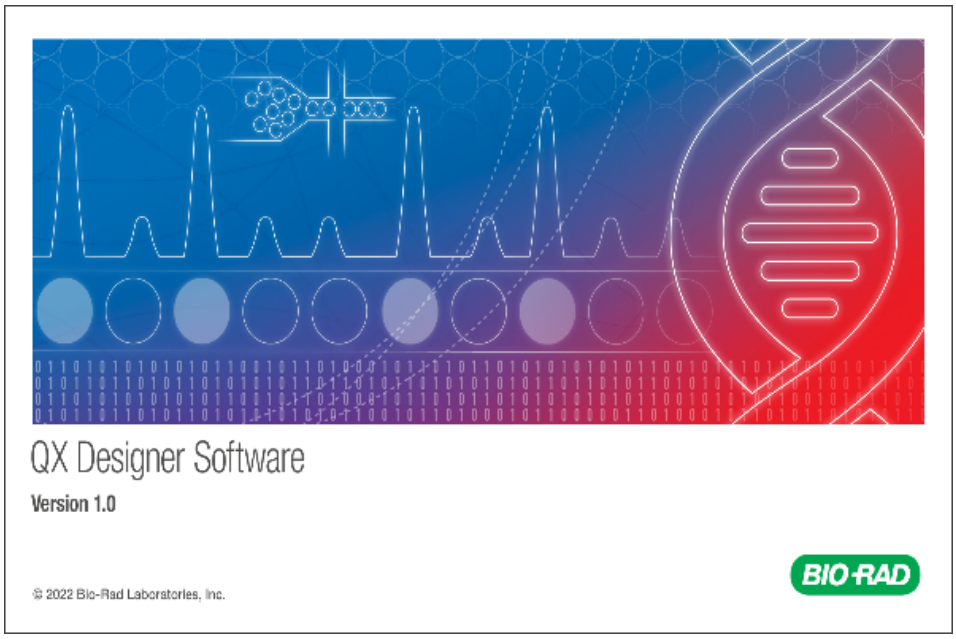
QX Designer Software

Assay Protocol File Configuration Guide

Version 1.0



BIO-RAD

QX Designer

Assay Protocol File Configuration Guide

Version 1.0

BIO-RAD

Bio-Rad Technical Support Department

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Legal Notices

Revision History

Document	Date	Description of Change
QX Designer Assay Protocol File Configuration Guide Software version 1.0 DIR No. 10000149015 Ver A	June 2023	New document explaining how to configure custom assay protocol files

Table of Contents

Chapter 1 Introduction

Assay protocol files (APFs) are static plate layout templates that contain detailed biological assay designs. APFs follow specific instructions and rules for qualitative or quantitative evaluations. QX Designer Software from Bio-Rad™ provides an interface for creating new APF packages, and editing existing packages, with combinations of values and settings that are used frequently in your laboratory. APFs are also connected to a specific results feature in QX Manager Software, Premium Edition (versions 2.0 and later), which facilitates viewing and analysis of targeted data.

This user guide contains instructions and procedures for creating and editing APFs by defining plate rules and definitions, kit lots and quality rules, custom variables and calculations for data analysis, and custom results and reports.

The content of this guide assumes you are proficient in QX Manager Software, Premium Edition, functions and options.

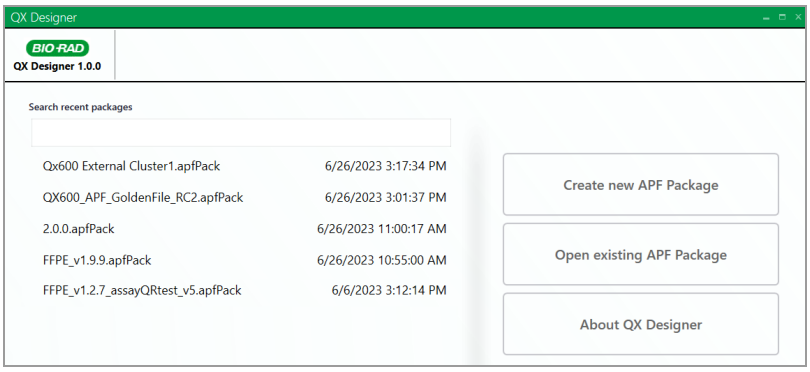
Opening QX Designer Software and Selecting an Option

When you open QX Designer Software, you can create a new APF package or you can edit an existing APF package.

To open the application and display the home screen

1. Double-click the QX Designer shortcut icon on the computer desktop.

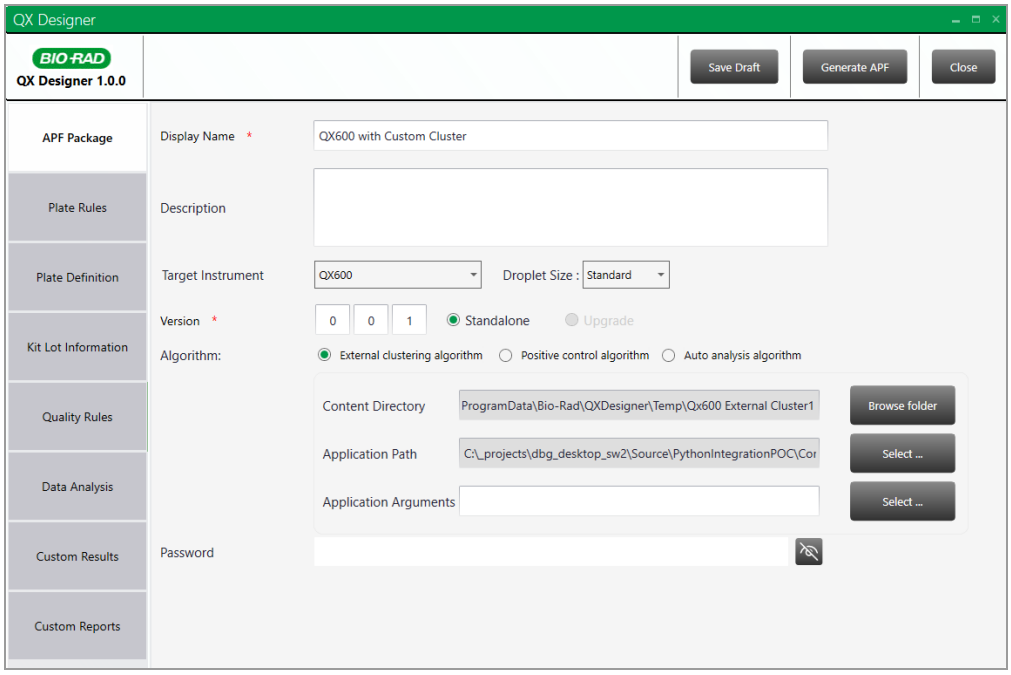
   The Home screen appears. As you create or upload APFs, the packages appear on the left.



2. Do one of the following:
   - To create a new configuration, click Create new APF Package.
   - To edit an existing configuration, click Open existing APF Package and navigate to the applicable storage location or click an APF package in the list on the left.
3. Continue to Configuration Screens Overview for information on the functionality available in QX Designer.

Configuration Screens Overview

When you click one of the options on the Home screen, the APF Package screen appears by default, as shown in the following graphic. Tabs to the other configuration screens appear on the left.



The screen associated with each tab is described in Table 1 below.

Table 1. QX Designer APF Configuration Screens

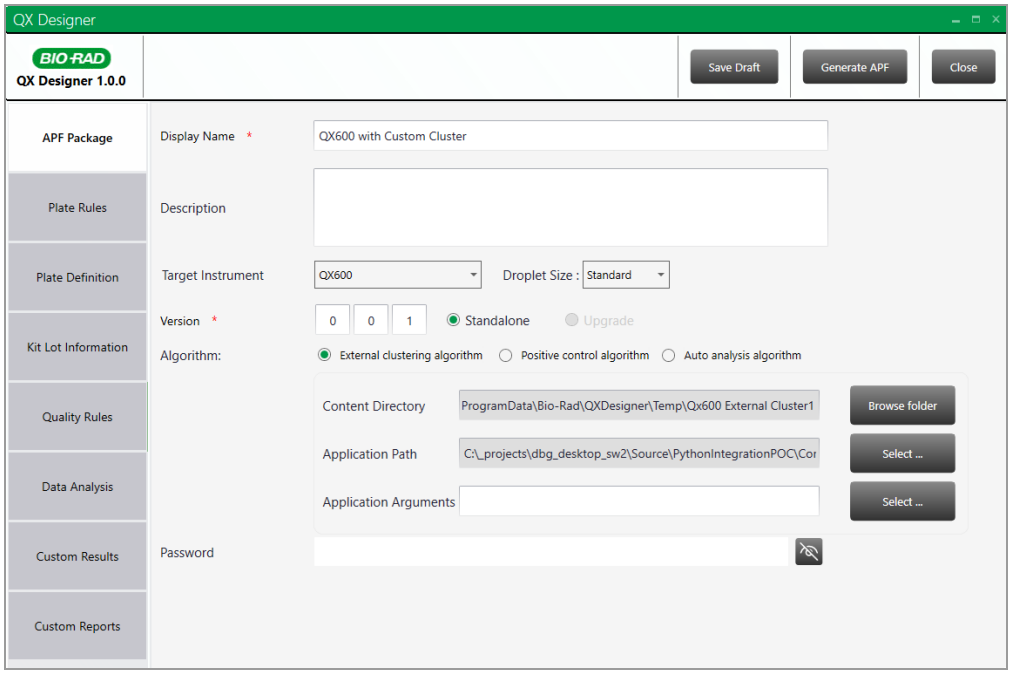
In this screen...	You can...
APF Package	Name and describe the APF, select the instrument, identify the APF version, select the algorithm, and identify the directory and path. Optionally, you can assign a password. See APF Package Screen on page 11.
Plate Rules	For each assay you define, select the experiment and assay types, and targets, signals, and sample types. See Plate Rules Screen on page 14.
Plate Definition	Identify the well assay types and sample types for fixed wells. See Plate Definition Screen on page 17 for
Kit Lot Information	Select to use specific lots for kits, consumables, and reagents. See See Kit Lots Screen.
Quality Rules	Define rules and criteria for calculations and data validation. See Quality Rules Screen on page 20.
Data Analysis	Define variables and their values, as well as custom calculations. See Data Analysis Screen on page 25.
Custom Results	Customize the APF results display in the Analysis Module. See Custom Results Screen on page 29.
Custom Reports	Generate reports from your customized results. See Custom Reports Screen on page 34.

Chapter 2 Creating and Editing APF Packages

This chapter provides more detailed information on options that are available for configuring an APF Package. You can create new packages or edit existing packages.

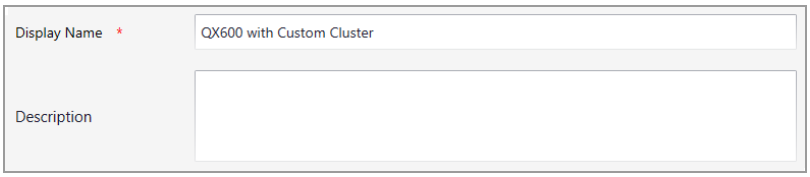
APF Package Screen

In the APF Package screen, you can enter general information regarding the APF. When you select an option to create or edit content from the Home window, the APF Package window appears by default.

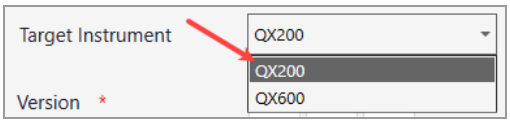


To add or update information in the APF Package screen

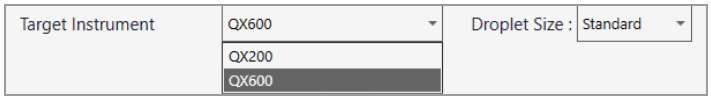
1. In the Display Name field, enter a file name for the APF and, optionally, add a brief description.

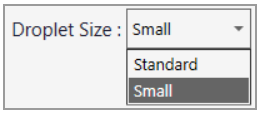


2. From the Target Instrument dropdown, select the instrument.



3. If you select the QX600, you must also specify the droplet size.





4. Do one of the following:

   - When creating a new APF, enter a version of three numbers.

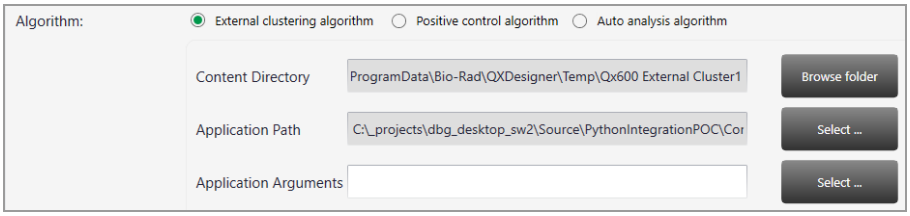
   - When updating an existing APF, change the version and select Standalone or Upgrade to further control your imported versions in QX Designer Software, Premium Edition.

     Note the following:

     - Standalone allows the import of multiple versions of the same APF
     - Upgrade allows the import of a single APF version only; therefore, importing a new version replaces the existing version.

5. Choose a calculation algorithm.

   Important: External clustering is currently supported for two channels only (Ch1 and Ch2), for the DropOff and Double Dropoff assay types. If you select the external clustering algorithm the section expands and displays additional fields, as shown in the graphic below. You must have a development application installed (Python or other developer language), and you must specify the path to the executable file and any arguments that are expected to pass through QX Manager Software, Premium Edition. For more information on external clustering algorithms, see Appendix A, External Clustering Algorithms.

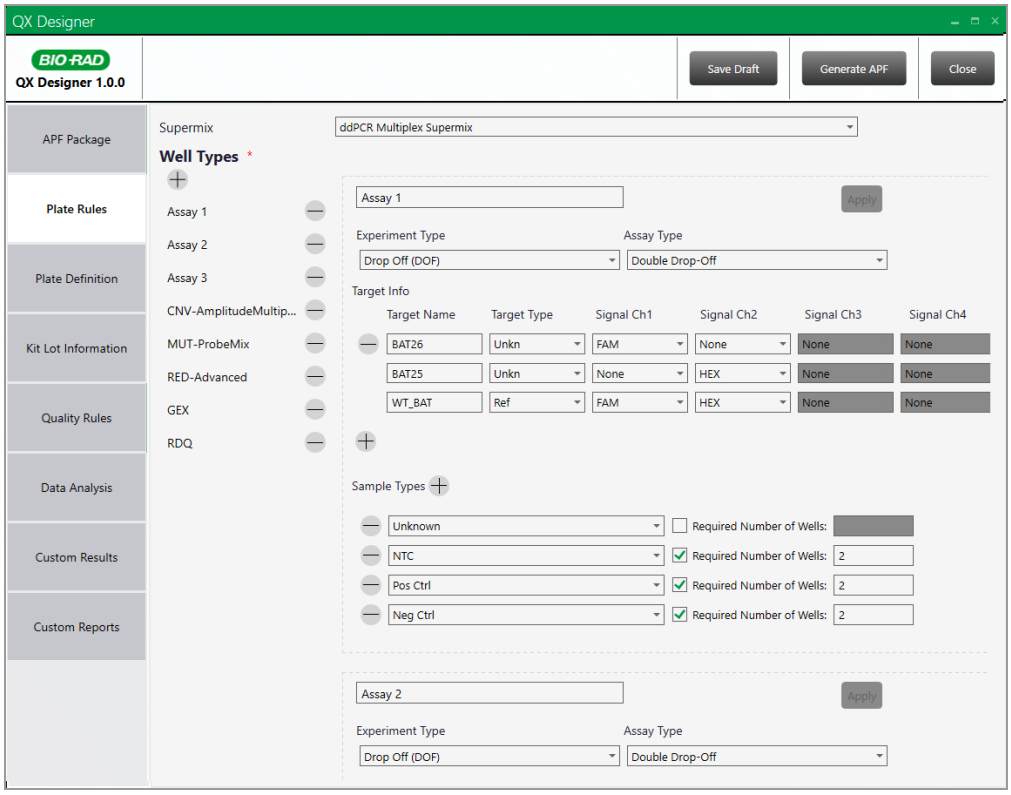


6. (Optional) To restrict access to the file, enter a password in the Password field. To show the password, click the icon on the right.

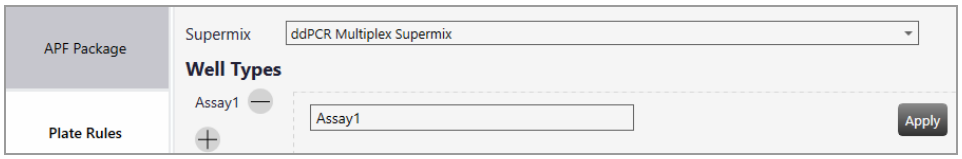
7. Continue to Plate Rules.

Plate Rules Screen

Using the Plate Rules screen, you can define different settings combinations as well types. As you click the ⊕ icons, fields and dropdown lists are enabled, depending on previous selections. Choices are identical to settings and options in QX Manager Software, Premium Edition.



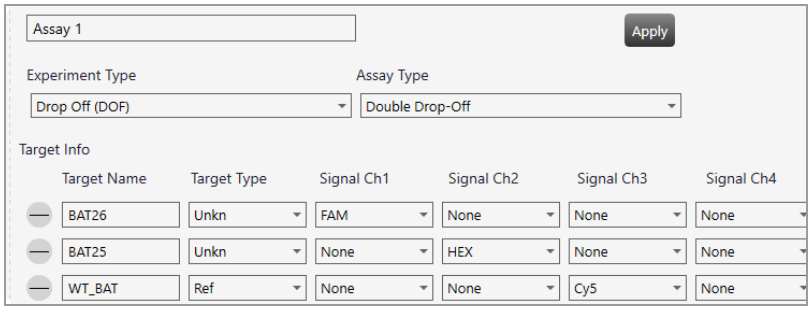
Well types begin as a blank screen, with the first supermix in the drop-down list selected.



To set up well types

1. Select the Plate Rules tab, and then select a different supermix if applicable.
2. Click the ⊕ icon under Well Types.

   a. Update Well Type 1 (default entry) with a new name (for example, Assay 1).



   b. From the Experiment Type dropdown list, select an experiment type.

      When you select the experiment type, corresponding assay types appear in the Assay Type drop-down list.

   c. From the Assay Type dropdown list, select or change the assay type.

      When you select an assay type, generic targets are defined under Target Info. If you change the assay type, the target information changes accordingly.

   d. Under Target Info, identify your targets and replace the default selections as applicable.

      You can change the target name, target type (unknown or reference), and select an alternate dye where applicable. For example, FAM to EvaGreen® or HEX to VIC.

e. Under Sample Types, select or change the sample type from the dropdown list (Unknown, NTC, Pos Ctrl, Neg Ctrl).

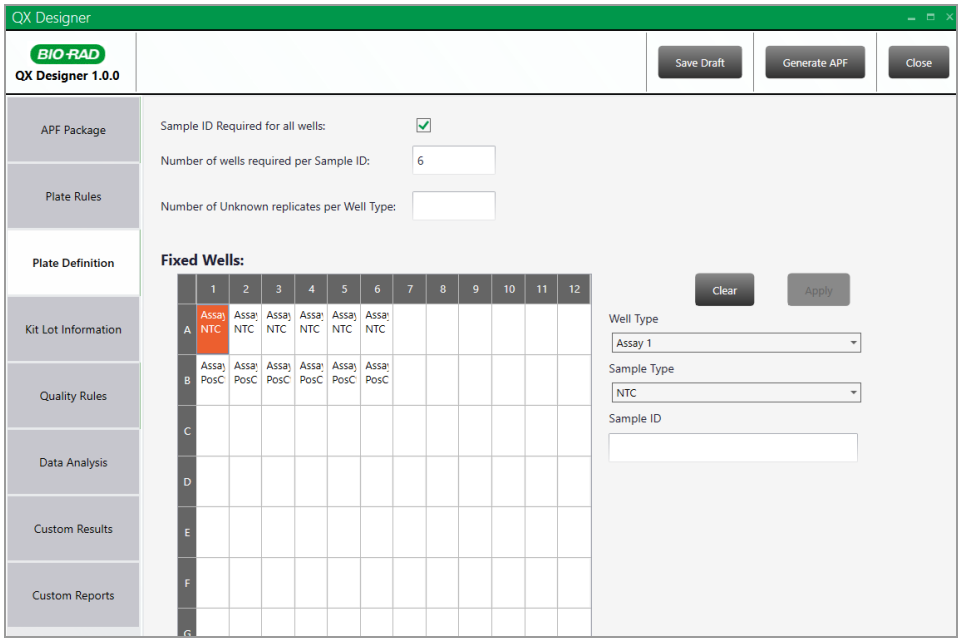
Tip: You can click the + icon next to Sample Types to add other sample types until the maximum is reached. You can also require a certain number of wells to inherit this well type configuration in the APF plate layout. Select the Required Number of Wells checkbox, and then enter the number.

f. Click Apply.

3. To add and configure another well type, click the + icon under Well Types and repeat this procedure.
4. When your well types are configured, continue to Plate Definition.

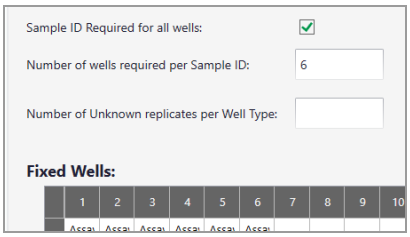
Plate Definition Screen

in the Plate Definition screen, you can select combinations of well types and sample types for your fixed wells, based on your configuration on the Plate Rules screen.



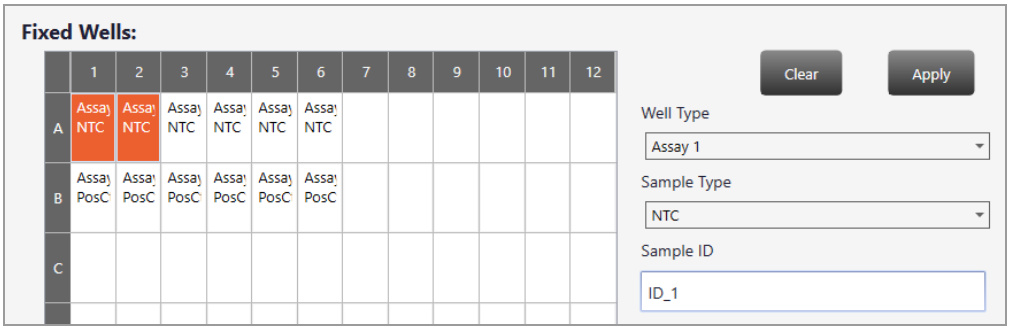
To define your plate

1. Select the Plate Definition tab.



2. (Optional) Above the Fixed Wells grid, enter information for the following:
   a. If a sample ID is required for all wells, select the checkbox.
   b. Enter the number of wells required for each sample ID.
   c. Enter the number of Unknown replicates per well type.

3. Select a group of wells in the grid.



4. From the Well Type drop-down list, select a well type that you configured in the Plate Rules screen.

   After you select the well type, the Sample Type dropdown list is enabled.

5. Select one of the sample types you configured in the Plate Rules screen.
6. If sample IDs are required, enter a sample ID in the corresponding field.
7. Click Apply.
8. Repeat for each group of wells you are setting up in the grid.

   These wells will always be read according to your configuration when you select the APF for a droplet reading run.

9. Continue to Kit Lot Information.

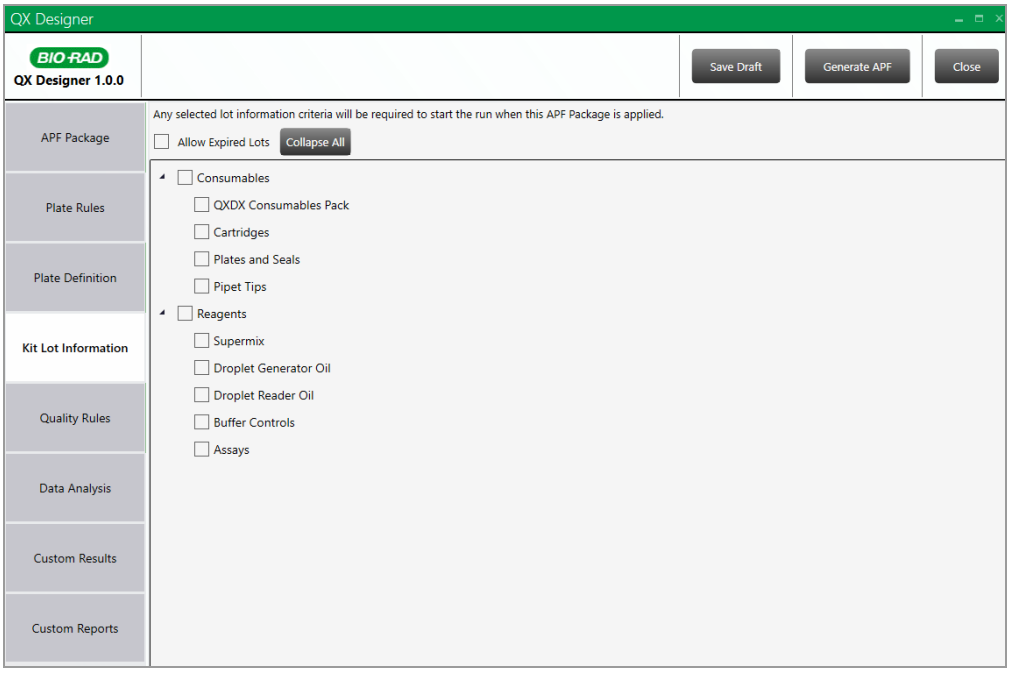
Kit Lots Screen

When you select the Kit Lot Information tab, the window expands with checkboxes for lot categories for consumables and reagents.

If you select a checkbox for a lot item, the item is required when you associate the APF with your run in QX Manager Software, Premium Edition. For example, if you select the Cartridges checkbox under Consumables in QX Designer Software, and then do not select a lot for cartridges in QX Manager Software, Premium Edition, QX Manager Software displays an error message.

To specify that lots will be used in the run

1. Click the arrow to the left of each category to expand the section and display an array of checkboxes.

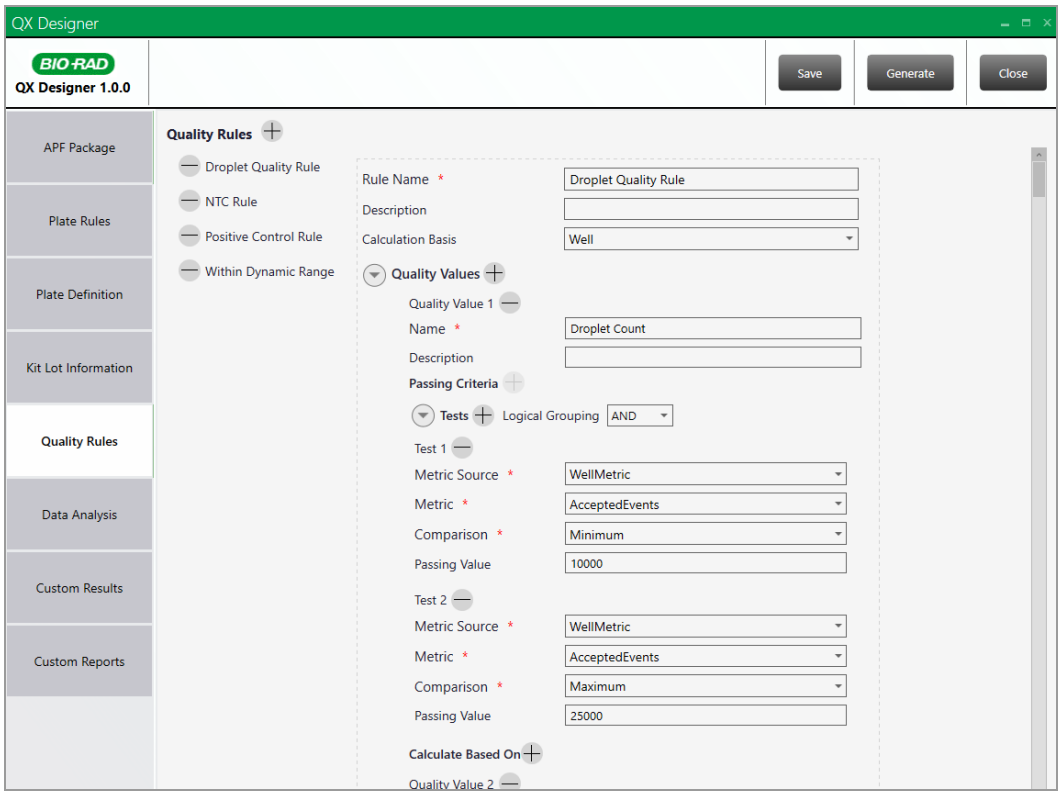


2. Select the corresponding checkboxes for lot items that should always be included in a plate run when the APF is assigned.
3. (Optional) If applicable, select the Allow Expired Lots checkbox.
4. Continue to Quality Rules.

Quality Rules Screen

Use the Quality Rules screen to establish Pass or Fail criteria for your wells, based on parameters you set up in the Plate Rules screen.

Within each quality rule, you specify one or more quality values, which are composed of tests that establish passing criteria and calculations, based on well metrics and/or quality rules. Sections and subsections expand to include applicable fields based on your selections.



To fully define a quality rule, you must name the rule and select a calculation basis type (Well, Sample, or Plate), and then define the associated quality values for the rule. Quality values are composed of one or more tests to validate droplet digital PCR™ (ddPCR™) data.

To create a quality rule

1. Select the Quality Rules tab, and then click the ⊕ icon next to Quality Rules in the upper-left corner to expand the display. QX Designer expands to display more fields as you click the ⊕ icons in different sections.

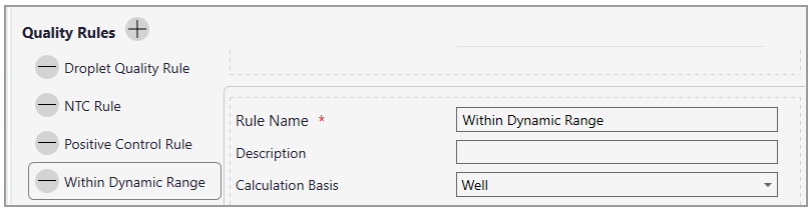
   The default entry of Quality Rule 1 is the starting point.

2. Under Quality Rule 1 do the following:

   a. Change the name in the Rule Name field to reflect the purpose of the quality rule.

   b. Optionally, enter a description for the rule in the Description field.

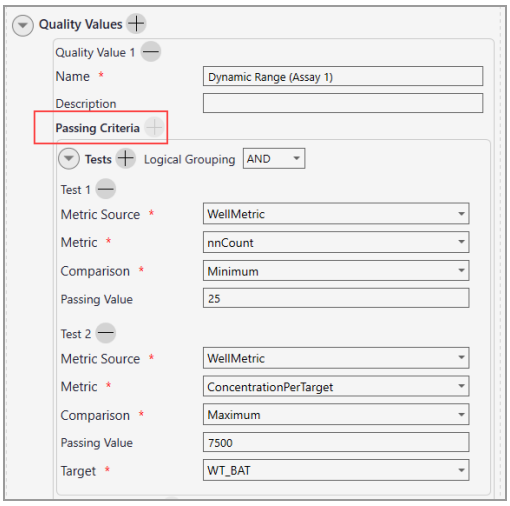
      Note: When you save the information, the Rule Name appears under Quality Rules so you can easily see the rules that are defined.

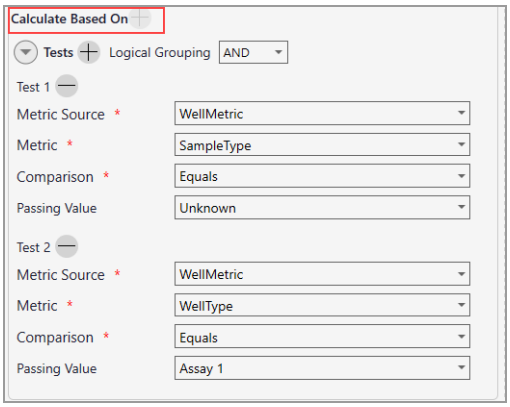


   c. Select a calculation basis type (Well, Sample, or Plate) by which the quality will be measured.

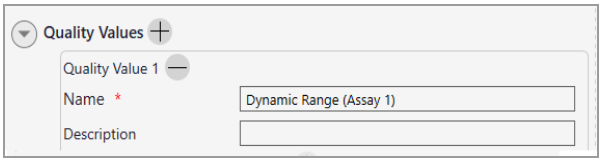
To define the quality values within the rule

Within the Quality Values section, you can set up one or more tests under Passing Criteria and Calculate Based On. You can set up one or both, and you can mix and match selections for a range of validation capabilities.

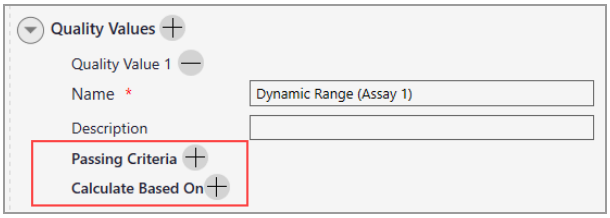




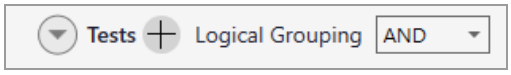
1. Click the + sign next to Quality Values to display Quality Value 1 section and enter a name and optionally, a description.



2. Click the + sign next to Passing Criteria or Calculate Based On. You can configure one or both within the same Quality Value.

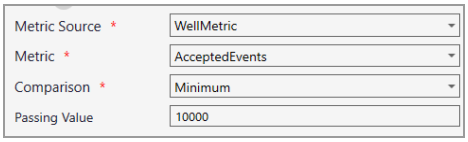


a. Click the ⊕ icon next to Tests and select an operator (AND, OR) from the Logical Grouping dropdown list . If you select AND, all rules must pass. If you select OR, a minimum of one rule must pass.

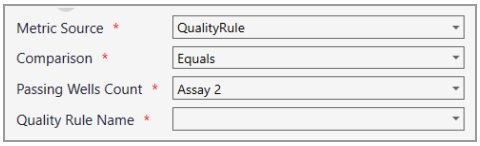


b. Select a Metric Source (Well Metric or Quality Rule) to set up validation testing. The section expands after you make your selection.

Well Metric — Use an analysis metric



Quality Rule —Use an existing quality rule



Important: Step 2c below describes the fields shown in the above graphics, but other fields might appear based on different selections for the Metric.

c. Select or enter the following:

Metric: Select a metric from the dropdown list; the dropdown list contains many options; for brief descriptions, see Appendix A. (applies to Well Metric only).

Comparison: Select from the following: (applies to Well Metric and Quality Rule).

- Minimum — must be a minimum of the Passing Value to pass.
- Maximum — can be a maximum of the Passing Value to pass.

Note: Set up two tests to define a range, one with the minimum value and the second with the maximum value.

- Precision Percentage — can pass if within a percentage defined in Passing Value (Well Metric only).
- Equals — metric can pass if it equals the Passing Value.
- Not Equals — metric can pass if it doesn't equal the Passing Value (Well Metric only).

Passing Value: Enter the applicable value. (Well Metric only).

Passing Wells Count: Enter the applicable value (Quality Rule only).

Quality Rule Name: Select an existing Quality Rule from the dropdown list (Quality Rule only).

d. Optionally, add another test.

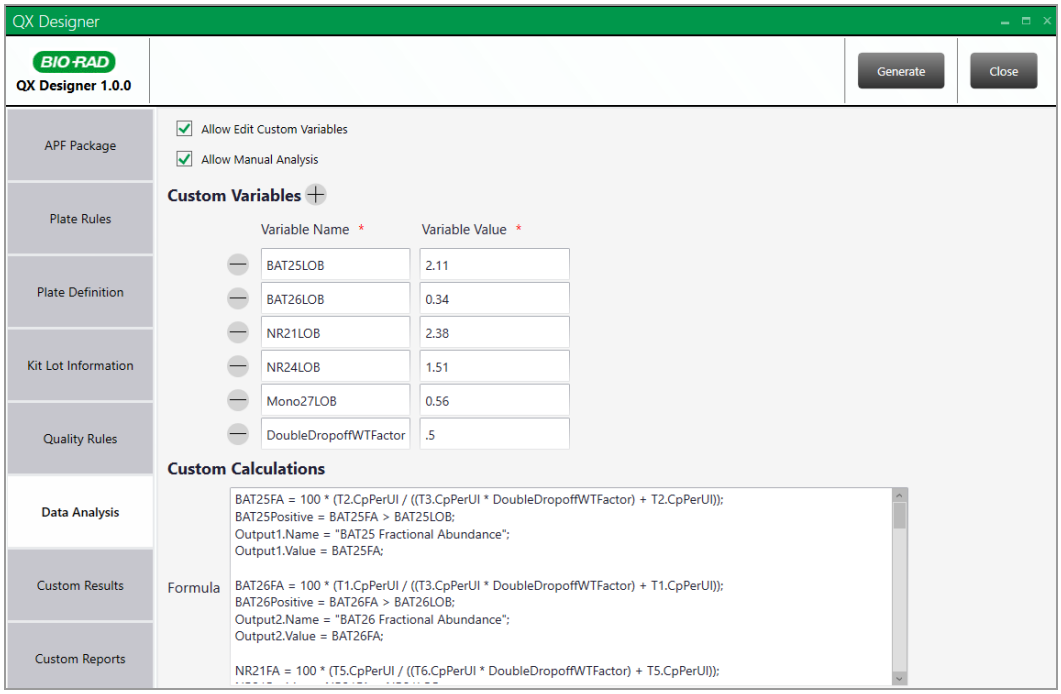
3. Repeat Step 2 to add another set of applicable tests for a Passing Criteria or Calculate Based on section.
4. Repeat all steps in this section to create another Quality Value within the Quality Rule.
5. Repeat both sections to create another Quality Rule.

Data Analysis Screen

Use the Data Analysis screen to define the analysis configuration for the Assay Protocol File. You can create custom variables and calculations to perform data analysis across well types for a given sample ID. For information on design, syntax, and variables see Appendix B, Tips for Custom Calculations.

Use the Data Analysis screen to do the following:

- Select checkboxes to enable system operator functions
- Define custom variables and calculation code that can be applied to each sample in the plate



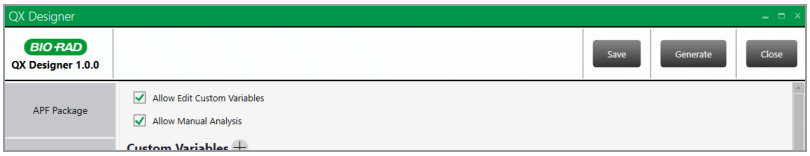
Data Analysis Checkbox Options

The checkboxes in the Data Analysis screen enable the system operator functions explained below:

- Allow Edit Custom Variables — Before starting the run, you can change the custom variable values in QX Manager Software, Premium Edition. The system uses the new values for the downstream calculations defined in the custom calculations script.
- Allow Manual Analysis —Before starting a run, you can manually set thresholds and clusters in QX Manager Software that the system uses to perform downstream calculations.

To enable editing and manual analysis

1. Select checkboxes to enable the corresponding functionality.

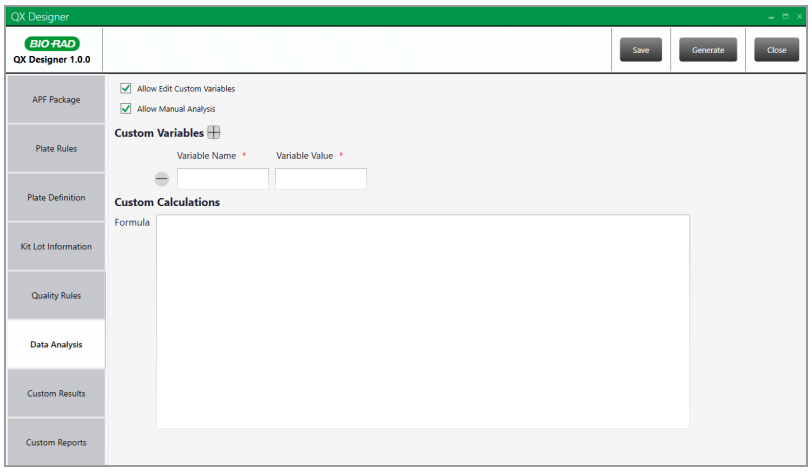


2. Click Save.

Defining Custom Variables

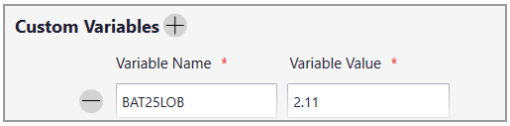
Custom variables are name-value pairs that you can define and use in your APF custom calculation scripts. For information, see Defining Custom Calculations on page 27.

Tip: If you enabled the Allow Edit Custom Variables checkbox in the previous section, you can edit the variable values in QX Manager Software, Premium Edition, before starting a droplet reading run.



To define custom variables

1. Click the ⊕ sign next to Custom Variables to display the Variable Name and Variable Value fields.
2. Enter a variable name and corresponding value.



3. Repeat until all variables are created.
4. Click Save.

Defining Custom Calculations

Custom calculations are scripted formulas that define output values in the Custom Results screen. They allow an Assay Protocol developer to perform post-ddPCR calculations on well data that are included as part of the APF.

Note: You can create custom calculations with or without custom variables. For information on custom variables, see Defining Custom Variables on page 26.

To define your custom calculation formulas

▶ Under Custom Calculations, enter a formula.

The following graphics show a formula example. For information on design, syntax, and variables you can use in your custom calculation formulas, see Appendix B, Tips for Custom Calculations.

Custom Calculations

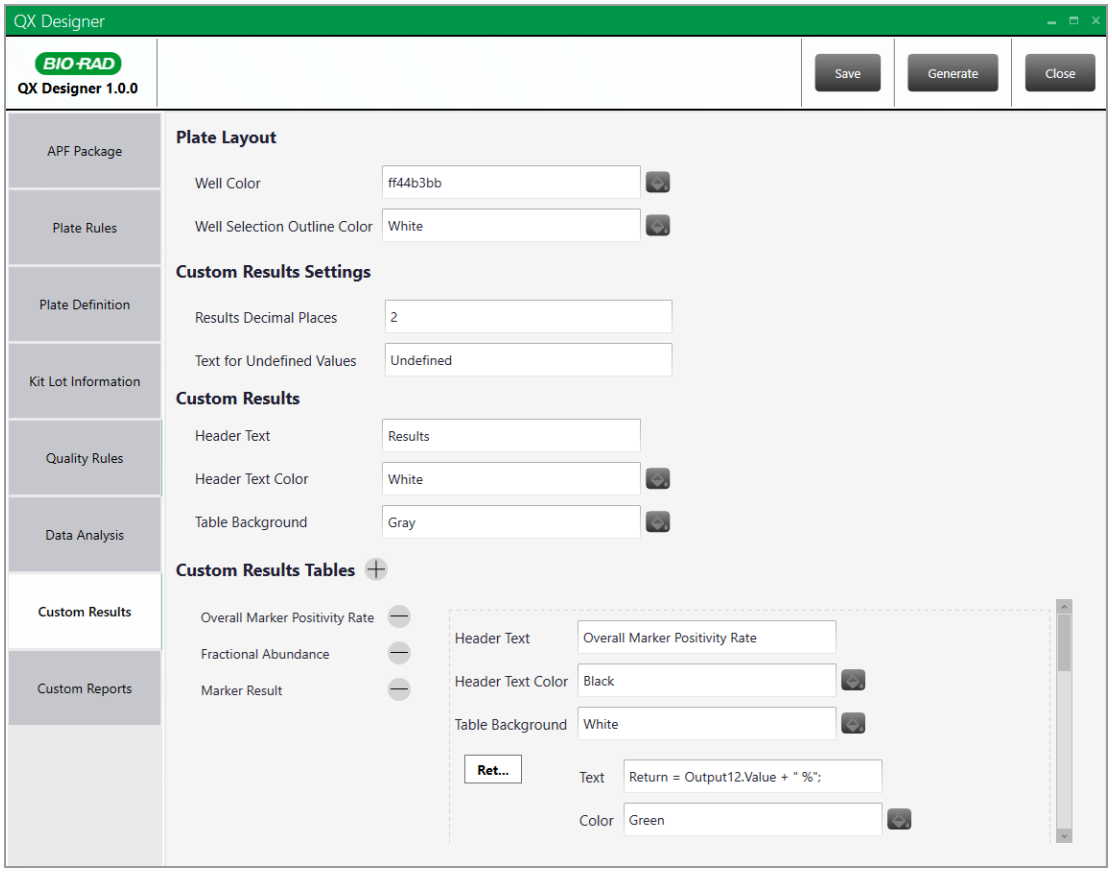
Formula

```
BAT25FA = 100 * (T2.CpPerUI / ((T3.CpPerUI * DoubleDropoffWTFactor) + T2.CpPerUI));
BAT25Positive = BAT25FA > BAT25LOB;
Output1.Name = "BAT25 Fractional Abundance";
Output1.Value = BAT25FA;

BAT26FA = 100 * (T1.CpPerUI / ((T3.CpPerUI * DoubleDropoffWTFactor) + T1.CpPerUI));
BAT26Positive = BAT26FA > BAT26LOB;
Output2.Name = "BAT26 Fractional Abundance";
Output2.Value = BAT26FA;

NR21FA = 100 * (T5.CpPerUI / ((T6.CpPerUI * DoubleDropoffWTFactor) + T5.CpPerUI));
NR21Positive = NR21FA > NR21LOB;
Output3.Name ="NR21 Fractional Abundance";
Output3.Value = NR21FA;

NR24FA = 100 * (T4.CpPerUI / ((T6.CpPerUI * DoubleDropoffWTFactor) + T4.CpPerUI));
NR24Positive = NR24FA > NR24LOB;
Output4.Name = "NR24 Fractional Abundance";
Output4.Value = NR24FA;

Mono27FA = 100 * (T7.CpPerUI / (T8.CpPerUI + T7.CpPerUI));
Mono27Positive = Mono27FA > Mono27LOB;
Output5.Name = "Mono27 Fractional Abundance";
Output5.Value = Mono27FA;

Output6.Name = "BAT25 Status";
Output6.Value = "Negative";
Output7.Name = "BAT26 Status";
Output7.Value = "Negative";
Output8.Name = "NR21 Status";
Output8.Value = "Negative";
Output9.Name = "NR24 Status";
Output9.Value = "Negative";
Output10.Name = "Mono27 Status";
Output10.Value = "Negative";

NumberPositive = 0;
if (BAT25Positive)
```

```
NumberPositive = 0;
if (BAT25Positive)
{
  NumberPositive = NumberPositive + 1;
  Output6.Value = "Positive";
}
if (BAT26Positive)
{
  NumberPositive = NumberPositive + 1;
  Output7.Value = "Positive";
}
if (NR21Positive)
{
  NumberPositive = NumberPositive + 1;
  Output8.Value = "Positive";
}
if (NR24Positive)
{
  NumberPositive = NumberPositive + 1;
  Output9.Value = "Positive";
}
if (Mono27Positive)
{
  NumberPositive = NumberPositive + 1;
  Output10.Value = "Positive";
}

Output11.Name = "Microsatellite Instability Status";
if (NumberPositive <= 1)
  Output11.Value = "MSS";
else
  Output11.Value = "MSI-H";

Output12.Name = "Marker Positivity";
Output12.Value = (NumberPositive/5)*100;
```

Custom Results Screen

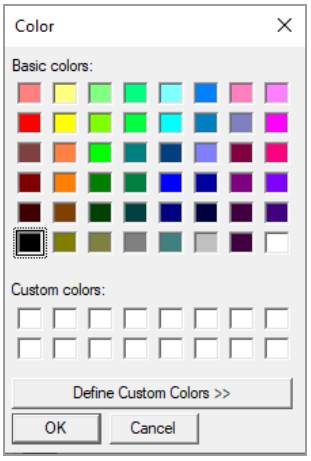
Use the Custom Results screen to colorize wells and define results tables in the QX Manager Software Analysis module APF Results screen. You can set up text, table, and background colors in any combination to design the custom Analysis layout, and to use in the Custom Reports design.



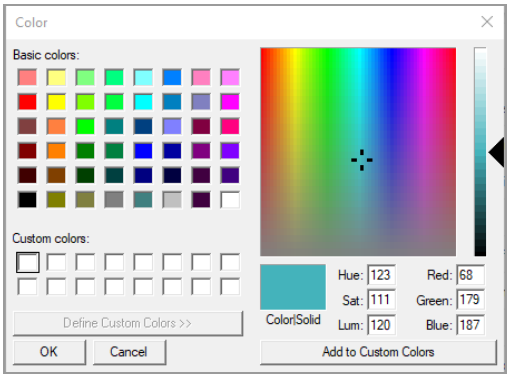
To specify Plate Layout settings

1. For Well Color and Well Selection Outline Color, click the respective icon on the right.

2. When the color palette appears, select a basic color for each field or click in a blank box under Custom colors and click Define Custom Colors and continue to Step 3.



3. Click a color in the rainbow palette and then slide the arrow on the far right down until the shade is acceptable.



4. Click Add to Custom Colors.

   The hexadecimal color code appears in the field in QX Designer Software.

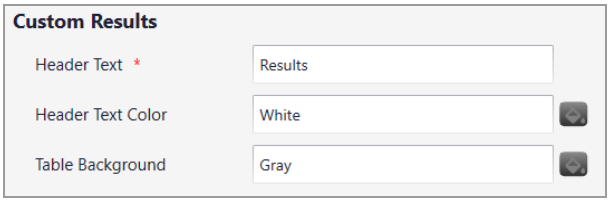
To specify Custom Results Settings

1. In the Results Decimal Places field, enter the number to expand the decimal display. For example, 2 results in two decimal places after the whole number (for example, 123.45).

2. In the Text for Undefined Values field, keep the default Undefined text or change it to a different entry.

To specify Custom Results text and colors

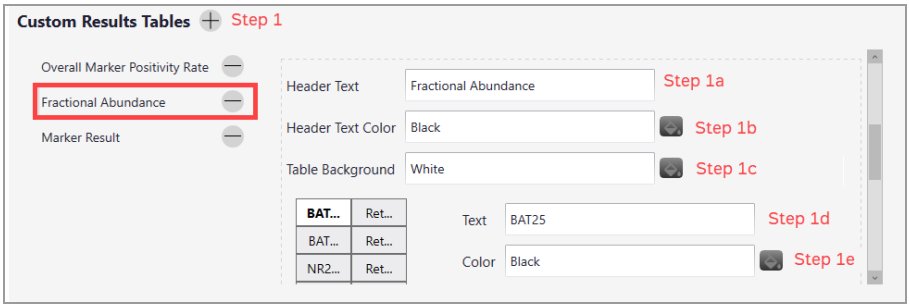
1. In the Header Text field, enter the text that should appear in the APF Results display.



2. In the Header Text Color field, click the icon on the right to open the color palette and select or create a color.
3. Repeat for the Table Background field.

To specify Custom Results Table text, colors, and formulas

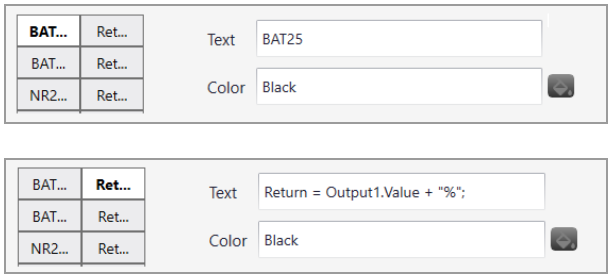
1. To create header text for different displays in the APF Results screen, click the + sign next to the Custom Results Tables heading.



   a. In the Header Text field, enter the heading name to be displayed in the custom report.
   b. In the Header Text Color field, click the icon on the right and select or create a color.

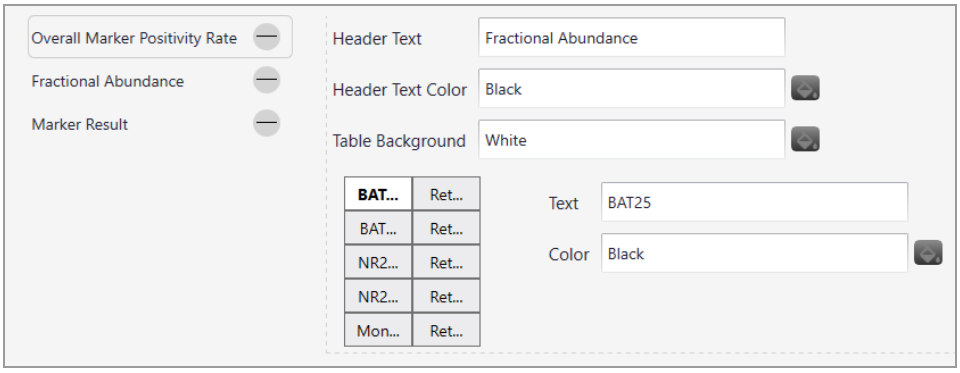
c. In the Table Background field, click the icon on the right and select or create a color.

d. In the Text field below Table Background, select a cell in the grid and enter the applicable text, value, or formula.

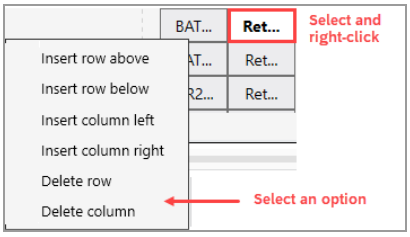


e. In the Color field, click the icon to select or create a corresponding color.

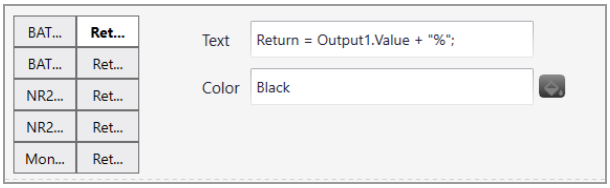
You can add multiple items, such as markers, to the grid and configure each item to appear in one or more corresponding colors.



2. To insert or delete a row or column, select a cell and right-click, and then select an option from the pop-up menu.



As you add to the grid, you can select a cell to configure text and color.



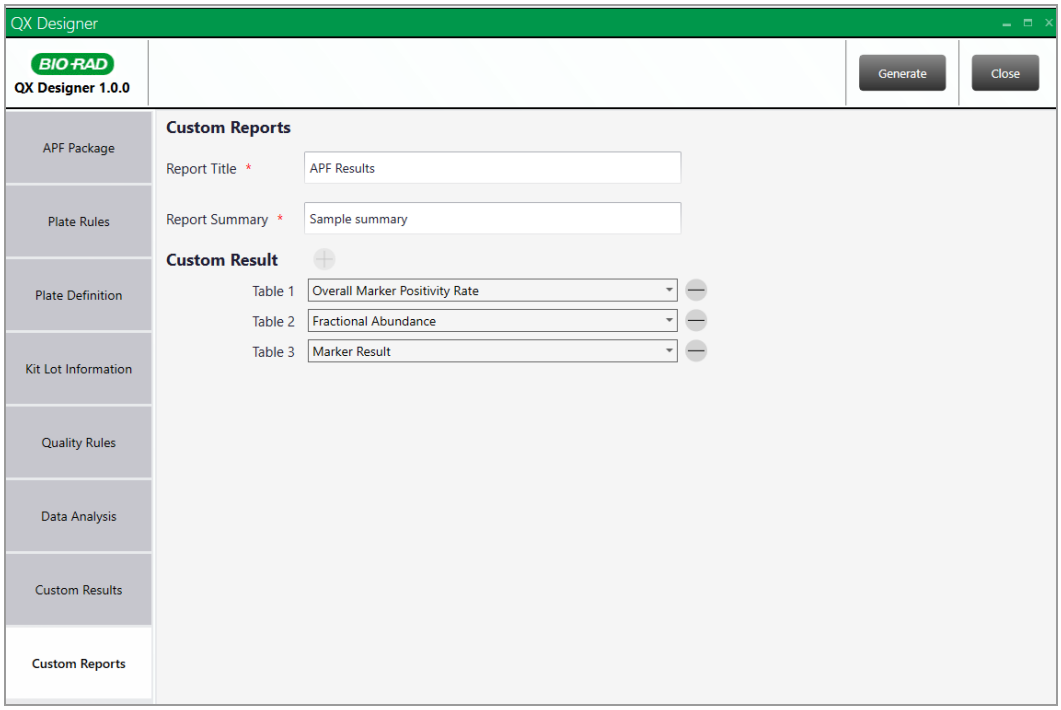
Note: As an Advanced feature, each of the color boxes in Custom Results accepts conditional formatting in similar syntax to Custom Calculations. For example, to present Output1.Value in a green color if the value is below 1, you can enter the conditional formatting as follows:

```
if (Output1.Value < 1 )
{
Return = "Green";
}
{
Return = "Black";
}
```

3. Repeat Step 4 to add another header definition.

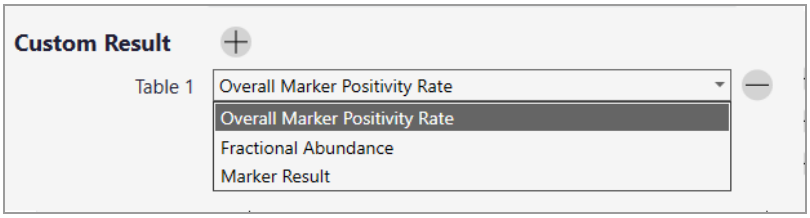
Custom Reports Screen

You can create custom reports to display data from the categories you created in Custom Results.



To create a custom report

1. Click the Custom Reports tab and enter a report title and a brief report summary description.
2. Click the ⊕ sign next to Custom Result and select from the custom results entries in the dropdown list.



3. Repeat Step 2 to add another custom report.

Appendix A External Clustering Algorithms

For customers interested in developing their own droplet clustering protocols, QX Designer Software supports the ability to integrate external, custom clustering scripts into APFs. This section explores the important components involved in integrating external custom cluster analysis with APFs, and by extension, QX Manager Software, Premium Edition. External clustering is currently supported for two channels only (Ch1 and Ch2), for the DropOff and Double Dropoff assay types..

Note: External Clustering Algorithm is an advanced feature that requires proficiency in programming and bioinformatics.

Generating Threshold Clusters

To perform droplet data clustering externally, the APF and QX Manager Software, Premium Edition, must be able to transfer data between the external application (such as Python) and QX Manager Software, Premium Edition. For APFs that utilize external clustering, QX Manager Software, Premium Edition, sets up a communication channel that allows the Python application to retrieve unclustered droplet data, assign clusters as determined by the user's algorithm, and then send the clustered droplet data back to QX Manager Software, Premium Edition, for downstream analysis.

To support this workflow, Bio-Rad provides two important files to assay developers:

- DataConnection.py
- BioRad.IPC.Shared.dll

DataConnection.py

DataConnection.py, which is stored on the QX Designer Software USB drive, is a critical component in the communication between QX Manager Software, Premium Edition, and the external Python script used for custom cluster analysis. The script is responsible for sending and receiving the ddPCR data, and establishes a communication channel using a shared memory space provided by the BioRad.IPC.Share.dll library. This communication channel is essential for transferring ddPCR data between the two applications, including the unclustered input data and the resulting output data from the custom clustering script.

DataConnection.py also performs data format conversions, as necessary, to ensure the data can be processed correctly by both applications. DataConnection.py converts the ddPCR data from the internal data format of QX Manager Software, Premium Edition, to a JSON format that can be read by the external Python script. Likewise, it converts the cluster analysis results generated by the customer's external script from JSON format to the format expected by QX Manager Software, Premium Edition.

BioRad.IPC.Shared.dll

BioRad.IPC.Shared.dll enables communication between QX Manager Software, Premium Edition, and the external Python script, allowing the exchange of data and the integration of custom analysis algorithms into QX Manager Software, Premium Edition.

BioRad.IPC.Shared.dll is a .NET assembly, which is included in QX Designer Software, Premium Edition, and is used in the communication mechanism between the software and the external Python script. Specifically, it provides an inter-process communication (IPC) mechanism to facilitate the transfer of data between the two applications. It can act as server as well as client, with bi-directional data transfer capabilities.

IPC allows multiple processes or applications to communicate with each other and share resources. In this case, BioRad.IPC.Shared.dll is used to establish a named pipe connection between QX Manager Software, Premium Edition, and the external Python script. This connection enables data to be passed securely between the two applications.

QX Manager Sotware, Premium Edition creates the communication channel using a unique channel name or identifier. The identifier is passed to the Python application as a required argument. With the help of DataConnection.py, the Python main script uses the identifier to create and connect to the named pipe and establishes the communication channel. Once the connection is established, data can be sent and received using the methods provided by DataConnection.py.

Python Startup Script

Assay developers use the two files, DataConnection.py and BioRad.IPC.Shared.dll, in their Python script, which is the starting point for the python application. In this script the following tasks are performed:

1. Importing the necessary modules and classes, including DataConnection.py
2. Creating an instance of the DataTransferConnection class to establish a connection with QX Manager Software, Premium Edition
3. Importing the data passed to the application through command-line arguments
4. Converting the data to a suitable format for performing the cluster task
5. Performing the cluster task to generate cluster groups
6. Converting the output cluster groups to a suitable format for exporting
7. Transfer the output cluster groups to QX Manager Software, Premium Edition, through the established connection.

Workflow

Following is the workflow for the Python application:

1 QX Manager Software, Premium Edition, extracts the cluster generation engine from the APFackage into a temporary location.

2 Before starting the cluster generation engine, QX Manager Software, Premium Edition, opens a communication pipe with a unique identifier. QX Manager Software, Premium Edition, uses the DataTransferConnection class inside the BioRad.IPC.Shared.dll, which has the necessary implementation for creating a pipe with the given unique name. This named pipe provides a way to communicate securely between processes.

Note: The unique pipe identifier is only accessible to an external Python process when it is launched by QX Manager Software, Premium Edition.

3 When QX Manager Software, Premium Edition, starts the external module executable engine in the temporary location, it passes the unique identifier of the communication pipe as an argument.

4 The Python application creates an instance of the DataTransferConnection class imported from BioRad.IPC.Shared.dll with the provided communication channel name, and then establishes a connection with QX Manager Software, Premium Edition.

5 QX Manager Software, Premium Edition, monitors the communication channel connection and the engine process during the execution period. Once the engine establishes a connection to the communication pipe, QX Manager Software, Premium Edition, prepares the required data to send through the pipe.

6 QX Manager Software, Premium Edition, passes the necessary data in JSON format to the Python application through the communication pipe.

7 The Python application imports the JSON data and converts it to a suitable format (such as NumPy) for performing the cluster task.

8 The Python application performs the cluster task to generate cluster groups for all the wells, and converts the newly clustered data back to the JSON format for transfer back to QX Manager Software, Premium Edition.

9 The Python application uses the DataConnection class methods to export the output cluster groups to QX Manager Software, Premium Edition, through the established connection.

10 QX Manager Software, Premium Edition, receives the data through the communication pipe from the external Python process module.

11 The Python process module then ends its execution and QX Manager Software, Premium Edition, and closes the communication pipe.

12 The generated cluster data is available as needed for the rest of the analysis process within QX Manager Software, Premium Edition.

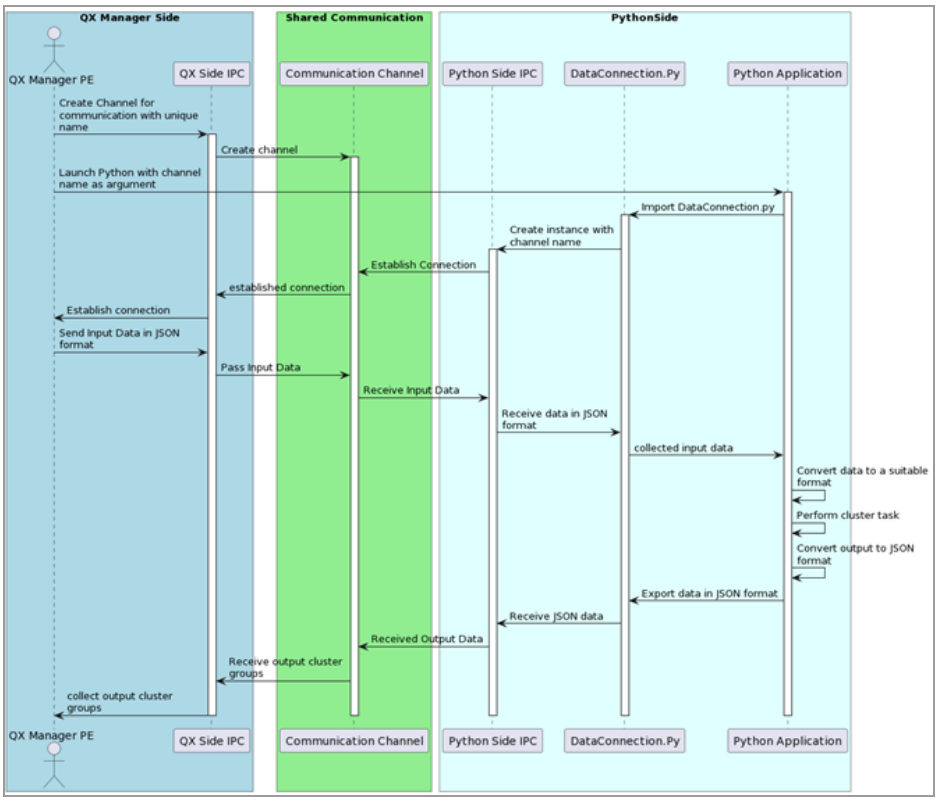


Fig. 1: Data pathway diagram

Error Handling

QX Manager Software, Premium Edition, handles errors by checking the return values from the subprocess calls and raising an exception if an error occurs. The errors are logged for further analysis. QX Manager Software, Premium Edition, also handle errors related to the communication pipe.

Data Formats

Following is an example JSON data structure when QX Manager Software, Premium Edition, sends the unclustered data to Python application.

Class: AutoClusteringInput

This class represents the input data for the auto clustering process, and contains the following properties:

- WellDescriptions — an array of WellMetaData objects. It provides information about the wells, including their metadata (sample type, target names, well index, and so forth). Each element in the array represents a specific well in the auto clustering process.
- WellDropletData — an array of WellDropletInputData objects. It contains the input data for each well, including the amplitudes of droplets in different channels and the well index. Each element in the array corresponds to a specific well.

```
"WellDescriptions": [
    {
        "SampleType": "Type A",
        "TargetNames": [
            "Target 1",
            "Target 2"
        ],
        "WellIndex": 0
    },
    {
        "SampleType": "Type B",
        "TargetNames": [
            "Target 1",
            "Target 2",
            "Target 3"
        ],
        "WellIndex": 1
    }
],
"WellDropletData": [
    {
        "Amplitudes": [
            [
                0.1,
                0.5,
                0.9
            ],
            [
                0.2,
                0.4,
                0.8
            ]
        ],
        "WellIndex": 0
    },
    {
        "Amplitudes": [
            [
                0.3,
                0.6,
                0.7
            ],
            [
                0.4,
                0.7,
                0.9
            ]
        ],
        "WellIndex": 1
    }
]
```


Fig. 2: Auto clustering input JSON

Class: WellMetaData

- SampleType — This property represents the type of sample present in a well. It is a string value that describes the sample in a human-readable format.
- TargetNames — This property is an array of strings that holds the assigned target names associated with the well. Each element in the array represents a target name, providing additional information about the samples present in the well.
- WellIndex — This property serves as a unique identifier for a well. It is an integer value ranging from 0 to 95, representing the different wells in the system.

Class: WellDropletInputData

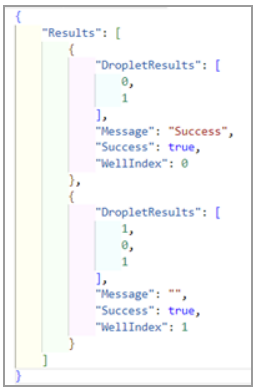
- Amplitudes — a two-dimensional array of floats. The first dimension represents the channels and the second dimension represents the droplets within each channel. For a given well, each channel might have multiple droplets, and their corresponding amplitudes are stored in this array. The size of the array varies, depending on the number of channels and droplets in each channel.
- WellIndex — Similar to the WellMetaData class, this property represents the unique identifier of a well and helps associate the input data with the corresponding well.

Following is an example JSON data structure when the Python application sends the clustered data back to QX Manager Software, Premium Edition.

Class: AutoClusteringOutput

This class represents the output data of the auto clustering process. It contains the following properties:

- Results — an array of WellDropletOutputData objects. It contains the clustering results for each well in the auto clustering process. Each element in the array corresponds to a specific well, providing the output data related to clustering.



```
{
  "Results": [
    {
      "DropletResults": [
        0,
        1
      ],
      "Message": "Success",
      "Success": true,
      "WellIndex": 0
    },
    {
      "DropletResults": [
        1,
        0,
        1
      ],
      "Message": "",
      "Success": true,
      "WellIndex": 1
    }
  ]
}
```


Fig. 3: Auto clustering output JSON

Class: WellDropletOutputData

- DropletResults — an array of integers that represents the clustering results for each droplet in a well. Each element in the array corresponds to a droplet and indicates its assigned cluster. The size of the array is the same as the number of droplets in the well.

  Cluster labels range from 0-3:

  All Negative = 0

  Fam Mutant = 1

  Wild Type = 2

  Hex Mutant = 3

  Important: Clustered data must be sent in the same target names order as specified in WellMetaData class above

- Message — a string that holds a message associated with the clustering results. When a well clustering process is unsuccessful, additional information indicates the reason for the failure. The message is empty when the cluster process is successful.
- Success — a boolean value that indicates the success or failure of the clustering process for a well. If the clustering process is successful, the value is True and if unsuccessful, the value is False.
- WellIndex — represents the unique identifier of a well to associate the clustering results with the corresponding well.

Conclusion

The use of external thresholding applications for generating cluster groups in QX Manager Software, Premium Edition, provides a flexible and powerful way to perform complex data analysis. The application can be customized and extended to perform a wide range of data analysis tasks, as needed, for the specific assay types analysis. This feature is a valuable addition to QX Manager Software, Premium Edition.

Appendix B Tips for Custom Calculations

QX Designer Software allows APF developers to create custom calculations using a simple scripting grammar that can be applied to the ddPCR data of your experiment. This section includes the syntax and operators supported by the Custom Calculation scripting language.

Supported Operators

You can use the operators specified in the following table in your custom formulas.

Table 2. Supported operators

Name	Operators
Assignment operator	=
Comparison operators	= = < > < > < = > =
Logical operators	| &
Math operators	+ - * / ^
log(x,y)	log base y of x
log2(x)	log base 2 of x
log10(x)	log base 10 of x
ln(x)	natural log of x
abs(x)	absolute value of x

Additionally, the following can apply:

Name	Operators
Keywords	IF, ELSE
Grouped expressions	{and}
Grouped statements	{ and } IF and ELSE clauses Statements end with a semicolon (;)
Boolean literals	TRUE FALSE
String literals	"Hello world" Character sequences enclosed by double quote
Number literals	1, 0, -8.44

Note: All math operators use standard order of operations and associativity. When in doubt, use parentheses to enforce your own order of operations.

Input Variables

Targets are identified by Tx, with the x replaced by 1, 2, 3, and so forth. The target identifier should precede the variable; for example, T1.CpPerµl.

Variable	Description
Positives	Number of droplets that contain the target
Negatives	Number of droplets that do not contain the target
Observed copies	Copies per droplet * accepted droplets
CpPerµLMax95	Copies per droplet * accepted droplets; max 95% confidence
CpPerµL	Well concentration in copies per µl
Ratio	Ratio of target over reference.
PoissonRatioMax	Maximum ratio of the unknown against the reference normalized for the high error bar of the droplet Poisson distribution for the 95% confidence interval

Variable	Description
PoissonRatioMin	Minimum ratio of the unknown against the reference normalized for the low error bar of the droplet Poisson distribution for the 95% confidence interval
FractionalAbundance	Calculation of fractional abundance of this unknown target vs. the reference target
PoissonFractionalAbundanceMax	Maximum fractional abundance normalized for the high error bar of the droplet Poisson distribution for the 95% confidence interval
PoissonFractionalAbundanceMin	Minimum fractional abundance normalized for the low error bar of the droplet Poisson distribution for the 95% confidence interval
PgPerul	Molecular weight
CopiesPer20 µL	Concentration of the target normalized to a volume of 20 µL
TotalConfMax	For merged wells the high error bar for the target concentration of the combined wells at a 95% confidence interval
TotalConfMin	For merged wells the low error bar for the target concentration of the combined wells at a 95% confidence interval
PoissonConfMax	Maximum target concentration normalized for the high error bar of the droplet Poisson distribution for the 95% confidence interval
PoissonConfMin	Minimum target concentration normalized for the low error bar of the droplet Poisson distribution for the 95% confidence interval
CNV	Copy number calculated for the target relative to the reference
TotalCNVMax	For merged wells, the high error bar for the copy number of the combined wells at a 95% confidence interval
TotalCNVMin	For merged wells, the low error bar for the copy number of the combined wells at a 95% confidence interval

Variable	Description
PoissonCNVMax	Maximum copy number normalized for the high error bar of the droplet Poisson distribution for the 95% confidence interval
PoissonCNVMin	Minimum copy number normalized for the low error bar of the droplet Poisson distribution for the 95% confidence interval
ReferenceCopies	Copy number identified for the reference target in the Plate Editor Default is 2, indicating 2 copies per diploid genome.
TotalRatioMax	For merged wells the high error bar for the ratio of the unknown against the reference of the combined wells at a 95% confidence interval
TotalRatioMin	For merged wells the low error bar for the ratio of the unknown against the reference of the combined wells at a 95% confidence interval
TotalFractionalAbundanceMax	For merged wells the high error bar for the fractional abundance of the combined wells at a 95% confidence interval
TotalFractionalAbundanceMin	For merged wells the low error bar for the fractional abundance of the combined wells at a 95% confidence interval
TotalConfidenceMax68	For merged wells the high error bar for the target concentration of the combined wells at a 68% confidence interval
TotalConfidenceMin68	For merged wells the low error bar for the target concentration of the combined wells at a 68% confidence interval
PoissonConfidenceMax68	Maximum target concentration normalized for the high error bar of the droplet Poisson distribution for the 68% confidence interval

Variable	Description
PoissonConfidenceMin68	Minimum target concentration normalized for the low error bar of the droplet Poisson distribution for the 68% confidence interval
TotalCNVMax68	For merged wells the high error bar for the copy number of the combined wells at a 68% confidence interval
TotalCNVMin68	For merged wells the low error bar for the copy number of the combined wells at a 68% confidence interval
PoissonCNVMax68	Maximum target concentration normalized for the high error bar of the droplet Poisson distribution for the 68% confidence interval
PoissonCNVMin68	Minimum target concentration normalized for the low error bar of the droplet Poisson distribution for the 68% confidence interval
TotalRatioMax68	For merged wells the high error bar for the ratio of the unknown against the reference of the combined wells at a 68% confidence interval
TotalRatioMin68	For merged wells the low error bar for the ratio of the unknown against the reference of the combined wells at a 68% confidence interval
PoissonRatioMax68	Maximum ratio of the unknown against the reference normalized for the high error bar of the droplet Poisson distribution for the 68% confidence interval
PoissonRatioMin68	Minimum ratio of the unknown against the reference normalized for the low error bar of the droplet Poisson distribution for the 68% confidence interval
TotalFractionalAbundanceMax68	For merged wells the high error bar for the fractional abundance of the combined wells at a 68% confidence interval
TotalFractionalAbundanceMin68	For merged wells the low error bar for the fractional abundance of the combined wells at a 68% confidence interval

Variable	Description
PoissonFractionalAbundanceMax68	Maximum fractional abundance normalized for the high error bar of the droplet Poisson distribution for the 68% confidence interval
PoissonFractionalAbundanceMin68	Minimum fractional abundance normalized for the low error bar of the droplet Poisson distribution for the 68% confidence interval plate list after correcting the issue

Well Type-Based

The following variables are Well Type-based have the following format: WT1.Variable (T1.AcceptedDroplets)

- Ch1PosCh2Pos
- Ch1NegCh2Pos
- Ch1PosCh2Neg
- Ch1NegCh2Neg
- AcceptedDroplets

Special Variables

The following variable is a special variable that can output calculation for linkage between any two targets within the same well type (example syntax = T1.LinkageWith.T2):

- LinkageWith

Outputs

Outputs from the custom calculations are indicated by the keyword Output. The term Output is always followed by a number, which should start with one and increment by one with each new output.

Each output has a Name and a Value, as shown below:

```
Output1.Name = “My Output One”;
Output1.Value = “Output One Value”;

Output2.Name = “My Output Two”;
Output2.Value = 3.14;
```

Note the following:

- The names must be configured as strings, and must be identical across all samples.
- The values can be numbers, strings, or booleans.
- For each specified output, one column is added to the export CSV, and is named as the output is named.
- Each line of the CSV that corresponds to a sample is given a value in that column, according to the value provided in the custom code.

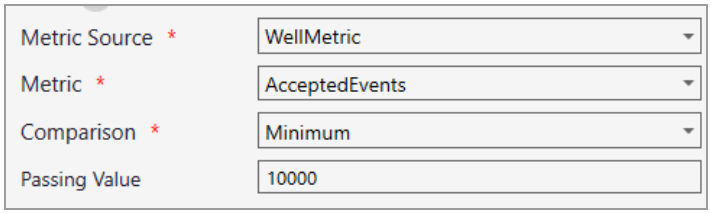
```
BAT25LOB = 2.11;

BAT26LOB = 0.34;

NR21LOB = 2.38;

NR24LOB = 1.51;

Mono27LOB = 0.56;

DoubleDropoffWTFactor = .5;

BAT25FA = T1.CpPerUl / ((T3.CpPerUl * DoubleDropoffWTFactor) + T1.CpPerUl);
BAT25Positive = BAT25FA > BAT25LOB;
Output1.Name = "BAT25 Fractional Abundance";
Output1.Value = BAT25FA;

BAT26FA = T2.CpPerUl / ((T3.CpPerUl * DoubleDropoffWTFactor) + T2.CpPerUl);
BAT26Positive = BAT26FA > BAT26LOB;
Output2.Name = "BAT26 Fractional Abundance";
Output2.Value = BAT26FA;
```

```
NR21FA = T4.CpPerUl / ((T6.CpPerUl * DoubleDropoffWTFactor) + T4.CpPerUl);
NR21Positive = NR21FA > NR21LOB;
Output3.Name ="NR21 Fractional Abundance";
Output3.Value = NR21FA;

NR24FA = T5.CpPerUl / ((T6.CpPerUl * DoubleDropoffWTFactor) + T5.CpPerUl);
NR24Positive = NR24FA > NR24LOB;
Output4.Name = "NR24 Fractional Abundance";
Output4.Value = NR24FA;

Mono27FA = T7.CpPerUl / (T8.CpPerUl + T7.CpPerUl);
Mono27Positive = Mono27FA > Mono27LOB;
Output5.Name = "Mono27 Fractional Abundance";
Output5.Value = Mono27FA;

Output6.Name = "BAT25 Status";
Output6.Value = "Negative";

Output7.Name = "BAT26 Status";
Output7.Value = "Negative";

Output8.Name = "NR21 Status";
Output8.Value = "Negative";

Output9.Name = "NR24 Status";
Output9.Value = "Negative";

Output10.Name = "Mono27 Status";
Output10.Value = "Negative";

NumberPositive = 0;
if (BAT25Positive)
{
NumberPositive = NumberPositive + 1;
Output6.Value = "Positive";
}
if (BAT26Positive)
{
NumberPositive = NumberPositive + 1;
Output7.Value = "Positive";
}
```

```
if (NR21Positive)
{
NumberPositive = NumberPositive + 1;
Output8.Value = "Positive";
}
if (NR24Positive)
{
NumberPositive = NumberPositive + 1;
Output9.Value = "Positive";
}
if (Mono27Positive)
{
NumberPositive = NumberPositive + 1;
Output10.Value = "Positive";
}
Output11.Name = "Microsatellite Instability Status";
if (NumberPositive <= 1)
Output11.Value = "MSS";
else
Output11.Value = "MSI-H";
```

Appendix C Well Metric Options in Quality Rules

As part of configuring quality rules, Table 3 explains the dropdown list options when Well Metric is selected as the Metric Source in the Quality Rules screen.



The metric you choose serves as a basis for calculations and their reliability.

Important: When selecting a Ch(x) well metric, the calculation is based on droplet or target counts, or by assay, in the selected channel.

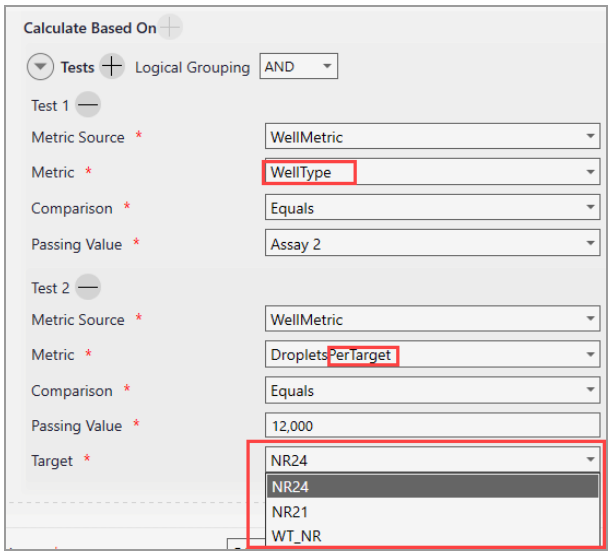
Table 3. Options in the Well Metric dropdown list

Selection	Calculation basis and outcome
None	No specified selection
SampleName	Groups and calculates by sample name
SampleType	Groups and calculates by the selected sample types: ■ Unknown ■ NTC (no template control) ■ Pos Ctrl ■ Neg Ctrl
WellType	Uses an overall well type for calculations (Plate, Well, or Sample)
AcceptedEvents	Total number of events accepted by the quality algorithm.
MeanAmplitudesCh(x)	Mean (average) amplitude value of targets
MeanAmplitudesCvCh(x)	Mean (average) amplitude value of targets, with standard deviation ratio (Cv) included
PositivePeaksCh(x)	Positive peak value
NegativePeaksCh(x)	Negative peak value
PositiveAmplitudeMeanCh(x)	Mean (average) positive amplitude
PositiveAmplitudeMedianCh(x)	Median (middle) positive amplitude
PositiveAmplitudeCvPctCh(x)	Positive amplitude with standard deviation percentage
NegativeAmplitudeMeanCh(x)	Mean (average) negative amplitude
NegativeAmplitudeMedianCh(x)	Median (middle) negative amplitude
NegativeAmplitudeCvPctCh(x)	Negative amplitude with standard deviation percentage
SValueCh(x)	Not available

Selection	Calculation basis and outcome
ConcentrationCh(x)	Concentration of the target molecules recorded as copies per microliter.
Ratio	The ratio of the targets against the identified reference
CnvCh(x)	Copy number variation value of targets
nnCount pnCount npCount ppCount	By count, where ■ Both targets are negative ■ Target 1 is positive and Target 2 is negative ■ Target 1 is negative and Target 2 is positive ■ Both targets are positive
nnMeanCh(x) pnMeanCh(x) npMeanCh(x) ppMeanCh(x)	By mean (average), where ■ Both targets are negative ■ Target 1 is positive and Target 2 is negative ■ Target 1 is negative and Target 2 is positive ■ Both targets are positive
nnCvPctCh(x) pnCvPctCh(x) npCvPctCh(x) ppCvPctCh(x)	By mean (average) standard deviation percentage for droplet concentration. where ■ Both targets are negative ■ Target 1 is positive and Target 2 is negative ■ Target 1 is negative and Target 2 is positive ■ Both targets are positive
nnSdCh(x) pnSdCh(x) pnSdCh(x) pnSdCh(x)	By standard deviation, where ■ Both targets are negative ■ Target 1 is positive and Target 2 is negative ■ Target 1 is negative and Target 2 is positive ■ Both targets are positive
DoubleNegShiftExternalNormCh(x)	Not available
SinglePosShiftExternalNormCh(x)	Not available
CpdCh(x)	Copies per droplet
ObservedCopiesCh(x)	Copies per droplet * accepted droplets

Selection	Calculation basis and outcome
DropletVolume	Partitioned volume of droplets, used to measure CNV
ThresholdStabilityCONFRoomAboveCh(x)	Not available
ThresholdStabilityCONFRoomBelowCh(x)	Not available
ThresholdStabilityCONFNormRoomAboveCh(x)	Not available
ThresholdStabilityCONFNormRoomBelowCh(x)	Not available
ConcentrationPerTarget (1)	Concentration of the target molecules recorded as copies per microliter
FractionalAbundancePerTarget (1)	Calculation of fractional abundance of the unknown target vs. the reference target
ObservedCopiesPerTarget (1)	Number of observed copies of the selected target
DropletsPerTarget (1)	Droplet count for each target

(1)To define a quality rule based on a PerTarget metric, you must create the rule under the Quality Value, Calculate Based On, calculation type. This filters your quality rule for Metric =WellType, allowing you to select a well type so the quality rule can populate the Target dropdown.



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