SOP pre-processing Methylight raw data qWID-CIN

Last updated: 10.01.2023, Charlotte Vavourakis

1. **Save the .eds file in the ZIDshare**
2. **Make a copy of the .eds file to your project folder and experiment subfolder in:**

\Dropbox\eutops\data\raw_data\methylight\

3. **Generate a samplesheet using the Shiny app**

- Prepare a sample sheet in Excel, first column should be labeled “Number”, second column should be labeled “Sample_name”. Up to 90 samples can be entered, mind the trailing “0” for the first 9 samples! Example:

	A	B	C
1	Number	Sample_name	
2	Sample_01	test1	
3	Sample_02	test2	
4	Sample_03	test3	
5	Sample_04	test4	
6	Sample_05	test5	
7	Sample_06	test6	
8	Sample_07	test7	
9	Sample_08	test8	
10	Sample_09	test9	
11	Sample_10	test10	
12	Sample_11	test11	
13	Sample_12	test12	
14	Sample_13	test13	

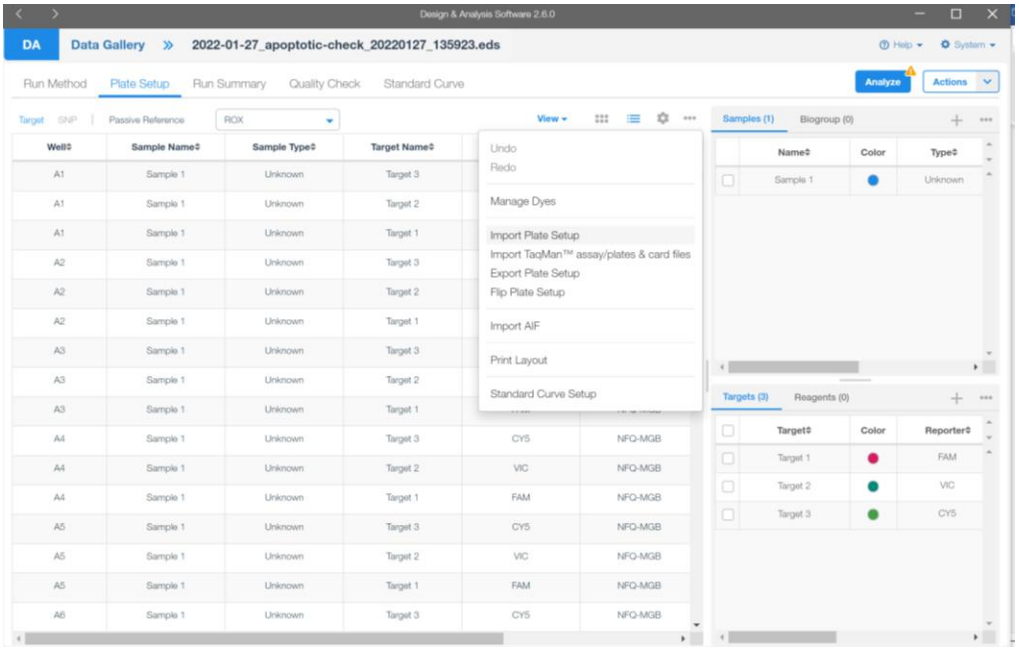
- Make sure R is installed and in your environment path. Libraries needed: shiny, readxl, stringr and dplyr.
- Navigate to \Dropbox\eutops\scripts\methylight\ML_WIDqGyn\shiny
- For Windows: Double-click GeneratePlatelayoutCIN.bat (you could make a shortcut to this file e.g. on your Desktop)
- Follow instructions in the browser.
- Please note that .bat files are specific for Windows OS.

4. **Save the resulting sample sheet, alongside the raw data (.eds file) both on the ZIDshare and the Dropbox**

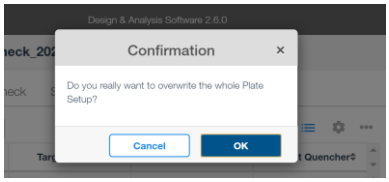
!!!!!!!!!! Make sure to do this BEFORE editing an .eds file !!!!!!!!!!

5. Using the Quantstudio Design & Analysis software 2.6.0, extract, normalize and export run results

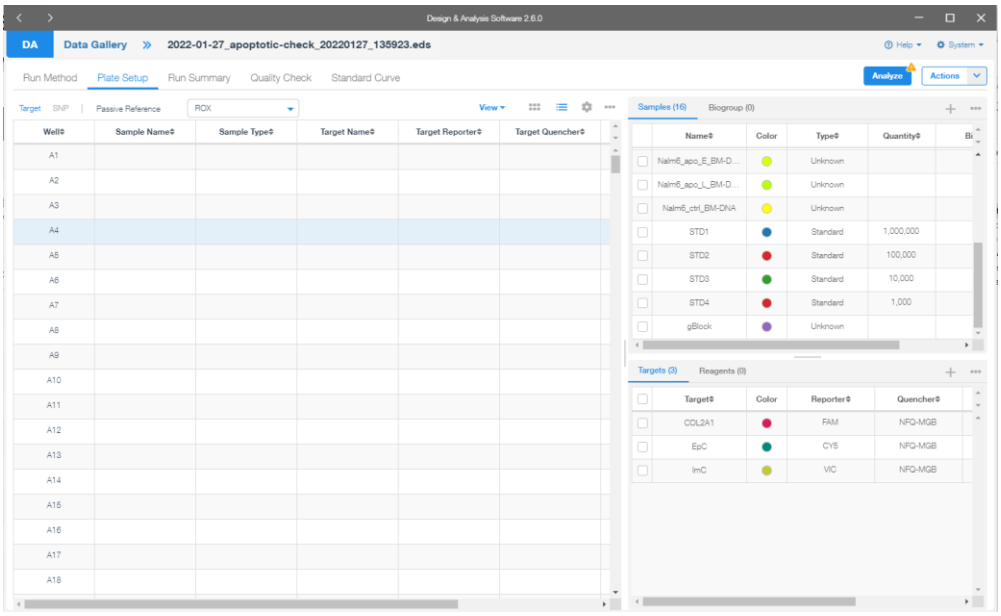
- Open .eds file
- Import your sample sheet under Plate setup > … > Import Plate Setup



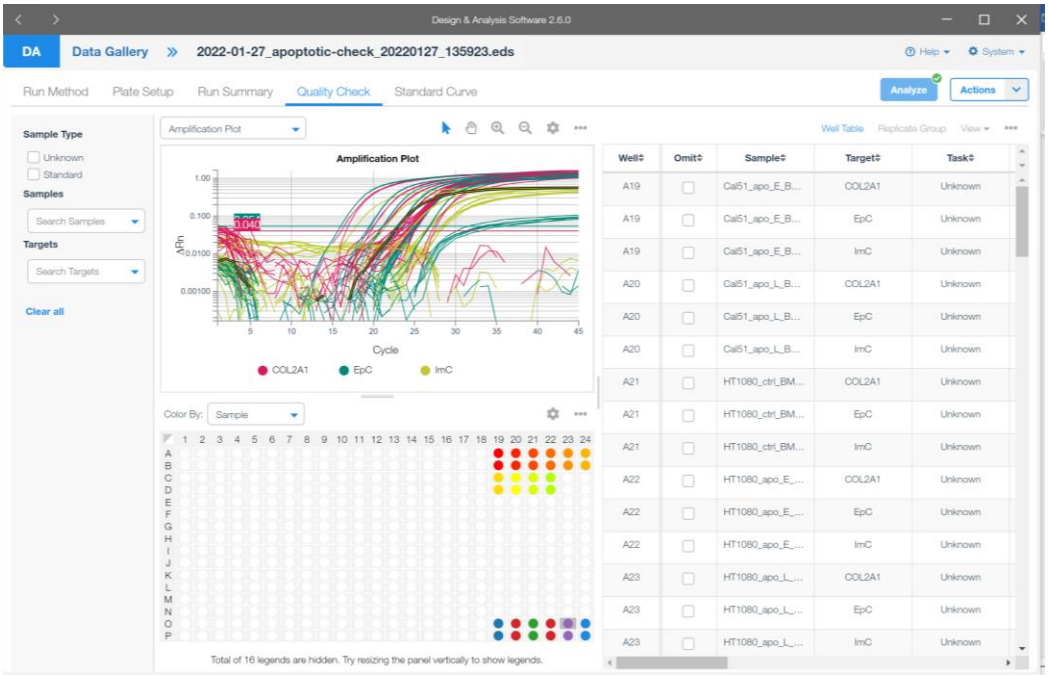
- Press ok, when prompted to overwrite Plate Setup



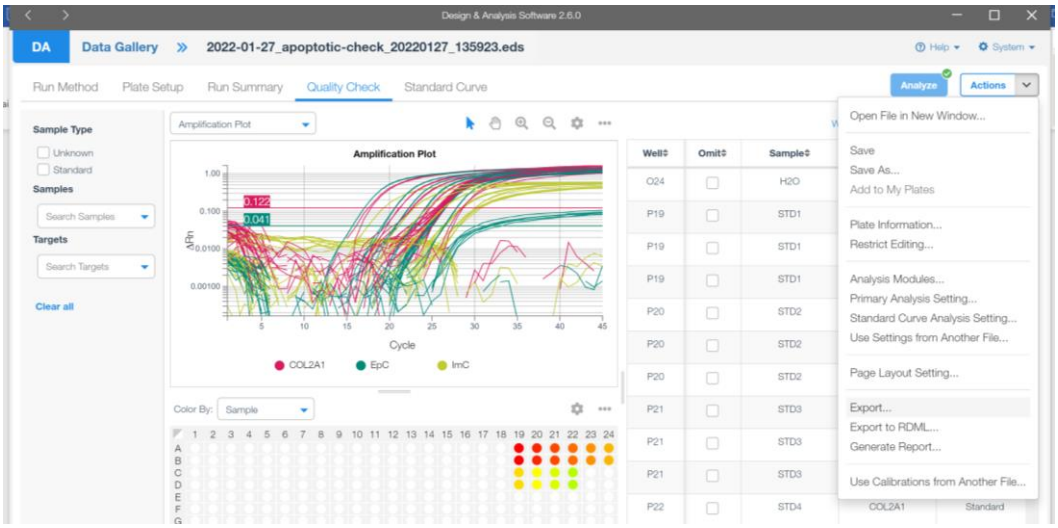
- Under Plate setup, Add the correct Quantities for the standards in the Sample list (example given for gBlocks)



- Press Analyze
- Under Quality check inspect the Amplification Plots
- You can select specific wells, checking minimally Standards, Positive and Negative controls.
- Delta Rn should be normalized on the Y-axis using the passive Reference dye ROX, showing a scale from 0 to 1



- Under Actions, Primary Analysis Settings, Set the Thresholds, and press Save:
  - COL2A1 (FAM) = 0,12
  - GSX1 (FAM) = 0.165
  - RALYL (CY5), DPP6 (CY5) = 0.04



- Under Actions export the result files (default setting)
- Save them to your project folder and experiment subfolder in:

\Dropbox\eutops\data\raw_data\methylight\

- Close and save the .eds file

6. Calculate PMR, WID-qEC and WID-qCIN using Shiny

- Navigate to \Dropbox\eutops\scripts\methylight\ML_WIDqGyn\shiny
- For Windows: double-click CalculatePMRGyn.bat (you could make a shortcut to this file e.g. on your Desktop)
- For Mac/Linux: double-click CalculatePMRGyn.sh (you could make a shortcut to this file e.g. on your Desktop)
- Follow instructions in the browser.

7. Please update “experiment_log” \Dropbox\eutops\data! For larger cohorts and studies