

SOP pre-processing MethyLight raw data qWID-GYN

Last updated: 14.12.2022, 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**

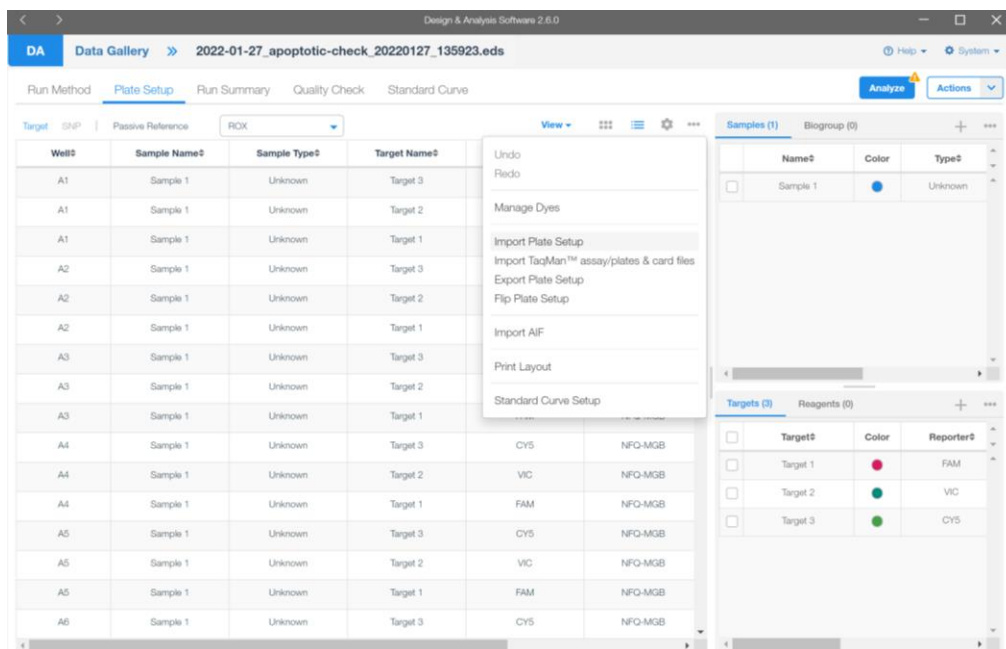
- Make sure R is installed and in your environment path. Libraries needed: shiny, readxl, stringr and dplyr.
- Navigate to \Dropbox\eutops\scripts\methyLight\ML_WIDqGyn\shiny
- For Windows: Double-click GeneratePlatelayouTGYN.bat (you could make a shortcut to this file e.g. on your Desktop)
- For Mac/Linux: Double-click GeneratePlatelayouTGYN.sh
- 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**

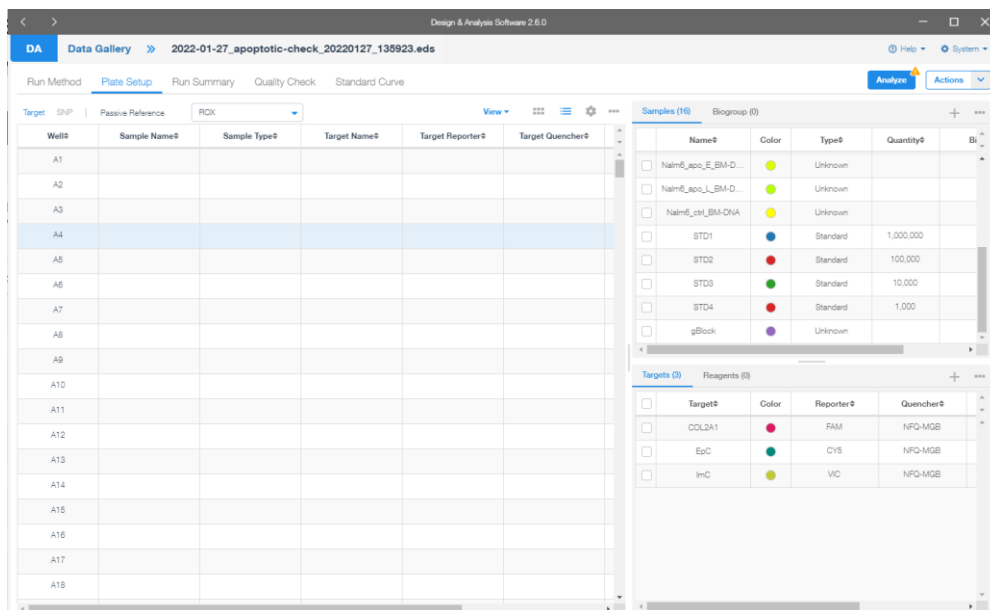
- Open .eds file
- Import your sample sheet under Plate setup > ... > Import Plate Setup



- Press ok, when prompted to overwrite Plate Setup

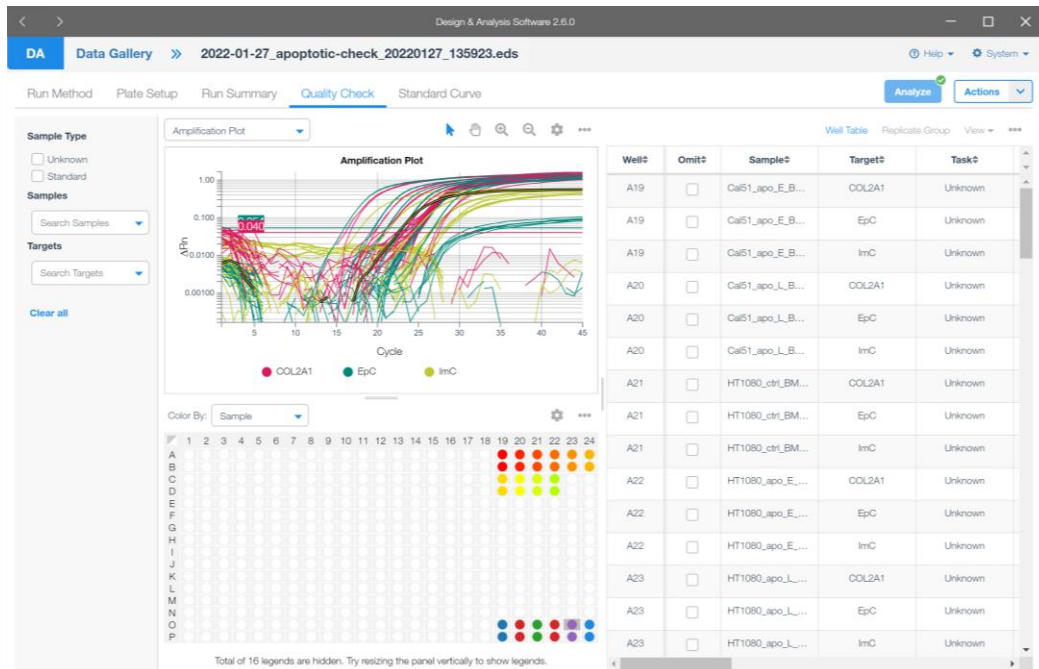


- Under Plate setup, Add the correct Quantities for the standards in the Sample list (example given for gBlocks)



- 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 = 0,12
 - ZSCAN12 (FAM) = 0.17
 - All other targets = 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