SOP pre-processing Methylight raw data qWID-EC

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 GeneratePlatelayoutEC.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.
- Note, your samples should be pipetted like this

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	EA-0001 ZSCANI2 GYPC1	EA-0009 ZSCANI2 GYPC1	EA-0019 ZSCANI2 GYPC1	EA-0027 ZSCANI2 GYPC1	EA-0035 ZSCANI2 GYPC1	EA-0043 ZSCANI2 GYPC1	EA-0051 ZSCANI2 GYPC1	EA-0059 ZSCANI2 GYPC1	EA-0067 ZSCANI2 GYPC1	EA-0075 ZSCANI2 GYPC1	EA-0085 ZSCANI2 GYPC1	Sample X ZSCANI2 GYPC1	EA-0001 GYPC2 COL2A1	EA-0009 GYPC2 COL2A1	EA-0019 GYPC2 COL2A1	EA-0027 GYPC2 COL2A1	EA-0035 GYPC2 COL2A1	EA-0043 GYPC2 COL2A1	EA-0051 GYPC2 COL2A1	EA-0059 GYPC2 COL2A1	EA-0067 GYPC2 COL2A1	EA-0075 GYPC2 COL2A1	EA-0085 GYPC2 COL2A1	Sample X GYPC2 COL2A1
B	EA-0001 ZSCANI2 GYPC1	EA-0009 ZSCANI2 GYPC1	EA-0019 ZSCANI2 GYPC1	EA-0027 ZSCANI2 GYPC1	EA-0035 ZSCANI2 GYPC1	EA-0043 ZSCANI2 GYPC1	EA-0051 ZSCANI2 GYPC1	EA-0059 ZSCANI2 GYPC1	EA-0067 ZSCANI2 GYPC1	EA-0075 ZSCANI2 GYPC1	EA-0085 ZSCANI2 GYPC1	Sample X ZSCANI2 GYPC1	EA-0001 GYPC2 COL2A1	EA-0009 GYPC2 COL2A1	EA-0019 GYPC2 COL2A1	EA-0027 GYPC2 COL2A1	EA-0035 GYPC2 COL2A1	EA-0043 GYPC2 COL2A1	EA-0051 GYPC2 COL2A1	EA-0059 GYPC2 COL2A1	EA-0067 GYPC2 COL2A1	EA-0075 GYPC2 COL2A1	EA-0085 GYPC2 COL2A1	Sample X GYPC2 COL2A1
C	EA-0002 ZSCANI2 GYPC1	EA-0011 ZSCANI2 GYPC1	EA-0020 ZSCANI2 GYPC1	EA-0028 ZSCANI2 GYPC1	EA-0036 ZSCANI2 GYPC1	EA-0044 ZSCANI2 GYPC1	EA-0052 ZSCANI2 GYPC1	EA-0060 ZSCANI2 GYPC1	EA-0068 ZSCANI2 GYPC1	EA-0077 ZSCANI2 GYPC1	EA-0086 ZSCANI2 GYPC1	Sample Y ZSCANI2 GYPC1	EA-0002 GYPC2 COL2A1	EA-0011 GYPC2 COL2A1	EA-0020 GYPC2 COL2A1	EA-0028 GYPC2 COL2A1	EA-0036 GYPC2 COL2A1	EA-0044 GYPC2 COL2A1	EA-0052 GYPC2 COL2A1	EA-0060 GYPC2 COL2A1	EA-0068 GYPC2 COL2A1	EA-0077 GYPC2 COL2A1	EA-0086 GYPC2 COL2A1	Sample Y GYPC2 COL2A1
D	EA-0002 ZSCANI2 GYPC1	EA-0011 ZSCANI2 GYPC1	EA-0020 ZSCANI2 GYPC1	EA-0028 ZSCANI2 GYPC1	EA-0036 ZSCANI2 GYPC1	EA-0044 ZSCANI2 GYPC1	EA-0052 ZSCANI2 GYPC1	EA-0060 ZSCANI2 GYPC1	EA-0068 ZSCANI2 GYPC1	EA-0077 ZSCANI2 GYPC1	EA-0086 ZSCANI2 GYPC1	Sample Y ZSCANI2 GYPC1	EA-0002 GYPC2 COL2A1	EA-0011 GYPC2 COL2A1	EA-0020 GYPC2 COL2A1	EA-0028 GYPC2 COL2A1	EA-0036 GYPC2 COL2A1	EA-0044 GYPC2 COL2A1	EA-0052 GYPC2 COL2A1	EA-0060 GYPC2 COL2A1	EA-0068 GYPC2 COL2A1	EA-0077 GYPC2 COL2A1	EA-0086 GYPC2 COL2A1	Sample Y GYPC2 COL2A1
E	EA-0003 ZSCANI2 GYPC1	EA-0012 ZSCANI2 GYPC1	EA-0021 ZSCANI2 GYPC1	EA-0029 ZSCANI2 GYPC1	EA-0037 ZSCANI2 GYPC1	EA-0045 ZSCANI2 GYPC1	EA-0053 ZSCANI2 GYPC1	EA-0061 ZSCANI2 GYPC1	EA-0069 ZSCANI2 GYPC1	EA-0078 ZSCANI2 GYPC1	EA-0087 ZSCANI2 GYPC1	STD1 ZSCANI2 GYPC1	EA-0003 GYPC2 COL2A1	EA-0012 GYPC2 COL2A1	EA-0021 GYPC2 COL2A1	EA-0029 GYPC2 COL2A1	EA-0037 GYPC2 COL2A1	EA-0045 GYPC2 COL2A1	EA-0053 GYPC2 COL2A1	EA-0061 GYPC2 COL2A1	EA-0069 GYPC2 COL2A1	EA-0078 GYPC2 COL2A1	EA-0087 GYPC2 COL2A1	STD1 GYPC2 COL2A1
F	EA-0003 ZSCANI2 GYPC1	EA-0012 ZSCANI2 GYPC1	EA-0021 ZSCANI2 GYPC1	EA-0029 ZSCANI2 GYPC1	EA-0037 ZSCANI2 GYPC1	EA-0045 ZSCANI2 GYPC1	EA-0053 ZSCANI2 GYPC1	EA-0061 ZSCANI2 GYPC1	EA-0069 ZSCANI2 GYPC1	EA-0078 ZSCANI2 GYPC1	EA-0087 ZSCANI2 GYPC1	STD1 ZSCANI2 GYPC1	EA-0003 GYPC2 COL2A1	EA-0012 GYPC2 COL2A1	EA-0021 GYPC2 COL2A1	EA-0029 GYPC2 COL2A1	EA-0037 GYPC2 COL2A1	EA-0045 GYPC2 COL2A1	EA-0053 GYPC2 COL2A1	EA-0061 GYPC2 COL2A1	EA-0069 GYPC2 COL2A1	EA-0078 GYPC2 COL2A1	EA-0087 GYPC2 COL2A1	STD1 GYPC2 COL2A1
G	EA-0004 ZSCANI2 GYPC1	EA-0013 ZSCANI2 GYPC1	EA-0022 ZSCANI2 GYPC1	EA-0030 ZSCANI2 GYPC1	EA-0038 ZSCANI2 GYPC1	EA-0046 ZSCANI2 GYPC1	EA-0054 ZSCANI2 GYPC1	EA-0062 ZSCANI2 GYPC1	EA-0070 ZSCANI2 GYPC1	EA-0079 ZSCANI2 GYPC1	EA-0088 ZSCANI2 GYPC1	STD2 ZSCANI2 GYPC1	EA-0004 GYPC2 COL2A1	EA-0013 GYPC2 COL2A1	EA-0022 GYPC2 COL2A1	EA-0030 GYPC2 COL2A1	EA-0038 GYPC2 COL2A1	EA-0046 GYPC2 COL2A1	EA-0054 GYPC2 COL2A1	EA-0062 GYPC2 COL2A1	EA-0070 GYPC2 COL2A1	EA-0079 GYPC2 COL2A1	EA-0088 GYPC2 COL2A1	STD2 GYPC2 COL2A1
H	EA-0004 ZSCANI2 GYPC1	EA-0013 ZSCANI2 GYPC1	EA-0022 ZSCANI2 GYPC1	EA-0030 ZSCANI2 GYPC1	EA-0038 ZSCANI2 GYPC1	EA-0046 ZSCANI2 GYPC1	EA-0054 ZSCANI2 GYPC1	EA-0062 ZSCANI2 GYPC1	EA-0070 ZSCANI2 GYPC1	EA-0079 ZSCANI2 GYPC1	EA-0088 ZSCANI2 GYPC1	STD2 ZSCANI2 GYPC1	EA-0004 GYPC2 COL2A1	EA-0013 GYPC2 COL2A1	EA-0022 GYPC2 COL2A1	EA-0030 GYPC2 COL2A1	EA-0038 GYPC2 COL2A1	EA-0046 GYPC2 COL2A1	EA-0054 GYPC2 COL2A1	EA-0062 GYPC2 COL2A1	EA-0070 GYPC2 COL2A1	EA-0079 GYPC2 COL2A1	EA-0088 GYPC2 COL2A1	STD2 GYPC2 COL2A1
I	EA-0005 ZSCANI2 GYPC1	EA-0014 ZSCANI2 GYPC1	EA-0023 ZSCANI2 GYPC1	EA-0031 ZSCANI2 GYPC1	EA-0039 ZSCANI2 GYPC1	EA-0047 ZSCANI2 GYPC1	EA-0055 ZSCANI2 GYPC1	EA-0063 ZSCANI2 GYPC1	EA-0071 ZSCANI2 GYPC1	EA-0080 ZSCANI2 GYPC1	EA-0089 ZSCANI2 GYPC1	STD3 ZSCANI2 COL2A1	EA-0005 GYPC2 COL2A1	EA-0014 GYPC2 COL2A1	EA-0023 GYPC2 COL2A1	EA-0031 GYPC2 COL2A1	EA-0039 GYPC2 COL2A1	EA-0047 GYPC2 COL2A1	EA-0055 GYPC2 COL2A1	EA-0063 GYPC2 COL2A1	EA-0071 GYPC2 COL2A1	EA-0080 GYPC2 COL2A1	EA-0089 GYPC2 COL2A1	STD3 GYPC2 COL2A1
J	EA-0005 ZSCANI2 GYPC1	EA-0014 ZSCANI2 GYPC1	EA-0023 ZSCANI2 GYPC1	EA-0031 ZSCANI2 GYPC1	EA-0039 ZSCANI2 GYPC1	EA-0047 ZSCANI2 GYPC1	EA-0055 ZSCANI2 GYPC1	EA-0063 ZSCANI2 GYPC1	EA-0071 ZSCANI2 GYPC1	EA-0080 ZSCANI2 GYPC1	EA-0089 ZSCANI2 GYPC1	STD3 ZSCANI2 GYPC1	EA-0005 GYPC2 COL2A1	EA-0014 GYPC2 COL2A1	EA-0023 GYPC2 COL2A1	EA-0031 GYPC2 COL2A1	EA-0039 GYPC2 COL2A1	EA-0047 GYPC2 COL2A1	EA-0055 GYPC2 COL2A1	EA-0063 GYPC2 COL2A1	EA-0071 GYPC2 COL2A1	EA-0080 GYPC2 COL2A1	EA-0089 GYPC2 COL2A1	STD3 GYPC2 COL2A1
K	EA-0006 ZSCANI2 GYPC1	EA-0016 ZSCANI2 GYPC1	EA-0024 ZSCANI2 GYPC1	EA-0032 ZSCANI2 GYPC1	EA-0040 ZSCANI2 GYPC1	EA-0048 ZSCANI2 GYPC1	EA-0056 ZSCANI2 GYPC1	EA-0064 ZSCANI2 GYPC1	EA-0072 ZSCANI2 GYPC1	EA-0081 ZSCANI2 GYPC1	EA-0090 ZSCANI2 GYPC1	STD4 ZSCANI2 GYPC1	EA-0006 GYPC2 COL2A1	EA-0016 GYPC2 COL2A1	EA-0024 GYPC2 COL2A1	EA-0032 GYPC2 COL2A1	EA-0040 GYPC2 COL2A1	EA-0048 GYPC2 COL2A1	EA-0056 GYPC2 COL2A1	EA-0064 GYPC2 COL2A1	EA-0072 GYPC2 COL2A1	EA-0081 GYPC2 COL2A1	EA-0090 GYPC2 COL2A1	STD4 GYPC2 COL2A1
L	EA-0006 ZSCANI2 GYPC1	EA-0016 ZSCANI2 GYPC1	EA-0024 ZSCANI2 GYPC1	EA-0032 ZSCANI2 GYPC1	EA-0040 ZSCANI2 GYPC1	EA-0048 ZSCANI2 GYPC1	EA-0056 ZSCANI2 GYPC1	EA-0064 ZSCANI2 GYPC1	EA-0072 ZSCANI2 GYPC1	EA-0081 ZSCANI2 GYPC1	EA-0090 ZSCANI2 GYPC1	STD4 ZSCANI2 GYPC1	EA-0006 GYPC2 COL2A1	EA-0016 GYPC2 COL2A1	EA-0024 GYPC2 COL2A1	EA-0032 GYPC2 COL2A1	EA-0040 GYPC2 COL2A1	EA-0048 GYPC2 COL2A1	EA-0056 GYPC2 COL2A1	EA-0064 GYPC2 COL2A1	EA-0072 GYPC2 COL2A1	EA-0081 GYPC2 COL2A1	EA-0090 GYPC2 COL2A1	STD4 GYPC2 COL2A1
M	EA-0007 ZSCANI2 GYPC1	EA-0017 ZSCANI2 GYPC1	EA-0025 ZSCANI2 GYPC1	EA-0033 ZSCANI2 GYPC1	EA-0041 ZSCANI2 GYPC1	EA-0049 ZSCANI2 GYPC1	EA-0057 ZSCANI2 GYPC1	EA-0065 ZSCANI2 GYPC1	EA-0073 ZSCANI2 GYPC1	EA-0082 ZSCANI2 GYPC1	EA-0091 ZSCANI2 GYPC1	gBlock ZSCANI2 GYPC1	EA-0007 GYPC2 COL2A1	EA-0017 GYPC2 COL2A1	EA-0025 GYPC2 COL2A1	EA-0033 GYPC2 COL2A1	EA-0041 GYPC2 COL2A1	EA-0049 GYPC2 COL2A1	EA-0057 GYPC2 COL2A1	EA-0065 GYPC2 COL2A1	EA-0073 GYPC2 COL2A1	EA-0082 GYPC2 COL2A1	EA-0091 GYPC2 COL2A1	gBlock GYPC2 COL2A1
N	EA-0007 ZSCANI2 GYPC1	EA-0017 ZSCANI2 GYPC1	EA-0025 ZSCANI2 GYPC1	EA-0033 ZSCANI2 GYPC1	EA-0041 ZSCANI2 GYPC1	EA-0049 ZSCANI2 GYPC1	EA-0057 ZSCANI2 GYPC1	EA-0065 ZSCANI2 GYPC1	EA-0073 ZSCANI2 GYPC1	EA-0082 ZSCANI2 GYPC1	EA-0091 ZSCANI2 GYPC1	gBlock ZSCANI2 GYPC1	EA-0007 GYPC2 COL2A1	EA-0017 GYPC2 COL2A1	EA-0025 GYPC2 COL2A1	EA-0033 GYPC2 COL2A1	EA-0041 GYPC2 COL2A1	EA-0049 GYPC2 COL2A1	EA-0057 GYPC2 COL2A1	EA-0065 GYPC2 COL2A1	EA-0073 GYPC2 COL2A1	EA-0082 GYPC2 COL2A1	EA-0091 GYPC2 COL2A1	gBlock GYPC2 COL2A1
O	EA-0008 ZSCANI2 GYPC1	EA-0018 ZSCANI2 GYPC1	EA-0026 ZSCANI2 GYPC1	EA-0034 ZSCANI2 GYPC1	EA-0042 ZSCANI2 GYPC1	EA-0050 ZSCANI2 GYPC1	EA-0058 ZSCANI2 GYPC1	EA-0066 ZSCANI2 GYPC1	EA-0074 ZSCANI2 GYPC1	EA-0084 ZSCANI2 GYPC1	EA-0092 ZSCANI2 GYPC1	H2O ZSCANI2 GYPC1	EA-0008 GYPC2 COL2A1	EA-0018 GYPC2 COL2A1	EA-0026 GYPC2 COL2A1	EA-0034 GYPC2 COL2A1	EA-0042 GYPC2 COL2A1	EA-0050 GYPC2 COL2A1	EA-0058 GYPC2 COL2A1	EA-0066 GYPC2 COL2A1	EA-0074 GYPC2 COL2A1	EA-0084 GYPC2 COL2A1	EA-0092 GYPC2 COL2A1	H2O GYPC2 COL2A1
P	EA-0008 ZSCANI2 GYPC1	EA-0018 ZSCANI2 GYPC1	EA-0026 ZSCANI2 GYPC1	EA-0034 ZSCANI2 GYPC1	EA-0042 ZSCANI2 GYPC1	EA-0050 ZSCANI2 GYPC1	EA-0058 ZSCANI2 GYPC1	EA-0066 ZSCANI2 GYPC1	EA-0074 ZSCANI2 GYPC1	EA-0084 ZSCANI2 GYPC1	EA-0092 ZSCANI2 GYPC1	H2O ZSCANI2 GYPC1	EA-0008 GYPC2 COL2A1	EA-0018 GYPC2 COL2A1	EA-0026 GYPC2 COL2A1	EA-0034 GYPC2 COL2A1	EA-0042 GYPC2 COL2A1	EA-0050 GYPC2 COL2A1	EA-0058 GYPC2 COL2A1	EA-0066 GYPC2 COL2A1	EA-0074 GYPC2 COL2A1	EA-0084 GYPC2 COL2A1	EA-0092 GYPC2 COL2A1	H2O GYPC2 COL2A1

4. **Save the resulting sample sheet, alongside the raw data (.eds file) both on the ZIDshare and the Dropbox**
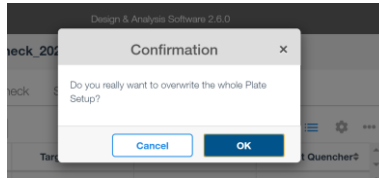
!!!!!!!!!! Make sure to do this BEFORE editing an .eds file !!!!!!!!!!

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
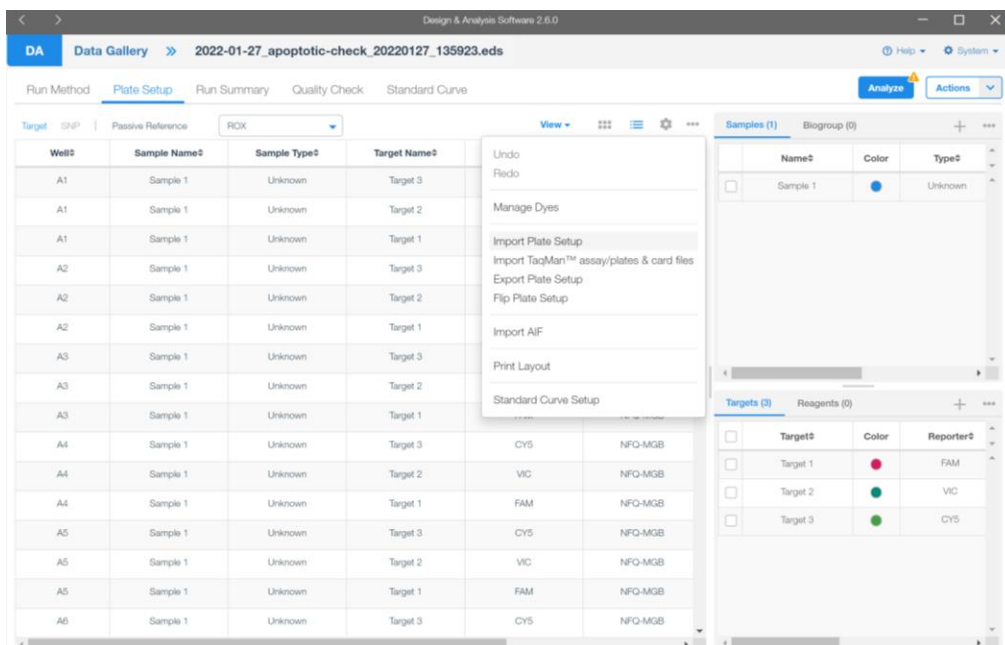
- Press ok, when prompted to overwrite Plate Setup

- 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
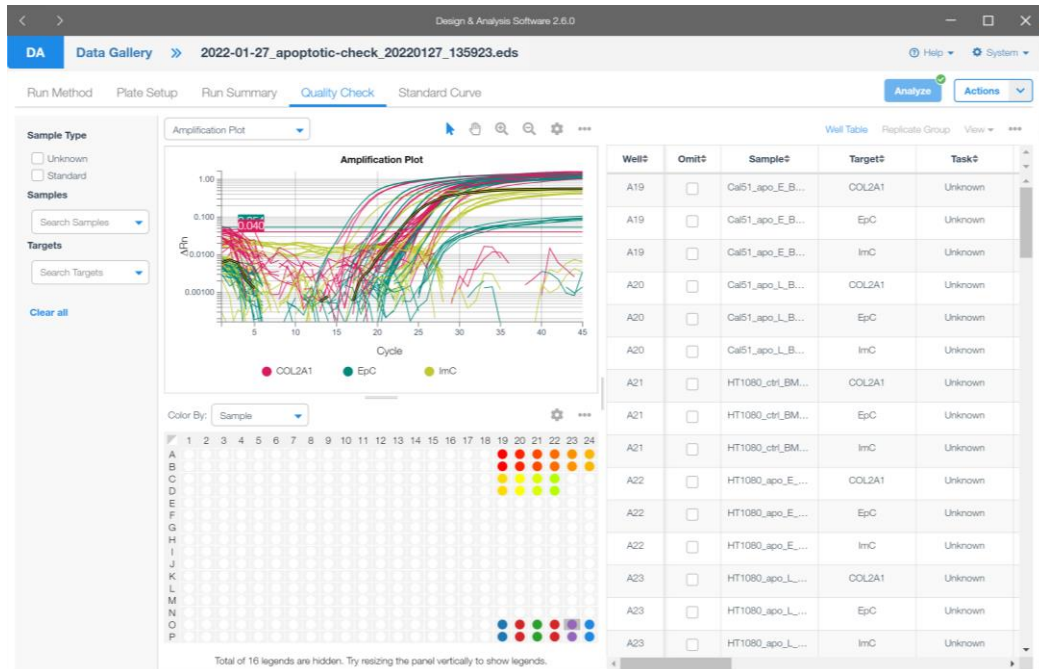
- Under Actions, Primary Analysis Settings, Set the Thresholds, and press Save:
  - COL2A1 (FAM) = 0,12
  - ZSCAN12 (FAM) = 0.17
  - GYPC1 (CY5), GYPC2 (CY5) = 0.04
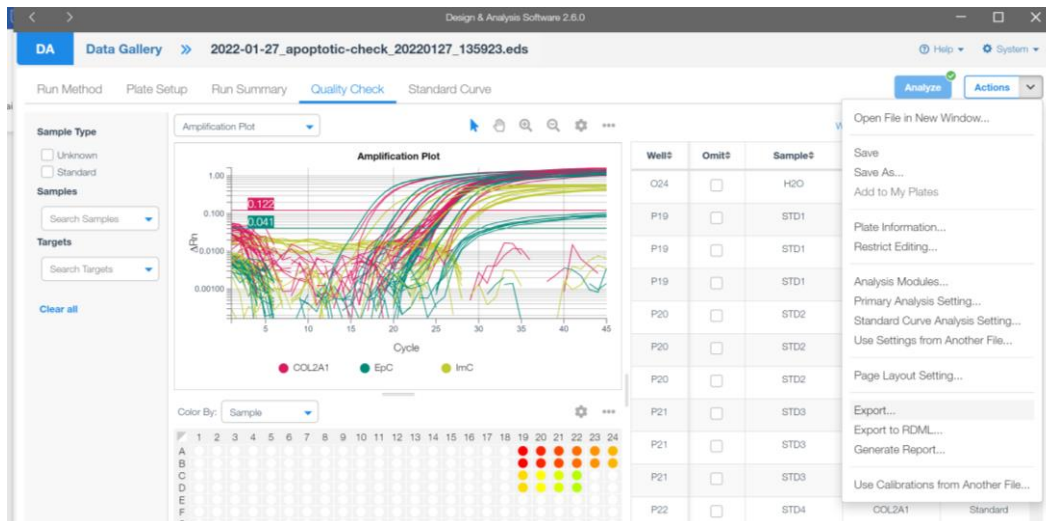
- Under Actions export the result files (default setting)
- Save them to your project folder and experiment subfolder in:

\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**