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\

1. 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:

Table

Description automatically generated

* 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

Table

Description automatically generated

1. 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 !!!!!!!!!!

1. 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

A screenshot of a computer

Description automatically generated

* Press ok, when prompted to overwrite Plate Setup

A screenshot of a computer

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

Graphical user interface, table

Description automatically generated

* 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 1A screenshot of a computer

  Description automatically generated
* 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

A screenshot of a computer

Description automatically generated

* 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

1. 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.

1. Please update “experiment\_log” \Dropbox\eutops\data! For larger cohorts and studies