

Use of the “RNA Negative Normalization” DSP DA script

Intended use

The RNA Negative Normalization DSP DA script is designed for data from the GeoMx DSP and is intended to support nCounter RNA analyses including those consisting of multiple panels and custom kits.

This script does the following:

1. Identifies negative control probes and associates them with their corresponding panel
2. Computes the negative normalization factor for each segment for each panel
 - a. Segment negative control probes count divided by geometric mean of all segment negative counts
3. Normalizes results for each panel by the corresponding negative normalization factor computed in step 2
 - a. Divide counts with corresponding negative normalization factor
4. Returns the negative normalized results as a target count matrix

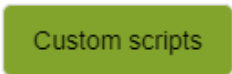
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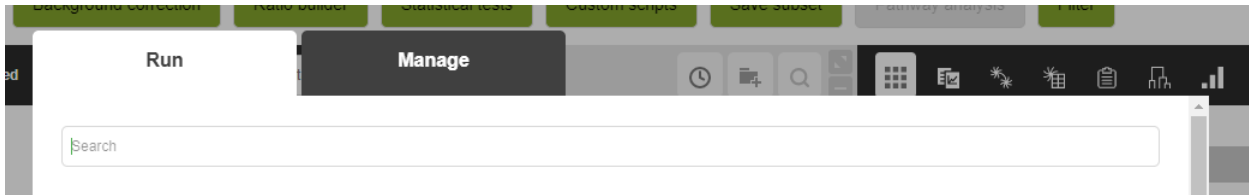
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Loading into the DSP-DA:

The RNANegativeNormalization.R file may be loaded into the custom scripts section of the DSP-DA after you have a QC'd dataset ready for analysis. To do so open the custom script section by clicking on the button shown below:



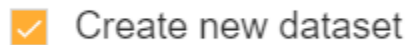
Select the "Manage" tab to open the area to load and edit scripts:



In the Management tab add a unique name for the script.

Name

Scroll down in the Management tab and select "Create new dataset".



Scroll to the bottom of the Management tab. Use the "+" button to add the RNANegativeNormalization.R file to the script and select the blue "Save" button.

Main script:

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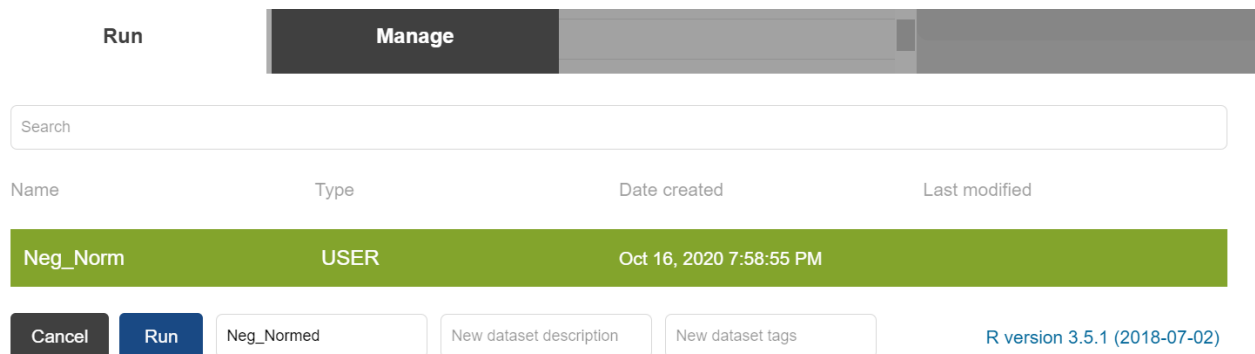
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Return to the Run tab. Select the newly added script and put in a unique name to give the resulting dataset. Press the blue "Run" button when ready.



Viewing normalized results:

Normalized results will show up as a new dataset in DSP-DA and can be downloaded as excel exports.

