GeoMx® DSP TCR Profiling Plug-in
Instructions for Use

The T Cell Receptor (TCR) Profiling Add-on assay for GeoMx® DSP enables users to understand the adaptive T cell immune response by profiling 146 variable and joining segments across all four human TCR loci. Data analysis is performed with a GeoMx DSP Data Analysis Suite software plug-in developed by NanoString, described here. The script outputs an Excel file with TCR genes above specified LOQ thresholds, Gini coefficients, and Shannon diversity scores.

Intended Use

The TCR Analysis plug-in was designed for data from GeoMx DSP NGS assays (Whole Transcriptome Atlas or Cancer Transcriptome Atlas) with TCR Profiling Add-on. Refer to the GeoMx DSP Manual Slide Preparation User Manual (MAN-10150) or Automated Slide Preparation User Manual (MAN-10151) for instructions to include the Add-on assay during slide preparation.

Load the plug-in into the GeoMx DSP Data Analysis Suite

1. Download the TCR_Analysis.R file from GitHub:

   a. Navigate to https://github.com/Nanostring-Biostats/DSPPlugins.

   b. Select the folder for TCRAnalysis.

   c. Click on TCR_Analysis.R.

   d. Click on the button ⎡Raw⎤.

   e. Right-click on the screen and select **Save As…** to save the .R file to your computer.

2. In the GeoMx DSP Data Analysis Suite, perform QC and Biological Probe QC in the study of interest. Refer to the GeoMx DSP Data Analysis User Manual (MAN-10154) for more information.

3. Select the BioProbeQC dataset (Figure 1), then click on the **Custom scripts** button in the toolbar.
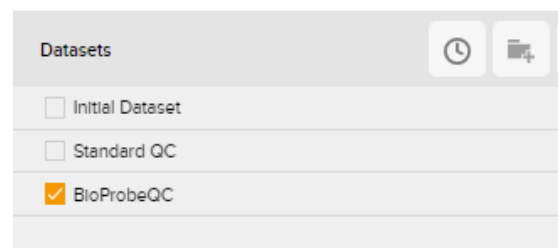

Figure 1.

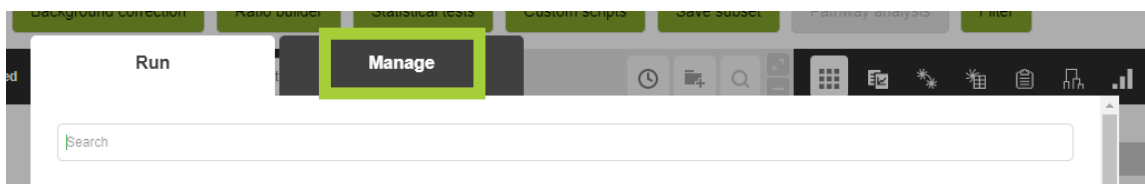4. In the Custom Script window, select the Manage tab (Figure 2).

Figure 2.

5. In the Manage tab, click **Add**.

   a. Fill in a name such as TCR Analysis.

   b. Optionally check the box to have the script **Create a new dataset** with the output.

   c. From the dropdown **Input data types** (Figure 3), select either Data frames or GeoMxSet (the script works with both).
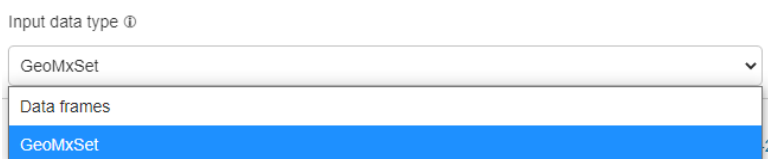
Figure 3.

   d. In the section **Main script**, click the + icon (Figure 4). Navigate to the previously downloaded TCR_Analysis.R file and click Open. The script text appears in the script text box (Figure 5).
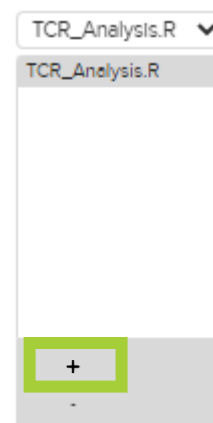
Figure 4.

6. The script parameters can be adjusted by editing the text in the User Options section within the text box (Figure 5). Parameters are described on page 4. Click **Save** to save any changes or **Revert** to return to the original text.

```
# in the DSPDA Annotation file
#         User Options          #
###############################

# option to filter out segments that fail QC
filter_segments = FALSE

# option to filter targets that occur in less than x% of AOIs (TCR
genes will not be filtered)
filter_targets = TRUE

if (filter_targets) {
    percent_thresh = .8 #e.g. 10%
}

# used to calculate detection of TCR probes over background
LOQ_thresholds = c(2, 2.5, 3)
```
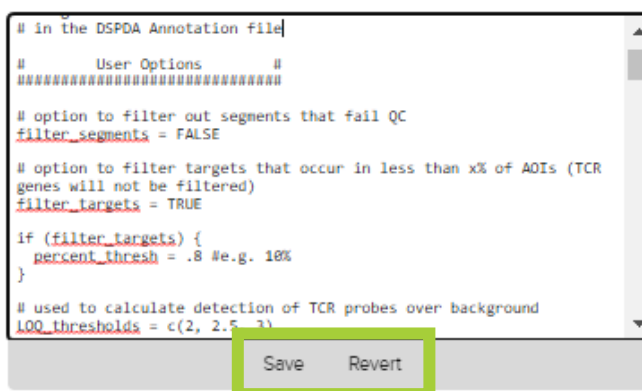
Figure 5.

7. When the script parameters are set as desired, scroll to the bottom of the Manage Custom Scripts window and click **Save** (Figure 6). The software will process the script.

8. Switch to the tab **Run** in the Custom Scripts window and navigate to the newly added script. (If you elected to **Create a new dataset**, you must give it a name in the text field to the right of the Run button.) Click **Run**.
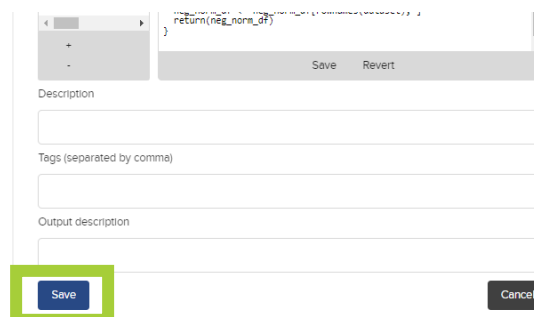

Figure 6.

9. Navigate to the **Dataset Summary** (clipboard icon) in the Datasets pane. Find the script output under **Attachments**.

10. The output Excel file includes a tab for each LOQ threshold specified in the script, indicating whether the segment is above (TRUE) or below (FALSE) that LOQ in each AOI (Figure 7). It also includes tabs for Gini coefficients, Gini coefficients using the quantile normalized data, and Shannon diversity scores for each AOI.

Figure 7.

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

3

Customizable Script Parameters

The following parameters can be adjusted by the user. Do not edit outside of the section marked `### User Options ###`.

- `filter_segments` – set to `TRUE` to exclude segments that fail QC. Set to `FALSE` to keep all segments.

- `filter_targets` – set to `TRUE` to exclude targets that occur in less than x% of AOIs (x is set by `percent_thresh` parameter, below). TCR genes will not be filtered. Set to `FALSE` to keep all targets.

   NOTE: If **Create new dataset** is checked (Step 5b, above), and GeoMxSet is selected as **input type** (Step 5c), and `filter_segments` or `filter_targets` is set to `TRUE`, be aware that the new dataset is comprised of unfiltered data. This is a known issue which will be resolved in an upcoming release. The script output (Excel file) is correctly comprised of filtered data.

- `percent_thresh` – if `filter_targets` = `TRUE`, set this to a numeral less than 1. For 10%, set to 0.1.

- `LOQ_thresholds` – set to a string of numeric LOQ thresholds that will be used to calculate detection of TCR probes over background. See examples below.

- `TCR_probes_bg_subtraction` – set to `TRUE` to subtract background from TCR probes.

- `background_method` – if `TCR_probes_bg_subtraction` = `TRUE`, set this to either "`geomean`" or "`LOQ`" to designate the background subtraction method to be used.

- `Bg_LOQ_thresh` – if `TCR_probes_bg_subtraction` = `TRUE` and `background_method` = "`LOQ`" - set to numeric of LOQ threshold to use in the background subtraction calculation.

Example 1: Do not filter segments or targets; show results of LOQ =2, 2.5, and 3; and do not perform background subtraction

```
filter_segments = FALSE
filter_targets = FALSE
LOQ_thresholds = c(2, 2.5, 3)
TCR_probes_bg_subtraction = FALSE
```

Example 2: Filter segments that failed QC; filter targets that occur in less than 10% of AOIs; show results of LOQ = 2, 2.5, and 3; perform background subtraction using LOQ of 2

```
filter_segments = TRUE
filter_targets = TRUE
percent_thresh = .1 #e.g. 10%
LOQ_thresholds = c(2, 2.5, 3)
TCR_probes_bg_subtraction = TRUE
background_method = "LOQ"
bg_LOQ_thresh = 2
```

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.