

Use of the “GeneralHeatmap” DSP DA script

Intended use

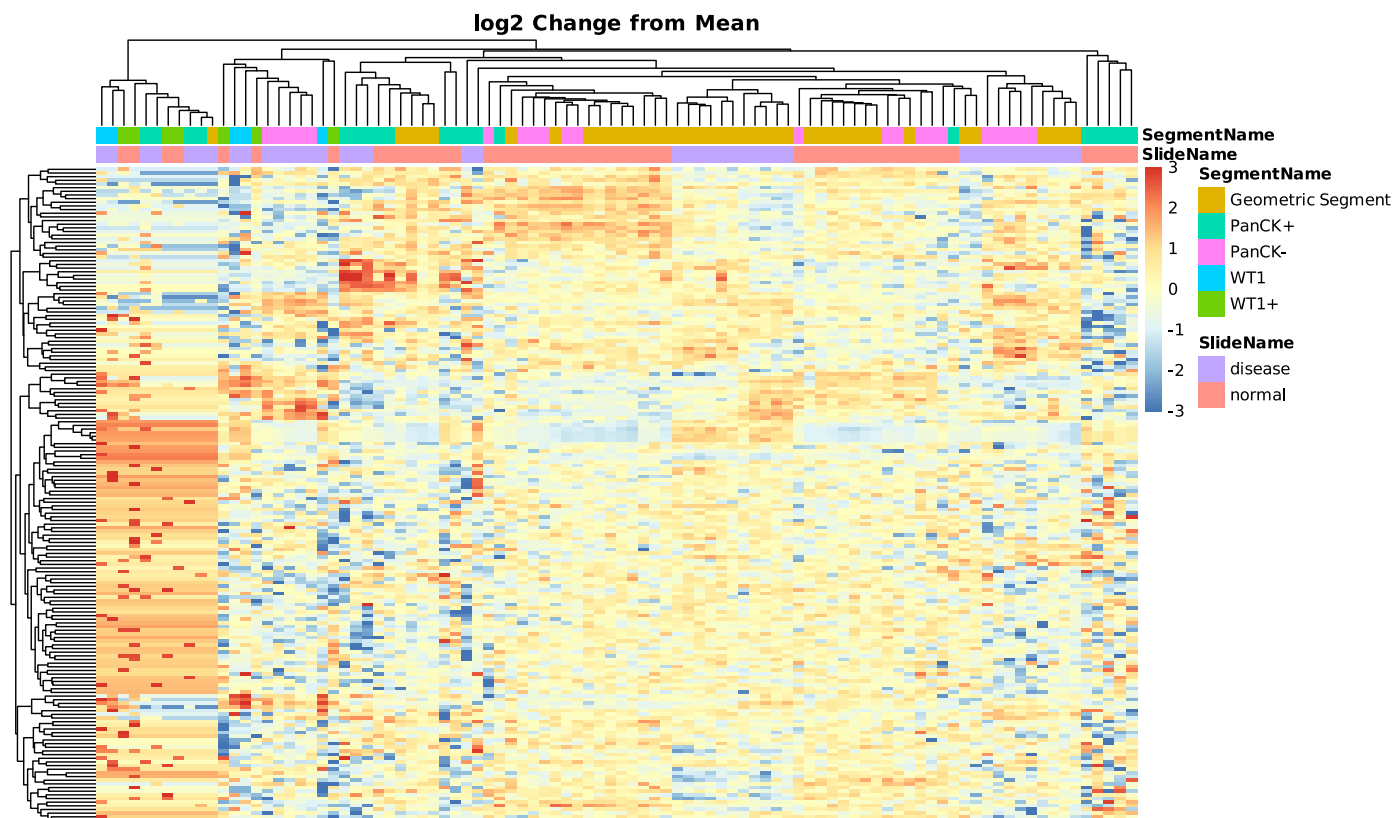
The General Heatmap DSP DA script was designed for data from the GeoMx nCounter (protein or RNA) or GeoMx NGS (CTA/WTA) readout applications. It creates publication ready labeled heatmaps based on user inputs.

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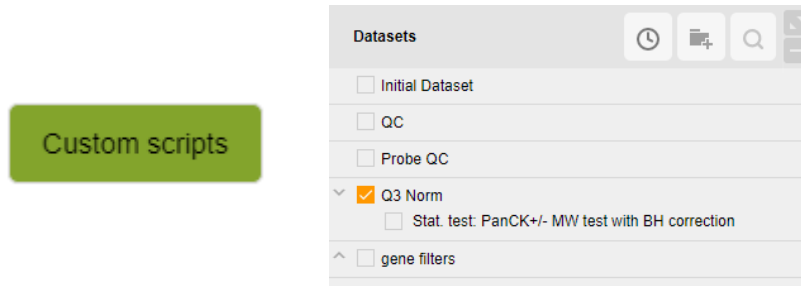
Page 5: Example Parameter Set-up



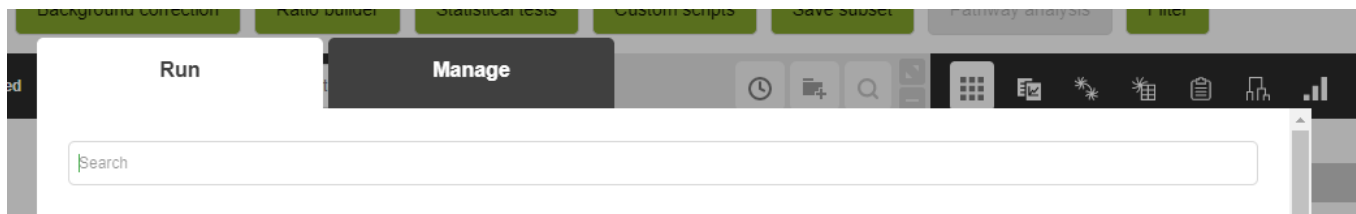
For more example graphs see page 5

Loading into the DSP DA

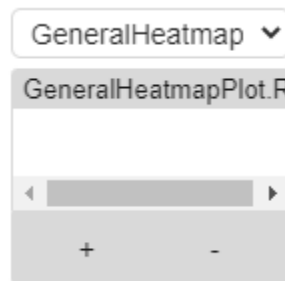
The GeneralHeatmap.R file may be loaded into the custom scripts section of the DSP DA after you have a dataset processed and ready for analysis. Filtering of targets and segments must happen before using this custom script. To do so open the custom script section by clicking on the button shown below with the statistical test dataset unmarked:



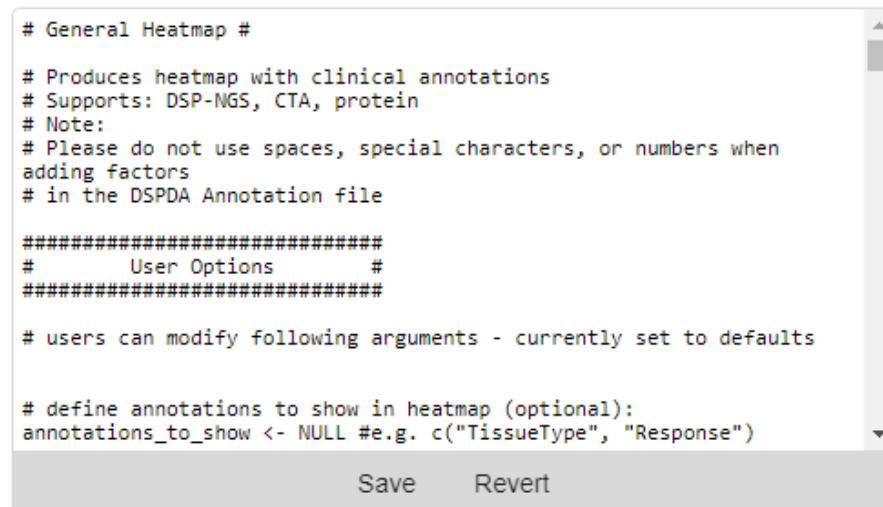
Select the "Manage" tab to open the area to load and edit scripts:



In the Management tab to add a new script and adjust parameters, fill out and then scroll to the bottom of the page. Use the "+" button to add the GeneralHeatmap.R file to the script:



Once added the parameters of the script can be adjusted by editing the top lines in the script and hitting the "Save" button. You do **not** need to check the Create new dataset button.



Setting User Parameters:

There are 15 settings that can be adjusted by the user at the top of the plug-in script. All parameters are optional. These include:

Files

1. *annotations_to_show*: (String) Segment annotations to show in heatmap, set to NULL if none needed
2. *custom_annotation_colors*: (Boolean) Should User defined colors be used? Otherwise heatmap defaults will be used
 - *annotation_colors*: (List) Color for each unique factor in each segment shown

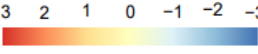
```
# example syntax
annotation_colors <- list(
  TissueType = c(
    "Normal" = "green",
    "Disease" = "red"),
  Response = c(
    "NR" = "blue",
    "R" = "orange"))
```

Annotation →

Options in Annotation →

3. *sort_by*: (String) If supervised clustering is wanted, which segment annotation should sorting be performed on. Set to NULL if unsupervised clustering is desired
4. *sort_order*: (String) If supervised clustering is wanted, what order should the annotations be ordered in. Set to NULL if unsupervised clustering is desired

Advanced User Options

5. *heatmap_colors*: (String) Color scheme for the heatmap, set to NULL if default is desired
 - example = c("navy", "white", "firebrick3")
6. *scale_cutoff*: (Numeric) Max (and absolute min) of heatmap color and legend scale
 - 
7. *show_sample_names*: (Boolean) Should sample names be shown on figure
8. *show_target_names*: (Boolean) Should target names be shown on figure
9. *clustering_distance*: (String) clustering distance for unsupervised clustering; default = Euclidean
 - options: "euclidean", "correlation" (Pearson's), "maximum", "manhattan", "canberra", "binary", or "minkowski"
10. *scale*: (String) axis to scale and center values on; default = row
 - options: "none", "row", "column"
11. *fontsize*: (Numeric) Font size for text in figure
12. *plot_title*: (String) Figure Title
13. *file_type*: (String) Desired output format for the figure
 - options: "pdf", "svg", "png", "tiff"
14. *plot_width*: (Numeric) Width of saved figure in inches
15. *plot_height*: (Numeric) Height of saved figure in inches

For color options:

Colors that can be recognized by R should be either named colors (e.g. "orange2") or hexadecimal colors ("#ABABAB"). See below for a cheat sheet of all named R colors.

Named R Colors:

coral3	deeppink4	gray27	gray87	gray99	lightpink1	mistyrose1	pink4	slategray1
coral2	deeppink3	gray26	gray86	gray98	lightpink	mistyrose	pink3	slategray
coral1	deeppink2	gray25	gray85	gray97	lightgrey	mintcream	pink2	slateblue4
coral	deeppink1	gray24	gray84	gray96	lightgreen	midnightblue	pink1	slateblue3
chocolate4	darkviolet	gray23	gray83	gray95	lightgray	mediumvioletred	pink	slateblue2
chocolate3	darkturquoise	gray22	gray82	gray94	lightgoldenrodyellow	mediumturquoise	peru	slateblue1
chocolate2	darkturquoise	gray21	gray81	gray93	lightgoldenrod4	mediumspringgreen	peachpuff4	slateblue
chocolate1	darkslategray	gray20	gray80	gray92	lightgoldenrod3	mediumslateblue	peachpuff3	skyblue4
chocolate	darkslategray4	gray19	gray79	gray91	lightgoldenrod2	mediumseagreen	peachpuff2	skyblue3
chartreuse4	darkslategray3	gray18	gray78	gray90	lightgoldenrod1	mediumpurple4	peachpuff1	skyblue2
chartreuse3	darkslategray2	gray17	gray77	gray89	lightgoldenrod	mediumpurple3	peachpuff	skyblue1
chartreuse2	darkslategray1	gray16	gray76	gray88	lightcyan4	mediumpurple2	papayawhip	skyblue
chartreuse1	darkslategray	gray15	gray75	gray87	lightcyan3	mediumpurple1	palevioletred4	sienna4
chartreuse	darkslateblue	gray14	gray74	gray86	lightcyan2	mediumpurple	palevioletred3	sienna3
cadetblue4	darkseagreen4	gray13	gray73	gray85	lightcyan1	mediumorchid4	palevioletred2	sienna2
cadetblue3	darkseagreen3	gray12	gray72	gray84	lightcyan	mediumorchid3	palevioletred1	sienna1
cadetblue2	darkseagreen2	gray11	gray71	gray83	lightcoral	mediumorchid2	palevioletred	sienna
cadetblue1	darkseagreen1	gray10	gray70	gray82	lightblue4	mediumorchid1	paleturquoise4	seashell4
cadetblue	darkseagreen	gray9	gray69	gray81	lightblue3	mediumorchid	paleturquoise3	seashell3
burlywood4	darksalmon	gray8	gray68	gray80	lightblue2	mediumblue	paleturquoise2	seashell2
burlywood3	darkred	gray7	gray67	gray79	lightblue1	mediumaquamarine	paleturquoise1	seashell1
burlywood2	darkorchid4	gray6	gray66	gray78	lightblue	maroon4	paleturquoise	seashell
burlywood1	darkorchid3	gray5	gray65	gray77	lemonchiffon4	maroon3	palegreen4	seagreen4
burlywood	darkorchid2	gray4	gray64	gray76	lemonchiffon3	maroon2	palegreen3	seagreen3
brown4	darkorchid1	gray3	gray63	gray75	lemonchiffon2	maroon1	palegreen2	seagreen2
brown3	darkorchid	gray2	gray62	gray74	lemonchiffon1	maroon	palegreen1	seagreen1
brown2	darkorange4	gray1	gray61	gray73	lemonchiffon	magenta4	palegreen	seagreen
brown1	darkorange3	gray0	gray60	gray72	lawngreen	magenta3	palegoldenrod	sandybrown
brown	darkorange2	gray	gray59	gray71	lavenderblush4	magenta2	orchid4	salmon4
blueviolet	darkorange1	goldenrod4	gray58	gray70	lavenderblush3	magenta1	orchid3	salmon3
blue4	darkolivegreen4	goldenrod3	gray57	gray69	lavenderblush2	magenta	orchid2	salmon2
blue3	darkolivegreen3	goldenrod2	gray56	gray68	lavenderblush1	linen	orchid1	salmon1
blue2	darkolivegreen2	goldenrod1	gray55	gray67	lavenderblush	limegreen	orchid	salmon
blue1	darkolivegreen1	goldenrod	gray54	gray66	lavender	lightyellow4	orangered4	saddlebrown
blanchedalmond	darkolivegreen	gold4	gray53	gray65	khaki4	lightyellow3	orangered3	royalblue4
black	darkmagenta	gold3	gray52	gray64	khaki3	lightyellow2	orangered2	royalblue3
bisque4	darkkhaki	gold2	gray51	gray63	khaki2	lightyellow1	orangered1	royalblue2
bisque3	darkkarey	gold1	gray50	gray62	khaki1	lightyellow	orangered	royalblue1
bisque2	darkgreen	ghostwhite	gray49	gray61	khaki	lightsteelblue4	orange4	royalblue
bisque1	darkgray	gainsboro	gray48	gray60	ivory4	lightsteelblue3	orange3	rosybrown4
bisque	darkgoldenrod4	forestgreen	gray47	gray59	ivory3	lightsteelblue2	orange2	rosybrown3
beige	darkgoldenrod3	floralwhite	gray46	gray58	ivory2	lightsteelblue1	orange1	rosybrown2
azure4	darkgoldenrod2	firebrick4	gray45	gray57	ivory1	lightsteelblue	orange	rosybrown1
azure3	darkgoldenrod1	firebrick3	gray44	gray56	ivory	lightslategray	olivedrab4	rosybrown
azure2	darkgoldenrod	firebrick2	gray43	gray55	indianred4	lightslategray	olivedrab3	red4
azure1	darkcyan	firebrick1	gray42	gray54	indianred3	lightslateblue	olivedrab2	red3
azure	darkblue	firebrick	gray41	gray53	indianred2	lightskyblue4	olivedrab1	red2
aquamarine4	cyan4	dodgerblue4	gray40	gray52	indianred1	lightskyblue3	olivedrab	red1
aquamarine3	cyan3	dodgerblue3	gray39	gray51	hotpink4	lightskyblue2	oldlace	red
aquamarine2	cyan2	dodgerblue2	gray38	gray50	hotpink3	lightskyblue1	navyblue	purple4
aquamarine1	cyan1	dodgerblue1	gray37	gray49	hotpink2	lightskyblue	navy	purple3
aquamarine	cyan	dodgerblue	gray36	gray48	hotpink1	lightseagreen	navajowhite4	purple2
antiquewhite4	cornsilk4	dimgray	gray35	gray47	hotpink	lightsalmon4	navajowhite3	purple1
antiquewhite3	cornsilk3	dimgray	gray34	gray46	honeydew4	lightsalmon3	navajowhite2	purple
antiquewhite2	cornsilk2	deepskyblue4	gray33	gray45	honeydew3	lightsalmon2	navajowhite1	powderblue
antiquewhite1	cornsilk1	deepskyblue3	gray32	gray44	honeydew2	lightsalmon1	navajowhite	plum4
antiquewhite	cornsilk	deepskyblue2	gray31	gray43	honeydew1	lightsalmon	moccasin	plum3
aliceblue	cornflowerblue	deepskyblue1	gray30	gray42	honeydew	lightpink4	mistyrose4	plum2
white	coral4	deepskyblue	gray29	gray41	honeydew	lightpink3	mistyrose3	plum1
			gray28	gray40	gray100	lightpink2	mistyrose2	plum
				gray88	gray100	lightpink2	mistyrose2	plum

Example Parameter Set-up

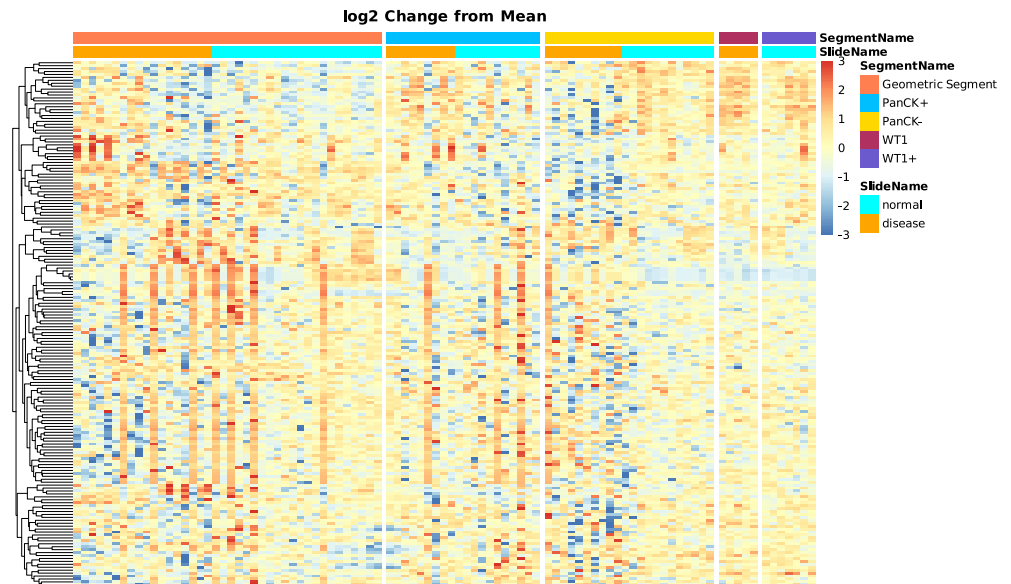
The GeneralHeatmap script outputs a typical heatmap figure with log2 change from mean expression.

Example figures with different input arguments.

Example 1:

```
sort_by <- "SegmentName"
```

```
annotation_colors <- list(  
  SlideName = c(  
    "normal" = "cyan",  
    "disease" = "orange"),  
  SegmentName = c(  
    "Geometric Segment" = "coral",  
    "PanCK+" = "deepskyblue",  
    "PanCK-" = "gold1",  
    "WT1" = "maroon",  
    "WT1+" = "slateblue"))
```



Example 2:

```
scale_cutoff <- 1
```

```
heatmap_colors <- viridis::viridis(5)
```

