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REPORT FOR NANOSTRING SERVICE

NanoString Analysis Report

Company X

February 2018

Summary

This Report contains both a NanoString QC Report and a NanoString Analysis Report. NanoString service was performed on the 12 RNA samples using the Human Pan Cancer Pathway Panel. The panel contains 770 genes from 13 cancer-associated canonical pathways including: MAPK, STAT, PI3K, RAS, Cell Cycle, Apoptosis, Hedgehog, WNT, DNA Damage Control, Transcriptional Regulation, Chromatin Modification, and TGF-beta pathways. The NanoString run was successful and the samples passed NanoString QC. Samples were normalized to the 40 housekeeping genes and then sample analysis was performed. Sample analysis, heat map analysis, and pathway mapping were performed on the data.

NanoString QC Report

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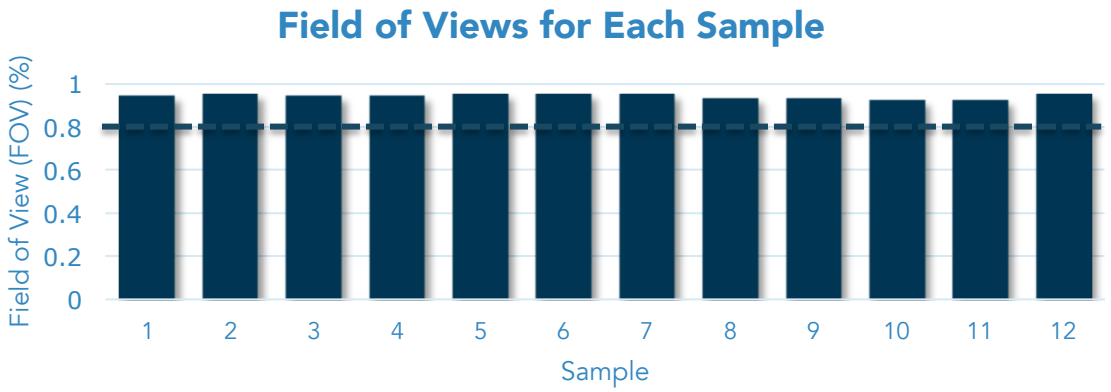
Field of Views



Imaging QC refers to the percentage of FOVs successfully counted by a digital Analyzer scan. Consistently reduced percentages can be indicative of an issue associated with the nCounter instrumentation. 80% is the Canopy Biosciences FOV cutoff for quality control.

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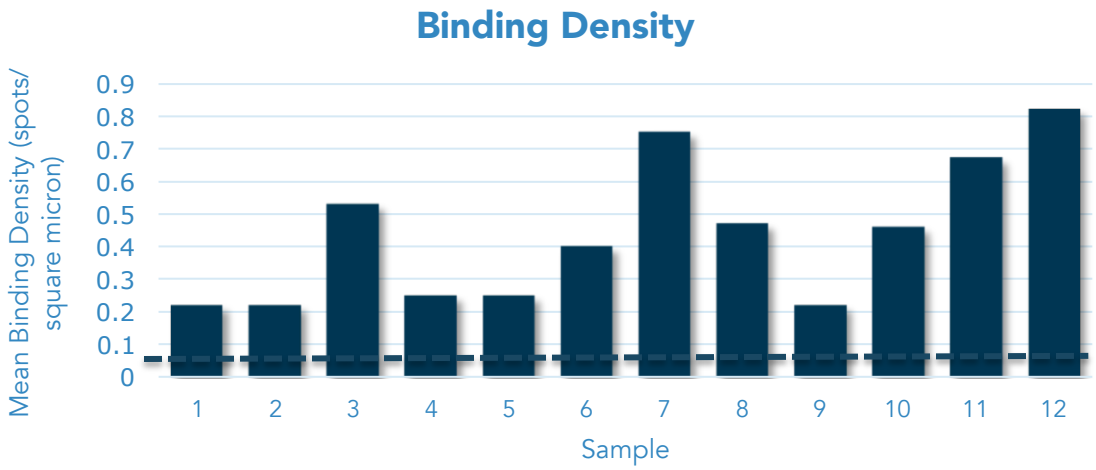
Binding Density



The mean binding density is measured in spots per square micron.
Acceptable probe count measurements are between 0.05 and 2.25 spots per square micron.
When too many probes are present, the Analyzer may not distinguish each individual probe accurately.

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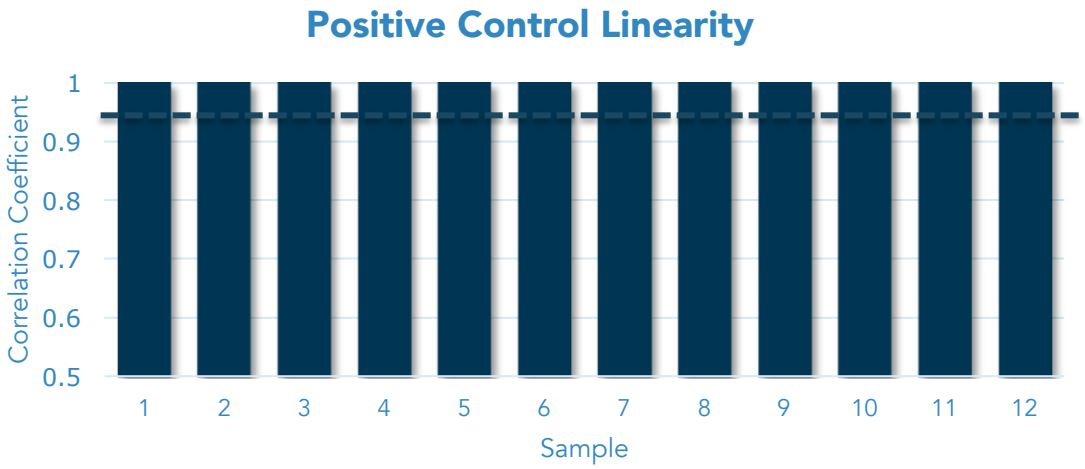
Positive Control Linearity



This assay contains a variety of positive control probes targeting molecules added during the production of the kit. Positive control linearity is a correlation analysis in log2 space between concentrations of added targets and the resulting counts. Low correlation values (below 0.95) may indicate an issue regarding hybridization.

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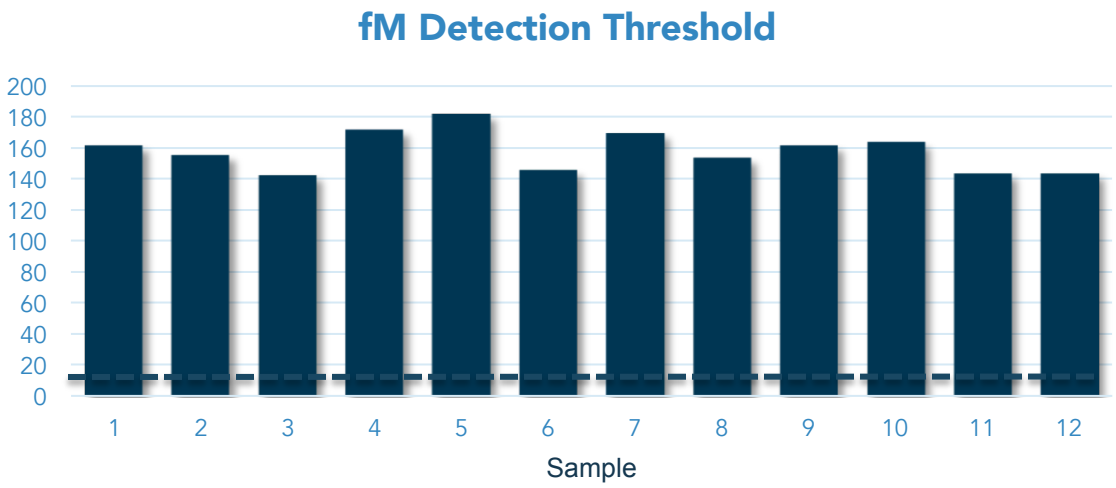
fM Detection Threshold



fM detection threshold is a calculation of limit of detection based on positive and negative control probes.
The 0.5 fM positive control probes must produce raw counts significantly higher than the mean of the negative control probes.
Detection threshold below the minimum value indicates hybridization difficulties.

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Controls

Positive Controls

	Class Name	Gene Name	Accession #	Average Count	Median	%CV	Standard Deviation
1	Positive	POS_A	ERCC_00117.1	42,203.42	41,920.5	0.07	2,968.15
2	Positive	POS_B	ERCC_00112.1	11,720.17	11,682.5	0.06	728.73
3	Positive	POS_C	ERCC_00002.1	3,247.92	3,290.5	0.07	225.19
4	Positive	POS_D	ERCC_00092.1	693.67	696.5	0.09	59.23
5	Positive	POS_E	ERCC_00035.1	157.25	158	0.08	12.66
6	Positive	POS_F	ERCC_00034.1	77.75	76	0.15	11.68

Negative Controls

	Class Name	Gene Name	Accession #	Average Count	Median	Standard Deviation
1	Negative	NEG_A	ERCC_00096.1	12.25	14	3.6
2	Negative	NEG_B	ERCC_00041.1	9.58	8.5	4.44
3	Negative	NEG_C	ERCC_00019.1	14.08	12	5.04
4	Negative	NEG_D	ERCC_00076.1	10.75	11.5	3.47
5	Negative	NEG_E	ERCC_00098.1	13.83	13.5	3.38
6	Negative	NEG_F	ERCC_00126.1	18.25	18	4.09
7	Negative	NEG_G	ERCC_00144.1	7.25	7.5	3.25
8	Negative	NEG_H	ERCC_00154.1	9.33	9	3.58

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Housekeeping Genes

	Class Name	Gene Name	Accession #	Average Count	Median	Standard Deviation
1	Housekeeping	ACAD9	NM_014049.4	520.42	490	241.75
2	Housekeeping	AGK	NM_018238.3	414.67	332.5	239
3	Housekeeping	AMMECR1L	NM_001199140.1	508.75	408.5	303.53
4	Housekeeping	C10orf76	NM_024541.2	237	224	116.12
5	Housekeeping	CC2D1B	NM_032449.2	19.33	18	9.54
6	Housekeeping	CNOT10	NM_001256741.1	471.83	487.5	170.97
7	Housekeeping	CNOT4	NM_001190848.1	527.08	402	335.81
8	Housekeeping	COG7	NM_153603.3	997.75	1,051.5	515.56
9	Housekeeping	DDX50	NM_024045.1	636.83	672.5	258.73
10	Housekeeping	DHX16	NM_001164239.1	239.92	264	98.82
11	Housekeeping	DNAJC14	NM_032364.5	148.58	144	79.06
12	Housekeeping	EDC3	NM_001142443.1	733	694	391.47
13	Housekeeping	EIF2B4	NM_172195.3	1,110.33	1,094	454.95
14	Housekeeping	ERCC3	NM_000122.1	166.25	161.5	60.68
15	Housekeeping	FCF1	NM_015962.4	1,278.25	1,144	486.04
16	Housekeeping	FTSJ2	NM_013393.1	753.42	686.5	417.98
17	Housekeeping	GPATCH3	NM_022078.2	48.75	42	20.74
18	Housekeeping	HDAC3	NM_003883.2	541.75	522	217.69
19	Housekeeping	MRPS5	NM_031902.3	897.92	1,011.5	372.02
20	Housekeeping	MTMR14	NM_022485.3	422.75	439	165.46
21	Housekeeping	NOL7	NM_016167.3	1,555.83	1,585	612.45
22	Housekeeping	NUBP1	NM_001278506.1	171.75	182	100.61
23	Housekeeping	PIAS1	NM_016166.1	336.67	342.5	100.94
24	Housekeeping	PIK3R4	NM_014602.1	464.5	410	227.71
25	Housekeeping	PRPF38A	NM_032864.3	1,072.75	936.5	546.65
26	Housekeeping	RBM45	NM_152945.2	109.75	112	43.84
27	Housekeeping	SAP130	NM_024545.3	530.83	513.5	236.95
28	Housekeeping	SF3A3	NM_006802.2	705.67	626	513.19
29	Housekeeping	SLC4A1AP	NM_018158.2	548.83	531	210.69
30	Housekeeping	TLK2	NM_006852.2	527.83	499	348.21
31	Housekeeping	TMUB2	NM_024107.2	280.17	251	168.02
32	Housekeeping	TRIM39	NM_021253.3	265.92	257	152.7
33	Housekeeping	TTC31	NR_027749.1	323.58	301	172.62
34	Housekeeping	USP39	NM_001256725.1	609.92	583	290.5
35	Housekeeping	VPS33B	NM_018668.3	146.58	132	73.81
36	Housekeeping	ZC3H14	NM_001160103.1	559.08	463.5	388.07
37	Housekeeping	ZKSCAN5	NM_014569.3	60.58	65	25.04
38	Housekeeping	ZNF143	NM_003442.5	287.33	274.5	126
39	Housekeeping	ZNF346	NM_012279.2	92.75	74	60.52
40	Housekeeping	ZNF384	NM_133476.3	548	571.5	223.86

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NanoString Analysis Report

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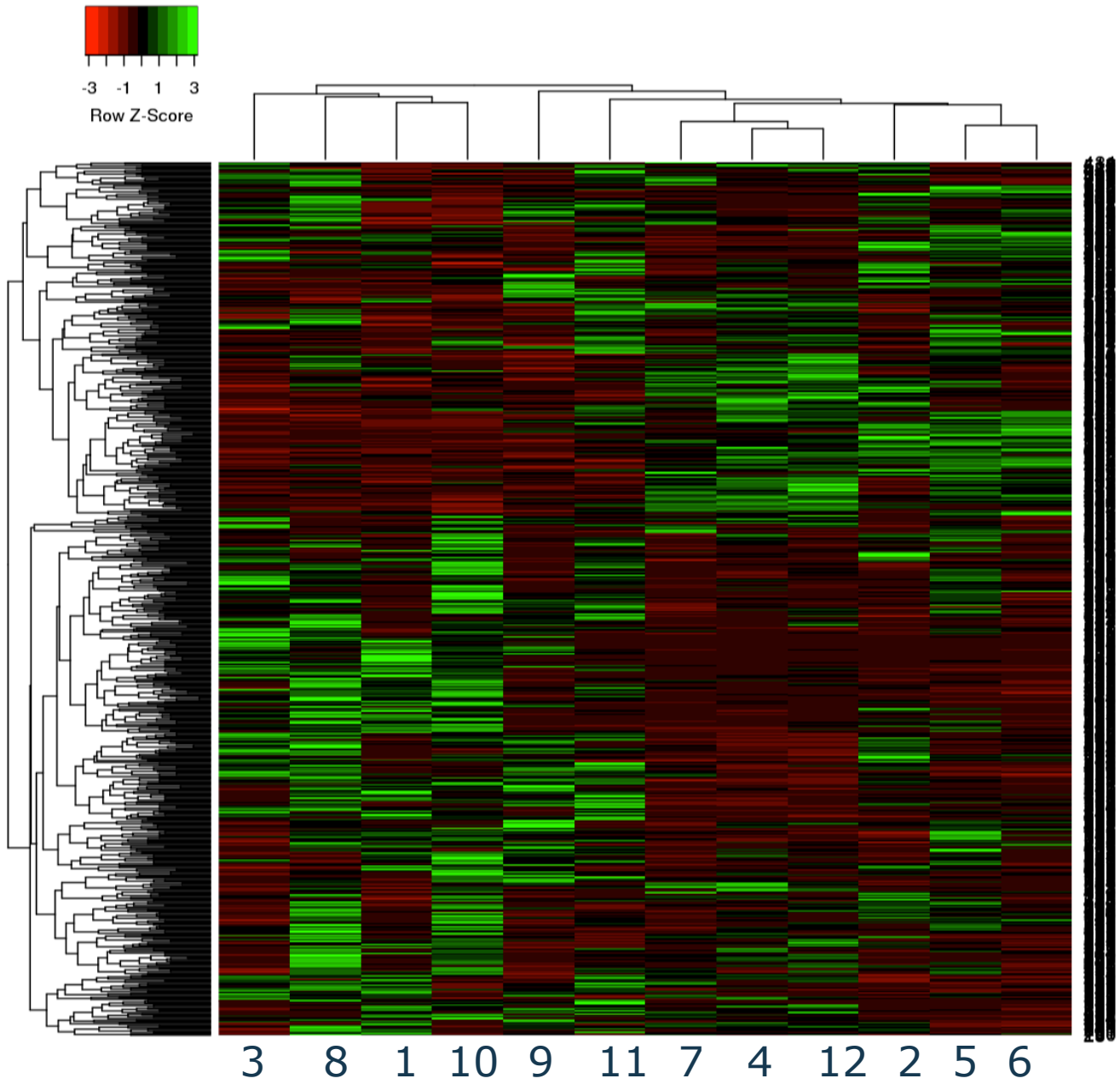
HEAT MAP ANALYSIS

Unbiased clustering was performed to generate a heat map analysis of the 12 samples. The average linkage clustering method and the kendall's tau distance method measurement were employed to generate the data.



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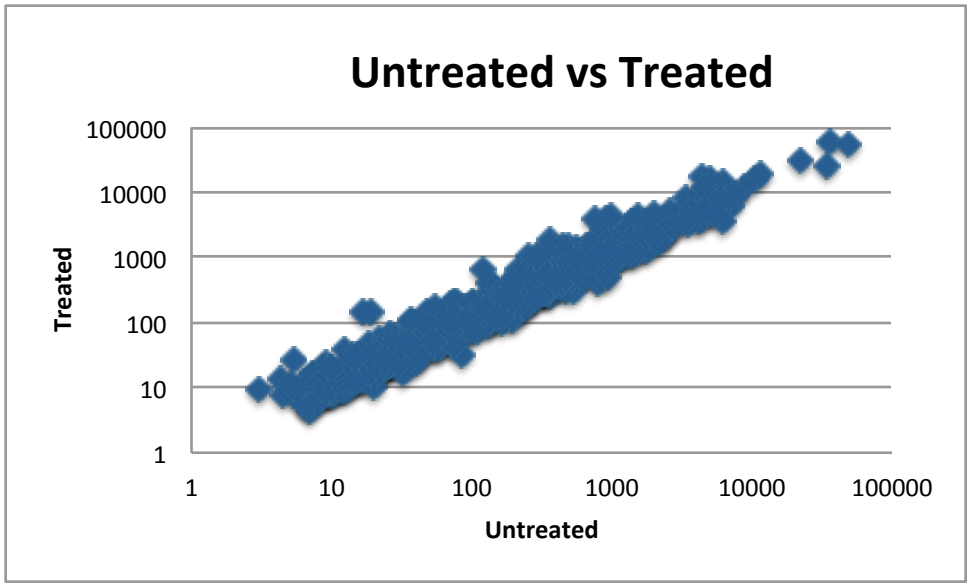
Untreated vs Treated Scatter Plot

A scatter plot was generated using the average of the untreated samples vs the average of treated samples.



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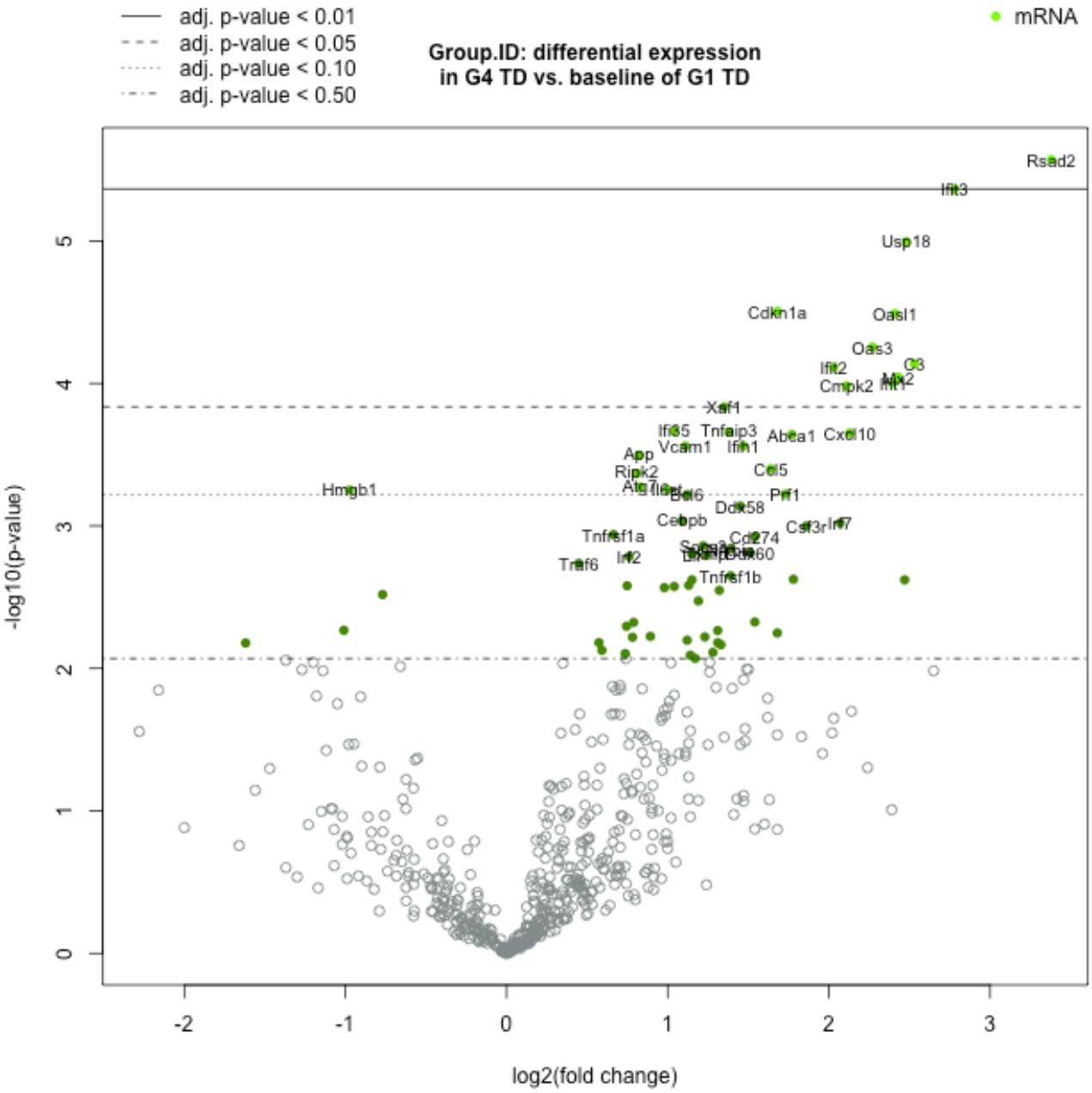
Untreated vs Treated Volcano Plot

A volcano plot was generated. Untreated samples were assigned to be the reference and treated samples were compared to untreated samples. The Benjamini-Yekutieli procedure, which controls the false discovery rate, was employed to generate an adjusted p-value.



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Untreated vs Treated Top Genes

The top 20 genes with a p-value of ≤0.05 and a log2 fold change of >1 or <-1 are listed in the table below. The Benjamini-Yekutieli procedure, which controls the false discovery rate, was employed to generate an adjusted p-value (BY.p.value).

Gene Name	Log2 Fold Change	P-value	BY.p.value
Rsad2	3.38	0.00000268	0.0088
Ifit3	2.78	0.00000431	0.0088
Usp18	2.48	0.0000101	0.0138
Cdkn1a	1.68	0.0000312	0.0264
Oasl1	2.41	0.0000323	0.0264
Oas3	2.27	0.0000555	0.0378
C3	2.53	0.0000725	0.0386
Ifit2	2.03	0.0000769	0.0386
Mx2	2.43	0.0000902	0.0386
Ifit1	2.4	0.0000994	0.0386
Cmpk2	2.11	0.000104	0.0386
Xaf1	1.35	0.000146	0.0497
Ifi35	1.04	0.000214	0.0581
Tnfaip3	1.38	0.000219	0.0581
Cxcl10	2.13	0.000223	0.0581
Abca1	1.77	0.000228	0.0581
Ifih1	1.47	0.000274	0.0623
Vcam1	1.11	0.000275	0.0623
Ccl5	1.64	0.000398	0.0812
Prf1	1.73	0.000595	0.0945

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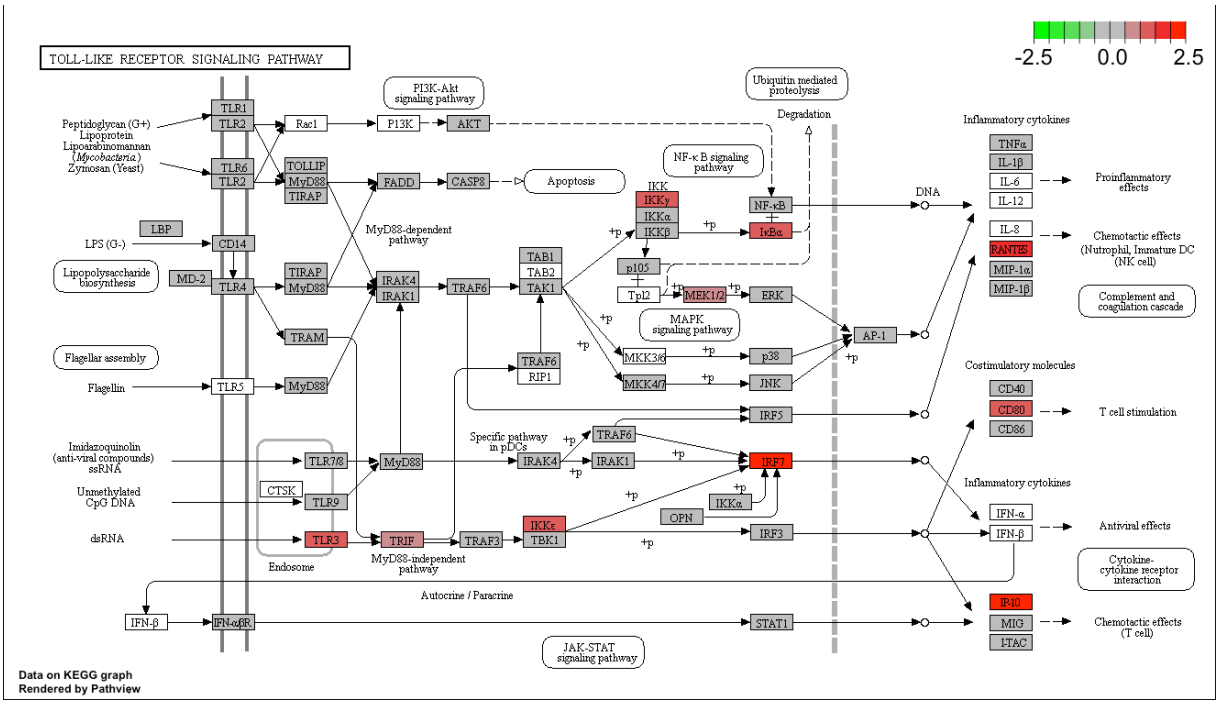
Untreated vs Treated Pathway Mapping

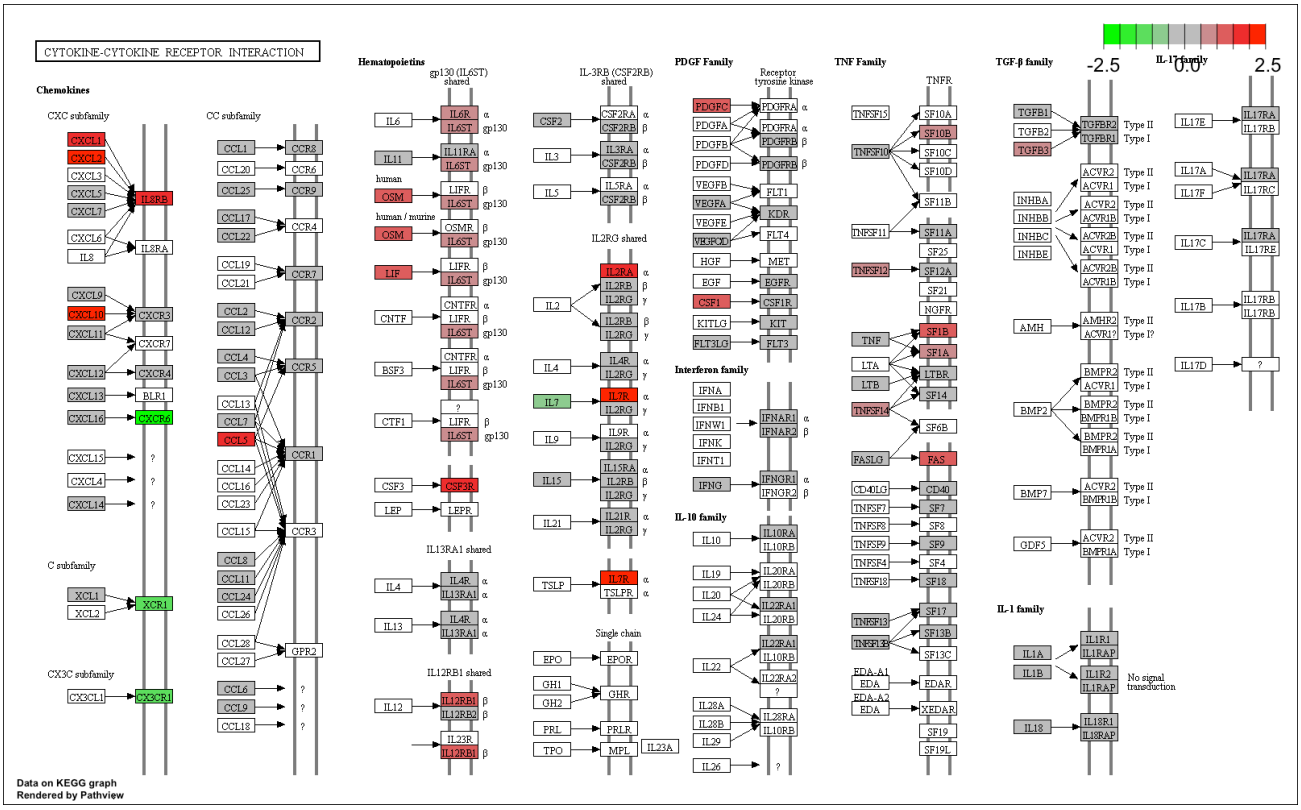
Untreated samples were assigned to be the reference and treated samples were compared to untreated samples. Pathway mapping was performed on genes with a p-value of ≤0.05. A significant pathway is defined as having three or more differentially regulated genes. Log2 fold change is shown in red (up-regulated) and green (down-regulated). Grey is no change in expression. White are genes not included in the NanoString panel. 20 pathways were analyzed: HTLV-1 infection, Herpes simplex infection, Tuberculosis, PI3K-Akt signaling pathway, Influenza A, Pathways in cancer, Jak-STAT signaling pathway, Hepatitis B, Toll-like receptor signaling pathway, Measles, Osteoclast differentiation, Epstein-Barr virus infection, Chagas disease, TNF signaling pathway, Cell adhesion molecules, Natural killer cell mediated cytotoxicity. Three pathways are highlighted below and on the following two slides.

Toll-Like Receptor Signaling Pathway



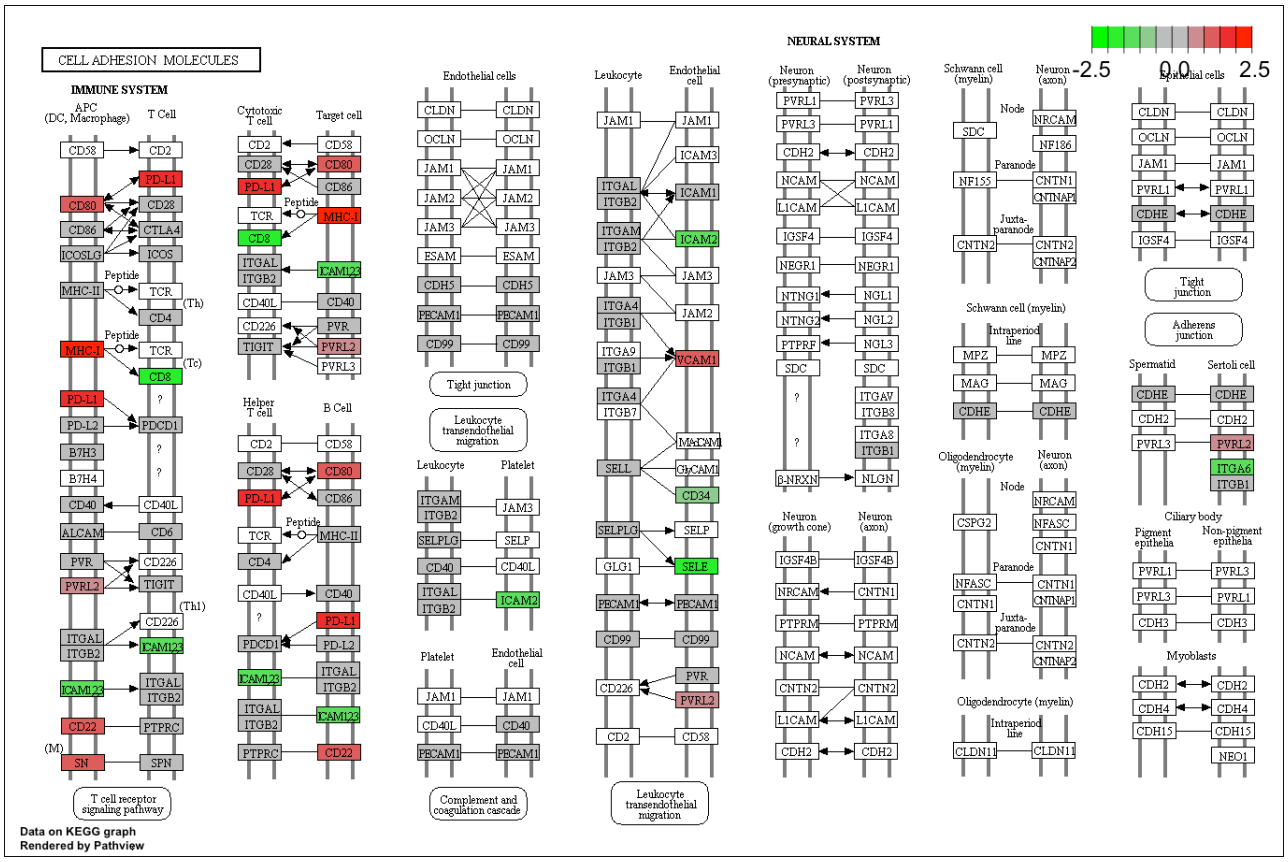
G1 vs G4 (Tumor D) Pathway Mapping

Cytokine-Cytokine Receptor Interaction Pathway



G1 vs G4 (Tumor D) Pathway Mapping

Cell Adhesion Molecules



CONCLUSIONS

NanoString service was performed on the 12 RNA samples using the Human Pan Cancer Pathway Panel. QC analysis and sample analysis was performed including heat maps, scatter plots, volcano plots, and pathway mapping.

For questions on the report please contact info@canopybiosciences.

For additional analysis please contact sales@canopybiosciences for a quote.

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