Project Name:	RUO PAM50 Demo
Experiment Name:	RUO PAM50 Demo Report 2
Date:	Mon Mar 16 15:25:16 PDT 2020
Investigator/Institution:	
Number of analysed samples:	48
Number of failed samples:	5
Number of borderline samples:	4
Number of passed samples:	39

The results from this report should be limited to Research Use Only (RUO) and should not be used in any medical decision making where results will go back to patients or patients' physicians, or used as inclusion/exclusion criteria or stratification in a prospective clinical trial.

The 48 sample dataset provided by Nanostring was analyzed using the RUO version of the NanoString RUO PAM50 algorithm to determine the molecular subtype of each sample. The RUO PAM50 algorithm measures the geometric mean of 8 housekeeping genes (HK geomean) to ensure RNA quality. Each sample meeting the QC threshold is reported as one of the four molecular subtypes, Luminal A, Luminal B, Her2-Enriched and Basal-Like. Subtypes are determined using PAM50 classification algorithm, which simultaneously measures the expression level of 50 genes, 8 housekeeping genes used for signal normalization, 6 positive control and 8 negative controls. Table 1 summarizes the QC test result of the samples.

Table 1. QC summary

	QC Status	Count	Percent
1	PASS	39	81
2	FAIL	5	10
3	BORDERLINE	4	8
4	Total	48	100

5 out of the 48 samples failed to meet the required RNA quality for the test (indicated as Fail). 4 of the 48 samples had borderline RNA quality for the test (indicated as Borderline). The remaining 39 samples met the QC thresholds (indicated as Pass) and subtype reported as Luminal A, Luminal B, Her2-Enriched or Basal-Like were provided by the algorithm. The subtype distribution of the 43 samples providing a borderline or passing RNA quality is shown in table 2 below.

Table 2. PAM50 subtype distribution

Pass and Borderline Subtype calls are represented in the table below, failures are excluded.

	Subtype	Count	Percent
1	Basal-Like	9	21
2	Her2-Enriched	10	23
3	Luminal A	13	30
4	Luminal B	11	26
5	Total	43	100

Table 3. ROR, subtype and QC results

QC status of each sample and subtype output for the samples with passing RNA quality are summarized in Table 3.

The ROR score is an integer value on a 0-100 scale that is related to an individual patient's probability of distant recurrence within 10 years for the defined intended use population. The ROR score is calculated by comparing the expression profile of 46 genes in an unknown sample with the expected profiles for the four intrinsic subtypes, to calculate four different correlation values. These correlation values are then combined with a proliferation score and the gross tumor size to calculate the ROR score.

	Lane Sample Name	Subtype	HK Geomean	QC Status	ROR	Risk
1	01	Luminal B	1928	PASS	74	High
2	02	Luminal B	1263	PASS	61	High
3	03	Luminal B	3132	PASS	62	High
4	04	Luminal B	2811	PASS	70	High
5	05	Luminal B	2535	PASS	74	N/A
6	06	Luminal A	3027	PASS	24	Low
7	07	Luminal A	3286	PASS	40	Low
8	08	Luminal A	3164	PASS	21	Low
9	09	Luminal B	2473	PASS	61	High
10	10	Luminal A	2433	PASS	26	Low
11	11	Luminal B	1738	PASS	84	High
12	12	N/A	2535	FAIL	N/A	N/A
13	13	Her2-Enriched	2996	PASS	73	High
14	74	Luminal A	983	PASS	38	Low
15	76	Luminal B	1197	PASS	79	High
16	78	Luminal A	312	BORDERLINE	36	Low
17	82	Basal-Like	1561	PASS	33	Low
18	92	Luminal A	321	BORDERLINE	32	Low
19	96	Her2-Enriched	1538	PASS	78	High
20	98	Her2-Enriched	403	BORDERLINE	79	N/A
21	100	Her2-Enriched	269	BORDERLINE	68	High
22	102	Her2-Enriched	417	PASS	55	High
23	112	Her2-Enriched	683	PASS	66	N/A

	Lane Sample Name	Subtype	HK Geomean	QC Status	ROR	Risk
24	116	Basal-Like	980	PASS	63	N/A
25	122	N/A	3142	FAIL	N/A	N/A
26	126	Luminal A	1346	PASS	27	N/A
27	130	Luminal A	912	PASS	35	Low
28	136	Her2-Enriched	1971	PASS	69	High
29	138	Luminal A	1433	PASS	33	Low
30	144	Basal-Like	3484	PASS	37	Low
31	146	Luminal B	3074	PASS	60	N/A
32	148	Basal-Like	4663	PASS	41	Intermediate
33	150	N/A	3598	FAIL	N/A	N/A
34	152	Basal-Like	3454	PASS	59	Intermediate
35	158	Luminal A	742	PASS	21	Low
36	160	Basal-Like	2368	PASS	32	Low
37	164	Luminal A	2243	PASS	39	Low
38	166	N/A	6039	FAIL	N/A	N/A
39	168	Her2-Enriched	4287	PASS	71	High
40	170	Luminal B	1238	PASS	80	High
41	172	Her2-Enriched	2020	PASS	64	High
42	174	Basal-Like	2049	PASS	37	Low
43	176	N/A	5	FAIL	N/A	N/A
44	178	Luminal B	2855	PASS	58	High
45	180	Luminal A	2419	PASS	31	Low
46	184	Basal-Like	5711	PASS	63	High
47	187	Basal-Like	3163	PASS	56	High
48	192	Her2-Enriched	3985	PASS	78	N/A

Table 4. Adjusted Risk Score cutoffs based on number of positive nodes. US Categories

Risk classification is also provided to allow interpretation of the ROR score by using cutoffs related to clinical outcome in tested patient populations.

	Risk Category	0 Positive Nodes	1-3 Positive Nodes	4+ Positive Nodes	Unknown Positive Nodes
1	Low	<= 40	0 - 40	N/A	N/A
2	Intermediate	41 - 60	-	N/A	N/A
3	High	> 60	41 - 100	N/A	N/A

Table 5. Raw data files used for analysis

	File Name
1	20180202_20180201HSD1_OR18398_06.RCC

	File Name
2	20180202_20180201HSD2_OR18326_10.RCC
3	20180209_20180208HSD1_OR18321_04.RCC
4	20180209_20180208HSD1_OR18371_10.RCC
5	20180209_20180208HSD1_OR18383_12.RCC
6	20180209_20180208HSD2_OR18307_08.RCC
7	20180209_20180208HSD2_OR18356_10.RCC
8	20180209_20180208HSD2_OR18358_02.RCC
9	20180209_20180208HSD2_OR18389_04.RCC
10	20180209_20180208HSD3_OR18388_04.RCC
11	20180209_20180208HSD4_OR18347_08.RCC
12	20180209_20180208HSD4_OR18351_02.RCC
13	20180209_20180208HSD4_OR18381_10.RCC
14	20180215_20180214HPM1_74_02.RCC
15	20180215_20180214HPM1_76_04.RCC
16	20180215_20180214HPM1_78_06.RCC
17	20180215_20180214HPM1_82_10.RCC
18	20180215_20180214HPM2_92_08.RCC
19	20180215_20180214HPM2_96_12.RCC
20	20180215_20180214HPM3_98_02.RCC
21	20180215_20180214HPM3_100_04.RCC
22	20180215_20180214HPM3_102_06.RCC
23	20180215_20180214HPM4_112_04.RCC
24	20180215_20180214HPM4_116_08.RCC
25	20180215_20180214HPM5_122_02.RCC
26	20180215_20180214HPM5_126_06.RCC
27	20180215_20180214HPM5_130_10.RCC
28	20180215_20180214HPM6_136_04.RCC
29	20180215_20180214HPM6_138_06.RCC
30	20180215_20180214HPM6_144_12.RCC
31	20180215_20180214HPM7_146_02.RCC
32	20180215_20180214HPM7_148_04.RCC
33	20180215_20180214HPM7_150_06.RCC
34	20180215_20180214HPM7_152_08.RCC
35	20180215_20180214HPM8_158_02.RCC
36	20180215_20180214HPM8_160_04.RCC
37	20180215_20180214HPM8_164_08.RCC

	File Name
38	20180215_20180214HPM8_166_10.RCC
39	20180215_20180214HPM8_168_12.RCC
40	20180215_20180214HPM9_170_02.RCC
41	20180215_20180214HPM9_172_04.RCC
42	20180215_20180214HPM9_174_06.RCC
43	20180215_20180214HPM9_176_08.RCC
44	20180215_20180214HPM9_178_10.RCC
45	20180215_20180214HPM9_180_12.RCC
46	20180215_20180214HPM10_184_04.RCC
47	20180215_20180214HPM10_187_07.RCC
48	20180215_20180214HPM10_192_12.RCC
49	20180109_20180108HSD2_BRS3_03.RCC
50	20180109_20180108HSD2_BRS4_04.RCC
51	20180109_20180108HSD2_BRS5_05.RCC
52	20180109_20180108HSD2_BRS6_06.RCC

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2. Sestak I, et al. Prediction of Late Distant Recurrence After 5 Years of Endocrine Treatment: A Combined Analysis of Patients From the Austrian Breast and Colorectal Cancer Study Group 8 and Arimidex, Tamoxifen Alone or in Combination Randomized Trials Using the PAM50 Risk of Recurrence Score. Journal of Clinical Oncology 2016, 33(8): 916-922
3. Parker JS, et al. Supervised Risk Predictor of Breast Cancer Based on Intrinsic Subtypes. Journal of Clinical Oncology 2009, 27(8): 1160-1167.
4. Geiss G, et al. Direct multiplexed measurement of gene expression with color-coded probe pairs Nature Biotechnology 2008; 26: 317-325.