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14. ABSTRACT

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RPPR Final Report
as of 01-Dec-2020

Agency Code:

Proposal Number: 75109LSDRP **Agreement Number: W911NF-19-2-0123**

INVESTIGATOR(S):

Name: Todd Michael
Email: tmichael@jcvi.org
Phone Number: 8582001864
Principal: Y

Organization: **J. Craig Venter Institute**
Address: 9704 Medical Center Drive, Rockville, MD 208503433
Country: USA

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Begin Performance Period: 02-Apr-2019 **End Performance Period:** 01-Apr-2020
Report Term: 0-Other
Submitted By: Harriet Danko Email: hdanko@jcvi.org
Phone: (000) 000-0000
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STEM Degrees: **STEM Participants:**

Major Goals: The major goal of this work is to generate longitudinal epigenomic datasets for a group of individuals going through an elite selection process. Blood will be drawn from individuals going through elite selection at two times points, peripheral blood mononuclear cells (PBMCs) will be isolated and frozen, and these samples will be sent to JCVI for DNA methylation sequencing. The PMCs will be counted and accessed for quality and viability. DNA will be extracted from PBMCs that pass quality control for DNA methylation sequencing. DNA will be bisulfite converted to detect methylated bases and sequenced. Serum samples will be used to measure the metabolome. DNA methylation calls and metabolic results will be delivered.

Accomplishments: 144 tubes containing frozen PBMCs were received at JCVI representing two time points called blood draw (BD) 3 and BD4 (from 2018 cohort; BD3_2018 and BD4_2018). Only one tube was sent with all of the PBMCs and no back up samples were collected by the air force (AF). Therefore, we generated test PBMCs at JCVI to build a splitting protocol that would allow us to split the PBMC samples so these precious singleton samples would not be lost. This protocol had to be specific to the conditions and buffers that the PBMCs were stored in by the AF, which differed from our protocols at JCVI. Once a splitting protocol was established, we measured the number of cells and whether they were alive for the 144 PBMCs (BD3_2018 and BD4_2018). The cell counting effort revealed that the number of PBMCs was very low for many samples and some had no cells at all (Table 1). DNA extraction and metabolomic work require greater than 8 million (M) cells and only 8% of the samples passed this criterion (Table 2). The PBMCs were also tested for viability (Table 3) and the live/total ratio was calculated (Table 4), which was also low and variable across the samples. Therefore, we reached out to DARPA and the AF to determine a path forward. There was a meeting between DARPA and JCVI where methodologies for optimizing PBMC isolation were discussed and then implemented by the AF in a new cohort. The AF also sent all samples from BD5 2018 (BD5_2018) for testing to see if these could be used for DNA methylation analysis. The first batch sent was serum samples in triplicate (not PBMCs) and some tubes were not sealed properly so they opened during shipping. The AF was contacted, and they sent the proper PMBC samples, which were received and checked in at JCVI. We tested BD5_2018 for cell counts and they ranged from 900,000 to 4,000,000 cells, which was still lowered than required for DNA methylation (Table 5). Also, the serum samples for

BD5_2018 were sent to JCVI and arrived with some tubes open making these serum samples useless for metabolic studies.
The AF took cell counts for new samples collected in 2019 and sent five (5) samples from BD1_2019 and five (5) samples from BD5_2019. The 10 samples were checked for cell count and viability (Table 5). The AF measured cell counts for BD5_2019, which were higher than previous samples but still under 8M cells. We rechecked these cell counts and found that our counts were about 25% of that reported by the AF (Table 5); this is consistent with the previous AF cell counts being off or a loss of cells in transit. Moreover, of the cells we found, only 20% of them were alive (Table 6). To address the low level of cells, we validated a new DNA methylation sequencing kit that would enable low input: Swift Accel-NGS® Methyl-Seq DNA Library Kit. Since we had not used this kit before we validated the kit. In addition, we determined how many PBMCs we would need to obtain the 100 pg DNA required to run through the Swift DNA methylation kit. We found that 80,000 cells were the minimum cells required to reliably obtain 100 pg of DNA (Figure 1).
We received three timepoints of PBMC samples from the 2019 cohort: BD2_2019, BD3_2019 and BD4_2019. Serum for the BD2_2019 and BD3_2019 were subjected to metabolic analysis. DNA was extracted for 56 samples from BD2_2019 and BD3_2019 each for a total of 112 DNA samples (Table 7). Samples that did not produce enough DNA for the Swift DNA methylation kit were re-extracted. DNA was subjected to bisulfite conversation and sequencing libraries were constructed with the Swift DNA methylation kit. Subsequent sequencing libraries were quality checked (QC), quantified and validated on an Illumina MiSeq run. Libraries passing QC were balanced and sequenced on the Illumina NovaSeq platform. Resulting sequence was subjected to QC, and read coverage was estimated to determine if additional sequence was required for any samples (based on quality and coverage). The sequence that was of high quality and of sufficient coverage was then it was processed through the standard bisulfite sequencing pipeline based on the BISMARK software package. The sequencing data was loaded onto an encrypted hard drive and mailed to LLNL. The metabolic data was shared with LLNL through a web portal.

Training Opportunities: Nothing to Report

Results Dissemination: The data was disseminated to the Lawrence Livermore National Lab (LLNL).

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

ARMY RESEARCH OFFICE (ARO)

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Accomplishments

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The following sections are required: (each section is limited to 8000 character plain text, images, tables, charts, or other graphics in support of the Accomplishments section may be uploaded as 1 combined attachment.)

What were the major goals and objectives of the project?

The major goal of this work is to generate longitudinal epigenomic datasets for a group of individuals going through an elite selection process. Blood will be drawn from individuals going through elite selection at two times points, peripheral blood mononuclear cells (PBMCs) will be isolated and frozen, and these samples will be sent to JCVI for DNA methylation sequencing. The PMCs will be counted and accessed for quality and viability. DNA will be extracted from PBMCs that pass quality control for DNA methylation sequencing. DNA will be bisulfite converted to detect methylated bases and sequenced. Serum samples will be used to measure the metabolome. DNA methylation calls and metabolic results will be delivered.

What was accomplished towards achieving these goals?

144 tubes containing frozen PBMCs were received at JCVI representing two time points called blood draw (BD) 3 and BD4 (from 2018 cohort; BD3_2018 and BD4_2018). Only one tube was sent with all of the PBMCs and no back up samples were collected by the air force (AF). Therefore, we generated test PBMCs at JCVI to build a splitting protocol that would allow us to split the PBMC samples so these precious singleton samples would not be lost. This protocol had to be specific to the conditions and buffers that the PBMCs were stored in by the AF, which differed from our protocols at JCVI. Once a

splitting protocol was established, we measured the number of cells and whether they were alive for the 144 PBMCs (BD3_2018 and BD4_2018). The cell counting effort revealed that the number of PBMCs was very low for many samples and some had no cells at all (Table 1). DNA extraction and metabolomic work require greater than 8 million (M) cells and only 8% of the samples passed this criterion (Table 2). The PBMCs were also tested for viability (Table 3) and the live/total ratio was calculated (Table 4), which was also low and variable across the samples. Therefore, we reached out to DARPA and the AF to determine a path forward. There was a meeting between DARPA and JCVI where methodologies for optimizing PBMC isolation were discussed and then implemented by the AF in a new cohort. The AF also sent all samples from BD5 2018 (BD5_2018) for testing to see if these could be used for DNA methylation analysis. The first batch sent was serum samples in triplicate (not PBMCs) and some tubes were not sealed properly so they opened during shipping. The AF was contacted, and they sent the proper PMBC samples, which were received and checked in at JCVI. We tested BD5_2018 for cell counts and they ranged from 900,000 to 4,000,000 cells, which was still lowered than required for DNA methylation (Table 5). Also, the serum samples for BD5_2018 were sent to JCVI and arrived with some tubes open making these serum samples useless for metabolic studies.

The AF took cell counts for new samples collected in 2019 and sent five (5) samples from BD1_2019 and five (5) samples from BD5_2019. The 10 samples were checked for cell count and viability (Table 5). The AF measured cell counts for BD5_2019, which were higher than previous samples but still under 8M cells. We rechecked these cell counts and found that our counts were about 25% of that reported by the AF (Table 5); this is consistent with the previous AF cell counts being off or a loss of cells in transit. Moreover, of the cells we found, only 20% of them were alive (Table 6). To address the low level of cells, we validated a new DNA methylation sequencing kit that would enable low input: Swift Accel-NGS® Methyl-Seq DNA Library Kit. Since we had not used this kit before we validated the kit. In addition, we determined how many PBMCs we would need to obtain the 100 pg DNA required to run through the Swift DNA methylation kit. We found that 80,000 cells were the minimum cells required to reliably obtain 100 pg of DNA (Figure 1).

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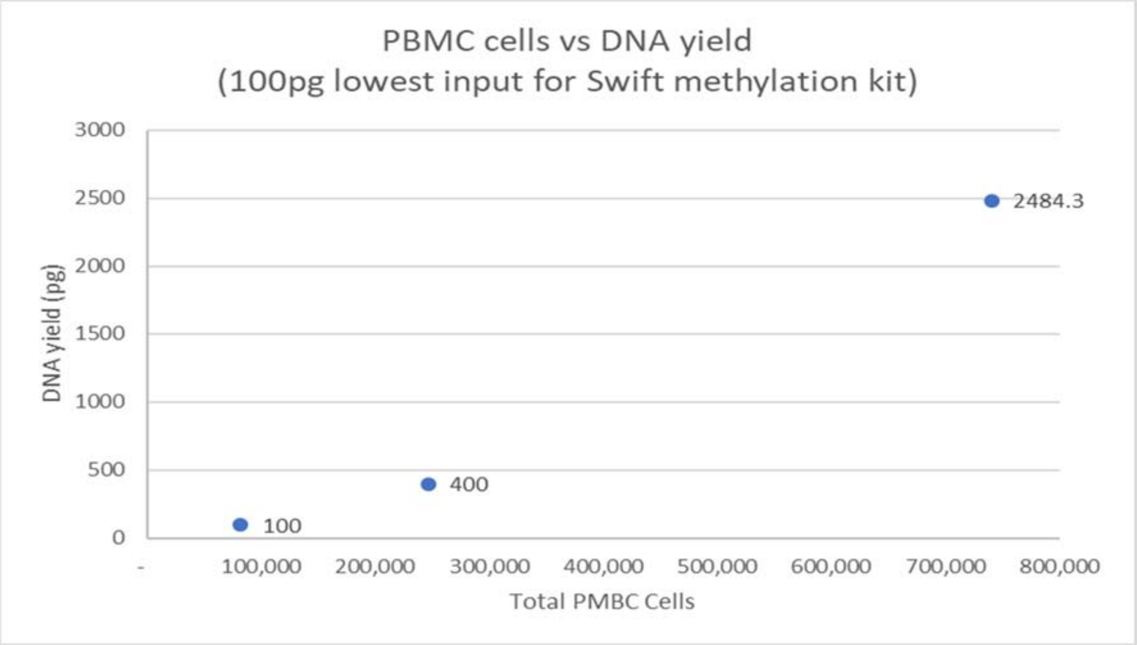

Figure 1. The number PBMCs required for DNA methylation sequencing. Test PBMCs were used to extract DNA at different concentrations revealing that at least 80,000 cells are required for 100 pg of DNA.

Table 2. Cell counts meeting requirement.

	# Cells				
	>8M	1-8M	0.1M-1M	<100K	
BD3	1	24	20	27	
BD4	10	37	21	1	
Total	11	61	41	28	141
% of samples	8%	43%	29%	20%	100%

Table 1. Cell counts.

Subject	Total cells	Total cells
1	-	
2	-	1,087,500
3	-	5,312,500
4	-	625,000
5	137,500	2,850,000
6	87,500	250,000
7	75,000	1,000,000
8	12,500	3,475,000
9	25,000	375,000
10	-	387,500
11	12,500	1,112,500
12	62,500	18,237,500
13	-	8,862,500
14	25,000	150,000
15	37,500	2,887,500
16	75,000	3,400,000
17	62,500	5,462,500
18	37,500	1,850,000
19	575,000	6,325,000
20	312,500	6,162,500
21	-	50,000
22	25,000	212,500
23	87,500	10,675,000
24	25,000	4,137,500
25	50,000	16,325,000
26	25,000	7,137,500
27	6,762,500	4,637,500
28	37,500	3,037,500
29	3,812,500	3,250,000
30	6,337,500	3,362,500
31	7,275,000	537,500
32	4,712,500	962,500
33	325,000	10,175,000
34	2,400,000	8,975,000
35	5,500,000	4,700,000
36	437,500	512,500
37	1,187,500	4,562,500
38	437,500	337,500
39	450,000	437,500
40	300,000	375,000
41	462,500	325,000
42	450,000	350,000
43	8,800,000	
44	787,500	875,000
45	1,800,000	325,000
46	6,412,500	5,575,000
47	6,187,500	1,487,500
48	2,300,000	4,800,000
49	800,000	2,750,000
50	1,400,000	8,937,500
51	6,662,500	2,150,000
52	962,500	2,962,500
53	1,087,500	3,600,000
54	7,100,000	
55	687,500	20,950,000
56	1,100,000	350,000
57	1,100,000	350,000
58	112,500	5,187,500
59	2,300,000	962,500
60	437,500	3,625,000
61	2,212,500	2,125,000
62	12,500	1,312,500
63	3,162,500	9,800,000
64	25,000	22,487,500
65	-	1,450,000
66	200,000	2,612,500
67	625,000	712,500
68	3,787,500	2,200,000
69	1,737,500	1,525,000
70	500,000	3,125,000
71	300,000	2,500,000
72	3,062,500	1,037,500

Table 3. Cell counts to determine the percent of live cells.

Subject	BD3 Live Cells	BD3 Total Cells Counted	BD4 Live Cells	BD4 Total Cells Counted
1	0	0		
2	0	0	56	87
3	0	0	284	425
4	0	0	28	50
5	2	11	172	228
6	1	7	4	20
7	1	6	57	80
8	1	1	222	278
9	0	2	18	30
10	0	0	22	31
11	0	1	71	89
12	1	5	952	1459
13	0	0	561	709
14	2	2	8	12
15	3	3	157	231
16	1	6	201	272
17	3	5	323	437
18	1	3	128	148
19	12	46	462	506
20	8	25	387	493
21	0	0	2	4
22	0	2	12	17
23	4	7	747	854
24	1	2	287	331
25	2	4	1155	1306
26	2	2	475	571
27	370	541	302	371
28	3	3	202	243
29	160	305	160	260
30	374	507	179	269
31	457	582	27	43
32	332	377	58	77
33	12	26	678	814
34	150	192	631	718
35	372	440	302	376
36	28	35	39	41
37	87	95	339	365
38	25	35	21	27
39	32	36	26	35
40	21	24	21	30
41	26	37	23	26
42	34	36	24	28
43	90	176		
44	58	63	48	70
45	123	144	24	26
46	471	513	411	446
47	447	495	100	119
48	170	184	323	384
49	60	64	185	220
50	108	112	710	715
51	516	533	146	172
52	63	77	212	237
53	65	87	272	288
54	51	142		
55	45	55	1672	1676
56	1	2	0	0
57	61	88	19	28
58	3	9	268	415
59	118	184	48	77
60	7	35	212	290
61	119	177	133	170
62	1	1	77	105
63	173	253	698	784
64	1	2	1586	1799
65	0	0	82	116
66	7	41	516	663
67	47	50	24	57
68	246	303	166	176
69	121	139	84	122
70	33	40	212	250
71	19	24	165	200
72	222	245	64	83

Table 4. Live to total cell ratio.

Subject	Bd3_ratio	Bd4_ratio
1	NA	NA
2	NA	64.4%
3	NA	66.8%
4	NA	56.0%
5	18.2%	75.4%
6	14.3%	20.0%
7	16.7%	71.3%
8	100.0%	79.9%
9	0.0%	60.0%
10	NA	71.0%
11	0.0%	79.8%
12	20.0%	65.3%
13	NA	79.1%
14	100.0%	66.7%
15	100.0%	68.0%
16	16.7%	73.9%
17	60.0%	73.9%
18	33.3%	86.5%
19	26.1%	91.3%
20	32.0%	78.5%
21	NA	50.0%
22	0.0%	70.6%
23	57.1%	87.5%
24	50.0%	86.7%
25	50.0%	88.4%
26	100.0%	83.2%
27	68.4%	81.4%
28	100.0%	83.1%
29	52.5%	61.5%
30	73.8%	66.5%
31	78.5%	62.8%
32	88.1%	75.3%
33	46.2%	83.3%
34	78.1%	87.9%
35	84.5%	80.3%
36	80.0%	95.1%
37	91.6%	92.9%
38	71.4%	77.8%
39	88.9%	74.3%
40	87.5%	70.0%
41	70.3%	88.5%
42	94.4%	85.7%
43	51.1%	NA
44	92.1%	68.6%
45	85.4%	92.3%
46	91.8%	92.2%
47	90.3%	84.0%
48	92.4%	84.1%
49	93.8%	84.1%
50	96.4%	99.3%
51	96.8%	84.9%
52	81.8%	89.5%
53	74.7%	94.4%
54	35.9%	NA
55	81.8%	99.8%
56	50.0%	NA
57	69.3%	67.9%
58	33.3%	64.6%
59	64.1%	62.3%
60	20.0%	73.1%
61	67.2%	78.2%
62	100.0%	73.3%
63	68.4%	89.0%
64	50.0%	88.2%
65	NA	70.7%
66	17.1%	77.8%
67	94.0%	42.1%
68	81.2%	94.3%
69	87.1%	68.9%
70	82.5%	84.8%
71	79.2%	82.5%
72	90.6%	77.1%

Table 5. BD5_2018 samples tested for cells.

Type	Subject	Stock Tube cells/mL	% Live	% Dead
BD5-18	1	572,314	2	98
BD5-18	5	1,575,000	10	90
BD5-18	6	888,172	15	85
BD5-18	7	267,871	17	83
BD5-18	8	631,821	11	89
BD5-18	11	613,560	29	71
BD5-18	12	318,750	13	87
BD5-18	13	137,065	39	61
BD5-18	15	1,607,931	7	93
BD5-18	16	841,537	9	91
BD5-18	17	3,800,000	30	70
BD5-18	18	881,347	9	91
BD5-18	19	1,070,183	10	90
BD5-18	23	2,742,188	5	95
BD5-18	24	244,471	17	83
BD5-18	25	396,311	9	91
BD5-18	26	1,500,000	6	94
BD5-18	27	2,719,444	9	91
BD5-18	28	473,390	12	88
BD5-18	29	719,298	31	69
BD5-18	31	4,412,500	25	75
BD5-18	32	923,529	14	86
BD5-18	33	2,111,464	7	93
BD5-18	34	1,786,458	10	90
BD5-18	36	1,721,324	19	81
BD5-18	37	3,090,744	14	86
BD5-18	39	3,100,000	14	86
BD5-18	40	85,294	40	60
BD5-18	41	1,850,168	17	83
BD5-18	43	2,975,830	10	90
BD5-18	44	2,966,602	5	95
BD5-18	46	2,051,361	6	94
BD5-18	47	3,937,500	13	87
BD5-18	48	743,356	7	93
BD5-18	49	1,716,765	9	91
BD5-18	50	858,350	15	85
BD5-18	51	842,434	10	90
BD5-18	52	1,600,160	10	90
BD5-18	53	3,347,018	8	92
BD5-18	55	571,565	7	93
BD5-18	56	359,297	15	85
BD5-18	57	590,087	8	92
BD5-18	58	544,319	8	92
BD5-18	59	235,432	27	73
BD5-18	60	374,269	11	89
BD5-18	61	973,684	5	95
BD5-18	62	1,212,500	23	77
BD5-18	63	2,940,337	7	93
BD5-18	64	2,821,256	16	84
BD5-18	65	707,009	5	95
BD5-18	68	962,500	22	78
BD5-18	69	1,068,755	7	93
BD5-18	70	743,501	20	80
BD5-18	71	688,000	17	83

Table 6. BD5 cell counts taken by AF.

Type	Subject	Stock Tube cells/mL	% Live	% Dead	AF cell counts	
BD1-19	1	2,671,535	35	65	NA	
BD1-19	7	7,170,704	33	67	NA	
BD1-19	8	9,402,174	29	71	NA	
BD1-19	31	19,325,714	35	65	NA	
BD1-19	45	996,820	25	75	NA	
						%
BD5-19	1	1,455,354	20	80	4,150,000	35.1%
BD5-19	7	654,444	22	78	3,900,000	16.8%
BD5-19	8	1,832,620	21	79	7,150,000	25.6%
BD5-19	31	1,202,924	26	74	5,600,000	21.5%
BD5-19	45	632,330	28	72	7,000,000	9.0%

NA= not availble

Table 7. DNA concentrations for BD3_2019 and BD4_2019.

BD #	Subject	Tube Name	Concentrations (ng/uL)	Volume (uL)	BD #	Subject	Tube Name	Concentrations (ng/uL)	Volume (uL)
BD2-19	1	BD2-19-1	5.7	69.7	BD3-19	1	BD3-19-1	Too Low	
BD2-19	2	BD2-19-2	6.2	69.7			BD3-19-1_2	0.2	19.0
		BD2-19-2_2	0.1	20.0	BD3-19	2	BD3-19-2	Too Low	
BD2-19	3	BD2-19-3	31.4	92.9			BD3-19-2_2	0.1	21.0
BD2-19	4	BD2-19-4	109.0	98.0	BD3-19	3	BD3-19-3	0.6	68.0
BD2-19	5	BD2-19-5	66.0	93.6	BD3-19	4	BD3-19-4	0.0	78.0
BD2-19	6	BD2-19-6	14.5	86.9	BD3-19	5	BD3-19-5	118.0	92.0
BD2-19	7	BD2-19-7	72.9	93.8	BD3-19	6	BD3-19-6	54.0	94.0
BD2-19	8	BD2-19-8	16.5	88.2	BD3-19	7	BD3-19-7	0.2	98.0
BD2-19	9	BD2-19-9	6.0	71.0	BD3-19	8	BD3-19-8	0.4	82.0
BD2-19	10	BD2-19-10	0.1	78.0	BD3-19	9	BD3-19-9	63.2	94.0
BD2-19	11	BD2-19-11	56.9	93.2	BD3-19	10	BD3-19-10	0.0	78.0
BD2-19	12	BD2-19-12	47.0	94.6	BD3-19	11	BD3-19-11	29.8	94.0
BD2-19	13	BD2-19-13	0.1	72.5	BD3-19	12	BD3-19-12	26.7	94.0
BD2-19	14	BD2-19-14	48.0	98.0	BD3-19	13	BD3-19-13	0.1	78.0
BD2-19	15	BD2-19-15	51.3	92.9	BD3-19	14	BD3-19-14	0.8	89.0
BD2-19	16	BD2-19-16	3.6	53.8	BD3-19	15	BD3-19-15	21.8	94.0
BD2-19	17	BD2-19-17	0.5	84.0	BD3-19	16	BD3-19-16	0.2	98.0
BD2-19	18	BD2-19-18	2.6	35.4	BD3-19	17	BD3-19-17	0.5	81.0
		BD2-19-18_2	0.5	84.0	BD3-19	18	BD3-19-18	0.4	89.0
BD2-19	19	BD2-19-19	55.6	98.0	BD3-19	19	BD3-19-19	0.2	98.0
BD2-19	20	BD2-19-20	0.5	84.0	BD3-19	20	BD3-19-20	0.2	89.0
BD2-19	21	BD2-19-21	11.1	83.5	BD3-19	21	BD3-19-21	206.0	92.0
BD2-19	22	BD2-19-22	0.3	87.0	BD3-19	22	BD3-19-22	10.7	81.5
BD2-19	23	BD2-19-23	10.9	85.3	BD3-19	23	BD3-19-23	0.2	93.0
BD2-19	24	BD2-19-24	21.4	90.5	BD3-19	24	BD3-19-24	2.2	94.0
BD2-19	25	BD2-19-25	0.2	98.0	BD3-19	25	BD3-19-25	48.4	94.0
BD2-19	26	BD2-19-26	9.3	94.0	BD3-19	26	BD3-19-26	12.4	94.0
BD2-19	27	BD2-19-27	30.2	94.0	BD3-19	27	BD3-19-27	0.5	89.0
BD2-19	28	BD2-19-28	Too Low		BD3-19	28	BD3-19-28	0.7	92.0
BD2-19		BD2-19-28_2	0.2	19.0	BD3-19	29	BD3-19-29	3.3	94.0
BD2-19	29	BD2-19-29	27.7	94.0	BD3-19	30	BD3-19-30	203.0	92.0
BD2-19	30	BD2-19-30	58.0	94.0	BD3-19	31	BD3-19-31	60.9	92.0
BD2-19	31	BD2-19-31	45.7	94.0	BD3-19	32	BD3-19-32	59.5	94.0
BD2-19	32	BD2-19-32	48.2	94.0	BD3-19	33	BD3-19-33	23.2	94.0
BD2-19	33	BD2-19-33	3.9	55.2	BD3-19	34	BD3-19-34	11.8	94.0
BD2-19	34	BD2-19-34	47.2	94.0	BD3-19	35	BD3-19-35	0.2	94.0
BD2-19	35	BD2-19-35	0.4	90.0	BD3-19	36	BD3-19-36	41.5	98.0
BD2-19	36	BD2-19-36	21.2	94.0	BD3-19	37	BD3-19-37	53.0	98.0
BD2-19	37	BD2-19-37	7.5	94.0	BD3-19	38	BD3-19-38	257.0	93.9
BD2-19	38	BD2-19-38	18.1	94.0	BD3-19	39	BD3-19-39	35.5	96.0
BD2-19	39	BD2-19-39	14.3	94.0	BD3-19	40	BD3-19-40	104.0	96.0
BD2-19	40	BD2-19-40	1.8	94.0	BD3-19	41	BD3-19-41	0.4	78.0
BD2-19	41	BD2-19-41	22.8	94.0	BD3-19	42	BD3-19-42	3.8	98.0
BD2-19	42	BD2-19-42	0.2	98.0	BD3-19	43	BD3-19-43	58.5	96.0
BD2-19	43	BD2-19-43	0.2	98.0	BD3-19	44	BD3-19-44	1.3	98.0
BD2-19	44	BD2-19-44	0.5	80.0	BD3-19	45	BD3-19-45	2.9	41.6
BD2-19	45	BD2-19-45	7.7	94.0	BD3-19	46	BD3-19-46	446.0	96.0
BD2-19	46	BD2-19-46	5.3	94.0	BD3-19	47	BD3-19-47	59.0	96.0
BD2-19	47	BD2-19-47	32.0	94.0	BD3-19	48	BD3-19-48	74.4	96.0
BD2-19	48	BD2-19-48	7.3	94.0	BD3-19	49	BD3-19-49	41.4	96.0
BD2-19	49	BD2-19-49	1.9	94.0	BD3-19	50	BD3-19-50	118.0	96.0
BD2-19	50	BD2-19-50	10.3	94.0	BD3-19	51	BD3-19-51	28.2	96.0
BD2-19	51	BD2-19-51	13.1	94.0	BD3-19	52	BD3-19-52	0.4	98.0
BD2-19	52	BD2-19-52	4.3	94.0	BD3-19	53	BD3-19-53	149.0	96.0
BD2-19	53	BD2-19-53	1.4	94.0	BD3-19	54	BD3-19-54	286.0	96.0
BD2-19	54	BD2-19-54	34.4	98.0	BD3-19	55	BD3-19-55	27.4	96.0
BD2-19	55	BD2-19-55	1.1	98.0	BD3-19	56	BD3-19-56	101.0	96.0
BD2-19	56	BD2-19-56	1.4	96.5					

Figure 8. DNA methylation sequencing statistics

DNA Sample Name	Library Sample Name	Input going in BS (ng)	75% recovery of BS DNA (ng)	Swift protocol suggested PCR cycles	PCR Cycles	qubit concentration for what we pooled (pM)	%PF	M PE Reads	Coverage
BD2-19-5	BD2_19_5-PE-D708D504-1	76?	n/a	n/a	5	1,634.5	85	460.4	46
BD2-19-33	BD2_19_33-PE-D708D501-1	131.4	98.55	5	9	1,994.0		508.1	51
BD2-19-7	BD2_19_7-PE-D709D504-1	104.8	78.6	8	12	5,582.7		921.6	93
BD2-19-8	BD2_19_8-PE-D709D505-1	103.6	77.7	8	12	6,683.5		643.4	65
BD3-19-38	BD3_19_38-PE-D711D507-1	100	75	8	12	2,769.8		401.0	40
BD2-19-11	BD2_19_11-PE-D709D507-1	98.4	73.8	8	12	3,682.8		642.1	65
BD2-19-9	BD2_19_9-PE-D709D506-1	98.2	73.65	8	12	3,283.8		1065.0	107
BD2-19-15	BD2_19_15-PE-D710D501-1	95.8	71.85	8	12	1,400.7		493.7	50
BD2-19-3	BD2_19_3-PE-D708D502-1	88.8	66.6	8	12	4,852.0		868.8	87
BD3-19-22	BD3_19_22-PE-D711D506-1	86.4	64.8	8	12	3,269.9		848.9	85
BD2-19-6	BD2_19_6-PE-D708D503-1	86.2	64.65	8	12	5,049.5		701.7	71
BD2-19-24	BD2_19_24-PE-D711D505-1	85.6	64.2	8	12	2,144.3		435.0	44
BD3-19-45	BD3_19_45-PE-D712D508-1	50	37.5	8	12	2,545.0		637.4	64
BD2-19-21	BD2_19_21-PE-D711D504-1	20.4	15.3	8	12	1,290.5		434.2	44
BD2-19-14	BD2_19_14-PE-D712D503-1	1.24	0.93	12	16	1,158.5		776.2	78
BD2-19-4	BD2_19_4-PE-D712D501-1	1.72	1.29	12	16	1,570.4		463.5	47
BD2-19-16	BD2_19_16-PE-D712D502-1	7.82	5.865	12	16	2,214.1		975.4	98
BD2-19-17	BD2_19_17-PE-D701D501-6	18.9	14.175	8	12	9,211.4	81	407.6	41
BD2-19-20	BD2_19_20-PE-D702D502-6	25.35	19.0125	8	12	6,462.2		378.3	38
BD2-19-22	BD2_19_22-PE-D703D503-6	16.2	12.15	8	12	3,154.3		337.9	34
BD2-19-35	BD2_19_35-PE-D704D504-6	21.45	16.0875	8	12	5,239.4		566.4	57
BD2-19-44	BD2_19_44-PE-D705D505-6	23.55	17.6625	8	12	6,617.0		383.3	39
BD3-19-3	BD3_19_3-PE-D706D506-6	22.65	16.9875	8	12	4,550.9		505.7	51
BD3-19-8	BD3_19_8-PE-D707D507-6	18.3	13.725	8	12	5,316.5		444.3	45
BD3-19-14	BD3_19_14-PE-D708D508-6	33.6	25.2	8	12	13,205.7		499.2	50
BD3-19-17	BD3_19_17-PE-D709D501-6	22.2	16.65	8	12	7,823.8		678.2	68
BD3-19-18	BD3_19_18-PE-D710D502-6	18.15	13.6125	8	12	11,920.5		494.4	50
BD3-19-27	BD3_19_27-PE-D711D503-6	20.1	15.075	8	12	11,859.7		513.1	52
BD3-19-28	BD3_19_28-PE-D712D504-6	29.25	21.9375	8	12	13,238.7		449.5	45
BD2-19-1	BD2_19_1-PE-D701D505-6	86.7	65.025	8	12	10,222.4		426.8	43
BD2-19-23	BD2_19_23-PE-D702D506-6	149.55	112.1625	8	12	11,780.4		595.3	60
BD2-19-26	BD2_19_26-PE-D703D507-6	90.6	67.95	8	12	15,552.9		381.4	38
BD2-19-27	BD2_19_27-PE-D704D508-6	79.8	59.85	8	12	6,234.3		419.2	42
BD2-19-29	BD2_19_29-PE-D705D501-6	83.7	62.775	8	12	11,927.4		540.8	54
BD2-19-30	BD2_19_30-PE-D706D502-6	83.4	62.55	8	12	6,097.8		480.6	48
BD2-19-31	BD2_19_31-PE-D707D503-6	82.2	61.65	8	12	6,036.6		572.6	58
BD2-19-32	BD2_19_32-PE-D708D504-6	79.8	59.85	8	12	9,965.0		358.8	36
BD2-19-34	BD2_19_34-PE-D709D505-6	82.2	61.65	8	12	15,176.7		341.2	34
BD2-19-36	BD2_19_36-PE-D710D506-6	85.65	64.2375	8	12	13,379.4		473.4	48
BD2-19-37	BD2_19_37-PE-D711D507-6	88.8	66.6	8	12	13,576.7		377.9	38
BD2-19-38	BD2_19_38-PE-D712D508-6	98.25	73.6875	8	12	14,458.2	87	530.9	53
BD2-19-39	BD2_19_39-PE-D701D501-6	609	456.75	8	12	6,339.6		65.3	7
BD2-19-40	BD2_19_40-PE-D702D502-6	90.15	67.6125	8	12	5,298.0		737.1	74
BD2-19-41	BD2_19_41-PE-D703D503-6	86.7	65.025	8	12	6,193.8		532.5	54
BD2-19-45	BD2_19_45-PE-D704D504-6	81.75	61.3125	8	12	4,086.5		667.3	67
BD2-19-46	BD2_19_46-PE-D705D505-6	98.7	74.025	8	12	3,268.0		669.1	67
BD2-19-47	BD2_19_47-PE-D706D506-6	77.7	58.275	8	12	6,006.3		473.8	48
BD2-19-48	BD2_19_48-PE-D707D507-6	91.95	68.9625	8	12	6,934.9		412.9	42
BD2-19-49	BD2_19_49-PE-D708D508-6	115.2	86.4	8	12	4,564.7		763.7	77
BD2-19-50	BD2_19_50-PE-D709D501-6	99.45	74.5875	8	12	13,259.1		569.2	57
BD2-19-51	BD2_19_51-PE-D710D502-6	106.05	79.5375	8	12	6,206.7		612.5	62
BD2-19-52	BD2_19_52-PE-D711D503-6	90.15	67.6125	8	12	6,061.6		417.2	42
BD3-19-5	BD3_19_5-PE-D712D504-6	127.95	95.9625	8	12	2,885.9		234.2	24
BD3-19-6	BD3_19_6-PE-D701D505-6	77.4	58.05	8	12	1,874.6		495.9	50
BD3-19-9	BD3_19_9-PE-D702D506-6	111.15	83.3625	8	12	1,960.5		672.8	68
BD3-19-11	BD3_19_11-PE-D703D507-6	81.3	60.975	8	12	10,960.3		218.0	22
BD3-19-12	BD3_19_12-PE-D704D508-6	75.75	56.8125	8	12	2,354.7		353.3	36
BD3-19-15	BD3_19_15-PE-D705D501-6	83.55	62.6625	8	12	7,450.8		406.9	41
BD3-19-21	BD3_19_21-PE-D706D502-6	132.45	99.3375	8	12	3,803.3		482.1	49
BD3-19-24	BD3_19_24-PE-D707D503-6	91.35	68.5125	8	12	9,354.1		493.7	50
BD3-19-25	BD3_19_25-PE-D708D504-6	78.6	58.95	8	12	13,913.0		162.9	16
BD3-19-26	BD3_19_26-PE-D709D505-6	93	69.75	8	12	14,466.1		404.4	41
BD3-19-29	BD3_19_29-PE-D710D506-6	72.45	54.3375	8	12	5,192.8		737.4	74

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How were the results disseminated to communities of interest?
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