Provenance Attestation of Human Cells Using Physical Unclonable Functions

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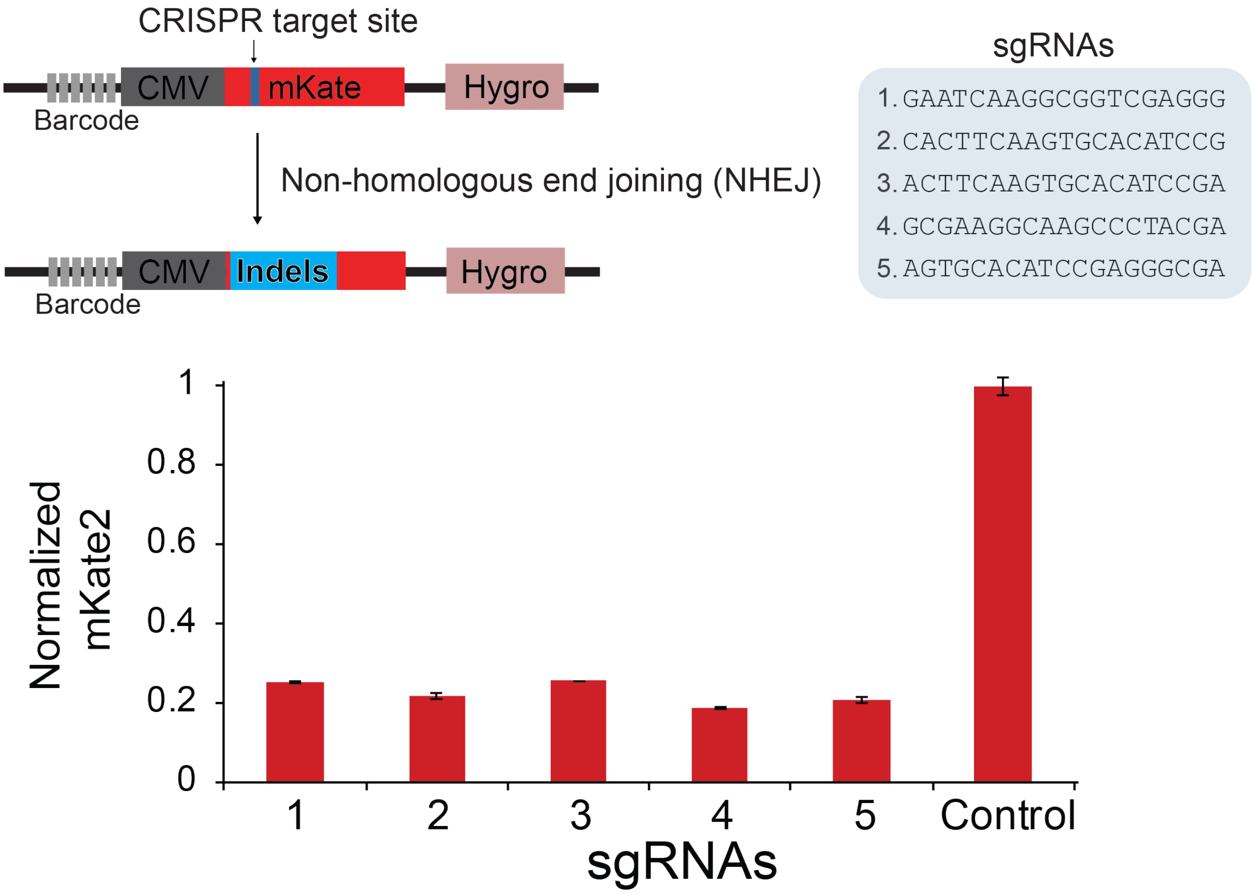
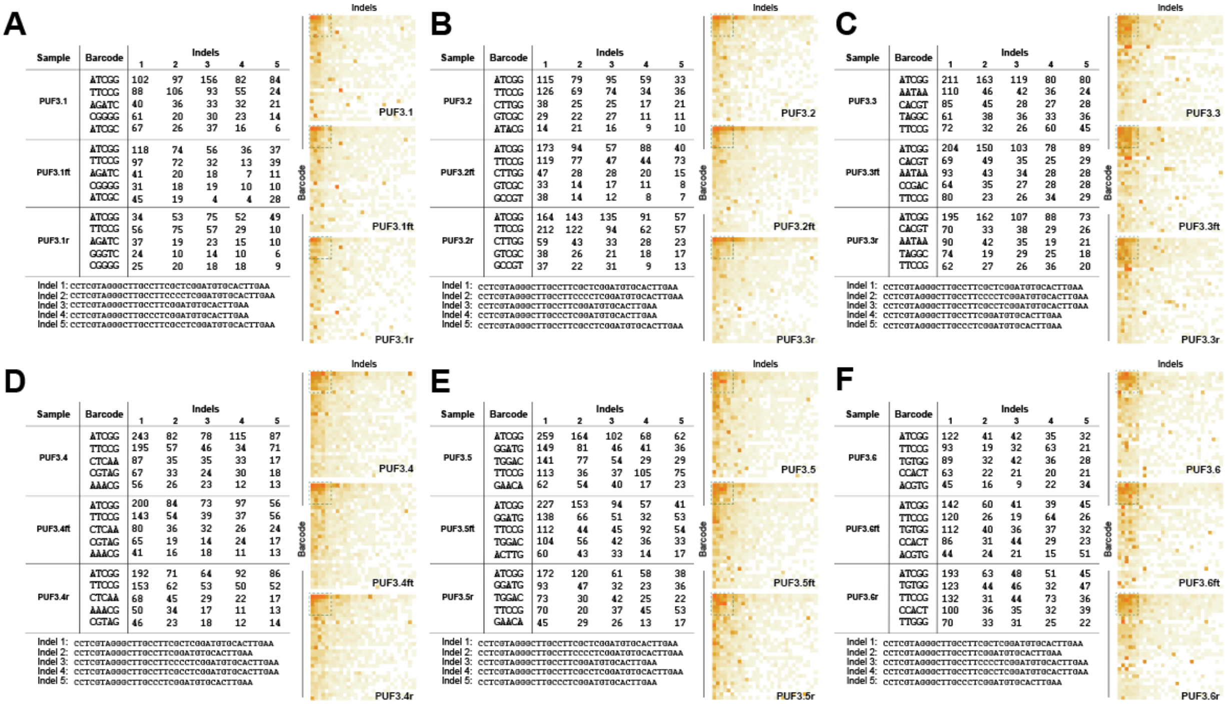


Supplementary Figure 1. Implementation of CREAM-PUFs in HEK293 cells. Five sgRNAs were designed to target the Open Reading Frames (ORFs) of the mKate2 construct, and demonstrated comparable efficiencies using *in vitro* fluorescence reporter assays.



A

Sample	Barcode	Indels 1	2	3	4	5
PUF3.1	ATCGG	102	97	156	82	84
	TTCCG	88	106	93	55	24
	AGATC	40	36	33	32	21
	CGGGG	61	20	30	23	14
	ATCGC	67	26	37	16	6
PUF3.1ft	ATCGG	118	74	56	36	37
	TTCCG	97	72	32	13	39
	AGATC	41	20	18	7	11
	CGGGG	31	18	19	10	10
	ATCGC	45	19	4	4	28
PUF3.1r	ATCGG	34	53	75	52	49
	TTCCG	56	75	57	29	10
	AGATC	37	19	23	15	10
	GGGTC	24	10	14	10	6
	CGGGG	25	20	18	18	9

Indel 1: CCTCGTAGGGCTTGCCTTCGCTCGGATGTGCACTTGAA
Indel 2: CCTCGTAGGGCTTGCCTTCCCCTCGGATGTGCACTTGAA
Indel 3: CCTCGTAGGGCTTGCCTTCGGATGTGCACTTGAA
Indel 4: CCTCGTAGGGCTTGCCCTCGGATGTGCACTTGAA
Indel 5: CCTCGTAGGGCTTGCCTTCGCCTCGGATGTGCACTTGAA

B

Sample	Barcode	Indels 1	2	3	4	5
PUF3.2	ATCGG	115	79	95	59	33
	TTCCG	126	69	74	34	36
	CTTGG	38	25	25	17	21
	GTCGC	29	22	27	11	11
	ATACG	14	21	16	9	10
PUF3.2ft	ATCGG	173	94	57	88	40
	TTCCG	119	77	47	44	73
	CTTGG	47	28	28	20	15
	GTCGC	33	14	17	11	8
	GCCGT	38	14	12	8	7
PUF3.2r	ATCGG	164	143	135	91	57
	TTCCG	212	122	94	62	57
	CTTGG	59	43	33	28	23
	GTCGC	38	26	21	18	17
	GCCGT	37	22	31	9	13

Indel 1: CCTCGTAGGGCTTGCCTTCGCTCGGATGTGCACTTGAA
Indel 2: CCTCGTAGGGCTTGCCTTCCCCTCGGATGTGCACTTGAA
Indel 3: CCTCGTAGGGCTTGCCTTCGGATGTGCACTTGAA
Indel 4: CCTCGTAGGGCTTGCCCTCGGATGTGCACTTGAA
Indel 5: CCTCGTAGGGCTTGCCTTCGCCTCGGATGTGCACTTGAA

C

Sample	Barcode	Indels 1	2	3	4	5
PUF3.3	ATCGG	211	163	119	80	80
	AATAA	110	46	42	36	24
	CACGT	85	45	28	27	28
	TAGGC	61	38	36	33	36
	TTCCG	72	32	26	60	45
PUF3.3ft	ATCGG	204	150	103	78	89
	CACGT	69	49	35	25	29
	AATAA	93	43	34	28	28
	CCGAC	64	35	27	28	28
	TTCCG	80	23	26	34	29
PUF3.3r	ATCGG	195	162	107	88	73
	CACGT	70	33	38	29	26
	AATAA	90	42	35	19	21
	TAGGC	74	19	29	25	18
	TTCCG	62	27	26	36	20

Indel 1: CCTCGTAGGGCTTGCCTTCGCTCGGATGTGCACTTGAA
Indel 2: CCTCGTAGGGCTTGCCTTCCCCTCGGATGTGCACTTGAA
Indel 3: CCTCGTAGGGCTTGCCTTCGCCTCGGATGTGCACTTGAA
Indel 4: CCTCGTAGGGCTTGCCTTCGGATGTGCACTTGAA
Indel 5: CCTCGTAGGGCTTGCCCTCGGATGTGCACTTGAA

D

Sample	Barcode	Indels 1	2	3	4	5
PUF3.4	ATCGG	243	82	78	115	87
	TTCCG	195	57	46	34	71
	CTCAA	87	35	35	33	17
	CGTAG	67	33	24	30	18
	AAACG	56	26	23	12	13
PUF3.4ft	ATCGG	200	84	73	97	56
	TTCCG	143	54	39	37	56
	CTCAA	80	36	32	26	24
	CGTAG	65	19	14	24	17
	AAACG	41	16	18	11	13
PUF3.4r	ATCGG	192	71	64	92	86
	TTCCG	153	62	53	50	52
	CTCAA	68	45	29	22	17
	AAACG	50	34	17	11	13
	CGTAG	46	23	18	12	14

Indel 1: CCTCGTAGGGCTTGCCTTCGCTCGGATGTGCACTTGAA
Indel 2: CCTCGTAGGGCTTGCCTTCGGATGTGCACTTGAA
Indel 3: CCTCGTAGGGCTTGCCTTCCCCTCGGATGTGCACTTGAA
Indel 4: CCTCGTAGGGCTTGCCTTCGCCTCGGATGTGCACTTGAA
Indel 5: CCTCGTAGGGCTTGCCCTCGGATGTGCACTTGAA

E

Sample	Barcode	Indels 1	2	3	4	5
PUF3.5	ATCGG	259	164	102	68	62
	GGATG	149	81	46	41	36
	TGGAC	141	77	54	29	29
	TTCCG	113	36	37	105	75
	GAACA	62	54	40	17	23
PUF3.5ft	ATCGG	227	153	94	57	41
	GGATG	138	66	51	32	53
	TTCCG	112	44	45	92	54
	TGGAC	104	56	42	36	33
	ACTTG	60	43	33	14	17
PUF3.5r	ATCGG	172	120	61	58	38
	GGATG	93	47	32	23	36
	TGGAC	73	30	42	25	22
	TTCCG	70	20	37	45	53
	GAACA	45	29	26	13	17

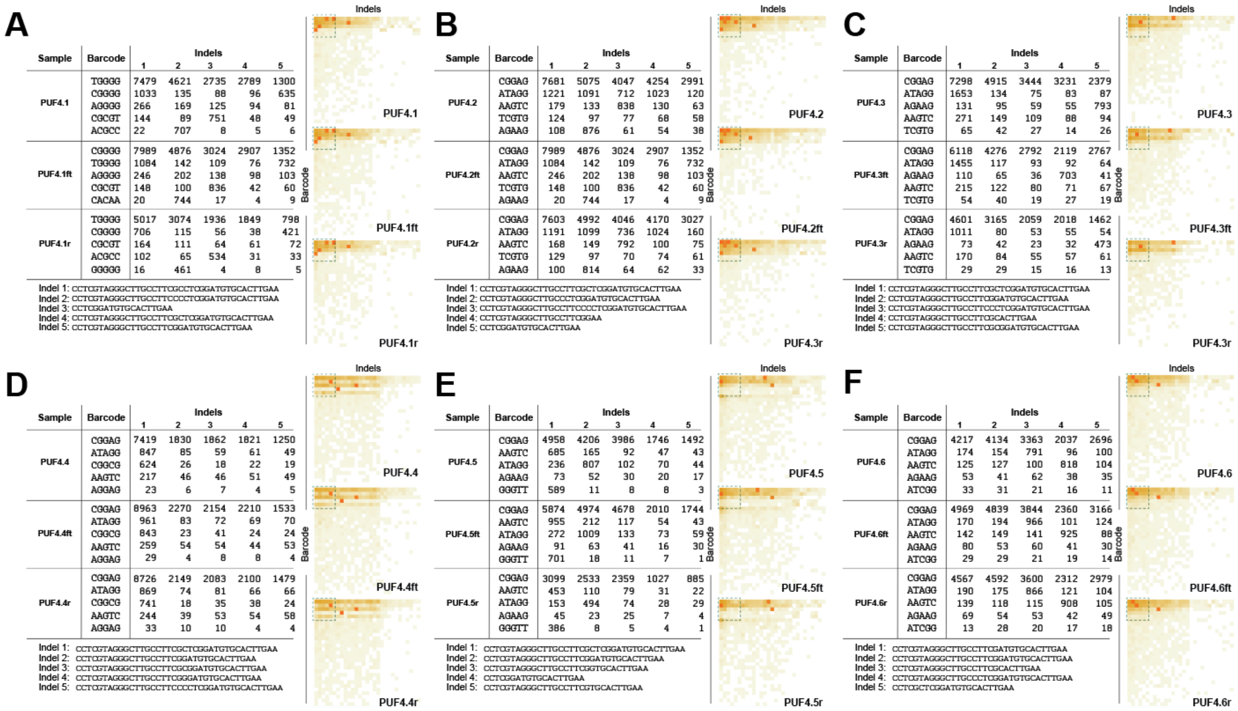
Indel 1: CCTCGTAGGGCTTGCCTTCGCTCGGATGTGCACTTGAA
Indel 2: CCTCGTAGGGCTTGCCTTCCCCTCGGATGTGCACTTGAA
Indel 3: CCTCGTAGGGCTTGCCTTCGGATGTGCACTTGAA
Indel 4: CCTCGTAGGGCTTGCCTTCGCCTCGGATGTGCACTTGAA
Indel 5: CCTCGTAGGGCTTGCCCTCGGATGTGCACTTGAA

F

Sample	Barcode	Indels 1	2	3	4	5
PUF3.6	ATCGG	122	41	42	35	32
	TTCCG	93	19	32	63	21
	TGTGG	89	32	42	36	28
	CCACT	63	22	21	20	21
	ACGTG	45	16	9	22	34
PUF3.6ft	ATCGG	142	60	41	39	45
	TTCCG	120	26	19	64	26
	TGTGG	112	40	36	37	32
	CCACT	86	31	44	29	23
	ACGTG	44	24	21	15	51
PUF3.6r	ATCGG	193	63	48	51	45
	TGTGG	123	44	46	32	47
	TTCCG	132	31	44	73	36
	CCACT	100	36	35	32	39
	TTGGG	70	33	31	25	22

Indel 1: CCTCGTAGGGCTTGCCTTCGCTCGGATGTGCACTTGAA
Indel 2: CCTCGTAGGGCTTGCCTTCGGATGTGCACTTGAA
Indel 3: CCTCGTAGGGCTTGCCTTCCCCTCGGATGTGCACTTGAA
Indel 4: CCTCGTAGGGCTTGCCTTCGCCTCGGATGTGCACTTGAA
Indel 5: CCTCGTAGGGCTTGCCCTCGGATGTGCACTTGAA

Supplementary Figure 2. Implementation of CREAM-PUFs in HCT116 cells. Qualitative assessment of CREAM-PUFs generated using HCT116. (A~E) Frequencies of barcode-indel addresses consisting of the 5 most commonly observed barcodes and indels (Left) and heatmap based on the same data but expanded to the top 30 most commonly observed barcodes and indels (Right) for a given PUF and its freeze-thaw counterparts and technical replicates. The green dashed square on the heatmap represents the data shown on the table. Data shown in (A) are barcode-indel addresses for PUF3.1 with their respective freeze-thaw counterpart and technical replicate. Data shown in (B~E) are for PUFs 3.2 to 3.6, respectively, which are produced identically to PUF3.1 using the same barcoded cell line and same sgRNA to introduce indels.



A

Sample	Barcode	Indels				
		1	2	3	4	5
PUF4.1	TGGGG	7479	4621	2735	2789	1300
	CGGGG	1033	135	88	96	635
	AGGGG	266	169	125	94	81
	CGCGT	144	89	751	48	49
	ACGCC	22	707	8	5	6
PUF4.1ft	CGGGG	7989	4876	3024	2907	1352
	TGGGG	1084	142	109	76	732
	AGGGG	246	202	138	98	103
	CGCGT	148	100	836	42	60
	CACAA	20	744	17	4	9
PUF4.1r	TGGGG	5017	3074	1936	1849	798
	CGGGG	706	115	56	38	421
	CGCGT	164	111	64	61	72
	ACGCC	102	65	534	31	33
	GGGGG	16	461	4	8	5

Indel 1: CCTCGTAGGGCTTGCCTTCGCCTCGGATGTGCACTTGAA
Indel 2: CCTCGTAGGGCTTGCCTTCCCCTCGGATGTGCACTTGAA
Indel 3: CCTCGGATGTGCACTTGAA
Indel 4: CCTCGTAGGGCTTGCCTTCGCTCGGATGTGCACTTGAA
Indel 5: CCTCGTAGGGCTTGCCTTCGGATGTGCACTTGAA

B

Sample	Barcode	Indels				
		1	2	3	4	5
PUF4.2	CGGAG	7681	5075	4047	4254	2991
	ATAGG	1221	1091	712	1023	120
	AAGTC	179	133	838	130	63
	TCGTG	124	97	77	68	58
	AGAAG	108	876	61	54	38
PUF4.2ft	CGGAG	7989	4876	3024	2907	1352
	ATAGG	1084	142	109	76	732
	AAGTC	246	202	138	98	103
	TCGTG	148	100	836	42	60
	AGAAG	20	744	17	4	9
PUF4.2r	CGGAG	7603	4992	4046	4170	3027
	ATAGG	1191	1099	736	1024	160
	AAGTC	168	149	792	100	75
	TCGTG	129	97	70	74	61
	AGAAG	100	814	64	62	33

Indel 1: CCTCGTAGGGCTTGCCTTCGCTCGGATGTGCACTTGAA
Indel 2: CCTCGTAGGGCTTGCCCTCGGATGTGCACTTGAA
Indel 3: CCTCGTAGGGCTTGCCTTCCCCTCGGATGTGCACTTGAA
Indel 4: CCTCGTAGGGCTTGCCTTCGGAA
Indel 5: CCTCGGATGTGCACTTGAA

C

Sample	Barcode	Indels				
		1	2	3	4	5
PUF4.3	CGGAG	7298	4915	3444	3231	2379
	ATAGG	1653	134	75	83	87
	AGAAG	131	95	59	55	793
	AAGTC	271	149	109	88	94
	TCGTG	65	42	27	14	26
PUF4.3ft	CGGAG	6118	4276	2792	2119	2767
	ATAGG	1455	117	93	92	64
	AGAAG	110	65	36	703	41
	AAGTC	215	122	80	71	67
	TCGTG	54	40	19	27	19
PUF4.3r	CGGAG	4601	3165	2059	2018	1462
	ATAGG	1011	80	53	55	54
	AGAAG	73	42	23	32	473
	AAGTC	170	84	55	57	61
	TCGTG	29	29	15	16	13

Indel 1: CCTCGTAGGGCTTGCCTTCGCTCGGATGTGCACTTGAA
Indel 2: CCTCGTAGGGCTTGCCTTCGGATGTGCACTTGAA
Indel 3: CCTCGTAGGGCTTGCCTTCCCTCGGATGTGCACTTGAA
Indel 4: CCTCGTAGGGCTTGCCTTCGCACTTGAA
Indel 5: CCTCGTAGGGCTTGCCTTCGCGGATGTGCACTTGAA

D

Sample	Barcode	Indels				
		1	2	3	4	5
PUF4.4	CGGAG	7419	1830	1862	1821	1250
	ATAGG	847	85	59	61	49
	CGGCG	624	26	18	22	19
	AAGTC	217	46	46	51	49
	AGGAG	23	6	7	4	5
PUF4.4ft	CGGAG	8963	2270	2154	2210	1533
	ATAGG	961	83	72	69	70
	CGGCG	843	23	41	24	24
	AAGTC	259	54	54	44	53
	AGGAG	29	4	8	8	4
PUF4.4r	CGGAG	8726	2149	2083	2100	1479
	ATAGG	869	74	81	66	66
	CGGCG	741	18	35	38	24
	AAGTC	244	39	53	54	58
	AGGAG	33	10	10	4	4

Indel 1: CCTCGTAGGGCTTGCCTTCGCTCGGATGTGCACTTGAA
Indel 2: CCTCGTAGGGCTTGCCTTCGGATGTGCACTTGAA
Indel 3: CCTCGTAGGGCTTGCCTTCGCGGATGTGCACTTGAA
Indel 4: CCTCGTAGGGCTTGCCTTCGGGATGTGCACTTGAA
Indel 5: CCTCGTAGGGCTTGCCTTCCCCTCGGATGTGCACTTGAA

E

Sample	Barcode	Indels				
		1	2	3	4	5
PUF4.5	CGGAG	4958	4206	3986	1746	1492
	AAGTC	685	165	92	47	43
	ATAGG	236	807	102	70	44
	AGAAG	73	52	30	20	17
	GGGTT	589	11	8	8	3
PUF4.5ft	CGGAG	5874	4974	4678	2010	1744
	AAGTC	955	212	117	54	43
	ATAGG	272	1009	133	73	59
	AGAAG	91	63	41	16	30
	GGGTT	701	18	11	7	1
PUF4.5r	CGGAG	3099	2533	2359	1027	885
	AAGTC	453	110	79	31	22
	ATAGG	153	494	74	28	29
	AGAAG	45	23	25	7	4
	GGGTT	386	8	5	4	1

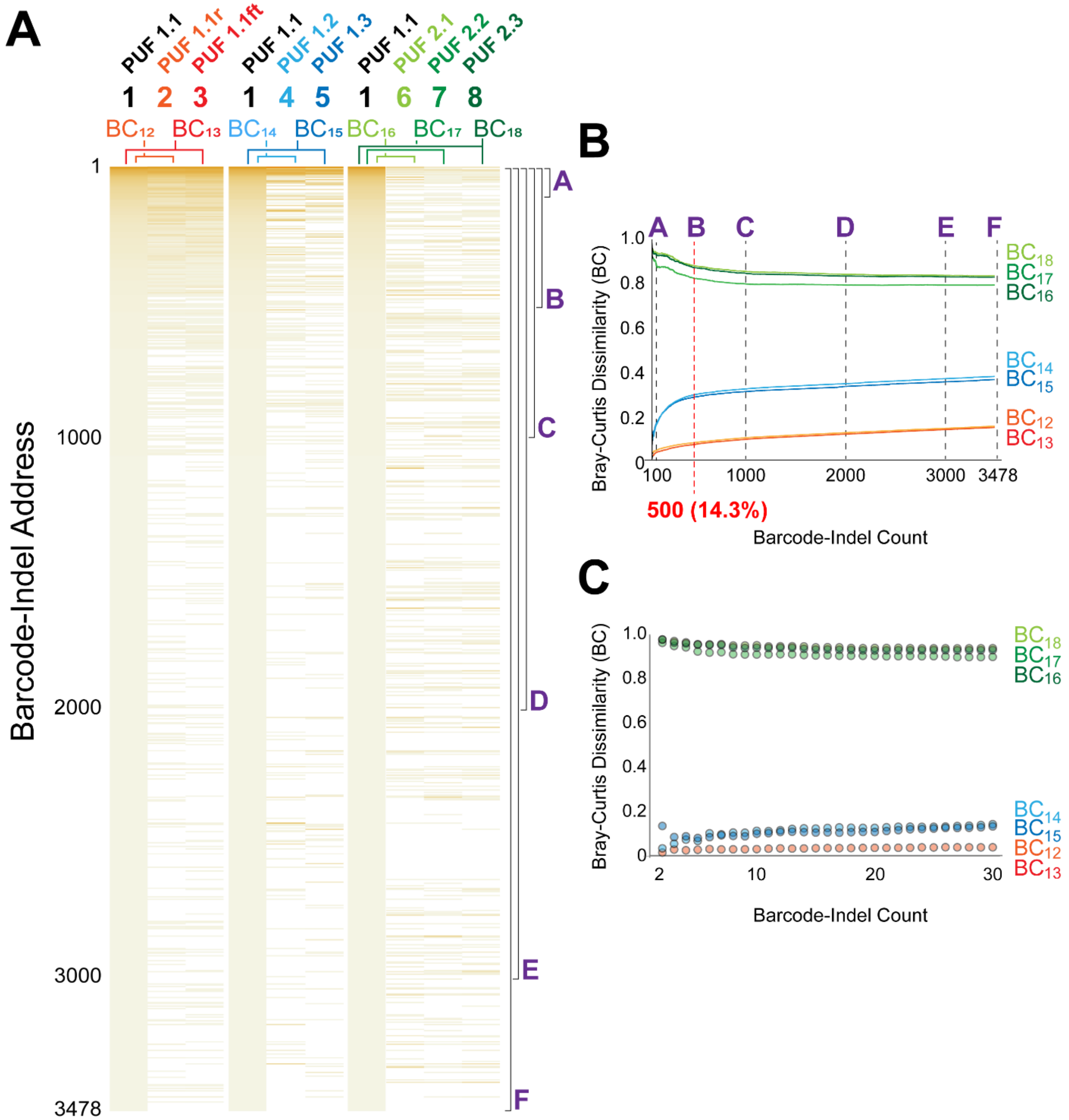
Indel 1: CCTCGTAGGGCTTGCCTTCGCTCGGATGTGCACTTGAA
Indel 2: CCTCGTAGGGCTTGCCTTCGGATGTGCACTTGAA
Indel 3: CCTCGTAGGGCTTGCCTTCGGTGCACTTGAA
Indel 4: CCTCGGATGTGCACTTGAA
Indel 5: CCTCGTAGGGCTTGCCTTCGTGCACTTGAA

F

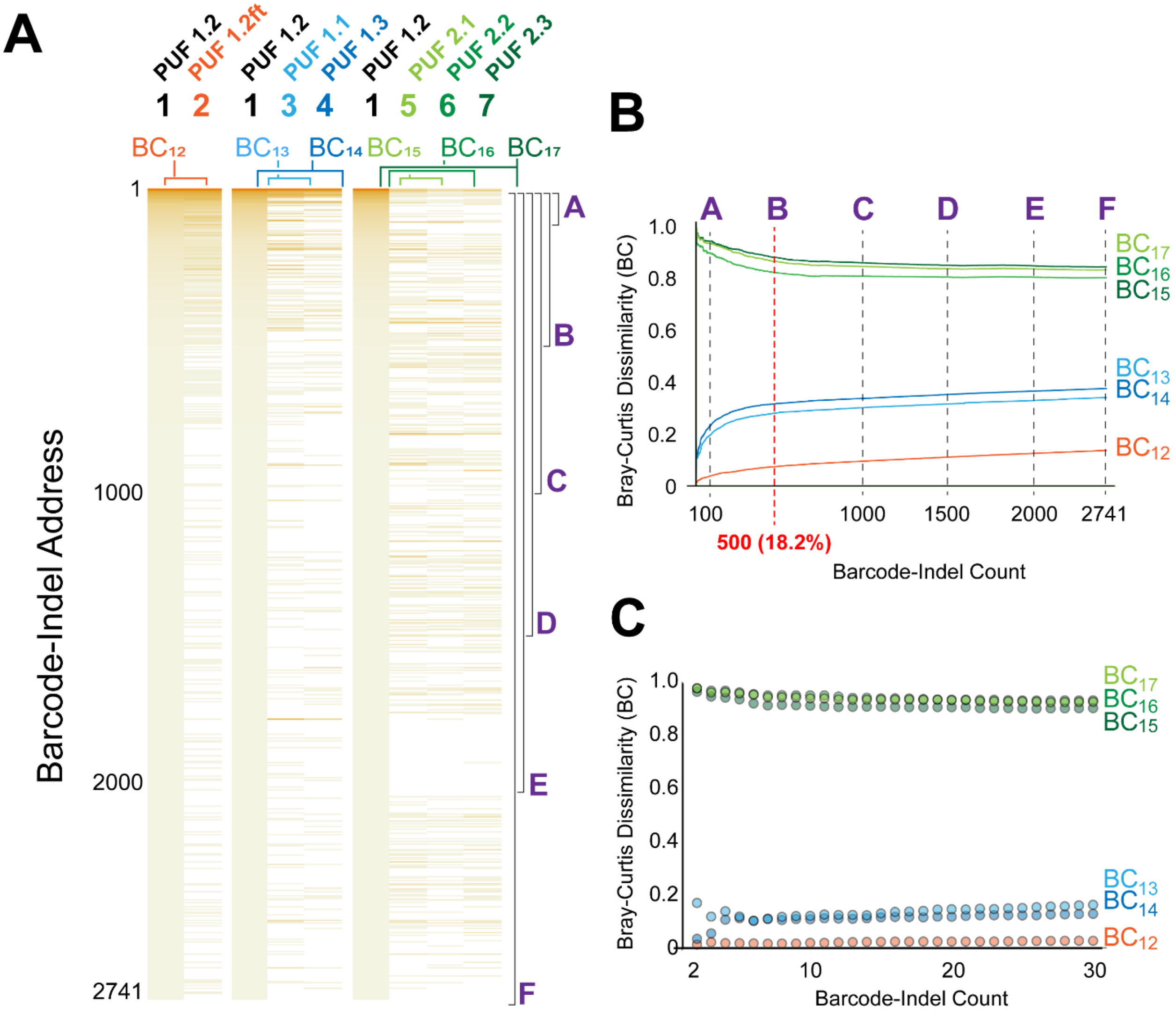
Sample	Barcode	Indels				
		1	2	3	4	5
PUF4.6	CGGAG	4217	4134	3363	2037	2696
	ATAGG	174	154	791	96	100
	AAGTC	125	127	100	818	104
	AGAAG	53	41	62	38	35
	ATCGG	33	31	21	16	11
PUF4.6ft	CGGAG	4969	4839	3844	2360	3166
	ATAGG	170	194	966	101	124
	AAGTC	142	149	141	925	88
	AGAAG	80	53	60	41	30
	ATCGG	29	29	21	19	14
PUF4.6r	CGGAG	4567	4592	3600	2312	2979
	ATAGG	190	175	866	121	104
	AAGTC	139	118	115	908	105
	AGAAG	69	54	53	42	49
	ATCGG	13	28	20	17	18

Indel 1: CCTCGTAGGGCTTGCCTTCGATGTGCACTTGAA
Indel 2: CCTCGTAGGGCTTGCCTTCGGATGTGCACTTGAA
Indel 3: CCTCGTAGGGCTTGCCTTCGCACTTGAA
Indel 4: CCTCGTAGGGCTTGCCCTCGGATGTGCACTTGAA
Indel 5: CCTCGCTCGGATGTGCACTTGAA

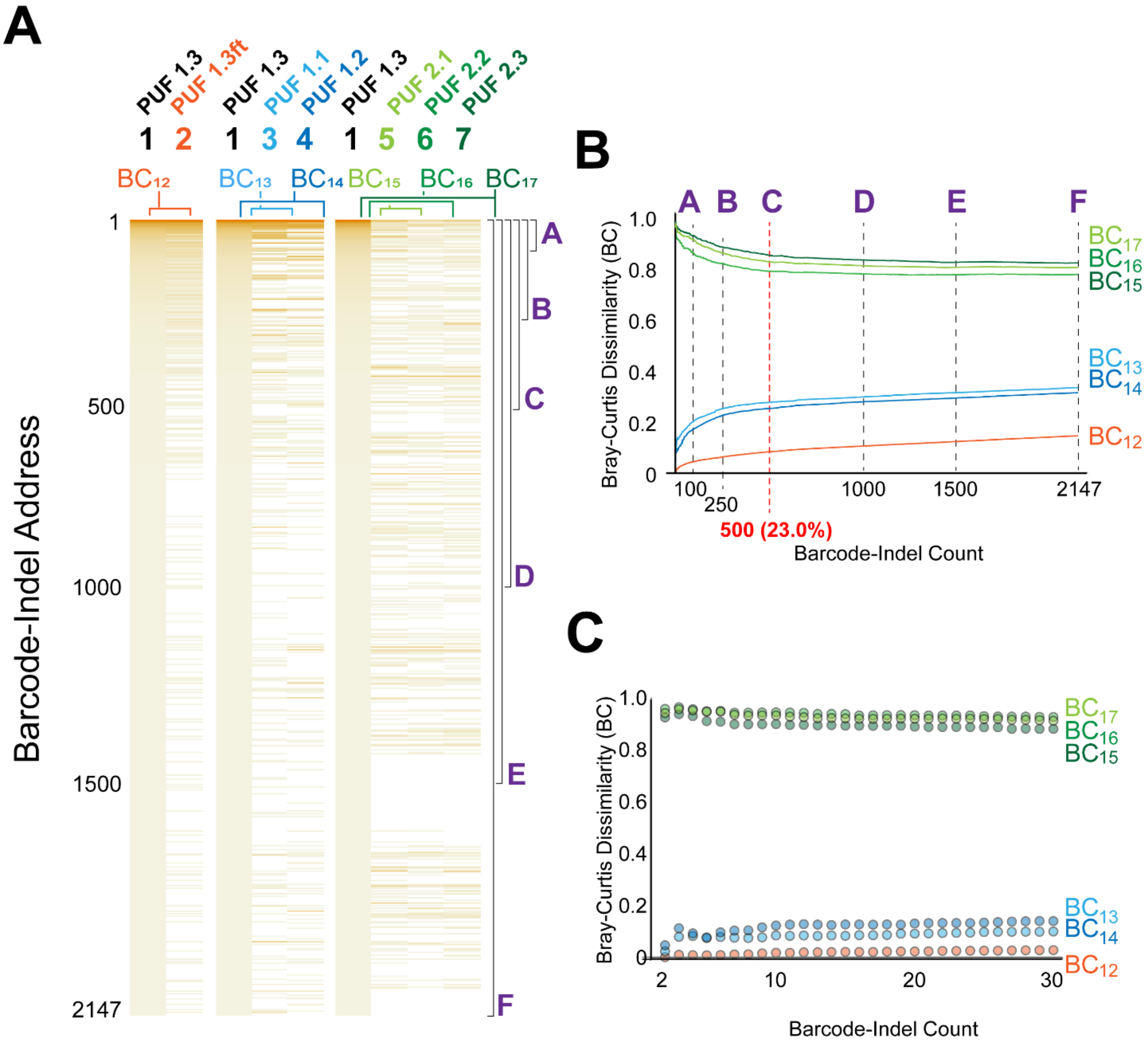
Supplementary Figure 3. Implementation of CREAM-PUFs in HeLa cells. Qualitative assessment of CREAM-PUFs generated using HeLa. See Supplementary Figure 2 for detailed description.



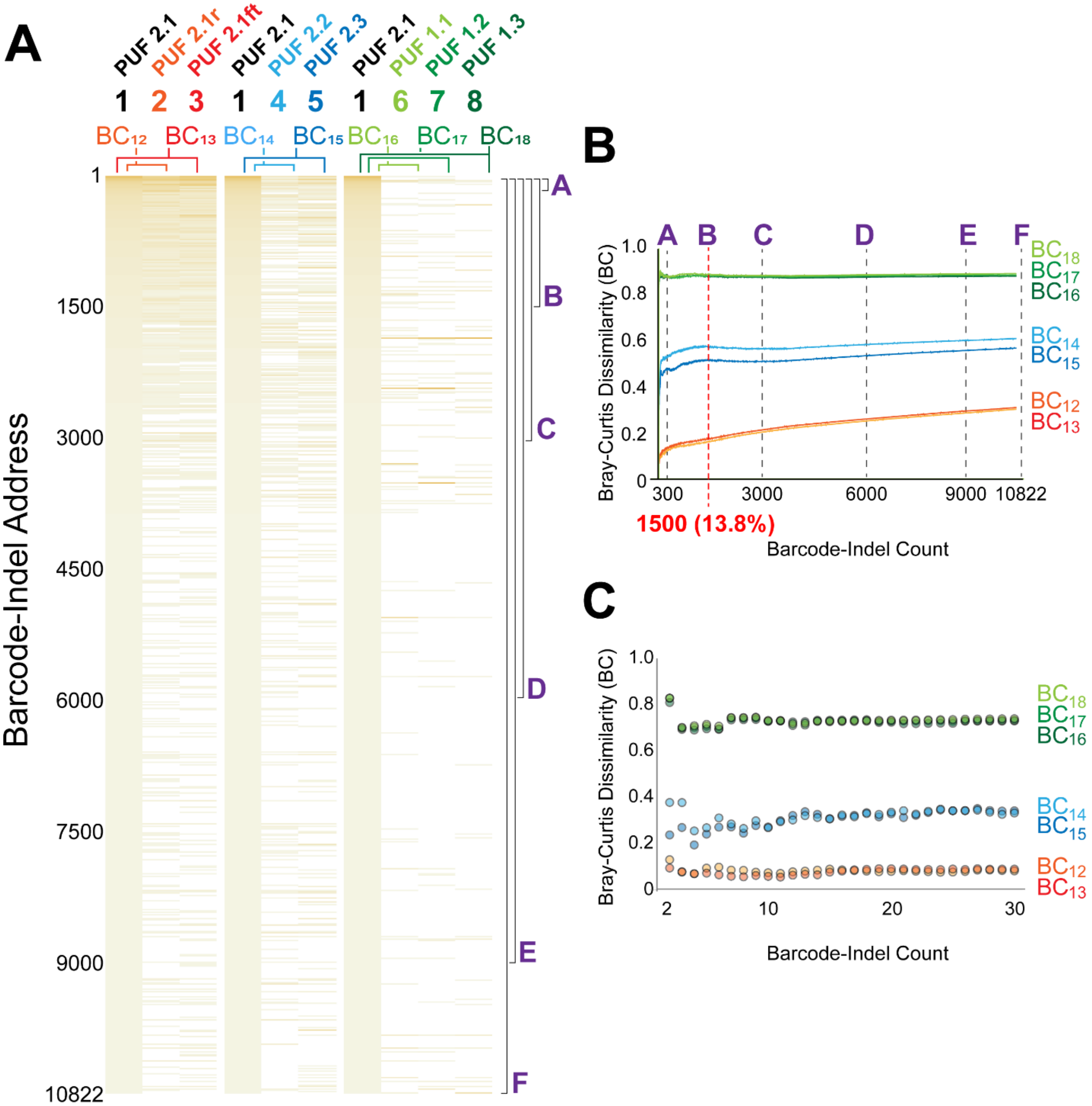
Supplementary Figure 4. Calculation of Bray-Curtis dissimilarities using PUF 1.1 as reference with varying sampling rate. (A) To calculate the Bray-Curtis value between 2 PUFs, the NGS results are first turned into an array of barcode-indel combinations. After sorting the array of the reference PUF based on frequency of occurrence, entries of the other arrays are then sorted to match this order. **(B)** The Bray-Curtis value between the reference and another PUF based on the size of the barcode-indel list used in the calculation, from 2 to the size of the reference sample. Purple letters indicate section of the array shown in **(A)** that corresponds to the visual representation of the list used in the calculation. The barcode-indel count shown in red indicates the list size used for analysis in the main text. **(C)** The Bray-Curtis dissimilarity based on the size of the barcode-indel list used to obtain the distance, from 2 to 30.



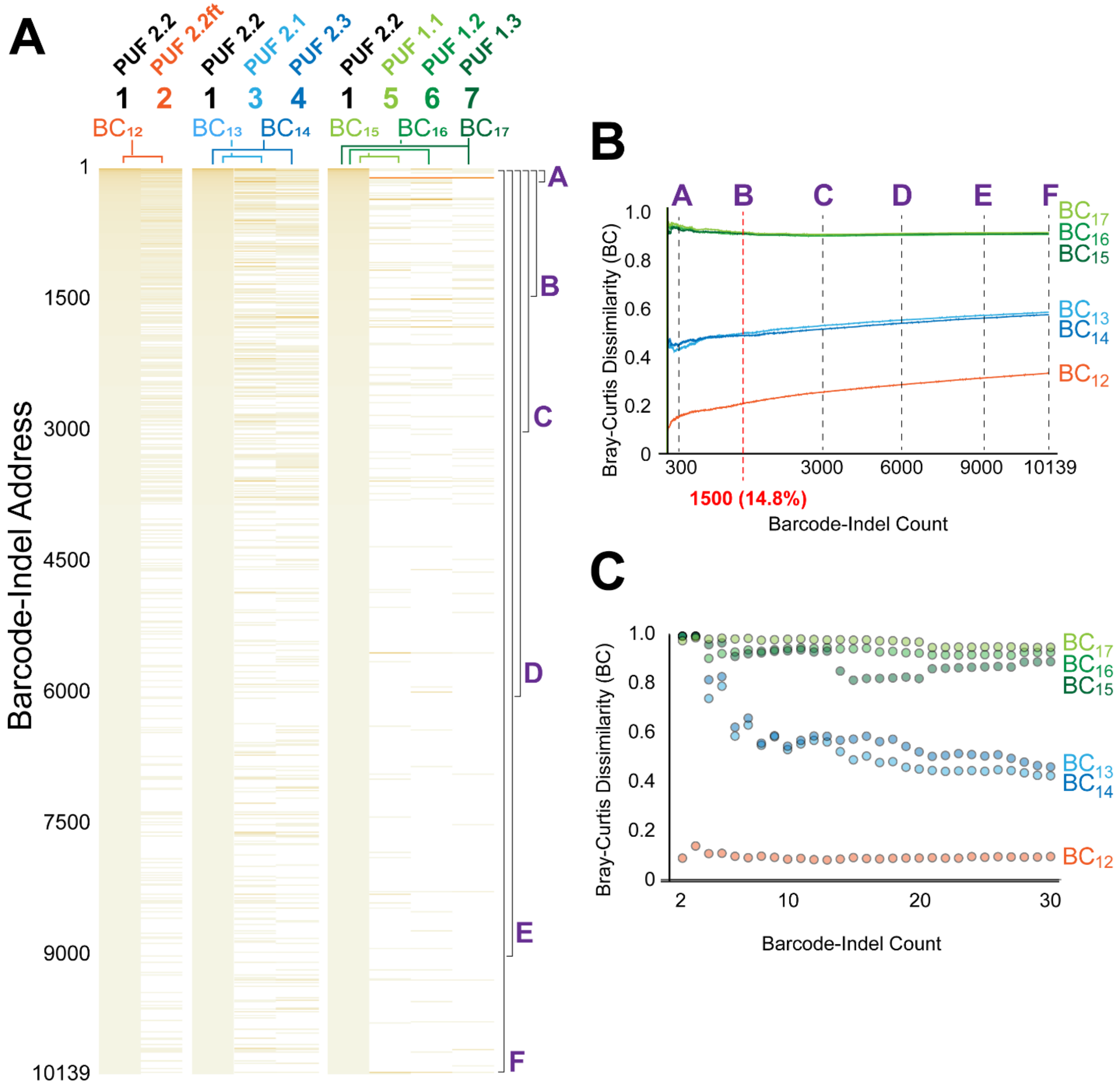
Supplementary Figure 5. Calculation of Bray-Curtis dissimilarities using PUF 1.2 as reference with varying sampling rate. Refer to **Supplementary Figure 4** for a detailed description.



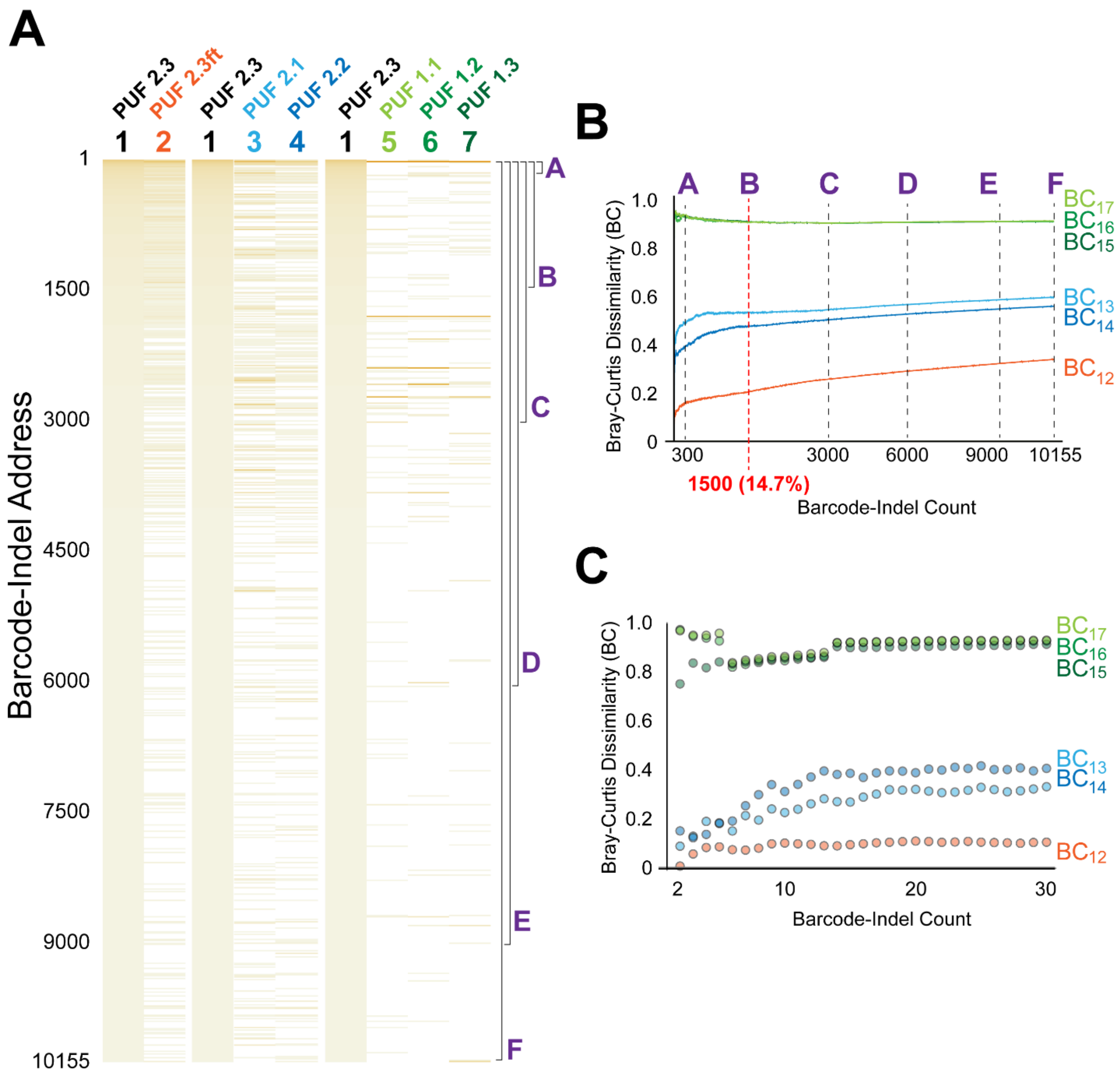
Supplementary Figure 6. Calculation of Bray-Curtis dissimilarities using PUF 1.3 as reference with varying sampling rate. Refer to **Supplementary Figure 4** for a detailed description.



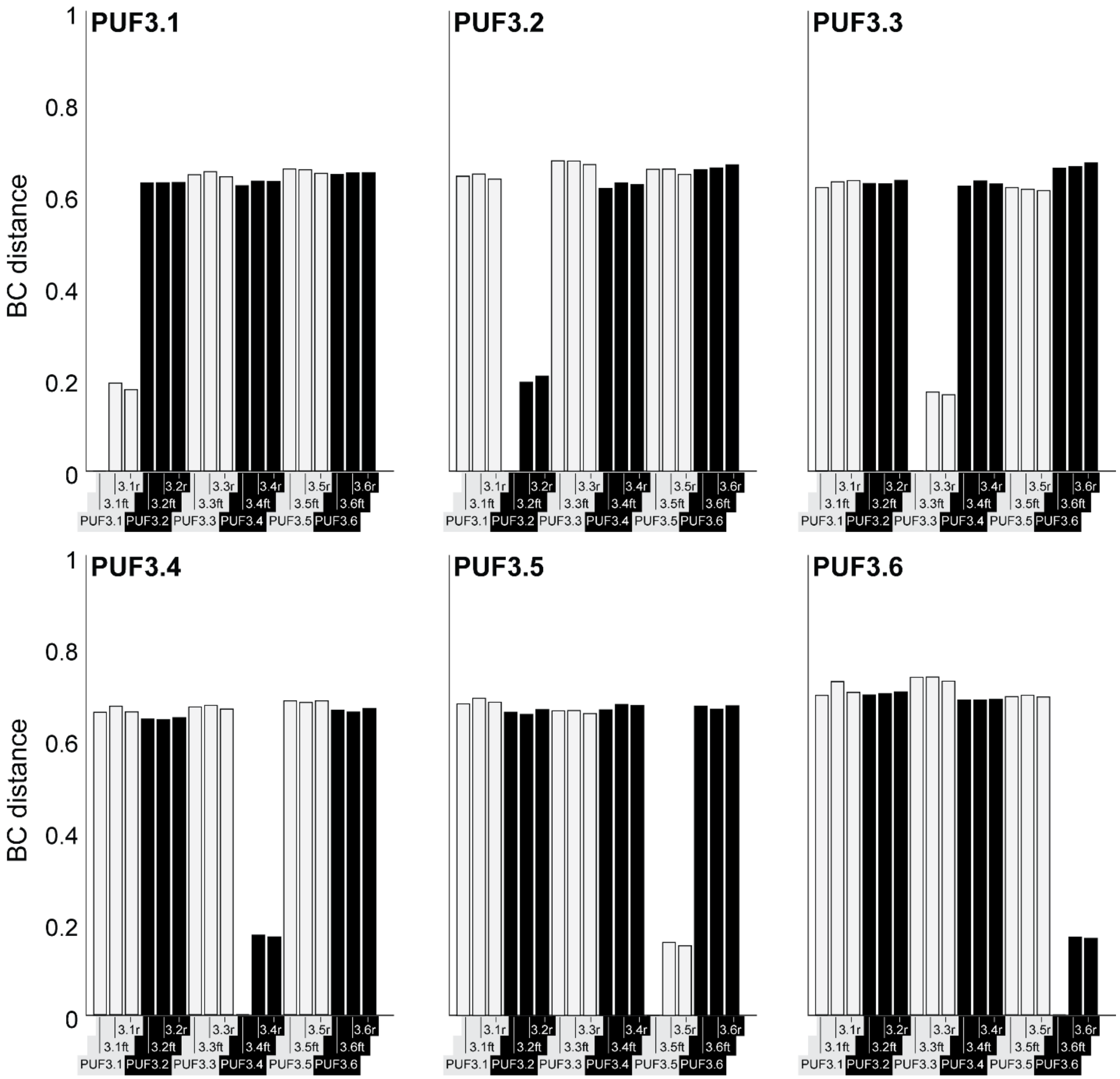
Supplementary Figure 7. Calculation of Bray-Curtis dissimilarities using PUF 2.1 as reference with varying sampling rate. Refer to **Supplementary Figure 4** for a detailed description.



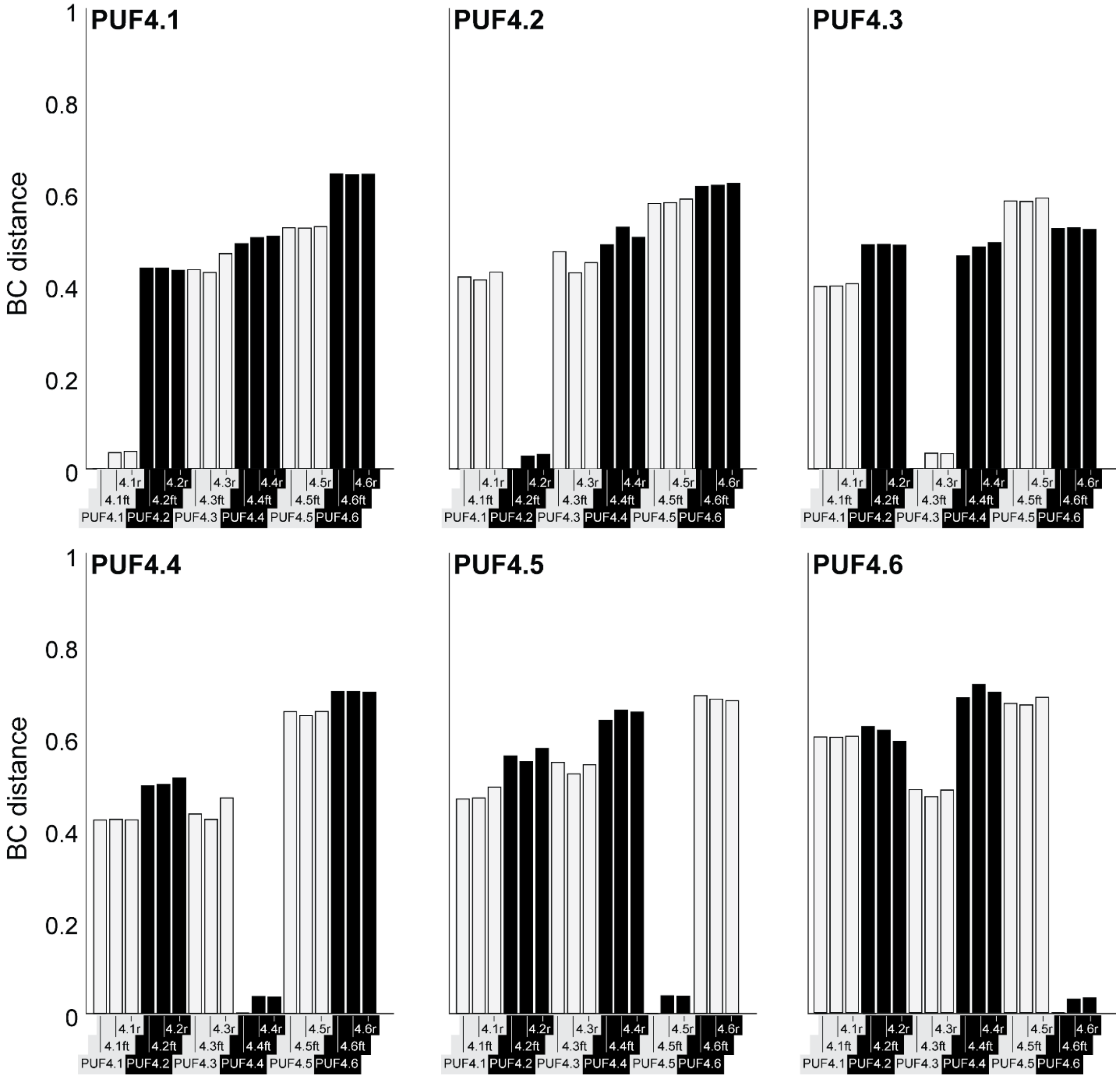
Supplementary Figure 8. Calculation of Bray-Curtis dissimilarities using PUF 2.2 as reference with varying sampling rate. Refer to **Supplementary Figure 4** for a detailed description.



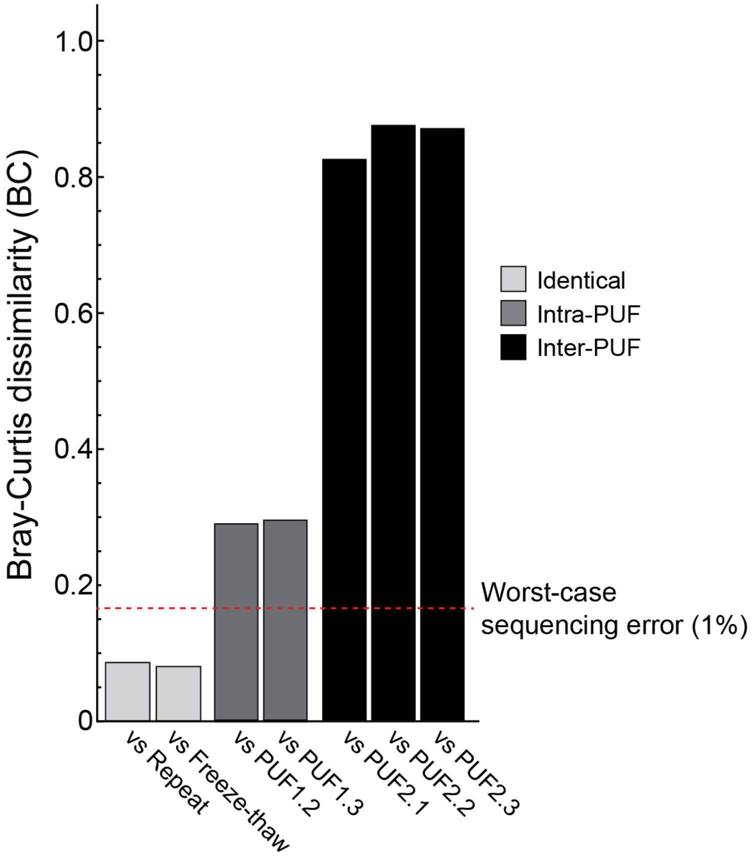
Supplementary Figure 9. Calculation of Bray-Curtis dissimilarities using PUF 2.3 as reference with varying sampling rate. Refer to **Supplementary Figure 4** for a detailed description.



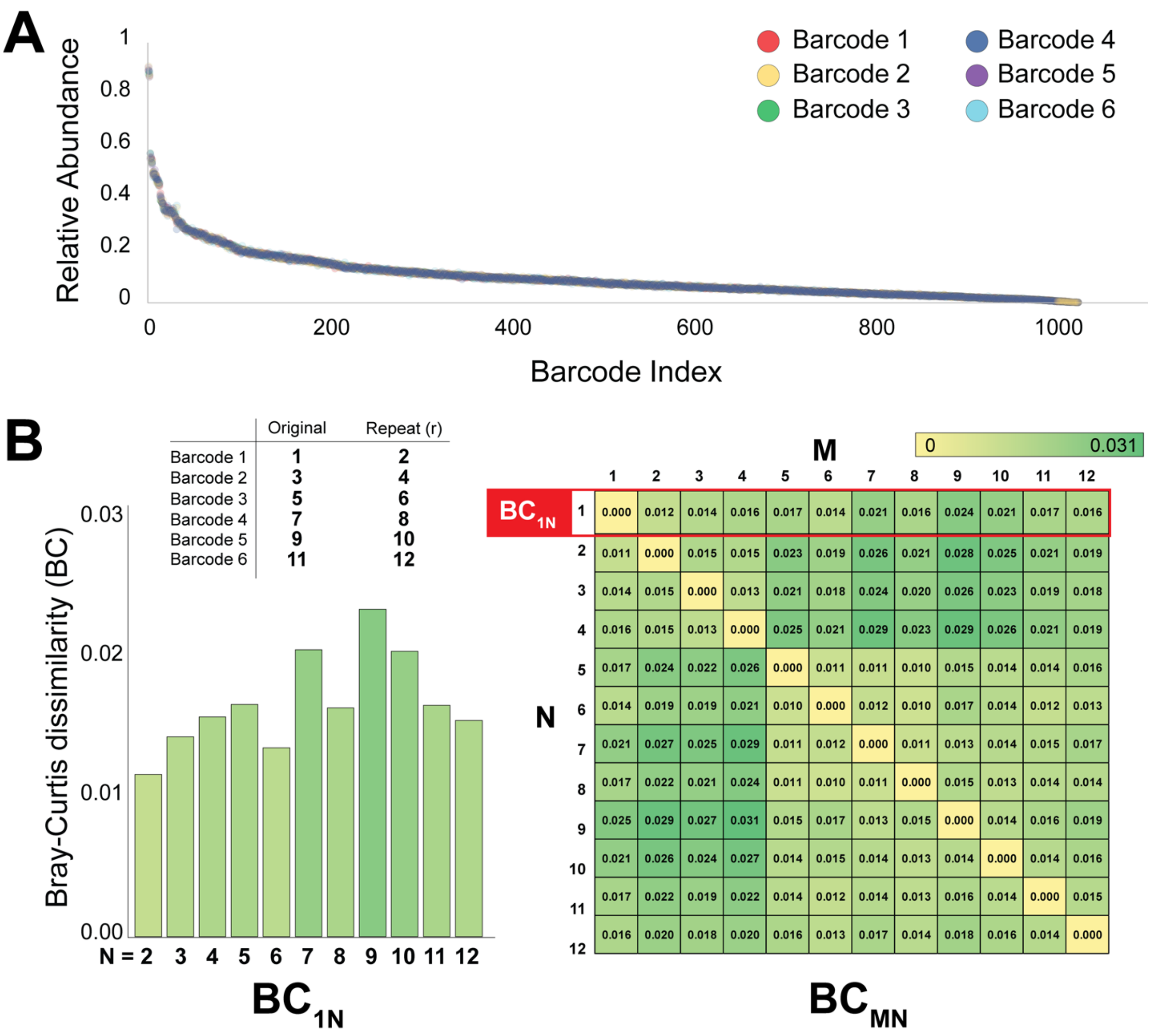
Supplementary Figure 10. Quantitative assessment of HCT116-derived CREAM-PUFs using Bray-Curtis dissimilarity. Comparison of Bray-Curtis dissimilarities for a single PUF3.i (i={1,2,3,4,5,6}) generated in HCT116 against 17 other PUFs generated in the same cell line.



Supplementary Figure 11. Quantitative assessment of HeLa-derived CREAM-PUFs using Bray-Curtis dissimilarity. Comparison of Bray-Curtis dissimilarities for a single PUF4.i (i={1,2,3,4,5,6}) generated in HeLa against 17 other PUFs generated in the same cell line.



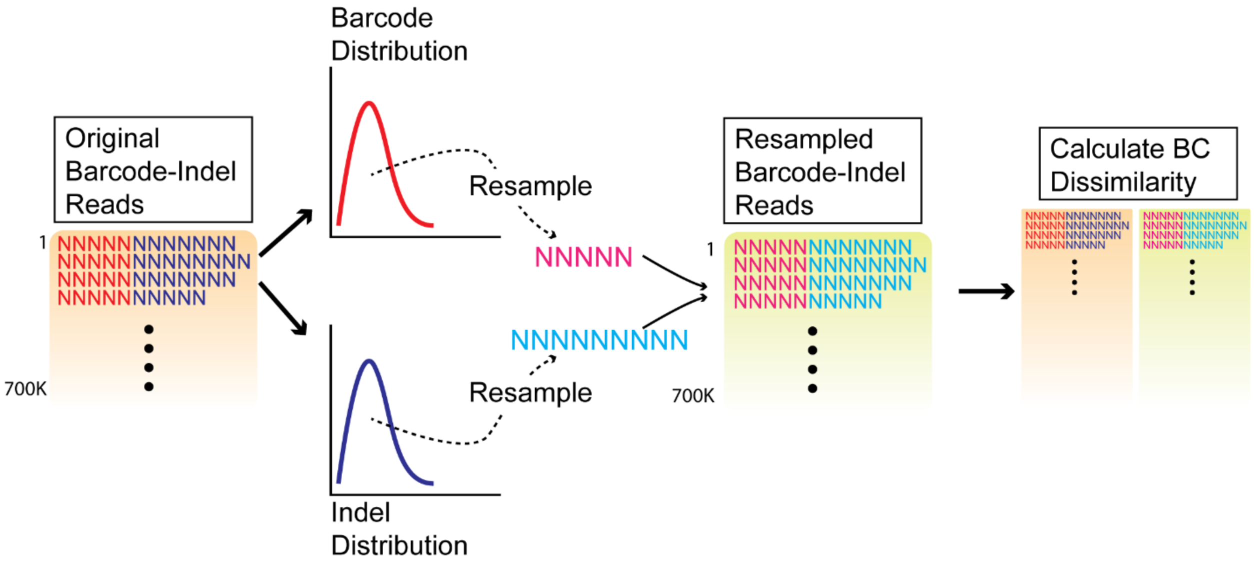
Supplementary Figure 12. Simulated maximum Bray-Curtis dissimilarity from sequencing error for PUFs. To obtain the worst-case Bray-Curtis values from sequencing error, each PUF barcode-indel sequencing data were mutated *in silico* using an error rate of 1% per base. The resulting dataset was then used to calculate the Bray-Curtis value against the original sequence and the technical replicates of the original sequence (repeat and freeze-thaw). The value shown for worst-case sequencing error is an average of 100 different simulations.



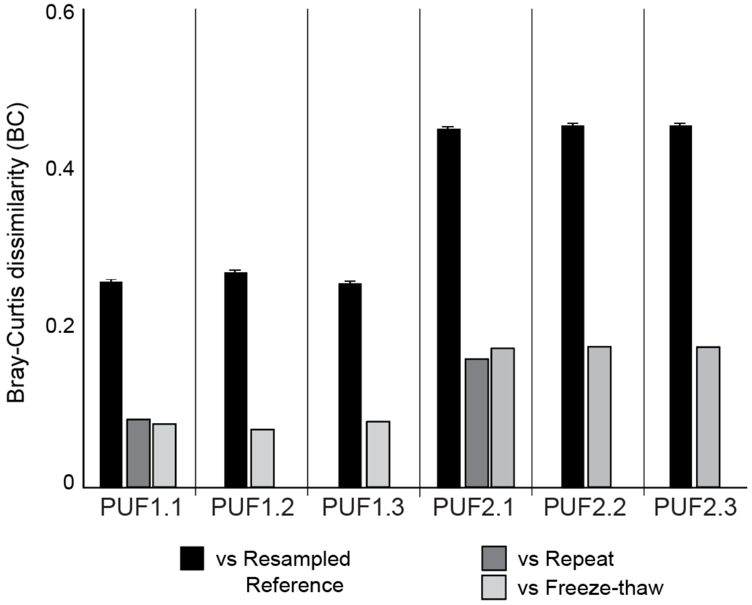
	Original	Repeat (r)
Barcode 1	1	2
Barcode 2	3	4
Barcode 3	5	6
Barcode 4	7	8
Barcode 5	9	10
Barcode 6	11	12

	1	2	3	4	5	6	7	8	9	10	11	12
1	0.000	0.012	0.014	0.016	0.017	0.014	0.021	0.016	0.024	0.021	0.017	0.016
2	0.011	0.000	0.015	0.015	0.023	0.019	0.026	0.021	0.028	0.025	0.021	0.019
3	0.014	0.015	0.000	0.013	0.021	0.018	0.024	0.020	0.026	0.023	0.019	0.018
4	0.016	0.015	0.013	0.000	0.025	0.021	0.029	0.023	0.029	0.026	0.021	0.019
5	0.017	0.024	0.022	0.026	0.000	0.011	0.011	0.010	0.015	0.014	0.014	0.016
6	0.014	0.019	0.019	0.021	0.010	0.000	0.012	0.010	0.017	0.014	0.012	0.013
7	0.021	0.027	0.025	0.029	0.011	0.012	0.000	0.011	0.013	0.014	0.015	0.017
8	0.017	0.022	0.021	0.024	0.011	0.010	0.011	0.000	0.015	0.013	0.014	0.014
9	0.025	0.029	0.027	0.031	0.015	0.017	0.013	0.015	0.000	0.014	0.016	0.019
10	0.021	0.026	0.024	0.027	0.014	0.015	0.014	0.013	0.014	0.000	0.014	0.016
11	0.017	0.022	0.019	0.022	0.014	0.012	0.014	0.013	0.016	0.014	0.000	0.015
12	0.016	0.020	0.018	0.020	0.016	0.013	0.017	0.014	0.018	0.016	0.014	0.000

Supplementary Figure 13. Barcode library alone does not satisfy the uniqueness requirement of PUFs. A 5-nucleotide barcode library was stably integrated into the AAVS1 locus of HEK293 cells in 6 parallel trials. **(A)** The relative abundances of stably integrated barcodes in 6 replicates. **(B)** The Bray-Curtis dissimilarity values between barcode 1 and all other 6 samples and their NGS sequencing replicates (left) and of any given pair of all barcodes (right). Note the *intra*-sample dissimilarities generally overlapped with those of *inter*-samples, thus violating the uniqueness requirement of PUFs.



Supplementary Figure 14. Procedure for generating resampled Barcode-Indel reads and corresponding BC dissimilarity



Supplementary Figure 15. Bray-Curtis dissimilarities for intra-PUFs and simulated inter-PUFs.

Supplementary Material

General cloning protocols

Q5 High-Fidelity 2X Master Mix (New England Biolabs) was used for all polymerase chain reactions (PCR) according to the manufacturer's protocol. All oligonucleotides were ordered from Sigma-Aldrich and were listed in **Supplementary Table 1**. The plasmids were constructed using PCR amplification, restriction digest (all restriction enzymes were ordered from New England Biolabs), and ligation with T4 DNA ligase (New England Biolabs). Gel purification and PCR purification were performed with QIAquick Gel Extraction and PCR Purification kits (Qiagen). Transformations were performed using NEB 5-alpha electrocompetent *Escherichia Coli* (New England Biolabs). The minipreps were performed using QIAprep Spin Miniprep kit (Qiagen). The final plasmids were confirmed by both restriction enzyme digestions and direct Sanger sequencings.

DNA constructs

Barcode-Truncated CMV-mKate-PGK1-hygromycin resistance gene: CMV-mKate-PGK1-hygromycin resistance gene (unpublished results) was used as the PCR template with primers P3 and P4. The purified PCR product was then cloned into CMV-mKate-PGK1-hygromycin resistance gene vector using AscI and SbfI sites.

CMV-SpCas9-U6-sgRNA1: CMV-SpCas9-U6-BRIP1-sgRNA (unpublished results) was used as the PCR template with primers P5 and P6. Next, the purified PCR product was used as the PCR template with primers P5 and P7. The purified PCR product was then cloned into CMV-SpCas9 (unpublished results) vector using KpnI and XbaI sites.

CMV-SpCas9-U6-sgRNA2: CMV-SpCas9-U6-BRIP1-sgRNA (unpublished results) was used as the PCR template with primers P5 and P8. Next, the purified PCR product was used as the PCR template with primers P5 and P7. The purified PCR product was then cloned into CMV-SpCas9 (unpublished results) vector using KpnI and XbaI sites.

CMV-SpCas9-U6-sgRNA3: CMV-SpCas9-U6-BRIP1-sgRNA (unpublished results) was used as the PCR template with primers P5 and P9. Next, the purified PCR product was used as the PCR template with primers P5 and P7. The purified PCR product was then cloned into CMV-SpCas9 (unpublished results) vector using KpnI and XbaI sites.

CMV-SpCas9-U6-sgRNA4: CMV-SpCas9-U6-BRIP1-sgRNA (unpublished results) was used as the PCR template with primers P5 and P10. Next, the purified PCR product was used as the PCR template with primers P5 and P7. The purified PCR product was then cloned into CMV-SpCas9 (unpublished results) vector using KpnI and XbaI sites.

CMV-SpCas9-U6-sgRNA5: CMV-SpCas9-U6-BRIP1-sgRNA (unpublished results) was used as the PCR template with primers P5 and P11. Next, the purified PCR product was used as the PCR template with primers P5 and P7. The purified PCR product was then cloned into CMV-SpCas9 (unpublished results) vector using KpnI and XbaI sites.

NGS (next generation sequencing)-based amplicon sequencing data analysis pipeline with sample commands

Step 1: extracting the 100-bp reads

```
awk 'NR%4 ==2' < f1.fastq | cat > f2.fastq
```

```
awk 'NR%4 ==2' < r1.fastq | cat > r2.fastq
```

Step 2: joining the paired-end reads

```
paste -d '\0' f2.fastq r2.fastq | cat > fr1.fastq
```

Step3: filtering out corrupted reads

```
grep “^CTTATATTCCCAGGGCCGGTTCGCGATCGCCCTGCAGG[A-Z][A-Z][A-Z][A-Z][A-
Z]TAGTTATTAATGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGACCG
CCCTCGACCGCCTTGATTCTCATGGTCTGGGTGC[A-Z]*GTGGTGGTTGTTCACGGTGCCCT”
< fr1.fastq | cat > fr2.fastq
```

Step 4: extracting the barcode and indel sequences

```
sed -e "s/CTTATATTCCCAGGGCCGGTTCGCGATCGCCCTGCAGG \(.*\)
TAGTTATTAATGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGACCGC
CCTCGACCGCCTTGATTCTCATGGTCTGGGTGC[A-Z]*GTGGTGGTTGTTCACGGTGCCCT
[A-Z]*/\1/"  < fr2.fastq | cat > barcode1.fastq
```

```
sed -e "s/CTTATATTCCCAGGGCCGGTTCGCGATCGCCCTGCAGG[A-Z][A-Z][A-Z][A-Z][A-
Z]TAGTTATTAATGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGACCG
CCCTCGACCGCCTTGATTCTCATGGTCTGGGTGC \(.*\) GTGGTGGTTGTTCACGGTGCCCT
[A-Z]* /\1/" <  fr2.fastq | cat > indel1.fastq
```

Step 5: joining the paired barcode and indel sequences

```
paste -d '\0' barcode1.fastq indel1.fastq | cat > fr3.fastq
```

Step 6: isolating indels containing insertions/deletions

```
grep -v –x '.\{45\}' fr3.fastq  | cat > fr4.fastq
```

Reverse Engineering a CREAM-PUF

The effort needed to reverse engineer a CREAM-PUF, i.e., to synthesize a population that produces an identical barcode-indel matrix, requires an insurmountable amount of time, effort, and cost. Indeed, doing so would necessitate that each individual barcode/indel sequence pair be individually integrated into the required cell line, followed by monoclonal verification and, ultimately, mixing of the individual cells in the right proportions to reproduce the same barcode/indel frequencies observed from the CREAM-PUF. Simply installing the barcode/indel sequence can, on average, take a single researcher up to seven attempts over 19 weeks with 472 hours of hands-on time and approximately $18,000 to complete a single CRISPR editing workflow[1], i.e., generation of the desired monoclonal cell line. Furthermore, outsourcing a CRISPR-mediated genetic knock-in, such as a barcode/indel sequence described in our CREAM-PUFs, can have a starting price of $18,000-$25,000[2,3] with a similar time of completion. This process would simply produce cells with the same barcode/indel sequences contained in an individual CREAM-PUF. For example, to replicate PUF1.1, one would need to create 500 cell lines, which would cost at least $9 million. Moreover, to dial in the right frequency of engineered cells to reproduce the CREAM-PUF, would largely be trial and error with no guarantee that it is even possible.

Barcode-Truncated CMV-mKate-PGK1-hygromycin resistance gene Sequence

```
TAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGGCCAGCTCCCATAGCTCAGTC
TGGTCTATCTGCCTGGCCCTGGCCATTGTCACTTTGCGCTGCCCTCCTCTCGCCCCCGAG
TGCCCTTGCTGTGCCGCCGGAACTCTGCCCTCTAACGCTGCCGTCTCTCTCCTGAGTCCG
GACCACTTTGAGCTCTACTGGCTTCTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCCAG
GCAGGTCCTGCTTTCTCTGACCTGCATTCTCTCCCCTGGGCCTGTGCCGCTTTCTGTCTGC
AGCTTGTGGCCTGGGTCACCTCTACGGCTGGCCCAGATCCTTCCCTGCCGCCTCCTTCAG
GTTCCGTCTTCCTCCACTCCCTCTTCCCCTTGCTCTCTGCTGTGTTGCTGCCCAAGGATGC
TCTTTCCGGAGCACTTCCTTCTCGGCGCTGCACCACGTGATGTCCTCTGAGCGGATCCTC
CCCGTGTCTGGGTCCTCTCCGGGCATCTCTCCTCCCTCACCCAACCCCATGCCGTCTTCA
CTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGGGGCCTGTGCCATCTCTCGTTTCTTAGGAT
GGCCTTCTCCGACGGATGTCTCCCTTGCGTCCCGCCTCCCCTTCTTGTAGGCCTGCATCAT
CACCGTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCTTTAGCCACCTCTCCATC
CTCTTGCTTTCTTTGCCTGGACACCCCGTTCTCCTGTGGATTCGGGTCACCTCTCACTCCT
TTCATTTGGGCAGCTCCCCTACCCCCCTTACCTCTCTAGTCTGTGCTAGCTCTTCCAGCCC
CCTGTCATGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACCCGGG
CCCCTATGTCCACTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCCGAGC
TGGGACCACCTTATATTCCCAGGGCCGGTTCGCGATCGCCCTGCAGGNNNNNTAGTTATT
AATGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGC
ACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGG
CGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGACCAGC
TAAGACACTGCCACGGTCAGATCCGCTAGCGCTACCGGTCGCCACCATGGTGAGCGAGCT
GATTAAGGAGAACATGCACATGAAGCTGTACATGGAGGGCACCGTGAACAACCACCACTT
CAAGTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAATCA
AGGCGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTTCATGT
ACGGCAGCAAAACCTTCATCAACCACACCCAGGGCATCCCCGACTTCTTTAAGCAGTCCTT
CCCCGAGGGCTTCACATGGGAGAGAGTCACCACATACGAAGACGGGGGCGTGCTGACCG
CTACCCAGGACACCAGCCTCCAGGACGGCTGCCTCATCTACAACGTCAAGATCAGAGGGG
TGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCTCCA
CCGAGACCCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGAGCCGACATGGCCCTGAAG
CTCGTGGGCGGGGGCCACCTGATCTGCAACTTGAAGACCACATACAGATCCAAGAAACCC
GCTAAGAACCTCAAGATGCCCGGCGTCTACTATGTGGACAGAAGACTGGAAAGAATCAAG
GAGGCCGACAAAGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATACTGCGA
CCTCCCTAGCAAACTGGGGCACAGAGGTGGAGGAGGTTCCGGATCTCACGGCTTCCCTCC
CGAGGTGGAGGAGCAGGCCGCCGGCACCCTGCCCATGAGCTGCGCCCAGGAGAGCGGC
ATGGATAGACACCCTGCTGCTTGCGCCAGCGCCAGGATCAACGTCTCTAGATAACTGATCA
TAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCC
CTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAAT
GGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCT
AGTTGTGGTTTGTCCAAACTCATCAATGTATCTTAACGCGTAAATTGGGCGCGCCCTTAAG
CTGGGACGGAGGCTTGTTTGCGAGGCCGCGGCCGGCCGAAGTTCCTATTCTCTAGAAAGT
ATAGGAACTTCTACCGGGTAGGGGAGGCGCTTTTCCCAAGGCAGTCTGGAGCATGCGCTT
TAGCAGCCCCGCTGGGCACTTGGCGCTACACAAGTGGCCTCTGGCCTCGCACACATTCCA
CATCCACCGGTAGGCGCCAACCGGCTCCGTTCTTTGGTGGCCCCTTCGCGCCACCTTCTA
CTCCTCCCCTAGTCAGGAAGTTCCCCCCCGCCCCGCAGCTCGCGTCGTGCAGGACGTGA
CAAATGGAAGTAGCACGTCTCACTAGTCTCGTGCAGATGGACAGCACCGCTGAGCAATGG
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AAGCGGGTAGGCCTTTGGGGCAGCGGCCAATAGCAGCTTTGCTCCTTCGCTTTCTGGGCT
CAGAGGCTGGGAAGGGGTGGGTCCGGGGGCGGGCTCAGGGGCGGGCTCAGGGGCGGG
GCGGGCGCCCGAAGGTCCTCCGGAGGCCCGGCATTCTGCACGCTTCAAAAGCGCACGTC
TGCCGCGCTGTTCTCCTCTTCCTCATCTCCGGGCCTTTCGACCTGCATCCATCTAGATCTC
GATCGAGCAGCTGAAGCTTACCGCAGGCTATGAAAAAGCCTGAACTCACCGCGACGTCTG
TCGAGAAGTTTCTGATCGAAAAGTTCGACAGCGTCTCCGACCTGATGCAGCTCTCGGAGG
GCGAAGAATCTCGTGCTTTCAGCTTCGATGTAGGAGGGCGTGGATATGTCCTGCGGGTAA
ATAGCTGCGCCGATGGTTTCTACAAAGATCGTTATGTTTATCGGCACTTTGCATCGGCCGC
GCTCCCGATTCCGGAAGTGCTTGACATTGGGGAATTCAGCGAGAGCCTGACCTATTGCAT
CTCCCGCCGTGCACAGGGTGTCACGTTGCAAGACCTGCCTGAAACCGAACTGCCCGCTGT
TCTGCAGCCGGTCGCGGAGGCCATGGATGCGATCGCTGCGGCCGATCTTAGCCAGACGA
GCGGGTTCGGCCCATTCGGACCGCAAGGAATCGGTCAATACACTACATGGCGTGATTTCA
TATGCGCGATTGCTGATCCCCATGTGTATCACTGGCAAACTGTGATGGACGACACCGTCAG
TGCGTCCGTCGCGCAGGCTCTCGATGAGCTGATGCTTTGGGCCGAGGACTGCCCCGAAG
TCCGGCACCTCGTGCACGCGGATTTCGGCTCCAACAATGTCCTGACGGACAATGGCCGCA
TAACAGCGGTCATTGACTGGAGCGAGGCGATGTTCGGGGATTCCCAATACGAGGTCGCCA
ACATCTTCTTCTGGAGGCCGTGGTTGGCTTGTATGGAGCAGCAGACGCGCTACTTCGAGC
GGAGGCATCCGGAGCTTGCAGGATCGCCGCGGCTCCGGGCGTATATGCTCCGCATTGGT
CTTGACCAACTCTATCAGAGCTTGGTTGACGGCAATTTCGATGATGCAGCTTGGGCGCAG
GGTCGATGCGACGCAATCGTCCGATCCGGAGCCGGGACTGTCGGGCGTACACAAATCGC
CCGCAGAAGCGCGGCCGTCTGGACCGATGGCTGTGTAGAAGTACTCGCCGATAGTGGAA
ACCGACGCCCCAGCACTCGTCCGAGGGCAAAGGAATAGGGGAGGCTAACTGAAGCTTCC
CGGGGGTACCAAATTCGTCGACAGATCTAACTTGTTTATTGCAGCTTATAATGGTTACAAAT
AAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTT
TGTCCAAACTCATCAATGTATCTTATGATGTCTGCATATGGAAGTTCCTATTCTCTAGAAAGT
ATAGGAACTTCGCGGCCGCTCCCACCCGCTCGTCCCCCCGCGCACCTTTGCTAGGAGCG
GGTCGCCCATGTGGCTCTCAGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCACAGTGG
GGCCACTAGGGACAGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCTAG
TCTCCTGATATTGGGTCTAACCCCCACCTCCTGTTAGGCAGATTCCTTATCTGGTGACACA
CCCCCATTTCCTGGAGCCATCTCTCTCCTTGCCAGAACCTCTAAGGTTTGCTTACGATGGA
GCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGGGGGTGGGAGGGAAGGGGGGGATG
CGTGACCTGCCCGGTTCTCAGTGGCCACCCTGCGCTACCCTCTCCCAGAACCTGAGCTGC
TCTGACGCGGCCGTCTGGTGCGTTTCACTGATCCTGGTGCTGCAGCTTCCTTACACTTCCC
AAGAGGAGAAGCAGTTTGGAAAAACAAAATCAGAATAAGTTGGTCCTGAGTTCTAACTTTG
GCTCTTCACCTTTCTAGTCCCCAATTTATATTGTTCCTCCGTGCGTCAGTTTTACCTGTGAG
ATAAGGCCAGTAGCCAGCCCCGTCCTGGCAGGGCTGTGGTGAGGAGGGGGGTGTCCGTG
TGGAAAACTCCCTTTGTGAGAATGGTGCGTCCTAGGTGTTCACCAGGTCGTGGCCGCCTC
TACTCCCTTTCTCTTTCTCCATCCTTCTTTCCTTAAAGAGTCCCCAGTGCTATCTGGGACAT
ATTCCTCCGCCCAGAGCAGGGTCCCGCTTCCCTAAGGCCCTGCTCTGGGCTTCTGGGTTT
GAGTCCTTGGCAAGCCCAGGAGAGGCGCTCAGGCTTCCCTGTCCCCCTTCCTCGTCCACC
ATCTCATGCCCCTGGCTCTCCTGCCCCTTCCCTACAGGGGTTCCTGGCTCTGCTCTTCAGA
CTGAGCCCCGTTCCCCTGCATCCCCGTTCCCCTGCATCCCCCTTCCCCTGCATCCCCCAG
AGGCCCCAGGCCACCTACTTGGCCTGGACCCCACGAGAGGCCACCCCAGCCCTGTCTAC
CAGGCTGCCTTTTGGGTGGATTCTCCTCCAACTGTGGGGTGACTGCTTGGCAAACTCACC
GGTACCCGGCCGCGACTCTAGATCATAATCAGCTCGAGCCTTAACAAGCTTCGAAACGATA
TGGGCTGAATACAAAAACGATATGGGCTGAATACAAAAACGATATGGGCTGAATACAAACC
GCTTGAAGTCTTTAATTAAACCGCTTGAAGTCTTTAATTAAACCGCTTGAAGTCTTTAATTAA
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AGGATCCACCGGATCTAGATAACTGATCATAATCGCGGCCGCACTCCTCAGGTGCAGGCT
GCCTATCAGAAGGTGGTGGCTGGTGTGGCCAATGCCCTGGCTCACAAATACCACTGAGAT
CTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTG
GCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCG
GAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTG
GCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTAT
ATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGAT
TTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGTTT
TACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTA
TGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTG
TGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAG
CCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTT
CCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTCAGCAACCATAGTC
CCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCC
CATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTAT
TCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTTGTTT
ATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTT
TTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATC
CGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTT
CCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCA
GCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAAC
ATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTT
TTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGG
CGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGC
TCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGC
GTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCA
AGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACT
ATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTA
ACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTA
ACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTT
CGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTT
TTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCT
TTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAG
ATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAA
AGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTC
AGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGA
TACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCAC
CGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTC
CTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAG
TTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGC
TCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGAT
CCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAA
GTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATG
CCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGT
GTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATA
GCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGAT
CTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCA
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TCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAA

Green: left homology arm

Red: 5-nucleotide barcode

Dark Red: truncated CMV promoter

Light Blue: mKate open reading frame

Purple: PGK1 promoter

Blue: hygromycin resistance gene open reading frame

Orange: right homology arm

Bray-Curtis and sequencing reads

Assume that a PUF sample contains N barcode-indel reads, the average length of each read is L, and the error rate per base is e. Thus, the total number of mutations is $N * L * e$.

When $N * L * e << N$, each mutation most likely will occur within a different read. We further assume that the mutation does not result in a sequence identical to one of the original reads. Thus, for the $(N – N * L * e)$ non-mutated reads, they will appear in both the original and in the mutated samples. In contrast, for the $(N * L * e)$ mutated reads, they will only appear in the original sample.

Therefore, the Bray-Curtis value will be: $(N * L * e) / (N + N – N * L * e) = (L * e) / (2 – L * e)$.

Since $L * e << 1$, the Bray-Curtis value is $(L * e) / 2$, therefore the BC values are directly related to the read size L.

Supplementary Tables

Supplementary Table 1. Primers used in this study

Supplementary Table 2. The list of individual barcodes and their frequencies for the pilot PUF.

Supplementary Table 3. The list of individual indels and their frequencies for the pilot PUF.

Supplementary Table 4. The PUF matrix for the pilot PUF.

Supplementary Table 5. The list of individual barcodes/indels and their frequencies for PUF1 samples.

Supplementary Table 6. The list of individual barcodes/indels and their frequencies for PUF2 samples.

Supplementary Table 7. The PUF matrices for PUF1 samples.

Supplementary Table 8. The PUF matrices for PUF2 samples.

Supplementary Table 9. The list of individual barcodes/indels and their frequencies for PUF3 samples.

Supplementary Table 10. The list of individual barcodes/indels and their frequencies for PUF4 samples.

Supplementary Table 11. The list of individual barcode-indel addresses and their frequencies for all PUF samples.

Supplementary Table 12. Total variation distances between PUF samples.

Supplementary Table 13. The Bray-Curtis dissimilarities between PUFs and their corresponding mutated samples.

Supplementary Table 14. The relative abundances of stably integrated barcodes in 6 replicates.

Supplementary Table 15. The Bray-Curtis dissimilarities between barcode replicates and their NGS sequencing replicates (denoted as r).

Supplementary Table 16. The Bray-Curtis dissimilarities between PUFs and their corresponding reshuffled samples.

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1. Synthego. CRISPR Benchmark Report. (2019).

2. CRISPR gene Editing Services-Genscript. Available at: https://www.genscript.com/CRISPR-genome-edited-mammalian-cell-lines.html.

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