2025.6.5 Amplicon library #2

Project: Hang_JW Lab

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Planned samples

	#	Treated on	Treatment	Incubation	RT ion	Index	i5	i7	RNA input (10ul RT rxn)
1	114	Live cells	Ctrl (5% DMSO)	40°C for 15min	Mn2+ (3mM)	i5-1 + i7-1	TAGATCGC	TCGCCTTA	60ng
2	115	Live cells	Ctrl (5% DMSO)	40°C for 15min	Mn2+ (3mM)	i5-1 + i7-2	TAGATCGC	CTAGTACG	60ng
3	116	Live cells	C12 (5mM final)	40°C for 15min	Mn2+ (3mM)	i5-1 + i7-3	TAGATCGC	TTCTGCCT	60ng
4	117	Live cells	C12 (5mM final)	40°C for 15min	Mn2+ (3mM)	i5-1 + i7-4	TAGATCGC	GCTCAGGA	60ng
5	118	Live cells	C30 (5mM final)	40°C for 15min	Mn2+ (3mM)	i5-1 + i7-5	TAGATCGC	AGGAGTCC	60ng
6	119	Live cells	C30 (5mM final)	40°C for 15min	Mn2+ (3mM)	i5-1 + i7-6	TAGATCGC	CATGCCTA	60ng
7	120	Live cells	Ctrl (5% DMSO)	50°C for 15min	Mn2+ (3mM)	i5-1 + i7-7	TAGATCGC	GTAGAGAG	60ng
8	121	Live cells	Ctrl (5% DMSO)	50°C for 15min	Mn2+ (3mM)	i5-1 + i7-8	TAGATCGC	CCTCTCTG	60ng
9	122	Live cells	C12 (5mM final)	50°C for 15min	Mn2+ (3mM)	i5-1 + i7-9	TAGATCGC	AGCGTAGC	60ng
10	123	Live cells	C12 (5mM final)	50°C for 15min	Mn2+ (3mM)	i5-1 + i7-10	TAGATCGC	CAGCCTCG	60ng
11	124	Live cells	C30 (5mM final)	50°C for 15min	Mn2+ (3mM)	i5-1 + i7-11	TAGATCGC	TGCCTCTT	60ng
12	125	Live cells	C30 (5mM final)	50°C for 15min	Mn2+ (3mM)	i5-1 + i7-12	TAGATCGC	TCCTCTAC	60ng
13	126	Live cells	C34 (1mM final)	50°C for 15min	Mn2+ (3mM)	i5-2 + i7-1	CTCTCTAT	TCGCCTTA	60ng
14	127	Live cells	C34 (1mM final)	50°C for 15min	Mn2+ (3mM)	i5-2 + i7-2	CTCTCTAT	CTAGTACG	60ng
15	128	Live cells	C12 (5mM final) + C34 (1mM final)	50°C for 15min	Mn2+ (3mM)	i5-2 + i7-3	CTCTCTAT	TTCTGCCT	60ng
16	129	Live cells	C12 (5mM final) + C34 (1mM final)	50°C for 15min	Mn2+ (3mM)	i5-2 + i7-4	CTCTCTAT	GCTCAGGA	60ng
17	130	Live cells	C30 (5mM final) + C34 (1mM final)	50°C for 15min	Mn2+ (3mM)	i5-2 + i7-5	CTCTCTAT	AGGAGTCC	60ng
18	131	Live cells	C30 (5mM final) + C34 (1mM final)	50°C for 15min	Mn2+ (3mM)	i5-2 + i7-6	CTCTCTAT	CATGCCTA	60ng
19	132	Live cells	Ctrl (5% DMSO)	60°C for 15min	Mn2+ (3mM)	i5-2 + i7-7	CTCTCTAT	GTAGAGAG	60ng

20	133	Live cells	Ctrl (5% DMSO)	60°C for 15min	Mn2+ (3mM)	i5-2 + i7-8	CTCTCTAT	CCTCTCTG	60ng
21	134	Live cells	C12 (5mM final)	60°C for 15min	Mn2+ (3mM)	i5-2 + i7-9	CTCTCTAT	AGCGTAGC	60ng
22	135	Live cells	C12 (5mM final)	60°C for 15min	Mn2+ (3mM)	i5-2 + i7-10	CTCTCTAT	CAGCCTCG	60ng
23	136	Live cells	C30 (5mM final)	60°C for 15min	Mn2+ (3mM)	i5-2 + i7-11	CTCTCTAT	TGCCTCTT	60ng
24	137	Live cells	C30 (5mM final)	60°C for 15min	Mn2+ (3mM)	i5-2 + i7-12	CTCTCTAT	TCCTCTAC	60ng
25	138	Live cells	C34 (1mM final)	60°C for 15min	Mn2+ (3mM)	i5-3 + i7-1	TATCCTCT	TCGCCTTA	60ng
26	139	Live cells	C34 (1mM final)	60°C for 15min	Mn2+ (3mM)	i5-3 + i7-2	TATCCTCT	CTAGTACG	60ng
27	140	Live cells	C12 (5mM final) + C34 (1mM final)	60°C for 15min	Mn2+ (3mM)	i5-3 + i7-3	TATCCTCT	TTCTGCCT	60ng
28	141	Live cells	C12 (5mM final) + C34 (1mM final)	60°C for 15min	Mn2+ (3mM)	i5-3 + i7-4	TATCCTCT	GCTCAGGA	60ng
29	142	Live cells	C30 (5mM final) + C34 (1mM final)	60°C for 15min	Mn2+ (3mM)	i5-3 + i7-5	TATCCTCT	AGGAGTCC	60ng
30	143	Live cells	C30 (5mM final) + C34 (1mM final)	60°C for 15min	Mn2+ (3mM)	i5-3 + i7-6	TATCCTCT	CATGCCTA	60ng
31	144	Live cells	Ctrl (5% DMSO)	70°C for 15min	Mn2+ (3mM)	i5-3 + i7-7	TATCCTCT	GTAGAGAG	60ng
32	145	Live cells	Ctrl (5% DMSO)	70°C for 15min	Mn2+ (3mM)	i5-3 + i7-8	TATCCTCT	CCTCTCTG	60ng
33	146	Live cells	C12 (5mM final)	70°C for 15min	Mn2+ (3mM)	i5-3 + i7-9	TATCCTCT	AGCGTAGC	60ng
34	147	Live cells	C12 (5mM final)	70°C for 15min	Mn2+ (3mM)	i5-3 + i7-10	TATCCTCT	CAGCCTCG	60ng
35	148	Live cells	C30 (5mM final)	70°C for 15min	Mn2+ (3mM)	i5-3 + i7-11	TATCCTCT	TGCCTCTT	60ng
36	149	Live cells	C30 (5mM final)	70°C for 15min	Mn2+ (3mM)	i5-3 + i7-12	TATCCTCT	TCCTCTAC	60ng
37	150	Live cells	Ctrl (5% DMSO)	37°C for 15min	Mn2+ (3mM)	i5-4 + i7-1	AGAGTAGA	TCGCCTTA	60ng
38	151	Live cells	Ctrl (5% DMSO)	37°C for 15min	Mn2+ (3mM)	i5-4 + i7-2	AGAGTAGA	CTAGTACG	60ng
39	152	Live cells	C30-FAI (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-4 + i7-3	AGAGTAGA	TTCTGCCT	60ng
40	153	Live cells	C30-FAI (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-4 + i7-4	AGAGTAGA	GCTCAGGA	60ng
41	154	Live cells	FAI-N3 (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-4 + i7-5	AGAGTAGA	AGGAGTCC	60ng

42	155	Live cells	FAI-N3 (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-4 + i7-6	AGAGTAGA	CATGCCTA	60ng
43	156	Live cells	Ctrl (5% DMSO)	80°C for 15min	Mn2+ (3mM)	i5-4 + i7-7	AGAGTAGA	GTAGAGAG	60ng
44	157	Live cells	Ctrl (5% DMSO)	80°C for 15min	Mn2+ (3mM)	i5-4 + i7-8	AGAGTAGA	CCTCTCTG	60ng
45	158	Live cells	C12 (5mM final)	80°C for 15min	Mn2+ (3mM)	i5-4 + i7-9	AGAGTAGA	AGCGTAGC	60ng
46	159	Live cells	C12 (5mM final)	80°C for 15min	Mn2+ (3mM)	i5-4 + i7-10	AGAGTAGA	CAGCCTCG	60ng
47	160	Live cells	C15 (5mM final)	80°C for 15min	Mn2+ (3mM)	i5-4 + i7-11	AGAGTAGA	TGCCTCTT	60ng
48	161	Live cells	C15 (5mM final)	80°C for 15min	Mn2+ (3mM)	i5-4 + i7-12	AGAGTAGA	TCCTCTAC	60ng
49	178	Live cells	C12 (5mM final) + C30 (5mM final)	80°C for 15min	Mn2+ (3mM)	i5-5 + i7-8	GTAAGGAG	CCTCTCTG	60ng
50	162	Live cells	C30 (5mM final)	80°C for 15min	Mn2+ (3mM)	i5-5 + i7-9	GTAAGGAG	AGCGTAGC	60ng
51	163	Live cells	C30 (5mM final)	80°C for 15min	Mn2+ (3mM)	i5-5 + i7-10	GTAAGGAG	CAGCCTCG	60ng
52	76	Live cells	Ctrl (5% DMSO)	37°C for 2h	Mn2+ (3mM)	i5-5 + i7-11	GTAAGGAG	TGCCTCTT	60ng
53	77	Live cells	Ctrl (5% DMSO)	37°C for 2h	Mn2+ (3mM)	i5-5 + i7-12	GTAAGGAG	TCCTCTAC	60ng
54	78	Live cells	C34-NM (1mM final)	37°C for 2h	Mn2+ (3mM)	i5-6 + i7-1	ACTGCATA	TCGCCTTA	60ng
55	79	Live cells	C34-NM (1mM final)	37°C for 2h	Mn2+ (3mM)	i5-6 + i7-2	ACTGCATA	CTAGTACG	60ng
56	80	Live cells	NM-only (1mM final)	37°C for 2h	Mn2+ (3mM)	i5-6 + i7-3	ACTGCATA	TTCTGCCT	60ng
57	81	Live cells	NM-only (1mM final)	37°C for 2h	Mn2+ (3mM)	i5-6 + i7-4	ACTGCATA	GCTCAGGA	60ng
58	82	Live cells	Ctrl (5% DMSO)	37°C for 30min	Mn2+ (3mM)	i5-6 + i7-5	ACTGCATA	AGGAGTCC	60ng
59	83	Live cells	Ctrl (5% DMSO)	37°C for 30min	Mn2+ (3mM)	i5-6 + i7-6	ACTGCATA	CATGCCTA	60ng
60	84	Live cells	C34-Squarate (1mM final)	37°C for 30min	Mn2+ (3mM)	i5-6 + i7-7	ACTGCATA	GTAGAGAG	60ng
61	85	Live cells	C34-Squarate (1mM final)	37°C for 30min	Mn2+ (3mM)	i5-6 + i7-8	ACTGCATA	CCTCTCTG	60ng
62	86	Live cells	Squarate-only (1mM final)	37°C for 30min	Mn2+ (3mM)	i5-6 + i7-9	ACTGCATA	AGCGTAGC	60ng
63	87	Live cells	Squarate-only (1mM final)	37°C for 30min	Mn2+ (3mM)	i5-6 + i7-10	ACTGCATA	CAGCCTCG	60ng

64	88	Fixed cells	Ctrl (5% DMSO)	37°C for 2h	Mn2+ (3mM)	i5-6 + i7-11	ACTGCATA	TGCCTCTT	60ng
65	89	Fixed cells	Ctrl (5% DMSO)	37°C for 2h	Mn2+ (3mM)	i5-6 + i7-12	ACTGCATA	TCCTCTAC	60ng
66	90	Fixed cells	C34-NM (1mM final)	37°C for 2h	Mn2+ (3mM)	i5-7 + i7-1	AAGGAGTA	TCGCCTTA	60ng
67	91	Fixed cells	C34-NM (1mM final)	37°C for 2h	Mn2+ (3mM)	i5-7 + i7-2	AAGGAGTA	CTAGTACG	60ng
68	92	Fixed cells	NM-only (1mM final)	37°C for 2h	Mn2+ (3mM)	i5-7 + i7-3	AAGGAGTA	TTCTGCCT	60ng
69	93	Fixed cells	NM-only (1mM final)	37°C for 2h	Mn2+ (3mM)	i5-7 + i7-4	AAGGAGTA	GCTCAGGA	60ng
70	94	Fixed cells	Ctrl (5% DMSO)	37°C for 30min	Mn2+ (3mM)	i5-7 + i7-5	AAGGAGTA	AGGAGTCC	60ng
71	95	Fixed cells	Ctrl (5% DMSO)	37°C for 30min	Mn2+ (3mM)	i5-7 + i7-6	AAGGAGTA	CATGCCTA	60ng
72	96	Fixed cells	C34-Squarate (1mM final)	37°C for 30min	Mn2+ (3mM)	i5-7 + i7-7	AAGGAGTA	GTAGAGAG	60ng
73	97	Fixed cells	C34-Squarate (1mM final)	37°C for 30min	Mn2+ (3mM)	i5-7 + i7-8	AAGGAGTA	CCTCTCTG	60ng
74	98	Fixed cells	Squarate-only (1mM final)	37°C for 30min	Mn2+ (3mM)	i5-7 + i7-9	AAGGAGTA	AGCGTAGC	60ng
75	99	Fixed cells	Squarate-only (1mM final)	37°C for 30min	Mn2+ (3mM)	i5-7 + i7-10	AAGGAGTA	CAGCCTCG	60ng
76	164	Fixed cells	Ctrl (5% DMSO)	37°C for 15min	Mn2+ (3mM)	i5-7 + i7-11	AAGGAGTA	TGCCTCTT	120ng
77	165	Fixed cells	Ctrl (5% DMSO)	37°C for 15min	Mn2+ (3mM)	i5-7 + i7-12	AAGGAGTA	TCCTCTAC	120ng
78	166	Fixed cells	C30-FAI (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-8 + i7-1	CTAAGCCT	TCGCCTTA	120ng
79	167	Fixed cells	C30-FAI (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-8 + i7-2	CTAAGCCT	CTAGTACG	120ng
80	168	Fixed cells	FAI-N3 (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-8 + i7-3	CTAAGCCT	TTCTGCCT	120ng
81	169	Fixed cells	FAI-N3 (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-8 + i7-4	CTAAGCCT	GCTCAGGA	120ng
82	170	Fixed cells	Ctrl (5% DMSO)	80°C for 15min	Mn2+ (3mM)	i5-8 + i7-5	CTAAGCCT	AGGAGTCC	120ng
83	171	Fixed cells	Ctrl (5% DMSO)	80°C for 15min	Mn2+ (3mM)	i5-8 + i7-6	CTAAGCCT	CATGCCTA	120ng
84	172	Fixed cells	C12 (5mM final)	80°C for 15min	Mn2+ (3mM)	i5-8 + i7-7	CTAAGCCT	GTAGAGAG	120ng
85	173	Fixed cells	C12 (5mM final)	80°C for 15min	Mn2+ (3mM)	i5-8 + i7-8	CTAAGCCT	CCTCTCTG	120ng

86	174	Fixed cells	C15 (5mM final)	80°C for 15min	Mn2+ (3mM)	i5-8 + i7-9	CTAAGCCT	AGCGTAGC	120ng
87	175	Fixed cells	C15 (5mM final)	80°C for 15min	Mn2+ (3mM)	i5-8 + i7-10	CTAAGCCT	CAGCCTCG	120ng
88	176	Fixed cells	C30 (5mM final)	80°C for 15min	Mn2+ (3mM)	i5-8 + i7-11	CTAAGCCT	TGCCTCTT	120ng
89	177	Fixed cells	C30 (5mM final)	80°C for 15min	Mn2+ (3mM)	i5-8 + i7-12	CTAAGCCT	TCCTCTAC	120ng

Not sequenced (chemical precipitation during incubation)

	#	Treated on	Treatment	Incubation	RT ion	Index	i5	i7	RNA input (10ul RT rxn)
1	100	Purified RNA	Ctrl (10% DMSO)	37°C for 2h	Mn2+ (3mM)	i5-7 + i7-11	AAGGAGTA	TGCCTCTT	60ng
2	101	Purified RNA	C34-NM (1mM final)	37°C for 2h	Mn2+ (3mM)	i5-7 + i7-12	AAGGAGTA	TCCTCTAC	60ng
3	102	Purified RNA	C34-NM (1mM final) + C34 (5mM final)	37°C for 2h	Mn2+ (3mM)	i5-8 + i7-1	CTAAGCCT	TCGCCTTA	60ng
4	103	Purified RNA	C34-NM (1mM final) + C34 (5mM final)	37°C for 2h	Mn2+ (3mM)	i5-8 + i7-2	CTAAGCCT	CTAGTACG	60ng
5	104	Purified RNA	NM-only (1mM final)	37°C for 2h	Mn2+ (3mM)	i5-8 + i7-3	CTAAGCCT	TTCTGCCT	60ng
6	105	Purified RNA	NM-only (1mM final) + C34 (5mM final)	37°C for 2h	Mn2+ (3mM)	i5-8 + i7-4	CTAAGCCT	GCTCAGGA	60ng
7	106	Purified RNA	NM-only (1mM final) + C34 (5mM final)	37°C for 2h	Mn2+ (3mM)	i5-8 + i7-5	CTAAGCCT	AGGAGTCC	60ng
8	107	Purified RNA	Ctrl (10% DMSO)	37°C for 30min	Mn2+ (3mM)	i5-8 + i7-6	CTAAGCCT	CATGCCTA	60ng
9	108	Purified RNA	C34-Squarate (1mM final)	37°C for 30min	Mn2+ (3mM)	i5-8 + i7-7	CTAAGCCT	GTAGAGAG	60ng
10	109	Purified RNA	C34-Squarate (1mM final) + C34 (5mM final)	37°C for 30min	Mn2+ (3mM)	i5-8 + i7-8	CTAAGCCT	CCTCTCTG	60ng
11	110	Purified RNA	C34-Squarate (1mM final) + C34 (5mM final)	37°C for 30min	Mn2+ (3mM)	i5-8 + i7-9	CTAAGCCT	AGCGTAGC	60ng
12	111	Purified RNA	Squarate-only (1mM final)	37°C for 30min	Mn2+ (3mM)	i5-8 + i7-10	CTAAGCCT	CAGCCTCG	60ng
13	112	Purified RNA	Squarate-only (1mM final) + C34 (5mM final)	37°C for 30min	Mn2+ (3mM)	i5-8 + i7-11	CTAAGCCT	TGCCTCTT	60ng
14	113	Purified RNA	Squarate-only (1mM final) + C34 (5mM final)	37°C for 30min	Mn2+ (3mM)	i5-8 + i7-12	CTAAGCCT	TCCTCTAC	60ng

THURSDAY, 6/5/2025

Amplicon PCR

First PCR - System

	Component	Vol (ul)	70x (ul)	Master mix (ul)	Note
1	cDNA from 60ng RNA (10ul rxn system)	1	1/EA		5%
2	5x Phusion HF buff	4	280		
3	10mM dNTP	0.4	28		
4	100uM F	0.1	7		CoV2_5UTR_P5_N6_Fw (345um stock)
5	100uM R	0.1	7		CoV2_5UTR_P7_N6_Rv (293um stock)
6	Water	14.2	994		
7	Phusion Pol	0.2	14	19/EA	
8	Total	20	20/EA		

First PCR - Program

	Temp	Time	Cycle
1	98°C	30s	
2	98°C	10s	
3	62°C	10s	
4	72°C	15s	15x
5	72°C	5min	
6	4°C	hold	

Bead cleanup

- 1.8x (36ul into 20ul)
- Wash twice with 150ul 70% EtOH
- Elute=30ul, transfer out 27ul

Indexed PCR

Second PCR - System

	Component	Vol (ul)	70x (ul)	Master mix (ul)	Note
1	First PCR product (purified)	27	27/EA		
2	5uM F+R	5	5/EA		P5/P7
3	5x Phusion HF buff	10	700		
4	10mM dNTP	1	70		
5	Water	6.5	455		
6	Phusion Pol	0.5	35	18/EA	
7	Total	50	50/EA		

Second PCR - Program

	Temp	Time	Cycle
1	98°C	30s	
2	98°C	10s	
3	61°C	10s	
4	72°C	15s	15x
5	72°C	5min	
6	4°C	hold	

Bead cleanup

- 1.8x (90ul into 50ul)
- Wash twice with 150ul 70% EtOH
- Elute=30ul, transfer out 27ul

Qubit

- Master mix: 1ul reagent + 199ul buffer (90x)
- S1: 10ul Standard #1 + 190ul master mix; 8 S1
- S2: 10ul Standard #2 + 190ul master mix; 8 S2
- Samples: 1ul sample + 199ul master mix; 65 samples
- Expect ~20ng/ul for each sample

Amplicon Qubit concentration (#2a - live cell samples)

	#	Qubit (ng/ul)	5ul PCR product into X ul water (dilute to 5.7ng/ul)	Pool
1	76	26.80	18.5	HC Amplicon lib #2a
2	77	26.20	18.0	HC Amplicon lib #2a
3	78	31.40	22.5	HC Amplicon lib #2a
4	79	32.20	23.2	HC Amplicon lib #2a
5	80	31.40	22.5	HC Amplicon lib #2a
6	81	30.00	21.3	HC Amplicon lib #2a
7	82	32.20	23.2	HC Amplicon lib #2a
8	83	32.00	23.1	HC Amplicon lib #2a
9	84	31.60	22.7	HC Amplicon lib #2a
10	85	29.20	20.6	HC Amplicon lib #2a
11	86	27.20	18.9	HC Amplicon lib #2a
12	87	26.60	18.3	HC Amplicon lib #2a
13	114	40.00	30.1	HC Amplicon lib #2a
14	115	38.60	28.9	HC Amplicon lib #2a
15	116	37.90	28.2	HC Amplicon lib #2a
16	117	36.80	27.3	HC Amplicon lib #2a
17	118	37.50	27.9	HC Amplicon lib #2a
18	119	35.90	26.5	HC Amplicon lib #2a
19	120	37.30	27.7	HC Amplicon lib #2a
20	121	36.10	26.7	HC Amplicon lib #2a
21	122	35.60	26.2	HC Amplicon lib #2a
22	123	35.90	26.5	HC Amplicon lib #2a
23	124	32.50	23.5	HC Amplicon lib #2a
24	125	31.90	23.0	HC Amplicon lib #2a
25	126	40.00	30.1	HC Amplicon lib #2a
26	127	38.60	28.9	HC Amplicon lib #2a
27	128	34.40	25.2	HC Amplicon lib #2a
28	129	31.10	22.3	HC Amplicon lib #2a
29	130	33.80	24.6	HC Amplicon lib #2a
30	131	34.50	25.3	HC Amplicon lib #2a
31	132	34.20	25.0	HC Amplicon lib #2a
32	133	34.50	25.3	HC Amplicon lib #2a
33	134	24.80	16.8	HC Amplicon lib #2a

34	135	25.60	17.5	HC Amplicon lib #2a
35	136	34.40	25.2	HC Amplicon lib #2a
36	137	35.30	26.0	HC Amplicon lib #2a
37	138	36.70	27.2	HC Amplicon lib #2a
38	139	37.30	27.7	HC Amplicon lib #2a
39	140	23.70	15.8	HC Amplicon lib #2a
40	141	20.30	12.8	HC Amplicon lib #2a
41	142	27.40	19.0	HC Amplicon lib #2a
42	143	28.70	20.2	HC Amplicon lib #2a
43	144	27.80	19.4	HC Amplicon lib #2a
44	145	30.80	22.0	HC Amplicon lib #2a
45	146	34.30	25.1	HC Amplicon lib #2a
46	147	36.50	27.0	HC Amplicon lib #2a
47	148	27.80	19.4	HC Amplicon lib #2a
48	149	30.70	21.9	HC Amplicon lib #2a
49	150	40.00	30.1	HC Amplicon lib #2a
50	151	39.90	30.0	HC Amplicon lib #2a
51	152	33.70	24.6	HC Amplicon lib #2a
52	153	33.10	24.0	HC Amplicon lib #2a
53	154	35.40	26.1	HC Amplicon lib #2a
54	155	37.10	27.5	HC Amplicon lib #2a
55	156	35.30	26.0	HC Amplicon lib #2a
56	157	34.40	25.2	HC Amplicon lib #2a
57	158	32.30	23.3	HC Amplicon lib #2a
58	159	33.90	24.7	HC Amplicon lib #2a
59	160	35.80	26.4	HC Amplicon lib #2a
60	161	34.70	25.4	HC Amplicon lib #2a
61	162	37.10	27.5	HC Amplicon lib #2a
62	163	38.40	28.7	HC Amplicon lib #2a
63	178	38.70	28.9	HC Amplicon lib #2a

- Batch #76-87 Mean ± SD = 29.7 ± 2.3
- Batch #114-178 Mean ± SD = 34.1 ± 4.4

Amplicon Qubit concentration (#2b - fixed cell samples)

	#	Qubit (ng/ul)	20ul PCR product into X ul water (dilute to 1.2ng/ul)	Pool
1	88	6.50	88.3	HC Amplicon lib #2b
2	89	7.38	103.0	HC Amplicon lib #2b
3	90	10.60	156.7	HC Amplicon lib #2b
4	91	15.70	241.7	HC Amplicon lib #2b
5	92	7.04	97.3	HC Amplicon lib #2b
6	93	6.90	95.0	HC Amplicon lib #2b
7	94	6.62	90.3	HC Amplicon lib #2b
8	95	6.86	94.3	HC Amplicon lib #2b
9	96	6.94	95.7	HC Amplicon lib #2b
10	97	7.70	108.3	HC Amplicon lib #2b
11	98	9.46	137.7	HC Amplicon lib #2b
12	99	13.40	203.3	HC Amplicon lib #2b
13	164	2.34	19.0	HC Amplicon lib #2b
14	165	2.15	15.8	HC Amplicon lib #2b
15	166	2.03	13.8	HC Amplicon lib #2b
16	167	1.85	10.8	HC Amplicon lib #2b
17	168	2.10	15.0	HC Amplicon lib #2b
18	169	2.05	14.2	HC Amplicon lib #2b
19	170	1.97	12.8	HC Amplicon lib #2b
20	171	2.18	16.3	HC Amplicon lib #2b
21	172	2.19	16.5	HC Amplicon lib #2b
22	173	2.04	14.0	HC Amplicon lib #2b
23	174	1.91	11.8	HC Amplicon lib #2b
24	175	1.67	7.8	HC Amplicon lib #2b
25	176	1.94	12.3	HC Amplicon lib #2b
26	177	1.29	1.5	HC Amplicon lib #2b

- Batch #88-99 Mean ± SD = 8.8 ± 2.9
- Batch #164-177 Mean ± SD = 2.0 ± 0.2

- For fixed cell samples, after pooling 10ul from each diluted sample, redo bead cleanup to concentrate:
  - 520 DNA, 1.8x bead = 936ul
  - two washes with 1.5ml 70% EtOH

- elute with 50ul water
- Qubit: 8.4ng/ul
- Dilute to 5.7ng/ul (20nM)

Not pooled for sequencing

	#	Qubit (ng/ul)	5ul PCR product into X ul water (dilute to 5.7ng/ul)
1	100	29.40	20.8
2	101	28.00	19.6
3	102	31.80	22.9
4	103	26.80	18.5
5	104	30.20	21.5
6	105	30.20	21.5
7	106	29.80	21.1
8	107	31.20	22.4
9	108	30.20	21.5
10	109	31.60	22.7
11	110	27.00	18.7
12	111	28.00	19.6
13	112	28.80	20.3
14	113	29.40	20.8

Normalization

- Based on the equation below, dilute each sample to **5.7ng/ul** (20nM), then take 5ul of each diluted sample to pool (for sequencing, the minimum requirement is 13ul of 10nM)

image.png

$$\text{nM} = \frac{(\text{ng}/\mu\text{L}) \times 10^6}{(\text{bp length}) \times 660}$$

Submission for AVITI-seq (Low Output flow cell, PE150)

- No BioAnalyzer report coming back
- Directly analyzed the fastq files

Note:

- For fixed cell samples, the second batch (#164-177) had significantly lower final PCR product than the first batch (#88-99), meaning there was more RNA damage in the second batch. I reduced the DSS fixing reagent down to half in the second batch, which seemed to work worse. Maybe higher concentration immobilized proteins faster and RNA was less accessible to DSS. If fixation turns out to be a necessary step, a more comprehensive testing (e.g., different concentrations, DSS vs DSP) will be needed.