2025.5.20 Amplicon library #1

Project: Hang_JW Lab

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MONDAY, 5/19/2025

Planned samples

	#	Treated on	Treatment	Incubation	RT ion	Index	i5	i7	RNA input (10ul RT rxn)
1	1	Cells	Ctrl (5% DMSO)	37°C for 15min on cells	Mg2+ (3mM)	i5-3 + i7-1	TATCCTCT	TCGCCTTA	60ng
2	16	Cells	Ctrl (5% DMSO)	37°C for 15min on cells	Mg2+ (3mM)	i5-3 + i7-2	TATCCTCT	CTAGTACG	60ng
3	17	Cells	Ctrl (5% DMSO)	37°C for 15min on cells	Mg2+ (3mM)	i5-3 + i7-3	TATCCTCT	TTCTGCCT	60ng
4	2	Cells	Ctrl (5% DMSO)	37°C for 15min on cells	Mn2+ (3mM)	i5-3 + i7-4	TATCCTCT	GCTCAGGA	60ng
5	18	Cells	Ctrl (5% DMSO)	37°C for 15min on cells	Mn2+ (3mM)	i5-3 + i7-5	TATCCTCT	AGGAGTCC	60ng
6	19	Cells	Ctrl (5% DMSO)	37°C for 15min on cells	Mn2+ (3mM)	i5-3 + i7-6	TATCCTCT	CATGCCTA	60ng
7	7	Cells	Ctrl (5% DMSO)	80°C for 15min on cells	Mg2+ (3mM)	i5-3 + i7-7	TATCCTCT	GTAGAGAG	60ng
8	22	Cells	Ctrl (5% DMSO)	80°C for 15min on cells	Mg2+ (3mM)	i5-3 + i7-8	TATCCTCT	CCTCTCTG	60ng
9	23	Cells	Ctrl (5% DMSO)	80°C for 15min on cells	Mg2+ (3mM)	i5-3 + i7-9	TATCCTCT	AGCGTAGC	60ng
10	8	Cells	Ctrl (5% DMSO)	80°C for 15min on cells	Mn2+ (3mM)	i5-3 + i7-10	TATCCTCT	CAGCCTCG	60ng
11	24	Cells	Ctrl (5% DMSO)	80°C for 15min on cells	Mn2+ (3mM)	i5-3 + i7-11	TATCCTCT	TGCCTCTT	60ng
12	25	Cells	Ctrl (5% DMSO)	80°C for 15min on cells	Mn2+ (3mM)	i5-3 + i7-12	TATCCTCT	TCCTCTAC	60ng
13	3	Cells	C12 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-4 + i7-1	AGAGTAGA	TCGCCTTA	60ng
14	28	Cells	C12 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-4 + i7-2	AGAGTAGA	CTAGTACG	60ng
15	29	Cells	C12 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-4 + i7-3	AGAGTAGA	TTCTGCCT	60ng
16	5	Cells	C12 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-4 + i7-4	AGAGTAGA	GCTCAGGA	60ng
17	34	Cells	C12 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-4 + i7-5	AGAGTAGA	AGGAGTCC	60ng
18	35	Cells	C12 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-4 + i7-6	AGAGTAGA	CATGCCTA	60ng
19	9	Cells	C12 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-4 + i7-7	AGAGTAGA	GTAGAGAG	60ng
20	40	Cells	C12 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-4 + i7-8	AGAGTAGA	CCTCTCTG	60ng

21	41	Cells	C12 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-4 + i7-9	AGAGTAGA	AGCGTAGC	60ng
22	11	Cells	C12 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-4 + i7-10	AGAGTAGA	CAGCCTCG	60ng
23	46	Cells	C12 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-4 + i7-11	AGAGTAGA	TGCCTCTT	60ng
24	47	Cells	C12 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-4 + i7-12	AGAGTAGA	TCCTCTAC	60ng
25	4	Cells	C15 (5mM final)	df_name <- "my_dataframe"	Mn2+ (3mM)	i5-5 + i7-1	GTAAGGAG	TCGCCTTA	60ng
26	30	Cells	C15 (5mM final)	df <- get(df_name)	Mn2+ (3mM)	i5-5 + i7-2	GTAAGGAG	CTAGTACG	60ng
27	31	Cells	C15 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-5 + i7-3	GTAAGGAG	TTCTGCCT	60ng
28	6	Cells	C15 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-5 + i7-4	GTAAGGAG	GCTCAGGA	60ng
29	36	Cells	C15 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-5 + i7-5	GTAAGGAG	AGGAGTCC	60ng
30	37	Cells	C15 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-5 + i7-6	GTAAGGAG	CATGCCTA	60ng
31	10	Cells	C15 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-5 + i7-7	GTAAGGAG	GTAGAGAG	60ng
32	42	Cells	C15 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-5 + i7-8	GTAAGGAG	CCTCTCTG	60ng
33	43	Cells	C15 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-5 + i7-9	GTAAGGAG	AGCGTAGC	60ng
34	12	Cells	C15 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-5 + i7-10	GTAAGGAG	CAGCCTCG	60ng
35	48	Cells	C15 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-5 + i7-11	GTAAGGAG	TGCCTCTT	60ng
36	49	Cells	C15 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-5 + i7-12	GTAAGGAG	TCCTCTAC	60ng
37	20	Cells	C30 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-6 + i7-1	ACTGCATA	TCGCCTTA	60ng
38	32	Cells	C30 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-6 + i7-2	ACTGCATA	CTAGTACG	60ng
39	33	Cells	C30 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-6 + i7-3	ACTGCATA	TTCTGCCT	60ng
40	21	Cells	C30 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-6 + i7-4	ACTGCATA	GCTCAGGA	60ng
41	38	Cells	C30 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-6 + i7-5	ACTGCATA	AGGAGTCC	60ng
42	39	Cells	C30 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)	i5-6 + i7-6	ACTGCATA	CATGCCTA	60ng

43	26	Cells	C30 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-6 + i7-7	ACTGCATA	GTAGAGAG	60ng
44	44	Cells	C30 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-6 + i7-8	ACTGCATA	CCTCTCTG	60ng
45	45	Cells	C30 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-6 + i7-9	ACTGCATA	AGCGTAGC	60ng
46	27	Cells	C30 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-6 + i7-10	ACTGCATA	CAGCCTCG	60ng
47	50	Cells	C30 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-6 + i7-11	ACTGCATA	TGCCTCTT	60ng
48	51	Cells	C30 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)	i5-6 + i7-12	ACTGCATA	TCCTCTAC	60ng
49	52	Purified RNA	Ctrl (10% DMSO)	37°C for 30min on RNA	Mg2+ (3mM)	i5-7 + i7-1	AAGGAGTA	TCGCCTTA	60ng
50	53	Purified RNA	Ctrl (10% DMSO)	37°C for 30min on RNA	Mg2+ (3mM)	i5-7 + i7-2	AAGGAGTA	CTAGTACG	60ng
51	54	Purified RNA	Ctrl (10% DMSO)	37°C for 30min on RNA	Mg2+ (3mM)	i5-7 + i7-3	AAGGAGTA	TTCTGCCT	60ng
52	55	Purified RNA	Ctrl (10% DMSO)	37°C for 30min on RNA	Mn2+ (3mM)	i5-7 + i7-4	AAGGAGTA	GCTCAGGA	60ng
53	56	Purified RNA	Ctrl (10% DMSO)	37°C for 30min on RNA	Mn2+ (3mM)	i5-7 + i7-5	AAGGAGTA	AGGAGTCC	60ng
54	57	Purified RNA	Ctrl (10% DMSO)	37°C for 30min on RNA	Mn2+ (3mM)	i5-7 + i7-6	AAGGAGTA	CATGCCTA	60ng
55	58	Purified RNA	Ctrl (10% DMSO)	37°C for 2h on RNA	Mg2+ (3mM)	i5-7 + i7-7	AAGGAGTA	GTAGAGAG	60ng
56	59	Purified RNA	Ctrl (10% DMSO)	37°C for 2h on RNA	Mg2+ (3mM)	i5-7 + i7-8	AAGGAGTA	CCTCTCTG	60ng
57	60	Purified RNA	Ctrl (10% DMSO)	37°C for 2h on RNA	Mg2+ (3mM)	i5-7 + i7-9	AAGGAGTA	AGCGTAGC	60ng
58	61	Purified RNA	Ctrl (10% DMSO)	37°C for 2h on RNA	Mn2+ (3mM)	i5-7 + i7-10	AAGGAGTA	CAGCCTCG	60ng
59	62	Purified RNA	Ctrl (10% DMSO)	37°C for 2h on RNA	Mn2+ (3mM)	i5-7 + i7-11	AAGGAGTA	TGCCTCTT	60ng
60	63	Purified RNA	Ctrl (10% DMSO)	37°C for 2h on RNA	Mn2+ (3mM)	i5-7 + i7-12	AAGGAGTA	TCCTCTAC	60ng
61	64	Purified RNA	C34-NM (1mM final)	37°C for 2h on RNA	Mn2+ (3mM)	i5-8 + i7-1	CTAAGCCT	TCGCCTTA	60ng
62	65	Purified RNA	C34-NM (1mM final)	37°C for 2h on RNA	Mn2+ (3mM)	i5-8 + i7-2	CTAAGCCT	CTAGTACG	60ng
63	66	Purified RNA	C34-NM (1mM final)	37°C for 2h on RNA	Mn2+ (3mM)	i5-8 + i7-3	CTAAGCCT	TTCTGCCT	60ng
64	67	Purified RNA	NM-only (1mM final)	37°C for 2h on RNA	Mn2+ (3mM)	i5-8 + i7-4	CTAAGCCT	GCTCAGGA	60ng

65	68	Purified RNA	NM-only (1mM final)	37°C for 2h on RNA	Mn2+ (3mM)	i5-8 + i7-5	CTAAGCCT	AGGAGTCC	60ng
66	69	Purified RNA	NM-only (1mM final)	37°C for 2h on RNA	Mn2+ (3mM)	i5-8 + i7-6	CTAAGCCT	CATGCCTA	60ng
67	70	Purified RNA	C34-Squarate (1mM final)	37°C for 30min on RNA	Mn2+ (3mM)	i5-8 + i7-7	CTAAGCCT	GTAGAGAG	60ng
68	71	Purified RNA	C34-Squarate (1mM final)	37°C for 30min on RNA	Mn2+ (3mM)	i5-8 + i7-8	CTAAGCCT	CCTCTCTG	60ng
69	72	Purified RNA	C34-Squarate (1mM final)	37°C for 30min on RNA	Mn2+ (3mM)	i5-8 + i7-9	CTAAGCCT	AGCGTAGC	60ng
70	73	Purified RNA	Squarate-only (1mM final)	37°C for 30min on RNA	Mn2+ (3mM)	i5-8 + i7-10	CTAAGCCT	CAGCCTCG	60ng
71	74	Purified RNA	Squarate-only (1mM final)	37°C for 30min on RNA	Mn2+ (3mM)	i5-8 + i7-11	CTAAGCCT	TGCCTCTT	60ng
72	75	Purified RNA	Squarate-only (1mM final)	37°C for 30min on RNA	Mn2+ (3mM)	i5-8 + i7-12	CTAAGCCT	TCCTCTAC	60ng

TUESDAY, 5/20/2025

Amplicon PCR

First PCR - System

	Component	Vol (ul)	75x (ul)	Master mix (ul)	Note
1	cDNA from 60ng RNA (10ul rxn system)	1	1/EA		5%
2	5x Phusion HF buff	4	300		
3	10mM dNTP	0.4	30		
4	100uM F	0.1	7.5		CoV2_5UTR_P5_N6_Fw (345um stock)
5	100uM R	0.1	7.5		CoV2_5UTR_P7_N6_Rv (293um stock)
6	Water	14.2	1065		
7	Phusion Pol	0.2	15	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)	74x (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	740		
4	10mM dNTP	1	74		
5	Water	6.5	481		
6	Phusion Pol	0.5	37	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 (92x)
- S1: 10ul Standard #1 + 190ul master mix; 8 S1
- S2: 10ul Standard #2 + 190ul master mix; 8 S2
- Samples: 1ul sample + 199ul master mix; 72 samples
- Expect ~20ng/ul for each sample

Amplicon Qubit concentration

	#	Qubit (ng/ul)	Gel check	5ul PCR product into X ul water (dilute to 11.4ng/ul)
1	1	30.4		8.33
2	16	28.8		7.63
3	17	28.6	Y	7.54
4	2	32.2		9.12
5	18	27.4		7.02
6	19	26.0	Y	6.40
7	7	30.8		8.51
8	22	28.0		7.28
9	23	28.8	Y	7.63
10	8	30.0		8.16
11	24	29.0		7.72
12	25	27.0	Y	6.84
13	3	29.0		7.72
14	28	28.0		7.28
15	29	30.8	Y	8.51
16	5	28.6		7.54
17	34	27.8		7.19
18	35	28.6	Y	7.54
19	9	27.6		7.11
20	40	26.0		6.40
21	41	24.2	Y	5.61
22	11	28.8		7.63
23	46	26.8		6.75
24	47	28.0	Y	7.28
25	4	29.2		7.81
26	30	27.4		7.02
27	31	29.2	Y	7.81
28	6	28.0		7.28
29	36	27.2		6.93
30	37	29.0	Y	7.72
31	10	29.6		7.98
32	42	30.4		8.33
33	43	30.4	Y	8.33

34	12	29.8		8.07
35	48	28.0		7.28
36	49	27.2	Y	6.93
37	20	22.8		5.00
38	32	23.6		5.35
39	33	23.0	Y	5.09
40	21	24.8		5.88
41	38	21.2		4.30
42	39	23.0	Y	5.09
43	26	20.6		4.04
44	44	22.4		4.82
45	45	23.4	Y	5.26
46	27	27.0		6.84
47	50	24.8		5.88
48	51	24.0	Y	5.53
49	52	26.8		6.75
50	53	26.4		6.58
51	54	27.2	Y	6.93
52	55	28.4		7.46
53	56	26.0		6.40
54	57	25.2	Y	6.05
55	58	22.6		4.91
56	59	27.8		7.19
57	60	27.2	Y	6.93
58	61	28.4		7.46
59	62	28.8		7.63
60	63	28.2	Y	7.37
61	64	30.0		8.16
62	65	30.2		8.25
63	66	26.4	Y	6.58
64	67	27.0		6.84
65	68	25.0		5.96
66	69	25.4	Y	6.14
67	70	26.8		6.75
68	71	29.4		7.89
69	72	28.0	Y	7.28
70	73	27.4		7.02

71	74	29.2		7.81
72	75	26.8	Y	6.75

- Mean ± SD = **27.2 ± 2.4 ng/ul**

Normalization

- Based on the equation below, dilute each sample to 11.4ng/ul (40nM), then take 5ul of each diluted sample to pool (for sequencing, the minimum requirement is 13ul of 10nM)

image.png

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

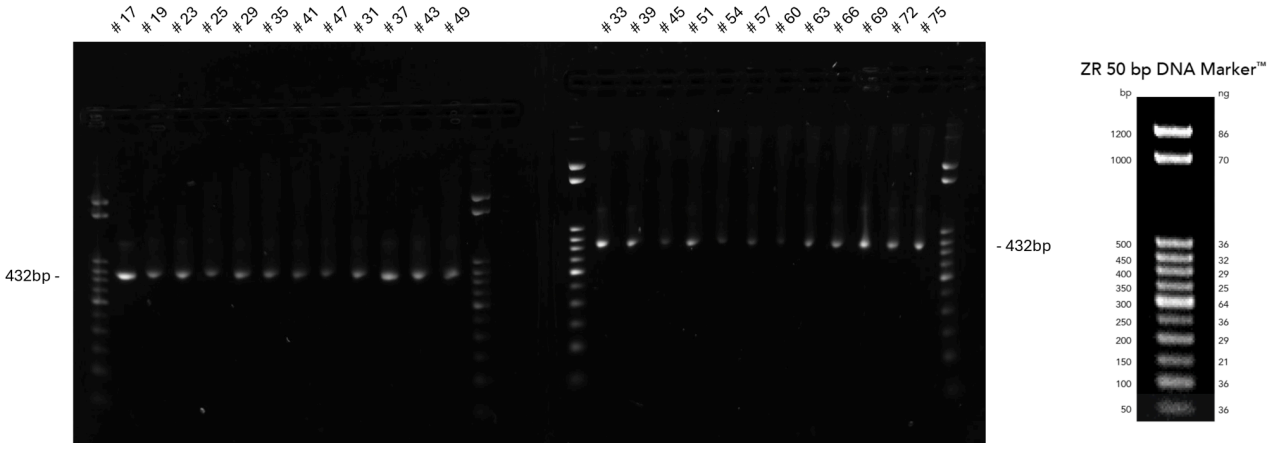
Size verification

- In each triplicate, pick one of the sample's final product, further amplify 25x, run on gel.

image.png

2025.5.20 Amplicon lib prep

- Input: 60ng treated RNA
- RT primer: CMV-5UTR-SHAPE-Rv
- First PCR primers: CoV2_5UTR_P5_N6_Fw + CoV2_5UTR_P7_N6_Rv
- Second PCR primers: P5-index-Fwd + P7-index-Rev
- Amplicon size: 432bp

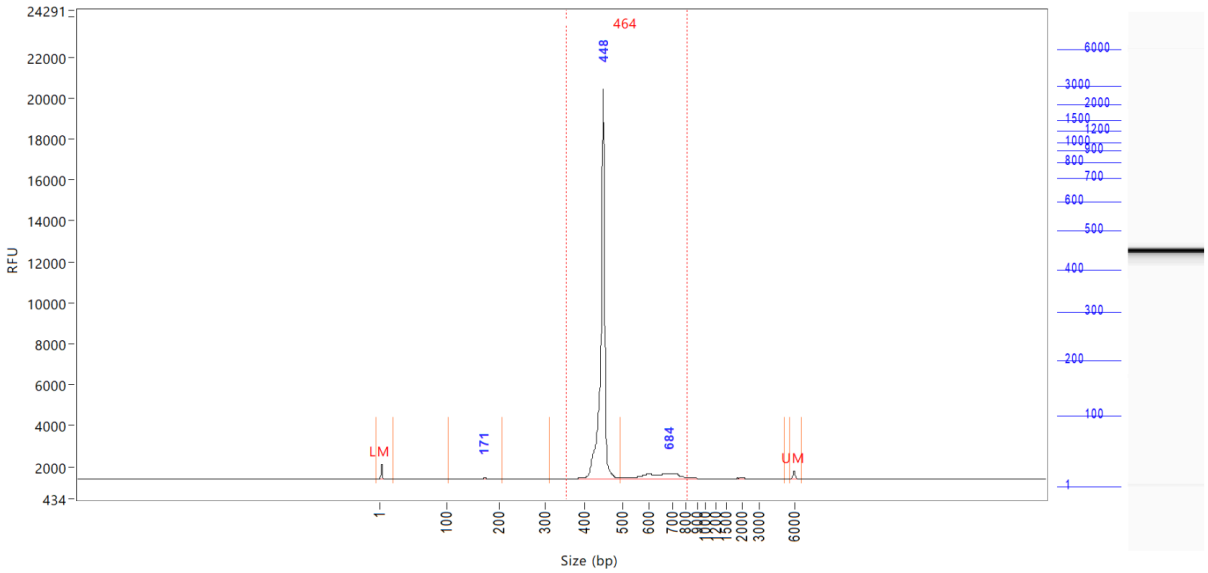


WEDNESDAY, 5/21/2025

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

BioAnalyzer

Sample: HC-Amplicon-Lib#1
Well location: E10
Created: Wednesday, May 21, 2025 11:41:58 AM



Sample Peak Width (sec): 50
Sample Min Peak Height: 25
Sample Baseline V to V?: Y
Sample Baseline V to V pts: 3
Sample filter: Binomial
Number of points for filter: 3
Sample start region (min): 0
Sample end region (min): 50
Manual baseline start (min): 10
Manual baseline end (min): 48
Marker peak width (sec): 5
Marker min peak height: 200
Marker baseline V to V?: Y
Marker baseline V to V points: 3
Lower marker selection: First peak > 200 RFU
Upper marker selection: Last peak > 200 RFU
Ladder size (bp)1, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1500, 2000, 3000, 6000
Quantification using: Ladder
Final concentration (ng/uL): 0.0415
Dilution factor: 24.0