2025.5.1-6 Pilot testing for C12 C15 reactivity in cells

Project: Hang_JW Lab
Author: Hang Chen
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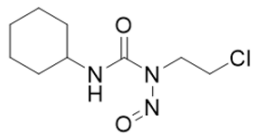
THURSDAY, 5/1/2025

Planned samples

	#	Covalent modifier	Covalent modifier (final)	Covalent rxn conditions	RT ion
1	1	DMSO (100%)	Ctrl (5% DMSO)	37°C for 15min on cells	Mg2+ (3mM)
2	2	DMSO (100%)	Ctrl (5% DMSO)	37°C for 15min on cells	Mn2+ (3mM)
3	3	C12 (100mM, stock)	C12 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)
4	4	C15 (100mM, stock)	C15 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)
5	5	C12 (10mM, 1:10 diluted)	C12 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)
6	6	C15 (10mM, 1:10 diluted)	C15 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)
7	7	DMSO (100%)	Ctrl (5% DMSO)	80°C for 15min on cells	Mg2+ (3mM)
8	8	DMSO (100%)	Ctrl (5% DMSO)	80°C for 15min on cells	Mn2+ (3mM)
9	9	C12 (100mM, stock)	C12 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)
10	10	C15 (100mM, stock)	C15 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)
11	11	C12 (10mM, 1:10 diluted)	C12 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)
12	12	C15 (10mM, 1:10 diluted)	C15 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)
13	13	DMSO ("D 1-1")	Ctrl	80°C for 5min on pure RNA	Mn2+ (3mM)
14	14	C12 ("12-2")	C12 (0.1mM final)	80°C for 5min on pure RNA	Mn2+ (3mM)
15	15	C15 ("15-2")	C15 (0.1mM final)	80°C for 5min on pure RNA	Mn2+ (3mM)

C12 Lomustine & C15 Nimustine



Lomustine

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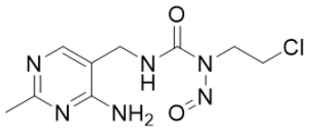

Nimustine

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Cell treatment with RNA covalent modifiers:

- Treat "SARS-CoV2 5UTR"cells (SL5 HEK293) with TrypLE Express at room temp
- Collect and wash cells with 1x PBS
- Count cells: **2.9x10^6 cells/ml**
- Passage at 1:20, keep the extra on ice
- Prepare 2x10^5 cells/rxn, 12 rxns
- Resuspend cells for each rxn in 47.5ul 1x PBS
- Add 2.5ul DMSO or covalent modifier in DMSO (total volume=50ul)
- Incubate at designated temp for **15min**
- Chill on ice for 2min
- Add 300ul RLT Plus buffer (total volume=350ul)
- Vortex for 1 min to homogenize
- Store lysate in -80

Note:

- When making 5mM solution with 1x PBS, C15 is transparent but C12 looks like emulsion at room temp, and both are transparent after 15min of 37°C or 80°C incubation.

FRIDAY, 5/2/2025

RNA extraction:

- Thaw cell lysate in RLT Plus buffer at RT
- Vortex for 1min to homogenize
- Transfer to DNA spin column, 8000xg 30s
- Add 350ul 70% EtOH to the flow-through
- Transfer to RNA spin column, 8000xg 15s, discard the flow-through
- Add 700ul RW1 buffer, 8000xg 15s, discard the flow-through
- Add 500ul 70% EtOH instead of RPE buffer, 8000xg 15s, discard the flow-through
- 500ul 80% EtOH, 8000xg 2min, discard the flow-through
- Full speed 5min
- Elute with **35ul** water

- Nanodrop:

RNA yield

	#	RNA conc (ng/ul)	RNA 260/280	100ng RNA (ul)	Water to 8ul (ul)
1	1	35.9	2.03	2.8	5.2
2	2	42.9	2.08	2.3	5.7
3	3	45.9	2.06	2.2	5.8
4	4	41.8	2.05	2.4	5.6
5	5	40.6	2.04	2.5	5.5
6	6	41.3	2.07	2.4	5.6
7	7	29.4	2.03	3.4	4.6
8	8	26.2	2.07	3.8	4.2
9	9	36.5	2.09	2.7	5.3
10	10	15	2.09	6.7	1.3
11	11	41.9	2.10	2.4	5.6
12	12	20.6	2.18	4.9	3.1

Reverse transcription:

- Input: 100ng total RNA

Annealing

	Component	vol (ul)	13x (ul)
1	CMV-5UTR-SHAPE-Rv (10uM)	1	13
2	dNTP (10mM)	1	13
3	100ng RNA+water	8	8/EA
4	Total	10	10/EA

- 65°C 5min, immediately put on ice.

Extension

	Component	vol (ul)	13x (ul)
1	Annealing product	10	10/EA
2	375mM Tris/500mM KCl (5x buffer)	4	52
3	100mM DTT (10x)	2	26
4	Protoscript II	0.5	6.5
5	RNaseIN	0.2	2.6
6	Water	1.3	16.9
7	30mM MgCl2 or MnCl2	2	2/EA
8	Total	20	20/EA

- 42°C 1h, 65°C 20min, 4°C hold.
- Store cDNA in -20.

MONDAY, 5/5/2025

PCR amplification of SL5:

System

	Component	Vol (ul)	13x (ul)	Note
1	5x Phusion HF buff	10	130	
2	10mM dNTP	1	13	
3	10uM F	2.5	32.5	COVID_6-28_F_56C
4	10uM R	2.5	32.5	CMV-5UTR_SHAPE_Rv
5	Water	23.5	305.5	
6	Phusion Pol	0.5	6.5	
7	cDNA	10	10/EA	Half RT product
8	Total	50	50/EA	

Program

	Temp	Time	Cycle
1	98°C	30s	
2	98°C	10s	
3	62°C	10s	
4	72°C	15s	32x
5	72°C	5min	
6	4°C	hold	

PCR purification:

- 250ul DNA Binding Buffer to 50ul PCR product (5:1)
- Transfer to Zymo-Spin Column, 10000xg 30s
- Add 200 ul DNA Wash Buffer, 10000xg 30s
- Repeat washing step
- Spin at full speed 2min
- Elute = 50ul water
- Nanodrop:

PCR purification

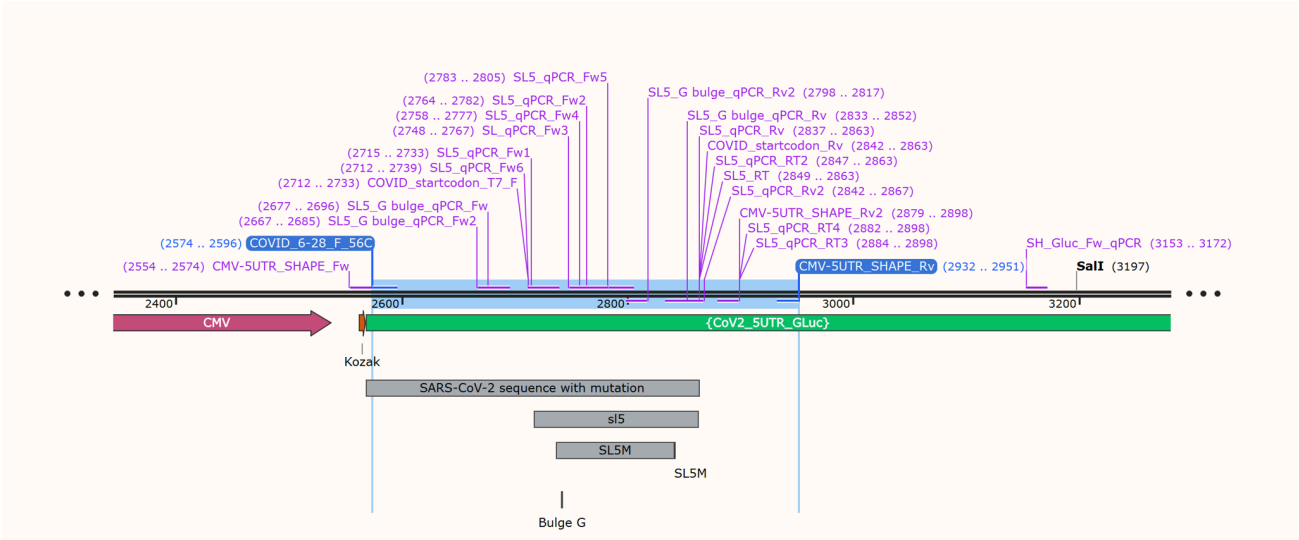
	#	DNA conc (ng/ul)	DNA 260/280	80ng= x ul	Waster to 20ul (ul)
1	1	21.1	2.06	3.8	16.2
2	2	12.5	2.02	6.4	13.6
3	3	12.0	2.14	6.7	13.3
4	4	11.1	2.06	7.2	12.8
5	5	15.2	1.85	5.3	14.7
6	6	11.7	2.01	6.8	13.2
7	7	24.8	1.90	3.2	16.8
8	8	12.0	1.87	6.7	13.3
9	9	12.2	2.02	6.6	13.4
10	10	10.5	2.13	7.6	12.4
11	11	12.6	2.07	6.3	13.7
12	12	13.1	2.08	6.1	13.9

Size verification:

- Gel: 1% agarose in 1x TBE, 1:20000 APExBIO Safe DNA Gel Stain
- Ladder: Zymo ZR 50bp DNA Marker

- Loading dye: Invitrogen 10x Blue Juice (9ul sample+1ul Blue Juice, or 7ul water+2ul 50bp Marker (1ug)+1ul Blue Juicer)
- Amplicon size=378bp
- Gel running: 100v 30min

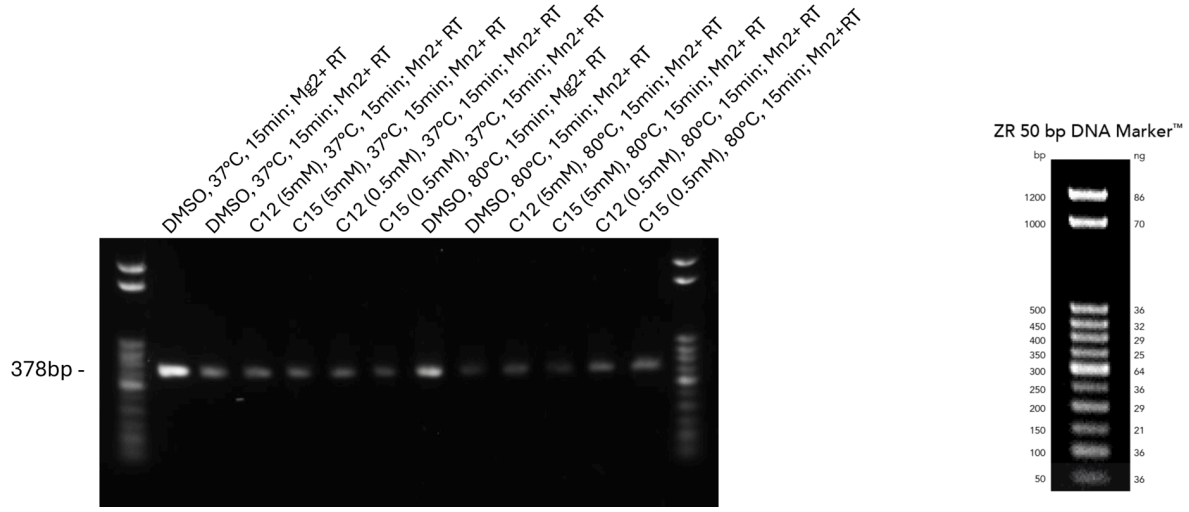
SL5 Amplicon



Gel result

2025.5.5 PCR Product

- Template: total RNA from SL5 HEK293 cells
- F primer: COVID_6-28_F_56C
- R primer: CMV-5UTR_SHAPE_Rv
- Expected size: 378bp



Note:

- After RT, the conc of total RNA should be 100ng/20ul=5ng/ul, given the LS5 RNA would be a small proportion, use high cDNA input for PCR.
- Mn2+ seemed to be less efficient than Mg2+ in terms of RT.

TUESDAY, 5/6/2025

Dr. Wang's feedback:

- Send all for Premium PCR sequencing, include positive controls from Zhichao
- Might be ideal to keep cDNA input no more than 5% for final PCR.
- Include C30 in the next next run.

Positive control samples (from Zhichao)

	#	Covalent modifier	Covalent modifier (final)	Covalent rxn temp	RT ion	cDNA conc (ul)	cDNA 260/280
1	13	DMSO ("D 1-1")	Ctrl	80°C (on naked RNA)	Mn2+ (3mM)	349.9	1.44
2	14	C12 ("12-2")	C12 (0.1mM)	80°C (on naked RNA)	Mn2+ (3mM)	385.2	1.50
3	15	C15 ("15-2")	C15 (0.1mM)	80°C (on naked RNA)	Mn2+ (3mM)	394.0	1.50

PCR amplification of positive controls:

System2

	Component	Vol (ul)	4x (ul)	Note
1	5x Phusion HF buff	10	40	
2	10mM dNTP	1	4	
3	2uM F+R	12.5	50	"148 PCR primer" (labeled on tube, from Zhichao); SMN2-GT-PCR-FW (GT2) + SMN1/2-GT-PCR-Rv (on map)
4	Water	23.5	94	
5	Phusion Pol	0.5	2	
6	cDNA	2.5	2.5/EA	5%
7	Total	50	50/EA	

Program2

	Temp	Time	Cycle
1	98°C	30s	
2	98°C	10s	35x
3	58°C	10s	
4	72°C	15s	
5	72°C	5min	
6	4°C	hold	

PCR purification:

- 250ul DNA Binding Buffer to 50ul PCR product (5:1)
- Transfer to Zymo-Spin Column, 10000xg 30s

- Add 200 ul DNA Wash Buffer, 10000xg 30s
- Repeat washing step
- Spin at full speed 2min
- Elute = **50ul** water
- Nanodrop:

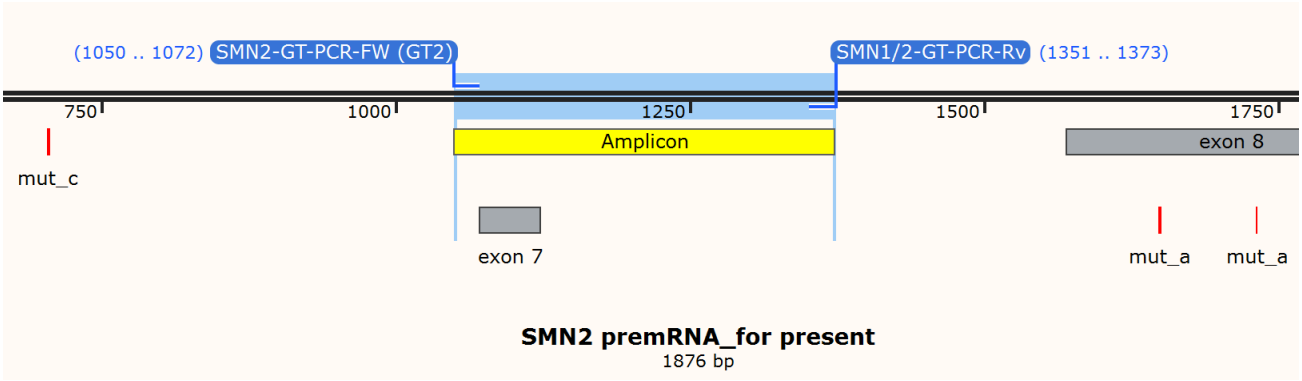
PCR purification2

	#	DNA conc (ng/ul)	DNA 260/280	80ng= x ul	Water to 20ul (ul)
1	13	31.5	1.85	2.5	17.5
2	14	35.3	1.85	2.3	17.7
3	15	38.5	1.87	2.1	17.9

Size verification:

- Gel: 1% agarose in 1x TBE, 1:20000 APExBIO Safe DNA Gel Stain
- Ladder: Zymo ZR 50bp DNA Marker
- Loading dye: Invitrogen 10x Blue Juice (200ng sample or 1ug 50bp Marker)
- Amplicon size=324bp
- Gel running: 100v 30min

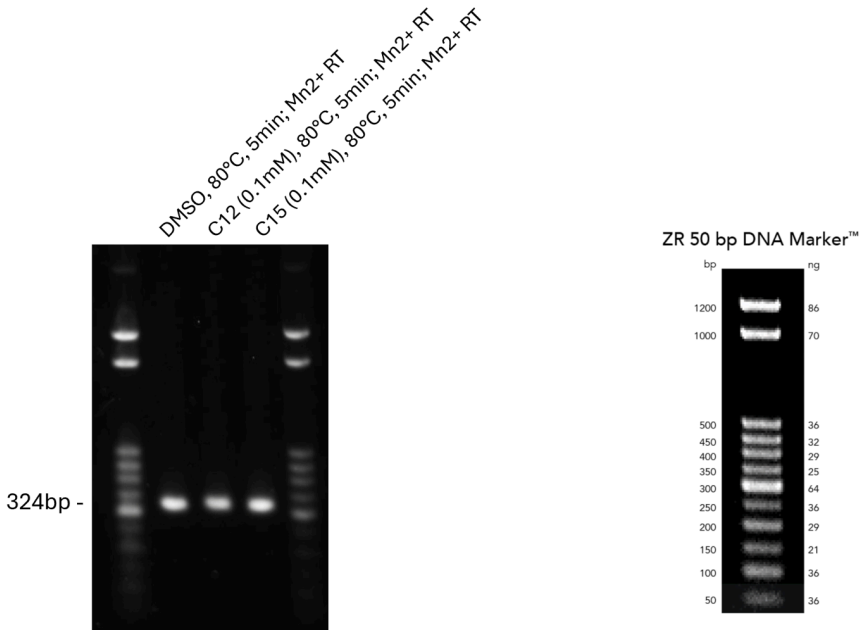
SMN2 exon7 amplicon



Gel result2

2025.5.6 PCR Product

- Template: cDNA from Zhichao (covalent modification on naked RNA)
- F/R primer: “148 PCR primer” from Zhichao
- Expected size: 324bp



Send for Premium PCR sequencing:

Premium_PCR_HC_20250506.pdf

TUESDAY, 5/13/2025

Note:

- We decided not to use Plasmidsaurus' Premium PCR (Nanopore) service going forward, which had high error rate. We will turn to AVITI-seq, PE150 on Low-Output flow cell (250M reads). **I will include these samples again in the multiplexed library prep.**

2025.5.8-12 Pilot testing for C30 reactivity in cells

Project: Hang_JW Lab

Author: Hang Chen

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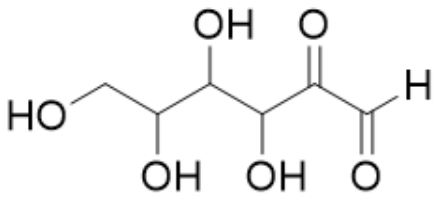
THURSDAY, 5/8/2025

Planned samples

	#	Covalent modifier	Covalent modifier (final)	Covalent rxn conditions	RT ion
1	16	DMSO (100%)	Ctrl (5% DMSO)	37°C for 15min on cells	Mg2+ (3mM)
2	17	DMSO (100%)	Ctrl (5% DMSO)	37°C for 15min on cells	Mg2+ (3mM)
3	18	DMSO (100%)	Ctrl (5% DMSO)	37°C for 15min on cells	Mn2+ (3mM)
4	19	DMSO (100%)	Ctrl (5% DMSO)	37°C for 15min on cells	Mn2+ (3mM)
5	20	C30 (100mM diluted from 1M stock)	C30 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)
6	21	C30 (10mM diluted from 100mM)	C30 (0.5mM)	37°C for 15min on cells	Mn2+ (3mM)
7	22	DMSO (100%)	Ctrl (5% DMSO)	80°C for 15min on cells	Mg2+ (3mM)
8	23	DMSO (100%)	Ctrl (5% DMSO)	80°C for 15min on cells	Mg2+ (3mM)
9	24	DMSO (100%)	Ctrl (5% DMSO)	80°C for 15min on cells	Mn2+ (3mM)
10	25	DMSO (100%)	Ctrl (5% DMSO)	80°C for 15min on cells	Mn2+ (3mM)
11	26	C30 (100mM diluted from 1M stock)	C30 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)
12	27	C30 (10mM diluted from 100mM)	C30 (0.5mM)	80°C for 15min on cells	Mn2+ (3mM)

C30 (2-Keto-D-Glucose)



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Cell treatment with RNA covalent modifiers:

- Treat "SARS-CoV2 5UTR"cells (SL5 HEK293) with TrypLE Express at room temp
- Collect and wash cells with 1x PBS
- Count cells: **3.925x10^6 cells/ml x 5ml**
- Passage at 1:20, keep the extra on ice
- Prepare **1x10^6cells/ml x 3ml** suspension in 1x PBS, aliquot **200ul**/1.5ml tube (2x10^5 cells/rxn), 12 rxns
- Resuspend cells for each rxn in 47.5ul 1x PBS
- Add 2.5ul DMSO or covalent modifier in DMSO (total volume=50ul), pipette to mix
- Incubate at designated temp for **15min**
- Chill on ice for 2min
- Add 300ul RLT Plus buffer (total volume=350ul)
- Vortex for 30s to homogenize
- Store lysate in -80

MONDAY, 5/12/2025

RNA extraction:

- Thaw cell lysate in RLT Plus buffer at RT, vortex to homogenize
- Transfer to DNA spin column, 8000xg 30s
- Add 350ul 70% EtOH to the flow-through
- Transfer to RNA spin column, 8000xg 15s, discard the flow-through
- Add 700ul RW1 buffer, 8000xg 15s, discard the flow-through
- Add 500ul 70% EtOH instead of RPE buffer, 8000xg 15s, discard the flow-through
- Add 500ul 80% EtOH, 8000xg 15s, discard the flow-through
- Full speed 5min
- Elute with **20ul** water
- Nanodrop:

RNA yield & dilution

	#	RNA conc (ng/ul)	RNA 260/280	150ng RNA (ul)	Water to 9.7ul (ul)	Note
1	16	96.5	2.07	1.6	8.1	
2	17	84.4	2.05	1.8	7.9	
3	18	113.3	2.08	1.3	8.4	
4	19	99.8	2.08	1.5	8.2	
5	20	102.4	2.05	1.5	8.2	
6	21	100.9	2.08	1.5	8.2	
7	22	25	2.08	6.0	3.7	
8	23	36.1	2.10	4.2	5.5	
9	24	27.7	2.10	5.4	4.3	
10	25	28.1	2.13	5.3	4.4	
11	26	5.3	2.08	9.7x2	0.0	very low yield
12	27	17.5	2.08	8.6	1.1	

- Store RNA in -80

TUESDAY, 5/13/2025

Note:

- I didn't proceed with COVID_6-28_F_56C and CMV-5UTR_SHAPE_Rv for amplicon PCR because we decided not to use Plasmidsaurus' Premium PCR (Nanopore) service going forward, which had high error rate. We will turn to AVITI-seq, PE150 on Low-Output flow cell (250M reads), therefore, **I will include these samples in the multiplexed library prep.**
- The #26 samples has very low yield, which has been observed by Dr. Wang previously. I will repeat this treatment with scaled up rxn system.

2025.5.15-19 Pilot testing for C12 C15 C30 reactivity in cells (repeat)

Project: Hang_JW Lab
Author: Hang Chen
Entry Created On: 14 May 2025 21:40:08 UTC
Entry Last Modified: 26 May 2025 14:56:19 UTC
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WEDNESDAY, 5/14/2025

Planned samples - Ctrl

	#	Covalent modifier	Covalent modifier (final)	Covalent rxn conditions	RT ion
1	1	DMSO (100%)	Ctrl (5% DMSO)	37°C for 15min on cells	Mg2+ (3mM)
2	16	DMSO (100%)	Ctrl (5% DMSO)	37°C for 15min on cells	Mg2+ (3mM)
3	17	DMSO (100%)	Ctrl (5% DMSO)	37°C for 15min on cells	Mg2+ (3mM)
4	2	DMSO (100%)	Ctrl (5% DMSO)	37°C for 15min on cells	Mn2+ (3mM)
5	18	DMSO (100%)	Ctrl (5% DMSO)	37°C for 15min on cells	Mn2+ (3mM)
6	19	DMSO (100%)	Ctrl (5% DMSO)	37°C for 15min on cells	Mn2+ (3mM)
7	7	DMSO (100%)	Ctrl (5% DMSO)	80°C for 15min on cells	Mg2+ (3mM)
8	22	DMSO (100%)	Ctrl (5% DMSO)	80°C for 15min on cells	Mg2+ (3mM)
9	23	DMSO (100%)	Ctrl (5% DMSO)	80°C for 15min on cells	Mg2+ (3mM)
10	8	DMSO (100%)	Ctrl (5% DMSO)	80°C for 15min on cells	Mn2+ (3mM)
11	24	DMSO (100%)	Ctrl (5% DMSO)	80°C for 15min on cells	Mn2+ (3mM)
12	25	DMSO (100%)	Ctrl (5% DMSO)	80°C for 15min on cells	Mn2+ (3mM)

Planned samples - C12 treatment

	#	Covalent modifier	Covalent modifier (final)	Covalent rxn conditions	RT ion
1	3	C12 (100mM, stock)	C12 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)
2	28	C12 (100mM, stock)	C12 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)
3	29	C12 (100mM, stock)	C12 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)
4	5	C12 (10mM, 1:10 diluted)	C12 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)
5	34	C12 (10mM, 1:10 diluted)	C12 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)
6	35	C12 (10mM, 1:10 diluted)	C12 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)
7	9	C12 (100mM, stock)	C12 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)
8	40	C12 (100mM, stock)	C12 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)
9	41	C12 (100mM, stock)	C12 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)
10	11	C12 (10mM, 1:10 diluted)	C12 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)
11	46	C12 (10mM, 1:10 diluted)	C12 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)
12	47	C12 (10mM, 1:10 diluted)	C12 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)

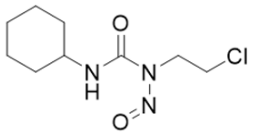
Planned samples - C15 treatment

	#	Covalent modifier	Covalent modifier (final)	Covalent rxn conditions	RT ion
1	4	C15 (100mM, stock)	C15 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)
2	30	C15 (100mM, stock)	C15 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)
3	31	C15 (100mM, stock)	C15 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)
4	6	C15 (10mM, 1:10 diluted)	C15 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)
5	36	C15 (10mM, 1:10 diluted)	C15 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)
6	37	C15 (10mM, 1:10 diluted)	C15 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)
7	10	C15 (100mM, stock)	C15 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)
8	42	C15 (100mM, stock)	C15 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)
9	43	C15 (100mM, stock)	C15 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)
10	12	C15 (10mM, 1:10 diluted)	C15 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)
11	48	C15 (10mM, 1:10 diluted)	C15 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)
12	49	C15 (10mM, 1:10 diluted)	C15 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)

Planned samples - C30 treatment

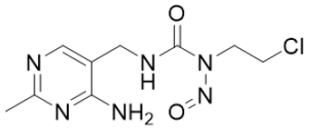
	#	Covalent modifier	Covalent modifier (final)	Covalent rxn conditions	RT ion
1	20	C30 (100mM, stock)	C30 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)
2	32	C30 (100mM, stock)	C30 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)
3	33	C30 (100mM, stock)	C30 (5mM final)	37°C for 15min on cells	Mn2+ (3mM)
4	21	C30 (10mM, 1:10 diluted)	C30 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)
5	38	C30 (10mM, 1:10 diluted)	C30 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)
6	39	C30 (10mM, 1:10 diluted)	C30 (0.5mM final)	37°C for 15min on cells	Mn2+ (3mM)
7	26	C30 (100mM, stock)	C30 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)
8	44	C30 (100mM, stock)	C30 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)
9	45	C30 (100mM, stock)	C30 (5mM final)	80°C for 15min on cells	Mn2+ (3mM)
10	27	C30 (10mM, 1:10 diluted)	C30 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)
11	50	C30 (10mM, 1:10 diluted)	C30 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)
12	51	C30 (10mM, 1:10 diluted)	C30 (0.5mM final)	80°C for 15min on cells	Mn2+ (3mM)

C12 Lomustine & C15 Nimustine



Lomustine

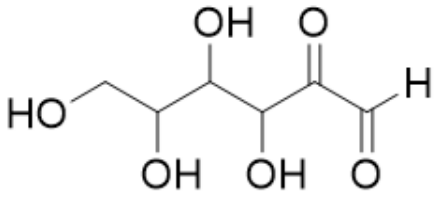
12



Nimustine

15

C30 (2-Keto-D-Glucose)



30

THURSDAY, 5/15/2025

Reverse transcription for existing RNA:

- 24 rxns (1 block)
- Input: 60ng total RNA

Existing RNA

	#	RNA conc (ng/ul)	60ng RNA (ul)	Water to 4.4ul (ul)	RT ion
1	1	35.9	1.67	2.73	Mg2+ (3mM)
2	2	42.9	1.40	3.00	Mn2+ (3mM)
3	3	45.9	1.31	3.09	Mn2+ (3mM)
4	4	41.8	1.44	2.96	Mn2+ (3mM)
5	5	40.6	1.48	2.92	Mn2+ (3mM)
6	6	41.3	1.45	2.95	Mn2+ (3mM)
7	7	29.4	2.04	2.36	Mg2+ (3mM)
8	8	26.2	2.29	2.11	Mn2+ (3mM)
9	9	36.5	1.64	2.76	Mn2+ (3mM)
10	10	15	4.00	0.40	Mn2+ (3mM)
11	11	41.9	1.43	2.97	Mn2+ (3mM)
12	12	20.6	2.91	1.49	Mn2+ (3mM)
13	16	96.5	0.62	3.78	Mg2+ (3mM)
14	17	84.4	0.71	3.69	Mg2+ (3mM)
15	18	113.3	0.53	3.87	Mn2+ (3mM)
16	19	99.8	0.60	3.80	Mn2+ (3mM)
17	20	102.4	0.59	3.81	Mn2+ (3mM)
18	21	100.9	0.59	3.81	Mn2+ (3mM)
19	22	25	2.40	2.00	Mg2+ (3mM)
20	23	36.1	1.66	2.74	Mg2+ (3mM)
21	24	27.7	2.17	2.23	Mn2+ (3mM)
22	25	28.1	2.14	2.26	Mn2+ (3mM)
23	27	17.5	3.43	0.97	Mn2+ (3mM)
24	SL5	498.8 dilute to 60	1.00	3.40	Mn2+ (3mM)

Annealing - existing RNA samples

	Component	vol (ul)	25x (ul)	Master mix (ul)
1	60ng RNA+water	4.4	4.4/EA	
2	CMV-5UTR-SHAPE-Rv (100uM)	0.05	1.25	
3	dNTP (10mM)	0.5	12.5	
4	Water	0.45	11.25	1/EA
5	Total	5.4	5.4/EA	

- 65°C 5min, immediately put on ice

Extension - existing RNA samples

	Component	vol (ul)	25x (ul)	Master mix (ul)
1	Annealing product	5.4	5.4/EA	
2	375mM Tris/500mM KCl (5x buffer)	2	50	
3	100mM DTT (10x)	1	25	
4	Protoscript II	0.5	12.5	
5	RNaseIN	0.1	2.5	3.6/EA
6	30mM MgCl2 or MnCl2	1	1/EA	
7	Total	10	10/EA	

- 42°C 1h, 65°C 20min, 4°C hold
- Store cDNA in -20

MONDAY, 5/19/2025

Cell treatment with RNA covalent modifiers:

- Treat "SARS-CoV2 5UTR"cells (SL5 HEK293) with TrypLE Express at room temp
- Collect and wash cells with 1x PBS
- Count cells: **2.05x10^6 cells/ml**
- Passage at 1:20, keep the extra on ice
- Prepare **2x10^5 cells/rxn x22 + 8x10^5 cells/rxn x3 = 6.8M** **(8x10^5 cells for C30 5mM final, 4x scale-up and multi-load)**
- Resuspend cells for each rxn in 47.5ul 1x PBS (for the 8x10^5 cells, resuspend cells for each rxn in 190ul)
- Add 2.5ul DMSO or covalent modifier in DMSO (total volume=50ul)
- Incubate at designated temp for **15min**

- Chill on ice for 2min
- Add 300ul RLT Plus buffer (total volume=350ul) (for 8x10^5 cells, add 1200ul RLT Plus)
- Vortex for 30s to homogenize
- Keep samples on ice and proceed to RNA extraction

Treatment

	#	Cell number	Covalent modifier	Final conc	Incubation
1	28	2x10^5	C12 (100mM, stock)	C12 (5mM final)	37°C for 15min on cells
2	29	2x10^5	C12 (100mM, stock)	C12 (5mM final)	37°C for 15min on cells
3	34	2x10^5	C12 (10mM, 1:10 diluted)	C12 (0.5mM final)	37°C for 15min on cells
4	35	2x10^5	C12 (10mM, 1:10 diluted)	C12 (0.5mM final)	37°C for 15min on cells
5	40	2x10^5	C12 (100mM, stock)	C12 (5mM final)	80°C for 15min on cells
6	41	2x10^5	C12 (100mM, stock)	C12 (5mM final)	80°C for 15min on cells
7	46	2x10^5	C12 (10mM, 1:10 diluted)	C12 (0.5mM final)	80°C for 15min on cells
8	47	2x10^5	C12 (10mM, 1:10 diluted)	C12 (0.5mM final)	80°C for 15min on cells
9	30	2x10^5	C15 (100mM, stock)	C15 (5mM final)	37°C for 15min on cells
10	31	2x10^5	C15 (100mM, stock)	C15 (5mM final)	37°C for 15min on cells
11	36	2x10^5	C15 (10mM, 1:10 diluted)	C15 (0.5mM final)	37°C for 15min on cells
12	37	2x10^5	C15 (10mM, 1:10 diluted)	C15 (0.5mM final)	37°C for 15min on cells
13	42	2x10^5	C15 (100mM, stock)	C15 (5mM final)	80°C for 15min on cells
14	43	2x10^5	C15 (100mM, stock)	C15 (5mM final)	80°C for 15min on cells
15	48	2x10^5	C15 (10mM, 1:10 diluted)	C15 (0.5mM final)	80°C for 15min on cells
16	49	2x10^5	C15 (10mM, 1:10 diluted)	C15 (0.5mM final)	80°C for 15min on cells
17	32	2x10^5	C30 (100mM, stock)	C30 (5mM final)	37°C for 15min on cells
18	33	2x10^5	C30 (100mM, stock)	C30 (5mM final)	37°C for 15min on cells
19	38	2x10^5	C30 (10mM, 1:10 diluted)	C30 (0.5mM final)	37°C for 15min on cells
20	39	2x10^5	C30 (10mM, 1:10 diluted)	C30 (0.5mM final)	37°C for 15min on cells
21	26	8x10^5	C30 (100mM, stock)	C30 (5mM final)	80°C for 15min on cells
22	44	8x10^5	C30 (100mM, stock)	C30 (5mM final)	80°C for 15min on cells
23	45	8x10^5	C30 (100mM, stock)	C30 (5mM final)	80°C for 15min on cells
24	50	2x10^5	C30 (10mM, 1:10 diluted)	C30 (0.5mM final)	80°C for 15min on cells
25	51	2x10^5	C30 (10mM, 1:10 diluted)	C30 (0.5mM final)	80°C for 15min on cells

RNA extraction:

- Thaw cell lysate in RLT Plus buffer at RT, vortex to homogenize
- Transfer to DNA spin column, 8000xg 30s

- Add 350ul 70% EtOH to the flow-through
- Transfer to RNA spin column, 8000xg 15s, discard the flow-through
- Add 700ul RW1 buffer, 8000xg 15s, discard the flow-through
- Add 500ul 70% EtOH instead of RPE buffer, 8000xg 15s, discard the flow-through
- Add 500ul 80% EtOH, 8000xg 15s, discard the flow-through
- Full speed 5min
- Elute with **20ul** water
- Nanodrop:

RNA yield

	#	RNA conc (ng/ul)	260/280	60ng RNA (ul)	Water to 4.4ul (ul)	RT ion
1	28	80.8	2.05	0.74	3.66	Mn2+ (3mM)
2	29	94.3	2.07	0.64	3.76	Mn2+ (3mM)
3	34	94.9	2.08	0.63	3.77	Mn2+ (3mM)
4	35	96.3	2.08	0.62	3.78	Mn2+ (3mM)
5	40	54.4	2.05	1.10	3.30	Mn2+ (3mM)
6	41	52.6	2.05	1.14	3.26	Mn2+ (3mM)
7	46	65.7	2.02	0.91	3.49	Mn2+ (3mM)
8	47	61.3	2.04	0.98	3.42	Mn2+ (3mM)
9	30	75.9	2.09	0.79	3.61	Mn2+ (3mM)
10	31	95.6	2.06	0.63	3.77	Mn2+ (3mM)
11	36	113.8	2.08	0.53	3.87	Mn2+ (3mM)
12	37	120.7	2.09	0.50	3.90	Mn2+ (3mM)
13	42	20.5	2.04	2.93	1.47	Mn2+ (3mM)
14	43	18.3	2.12	3.28	1.12	Mn2+ (3mM)
15	48	27.3	2.16	2.20	2.20	Mn2+ (3mM)
16	49	28.9	2.11	2.08	2.32	Mn2+ (3mM)
17	32	125.7	2.09	0.48	3.92	Mn2+ (3mM)
18	33	114.2	2.10	0.53	3.87	Mn2+ (3mM)
19	38	101.7	2.06	0.59	3.81	Mn2+ (3mM)
20	39	88.6	2.09	0.68	3.72	Mn2+ (3mM)
21	26	34.5	2.00	1.74	2.66	Mn2+ (3mM)
22	44	47.0	1.98	1.28	3.12	Mn2+ (3mM)
23	45	43.4	1.97	1.38	3.02	Mn2+ (3mM)
24	50	29.7	1.99	2.02	2.38	Mn2+ (3mM)
25	51	21.6	2.04	2.78	1.62	Mn2+ (3mM)

- Store RNA in -80

Reverse transcription:

- 25 rxns (1 block)
- Input: 60ng total RNA

Annealing

	Component	vol (ul)	26x (ul)	Master mix (ul)
1	60ng RNA+water	4.4	4.4/EA	
2	CMV-5UTR-SHAPE-Rv (100uM)	0.05	1.3	
3	dNTP (10mM)	0.5	13	
4	Water	0.45	11.7	1/EA
5	Total	5.4	5.4/EA	

- 65°C 5min, immediately put on ice

Extension

	Component	vol (ul)	26x (ul)	Master mix (ul)
1	Annealing product	5.4	5.4/EA	
2	375mM Tris/500mM KCl (5x buffer)	2	52	
3	100mM DTT (10x)	1	26	
4	Protoscript II	0.5	13	
5	RNaseIN	0.1	2.6	
6	30mM MnCl2	1	26	4.6/EA
7	Total	10	10/EA	

- 42°C 1h, 65°C 20min, 4°C hold
- Store cDNA in -20

Note:

- **I will include these samples in the multiplexed library prep.**

2025.5.14-15 Pilot testing for C34-NM C34-squarate in activity-based RNA profiling (on purified RNA)

Project: Hang_JW Lab

Author: Hang Chen

Entry Created On: 14 May 2025 16:50:21 UTC

Entry Last Modified: 02 Jun 2025 17:20:19 UTC

Export Generated On: 02 Jun 2025 17:23:28 UTC

WEDNESDAY, 5/14/2025

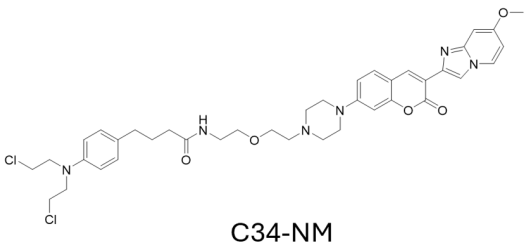
Planned samples - Ctrl

	#	Covalent modifier	Covalent modifier (final)	Covalent rxn conditions	RT ion
1	52	DMSO (100%)	Ctrl (10% DMSO)	37°C for 30min on purified RNA	Mg2+ (3mM)
2	53	DMSO (100%)	Ctrl (10% DMSO)	37°C for 30min on purified RNA	Mg2+ (3mM)
3	54	DMSO (100%)	Ctrl (10% DMSO)	37°C for 30min on purified RNA	Mg2+ (3mM)
4	55	DMSO (100%)	Ctrl (10% DMSO)	37°C for 30min on purified RNA	Mn2+ (3mM)
5	56	DMSO (100%)	Ctrl (10% DMSO)	37°C for 30min on purified RNA	Mn2+ (3mM)
6	57	DMSO (100%)	Ctrl (10% DMSO)	37°C for 30min on purified RNA	Mn2+ (3mM)
7	58	DMSO (100%)	Ctrl (10% DMSO)	37°C for 2h on purified RNA	Mg2+ (3mM)
8	59	DMSO (100%)	Ctrl (10% DMSO)	37°C for 2h on purified RNA	Mg2+ (3mM)
9	60	DMSO (100%)	Ctrl (10% DMSO)	37°C for 2h on purified RNA	Mg2+ (3mM)
10	61	DMSO (100%)	Ctrl (10% DMSO)	37°C for 2h on purified RNA	Mn2+ (3mM)
11	62	DMSO (100%)	Ctrl (10% DMSO)	37°C for 2h on purified RNA	Mn2+ (3mM)
12	63	DMSO (100%)	Ctrl (10% DMSO)	37°C for 2h on purified RNA	Mn2+ (3mM)

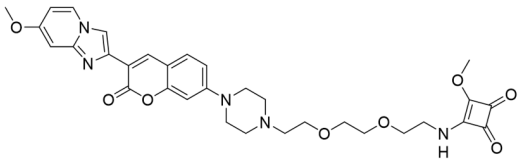
Planned samples - treatment

	#	Covalent modifier	Covalent modifier (final)	Covalent rxn conditions	RT ion
1	64	C34-NM (10mM, stock)	C34-NM (1mM final)	37°C for 2h on purified RNA	Mn2+ (3mM)
2	65	C34-NM (10mM, stock)	C34-NM (1mM final)	37°C for 2h on purified RNA	Mn2+ (3mM)
3	66	C34-NM (10mM, stock)	C34-NM (1mM final)	37°C for 2h on purified RNA	Mn2+ (3mM)
4	67	NM-only (10mM, stock)	NM-only (1mM final)	37°C for 2h on purified RNA	Mn2+ (3mM)
5	68	NM-only (10mM, stock)	NM-only (1mM final)	37°C for 2h on purified RNA	Mn2+ (3mM)
6	69	NM-only (10mM, stock)	NM-only (1mM final)	37°C for 2h on purified RNA	Mn2+ (3mM)
7	70	C34-Squarate (10mM, stock)	C34-Squarate (1mM final)	37°C for 30min on purified RNA	Mn2+ (3mM)
8	71	C34-Squarate (10mM, stock)	C34-Squarate (1mM final)	37°C for 30min on purified RNA	Mn2+ (3mM)
9	72	C34-Squarate (10mM, stock)	C34-Squarate (1mM final)	37°C for 30min on purified RNA	Mn2+ (3mM)
10	73	Squarate-only (10mM, stock)	Squarate-only (1mM final)	37°C for 30min on purified RNA	Mn2+ (3mM)
11	74	Squarate-only (10mM, stock)	Squarate-only (1mM final)	37°C for 30min on purified RNA	Mn2+ (3mM)
12	75	Squarate-only (10mM, stock)	Squarate-only (1mM final)	37°C for 30min on purified RNA	Mn2+ (3mM)

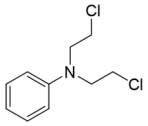
Chemicals



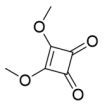
C34-NM



WUXI36
C34-squarate



NM-only



RM26
Squarate-only

Cell collection:

- Treat "SARS-CoV2 5UTR"cells (SL5 HEK293) with TrypLE Express at room temp
- Collect and wash cells with 1x PBS
- Count cells: **2.935x10^6 cells/ml x5ml**
- Passage at 1:10
- Prepare **5.5x10^5 x24 = 13.2M** cells suspension in 1x PBS
- Pellet cells, remove supernatant, resuspend/lyse cells in **8400ul** RLT Plus buffer
- Vortex for 1min to homogenize
- Keep on ice

RNA extraction:

- Prepare 12x RNA extraction columns
- Transfer 700ul lysate to DNA spin column, 8000xg 30s, keep the flow-through
- Add 700ul 70% EtOH to the flow-through
- Transfer 700ul mixture to RNA spin column, 8000xg 15s, discard the flow-through
- Repeat previous step one more time
- Add 700ul RW1 buffer, 8000xg 15s, discard the flow-through
- Add 500ul 70% EtOH instead of RPE buffer, 8000xg 15s, discard the flow-through
- Add 500ul 80% EtOH, 8000xg 15s, discard the flow-through
- Full speed 5min
- Elute with **40ul water/column**
- Nanodrop: **498.8ng/ul**, 260/280=2.14
- Store RNA in -80

Making buffer:

5x RNA folding buffer

	Component	Vol	Stock conc (5x)	Final conc
1	1M HEPES (pH 7.3)	600ul	500mM	100mM
2	3M KCl	200ul	500mM	100mM
3	1M MgCl2	36ul	30mM	6mM
4	Water	364ul		
5	Total	1200ul		

- Stored buffer in -20

THURSDAY, 5/15/2025

RNA folding:

- Perform in 1.5ml Eppendorf tube
- Fully **unfold purified RNA at 95C for 3min**, immediately put on ice for 2min
- Then add RNA folding buffer

RNA folding system

	Component	Vol (ul)	two batches of 13x (ul)	Master mix (ul)
1	5ug total RNA	10	10/EA	
2	Water	7.5	97.5	
3	5x RNA folding buffer	5	65	12.5/EA
4	Total	22.5	45/EA	

RNA folding program

	Temp	Time
1	37°C	30min
2	on ice	2min

RNA covalent modification:

- Perform in the same 1.5ml Eppendorf tube

- Because the C34 conjugates in DMSO freeze on ice, DMSO/C34 conjugates were added to RNA at room temp and transferred to heat block as quickly as possible

RNA covalent modifying system

	Component	Vol (ul)
1	5ug folded RNA	22.5/EA
2	DMSO/10mM C34 conjugates	2.5/EA
3	Total	25/EA

RNA covalent modifying program

	Temp	Time
1	37°C	30min or 2h
2	on ice	2min

RNA clean & concentrator-5:

- Add 25ul water to each sample
- Add 100ul RNA Binding Buffer to each 50ul diluted sample
- Add 150ul pure EtOH
- Transfer 300ul to Zymo-Spin IC Column, 10,000xg 30s
- Add 400 µl RNA Prep Buffer, 10,000xg 30s
- Add 700 µl RNA Wash Buffer, 10,000xg 30s
- Add 400 µl RNA Wash Buffer, 10,000xg 30s
- Spin at full speed for 2min
- Elute=**8ul** water

RNA clean & concentrate

	#	RNA conc (ng/ul)	260/280	Dilute to 60ng/ul with water (ul)	60ng RNA (ul)	Water to 4.4ul (ul)
1	52	533.8	2.10	55.3	1	3.4
2	53	673.1	2.11	71.5	1	3.4
3	54	614.6	2.14	64.7	1	3.4
4	55	605.9	2.13	63.7	1	3.4
5	56	493.3	2.13	50.6	1	3.4
6	57	656.8	2.10	69.6	1	3.4
7	58	604.6	2.09	63.5	1	3.4
8	59	638.1	2.13	67.4	1	3.4
9	60	531.2	2.14	55.0	1	3.4
10	61	676.6	2.13	71.9	1	3.4
11	62	689.9	2.15	73.5	1	3.4
12	63	522.7	2.13	54.0	1	3.4
13	64	626.3	2.11	66.1	1	3.4
14	65	539.1	2.11	55.9	1	3.4
15	66	632.9	2.11	66.8	1	3.4
16	67	559.6	2.10	58.3	1	3.4
17	68	490.9	2.14	50.3	1	3.4
18	69	488.8	2.14	50.0	1	3.4
19	70	487.2	2.13	49.8	1	3.4
20	71	696.6	2.08	74.3	1	3.4
21	72	544.3	2.10	56.5	1	3.4
22	73	540.8	2.10	56.1	1	3.4
23	74	662.6	2.10	70.3	1	3.4
24	75	607.9	2.07	63.9	1	3.4

- Store RNA in -80

Reverse transcription:

- Input: 60ng total RNA

Annealing

	Component	vol (ul)	25x (ul)	Master mix (ul)
1	60ng RNA+water	1	1/EA	
2	CMV-5UTR-SHAPE-Rv (100uM)	0.05	1.25	
3	dNTP (10mM)	0.5	12.5	
4	Water	3.85	96.25	4.4/EA
5	Total	5.4	5.4/EA	

- 65°C 5min, immediately put on ice

Extension

	Component	vol (ul)	25x (ul)	Master mix (ul)
1	Annealing product	5.4	5.4/EA	
2	375mM Tris/500mM KCl (5x buffer)	2	50	
3	100mM DTT (10x)	1	25	
4	Protoscript II	0.5	12.5	
5	RNaseIN	0.1	2.5	3.6/EA
6	30mM MgCl2 or MnCl2	1	1/EA	
7	Total	10	10/EA	

- 42°C 1h, 65°C 20min, 4°C hold
- Store cDNA in -20

Note:

- I was going to use 10ug in a 50ul system, but there wasn't enough compound, so I scaled down by half.
- In Zhichao's original protocol, he used 5ug RNA in a 50ul rxn system.