2025.6.2-4 C12 C30 Temperature gradient; C34 Protection (on live cells)

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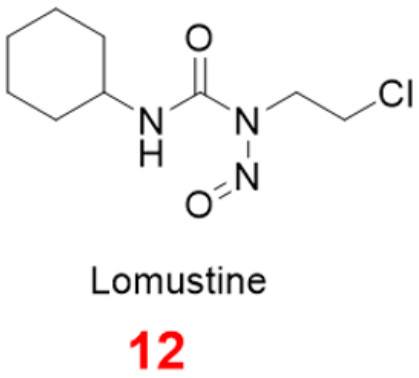
MONDAY, 6/2/2025

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

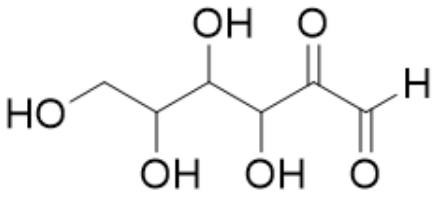
C12_Lomustine.png



Lomustine

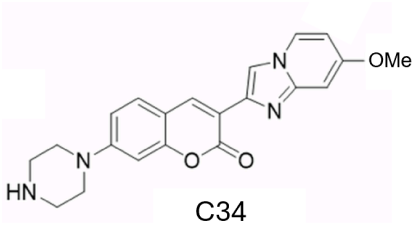
12

C30 (2-Keto-D-Glucose)



30

C34 (SL5 ligand)



C34

TUESDAY, 6/3/2025

Cell treatment batch-1

	#	Cell number	Treatment	Incubation
1	114	2x10^5	Ctrl (5% DMSO)	40°C for 15min
2	115	2x10^5	Ctrl (5% DMSO)	40°C for 15min
3	116	2x10^5	C12 (5mM final)	40°C for 15min
4	117	2x10^5	C12 (5mM final)	40°C for 15min
5	118	2x10^5	C30 (5mM final)	40°C for 15min
6	119	2x10^5	C30 (5mM final)	40°C for 15min
7	120	2x10^5	Ctrl (5% DMSO)	50°C for 15min
8	121	2x10^5	Ctrl (5% DMSO)	50°C for 15min
9	122	2x10^5	C12 (5mM final)	50°C for 15min
10	123	2x10^5	C12 (5mM final)	50°C for 15min
11	124	4x10^5	C30 (5mM final)	50°C for 15min
12	125	4x10^5	C30 (5mM final)	50°C for 15min
13	126	2x10^5	C34 (1mM final)	50°C for 15min
14	127	2x10^5	C34 (1mM final)	50°C for 15min
15	128	2x10^5	C12 (5mM final) + C34 (1mM final)	50°C for 15min
16	129	2x10^5	C12 (5mM final) + C34 (1mM final)	50°C for 15min
17	130	4x10^5	C30 (5mM final) + C34 (1mM final)	50°C for 15min
18	131	4x10^5	C30 (5mM final) + C34 (1mM final)	50°C for 15min

Cell treatment batch-2

	#	Cell number	Treatment	Incubation
1	132	2x10^5	Ctrl (5% DMSO)	60°C for 15min
2	133	2x10^5	Ctrl (5% DMSO)	60°C for 15min
3	134	2x10^5	C12 (5mM final)	60°C for 15min
4	135	2x10^5	C12 (5mM final)	60°C for 15min
5	136	6x10^5	C30 (5mM final)	60°C for 15min
6	137	6x10^5	C30 (5mM final)	60°C for 15min
7	138	2x10^5	C34 (1mM final)	60°C for 15min
8	139	2x10^5	C34 (1mM final)	60°C for 15min
9	140	2x10^5	C12 (5mM final) + C34 (1mM final)	60°C for 15min
10	141	2x10^5	C12 (5mM final) + C34 (1mM final)	60°C for 15min
11	142	6x10^5	C30 (5mM final) + C34 (1mM final)	60°C for 15min
12	143	6x10^5	C30 (5mM final) + C34 (1mM final)	60°C for 15min
13	144	2x10^5	Ctrl (5% DMSO)	70°C for 15min
14	145	2x10^5	Ctrl (5% DMSO)	70°C for 15min
15	146	2x10^5	C12 (5mM final)	70°C for 15min
16	147	2x10^5	C12 (5mM final)	70°C for 15min
17	148	8x10^5	C30 (5mM final)	70°C for 15min
18	149	8x10^5	C30 (5mM final)	70°C for 15min

- For C12/C30 (5mM final) + C34 (1mM final):
  - 1x: 2.5ul 100mM C12/C30, 0.25ul 200mM C34, into 47.5ul cells in PBS
  - 4x: 10ul 100mM C12, 1ul 1M C30, into 190ul cells in PBS

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
- For batch-1,
  - Count cells: **1.63x10^6 cells/ml**
  - prepare **2x10^5 cells/rxn x25 = 5M**
- For batch-2,
  - Count cells: **3.5x10^6 cells/ml**
  - prepare **2x10^5 cells/rxn x35 = 7M**

- Resuspend cells:
  - for 2x10^5 cells, resuspend in 47.5ul
  - for 4x10^5 cells, resuspend in 95ul
  - for 6x10^5 cells, resuspend in 142.5ul
  - for 8x10^5 cells, resuspend in 190ul
- Add DMSO or covalent modifier:
  - for 2x10^5 cells, add 2.5ul
  - for 4x10^5 cells, add 5ul
  - for 6x10^5 cells, add 7.5ul
  - for 8x10^5 cells, add 10ul
- 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
- Store lysate in -80

WEDNESDAY, 6/4/2025

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 350ul flow-through
- Transfer to Zymo Spin IC 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 2min
- Elute with **15ul** water
- Nanodrop:

RNA yield batch-1

	#	RNA conc (ng/ul)	260/280	Add x ul water to dilute to 60ng/ul	60ng RNA (ul)	Water to 4.4ul (ul)	RT ion
1	114	89.0	1.93	6.8	1.00	3.40	Mn2+ (3mM)
2	115	141.1	1.94	18.9	1.00	3.40	Mn2+ (3mM)
3	116	161.6	2.03	23.7	1.00	3.40	Mn2+ (3mM)
4	117	167.0	2.06	23.2	1.00	3.40	Mn2+ (3mM)
5	118	131.0	1.99	16.6	1.00	3.40	Mn2+ (3mM)
6	119	158.1	2.04	22.9	1.00	3.40	Mn2+ (3mM)
7	120	133.3	1.98	17.1	1.00	3.40	Mn2+ (3mM)
8	121	181.2	1.99	28.3	1.00	3.40	Mn2+ (3mM)
9	122	147.7	2.03	20.5	1.00	3.40	Mn2+ (3mM)
10	123	165.7	2.03	24.7	1.00	3.40	Mn2+ (3mM)
11	124	331.5	2.07	58.8	1.00	3.40	Mn2+ (3mM)
12	125	330.7	2.04	63.2	1.00	3.40	Mn2+ (3mM)
13	126	134.3	2.01	17.3	1.00	3.40	Mn2+ (3mM)
14	127	170.0	1.99	25.7	1.00	3.40	Mn2+ (3mM)
15	128	175.0	2.02	26.8	1.00	3.40	Mn2+ (3mM)
16	129	160.2	1.99	23.4	1.00	3.40	Mn2+ (3mM)
17	130	361.8	2.02	70.4	1.00	3.40	Mn2+ (3mM)
18	131	375.2	2.05	73.5	1.00	3.40	Mn2+ (3mM)

RNA yield batch-2

	#	RNA conc (ng/ul)	260/280	Add x ul water to dilute to 60ng/ul	60ng RNA (ul)	Water to 4.4ul (ul)	RT ion
1	132	101.5	2.00	9.7	1.00	3.40	Mn2+ (3mM)
2	133	93.0	1.98	7.7	1.00	3.40	Mn2+ (3mM)
3	134	142.2	1.77	19.2	1.00	3.40	Mn2+ (3mM)
4	135	159.4	1.98	23.2	1.00	3.40	Mn2+ (3mM)
5	136	425.8	2.03	85.4	1.00	3.40	Mn2+ (3mM)
6	137	425.4	2.02	85.3	1.00	3.40	Mn2+ (3mM)
7	138	80.5	2.01	4.8	1.00	3.40	Mn2+ (3mM)
8	139	95.5	2.03	8.3	1.00	3.40	Mn2+ (3mM)
9	140	140.0	2.02	18.7	1.00	3.40	Mn2+ (3mM)
10	141	145.7	2.09	20.0	1.00	3.40	Mn2+ (3mM)
11	142	276.7	2.01	50.6	1.00	3.40	Mn2+ (3mM)
12	143	290.0	1.99	53.7	1.00	3.40	Mn2+ (3mM)
13	144	126.1	1.94	15.4	1.00	3.40	Mn2+ (3mM)
14	145	123.6	1.90	14.8	1.00	3.40	Mn2+ (3mM)
15	146	127.4	1.93	15.7	1.00	3.40	Mn2+ (3mM)
16	147	134	1.92	17.3	1.00	3.40	Mn2+ (3mM)
17	148	120.4	1.96	13.1	1.00	3.40	Mn2+ (3mM)
18	149	123.6	1.91	14.8	1.00	3.40	Mn2+ (3mM)

- Store RNA in -80

Reverse transcription:

- Input: 60ng total RNA

Annealing

	Component	vol (ul)	40x (ul)	Master mix (ul)
1	60ng RNA+water	4.4	4.4/EA	
2	CMV-5UTR-SHAPE-Rv (100uM)	0.05	2	
3	dNTP (10mM)	0.5	20	
4	Water	0.45	18	1/EA
5	Total	5.4	5.4/EA	

- 65°C 5min, immediately put on ice

Extension

	Component	vol (ul)	40x (ul)	Master mix (ul)
1	Annealing product	5.4	5.4/EA	
2	375mM Tris/500mM KCl (5x buffer)	2	80	
3	100mM DTT (10x)	1	40	
4	Protoscript II	0.5	20	
5	RNaseIN	0.1	4	
6	30mM MnCl2	1	40	4.6/EA
7	Total	10	10/EA	

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