2025.6.4 C30-FAI (on live and fixed 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	150	Live cells	Ctrl (5% DMSO)	37°C for 15min	Mn2+ (3mM)	i5-4 + i7-1	AGAGTAGA	TCGCCTTA	60ng
2	151	Live cells	Ctrl (5% DMSO)	37°C for 15min	Mn2+ (3mM)	i5-4 + i7-2	AGAGTAGA	CTAGTACG	60ng
3	152	Live cells	C30-FAI (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-4 + i7-3	AGAGTAGA	TTCTGCCT	60ng
4	153	Live cells	C30-FAI (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-4 + i7-4	AGAGTAGA	GCTCAGGA	60ng
5	154	Live cells	FAI-N3 (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-4 + i7-5	AGAGTAGA	AGGAGTCC	60ng
6	155	Live cells	FAI-N3 (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-4 + i7-6	AGAGTAGA	CATGCCTA	60ng
7	164	Fixed cells	Ctrl (5% DMSO)	37°C for 15min	Mn2+ (3mM)	i5-7 + i7-11	AAGGAGTA	TGCCTCTT	120ng
8	165	Fixed cells	Ctrl (5% DMSO)	37°C for 15min	Mn2+ (3mM)	i5-7 + i7-12	AAGGAGTA	TCCTCTAC	120ng
9	166	Fixed cells	C30-FAI (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-8 + i7-1	CTAAGCCT	TCGCCTTA	120ng
10	167	Fixed cells	C30-FAI (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-8 + i7-2	CTAAGCCT	CTAGTACG	120ng
11	168	Fixed cells	FAI-N3 (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-8 + i7-3	CTAAGCCT	TTCTGCCT	120ng
12	169	Fixed cells	FAI-N3 (1mM final)	37°C for 15min	Mn2+ (3mM)	i5-8 + i7-4	CTAAGCCT	GCTCAGGA	120ng

TUESDAY, 6/3/2025

Cell treatment batch-3

	#	Cell number	Treatment	Incubation
1	150	2x10^5	Ctrl (5% DMSO)	37°C for 15min
2	151	2x10^5	Ctrl (5% DMSO)	37°C for 15min
3	152	2x10^5	C30-FAI (1mM final)	37°C for 15min
4	153	2x10^5	C30-FAI (1mM final)	37°C for 15min
5	154	2x10^5	FAI-N3 (1mM final)	37°C for 15min
6	155	2x10^5	FAI-N3 (1mM final)	37°C for 15min

- For C12 (5mM final) + C30 (5mM final):
  - 1x: 2.5ul 100mM C12, 0.25ul 1M C30, 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
- Count cells: **3.65x10^6 cells/ml**
- Prepare **2x10^5 cells/rxn x25 = 5M**
- 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

Cell treatment batch-4

	#	Cell number	Treatment	Incubation
1	164	2.5x10^5	Ctrl (5% DMSO)	37°C for 15min
2	165	2.5x10^5	Ctrl (5% DMSO)	37°C for 15min
3	166	2.5x10^5	C30-FAI (1mM final)	37°C for 15min
4	167	2.5x10^5	C30-FAI (1mM final)	37°C for 15min
5	168	2.5x10^5	FAI-N3 (1mM final)	37°C for 15min
6	169	2.5x10^5	FAI-N3 (1mM final)	37°C for 15min

Making buffers right before the experiment:

1x PBS Plus (keep on ice)

- for cell resuspension and fixation

1x PBS Plus

	Component	Vol (1ml)	Vol (15ml)	Final conc
1	1x PBS	1ml	15ml	1x
2	1M MgCl2	3ul	45ul	3mM
3	10% (w/v) BSA/PBS	8ul	120ul	0.08%
4	Total	1ml	15ml	

Fixing buffer (keep on ice)

- for cell fixation
- add at 4:1 ratio to cell suspension, e.g., 600ul fixing buffer to 150ul cells in washing buffer

Fixing buffer

	Component	Vol	Vol (16x)	Final conc	Note
1	50x DSS	7.5ul	120ul	0.5mg/ml	Final conc if added to cell solution at 4:1 ratio
2	Methanol (-20℃)	600ul	9.6ml	80%	Final conc if added to cell solution at 4:1 ratio

Washing buffer (keep on ice)

- for both cell washing and later covalent modification reactions

Washing buffer

	Component	Vol (1ml)	Vol (30ml)	Final conc	E
1	1x PBS	940ul	28.2ml	1x	
2	1M MgCl2	3ul	90ul	3mM	
3	10% (w/v) BSA/PBS	50ul	1500ul	0.5%	
4	10% (v/v) Triton X100	10ul	300ul	0.1%	
5	*RNaseIn*	*1ul*	*30ul*	*0.1%*	*Optional*
6	Total	1ml	30ml		

Cell collection:

- Treat "SARS-CoV2 5UTR"cells (SL5 HEK293) with TrypLE Express at room temp
- Collect and wash cells with **1x PBS Plus**
- Count cells: **4.9x10^6 cells/ml**
- Prepare **2.5x10^5 cells/rxn x16 = 4x10^6** cells in **2.4ml** 1x PBS Plus in a new 15ml tube (labeled as "fixed cell samples")

Cell fixation:

- Add **9.6ml** fixing buffer to the **2.4ml** "fixed cell samples"
- Incubate on ice for 15min, invert tube every 2min
- Add **3ml** wash buffer, 300xg 3min
- Add **10ml** wash buffer, 300xg 3min
- Add **10ml** wash buffer, 300xg 3min
- Resuspend in **760ul** wash buffer
- Aliquot **47.5ul** to 14 tubes

Cell treatment with covalent modifiers:

- Add **2.5ul** DMSO or covalent modifier in DMSO (total volume=50ul) to each sample **at room temp**, pipette to mix
- Incubate at designated temp for **15min**
- Chill on ice for 2min
- For fixed cell samples:
  - Add **1.5ul** proteinase K (NEB P8107S), incubate at 37°C for **10min, pipette to mix at 5min**
  - Add 300ul RLT Plus buffer (total volume=350ul)
  - Vortex for 30s to homogenize
- Store lysate in -80

Note:

- Sample #174 and #175 had white precipitation after 15min incubation and chilling on ice, which is not observed on the corresponding live cell samples (#160 and #161).

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-3

	#	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	150	142.0	1.95	19.1	1.00	3.40	Mn2+ (3mM)
2	151	141.8	2.01	19.1	1.00	3.40	Mn2+ (3mM)
3	152	152.4	1.96	21.6	1.00	3.40	Mn2+ (3mM)
4	153	170.4	1.99	25.8	1.00	3.40	Mn2+ (3mM)
5	154	139.3	1.97	18.5	1.00	3.40	Mn2+ (3mM)
6	155	132.9	1.99	17.0	1.00	3.40	Mn2+ (3mM)

RNA yield batch-4

	#	RNA conc (ng/ul)	260/280	Add x ul water to dilute to 60ng/ul	120ng RNA (ul)	Water to 4.4ul (ul)	RT ion
1	164	61.2	2.05	0.28	2.00	2.40	Mn2+ (3mM)
2	165	67.8	1.98	1.82	2.00	2.40	Mn2+ (3mM)
3	166	57.5	1.99	NA	2.09	2.31	Mn2+ (3mM)
4	167	59.0	1.97	NA	2.03	2.37	Mn2+ (3mM)
5	168	49.9	1.94	NA	2.40	2.00	Mn2+ (3mM)
6	169	65.6	1.95	1.31	2.00	2.40	Mn2+ (3mM)

- Store RNA in -80

Reverse transcription:

- Input: 60ng total RNA for live cell samples, 150ng total RNA for fixed cell samples

Annealing

	Component	vol (ul)	13x (ul)	Master mix (ul)
1	60ng or 150ng RNA+water	4.4	4.4/EA	
2	CMV-5UTR-SHAPE-Rv (100uM)	0.05	0.65	
3	dNTP (10mM)	0.5	6.5	
4	Water	0.45	5.85	1/EA
5	Total	5.4	5.4/EA	

- 65°C 5min, immediately put on ice

Extension

	Component	vol (ul)	13x (ul)	Master mix (ul)
1	Annealing product	5.4	5.4/EA	
2	375mM Tris/500mM KCl (5x buffer)	2	26	
3	100mM DTT (10x)	1	13	
4	Protoscript II	0.5	6.5	
5	RNaseIN	0.1	1.3	
6	30mM MnCl2	1	13	4.6/EA
7	Total	10	10/EA	

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

Note:

- Done in the same batches with "2025.6.2-4 C12 C15 C30 (on live/fixed cells); C12+C30 co-editing (on live cells)"