ATAC-seq

FRIDAY, 5/10/2024

I am repeating the entire ATAC-seq run because the library prep was not working before. I made the mistake of ordering primers that are not compatible with the adaptors on the transposes.

Cell lines for ATACseq1

	Quartzy name	Shortened name	Media	Rack	Box	Location	Date drawn	Thaw out date	Change media	First passage	Second passage	Seeding	Seeding time	ATACseq cell lysis and transposition	Start time	PCR amplification and size selection	Start time
1	COLO858 WT	WT	RPMI 1640	4	8	E1	04/14/2023	05/30/2024	06/01/2024	06/03/2024		06/05/2024	6:00 PM	06/08/2024	7:30 PM	07/01/2024	2:00 PM
2	COLO858 Cas9 Control	Cas9 Control	RPMI 1640	2	7	D1	05/03/2022	06/03/2024		06/05/2024	06/08/2024	06/10/2024	3:30 PM	06/13/2024	2:30 PM	07/01/2024	2:00 PM
3	COLO858 Cas9 FOS KO-BB5	FOS KO-1	RPMI 1640	2	10	I4	03/27/2023	06/03/2024	06/05/2024	06/07/2024		06/10/2024	3:30 PM	06/13/2024	2:30 PM	07/01/2024	2:00 PM
4	COLO858 Cas9 FOSL1 KO-E25	FOSL1 KO-1	RPMI 1640	8	8	F3	04/13/2023	06/03/2024	06/05/2024	06/07/2024		06/10/2024	3:30 PM	06/13/2024	2:30 PM	07/01/2024	2:00 PM
5	COLO858 Cas9 FOSL2 KO-A25	FOSL2 KO	RPMI 1640	2	10	D1	11/07/2023	06/03/2024	06/05/2024	06/08/2024	06/18/2024	06/10/2024	3:30 PM	06/13/2024	2:30 PM	07/01/2024	2:00 PM
6	COLO858 Cas9 / Positive GBF Control	Cas9 / Positive GBF Control	RPMI 1640	4	4	E4	09/09/2023	05/30/2024	06/01/2024	06/03/2024		06/05/2024	6:00 PM	06/08/2024	7:30 PM	07/01/2024	2:00 PM
7	COLO858 Cas9 FOSL1 KO-A25 / FOSL1 OE	FOSL1 KO-1 / FOSL1 OE	RPMI 1640	8	5	F4	05/18/2024	05/30/2024	06/01/2024	06/03/2024		06/05/2024	6:00 PM	06/08/2024	7:30 PM	07/01/2024	2:00 PM
8	COLO858 Positive GBF Control	Positive GBF Control	RPMI 1640 + 5 ug/mL blasticidin	5	11	D9	03/06/2023	06/10/2024	06/11/2024	06/14/2024	06/18/2024	06/20/2024	5:00 PM	06/23/2024	6:30 PM	07/02/2024	3:00 PM
9	COLO858 FOS OE	FOS OE	RPMI 1640 + 5 ug/mL blasticidin	5	11	F7	03/07/2023	06/10/2024	06/11/2024	06/14/2024	06/18/2024	06/20/2024	5:00 PM	06/23/2024	6:30 PM	07/02/2024	3:00 PM
10	COLO858 FOSL2 OE	FOSL2 OE	RPMI 1640 + 5 ug/mL blasticidin	5	11	C4	03/03/2023	06/10/2024	06/11/2024	06/14/2024	06/18/2024	06/20/2024	5:00 PM	06/23/2024	6:30 PM	07/02/2024	3:00 PM
11	COLO858 JUN OE	JUN OE	RPMI 1640 + 5 ug/mL blasticidin	2	6	E9	03/07/2023	06/10/2024	06/11/2024	06/14/2024	06/18/2024	06/20/2024	5:00 PM	06/23/2024	6:30 PM	07/02/2024	3:00 PM
12	COLO858 NTC shRNA #2 Control	NTC KD-2 Control	RPMI 1640 + 0.5 ug/mL puromycin	1	1	C7	09/23/2022	05/20/2024		05/21/2024		05/24/2024	4:00 PM	05/27/2024	4:00 PM	07/02/2024	3:00 PM
13	COLO858 JUN shRNA #3	JUN KD	RPMI 1640 + 0.5 ug/mL puromycin	8	6	I6	05/15/2023	05/20/2024		05/21/2024		05/24/2024	4:00 PM	05/27/2024	4:00 PM	07/02/2024	3:00 PM
14	COLO858 JUNB shRNA #1	JUNB KD-1	RPMI 1640 + 0.5 ug/mL puromycin	8	6	G4	05/15/2023	05/20/2024		05/21/2024		05/24/2024	4:00 PM	05/27/2024	4:00 PM	07/02/2024	3:00 PM
15	COLO858 JUND shRNA #1	JUND KD	RPMI 1640 + 0.5 ug/mL puromycin	1	6	G8	02/02/2023	05/20/2024		05/21/2024		05/24/2024	4:00 PM	05/27/2024	4:00 PM	07/02/2024	3:00 PM
16	COLO858 Cas9 / NTC shRNA #2 Control	Cas9 / NTC KD-2 Control	RPMI 1640 + 0.5 ug/mL puromycin	4	10	C2	07/13/2023	06/11/2024	06/12/2024	06/14/2024		06/17/2024	8:30 PM	06/20/2024	8:00 PM	07/03/2024	2:00 PM
17	COLO858 Cas9 FOSL2 KO-A25 / JUN shRNA #3	FOSL2 KO / JUN KD	RPMI 1640 + 0.5 ug/mL puromycin	4	9	C7	09/18/2023	06/11/2024	06/12/2024	06/14/2024		06/17/2024	8:30 PM	06/20/2024	8:00 PM	07/03/2024	2:00 PM
18	COLO858 Cas9 FOS KO-BB5 / JUNB shRNA #1	FOS KO-1 / JUNB KD-1	RPMI 1640 + 0.5 ug/mL puromycin	4	9	E3	09/18/2023	06/11/2024	06/12/2024	06/14/2024		06/17/2024	8:30 PM	06/20/2024	8:00 PM	07/03/2024	2:00 PM
19	COLO858 Cas9 FOS KO-BB5 / JUND shRNA #1	FOS KO-1 / JUND KD	RPMI 1640 + 0.5 ug/mL puromycin	4	8	I3	04/18/2023	06/11/2024	06/12/2024	06/14/2024		06/17/2024	8:30 PM	06/20/2024	8:00 PM	07/03/2024	2:00 PM
20	COLO858 Cas9 JUN KO-A4 / JUND shRNA #1	JUN KO / JUND KD	RPMI 1640 + 0.5 ug/mL puromycin	4	9	E9	09/18/2023	06/11/2024	06/12/2024	06/14/2024		06/17/2024	8:30 PM	06/20/2024	8:00 PM	07/03/2024	2:00 PM

Today I passaged NTC shRNA #2 and JUND shRNA #1 1:5 fold into T75 flasks. I also seeded NTC shRNA #2 at 2:5 dilution in another T75 flask for a trial run of ATACseq library preparation. Additionally I thawed out JUNB shRNA #1 , JUN shRNA #3, and COLO858 WT P12.

WEDNESDAY, 5/15/2024

I am trying different Tn5 enzyme to nuclei ratios. The PCR amplification was done with 8 cycles for all samples.

Qubit concentrations (ng/mL)

	Sample	1.8x bead elution; 100 bp < DNA < 1000 bp	1.8x supernatant; DNA < 100 bp	0.5x bead elution; DNA > 1000 bp	E	F	G	H	I
1	0 uL Tn5 enzyme	119	544	34					
2	1.25 uL Tn5 enzyme	8510	644	7560					
3	2.5 uL Tn5 enzyme	10800	876	9200					
4	3.75 uL Tn5 enzyme	18700	768	10560					
5	0 uL Tn5 enzyme and no buffer	230	387	145					

Agilent High Sensitivity D5000 ScreenTape:
- Sizing range: 100-5000 bp
- Typical resolution: 400-5000 bp: 15%
- Sensitivity: 5 pg/uL
- Quantification range: 10 - 1000 pg/uL

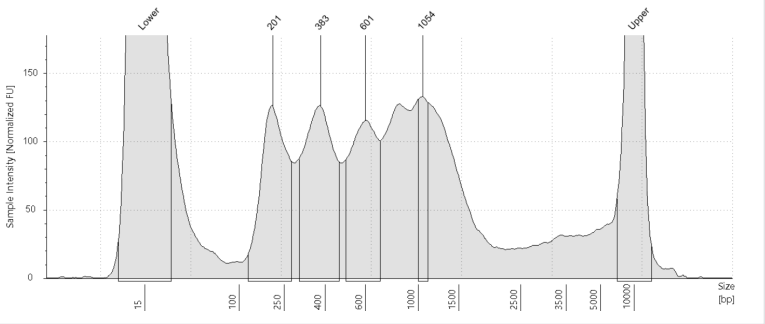
TapeStation dilutions

	Well	Sample	Portion	Qubit conc. (pg/uL)	Fold dilution factor	Final conc. (pg/uL)
1	B1	0 uL Tn5 enzyme	1.8x bead elution; 100 bp < DNA < 1000 bp	119	1	119
2	C1	1.25 uL Tn5 enzyme	1.8x bead elution; 100 bp < DNA < 1000 bp	8510	20	425.5
3	D1	2.5 uL Tn5 enzyme	1.8x bead elution; 100 bp < DNA < 1000 bp	10800	20	540
4	E1	3.75 uL Tn5 enzyme	1.8x bead elution; 100 bp < DNA < 1000 bp	18700	20	935
5	F1	0 uL Tn5 enzyme	1.8x supernatant; DNA < 100 bp	544	1	544
6	G1	1.25 uL Tn5 enzyme	1.8x supernatant; DNA < 100 bp	644	1	644
7	H1	2.5 uL Tn5 enzyme	1.8x supernatant; DNA < 100 bp	876	1	876
8	A2	3.75 uL Tn5 enzyme	1.8x supernatant; DNA < 100 bp	768	1	768
9	B2	0 uL Tn5 enzyme	0.5x bead elution; DNA > 1000 bp	34	1	34
10	C2	1.25 uL Tn5 enzyme	0.5x bead elution; DNA > 1000 bp	7560	20	378
11	D2	2.5 uL Tn5 enzyme	0.5x bead elution; DNA > 1000 bp	9200	20	460
12	E2	3.75 uL Tn5 enzyme	0.5x bead elution; DNA > 1000 bp	10560	20	528

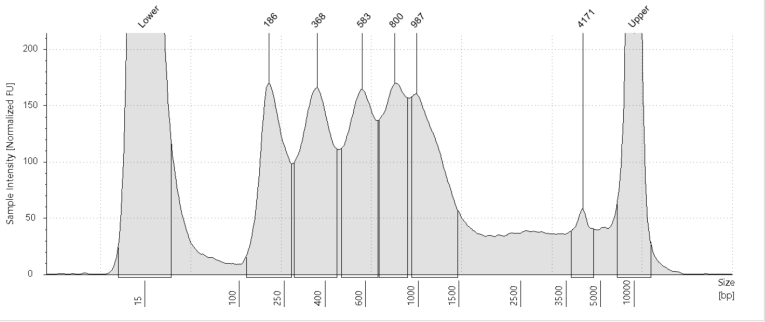
2024-05-16 - 12.51.42.pdf

2024-05-16 - 12.51.42_2.pdf

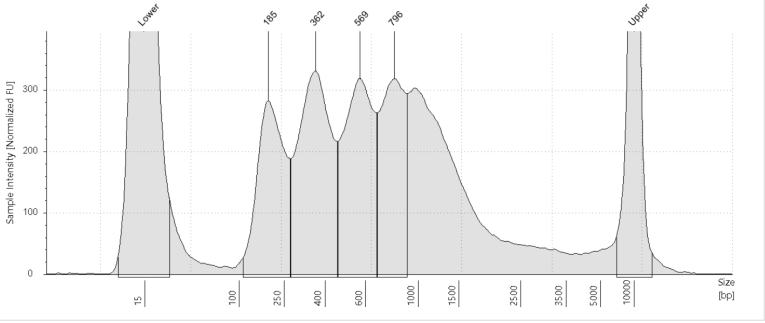
C1 scaled to sample (1.25 ul of Tn5 enzyme; 50,000 cells)



D1 scaled to sample (2.5 ul of Tn5 enzyme; 50,000 cells)



E1 scaled to sample (3.75 ul of Tn5 enzyme; 50,000 cells)



FRIDAY, 5/24/2024

Plate coating:

Coating: Human Plasma Fibronectin
Vendor: EMD Millipore
Catalog: FC010

Protocol:

1. Make the working solution of fibronectin in 1xDPBS.
2. Add 1.5 mL of the working solution to each well.
3. Incubate at room temperature inside of the culture hood for 1 hour.
4. Aspirate the working solution.
5. Add 2 mL of 1xDPBS to each well and aspirate. Repeat for a total of three washes.

Plate coating

	A	B
1	Stock solution concentration (ug/mL)	1000
2	Working sollution concentration (ug/mL)	10
3	Number of wells	20
4	Volume per well (mL)	1.5
5	Extra volume (mL)	1
6		
7	Volume of working dilution needed (mL)	31
8	Volume of stock solution (uL)	310
9	Volume of 1x DPBS (mL)	30.69

Cell seeding:

Seeding density in the 6-well plate: 200,000 cells/well; 4 wells: three biological replicates + one wells for counting cells
Start ATAC-seq library prep two days after seeding.

Cell Seeding calculations

	Cell line	Media	Seeding density (cells/well)	Total number of wells	Volume per well (mL)	Extra factor	Total number of cells needed	Total volume (mL)	Live cell count #1 (cells/mL)	% live	Live cell count #2 (cells/mL)	% live	Avg. live cell count (cells/mL)	Volume of cell suspension (mL)	Volume of media (mL)
1	WT	RPMI 1640	75000	4	3	1.1	330000	13.2	1030000	100	965000	99	997500	0.331	12.9
2	Cas9 Control	RPMI 1640	75000	4	3	1.1	330000	13.2	976000	99	818000	100	897000	0.368	12.8
3	FOS KO-1	RPMI 1640	75000	4	3	1.1	330000	13.2	747000	100	720000	100	733500	0.450	12.8
4	FOSL1 KO-1	RPMI 1640	100000	4	3	1.1	440000	13.2	354000	100	464000	100	409000	1.076	12.1
5	FOSL2 KO	RPMI 1640	75000	4	3	1.1	330000	13.2	676000	99	578000	98	627000	0.526	12.7
6	Cas9 / Positive ORF Control	RPMI 1640	75000	4	3	1.1	330000	13.2	1290000	100	1270000	100	1280000	0.258	12.9
7	FOSL1 KO-1 / FOSL1 OE	RPMI 1640	75000	4	3	1.1	330000	13.2	709000	99	862000	100	785500	0.420	12.8
8	Positive ORF Control	RPMI 1640 + 5 ug/mL blasticidin	75000	4	3	1.1	330000	13.2	660000	98	682000	98	671000	0.492	12.7
9	FOS OE	RPMI 1640 + 5 ug/mL blasticidin	100000	4	3	1.1	440000	13.2	1030000	100	982000	99	1006000	0.437	12.8
10	FOSL2 OE	RPMI 1640 + 5 ug/mL blasticidin	75000	4	3	1.1	330000	13.2	823000	100	785000	99	804000	0.410	12.8
11	JUN OE	RPMI 1640 + 5 ug/mL blasticidin	100000	4	3	1.1	440000	13.2	698000	99	703000	100	700500	0.628	12.6
12	NTC KD-2 Control	RPMI 1640 + 0.5 ug/mL puromycin	75000	4	3	1.1	330000	13.2	665000	97	709000	99	687000	0.480	12.7
13	JUN KD	RPMI 1640 + 0.5 ug/mL puromycin	75000	4	3	1.1	330000	13.2	518000	99	529000	100	523500	0.630	12.6
14	JUNB KD-1	RPMI 1640 + 0.5 ug/mL puromycin	75000	4	3	1.1	330000	13.2	594000	99	627000	98	610500	0.541	12.7
15	JUND KD	RPMI 1640 + 0.5 ug/mL puromycin	75000	4	3	1.1	330000	13.2	894000	99	665000	98	779500	0.423	12.8
16	Cas9 / NTC KD-2 Control	RPMI 1640 + 0.5 ug/mL puromycin	75000	4	3	1.1	330000	13.2	916000	100	916000	99	916000	0.360	12.8
17	FOSL2 KO / JUN KD	RPMI 1640 + 0.5 ug/mL puromycin	75000	4	3	1.1	330000	13.2	927000	99	763000	99	845000	0.391	12.8
18	FOS KO-1 / JUNB KO-1	RPMI 1640 + 0.5 ug/mL puromycin	75000	4	3	1.1	330000	13.2	414000	99	256000	100	335000	0.985	12.2
19	FOS KO-1 / JUND KO	RPMI 1640 + 0.5 ug/mL puromycin	75000	4	3	1.1	330000	13.2	638000	100	714000	99	676000	0.488	12.7
20	JUN KO / JUND KO	RPMI 1640 + 0.5 ug/mL puromycin	75000	4	3	1.1	330000	13.2	409000	100	453000	100	431000	0.766	12.4

MONDAY, 5/27/2024

Solutions and reagent setup

- [ ] Prepare ATAC-seq resuspension buffer (ATAC-RSB). Filter steralixe using a 0.22 um filter. Store at 4 deg C for up to 6 months.

ATAC-seq resuspension buffer

	Reagent	Volume for 100 mL buffer (mL)	Final conc. (mM)
1	1 M Tris-HCl pH 7.5	1	10
2	5 M NaCl	0.2	10
3	1 M MgCl2	0.3	3
4	UltraPure distilled water	98.5	
5	Total	100	

- [ ] Digitonin: The recommended digitonin from Promega is supplied at 2% (wt/vol) concentration in DMSO. Dilute this digitonin 1:1 with water to make a 1% (wt/vol) (100x) stock solution. This DMSO-water mixture will no longer freeze at -20 deg C. Store at -20 deg C for up to 6 months.

Prepare fresh buffers

- [ ] Make the ATAC-seq Lysis Buffer and ATAC-seq Wash Buffer and keep them and DPBS on ice. Be sure to use freshly made ATAC-seq Lysis Buffer and ATAC-seq Wash Buffer each time.

ATAC-seq Lysis Buffer

	Reagent	Volume per sample (ul)	Final conc.	Master Mix (ul) 3 cell lines	Master Mix (ul) 4 cell lines	Master Mix (ul) 5 cell lines
1	Cold ATAC-RSB	48.5		480.15	640.2	800.25
2	10% (wt/vol) NP40	0.5	0.1% (wt/vol)	4.95	6.6	8.25
3	10% (wt/vol) Tween-20	0.5	0.1% (wt/vol)	4.95	6.6	8.25
4	1% (wt/vol) digitonin	0.5	0.01% (wt/vol)	4.95	6.6	8.25
5	Total	50		495	660	825

ATAC-seq Wash Buffer

	Reagent	Volume per sample (ul)	Final conc.	Master Mix (ul) 3 cell lines	Master Mix (ul) 4 cell lines	Master Mix (ul) 5 cell lines
1	Cold ATAC-RSB	990		9801	13068	16335
2	10% (wt/vol) Tween-20	10	0.1% (wt/vol)	99	132	165
3	Total	1000		9900	13200	16500

Cell counting

1. Using the plate for cell counting, trypsinize cells and wash them once with cold 1xDPBS. Resuspend cells in 1 mL of ice cold 1x DPBS.
2. Count the cells using the cell counter. Note the total number of cells in the plate and calculate how much volume to transfer from each well such that we use 50,000 cells per reaction. For example, if we determine that there are 500,000 cells/mL, we will transfer 100 uL in the next section.

Cell counts

	Sample	Live cell count #1 (cells/mL)	% live	Live cell count #2 (cells/mL)	% live	Live cell count #3 (cells/mL)	% live	Live cell count #4 (cells/mL)	% live	Avg. live cell count (cells/mL)	# of cells needed	Volume of cell suspension (ul)
1	WT	344000	98	404000	99					374000	50000	133.69
2	Cas9 Control	436000	96	431000	96	311000	100	322000	98	375000	50000	133.33
3	FOS KO-1	202000	100	164000	100	284000	98	289000	90	234750	50000	212.99
4	FOSL1 KO-1	131000	100	136000	96	207000	93	344000	94	204500	50000	244.50
5	FOSL2 KO	267000	98	420000	99	398000	97	360000	99	361250	50000	138.41
6	Cas9 / Positive ORF Control	431000	98	398000	99					414500	50000	120.63
7	FOSL1 KO-1 / FOSL1 OE	245000	100	196000	95					220500	50000	226.76
8	Positive ORF Control	311000	100	382000	100	267000	100	409000	96	342250	50000	146.09
9	FOS OE	474000	98	387000	99	245000	98	474000	99	395000	50000	126.58
10	FOSL2 OE	365000	99	458000	97	480000	100	442000	100	436250	50000	114.61
11	JUN OE	398000	92	480000	100	496000	98	404000	96	444500	50000	112.49
12	NTC KD-2 Control	338000	97	294000	96					316000	50000	158.23
13	JUN KD	540000	100	502000	98					521000	50000	95.97
14	JUNB KD-1	234000	93	273000	100					253500	50000	197.24
15	JUND KD	311000	98	218000	98	333000	94	267000	98	282250	50000	177.15
16	Cas9 / NTC KD-2 Control	284000	95	305000	98	365000	100	327000	92	320250	50000	156.13
17	FOSL2 KO / JUN KD	147000	96	245000	98	256000	87	224000	95	218000	50000	229.36
18	FOS KO-1 / JUNB KD-1	142000	90	245000	98	125000	96	131000	100	160750	50000	311.04
19	FOS KO-1 / JUND KD	289000	100	251000	98	185000	100	175000	100	225000	50000	222.22
20	JUN KO / JUND KD	267000	96	224000	93	229000	98	273000	98	248250	50000	201.41

Cell lysis and transposition

1. Move the second 6-well plate and aspirate the media.
2. Trypsinize cells and wash them once with cold 1xDPBS. Resuspend cells in 1 mL of ice cold 1x DPBS.
3. Transfer 50,000 cells into a 1.5 mL DNA LoBind tube (volume determined above).
4. Pellet the cells at 500g for 5 min at 4 deg C in a fixed-angle microcentrifuge.
5. Aspirate all the supernatant using two pipetting steps. First, aspirate down to 100 uL with a p1000 pipette. Then, remove the final 100 uL with a p200 pipette.
   [CRITICAL] Make sure to avoid the visible cell pellet when pipetting. Optimal removal of the supernatant and minimal disruption of the cell pellet is attained when the removal of the final 100 uL is performed in a consistent and fluid motion.
6. Resuspend the cell pellet in 50 uL of ATAC-seq Lysis Buffer by pipetting up and down three times. ATAC-seq Lysis Buffer should be made fresh each time and mixed thoroughly prior to use.
7. Incubate on ice for 3 min. If lysing multiple samples, make sure that all samples are lysed for the same total amount of time by proceeding to Step 8 after 3 min.
8. Add 1 mL of ATAC-seq Wash Buffer to dilute the lysis reagents. Invert the tube five times to mix. ATAC-seq Wash Buffer should be made fresh each time and mixed thoroughly prior to use.
9. Pellet nuclei at 500g for 10 min at 4 deg C in a fixed-angle microcentrifuge. During this time prepare the Transposition Mix that will be used in step 11 and preheat the thermomixer.
10. Aspirate all supernatant using two pipting steps as above.
    [CRITICAL] Make sure to avoid the visible cell pellet when pipetting. Optimal removal of the supernatant and minimal disruption of the cell pellet is attained when the removal of the final 100 uL is performed in a consistent and fluid motion.
11. Resuspend the cell pellet in 50 uL of Transposition Mix by pipetting up and down six times. Transposition Mix should be made fresh each time and mixed thoroughly prior to use.=

Transposition reaction

	Reagent	Volume per sample (ul)	Final conc.	Master Mix (ul) 3 cell lines	Master mix (ul) 4 cell lines	Master Mix (ul) 5 cell lines
1	2x TD buffer	25	1x	236.25	330	412.5
2	PBS	16.5		155.925	217.8	272.25
3	UltraPure distilled water	5	0.1% (wt/vol)	47.25	66	82.5
4	1% (wt/vol) digitonin	0.5	0.01% (wt/vol)	4.725	6.6	8.25
5	10% (wt/vol) Tween-20	0.5	0.1% (wt/vol)	4.725	6.6	8.25
6	TDE1 TD enzyme (Tn5 transposase)	2.5		23.625	33	41.25
7	Total	50		472.5	660	825

1. Incubate reaction at 37 deg C for 30 min in a thermomixer with 1,000 rpm mixing.

2. Remove the tubes from the thermomixer, and immediately terminate the transposition reaction by adding 250 uL (five volumes) of DNA Binding Buffer from the DNA Clean and Concentrator-5 kit and mix well by pipetting or inversion.
3. Pulse centrifuge to collect solution in the bottom of the tube.
   [PAUSE] This solution can be stored at -20 deg C for up to 3 weeks. Allow this mixture to warm back to room temperature and mix throughly before proceeding.
4. Clean up the transposition reaction using the DNA Clean and Concentrator-5 kit. Transfer each sample, mixed with the DNA Binding Buffer to a Zymo-Spin Column in a collection tube. Centrifuge at RT for 30 sec at 10,000g and discard the flow through.
5. Add 500 ul of DNA Wash Buffer to the column and centrifuge at RT for 30 sec at 10,000g.
6. Repeat this wash for a total of two wash steps.
7. Perform a final 'dry spin' after the second wash step to remove any traces of residual wash buffer from the column. To do this remove any flowthrough from the collection tube and centrifuge the column and collection tube at RT for 1 min at >13,000g.
8. Transfer the column to a clean prelabeled 2 mL DNA LoBind tube. Pipette 23 ul of Elution Buffer directly onto the column membrane and wait for 1 min.
9. Centrifuge the column at RT for 1 min at 13,000g to elute the DNA. Use 1 ul of the elution to measure the concentration using Qubit.
   [PAUSE] This solution can be stored at -20 deg C for as long as necessary

Barcoding of the transposed fragments

1. Assign each sample in the study to a unique pair of adapters:

Custom Barcodes Adapter 1 (Index i5)

	Adapter Name	Adapter Sequence	SampleSheet Barcode - Forward Strand Workflow	SampleSheet Barcode - Reverse Strand Workflow
1	Ad1.1	AATGATACGGCGACCACCGAGATCTACACTAGATCGCTCGTCGGCAGCGTCAGATGTGTAT	TAGATCGC	GCGATCTA
2	Ad1.2	AATGATACGGCGACCACCGAGATCTACACCTCTCTATTCGTCGGCAGCGTCAGATGTGTAT	CTCTCTAT	ATAGAGAG
3	Ad1.3	AATGATACGGCGACCACCGAGATCTACACTATCCTCTTCGTCGGCAGCGTCAGATGTGTAT	TATCCTCT	AGAGGATA
4	Ad1.4	AATGATACGGCGACCACCGAGATCTACACAGAGTAGATCGTCGGCAGCGTCAGATGTGTAT	AGAGTAGA	TCTACTCT
5	Ad1.5	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGTCGTCGGCAGCGTCAGATGTGTAT	GTAAGGAG	CTCCTTAC
6	Ad1.6	AATGATACGGCGACCACCGAGATCTACACACTGCATATCGTCGGCAGCGTCAGATGTGTAT	ACTGCATA	TATGCAGT
7	Ad1.7	AATGATACGGCGACCACCGAGATCTACACAAGGAGTATCGTCGGCAGCGTCAGATGTGTAT	AAGGAGTA	TACTCCTT
8	Ad1.8	AATGATACGGCGACCACCGAGATCTACACCTAAGCCTTCGTCGGCAGCGTCAGATGTGTAT	CTAAGCCT	AGGCTTAG

Custom Barcodes Adapter 2 (Index i7)

	Adapter Name	Adapter Sequence	SampleSheet Barcode - All Sequencers
1	Ad2.1	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGTG	TAAGGCGA
2	Ad2.2	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGTG	CGTACTAG
3	Ad2.3	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGTG	AGGCAGAA
4	Ad2.4	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGTG	TCCTGAGC
5	Ad2.5	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGTG	GGACTCCT
6	Ad2.6	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGTG	TAGGCATG
7	Ad2.7	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGTG	CTCTCTAC
8	Ad2.8	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGTG	CAGAGAGG

Table3

	Sample name	Rep #	Ad1 (i5)	SampleSheet Barcode - Forward Strand Workflow	SampleSheet Barcode - Reverse Strand Workflow	Ad2 (i7)	SampleSheet Barcode - All Sequencers
1	WT	1	1	TAGATCGC	GCGATCTA	1	TAAGGCGA
2	WT	2	1	TAGATCGC	GCGATCTA	2	CGTACTAG
3	WT	3	1	TAGATCGC	GCGATCTA	3	AGGCAGAA
4	Cas9 Control	1	1	TAGATCGC	GCGATCTA	4	TCCTGAGC
5	Cas9 Control	2	1	TAGATCGC	GCGATCTA	5	GGACTCCT
6	Cas9 Control	3	1	TAGATCGC	GCGATCTA	6	TAGGCATG
7	FOS KO-1	1	1	TAGATCGC	GCGATCTA	7	CTCTCTAC
8	FOS KO-1	2	1	TAGATCGC	GCGATCTA	8	CAGAGAGG
9	FOS KO-1	3	2	CTCTCTAT	ATAGAGAG	1	TAAGGCGA
10	FOSL1 KO-1	1	2	CTCTCTAT	ATAGAGAG	2	CGTACTAG
11	FOSL1 KO-1	2	2	CTCTCTAT	ATAGAGAG	3	AGGCAGAA
12	FOSL1 KO-1	3	2	CTCTCTAT	ATAGAGAG	4	TCCTGAGC
13	FOSL2 KO	1	2	CTCTCTAT	ATAGAGAG	5	GGACTCCT
14	FOSL2 KO	2	2	CTCTCTAT	ATAGAGAG	6	TAGGCATG
15	FOSL2 KO	3	2	CTCTCTAT	ATAGAGAG	7	CTCTCTAC
16	Cas9 / Positive ORF Control	1	2	CTCTCTAT	ATAGAGAG	8	CAGAGAGG
17	Cas9 / Positive ORF Control	2	3	TATCCTCT	AGAGGATA	1	TAAGGCGA
18	Cas9 / Positive ORF Control	3	3	TATCCTCT	AGAGGATA	2	CGTACTAG
19	FOSL1 KO-1 / FOSL1 OE	1	3	TATCCTCT	AGAGGATA	3	AGGCAGAA
20	FOSL1 KO-1 / FOSL1 OE	2	3	TATCCTCT	AGAGGATA	4	TCCTGAGC
21	FOSL1 KO-1 / FOSL1 OE	3	3	TATCCTCT	AGAGGATA	5	GGACTCCT
22	Positive ORF Control	1	3	TATCCTCT	AGAGGATA	6	TAGGCATG
23	Positive ORF Control	2	3	TATCCTCT	AGAGGATA	7	CTCTCTAC
24	Positive ORF Control	3	3	TATCCTCT	AGAGGATA	8	CAGAGAGG
25	FOS OE	1	4	AGAGTAGA	TCTACTCT	1	TAAGGCGA
26	FOS OE	2	4	AGAGTAGA	TCTACTCT	2	CGTACTAG
27	FOS OE	3	4	AGAGTAGA	TCTACTCT	3	AGGCAGAA
28	FOSL2 OE	1	4	AGAGTAGA	TCTACTCT	4	TCCTGAGC
29	FOSL2 OE	2	4	AGAGTAGA	TCTACTCT	5	GGACTCCT
30	FOSL2 OE	3	4	AGAGTAGA	TCTACTCT	6	TAGGCATG
31	JUN OE	1	4	AGAGTAGA	TCTACTCT	7	CTCTCTAC
32	JUN OE	2	4	AGAGTAGA	TCTACTCT	8	CAGAGAGG
33	JUN OE	3	5	GTAAGGAG	CTCCTTAC	1	TAAGGCGA
34	NTC KD-2 Control	1	5	GTAAGGAG	CTCCTTAC	2	CGTACTAG
35	NTC KD-2 Control	2	5	GTAAGGAG	CTCCTTAC	3	AGGCAGAA
36	NTC KD-2 Control	3	5	GTAAGGAG	CTCCTTAC	4	TCCTGAGC
37	JUN KD	1	5	GTAAGGAG	CTCCTTAC	5	GGACTCCT
38	JUN KD	2	5	GTAAGGAG	CTCCTTAC	6	TAGGCATG
39	JUN KD	3	5	GTAAGGAG	CTCCTTAC	7	CTCTCTAC
40	JUNB KD-1	1	5	GTAAGGAG	CTCCTTAC	8	CAGAGAGG
41	JUNB KD-1	2	6	ACTGCATA	TATGCAGT	1	TAAGGCGA
42	JUNB KD-1	3	6	ACTGCATA	TATGCAGT	2	CGTACTAG
43	JUND KD	1	6	ACTGCATA	TATGCAGT	3	AGGCAGAA
44	JUND KD	2	6	ACTGCATA	TATGCAGT	4	TCCTGAGC
45	JUND KD	3	6	ACTGCATA	TATGCAGT	5	GGACTCCT
46	Cas9 / NTC KD-2 Control	1	6	ACTGCATA	TATGCAGT	6	TAGGCATG
47	Cas9 / NTC KD-2 Control	2	6	ACTGCATA	TATGCAGT	7	CTCTCTAC
48	Cas9 / NTC KD-2 Control	3	6	ACTGCATA	TATGCAGT	8	CAGAGAGG
49	FOSL2 KO / JUN KD	1	7	AAGGAGTA	TACTCCTT	1	TAAGGCGA
50	FOSL2 KO / JUN KD	2	7	AAGGAGTA	TACTCCTT	2	CGTACTAG
51	FOSL2 KO / JUN KD	3	7	AAGGAGTA	TACTCCTT	3	AGGCAGAA
52	FOS KO-1 / JUNB KD-1	1	7	AAGGAGTA	TACTCCTT	4	TCCTGAGC
53	FOS KO-1 / JUNB KD-1	2	7	AAGGAGTA	TACTCCTT	5	GGACTCCT
54	FOS KO-1 / JUNB KD-1	3	7	AAGGAGTA	TACTCCTT	6	TAGGCATG
55	FOS KO-1 / JUND KD	1	7	AAGGAGTA	TACTCCTT	7	CTCTCTAC
56	FOS KO-1 / JUND KD	2	7	AAGGAGTA	TACTCCTT	8	CAGAGAGG
57	FOS KO-1 / JUND KD	3	8	CTAAGCCT	AGGCTTAG	1	TAAGGCGA
58	JUN KO / JUND KD	1	8	CTAAGCCT	AGGCTTAG	2	CGTACTAG
59	JUN KO / JUND KD	2	8	CTAAGCCT	AGGCTTAG	3	AGGCAGAA
60	JUN KO / JUND KD	3	8	CTAAGCCT	AGGCTTAG	4	TCCTGAGC

1. Transfer each cleaned-up transposed DNA sample to a 200 uL PCR tube.
2. Add 25 uL of NEBNext Ultra II Q5 2x Master Mix to each tube.
3. Add 2.5 uL of Ad1 and Ad2 to each sample.
4. Cap tubes, vortex and spin down to collect all liquid at the bottom of the tube. When completed, each reaction should contain the following:

PCR reaction

	Reagent	Volume per sample (ul)	Final conc.
1	Transposed sample	20	
2	NEBNext Ultra II Q5 2x Master Mix	25	1x
3	5 uM Adapter Ad1 (sample specific)	2.5	0.25 uM
4	5 uM Adapter Ad2 (sample specific)	2.5	0.25 uM
5	Total volume	50	

5. Run the barcodeing PCR reactions according to the following cycling conditions.

[CRITICAL] The initial 5 min incuabtion at 72 deg C is critical for the success of the amplification reaction. This is because (i) transposed DNA contains nicks and overhangs that must be filled in prior to denaturation and (ii) the polymerase enzyme in the NEBNext Ultra II 6. Q5 2x Master Mix is a hot-start polymerase that becomes active at 45 deg C.

Thermocycler settings

	Step	Temperature (deg C)	Time	# of cycles
1	Initial Extention	72	5 min	1
2	Initial denaturation	98	30 sec	1
3	Denaturation	98	10 sec	7
4	Anealing	68	30 sec	
5	Extension	68	45 sec	
6	Hold	4		

7. Remove tubes from the thermocycler, and store on ice. Proceed to the next step immediately.

Library Purification and Size Selection

1. Vortex SPRI select beads for 15 sec to resuspend.
2. Add 0.5X (22.5 uL) volume of SPRI select beads to fresh 200 uL PCR tubes.
3. Add 45 uL of the PCR reaction to the beads and mix well by pipetting up and down 10x.
4. Incubate at room temperature for 10 min.
5. Place the tubes in the magnetic rack for 5 min.
6. Transfer 60 ul of supernatant to new PCR tube.
7. Add 1.8X volume (108 ul) SPRI select beads, pipet up and down 10x to mix throughly.
8. Incubate at room temperature for 10 min.
9. Place the tubes in the magentic rack for 5 min.
10. Discard the supernatant.
11. Wash beads with 200 uL of 80% EtOH (freshly made). Incubate for 30 sec and remove and discard the supernatant. Repeat for a total of three washes. After the last wash ensure all EtOH is removed by using 10 ul pipette tips.
12. Leave the tubes on the magnet with the caps open to allow the EtOH evaporate. It should take approx. 7-10 min.
13. Resuspend beads in 20 uL of nuclease-free H2O, pipet up and down 10x to mix throughly.
14. Incubate at room temperature for 2 min, then quickly spin the tubes down.
15. Place the tubes in the magnetic rack for 5 min.
16. Transfer to new microcentrifuge tube.
    [PAUSE] Can store samples at -20 deg C.

Library concentration determination

- Qubit
- TapeStation to check the size of the fragments
  Agilent High Sensitivity D5000 ScreenTape:
  - Sizing range: 100-5000 bp
  - Typical resolution: 400-5000 bp: 15%
  - Sensitivity: 5 pg/uL
  - Quantification range: 10 - 1000 pg/uL
- qPCR

Library concentrations and molarity

	Cell line	Rep	Notes	dsDNA Qubit before PCR amplification (ng/mL)	Qubit dsDNA after PCR amplification and size selection (ng/mL)	TapeStation (pmol/l); 2-fold diluted	TapeStation Molarity (nM)	qPCR Molarity (nM)	Volume for pooling (ul)	Total volume of pooled library (ul)
1	WT	1		6280	5620	6540	13.08	5.67	0.7054673721	45.4148952102
2		2		6140	3240	5700	11.4	4.02	0.9950248756	
3		3		6220	3580	7570	15.14	6.27	0.6379585327	
4	Cas9 Control	1		6360	4180	8320	16.64	7.01	0.5706134094	
5		2		8020	4680	9620	19.24	7.87	0.5082592122	
6		3		4680	2720	5820	11.64	3.46	1.1560693642	
7	FOS KO-1	1		7160	4940	9980	19.96	7.62	0.5249343832	
8		2		8720	5700	9830	19.66	7.96	0.5025125628	
9		3		6640	4780	5860	11.72	4.18	0.956937799	
10	FOSL1 KO-1	1		5720	4540	7540	15.08	5.16	0.7751937984	
11		2		5460	5060	8760	17.52	7.53	0.5312084993	
12		3		6460	3700	9140	18.28	4.62	0.8658008658	
13	FOSL2 KO	1		5440	4540	8870	17.74	6.56	0.6097560976	
14		2		6260	3900	7060	14.12	6.16	0.6493506494	
15		3	I did not make enough transposition mix and I had to remake it specifically for this sample	7520	5440	8620	17.24	7.67	0.5215123859	
16	Cas9 / Positive ORF Control	1		4600	3440	6150	12.3	4.13	0.9685230024	
17		2		3580	3100	4450	8.9	3.03	1.3201320132	
18		3		4860	3140	4800	9.6	3.37	1.1869436202	
19	FOSL1 KO-1 / FOSL1 OE	1		9600	4740	6860	13.72	5.64	0.7092198582	
20		2		8180	8180	10300	20.6	9.26	0.4319654428	
21		3		9040	4100	7290	14.58	5.48	0.7299270073	
22	Positive ORF Control	1		13000	6060	7280	14.56	6.03	0.6633499171	
23		2		13340	7520	12500	25	10.83	0.3693444137	
24		3		8420	5600	10300	20.6	5.90	0.6779661017	
25	FOS OE	1		6800	5660	6390	12.78	6.30	0.6349206349	
26		2		9660	6200	9160	18.32	7.05	0.5673758865	
27		3		6800	5700	8440	16.88	5.62	0.7117437722	
28	FOSL2 OE	1		6220	5180	7700	15.4	4.45	0.8988764045	
29		2		6940	5300	8700	17.4	5.90	0.6779661017	
30		3		6460	5040	8880	17.76	5.27	0.7590132827	
31	JUN OE	1		6100	5340	6970	13.94	5.57	0.7181328546	
32		2		6080	6140	8840	17.68	5.73	0.6980802792	
33		3		4139	5260	9500	19	7.09	0.5641748942	
34	NTC KD-2 Control	1		5717	4400	6150	12.3	3.83	1.044386423	
35		2		7670	4420	6430	12.86	4.33	0.9237875289	
36		3		6557	4460	6570	13.14	4.06	0.9852216749	
37	JUN KD	1		6447	5200	7720	15.44	5.66	0.7067137809	
38		2		4685	3120	3860	7.72	3.64	1.0989010989	
39		3		6096	4260	6170	12.34	4.27	0.9367681499	
40	JUNB KD-1	1		6625	5500	7480	14.96	5.62	0.7117437722	
41		2		5555	4980	6050	12.1	5.60	0.7142857143	
42		3		4883	4460	6400	12.8	4.04	0.9900990099	
43	JUND KD	1		8394	7000	8780	17.56	8.51	0.4700352526	
44		2		6759	4820	5900	11.8	5.55	0.7207207207	
45		3		7458	5020	8940	17.88	5.75	0.6956521739	
46	Cas9 / NTC KD-2 Control	1		7393	4120	5640	11.28	4.90	0.8163265306	
47		2		7792	4240	6930	13.86	5.88	0.6802721088	
48		3		9020	6460	10600	21.2	8.86	0.4514672686	
49	FOSL2 KO / JUN KD	1		8522	6920	7890	15.78	9.55	0.4188481675	
50		2		9923	7100	11800	23.6	7.67	0.5215123859	
51		3		12343	8320	13500	27	10.83	0.3693444137	
52	FOS KO-1 / JUNB KD-1	1		8667	4940	7650	15.3	3.51	1.1396011396	
53		2		6479	4600	6300	12.6	5.40	0.7407407407	
54		3		9874	4780	5710	11.42	4.10	0.9756097561	
55	FOS KO-1 / JUND KD	1		7108	4520	7920	15.84	5.32	0.7518796992	
56		2		8485	3740	5400	10.8	2.52	1.5873015873	
57		3		6636	4740	7940	15.88	5.99	0.6677796327	
58	JUN KO / JUND KD	1		4715	3380	5940	11.88	3.51	1.1396011396	
59		2		5787	4280	8250	16.5	4.30	0.9302325581	
60		3		6540	6380	15700	31.4	9.35	0.4278074866	

FRIDAY, 7/12/2024

TapeStation

ATACseq run D5000 High sensitivity tapestation with two fold dilution.

TapeStation Sample Layout (numbers correspond to the sample numbers in the table above)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57				
B	2	10	18	26	34	42	50	58				
C	3	11	19	27	35	43	51	59				
D	4	12	20	28	36	44	52	60				
E	5	13	21	29	37	45	53					
F	6	14	22	30	38	46	54					
G	7	15	23	31	39	47	55					
H	8	16	24	32	40	48	56					

Results:

2024-07-12 - 13.50.38_scale_to_sample.pdf

2024-07-12 - 13.50.38.pdf

qPCR

Plan and results are in this excel file:

20240712_ATACseq_qPCR.xlsx

MONDAY, 9/2/2024

miSeq Results

Analysis:

- FASTQC (version 0.11.5) was used to determine the quality of sequencing and to check for presence of adapters. The quality scores across bases are good and there is some adapter content matching Nextera Transposase Sequence towards the ends of the reads.
- Adapter trimming was performed using Trim Galore (version 0.6.4) using the following arguments: --quality 20 --nextera --length 20 --paired --fastqc_args "--outdir $FASTQC_OUTDIR" --output_dir $OUTDIR/ $FILE_FOR $FILE_REV. The FASTQC files indicated that the trimming successfully removed adapters.
- Bowtie2 (version 2.5.1) was used for alignement. The reference genome index was build using GRCh38 primary assembly genome pasta and annotation from gencode release version 46. The following arguments were used for read alignment: --end-to-end --very-sensitive --no-mixed --phred33 -X 1000. I set the X argument to 1000, because I am interested in plotting fragment length distribution for each sample and if there are a lot of long reads I want to capture them.
- The sam files were sorted by coordinate using Samtools (version 1.17) sort function.
- The percentage of reads that are PCR duplicates was determined using Picard (version 2.27.5).
- To extract the fragment lengths for each sample, I used Samtools view function: "samtools view -@ 4 -F 0x04 $FILE | awk -F'\t' 'function abs(x){return ((x<0.0) ? -x : x)} {print abs($9)}' | sort | uniq -c | awk -v OFS="\t" '{print $2, $1/2}' > $OUTPUT_PATH/${SAMPLE_ID}_fragmentLen.txt". To plot the fragment length distribution, I imported the txt files to R and made fragment length vs read count line plots using ggplot2.
- To calculate the % of mitochondrial reads I used ATACseqQC R package.
- The heat maps over transcription start site were plotted using deep tools. First, I generated normalized BigWig files for each sample using the following arguments: bamCoverage -b $FILE -p 4 --normalizeUsing CPM --ignoreForNormalization chrM -o $OUTPUT_PATH/$SAMPLE_ID.bw. Next, I created a matrix file using the following function: computeMatrix scale-regions -S $OUTPUT_PATH/$SAMPLE_ID.bw -R $PROJECT_PATH/Hg38_gencode_v46_gtf/gencode.v46.primary_assembly.annotation.gtf --beforeRegionStartLength 3000 --regionBodyLength 5000 --afterRegionStartLength 3000 --skipZeros -o $SAMPLE_ID.matrix_gene.mat.gz -p 4. The heat map was plotted using the following function: plotHeatmap -m ${SAMPLE_ID}.matrix_gene.mat.gz -out ${SAMPLE_ID}.eps --sortUsing sum.

% reads identified

The percent reads identified is not equal across samples. It ranges from 0.5 to 2.2 %. I will repeat pooling of the libraries to attempt to get a more equal coverage of the libraries.

Read alignment (Bowtie2)

Percentage of reads aligned concordantly exactly 1 time ranges from 77.5 to 84.6%. The majority of the remaining reads were aligned concordantly > 1 times.

PCR duplicates (Picard)

The percentage of duplicates is very low across all of the samples. I used a very low number of PCR cycles.

Percentage of reads that map to mitochondrial DNA (ATACseqQC R package)

The percentage of reads that map to mitochondrial DNA ranges from 1.3 to 7.05%, which I think is considered low.

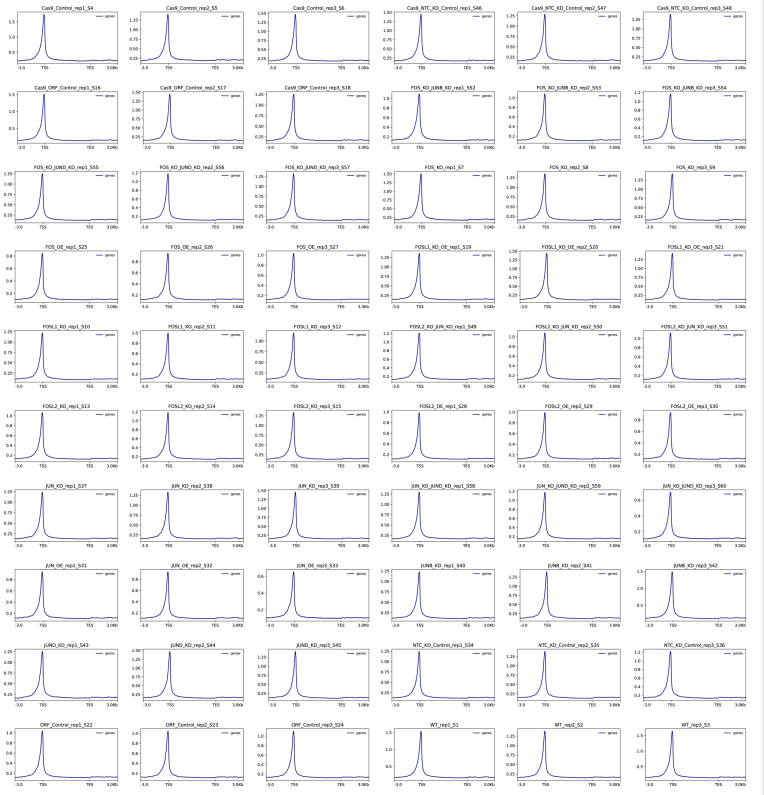
Fragment length distribution

The samples have a distinct nucleosome-free and mononucleosome peaks. Approximately 40% of reads have lengths that are less than 150 nt long, which corresponds to nucleosome-free region.

Fragment_length.png

Chromatin accessibility around transcription start site (TSS)

all_samples_profile_across_TSS.png



miSeq statistics

	Library name	% reads identified (PF)	Bowtie2: number of read pairs examined	Bowtie2: Aligned concordantly exactly 1 time (%)	Picard: Number of reads examined	Picard: Percent duplicates (%)	AtacQC: Total number of read pairs	AtacQC: Mitochondria rate	AtacQC: Percent of reads that map to mitochondrial DNA (%)	Number of reads after removal of mitochondrial reads	% of total reads
1	WT_rep1	1.0027	137694	82.27	136307	0.19	136307	0.047	4.73	129865	1.06
2	WT_rep2	1.4087	193450	81.55	191759	0.23	191759	0.048	4.80	182561	1.49
3	WT_rep3	1.0783	148078	83.19	146709	0.16	146709	0.037	3.73	141244	1.16
4	Cas9_Control_rep1	0.5546	76146	81.26	75346	0.12	75346	0.054	5.40	71281	0.58
5	Cas9_Control_rep2	1.1037	151572	79.34	149999	0.34	149999	0.071	7.05	139423	1.14
6	Cas9_Control_rep3	1.2084	165924	79.88	164337	0.32	164337	0.065	6.55	153578	1.26
7	FOS_KO_rep1	0.7815	107299	82.04	106140	0.14	106140	0.043	4.32	101552	0.83
8	FOS_KO_rep2	1.3583	186496	81.46	184549	0.24	184549	0.045	4.48	176272	1.44
9	FOS_KO_rep3	1.8332	251639	81.27	248449	0.38	248449	0.052	5.15	235642	1.93
10	FOSL1_KO_rep1	1.5088	207114	83.29	204635	0.14	204635	0.013	1.34	201890	1.65
11	FOSL1_KO_rep2	1.3704	188147	81.71	185905	0.13	185905	0.015	1.46	183184	1.50
12	FOSL1_KO_rep3	1.8197	249836	82.49	247325	0.16	247325	0.017	1.71	243097	1.99
13	FOSL2_KO_rep1	1.8454	253393	80.60	250485	0.24	250485	0.036	3.63	241399	1.98
14	FOSL2_KO_rep2	1.3199	181186	80.31	179053	0.23	179053	0.042	4.16	171596	1.41
15	FOSL2_KO_rep3	1.1567	158773	81.39	156551	0.17	156551	0.032	3.19	151559	1.24
16	Cas9_ORF_Control_rep1	1.335	183294	82.65	181167	0.21	181167	0.038	3.79	174302	1.43
17	Cas9_ORF_Control_rep2	1.5932	218695	81.44	215498	0.26	215498	0.045	4.47	205870	1.69
18	Cas9_ORF_Control_rep3	1.5861	217717	79.69	214495	0.36	214495	0.058	5.77	202122	1.66
19	FOSL1_KO_OE_rep1	1.3772	189046	83.09	186591	0.22	186591	0.034	3.41	180234	1.48
20	FOSL1_KO_OE_rep2	1.1578	158951	84.62	156892	0.14	156892	0.017	1.70	154228	1.26
21	FOSL1_KO_OE_rep3	1.4981	205721	84.58	203583	0.16	203583	0.024	2.37	198757	1.63
22	ORF_Control_rep1	1.5981	219410	80.73	216622	0.19	216622	0.028	2.81	210531	1.72
23	ORF_Control_rep2	1.7347	238160	80.30	235696	0.21	235696	0.030	3.04	228526	1.87
24	ORF_Control_rep3	2.028	278423	80.10	275378	0.27	275378	0.037	3.70	265186	2.17
25	FOS_OE_rep1	1.9798	271722	80.27	268198	0.16	268198	0.020	2.02	262773	2.15
26	FOS_OE_rep2	1.6975	232986	80.07	230172	0.19	230172	0.029	2.95	223386	1.83
27	FOS_OE_rep3	1.8578	254980	80.27	251690	0.22	251690	0.031	3.08	243927	2.00
28	FOSL2_OE_rep1	2.2033	302431	79.72	298913	0.28	298913	0.037	3.69	287887	2.36
29	FOSL2_OE_rep2	1.8449	253311	78.97	250370	0.26	250370	0.040	4.01	240340	1.97
30	FOSL2_OE_rep3	1.8471	253531	79.36	250392	0.21	250392	0.035	3.45	241748	1.98
31	JUN_OE_rep1	1.6124	221323	80.40	218687	0.18	218687	0.029	2.86	212430	1.74
32	JUN_OE_rep2	2.1413	293909	80.49	290013	0.19	290013	0.025	2.48	282816	2.32
33	JUN_OE_rep3	1.7583	241382	78.93	238446	0.18	238446	0.023	2.28	233013	1.91
34	NTC_KD_Control_rep1	1.7877	245390	81.83	241799	0.21	241799	0.032	3.16	234156	1.92
35	NTC_KD_Control_rep2	1.7907	245799	81.38	242147	0.31	242147	0.042	4.24	231874	1.90
36	NTC_KD_Control_rep3	1.41	193626	81.78	191224	0.18	191224	0.036	3.58	184386	1.51
37	JUN_KD_rep1	1.5735	216052	81.37	213355	0.22	213355	0.037	3.65	205563	1.68
38	JUN_KD_rep2	1.6533	226928	79.93	223408	0.36	223408	0.053	5.34	211478	1.73
39	JUN_KD_rep3	1.6469	226088	77.54	214219	0.31	214219	0.051	5.06	203377	1.67
40	JUNB_KD_rep1	1.4606	200521	82.16	197595	0.18	197595	0.023	2.31	193037	1.58
41	JUNB_KD_rep2	1.6441	225671	82.43	222355	0.19	222355	0.028	2.79	216143	1.77
42	JUNB_KD_rep3	1.662	228168	83.04	224922	0.18	224922	0.025	2.47	219361	1.80
43	JUND_KD_rep1	1.3293	182428	79.46	179905	0.34	179905	0.059	5.92	169257	1.39
44	JUND_KD_rep2	1.1834	162435	81.39	160073	0.21	160073	0.042	4.16	153416	1.26
45	JUND_KD_rep3	1.8503	254068	82.22	250830	0.26	250830	0.034	3.39	242315	1.98
46	Cas9_NTC_KD_Control_rep1	1.4237	195456	80.10	193497	0.34	193497	0.061	6.06	181776	1.49
47	Cas9_NTC_KD_Control_rep2	1.4513	199267	79.68	197214	0.36	197214	0.056	5.58	186212	1.52
48	Cas9_NTC_KD_Control_rep3	1.4423	197991	81.21	195779	0.28	195779	0.043	4.28	187390	1.53
49	FOSL2_KO_JUN_KD_rep1	1.4159	194380	80.70	191650	0.18	191650	0.034	3.39	185155	1.52
50	FOSL2_KO_JUN_KD_rep2	1.573	215920	81.21	213276	0.17	213276	0.026	2.62	207687	1.70
51	FOSL2_KO_JUN_KD_rep3	1.4996	205858	81.00	203374	0.18	203374	0.025	2.54	198209	1.62
52	FOS_KO_JUNB_KD_rep1	0.9673	132806	83.27	131418	0.09	131418	0.023	2.28	128418	1.05
53	FOS_KO_JUNB_KD_rep2	1.7642	242239	81.63	238677	0.17	238677	0.022	2.19	233448	1.91
54	FOS_KO_JUNB_KD_rep3	1.7686	242795	83.07	240233	0.17	240233	0.023	2.26	234802	1.92
55	FOS_KO_JUND_KD_rep1	1.5166	208212	81.95	206031	0.19	206031	0.033	3.33	199160	1.63
56	FOS_KO_JUND_KD_rep2	2.1687	297730	82.17	294407	0.20	294407	0.028	2.76	286278	2.34
57	FOS_KO_JUND_KD_rep3	1.4432	198176	81.71	196220	0.26	196220	0.044	4.37	187654	1.54
58	JUN_KO_JUND_KD_rep1	1.7842	244949	79.35	242397	0.50	242397	0.064	6.38	226937	1.86
59	JUN_KO_JUND_KD_rep2	2.1485	294972	78.98	291644	0.48	291644	0.056	5.58	275359	2.25
60	JUN_KO_JUND_KD_rep3	1.9624	269464	78.06	266287	0.28	266287	0.035	3.45	257097	2.11