Lab_Notebook_2024

Callum Malcolm

Contents

January 2024

Tuesday 16-01-2024

WILDseq - Mouse Experiment 1: PCR 1 Samples 1-8 / PCR 2 Sample 5

Overview:

- PCR tests to see if PCR would work for WS-ME1 cDNA previously made
- Started with samples 1-8 so as not to waste sample/reagents if PCR 1 did not work

Sample List

Sample ID	Treatment/Sample	ng/uL	i7 index	i5 index
1	Cyclophosphamide	773.7	N701	S502
2	Cyclophosphamide	545.3	N702	S502
3	Cyclophosphamide	903.6	N703	S502
4	Cyclophosphamide	1056.1	N704	S502
5	Cyclophosphamide	959.1	N705	S502
6	Combination	730.1	N706	S502
7	Combination	602.3	N707	S502
8	Combination	449.6	N710	S502
9	Combination	1002.1	N701	S503
10	Combination	1929.2	N702	S503
11	Methotrexate	861.5	N703	S503
12	Methotrexate	1110.4	N704	S503
13	Methotrexate	1171.0	N705	S503
14	Methotrexate	1347.8	N706	S503
15	Methotrexate	891.4	N707	S503
16	Vehicle	374.5	N710	S503
17	Vehicle	911.2	N701	S505
18	Vehicle	829.8	N702	S505
19	Vehicle	600.1	N703	S505
20	Vehicle	750.3	N704	S505
21	Baseline	401.5	N705	S505
22	Baseline	443.3	N706	S505
23	Baseline	373.4	N707	S505
24	Baseline	444.5	N710	S505
25	Baseline	267.6	N701	S506
26	BLLW 14K	378.7	N702	S506
27	BLLW 2K	362.7	N703	S506
28	BLLW 1K	563.8	N704	S506
29	Methotrexate (outlier)	348.1	N705	S506
30	Combo	380.8	N706	S506
31	RBL2P 2K	173.3	N707	S506
32	RBL2P 7K	2708.0	N7010	S506
33	RBL2P 250K	1418.5	N701	S507
34	Mock_direct_1	-	N702	S507
35	Mock_direct_2	-	N703	S507
36	Mock_direct_3	-	N704	S507
37	Mock_culture_1	-	N705	S507
38	Mock_culture_2	-	N706	S507
39	Mock_culture_3	-	N707	S507

PCR 1: Samples 1-8

- Checked [cDNA] on NanoDrop of sample 8 to make sure samples were still intact
  - Sample 8 ng/μL = 1042.3
  - It is assumed all other cDNA is of similar quality

1. Made a master mix of PCR1 reagents

- Made enough for 31 samples
- Primer mix was made earlier

Component	Volume	Master Mix
10uM WS PCR1 Primer Mix	3 μL	93 μL
DNAse/RNAse H20	12 μL	372 μL
Kapa Hifi HotStart Ready Mix (2X)	25 μL	775 μL

2. Add following components to tubes

Component	Volume
PCR1 MasterMix	40 μL
cDNA	10 μL

3. Performed PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	**Step 2: 98C**	**20s**
Annealing	**Step 3: 60C**	**15s**
Extension	**Step 4: 72C**	**15s**
Final Extension	Step 5: 72C	1 min
Hold Step	Step 6: 12C	Hold

10-25 cycles of steps 2-4

Bead Clean Up

Overview

- Clean PCR reaction by removing leftover primers
- This prevents false reads, cleaner indexing in later steps, and higher quality samples

Materials

- For 40 samples

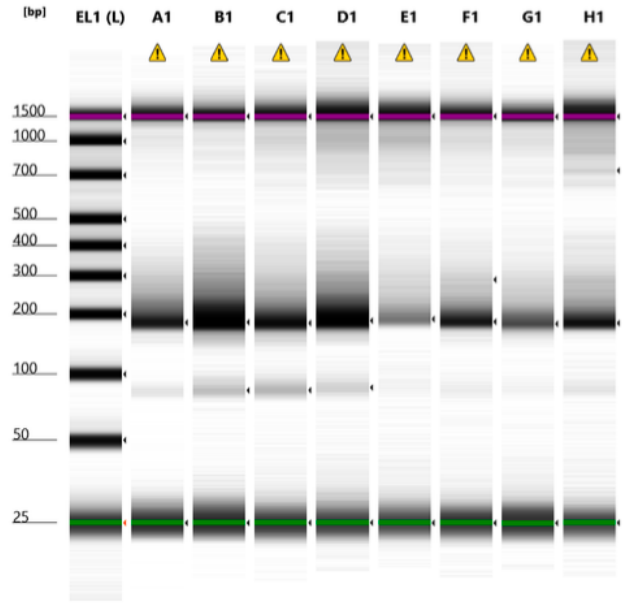
Component	Expected Volume/experiment	Material ID
Ampure Bead Kit	200 μL	

Protocol

1. Equilibrate Ampure beads to room temp (available from supplies cold room)
2. Mix well and vortex for 30sec to ensure uniform distribution.
3. Add 90ul (1.8X volume) to PCR reaction and mix thoroughly by pipetting.

4. Incubate 5min, room temp
5. Place on magnet for 2 min and keep on magnet until final elution
6. Remove supernatant and add 200ul 70% ethanol (make fresh)
7. Incubate 30sec and remove
8. Repeat 70% ethanol wash
9. Air dry beads 5-10min room temp. Once dry the beads will go from shiny to matt in appearance. Avoid over drying (cracked appearance).
10. Add 15 ul EB buffer and resuspend beads by pipetting
11. Incubate 2min room temp
12. Place on magnet and remove eluate
13. Assess PCR product size, contamination and concentration on tapestation.
    Expected size = 165-172bp

Tapestation PCR 1 - Samples 1-8



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Sample Info

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
EL1	2350	Electronic Ladder		Ladder
A1	310	1 PCR1	⚠	Caution! Expired ScreenTape device
B1	631	2 PCR1	⚠	Caution! Expired ScreenTape device
C1	333	3 PCR1	⚠	Caution! Expired ScreenTape device
D1	369	4 PCR1	⚠	Caution! Expired ScreenTape device
E1	90.0	5 PCR1	⚠	Caution! Expired ScreenTape device
F1	266	6 PCR1	⚠	Caution! Expired ScreenTape device
G1	181	7 PCR1	⚠	Caution! Expired ScreenTape device
H1	206	8 PCR1	⚠	Caution! Expired ScreenTape device

Initial Test PCR 1

PCR 2: Samples 5

Overview

- Yield from PCR1 is low (.09 - .631 ng/μL)
- Sample with lowest concentration was taken ahead to PCR 2 (E1 - Sample 5)
- PCR to index samples for sequencing by attaching UMI

Materials

Component	Expected Volume/experiment	Material ID
10uM Nxxx Nextera i7 adapter	-	-
10uM Sxxx Nextera i5 adapter	-	-
DNAse/RNAse H20	1000 μL	
Kapa Hifi HotStart Ready Mix (2X)	1200 μL	KK2601

Protocol

1. Prepared samples according to the following table:

- Sample - Adaptor pairs in table above

Component	Volume
10uM N705 Nextera i7 adapter	1.5 μL
10uM S502 Nextera i5 adapter	1.5 μL
DNAse/RNAse H20	21 μL
Kapa Hifi HotStart Ready Mix (2X)	25 μL
10ng/μL PCR1	1 μL

2. Performed PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	**Step 2: 98C**	**20s**
Annealing	**Step 3: 55C**	**15s**
Extension	**Step 4: 72C**	**15s**
Final Extension	Step 5: 72C	2 min
Hold Step	Step 6: 12C	Hold

8 cycles of steps 2-4

3. Performed Ampure bead clean up as above with 90ul beads per 50ul PCR reaction
4. Checked size and concentration on tapestation

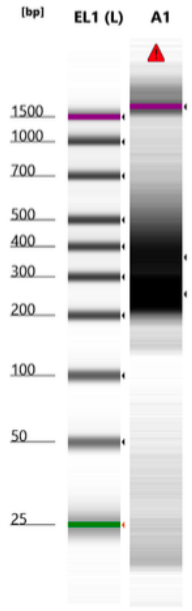
Tapestation PCR 2

Review

- Contamination/PCR bubble?
- Yield is adequate (assuming it is representative of the correct peak)
- Chris wants to repeat PCR1 for remaining samples, take PCR2 ahead, and re-run sames with D1000 tape (non High-Sensitivity)

Plan for tomorrow

- PCR 1 samples 9-30
- Bead Clean Up
- Tape Station



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Sample Info

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
EL1	2350	Electronic Ladder		Ladder
A1	3180	5 PCR2	▲	Marker(s) not detected; Caution! Expired ScreenTape device

Figure 1: 2024-01-16 Tapestation PCR 2 Test Samples 5

Wednesday 17-01-2024

WILDseq - Mouse Experiment 1: PCR 1 Samples 9-30

Overview:

- Completed PCR 1 for Samples 9-30

PCR 1: Samples 9-30

1. Used master mix of PCR1 reagents made 2024-01-16
2. Add following components to tubes |Component |Volume| |:————|—-| | PCR1 MasterMix | 40 μL | | cDNA | 10 μL |
3. Performed PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	**Step 2: 98C**	**20s**
Annealing	**Step 3: 60C**	**15s**
Extension	**Step 4: 72C**	**15s**
Final Extension	Step 5: 72C	1 min
Hold Step	Step 6: 12C	Hold

10-25 cycles of steps 2-4

Bead Clean Up

Overview

- Clean PCR reaction by removing leftover primers
- This prevents false reads, cleaner indexing in later steps, and higher quality samples

Materials

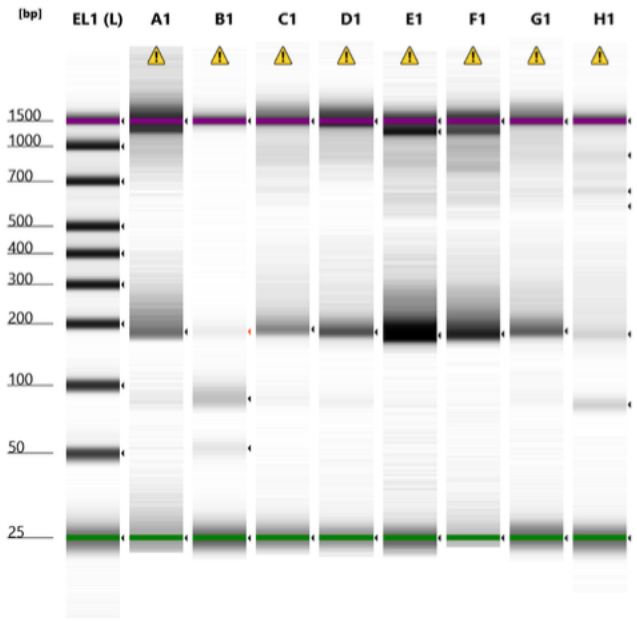
- For 40 samples

Component	Expected Volume/experiment	Material ID
Ampure Bead Kit	200 μL	

Protocol

1. Equilibrate Ampure beads to room temp (available from supplies cold room)
2. Mix well and vortex for 30sec to ensure uniform distribution.
3. Add 90ul (1.8X volume) to PCR reaction and mix thoroughly by pipetting.
4. Incubate 5min, room temp
5. Place on magnet for 2 min and keep on magnet until final elution
6. Remove supernatant and add 200ul 70% ethanol (make fresh)
7. Incubate 30sec and remove
8. Repeat 70% ethanol wash
9. Air dry beads 5-10min room temp. Once dry the beads will go from shiny to matt in appearance. Avoid over drying (cracked appearance).
10. Add 15 ul EB buffer and resuspend beads by pipetting
11. Incubate 2min room temp

12. Place on magnet and remove eluate
13. Assess PCR product size, contamination and concentration on tapestation.
    Expected size = 165-172bp



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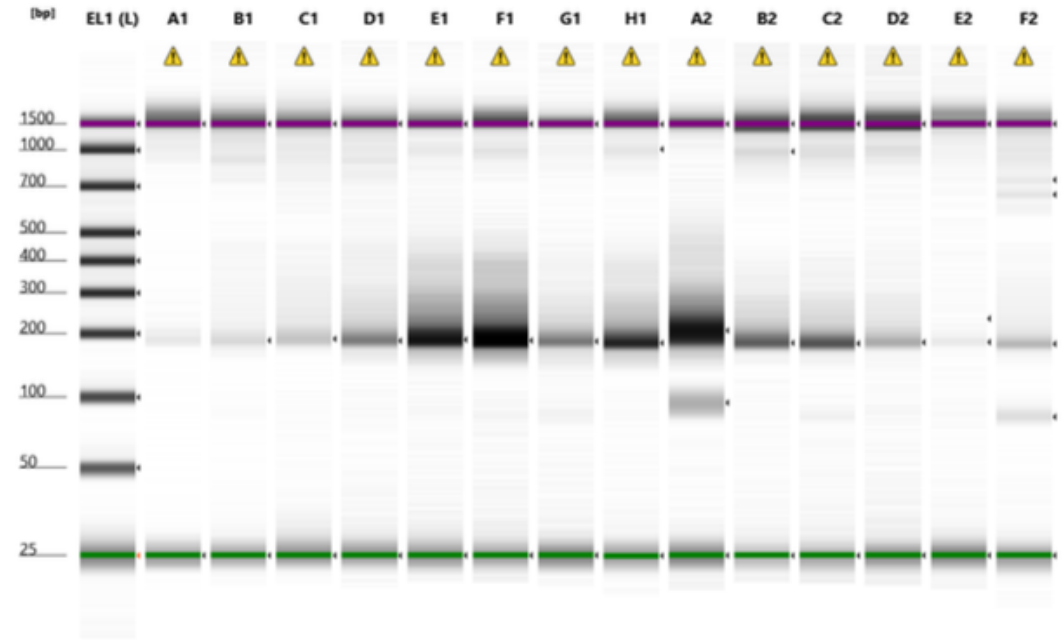
Sample Info

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
EL1	2350	Electronic Ladder		Ladder
A1	88.4	9 PCR 1	⚠	Caution! Expired ScreenTape device
B1	164	10 PCR 1	⚠	Caution! Expired ScreenTape device
C1	142	11 PCR 1	⚠	Caution! Expired ScreenTape device
D1	140	12 PCR 1	⚠	Caution! Expired ScreenTape device
E1	1070	13 PCR 1	⚠	Caution! Expired ScreenTape device
F1	354	14 PCR 1	⚠	Caution! Expired ScreenTape device
G1	203	15 PCR 1	⚠	Caution! Expired ScreenTape device
H1	203	16 PCR 1	⚠	Caution! Expired ScreenTape device

Figure 2: 2024-01-17 Tapestation PCR 1 Samples 9-16

Tapestation PCR 1

Tapestation PCR 1



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Sample Info

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
EL1	2350	Electronic Ladder		Ladder
A1		17 PCR 1	⚠	Caution! Expired ScreenTape device
B1	40.7	18 PCR 1	⚠	Caution! Expired ScreenTape device
C1	84.0	19 PCR 1	⚠	Caution! Expired ScreenTape device
D1	226	20 PCR 1	⚠	Caution! Expired ScreenTape device
E1	665	21 PCR 1	⚠	Caution! Expired ScreenTape device
F1	820	22 PCR 1	⚠	Caution! Expired ScreenTape device
G1	270	23 PCR 1	⚠	Caution! Expired ScreenTape device
H1	423	24 PCR 1	⚠	Caution! Expired ScreenTape device
A2	1290	25 PCR 1	⚠	Caution! Expired ScreenTape device
B2	223	26 PCR 1	⚠	Caution! Expired ScreenTape device
C2	170	27 PCR 1	⚠	Caution! Expired ScreenTape device
D2	71.5	28 PCR 1	⚠	Caution! Expired ScreenTape device
E2	30.5	29 PCR 1	⚠	Caution! Expired ScreenTape device
F2	157	30 PCR 1	⚠	Caution! Expired ScreenTape device

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Review

- Samples 10/17/18/29 don't have adequate yield
  - Will need to redo PCR 1
  - Sample 10 has acceptable PCR1 sample from previous run
- Yield is adequate for the rest to move ahead

Plan for tomorrow

- PCR 1 samples 17/18/29
  - Bead Clean Up
  - Tape Station
- PCR 2 for all samples
  - Double check reagents amounts
  - Running low on KAPA (re-ordered) and Nextera Primers

- Chris wants to add 2 additional samples
  - To Do - RNA Extraction / RT / PCR 1 / PCR 2
  - Wait until majority of samples have acceptable PCR 2 product

Thursday 18-01-2024

WILDseq - Mouse Experiment 1: PCR 1 Samples 17/18/29

Overview:

- Repeated PCR 1 for Samples 17/18/29

PCR 1: Samples 17/18/29

1. Used master mix of PCR1 reagents made 2024-01-16
2. Add following components to tubes |Component |Volume| |:————:|:—-:| | PCR1 MasterMix | 40 μL | | cDNA | 10 μL |
3. Performed PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	**Step 2: 98C**	**20s**
Annealing	**Step 3: 60C**	**15s**
Extension	**Step 4: 72C**	**15s**
Final Extension	Step 5: 72C	1 min
Hold Step	Step 6: 12C	Hold

10-25 cycles of steps 2-4

Bead Clean Up

Overview

- Clean PCR reaction by removing leftover primers
- This prevents false reads, cleaner indexing in later steps, and higher quality samples

Materials

- For 40 samples

Component	Expected Volume/experiment	Material ID
Ampure Bead Kit	200 μL	

Protocol

1. Equilibrate Ampure beads to room temp (available from supplies cold room)
2. Mix well and vortex for 30sec to ensure uniform distribution.
3. Add 90ul (1.8X volume) to PCR reaction and mix thoroughly by pipetting.
4. Incubate 5min, room temp
5. Place on magnet for 2 min and keep on magnet until final elution
6. Remove supernatant and add 200ul 70% ethanol (make fresh)
7. Incubate 30sec and remove
8. Repeat 70% ethanol wash

9. Air dry beads 5-10min room temp. Once dry the beads will go from shiny to matt in appearance. Avoid over drying (cracked appearance).
10. Add 15 ul EB buffer and resuspend beads by pipetting
11. Incubate 2min room temp
12. Place on magnet and remove eluate
13. Assess PCR product size, contamination and concentration on tapestation.
    Expected size = 165-172bp

Tapestation PCR 1

Review

- Samples 10/17/18/29 don't have adequate yield
  - Will need to redo PCR 1
  - Sample 10 has acceptable PCR1 sample from previous run
- Yield is adequate for the rest to move ahead

Plan for tomorrow

- PCR 1 samples 17/18/29
  - Bead Clean Up
  - Tape Station
- PCR 2 for all samples
  - Double check reagents amounts
  - Running low on KAPA (re-ordered) and Nextera Primers
- Chris wants to add 2 additional samples
  - To Do - RNA Extraction / RT / PCR 1 / PCR 2
  - Wait until majority of samples have acceptable PCR 2 product

Friday 19-01-2024

WILDseq - Mouse Experiment 1: RNA Extraction/RT Samples 31-33

Overview: Extra samples given by Chris for whitelist

RNA extraction Samples 31-33

- Performed RNA extraction of tissue/cell samples according to the following kit:

RT Protocol Samples 31-33

1. In PCR strip tubes, prepared 5 μg of RNA in a total volume of 10 μl of RNAse/DNAase-free water.
2. Add 1 μl of WS_RT_UMI_NexteraR2 primer (2μM stock)
3. Add 1 μl dNTPs (10 mM each).
4. Denature at 65 C for 5 mins in the PCR machine, then straight onto ice for at least 2 mins, spin briefly to get liquid to the bottom of the tube.
5. Used Master Mix previously made
6. Add 7 μl of RT MM prepared above to each sample and mix, spin briefly to get liquid to the bottom of the tube.
   - Spin briefly to get liquid to bottom of the tube
7. In the PCR machine incubate at 53 C for 10 mins followed by 80 C for 10 mins.
8. Add 1 μl Themolabile Exonuclease I (NEB M0568) to remove excess RT primer.

Component	Volume	MM volume
Thermolabile Exonuclease I	1 μL	
NEBuffer r3.1*	2 μL	

Most PCR buffers are compatible

9. In PCR Machine: heat at 37C for 4 mins followed by 80C for 1 min
    - Spin briefly to get liquid to bottom of the tube
10. Add 1 μl of RNAse H and incubate at 37 C for 20 mins.
11. Checked [cDNA] on NanoDrop

- Added to Sample List

Monday 22-01-2024

PCR 1: Samples 31-33

- Included negative control (MM + H20)

1. Used master mix of PCR1 reagents made 2024-01-16
2. Add following components to tubes

Component	Volume
PCR1 MasterMix	40 μL
cDNA	10 μL

3. Performed PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	**Step 2: 98C**	**20s**
Annealing	**Step 3: 60C**	**15s**
Extension	**Step 4: 72C**	**15s**
Final Extension	Step 5: 72C	1 min
Hold Step	Step 6: 12C	Hold

10-25 cycles of steps 2-4

Bead Clean Up

Overview

- Clean PCR reaction by removing leftover primers
- This prevents false reads, cleaner indexing in later steps, and higher quality samples

Materials

- For 40 samples

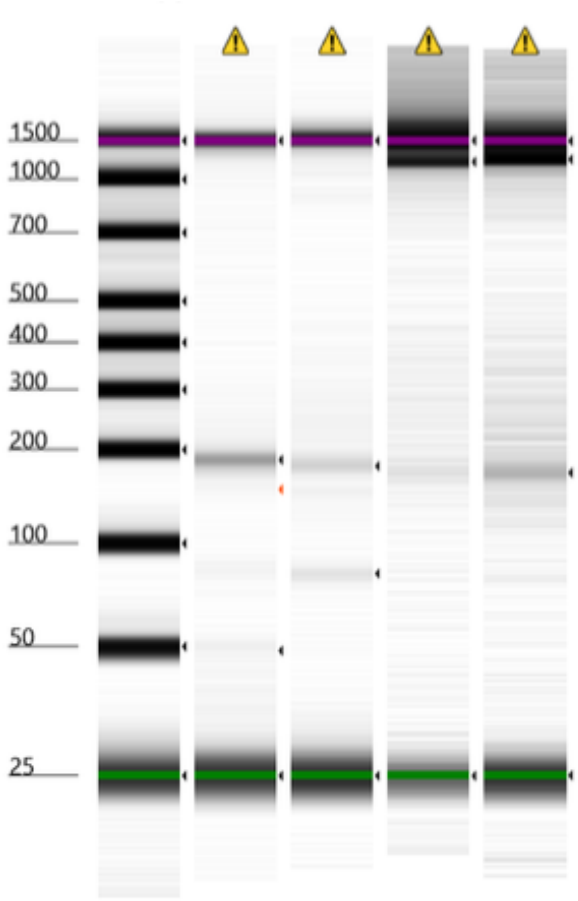
Component	Expected Volume/experiment	Material ID
Ampure Bead Kit	200 μL	

Protocol

1. Equilibrate Ampure beads to room temp (available from supplies cold room)
2. Mix well and vortex for 30sec to ensure uniform distribution.
3. Add 90ul (1.8X volume) to PCR reaction and mix thoroughly by pipetting.
4. Incubate 5min, room temp
5. Place on magnet for 2 min and keep on magnet until final elution
6. Remove supernatant and add 200ul 70% ethanol (make fresh)
7. Incubate 30sec and remove
8. Repeat 70% ethanol wash
9. Air dry beads 5-10min room temp. Once dry the beads will go from shiny to matt in appearance. Avoid over drying (cracked appearance).
10. Add 15 ul EB buffer and resuspend beads by pipetting
11. Incubate 2min room temp
12. Place on magnet and remove eluate
13. Assess PCR product size, contamination and concentration on tapestation.
    Expected size = 165-172bp

Tapestation PCR 1 31-33

Tapestation PCR 1



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Sample Info

Well	Conc. [pg/µl]	Sample Description
EL1	2350	Electronic Ladder
A1	89.4	-RT Control
B1	46.1	31 PCR 1
C1	87.3	32 PCR 1
D1	195	33 PCR 1

!!!! Band present in - Control lane !!!!

PCR 1: Negative Control

- Remade negative control (MM + H20)

1. Used master mix of PCR1 reagents made 2024-01-16
2. Add following components to tubes

Component	Volume
PCR1 MasterMix	40 μL
cDNA	10 μL

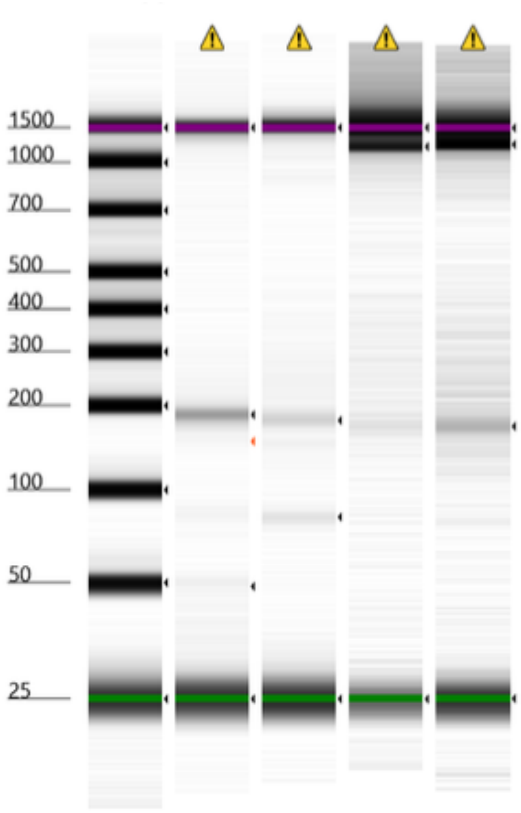
3. Performed PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	**Step 2: 98C**	**20s**
Annealing	**Step 3: 60C**	**15s**
Extension	**Step 4: 72C**	**15s**
Final Extension	Step 5: 72C	1 min
Hold Step	Step 6: 12C	Hold

10-25 cycles of steps 2-4

Tapestation Control Test 1

- Compared New -Control to old
  - Wanted to determine if this was a pipetting error



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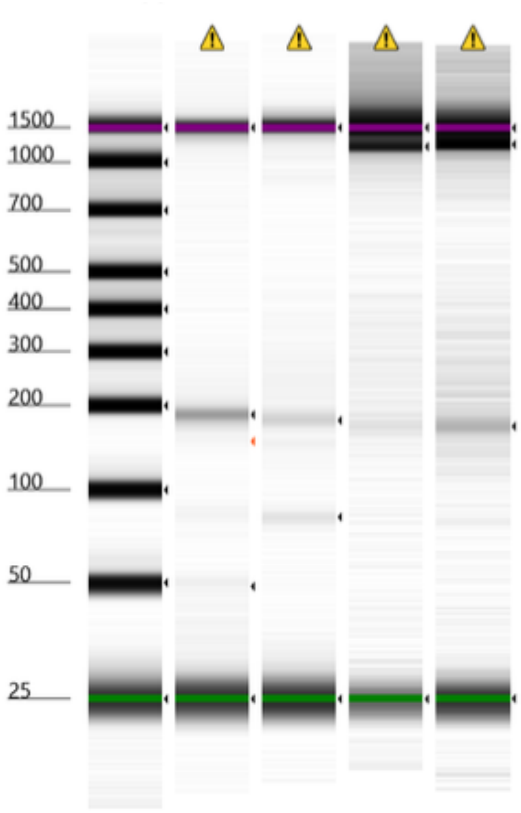
Sample Info

Well	Conc. [pg/µl]	Sample Description
EL1	2350	Electronic Ladder
A1	89.4	-RT Control
B1	46.1	31 PCR 1
C1	87.3	32 PCR 1
D1	195	33 PCR 1

Tapestation Control Test 2

- Compared New -Control to H20
  - Wanted to determine if water was contaminated



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Sample Info

Well	Conc. [pg/µl]	Sample Description
EL1	2350	Electronic Ladder
A1	89.4	-RT Control
B1	46.1	31 PCR 1
C1	87.3	32 PCR 1
D1	195	33 PCR 1

Tuesday 23-01-2024

Made New Media: AR-5

1. Made new media: AR-5

Solution	ID code	Volume	% Total volume
Advanced RMPI	—	500 mL	78%
Glutamax	—	6.5 mL	20%
Pen-Strep	—	6.5 mL	1%
FBS	—	128 mL	1%

Cell Culture

A20

- Thawed A20 and seeded in T25
- Spun down CS to remove DMSO, cell pellet small
- Cells didn't look super healthy
- Monitor tomorrow

HEK

- Got T75 flask from Chris
- 70% confluent
- Added 10mL of trypsin and tapped flask until cells were dislodged
- Added 10mL of DMEM (20% FBS) and added to 50mL tube
- Added 25mL of DMEM to 5 T75 flasks
- Mixed CS to get single cell suspension and remvoe clumps
- Transferred 4mL of CS to each flask
- Will grow up for 1-2 days and freeze down 4 T75 flasks
  - Other flask will be used to gorw WILDseq library virus for A20's

WS-ME1 Library Prep: Control Test 3

Overview: question around contaminated primers - Ran PCR1 with new primer/PCR 1 mix

PCR 1: Negative Control

- Remade negative control (MM + H20)
- Remade Primer mix and PCR1 MM

PCR1 Primer Mix

- WILDseq protocol from Kirsty dictates 8 fwd primers mixed in equal amounts with equivilant amount of rev priemr
- Primers were reconsituted at 100 μM so needed to be diluted to 10 μ

Component	Volume
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	16 μL
DNAse/RNAse H20	320 μL

PCR1 MM

Component	Volume	Master Mix
10uM WS PCR1 Primer Mix	3 μL	
DNAse/RNAse H20	12 μL	
Kapa Hifi HotStart Ready Mix (2X)	25 μL	

1. Add following components to tubes

Component	Volume
PCR1 MasterMix	40 μL
H2O	10 μL

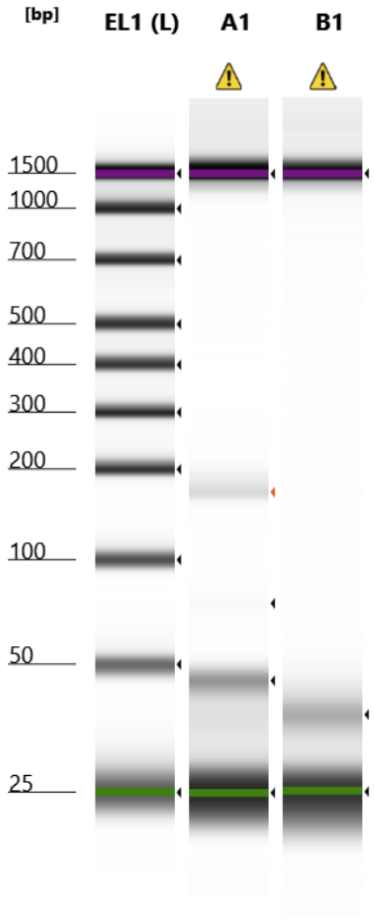
2. Performed PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	**Step 2: 98C**	**20s**
Annealing	**Step 3: 60C**	**15s**
Extension	**Step 4: 72C**	**15s**
Final Extension	Step 5: 72C	1 min
Hold Step	Step 6: 12C	Hold

10-25 cycles of steps 2-4

Tapestation Control Test 3

- Compared New -Control to old -control
  - Wanted to determine if water was contaminated



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Sample Info

Well	Conc. [ng/µl]	Sample Description
EL1	20.3	Electronic Ladder
A1	2.13	Control 1
B1	2.01	Control 2

Wednesday 24-01-2024

Cell Culture

A20

- Checked cells ~10% confluent
- Cells looked healthy but sparse
- Grow up for inection
- Ask Chris about cell numbers needed for injecting into 12 mice

HEK

- 20% confluent
- Check tomorrow and split or Friday

WILDseq - Mouse Experiment 1: Library Prep Attempt 5 - RT

- Decided to repeat the entire library prep process using RNA extracted previously
- Everything done in PCR room RNA hood

RT Protocol

1. In PCR strip tubes, prepared 5 μg of RNA in a total volume of 10 μl of RNAse/DNAase-free water.
2. Add 1 μl of WS_RT_UMI_NexteraR2 primer (2μM)

- Diluted primer from stock (100μM)
- Added 1 μL RT-Primer stock into 49μL water

3. Add 1 μl dNTPs (10 mM each).
4. Denature at 65 C for 5 mins in the PCR machine, then straight onto ice for at least 2 mins, spin briefly to get liquid to the bottom of the tube.
5. Prepare mastermix of RT enzyme and buffers. 1.1x the number of samples.

Component	Volume	MM volume
5x SSIV Buffer	4 μL	134 μL
SSIV RT	1 μL	33.5 μL
100 mM DTT	1 μL	33.5 μL
RNAse Out	1 μL	33.5 μL

6. Add 7 μl of RT MM prepared above to each sample and mix, spin briefly to get liquid to the bottom of the tube.
   - Spin briefly to get liquid to bottom of the tube
7. In the PCR machine incubate at 53 C for 10 mins followed by 80 C for 10 mins.
8. Add 1 μl Themolabile Exonuclease I (NEB M0568) to remove excess RT primer.

Component	Volume	MM volume
Thermolabile Exonuclease I	1 μL	33.5 μL
NEBuffer r3.1*	2 μL	67 μL

Most PCR buffers are compatible

9. In PCR Machine: heat at 37C for 4 mins followed by 80C for 1 min
   - Spin briefly to get liquid to bottom of the tube
10. Add 1 μl of RNAse H and incubate at 37 C for 20 mins.
    - Stored at -4C

Plan for tomorrow

- PCR 1 (check for KAPA HIFI amount)
  - Bead Clean Up
  - Tape Station
- Check on cells
- Plan cell numbers needed for A20 mouse injections

Thursday 25-01-2024

Cell Culture

A20

- Checked cells ~50% confluent
- Cells looked healthy but sparse
- Added 10ml of AR-5
- Grow up for injection
- Ask Chris about cell numbers needed for injecting into 12 mice

HEK

- Collected all T175 and froze down
- Left one flask going at 20% confluency

WS-ME1 Library Prep - Attempt 5 PCR 1

Ran PCR1 for attempt 5

PCR 1: Negative Control

- Remade negative control (MM + H20)
- Remade Primer mix and PCR1 MM

PCR1 Primer Mix

- WILDseq protocol from Kirsty dictates 8 fwd primers mixed in equal amounts with equivalent amount of rev primer
- Primers were reconsituted at 100 μM so needed to be diluted to 10 μM

Component	Volume
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	2 μL
100uM WS PCR1 Primer Mix	16 μL
DNAse/RNAse H20	144 μL

PCR1 MM

Component	Volume	Master Mix
10uM WS PCR1 Primer Mix	3 μL	
DNAse/RNAse H20	12 μL	
Q5 Master Mix	25 μL	

- Used Q5 instead of KAPA

1. Add following components to tubes

Component	Volume
PCR1 MasterMix	40 μL
H2O	10 μL

2. Performed PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	**Step 2: 98C**	**20s**
Annealing	**Step 3: 60C**	**15s**
Extension	**Step 4: 72C**	**15s**
Final Extension	Step 5: 72C	1 min
Hold Step	Step 6: 12C	Hold

10-25 cycles of steps 2-4

Bead Clean Up

Overview

- Clean PCR reaction by removing leftover primers
- This prevents false reads, cleaner indexing in later steps, and higher quality samples

Materials

- For 40 samples

Component	Expected Volume/experiment	Material ID
Ampure Bead Kit	200 μL	

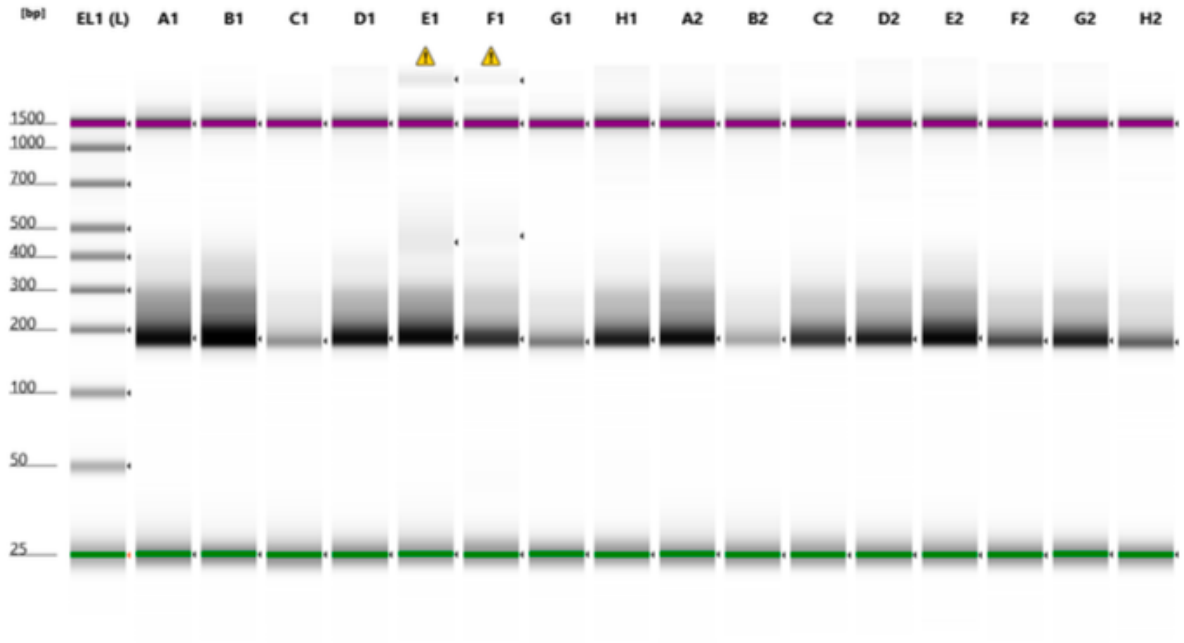
Protocol

1. Equilibrate Ampure beads to room temp (available from supplies cold room)
2. Mix well and vortex for 30sec to ensure uniform distribution.
3. Add 90ul (1.8X volume) to PCR reaction and mix thoroughly by pipetting.
4. Incubate 5min, room temp
5. Place on magnet for 2 min and keep on magnet until final elution
6. Remove supernatant and add 200ul 70% ethanol (make fresh)
7. Incubate 30sec and remove
8. Repeat 70% ethanol wash
9. Air dry beads 5-10min room temp. Once dry the beads will go from shiny to matt in appearance. Avoid over drying (cracked appearance).
10. Add 15 ul EB buffer and resuspend beads by pipetting
11. Incubate 2min room temp
12. Place on magnet and remove eluate
13. Assess PCR product size, contamination and concentration on tapestation.
    Expected size = 165-172bp

Tapestation PCR 1 Samples 1-33

Tapestation PCR 1 1-16

Tapestation PCR 1



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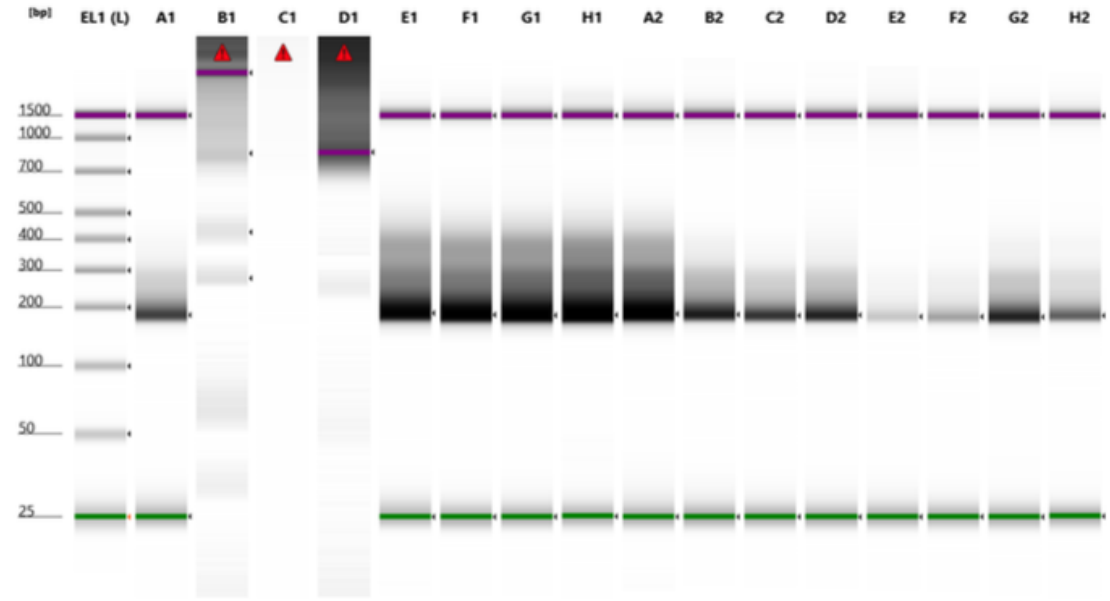
Sample Info

Well	Conc. [ng/µl]	Sample Description	Alert	Observations
EL1	20.3	Electronic Ladder		Ladder
A1	18.8	1 PCR 1		
B1	28.9	2 PCR 1		
C1	4.49	3 PCR 1		
D1	17.5	4 PCR 1		
E1	19.4	5 PCR 1	⚠	Peak out of Sizing Range
F1	12.7	6 PCR 1	⚠	Peak out of Sizing Range
G1	5.42	7 PCR 1		
H1	13.6	8 PCR 1		
A2	15.8	9 PCR 1		
B2	3.52	10 PCR 1		
C2	12.2	11 PCR 1		
D2	13.0	12 PCR 1		
E2	18.8	13 PCR 1		
F2	8.74	14 PCR 1		
G2	13.2	15 PCR 1		
H2	6.68	16 PCR 1		

Figure 3: 2024-01-25 Tapestation PCR 1 Samples 1-16

Tapestation PCR 1 1-32



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Sample Info

Well	Conc. [ng/µl]	Sample Description	Alert	Observations
EL1	20.3	Electronic Ladder		Ladder
A1	15.5	17 PCR 1		
B1	2.76	18 PCR 1	▲	Marker(s) not detected
C1		19 PCR 1	▲	Marker(s) not detected
D1		20 PCR 1	▲	Marker(s) not detected
E1	39.1	21 PCR 1		
F1	43.3	22 PCR 1		
G1	46.2	23 PCR 1		
H1	49.7	24 PCR 1		
A2	47.3	25 PCR 1		
B2	20.2	26 PCR 1		
C2	15.7	27 PCR 1		
D2	18.5	28 PCR 1		
E2	2.85	29 PCR 1		
F2	5.29	30 PCR 1		
G2	20.4	31 PCR 1		
H2	10.8	32 PCR 1		

Figure 4: 2024-01-25 Tapestation PCR 1 Samples 17-32

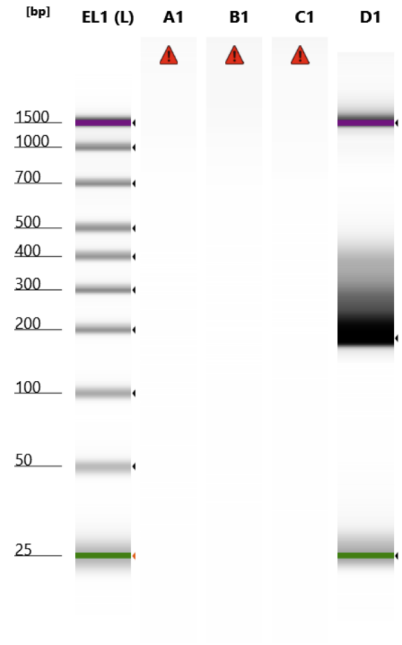
Tapestation PCR 1

- Samples 18, 19, and 20 DNW

Tapestation PCR 1 18-20, 33

Tapestation PCR 1

- Samples 18, 19, and 20 DNW again



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Sample Info

Well	Conc. [ng/µl]	Sample Description	Alert	Observations
EL1	20.3	Electronic Ladder		Ladder
A1		17 PCR 1 v2	⚠	Marker(s) not detected
B1		18 PCR 1 v2	⚠	Marker(s) not detected
C1		20 PCR 1 v2	⚠	Marker(s) not detected
D1	36.1	33 PCR 1		

Figure 5: 2024-01-25 Tapestation PCR 1 Samples 33 and redo 18-20

Friday 26-01-2024

Cell Culture

A20

- Expanded to T175
- Grow up for injection
- Ask Chris about cell numbers needed for injecting into 12 mice

HEK

- Split 1/2
- DMEM

WS-ME1 Library Prep - Attempt 5 PCR 1 repeat and PCR 2 prep

- Reran PCR1 for samples 18,19,20
- Prepped PCR2 primers

PCR2 Prep

- Reconstituted PCR2 primers bought by Chris
  - Used DNAse/RNAse Free H20 and diluted to 100 μM
- Diluted into strip tubes working stock (10μM)
  - 90 μL H20 + 10 μL primer stock

PCR1 Samples 18-20

PCR1 Protocol

- Used Primer Mix previously made on 25-01-2024

Component	Volume	Master Mix
10uM WS PCR1 Primer Mix	3 μL	
DNAse/RNAse H20	12 μL	
Q5 Master Mix	25 μL	

- Used Q5 instead of KAPA

1. Add following components to tubes

Component	Volume
PCR1 MasterMix	40 μL
H2O	10 μL

2. Performed PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	**Step 2: 98C**	**20s**
Annealing	**Step 3: 60C**	**15s**
Extension	**Step 4: 72C**	**15s**
Final Extension	Step 5: 72C	1 min

Step Name	Steps	Time
Hold Step	Step 6: 12C	Hold

10-25 cycles of steps 2-4

Bead Clean Up

Overview

- Clean PCR reaction by removing leftover primers
- This prevents false reads, cleaner indexing in later steps, and higher quality samples

Materials

- For 40 samples

Component	Expected Volume/experiment	Material ID
Ampure Bead Kit	200 μL	

Protocol

1. Equilibrate Ampure beads to room temp (available from supplies cold room)
2. Mix well and vortex for 30sec to ensure uniform distribution.
3. Add 90ul (1.8X volume) to PCR reaction and mix thoroughly by pipetting.
4. Incubate 5min, room temp
5. Place on magnet for 2 min and keep on magnet until final elution
6. Remove supernatant and add 200ul 70% ethanol (make fresh)
7. Incubate 30sec and remove
8. Repeat 70% ethanol wash
9. Air dry beads 5-10min room temp. Once dry the beads will go from shiny to matt in appearance. Avoid over drying (cracked appearance).
10. Add 15 ul EB buffer and resuspend beads by pipetting
11. Incubate 2min room temp
12. Place on magnet and remove eluate
13. Assess PCR product size, contamination and concentration on tapestation.
    Expected size = 165-172bp

Tapestation PCR 1 Samples 18-20

Tapestation PCR 1 18-20

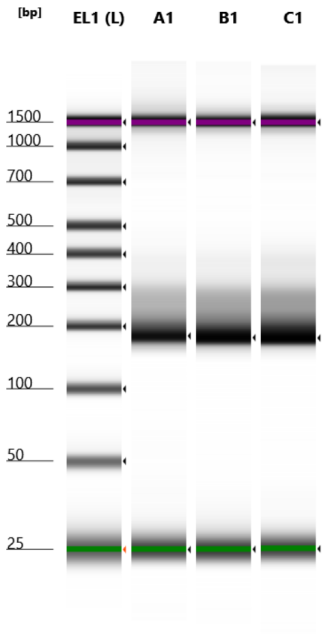
Tapestation PCR 1

Monday 29-01-2024

Cell Culture

A20

- Split 1:6
- AR-5
- Ask Chris about cell numbers needed for injecting into 12 mice



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Sample Info

Well	Conc. [ng/µl]	Sample Description	Alert	Observations
EL1	20.3	Electronic Ladder		Ladder
A1	8.30	18 PCR 1		
B1	9.76	19 PCR 1		
C1	10.3	20 PCR 1		

Figure 6: 2024-01-26 Tapestation PCR 1 Samples 18-20

HEK

- Split 1/6
- DMEM

PCR Step 2 - Samples 1-24

Overview

- PCR to index samples for sequencing by attaching UMI
- Didn't have enough Q5 to complete all the samples

PCR2 Protocol

1. Prepare samples according to the following table:

- Keep careful note of which adaptors are added to which sample

Component	Volume
10uM Nxxx Nextera i7 adapter	1.5 μL
10uM Sxxx Nextera i5 adapter	1.5 μL
DNAse/RNAse H20	21 μL
Q5	25 μL
10ng/μL PCR1	1 μL

2. Perform PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	**Step 2: 98C**	**20s**
Annealing	**Step 3: 55C**	**15s**
Extension	**Step 4: 72C**	**15s**
Final Extension	Step 5: 72C	2 min
Hold Step	Step 6: 12C	Hold

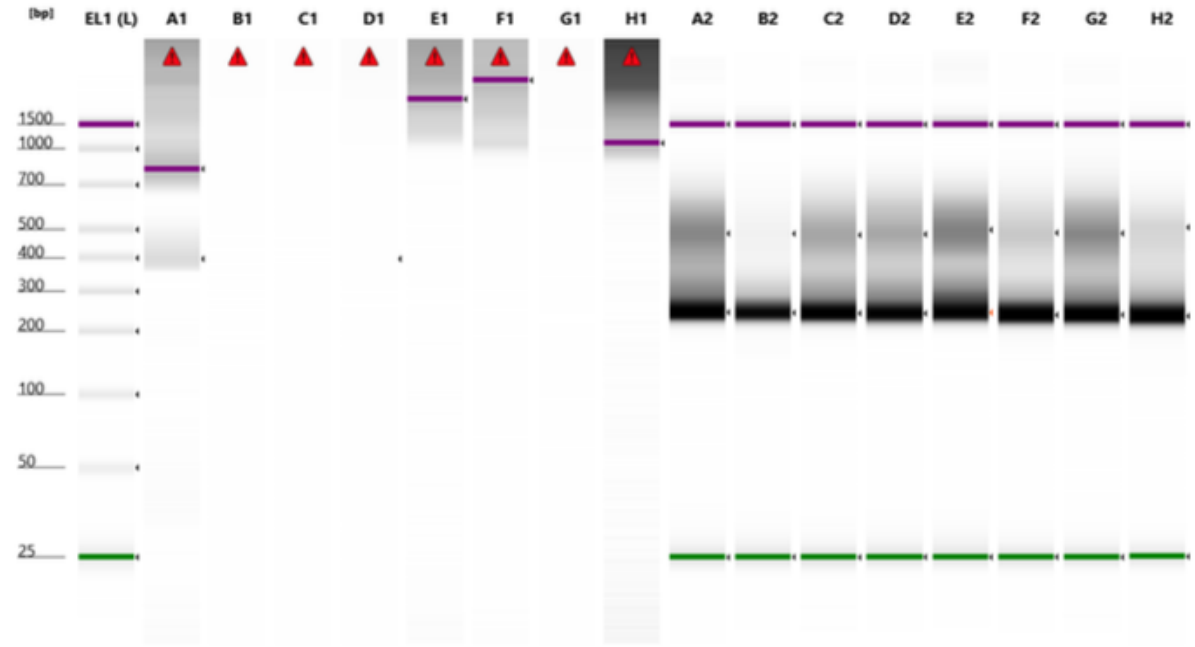
8 cycles of steps 2-4

3. Perform Ampure bead clean up as above with 90ul beads per 50ul PCR reaction.

- If you don't have a crazy number of samples you might also want to consider doing up to 4 PCR2 reactions per sample and pooling them afterwards just to make sure all your sequences are captured.

4. Check size and concentration on tapestation. Dilute and pool samples for sequencing.

Tapestation



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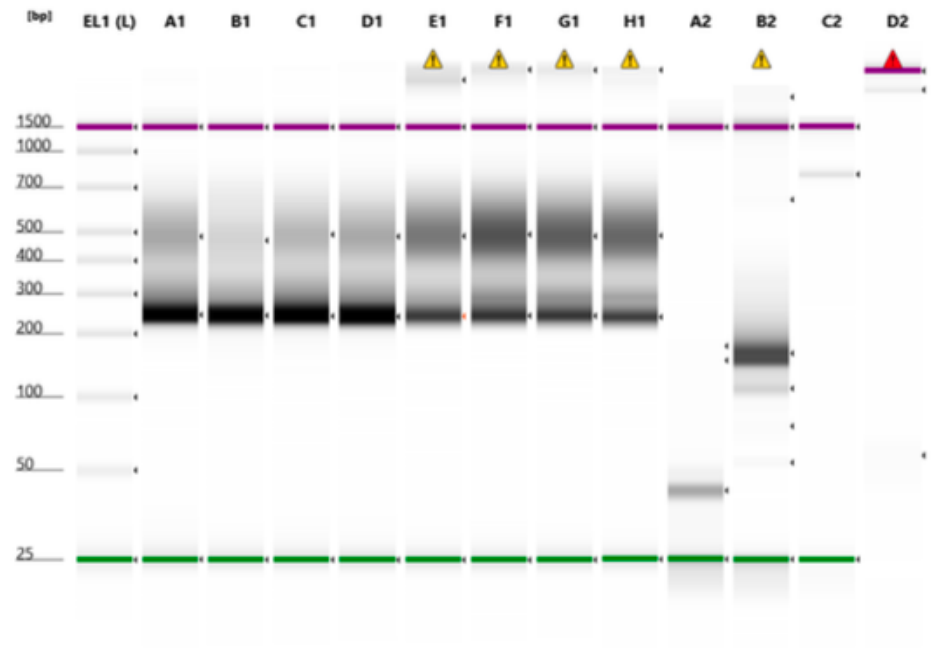
Sample Info

Well	Conc. [ng/µl]	Sample Description	Alert	Observations
EL1	20.3	Electronic Ladder		Ladder
A1	2.72	1 PCR 2	▲	Marker(s) not detected
B1		2 PCR 2	▲	Marker(s) not detected
C1		3 PCR 2	▲	Marker(s) not detected
D1		4 PCR 2	▲	Marker(s) not detected
E1		5 PCR 2	▲	Marker(s) not detected
F1		6 PCR 2	▲	Marker(s) not detected
G1		7 PCR 2	▲	Marker(s) not detected
H1		8 PCR 2	▲	Marker(s) not detected
A2	206	9 PCR 2		
B2	88.9	10 PCR 2		
C2	173	11 PCR 2		
D2	159	12 PCR 2		
E2	160	13 PCR 2		
F2	164	14 PCR 2		
G2	214	15 PCR 2		
H2	149	16 PCR 2		

- 1-8 PCR DNW (suspected issue with bead clean-up)



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Sample Info

Well	Conc. [ng/µl]	Sample Description	Alert	Observations
EL1	20.3	Electronic Ladder		Ladder
A1	167	17 PCR 2		
B1	155	18 PCR 2		
C1	186	19 PCR 2		
D1	200	20 PCR 2		
E1	177	21 PCR 2	⚠	Peak out of Sizing Range
F1	224	22 PCR 2	⚠	Peak out of Sizing Range
G1	210	23 PCR 2	⚠	Peak out of Sizing Range
H1	184	24 PCR 2	⚠	Peak out of Sizing Range
A2	13.5			
B2	37.3		⚠	Peak out of Sizing Range
C2	2.29			
D2	3.24		▲	Marker(s) not detected

-

- Last 4 samples are from Chris (seperate experiment)

Tuesday 30-01-2024

Cell Culture

HEK

- Unhealthy
- Discarded

A20

- Split 1/6

February 2024

Thursday 01-02-2024

PCR Step 2 - Samples 1-8, 25-39

- Repeated PCR2 for samples 1-8

Overview

- PCR to index samples for sequencing by attaching UMI
- Didn't have enough Q5 to complete all the samples

PCR2 Protocol

1. Prepare samples according to the following table:

- Keep careful note of which adaptors are added to which sample

Component	Volume
10uM Nxxx Nextera i7 adapter	1.5 μL
10uM Sxxx Nextera i5 adapter	1.5 μL
DNAse/RNAse H20	21 μL
Q5	25 μL
10ng/μL PCR1	1 μL

2. Perform PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	**Step 2: 98C**	**20s**
Annealing	**Step 3: 55C**	**15s**
Extension	**Step 4: 72C**	**15s**
Final Extension	Step 5: 72C	2 min
Hold Step	Step 6: 12C	Hold

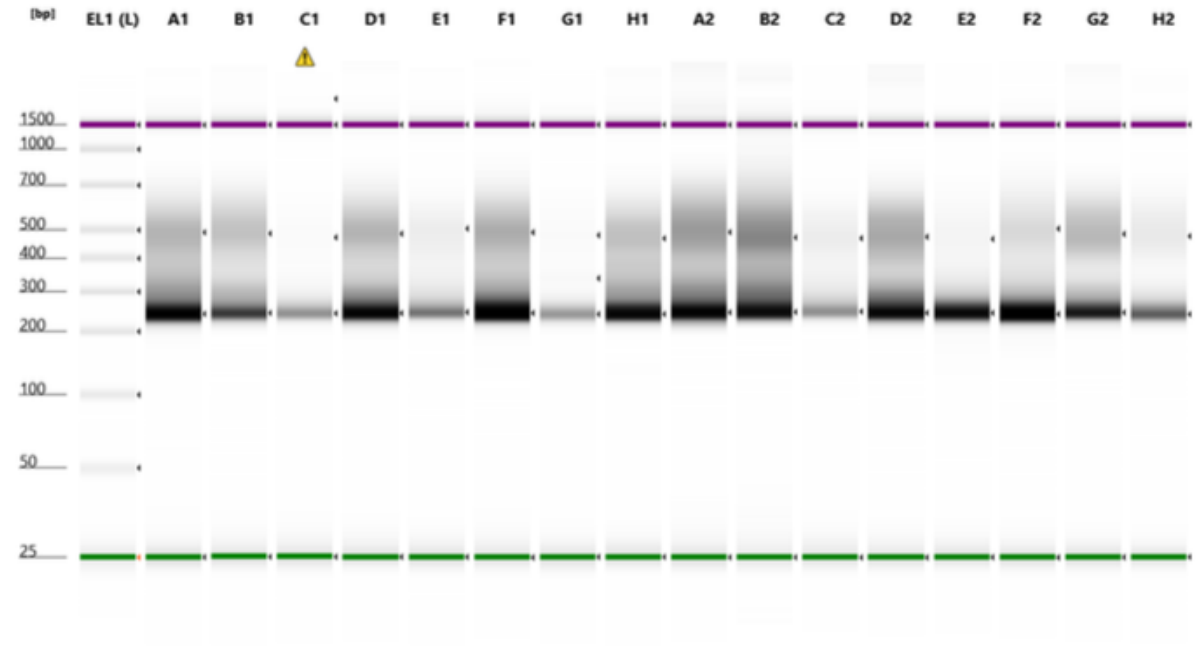
8 cycles of steps 2-4

3. Perform Ampure bead clean up as above with 90ul beads per 50ul PCR reaction.

- If you don't have a crazy number of samples you might also want to consider doing up to 4 PCR2 reactions per sample and pooling them afterwards just to make sure all your sequences are captured.

4. Check size and concentration on tapestation. Dilute and pool samples for sequencing.

Tapestation



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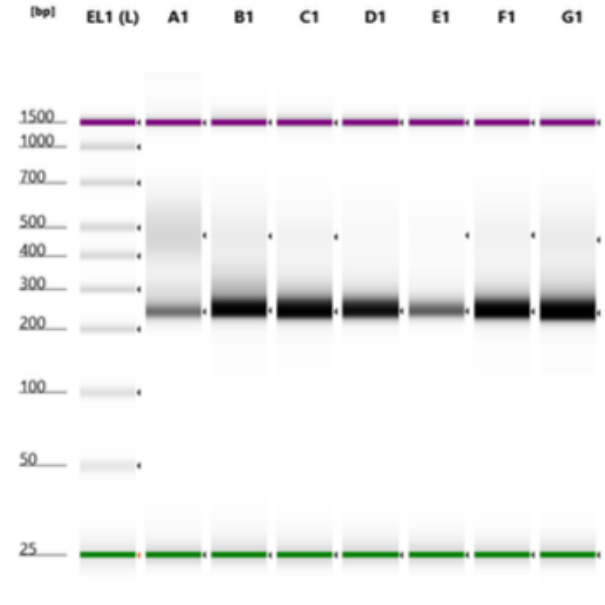
Sample Info

Well	Conc. [ng/µl]	Sample Description	Alert	Observations
EL1	20.3	Electronic Ladder		Ladder
A1	133	1 PCR 2		
B1	83.8	2 PCR 2		
C1	20.0	3 PCR 2	⚠	Peak out of Sizing Range
D1	130	4 PCR 2		
E1	34.0	5 PCR 2		
F1	166	6 PCR 2		
G1	21.2	7 PCR 2		
H1	94.9	8 PCR 2		
A2	153	25 PCR 2		
B2	147	26 PCR 2		
C2	29.1	27 PCR 2		
D2	149	28 PCR 2		
E2	81.6	29 PCR 2		
F2	141	30 PCR 2		
G2	104	31 PCR 2		
H2	45.9	32 PCR 2		



Monday 05-02-2024

Cell Culture

Made DMEM

Solution	ID code	Volume	% Total volume
DMEM	—-	500 mL	78%
Pen-Strep	—-	5.5 mL	1%
FBS	—-	55 mL	10%

HEK - Seeded

- Seeded New HEK
  - HEK stock are in CM Box 1 -80

1. Thawed vial
2. Added 1mL DMEM to vial
3. Transferred to 15mL falcon tube
4. Slowly added 5mL DMEM
5. Spun down at 650rpm for 4min
6. Removed supernant and resuspended vial flicking
7. Added 1mL DMEM and pipetted multiple times to create single cell suspension
8. Added to T25 flask
9. Added 14mL of DMEM
10. Placed in incubator

A20 - Split

- Split 1/6
- Spliut T25 vial for Emily James (EJ)

Tuesday 06-02-2024

WS-ME1 Pooling for Submission

- Genomics core indicated that library should be submitted at 5nM-10nM in a volume of 20-30uL
- Cubit PCR2 Samples to determine concentration

Sample ID	Submission ID	Nextera Adaptor Indices	Desired ng/uL	Base Pair	[Final] nM	Sample Volume (uL)	H2O (uL)
1	1_Cyclophosphamide	i701-i502	1.5	241	9.43	1	29.60
2	2_Cyclophosphamide	i702-i502	1.5	241	9.43	1	19.20
3	3_Cyclophosphamide	i703-i502	1.5	241	9.43	1	4.52
4	4_Cyclophosphamide	i704-i502	1.5	241	9.43	1	25.90
5	5_Cyclophosphamide	i705-i502	1.5	241	9.43	1	6.40
6	6_Combination	i706-i502	1.5	241	9.43	1	31.90
7	7_Combination	i707-i502	1.5	241	9.43	1	4.50
8	8_Combination	i710-i502	1.5	241	9.43	1	24.70
9	9_Combination	i701-i503	1.5	241	9.43	1	45.70
10	10_Combination	i702-i503	1.5	241	9.43	1	22.30
11	11_Methotrexate	i703-i503	1.5	241	9.43	1	43.90
12	12_Methotrexate	i704-i503	1.5	241	9.43	1	32.70

Sample ID	Submission ID	Nextera Adaptor Indices	Desired ng/uL	Base Pair	[Final] nM	Sample Volume (uL)	H2O (uL)
13	13_Methotrexate	i705-i503	1.5	241	9.43	1	41.20
14	14_Methotrexate	i706-i503	1.5	241	9.43	1	34.80
15	15_Methotrexate	i707-i503	1.5	241	9.43	1	46.10
16	16_Vehicle	i710-i503	1.5	241	9.43	1	28.30
17	17_Vehicle	i701-i505	1.5	241	9.43	1	39.70
18	18_Vehicle	i702-i505	1.5	241	9.43	1	32.00
19	19_Vehicle	i703-i505	1.5	241	9.43	1	34.90
20	20_Vehicle	i704-i505	1.5	241	9.43	1	34.00
21	21_Baseline	i705-i505	1.5	241	9.43	1	34.10
22	22_Baseline	i706-i505	1.5	241	9.43	1	34.30
23	23_Baseline	i707-i505	1.5	241	9.43	1	28.90
24	24_Baseline	i710-i505	1.5	241	9.43	1	31.60
25	25_Baseline	i701-i506	1.5	241	9.43	1	27.60
26	26_BLLW 14K	i702-i506	1.5	241	9.43	1	24.10
27	27_BLLW 2K	i703-i506	1.5	241	9.43	1	5.20
28	28_BLLW 1K	i704-i506	1.5	241	9.43	1	30.50
29	29_Methotrexate (outlier)	i705-i506	1.5	241	9.43	1	14.00
30	30_Combo	i706-i506	1.5	241	9.43	1	28.10
31	31_RBL2P_2K	i707-i506	1.5	241	9.43	1	15.10
32	32_RBL2P_7K	i710-i506	1.5	241	9.43	1	49.40
33	33_RBL2P_250K	i701-i507	1.5	241	9.43	1	41.50
34	34_Mock_direct_1	i702-i507	1.5	241	9.43	1	15.30
35	35_Mock_direct_2	i703-i507	1.5	241	9.43	1	15.90
36	36_Mock_direct_3	i704-i507	1.5	241	9.43	1	10.90
37	37_Mock_culture_1	i705-i507	1.5	241	9.43	1	4.48
38	38_Mock_culture_2	i706-i507	1.5	241	9.43	1	18.00
39	39_Mock_culture_3	i707-i507	1.5	241	9.43	1	17.70

- Used IDT Library Concentration Conversion Calculator
  - Link: https://eu.idtdna.com/Calc/library-concentration-conversion

Wednesday 07-02-2024

Cell Culture

HEK

- Decided to wait till Monday to start making virus
- Discarded HEK
- Will reseed HEK Sunday

A20

- Split 1/6
- Keeping going for NSG/BALBc injection
  - Issue with procedure room in AMB, waiting for that to be resolved
- Also plan to incorporate WILDseq

Friday 09-02-2024

Cell Culture

A20

- Split 1/6
- Keeping going for NSG/BALBc injection
  - Issue with procedure room in AMB, waiting for that to be resolved
- Also plan to incorporate WILDseq

Monday 12-02-2024

Cell Culture

Made DMEM-V1

- DMEM for virus prodution
- Does not have Pen/Strep

Solution	ID code	Volume	% Total volume
DMEM	—-	500 mL	78%
FBS	—-	128 mL	20%
Glutamax	—-	6.5 mL	1%

Thawed HEK

A20

- Split 1/6
- Keeping going for NSG/BALBc injection
  - Planning to inject with Chris tomorrow
- Also plan to incorporate WILDseq once virus is made

Processing scRNAseq

Tuesday 13-02-2024

Cell Culture

HEK

- Morphology looked weird
- 20% FBS is probably too high
- Threw away will start next week

Processing scRNAseq

Wednesday 14-02-2024

Cell Culture

A20

- Split 1/6
- Keeping going for NSG/BALBc injection

    - Planning to inject with Chris tomorrow
- Also plan to incorporate WILDseq once virus is made

Processing scRNAseq

Friday 16-02-2024

A20 Xenograft EXP 1

Cell Prep

1. Thawed 2.5mL of Matrigel on ice ~3hours before
2. Transferred 12x10^6 cells to a 15mL eppendorf
3. Pelleted CS
4. Resuspended in 2.5mL of PBS
5. Transferred 400μL CS to 6 different 1mL eppendorfs
6. Added 400μL of Matrigel, mixed gentlely and placed back on ice
7. Brought materials over to the AMB for injection

Injection

- Chris Injected
- Study Plan: SP140164

Mouse ID	Earmark	Genotype	Injection Location
TUAD36.2h	NM	NSG	IP
TUAD36.2i	1R	NSG	IP
TUAD36.2k	2R	NSG	IP
TUAD36.2a	NM	NSG	SC
TUAD36.2b	1L	NSG	SC
TUAD36.2c	1R	NSG	SC
TUAP3.1e	1L	BALB/c	IP
TUAP3.1a	NM	BALB/c	IP
TUAP4.1a	2L	BALB/c	IP
TUAP3.1b	1L	BALB/c	SC
TUAP4.1b	2R	BALB/c	SC
TUAP3.1c	1R	BALB/c	SC

- Each mouse received ~1x10^6 cells
- Mice will be monitored over the coming weeks for tumour development

Monday 19-02-2024

Cell Culture

A20

- Split 1/6
- Used AR-5

Wednesday 21-02-2024

Cell Culture

A20

- Split 1/6
- Used AR-5

HEK

- Seeded New HEK
  - HEK stock are in CM Box 1 -80
- Used DMEM-V1

1. Thawed vial
2. Added 1mL DMEM to vial
3. Transferred to 15mL falcon tube
4. Slowly added 5mL DMEM
5. Spun down at 650rpm for 4min
6. Removed supernant and resuspended vial flicking
7. Added 1mL DMEM and pipetted multiple times to create single cell suspension
8. Added to T25 flask
9. Added 14mL of DMEM
10. Placed in incubator

Friday 23-02-2024

Cell Culture

A20

- Split 1/6
- Used AR-5

HEK

- Split 1/6
- Used DMEM-V1

Monday 26-02-2024

Cell Culture

A20

- Split into T25
- Froze down 4 vials
- $4x10^6$ cells per vial
- Used AR-5

HEK

- Froze down 7 vials
- $6x10^6$ cells per vial

WILDseq Virus Production Day 1

- Seeded 2 10cm dishes of HEK cells
  - Coated with Poly-D-Lysine (covered plate, removed liquid, let sit for 10 min, washed with PBS)
  - 4.5x10^6 cells seeded per dish (seeded at 13:00)

Wednesday 06-03-2024

- Collected SC tumours
- HEK infection
  - binned A20/HEK

Wednesday 13-03-2024

- Made media
- Seededin N2-BC4
- Froze down A20
- Collected 3 IP NSG tumours

Thursday 14-03-2024

- Changed media (N2-BC4)

Friday 15-03-2024

Cell Culture

N2-BC4 RTX CDC Assay - version 1

Monday 18-03-2024

Cell Culture - N2 -C4

- Split: seeded 5x10^6 cells

N2-BC4 RTX CDC Assay - version 2

Overview: Trying to optimise RTX CDC assay - Using 0% serum, since there are no complement factors in the media the cell live/dead reading should not change - Next step: add varying amounts of human serum

Friday 22-03-2024

Cell Culture

N2-BC4

- Cells look healthy
- Feeders in flask are also healthly
- Split 5x10^6 back into T175 with 30mL of AR-6

EC50 RTX N2-BC4 22324 - Seeding

- Seeded 96-well plate with N2-BC4 and treated with RTX concentration range with or with out 10% Human Serum (HS)

- Plate seeding protocol:
    1. Diluted cell suspension to seed 20000 cells/well in 50μL amounts

Well Number	Cells total	Volume total	CS cells/mL	Flask cell count	**Flask Volume**	**Media Volume**
60 wells	1.2x10^6 cells	3 mL	4x10^5	3.06x10^6	**400μL**	**2.6 mL**

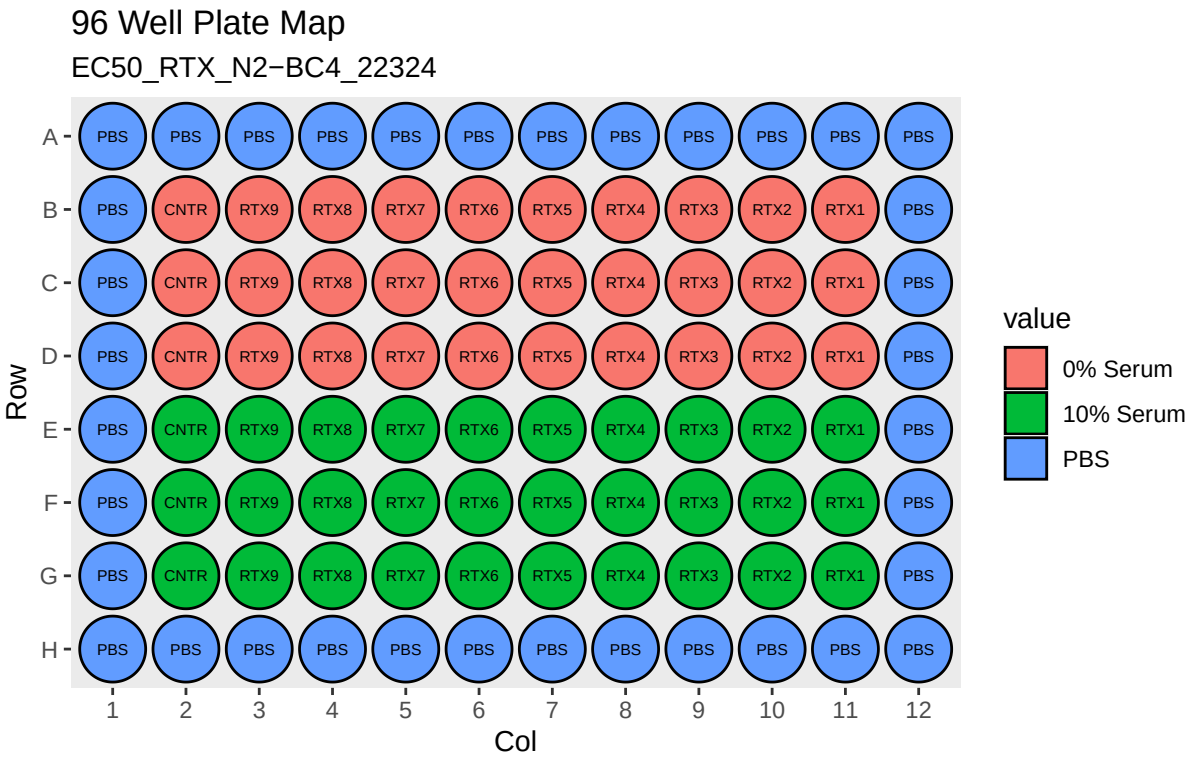
2. Made RTX dilutions and added to respective wells in 50μL
   - [RTX stock] = 10.3 mg/mL - Drug volumes are added in triplicate - Drug volumes are being added consititute 1/4 of well volume: [RTX working] needs to be 4x [RTX well]
   - 6 wells per condition, 50μL per well ~ minimum of 300μL per condition needed (recommend 500μL)

Dilution ID	[RTX Well]	Media Volume	Source	[RTX WS]
RTX 1	100 μg/mL	1 mL	50μL stock	400 μg/mL
RTX 2	50 μg/mL	500 mL	500μL RTX 1	200 μg/mL
RTX 3	25 μg/mL	500 mL	500μL RTX 2	100 μg/mL
RTX 4	12.5 μg/mL	500 mL	500μL RTX 3	50 μg/mL
RTX 5	6.25 μg/mL	500 mL	500μL RTX 4	25 μg/mL
RTX 6	3.125 μg/mL	500 mL	500μL RTX 5	12.5 μg/mL
RTX 7	1.56 μg/mL	500 mL	500μL RTX 6	6.25 μg/mL
RTX 8	.78 μg/mL	500 mL	500μL RTX 7	3.125 μg/mL
RTX 9	.39 μg/mL	500 mL	500μL RTX 8	1.56 μg/mL
CNTR	0 μg/mL	500 mL	-	0 μg/mL

3. Added 10% serum or media control

- This provides complement factors to bind BL-bound RTX and initiate CDC
- Serum/media amounts added at 100 μL/well
- Serum volume is added in 1:4 ratio (20μL serum in 200μL final well volume)
- Serum stock mix is 1.2mL HS : 2.4 mL media / plate

4. Plate is incubated for 72 hrs at 37C



Monday 25-03-2024

EC50 RTX N2-BC4 22324 - Collection

- Collected plate seeded on 22-03-2025
- EC Plate collection protocol:
  1. Added 40μL Cell Titre Blue (CTB) to each conditioned well
  − 20μL CTB/100μL of conditioned well recommended by manufacturer
  2. Incubated for 1hr at 37C
  3. Read on plate reader according to Cell Titre Blue Protocol
- Results:

EC50 RTX N2-BC4 25324 - Seeding (DNW)

- Seeded an EC50 experiement comparing the effects of RTX on N2-BC4 with or without 10% serum
- Decreased overall well volume to reduce CTB being used
- Used RTX provided by Jamie
- Plate seeding protocol:
  1. Diluted cell suspension to seed 20000 cells/well in 25μL amounts

Well Number	Cells total	Volume total	CS cells/mL	Flask cell count	**Flask Volume**	**Media Volume**
60 wells	1.2x10^6 cells	1.5 mL	8x10^5	2.92x10^6	**410μL**	**1.1 mL**

2. Made RTX dilutions and added to respective wells in 50μL
- [RTX stock] = 10.3 mg/mL - Drug volumes are added in triplicate - Drug volumes are being added consititute 1/4 of well volume: [RTX working] needs to be 4x [RTX well]

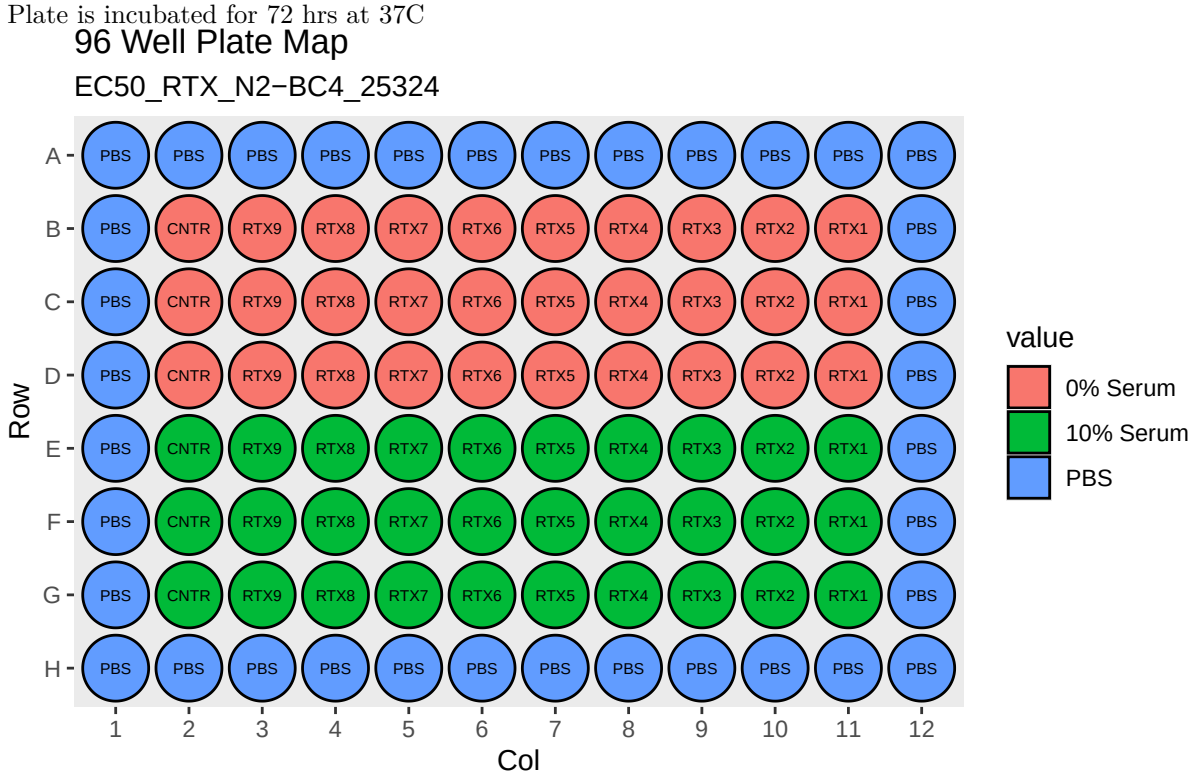
- 6 wells per condition, 25μL per well ~ minimum of 150μL per condition needed (recommend 200μL)

Dilution ID	[RTX Well]	Media Volume	Source	[RTX WS]
RTX 1	100 μg/mL	400μL	15.5μL stock	400 μg/mL
RTX 2	50 μg/mL	200 mL	200μL RTX 1	200 μg/mL
RTX 3	25 μg/mL	200 mL	200μL RTX 2	100 μg/mL
RTX 4	12.5 μg/mL	200 mL	200μL RTX 3	50 μg/mL
RTX 5	6.25 μg/mL	200 mL	200μL RTX 4	25 μg/mL
RTX 6	3.125 μg/mL	200 mL	200μL RTX 5	12.5 μg/mL
RTX 7	1.56 μg/mL	200 mL	200μL RTX 6	6.25 μg/mL
RTX 8	.78 μg/mL	200 mL	200μL RTX 7	3.125 μg/mL
RTX 9	.39 μg/mL	200 mL	200μL RTX 8	1.56 μg/mL
CNTR	0 μg/mL	500 mL	-	0 μg/mL

3. Added 10% serum or media control

- This provides complement factors to bind BL-bound RTX and initiate CDC
- Serum/media amounts added at 50 μL/well
- Serum volume is added in 1:4 ratio (10μL serum in 100μL final well volume)
- Serum stock mix is 600μL HS : 1.2 mL media / plate

4. Plate is incubated for 72 hrs at 37C



Cell Culture

N2-BC4

- Cells look healthy
- Feeders in flask are also healthly
- Split 5x10^6 back into T175 with 30mL of AR-6

Wednesday 27-03-2024

Thursday 28-03-2024

Friday 29-03-2024

EC50 RTX N2-BC4/RAMOS-BC5 25324 - Seeding (DNW)

- Seeded an EC50 experiement comparing the effects of RTX on N2-BC4 and RAMOS-BC5 with or without 10% serum
- Decreased overall well volume to reduce CTB being used
- Used RTX provided by Jamie
- Plate seeding protocol:
    1. Diluted cell suspension to seed 20000 cells/well in 25μL amounts

Cell Line	Well Number	Cells total	Volume total	CS cells/mL	Flask cell count	**Flask Volume**
N2-BC4	60 wells	1.2x10^6 cells	1.5 mL	8x10^5	2.92x10^6	**410μL**
RAMOS-BC5	60 wells	1.2x10^6 cells	1.5 mL	8x10^5	2.92x10^6	**410μL**

2. Made RTX dilutions and added to respective wells in 50μL
   - [RTX stock] = 10.3 mg/mL - Drug volumes are added in triplicate - Drug volumes are being added consititute 1/4 of well volume: [RTX working] needs to be 4x [RTX well]
   - 6 wells per condition, 25μL per well ~ minimum of 150μL per condition needed (recommend 200μL)

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RTX 1	100 μg/mL	400μL	15.5μL stock	400 μg/mL
RTX 2	50 μg/mL	200 mL	200μL RTX 1	200 μg/mL
RTX 3	25 μg/mL	200 mL	200μL RTX 2	100 μg/mL
RTX 4	12.5 μg/mL	200 mL	200μL RTX 3	50 μg/mL
RTX 5	6.25 μg/mL	200 mL	200μL RTX 4	25 μg/mL
RTX 6	3.125 μg/mL	200 mL	200μL RTX 5	12.5 μg/mL
RTX 7	1.56 μg/mL	200 mL	200μL RTX 6	6.25 μg/mL
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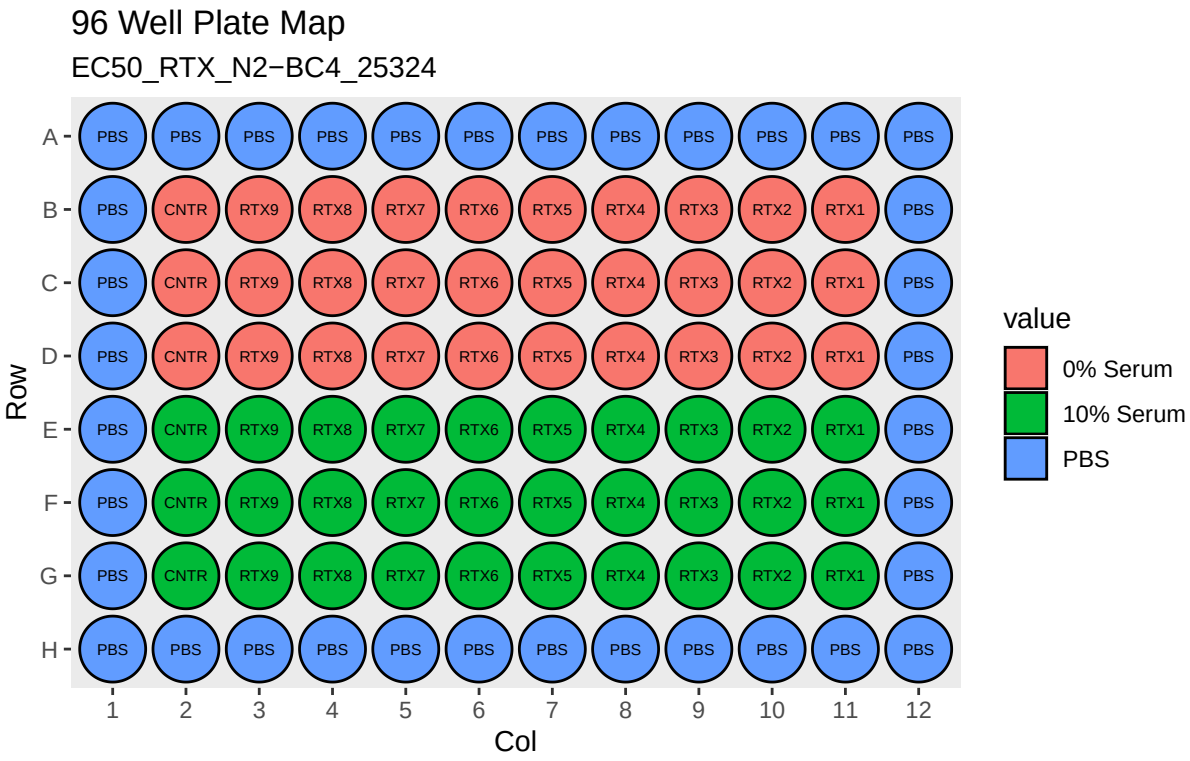
96 Well Plate Map
EC50_RTX_N2−BC4_25324
Row
A
B
C
D
E
F
G
H
PBS
CNTR
RTX9
RTX8
RTX7
RTX6
RTX5
RTX4
RTX3
RTX2
RTX1
1
2
3
4
5
6
7
8
9
10
11
12
Col
value
0% Serum
10% Serum
PBS