# $Lab\_Notebook\_2024$

# Callum Malcolm

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# January 2024

### Tuesday 16-01-2023

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

- $\bullet\,$  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/ $\mu$ L = 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	$3~\mu L$	$93~\mu L$
DNAse/RNAse H20	$12 \ \mu L$	$372~\mu L$
Kapa Hifi HotStart Ready Mix $(2X)$	$25 \ \mu L$	$775~\mu\mathrm{L}$

2. Add following components to tubes

Component	Volume
PCR1 MasterMix	$40~\mu L$
cDNA	$10~\mu L$

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	$200~\mu \rm L$	

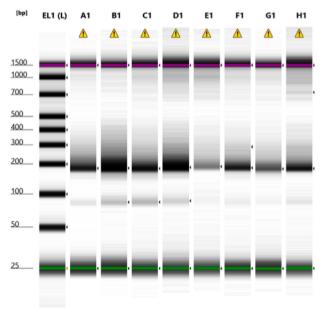
#### Protocol

- 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	<u> </u>	Caution! Expired ScreenTape device
B1	631	2 PCR1	<u> </u>	Caution! Expired ScreenTape device
Cl	333	3 PCR1	<u> </u>	Caution! Expired ScreenTape device
D1	369	4 PCR1	<u> </u>	Caution! Expired ScreenTape device
E1	90.0	5 PCR1	<u> </u>	Caution! Expired ScreenTape device
F1	266	6 PCR1	<u> </u>	Caution! Expired ScreenTape device
Gl	181	7 PCR1	<u> </u>	Caution! Expired ScreenTape device
H1	206	8 PCR1	<u>^</u>	Caution! Expired ScreenTape device

Inital Test PCR 1

### PCR 2: Samples 5

#### Overview

- Yield from PCR1 is low (.09 .631 ng/ $\mu$ L)
- 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	$1000~\mu\mathrm{L}$	
Kapa Hifi HotStart Ready Mix (2X)	$1200~\mu\mathrm{L}$	KK2601

#### Protocol

- 1. Prepared samples according to the following table:
- Sample Adaptor pairs in table above

Component	Volume
10uM N705 Nextera i7 adapter	$1.5 \mu L$
10uM S502 Nextera i5 adapter	$1.5~\mu\mathrm{L}$
DNAse/RNAse H20	$21 \ \mu L$
Kapa Hifi HotStart Ready Mix (2X)	$25 \ \mu L$
$10 \text{ng}/\mu\text{L PCR}1$	$1~\mu { m L}$

2. Performed PCR using the following parameters:

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

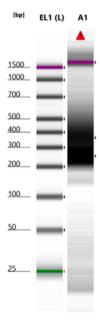
#### Tapestation PCR 2

#### 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
Al	3180	5 PCR2		Marker(s) not detected; Caution! Expired ScreenTape device

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

### Wednesday 17-01-2023

### 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  $\mu$ L | | cDNA | 10  $\mu$ L |
- 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	$\operatorname{Hold}$

#### 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

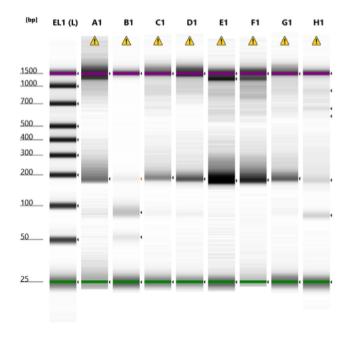
Component	Expected Volume/experiment	Material ID
Ampure Bead Kit	$200~\mu \rm L$	

#### 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	<u> </u>	Caution! Expired ScreenTape device
Bl	164	10 PCR 1	<u> </u>	Caution! Expired ScreenTape device
Cl	142	11 PCR 1	<u> </u>	Caution! Expired ScreenTape device
D1	140	12 PCR 1	<u> </u>	Caution! Expired ScreenTape device
El	1070	13 PCR 1	<u> </u>	Caution! Expired ScreenTape device
F1	354	14 PCR 1	<u> </u>	Caution! Expired ScreenTape device
Gl	203	15 PCR 1	<u> </u>	Caution! Expired ScreenTape device
H1	203	16 PCR 1	<u> </u>	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	<u> </u>	Caution! Expired ScreenTape device
B1	40.7	18 PCR 1	<u> </u>	Caution! Expired ScreenTape device
C1	84.0	19 PCR 1	<u> </u>	Caution! Expired ScreenTape device
D1	226	20 PCR 1	<u> </u>	Caution! Expired ScreenTape device
E1	665	21 PCR 1	<u> </u>	Caution! Expired ScreenTape device
F1	820	22 PCR 1	<u> </u>	Caution! Expired ScreenTape device
Gl	270	23 PCR 1	<u> </u>	Caution! Expired ScreenTape device
HI	423	24 PCR 1	<u> </u>	Caution! Expired ScreenTape device
A2	1290	25 PCR 1	<u> </u>	Caution! Expired ScreenTape device
B2	223	26 PCR 1	<u> </u>	Caution! Expired ScreenTape device
C2	170	27 PCR 1	<u> </u>	Caution! Expired ScreenTape device
D2	71.5	28 PCR 1	<u> </u>	Caution! Expired ScreenTape device
E2	30.5	29 PCR 1	<u> </u>	Caution! Expired ScreenTape device
F2	157	30 PCR 1	<u> </u>	Caution! Expired ScreenTape device

#### 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 18-01-2023

### 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  $\mu$ L | | cDNA | 10  $\mu$ L |
- 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

 $\bullet$  For 40 samples

Component	Expected Volume/experiment	Material ID
Ampure Bead Kit	$200~\mu L$	

#### 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 19-01-2023

### 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  $\mu g$  of RNA in a total volume of 10  $\mu l$  of RNAse/DNAase-free water.
- 2. Add 1  $\mu$ l of WS\_RT\_UMI\_NexteraR2 primer (2 $\mu$ M stock)
- 3. Add 1  $\mu l$  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  $\mu$ l 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  $\mu l$  The molabile Exonuclease I (NEB M0568) to remove excess RT primer.

Component	Volume	MM volume
Thermolabile Exonuclease I	$1~\mu { m L}$	
NEBuffer r3.1*	$2 \mu L$	

#### 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  $\mu$ l of RNAse H and incubate at 37 C for 20 mins.
- 11. Checked [cDNA] on NanoDrop
- Added to Sample List

### Monday 22-01-2023

#### 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  $\mu \rm L$  | cDNA | 10  $\mu \rm L$  |
- 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	$200~\mu { m L}$	

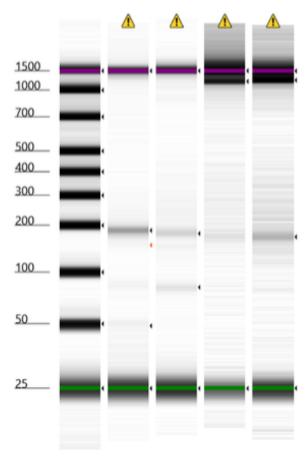
#### Protocol

- 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
Al	89.4	-RT Control
Bl	46.1	31 PCR 1
Cl	87.3	32 PCR 1
Dl	195	33 PCR 1

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

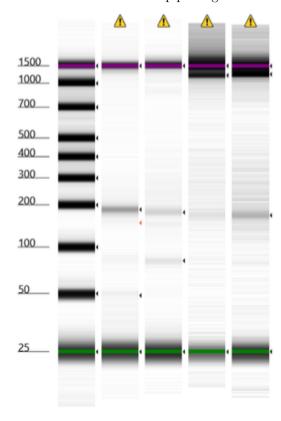
### PCR 1: Negative Control

- 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  $\mu L$  | cDNA | 10  $\mu L$  |
- $3.\ \,$  Performed PCR using the following parameters:

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

### Tapestation Control Test 1

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



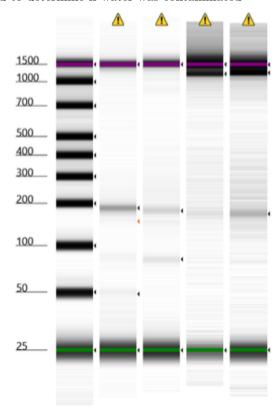
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### Sample Info

Well	Conc. [pg/µl]	Sample Description
EL1	2350	Electronic Ladder
Al	89.4	-RT Control
Bl	46.1	31 PCR 1
Cl	87.3	32 PCR 1
DI	195	33 PCR 1

### Tapestation Control Test 2

- Wanted to determine if water was contaminated



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### Sample Info

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

# Tuesday 23-01-2023

Made New Media: AR-5

1. Made new media: AR-5

Solution	ID code	Volume	% Total volume
Advanced RMPI		$500~\mathrm{mL}$	78%
Glutamax	<del></del> -	$6.5~\mathrm{mL}$	20%
Pen-Strep	—-	$6.5~\mathrm{mL}$	1%
FBS		$128~\mathrm{mL}$	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  $\mu\mathrm{M}$  so needed to be diluted to 10  $\mu$

Component	Volume
100uM WS PCR1 Primer Mix	$2 \mu L$
100uM WS PCR1 Primer Mix	$2 \mu L$
100uM WS PCR1 Primer Mix	$2 \mu L$
100uM WS PCR1 Primer Mix	$2 \mu L$
100uM WS PCR1 Primer Mix	$2~\mu L$
100uM WS PCR1 Primer Mix	$2 \mu L$
100uM WS PCR1 Primer Mix	$2 \mu L$
100uM WS PCR1 Primer Mix	$2 \mu L$
100uM WS PCR1 Primer Mix	$16 \ \mu L$
DNAse/RNAse~H20	$320~\mu L$

#### PCR1 MM

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

1. Add following components to tubes

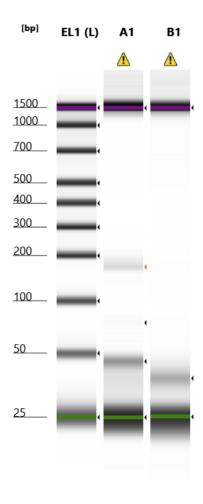
Component	Volume
PCR1 MasterMix H2O	$\begin{array}{c} 40~\mu\mathrm{L} \\ 10~\mu\mathrm{L} \end{array}$

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

### Tapestation Control Test 3

- $\bullet$  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 24-01-2023

### Cell Culture

#### **A20**

- Checked cells  $\sim 10\%$  confluent
- Cells looked healthy but sparse
- Grow up for inection
- $\bullet\,$  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  $\mu g$  of RNA in a total volume of 10  $\mu l$  of RNAse/DNAase-free water.
- 2. Add 1  $\mu$ l of WS RT UMI NexteraR2 primer (2 $\mu$ M)
- Diluted primer from stock ( $100\mu M$ )
- Added 1  $\mu$ L RT-Primer stock into  $49\mu$ L water
- 3. Add 1  $\mu$ l 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	$4~\mu L$	$134~\mu L$
SSIV RT	$1~\mu L$	$33.5~\mu\mathrm{L}$
$100~\mathrm{mM}~\mathrm{DTT}$	$1~\mu L$	$33.5~\mu\mathrm{L}$
RNAse Out	$1~\mu L$	$33.5~\mu\mathrm{L}$

- 6. Add 7  $\mu$ l 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  $\mu$ l Themolabile Exonuclease I (NEB M0568) to remove excess RT primer.

Component	Volume	MM volume
Thermolabile Exonuclease I NEBuffer r3.1*	$\begin{array}{cc} 1~\mu L \\ 2~\mu L \end{array}$	$33.5 \ \mu L$ $67 \ \mu L$

<sup>\*\*</sup>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  $\mu$ l 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
- $\bullet\,$  Plan cell numbers needed for A20 mouse injections