Lab_Notebook_2024

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Contents

January 2024

Tuesday 16-01-2023

WILDseq - Mouse Experiment 1

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

PCR 1: Samples 1-8

- Checked [cDNA] on NanoDrop of sample 8 to make sure samples were still intact
  - 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

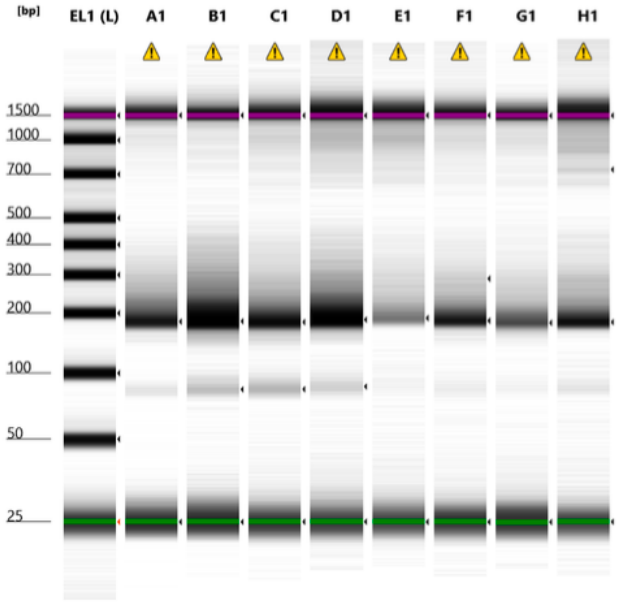
Materials

- 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



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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

Figure 1: 2024-01-16 Tapestation PCR 1 Results Samples 1-8

Tapestation 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

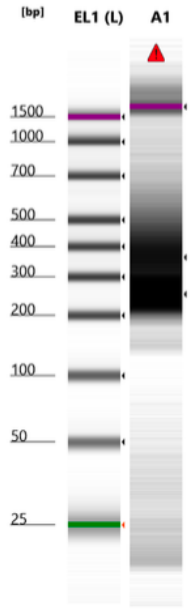
8 cycles of steps 2-4

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)



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

Plan for tomorrow

- PCR 1 samples 9-30
- Bead Clean Up
- Tape Station
- Ask Chris about HEK
- Find A20 for Rahul

Wednesday 17-01-2023