

# Lab Notebook 2024

Callum Malcolm

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# LN Repository

## Rack 1

- Location: Tank 2, Rack 1, Row H (Bottom)

Location	Cap ID	Description	Date
1	Grey	Empty - Marker	-
2	Ramos BC 1	Ramos RTX CDC Baseline	12/06/2024
3	Ramos BC 1	Ramos RTX CDC Baseline	12/06/2024
4	Ramos BC 1	Ramos RTX CDC Baseline	12/06/2024
5	C4 DP2	Ramos RTX CDC C4-DP2	-
6	C5 DP2	Ramos RTX CDC C5-DP2	-
7	C1 DP2	Ramos RTX CDC C1-DP2	-
8	R3 DP2	Ramos RTX CDC R3-DP2	-
9	C6 DP2	Ramos RTX CDC C6-DP2	-
10	C3 DP2	Ramos RTX CDC C3-DP2	-
11	C2 DP2	Ramos RTX CDC C2-DP2	-
12	-	-	-
13	-	-	-
14	-	-	-
15	-	-	-
16	-	-	-
17	-	-	-
18	-	-	-
19	-	-	-
20	-	-	-
21	-	-	-
22	-	-	-
23	-	-	-
24	-	-	-
25	-	-	-
26	-	-	-
27	-	-	-
28	-	-	-
29	-	-	-
30	-	-	-
31	Ramos BC 1	Ramos Barcode Pool 1	12/06/2024
32	Ramos BC 1	Ramos Barcode Pool 1	04/01/2023
33	Ramos BC 1	Ramos Barcode Pool 1 *	16/07/2024
34	Ramos BC 3	Ramos Barcode Pool 3	12/06/2024
35	Ramos BC 3	Ramos Barcode Pool 3	04/06/2024
36	Ramos BC 3	Ramos Barcode Pool 3	12/06/2024
37	Ramos BC 5	Ramos BC Pool 5	12/06/2024
38	Ramos BC 5	Ramos Barcode Pool 5	04/16/2024
39	Ramos BC 6	Ramos Barcode Pool 6	04/16/2024
40	-	-	-
41	-	-	-
42	-	-	-
43	-	-	-
44	-	-	-
45	-	-	-
46	-	-	-
47	-	-	-

Location	Cap ID	Description	Date
48	-	-	-
49	-	-	-
50	-	-	-
51	RBL1	RBL1 PDX	31/07/2023
52	RBL1	RBL1 PDX	31/07/2024
53	RBL1 PDX	RBL1 PDX	31/07/2024
54	BLLW	BLLW PDX Pool	31/07/2024
55	BLLW	BLLW PDX Pool	31/07/2024
56	N4	N4 PDX pool	07/11/2023
57	N4	N4 PDX pool	07/11/2023
58	N2 BC	N2 Barcoded pool	11/05/2023
59	N2 BC 5	N2 barcode pool 5	29/04/2024
60	A20	A20 Cell Pool	13/10/2024
61	A20	A20 Stock	13/10/2024
62	-	-	-
63	-	-	-
64	-	-	-
65	-	-	-
66	-	-	-
67	-	-	-
68	-	-	-
69	-	-	-
70	-	-	-
71	-	-	-
72	-	-	-
73	-	-	-
74	-	-	-
75	-	-	-
76	-	-	-
77	-	-	-
78	-	-	-
79	-	-	-
80	-	-	-
81	-	-	-
82	-	-	-
83	-	-	-
84	-	-	-
85	-	-	-
86	-	-	-
87	-	-	-
88	-	-	-
89	-	-	-
90	-	-	-
91	NA	NA	NA
92	NA	NA	NA
93	NA	NA	NA
94	A20 ME	B-IP-724-1L	-
95	A20 ME	B-IP-723 NM	-
96	A20 ME	B-IP-723-2L	-
97	A20 ME	723-2R	-
98	A20 ME	723-1L	-
99	A20 ME	710 NM - A20 Mouse Experiment	-

Location	Cap ID	Description	Date
100	NA	NA	NA

## 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

Sample ID	Treatment/Sample	ng/uL	i7 index	i5 index
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 $\mu$ 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	<b>Step 2: 98C</b>	<b>20s</b>
Annealing	<b>Step 3: 60C</b>	<b>15s</b>
Extension	<b>Step 4: 72C</b>	<b>15s</b>
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

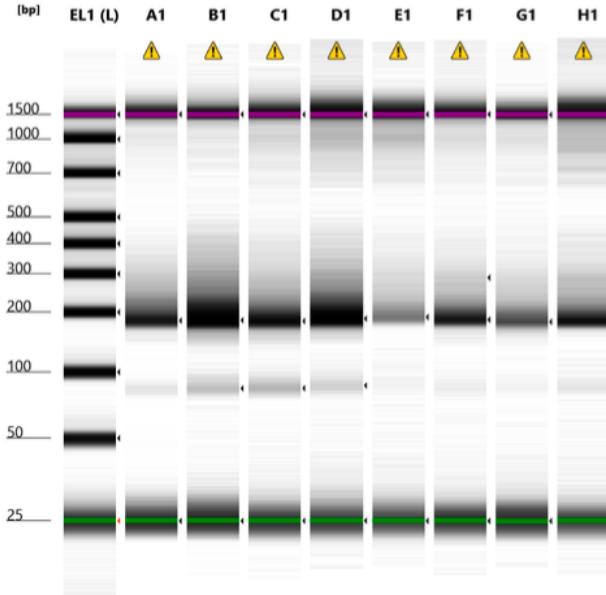
Component	Expected Volume/experiment	Material ID
Ampure Bead Kit	200 $\mu$ L	

## 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/ $\mu$ L]	Sample Description	Alert	Observations
EL1	2350	Electronic Ladder		Ladder
A1	310	1 PCRI	⚠️	Caution! Expired ScreenTape device
B1	631	2 PCRI	⚠️	Caution! Expired ScreenTape device
C1	333	3 PCRI	⚠️	Caution! Expired ScreenTape device
D1	369	4 PCRI	⚠️	Caution! Expired ScreenTape device
E1	90.0	5 PCRI	⚠️	Caution! Expired ScreenTape device
F1	266	6 PCRI	⚠️	Caution! Expired ScreenTape device
G1	181	7 PCRI	⚠️	Caution! Expired ScreenTape device
H1	206	8 PCRI	⚠️	Caution! Expired ScreenTape device

## Initial 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 Nxxxx Nextera i7 adapter	-	-
10uM Sxxxx Nextera i5 adapter	-	-
DNAse/RNase H20	1000 $\mu$ L	
Kapa Hifi HotStart Ready Mix (2X)	1200 $\mu$ 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 $\mu$ L
DNAse/RNase H20	21 $\mu$ L
Kapa Hifi HotStart Ready Mix (2X)	25 $\mu$ L
10ng/ $\mu$ L PCR1	1 $\mu$ L

2. Performed PCR using the following parameters:

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

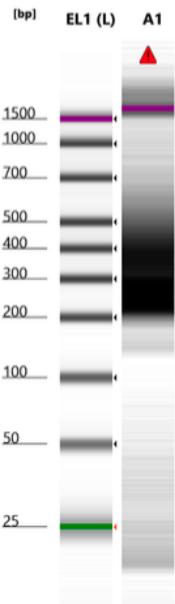
#### 8 cycles of steps 2-4

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

## Plan for tomorrow

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

**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  $\mu$ 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

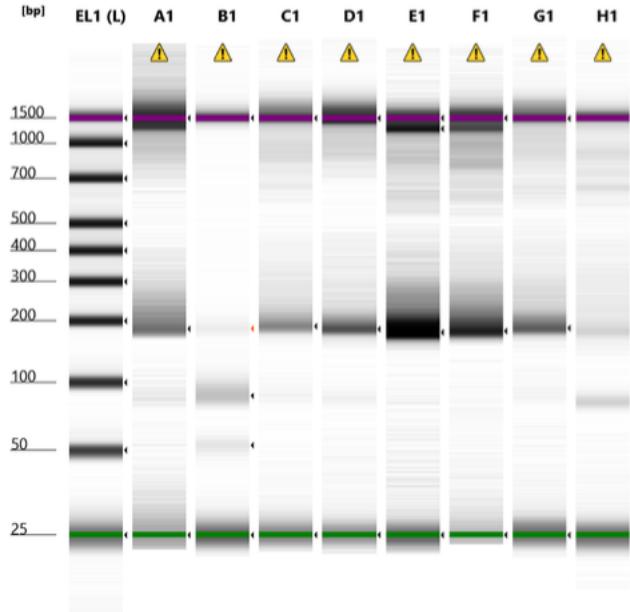
Component	Expected Volume/experiment	Material ID
Ampure Bead Kit	200 $\mu$ L	

### 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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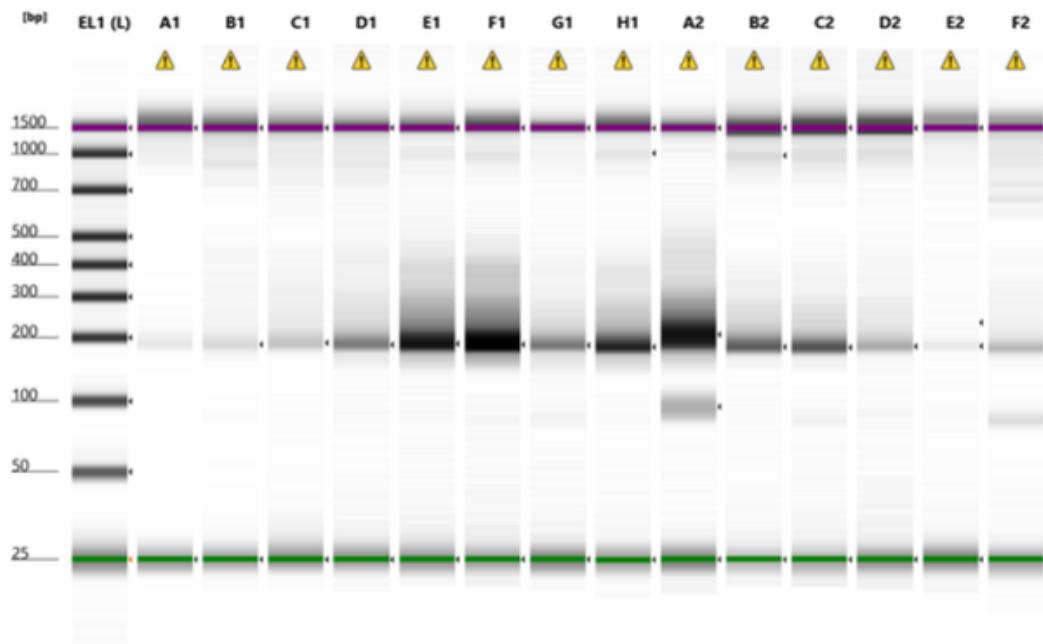
#### Sample Info

Well	Conc. [pg/ $\mu$ l]	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  $\mu$ 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 Themolabile Exonuclease I (NEB M0568) to remove excess RT primer.

Component	Volume	MM volume
Thermolabile Exonuclease I	1 $\mu$ 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 22-01-2024

### PCR 1: Samples 31-33

- Included negative control (MM + H2O)
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	<b>Step 2: 98C</b>	<b>20s</b>
Annealing	<b>Step 3: 60C</b>	<b>15s</b>
Extension	<b>Step 4: 72C</b>	<b>15s</b>
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 $\mu$ L	

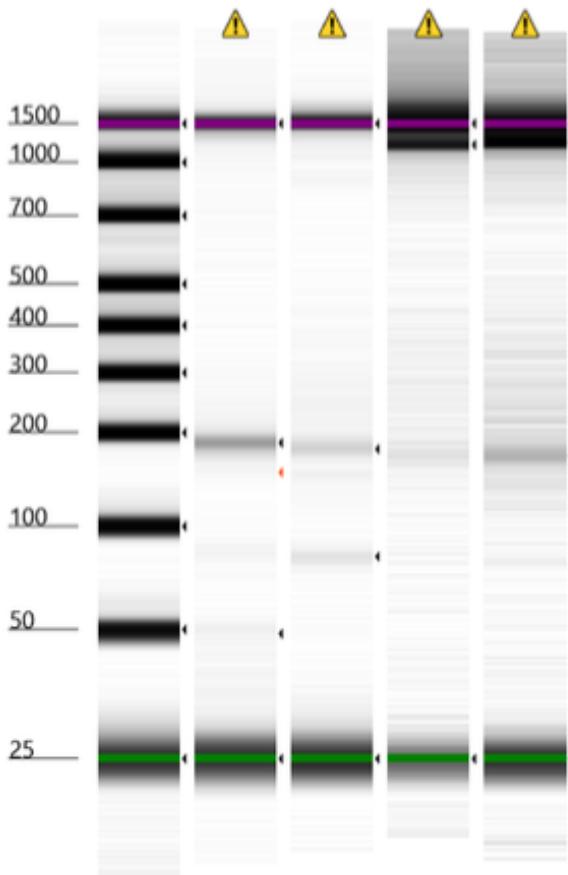
## **Protocol**

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/ $\mu$ l]	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 + H<sub>2</sub>O)
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

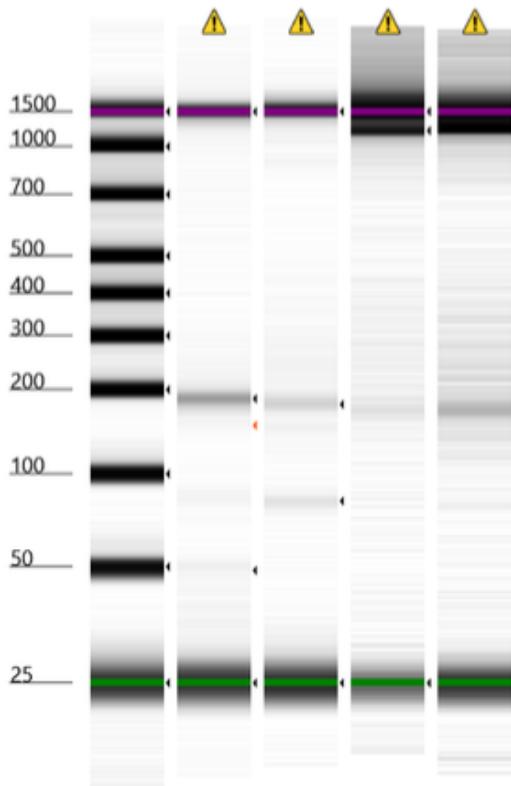
3. Performed PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	<b>Step 2: 98C</b>	<b>20s</b>
Annealing	<b>Step 3: 60C</b>	<b>15s</b>
Extension	<b>Step 4: 72C</b>	<b>15s</b>
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



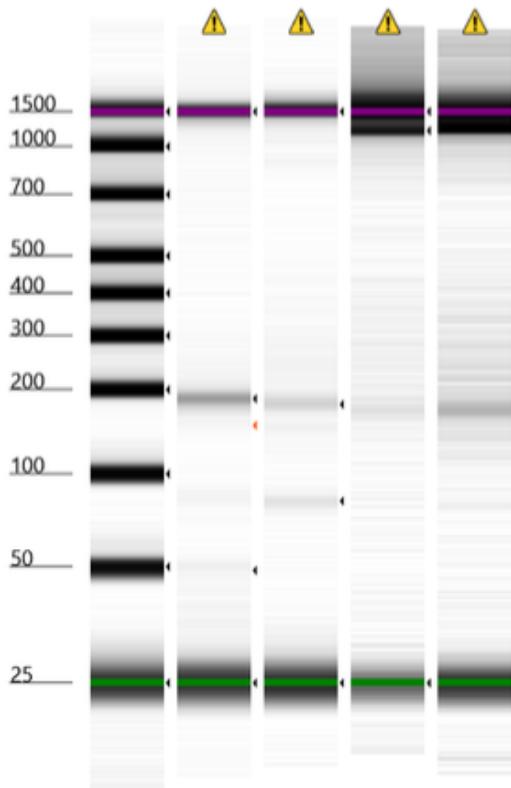
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### Sample Info

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 H2O
  - Wanted to determine if water was contaminated



Default image (Contrast 100%)

### 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 remove 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 grow 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 + H<sub>2</sub>O)
- 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 priemr
- Primers were reconstituted 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	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 $\mu\text{L}$
H <sub>2</sub> O	10 $\mu\text{L}$

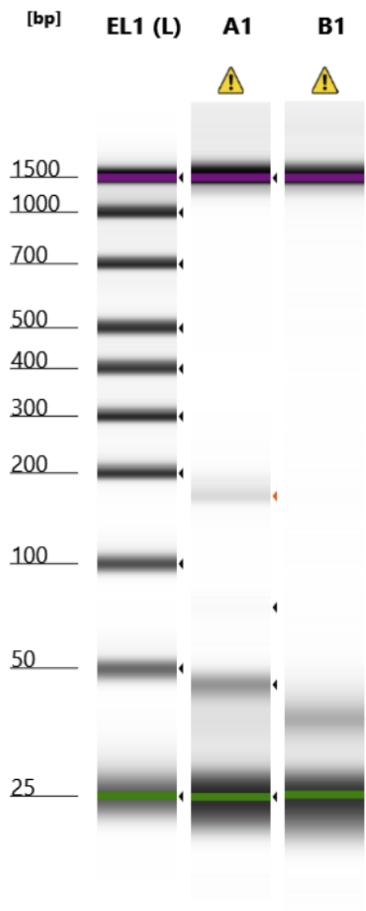
2. Performed PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	<b>Step 2: 98C</b>	<b>20s</b>
Annealing	<b>Step 3: 60C</b>	<b>15s</b>
Extension	<b>Step 4: 72C</b>	<b>15s</b>
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



Default image (Contrast 100%)

### Sample Info

Well	Conc. [ng/ $\mu$ l]	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 injection
- 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 $\mu$ L
100 mM DTT	1 $\mu$ L	33.5 $\mu$ L
RNase Out	1 $\mu$ L	33.5 $\mu$ 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	1 $\mu$ L	33.5 $\mu$ L
NEBuffer r3.1*	2 $\mu$ L	67 $\mu$ 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.
  - 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 + H2O)
- 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 reconstituted at 100  $\mu$ M so needed to be diluted to 10  $\mu$ M

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	16 $\mu$ L
DNase/RNase H2O	144 $\mu$ L

### PCR1 MM

Component	Volume	Master Mix
10uM WS PCR1 Primer Mix	3 $\mu$ L	
DNase/RNase H2O	12 $\mu$ L	
Q5 Master Mix	25 $\mu$ L	

- Used Q5 instead of KAPA
- 1. Add following components to tubes

Component	Volume
PCR1 MasterMix	40 $\mu$ L
H2O	10 $\mu$ L

2. Performed PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	<b>Step 2: 98C</b>	<b>20s</b>
Annealing	<b>Step 3: 60C</b>	<b>15s</b>
Extension	<b>Step 4: 72C</b>	<b>15s</b>
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 $\mu$ L	

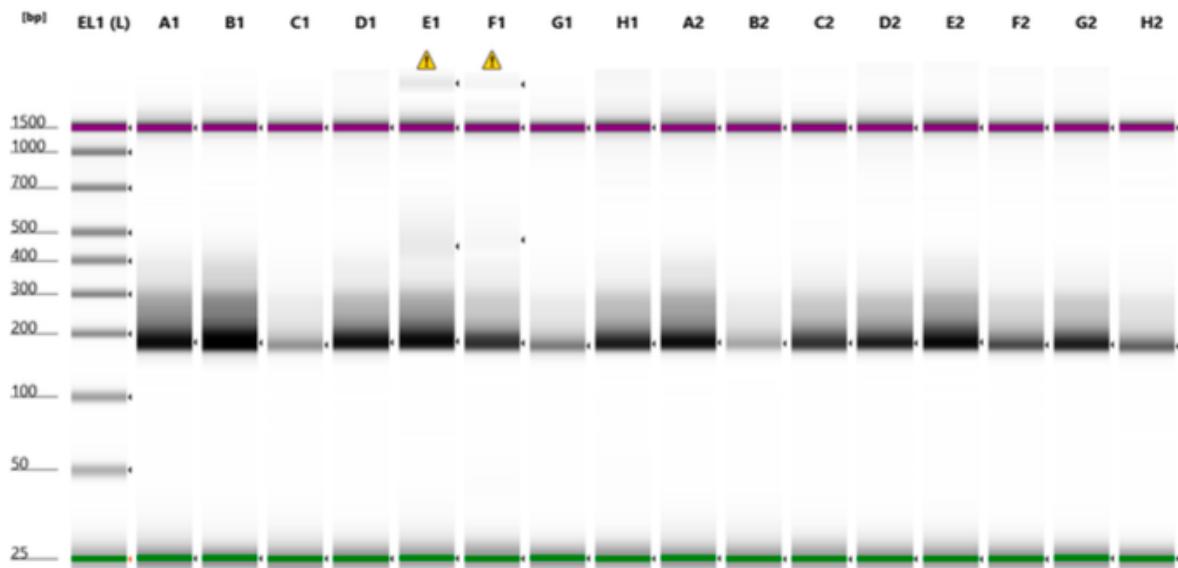
### Protocol

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 1-33

### Tapestation PCR 1 1-16

### Tapestation PCR 1



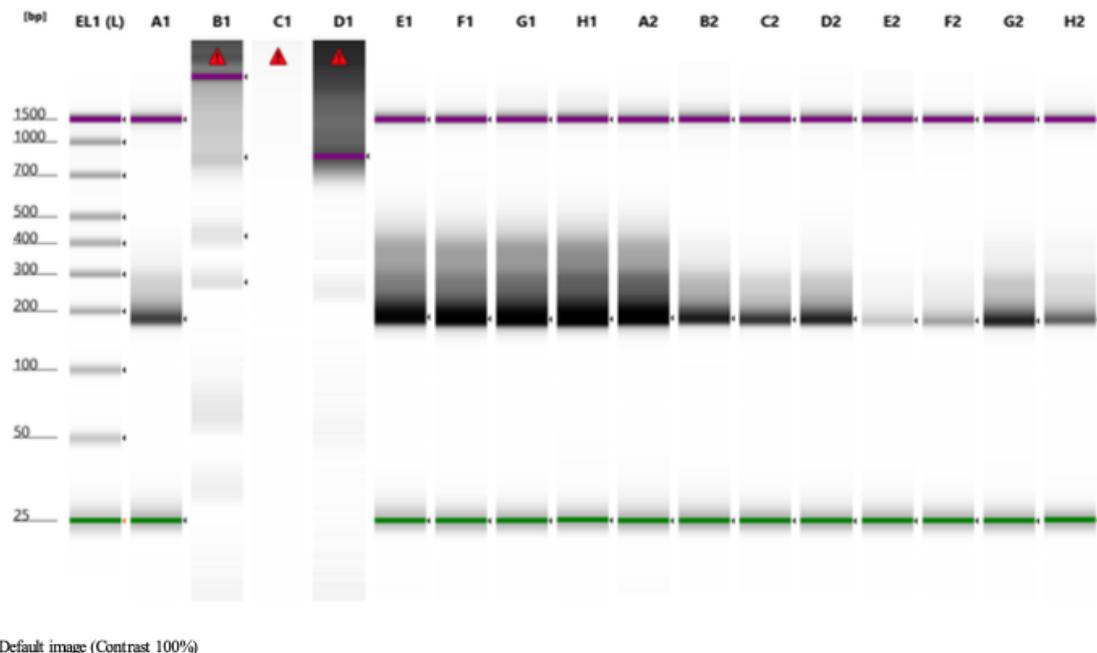
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#### Sample Info

Well	Conc. [ng/ $\mu$ l]	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	<span style="color: yellow;">⚠</span>	Peak out of Sizing Range
F1	12.7	6 PCR 1	<span style="color: yellow;">⚠</span>	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



### Sample Info

Well	Cone. [ng/ $\mu$ l]	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

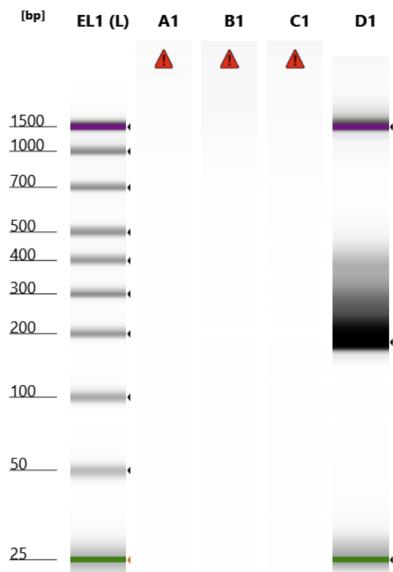
## Tapestation PCR 1

- 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/ $\mu$ l]	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 H2O and diluted to 100  $\mu$ M
- Diluted into strip tubes working stock (10 $\mu$ M)
  - 90  $\mu$ L H2O + 10  $\mu$ L 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 $\mu$ L	
DNase/RNase H2O	12 $\mu$ L	
Q5 Master Mix	25 $\mu$ L	

- Used Q5 instead of KAPA

1. Add following components to tubes

Component	Volume
PCR1 MasterMix	40 $\mu$ L
H2O	10 $\mu$ L

2. Performed PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	<b>Step 2: 98C</b>	<b>20s</b>
Annealing	<b>Step 3: 60C</b>	<b>15s</b>
Extension	<b>Step 4: 72C</b>	<b>15s</b>
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 $\mu$ 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 Samples 18-20

### Tapestation PCR 1 18-20

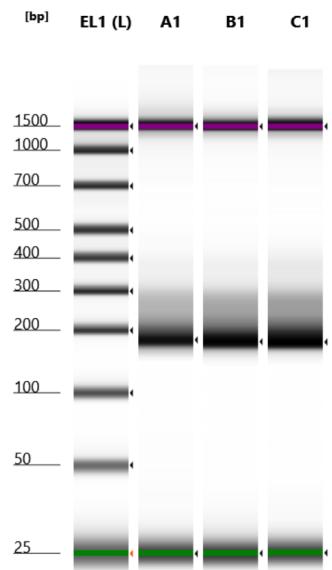
### Tapestation PCR 1

**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/ $\mu$ l]	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 Nxxxx Nextera i7 adapter	1.5 $\mu$ L
10uM Sxxxx Nextera i5 adapter	1.5 $\mu$ L
DNAse/RNase H20	21 $\mu$ L
Q5	25 $\mu$ L
10ng/ $\mu$ L PCR1	1 $\mu$ L

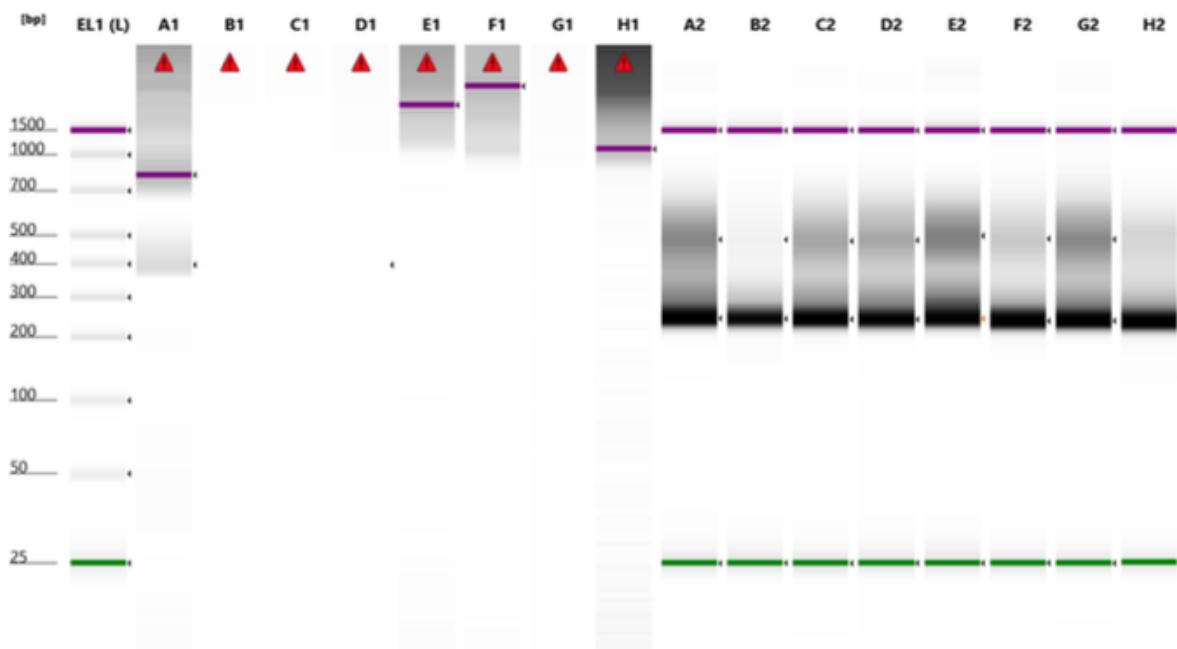
2. Perform PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	<b>Step 2: 98C</b>	<b>20s</b>
Annealing	<b>Step 3: 55C</b>	<b>15s</b>
Extension	<b>Step 4: 72C</b>	<b>15s</b>
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

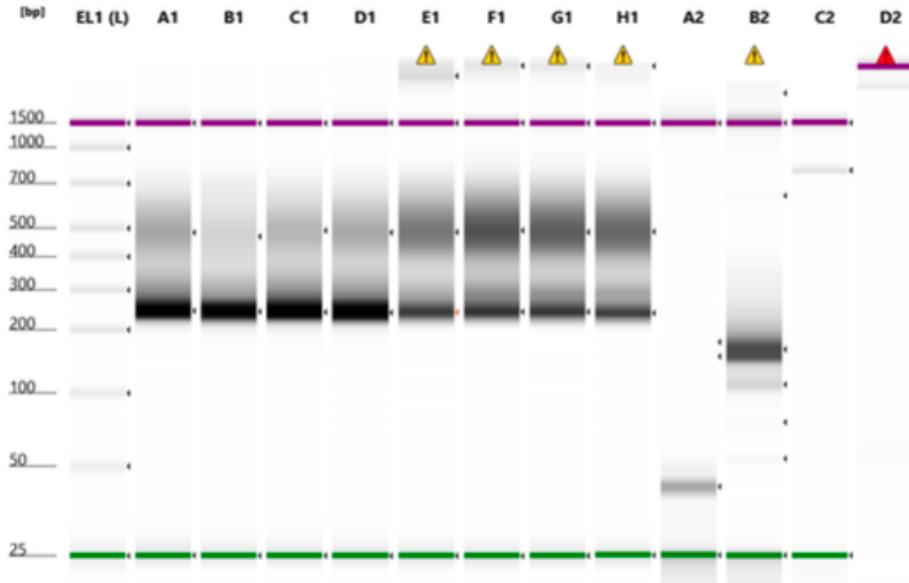


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## Sample Info

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 Nxxxx Nextera i7 adapter	1.5 $\mu$ L
10uM Sxxxx Nextera i5 adapter	1.5 $\mu$ L
DNAse/RNase H20	21 $\mu$ L
Q5	25 $\mu$ L
10ng/ $\mu$ L PCR1	1 $\mu$ L

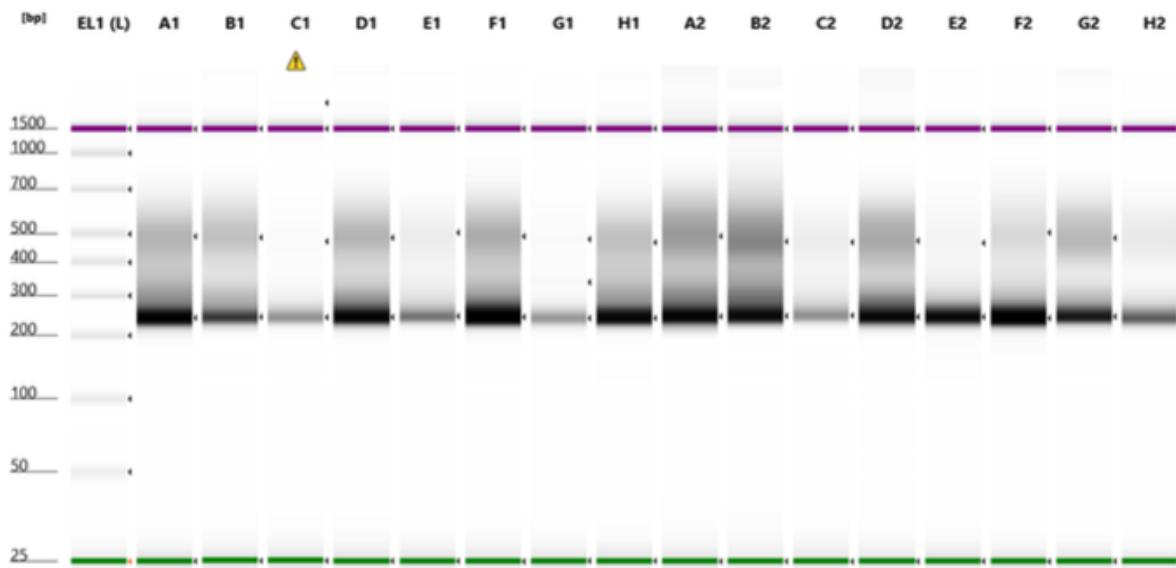
2. Perform PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	<b>Step 2: 98C</b>	<b>20s</b>
Annealing	<b>Step 3: 55C</b>	<b>15s</b>
Extension	<b>Step 4: 72C</b>	<b>15s</b>
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.

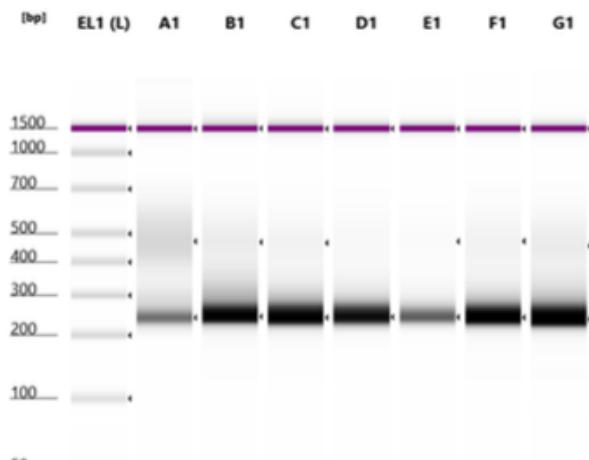
## Tapestation



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### Sample Info

Well	Cone. [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
- Split 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 production
- 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 scRNAsq

**Tuesday 13-02-2024**

## Cell Culture

HEK

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

## Processing scRNAsq

**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<sup>6</sup> 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 gently 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<sup>6</sup> 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
- $4 \times 10^6$  cells per vial
- Used AR-5

#### **HEK**

- Froze down 7 vials
- $6 \times 10^6$  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)
  - $4.5 \times 10^6$  cells seeded per dish (seeded at 13:00)

## **March 2024**

### **Wednesday 06-03-2024**

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

### **Wednesday 13-03-2024**

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

### **Thursday 14-03-2024**

#### **Cell Culture - N2**

- 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  $5 \times 10^6$  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 healthy
- Split  $5 \times 10^6$  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 without 10% Human Serum (HS)
  - Used Heat-Inactivated Serum which does not have functional complement which is why this experiment did not work
- Plate seeding protocol:
  1. Diluted cell suspension to seed 20000 cells/well in 50 $\mu$ L amounts

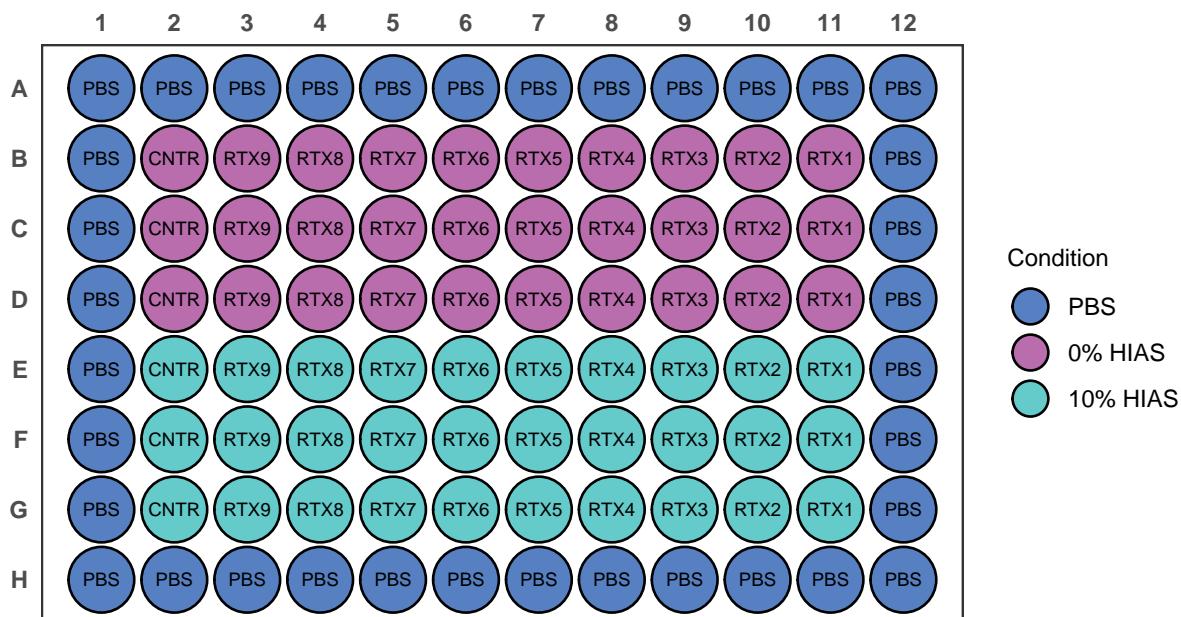
Well Number	Cells total	Volume total	CS cells/mL	Flask cell count	Flask Volume	Media Volume
60 wells	1.2x10 <sup>6</sup> cells	3 mL	4x10 <sup>5</sup>	3.06x10 <sup>6</sup>	400 $\mu$ L	2.6 mL

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

Dilution ID	Well [RTX] ( $\mu$ g/mL)	RTX Source	Source Volume ( $\mu$ L)	Media Volume ( $\mu$ L)	Working Stock [RTX] ( $\mu$ L/mL)
RTX 1	93.0722892	Stock	15	400	372.289157
RTX 2	46.5361446	RTX 1	200	200	186.144578
RTX 3	23.2680723	RTX 2	200	200	93.072289
RTX 4	11.6340361	RTX 3	200	200	46.536145
RTX 5	5.8170181	RTX 4	200	200	23.268072
RTX 6	2.9085090	RTX 5	200	200	11.634036
RTX 7	1.4542545	RTX 6	200	200	5.817018
RTX 8	0.7271273	RTX 7	200	200	2.908509
RTX 9	0.3635636	RTX 8	200	200	1.454255
CNTR	0.0000000	-	-	400	0.000000

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  $\mu$ L/well
  - Serum volume is added in 1:4 ratio (20 $\mu$ L serum in 200 $\mu$ L final well volume)
  - Serum stock mix is 1.2mL HS : 2.4 mL media / plate
4. Plate is incubated for 72 hrs at 37C

## EC50\_RTX\_N2-BC4\_250322



**Monday 25-03-2024**

### EC50 RTX N2-BC4 22324 - Collection

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

### EC50 RTX N2-BC4 25324 - Seeding (DNW)

- Seeded an EC50 experiment comparing the effects of RTX on N2-BC4 with or without 10% serum
  - Used Heat-Inactivated Serum which does not have functional complement which is why this experiment did not work
- 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 25 $\mu$ L amounts

Well Number	Cells total	Volume total	CS cells/mL	Flask cell count	Flask Volume	Media Volume
60 wells	1.2x10 <sup>6</sup> cells	1.5 mL	8x10 <sup>5</sup>	2.92x10 <sup>6</sup>	410 $\mu$ L	1.1 mL

2. Made RTX dilutions and added to respective wells in 50 $\mu$ L
  - [RTX stock] = 10.3 mg/mL
  - Drug volumes are added in triplicate

- Drug volumes are being added constitute 1/4 of well volume: [RTX working] needs to be 4x [RTX well]
- 6 wells per condition,  $25\mu\text{L}$  per well ~ minimum of  $150\mu\text{L}$  per condition needed (recommend  $200\mu\text{L}$ )

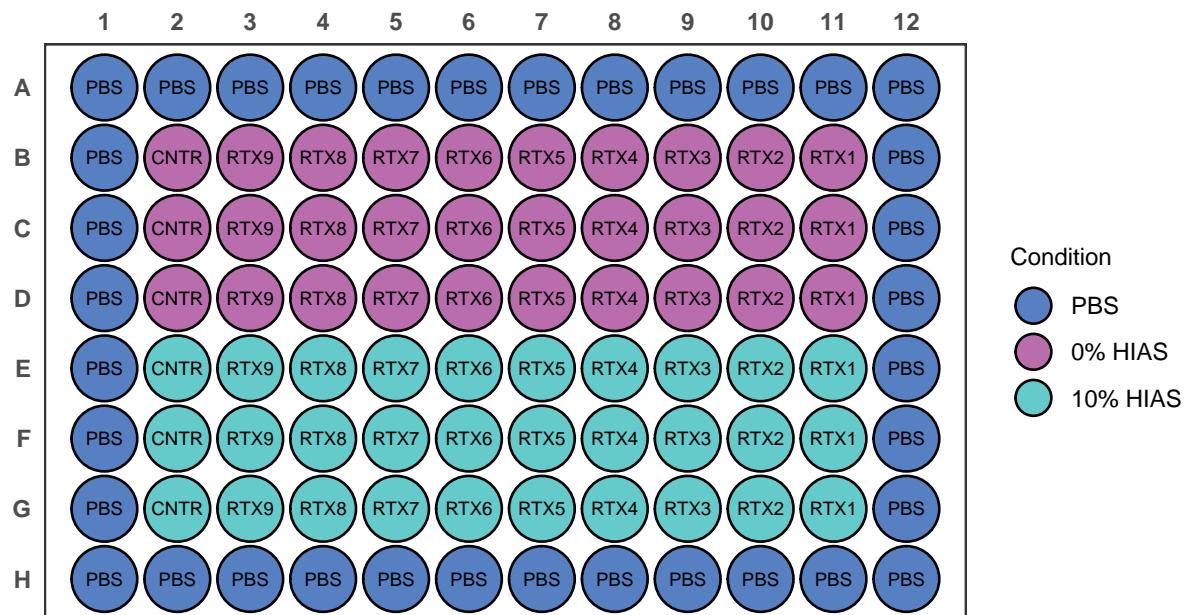
Dilution ID	Well [RTX] ( $\mu\text{g/mL}$ )	RTX Source	Source Volume ( $\mu\text{L}$ )	Media Volume ( $\mu\text{L}$ )	Working Stock [RTX] ( $\mu\text{L/mL}$ )
RTX 1	93.0722892	Stock	15	400	372.289157
RTX 2	46.5361446	RTX 1	200	200	186.144578
RTX 3	23.2680723	RTX 2	200	200	93.072289
RTX 4	11.6340361	RTX 3	200	200	46.536145
RTX 5	5.8170181	RTX 4	200	200	23.268072
RTX 6	2.9085090	RTX 5	200	200	11.634036
RTX 7	1.4542545	RTX 6	200	200	5.817018
RTX 8	0.7271273	RTX 7	200	200	2.908509
RTX 9	0.3635636	RTX 8	200	200	1.454255
CNTR	0.0000000	-	-	400	0.000000

### 3. Added 10% serum or media control

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

### 4. Plate is incubated for 72 hrs at 37°C

**EC50\_RTX\_N2-BC4\_25324**



## Cell Culture

### N2-BC4

- Cells look healthy
- Feeders in flask are also healthy
- Split  $5 \times 10^6$  back into T175 with 30mL of AR-6

## Wednesday 27-03-2024

### Cell Culture

- Got Ramos BC 5 split from Jamie
- Cultured in T75

## Thursday 28-03-2024

### EC50 RTX N2-BC4 25325 - Collection

- Collected plate seeded on 25-03-2025
- EC Plate collection protocol:
  1. Added 40 $\mu$ L Cell Titre Blue (CTB) to each conditioned well
    - 20 $\mu$ L CTB/100 $\mu$ L of conditioned well recommended by manufacturer
  2. Incubated for 1hr at 37C
  3. Read on plate reader according to Cell Titre Blue Protocol
- Results: DNW

## Friday 29-03-2024

### EC50 RTX N2-BC4/RAMOS-BC5 25329 - Seeding (DNW)

- Seeded an EC50 experiment comparing the effects of RTX on N2-BC4 and RAMOS-BC5 with or without 10% serum
  - Used Heat-Inacted Serum which does not have functional complement which is why this experiment did not work
- 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 25 $\mu$ L amounts

Cell Line	Well Number	Cells total	Volume total	CS cells/mL	Flask cell count	Flask Volume
N2-BC4	60 wells	1.2x10 <sup>6</sup> cells	1.5 mL	8x10 <sup>5</sup>	2.92x10 <sup>6</sup>	410 $\mu$ L
RAMOS-BC5	60 wells	1.2x10 <sup>6</sup> cells	1.5 mL	8x10 <sup>5</sup>	2.92x10 <sup>6</sup>	410 $\mu$ L

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

Dilution ID	Well [RTX] ( $\mu$ g/mL)	RTX Source	Source Volume ( $\mu$ L)	Media Volume ( $\mu$ L)	Working Stock [RTX] ( $\mu$ L/mL)
RTX 1	95.8600770	Stock	58	1500	383.440308
RTX 2	47.9300385	RTX 1	750	750	191.720154
RTX 3	23.9650193	RTX 2	750	750	95.860077
RTX 4	11.9825096	RTX 3	750	750	47.930039
RTX 5	5.9912548	RTX 4	750	750	23.965019
RTX 6	2.9956274	RTX 5	750	750	11.982510

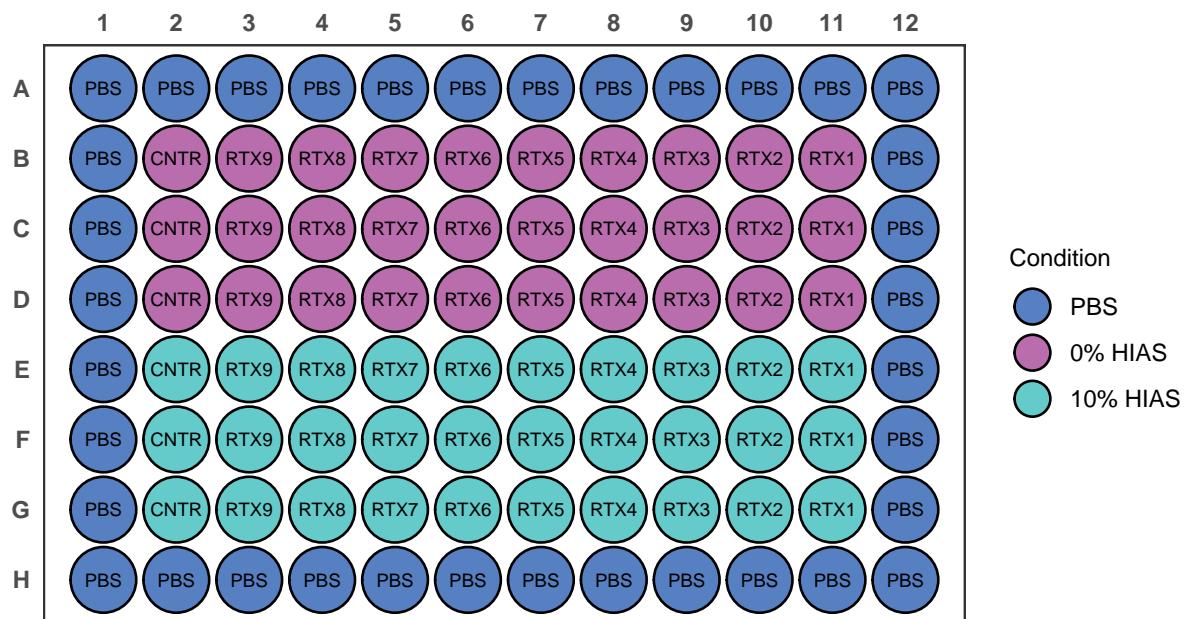
Dilution ID	Well [RTX] (µg/mL)	RTX Source	Source Volume (µL)	Media Volume (µL)	Working Stock [RTX] (µL/mL)
RTX 7	1.4978137	RTX 6	750	750	5.991255
RTX 8	0.7489069	RTX 7	750	750	2.995627
RTX 9	0.3744534	RTX 8	750	750	1.497814
CNTR	0.0000000	-	-	750	0.000000

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

**EC50\_RTX\_N2-BC4\_250324**



**Sunday 31-03-2024**

**Cell Culture**

**N2-BC4**

- Healthy, split

**Changed FDC:**

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 T175 flask
9. Added 30mL of DMEM
10. Added  $5 \times 10^5$  N2 cells from previous flask
11. Placed in incubator

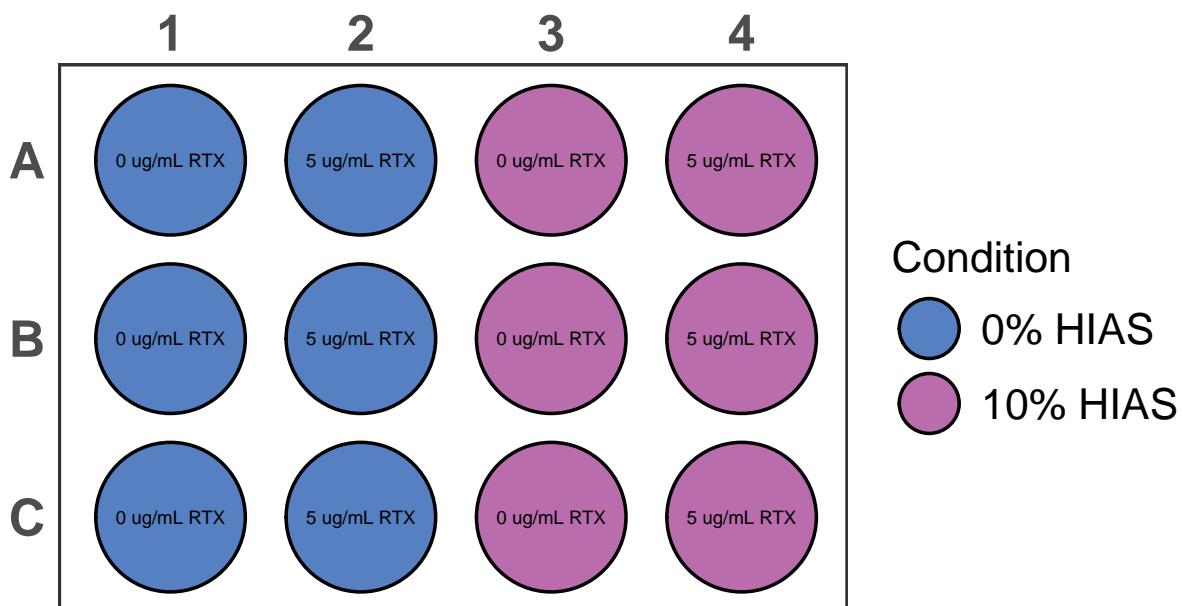
### Ramos-BC5

- Healthy, split
- Count:  $2.18 \times 10^6$  cells/mL
- Split:  $7 \times 10^5$  cells in 15 mL

### Ramos CDC Testing

- Tested CDC assay adapted from [Ge et al., 2019](#)
  - CDC protocol
1. Seed 250k cells 500uL volumes per well of a 12-well plate
  2. Add RTX or media in 250uL volumes per well
    - [RTX] working needs to be 4x final desired well volume
  3. Add Serum or media in 250uL volumes per well
    - Serum amount should be 4x final % desired

**EC50\_RTDX\_N2-BC4\_250331**



### EC50 RTX N2-BC4/RAMOS-BC5 24331 - Seeding (DNW)

- Seeded an EC50 experiment 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
- Only incubated for 24hr
- Plate seeding protocol:
  1. Diluted cell suspension to seed 20000 cells/well in 25 $\mu$ L amounts

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

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 constitute 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)

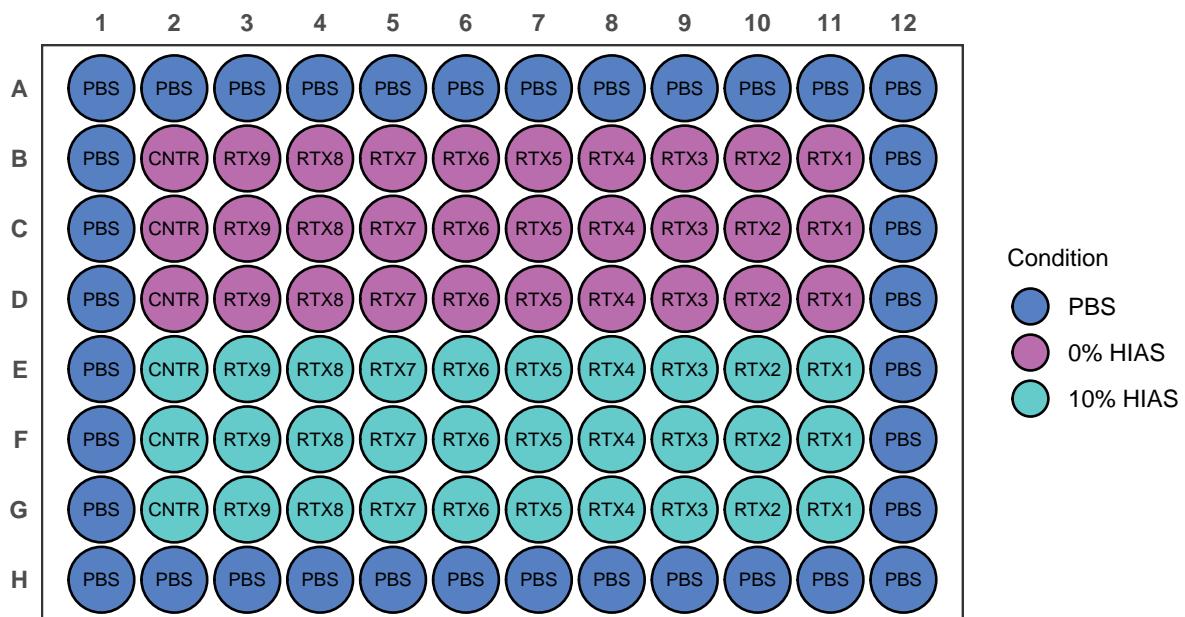
Dilution ID	Well [RTX] (μg/mL)	RTX Source	Source Volume (μL)	Media Volume (μL)	Working Stock [RTX] (μL/mL)
RTX 1	95.8600770	Stock	58	1500	383.440308
RTX 2	47.9300385	RTX 1	750	750	191.720154
RTX 3	23.9650193	RTX 2	750	750	95.860077
RTX 4	11.9825096	RTX 3	750	750	47.930039
RTX 5	5.9912548	RTX 4	750	750	23.965019
RTX 6	2.9956274	RTX 5	750	750	11.982510
RTX 7	1.4978137	RTX 6	750	750	5.991255
RTX 8	0.7489069	RTX 7	750	750	2.995627
RTX 9	0.3744534	RTX 8	750	750	1.497814
CNTR	0.0000000	-	-	750	0.000000

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

## EC50\_RTX\_N2-BC4\_250331



**April**

**Monday 01-04-2024**

**Cell Culture**

**N2**

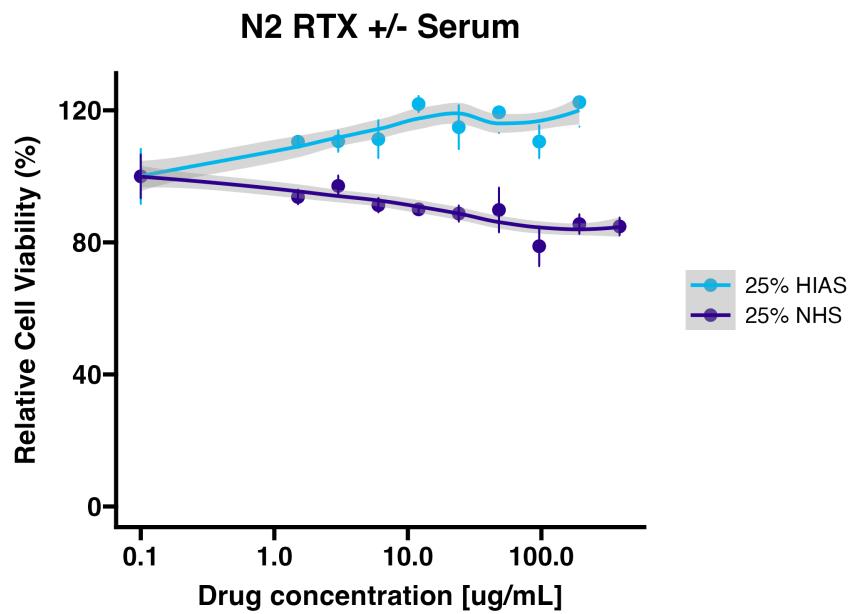
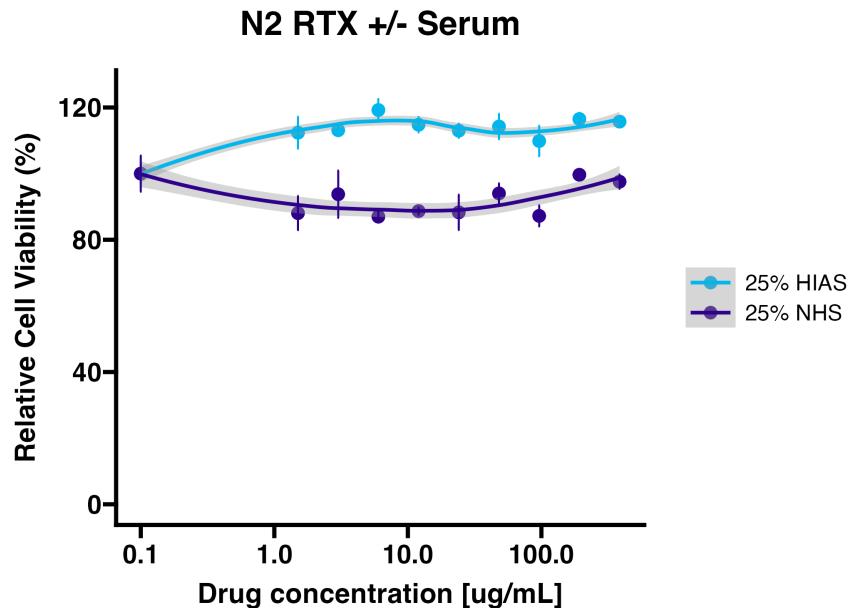
- Not ready to split
- Added 5mL media

**Ramos BC 5**

- Count:  $1.6 \times 10^6$  cells/mL
- Seeded:  $5 \times 10^5$  cells in 15mL

**EC50 Collection: 24329/24331**

- Collected plates seeded on 24-03-2024 and 31-3-2024
- EC Plate collection protocol:
  1. Added  $40\mu\text{L}$  Cell Titre Blue (CTB) to each conditioned well  
–  $20\mu\text{L}$  CTB/ $100\mu\text{L}$  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/RAMOS-BC5 24401 - Seeding (DNW)

- Seeded an EC50 experiment comparing the effects of RTX on N2-BC4 and RAMOS-BC5 with or without 10% serum
- Increased [RTX] to 1mg/mL (closer to Sorcha EC50)
- Used RTX provided by Jamie
- Plate seeding protocol:
  - Diluted cell suspension to seed 20000 cells/well in 25 $\mu\text{L}$  amounts

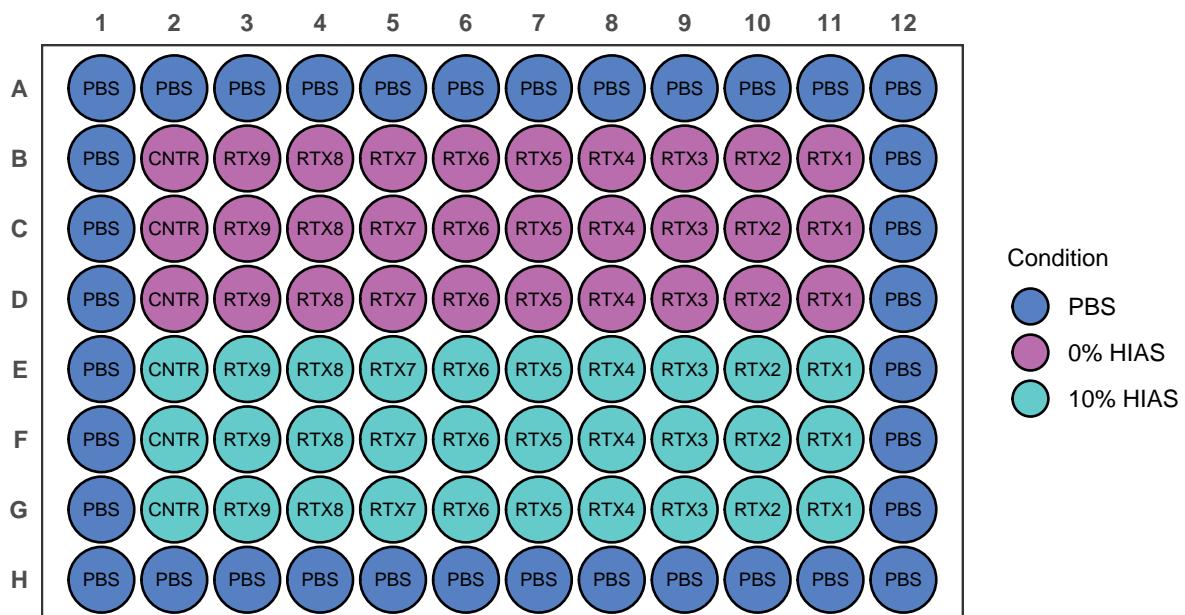
Cell Line	Well Number	Required Cell / Volume total	Required cells/mL	Stock cells/mL	Stock CS Volume
RAMOS-BC5	60 wells	1.6x10 <sup>6</sup> cells in 2 mL	8x10 <sup>5</sup>	1.6x10 <sup>6</sup>	1 mL

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

Dilution ID	Well [RTX] ( $\mu$ g/mL)	RTX Source	Source Volume ( $\mu$ L)	Media Volume ( $\mu$ L)	Working Stock [RTX] ( $\mu$ L/mL)
RTX 1	1030.000000	Stock	360	540	4120.00000
RTX 2	515.000000	RTX 1	450	450	2060.00000
RTX 3	257.500000	RTX 2	450	450	1030.00000
RTX 4	128.750000	RTX 3	450	450	515.00000
RTX 5	64.375000	RTX 4	450	450	257.50000
RTX 6	32.187500	RTX 5	450	450	128.75000
RTX 7	16.093750	RTX 6	450	450	64.37500
RTX 8	8.046875	RTX 7	450	450	32.18750
RTX 9	4.023438	RTX 8	450	450	16.09375
CNTR	0.000000	-	-	900	0.00000

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  $\mu$ L/well
  - Serum volume is added in 1:4 ratio (10 $\mu$ L serum in 100 $\mu$ L final well volume)
  - Serum stock mix is 600 $\mu$ L HS : 1.2 mL media / plate
4. Plate is incubated for 72 hrs at 37C

## EC50\_RTG\_N2-BC4\_240401



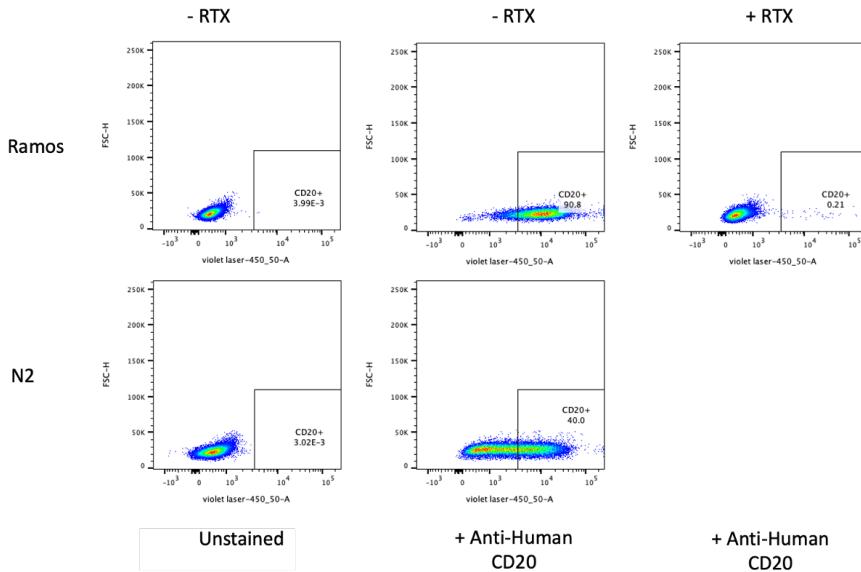
**Tuesday 02-04-2024**

### N2/Ramos CD20 Flow Cytometry

- Got antibody from Chris for CD20
- Cat#: 562873

Cell Stain Protocol:

1. Resuspend cells to  $1 \times 10^6$  cells in 1mL
2. Distribute CS into 3 wells of a 96-well plate
3. Spin down @ 1500g for 2min
4. Flick media out
5. Make Cell Stain:
  - 500uL PBS + 2uL Stain -Make this while cells are spinning down
6. Resuspend w/ 100uL in the first well and then resuspend the next 2 wells in the same stain volume
  - Consolidate into 1 well/sample
7. incubate in the fridge for 20min
8. Spin down @ 1500g for 2 min
9. Transfer to FACS tube and run
  - Results:



**Thursday 04-04-2024**

### Cell Culture

#### N2

- Count:  $2.1 \times 10^6$  cells/mL
- Seeded:  $5 \times 10^5$  cells in 15mL

#### Ramos

- Count:  $1.9 \times 10^6$  cells/mL
- Seeded:  $5 \times 10^5$  cells in 15mL

### EC50 Collection: 240401

- Collected plate seeded on 01-04-2024
- EC Plate collection protocol:
  1. Added  $40\mu\text{L}$  Cell Titre Blue (CTB) to each conditioned well  
–  $20\mu\text{L}$  CTB/ $100\mu\text{L}$  of conditioned well recommended by manufacturer
  2. Incubated for 1hr at 37C
  3. Read on plate reader according to Cell Titre Blue Protocol
- Results:DNW

**Monday 08-04-2024**

### Cell Culture

#### N2

- Count:  $1.4 \times 10^6$  cells/mL
- Seeded:  $3 \times 10^5$  cells in 15mL

#### Ramos

- Count:  $2.9 \times 10^6$  cells/mL

- Seeded:  $3 \times 10^5$  cells in 15mL

## Tuesday 09-04-2024

### Perla Drug Randomizer

- Randomized drugs for Perla Pucci mouse experiment

Drug	Identification
Vehicle	B
Beta 30	F
Beta 10	D
Plo 30	A
Plo 10	E
TESA 4	C
TESA0,4	G

## Saturday 14-04-2024

### Cell Culture

#### N2-BC4

- Count:  $6.7 \times 10^5$  cells/mL
- Seeded:  $3 \times 10^5$  cells in 15mL

#### Ramos-BC5

- Count:  $6.0 \times 10^5$  cells/mL
- Seeded:  $3 \times 10^5$  cells in 15mL

### EC50 RTX N2-BC4/RAMOS-BC5 24414 - Seeding (DNW)

- Seeded an EC50 experiment comparing the effects of RTX on N2-BC4 and RAMOS-BC5 with or without 10% serum
- Increased [RTX] to 1mg/mL (closer to Sorcha EC50)
- Used RTX provided by Jamie
- Looked at cell-intrinsic effects of RTX (no serum)
- Plate seeding protocol:
  1. Diluted cell suspension to seed 20000 cells/well in  $50\mu\text{L}$  amounts

Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	Stock CS cells/mL	Stock Volume	Media Volume
Plate 1	RAMOS	$6.00 \times 10^5$	$6.00 \times 10^5$	$1.50 \times 10^3$	$4.00 \times 10^5$	$1.00 \times 10^0$	$5.00 \times 10^{-1}$
Plate 2	N2-BC	$6.00 \times 10^5$	$6.00 \times 10^5$	$1.50 \times 10^3$	$4.00 \times 10^5$	$1.00 \times 10^0$	$5.00 \times 10^{-1}$

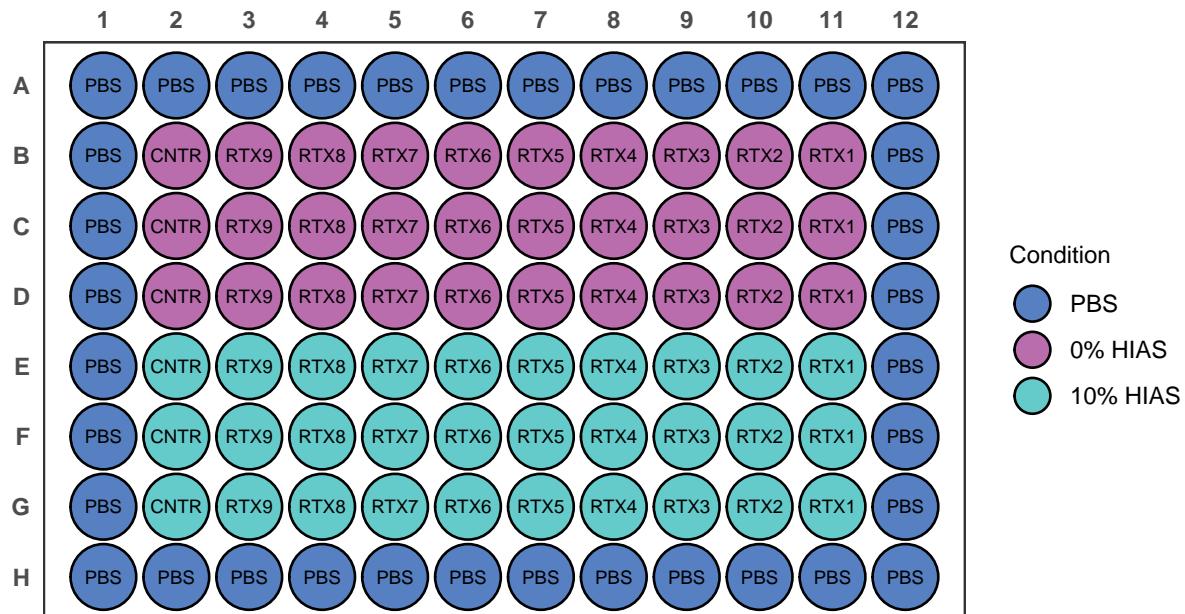
2. Made RTX dilutions and added to respective wells in  $25\mu\text{L}$ 
  - [RTX stock] = 10.3 mg/mL
  - Drug volumes are added in triplicate
  - Drug volumes are being added constitute 1/4 of well volume: [RTX working] needs to be 4x [RTX well]

- 6 wells per condition, 25 $\mu$ L per well ~ minimum of 150 $\mu$ L per condition needed (recommend 200 $\mu$ L)

Dilution ID	Well [RTX] ( $\mu$ g/mL)	RTX Source	Source Volume ( $\mu$ L)	Media Volume ( $\mu$ L)	Working Stock [RTX] ( $\mu$ L/mL)
RTX 1	515.000000	Stock	200	800	2060.000000
RTX 2	257.500000	RTX 1	500	500	1030.000000
RTX 3	128.750000	RTX 2	500	500	515.000000
RTX 4	64.375000	RTX 3	500	500	257.500000
RTX 5	32.187500	RTX 4	500	500	128.750000
RTX 6	16.093750	RTX 5	500	500	64.375000
RTX 7	8.046875	RTX 6	500	500	32.187500
RTX 8	4.023438	RTX 7	500	500	16.093750
RTX 9	2.011719	RTX 8	500	500	8.046875
CNTR	0.000000	-	-	1000	0.000000

3. Plate is incubated for 72 hrs at 37C

### EC50\_RTX\_N2-BC4\_240414



Wednesday 17-04-2024

### EC50 Collection: 240414

- Collected plate seeded on 14-04-2024
- EC Plate collection protocol:
  - Added 40 $\mu$ L Cell Titre Blue (CTB) to each conditioned well
    - 20 $\mu$ L CTB/100 $\mu$ L of conditioned well recommended by manufacturer
  - Incubated for 1hr at 37C
  - Read on plate reader according to Cell Titre Blue Protocol
- Results:DNW

## **Monday 22-04-2024**

### **Cell Culture**

#### **N2-BC4**

- Seeded:  $3 \times 10^5$  cells in 15mL

#### **Ramos-BC5**

- Seeded:  $3 \times 10^5$  cells in 15mL

### **Thawed NK-92**

- Obtained from Chris Steele
- Cultured in Advanced RPMI
- Added 200u/mL of IL-2 (#78036.1)
  - Obtained from Emily
  - Product listed as  $4.1 \times 10^4$  IU/ug
  - Stock diluted to 10ug/mL
  - Added .48 uL stock/mL of media

## **Wednesday 24-04-2024**

### **Cell Culture**

#### **N2-BC4**

- Seeded:  $3 \times 10^5$  cells in 15mL

#### **Ramos-BC5**

- Seeded:  $3 \times 10^5$  cells in 15mL

#### **NK-92**

- Looked very unhealthy
- Lots of dead cells/debris in the media
- Spun down, resuspended in 1mL of media
- Split into 4 wells of a 48 well plate with increasing amounts of IL-2

## **Friday 26-04-2024**

### **Cell Culture**

#### **N2-BC4**

- Seeded:  $3 \times 10^5$  cells in 15mL

#### **Ramos-BC5**

- Seeded:  $3 \times 10^5$  cells in 15mL

#### **NK-92**

- Cells still look unhealthy
- Tried to consolidate in single well of 24 well plate

- Spun down in eppendorf and resuspended in 1mL of media

## Monday 29-04-2024

### Cell Culture

#### N2-BC4

- Seeded:  $3 \times 10^5$  cells in 15mL

#### Ramos-BC5

- Seeded:  $3 \times 10^5$  cells in 15mL

## Tuesday 30-04-2024

### Cell Culture

#### N2-BC4

- Count:  $3 \times 10^5$  cells/mL
- Seeded:  $3 \times 10^5$  cells in 12mL
- Reseeded FDC cells in T75

#### Ramos-BC5

- Count:  $4 \times 10^5$  cells/mL
- Seeded:  $4 \times 10^5$  cells in 12mL

### Human Serum Receipt

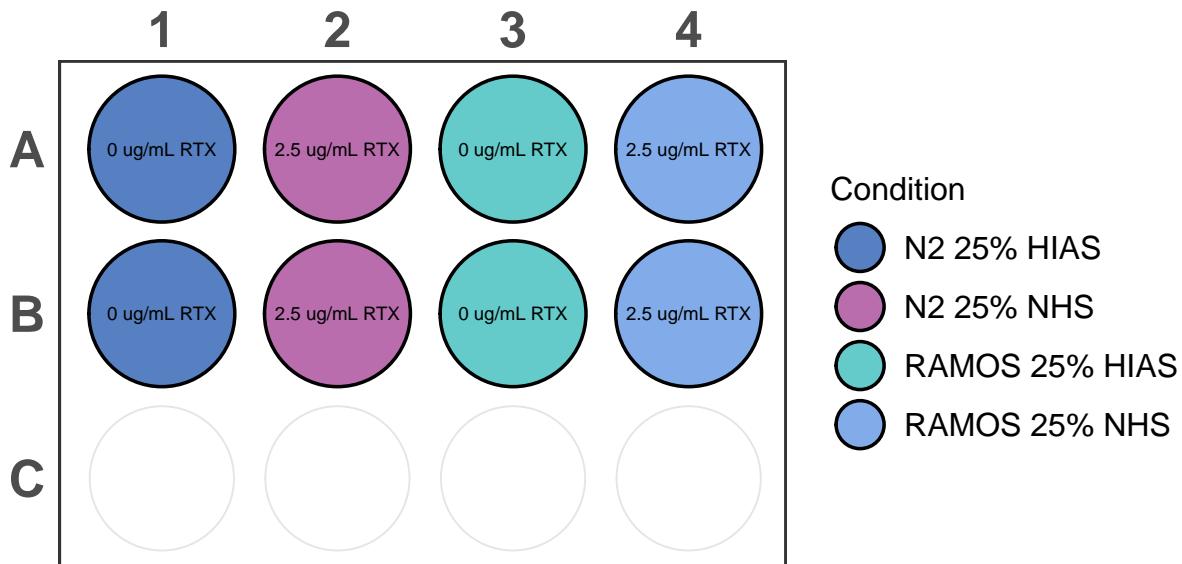
- Serum from 3 donors ordered from Cambridge Biosciences
- Serum mixed together, aliquoted, and stored at -80

Product	Product ID	Barcode ID	Volume
Human Serum - Fresh Frozen	SERSKF2SIL10-FSXX	PR24C441891	10 mL
Human Serum - Fresh Frozen	SERSKF2SIL10-FSXX	PR23K435425	10 mL
Human Serum - Fresh Frozen	SERSKF2SIL10-MSXX	PR23D435392	10 mL

### Ramos/N2 CDC Testing

- Tested CDC assay adapted from [Ge et al., 2019](#)
  - CDC protocol
1. Seed 250k cells 500uL volumes per well of a 12-well plate
  2. Added RTX or media in 250uL volumes per well
    - [RTX Stock] = 10mg/mL
    - [RTX] working needs to be 4x final desired well volume
    - 1uL RTX stock added to 1mL Media
  3. Add Serum or media in 250uL volumes per well
    - Serum amount should be 4x final % desired

# CDC Test N2 + RAMOS 240430



## Results: DNW

- No clear differences between +HIAS/+RTX and +NHS/+RTX
- Seemed relatively healthy
- Possible fixes:
  - Increase RTX dose
  - Increase Serum %
  - Increase the amount of time
  - Add at the same time instead of pre-incubating with RTX

## EC50 RTX RAMOS-BC5 24430 - Seeding

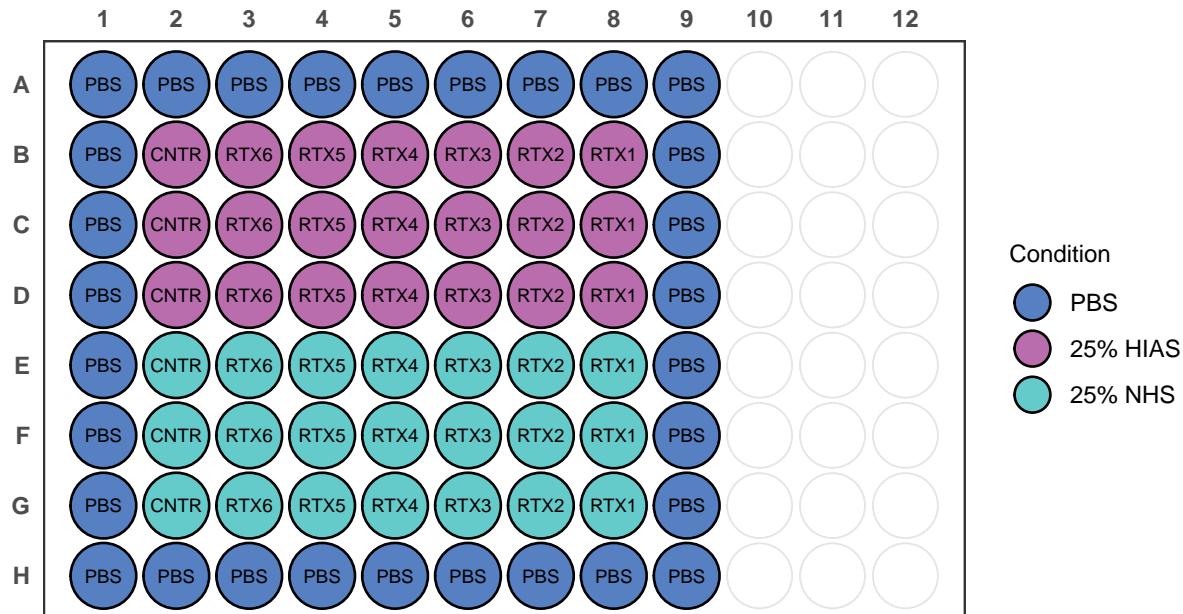
- Seeded an EC50 experiment comparing the effects of RTX on N2-BC4 and RAMOS-BC5 with or without 10% serum
- Increased [RTX] to 1mg/mL (closer to Sorcha EC50)
- Used RTX provided by Jamie
- Plate seeding protocol:
  - Diluted cell suspension to seed 20000 cells/well in 50 $\mu$ L amounts
  - Made RTX dilutions and added to respective wells in 25 $\mu$ L
    - [RTX stock] = 10.3 mg/mL
    - Drug volumes are added in triplicate
    - Drug volumes are being added constitute 1/4 of well volume: [RTX working] needs to be 4x [RTX well]
    - 6 wells per condition, 25 $\mu$ L per well ~ minimum of 150 $\mu$ L per condition needed (recommend 200 $\mu$ L)

Dilution ID	Well [RTX] ( $\mu$ g/mL)	RTX Source	Source Volume ( $\mu$ L)	Media Volume ( $\mu$ L)	Working Stock [RTX] ( $\mu$ L/mL)
RTX 1	257.500000	Stock	60	540	1030.0000
RTX 2	128.750000	RTX 1	300	300	515.0000
RTX 3	64.375000	RTX 2	300	300	257.5000
RTX 4	32.187500	RTX 3	300	300	128.7500
RTX 5	16.093750	RTX 4	300	300	64.3750

Dilution ID	Well [RTX] (µg/mL)	RTX Source	Source Volume (µL)	Media Volume (µL)	Working Stock [RTX] (µL/mL)
RTX 6	8.046875	RTX 5	300	300	32.1875
CNTR	0.000000	-	-	600	0.0000

3. Plate is incubated for 72 hrs at 37C

### EC50 CDC Test N2 + RAMOS 240430



May

Wednesday 01-05-2024

### Cell Culture

#### N2-BC4

- Count:  $3 \times 10^5$  cells/mL
- Seeded:  $3 \times 10^5$  cells in 12mL
- Reseeded FDC cells in T75

#### Ramos-BC5

- Count:  $4 \times 10^5$  cells/mL
- Seeded:  $4 \times 10^5$  cells in 12mL

June

Monday 10-06-2024

### Cell Culture

- Split cells

Cell Line Name	Count	Seeding Density
Ramos BC 1	3.3x10 <sup>6</sup> cells/mL	6.5x10 <sup>4</sup> cells/mL
Ramos BC 3	3.3x10 <sup>6</sup> cells/mL	6.5x10 <sup>4</sup> cells/mL
Ramos BC 5	3.6x10 <sup>6</sup> cells/mL	6.5x10 <sup>4</sup> cells/mL

## EC50 RTX RAMOS-BC5 240610 - Seeding

- Seeded an EC50 experiment comparing the effects of RTX on N2-BC4 and RAMOS-BC5 with or without 10% serum
- Seeded 3 plates each with a different RAMOS barcode population

Plate ID	Cell Line
Plate 1	RAMOS BC 1
Plate 2	RAMOS BC 3
Plate 3	RAMOS BC 5

- Increased [RTX] to >1mg/mL (closer to Sorcha EC50)
- Used RTX provided by Jamie
- Plate seeding protocol:
  1. Diluted cell suspension to seed 10000 cells/well in 50 $\mu$ L amounts

Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	CS cells/mL	Stock Volume (uL)	Media Volume (mL)
Plate 1	RAMOS BC 1	$3.31 \times 10^6$	$6.00 \times 10^5$		$3 \times 1.10 \times 10^6$	181.2689	2.818731
Plate 2	RAMOS BC 3	$3.30 \times 10^6$	$6.00 \times 10^5$		$3 \times 1.10 \times 10^6$	181.8182	2.818182
Plate 3	RAMOS BC 5	$3.60 \times 10^6$	$6.00 \times 10^5$		$3 \times 1.20 \times 10^6$	166.6667	2.833333

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

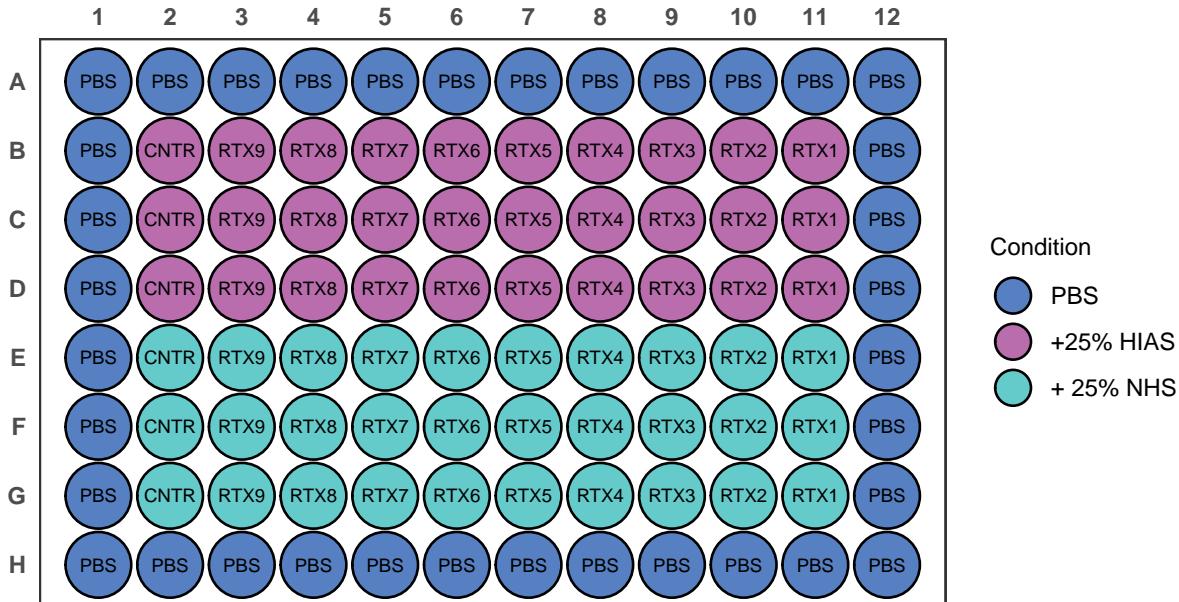
Dilution ID	Well [RTX] ( $\mu$ g/mL)	RTX Source	Source Volume ( $\mu$ L)	Media Volume ( $\mu$ L)	Working Stock [RTX] ( $\mu$ L/mL)
RTX 1	1030.000000	Stock	800	1200	4120.000000
RTX 2	515.000000	RTX 1	1000	1000	2060.000000
RTX 3	257.500000	RTX 2	1000	1000	1030.000000
RTX 4	128.750000	RTX 3	1000	1000	515.000000
RTX 5	64.375000	RTX 4	1000	1000	257.500000
RTX 6	32.187500	RTX 5	1000	1000	128.750000
RTX 7	16.093750	RTX 6	1000	1000	64.375000
RTX 8	8.046875	RTX 7	1000	1000	32.187500
RTX 9	4.023438	RTX 8	1000	1000	16.093750
CNTR	0.000000	-	-	1000	0.000000

3. Added HIAS/NHS to indicated wells

- $25\mu\text{L}/\text{well}$
- Final well volume = 25% Serum (HIAS/NHS)

4. Plate is incubated for 48 hrs at 37C

### EC50 CDC Test N2 + RAMOS 240430



**Wednesday 13-06-2024**

### Cell Culture

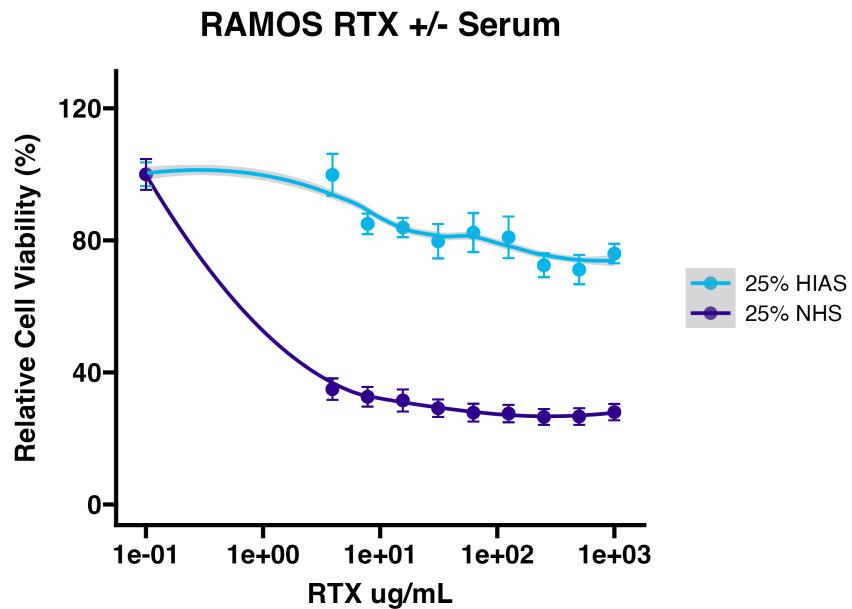
- Split cells

Cell Line Name	Count	Seeding Density	Flask	Flask Volume
Ramos BC 1	$3.3 \times 10^6$ cells/mL	$6.5 \times 10^4$ cells/mL	T75	20mL

### EC50 Collection: EC50\_RTDX\_N2-BC4\_10064

- Collected plates seeded on 10-06-2024
- EC Plate collection protocol:
  1. Added  $40\mu\text{L}$  Cell Titre Blue (CTB) to each conditioned well  
–  $20\mu\text{L}$  CTB/ $100\mu\text{L}$  of conditioned well recommended by manufacturer
  2. Incubated for 1hr at 37C
  3. Read on plate reader according to Cell Titre Blue Protocol

Results:



**Thursday 13-06-2024**

#### Cryopreservation - RAMOS-BC 1/3/5

- Cryopreserved 3 vials of the following cell lines:
- Ramos-BC 1
- Ramos-BC 3
- Ramos-BC 5
- ~ $2 \times 10^6$  cells/vial
- Freezing media: FBS + 10% DMSO

#### Protocol

1.

**Monday 24-06-2024**

#### Cell Culture

- Transferred RAMOS BC 3 to RPMI-1640

#### Making RPMI

- RPMI\_1

Solution	ID code	Volume	% Total volume
RPMI 1640	—	500 mL	89%
FBS	—	56 mL	10%
Glutamax	—	5.6 mL	1%

### Splitting Ramos BC 3

Cell Line Name	Count	Seeding Density	Flask	Flask Volume
Ramos BC 1	2x10 <sup>6</sup> cells/mL	1x10 <sup>5</sup> cells/mL	T75	20mL

1. Transferred CS to 50mL flask
2. Spun down RAMOS BC 3
3. Removed media and resuspended in 5mL of RPMI\_1

### RAMOS RTX CDC Testing 240625 - Seeding

- Set up 6 well plates and incubated for various amounts of time to look at RTX-CDC in an expanded format
- Sample Plate setup:

## EC50 CDC Test RAMOS 240624

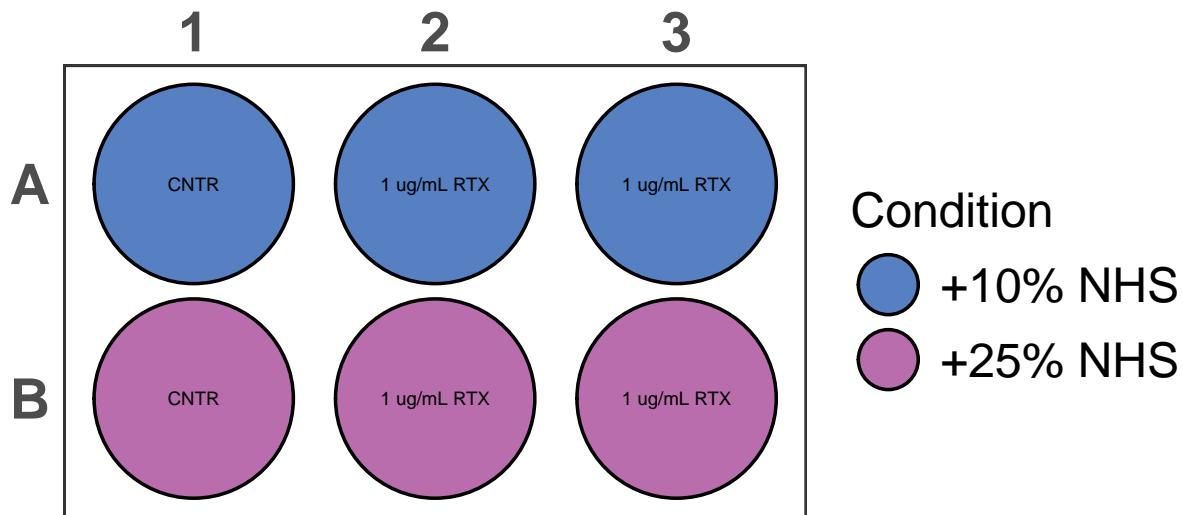


Plate Set Up: 1. Make working CS

**Tuesday 25-06-2024**

### Cell Culture

- Split RAMOS
- Spun down flask and counted
  - Count: 2x10<sup>6</sup> cells/mL
  - Added 1mL of CS in 19mL media (1x10<sup>5</sup>cells/mL)

### RAMOS RTX CDC Testing 240625 - Collection

- Assesed plates for cell death
- Used Trypan Blue Exclusion assay

#### Trypan Blue Protocol:

1. Resuspend cells in each well 2. Take 10uL sample from each well and add to respective 0.2mL tube 3. Added 10uL Trypan blue to each 0.2mL tube and mix well 4. Added 10uL sample from tube to haemocytometer 5. Counted both live/dead in all 4 squares

## Results

Well	Condition	Live	Dead	Viability %
1.A1	-RTX / +10% NHS (CNTR)	178	39	82.02765
1.A2	+RTX / +10% NHS	62	19	76.54321
1.A3	+RTX / +10% NHS	91	49	65.00000
1.B1	-RTX / +25% NHS (CNTR)	131	18	87.91946
1.B2	+RTX / +25% NHS	67	61	52.34375
1.B3	+RTX / +25% NHS	36	59	37.89474

**July**

**Wednesday 10-07-2024**

### Cell Culture

- Split RAMOS BC 1
- Count:  $1.65 \times 10^6$
- Added 1.5mL CS into 18.5media

### RAMOS - RTX In Vitro CDC Drug Pressure Experiment

- Began RTX CDC In Vitro experiments
- Seeded RAMOS BC 1 into 2x 6 well plates
- Froze down 3 vials of RAMOS BC 1
  - These can be considered Day 0/Baseline pools
  - Marked with black mark on top of caps

### Freezing Down Cells Protocol

1. Counted cells in suspension
2. Took volume of cell suspension such that each vial would contain at least  $2 \times 10^6$  cells
3. Spun down CS @ 300 rcf for 5min
4. Discarded supernatant and resuspended in freezing media such that 1ml of freezing media contains  $2 \times 10^6$  cells - Freezing media: FBS + 10% DMSO
5. Added 1mL CS in freezing media per cryovial
6. Cryovials were immediately put into freezing caddy and placed in -80 freezer
7. After 24hrs vials removed from freezing caddy and placed in Liquid Nitrogen
  - Location: Tank 1, Rack 5, Box 6

### RAMOS RTX DP - Dose 1

- Began RTX CDC In Vitro dosing
- Seeded RAMOS BC 1 into 2x 6 well plates

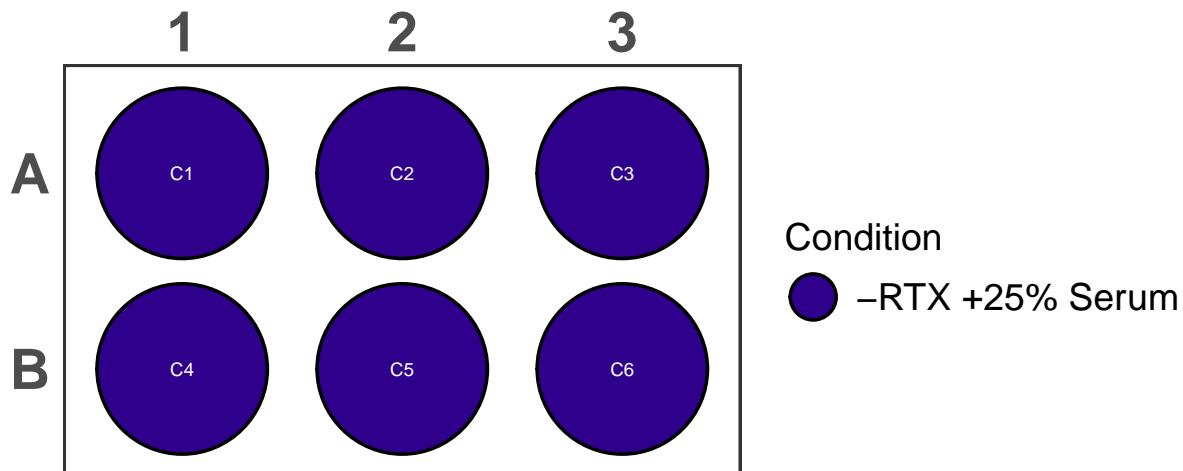
### Dosing Protocol

1. Count CS and dilute to  $1 \times 10^5$  cells in 1 mL
  - If cell count is below either re-culture or add required CS amount, spin down, and resuspend in 1mL
2. Add 1mL of cell suspension containing  $1 \times 10^5$  cells to respective wells of 6-well plate
3. Made RTX dilutions and added to respective Rx wells in  $500 \mu\text{L}$ 
  - [RTX stock] = 10.3 mg/mL

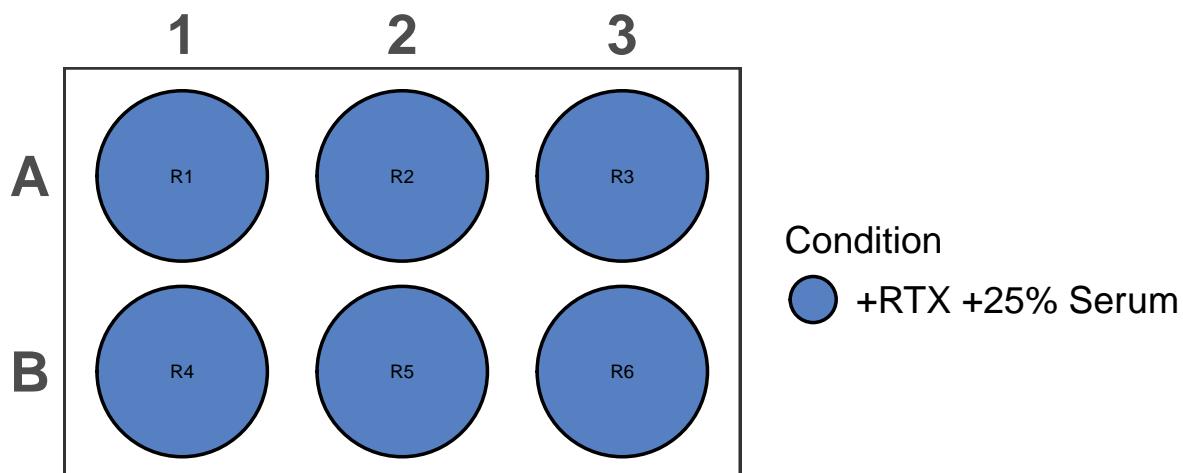
- Drug volumes are being added constitute 1/4 of well volume:
    - [RTX working] needs to be 4x [RTX well]
  - 6 wells per RTX dosing,  $500\mu\text{L}$  per well ~ minimum of  $3000\mu\text{L}$  per condition needed (recommend  $3500\mu\text{L}$ )
  - $500\mu\text{L}$  media added to Cx wells
4. Added NHS to all wells
- $500\mu\text{L}/\text{well}$
  - Final well volume = 25% Serum (NHS)
4. Plates incubated for 24 hrs at 37C

**Plate Layout**

**RAMOS RTX DP1 Control 240724**



**RAMOS RTX DP1 Rituximab 240724**



## **Wednesday 17-07-2024**

### **Cell Culture**

#### **RAMOS BC 1 - Baseline**

- Split 1/20
- RPMI/10% FBS in T75

#### **RAMOS BC 1 - Cx/DP2**

- Split  $0.7 \times 10^6$  cells/flask
- Use for RTX CDC EC50
- RPMI/10% FBS in T25

#### **RAMOS BC 1 - Rx/DP2**

- Split  $0.7 \times 10^6$  cells/flask
- Use for RTX CDC EC50
- RPMI/10% FBS in T25

## **Friday 19-07-2024**

### **Cell Culture**

#### **RAMOS BC 1 - Baseline**

- Split 1/20
- RPMI/10% FBS in T75

### **RAMOS - RTX In Vitro CDC Drug Pressure Experiment**

#### **RAMOS BC 1 - Cx/DP2 and Rx/DP2**

- Cells from RAMOS RTX CDC DP
- Following indicator will be used:
  - Cx - Control RAMOS lines 1-6
  - Rx - Rituximab treated RAMOS lines 1-6
  - 2nd Dose of RTX (DP2) - 10ug/mL
- Cells were expanded from 6-well plates to individual T25 flasks in 6mL of media
  - Cx-DP2 were expanded by taking 1/2 of 6-well CS
  - Rx-DP2 were fully expanded

### **EC50 RTX RAMOS-BC5 240719 - Seeding**

- Seeded an EC50 experiment comparing the effects of RTX on Cx/Rx-DP1 cell lines in the presence of 25% NHS
- Seeded 3 plates each with a different RAMOS-DP line
- Used Rixathon (Catalogue#: )

#### **Plate seeding protocol:**

1. Diluted cell suspension to seed 10000 cells/well in  $50\mu\text{L}$  amounts

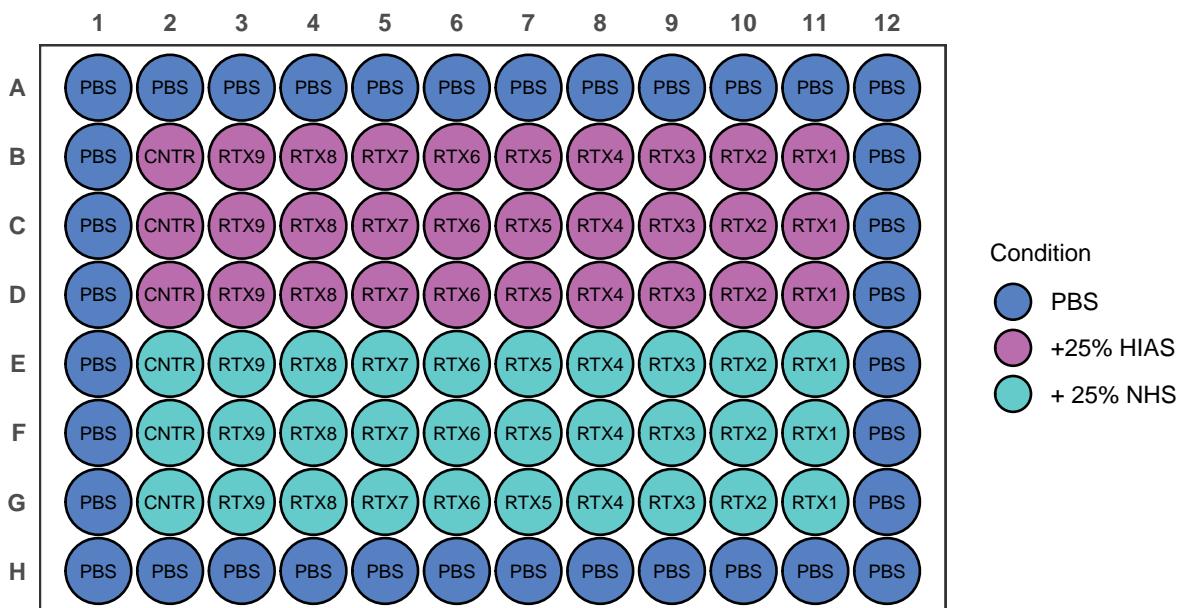
Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	CS cells/mL	Stock Volume (uL)	Media Volume (mL)
Plate 1 - Top	R1-DP1	$5.23 \times 10^5$	$3.00 \times 10^5$	1.5	$3.49 \times 10^5$	573.6	0.9264
Plate 1 - Bottom	R2-DP1	$3.61 \times 10^5$	$3.00 \times 10^5$	1.5	$2.41 \times 10^5$	831.0	0.6690
Plate 2 - Top	R6-DP1	$4.87 \times 10^5$	$3.00 \times 10^5$	1.5	$3.25 \times 10^5$	616.0	0.8840
Plate 2 - Bottom	C1-DP1	$1.28 \times 10^6$	$3.00 \times 10^5$	1.5	$8.53 \times 10^5$	234.3	1.2657
Plate 3 - Top	C2-DP1	$1.17 \times 10^6$	$3.00 \times 10^5$	1.5	$7.80 \times 10^5$	256.4	1.2436
Plate 3 - Bottom	C6-DP1	$9.42 \times 10^5$	$3.00 \times 10^5$	1.5	$6.28 \times 10^5$	318.4	1.1816

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

Dilution ID	Well [RTX] ( $\mu\text{g/mL}$ )	RTX Source	Source Volume ( $\mu\text{L}$ )	Media Volume ( $\mu\text{L}$ )	Working Stock [RTX] ( $\mu\text{L/mL}$ )
RTX 1	1030.000000	Stock	400	600	4120.00000
RTX 2	515.000000	RTX 1	500	500	2060.00000
RTX 3	257.500000	RTX 2	500	500	1030.00000
RTX 4	128.750000	RTX 3	500	500	515.00000
RTX 5	64.375000	RTX 4	500	500	257.50000
RTX 6	32.187500	RTX 5	500	500	128.75000
RTX 7	16.093750	RTX 6	500	500	64.37500
RTX 8	8.046875	RTX 7	500	500	32.18750
RTX 9	4.023438	RTX 8	500	500	16.09375
CNTR	0.000000	-	-	1000	0.00000

3. Added HIAS/NHS to indicated wells
  - $25\mu\text{L}/\text{well}$
  - Final well volume = 25% Serum (HIAS/NHS)
4. Plate is incubated for 48 hrs at 37C

## EC50 CDC Test N2 + RAMOS 240719



**Sunday 21-07-2024**

### Cell Culture

#### RAMOS BC 1 - Baseline

- Split 1/20
- RPMI/10% FBS in T75

#### RAMOS BC 1 - Cx/DP2

- Split 1/6
- RPMI/10% FBS in T25

#### RAMOS BC 1 - Rx/DP2

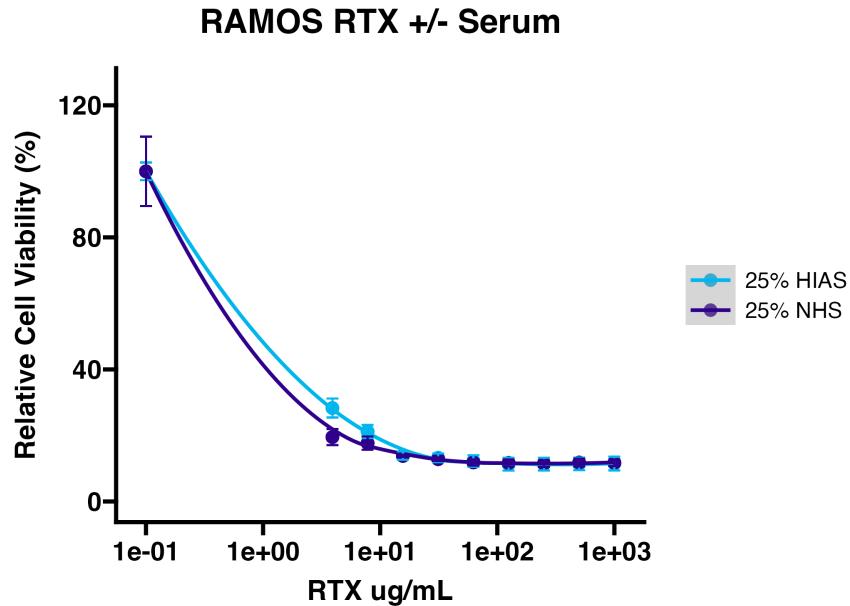
- Not Growing well, still very sparse
  - Transferred to 15mL Eppendorf tubes
  - Spun down @ 300rcf for 5 min
  - Resuspended in 1mL RPMI/10% FBS
  - Added to separate wells of 12 well plate
- RPMI/10% FBS in 12-well plate

#### EC50 Collection: EC50\_RTX\_N2-BC4\_240721

- Collected plates seeded on 21-07-2024
- EC Plate collection protocol:
  1. Added 40 $\mu$ L Cell Titre Blue (CTB) to each conditioned well
    - 20 $\mu$ L CTB/100 $\mu$ L of conditioned well recommended by manufacturer
  2. Incubated for 1hr at 37C
  3. Read on plate reader according to Cell Titre Blue Protocol

**Results:**

- Dosages too high
- Need to decrease amounts for next EC50
  - RTX1 should be ~20ug/mL



**Tuesday 23-07-2024**

**RAMOS BC 1 - Baseline**

- Split 1/20
- RPMI/10% FBS in T75

**RAMOS BC 1 - Cx/DP2**

- Split 1/6
- RPMI/10% FBS in T25

**RAMOS BC 1 - Rx/DP2**

- Expanded into T25
- RPMI/10% FBS in T25

**Wednesday 24-07-2024**

**Cell Culture**

**RAMOS BC 1 - Baseline**

- Split 1/3
- RPMI/10% FBS in T75

**RAMOS BC 1 - Cx/DP2**

- Expanded 1/2 into T75

- RPMI/10% FBS in T75

### **RAMOS BC 1 - Rx/DP2**

- Expanded into T75
- RPMI/10% FBS in T75

### **Made Media - RPMI**

- Media recipe

Solution	ID code	Volume	% Total volume
RPMI 1640	—	500 mL	89%
FBS	—	56 mL	10%
Glutamax	—	5.6 mL	1%
Pen/Strep	—	5.6 mL	1%

## **Friday 26-07-2024**

### **RAMOS BC 1 - Baseline**

- Split 1/6
- RPMI/10% FBS in T75

### **RAMOS BC 1 - Cx/DP2**

- Split 1/6
- RPMI/10% FBS in T75

### **RAMOS BC 1 - Rx/DP2**

- Added 2-3mL of fresh media

## **Monday 29-07-2024**

### **RAMOS BC 1 - Baseline**

- Split 1/3
- RPMI/10% FBS in T75

### **RAMOS BC 1 - Cx/DP2**

- Add 5mL of RPMI/10% FBS

### **RAMOS BC 1 - Rx/DP2**

- Cells still look unhealthy/too sparse to use
- Spun down and resuspended in 6mL of media in new T25 flask
- RPMI/10% FBS in T75

Cell Line	Cell Count	Media Volume
R1-DP2	383333.3333333331	6 mL
R2-DP2	193333.3333333334	6 mL

Cell Line	Cell Count	Media Volume
R3-DP2	444000	10 mL
R4-DP2	942000	5 mL
R5-DP2	Expanded to 6 well plate	***
R6-DP2	220000	6 mL

**Tuesday 30-07-2024**

### RAMOS RTX DP - Dose 3

- Began RTX CDC In Vitro dosing
- Seeded RAMOS BC 1 into 2x 6 well plates

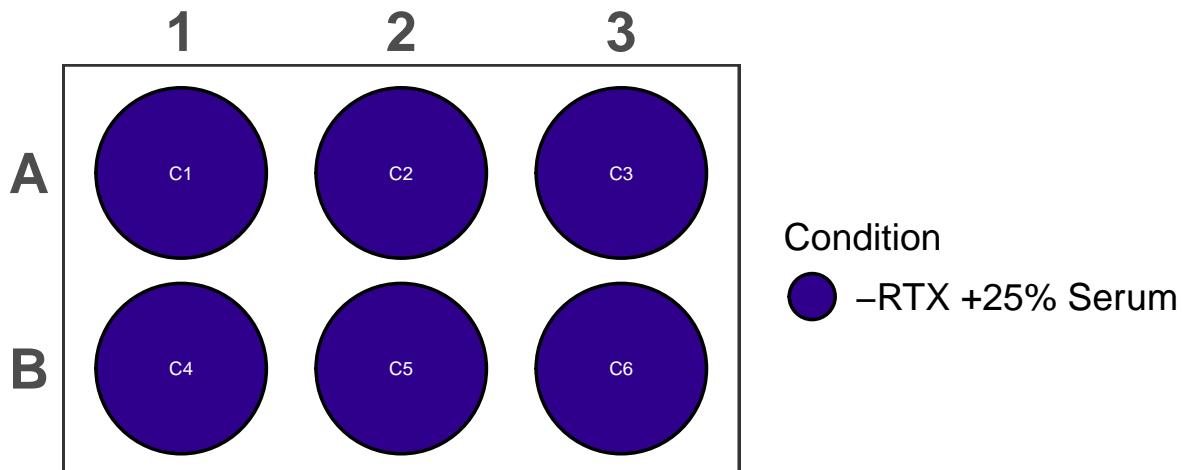
#### Dosing Protocol

1. Count CS and dilute to  $2 \times 10^5$  cells in 1 mL
  - If cell count is below either re-culture or add required CS amount, spin down, and resuspend in 1mL
2. Add 1mL of cell suspension containing  $2 \times 10^5$  cells to respective wells of 6-well plate
3. Made RTX dilutions and added to respective Rx wells in  $500\mu\text{L}$ 
  - [RTX stock] = 10.3 mg/mL
  - Drug volumes are being added constitute 1/4 of well volume:
    - [RTX working] needs to be 4x [RTX well]
  - 6 wells per RTX dosing,  $500\mu\text{L}$  per well ~ minimum of  $3000\mu\text{L}$  per condition needed (recommend  $3500\mu\text{L}$ )
  - $500\mu\text{L}$  media added to Cx wells
4. Added NHS to all wells
  - $500\mu\text{L}/\text{well}$
  - Final well volume = 25% Serum (NHS)
4. Plates incubated for 24 hrs at 37C

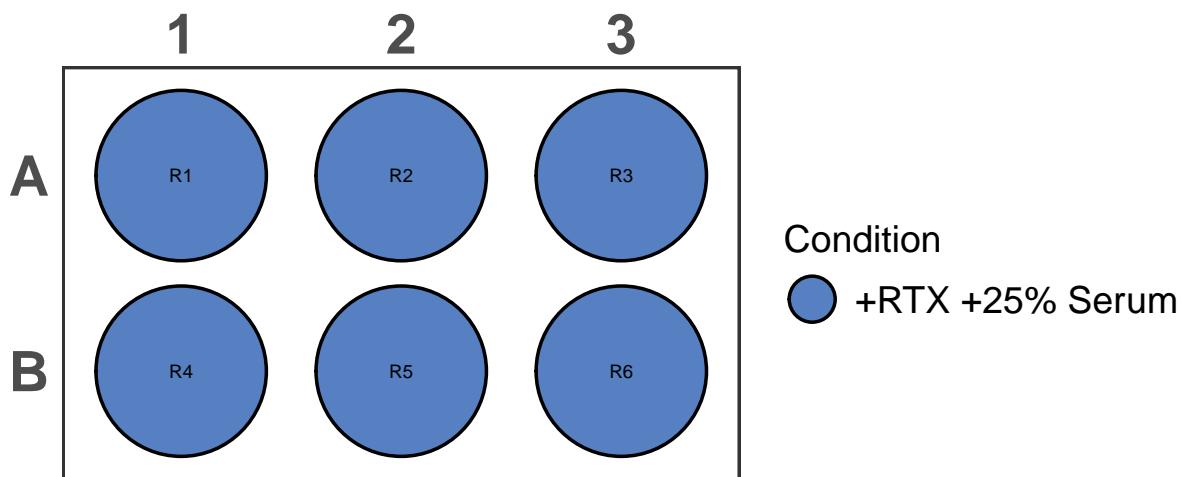
#### Plate Layout Cell Count

Plate	Cell Line	Cell Count	CS Volume	Media Volume	Final cells/well
Plate 1 - A1	R1-DP2	$5.65 \times 10^5$	353.98230088495575	646.01769911504425	$2.00 \times 10^5$
Plate 1 - A2	R2-DP2	$2.30 \times 10^5$	869.56521739130437	130.43478260869563	$2.00 \times 10^5$
Plate 1 - A3	R3-DP2	$8.89 \times 10^5$	224.97187851518561	775.02812148481439	$2.00 \times 10^5$
Plate 1 - B1	R4-DP2	$6.80 \times 10^4$	***	***	$2.00 \times 10^5$
Plate 1 - B2	R5-DP2	$3.14 \times 10^4$	***	***	$2.00 \times 10^5$
Plate 1 - B3	R6-DP2	$4.92 \times 10^5$	406.5040650406504	593.4959349593496	$2.00 \times 10^5$
Plate 2 - A1	C1-DP2	$6.23 \times 10^5$	321.02728731942216	678.97271268057784	$2.00 \times 10^5$
Plate 2 - A2	C2-DP2	$4.24 \times 10^5$	471.69811320754718	528.30188679245282	$2.00 \times 10^5$
Plate 2 - A3	C3-DP2	$3.24 \times 10^5$	617.28395061728395	382.71604938271605	$2.00 \times 10^5$
Plate 2 - B1	C4-DP2	$2.51 \times 10^5$	796.81274900398398	203.18725099601602	$2.00 \times 10^5$
Plate 2 - B2	C5-DP2	$4.34 \times 10^5$	460.82949308755758	539.17050691244242	$2.00 \times 10^5$
Plate 2 - B3	C6-DP1	$2.67 \times 10^5$	749.06367041198507	250.93632958801493	$2.00 \times 10^5$

## RAMOS RTX DP3 Control 240726



## RAMOS RTX DP3 Rituximab 2407246



## EC50 RTX RAMOS-DP2 240730 - Seeding

- Seeded an EC50 experiment comparing the effects of RTX on Cx/Rx-DP1 cell lines in the presence of 25% NHS
- Seeded 3 plates each with a different RAMOS-DP line
- Used Rixathon (Catalogue#: )

### Plate seeding protocol:

- Diluted cell suspension to seed 10000 cells/well in 50 $\mu$ L amounts

Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	CS cells/mL	Stock Volume (uL)	Media Volume (mL)
Plate 1 - Top	R1-DP2	$5.65 \times 10^5$	$4.00 \times 10^5$		$2.82 \times 10^5$	707.9	1.2921
Plate 2 - Bottom	R3-DP2	$8.89 \times 10^5$	$4.00 \times 10^5$		$4.44 \times 10^5$	449.9	1.5501

Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	CS cells/mL	Stock Volume (uL)	Media Volume (mL)
Plate 3 - Top	R6-DP2	$4.92 \times 10^5$	$4.00 \times 10^5$	2	$2.46 \times 10^5$	813.0	1.1870
Plate 4 - Top	C1-DP2	$6.23 \times 10^5$	$4.00 \times 10^5$	2	$3.12 \times 10^5$	642.0	1.3580
Plate 5 - Top	C3-DP2	$3.24 \times 10^5$	$4.00 \times 10^5$	2	$1.62 \times 10^5$	1234.5	0.7655
Plate 6 - Bottom	C6-DP1	$2.67 \times 10^5$	$4.00 \times 10^5$	2	$1.34 \times 10^5$	1498.1	0.5019

2. Made RTX dilutions and added to respective wells in  $25\mu\text{L}$

- [RTX stock] = 10.3 mg/mL
- Drug volumes are added in triplicate
- Drug volumes are being added constitute 1/4 of well volume:
  - [RTX working] needs to be 4x [RTX well]
    - 6 wells per condition,  $25\mu\text{L}$  per well ~ minimum of  $150\mu\text{L}$  per condition needed (recommend  $200\mu\text{L}$ )

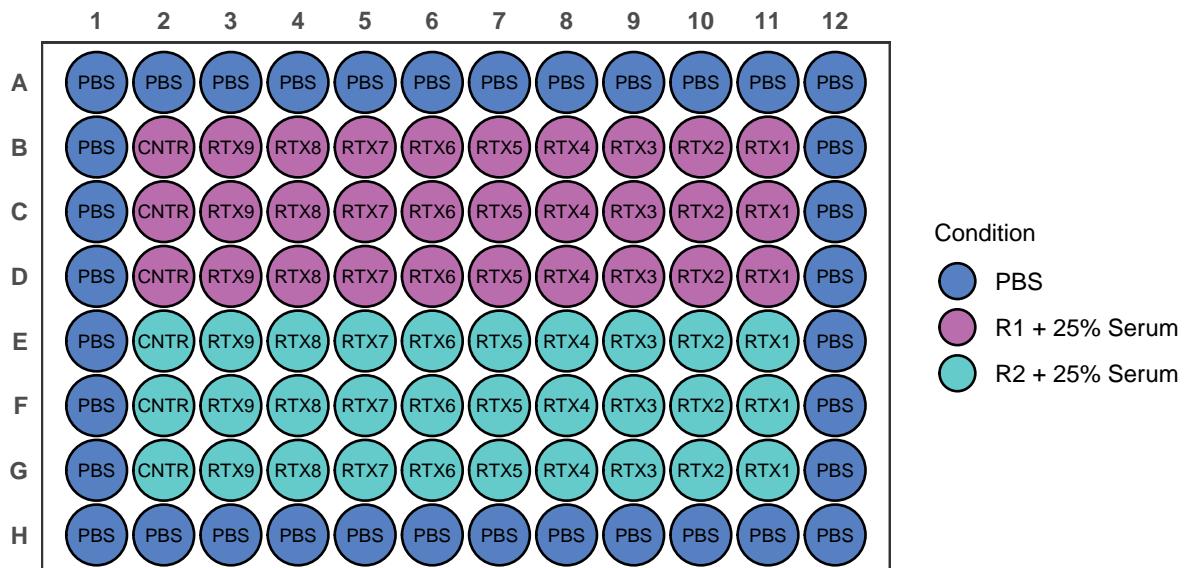
Dilution ID	Well [RTX] (µg/mL)	RTX Source	Source Volume (µL)	Media Volume (µL)	Working Stock [RTX] (µg/mL)
RTX 1	20.0	Stock	15.5	1984.5	79.8
RTX 2	10.0	RTX 1	1000	1000.0	39.9
RTX 3	5.0	RTX 2	1000	1000.0	20.0
RTX 4	2.5	RTX 3	1000	1000.0	10.0
RTX 5	1.2	RTX 4	1000	1000.0	5.0
RTX 6	0.6	RTX 5	1000	1000.0	2.5
RTX 7	0.3	RTX 6	1000	1000.0	1.2
RTX 8	0.2	RTX 7	1000	1000.0	0.6
RTX 9	0.1	RTX 8	1000	1000.0	0.3
CNTR	0.0	-	-	1000.0	0.0

3. Added HIAS/NHS to indicated wells

- $25\mu\text{L}$ /well
- Final well volume = 25% Serum (HIAS/NHS)

4. Plate is incubated for 48 hrs at 37C

## EC50 CDC Test N2 + RAMOS 240730



## RAMOS RTX CDC DP2 - CD20 Flow Cytometry

- Checked CD20 expression between Cx and Rx DP2
- CD20:
- Samples tested:
  - Baseline RAMOS
  - R1
  - R3
  - R6
  - C1
  - C3
  - C6

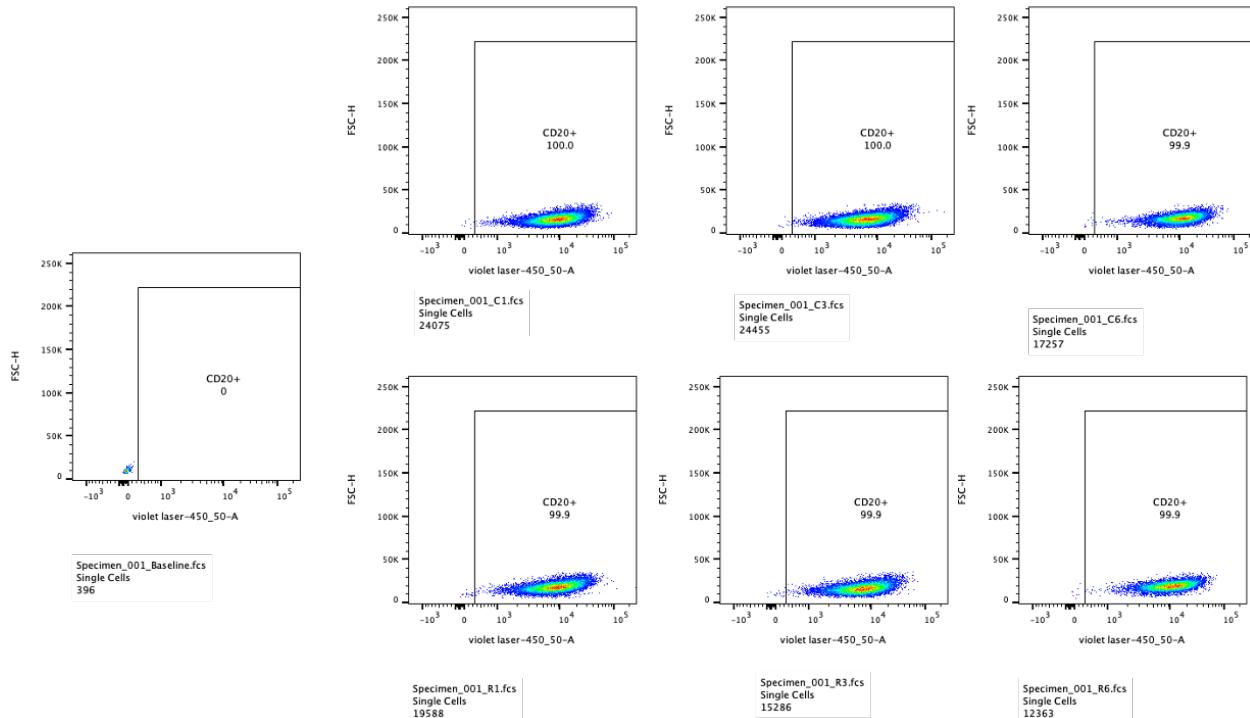
### CD20 Flow Protocol

- Prior to starting: Make cell stain
    - $500\mu\text{L}$  PBS +  $2\mu\text{L}$  Stain
    - $100\mu\text{L}$  of stain used/condition
  - Cell stain made:  $2000 \mu\text{L}$  and  $8 \mu\text{L}$
1. Resuspend cells and transferred to 3 wells of a 96 well plate
  2. Spun down at 1500g for 2min
  3. Flick media out
  4. Resuspend w/  $100\mu\text{L}$  stain in well and consolidate in 1 well
    - Add  $100\mu\text{L}$  to bottom well and mix until cells resuspended
    - Take  $100\mu\text{L}$  CS-stain mix and add to well below and mix
    - Repeat for third well and add the total CS-Stain mix to top well
  5. Incubate in fridge for 25min
    - In the dark
  6. Spin down at 1500g for 2min
  7. Flick out media
  8. Resuspend in PBS and add to FACS tube

- Add an additional volume of PBS to dilute cells appropriately for flow
- Usually make it up to about 300-400 $\mu$ L CS-PBS in the tube

## Results

- No apparent change in CD20 expression
- This is interesting based on strong evidence which suggests loss of CD20 is the primary mechanism of RTX resistance
- Will need to compare to EC50\_240730 results
  - Are Rx-DP2 actually resistant compared to Cx-DP2?



**Wednesday 31-07-2024**

### RAMOS BC 1 - Baseline

- Split 1/6
- RPMI/10% FBS in T75

### RAMOS BC 1 - Rx/DP2

- Expanded remaining Rx/DP2 into T75
- RPMI/10% FBS in T75

DP Line	CS Volume	Media Volume
R1-DP2	10 mL	10 mL
R2-DP2	10 mL	10 mL
R4-DP2	5 mL	5 mL
R6-DP2	8 mL	10 mL

## **RAMOS RTX DP - Dose 3 Collection**

- Decided to collect after 24hrs due to high amount of cell killing
  - Most examples in the literature do 24hr collection

### **Collection Protocol**

#### **Rx-DP3:**

1. Well volume transferred to 15ml Eppendorf
2. Eppendorfs supn down at 200 ref for 6 min
3. Supernatant discarded and R1-4, R6 resuspended in 1ml
  - R5 resuspended in 500 $\mu$ L due to small pellet
4. Collected cells added to individual wells of 12-well plate
  - R5 added to 24-well plate

#### **Cx-DP3**

- Good viability for all wells
- Entire CS transferred to individual T75s and topped up with 8mL of RPMI/10% FBS in T75

**Thursday 01-08-2024**

### **Rx-DP3 Culture**

- Cells are growing but lots of dead cells as well
- Added 1mL to all wells
  - 500 $\mu$ L to R5

### **Cx-RP3 Culture**

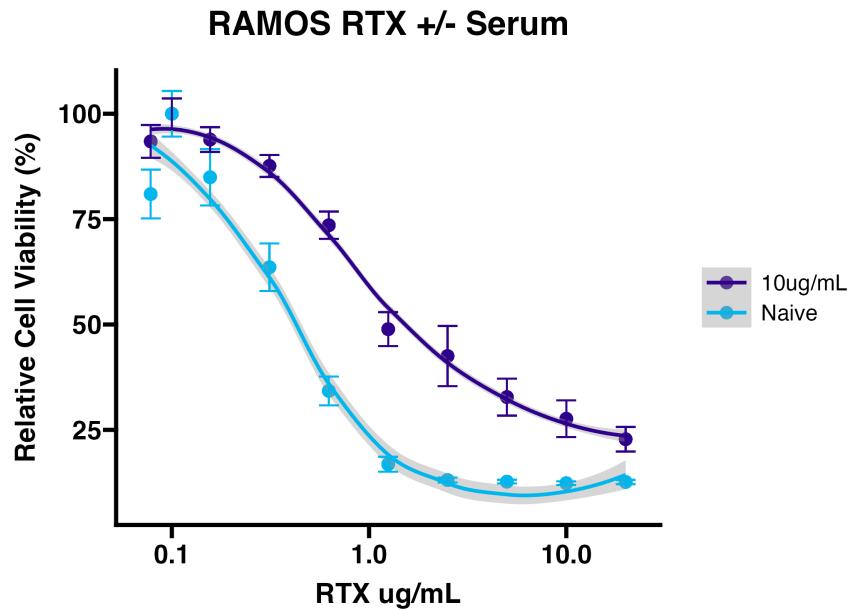
- Look healthy but still growing, no split

## **EC50\_240730 Collection - RAMOS RTX CDC DP2**

- Collected plates seeded on 21-07-2024
- EC Plate collection protocol:
  1. Added 20 $\mu$ L Cell Titre Blue (CTB) to each conditioned well
    - 20 $\mu$ L CTB/100 $\mu$ L of conditioned well recommended by manufacturer
  2. Incubated for 2hr at 37C
  3. Read on plate reader according to Cell Titre Blue Protocol

### **Results:**

- Less response for Rx-DP2 vs Cx-DP2
- Not sure its fully “resistant”, still a robust response to RTX dosing for Rx-DP2



**Friday 02-08-2024**

#### Rx-DP3 Culture

- Cells growing, expanded to 6 well plate
- R5 unhealthy
  - Spun down and transferred to single well of 48 well plate

#### Cx-DP3 Culture

- Split 1/2 and added 10ml
- Collected samples for western/barcoding
  1. Collected  $1 \times 10^6$  cells in 15 mL tube
  2. Spun down @ 300 rcf for 5min
  3. Resuspended in 500 $\mu$ L PBS and transferred to 1.5mL Eppendorf
  4. Spun down @ max speed for 5 min at 4C
  5. Remove supernatant
  6. Snap freeze cell pellet and transfer to -80C

#### Ramos Baseline

- Split 1/6
- Gave aliquot to Chris
- Count:  $3.68 \times 10^6$  cells/mL

**Saturday 03-08-2024**

#### Cell Culture

- Add media to all

**Sunday 04-08-2024**

### Cx-DP3 Culture

- Split 1/2 and added 10ml
- Collected samples for western/barcoding

### EC50 RTX RAMOS-DP3 240804 - Seeding

- Seeded an EC50 experiment comparing the effects of RTX + 25% NHS vs 25% HIAS on Cx-DP3 cell lines
  - 3x lines (C1, C2, C5) exposed to RTX + 25% HIAS while 1x line (C6) exposed to RTX + 25% NHS
- Seeded 2 plates each section with a different Cx-DP3 line
- Used Rixathon (Catalogue#: )

#### Plate seeding protocol:

1. Diluted cell suspension to seed 10000 cells/well in 50 $\mu$ L amounts

Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	CS cells/mL	Stock Volume (uL)	Media Volume (mL)
Plate 1 - Top	C1-DP3	$1.23 \times 10^7$	$4.00 \times 10^5$		$6.15 \times 10^6$	32.5	1.9675
Plate 2 - Bottom	C2-DP3	$1.31 \times 10^7$	$4.00 \times 10^5$		$6.55 \times 10^6$	30.5	1.9695
Plate 3 - Top	C5-DP3	$1.15 \times 10^7$	$4.00 \times 10^5$		$5.75 \times 10^6$	34.7	1.9653
Plate 4 - Top	C6-DP3	$1.12 \times 10^7$	$4.00 \times 10^5$		$5.60 \times 10^6$	35.7	1.9643

2. Made RTX dilutions and added to respective wells in 25 $\mu$ L

- [RTX stock] = 10.3 mg/mL
- Drug volumes are added in triplicate
- Drug volumes are being added constitute 1/4 of well volume:
  - [RTX working] needs to be 4x [RTX well]
    - 6 wells per condition, 25 $\mu$ L per well ~ minimum of 150 $\mu$ L per condition needed (recommend 200 $\mu$ L)

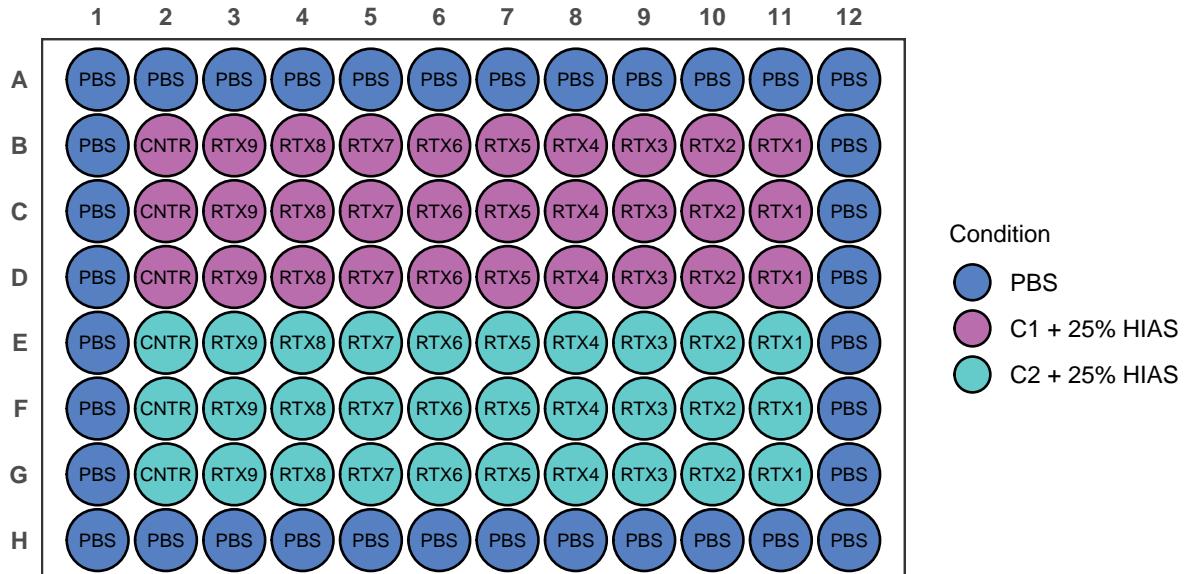
Dilution ID	Well [RTX] (pg/mL)	RTX Source	Source Volume (uL)	Media Volume (uL)	Working Stock [RTX] (pg/mL)
RTX 1	20.0	Stock	11.65	1488.3	80.0
RTX 2	10.0	RTX 1	750	750.0	40.0
RTX 3	5.0	RTX 2	750	750.0	20.0
RTX 4	2.5	RTX 3	750	750.0	10.0
RTX 5	1.2	RTX 4	750	750.0	5.0
RTX 6	0.6	RTX 5	750	750.0	2.5
RTX 7	0.3	RTX 6	750	750.0	1.2
RTX 8	0.2	RTX 7	750	750.0	0.6
RTX 9	0.1	RTX 8	750	750.0	0.3
CNTR	0.0	-	-	1000.0	0.0

3. Added HIAS/NHS to indicated wells

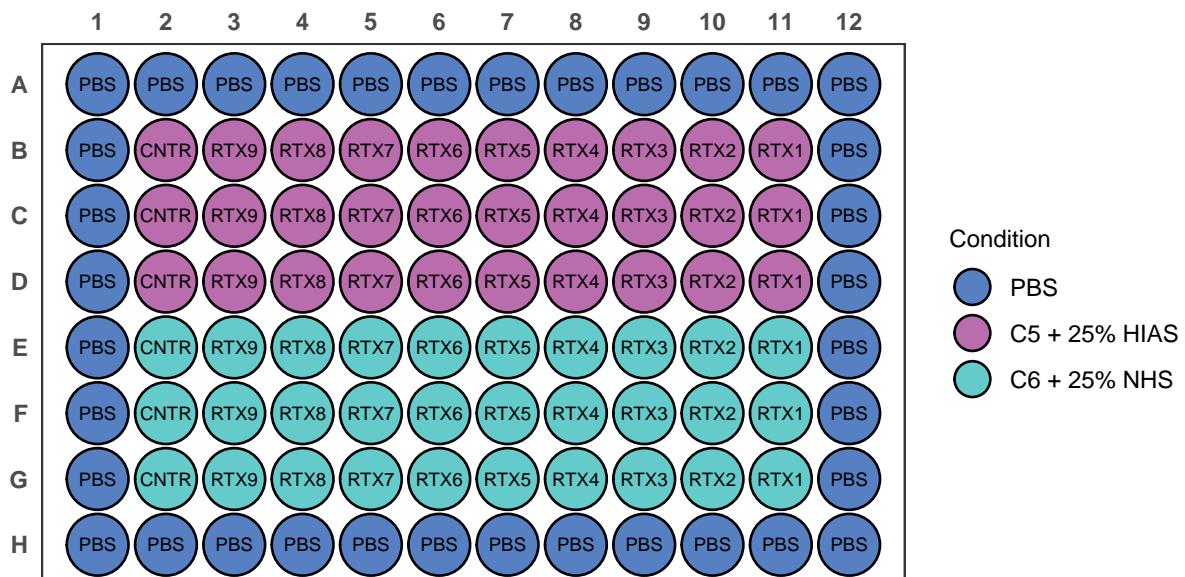
- $25\mu\text{L}/\text{well}$
- Final well volume = 25% Serum (HIAS/NHS)

4. Plate is incubated for 48 hrs at 37C

### EC50 240804 Plate 1



### EC50 240804 Plate 2



Tuesday 06-08-2024

### RAMOS Baseline Culture

- Split 1/6

### Cx-DP3 Culture

- Split 1/6 and added 10ml

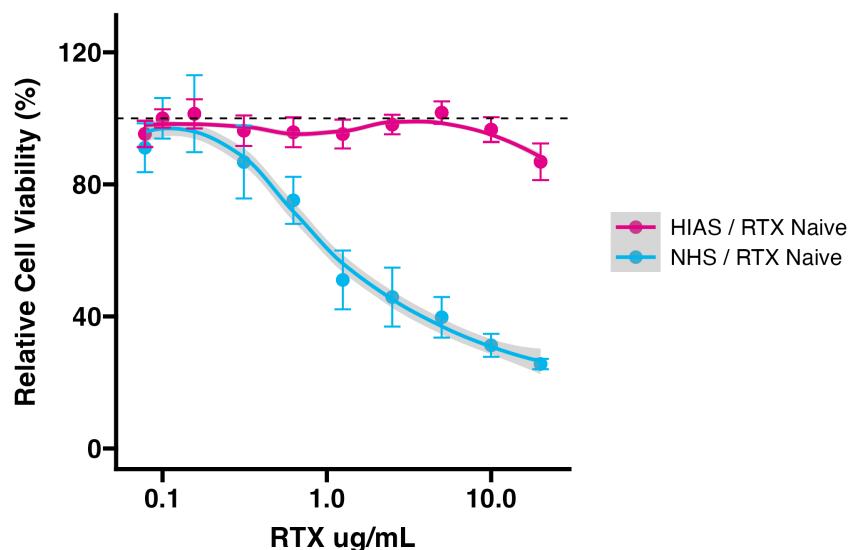
## EC50\_240804 Collection - RAMOS RTX CDC DP3

- Collected plates seeded on 06-08-2024
- EC Plate collection protocol:
  1. Added 20 $\mu$ L Cell Titre Blue (CTB) to each conditioned well
    - 20 $\mu$ L CTB/100 $\mu$ L of conditioned well recommended by manufacturer
  2. Incubated for 2hr at 37C
  3. Read on plate reader according to Cell Titre Blue Protocol

### Results:

- HIAS impact on RTX CDC similar to previous experiments
- NHS impact on RTX CDC is consistent with previous experiments

### RAMOS RTX +/- Serum



## EC50 RTX RAMOS-DP3 240806 - Seeding

- Seeded an EC50 experiment comparing the effects of RTX + 25% NHS on Rx-DP3 cell lines
  - 2x lines (R1 + R3) exposed to RTX + 25% NHS
- Seeded 1 plate, each section with a different Rx-DP3 line
- Used Rixathon (Catalogue#: )

### Plate seeding protocol:

1. Diluted cell suspension to seed 10000 cells/well in 50 $\mu$ L amounts

Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	CS cells/mL	Stock Volume (uL)	Media Volume (mL)
Plate 1 - Top	R1-DP3	$5.55 \times 10^5$	$4.00 \times 10^5$		$2.78 \times 10^5$	720.7	1.2793
Plate 1 - Bottom	R3-DP3	$5.55 \times 10^5$	$4.00 \times 10^5$		$2.78 \times 10^5$	720.7	1.2793

2. Made RTX dilutions and added to respective wells in 25 $\mu$ L

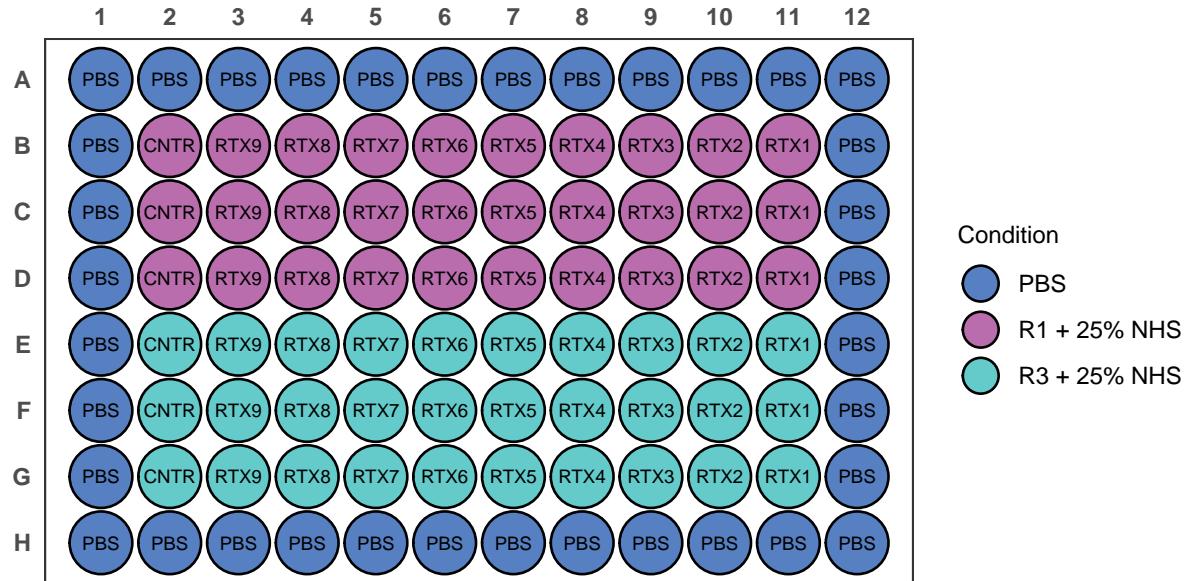
- [RTX stock] = 10.3 mg/mL
- Drug volumes are added in triplicate
- Drug volumes are being added constitute 1/4 of well volume:
  - [RTX working] needs to be 4x [RTX well]
    - 6 wells per condition, 25 $\mu$ L per well ~ minimum of 150 $\mu$ L per condition needed (recommend 200 $\mu$ L)

Dilution ID	Well [RTX] ( $\mu$ g/mL)	RTX Source	Source Volume ( $\mu$ L)	Media Volume ( $\mu$ L)	Working Stock [RTX] ( $\mu$ g/mL)
RTX 1	20.0	Stock	7.76	992.2	79.9
RTX 2	10.0	RTX 1	500	500.0	40.0
RTX 3	5.0	RTX 2	500	500.0	20.0
RTX 4	2.5	RTX 3	500	500.0	10.0
RTX 5	1.2	RTX 4	500	500.0	5.0
RTX 6	0.6	RTX 5	500	500.0	2.5
RTX 7	0.3	RTX 6	500	500.0	1.2
RTX 8	0.2	RTX 7	500	500.0	0.6
RTX 9	0.1	RTX 8	500	500.0	0.3
CNTR	0.0	-	-	1000.0	0.0

3. Added HIAS/NHS to indicated wells
  - 25 $\mu$ L/well
  - Final well volume = 25% Serum (HIAS/NHS)

4. Plate is incubated for 48 hrs at 37C

### EC50 240806 Plate 1



Wednesday 07-08-2024

### Cell Culture

- All cells growing fine, not ready to split/use experimentally
  - RAMOS Baseline (BC 1)

- Cx-DP3
- Rx-DP3

**Thursday 08-08-2024**

### Ramos Baseline Culture

- Split 1/2

### Cx-DP3 Culture

- Split 1/2

### Rx-DP3 Culture

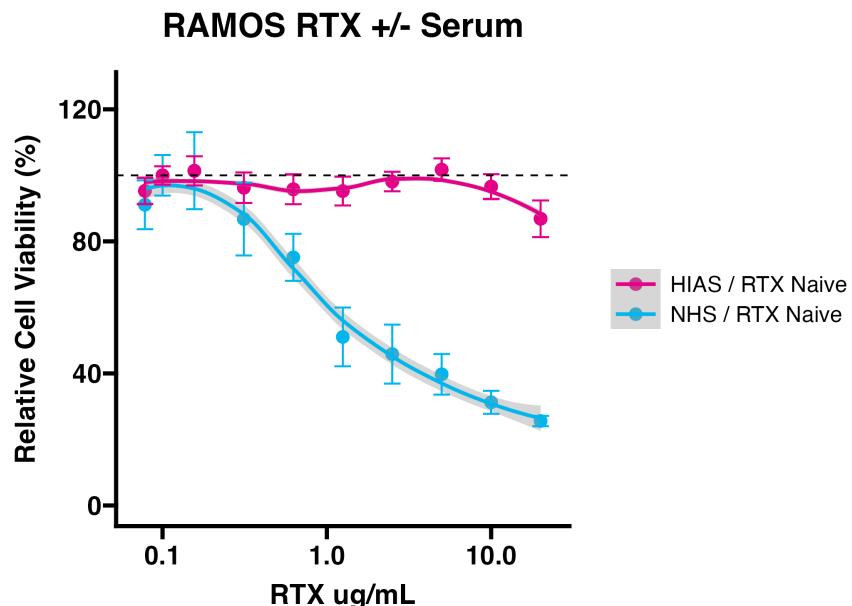
- Added media to Rx-DP3 cultures
  - R1/R3/R4/R6 added 8mL media
  - R2 added 4mL

### EC50\_240806 Collection - RAMOS RTX CDC DP3

- Collected plates seeded on 06-08-2024
- EC Plate collection protocol:
  1. Added 20 $\mu$ L Cell Titre Blue (CTB) to each conditioned well
    - 20 $\mu$ L CTB/100 $\mu$ L of conditioned well recommended by manufacturer
  2. Incubated for 2hr at 37C
  3. Read on plate reader according to Cell Titre Blue Protocol

### Results:

- HIAS impact on RTX CDC similar to previous experiments
- NHS impact on RTX CDC is consistent with previous experiments



## **RAMOS RTX CDC DP3 - CD20 Flow Cytometry**

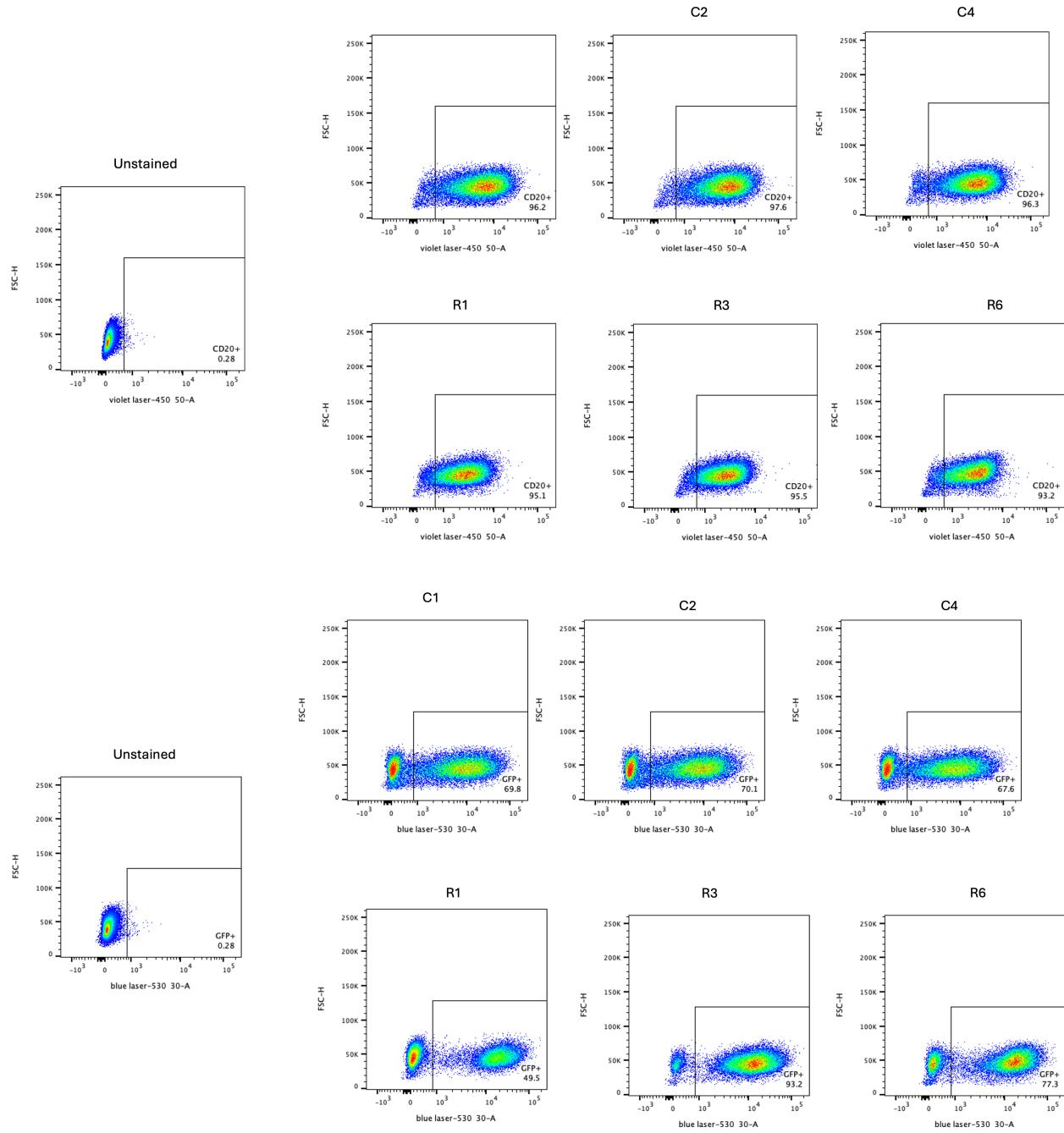
- Checked CD20 expression between Cx and Rx DP4
- CD20:
- Samples tested:
  - Baseline RAMOS
  - R2
  - R3
  - R4
  - C1
  - C2
  - C6

### **CD20 Flow Protocol**

- Prior to starting: Make cell stain
    - $500\mu\text{L}$  PBS +  $2\mu\text{L}$  Stain
    - $100\mu\text{L}$  of stain used/condition
  - Cell stain made:  $2000\mu\text{L}$  and  $8\mu\text{L}$
1. Resuspend cells and transferred to 3 wells of a 96 well plate
  2. Spun down at  $1500\text{g}$  for 2min
  3. Flick media out
  4. Resuspend w/  $100\mu\text{L}$  stain in well and consolidate in 1 well
    - Add  $100\mu\text{L}$  to bottom well and mix until cells resuspended
    - Take  $100\mu\text{L}$  CS-stain mix and add to well below and mix
    - Repeat for third well and add the total CS-Stain mix to top well
  5. Incubate in fridge for 25min
    - In the dark
  6. Spin down at  $1500\text{g}$  for 2min
  7. Flick out media
  8. Resuspend in PBS and add to FACS tube
    - Add an additional volume of PBS to dilute cells appropriately for flow
    - Usually make it up to about  $300\text{-}400\mu\text{L}$  CS-PBS in the tube

### **Results**

- No apparent change in CD20 expression
- This is interesting based on strong evidence which suggests loss of CD20 is the primary mechanism of RTX resistance
- GFP+% is not ideal



**Friday 09-08-2024**

### Ramos Baseline Culture

- Split 1/6

### Cx-DP3 Culture

- Split 1/6

### **Rx-DP3 Culture**

- Split 1/2

## **Monday 12-08-2024**

### **Ramos Baseline Culture**

- Split 1/2

### **Cx-DP3 Culture**

- Split 1/2

### **Rx-DP3 Culture**

- Split 1/2
- Froze Down R4-DP2
  1. Counted cells in suspension
  2. Took volume of cell suspension such that each vial would contain at least  $2 \times 10^6$  cells
  3. Spun down CS @ 300 rcf for 5min
  4. Discarded supernatant and resuspended in freezing media such that 1ml of freezing media contains  $2 \times 10^6$  cells
    - Freezing media: FBS + 10% DMSO
  5. Added 1mL CS in freezing media per cryovial
  6. Cryovials were immediately put into freezing caddy and placed in -80 freezer
  7. After 24hrs vials removed from freezing caddy and placed in Liquid Nitrogen
    - Location: Tank 1, Rack 5, Box 6

## **Wednesday 14-08-2024**

### **Ramos Baseline Culture**

- Split 1/2

### **DP3 Viable Freezing**

- Viably Froze R4-DP2/Cx-DP3/Rx-DP3

### **Freezing Down Cells Protocol**

1. Counted cells in suspension
2. Took volume of cell suspension such that each vial would contain at least  $2 \times 10^6$  cells
3. Spun down CS @ 300 rcf for 5min
4. Discarded supernatant and resuspended in freezing media such that 1ml of freezing media contains  $2 \times 10^6$  cells - Freezing media: FBS + 10% DMSO
5. Added 1mL CS in freezing media per cryovial
6. Cryovials were immediately put into freezing caddy and placed in -80 freezer
7. After 24hrs vials removed from freezing caddy and placed in Liquid Nitrogen
  - Location: Tank 1, Rack 5, Box 6

### **Dosing RAMOS-DP4 RTX CDC**

- Began RTX CDC In Vitro dosing
- Seeded Cx/Rx-DP2 into 2x 6 well plates

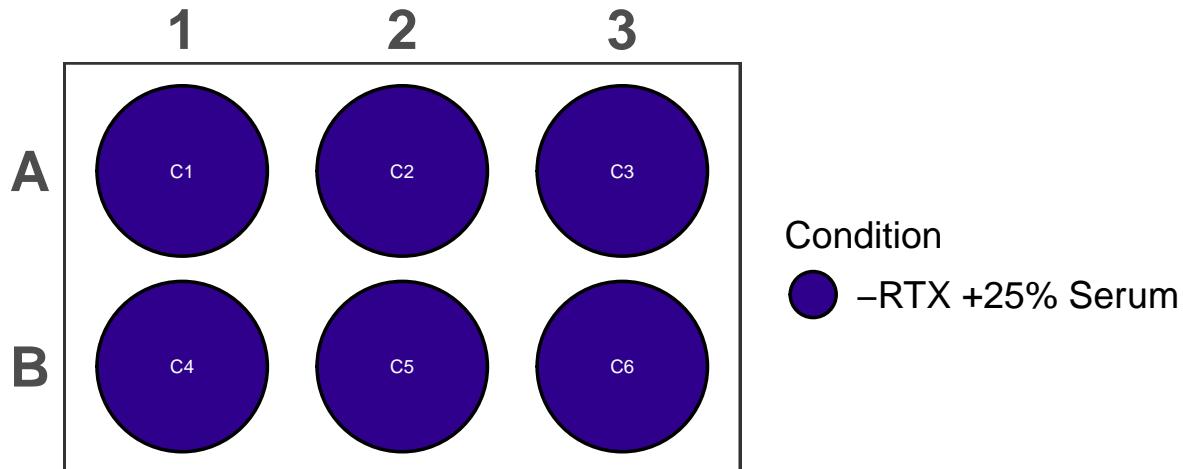
## Dosing Protocol

1. Count CS and diluted to  $2 \times 10^5$  cells in 1 mL
  - If cell count is below either re-culture or add required CS amount, spin down, and resuspend in 1mL
2. Add 1mL of cell suspension containing  $2 \times 10^5$  cells to respective wells of 6-well plate
3. Made RTX dilutions and added to respective Rx wells in  $500\mu\text{L}$ 
  - [RTX stock] = 10.3 mg/mL
  - [RTX Final] =  $50\mu\text{g}/\text{mL}$
  - Drug volumes are being added constitute 1/4 of well volume:
    - [RTX working] needs to be 4x [RTX well]
  - 6 wells per RTX dosing,  $500\mu\text{L}$  per well ~ minimum of  $3000\mu\text{L}$  per condition needed (recommend  $3500\mu\text{L}$ )
  - $500\mu\text{L}$  media added to Cx wells
4. Added NHS to all wells
  - $500\mu\text{L}/\text{well}$
  - Final well volume = 25% Serum (NHS)
4. Plates incubated for 24 hrs at 37C

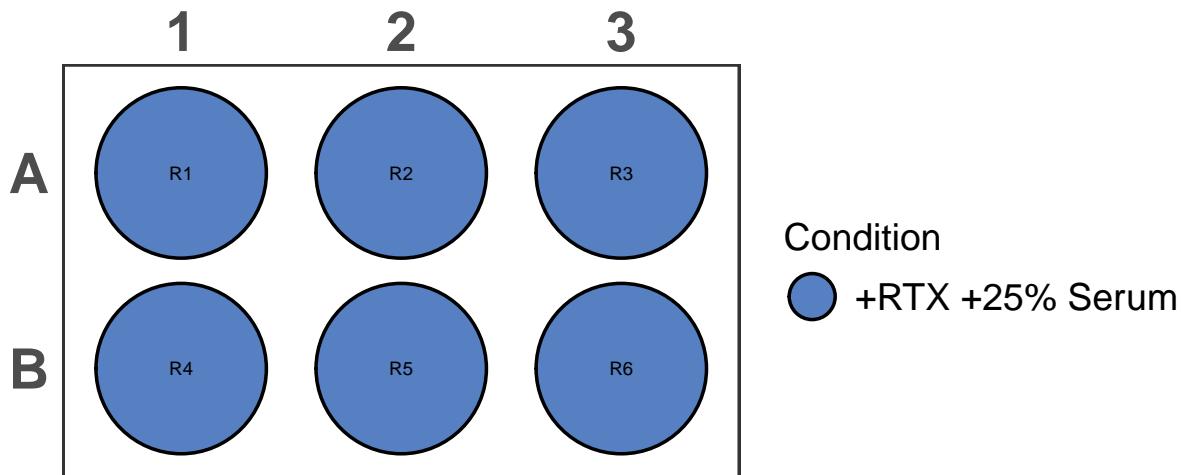
## Plate Layout Cell Count

Plate	Cell Line	Cell Count	CS Volume	Media Volume	Final cells/well
Plate 1 - A1	R1-DP2	466000	429.18454935622321	570.81545064377679	$2.00 \times 10^5$
Plate 1 - A2	R2-DP2	507000	394.47731755424064	605.5226824457593	$2.00 \times 10^5$
Plate 1 - A3	R3-DP2	293000	682.59385665529021	317.40614334470979	$2.00 \times 10^5$
Plate 1 - B1	R4-DP2	429000	466.20046620046617	533.79953379953383	$2.00 \times 10^5$
Plate 1 - B2	***	***	***	***	$2.00 \times 10^5$
Plate 1 - B3	R6-DP2	319000	626.95924764890287	373.04075235109713	$2.00 \times 10^5$
Plate 2 - A1	C1-DP2	916000	218.34061135371181	781.65938864628822	$2.00 \times 10^5$
Plate 2 - A2	C2-DP2	1200000	166.66666666666666	833.33333333333337	$2.00 \times 10^5$
Plate 2 - A3	C3-DP2	1200000	166.66666666666666	833.33333333333337	$2.00 \times 10^5$
Plate 2 - B1	C4-DP2	400000	500	500	$2.00 \times 10^5$
Plate 2 - B2	C5-DP2	361000	554.016620498615	445.983379501385	$2.00 \times 10^5$
Plate 2 - B3	C6-DP1	366000	546.44808743169403	453.55191256830597	$2.00 \times 10^5$

## RAMOS RTX DP3 Control 240726



# RAMOS RTX DP3 Rituximab 2407246



## EC50 RTX RAMOS-DP3 240814 - Seeding

- Seeded an EC50 experiment comparing the effects of RTX + 25% NHS vs 25% HIAS on Cx-DP3 cell lines
  - 2x Rx-DP3 lines (R1 + R4) and 2x Cx-DP3 (C2 + C4) exposed to RTX + 25% HIAS
- Seeded 2 plates each section with a different Ramos conditioned lines
  - Plate 1: R1 + R4
  - Plate 2: C2 + C4
- Used Rixathon (Catalogue#: )

### Plate seeding protocol:

- Diluted cell suspension to seed 10000 cells/well in 50 $\mu$ L amounts

Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	CS cells/mL	Stock Volume (uL)	Media Volume (mL)
Plate 1 - Top	R2-DP3	$5.07 \times 10^5$	$4.00 \times 10^5$	2	$2.54 \times 10^5$	788.9	1.2111
Plate 1 - Bottom	R4-DP3	$4.29 \times 10^5$	$4.00 \times 10^5$	2	$2.14 \times 10^5$	932.4	1.0676
Plate 2 - Top	C2-DP3	$1.20 \times 10^6$	$4.00 \times 10^5$	2	$6.00 \times 10^5$	333.3	1.6667
Plate 2 - Bottom	C4-DP3	$4.00 \times 10^5$	$4.00 \times 10^5$	2	$2.00 \times 10^5$	1000.0	1.0000

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

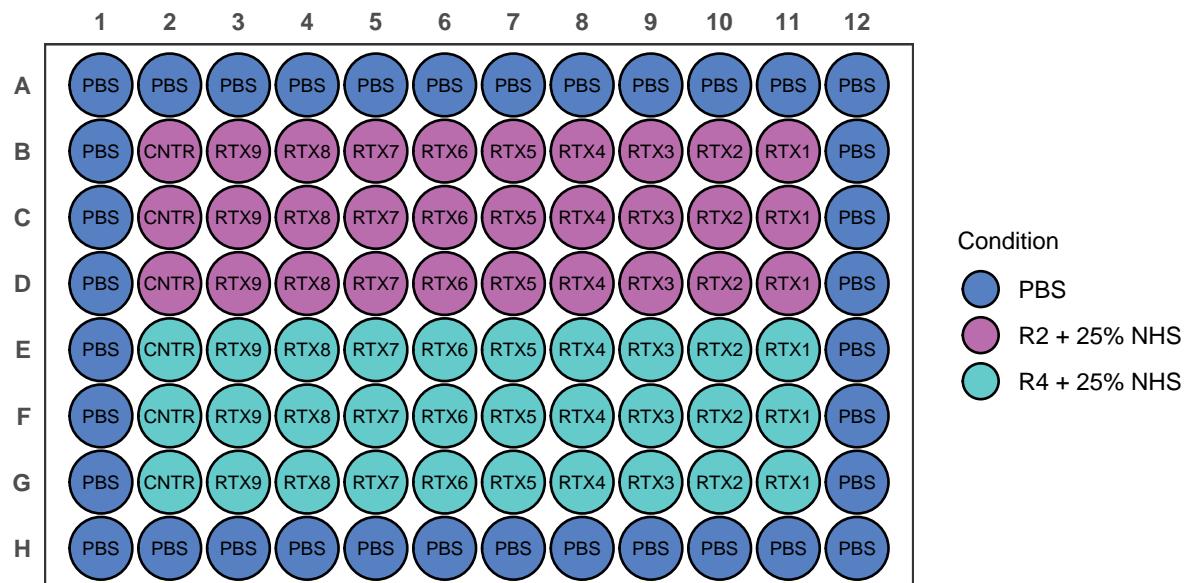
Dilution ID	Well [RTX] (µg/mL)	RTX Source	Source Volume (µL)	Media Volume (µL)	Working Stock [RTX] (µg/mL)
RTX 1	20.0	Stock	15.56	1984.4	80.1
RTX 2	10.0	RTX 1	1000	1000.0	40.1
RTX 3	5.0	RTX 2	1000	1000.0	20.0
RTX 4	2.5	RTX 3	1000	1000.0	10.0
RTX 5	1.3	RTX 4	1000	1000.0	5.0
RTX 6	0.6	RTX 5	1000	1000.0	2.5
RTX 7	0.3	RTX 6	1000	1000.0	1.3
RTX 8	0.2	RTX 7	1000	1000.0	0.6
RTX 9	0.1	RTX 8	1000	1000.0	0.3
CNTR	0.0	-	-	1000.0	0.0

3. Added HIAS/NHS to indicated wells

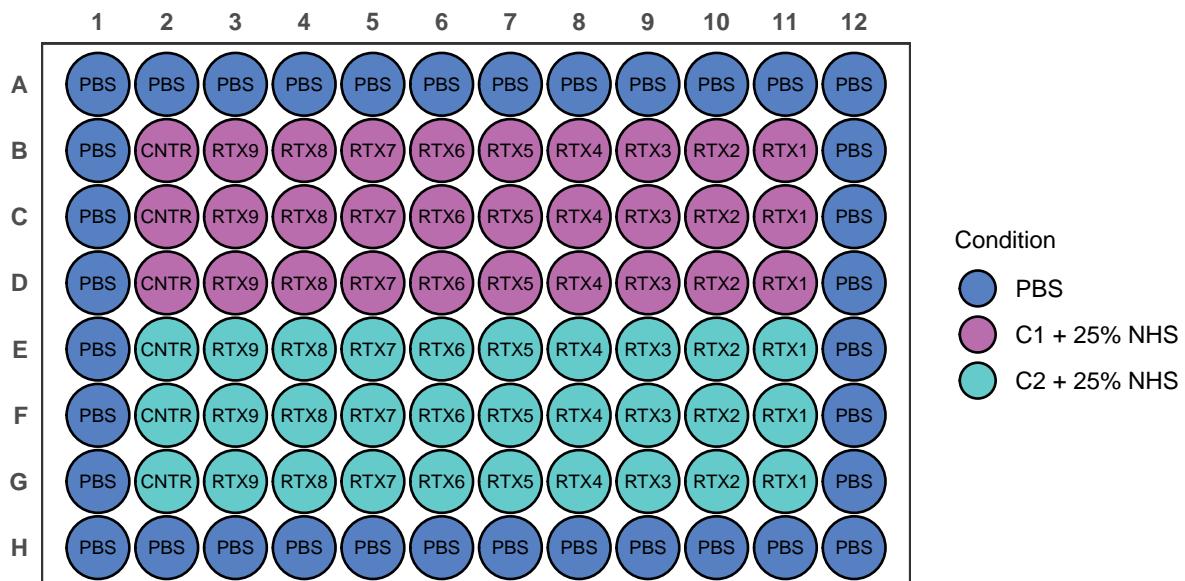
- 25µL/well
- Final well volume = 25% Serum (HIAS/NHS)

4. Plate is incubated for 48 hrs at 37C

### EC50 240814 Plate 1



## EC50 240814 Plate 2



**Friday 16-08-2024**

**Ramos Baseline - BC 1**

- Split 1/10

**RAMOS RTX-DP4**

- Expanded Cx to T25s
- Spun down Rx-DP4 t+ added to 12 well plate

**Collect EC50 240814**

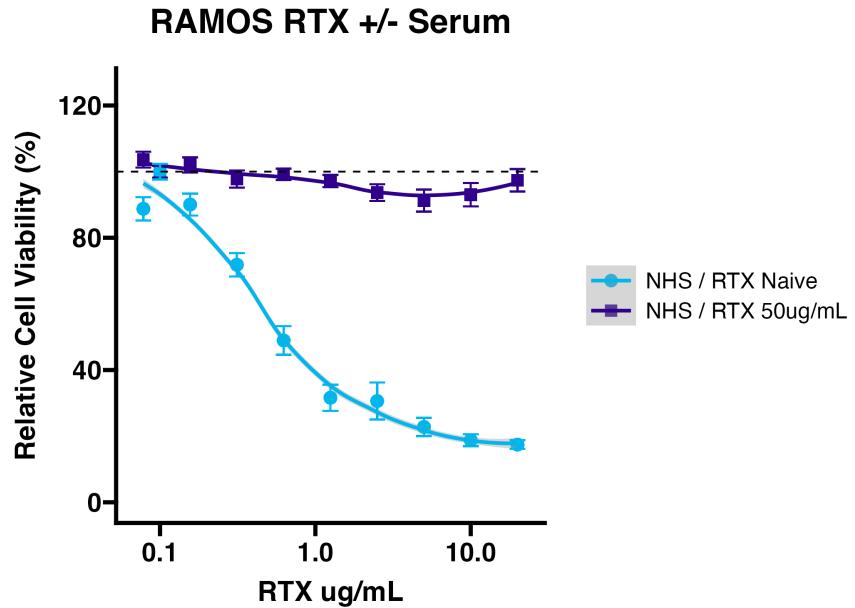
- Add script here

**EC50\_240814 Collection - RAMOS RTX CDC DP3**

- Collected plates seeded on 14-08-2024
- EC Plate collection protocol:
  1. Added 20µL Cell Titre Blue (CTB) to each conditioned well
    - 20µL CTB/100µL of conditioned well recommended by manufacturer
  2. Incubated for 2hr at 37C
  3. Read on plate reader according to Cell Titre Blue Protocol

**Results:**

- HIAS impact on RTX CDC similar to previous experiments
- NHS impact on RTX CDC is consistent with previous experiments



**Monday 19-08-2024**

#### RAMOS Rx-DP4

- Expanded Rx to T25s

**Tuesday 20-08-2024**

#### RAMOS RTX-DP4

- Expanded Rx-DP4 to T25s

#### EC50 RTX RAMOS-DP3 240814 - Seeding

- Seeded an EC50 experiment comparing the effects of RTX + 25% NHS vs 25% HIAS on Cx-DP3 cell lines
  - 3x Rx-DP3 lines (R2,R3,R4) exposed to RTX + 25% HIAS
- Seeded 2 plates each section with a different Ramos conditioned lines
  - Plate 1: R2 + R3
  - Plate 2: R4
- Used Rixathon (Catalogue#: )

#### Plate seeding protocol:

1. Diluted cell suspension to seed 10000 cells/well in 50 $\mu$ L amounts

Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	CS cells/mL	Stock Volume (uL)	Media Volume (mL)
Plate 1 - Top	R2-DP3	$8.75 \times 10^5$	$4.00 \times 10^5$		$4.38 \times 10^5$	457.1	1.5429
Plate 1 - Bottom	R3-DP4	$5.02 \times 10^5$	$4.00 \times 10^5$		$2.51 \times 10^5$	796.8	1.2032

Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	CS cells/mL	Stock Volume (uL)	Media Volume (mL)
Plate 2 - Top	R4-DP3	$8.75 \times 10^5$	$4.00 \times 10^5$	2	$4.38 \times 10^5$	457.1	1.5429

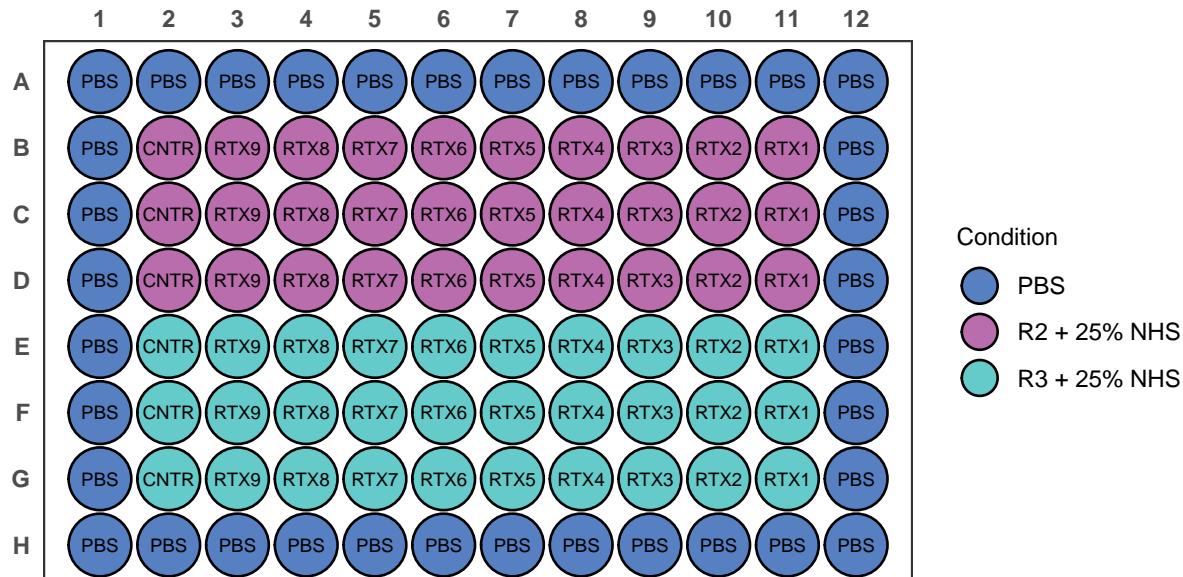
2. Made RTX dilutions and added to respective wells in  $25\mu\text{L}$
- [RTX stock] = 10.3 mg/mL
  - Drug volumes are added in triplicate
  - Drug volumes are being added constitute 1/4 of well volume:
    - [RTX working] needs to be 4x [RTX well]
      - 6 wells per condition,  $25\mu\text{L}$  per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

Dilution ID	Well [RTX] ( $\mu\text{g/mL}$ )	RTX Source	Source Volume ( $\mu\text{L}$ )	Media Volume ( $\mu\text{L}$ )	Working Stock [RTX] ( $\mu\text{g/mL}$ )
RTX 1	20.0	Stock	9.300000000000000007	1190.7	79.8
RTX 2	10.0	RTX 1	600	600.0	39.9
RTX 3	5.0	RTX 2	600	600.0	20.0
RTX 4	2.5	RTX 3	600	600.0	10.0
RTX 5	1.2	RTX 4	600	600.0	5.0
RTX 6	0.6	RTX 5	600	600.0	2.5
RTX 7	0.3	RTX 6	600	600.0	1.2
RTX 8	0.2	RTX 7	600	600.0	0.6
RTX 9	0.1	RTX 8	600	600.0	0.3
CNTR	0.0	-	-	1000.0	0.0

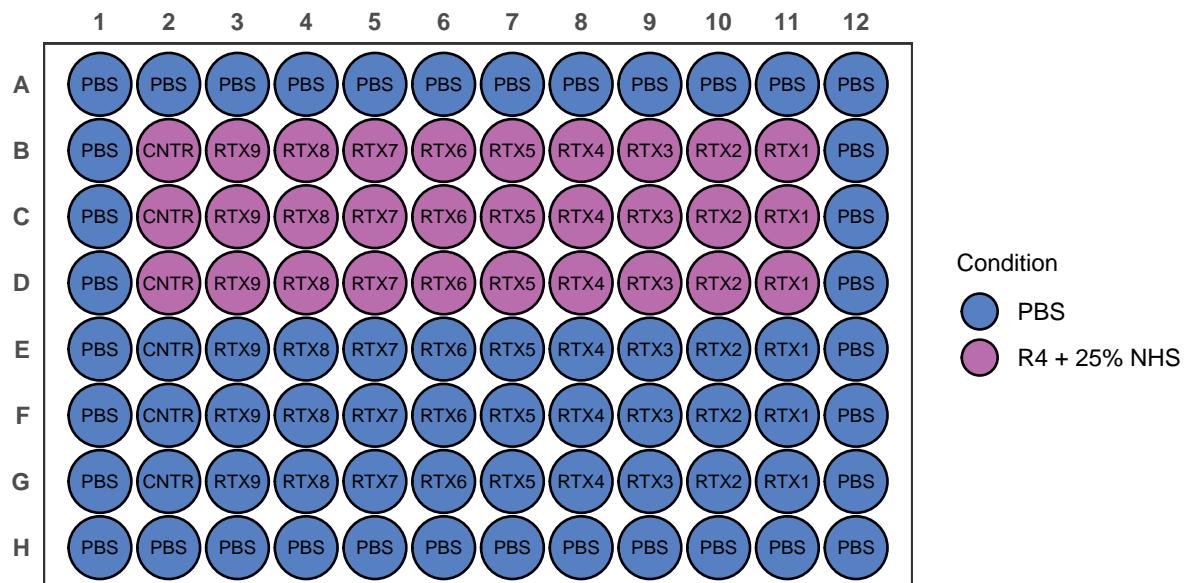
3. Added HIAS/NHS to indicated wells
- $25\mu\text{L}$ /well
  - Final well volume = 25% Serum (HIAS/NHS)

4. Plate is incubated for 48 hrs at 37C

## EC50 240820 Plate 1



## EC50 240820 Plate 2



Wednesday 21-08-2024

### RAMOS RTX CDC DP4 - CD20 Flow Cytometry

- Checked CD20 expression between Cx and Rx DP4
- CD20:
- Samples tested:
  - Baseline RAMOS
  - R2
  - R3
  - R4
  - C1
  - C2
  - C6

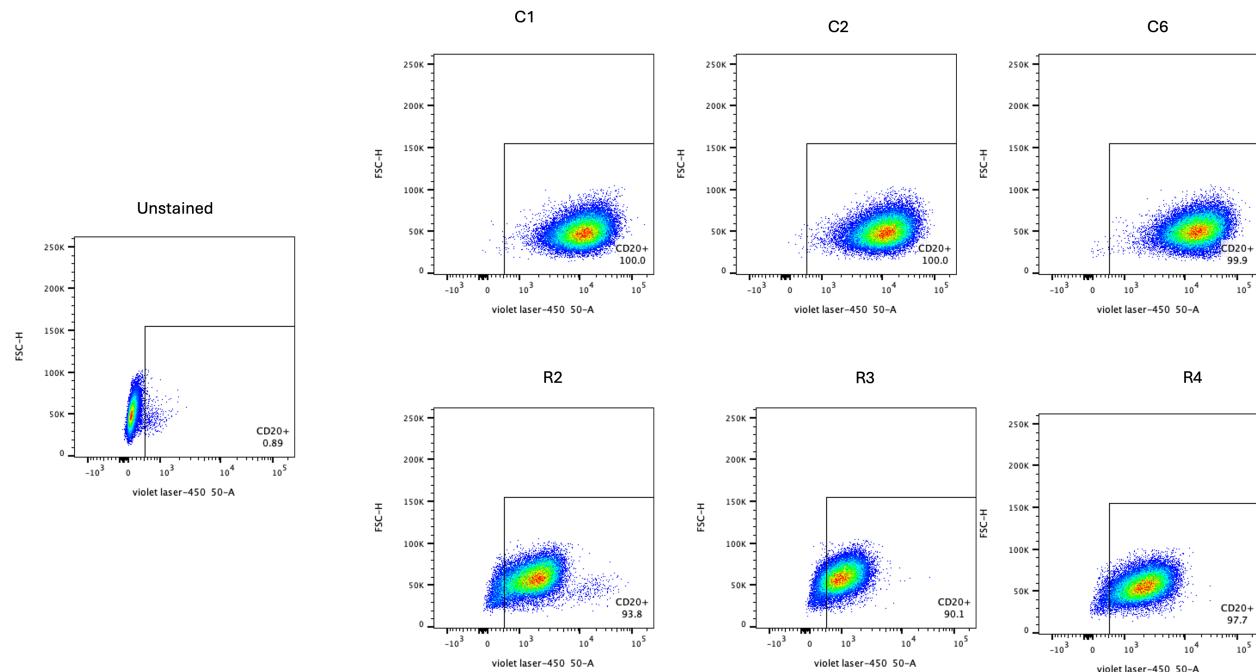
### CD20 Flow Protocol

- Prior to starting: Make cell stain
    - 500 $\mu$ L PBS + 2 $\mu$ L Stain
    - 100 $\mu$ L of stain used/condition
  - Cell stain made: 2000  $\mu$ L and 8  $\mu$ L
1. Resuspend cells and transferred to 3 wells of a 96 well plate
  2. Spun down at 1500g for 2min
  3. Flick media out
  4. Resuspend w/ 100 $\mu$ L stain in well and consolidate in 1 well
    - Add 100 $\mu$ L to bottom well and mix until cells resuspended
    - Take 100 $\mu$ L CS-stain mix and add to well below and mix
    - Repeat for third well and add the total CS-Stain mix to top well
  5. Incubate in fridge for 25min
    - In the dark

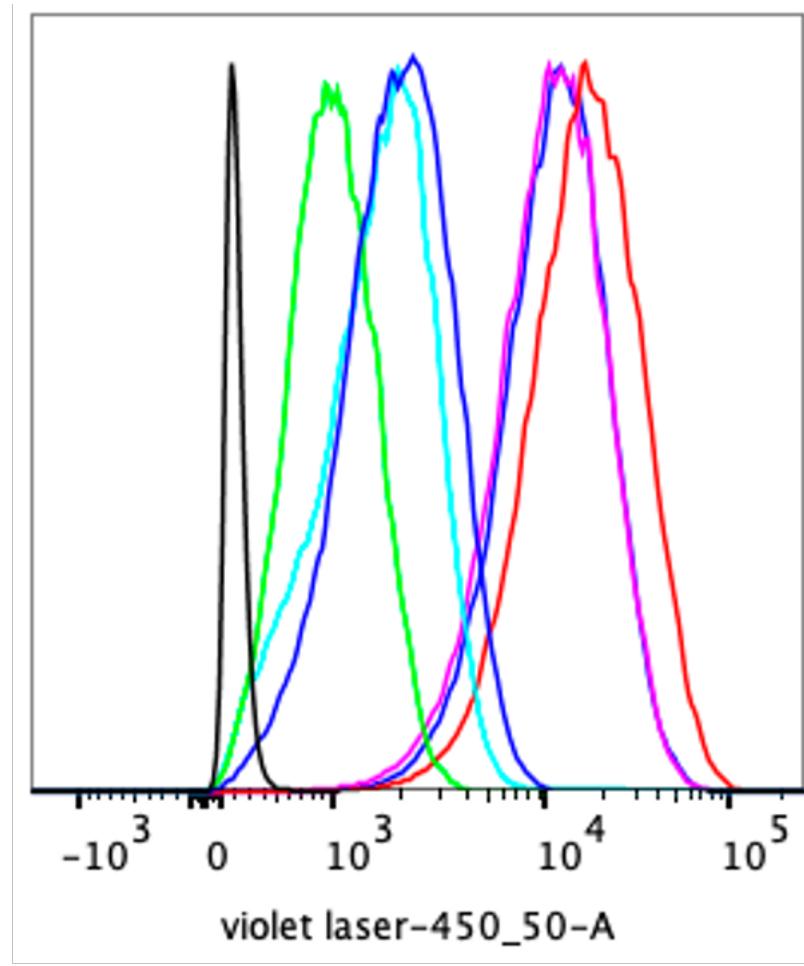
6. Spin down at 1500g for 2min
7. Flick out media
8. Resuspend in PBS and add to FACS tube
  - Add an additional volume of PBS to dilute cells appropriately for flow
  - Usually make it up to about 300-400 $\mu$ L CS-PBS in the tube

## Results

- No apparent change in CD20 expression
- This is interesting based on strong evidence which suggests loss of CD20 is the primary mechanism of RTX resistance



- Histogram analysis is interesting
  - CD20 staining is not as strong
- This could be due to RTX exposure
  - CD20 Ab competing with bound RTX
  - Expression is not necessarily different
- Colours:
  - Rx-DP4: Green, Light blue, Dark Blue
  - Cx-DP4: Purple, Blue, Red



Thursday 22-08-2024

## DP4 Collections

### Viable Freezing

- Viably froze the following for splitseq
  - R1-DP4
  - R2-DP4
  - R3-DP4
  - R4-DP4
  - C1-DP4
  - C2-DP4
  - C3-DP4
  - C4-DP4

### Protocol

- Need ~500k cells for each sample
  - Ex. one well from a 6 well plate
- Freeze down a few extra samples in order to test PCR cycles for split-seq

### PBS + RI

- Make up fresh and store at 4C

Component	Volume/sample	Volume/20 samples
PBS (TC Grade)	2 mL	42 mL
RNase OUT	40 U/ $\mu$ L	840 U/ $\mu$ L
SUPERase Inhibitor	20 U/ $\mu$ L	420 U/ $\mu$ L

### PBS + Formaldehyde

- Make up fresh on the day and chill

Component	Volume/sample	Volume/20 samples
PBS (TC Grade)	2.75 mL	57.75 mL
16% Formaldehyde (Pierce cat.28906)	0.25 mL	5.25 mL

### Protocol

1. Collect ~500k cells from cell suspension
2. Spin down at 180 x g (RCF)
3. Resuspend pellet in 1mL of PBS + RI
4. Add 3mL of ice cold PBS + 1.33% formaldehyde and mix gently by pipetting up and down 2-3 times
5. Incubate on ice for 10min
6. Spin 300g for 3min at RT
7. Wash pellet with 1mL PBS + RI and respin 300g for 3min at RT
8. Remove supernatant and snap freeze pellet on dry ice
9. Store pellet at -80C #### Freezing Down Cells Protocol
10. Counted cells in suspension
11. Took volume of cell suspension such that each vial would contain at least  $2 \times 10^6$  cells
12. Spun down CS @ 300 rcf for 5min
13. Discarded supernatant and resuspended in freezing media such that 1ml of freezing media contains  $2 \times 10^6$  cells - Freezing media: FBS + 10% DMSO
14. Added 1mL CS in freezing media per cryovial
15. Cryovials were immediately put into freezing caddy and placed in -80 freezer
16. After 24hrs vials removed from freezing caddy and placed in Liquid Nitrogen
  - Location: Tank 1, Rack 5, Box 6