Turner Lab Notebook

Callum Malcolm

Contents

MARCH 2023

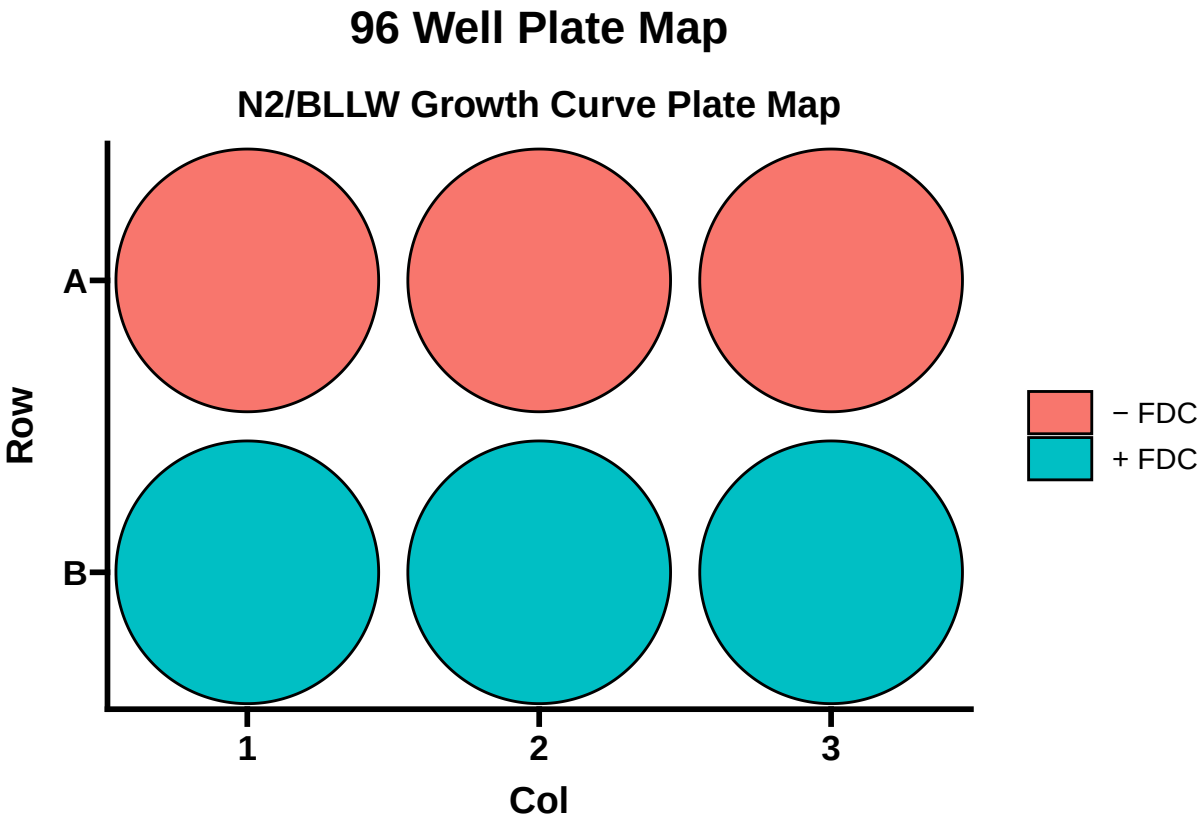
Wednesday 15-03-2023

Cell Culture

- Given N2/RBL1/BLLW PDX line from Chris Steele

N2 Growth Curve

Looking to compare N2 growth on and off feeders
1. Seeded 2mL of N2/BLLW cell suspension at 0.25x10^{6}cells/mL



Thursday 16-03-2023

N2/BLLW Growth Curve Day 1

- Cells gently mixed
- 30 μL cell suspension taken and counted
- Only counted 1 square and multiplied by 4

Cell Line	FDC Status	Count	Cell Number
N2	+ FDC	25	2000000
N2	+ FDC	26	2080000
N2	+ FDC	12	960000
N2	- FDC	15	1200000
N2	- FDC	14	1120000
N2	- FDC	6	480000
BLLW	+ FDC	6	480000
BLLW	+ FDC	6	480000
BLLW	+ FDC	5	400000
BLLW	- FDC	3	240000
BLLW	- FDC	3	240000
BLLW	- FDC	6	480000

Friday 17-03-2023

N2/BLLW Growth Curve Day 2

- Cells gently mixed
- 30 μL cell suspension taken and counted
- Only counted 1 square and multiplied by 4

Cell Line	FDC Status	Count	Cell Number
N2	+ FDC	36	2880000
N2	+ FDC	28	2240000
N2	+ FDC	38	3040000
N2	- FDC	61	4880000
N2	- FDC	38	3040000
N2	- FDC	16	1280000
BLLW	+ FDC	3	240000
BLLW	+ FDC	5	400000
BLLW	+ FDC	5	400000
BLLW	- FDC	1	80000
BLLW	- FDC	0	0
BLLW	- FDC	1	80000

Split Cells

Cell Line	Cells/mL	Cells/mL Seeded	CS volume	Final Volume	Seeded Cells/mL
N2	$8.8x10^5$	$3x10^5$.	6.8 mL	12 mL	$3x10^5$
RBL1	$8.6x10^5$	$3x10^5$.	11.6 mL	12 mL	$5x10^5$

Saturday 18-03-2023

N2/BLLW Growth Curve Day 3

- Counted by Chris

Sunday 18-03-2023

N2/BLLW Growth Curve Day 4

- Counted by Chris

Monday 20-03-2023

N2 Growth Curve Day 5

- BLLW were all dead, experiment stopped
- Cells gently mixed
- 30 μL cell suspension taken and counted
- Only counted 1 square and multiplied by 4

Cell Line	FDC Status	Count	Cell Number
N2	+ FDC	146	11680000
N2	+ FDC	112	8960000
N2	+ FDC	132	10560000
N2	- FDC	112	8960000
N2	- FDC	155	12400000
N2	- FDC	141	11280000

Split Cells

- Split cell lines N2/RBL1/JIRE
- Dirty split 1:2

Seed BLLW

- Given BLLW aliquot from Chris Steel
- Thawed and resuspended in 12 mL media
- Added to T75 pre-seeded with FDC cells (from Chris Steel)

Tuesday 21-03-2023

Large Infection in TC
- N2 Growth Curve stopped

Friday 24-03-2023

Seed Cells - N2/RBL1/JIRE

- Vials given by Chris Steel
- Thawed and resuspended in 12 mL media
- Added to T75 pre-seeded with FDC cells (from Chris Steel)

Monday 27-03-2023

Split Cells

- Split cell lines N2/RBL1/JIRE
- Dirty split 1:3

Wednesday 29-03-2023

Split Cells

- Split by Chris Steel.

Friday 31-03-2023

Split Cells

- Split cell lines N2/RBL1/JIRE
- Dirty split 1:4

APRIL 2023

Monday 03-04-2023

Large Infection in TC

Seed Cells - N2/RBL1/JIRE

- Vials given by Chris Steel
- Thawed and resuspended in 12 mL media
- Added to T75 pre-seeded with FDC cells (from Chris Steel)

Seed EC50

DNW - N2/JIRE/RBL1
- Vin/Dactolisib

Wednesday 05-04-2023

Seed Cells - N2/RBL1/JIRE

- Vials given by Chris Steel
- Thawed and resuspended in 12 mL media
- Added to T75 pre-seeded with FDC cells (from Chris Steel)

Thursday 06-04-2023

Collect EC50 Plate

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- Plate ID: EC50_230619_Plate1
- DNW
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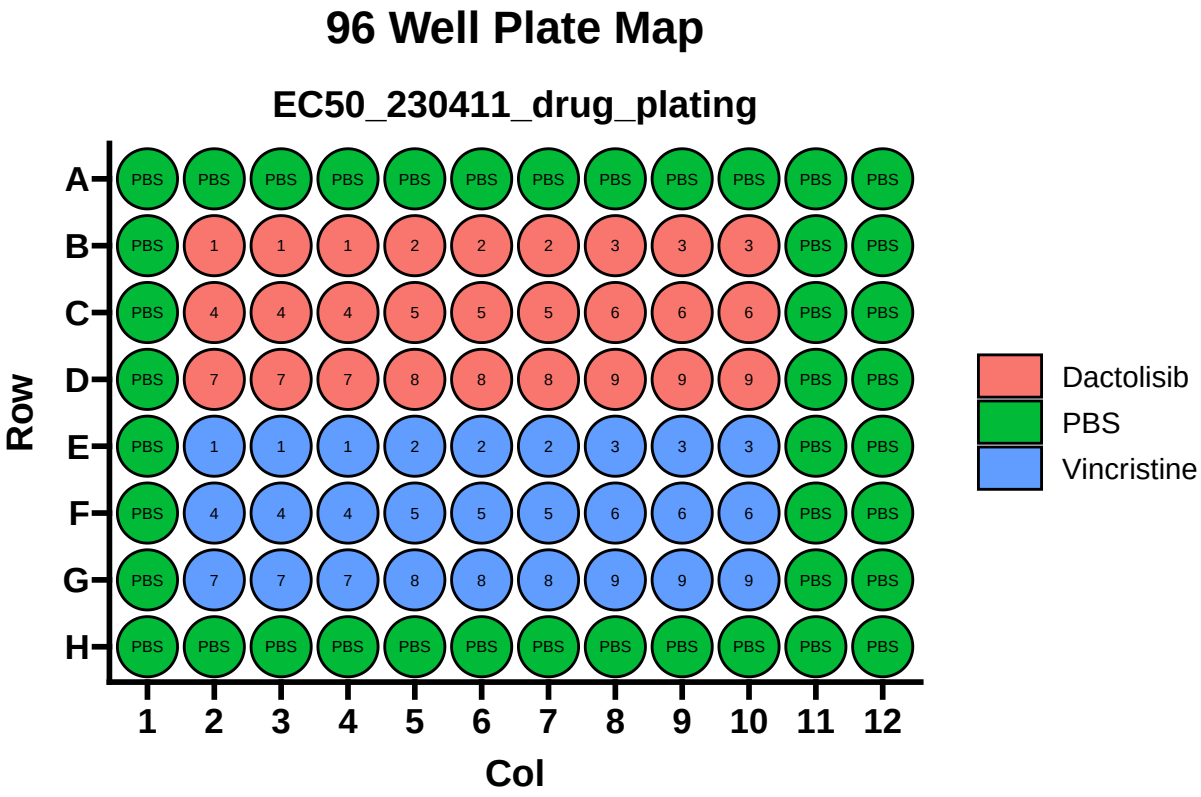
Tuesday 11-04-2023

Seed EC50_230411



Drug Dilutions

Drug	Dilution	Concentration	Units	Starting_Concentration
Dactolisib	1	20	uM	20000
Dactolisib	2	2	uM	2000
Dactolisib	3	0.2	uM	200
Dactolisib	4	0.02	uM	20
Dactolisib	5	0.002	uM	2
Dactolisib	6	0.0002	uM	0.2
Dactolisib	7	2e-05	uM	0.02
Dactolisib	8	2e-06	uM	0.002
Dactolisib	9	2e-07	uM	0.0002

Drug	Dilution	Concentration	Units	Starting_Concentration
Vincristine	1	30	nM	30000
Vincristine	2	3	nM	3000
Vincristine	3	0.3	nM	300
Vincristine	4	0.03	nM	30
Vincristine	5	0.003	nM	3
Vincristine	6	0.0003	nM	0.3
Vincristine	7	3e-05	nM	0.03
Vincristine	8	3e-06	nM	0.003
Vincristine	9	3e-07	nM	0.0003

- 3 plates seeded
  - Plate 1: N2

  - Plate 2: JIRE
  - Plate 3: RBL1

Wednesday 12-04-2023

Cell Culture

- Thaw RBL1

Side Population - N2_230412

- Hoescht
  - 2.5 / 2.75 / 3

  - DNW

MAY 2023

Tuesday 02-05-2023

Seeding Cells - N2/RBL1/RAMOS

1. Thawed N2, RBL1, and RAMOS into T75 flasks
   Note: N2 and RBL1 flaskes seeded with feeders

Wednesday 03-05-2023

Split Cells

Cell Line	Cells/mL	Cells/mL Seeded	CS volume	Final Volume	Last day split	P number
N2	$8.93x10^5$	$3x10^5$.	6.71 mL	12 mL	Tues 02-05-23	2
RBL1	$7.73x10^5$	$3x10^5$.	7.76 mL	12 mL	Tues 02-05-23	2

2. Expanded N2-Barcodes to T175
3. Seeded feeders into 96-well
   a. Diluted feeders to $3.2x10^4$ cells/mL
   b. Add 100μL cell suspension to each well

Thursday 04-05-2023

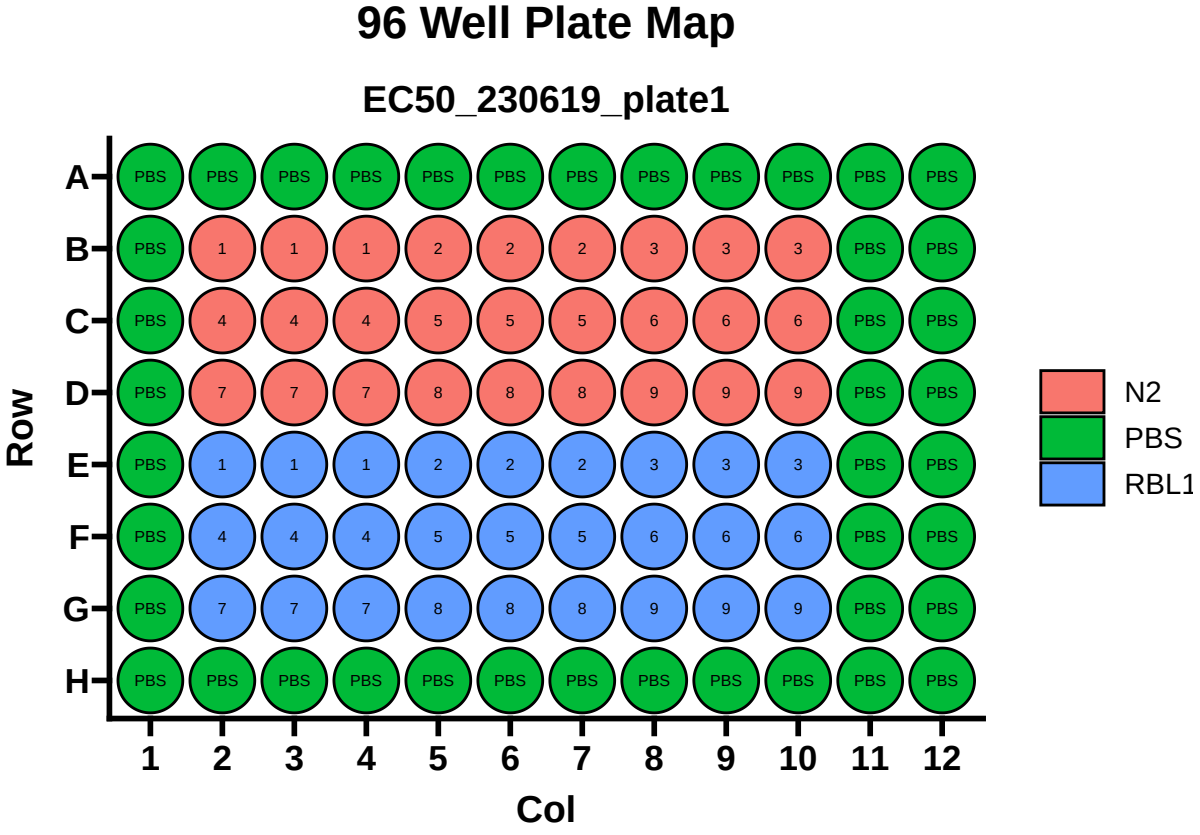
Monday 19-06-2023

Split Cells

- Split N2/RBL1/JIRE
- Added 10 mL media to BLLW
  - Lots of dead cells observed

Seed EC50

- Plate ID: EC50_230619_Plate1
- Cylophosphamide (Stock: 20mM) EC50 for N2 and RBL1



Drug Dilutions

Drug	Dilution	Concentration	Units	Starting_Concentration
Cyclophosphamide	1	20	uM	20000
Cyclophosphamide	2	2	uM	2000
Cyclophosphamide	3	0.2	uM	200
Cyclophosphamide	4	0.02	uM	20
Cyclophosphamide	5	0.002	uM	2
Cyclophosphamide	6	0.0002	uM	0.2
Cyclophosphamide	7	2e-05	uM	0.02
Cyclophosphamide	8	2e-06	uM	0.002
Cyclophosphamide	9	2e-07	uM	0.0002

Saturday 20-05-2023

JUNE 2023

Tuesday 20-06-2023

RNA Extraction for SP Test 3 Sample

- Sample collected:

- Low number of cells so used Pico-Pure Kit
- 1 sample (n = 1)

Note before starting:
- Pre-heat block to 42C
- Pre-make DNAse treatment master mix (1.1n)

Component	Volume	MM volume
DNAse I Stock	5 μL	5.5 μL
DNA Disgestion Buffer	35 μL	38.5 μL

RNA Extraction Pico-Pure Protocol

1. Extract cells with 100 μL of Extraction Buffer (XB)
   - Resuspend the cell pellet gently by pipetting
   - DO NOT VORTEX

2. Incubate at 42C for 30min

3. Centrifuge sample at 3000xg for two minutes

4. Collect supernatant
   - Can stop here and freeze RNA at 70C

5. Pre-condition the RNA Purification Column
   - Add 250 μL Conditioning Buffer onto the purification column filter membrane
   - Incubate RNA Purification Column with CB for 5min at RT
   - Centrifuge purification column at 16000xg for 1 min

6. Pipette 100 μL of 70% Ethanol (EtOH) into cell extract
   - Mix well by pipetting
   - DO NOT VORTEX

7. Add cell extract to column

8. Centrifuge column for 2 minutes at 100 x g and then 16000 x g for 30s

9. Pipette 100 μL Wash Buffer 1 (W1) into the purification column and centrifuge for 1min at 8000 x g

10. Add 40 μL of DNAse treatment master mix to purification tube
    - Add directly onto membrane

11. Incubate at RT for 15 min

12. Pipette 40 μL Wash Buffer 1 (W1) onto purification column membrane and centrifuge at 8000 x g for 15 seconds

13. Pipette 100 μL Wash Buffer 1 (W2) into purification column membrane and centriguge for one minute at 8000 x g

14. Pipette 100 μL Wash Buffer 1 (W2) into purification column membrane and centriguge for two minutes at 16000 x g
    - Check if any wash buffer remains in column and centrifuge again at 16000 x g for one minute to clear

all the liquid

15. Transfer purification column to new 0.5mL tube
    - Insert purification column/0.5mL assembly into 1.5mL tube for centrifugation

16. Pipette EB directly onto membrane of the purification column
    - Gently touch tip to surface of membrane to ensure maximum absorption of EB into membrane
    - Use 11 μL to 30 μL EB

17. Incubate purification column for one minute at room temperature

18. Centrifuge column for one minute at 1000 x g to distribute EB in column

19. Centrifuge column for one minute at 16000 x g to elute RNA

20. Started PCR and completed Tapestation

Tapestation_230620

High-Sensitivity RNA ScreenTape Protocol

Results
- Inconclusive, proceeded with PCR

Reverse Transcription

Notes Before Starting:

- Label PCR strip tubes
- Thaw RT reagents
- Uses WILDseq specific RT primer
- Dilute RT primer to 2μM
  - Do this in a PCR hood
  - **IMPORTANT** Do not mix primers and carefully note which RT primer is added to which sample
- Make RT and ExoI master mixes (MM) before starting
  - MM amounts are 1.1n

Protocol:

Note: Used "rt" protocol in callum folder on thermocycler for heating steps

1. In PCR strip tubes diluted 1-5ug RNA in a total volume of 10 μL of RNAse/DNAse-free water

2. Added 1 ul of RT primer

3. Added 1 ul of dNTPs
4. Denatured at 65 C for 5 mins in PCR machine

5. Added straight to ice for 2 mins

6. Spun to get liquid to bottom of tube

7. Prepared master mix of RT enzyme and buffers (Made prior to starting)

Component	Volume	MM volume
5x SSIV Buffer	4 μL	4.4 μL

Component	Volume	MM volume
SSIV RT	1 μL	1.1 μL
100 mM DTT	1 μL	1.1 μL
RNAse Out	1 μL	1.1 μL

8. Added 7 μL of RT MM prepared above to each sample and mix
    - Spun briefly to get liquid to bottom of the tube
9. In PCR Machine: incubated at 53C for 10 mins followed by 80C for 10 mins
10. Added 3 μL of ExoI MM to each sample

Component	Volume	MM volume
Thermolabile Exonuclease I	1 μL	1.1 μL
NEBuffer r3.1*	2 μL	2.2 μL

- Most PCR buffers are compatible

11. 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
12. Add 1 μL of RNAse H and incubate at 37C for 20 mins
13. Dilute final cDNA 1 in 2 with DNAse/RNAse-free water

PCR Amplification of cDNA

1. Prepare mastermix of below reagents at 1.1x the number of samples

Component	Volume	MM volume
Q5 Master Mix	25 μL	
P5_R1_fwd (100μM)	2.5 μL	
P7_rev	2.5 μL	
DNAse/RNAse H20	13.5 μL	
Q5 polymerase	0.5 μL	
Total Master Mix volume	**40 μL**	
cDNA from above	10 μL	

2. Perform PCR using the following parameters:

Steps	Time
Step 1: 98C	30s
Step 2: 98C	**10s**
Step 3: 61C	**30s**
Step 4: 72C	**30s**
Step 5: 72C	2 min
Step 6: 12C	Hold

20-25 cycles of steps 2-4

Ran overnight and left at 12C

Wednesday 21-06-2023

Tapestation_230619_SPTest3_cDNA

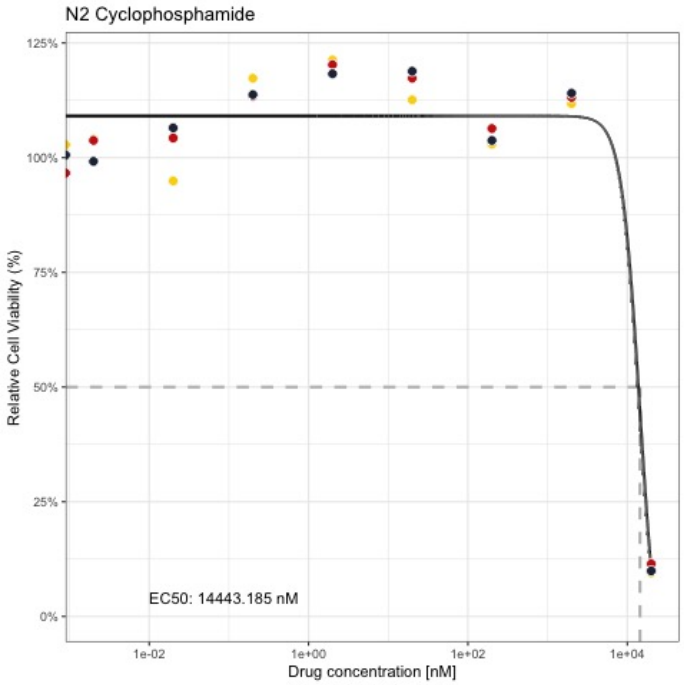
High-Sensitivity DNA ScreenTape Protocol

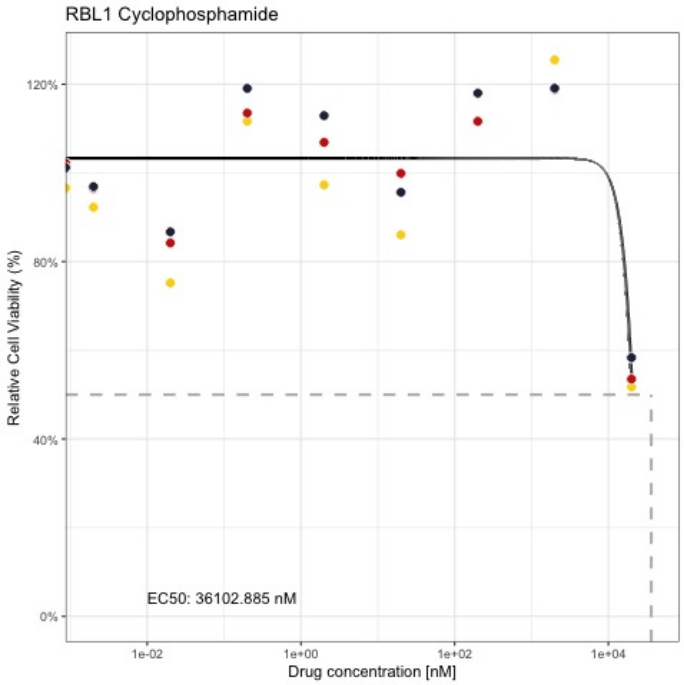
Results
* Did not work, bands present but faint and not at 200bp
* Potential solutions:
- Repeat PCR with increased cycles
- Repeat RNA extration with trizol protocol (Anna)

Thursday 22-06-2023

Collect EC50 Plate

- Plate ID: EC50_230619_Plate1





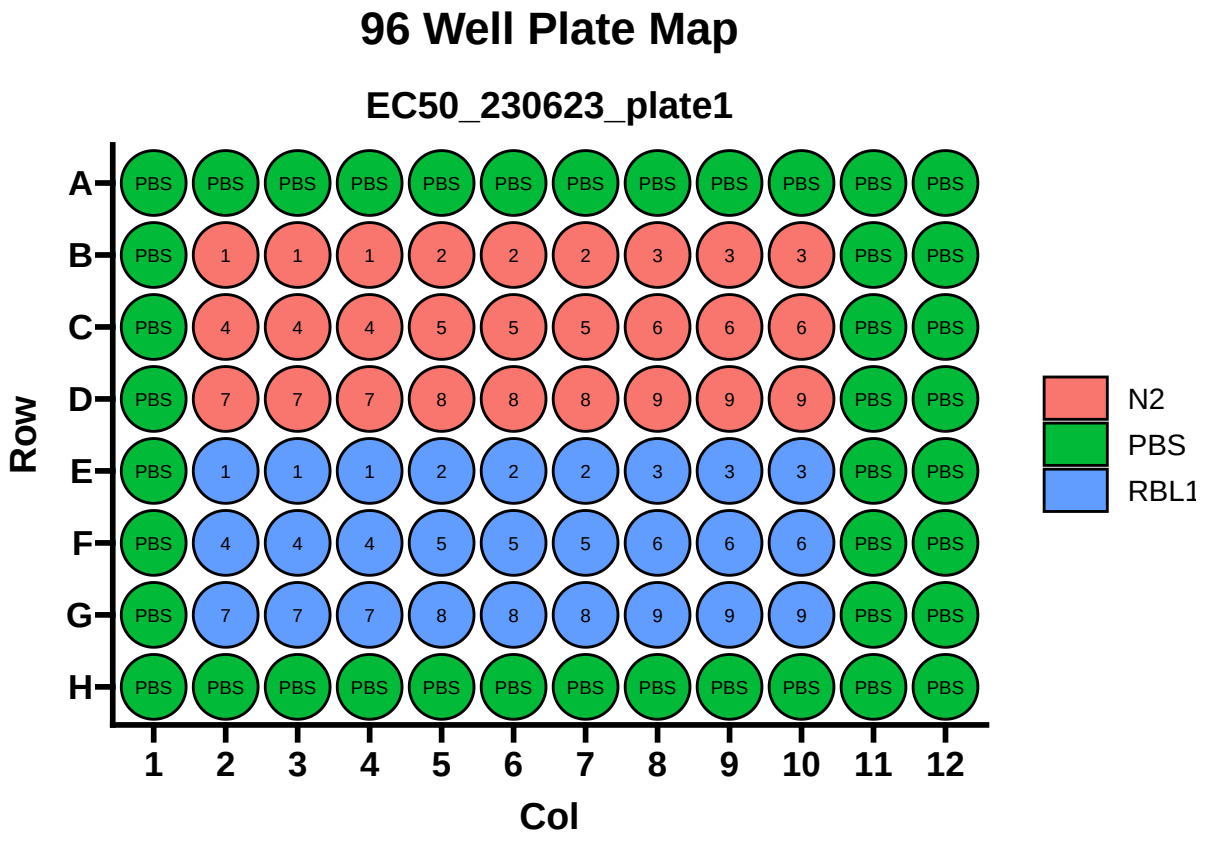
Friday 23-06-23

Split Cells

1. Threw out BLLW (all dead)
2. Split JIRE 1/2
3. Split N2/RBL1 1/4

Seed EC50

- EC50 Plate ID: EC50_230623_Plate1



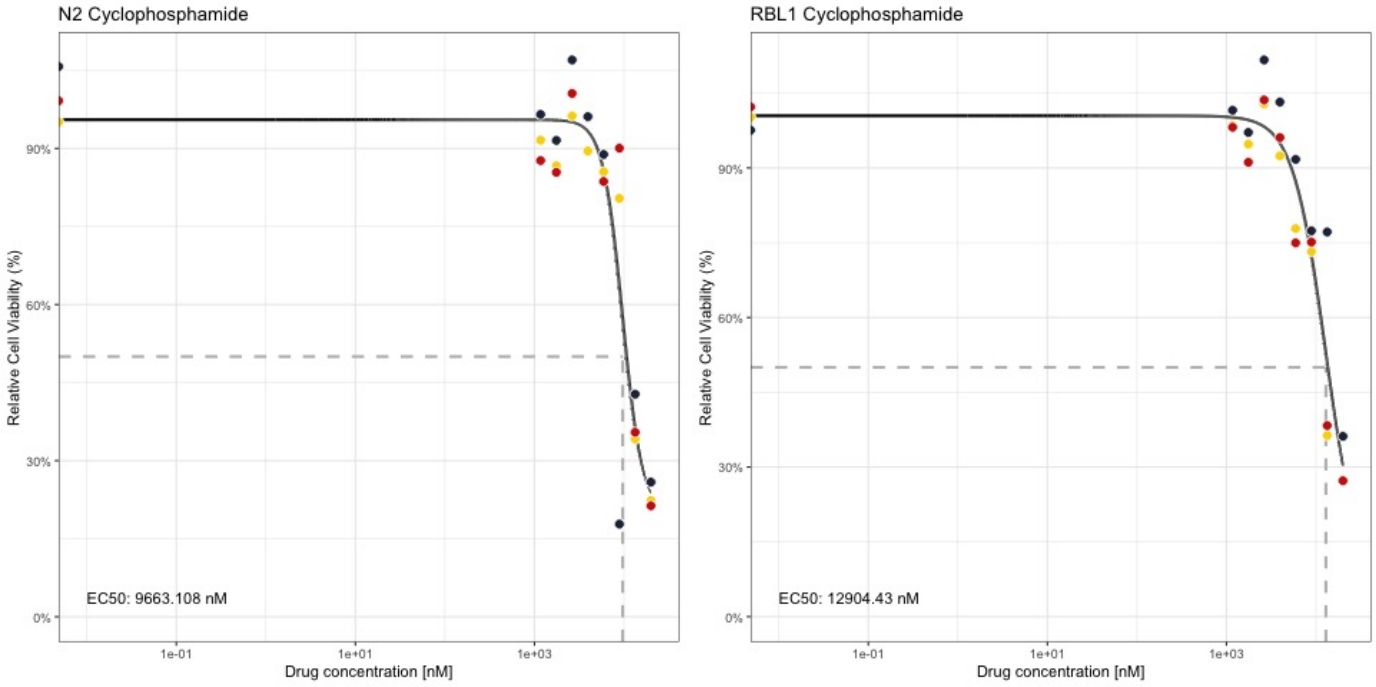
Drug Dilutions

Drug	Dilution	Concentration	Units	Starting_Concentration
Cyclophosphamide	1	20	uM	20000
Cyclophosphamide	2	13.3	uM	13300
Cyclophosphamide	3	8.89	uM	8890
Cyclophosphamide	4	5.93	uM	5930
Cyclophosphamide	5	3.95	uM	3950
Cyclophosphamide	6	2.63	uM	2630
Cyclophosphamide	7	1.76	uM	1760
Cyclophosphamide	8	1.17	uM	1170
Cyclophosphamide	9	0.78	uM	780

Monday 26-06-23

Collect EC50 Plate

- Plate ID: EC50_230623_Plate1



Break for writing MRes Report: 27/06/2023 - 24/07/2023

JULY 2023

Monday 24-07-2023

Seeding Cells - RAMOS/FDC

Made New Media: AR-1

1. Made new media: AR-1

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%

2. Seeded Feeders

- 2 vial of FDC cells thawed
- Resuspended in 12 mL of media
- 6 mL of FDC cell suspension were added to 2 seperate T75 flasks (Seeding RBL1/BLLW on 25-07-2023)
- Remaining 12 mL of Media were added to T175 (Seeding N2 on 25-07-2023)

3. Seeded Ramos WILDseq Barcode Pool 2

- Thawed a vial of RAMOS pool 2 (previously barcorded DATE)
- Resuspended in 5 mL
- Spun down 90* for 5min

- Resuspended in 12 mL of AR-1 media
- Seeded into T75

Experimental Plans

1. Barcode BLLW/RBL1
2. Set up Drug pressure experiement for RAMOS barcoded cells

- EC50's for cyclophosphamide and methotrexate need to be completed
- Drugs to test (EC50):

Drug	Concentration	Replicates
DMSO	1%	3
Doxorubicin	200nM	3
Methotrexate	100nM	3
Vincristine	1nM	3

3. Do Side Population Experiement for N2 Barcoded Cells

- Grow up sorted side pop to avoid issues with low cell numbers

Tuesday 25-07-2023

Seed Cells - N2-BC4/RBL1/BLLW/RAMOS-BC1

- Got vials from Chris
- Thawed, resuspended in 6 mL AR-1
- Spun down at 90* for 5 min
- Discarded supernatant and resuspended pellet in 12 mL of AR-1
- Added RBL1/BLLW to T75's pre-seeded with FDC (24-07-2023)
- Added RAMOS-BC1 to new T75
  - RAMOS-BC2 from 24-07-2023 was dead
  - Was taken from -80, New RAMOS vial from LN
- Added N2 to T125 seeded with FDC (24-07-2023)

Wednesday 26-07-2023

Cell Culture

- Checked cells, all flasks looked healthy
- Added media
  - N2 - 20ml of AR-1
  - RAMOS - 10 mL of AR-1
  - RBL1 - 10ml of AR-1
  - BLLW - 10ml of AR-1

Thursday 27-07-2023

Split Cells - RAMOS

- Split Ramos 1:2
- Added 10ml of AR-1 to N2
- Plan to split RBL1/BLLW tomorrow

Lucy Drug Randomizer

- Randomized drugs for Lucy Hare mouse experiment

Drug	Identification
Alectinib	A
Erda	B
Vehicle	C
Alectinib + Erda	D

Friday 28-07-2023

Drug	Dilution	Concentration	Units	Starting_Concentration
Cyclophosphamide	1	20	uM	20000
Cyclophosphamide	2	13.3	uM	13300
Cyclophosphamide	3	8.89	uM	8890
Cyclophosphamide	4	5.93	uM	5930
Cyclophosphamide	5	3.95	uM	3950
Cyclophosphamide	6	2.63	uM	2630
Cyclophosphamide	7	1.76	uM	1760
Cyclophosphamide	8	1.17	uM	1170
Cyclophosphamide	9	0.78	uM	780

OCTOBER 2023

Friday 06-10-2023

Cell Culture

Made New Media: AR-2

1. Made new media: AR-2

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%

Monday 09-10-2023

Cell Culture

Seeded A20

- Got A20 from Swetha box: 2nd rack in shared -80
- AR-2

Seeded FDC

- Seeded feeders, taken from feeder box, irradiated by Chris
- Will seed N4 PDX tomorrow

Tuesday 10-10-2023

Cell Culture

Seeded N4

- Given by Chris -AR-2
- Seeded onto FDC cells from 09-10
- Future experiments:
  - Barcode PDX
  - EC50: Vincristine/Doxorubicin/Methotrexate/Cyclophosphamide (Active)

Seeded RAMOS-BC

- Seeded barcoded Ramos cells - Pool #4
  - From Chris
  - LN tank 1 - Rack 5 - box 2nd from Bottom
- AR-2
- Future Experiments:
  - Combination Drug pressure: Vin/Dox/Meth

Checked A20

- Look healthy
- ~40% confluency

Wednesday 11-10-2023

Cell Culture

A20 Culture

- ~70% confluent
- Split A20 in half
  - Half in 1xT75 and other half in 1xT175
  - T175 to be frozen down

RAMOS-BC4 Culture

- ~60% confluent
- Spun down at 400xg for 5 min and resuspended in 20ml AR-2

N4 Culture

- Look healthy, attached to feeders with some floaters
- Will add media tomorrow
- Plans:
  - Freeze down aliqouts (2-3x10^6 per vial needed, ideally freeze down 3x vials)
  - Plan barcoding experiment

Thursday 12-10-2023

Cell Culture

Ramos-BC4

- Infected, will need to grab another vial
- Is the Stock infected? Will thaw 2 different pools this time

A20

- 1 x T75 + 1 x T175 both about ~60% confluent
- Media is slughtly turbid, Jamie did a visual check and cells seem clean (A20 have a tendency towards slight turbidity - Jamie)
- Will add 10mL of AR-2 and observe till tomorrow
- If clean:
  - Freeze down 3 vials from T175
  - Set up EC50s with left over cells (Vin/Dox/Meth)
  - Split

N4

- Look healthy, media clean
- About ~40-50% confluent
- Will add 10mL of AR-2 and observe on Friday

Friday 13-10-2023

Cell Culture

A20

- Freeze down (10% DMSO in FBS)
- A20 count: $2.91 \text{x} 10^6$/mL
  - 2mL + 3mL Media
- 3 vials frozen and put in LN Tank 6, Rack 5, Box 6

EC50_231013

EC50 Plate ID: EC50_231013_Plate1

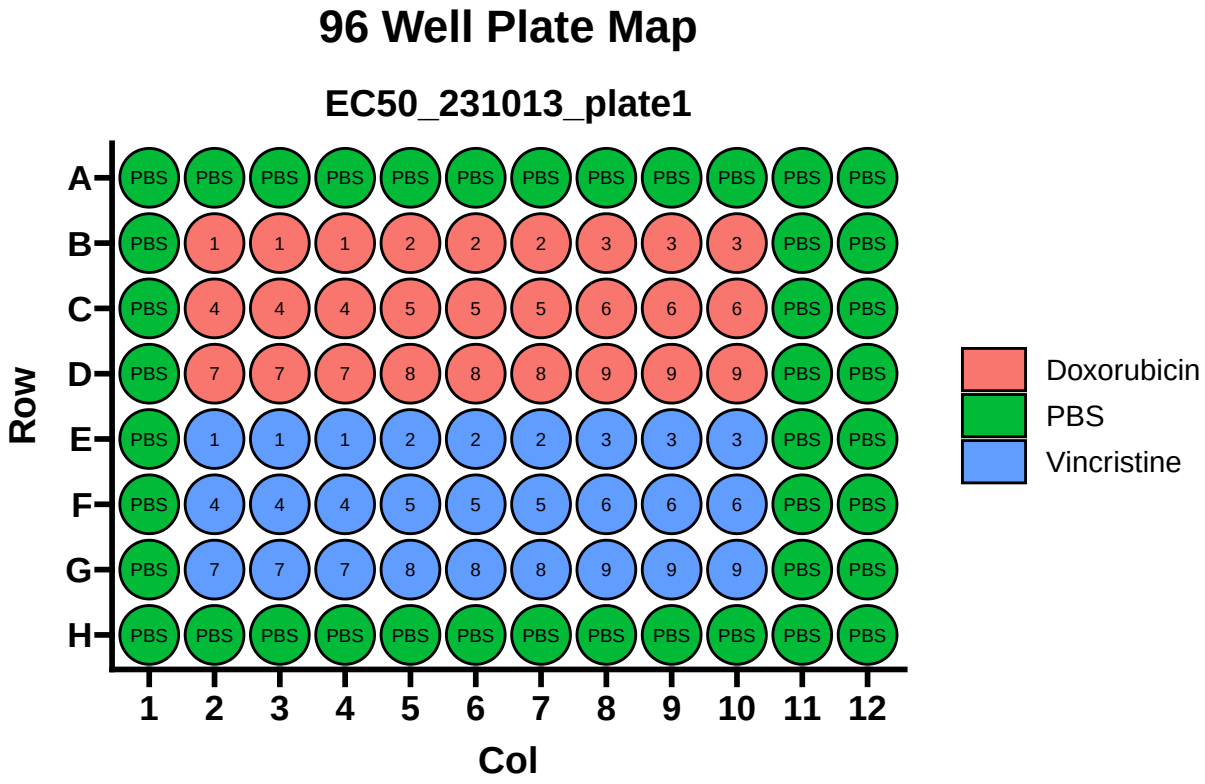
- Doxorubicin top/Vincristine bottom

Doxorubicin Stock: 10mM DMSO

Drug	Dilution	Concentration	Units	Starting_Concentration
Doxorubicin	1	300	nM	300000
Doxorubicin	2	200	nM	200000
Doxorubicin	3	133	nM	133000
Doxorubicin	4	88.9	nM	88900
Doxorubicin	5	59.3	nM	59300
Doxorubicin	6	39.5	nM	39500
Doxorubicin	7	26.3	nM	26300
Doxorubicin	8	17.6	nM	17600
Doxorubicin	9	11.7	nM	11700

Vincristine stock: 30μM

Drug	Dilution	Concentration	Units	Starting_Concentration
Vincristine	1	10	nM	10000
Vincristine	2	7.14	nM	7140
Vincristine	3	5.1	nM	5100
Vincristine	4	3.64	nM	3640
Vincristine	5	2.6	nM	2600
Vincristine	6	1.86	nM	1860
Vincristine	7	1.33	nM	1330
Vincristine	8	0.949	nM	949
Vincristine	9	0.678	nM	678



EC50 Plate ID: EC50_231013_Plate2

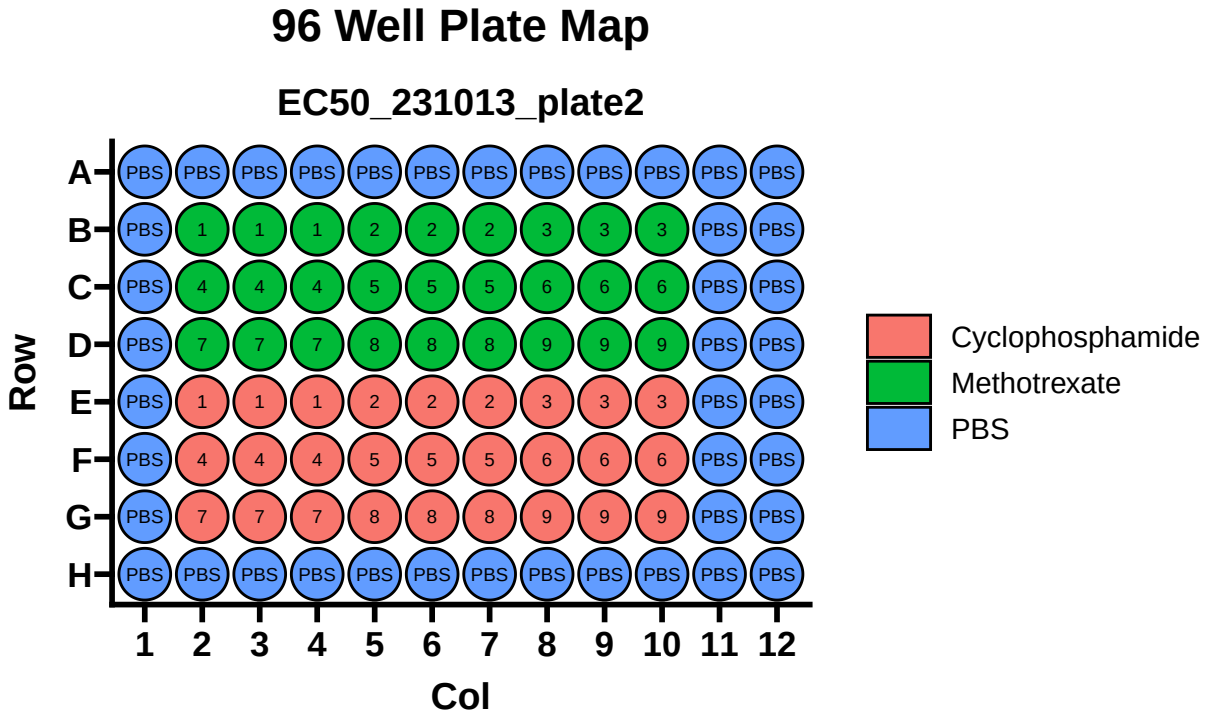
- Methotrexate top/Cyclophosphamide bottom

Methotrexate Stock: 10mM DMSO

Drug	Dilution	Concentration	Units	Starting_Concentration
Methotrexate	1	500	nM	500000
Methotrexate	2	250	nM	250000
Methotrexate	3	125	nM	125000
Methotrexate	4	62.5	nM	62500
Methotrexate	5	31.2	nM	31200
Methotrexate	6	15.6	nM	15600
Methotrexate	7	7.81	nM	7810
Methotrexate	8	3.91	nM	3910
Methotrexate	9	1.95	nM	1950

Cyclophosphamide stock: 20 mM DMSO

Drug	Dilution	Concentration	Units	Starting_Concentration
Cyclophosphamide	1	20	uM	20000
Cyclophosphamide	2	14.3	uM	14300
Cyclophosphamide	3	10.2	uM	10200
Cyclophosphamide	4	7.29	uM	7290
Cyclophosphamide	5	5.21	uM	5210
Cyclophosphamide	6	3.72	uM	3720
Cyclophosphamide	7	2.66	uM	2660
Cyclophosphamide	8	1.9	uM	1900
Cyclophosphamide	9	1.36	uM	1360



Monday 16-10-2023

Cell Culture

A20

- Split 1/5
- AR-2

N4

- Split 1/5
- AR-2

Collect EC50_231013

Plate ID: EC50_231013_Plate1

- Vincristine DNW

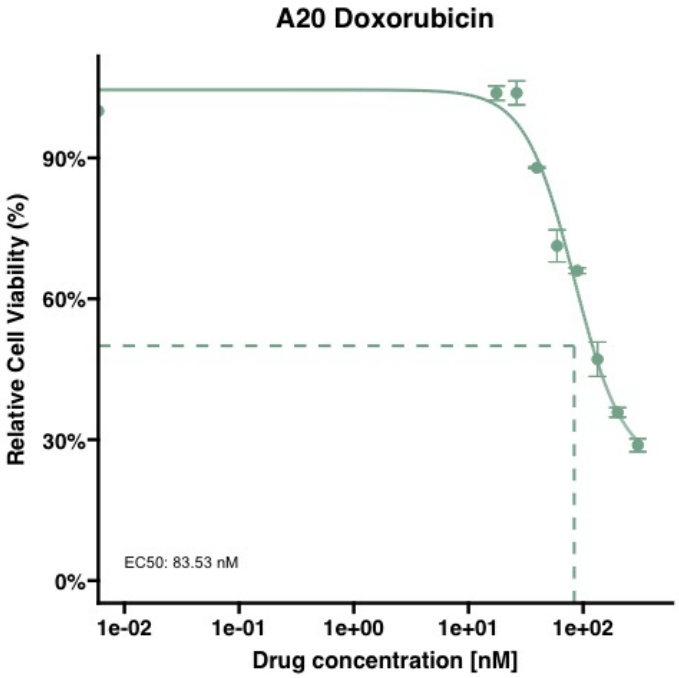


Figure 1: A20 Doxorubicin Results

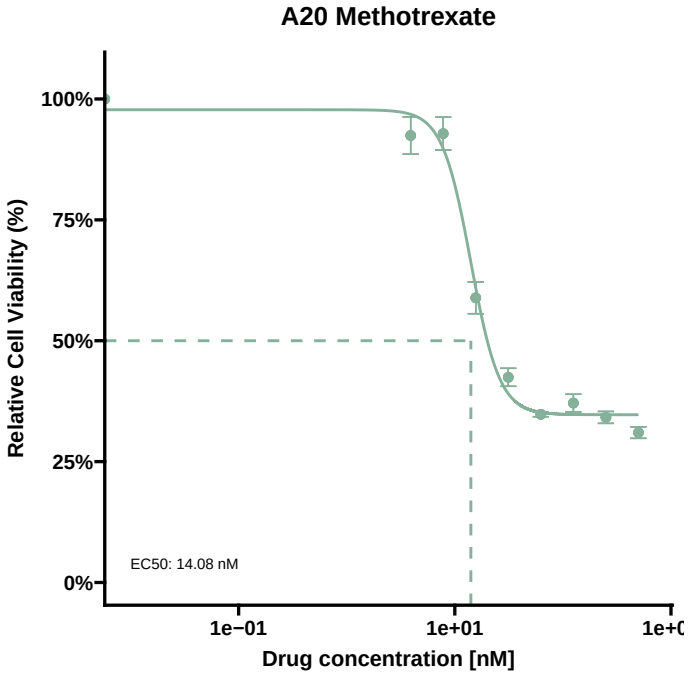


Figure 2: A20 Methotrexate Results

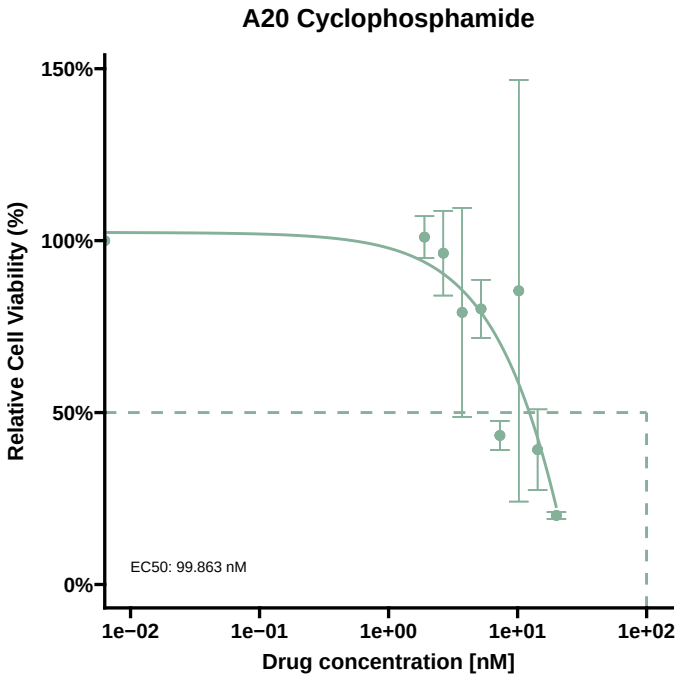


Figure 3: A20 Cyclophosphamide Results

Plate ID: EC50_231013_Plate2

Thursday 19-10-2023

Cell Culture

A20

- Split 1/5
- AR-2

N4

- Split 1/5
- AR-2

Friday 20-10-2023

Cell Culture

A20

- Split 1/5
- AR-2

N4

- Split 1/5
- AR-2

Sunday 22-10-2023

Cell Culture

RAMOS Barcode

- LN Tank 6, Rack 5, Box 6
- Thawed Ramos-BC 1, 5, 6

Monday 23-10-2023

Cell Culture

N4

- ~40-50% confluent
- Added 10mL AR-2

A20

- ~60-70% confluent
- Added 10mL AR-2

Ramos-BC 1

- ~50% confluent
- Added 10mL AR-2

Ramos-BC 5

- ~50% confluent
- Added 10mL AR-2

Ramos-BC 6

- ~50% confluent
- Added 10mL AR-2

Seed Feeders (FDC)

- Count: $4.19 \text{x} 10^5$
- Dilute to $4.8 \text{x} 10^4$
- Resuspended in 5mL AR-2, added 3.73 mL
- Seeded 1x 6-well plate
  - 2 mL/well
- Seeded 1x 96-well plate (15 wells)
  - 90 μL/well

Tuesday 23-10-2023

Cell Culture

N4

- No split, maintained

A20

- No split, maintained

Ramos-BC 1

- ~60% confluent
- Split 1/3
- AR-2

Ramos-BC 5

- ~60% confluent
- Split 1/3
- AR-2

Ramos-BC 6

- ~60% confluent
- Split 1/3
- AR-2

FDC

- Looked attached, healthy
- Media fine

Transduce A20/N4 with WILDseq Library

1. Made 2mL cell suspension of $4x10^6$ cells/mL

- A20: $1.91x10^6$ count
- N4: $1.3x10^5$ count (took 2 mL of CS from flask)

2. Add 0.5mL of cell suspension to 4 wells of 12 well plate
3. Add virus mixture (listed below) to respective wells:

Well ID	Virus	Media	HEPES
A1	25 μL	900 μL	50 μL
A2	10 μL	930 μL	50 μL
A3	1 μL	948 μL	50 μL
A4	0 μL	950 μL	50 μL

4. Centrifuged cells at 600 x g for 1.5 hours at 32C
5. Resuspended cells and transferred to 6 well plate

- N4 were put on feeder plate which was seeded 23-10-2023

Wednesday 25-10-23

Attempt 1: A20/N4 Barcoding - Media change

- Changed media
- AR-2

Made New Media: AR-3

1. Made new media: AR-3

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%

Thursday 26-10-23

Attempt 1: A20/N4 Barcoding - Sort

- DNW
- No cells were positive
- Possible causes:
  - Virus volume too low
  - Spin speed too low
  - packaging envelope incorrect for mouse cells (GALV)

Friday 27-10-23

Cell Culture

A20

- Maintained
- No Split

N4

- Split 1/4
- AR-3

Ramos-BC 1

- ~60% confluent
- Split 1/4
- AR-3

Ramos-BC 5

- ~60% confluent
- Split 1/4
- AR-3

Ramos-BC 6

- ~60% confluent
- Split 1/4
- AR-3

EC50_231027

Overview

- Treated A20 with Doxorubicin, Vincristine, Cyclophosphamide, Methotreaxte
- Concentrations were the same as EC50_231013
- Repeat for biological replicates
- Made an error for plate 1

EC50 Protocol:

1. Create 12 ml cell suspension of 2.5x10^6 cells

- A20 Cell count: 2.5x10^6
- 8.33mL AR-3 added to 1.66mL of flask CS

2. Add 90 μL of CS to respective wells of a 96-well plate
3. Dilute drug to starting concentration indicated in DMSO
4. Serial dilute into DMSO 8 times
5. Add 10uL of diluted drug to respective eppendorf containing 990uL media
6. Add 10μL of drugged media to respective well

EC50 Plate ID: EC50_231027_Plate1

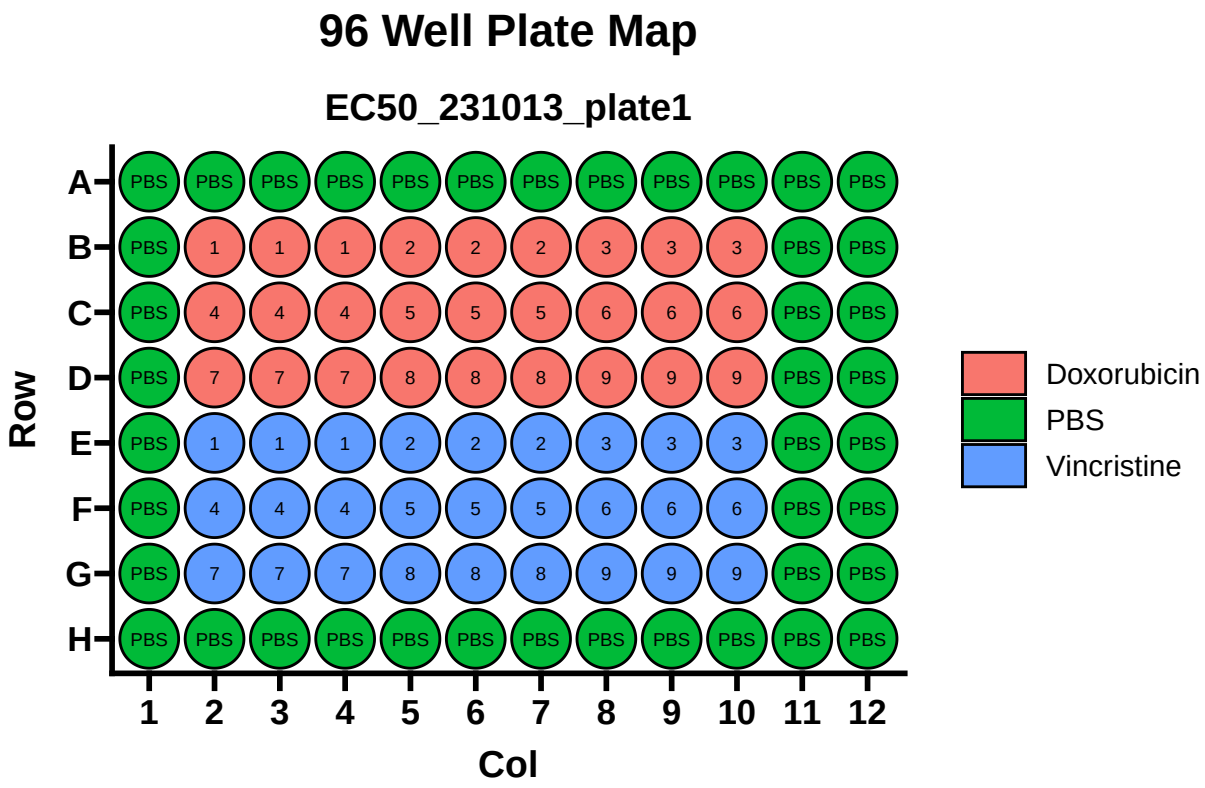
- Doxorubicin top/Vincristine bottom

Doxorubicin Stock: 10mM DMSO

Drug	Dilution	Concentration	Units	Starting_Concentration
Doxorubicin	1	300	nM	300000
Doxorubicin	2	200	nM	200000
Doxorubicin	3	133	nM	133000
Doxorubicin	4	88.9	nM	88900
Doxorubicin	5	59.3	nM	59300
Doxorubicin	6	39.5	nM	39500
Doxorubicin	7	26.3	nM	26300
Doxorubicin	8	17.6	nM	17600
Doxorubicin	9	11.7	nM	11700

Vincristine stock: 30μM DMSO

Drug	Dilution	Concentration	Units	Starting_Concentration
Vincristine	1	10	nM	10000
Vincristine	2	7.14	nM	7140
Vincristine	3	5.1	nM	5100
Vincristine	4	3.64	nM	3640
Vincristine	5	2.6	nM	2600
Vincristine	6	1.86	nM	1860
Vincristine	7	1.33	nM	1330
Vincristine	8	0.949	nM	949
Vincristine	9	0.678	nM	678



NOTE: Made an error by adding Methotrexate and Cyclophosphamide treatments to PLate 1 (as well as listed treatments) - Disregard results

EC50 Plate ID: EC50_231027_Plate2

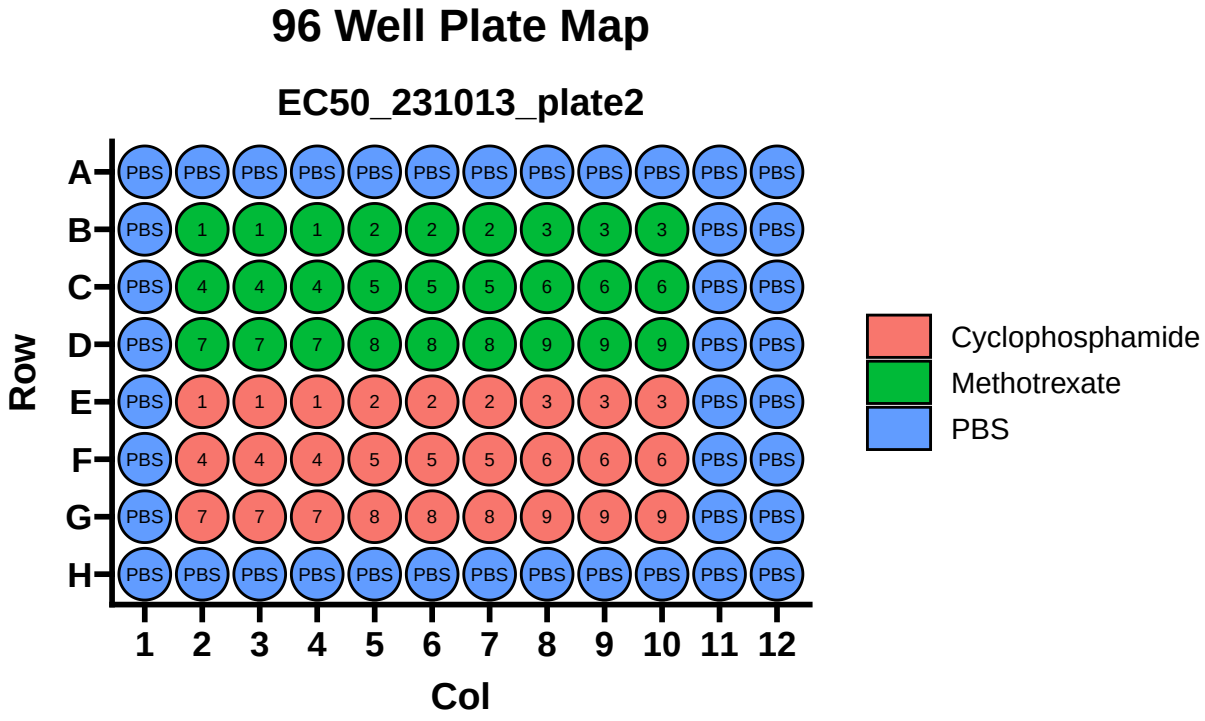
- Methotrexate top/Cyclophosphamide bottom

Methotrexate Stock: 10mM DMSO

Drug	Dilution	Concentration	Units	Starting_Concentration
Methotrexate	1	500	nM	500000
Methotrexate	2	250	nM	250000
Methotrexate	3	125	nM	125000
Methotrexate	4	62.5	nM	62500
Methotrexate	5	31.2	nM	31200
Methotrexate	6	15.6	nM	15600
Methotrexate	7	7.81	nM	7810
Methotrexate	8	3.91	nM	3910
Methotrexate	9	1.95	nM	1950

Cyclophosphamide stock: 20 mM DMSO

Drug	Dilution	Concentration	Units	Starting_Concentration
Cyclophosphamide	1	20	uM	20000
Cyclophosphamide	2	14.3	uM	14300
Cyclophosphamide	3	10.2	uM	10200
Cyclophosphamide	4	7.29	uM	7290
Cyclophosphamide	5	5.21	uM	5210
Cyclophosphamide	6	3.72	uM	3720
Cyclophosphamide	7	2.66	uM	2660
Cyclophosphamide	8	1.9	uM	1900
Cyclophosphamide	9	1.36	uM	1360



Monday 27-10-23

Cell Culture

A20

- Split 1/2
- AR-3

N4

- Split 1/2
- AR-3

Ramos-BC 1

- Split 1/2
- AR-3

Ramos-BC 5

- Split 1/2
- AR-3

Ramos-BC 6

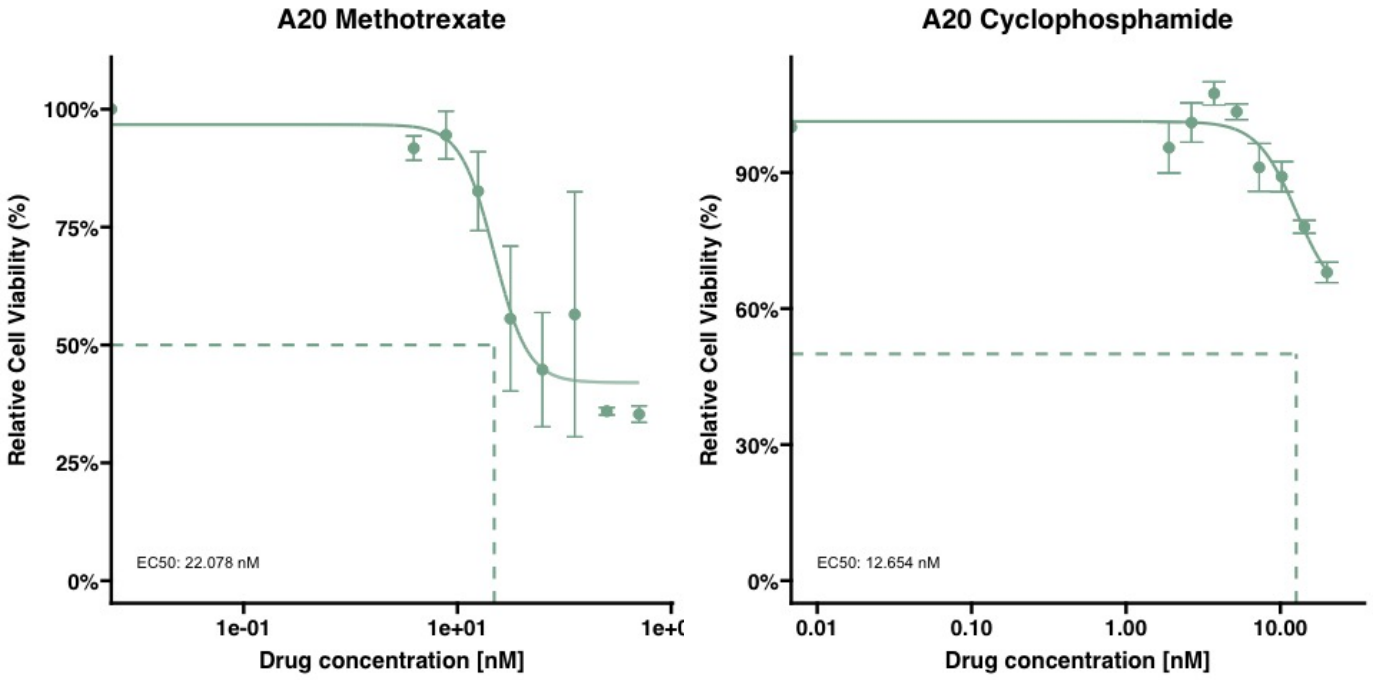
- Split 1/2
- AR-3

Seed Feeders

- Repeating barcoding of A20/N4
- Count: 3.64×10^5
- Dilute to 4.8×10^4
- Resuspended in 5mL AR-2, added 2.58 mL
- Seeded 1x 6-well plate
  - 2 mL/well
- Seeded 1x 96-well plate (15 wells)
  - 90 μL/well

Collect EC50_231027

Plate ID: EC50_231027_Plate



Tuesday 31-10-2023

Attempt 2: Transduce A20/N4 with WILDseq Library

1. Made 2mL cell suspension of 4×10^6 cells/mL

- A20: 2.18×10^6 count
- N4: 3.06×10^5 count (took 2 mL of CS from flask)

2. Add 0.5mL of cell suspension to 4 wells of 12 well plate
3. Add virus mixture (listed below) to respective wells:

Well ID	Virus	Media	HEPES
A1	250 μL	225 μL	25 μL
A2	150 μL	150 μL	25 μL
A3	50 μL	50 μL	25 μL
A4	0 μL	0 μL	25 μL

4. Centrifuged cells at 1000 x g for 1.5 hours at 0C

- Mistakenly set centrifuge to 0C

5. Resuspended cells and transferred to 6 well plate

- N4 were put on feeder plate which was seeded 23-10-2023

Drug Pressure Experiment 1: RAMOS Day 1

Overview

- Designing combination treatment to reflect common BL treatments in clinic
- Plan to analyse differences in barcode represetnation between baseline, DMSO, and treatment-resistant
- Drug concentrations:

Drug	Treatment Day	Concentration	Stock
Doxorubicin	Day 2	80nM	100 μM working stock
Vincristine	Day 1	10nM	30μM
Methotrexate	Day 1	20nM	100 μM working stock
Cyclophosphamide	Day 2/3/4	20 μM	20μM working stock

- Plan:

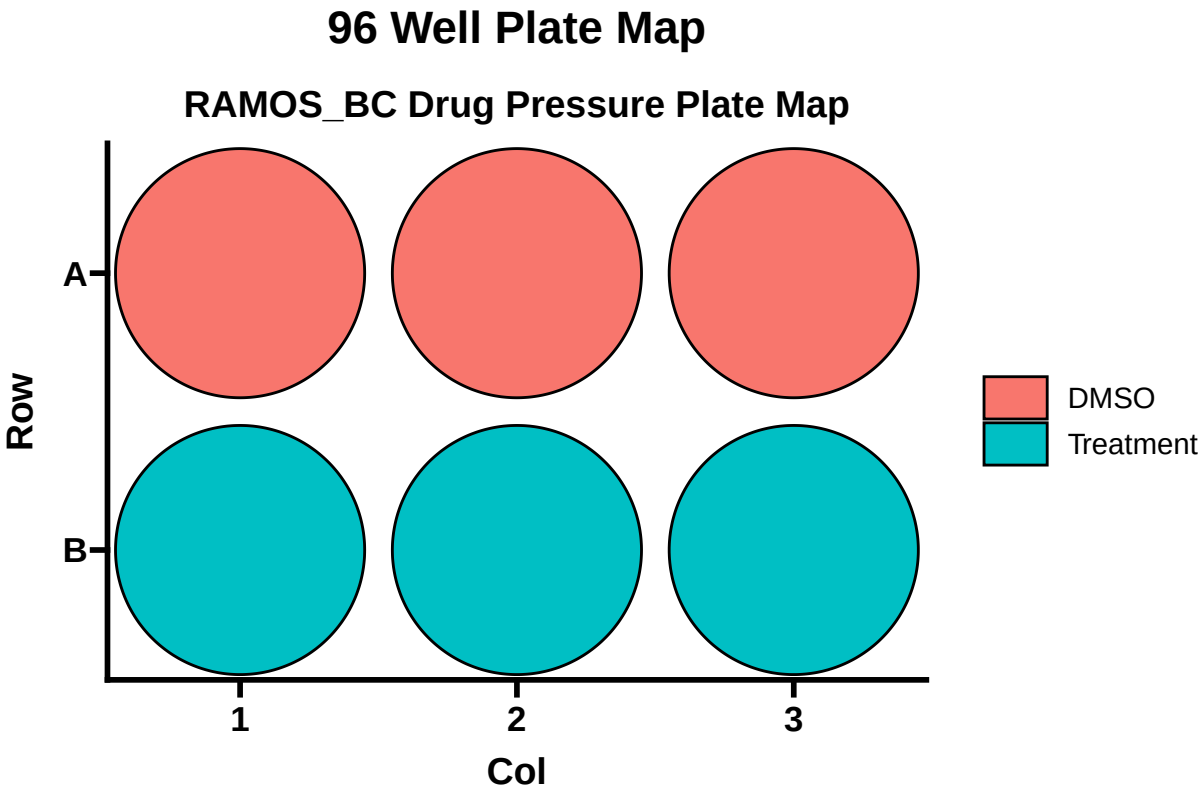
1. Collect sample at baseline
2. Complete 4 day treatment course of RAMOS-BC cells (with corresponding DMSO controls)
3. Allow treatment(T) cells to recover and collect sample
4. Complete 4 day treatment course again and collect sample

- Possible future experiments:
  - EC50's of T vs WT cells (are T RAMOS cells drug resistant?)

Day 1

Seeded RAMOS BC

- Seeded 3x 6 well plates with RAMOS-BC 1, 5, 6 respectively



- Seeded 0.25x10^6 cells/well in 3mL AR-3
- Treatment wells

Drug	Treatment Day	Concentration	Stock	Stock Volume	Media Volume
DMSO	Day 1	-	-	14.4 μL	27 mL AR-3
Vincristine	Day 1	10nM	30μM	9 μL	27 mL AR-3
Methotrexate	Day 1	20nM	100 μM working stock	5.4 μL of WS	27 mL AR-3

Collected RAMOS Baseline

- Took 1mL CS from respective flask and transferred to eppendorf
- Spun down 2500 x g at 4C
- Removed Media
- Snap froze in LN
- Put in -80 CM Box 1

NOVEMBER 2023

Wednesday 01-11-2023

Drug Pressure Experiment 1: RAMOS Day 2

- Changed media
- Spun down at 600 x g for 5 min
- Resuspended in Day 2 Treatment:

Drug	Treatment Day	Concentration	Stock	Stock Volume	Media Volume
DMSO	Day 1	-	-	48.60 μL	27 mL AR-3
Doxorubicin	Day 1	80nM	100 μM working stock	21.60 μL of working stock	27 mL AR-3
Cyclophosphamide	Day 1	20μM	20mM	27 μL	27 mL AR-3

Attempt 2: Barcoding A20/N4

- Sanity check
- Ran samples on E6 flow
- A20 negative
- Suggests envelope protein is not compatible with mouse cells
- N4 dead
- Re-make WILDseq virus with VSVG packaging envelope

Thursday 02-11-2023

Cell Culture

A20

- Split 1/2
- AR-3

N4

- Split 1/2
- AR-3

Ramos-BC 1

- Split 1/4
- AR-3

Ramos-BC 5

- Split 1/4
- AR-3

Ramos-BC 6

- Split 1/4
- AR-3

Seeded Feeders

- 1 vial
- Split into 2 T75 flasks

Drug Pressure Experiment 1: RAMOS Day 3

- Changed media
- Spun down at 600 x g for 5 min
- Resuspended in Day 3 Treatment:

Drug	Treatment Day	Concentration	Stock	Stock Volume	Media Volume
DMSO	Day 1	-	-	27 μL	27 mL AR-3
Cyclophosphamide	Day 1	20μM	20mM	27 μL	27 mL AR-3

Friday 03-11-2023

Cell Culture

A20

- Split 1/2
- AR-3

N4

- Split into two new feeder flasks
  - Seeded 02-11-2023
  - N4.1 / N4.2
  - 1 flask to transdue, 1 flask to freeze down
- AR-3

Ramos-BC 1

- Add 10mL AR-3

Ramos-BC 5

- Add 10mL AR-3

Ramos-BC 6

- Add 10mL AR-3

Drug Pressure Experiment 1: RAMOS Day 4

- Treatment cells too sick to continue
- Changed media
- Spun down at 600 x g for 5 min
- Resuspended both DMSO and Treatment cells in fresh AR-3
- Allow to grow

Monday 06-11-2023

Cell Culture

A20

- Split 1/2
- AR-3

N4.1

- Healthy/growing
- Leave till tomorrow

N4.2

- Healthy/growing
- Leave till tomorrow

Ramos-BC 1

- Froze down
- 3x vials
- FBS +10% DMSO

Ramos-BC 5

- Froze down
- 3x vials
- FBS +10% DMSO

Ramos-BC 6

- Froze down
- 3x vials
- FBS +10% DMSO

Seed Feeders

- For WILDseq transduction of N4 (tuesday?)
- 1x 4 wells of 6-well plate, 15wells of 96-well plate
- Dilute in 8ml
- 2mL/well of 6well
- 90μL/well of 96well plate

Drug Pressure Experiment 1: RAMOS Day 7

- Split DMSO cells 1/6
- Add 1mL AR-3 to treatment cells
  - treatment cells still sick, allow to grow for a few more days
  - Perhaps transfer to smaller well?

Tuesday 07/11/2023

Made New Media: AR-4

1. Made new media: AR-4

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

- Split 1/6
- AR-4

N4.1

- Healthy/growing
- Leave till tomorrow

N4.2

- Froze down
- 2x vials
- FBS +10% DMSO

Drug Pressure Experiment 1: RAMOS Day 5

- Split D 1/6

Attempt 3: Transduce N4 with WILDseq Library

1. Made 2mL cell suspension of 4x10^6 cells/mL

- N4: took 10ml of 20ml CS, spun down and resuspended in 2mL AR-4

2. Add 0.5mL of cell suspension to 4 wells of 12 well plate
3. Add virus mixture (listed below) to respective wells:

Well ID	Virus	Media	HEPES
A1	250 μL	225 μL	25 μL
A2	150 μL	150 μL	25 μL
A3	50 μL	50 μL	25 μL
A4	0 μL	0 μL	25 μL

4. Centrifuged cells at 1000 x g for 1.5 hours at 32C
5. Resuspended cells and transferred to 6 well plate

- N4 were put on feeder plate which was seeded 06-11-2023

Wednesday 08/11/2023

Attempt 3: Barcoding N4

- Changed Media
- Cells look healthy ## Drug Pressure Experiment 1: RAMOS Day 6
- Checked treatment cells with trypan blue
- Everything was dead
- Ended experiment: will reconsider approach
  - Treat Ramos with individual agents as well as combination
  - Treat for 3 days and recover or 12 days and recover(?)

Thursday 09/11/2023

Attempt 3: Barcoding N4

- Booked sort for tomorrow
- Changed media
- Sanity check on E6 Fortessa (very low MOI) ## Cell Culture

A20

- Split 5mL CS into 15mL of AR-4 ### N4
- Split 5mL CS into 30mL of AR-4

Friday 10/11/2023

Attempt 3: Barcoding N4

- Sorted N4
- 1000 cells into wells A1-A3 of 96-well plate seeded with feeders
  - Seeded 06/11/2023
- 500 cells into wells B1-B3
  - Seeded 06/11/2023

Cell Culture

RAMOS-BC1

- Thawed vial of RAMOS-BC1 into 15mL of AR-4 ### N4
- Threw away ### A20
- Threw away

Monday 13/11/2023

Start RAMOS-Drug Pressure v2

1. Count RAMOS-BC1 CS

- CS: 1x10^6 cells/mL

2. Add 122.8μL CS per well
3. Make drug dilutions according to following table:

- Make working stock of Doxorubicin and Methotrexate
- Dilute 10mM stock to 100μM
  - 10μL stock in 990 μL DMSO

DMSO: 16.67 μL DMSO in 10mL Combo: Added doxorubicin, vincristine, methotrexate, and cyclophosphamide to 10mL at same volumes indicated below

Drug	Concentration	Stock	Dilution
Doxorubicin	80nM	100 μM working stock (10mM Stock)	5 μL WS in 10mL
Vincristine	10nM	30μM	1.67 μL stock in 10mL
Methotrexate	20nM	100 μM working stock (10mM Stock)	5μL WS in 10mL
Cyclophosphamide	20 μM	20μM working stock	5 μL stock in 10mL

4. Add 3 mL of drugged media to respective wells

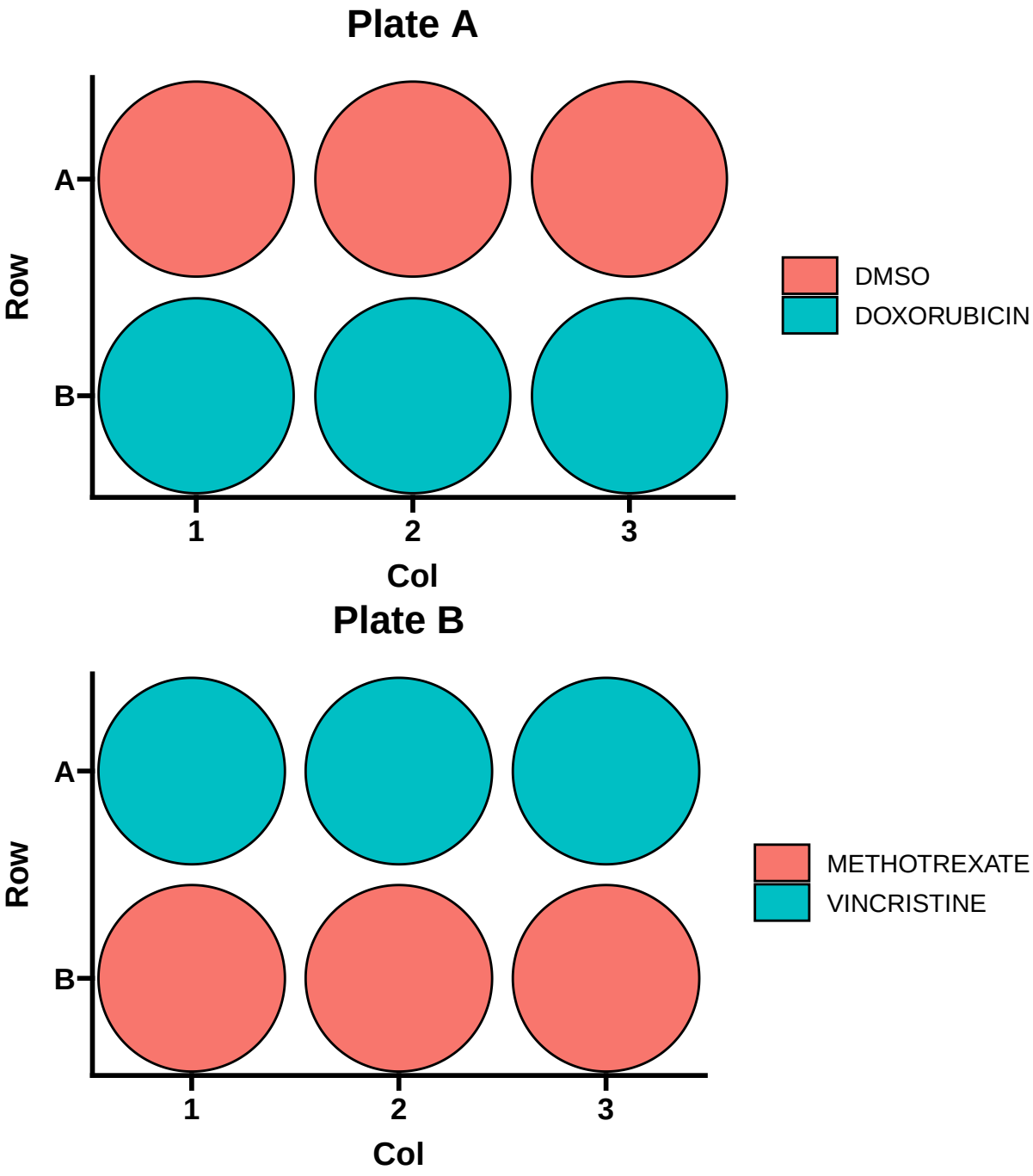
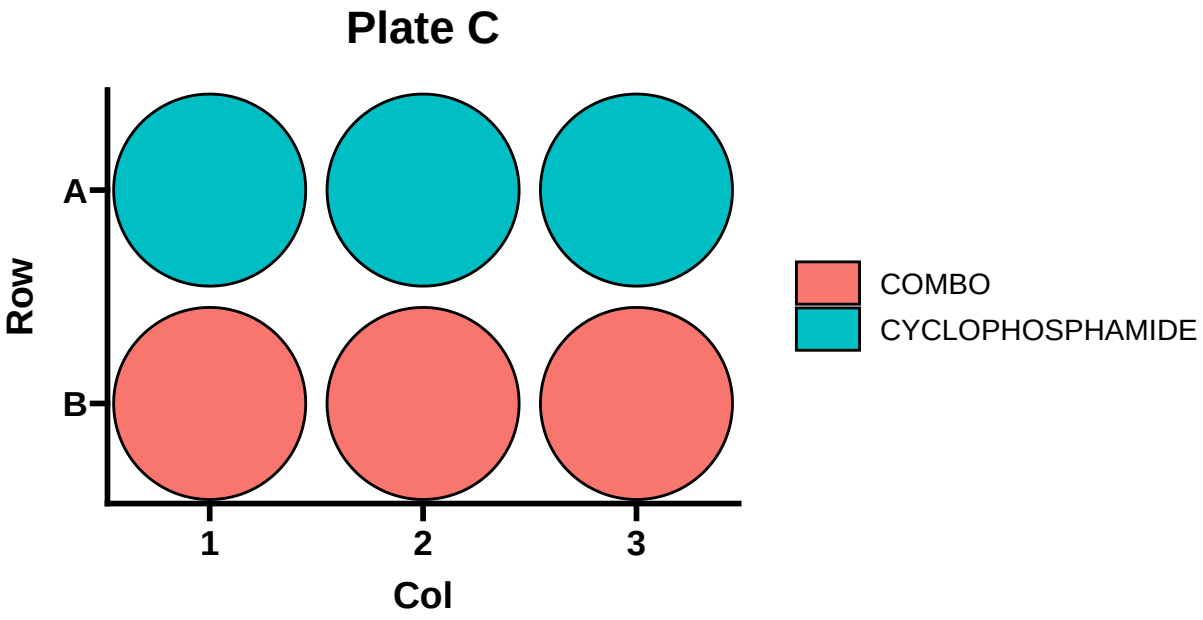


Plate A
Row
A
B
1
2
3
Col
DMSO
DOXORUBICIN
Plate B
Row
A
B
1
2
3
Col
METHOTREXATE
VINCRISTINE



Cell Culture

N4-BC Attempt 3

- Add media to wells with cells
- Lots of death, some clusters of live cells ### RAMOS-BC1
- Split 4mL CS into 20mL AR-4

PIL COURSE

Thursday 13/11/2023

RAMOS-DPv2

Change media: 1. Transfer into 15ml Falcon 2. Spun down @ 500xg for 6min 3. Resuspended in 1mL 4. Transferred into new 12-well plate

Friday 17/11/2023

Cell Culture

INFECTION - RAMOS-BC1 and RAMOS-DPv2 infected - Threw out cultures and AR-4 - N4 seemingly unaffected

Monday 20/11/2023

WILDseq - Mouse Experiment 1

Overview

- Chris Steel injected mice with barcoded N2 cells
- He applied various treatment conditions and tracked tumour growth over time
- Collected tumours at humane endpoint for barcode analysis and scRNA-seq ### RNA extraction WILDseq-ME1
- Performed RNA extraction of tissue/cell samples according to the following kit:

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