Turner Lab Notebook

Callum Malcolm

Contents

MARCH 2023

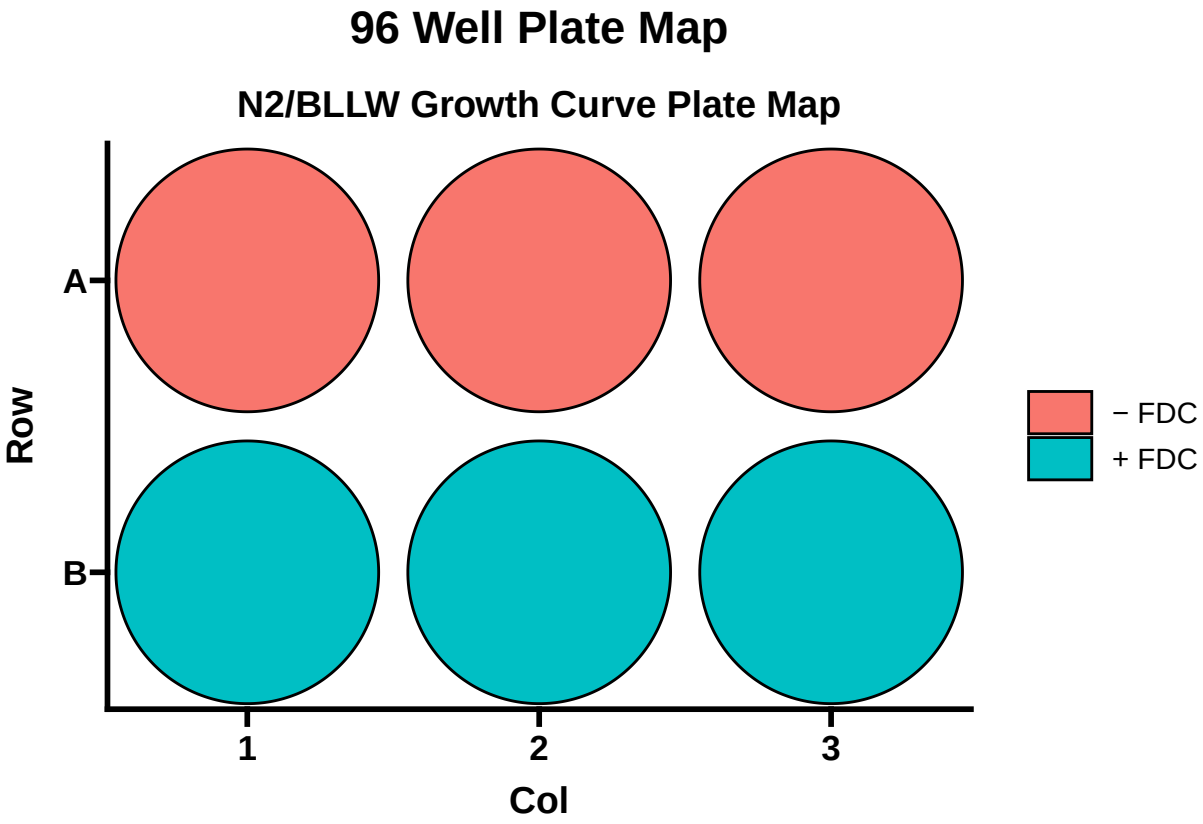
Wednesday 15-03-2023

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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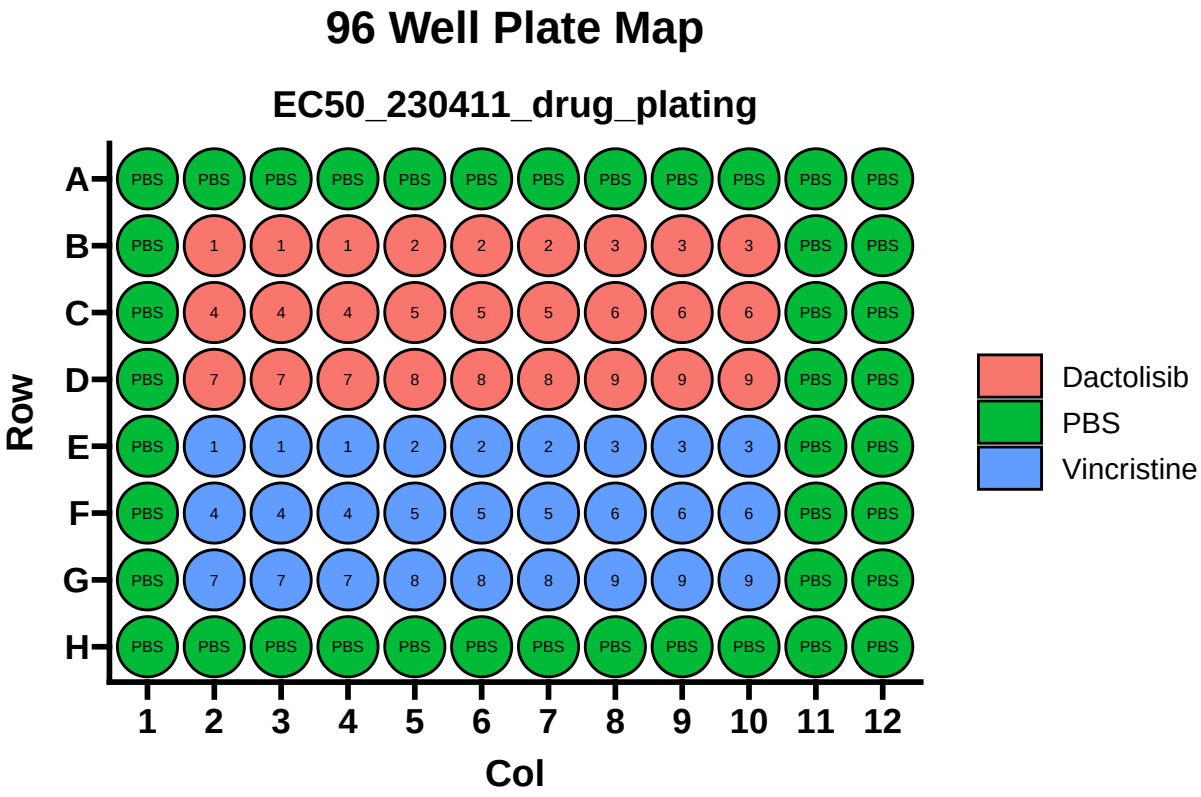
Tuesday 11-04-2023

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

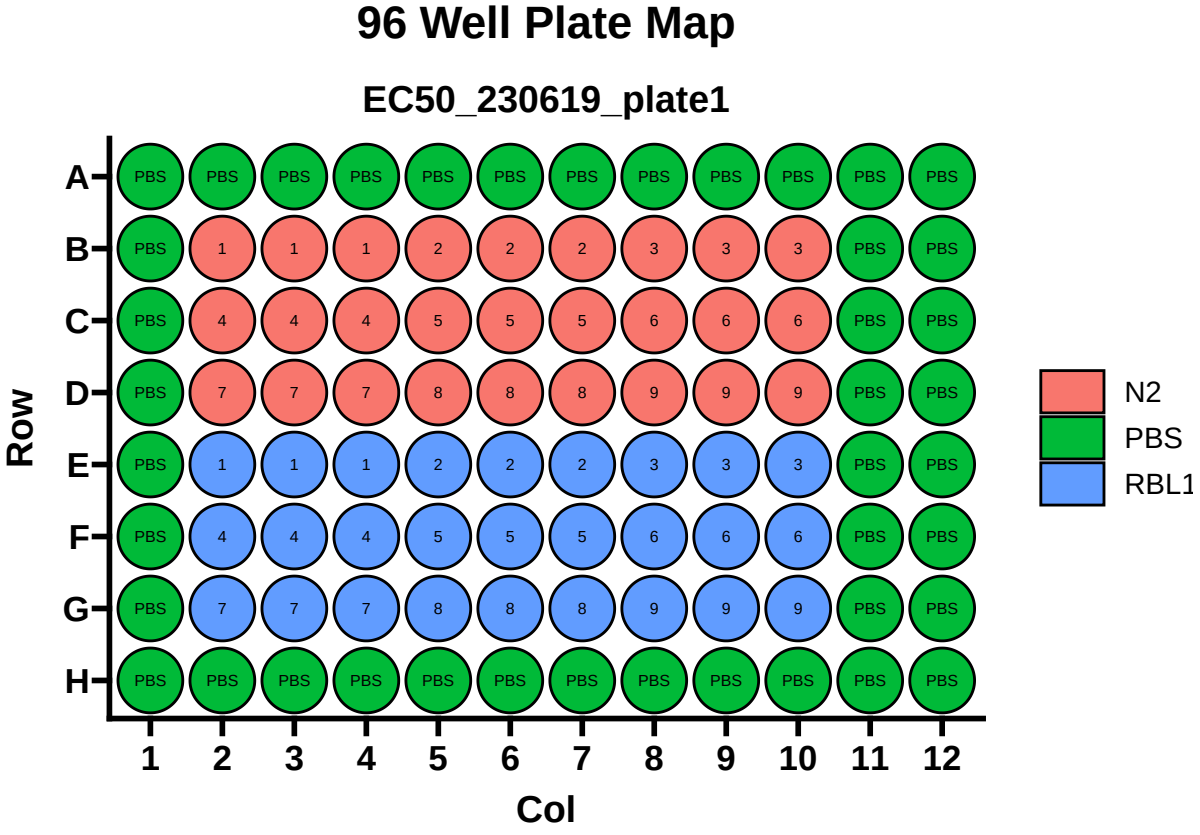
Monday 19-06-2023

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

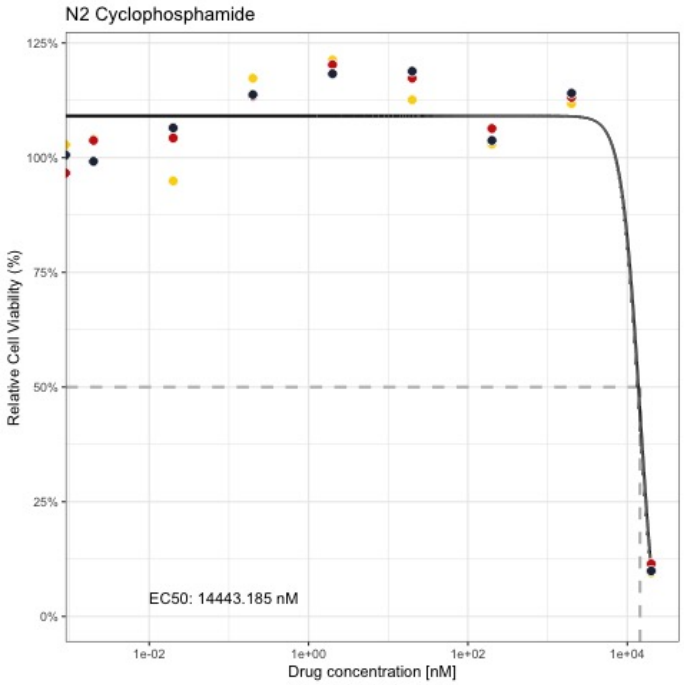
High-Sensitivity DNA ScreenTape Protocol

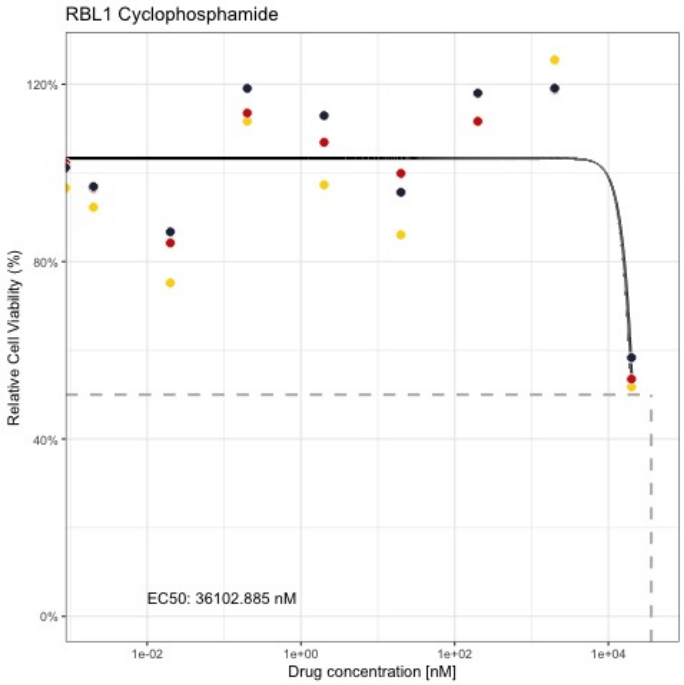
Results
* Did not work, bands present but faint and not at 200bp
* Potential solutions:
- Repeat PCR with increased cycles
- Repeat RNA extration with trizol protocol (Anna)

Thursday 22-06-2023

Collect EC50 Plate

- Plate ID: EC50_230619_Plate1





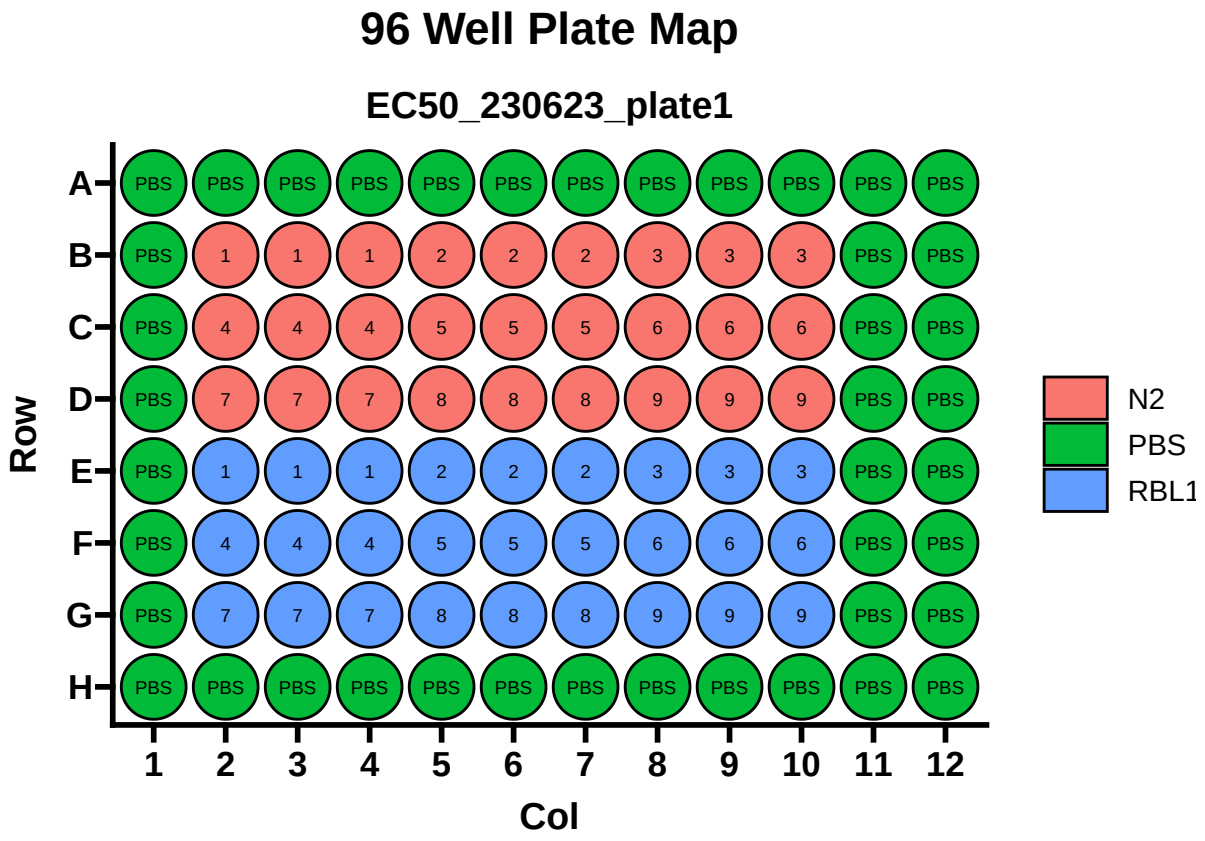
Friday 23-06-23

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



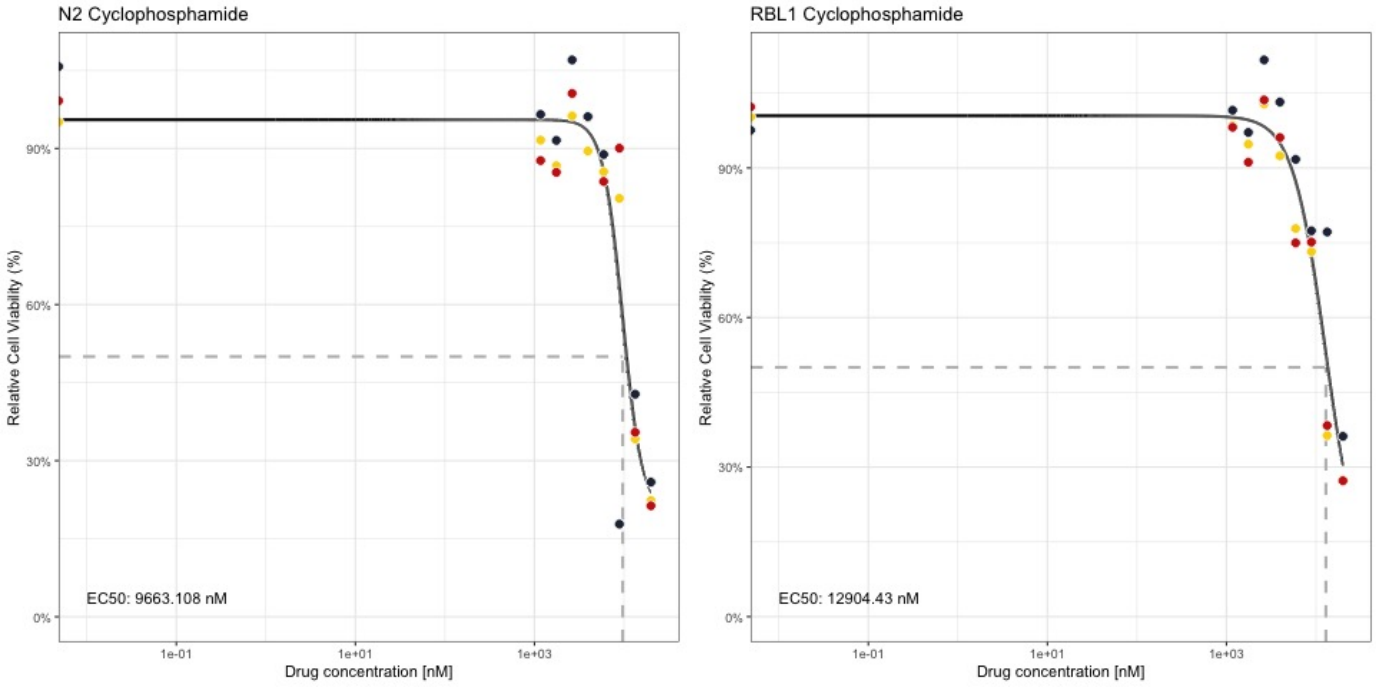
Drug Dilutions

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

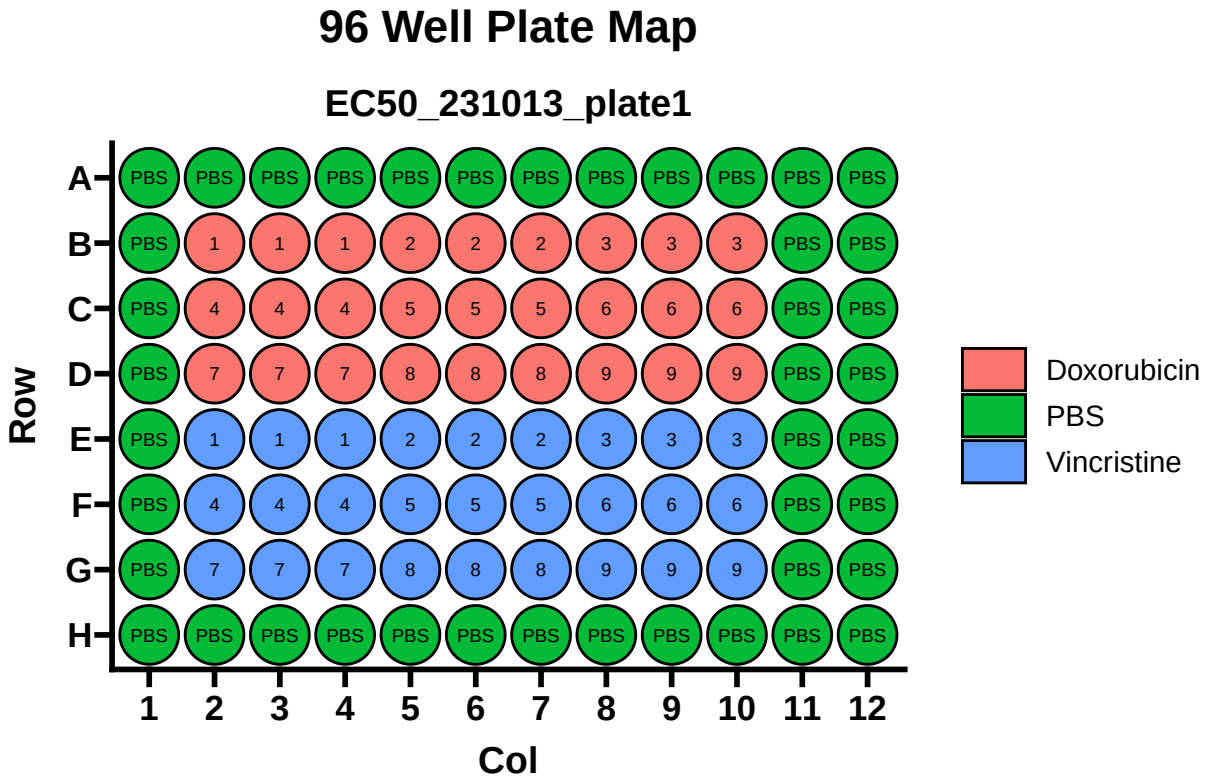
- Doxorubicin top/Vincristine bottom

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

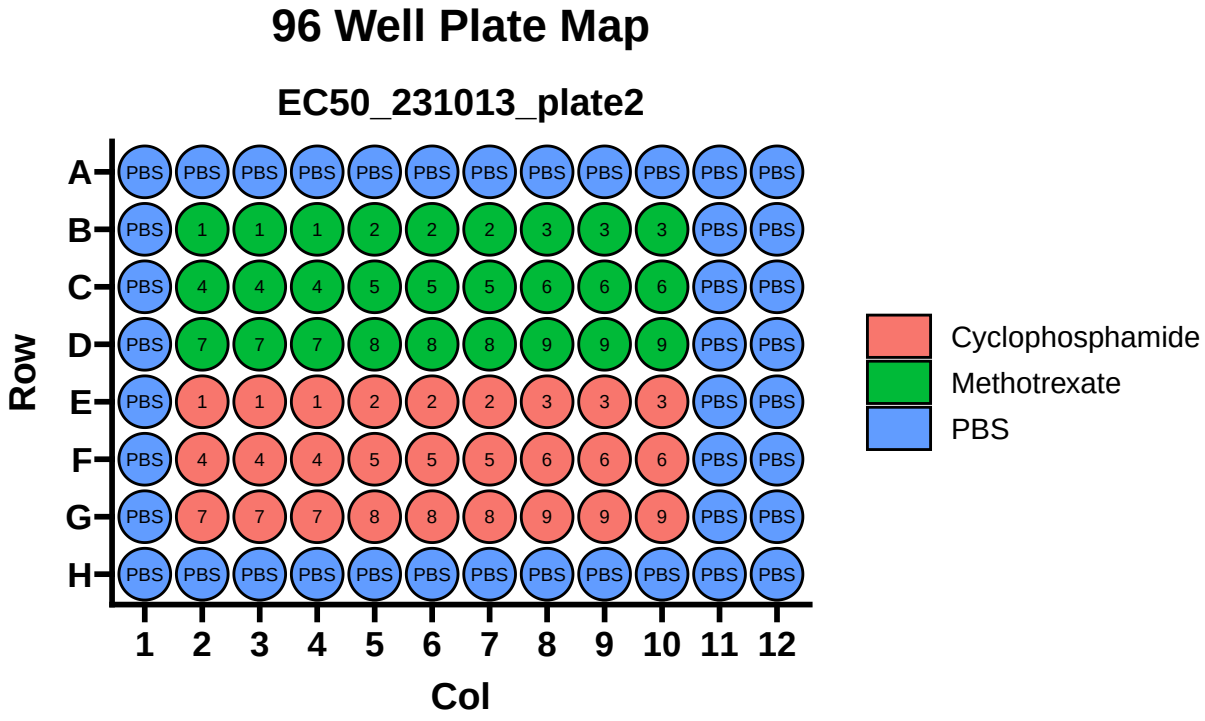
- Methotrexate top/Cyclophosphamide bottom

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

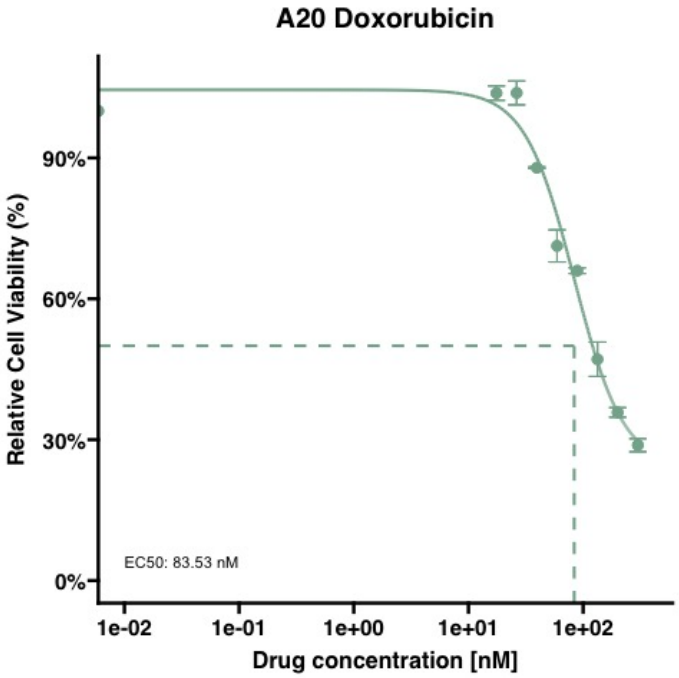


Figure 1: A20 Doxorubicin Results

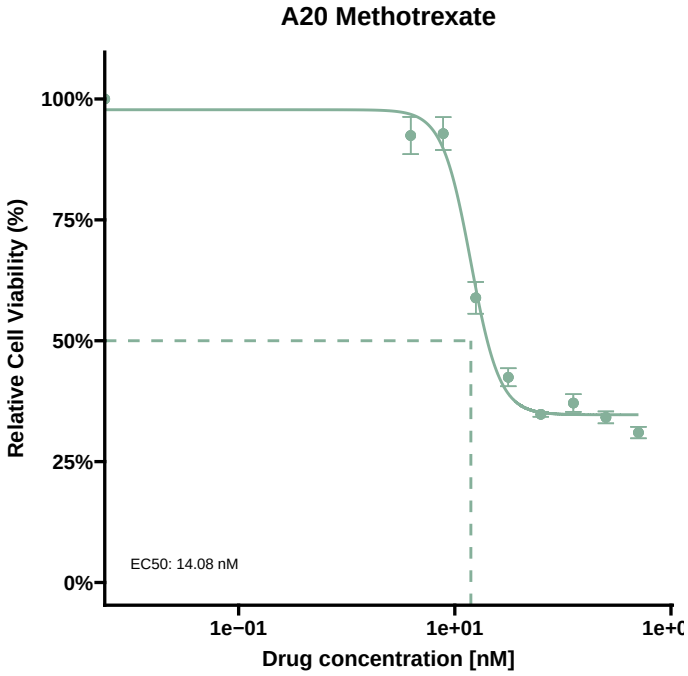


Figure 2: A20 Methotrexate Results

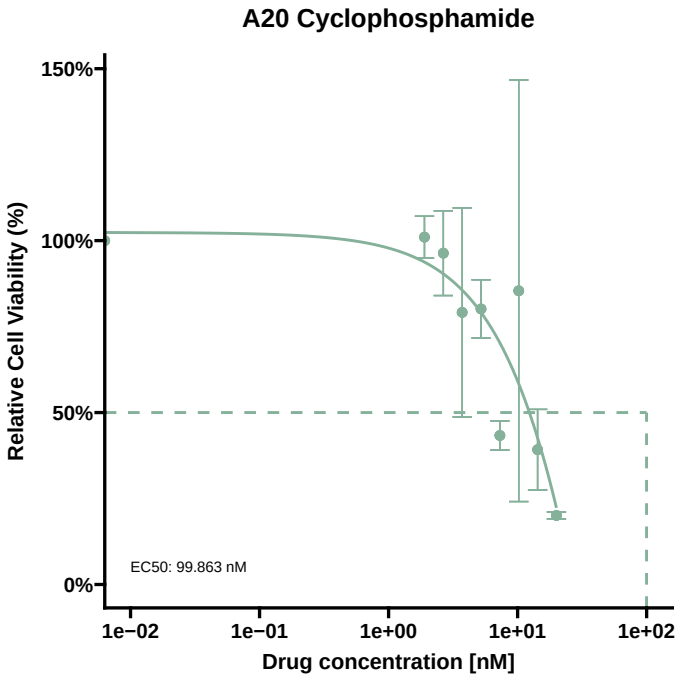


Figure 3: A20 Cyclophosphamide Results

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

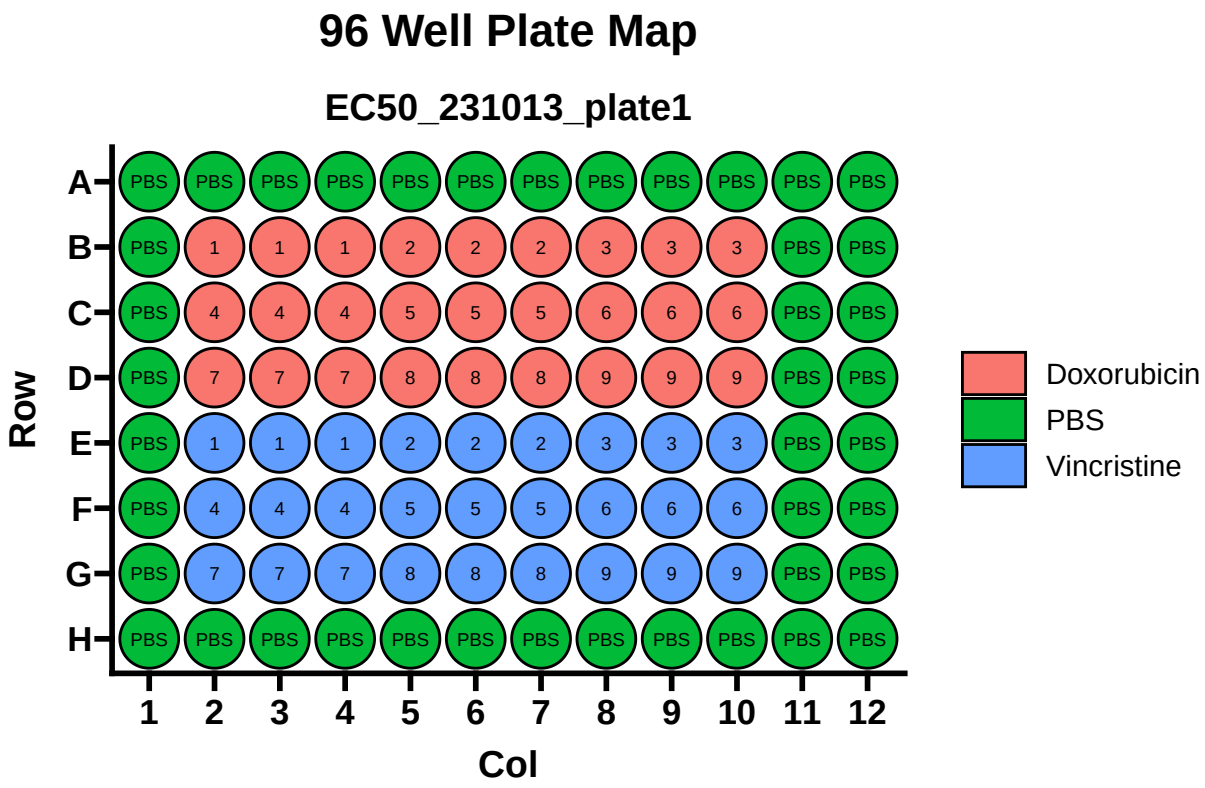
- Doxorubicin top/Vincristine bottom

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

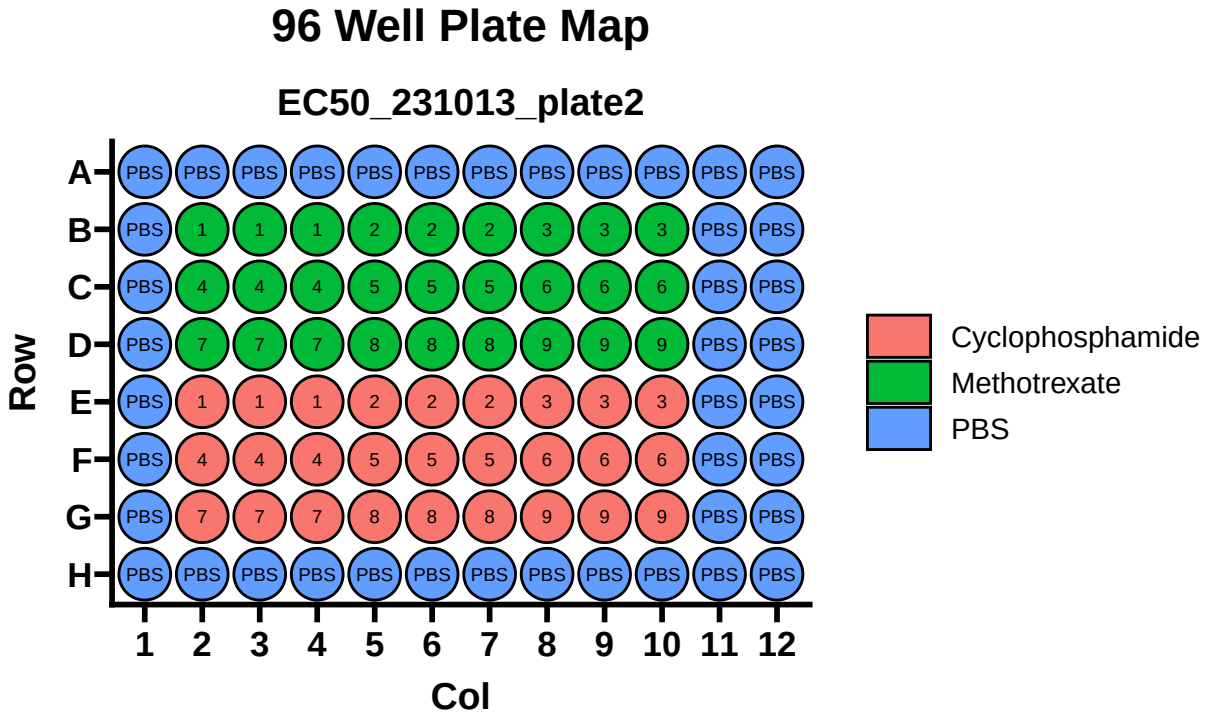
- Methotrexate top/Cyclophosphamide bottom

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

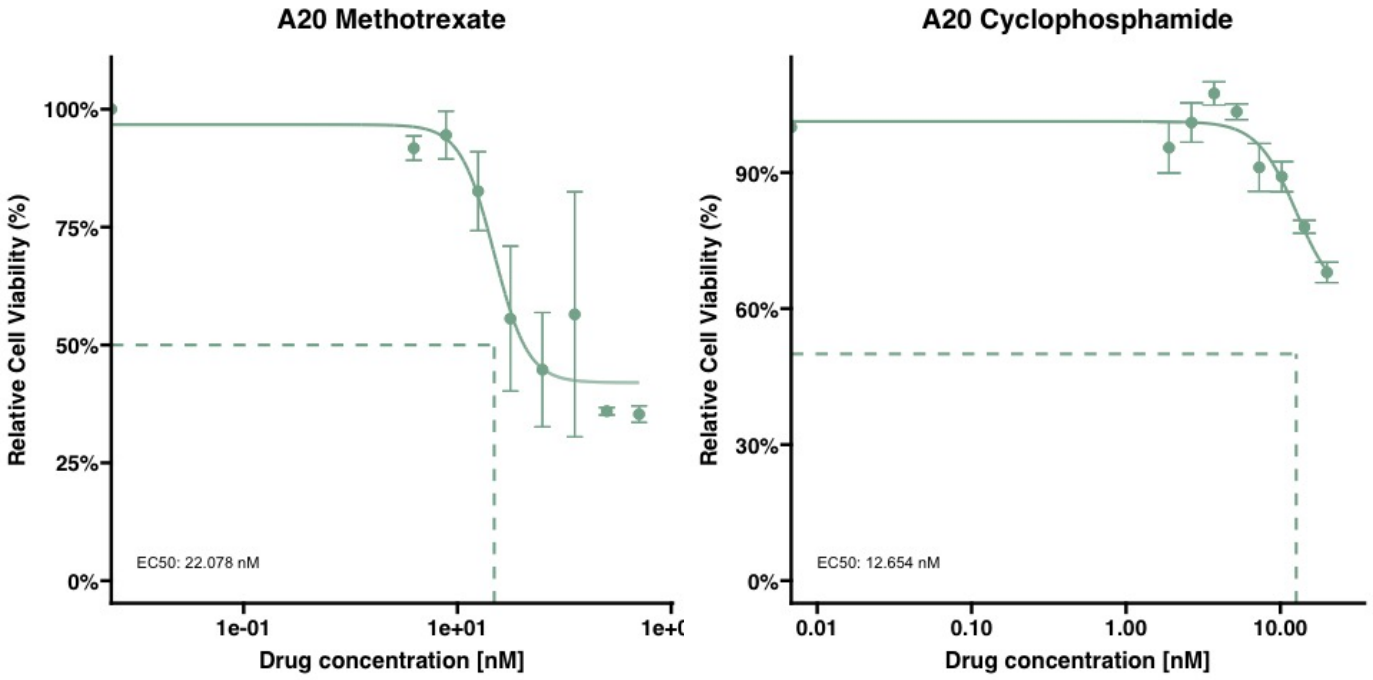
- Split 1/2
- AR-3

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:

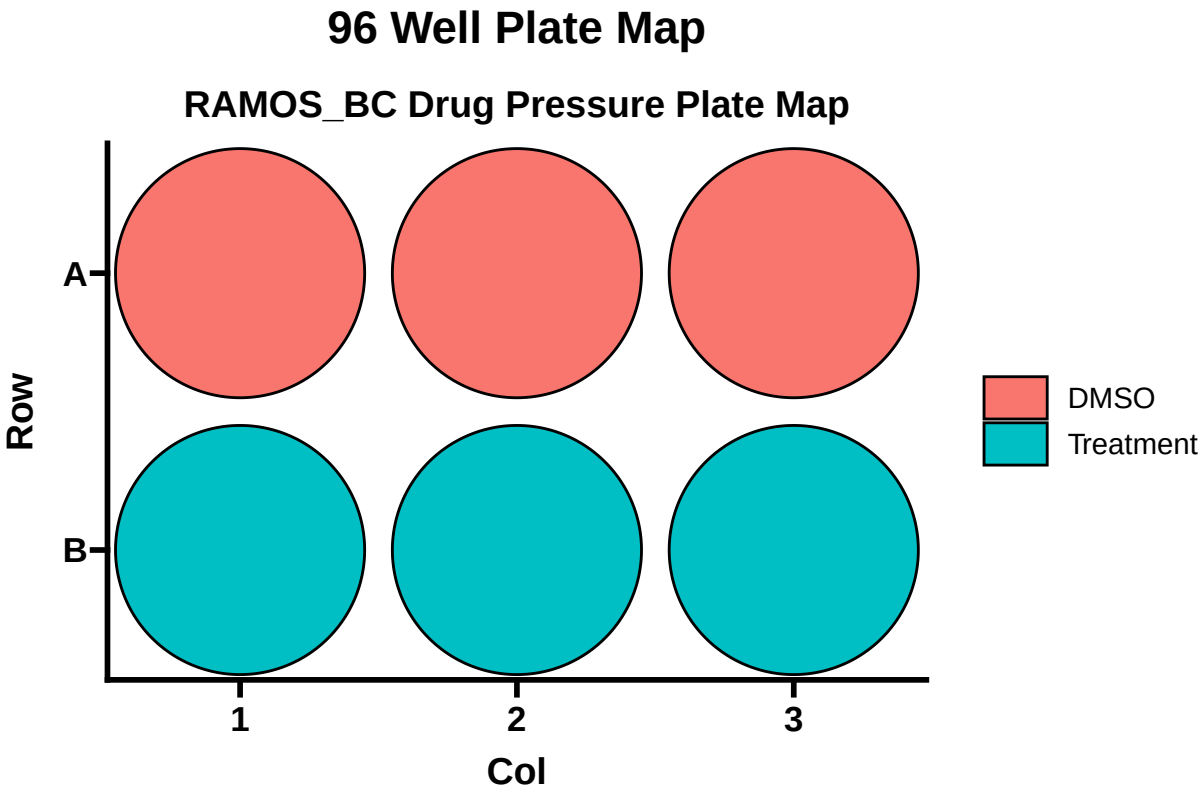
1. Collect sample at baseline
2. Complete 4 day treatment course of RAMOS-BC cells (with corresponding DMSO controls)
3. Allow treatment(T) cells to recover and collect sample
4. Complete 4 day treatment course again and collect sample

- 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

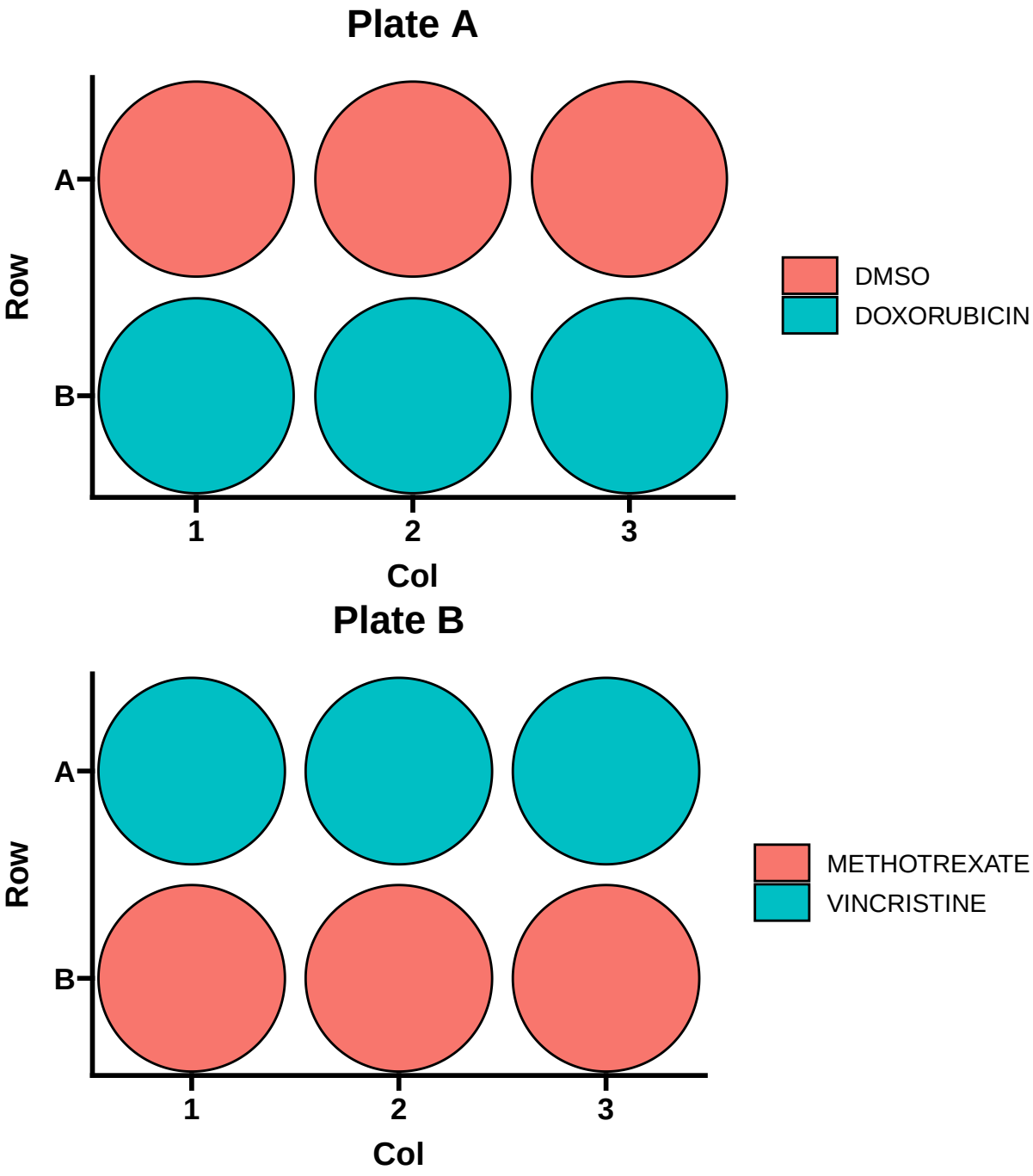
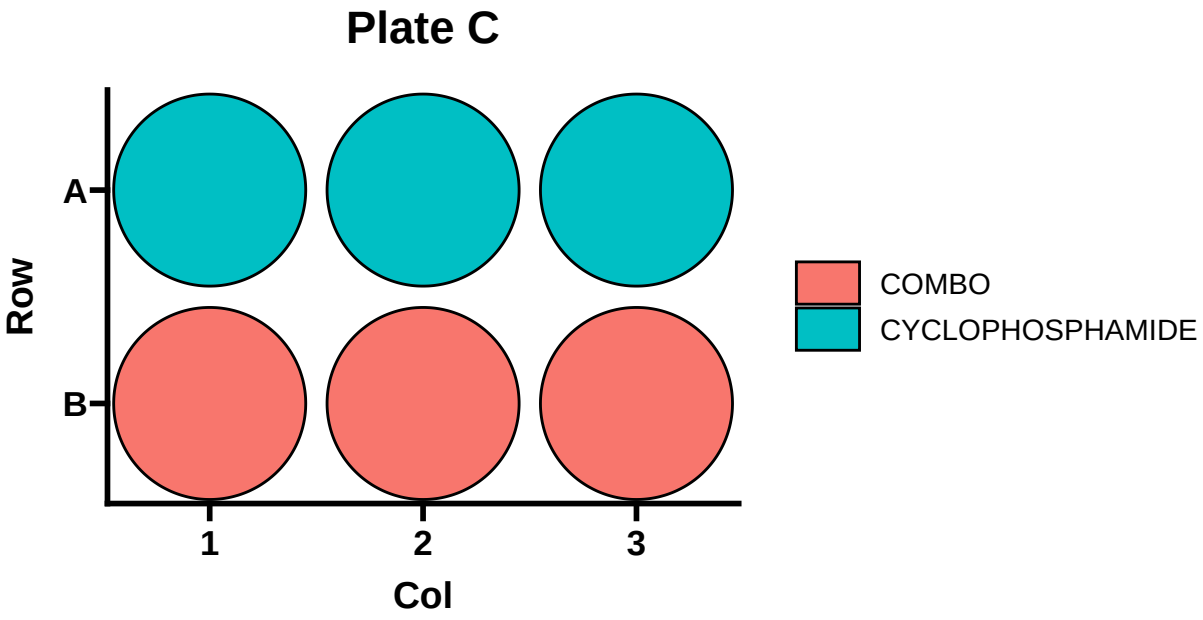


Plate A
Row
A
B
1
2
3
Col
DMSO
DOXORUBICIN
Plate B
Row
A
B
1
2
3
Col
METHOTREXATE
VINCRISTINE



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 14/11/2023

Cell Cutlure

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