# Turner Lab Notebook

Callum Malcolm

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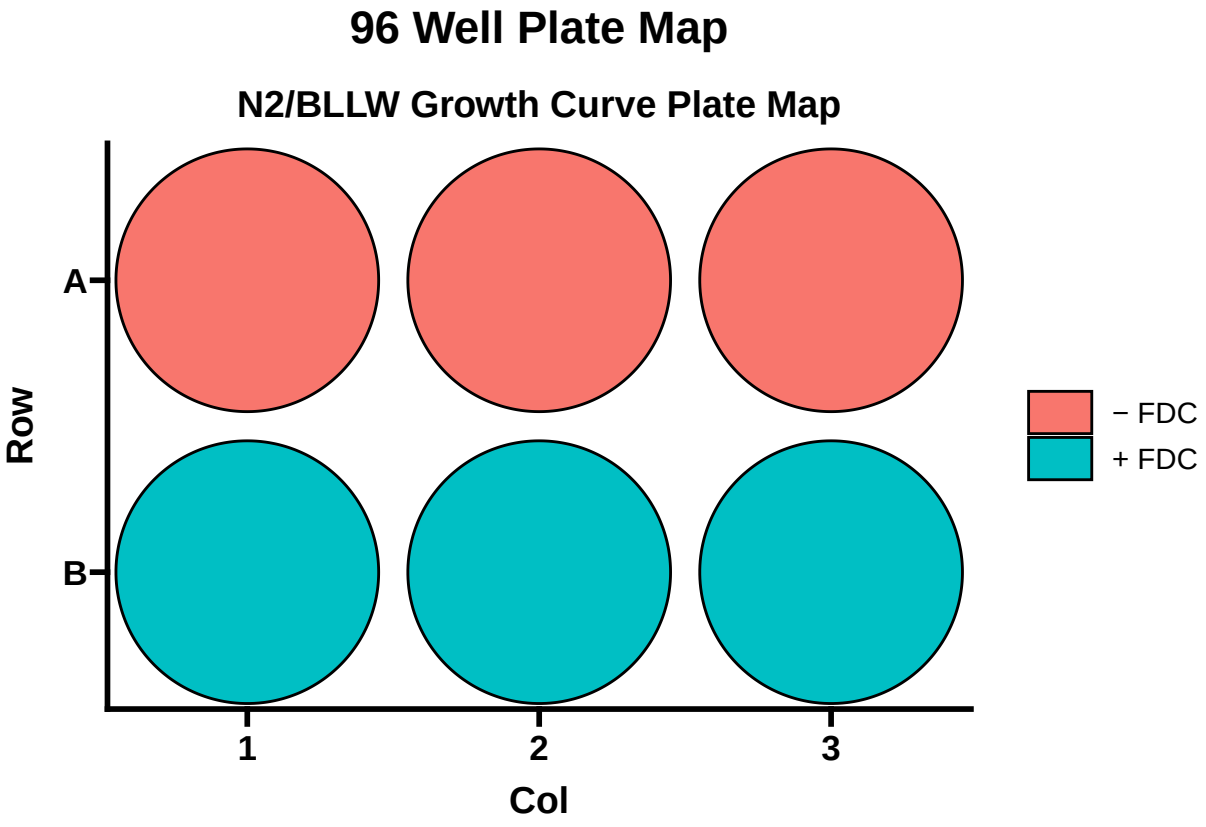
## Wednesday 15-03-2023

### 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 0.25x$10^6$cells/mL




## Thursday 16-03-2023

### N2/BLLW Growth Curve Day 1

- Cells gently mixed
- 30 $\mu$L 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 $\mu$L 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 $\mu$L 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

# 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)

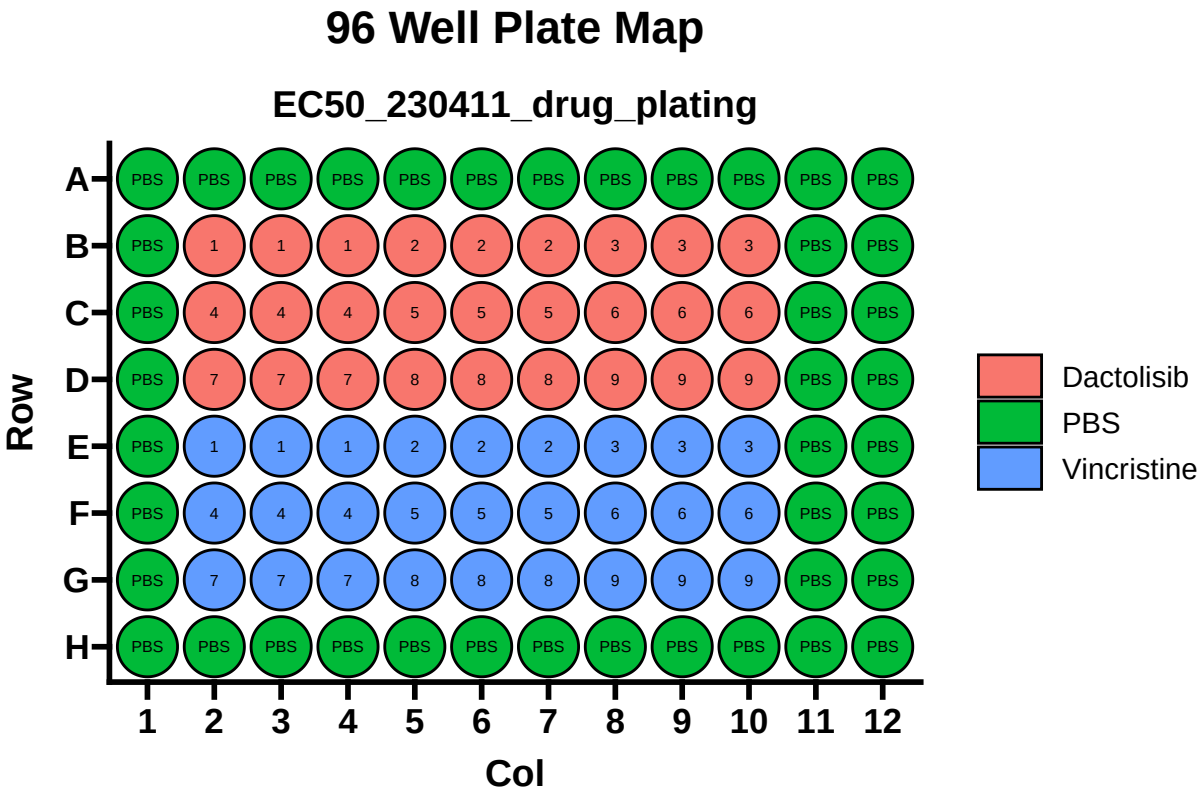
# Thursday 06-04-2023

## Collect EC50 Plate

```
- Plate ID: EC50_230619_Plate1
- DNW
```

## 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

# 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 | $8.93 \times 10^5$ | $3 \times 10^5$. | 6.71 mL | 12 mL | Tues 02-05-23 | 2 |
| RBL1 | $7.73 \times 10^5$ | $3 \times 10^5$. | 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 $3.2 \times 10^4$ cells/mL
   b. Add 100$\mu$L cell suspension to each well

# Thursday 04-05-2023

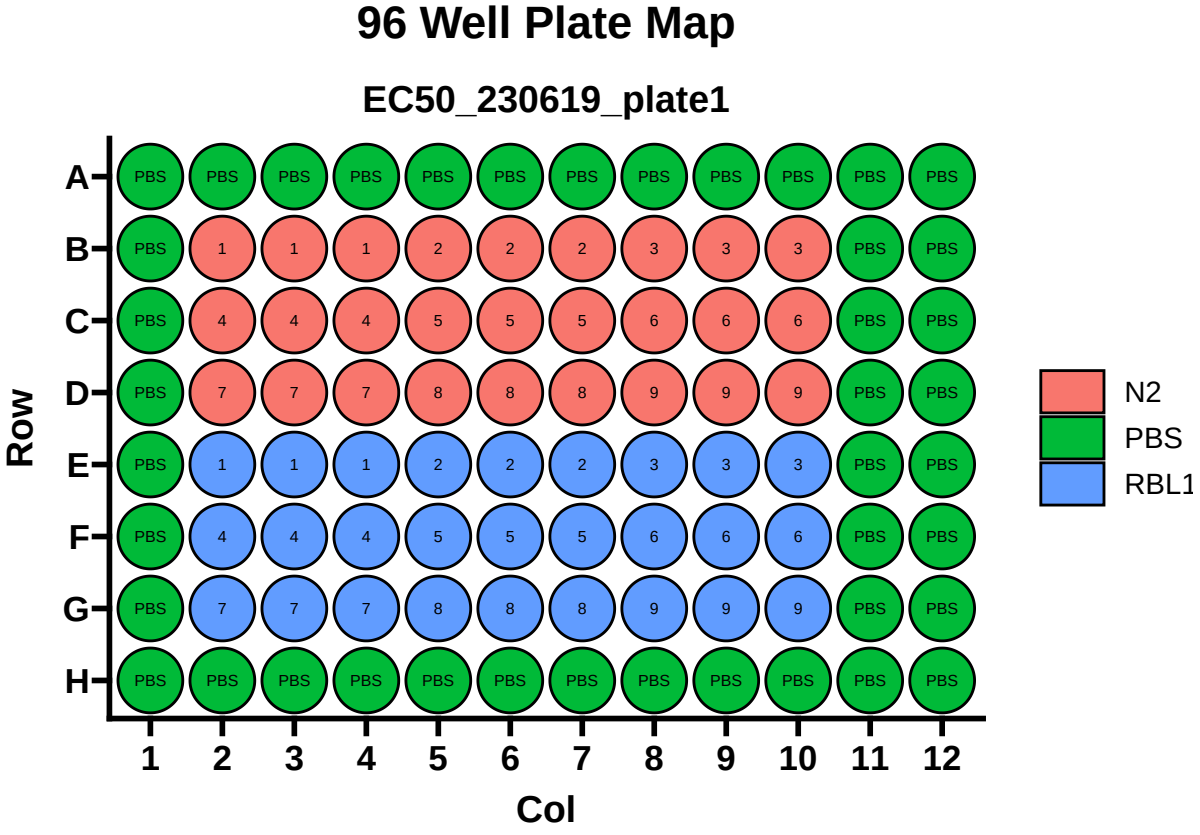
# Monday 19-06-2023

## 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

## 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 $\mu$L | 5.5 $\mu$L |
| DNA Disgestion Buffer | 35 $\mu$L | 38.5 $\mu$L |

**RNA Extraction Pico-Pure Protocol**

1. Extract cells with 100 $\mu$L 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 $\mu$L 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 $\mu$L 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 $\mu$L Wash Buffer 1 (W1) into the purification column and centrifuge for 1min at 8000 x g

10. Add 40 $\mu$L of DNAse treatment master mix to purification tube

- Add directly onto membrane

11. Incubate at RT for 15 min

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

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

14. Pipette 100 $\mu$L 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 $\mu$L to 30 $\mu$L 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 2$\mu$M
  - 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 $\mu$L 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 $\mu$L | 4.4 $\mu$L |
| SSIV RT | 1 $\mu$L | 1.1 $\mu$L |
| 100 mM DTT | 1 $\mu$L | 1.1 $\mu$L |

| Component | Volume | MM volume |
|---|---|---|
| RNAse Out | 1 $\mu$L | 1.1 $\mu$L |

8. Added 7 $\mu$L 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 $\mu$L of ExoI MM to each sample

| Component | Volume | MM volume |
|---|---|---|
| Thermolabile Exonuclease I | 1 $\mu$L | 1.1 $\mu$L |
| NEBuffer r3.1* | 2 $\mu$L | 2.2 $\mu$L |

- 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 $\mu$L 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 $\mu$L | |
| P5_R1_fwd (100$\mu$M) | 2.5 $\mu$L | |
| P7_rev | 2.5 $\mu$L | |
| DNAse/RNAse H20 | 13.5 $\mu$L | |
| Q5 polymerase | 0.5 $\mu$L | |
| **Total Master Mix volume** | **40 $\mu$L** | |
| cDNA from above | 10 $\mu$L | |

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

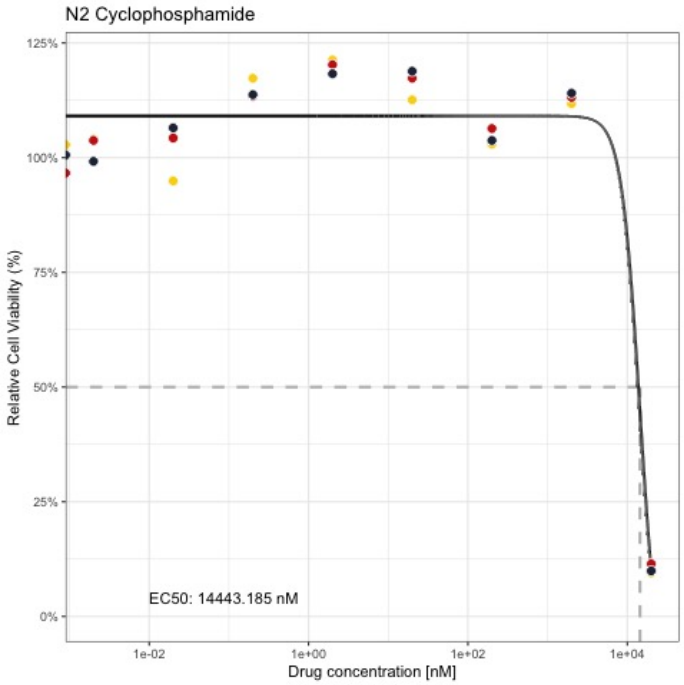
High-Sensitivity DNA ScreenTape Protocol

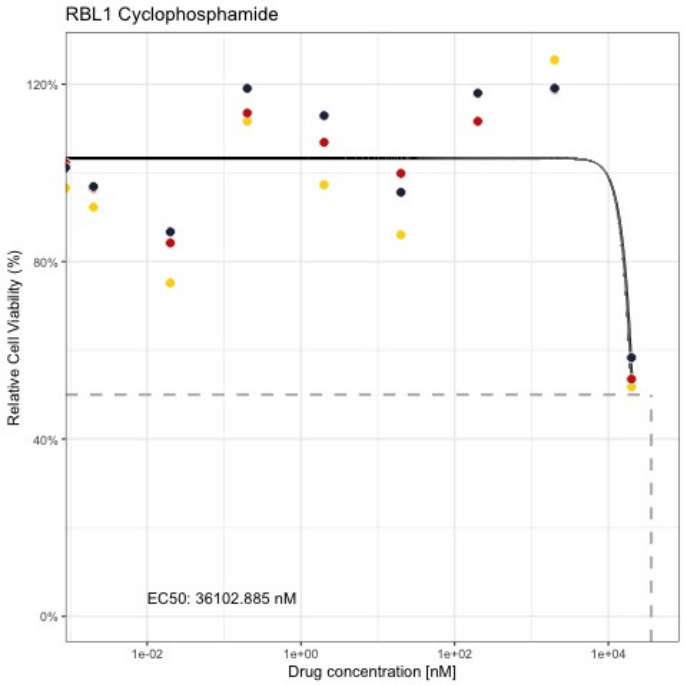
Results
* Did not work, bands present but faint and not at 200bp
* Potential solutions:
- Repeat PCR with increased cycles
- Repeat RNA extration with trizol protocol (Anna)

# Thursday 22-06-2023

## Collect EC50 Plate

- Plate ID: EC50_230619_Plate1



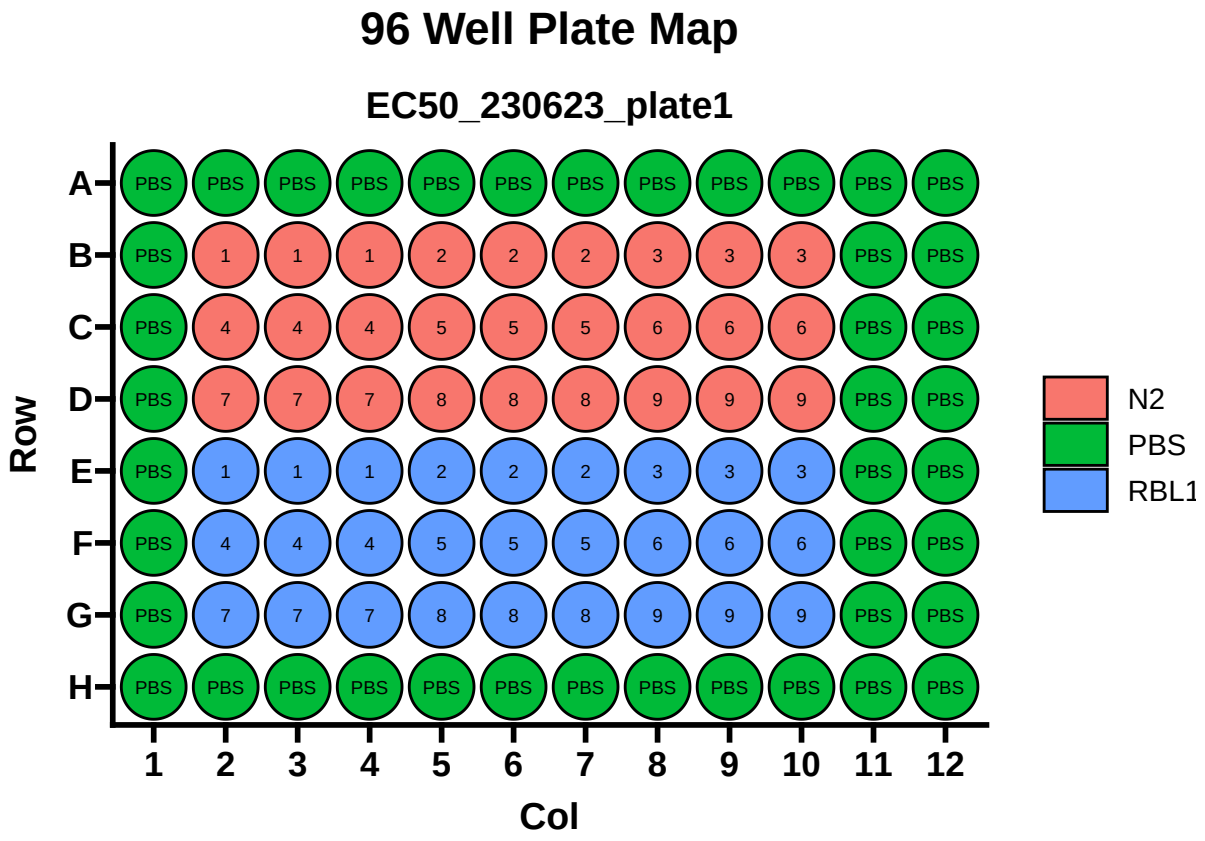

# Friday 23-06-23

### 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



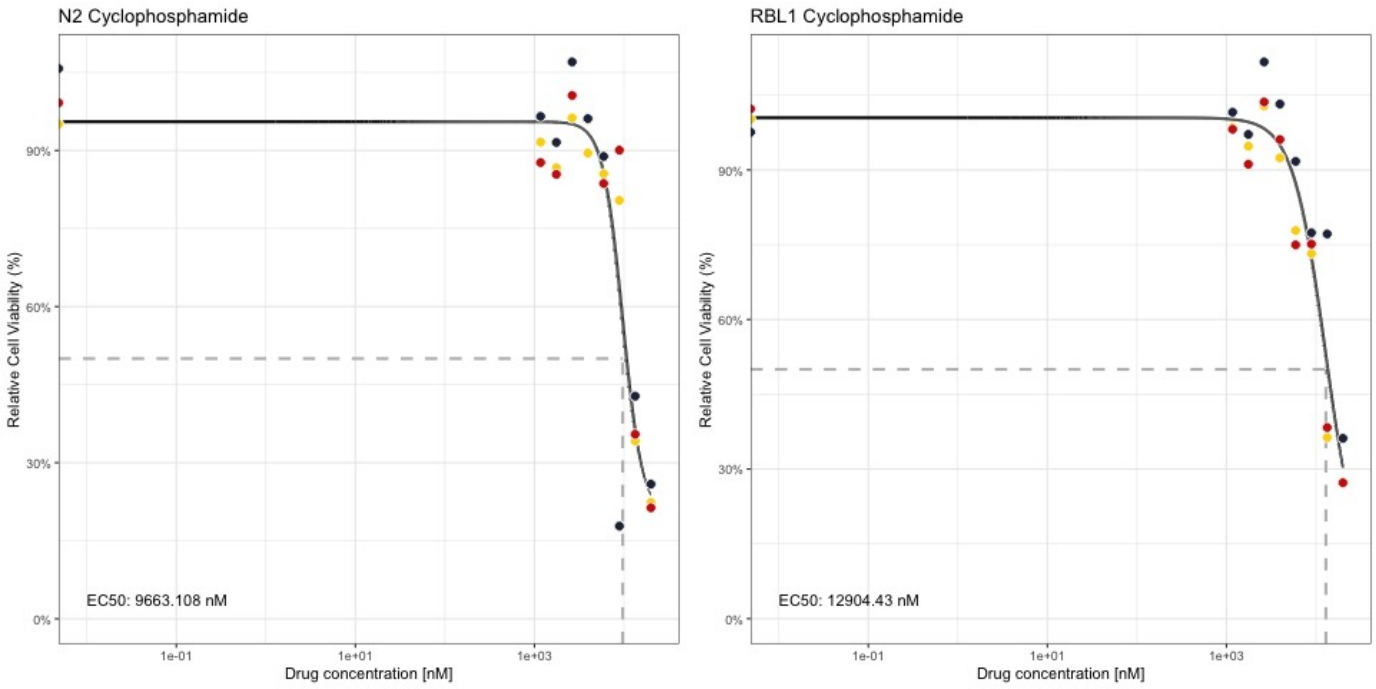

Drug Dilutions

| 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**

# 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 |

## 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**

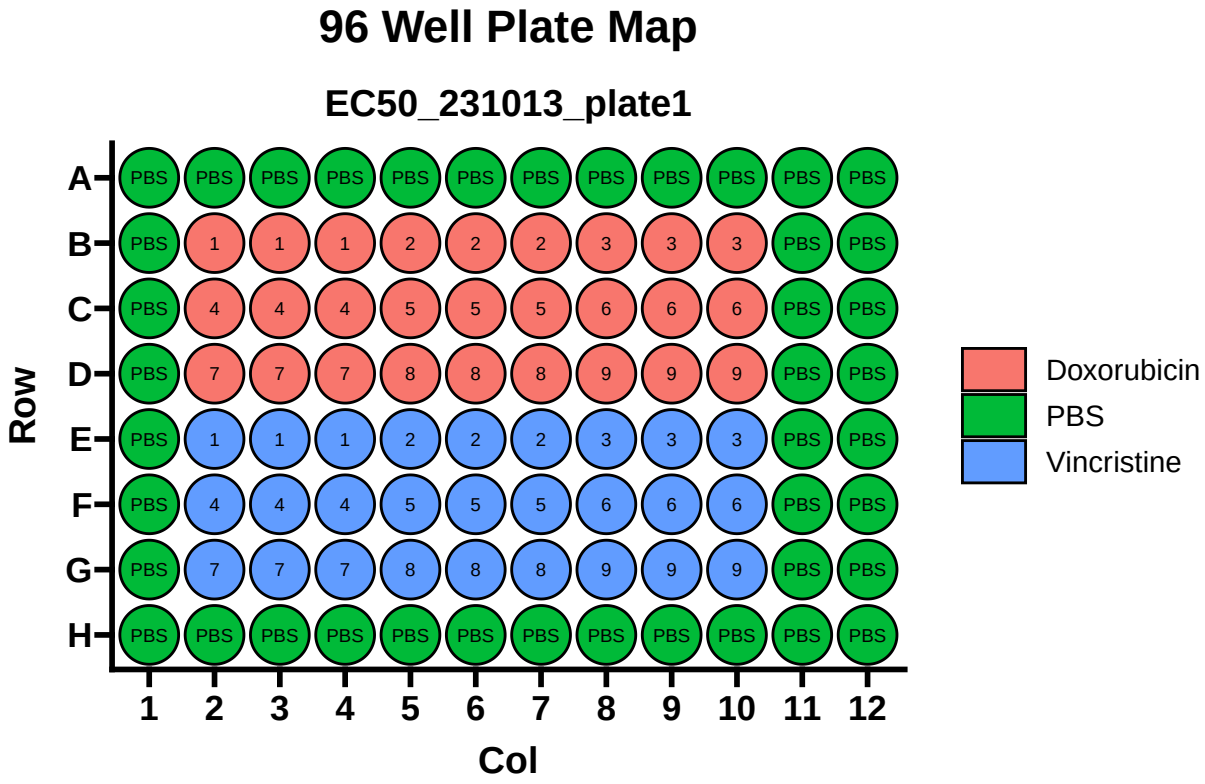
- Doxorubicin top/Vincristine bottom

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: 30$\mu$M

| 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**

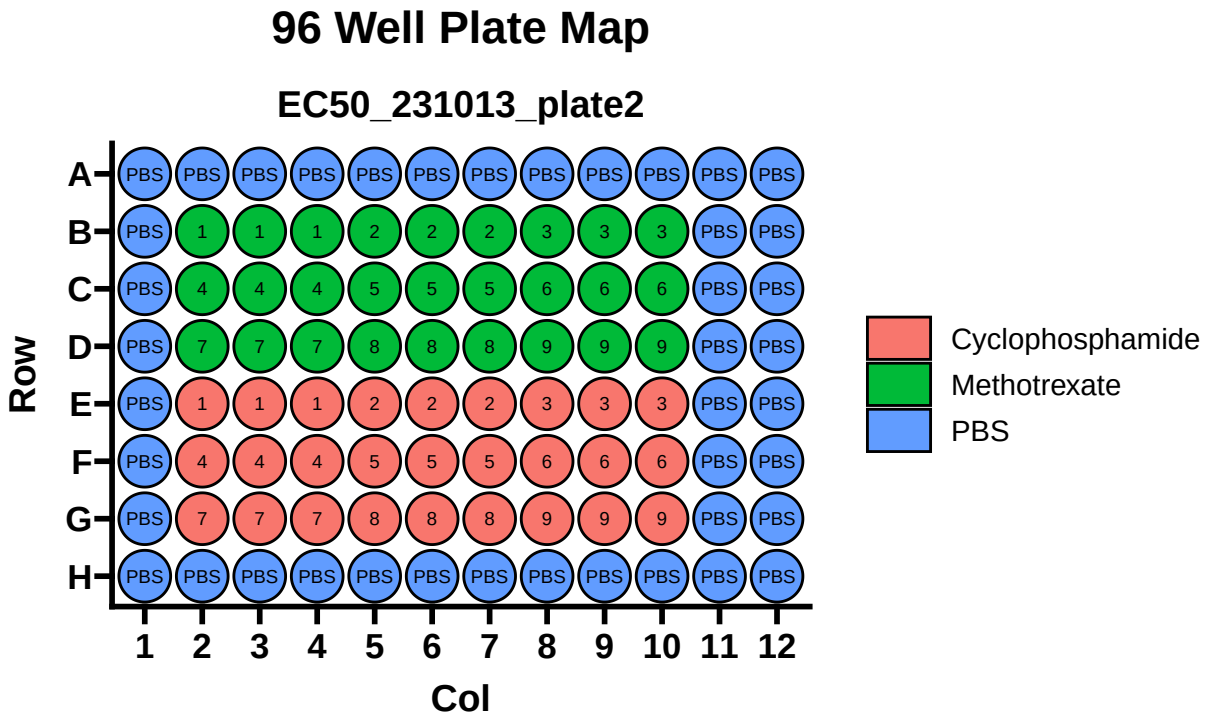
- Methotrexate top/Cyclophosphamide bottom

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

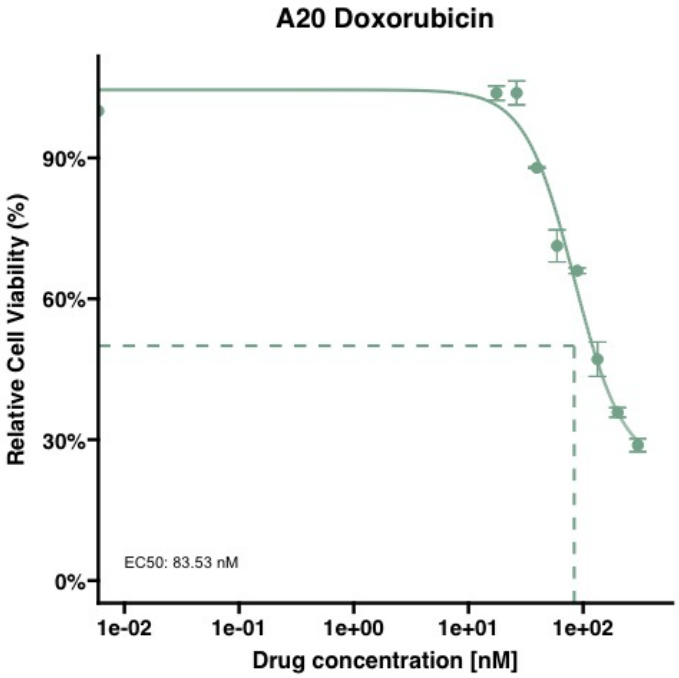



Figure 1: A20 Doxorubicin Results

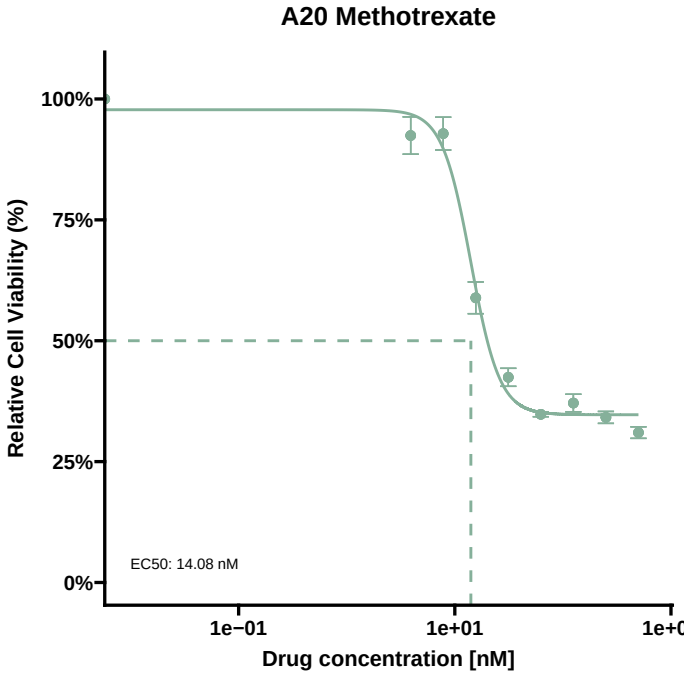



Figure 2: A20 Methotrexate Results

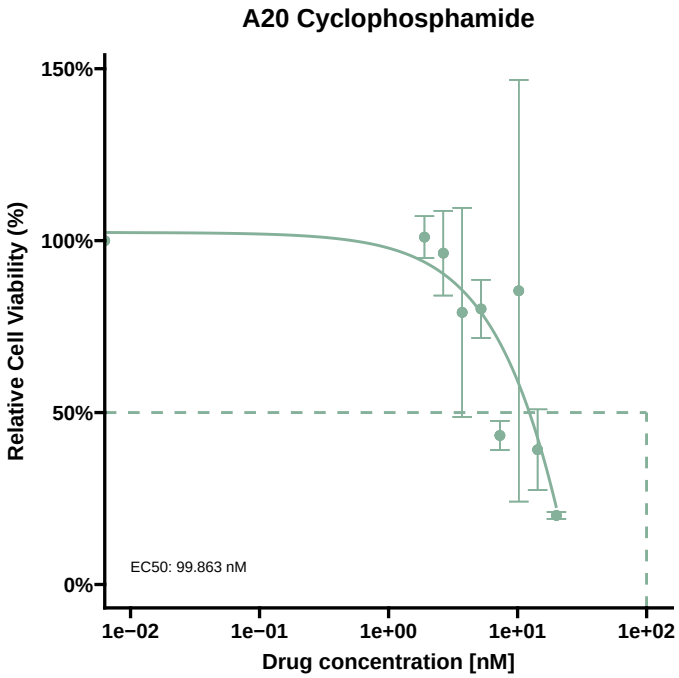



Figure 3: A20 Cyclophosphamide Results

**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 $\mu$L/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 $\mu$L | 900 $\mu$L | 50 $\mu$L |
| A2 | 10 $\mu$L | 930 $\mu$L | 50 $\mu$L |
| A3 | 1 $\mu$L | 948 $\mu$L | 50 $\mu$L |
| A4 | 0 $\mu$L | 950 $\mu$L | 50 $\mu$L |

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 2.5x$10^6$ cells

- A20 Cell count: 2.5x$10^6$
- 8.33mL AR-3 added to 1.66mL of flask CS

2. Add 90 $\mu$L 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 10$\mu$L of drugged media to respective well

### EC50 Plate ID: EC50_231027_Plate1

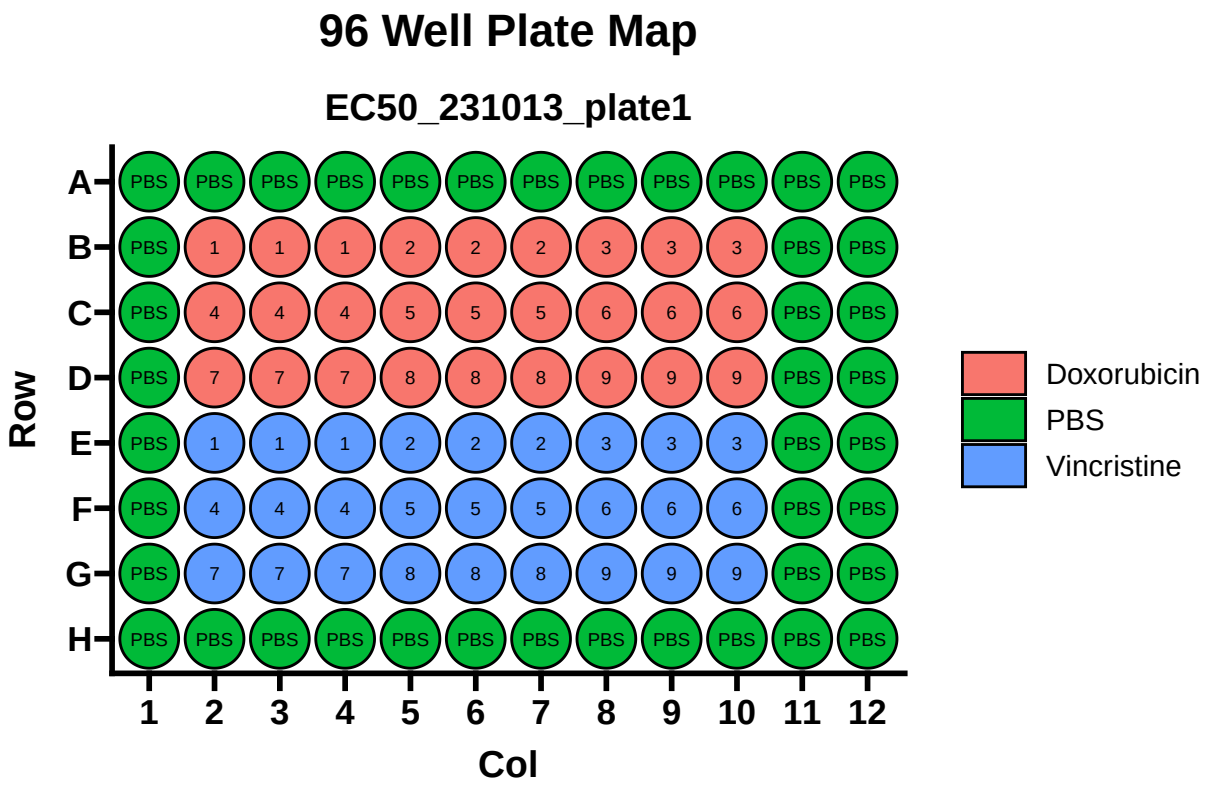
- Doxorubicin top/Vincristine bottom

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: 30$\mu$M 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**

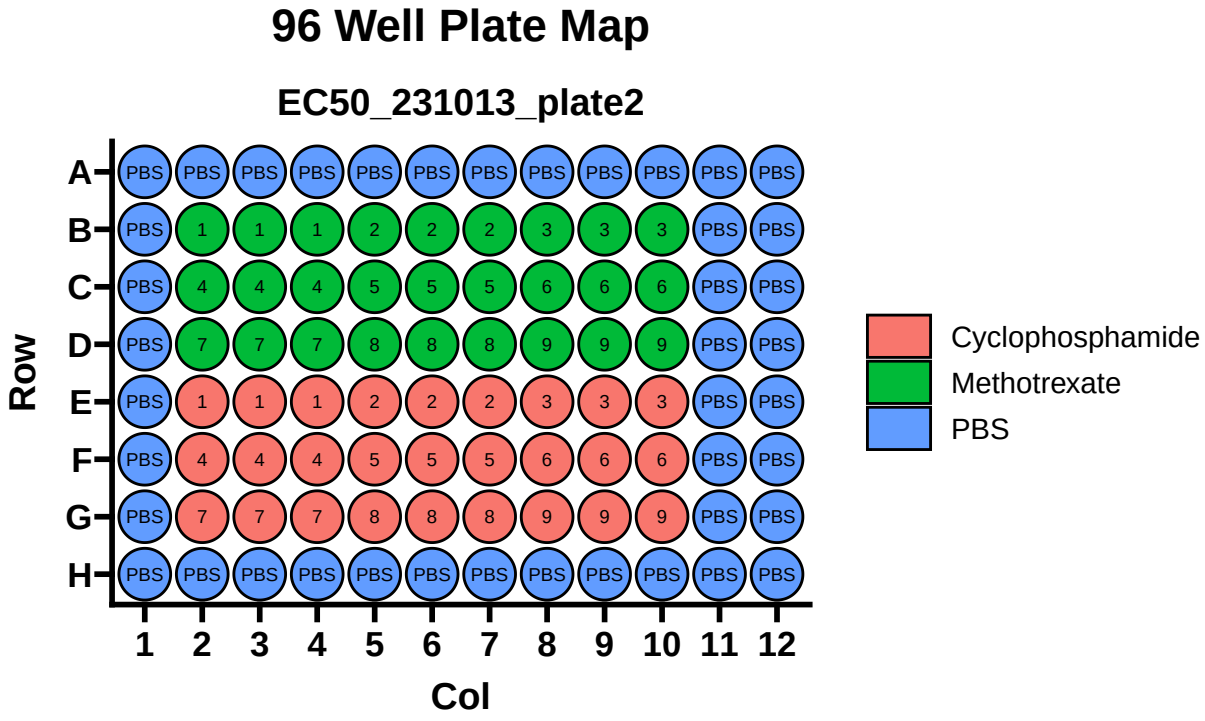
- Methotrexate top/Cyclophosphamide bottom

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**

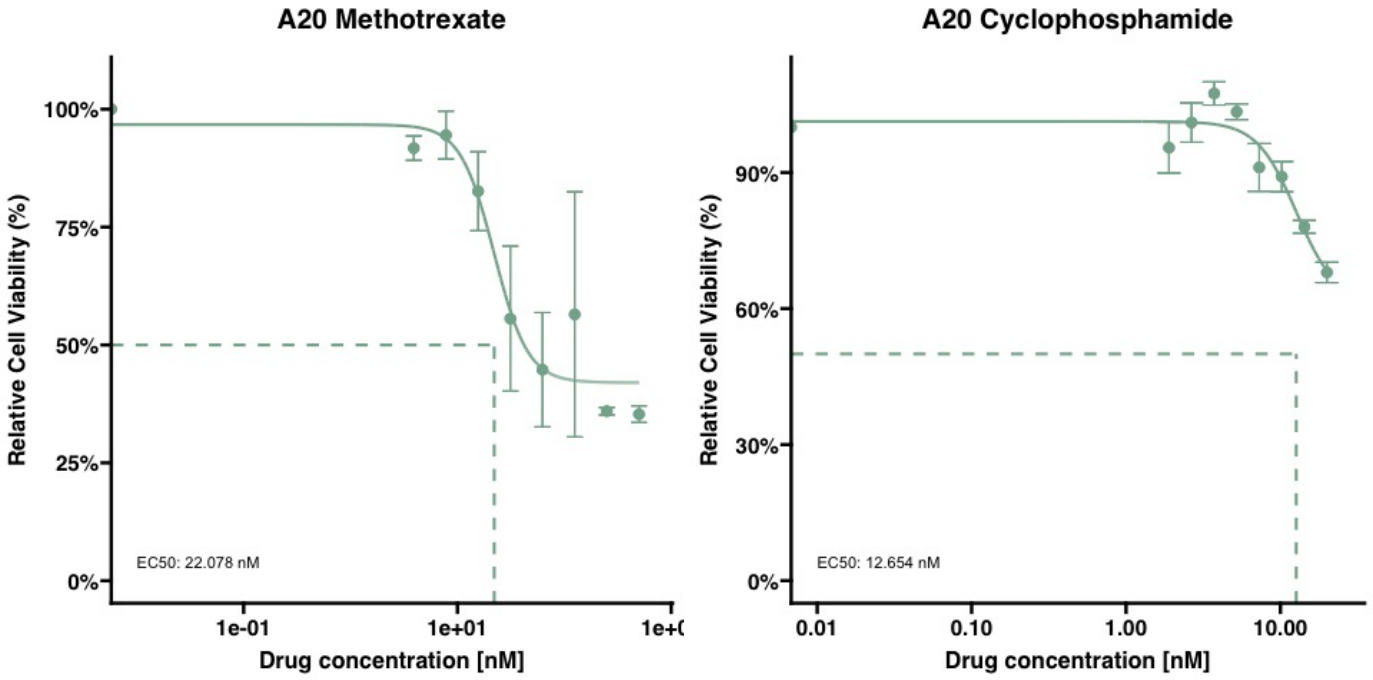
- Split 1/2
- AR-3

## Seed Feeders

- Repeating barcoding of A20/N4
- Count: $3.64 \times 10^5$
- Dilute to $4.8 \times 10^4$
- 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 $\mu$L/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 $4 \times 10^6$ cells/mL

- A20: $2.18 \times 10^6$ count
- N4: $3.06 \times 10^5$ 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 $\mu$L | 225 $\mu$L | 25 $\mu$L |
| A2 | 150 $\mu$L | 150 $\mu$L | 25 $\mu$L |
| A3 | 50 $\mu$L | 50 $\mu$L | 25 $\mu$L |
| A4 | 0 $\mu$L | 0 $\mu$L | 25 $\mu$L |

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 $\mu$M working stock |
| Vincristine | Day 1 | 10nM | 30$\mu$M |
| Methotrexate | Day 1 | 20nM | 100 $\mu$M working stock |
| Cyclophosphamide | Day 2/3/4 | 20 $\mu$M | 20$\mu$M working stock |

- Plan:

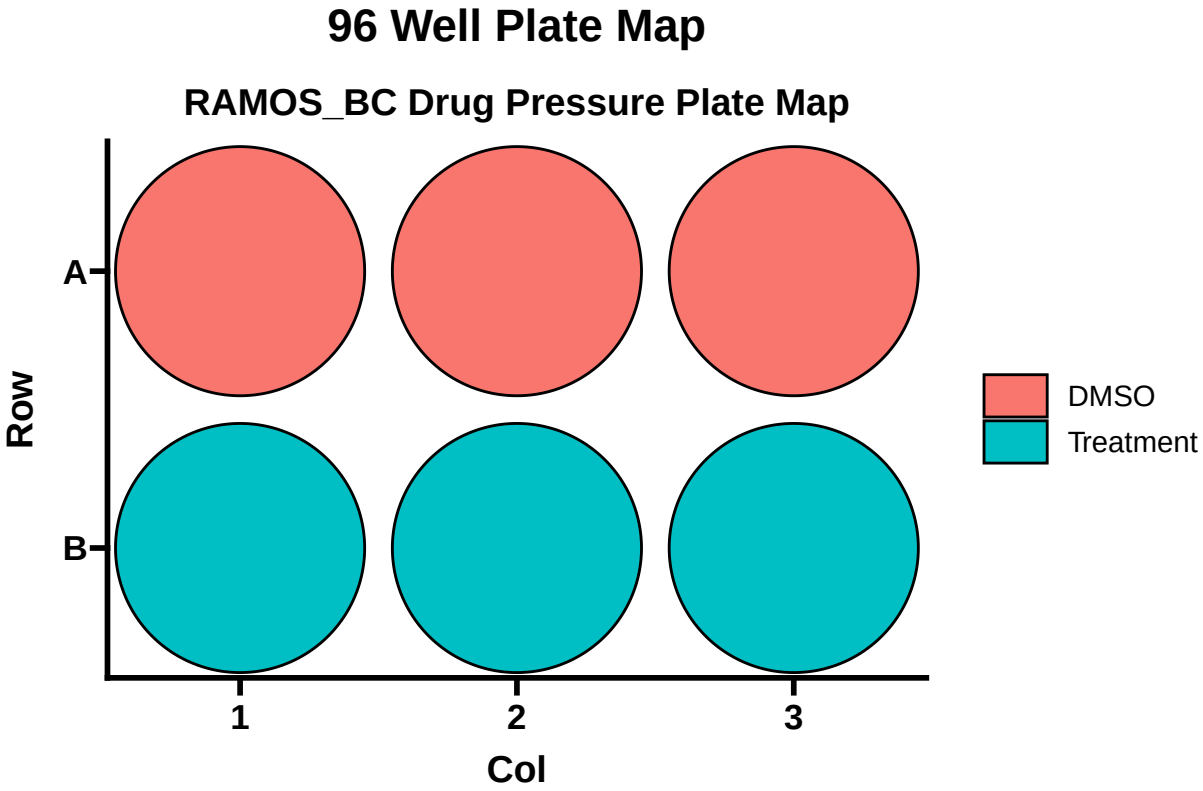
1. Collect sample at baseline
2. Complete 4 day treatment course of RAMOS-BC cells (with corresponding DMSO controls)
3. Allow treatment(T) cells to recover and collect sample
4. Complete 4 day treatment course again and collect sample

- 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 0.25x$10^6$ cells/well in 3mL AR-3
- Treatment wells

| Drug | Treatment Day | Concentration | Stock | Stock Volume | Media Volume |
|---|---|---|---|---|---|
| DMSO | Day 1 | - | - | 14.4 $\mu$L | 27 mL AR-3 |
| Vincristine | Day 1 | 10nM | 30$\mu$M | 9 $\mu$L | 27 mL AR-3 |
| Methotrexate | Day 1 | 20nM | 100 $\mu$M working stock | 5.4 $\mu$L 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

# 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 $\mu$L | 27 mL AR-3 |
| Doxorubicin | Day 1 | 80nM | 100 $\mu$M working stock | 21.60 $\mu$L of working stock | 27 mL AR-3 |
| Cyclophosphamide | Day 1 | 20$\mu$M | 20mM | 27 $\mu$L | 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 $\mu$L | 27 mL AR-3 |
| Cyclophosphamide | Day 1 | 20$\mu$M | 20mM | 27 $\mu$L | 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