Turner Lab Notebook

Callum Malcolm

Contents

Tuesday 02-05-2023

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

Wednesday 03-05-2023

1. Splitting cells

Cell Line	Cells/mL	Cells/mL Seeded	CS volume	Final Volume	Last day split	P number
N2	8.93×10^5	3×10^5.	6.71 mL	12 mL	Tues 02-05-23	2
RBL1	7.73×10^5	3×10^5.	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 3.2×10^4 cells/mL
   b. Add 100μL cell suspension to each well

Monday 19-06-2023

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

Seed EC50

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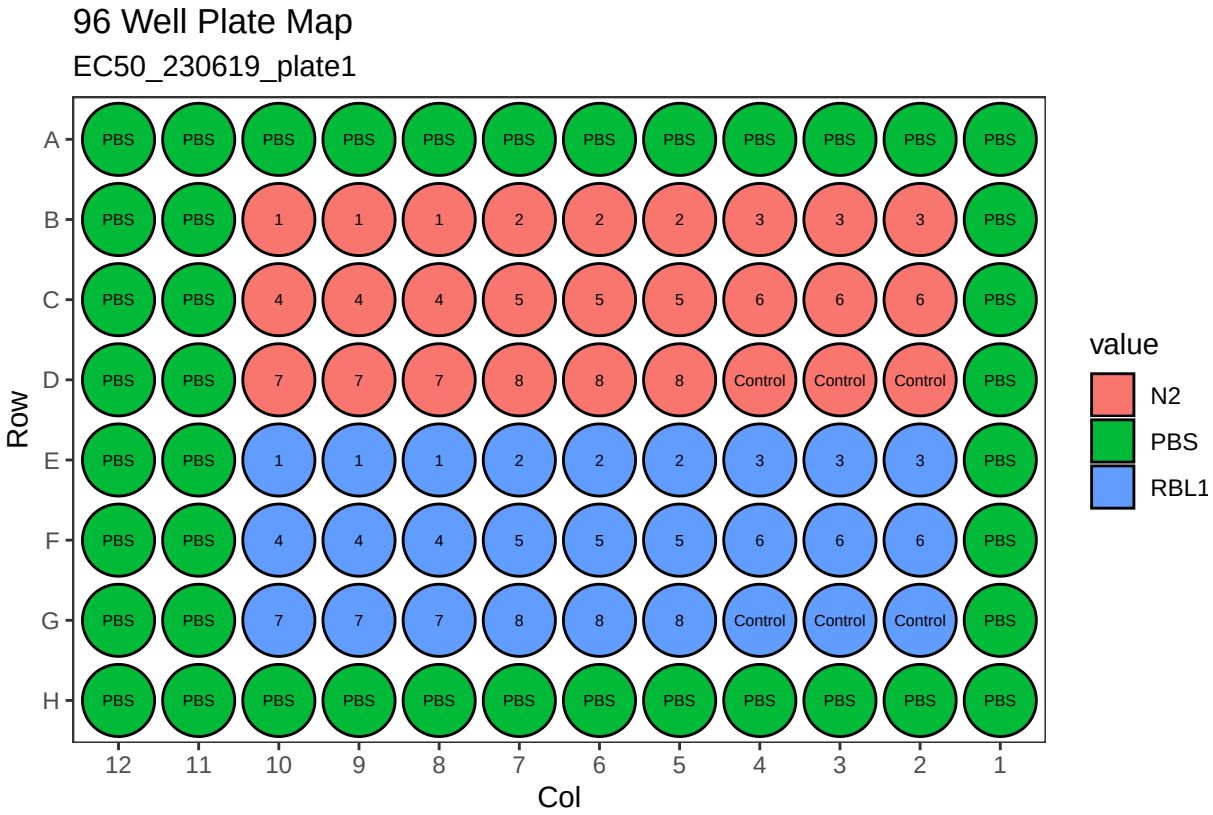


Drug Dilutions

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

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
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**

Steps	Time
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

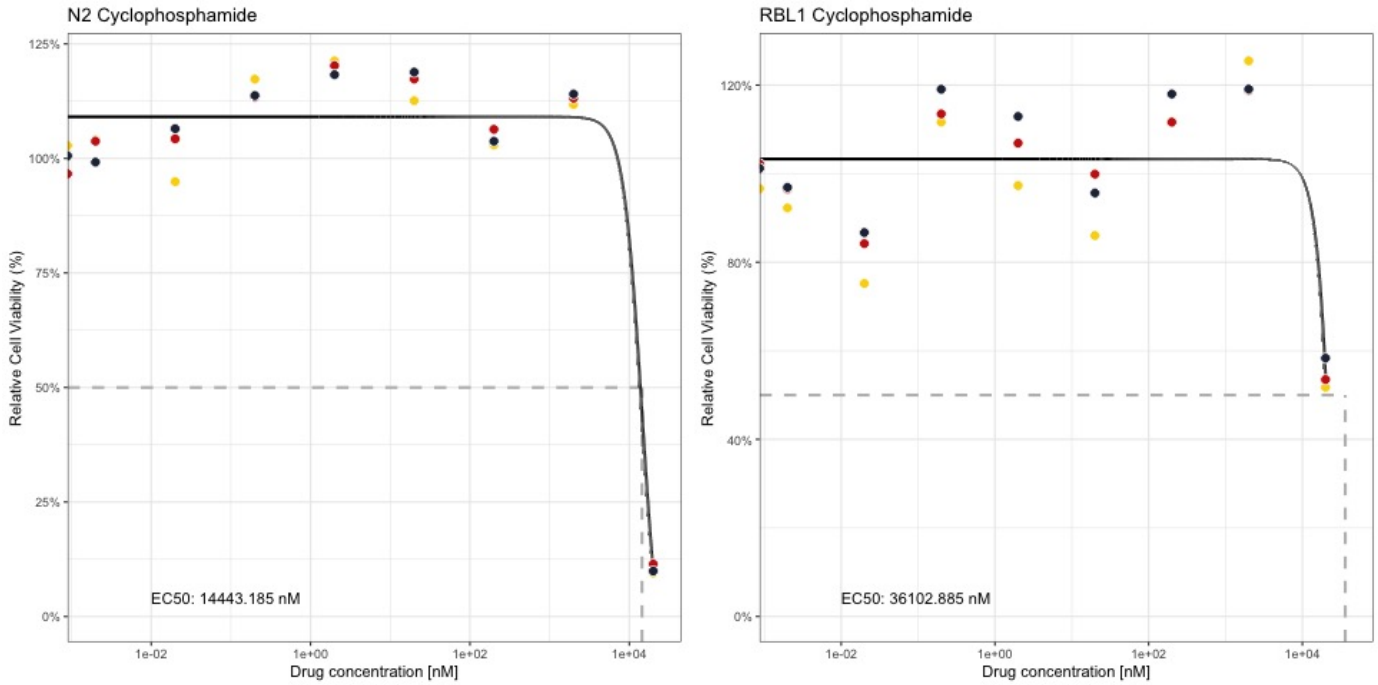
High-Sensitivity DNA ScreenTape Protocol

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

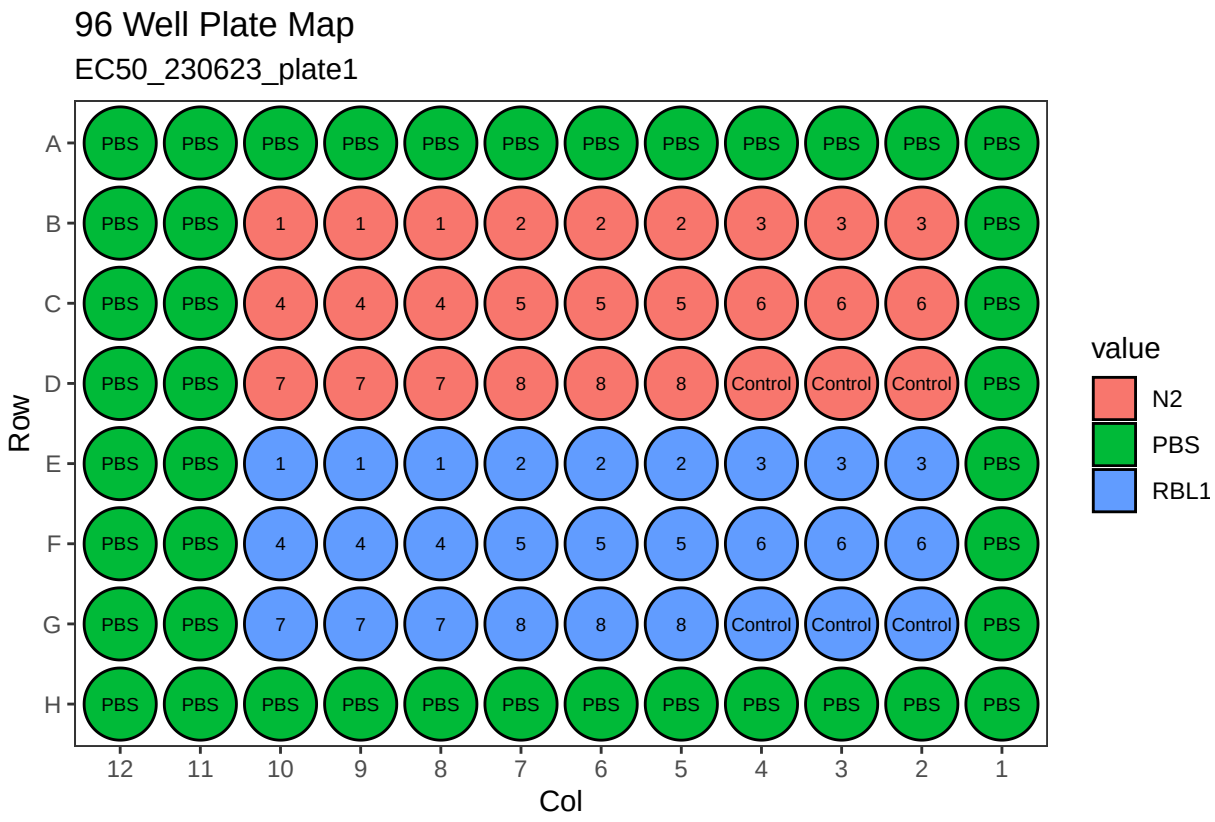
Split Cells

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