Turner Lab Notebook

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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

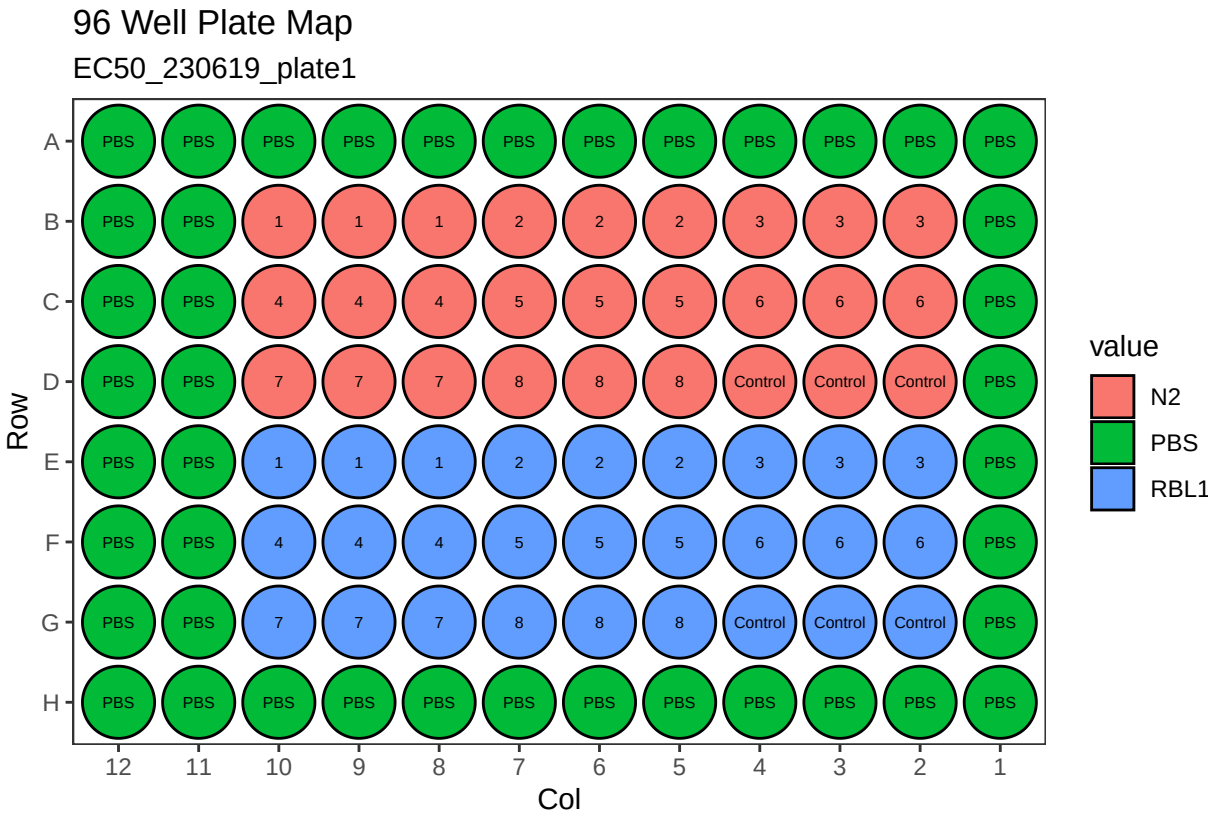
1. Splitting cells

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
2. EC50 - ID:
   - Cylophosphamide 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

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	5.1 μL
DNA Disgestion Buffer	35 μL	38.5 μL

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. Start RT immediately or store at -80C until use