PRISM Screening SOP

MATERIALS

Mammalian cell culture

- RPMI 1640, No Phenol Red (Gibco, cat. no. 11835055)
- FBS, sterile, heat inactivated, (Sigma, cat. no. F4135)
- PBS, pH 7.4, (Gibco, cat. no. 10010049)
- 70% Ethanol
- Recovery Cell Culture Freezing Medium (Thermo Fisher Scientific, cat. no. 12648010)
- Trypsin-EDTA (0.25% w/v), Phenol Red (Gibco, cat. no. 25200056)
- Trypan Blue Solution (0.4% w/v), Corning (VWR, cat. no. 45000-717)

PRISM Assay

- RPMI 1640, No Phenol Red (Gibco, cat. no. 11835055)
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- Bortezomib (Selleck Chem cat. no. S1013)
- DMSO (Sigma-Aldrich, cat. no. D2438)
- 50mL conical tubes
- Cellometer Slides (Nexcelom, cat. no. CHT4-SD100-002)
- 500mL or 1L sterile bottle
- Cassette for Multidrop Combi (ThermoFisher, cat. no. 24072671)
- 70% Ethanol
- White, flat bottom, low flange, TC treated 384-well plates with labels (Corning, cat. No. 8867BC)
- Aluminum seals (Bio Rad Laboratories, cat. no. MSF-1001)
- TCL Buffer (Qiagen, cat. no. 1031576)

REAGENT SETUP

RPMI 1640 normal growth medium Add 10% heat inactivated FBS to a 500 mL bottle RPMI 1640 Medium, no phenol red, to make up RPMI 1640 /10% FBS normal growth medium. Sterile filter all components and store at 4 ^{o}C for up to 1 month.

PROCEDURE

PRISM Assay ● **Timing: 4 weeks, hands on 15 days**

1| *Thaw Pooled Cell Vials:* Set up one 50 mL conical tube per mini-pool (5 cell lines) with 10mL PBS. Transfer cells from freezer on dry ice and thaw vials of cells in 37°C water bath for ~2 mins. Transfer thawed cells from cryovials to the conical tubes containing PBS. Use some of the PBS to wash the cryovials to ensure that all cells are collected and add back to the PBS tube. Spin the conical tube for 5 mins @ 1250rpm. Aspirate supernatant then resuspend the cells in 10mL normal growth media. Thoroughly mix cells and count by mixing 20uL cell suspension with 20ul trypan blue and pipette 20ul of the mixture onto a cellometer slide. Count using the cellometer (dilution factor 2). Dilute the cells to 1,250 cells/50ul/well (50 cells/cell line/well)

(25,000 cells/ml) in normal growth media in a sterile bottle. Always make >20% extra. Pool 5 mini-pools together in equal volumes for 25 cell line suspensions and mix well.

2| *Plate cells:* Plate 1250 cells/well using a Thermo Fisher Multidrop Combi. Attach the cassette to the combi. Clean the cassette by priming first with ~25mL of 70% EtOH, then ~25mL ddH_2O. Prime until all of the liquid has been removed, then prime using the cell suspension. Set the combi to the default protocol, full plate, 50ul/well. Re-lid the plates once complete and place the plates in a metal microplate rack, then in the 37°C incubator. Clean the cassette by priming first with ~25mL ddH_2O, then ~25mL of 70% EtOH.

3| *Treat cells:* Cells can be treated either by seeding cells directly onto assay ready plates, or by pin transfers the day after cells are plated. Assay ready plates are made by using an Echo to acoustically transfer 100nL of compound to the source plate. Compounds are pinned using a CyBio® Well vario, with a 100nL pin head.

4| *Lyse cells:* After 5 days of treatment, lyse the cells. Remove 384w plates containing the treated cells from the 37°C incubator. Remove lids and place cell plates in Biotek EL406 platewasher. Aspirate majority of medium, then add 30uL of TCL buffer using peri pump. Seal the plate with aluminum seal manually, or with a PlateLoc at 174C for 1.4sec. Spin plates down at 1000rpm for 1 minute, leave plates at room temperature for 30 minutes, then store in -80 freezer.

PRISM mRNA Protocol for 20 Pool Lysate Collapse ‘PR500’

Materials:

Item	Vendor	Cat #
Sera-Mag Magnetic Oligo(dT) particles	GE Healthcare	38152103010350
SuperScript IV Reverse Transcriptase	Invitrogen	EP145C001 (Kit)
SuperScript IV Reverse Transcriptase Buffer	Invitrogen	EP145C001 (Kit)
0.1M DTT	Invitrogen	EP145C001 (Kit)
25mM dNTP	Life Technologies, Inc.	N8080261
Platinum Taq DNA Polymerase	Invitrogen	102689 (Kit)
10X PCR Buffer (-MgCl2)	Invitrogen	102689 (Kit)

50 mM MgCl2	Invitrogen	102689 (Kit)
100uM reverse primer	Integrated DNA Technologies	Custom order
100uM forward primer	Integrated DNA Technologies	Custom order
DEPC Treated Nuclease Free Water	GrowCells	UPW-1000
Nuclease Free Water	Qiagen	1039480
Stain Buffer	Broad internal	Broad internal
Lithium Chloride	SIGMA-ALDRICH Inc.	L7026-1L
0.5M EDTA	Life Technologies, Inc.	15575020
1.0M Tris HCl	Life Technologies, Inc.	15567027
Bovine Serum Albumin	Seracare Life Sciences Inc.	AP-4510-80
Streptavidin, R-Phycoerythrin Conjugate (SAPE) - premium grade	Invitrogen	S21388
Antibody, Anti-Streptavidin	Vector Laboratories Inc	BA-0500

Automation Protocol Descriptions:

Protocol Name	Description	Device
PRISM_COLLAPSE_20PLATE	Collapses 5ul from each 384 well lysate plate (20) + 5ul from control pool into deep 384 well source plate, then mixes 10 times	JANUS
PRISM_deepwell_to_PCR	Transfers 30ul of collapsed lysate from deep well to Eppendorf PCR plates	JANUS
PRISM bead to PCR transfer	Transfers 5ul Seramag dT beads into PCR plate containing collapsed lysate.	JANUS
15ul Master mix addition high dispense	Adds 15ul volume from reagent reservoir to 384 well plate	JANUS

postmag_cleanup	Removes all liquid volume from plates, then washes once with DEPC or nuclease free treated water. Protocol finishes with only bead in well.	JANUS
Bead Addition	Adds 30ul pooled PR500 bead set into 384 well plate.	Tecan Fluent
Hybridize dT Bead	Transfers 15ul from amplicon plate to bead plate.	Tecan Fluent
PRISM Cleanup	Removes unbound amplicon, adds SAPE stain, washes. (detailed steps below)	Tecan Fluent
Hybridize	Denatures at 90 ℃, then holds at 45 ℃.	ProFlex Thermal cycler

Amplification and Detection:

1| *Collapse:* Thaw Lysate plates (20) and "Lysate control" pool for 45 min on benchtop. Remove seal and place thawed lysate plates onto Janus deck. Place deep well plate (costar 3342) in correct position on deck. Place reservoir with 'control lysate' pool in correct position on deck. Run protocol: PRISM_COLLAPSE_20PLATE. After protocol runs, seal deep well plate and spin 1000rpm 30 seconds. Store on ice until ready for capture step.

2| *Capture:* Wash SeraMag beads with 1:1 ratio using 'Binding buffer' and dilute to a final 2:1 ratio using 'Binding buffer.' Place deep well plates and PCR plates onto JANUS deck according to 'PRISM_deepwell_to_PCR' protocol. Remove seals from deep well plates. Run protocol 'PRISM_deepwell_to_PCR.' When complete, Place plates on JANUS deck in appropriate position for bead transfer. Pour SeraMag beads into reservoir at appropriate position on deck. Run protocol 'PRISM bead to PCR transfer.' Seal plates, and vortex to distribute bead. Place plates on shaker, turn shaker on and adjust speed to level 10, then lower speed to level 3 for 10 minutes.

3| *Wash:* Remove seal and place plates on post magnet. Place reservoir for waste on deck. Place DEPC and Nuclease-free water on correct deck positions according to 'postmag_cleanup' protocol. Select # plates. Select DEPC. Run protocol. Remove plates and proceed to RT.

4| *Reverse Transcription:* Pour RT mix into reservoir and lay plates on Janus deck according to '15ul Master mix addition high dispense.' Run protocol '15ul Master mix addition high dispense.' Seal plates and vortex upside down until beads are re-suspended in master mix. Vortex right-side up to knock liquid back to bottom of well. Lock into compression rack and submerge in 55°C water bath for 10 min reaction.

	1 well	**1 plate**
DEPC water	**11.625ul**	**4,882.5ul**
5x Buffer	**3.0ul**	**1260ul**
25mM dNTP	**0.1ul**	**42ul**

DTT	0.25ul	105ul
SSIV	0.025ul	10.5ul

5| *Wash:* Remove seal and place plates on post magnet. Place reservoir for waste on deck. Place DEPC and Nuclease-free water on correct deck positions according to 'postmag_cleanup' protocol. Select # plates. Select Nuclease-free. Run protocol. Remove plates and proceed to PCR.

6| *PCR:* Pour PCR mix into reservoir and lay plates on Janus deck according to '15ul Master mix addition high dispense' and Run protocol '15ul Master mix addition high dispense.' Seal plates and vortex upside down until beads are re-suspended in master mix. Vortex right-side up to knock liquid back to bottom of well. Load plates into cyclers and run 'PRISM Plat Taq 31cycles.'

	1 well	1 plate
Nuclease free water	12.35ul	5,187ul
10x PCR buffer	1.5ul	630ul
50mM MgCl2	0.75ul	315ul
25mM dNTP	0.2ul	84ul
100uM forward primer	0.05ul	21ul
100uM reverse primer	0.05ul	21ul
Platinum Taq	0.1ul	42ul

7| *Hybridization:* Label 384 bead plates with names corresponding to assay plates and place plates on Tecan deck according to worktable in 'Bead Addition' protocol. Place reservoir on deck according to worktable in 'Bead Addition' protocol and pour bead into the reservoir. Set parameters to 30uL of bead per well and run protocol to add bead mixture to plates. Vortex assay plates to resuspend bead. Place plates on post magnet for minimum of 2 minutes. Place plates on magnets on deck and load hotel with bead plates per protocol. Run 'Hybridize dT bead' protocol, transferring total 15uL of amplicon Seal and vortex bead plates once amplicon has been transferred. Discard assay plates, ensuring all volume has been removed. Place plates in thermocyclers on 'Hybridize' Protocol

8| *Wash and Stain:* Next day, remove plates from thermocyclers. Remove seal and place on Tecan tower per protocol. Add stains to reservoirs and run 'PRISM cleanup.' Seal, vortex, and detect on Luminex FLEXMAP 3D.

Stain 1&3

	1 plate
Milli-Q water	11.7mL

2x stain buffer	13.5mL
50mg/ml BSA	1.44mL
Premium SAPE	0.36mL

Stain 2

	1 plate
Milli-Q water	5.922mL
2x stain buffer	6.75mL
50mg/ml BSA	0.72mL
antiSAPE	0.108mL

PRISM Cleanup Procotol Steps

a. Washes Luminex beads with low stringent buffer
b. Washes Luminex beads with high stringent buffer
c. Adds 'stain 1' to each well of plate
d. Washes Luminex beads with low stringent buffer
e. Adds 'stain 2' to each well of plate
f. Washes Luminex beads with low stringent buffer
g. Adds 'stain 3' to each well of plate
h. Washes Luminex beads with low stringent buffer
i. Washes Luminex beads with Sheath fluid buffer
j. Fills well with 40ul sheath fluid buffer
k. Seals plate

Control Barcode Pool: This is a pool of cell lines containing 25 different barcode sequences. The sequences are pre-measured to span the range of the assay.

Binding Buffer 100mL

7.5M LiCL	13mL
0.5M EDTA	0.4mL
1M Tris HCl	2mL
DEPC treated water	84.6mL