**10.27.2015 Dot Blot HS' RNAse Experiment**

On 10/22/2015 HS performed this experiment. I'll be dot blotting it.

| ng/w | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 96 wp | U2OS | U2OS | U2OS | U2OS | U2OS | U2OS | U2OS | U2OS | U2OS | U2OS | controls | |
| A | 512 | 512 | 512 | 512 | 512 | 512 | 512 | 512 | 512 | 512 | blanks | C96 |
| B | 256 | 256 | 256 | 256 | 256 | 256 | 256 | 256 | 256 | 256 | blanks | C96 |
| C | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | blanks | C96 |
| D | 64 | 64 | 64 | 64 | 64 | 64 | 64 | 64 | 64 | 64 | blanks | C96 |
| E | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | blanks | DAG |
| F | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | blanks | DAG |
| G | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | blanks | TAG |
| H | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | blanks | TAG |

1, 2 & 5-11 & 12A-D have 20 uL volumes. 12E-H don't have anything; I'm adding controls. Columns 3 & 4 have 30 uL volumes.

1. Thaw CC probe, TAG, DAG & HS 10/22/2015 plate
2. 37C 15 mL of prehyb
3. Prepare TAG
   a. Do a 1/10 $^{th}$ dilution of the TAG (2 uL TAG, 18 uL PCR water)
   b. mix 3.35 uL of 1/10 $^{th}$ TAG and 776.65 uL 2X SSC
4. Prepare DAG
   a. 2.38 uL DAG + 777.62uL 2XSSC
5. Add 150 uL of TAG & DAG to selected wells
6. Add 120 uL of 2X SSC to columns 3 & 4
7. Add 130 uL of 2X SSC to columns 1, 2 & 5-11 AND wells 12A-D
8. Run dot blot protocol
9. 14.75 uL of CC probe per blot

Wells 12E and F didn't seem to hold liquid well (prior to vacuum); the liquid appeared to flow through the lower channel & stay there. I didn't see this issue for any of the other samples. I had trouble w/ clogging for every blotting step (the ones w/ vacuum). I had to pipette loose the samples. 7pm was when I crosslinked. 7pm was prehybridization. 730pm was the start of the CC probe.

**10.27.2015 BLM siRNA Treatment of U2OS**

On 10.14.2015 I performed a very similar experiment (covering the APH), so I'll be copying a bit from there. I want a serial dilution of 60 uM down to 3.75 uM with a final volume of 800 uL per dilution. The stock concentration is 30 mM in pure DMSO.

<u>How much of the top SD do I need?</u>

200 uL X 4 wells X 2 (SD loss) X 1.3 (+30%)= 2,080 uL

<u>How to make top SD APH?</u>

30 mmole/L * V = 60 umole/L X mmole/10^3 ummole X 2,080 uL X L/10^6 uL=> 4.16 uL APH + 2075.84

4.16 uL X 100 / 2,080 ul => 0.2% DMSO

Will be diluted w/ halvings of 1040 uL. I need 1,040 uL X 4 dilutions X 1.3 = 5,408 uL of diluent required

How do I make the 1 mM HU?

I have 877 mM & 1600 mM stocks available. I want 800 (1040 +30%) uL total w/ 0.2% DMSO

877 mM X V = 1 mM X 1,040 uL => 1.18 uL of 877 mM HU,

1,040 X 0.2/100 = 2.08 total uL of DMSO required. Therefore: 0.9 uL of DMSO, 1.18 uL of 877 mM HU, 1,037.92 uL of media. X2 that to get into a pipettable range: 1.8 uL DMSO, 2.36 uL 877 mM HU, 2075.84

How much 0.2% DMSO do I need?

5,408 uL (APD SDs) + 4X200 uL X 1.3 (DMSO controls) = 1,040 + 5,408 = 6,448 uL +30%=> 8,328.4

100% X V = 0.2% X 8,328.4 uL=> 16.76 uL DMSO, 8,311.64 uL media

How do I make the siRNAs?

Stock concentration is: 0.75 nmole/625 uL X 10^6 uL/L = 1,200 nM = 1.2 uM

50 nM X 4 wells X 200 uL X2 (SD) X1.3 (30%)= 1,200 nM X V => 86.66 uL siRNA & 1,9993.3

86.66 uL X 100/2,080 = 4.16% water

How much 4.16% water-media do I need?

(200 uL X 4 wells)/conc. 4 dilutions/siRNA X3 siRNAs X1.3 (30% excess) = 12,480 uL

100% X V

**10.27.2015 BLM siRNA Treatment of U2OS**

Protocol

1. Thaw 4X0.5 mL tubes of each siRNA, 877 uM HU & APH
2. 37C >27.95 mL McCoy's
3. Prepare serial dilutions & treatments in single wells of a deep 96WP (see figure)
   a. 1 mM HU 6E: 1.8 uL DMSO, 2.36 uL 877 mM HU, 2,075.84 uL McCoy's
   b. 60 uM APH 7E: 4.16 uL 30 mM APH, 2075.84 uL McCoy's
   c. siRNA (scrambled, 2 & 4)1A, 1E, 7A: 86.66 uL sIRNA & 1,9993.3 uL McCoy's
4. Prepare diluents in 15 mL conicals
   a. 0.2% DMSO: 16.76 uL DMSO, 8,311.64 uL media
   b. 4.16% water: 519.2 uL qPCR water, 11,960.8 uL McCoy's
5. Add 1,040 uL of 4.16% water media to: 2A-5A, 7A-11A, 2E-5E
6. Add 1,040 uL of 0.2% DMSO media to: 8E-11E
7. Serially dilute siRNAs and APH
   a. 1,040 uL volume dilutions
   b. 1 minute wait between each
8. Replace single columns of the plate at a time
   a. Multichannel aspirate 200 uL from wells A-H
   b. Repeater mode transfer 200 uL of treatment to well

Here's how I'll serially dilute

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 50 nM Scramble | 25 nM Scramble | 12.5 nM Scramble | 6.25 nM Scramble | 3.125 nM Scramble | DMSO Control | 50 nM BLM4 | 25 nM BLM4 | 12.5 nM BLM4 | 6.25 nM BLM4 | 3.125 nM BLM4 |
| B | | | | | | | | | | | |
| C | | | | | | | | | | | |
| D | | | | | | | | | | | |
| E | 50 nM BLM2 | 25 nM BLM2 | 12.5 nM BLM2 | 6.25 nM BLM2 | 3.125 nM BLM2 | HU 1 mM | 60 uM APH | 30 uM APH | 15 uM APH | 7.5 uM APH | 3.75 uM APH |
| F | | | | | | | | | | | |
| G | | | | | | | | | | | |
| H | | | | | | | | | | | |

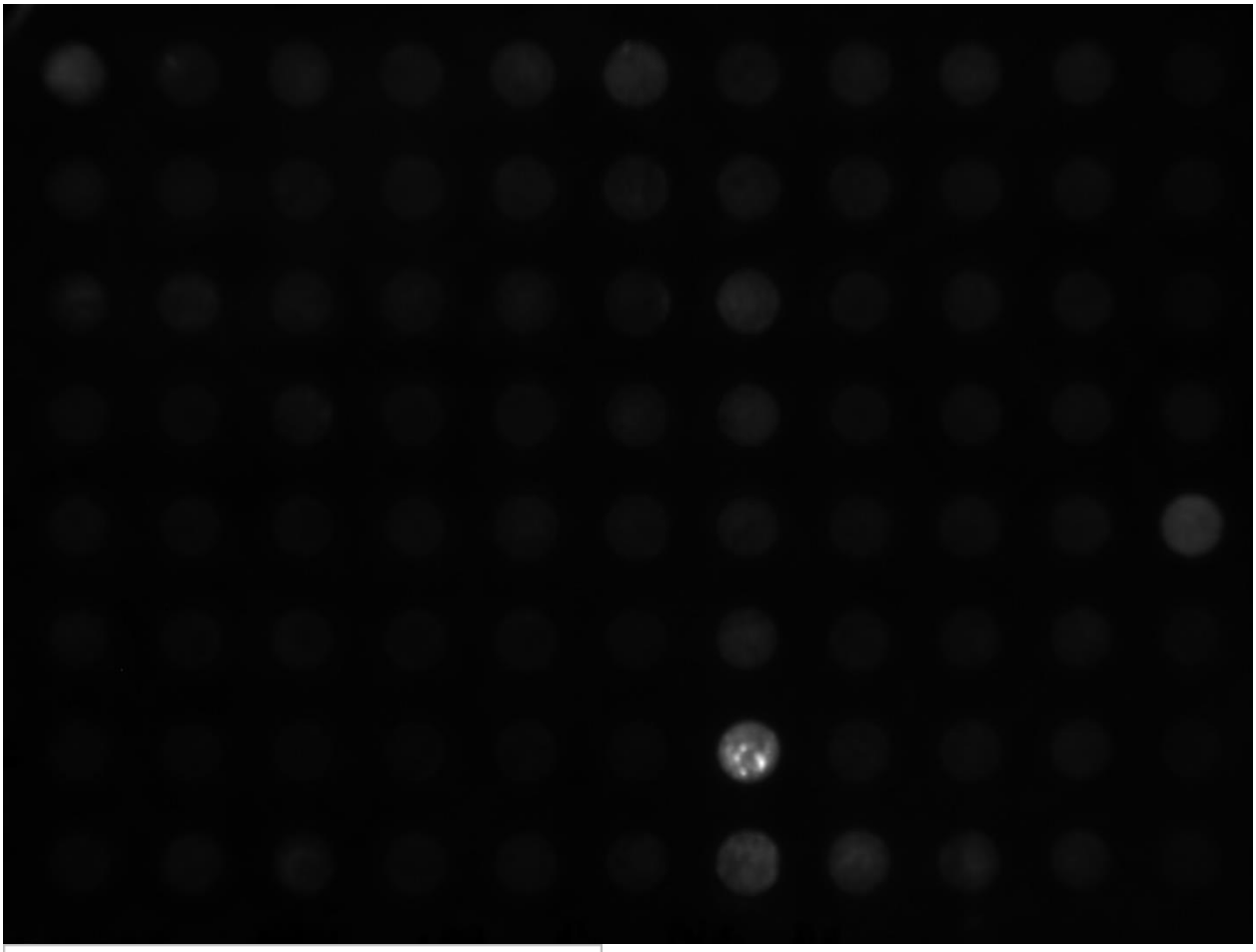
Here's the final treatment plan:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 50 uM Scramble | 25 uM Scramble | 12.5 uM Scramble | 6.25 uM Scramble | 3.125 uM Scramble | DMSO Control | 50 uM BLM4 | 25 uM BLM4 | 12.5 uM BLM4 | 6.25 uM BLM4 | 3.125 uM BLM4 | Media |
| B | | | | | | | | | | | | Media |
| C | | | | | | | | | | | | Media |
| D | | | | | | | | | | | | Media |
| E | 50 uM BLM2 | 25 uM BLM2 | 12.5 uM BLM2 | 6.25 uM BLM2 | 3.125 uM BLM2 | HU 1 mM | 60 uM APH | 30 uM APH | 15 uM APH | 7.5 uM APH | 3.75 uM APH | Media |
| F | | | | | | | | | | | | Media |
| G | | | | | | | | | | | | Media |
| H | | | | | | | | | | | | Media |

I didn't have enough media water for every dilution initially (I had to make more). 11A was the only well that I didn't have enough for. Making more solved the problem. I followed the protocol and didn't have any issues. I finished all of the media changes at 650 pm.

**Alu development of APH Blot**

Update from 11/10/2015. I've mislabeled some of these files. Presat for 12H is not correct; it's actually 7H. 7G reached saturation earlier on and should be discounted.



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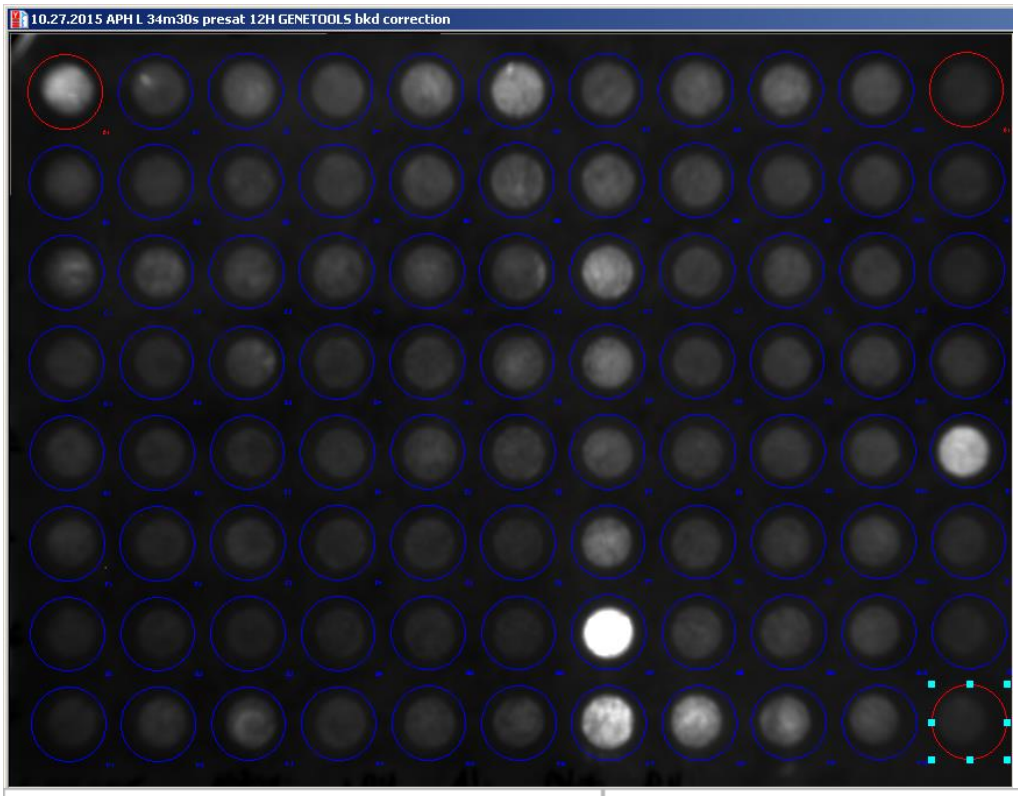
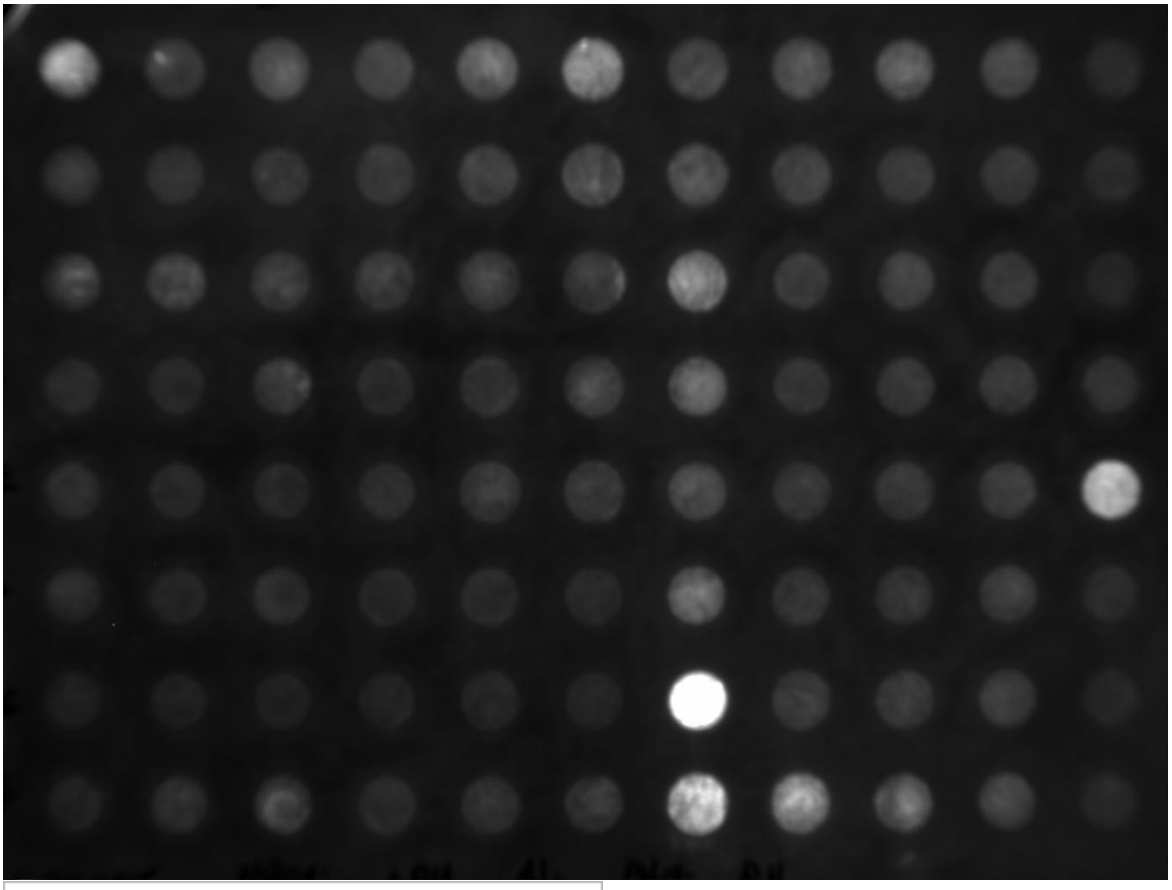
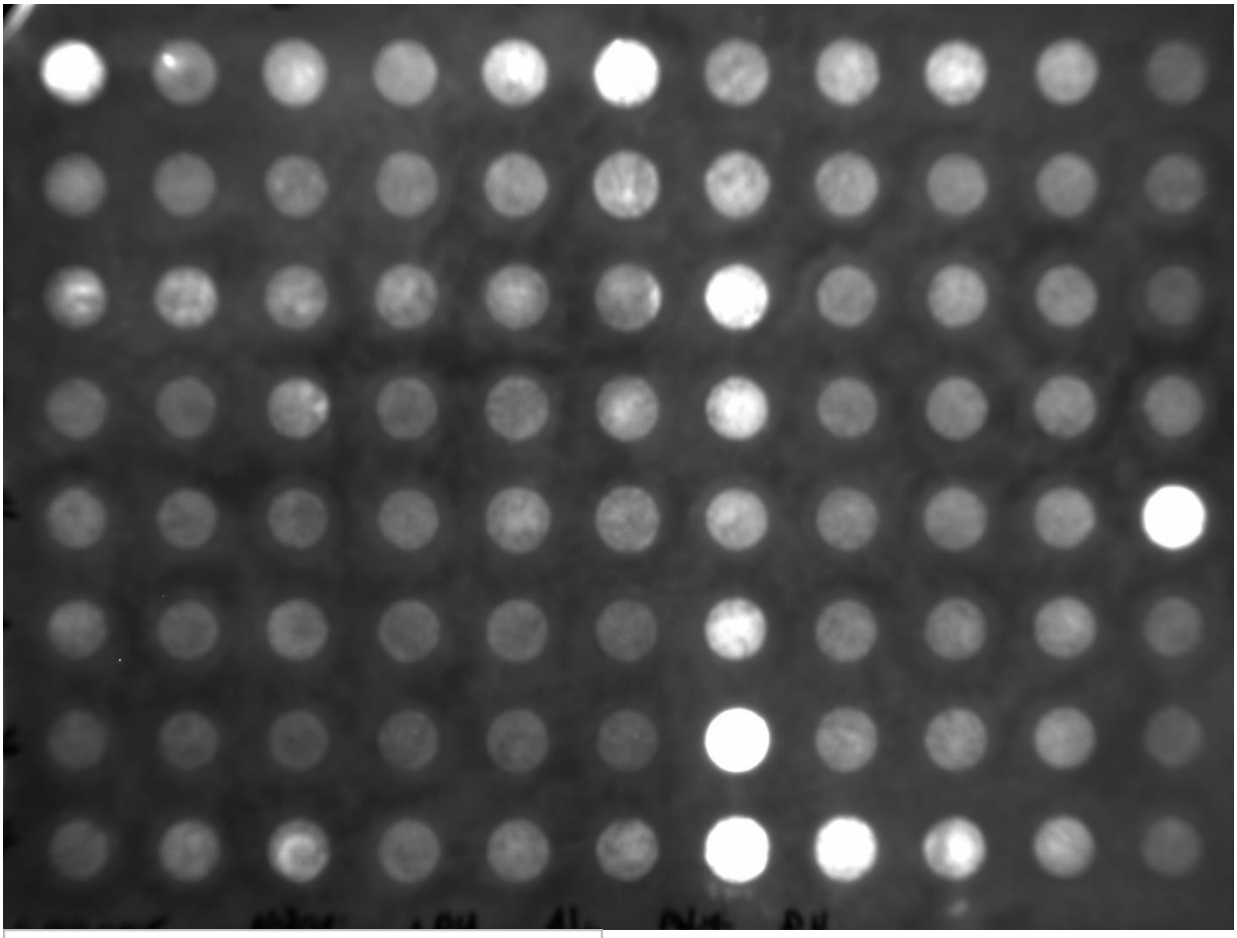
7/22/2018 9:22 PM, 7.8 MB



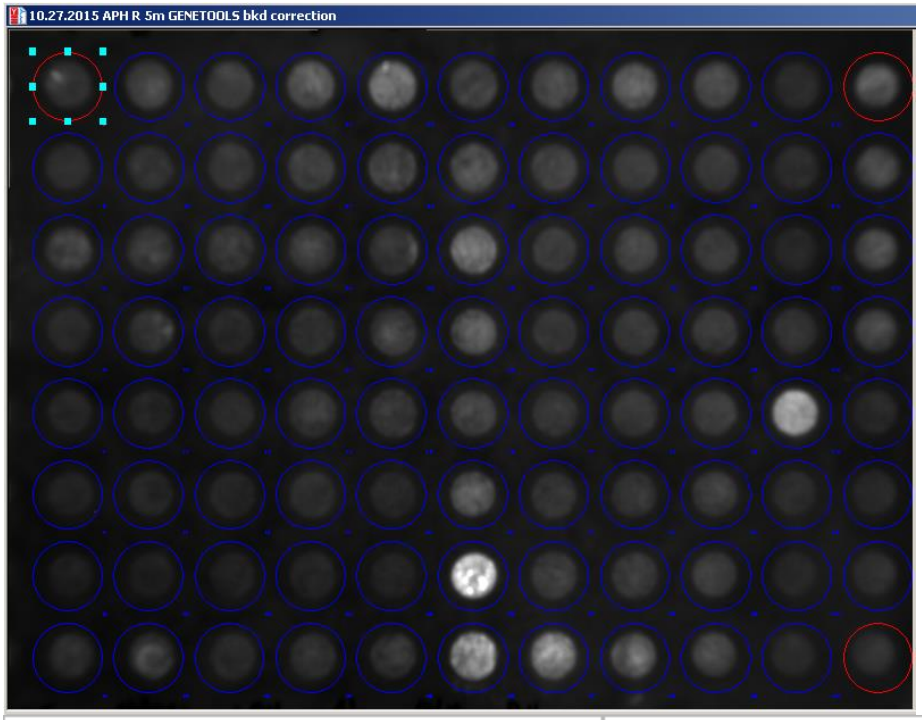

| | |
|---|---|
| 10.27.2015 APH L 34m30s presat 12H GENETO...<br>7/22/2018 9:22 PM, 7.8 MB | 10.27.2015 APH L 34m30s presat 12H GENETO...<br>7/22/2018 9:22 PM, 12.6 KB |
| 10.27.2015 APH L 34m30s presat 12H GENETO...<br>7/22/2018 9:22 PM, 7.8 MB | 10.27.2015 APH L 34m30s presat 12H GENETO...<br>7/22/2018 9:22 PM, 12.0 KB |



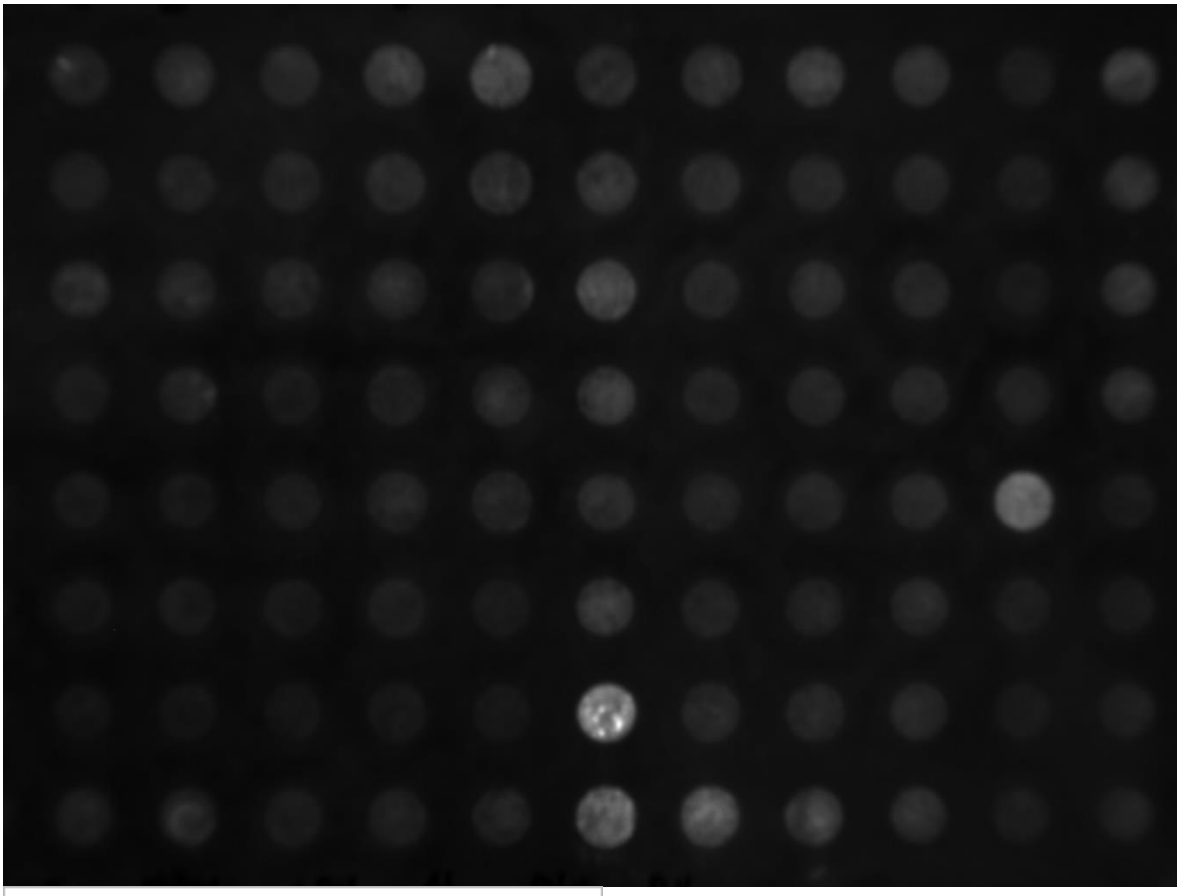
10.27.2015 APH L 34m30s presat 12H.sgd
7/22/2018 9:22 PM, 7.8 MB



10.27.2015 APH L 60m.sgd
7/22/2018 9:22 PM, 7.8 MB




| | |
|---|---|
| 10.27.2015 APH R 5m GENETOOLS bkd correct...<br>7/22/2018 9:22 PM, 7.8 MB | 10.27.2015 APH R 5m GENETOOLS bkd correct...<br>7/22/2018 9:22 PM, 12.8 KB |
| 10.27.2015 APH R 5m GENETOOLS no bkd cor...<br>7/22/2018 9:22 PM, 7.8 MB | 10.27.2015 APH R 5m GENETOOLS no bkd cor...<br>7/22/2018 9:22 PM, 12.0 KB |



10.27.2015 APH R 5m.sgd
7/22/2018 9:22 PM, 7.8 MB

**3/30/2016 To Do List**

- Transfer cryovials to LN2, Check on cells, 5 liquid nitrogen/5 -80
- Clean stuff:
    - Create boxes of plates, 4C plate garbages?, Consolidate boxes, Clean up castle section
- Analyze 3/25/16 PG of JS 1/10 th dilution
    - Plan Phi29 expansion
- HL lyse U2OS, 143B yesterday seeded
    - 384WP (192 wells) 12 columns of each +/- PG Phi29. Do 0.5U of Phi29
    - 96WP smaller version. Doesn't need to be alternating

**Alu re-probe BLM ½ Plate**

3 2X SSC washes, new box, 3 2X SSC washes, new box, 30 m perfect hyb, 10.35 uL Alu

**Cell Culture**

143B 15%
293T 30%
U2OS 35%

**3.30.2016 PicoGreen Read Henson Lysis-Treated U2OS & 143B Cell Serial Dilution**

See yesterday's note for the mismatched signal for U2OS cell seeding serial dilution. I'll be re-doing that today. This was the planned layout. I didn't realize at the time it wouldn't be possible because of the distance between tips, so I actually arrayed it as seen below this layout.

| | Cell Type | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | U2OS | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | 143B gDNA SD in HL 2/2/2016 A->H; Top->Bot | 143B gDNA SD in HL 2/2/2016 A->H; Top->Bot | U2OS gDNA SD in HL 2/2/2016 A->H; Top->Bot | U2OS gDNA SD in HL 2/2/2016 A->H; Top->Bot |
| B | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | | | | |
| C | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | | | | |
| D | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | | | | |
| E | 143B | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | | | | |
| F | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | | | | |
| G | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | | | | |
| H | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | | | | |

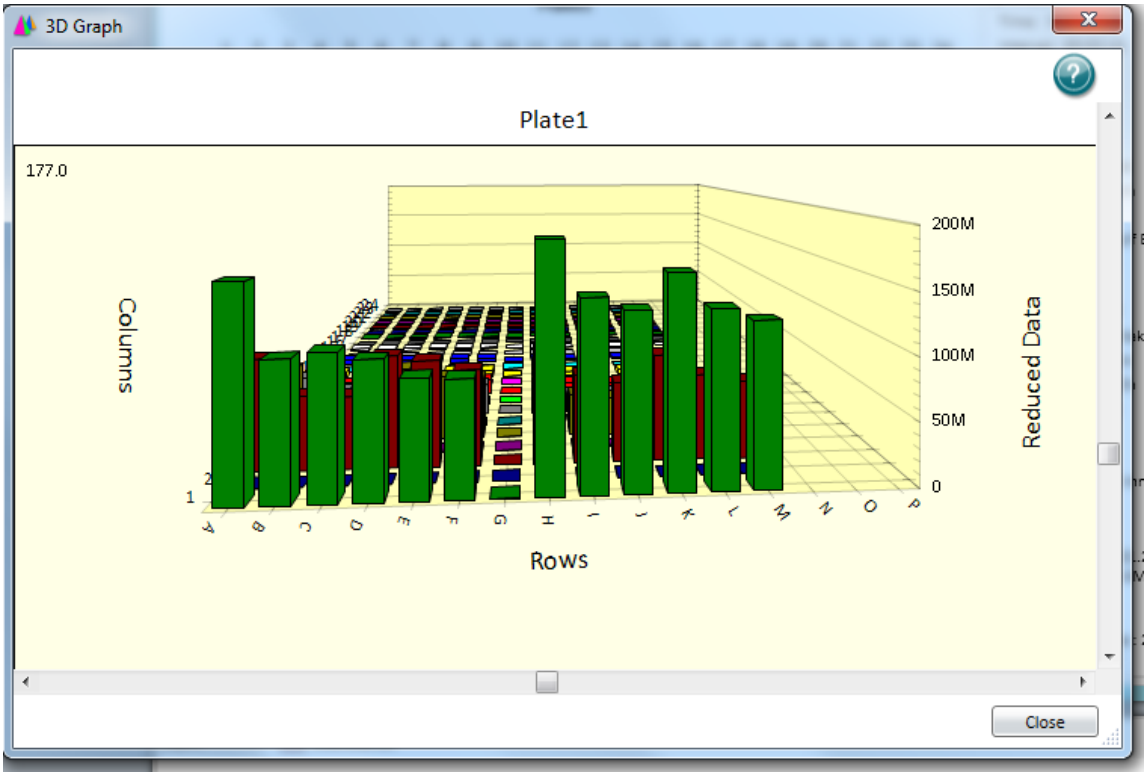
| | Cell Type | 1 | 3 | 5 | 7 | 9 | 11 | 13 | 15 | 17 | 19 | 21 | 23 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | U2OS | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 |
| B | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 |
| C | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 |
| D | 143B | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 |
| E | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 |
| F | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 |
| G | Blanks | HI HL | HI HL | HI HL | HI HL | HI HL | HI HL | HI HL | HI HL | HI HL | HI HL | HI HL | HI HL |
| H | 143B Scur | 80 | 40 | 20 | 10 | 5 | 2.5 | 1.25 | 0 | | | | |
| I | 143B Scur | 80 | 40 | 20 | 10 | 5 | 2.5 | 1.25 | 0 | | | | |
| J | U2OS Scu | 80 | 40 | 20 | 10 | 5 | 2.5 | 1.25 | 0 | | | | |
| K | U2OS Scu | 80 | 40 | 20 | 10 | 5 | 2.5 | 1.25 | 0 | | | | |

| | |
|---|---|
| # wells | 116 |
| plus 1.2% | 139.2 |
| uL PG | 86.304 |
| uL 1X TE | 13137.7 |
| uL 20X TE | 656.8848 |
| uL H2O | 12480.81 |

1. Thaw deep 96WP HL samples (Row 1 U2OS; Row H 143B)
2. Thaw HI HL, PG, U2OS & 143B gDNA HLSD & acquire TE and 384WP
3. Prepare PicoGreen solution
4. Thoroughly mix parental
5. Transfer samples into 384WP @5 uL each
6. Add 95 uL of PG solution to all wells
7. Spin at 1,000 rpm >10 seconds
8. Incubate RT 5 minutes
9. PG read plate

Here's that data. I'm experiencing the same issue of replicates having different signal intensities. For every case, the first sample added has a higher signal than the others. I'll add more details after HS' talk.


I was very thorough with the mixing this time out of concern for volume reproducibility. I mixed each sample 6 times w/ a volume of 400 uL before transferring. I actually made enough PG solution for 116 wells (the protocol only calls for 92). The read was at 1024 23C H1 used for read height of 3.75 mm. I had a problem with reproducibility again. Every 1st replicate of a sample had higher signal than all others. See the figure below for an example of the highest concentration samples (column 1). It's likely to be excess liquid on the tip of the pipette OR that a different volume is being added for that 1st volume. I'll repeat this protocol and eject all liquid volumes (pre-sample addition) while the tip is submerged. Also, I'll set the pipette to dispense n+1 volumes of sample and get rid of that before transferring sample.




## 3.30.2016 PicoGreen ***RETRY READ*** Henson Lysis-Treated U2OS & 143B Cell Serial Dilution

I did this earlier today w/ issues I'll describe later. I'm re-doing it w/ the main change of withdrawing 4X and dumping that (and the initial dump) w/ the tip still submerged.

| | Cell Type | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | U2OS | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | 143B gDNA SD in HL 2/2/2016 A-> H; Top->Bot | 143B gDNA SD in HL 2/2/2016 A-> H; Top->Bot | U2OS gDNA SD in HL 2/2/2016 A-> H; Top->Bot | U2OS gDNA SD in HL 2/2/2016 A-> H; Top->Bot |
| B | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | | | | |
| C | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | | | | |
| D | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | | | | |
| E | 143B | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | | | | |
| F | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | | | | |
| G | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | | | | |
| H | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 | HI HL | | | | |

| | Cell Type | 1 | 3 | 5 | 7 | 9 | 11 | 13 | 15 | 17 | 19 | 21 | 23 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | U2OS | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 |
| B | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 |
| C | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 |
| D | 143B | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 |
| E | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 |
| F | | 160000 | 80000 | 40000 | 20000 | 10000 | 5000 | 2500 | 1250 | 625 | 312.5 | 156.25 | 0 |
| G | Blanks | HI HL | HI HL | HI HL | HI HL | HI HL | HI HL | HI HL | HI HL | HI HL | HI HL | HI HL | HI HL |
| F | 143B Scur | 80 | 40 | 20 | 10 | 5 | 2.5 | 1.25 | 0 | | | | |
| G | 143B Scur | 80 | 40 | 20 | 10 | 5 | 2.5 | 1.25 | 0 | | | | |
| H | 143B Scur | 80 | 40 | 20 | 10 | 5 | 2.5 | 1.25 | 0 | | | | |
| I | U2OS Scu | 80 | 40 | 20 | 10 | 5 | 2.5 | 1.25 | 0 | | | | |
| J | U2OS Scu | 80 | 40 | 20 | 10 | 5 | 2.5 | 1.25 | 0 | | | | |
| K | U2OS Scu | 80 | 40 | 20 | 10 | 5 | 2.5 | 1.25 | 0 | | | | |

| 100 uL fin vol | |
|---|---|
| # wells | 132 |
| plus 1.2% | 158.4 |
| uL PG | 98.208 |
| uL 1X TE | 14949.79 |
| uL 20X TE | 747.4896 |
| uL H2O | 14202.3 |

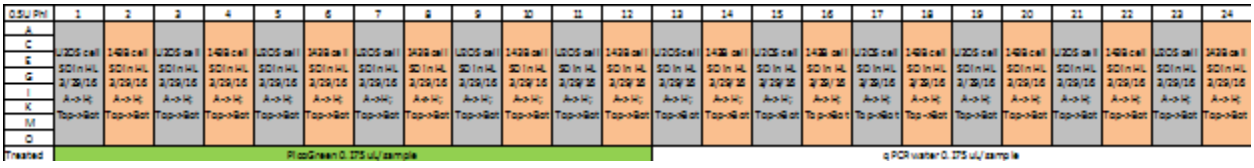
1. Thaw deep 96WP HL samples (Row 1 U2OS; Row H 143B)
2. Thaw HI HL, PG, U2OS & 143B gDNA HLSD & acquire TE and 384WP
3. Prepare PicoGreen solution
4. Thoroughly mix parental
5. Transfer samples into 384WP @5 uL each
6. Add 95 uL of PG solution to all wells
7. Spin at 1,000 rpm >10 seconds
8. Incubate RT 5 minutes
9. PG read plate


^That excel is the data and the analysis. The read was at 236pm and 23.5C. H1 was used for read height of 4.39 mm. Note that I had the CSDHL sample plate inverted when I was transferring samples, so the sample ordering is backwards (both vertically & horizontally). I adjusted for that in the Excel. See the final gDNA ng/8 uL estimates in the next section (planning for the massive Phi29 expansion).

### 3.30.2016 Phi29 +/-PG New U2OS & 143B CSDHL (qPCR & thermomixer)

143B vs. U2OS blanks had been slightly different previously. I prepared them both in the same way & today'll be Phi29 +/-PG for HS' Exo assay and my dot blot method. Figures are gDNA reads (ng/8 uL), 384WP layout, qPCR layout.

| ng/8uL | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 96 layout | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| 384 layou | 1 | 3 | 5 | 7 | 9 | 11 | 13 | 15 | 17 | 19 | 21 | 23 |
| 143B Ave | 0.0488033 | 0.23761 | 0.386217 | 1.133753 | 1.92138 | 4.104415 | 8.075665 | 13.87277 | 29.33644 | 52.46711 | 77.41342 | 90.22101 |
| 143B Sde | 0.1021425 | 0.134819 | 0.071233 | 0.577111 | 0.16851 | 0.470379 | 1.96537 | 0.369962 | 3.607548 | 2.432111 | 2.125583 | 4.233495 |
| U2OS Av | -0.005759 | 0.153211 | 0.391162 | 0.965067 | 1.788498 | 3.663594 | 7.324711 | 13.05321 | 24.90179 | 39.7754 | 56.57459 | 100.562 |
| U2OS Sd | 0.004725 | 0.009523 | 0.024304 | 0.108285 | 0.061903 | 0.356869 | 1.131036 | 0.483812 | 2.325293 | 4.52552 | 6.45967 | 9.205371 |



| 0.5U Phi | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | U2OS cell SD in HL 3/29/16 A-> H; Top->Bot | U2OS cell SD in HL 3/29/16 A-> H; Top->Bot | U2OS cell SD in HL 3/29/16 A-> H; Top->Bot | 143B cell SD in HL 3/29/16 A-> H; Top->Bot | 143B cell SD in HL 3/29/16 A-> H; Top->Bot | 143B cell SD in HL 3/29/16 A-> H; Top->Bot | U2OS cell SD in HL 3/29/16 A-> H; Top->Bot | U2OS cell SD in HL 3/29/16 A-> H; Top->Bot | U2OS cell SD in HL 3/29/16 A-> H; Top->Bot | 143B cell SD in HL 3/29/16 A-> H; Top->Bot | 143B cell SD in HL 3/29/16 A-> H; Top->Bot | 143B cell SD in HL 3/29/16 A-> H; Top->Bot |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |
| Treated | PicoGreen 0.175 uL/sample | | | | | | qPCR water 0.175 uL/sample | | | | | |

| +2uL H2O & -Tween | | | | |
|---|---|---|---|---|
| # Rxns | 288 | | | |
| #rxns +80% | 518.4 | | | |
| Volume/rxn | 10 | | | |
| Total Soup w/o +2/rxn | 5184 | | | |
| Total Rxn Volume | 10368 | | | |
| Soup +2uL/rxn | 6220.8 | | | |
| Pre-phi29 volume | 6194.88 | | | |
| | Conc. | #/ rxn | uL/1rxn | Tot uL |
| Phi29 Polymerase | 10 | 0.5 | 0.05 | 25.92 |
| dNTP (mM) | 10 | 1 | 1 | 518.4 |
| BSA (mg/mL) | 20 | 0.2 | 0.1 | 51.84 |
| Tween (%) | 1 | 0 | 0 | 0 |
| phi29 buffer (X) | 10 | 1 | 1 | 518.4 |
| DTT (mM) | 100 | 4 | 0.4 | 207.36 |
| ~~PicoGreen (X)~~ | ~~200~~ | | ~~0.175~~ | ~~90.72~~ |
| Rnase Free Water (uL) | | | 7.45 | 4808.16 |
| Total Volume | | | | 6220.8 |


## 3.30.2016 Phi29 +/-PG New U2OS & 143B CSDHL (qPCR & thermomixer)

1. Thaw Phi29 buffer, DTT, dNTP, BSA, and BLM siRNA parental plate
2. Acquire & label a qPCR plate (for expansion) & 384WP (for expansion)
3. Prepare Phi29 expansion solution in 15 mL conical tube
    1. Split 2554.2 uL into two 15 mL conicals
    2. Add 37.8 uL PG to + and 27.8 uL qPCR water to –
    3. Transfer 270 uL of each type into 8 wells of a strip tube
4. Transfer gDNA into destination plates
    1. 143B 3-9 & 12; U2OS 2-8 & 12
5. 8 channel, repeater mode transfer **12 uL of PG Phi29** into plate
6. Add qPCR film covers

7. Spin plates at 1,000 rpm for 10 seconds
8. Run qPCR Phi29 expansion **WITH PG reads enabled** & thermomixer run

The qPCR program crashed while I was trying to download the previous set of data. I had to restart both the program and the qPCR machine to get it working. I didn't have any issues with using the thermomixer.