LAB NOTEBOOK ENTRY EXAMPLE

Wednesday, March 2, 2016
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20160302_chandy_SNVMutationDetection_TP53ddPCRmultiplex

Temperature Gradient of Multiplexed TP53 ddPCR Primers

Purpose:

Christina Wood tested tailed primers for TP53 and saw that when tails were added to the wild-type primers, the wild-type droplet cluster moved up as was expected, but when tails were added to the mutant primers, the droplet clusters moved down. She has asked me to run this temperature gradient test to see if the change in temperature moves the WT tailed population at all.

Experimental Notes:

- Resuspend primers in 10 x the nmol value on the tube of ddH_2O
- Combine forward primer with each of the reverse primers to 10µM
  - 80 µL water
  - 10 µL F primer
  - 10 µL R primer
- LS123 DNA was extracted and digested from the cell line by Christina on 2/11/16
  - 146 c/µL quantified using RPP30 medium primer set
    - Make 1:2 dilution in water
- 18507 was digested by Christina on 1/19/16
  - 712 c/µL quantified using RPP30 medium primer set
    - Make 1:10 dilution in water

Primers

24-Feb-2016	TP53_R175H_F144_MA
04-Jan-2016	TP53_R175H_R_W
04-Jan-2016	TP53_R175H_R_M
19-Jan-2016	TP53_R175H_W100T
19-Jan-2016	TP53_R175H_M100T

ddPCR

Conditions of ddPCR
1. 18507 WT DNA
2. LS123 Mutant DNA
3. 18507 & LS123
4. Water (NTC)

Reaction setup

11 µL	**ddPCR Supermix for EvaGreen**
0.22 µL	**WT primers (F & R)**
0.22 µL	**Mutant primers (F & R)**
9.56 µL	**Water**
[1 µL	**70 copies DNA]**

	67°C		68°C		69°C		70°C		71°C		72°C	
	1	2	3	4	5	6	7	8	9	10	11	12
A		**18507** *WT* 100bpTail		**18507** *WT* 100bpTail		**18507** *WT* 100bpTail	**18507*** *WT* 100bpTail	**18507** *WT* 100bpTail		**18507** *WT* 100bpTail		**18507** *WT* 100bpTail
B		**LS123** *WT* 100bpTail		**LS123** *WT* 100bpTail		**LS123** *WT* 100bpTail	**LS123*** *WT* 100bpTail	**LS123** *WT* 100bpTail		**LS123** *WT* 100bpTail		**LS123** *WT* 100bpTail
C		**1:1 Mix** *WT* 100bpTail		**1:1 Mix** *WT* 100bpTail		**1:1 Mix** *WT* 100bpTail	**1:1 Mix*** *WT* 100bpTail	**1:1 Mix** *WT* 100bpTail		**1:1 Mix** *WT* 100bpTail		**1:1 Mix** *WT* 100bpTail
D		**NTC** *WT* 100bpTail		**NTC** *WT* 100bpTail		**NTC** *WT* 100bpTail	**NTC*** *WT* 100bpTail	**NTC** *WT* 100bpTail		**NTC** *WT* 100bpTail		**NTC** *WT* 100bpTail
E		**18507** *Mutant* 100bpTail		**18507** *Mutant* 100bpTail		**18507** *Mutant* 100bpTail	**18507*** *Mutant* 100bpTail	**18507** *Mutant* 100bpTail		**18507** *Mutant* 100bpTail		**18507** *Mutant* 100bpTail
F		**LS123** *Mutant* 100bpTail		**LS123** *Mutant* 100bpTail		**LS123** *Mutant* 100bpTail	**LS123*** *Mutant* 100bpTail	**LS123** *Mutant* 100bpTail		**LS123** *Mutant* 100bpTail		**LS123** *Mutant* 100bpTail
G		**1:1 Mix** *Mutant* 100bpTail		**1:1 Mix** *Mutant* 100bpTail		**1:1 Mix** *Mutant* 100bpTail	**1:1 Mix*** *Mutant* 100bpTail	**1:1 Mix** *Mutant* 100bpTail		**1:1 Mix** *Mutant* 100bpTail		**1:1 Mix** *Mutant* 100bpTail
H		**NTC** *Mutant* 100bpTail		**NTC** *Mutant* 100bpTail		**NTC** *Mutant* 100bpTail	**NTC*** *Mutant* 100bpTail	**NTC** *Mutant* 100bpTail		**NTC** *Mutant* 100bpTail		**NTC** *Mutant* 100bpTail

*Droplets pipetted manually

- Make droplets
  - 20 µL of sample into middle row of cartridge
  - 70 µL of probe oil into bottom well
  - Place gasket onto cartridge and align cartridge holder in droplet generator
  - After droplet generation SLOWLY pick up 42.5 µL of droplets from the top row
  - Transfer droplets to twin tech semi-skirted PCR plate, dispense SLOWLY
  - Seal the plate with a foil seal

SM Lot: 64025627
Oil Lot: 64040820
Cartridges: C000058097
Cycler: Princess Peach
Reader: Mario

- Thermocycling conditions

		I-40 cycles-I				
Temp (°C)	95	95	67-72	4	90	4
Time (min)	5:00	0:30	1:00	5:00	5:00	∞
Ramp Rate (%)	100	100	100	100	100	100

- After thermocycling, transfer the plate to the droplet reader

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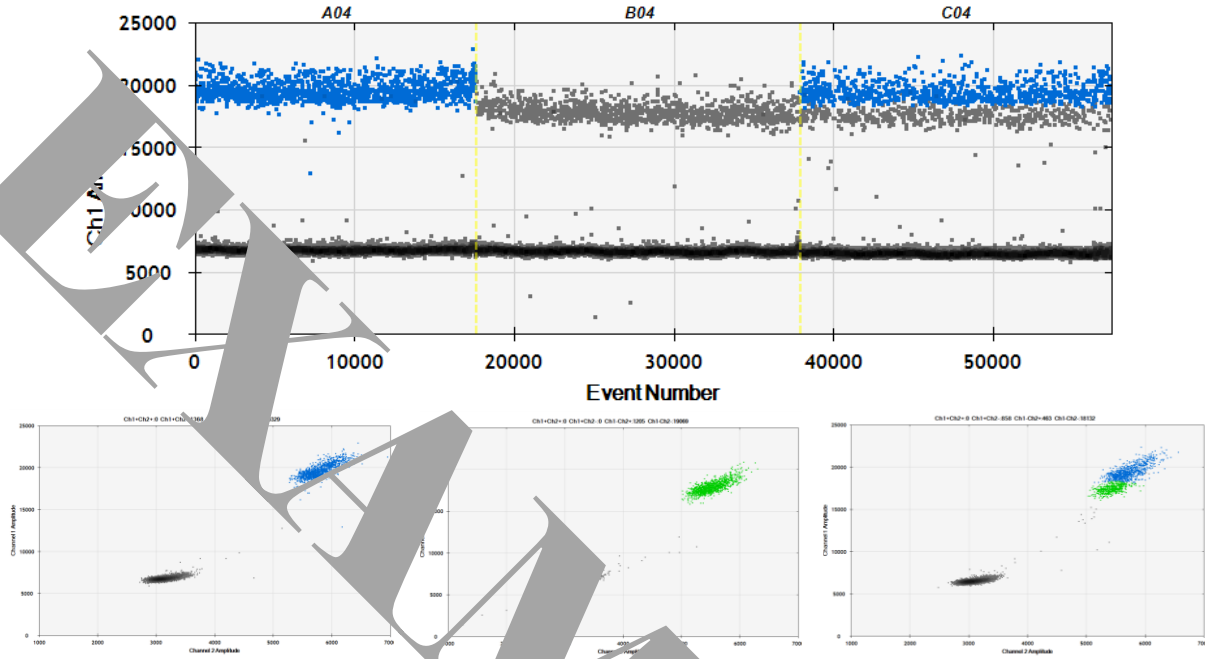
Results:

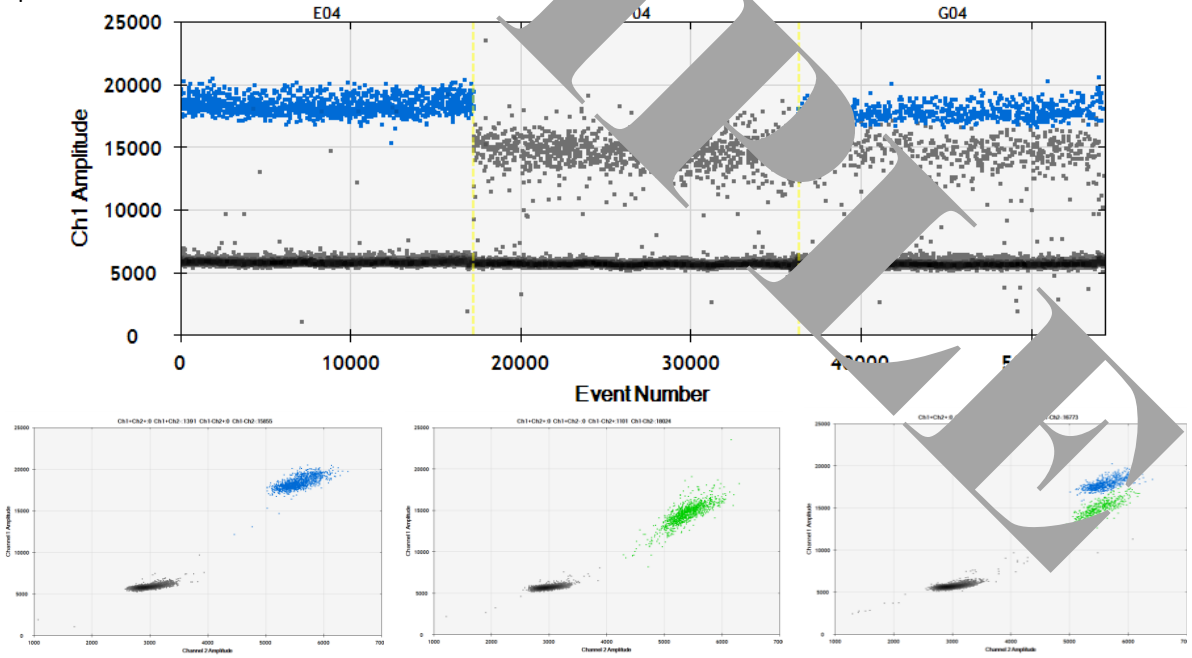
*The Mario droplet reader showed "Error 301" after pulling the droplets from the first well. The error related to the connection between the reader and the computer and resulted in a complete loss of data from well A2.

QuantaSoft File: 20160303_chandy_SNVMutationDetection_TP53Multiplex.qlp

WT 100bp Tail at 68°C



Mutant 100bp Tail at 68°C



Conclusions:

The best separation was seen from the mutant tailed primer at 68°C even though the tailed primer caused the cluster to move down on the y-axis. This result confirms Christina's previous findings. The mutant tailed cluster begins degrading at 69°C and only negative clusters are seen at 72°C. Moving forward, Christina will redesign the primers based on Hanlee's suggestion so that the forward primer is the detecting primer and the reverse primer is common. This will hopefully result in the mutant tail causing the cluster to move up on the y-axis rather than down.