

BioRad

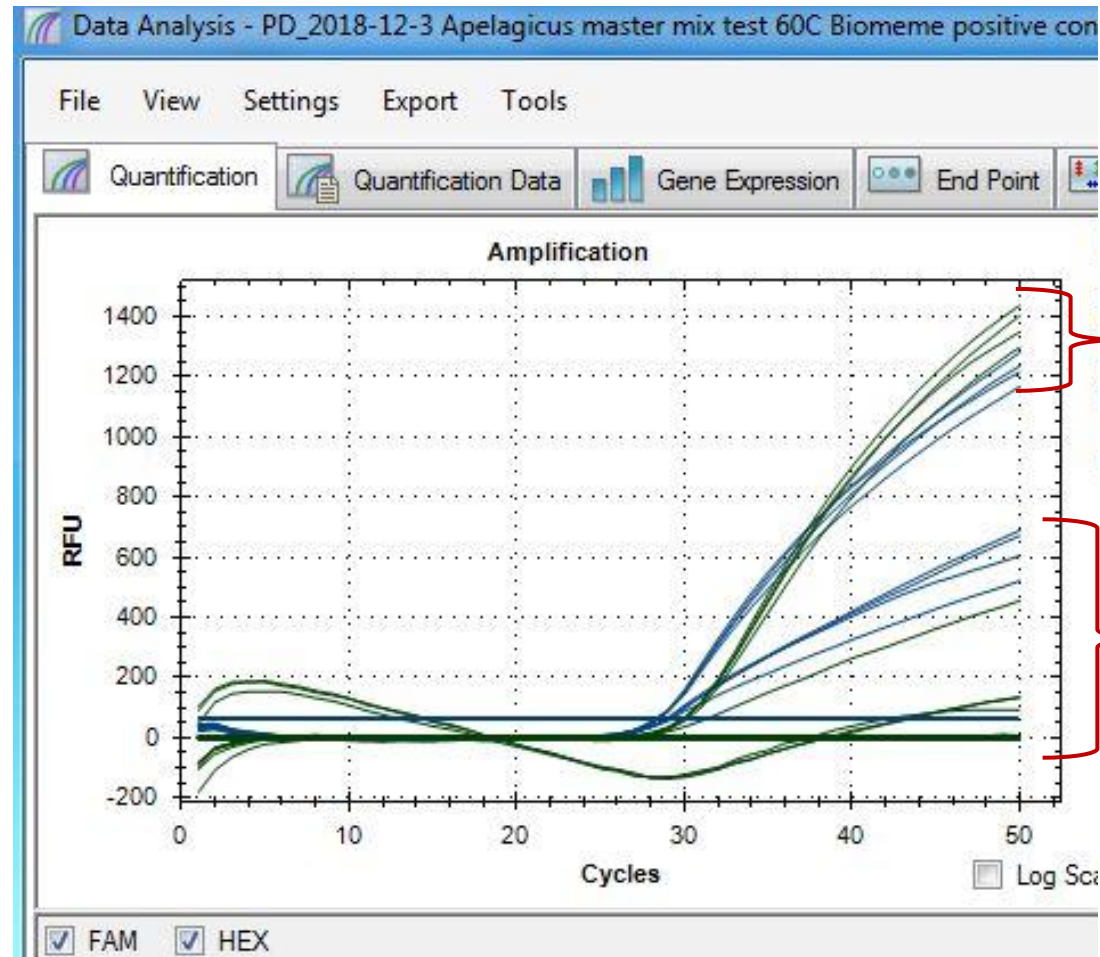
A. pelagicus – Biomeme positive control master mix test

A. pelagicus

Approx. 1000 copies of A. pelagicus positive control (Whatman paper punch provided by Biomeme)

Samples 1-4 are replicates, 5 is the negative control (water)

**Be careful to not use too much Taq polymerase



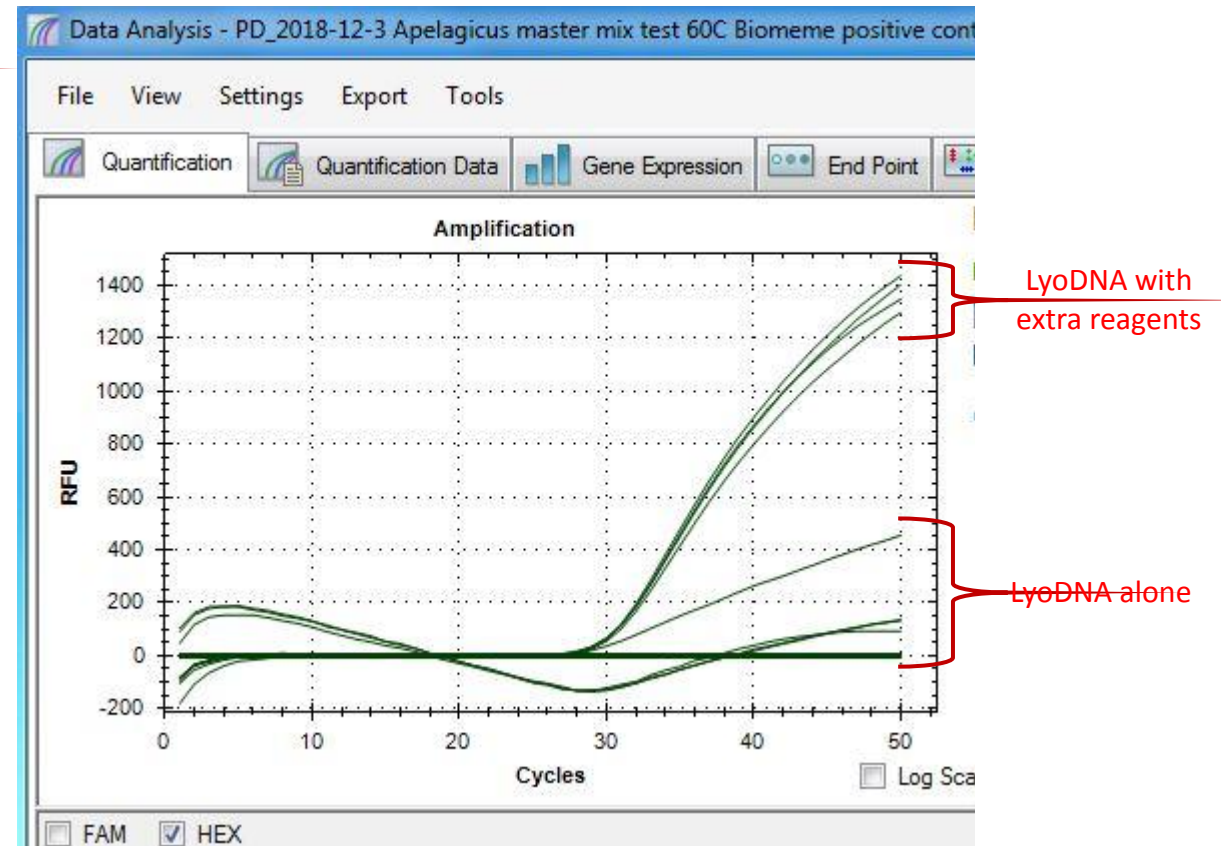
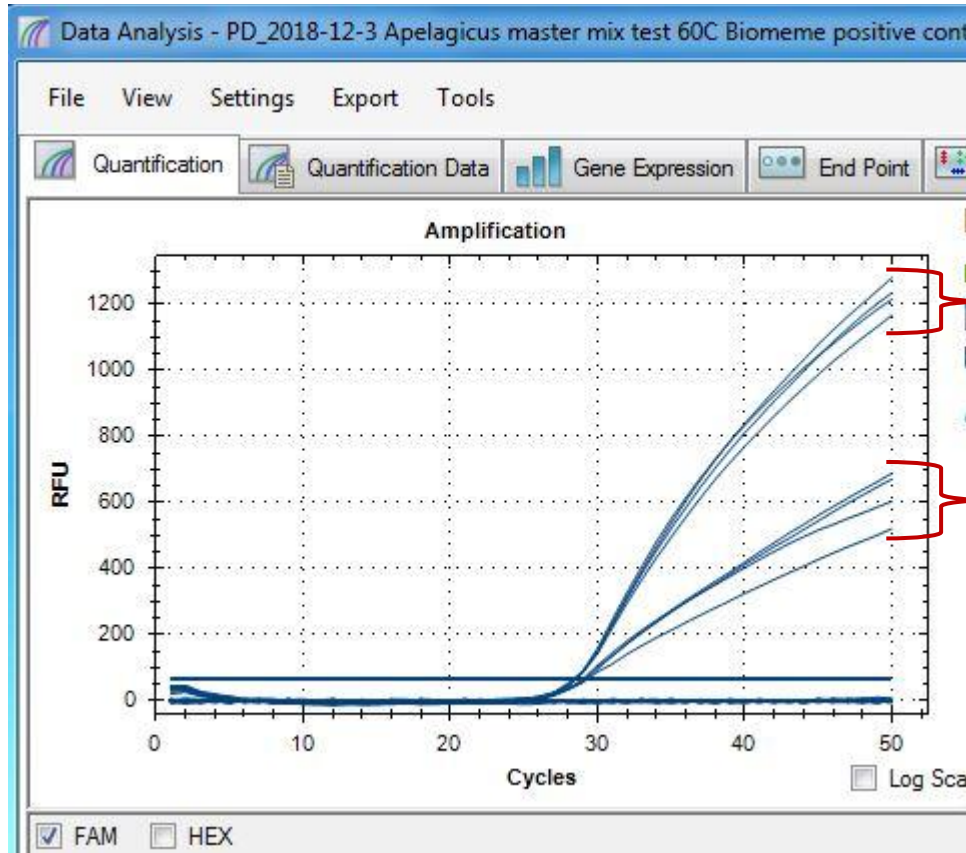
5X LyoDNA alone:

	1	2	3	4	5
FAM	29.22	29.04	28.92	29.16	NA
HEX	37.30	NA	NA	NA	NA

5X LyoDNA with extra reagents (Taq, MgCl₂, dNTPs):

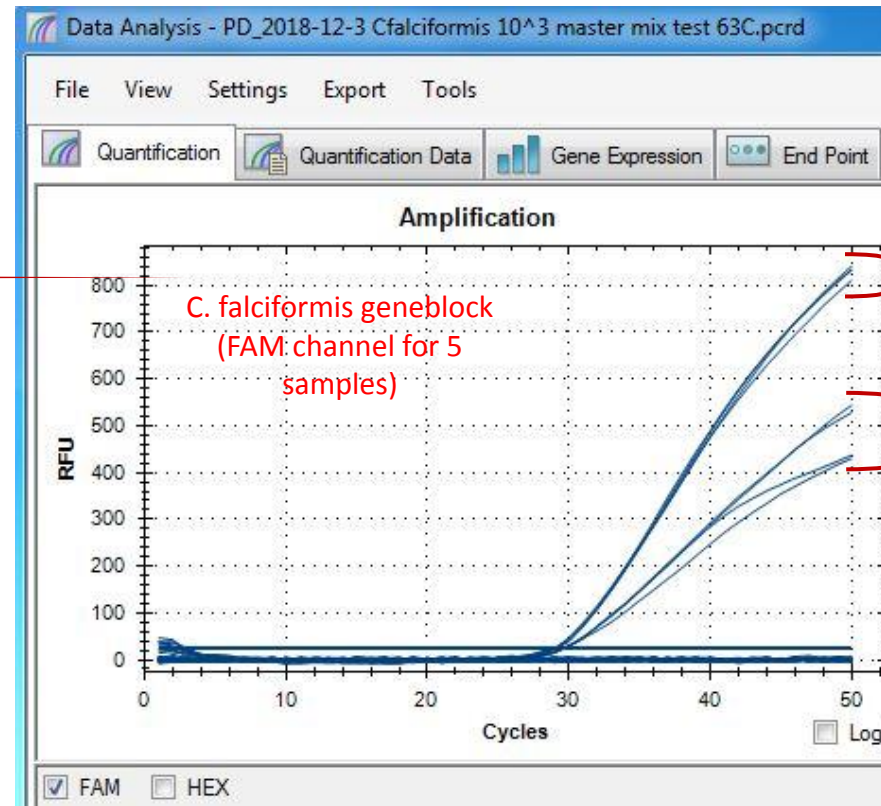
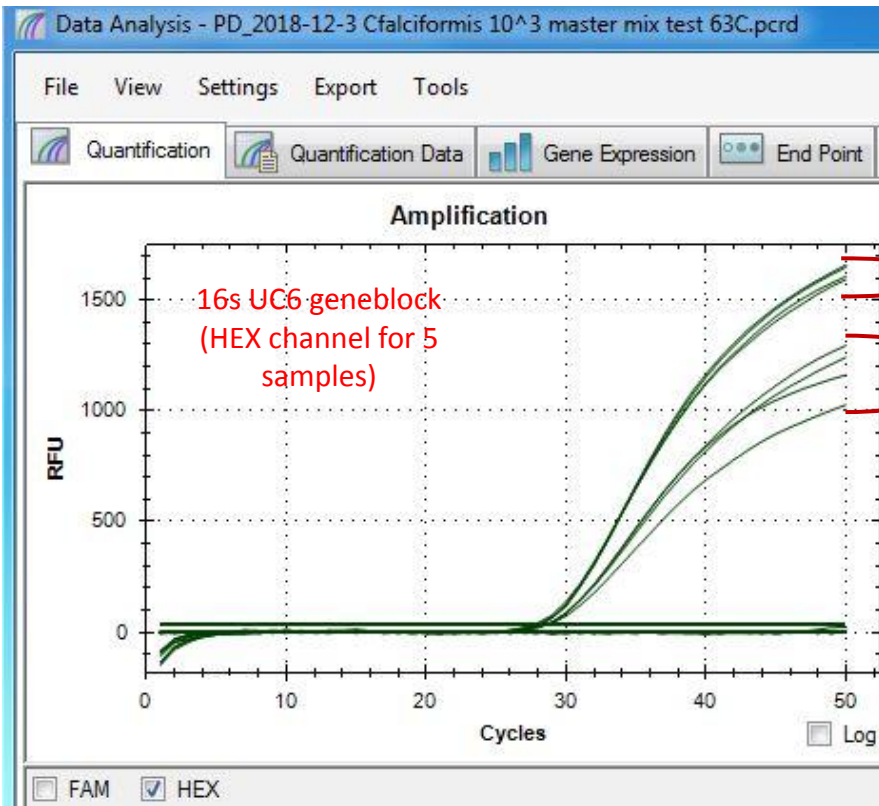
	1	2	3	4	5
A. pelagicus FAM	28.46	28.38	28.42	28.24	NA
16S UC6 HEX	32.43	32.22	32.09	32.21	NA

A. pelagicus (Continued from previous slide)



C. falciformis master mix test (using 6500 copies)

C. falciformis (63°C)



Samples 1-4 are replicates = 1 ul of 6.56×10^3 copies of *C. falciformis* geneblock (6500 copies) + 1 ul of 4.54×10^3 copies of 16S UC6 geneblock (4500 copies)
 Sample 5 is the negative control (water)

5X LyoDNA alone:

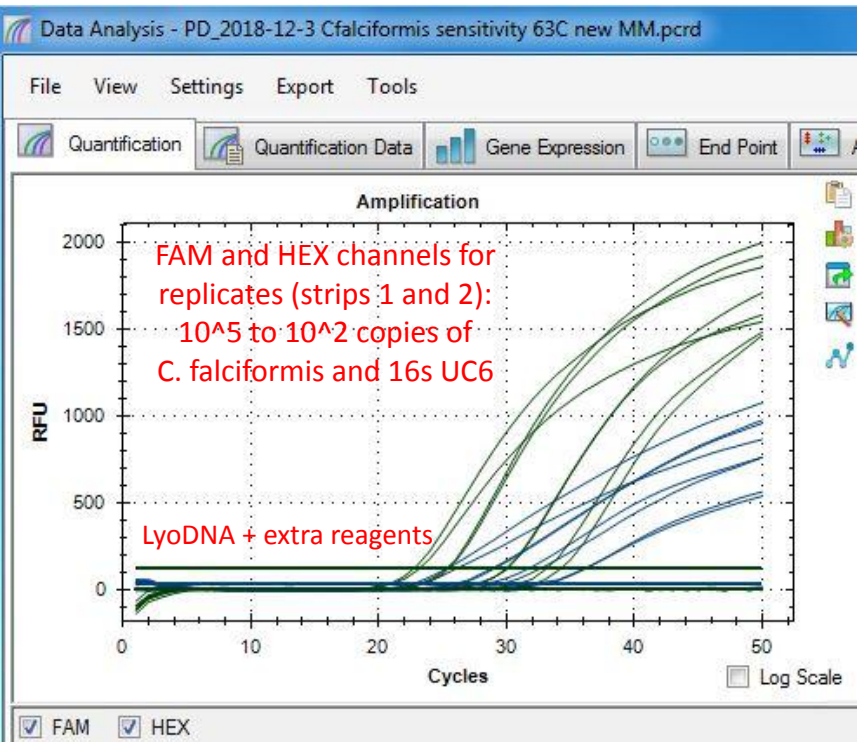
	1	2	3	4	5
FAM	29.57	29.41	29.61	29.59	NA
HEX	28.89	28.73	28.72	28.61	NA

C. falciformis
 16S UC6

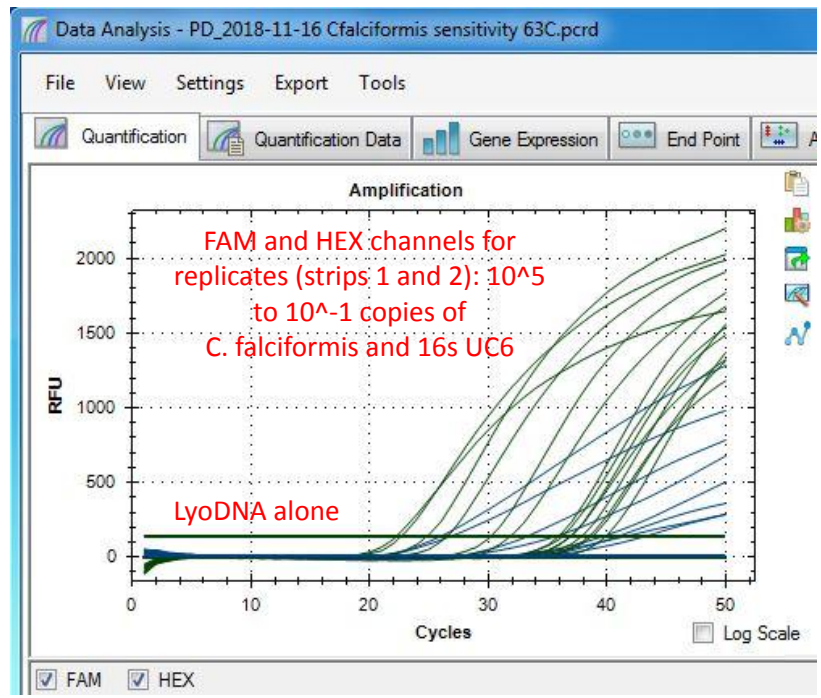
5X LyoDNA with extra reagents (Taq, MgCl₂, dNTPs):

	1	2	3	4	5
FAM	28.93	29.01	28.88	28.96	NA
HEX	28.17	28.20	27.82	28.20	NA

C. falciformis sensitivity assay using new master mix



C. falciformis sensitivity (63°C) – 5x LyoDNA with extra reagents



FOR COMPARISON

Strips 1 and 2 are replicates = 1 ul of *C. falciformis* geneblock (10^5 to 10^2) + 1 ul 16S UC6 geneblock (10^5 to 10^2)
 Sample 5 is the negative control (water)

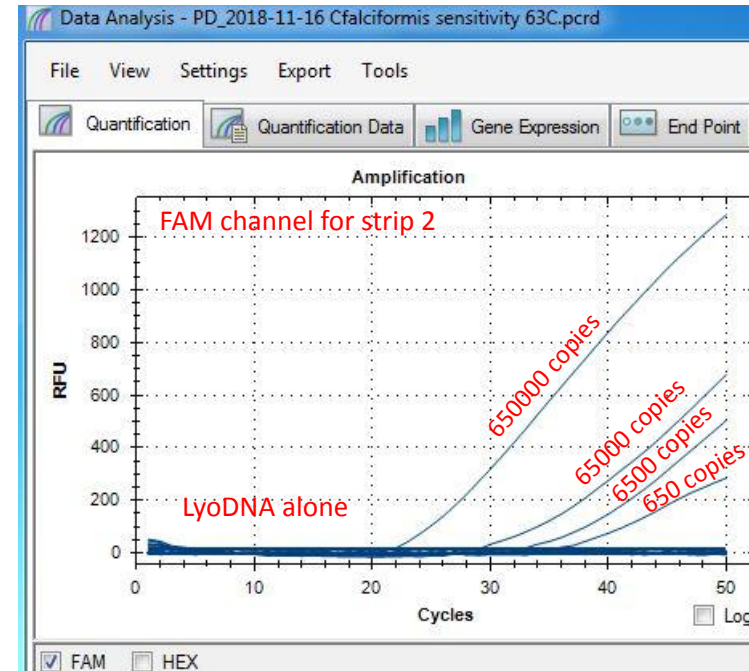
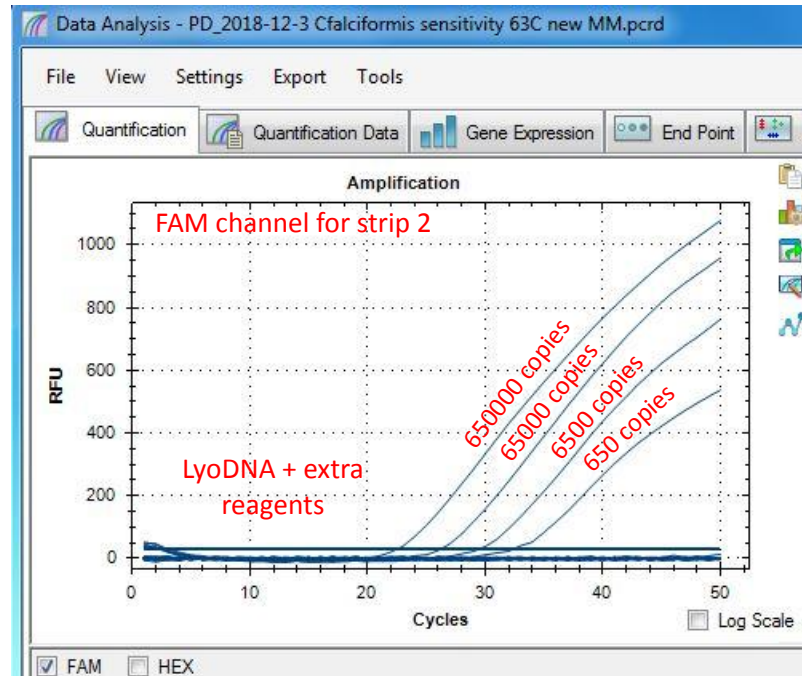
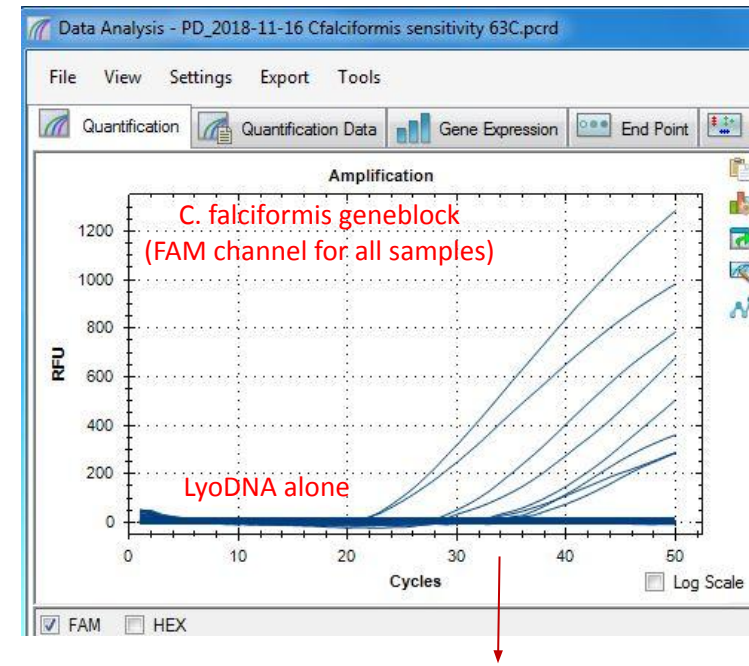
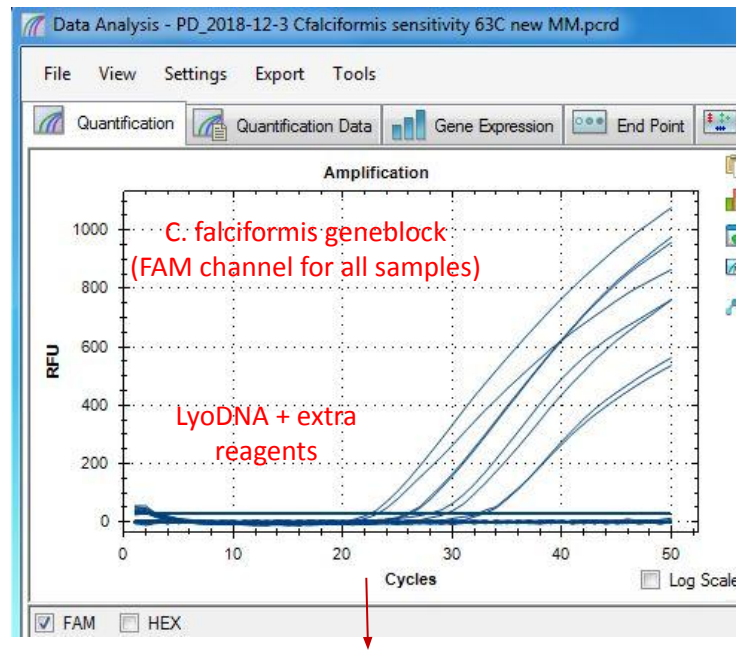
Strip 1 (NEW Master Mix):

Tubes:	1	2	3	4	5
Cfalc copies	650000	65000	6500	650	Neg
16S copies	454000	45400	4540	454	Neg
FAM	23.21	25.89	27.97	32.95	NA
HEX	23.18	25.46	30.10	34.24	NA

Strip 2 (NEW Master Mix):

Tubes:	1	2	3	4	5
Cfalc copies	650000	65000	6500	650	Neg
16S copies	454000	45400	4540	454	Neg
FAM	22.53	26.20	29.48	32.40	NA
HEX	22.75	25.31	29.97	33.12	NA

C. falciformis sensitivity (63°C) – 5x
LyoDNA with extra reagents-
Continued from previous slide - FAM

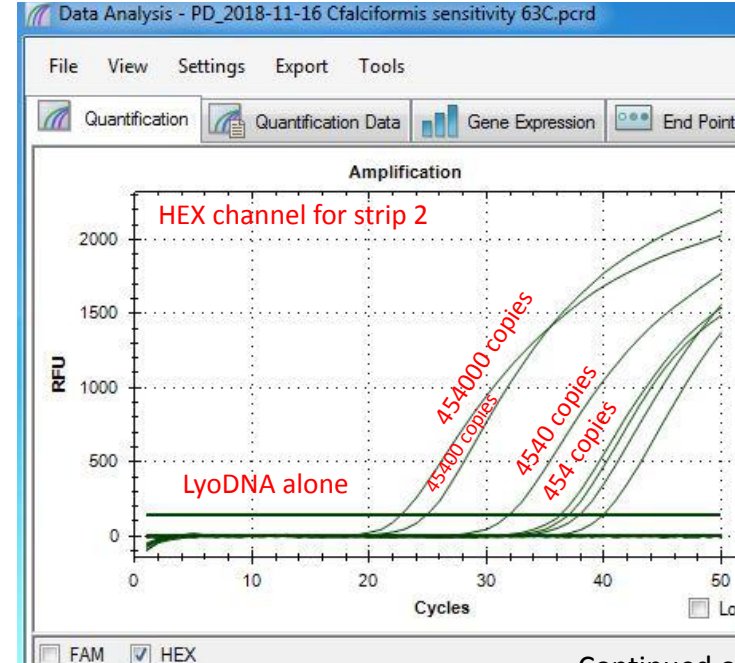
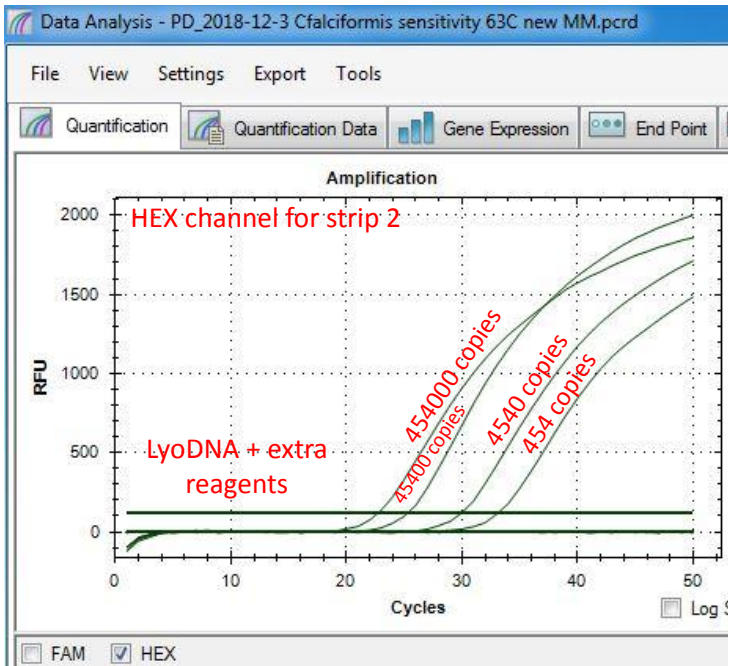
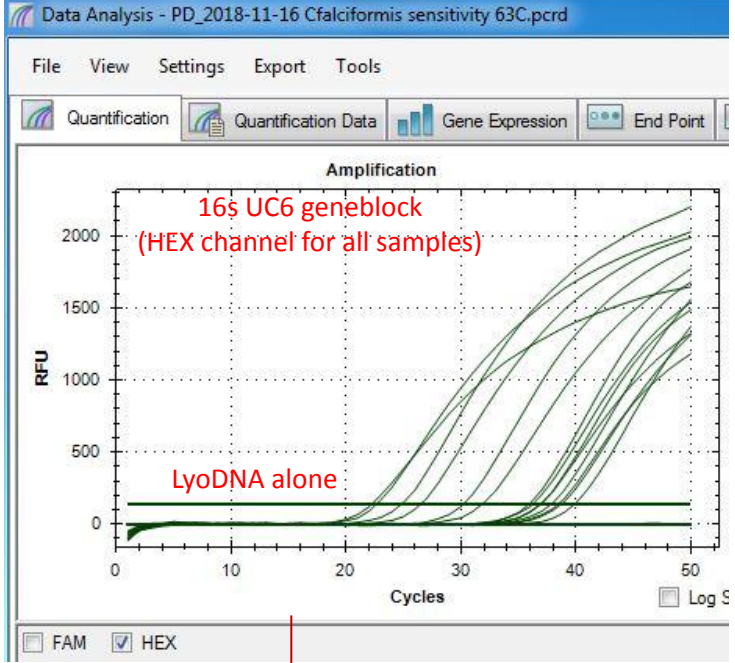
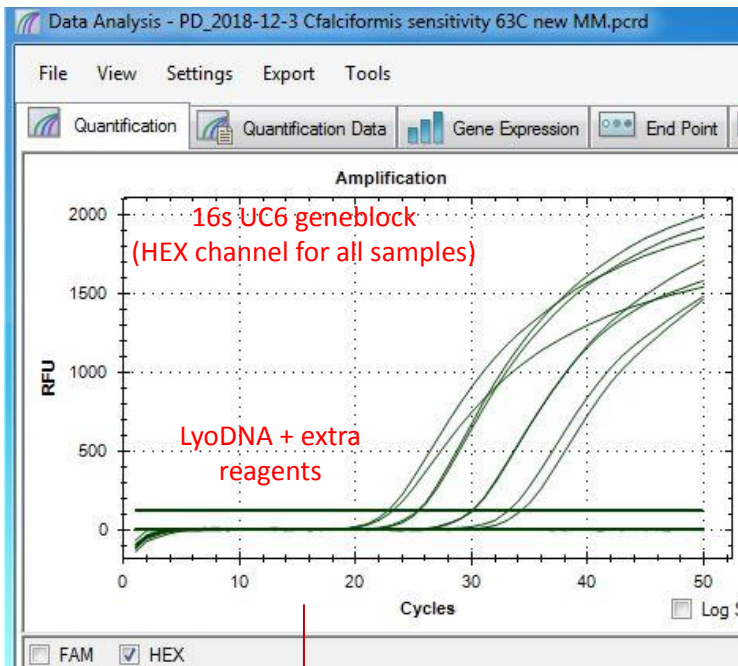


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C. falciformis sensitivity (63°C) – 5x LyoDNA with extra reagents- Continued from previous slides – FAM highlighted

Tube	Copy number	<i>LyoDNA alone</i>		<i>LyoDNA + extra reagents</i>	
		1	2	3	4
1	Cfalc - 650000	23.39	23.11	23.21	22.53
	16S - 454000	22.22	22.61	23.18	22.75
2	Cfalc - 65000	29.78	31.06	25.89	26.20
	16S - 45400	26.45	24.78	25.46	25.31
3	Cfalc - 6500	36.09	35.52	27.97	29.48
	16S - 4540	30.17	31.81	30.10	29.97
4	Cfalc - 650	37.16	38.14	32.95	32.40
	16S - 454	36.89	36.20	34.24	33.12

C. falciformis sensitivity (63°C) – 5x
 LyoDNA with extra reagents – Continued
 from previous slides - HEX



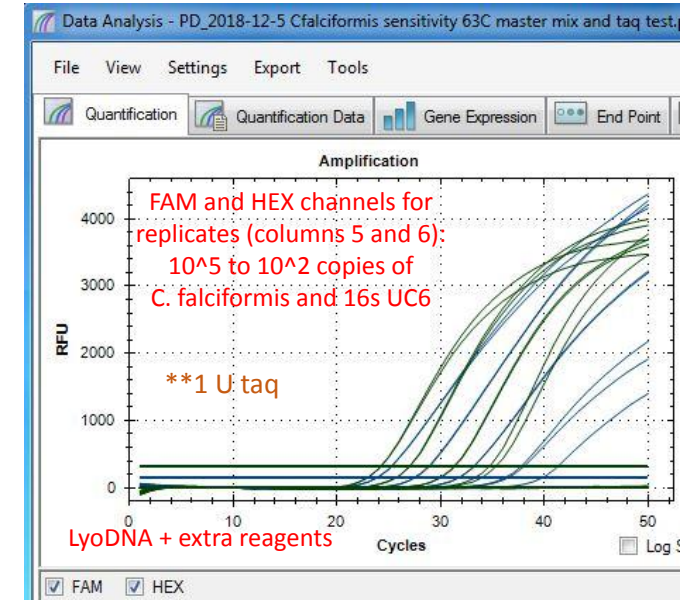
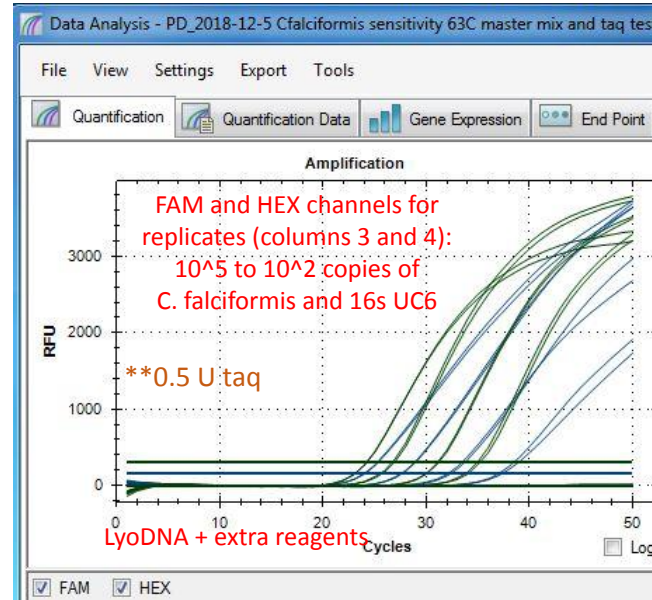
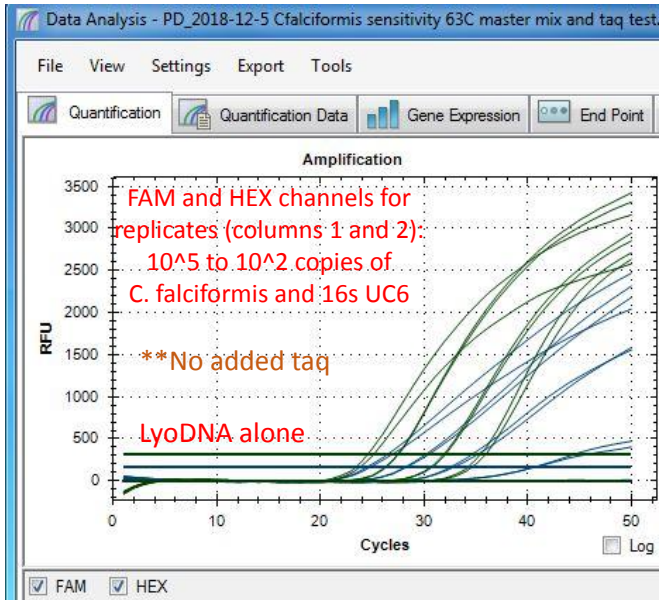
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C. falciformis sensitivity (63°C) – 5x LyoDNA with extra reagents – Continued from previous slides – HEX highlighted

Tube	Copy number	<i>LyoDNA alone</i>		<i>LyoDNA + extra reagents</i>	
		1	2	3	4
1	Cfalc - 650000	23.39	23.11	23.21	22.53
	16S - 454000	22.22	22.61	23.18	22.75
2	Cfalc - 65000	29.78	31.06	25.89	26.20
	16S - 45400	26.45	24.78	25.46	25.31
3	Cfalc - 6500	36.09	35.52	27.97	29.48
	16S - 4540	30.17	31.81	30.10	29.97
4	Cfalc - 650	37.16	38.14	32.95	32.40
	16S - 454	36.89	36.20	34.24	33.12

C. falciformis sensitivity assay 63°C comparing master mix and TAQ

C. falciformis sensitivity (63°C) – 5x LyoDNA/Lyo + Extras (with correct Taq)/Lyo + Extras (with double Taq)



1 ul of C. falciformis geneblock (10⁵ to 10²) + 1 ul 16S UC6 geneblock (10⁵ to 10²)
Sample E is the negative control (water)

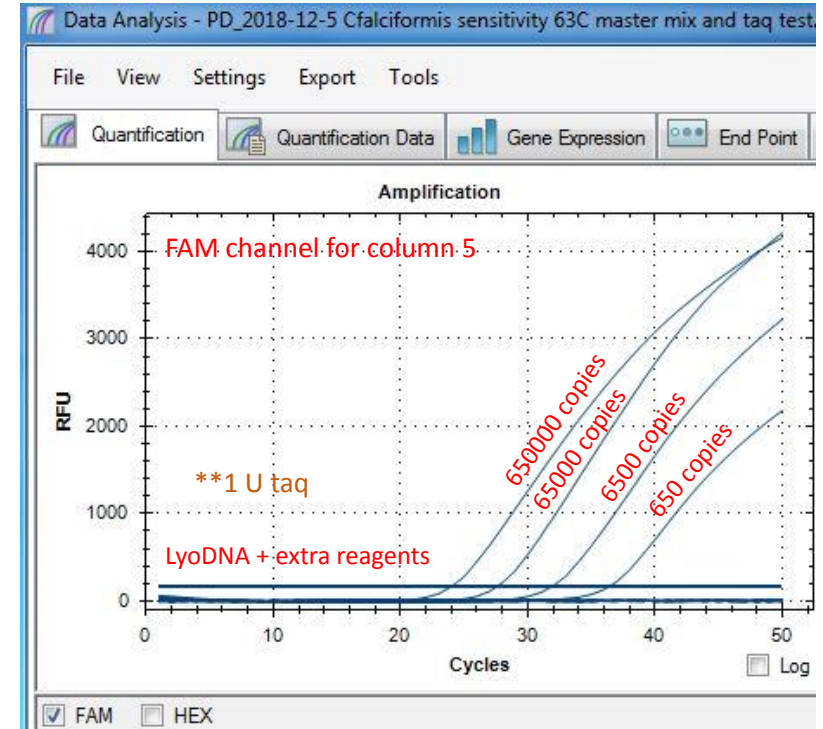
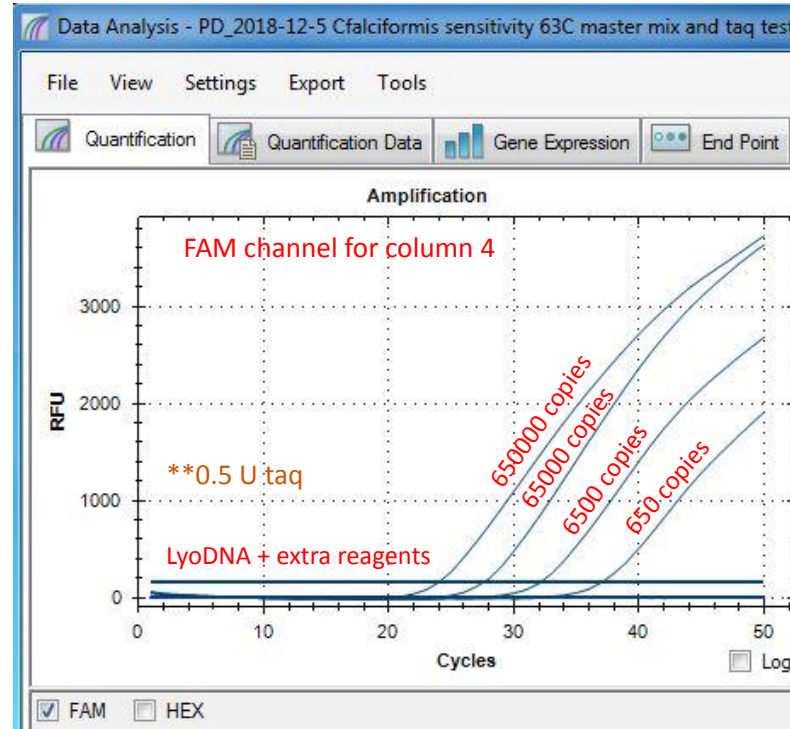
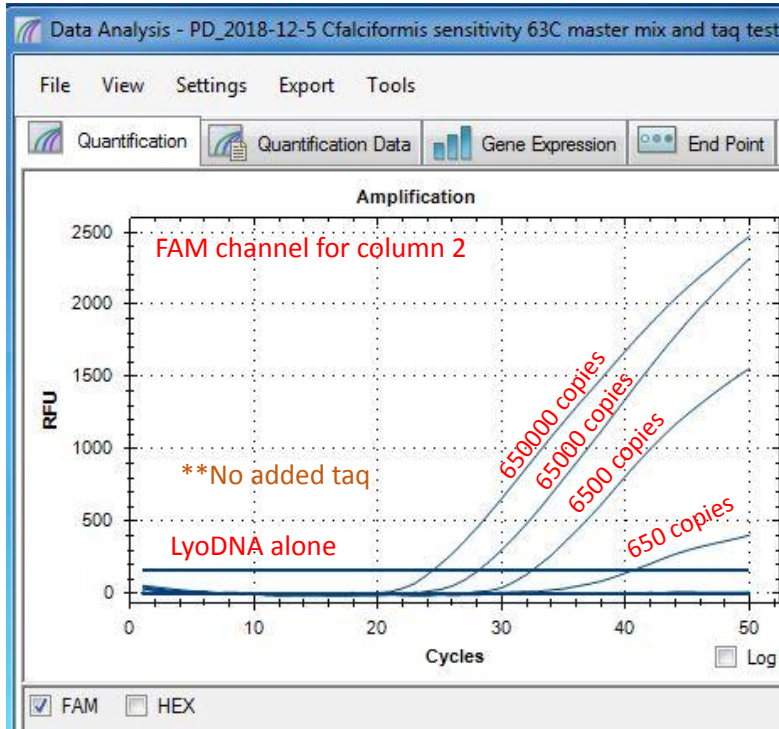
Columns 1 and 2 = no added reagents, just 5X LyoDNA

Columns 3 and 4 = 1 ul of 0.5 U/ ul in a single 20 ul PCR reaction

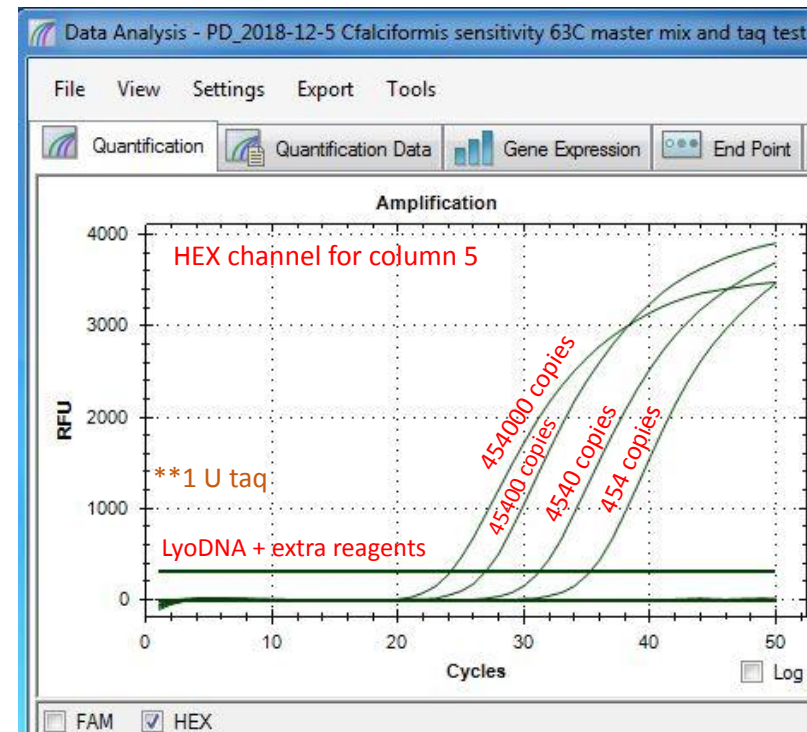
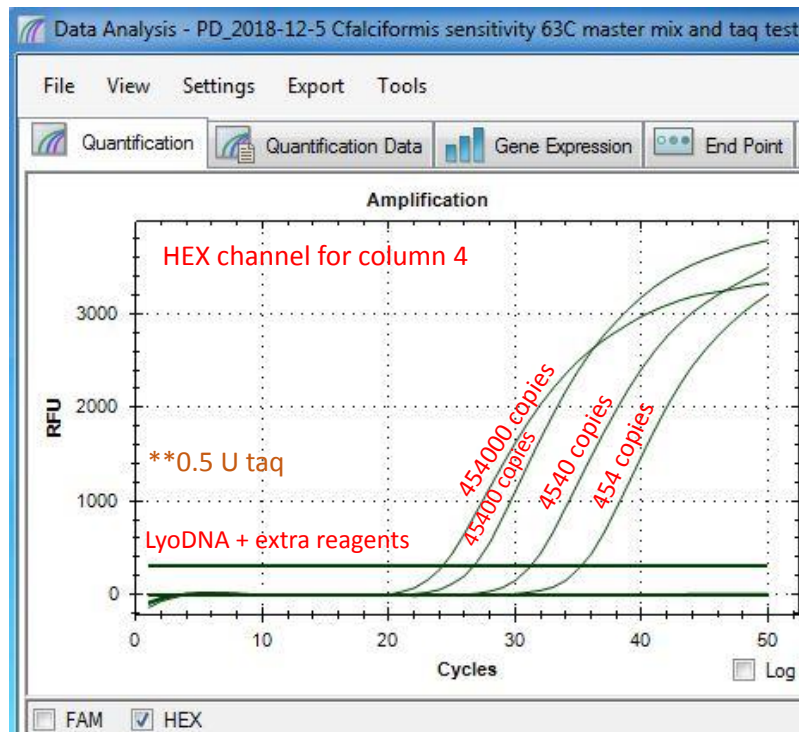
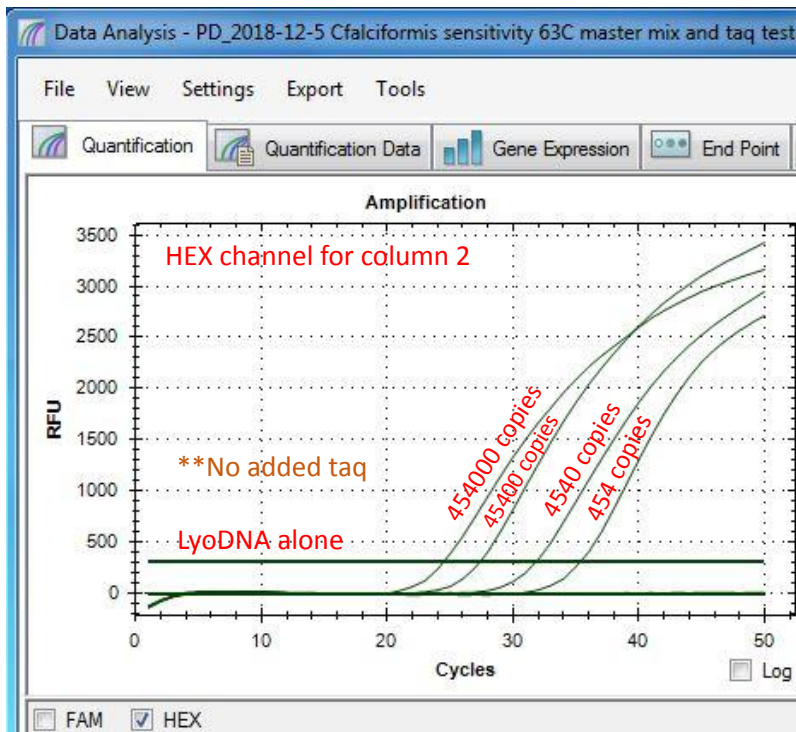
Columns 5 and 6 = 2 ul of 0.5 U/ ul in a single 20 ul PCR reaction

		1	2	3	4	5	6
A	Cfalc - 650000	24.70	24.38	24.07	23.98	23.91	23.98
	16S - 454000	25.07	24.47	24.21	24.25	24.16	24.15
B	Cfalc - 65000	28.15	28.08	27.63	27.54	27.48	27.57
	16S - 45400	27.42	27.35	26.92	26.69	26.92	26.92
C	Cfalc - 6500	32.75	32.41	32.36	32.12	31.81	31.84
	16S - 4540	32.01	31.82	31.11	31.21	31.19	31.27
D	Cfalc - 650	40.39	40.50	37.35	37.02	36.30	36.56
	16S - 454	35.86	35.34	34.96	35.20	35.22	34.58
E	Cfalc - Negative	NA	NA	NA	NA	NA	39.91
	16S - Negative	NA	NA	NA	NA	NA	NA

C. falciformis sensitivity (63°C) – 5x LyoDNA/Lyo + Extras (with correct Taq)/Lyo + Extras (with double Taq) – Continued from previous slide - FAM



C. falciformis sensitivity (63°C) – 5x LyoDNA/Lyo + Extras (with correct Taq)/Lyo + Extras (with double Taq) – Continued from previous 2 slides - HEX



Biomeme

A. pelagicus – Biomeme positive control

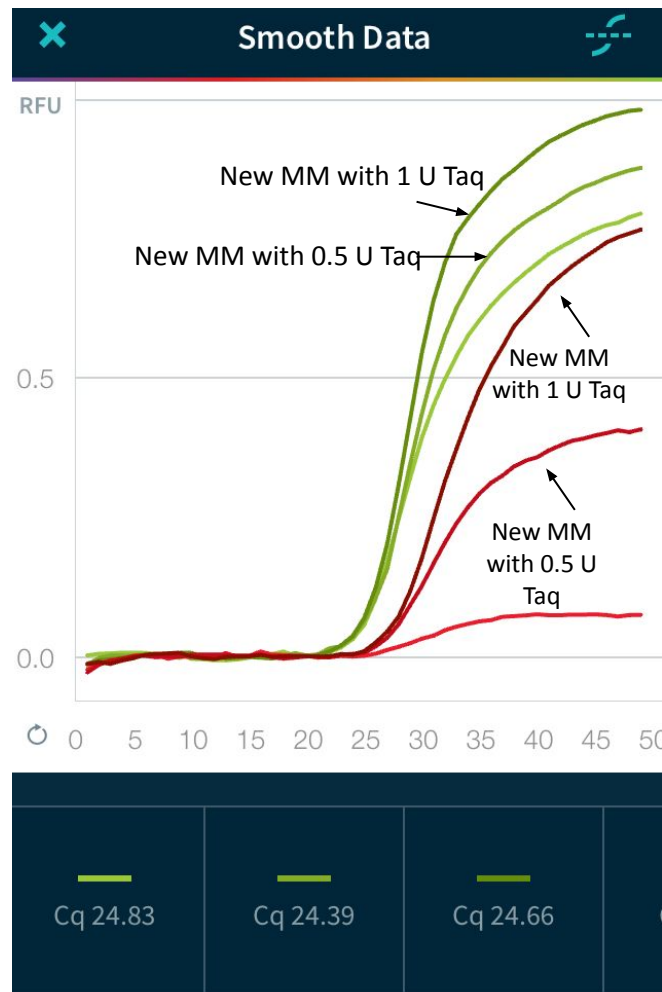
Approx. 5000 copies of A. pelagicus positive control
(Whatman paper punch provided by Biomeme)

Strip 1 and 2 are replicates

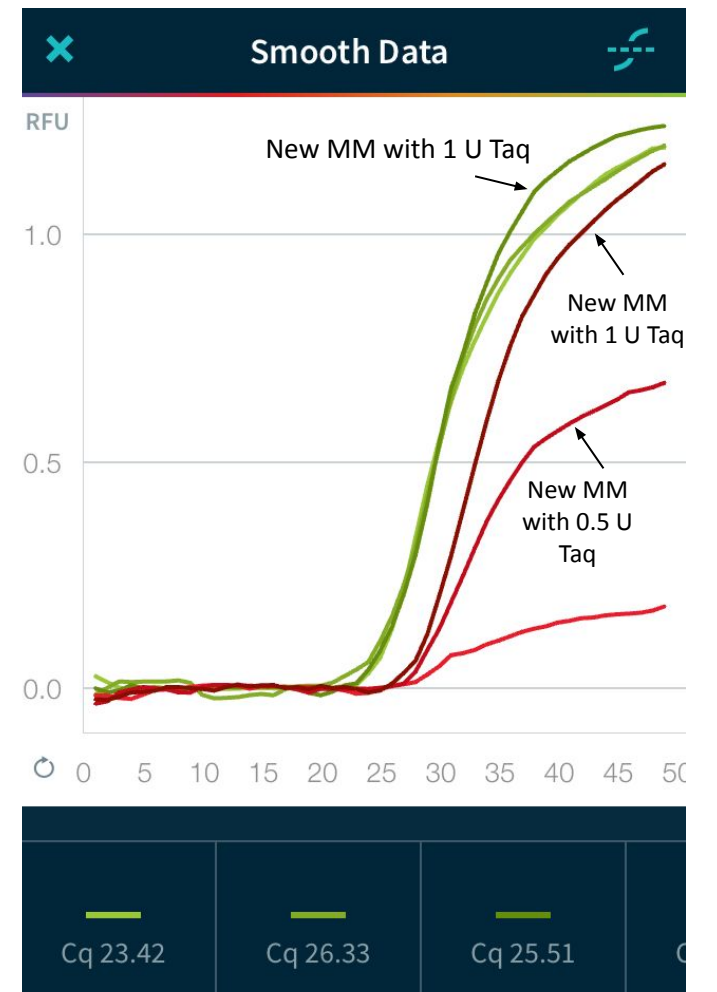
Tube 1 – Old MM (5X LyoDNA alone)

Tube 2 – New MM (Extra reagents with 0.5 U Taq)

Tube 3 – New MM (Extra reagents with 1 U Taq)



Strip 1



Strip 2

Strip 1:

	Tube 1 Old MM	Tube 2 New MM (0.5 U Taq)	Tube 3 New MM (1 U Taq)
FAM	24.83	24.39	24.66
HEX	0	26.58	26.53

Strip 2:

	Tube 1 Old MM	Tube 2 New MM (0.5 U Taq)	Tube 3 New MM (1 U Taq)
FAM	23.42	26.33	25.51
HEX	30.55	28.37	27.69

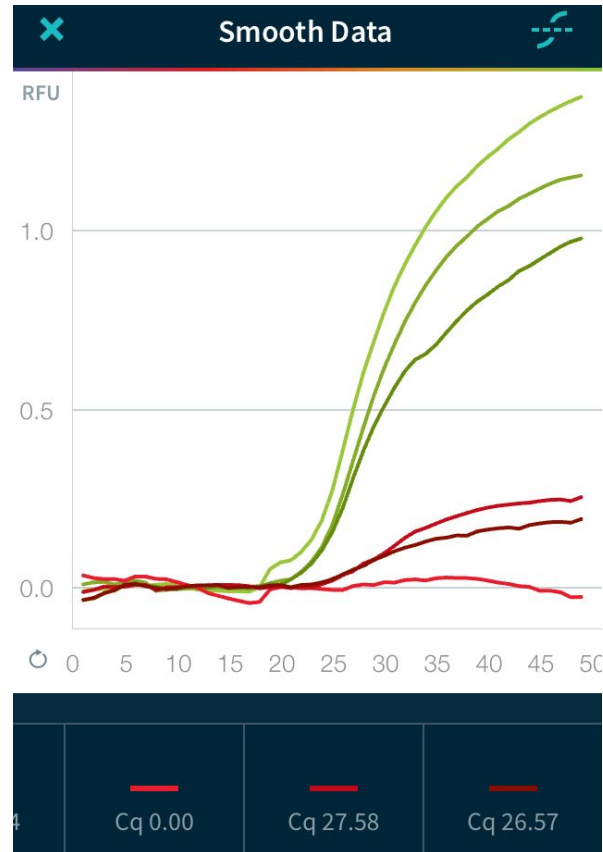
A. pelagicus – Biomeme positive control

Approx. 10,000 copies of A. pelagicus positive control
(Whatman paper punch provided by Biomeme)

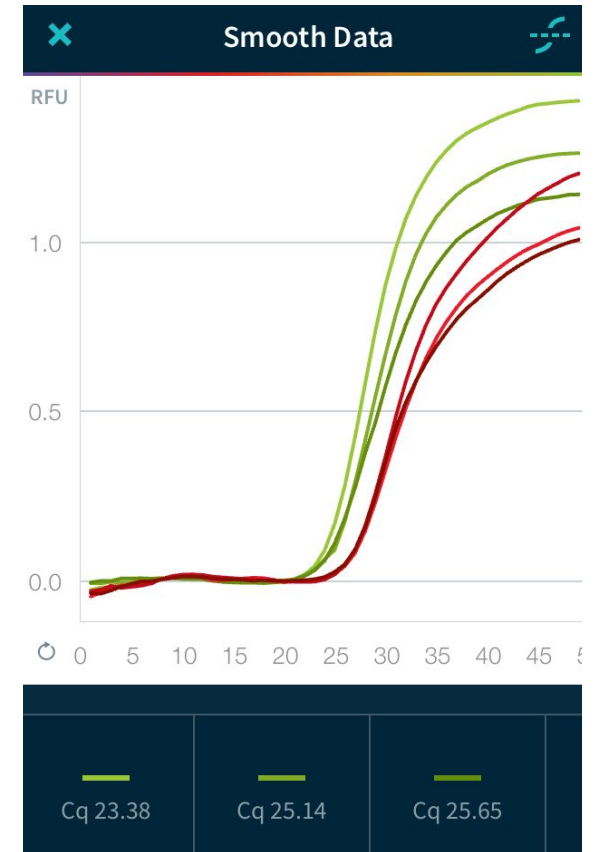
Tubes 1-3 are replicates.

Strip 1 – old mm (5X LyoDNA alone)

Strip 2 – new MM (Extra reagents with 1 U Taq)



Strip 1



Strip 2

Strip 1 – Old MM:

	1	2	3
FAM	24.54	23.81	23.14
HEX	0	27.58	26.57

Strip 2 – new MM with 1 U Taq:

	1	2	3
FAM	23.38	25.14	25.65
HEX	26.94	27.17	26.19

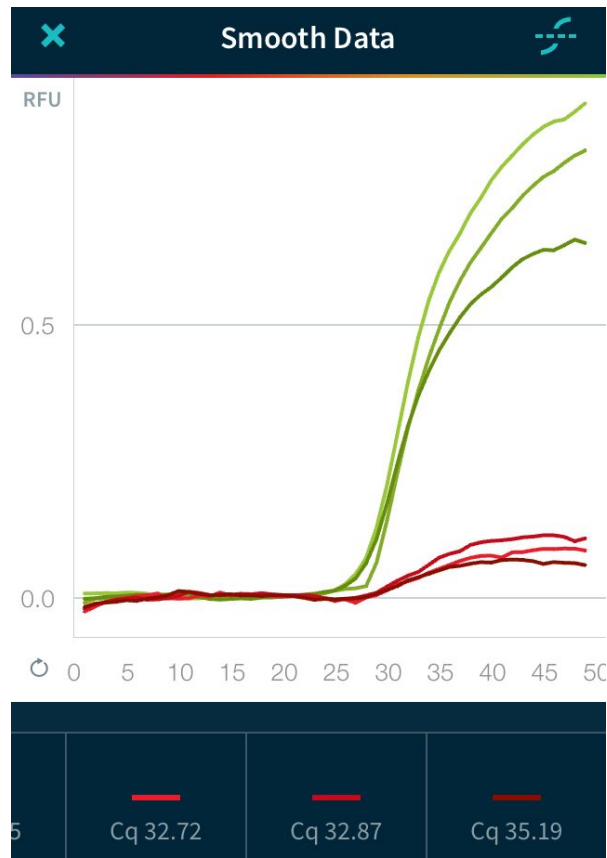
A. pelagicus – Biomeme positive control

Approx. 1,000 copies of A. pelagicus positive control
(Whatman paper punch provided by Biomeme)

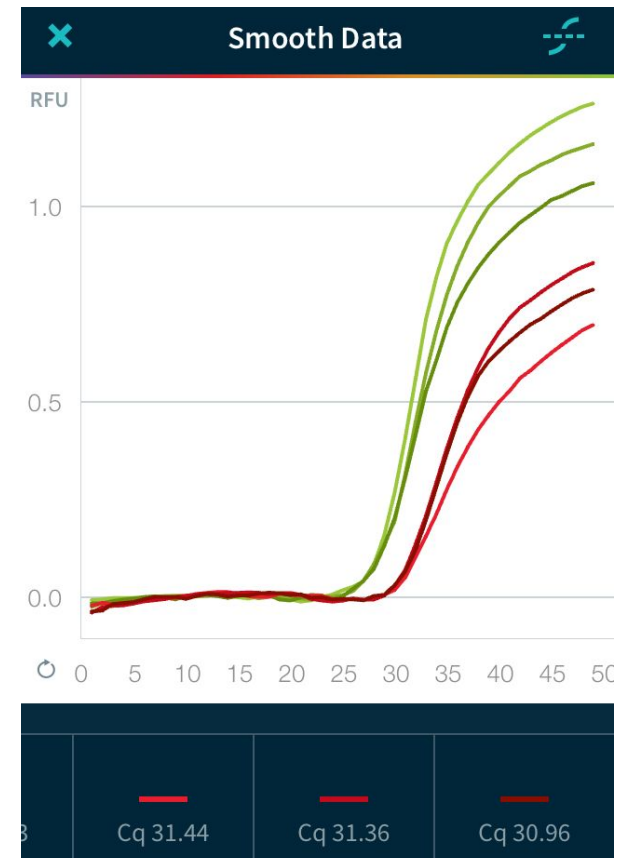
Tubes 1-3 are replicates.

Strip 1 – old mm (5X LyoDNA alone)

Strip 2 – new MM (Extra reagents with 1 U Taq)



Strip 1



Strip 2

Strip 1 – Old MM:

	1	2	3
FAM	27.07	28.37	27.45
HEX	32.72	32.87	35.19

Strip 2 – new MM with 1 U Taq:

	1	2	3
FAM	27.99	27.07	27.83
HEX	31.44	31.36	30.96

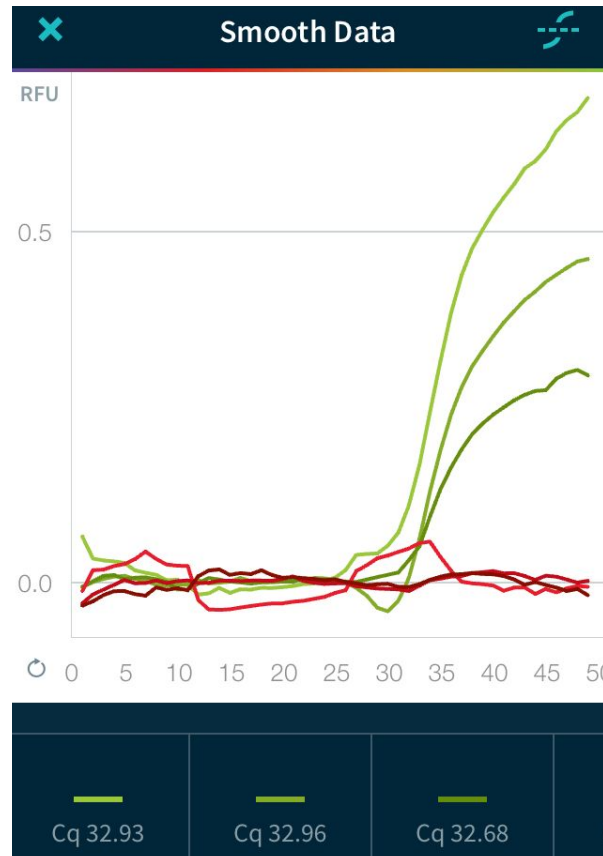
A. pelagicus – Biomeme positive control

Approx. 100 copies of A. pelagicus positive control
(Whatman paper punch provided by Biomeme)

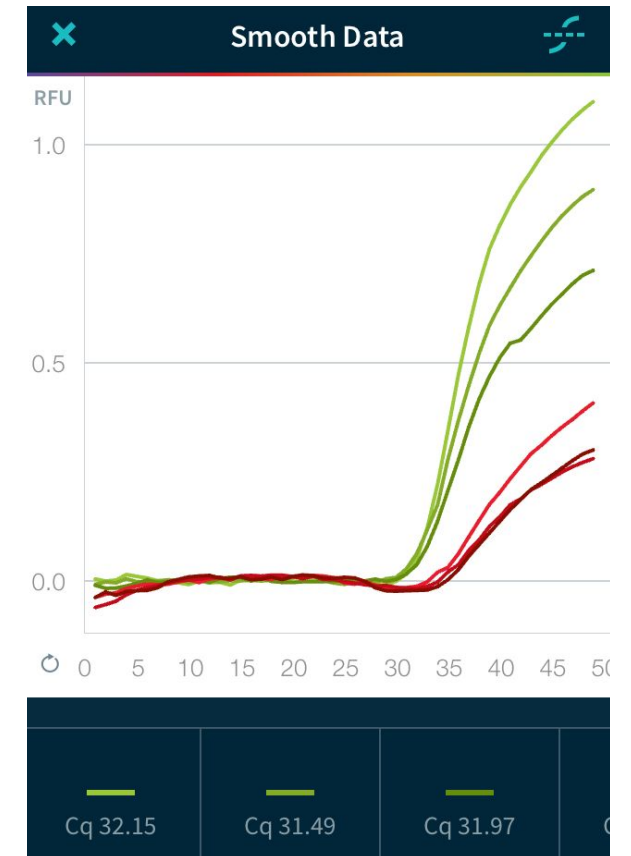
Tubes 1-3 are replicates.

Strip 1 – old mm (5X LyoDNA alone)

Strip 2 – new MM (Extra reagents with 1 U Taq)



Strip 1



Strip 2

Strip 1 – Old MM:

	1	2	3
FAM	32.93	32.96	32.68
HEX	0	0	0

Strip 2 – new MM with 1 U Taq:

	1	2	3
FAM	32.15	31.49	31.97
HEX	36.81	38.38	39.73