

Droplet Digital PCR

Droplets generated on a Bio-Rad Manual Droplet Generator.

20 uL of template, primers and EvaGreen supermix and 70 uL of EvaGreen droplet generation oil added to cartridge sample and oil wells respectively.

Makeup of samples:

Solution	Concentration	Volume added	Final Concentration
Template DNA	1:100 or 1:10	1 uL	1:2000 or 1:200
Forward Primer	2 uM	1 uL	100 nM
Reverse Primer	2 uM	1 uL	100 nM
EvaGreen Supermix	2X	10 uL	1X
Water	-	7 uL	-

Droplets were transferred to a 96-well PCR plate, heat sealed with foil and added to a Bio-Rad C1000 Touch thermal cycler.

PCR cycling protocol:

Cycling Step	Temperature	Time	Ramp Rate	# Cycles
Enzyme activation	95 C	5 min	~2C/sec	1
Denaturation	95 C	30 sec		40
Annealing/extension	60 C	1 min		
Signal stabilization	4 C	5 min		1
	90 C	5 min		1
Hold	4 C	Infinite		1

Droplets were subsequently read on a Bio-Rad QX200 droplet reader. Samples had between 9000-18000 droplets and exhibited good differentiation between positive and negative droplets.

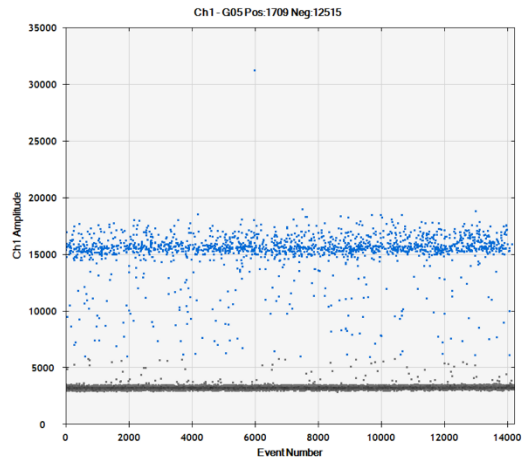
We used the RPPH1 gene as a well-established single copy reference against which to compare the puromycin resistance gene found in our viral construct. (See *PMIDs: 26874951, 2308839, 31164119*). Template DNA from unattenuated control cell line. Primers used are listed below, and following data illustrate copies of puromycin are found in relative equivalence to single-copy RPPH1 gene.

1	GCAACCTCCCCTTCTACGAGC	Puro C-terminal Forward
2	GGTCTTAAAGGTACCTCAGGC	Puro C-terminal Reverse1
3	GGCTAAGATCTACAGCTGCC	Puro C-terminal Reverse2
4	CGCGCGAGGTCAGACT	RPPH1 Reference Gene Forward
5	GGTACCTCACCTCAGCCATT	RPPH1 Reference Gene Reverse

Primer Set	Description	Using 1 uL 1:100 dilution	Using 1uL 1:10 dilution	From 1:100 dilution	From 1:10 dilution
		# of copies/ 20uL well	# of copies/ 20uL well	# of copies/ uL of stock	# of copies/ uL of stock
4+5	Reference	14.3	162	1430	1620
1+2	Puromycin	11.8	151	1180	1510
1+3	Puromycin	13	145	1300	1450

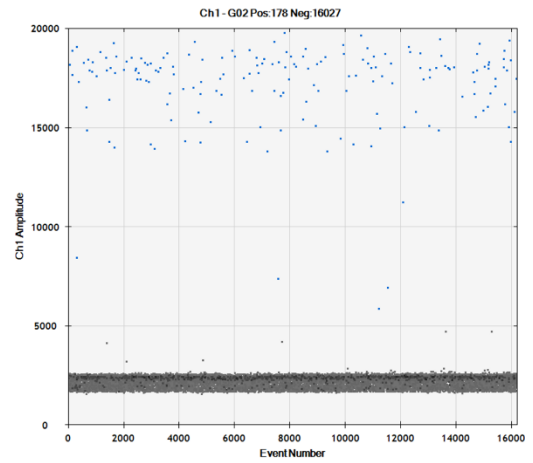
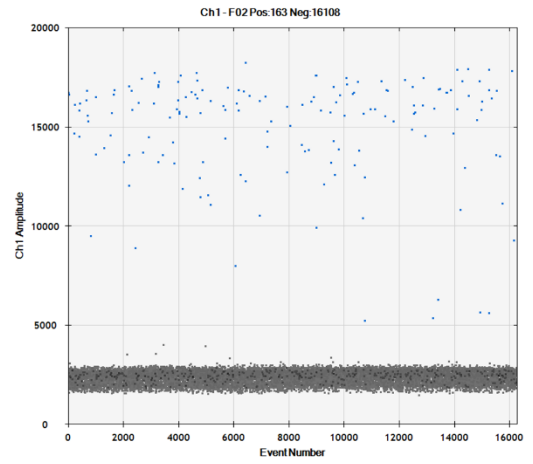
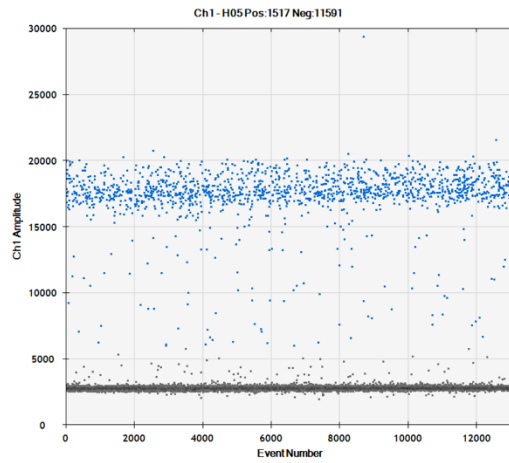
1:10 template dilution

puromycin
primers 1+2



1:100 template dilution

puromycin
primers 1+3



RPPH1
primers 4+5

