**Droplet Digital PCR**

Droplets generated on a Bio-Rad Manual Droplet Generator. 20 µL of template, primers and EvaGreen supermix and 70 µL 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 µL         | 1:2000 or 1:200     |
| Forward Primer    | 2 uM          | 1 µL         | 100 nM              |
| Reverse Primer    | 2 uM          | 1 µL         | 100 nM              |
| EvaGreen Supermix | 2X            | 10 µL        | 1X                  |
| Water             | -             | 7 µL         | -                   |

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. GCAACCTCCCTTCTACGAGC Puro C-terminal Forward
2. GGTCTTAAAGGTACCTCAGGC Puro C-terminal Reverse1
3. GGCTAAGATCTACAGCTGCC Puro C-terminal Reverse2
4. CGCGCCGCGTCAGACT RPPH1 Reference Gene Forward
5. GGTACCTCACCTCAGCCATT RPPH1 Reference Gene Reverse

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