**AL27: Deep Sequencing ZIKV Stocks**

Day 1 – isolating viral RNA:

1. Pipet 1.68ml Buffer AVL with carrier RNA into clean 50ml conicals (one per sample)
   1. Make sure carrier RNA (reconstituted in Buffer AVE) is added to Buffer AVL before use – this should be made fresh
      1. Add 310ul Buffer AVE to 310ug carrier RNA, aliquot, and store at -20C
   2. For 5 samples of 420ul, add 84ul reconstituted carrier RNA to 8.4ml Buffer AVL and mix by inverting 10 times
2. Inside tissue culture hood, syringe filter each of the virus stocks to be sequenced to remove any contaminants
   1. Use 0.22um syringe filters and 1ml syringes
3. Add 420ul of samples to Buffer AVL/carrier RNA mixture and mix by pulse vortexing for 30sec.
4. Incubate samples at room temperature for 10min.
5. Centrifuge conicals briefly and bring samples out of TC
6. Add 1.68ml of 100% ethanol to each sample and mix by pulse vortexing for 30sec.
7. Centrifuge conicals briefly
8. Add 630ul of each sample to QIAamp mini column and centrifuge at 6000xg for 1 min.
   1. Repeat until all of the sample has been loaded onto spin column
9. Discard flow through and place mini columns into clean collection tubes
10. Add 500ul Buffer AW1 to each column and centrifuge at 6000xg for 1 min.
11. Discard flow through and place mini columns into clean collection tubes
12. Add 500ul Buffer AW2 to each column and centrifuge at full speed for 3 min.
13. Discard flow through and place columns into clean collection tubes
14. Centrifuge at full speed for 1 min.
15. Discard flow through and place columns into clean Eppendorf tubes
16. Add 60ul of Buffer AVE (at room temperature) to each column and incubate at room temperature for 1min.
17. Centrifuge columns at 6000xg for 1min.
18. Measure RNA concentrations using NanoDrop and store isolated RNA at -80C

Day 2 – purifying viral RNA:

1. Add 10ul Buffer RDD and 2.5ul DNase I to 50ul of viral RNA for each sample
2. Add dH2O up to 100ul total volume
3. Incubate samples at room temperature for 10min.
4. Add 350ul Buffer RLT to each sample and pipette to mix
5. Add 250ul of 100% ethanol to each sample and pipette to mix
6. Centrifuge for 30sec. at 8000xg and discard flow through
7. Add 500ul Buffer RPE to columns and centrifuge for 30sec. at 8000xg
8. Discard flow through and add 500ul Buffer RPE to columns
9. Centrifuge for 2min. at 8000xg and discard flow through
10. Place columns into clean collection tubes and centrifuge at full speed for 1 min.
11. Discard flow through and place columns in clean Eppendorf tubes
12. Add 30ul dH2O to columns and centrifuge for 1min. at 8000xg
13. Measure RNA concentration using NanoDrop and store at -80C