***Viral cDNA Synthesis for Sequencing***

*Walker Orr, 08/15/202*

## First Strand Synthesis

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| 1. Use the reverse primer corresponding to the region of the genome you will be synthesizing (WB126 for capsid, WB284 for nSP). Set up each reaction as follows. If doing multiple reactions, you can set up a master mix with common components, keeping in mind that RNA volumes might be different for each sample based on concentration. This protocol uses NEB ProtoScript II First Strand Synthesis kit. |
| |  |  | | --- | --- | | **Component** | **Volume** | | Template RNA | (1 ug) | | Reverse primer | 2 ul | | 2x ProtoScript Buffer | 10 ul | | 10x ProtoScript Enzyme Mix | 2 ul | | Nuclease-free water | To 20 ul | |
| 1. Perform Q5 PCR for sequencing prep, per SOP (4x 50 ul reactions). Use 2 ul first strand synthesis product per reaction, or 10 ul per 5x master mix. |
| 1. Gel purify 50 ul of pooled PCR product. |