***In vitro transcription of viral RNA***

*Walker Orr, 08/15/202*

## Guidelines for Working with RNA

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| When working with RNA, it is very important to prevent nuclease contamination in your work areas, always keep RNA on ice, and store it at -80C.  Start by creating an RNAZone on your bench that will function similarly to a hood. Tape out an area of your bench where you will keep all your supplies that will be used to prepare the RNA. Think carefully about everything you will need—if you are spinning down RNA, for instance, your mySpin will need to end up in the RNAZone.  Use a different lab coat than the one you use for bacterial work, routine molecular biology, etc. Be liberal with glove changes; you can also spray your gloves down with RNAse-ZAP.  Take everything out of the RNA zone. Clean the area well with ethanol and then apply RNAse-ZAP. This surfactant kills RNAse enzymes that can degrade your RNA. Clean all tools (i.e., pipettes), scaffolding (i.e., tube racks, ice bucket, BioScion tube holders), and supplies (i.e., pipette tip boxes) with RNAse ZAP before bringing them into the RNAZone. Wipe off excess RNAse-ZAP with KimWipes.  When using microcentrifuge tubes, PCR strips, etc., take them from a freshly-opened bag of certified nuclease-free tubes. The DNA Lo Bind tubes, for instance, come in large bags of ~500 tubes and in smaller bags of <50. For working with RNA, use the smaller bags, as these will have less chance to experience contamination.  The quality of RNA preps can be readily assessed on a TapeStation RNA Screen Tape device. Make sure to use the correct buffer and ladder; there exist both High Sensitivity and standard RNA devices. |

## Linearization of DNA Template

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## *In vitro* transcription

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| 1. Perform DNA Cleanup on the linearized DNA. (NEB Monarch PCR & DNA Cleanup; SOP #)   Recall that it is a large fragment, so 2:1 binding buffer and 50C water for elution. |
| 1. Thaw components and keep on ice. Make a master mix with equal volumes of 10x reaction buffer and each NTP. |
| 1. Set up reactions in PCR strip tubes:   10 ul Master Mix  2 ul T7 RNA Polymerase  Template DNA: 1 ug.  Nuclease-free water to 20 ul. |
| 1. Mix thoroughly, spin down. Incubate 37C for 2 hours in a thermocycler. |

## DNAseI Treatment

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| 1. Make a Master Mix with the following components (1x):   5 ul 10x DNAseI reaction buffer  2.5 ul DNAseI  22.5 ul Nuclease-free water   1. Set up DNAseI reactions:   30 ul Master Mix  20 ul IVT reaction (i.e., all) |
| 1. Incubate at 37C for 10 minutes. |

## Cleanup and QC

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| 1. Purify RNA using Monarch RNA Cleanup Kit. |
| 1. Analyze RNA quality using TapeStation 4200. |