RNAPath Target Identification via Fluorescent Hybridization

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Molecular Instruments, COVID, Microscopy, Hybridization, Fluorescence, RNA

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BEFORE STARTING

Ensure you have cleaned the workspace with an appropriate RNAse away solution to prevent RNA degradation.

Sample Preparation

1. Suspend RNA pellet in 2 µl TE Buffer
   - TE Buffer Thermo
   - Fisher Catalog #12090015

Hybridization

2. Add 2 µl Molecular Instruments COVID Probe Set to the RNA suspension
   - Molecular Instruments HCR COVID Probe Set

3. Place Molecular Instruments Alexa Flour 488 B1 HCR Amplifier in 90 °C for 00:02:00

4. Remove Molecular Instruments Alexa Flour 488 B1 HCR Amplifiers and place at Room temperature for 00:30:00

5. Add 2 µl of each Molecular Instruments Alexa Flour 488 B1 HCR Amplifier H1 and H2 to the RNA suspension

6. Dilute 100 µl of 20x SSC Buffer to 5x SSC Buffer
   - SSC Buffer, 20X,
   - Promega Catalog #V4261

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7 Add 4 µl of 5x SSC Buffer to the RNA Suspension

8 Incubate at 37 °C for 12:00:00

Imaging

9 Remove sample from incubation and pipette 1 µl onto a glass slide with a coverslip

10 Image slide under a fluorescent microscope with filter cubes for FITC or Alexa Flour 488