The Alkek Center for Metagenomics and Microbiome Research – CMMR
Department of Molecular Virology and Microbiology
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Virome Methods

Approximately 0.5g of stool was mixed with an equal volume of 1xPBS. This slurry was then spun for 5min at 17,000xg to remove debris. The supernatant was transferred to an Ambion® MagMAX™ Total Nucleic Acid Isolation Kit bead tube and kit instructions were followed from there. Extracted nucleic acids were stored at -20°C until reverse transcription.

For reverse transcription, we added 9.5 µL of the extracted RNA/DNA, 3 µL of 25 µM random hexamer primer, and 1 µL of 10 mM dNTPs to each reaction. This mixture was incubated at 65°C for 5 minutes, then cooled to 4°C and incubated for 2 minutes. To this mixture we added 4 µL of 5x First Strand Buffer [250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM $MgCl_2$], 2 µL of 0.1 M dithiothreitol, and 0.5 µL of SuperScript® II reverse transcriptase. This mixture is then incubated at 25°C for 10 minutes, 42°C for 50 minutes, and inactivated at 70°C for 15 minutes.

The resulting mixture of single stranded cDNA and double stranded DNA (carried through from the extraction) was purified using the ChargeSwitch® PCR Clean-Up Kit to remove remaining random primer and other very short DNA fragments. The entire 20 µL RT-PCR reaction was mixed with an equal volume of ChargeSwitch® purification buffer (N5 CS11210). A 10 µL volume of ChargeSwitch® magnetic bead suspension [25 mg/mL beads in 10 mM MES, pH 5.0, 10 mM NaCl, 0.1% Tween 20] was then added, mixed, and incubated for 1 minute. The beads were then captured via a magnet, and the liquid removed. The beads were twice washed by resuspension in 150 µL ChargeSwitch® wash buffer (W12 CS12102) followed by bead recapture and buffer removal. Finally, washed beads were resuspended in 35 µL of ChargeSwitch® Elution Buffer [10 mM Tris-HCl, pH 8.5] and incubated for 1 minute. Beads were magnetically captured and the eluted nucleic acids were transferred to a new tube. This purified DNA/cDNA mix was stored at -20°C until amplification.

For amplification, 10 µL of the RT-PCR reaction was mixed with 3 µL of the BC-V_8A_2 primer (10 µM) (unique barcode (BC) for each sample), 2.5 µL AccuPrime™ Buffer 1, 0.5 µL AccuPrime™ *Taq*, and 9 µL of water. This mix was subjected to the following PCR program: 95°C for 2 minutes followed by 45 cycles of 94°C for 30 seconds, 33°C for 1 second then ramping at 8% to 58°C, and 58°C for 2 minutes. All PCR reactions were carried out in an Eppendorf Mastercycler® pro S. After amplification, the entire 25 µL barcoded semi-random amplification reaction was mixed with an equal volume of ChargeSwitch® purification buffer (N5 CS11210). A 10 µL volume of ChargeSwitch® magnetic bead suspension [25 mg/mL beads in 10 mM MES,

pH 5.0, 10 mM NaCl, 0.1% Tween 20] was then added, mixed, and incubated for 1 minute. The beads were then captured via a magnet, and the liquid removed. The beads were twice washed by resuspension in 150 µL ChargeSwitch® wash buffer (W12 CS12102) followed by bead recapture and buffer removal. Finally, washed beads were resuspended in 35 µL of ChargeSwitch® Elution Buffer [10 mM Tris-HCl, pH 8.5] and incubated for 1 minute. Beads were magnetically captured and the eluted nucleic acids were transferred to a new tube and stored at -20°C until sequencing library generation.