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Client: Samuel White University of Washington Fishery Sciences 116 1122 NE Boat St. Seattle, Washington 98105 United States +1-2066853273 samwhite@uw.edu	**Tracking Number: 30-852430235** **Order Name: A.poculata sRNA for H.putnam** Quotation Effective Date: 05/Apr/2023 Quotation Expiration Date: 05/Jul/2023 Payment Term: NET 30 Shipment: Prepaid and Add **Contact:** NGS@azenta.com +1-877-(436-3949) ex 1 Account Manager: Michael Bukolt Account Manager Email: michael.bukolt@azenta.com

RNA library preparation and sequencing was conducted at Azenta Life Sciences (South Plainfield, NJ, USA) as follows:

Library Preparation and Sequencing

RNA samples were quantified using Qubit 2.0 Fluorometer (ThermoFisher Scientific, Waltham, MA, USA) and RNA integrity was checked with 4200 TapeStation (Agilent Technologies, Palo Alto, CA, USA).

Small RNA sequencing libraries were prepared by using NEB Small RNA library Prep Kit (New England Biolabs, Ipswich, MA, USA). In brief, Illumina 3' and 5' adapter was added to RNA molecules with a 5'-phosphate and a 3'-hydroxyl group sequentially. A reverse transcription reaction was used to create single stranded cDNA. The cDNA was then PCR amplified using a common primer and a primer containing index sequence. Amplified cDNA construct was purified by polyacrylamide gel electrophoresis, and the correct band (~145 – 160 bp) was excised from the gel and eluted with water. The eluted cDNA was concentrated by EtOH precipitation, and this was the final sequencing library. The sequencing library was validated on the Agilent TapeStation (Agilent Technologies, Palo Alto, CA, USA), and quantified by using Qubit 2.0 Fluorometer (ThermoFisher Scientific, Waltham, MA, USA) as well as by quantitative PCR (KAPA Biosystems, Wilmington, MA, USA).

The sequencing libraries were multiplexed and clustered onto a flowcell. After clustering, the flowcell was loaded onto the Illumina HiSeq (4000 or equivalent) instrument according to manufacturer's instructions. The samples were sequenced using a 2x150bp Paired End (PE) configuration. Image analysis and base calling were conducted by the HiSeq Control Software (HCS). Raw sequence data (.bcl files) generated from Illumina HiSeq was converted into fastq files and de-multiplexed using Illumina bcl2fastq 2.20 software. One mis-match was allowed for index sequence identification.