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JAX-Sen: Watchmaker RNA Library Prep Kits with Polaris Depletion

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We use this protocol and it's working

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Abstract

This protocol describes the library preparation using the RNA Library Prep Kit with Polaris Depletion protocol followed by sequencing on the Illumina NovaSeq X-Plus platform using patterned flow cells and two-color chemistry.

Materials and Reagents:

1 Instruments:

- Qubit Flex Fluorometer, Thermo Fisher Scientific
- TapeStation 4200, Agilent Technologies
- Thermal Cycler, Bio-Rad
- NovaSeq X-Plus, Illumina

Procedure:

- 2 The concentration and integrity of RNA and sequencing libraries were assessed using the Qubit Flex Fluorometer (Thermo Fisher Scientific) and the Agilent TapeStation 4200 (Agilent Technologies).

3 Library preparation:

RNA-Seq libraries were prepared using the *RNA Library Prep Kit with Polaris Depletion* (Watchmaker, Cat# 7BK0002-096) following a modified version of the manufacturer's protocol (**Watchmaker protocol**). Modifications included the addition of ERCC spike-in to each sample, a 0.8× bead clean-up after ligation, and a 0.7× bead clean-up after PCR amplification.

Indexes used were Illumina xGenTM UDI-UMI Adapters, 1-96 (10005903) and special orders from IDT.

4 Sequencing:

Sequencing was performed on the Illumina NovaSeq X-Plus platform using patterned flow cells and two-color chemistry (green and blue channels). Libraries were sequenced on either a 10B or 25B flow cell, each containing eight lanes. Runs were carried out with 300-cycle kits, which include an additional 38 cycles for index reads. The expected yield per lane is approximately 1,250 million reads on a 10B flow cell and 3,250 million reads on a 25B flow cell.