Comparative Analysis of RNA-Seq Library Prep Kits for Shared Research Resource Facilities

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Accessibility to the genetic code continues to improve as manufacturing companies across the globe develop their sequencing techniques. With so many options, all claiming that their sequencing library preparation kits are best, it can be hard to make a decision. What makes a kit the "best"? Due to the inherently high cost of reagents and computing/processing power, the optimization of sequencing runs is critical. Identifying library prep kits that deliver the highest quality and quantity of sequencing data is key, but there are additional considerations to be made, such as cost, hands-on processing time, shelf life, and more. Our goal was to find a RNA-Seq library prep kit that can process a wide range of sample quality and quantity, as users within our network have expressed interest in sequencing low-quality samples of low-quantity. We used the Illumina NextSeq2000 to sequence libraries prepared by a total of five different Stranded RNA-Seq Library Prep kits from New England Biolabs, Illumina, and Takara, utilizing either rRNA depletion or mRNA magnetic isolation methods. Based on our criteria of data output, aligned reads, reagent shelf-life, and ease of hands-on sample processing, the SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian outcompeted the others. While cost exceeds the other options, the amount of data generated from these prepared libraries will likely eliminate the need to process additional sample to increase sequencing coverage.