NEBNext UltraExpress™ RNA Library Prep Kit

NEB #E3330

FIGURE 1: **NEBNext UltraExpress RNA Library Prep Kit workflow**

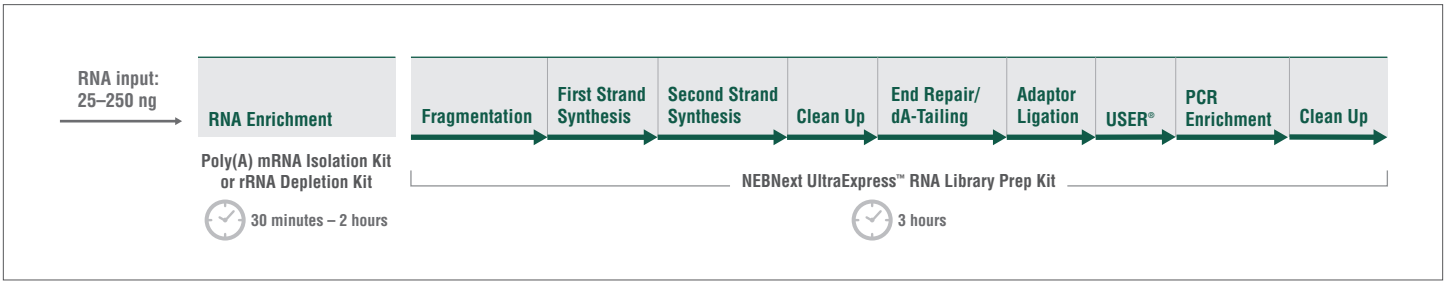
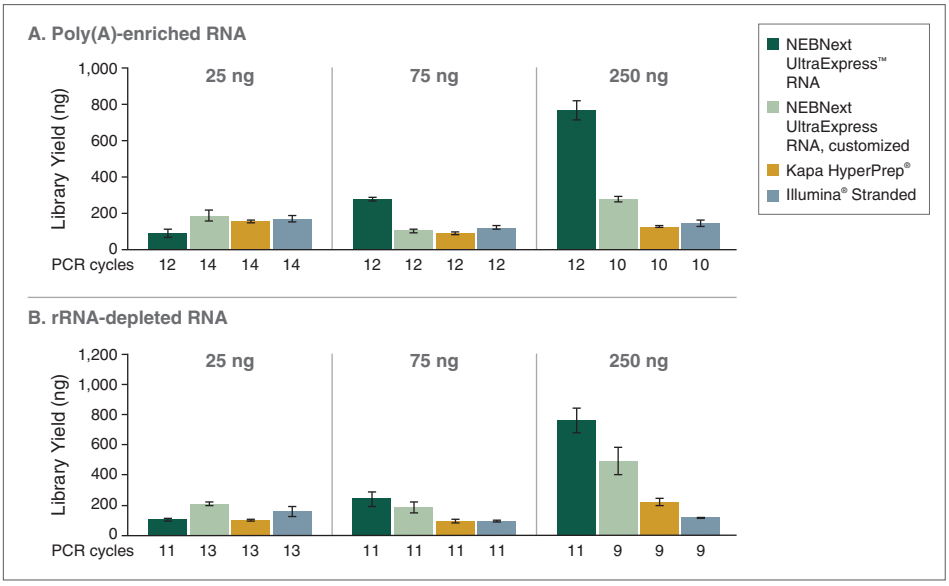


FIGURE 2: **The NEBNext UltraExpress RNA Library Prep Kit produces high library yields across a range of inputs**

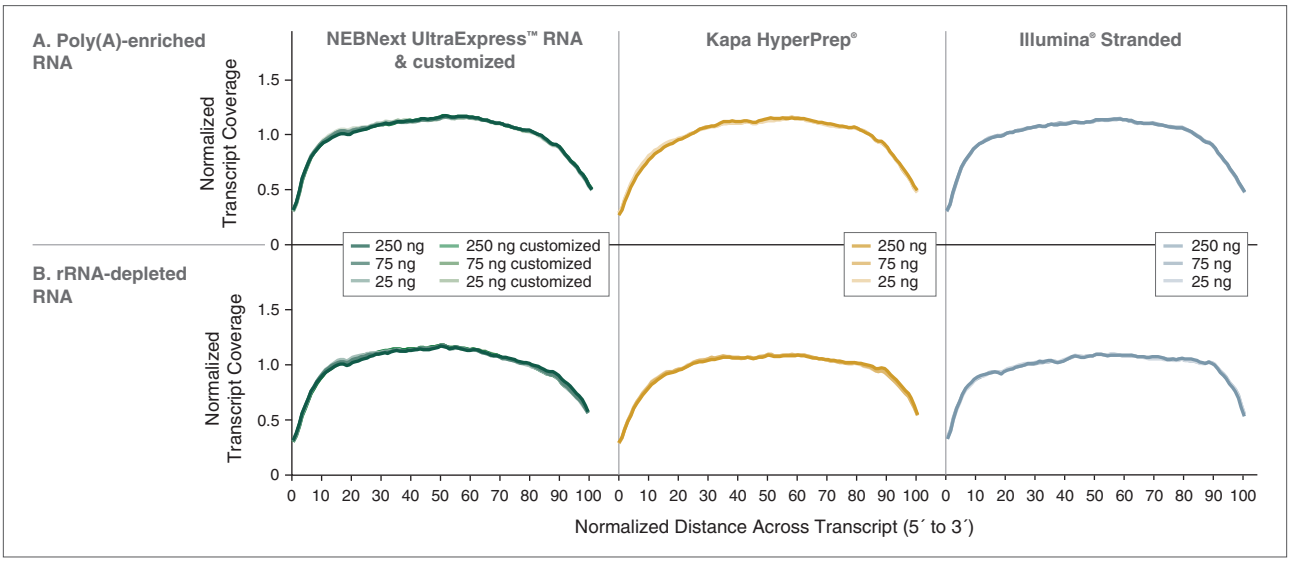


A. Poly(A)-containing mRNA was isolated from Universal Human Reference RNA (UHRR) (Agilent®), using the NEBNext® Poly(A) mRNA Magnetic Isolation Module (NEB #E7490). Libraries were prepared using the NEBNext UltraExpress RNA Library Prep Kit, Kapa mRNA HyperPrep® Kit or Illumina® Stranded mRNA Kit. The NEBNext UltraExpress RNA Library Prep Kit was used with a single adaptor dilution (50X) and 12 PCR cycles for all inputs, or with customized adaptor dilutions (20X for 76–250ng, 100X for 25–75 ng) and PCR cycle numbers (listed in figure).

B. Ribosomal RNA (rRNA) was depleted from UHRR, and libraries were prepared using the UltraExpress RNA Library Prep Kit (preceded by the NEBNext rRNA Depletion Kit (Human/Mouse/Rat – NEB #E7400), Kapa HyperPrep Kit with RiboErase, or Illumina Stranded Total RNA Library Prep Kit with Ribo-Zero® Plus. The NEBNext UltraExpress RNA Library Prep Kit was used with a single adaptor dilution (50X) and 11 PCR cycles for all inputs, or with customized adaptor dilutions (20X for 76–250 ng, 100X for 25–75 ng) and PCR cycle numbers (listed in figure).

The total RNA input amount and number of PCR cycles are indicated. Library yields calculated from an average of three replicates are shown with error bars indicating the standard deviation between replicates.

FIGURE 3: **The NEBNext UltraExpress RNA Library Prep Kit provides consistent transcript coverage**

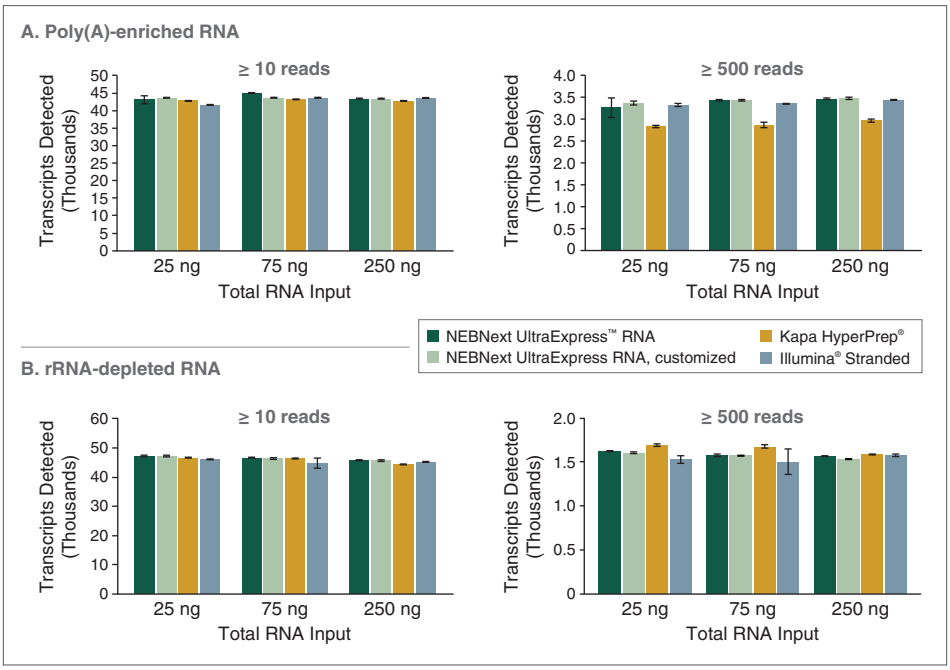


A. Poly(A)-containing mRNA was isolated from Universal Human Reference RNA (UHRR) (Agilent), using the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490). Libraries were prepared using the NEBNext UltraExpress RNA Library Prep Kit, Kapa mRNA HyperPrep Kit or Illumina Stranded mRNA Kit. Poly(A)-containing mRNA was isolated from Universal Human Reference RNA (UHRR) (Agilent), and libraries were made using the UltraExpress RNA Library Prep Kit, Kapa mRNA HyperPrep Kit and Illumina Stranded mRNA Kit. The NEBNext UltraExpress RNA Library Prep Kit was used with a single adaptor dilution (50X) and 12 PCR cycles for all inputs, or with customized adaptor dilutions and PCR cycle numbers (20X adaptor dilution with 10 PCR cycles for 250 ng input, 100X adaptor dilution with 12 PCR cycles (75 ng) and 14 PCR cycles (25 ng)).

B. Ribosomal RNA (rRNA) was depleted from UHRR, and libraries were prepared using the UltraExpress RNA Library Prep Kit (preceded by the NEBNext rRNA Depletion Kit (Human/Mouse/Rat – NEB #E7400), KAPA HyperPrep Kit with RiboErase, or Illumina Stranded Total RNA Library Prep Kit with Ribo-Zero Plus. The NEBNext UltraExpress RNA Library Prep Kit was used with a single adaptor dilution (50X) and 12 PCR cycles for all inputs, or with customized adaptor dilutions and PCR cycles (20X adaptor dilution with 9 PCR cycles for 250 ng input, 100X adaptor dilution with 11 PCR cycles (75 ng) and 13 PCR cycles (25 ng)).

Libraries were sequenced on an Illumina NextSeq® 500 (2 x 75 bases). 10 million reads were sampled and mapped to the hg38 reference genome using RNA STAR v2.7.8a and 5´ to 3´ Transcript coverage was calculated from the top 1,000 transcripts using the CollectRnaSeqMetrics (Picard) tool v2.18.2.2.

FIGURE 4: **The NEBNext UltraExpress RNA Library Prep Kit provides greater sensitivity of transcript detection**



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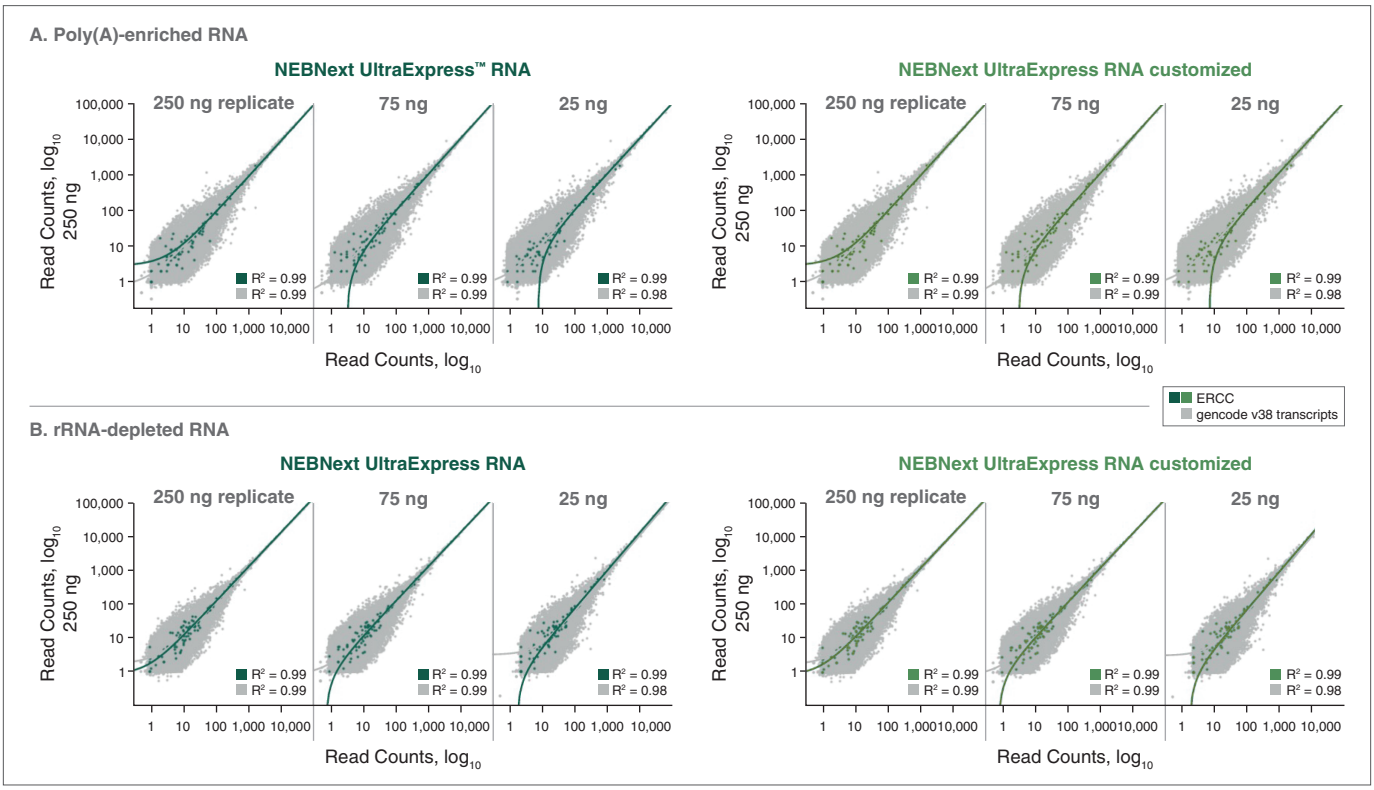
Libraries were sequenced on an Illumina NextSeq 500 (2 x 75 bases). 10 million reads were sampled and average transcript abundance was assessed for transcripts detected with ≤ 10 reads and ≤ 500 reads, respectively. Error bars indicate standard deviation for 3 replicates. Salmon v1.5.1 was used for mapping and quantification of all gencode v38 transcripts and ERCCs.

FIGURE 5: **The NEBNext UltraExpress RNA Library Prep Kit provides excellent transcript correlation between inputs and replicates**

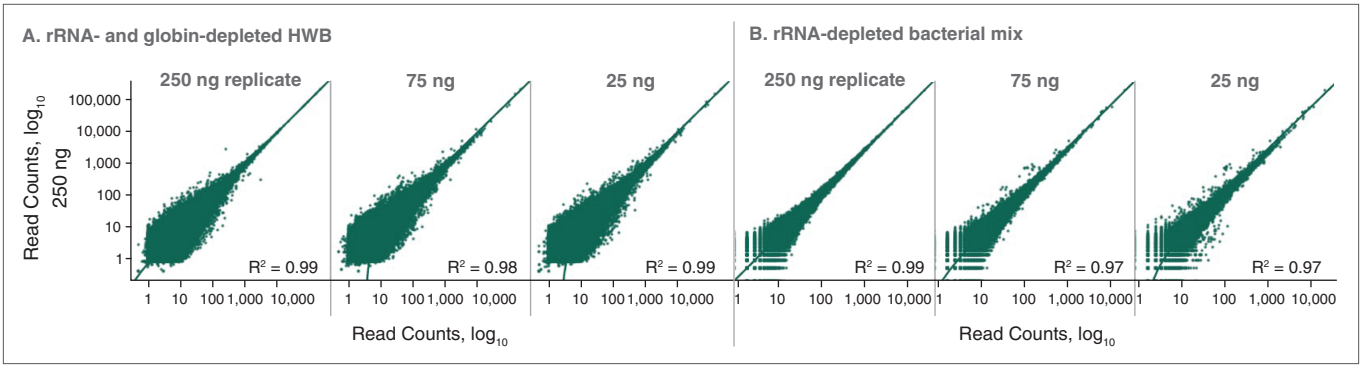


A. Poly(A)-containing mRNA was isolated from Universal Human Reference RNA (UHRR) (Agilent), using the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490). Libraries were prepared using the NEBNext UltraExpress RNA Library Prep with a single adaptor dilution (50X) and 12 PCR cycles for all inputs, or with customized adaptor dilutions and PCR cycle numbers (20X adaptor dilution with 10 PCR cycles for 250 ng input, 100X adaptor dilution with 12 PCR cycles (75 ng) and 14 PCR cycles (25 ng)).

B. Ribosomal RNA (rRNA) was depleted from UHRR using the NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat – NEB #E7400) and libraries. The NEBNext UltraExpress RNA Library Prep Kit was used with a single adaptor dilution (50X) and 11 PCR cycles for all inputs, or with customized adaptor dilutions and PCR cycle numbers (20X adaptor dilution with 9 PCR cycles for 250 ng input, 100X adaptor dilution with 11 PCR cycles (75 ng) and 13 PCR cycles (25 ng)).

Libraries were sequenced on an Illumina NextSeq 500 (2 x 75 bases). The data was sampled from 10 million reads and libraries were correlated for transcript expression levels across inputs using Salmon v1.5.1 quantification of all gencode v38 transcripts and ERCCs. Each data point represents a transcript, with the $\log_{10}$ abundance in Number of Reads with 250 ng total RNA input on the y-axis compared to a replicate at 250 ng followed by 75 and 25 ng on the x-axis.

FIGURE 6: **The NEBNext UltraExpress RNA Library Prep Kit provides excellent transcript correlation across sample types**



RNA from human whole blood (HWB) was depleted of globin mRNA and rRNA (A) using the NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat – NEB #E7750) or rRNA was depleted from a pool of 20 different bacterial organisms (ATCC® #MSA-2002) (B) using the NEBNext rRNA Depletion Kit (Bacteria – NEB #E7850). Libraries were prepared using the NEBNext UltraExpress RNA Library Prep Kit, with customized adaptor dilution and PCR cycles per input (20X adaptor dilution with 9 PCR cycles for 250 ng input, 100X adaptor dilution with 11 PCR cycles (75 ng) and 13 PCR cycles (25 ng)). Libraries were sequenced on an Illumina NextSeq 500 (2 x 75 bases). The data was sampled from 10 million reads and libraries were correlated for transcript expression levels across inputs using Salmon v1.5.1 quantification of all gencode v38 transcripts (HWB) and a composite genome (Bacterial). Each data point represents a transcript, with the $\log_{10}$ abundance in number of Reads with 250 ng total RNA input on the y-axis compared to a replicate at 250 ng followed by 75 and 25 ng on the x-axis.