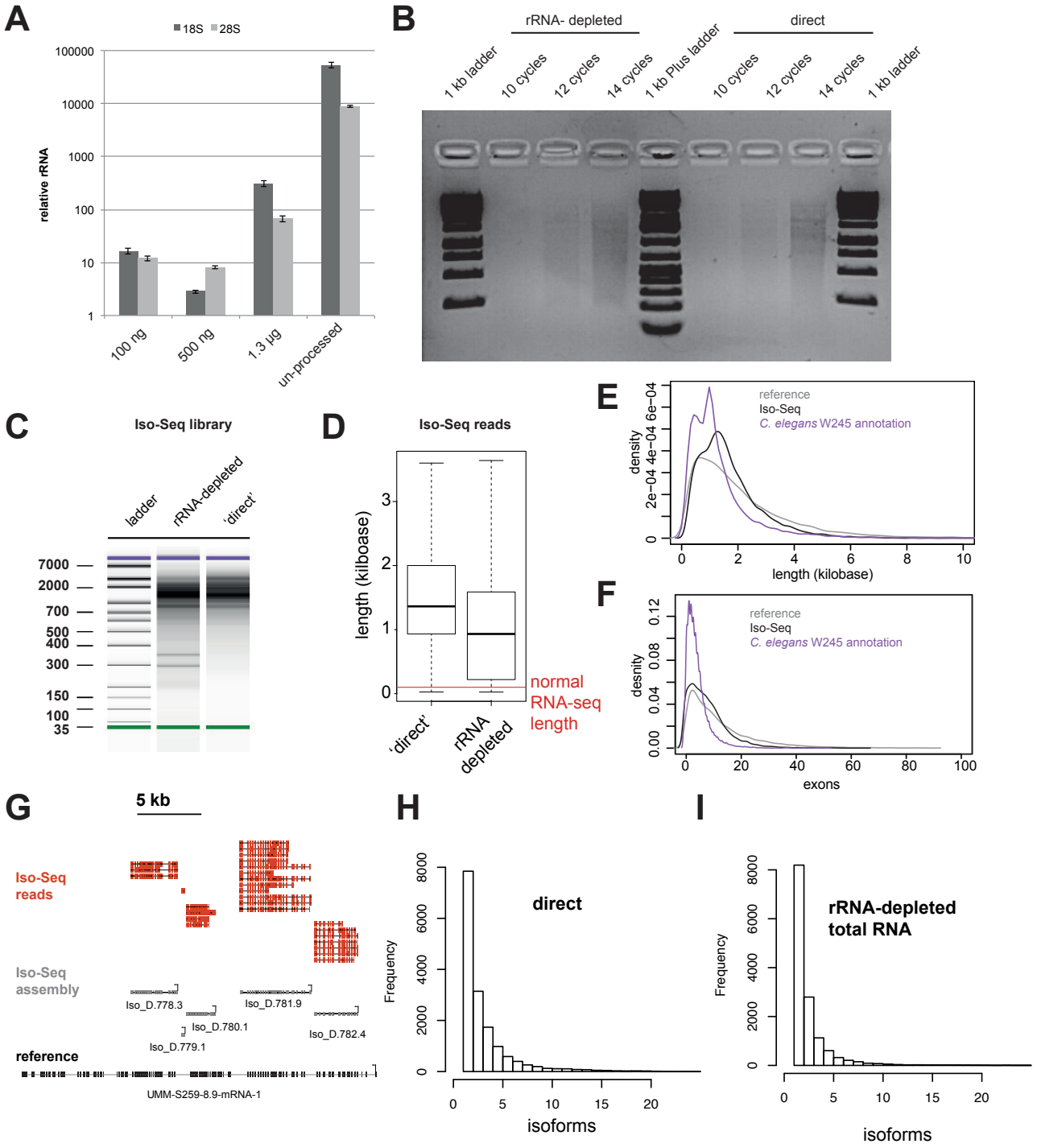

Supplemental Figure S1. Iso-seq methodology and quality control. (*A*) qPCR of rRNA (18 and 28S) from rRNA-depleted or un-processed cDNA, normalized to *Ppa-cdc-42*. Y-axis is the ratio of $2^{\Delta Ct}$ in log10-scale, and error bars represent the propagated standard deviation of four technical replicates. Based on this analysis, 500 ng of RNA input was used for Iso-seq rRNA-depletion with the Ribo-Zero rRNA removal kit (Human/Mouse/Rat) (Illumina), which yielded a 1,105-fold decrease in 28S and 19,383-fold decrease in 18S rRNA. (*B*) Agarose gel of Iso-seq library with indicated number of cycles, which was used to determine the appropriate cycle number without over-amplification (12 cycles). (*C*) Bioanalyzer trace of Iso-seq libraries showing size distribution (bp). (*D*) Actual read-length distribution of Iso-seq sequences (kb). Red line indicates standard RNA-seq read length of 0.1 kb. (*E*) Density distribution of gene length, and (*F*) exon number for *P. pacificus* reference (El Paco), Iso-seq 'direct', and *C. elegans* Ref-seq gene annotations, produced in R using the density function. (*G*) Example locus comparing the direct Iso-seq annotation that includes five genes, to the reference annotation that has one long gene, and accompanying Iso-seq reads. (*H*) Frequency distribution of isoform number from direct and total Iso-seq. Isoform annotations determined from Stringtie assembly.