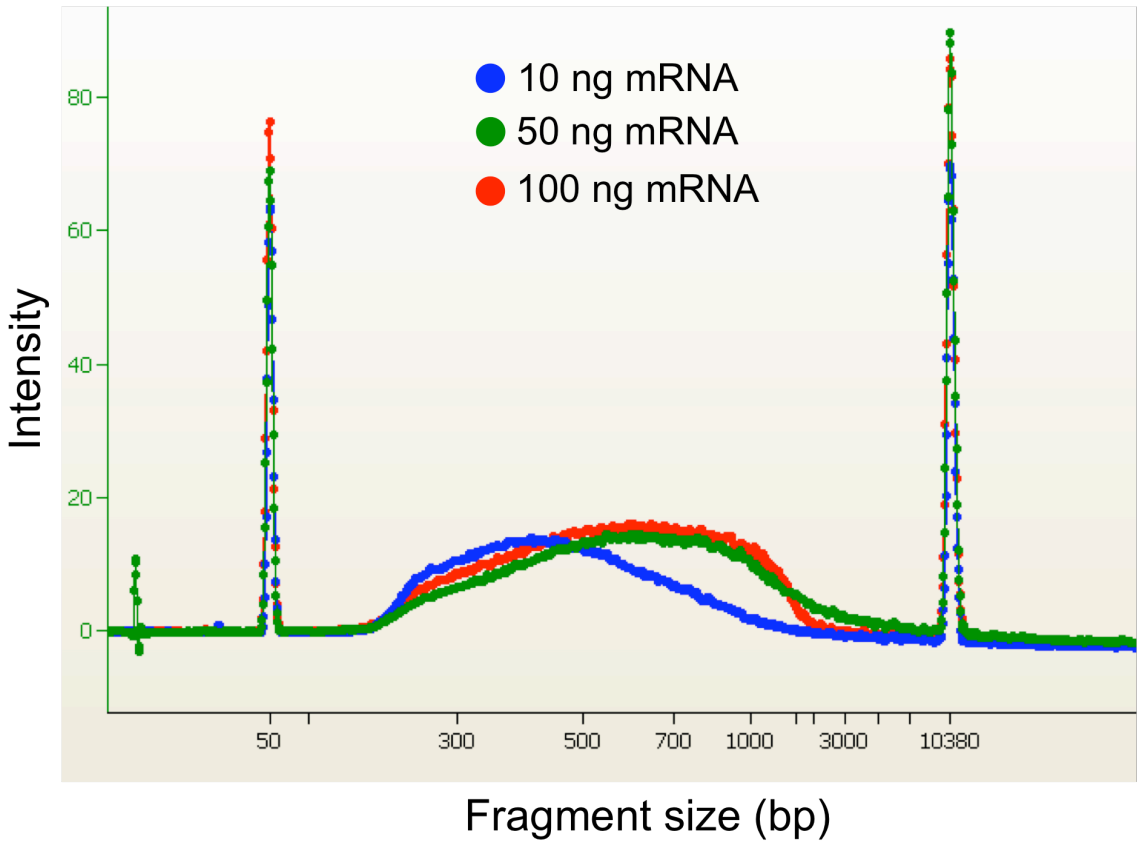
Supplementary Information

Supplemental Table S1. Low input Tn-RNA-seq comparison

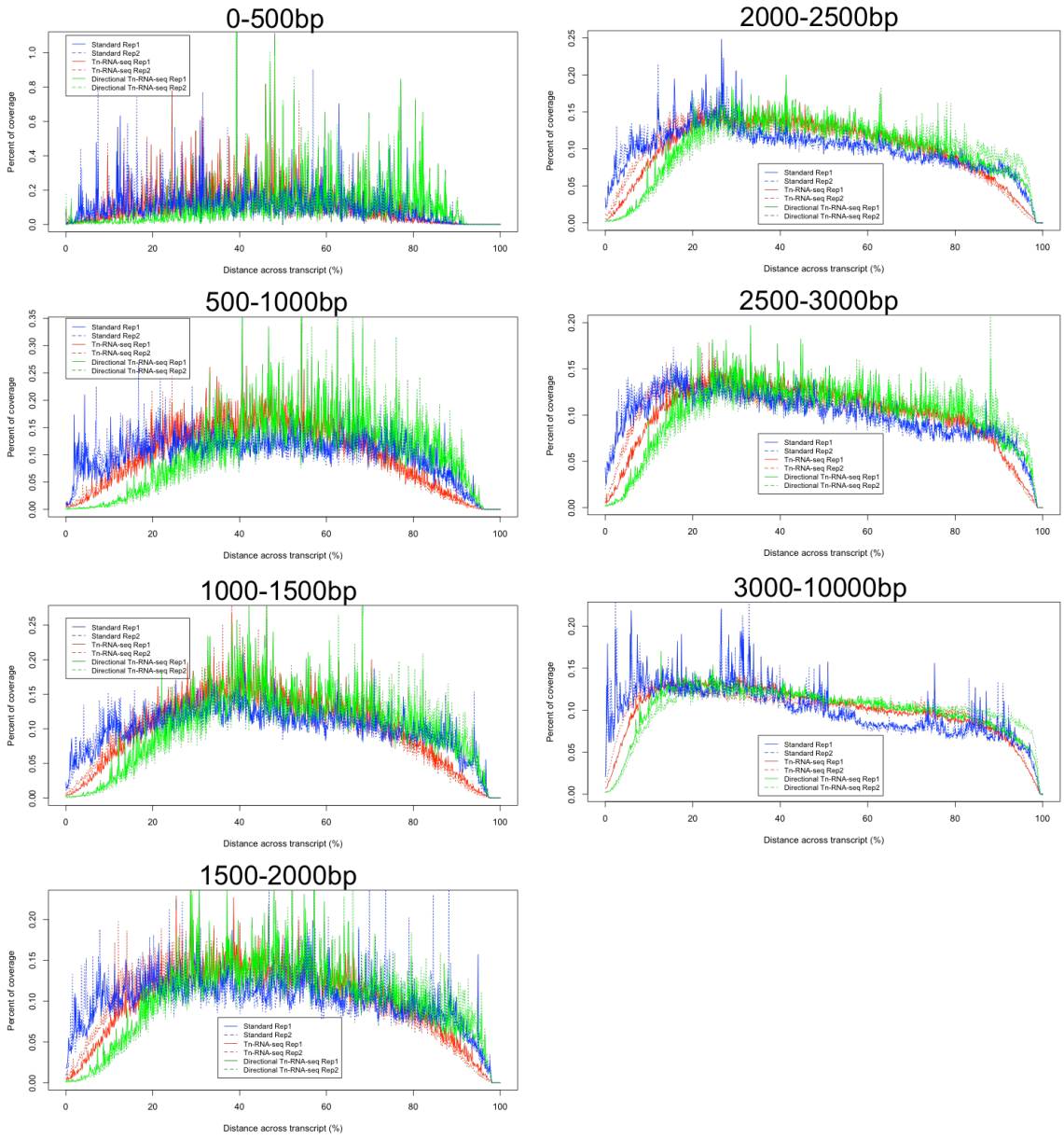
Amount of mRNA	Reads aligned to genome	Percent of aligned reads that map to RefSeq transcripts	Complexity	Rank Correlation with 50 ng library
50 ng	18,513,940	81.3%	85.20%	
10 ng	22,728,335	81.9%	89.68%	0.99
5 ng	20,703,840	72.9%	89.93%	0.98
1 ng	17,474,862	74.3%	90.22%	0.98
0.5 ng	18,742,632	73.2%	91.21%	0.98
0.1 ng	65,270,974	74.2%	91.38%	0.98
10 pg	44,001,319	73.1%	90.43%	0.96
1 pg	1,218,201	62.1%	77.70%	0.84

Supplemental Table S2. Primer and adapter sequences for Tn-RNA-seq library construction

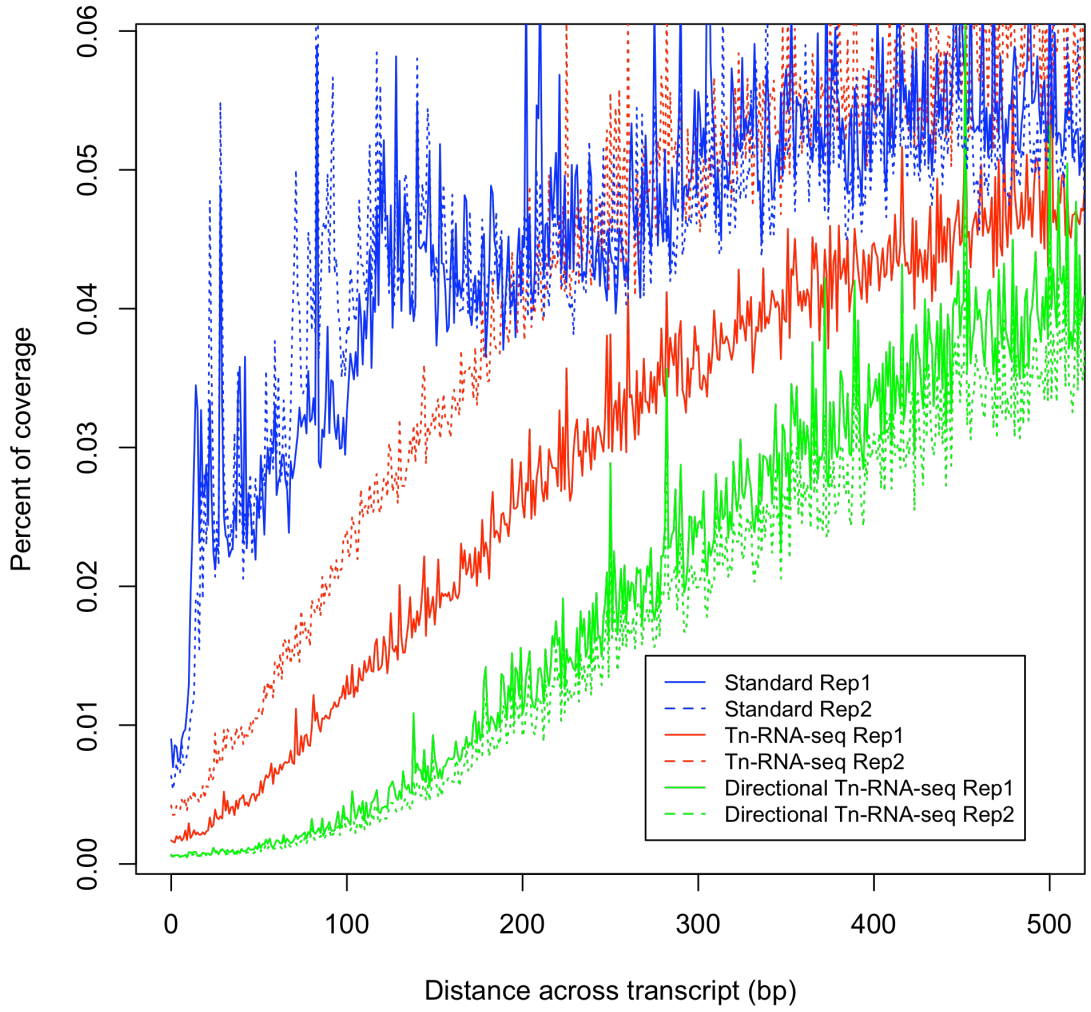
Primer	Sequence
Tn-RNA-seq Primer cocktail 1	AATGATACGGCGACCACCGA
Tn-RNA-seq Primer cocktail 2	AATGATACGGCGACCACCGAGATCTACACGCCTCCCTCGCGCCATCAG
Tn-RNA-seq Primer cocktail 3	CAAGCAGAAGACGGCATACGA
Tn-RNA-seq Adapter 2	CAAGCAGAAGACGGCATACGAGATCGGTCTGCCTTGCCAGCCCGCTCAG
Tn-RNA-seq Read 1 primer	GCCTCCCTCGCGCCATCAGAGATGTGTATAAGAGACAG
Directional Tn-RNA-seq Adapter	AATGATACGGCGACCACCGAGATCTACACAGATGTGTATAAGAGACAG
Directional Tn-RNA-seq Primer 1	AATGATACGGCGACCACCGA
Directional Tn-RNA-seq Primer 2	CAAGCAGAAGACGGCATACGAGA
Directional Tn-RNA-seq Read 1 primer	CGACCACCGAGATCTACACAGATGTGTATAAGAGACAG



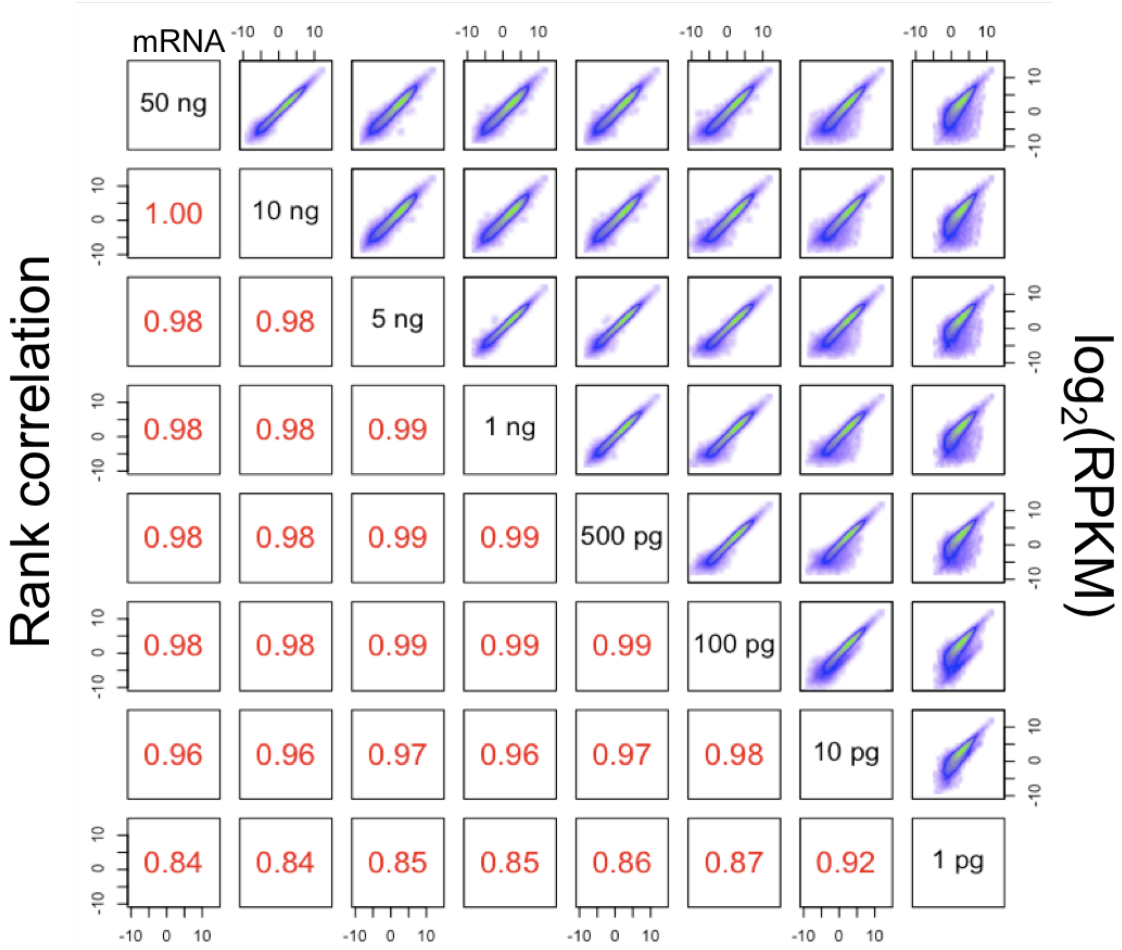
Supplemental Figure S1. Library size is influenced by amount of starting material. Bioanalyzer traces for Tn-RNA-seq libraries constructed from 10 (blue), 50 (green) and 100 ng (red) of mRNA are shown as intensity versus size in basepairs. Intensity spikes at 50 bp and 10,380 bp represent internal controls.



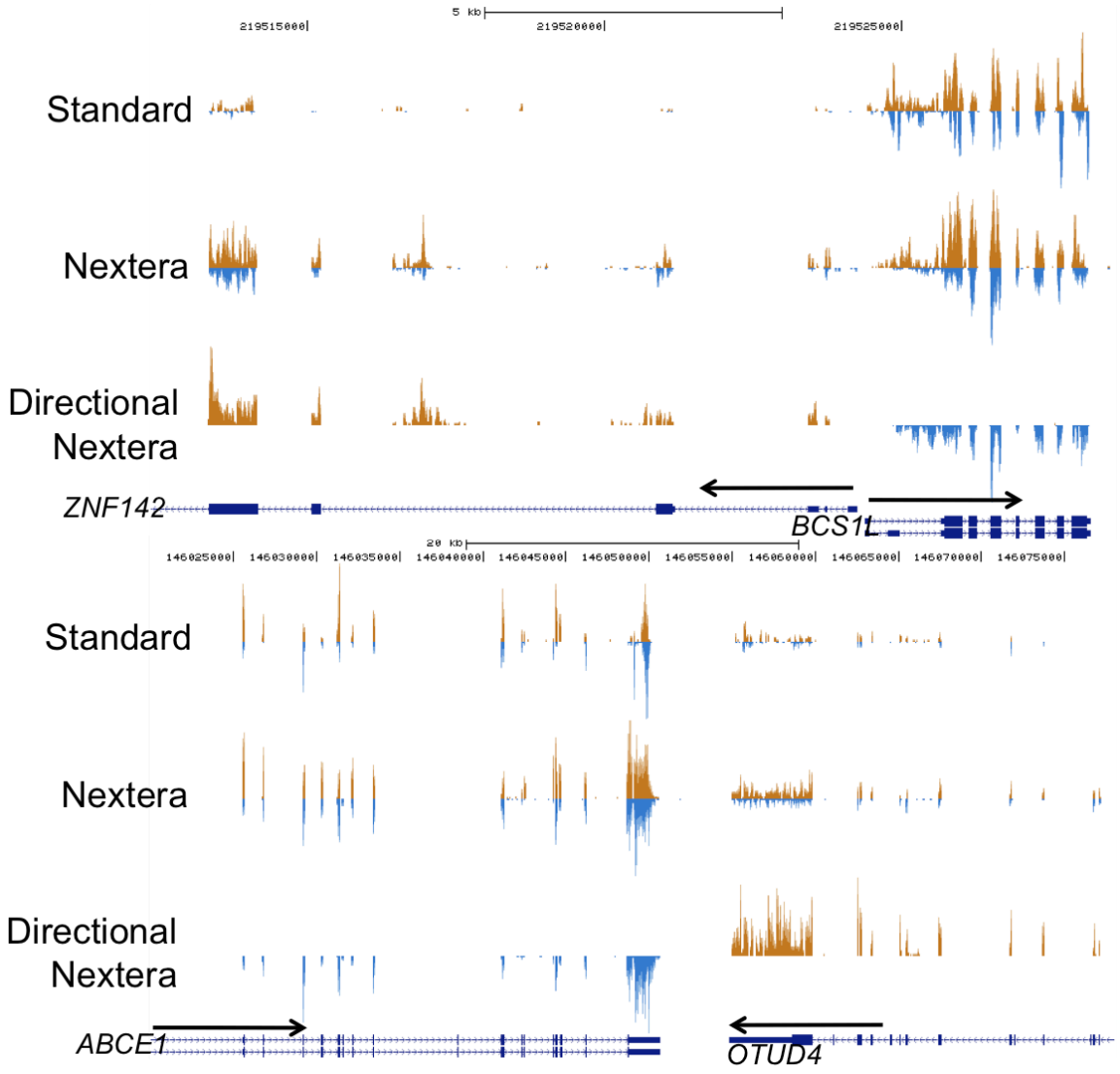
Supplemental Figure S2. Coverage across transcripts broken up by transcript length. For each graph, the percentage of coverage (y-axis), averaged across all transcripts, is plotted as a function of distance across the transcripts (x-axis). 0% corresponds to the 5' ends and 100% corresponds to the 3' ends of transcripts.



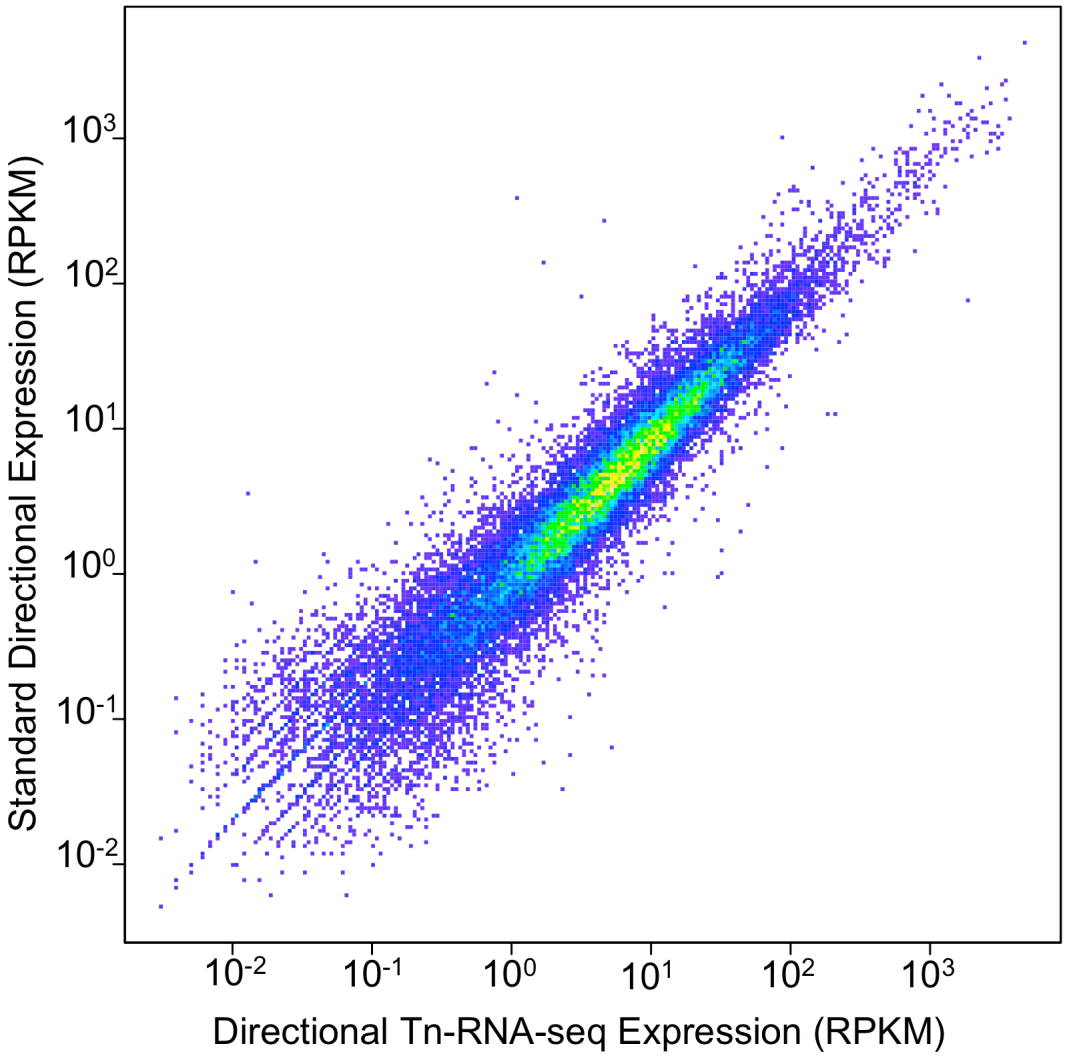
Supplemental Figure S3. Coverage across transcripts with base pair scale. For each graph, the percentage of coverage (y-axis), averaged across all transcripts greater than 1,000 bp, is plotted as a function of distance across the transcripts from the 5' end (x-axis).



Supplemental Figure S4. Changes in the amount of starting material do not alter gene expression measurements for libraries made with at least 10 pg mRNA. Expression measurements from Tn-RNA-seq libraries made with between 50 ng and 1 pg mRNA are shown as scatter plots in the upper right half. Spearman rank correlations are displayed in red in the lower left half.



Supplemental Figure S5. Examples of the Directional Tn-RNA-seq method's strand specificity. Aligned reads for the standard, Tn-RNA-seq and Directional Tn-RNA-seq method are displayed for two genomic loci. Reads mapping to the positive strand are shown in orange and reads mapping to the negative strand are shown in blue. Arrows indicate the direction of transcription for each RefSeq gene.



Supplemental Figure S6. Expression values are consistent between the Directional Tn-RNA-seq and the RNA ligation (Standard Directional) methods. Scatterplot showing expression values for Standard Directional RNA-seq library construction (y-axis) and the Directional Tn-RNA-seq library construction (x-axis). The Pearson correlation between the protocols is 0.947 and the Spearman rank correlation is 0.96.