

Figure S1. Potential applications of single-bacterium total transcript amplification methods. Many current paradigms of bacterial pathogenesis and physiology are confined within a ‘box’ based on transcriptome analyses of multiple microbial cells and, therefore, lack the resolution necessary to acquire deeper knowledge into detailed mechanisms, attainable only at the single-cell level. Hence, novel and innovative methods to isolate and define conditions for transcript amplification of single (or a few) bacterium could profoundly shift, transcend, and even establish many new paradigms.

Figure S2. Correlation of gene expression levels among amplified replicates and amplified versus non-amplified samples. (A) and (B), comparison of the gene expression levels from amplified single cells versus nonamplified sample when grown in MG. The number at the bottom right corner indicates the percentage of transcripts that were missing in the single cell compared to nonamplified pool. The Pearson correlation coefficient between the gene expression levels is shown at the upper left corner for each plot, indicating a higher correlation among amplified replicates than amplified versus non-amplified samples. (C) and (D), scatter plots of the gene expression levels from independently amplified single cells grown in MG. The number at the bottom right corner indicates the percentage of transcripts that were only detected in one of the single cell compared to all detected transcripts.

Figure S3. Construction of the cDNA libraries from amplified single cells. The amplified transcripts were fragmented with DNaseI and blunt-ended, and ds-DNA of two different size ranges (A) were purified and cloned into the SmaI site of the cloning vector pUC19

(B). Primers that anneal outside of the MCS (multiple cloning site), as indicated by blue arrows in (B), were used to confirm the representation of cDNA clones of different sizes as shown in (C). Abbreviations: C, control with pUC19 as PCR template; E and U, enriched and unenriched samples, respectively; M, 1 kb DNA marker from New England BioLabs.