

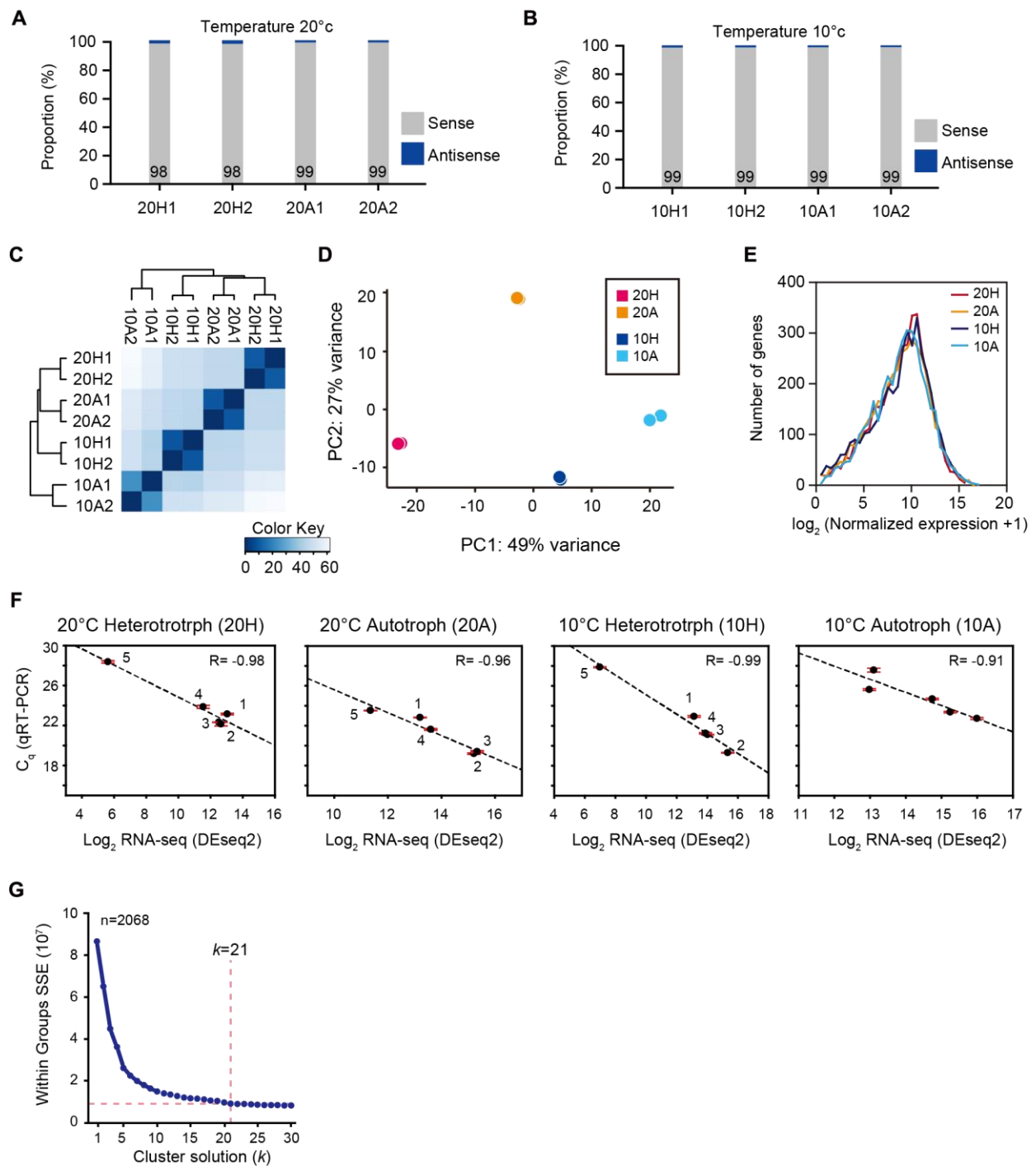


Figure S1. Reproducibility and quality of ssRNA-seq data. (A–B) Strand-specificity of ssRNA-seq data at (A) optimal temperature (20 °C) and (B) low temperature (10 °C). (C) Relationship of ssRNA-seq data among the data sets. Heat map of the sample-to-sample distance matrix is shown with hierarchical clustering using rlog-transformed read counts. (D) Principal component analysis (PCA) of ssRNA data. (E) Distribution of normalized mRNA transcript level among the four growth conditions. (F) Concordance between RNA-seq and qRT-PCR data. qRT-PCR was performed using RNA samples extracted independently from cultures grown in the same condition. The relationship between the normalized expression value from ssRNA-seq and the C_q value from qRT-PCR was analyzed for five genes. All plots show that the expression pattern is similar between ssRNA-seq and qRT-PCR data. Selected genes are represented by number: 1, ABAKI_c25770 encoding protein translocase subunit *secA*; 2, ABAKI_c13120 encoding corrinoid/iron-sulfur protein, small subunit *acsD* (from carbonyl-branch of WLP); 3, ABAKI_c24840 encoding methylenetetrahydrofolate reductase large subunit *metF*

(from the methyl-branch of the WLP); 4, ABAKI_c09070 encoding iron hydrogenase *hydA2* (Formate dehydrogenase cluster); 5, ABAKI_c24310 encoding electron transfer flavoprotein beta-subunit *etfB* (lactate dehydrogenase operon). (**G**) Error Sum of Squares (SSE) plot for estimating the appropriate K-means cluster number. SSE, which is defined as the sum of the squared distance among data points, was calculated by the following equation.

$$SSE = \sum_{j=1}^p \sum_{t=1}^k \sum_{i \in C_t} (x_{ij} - \bar{x}_j^{(t)})^2$$

Based on the SSE plot, a local minimum (elbow at the 21 cluster solution) of SSE was used as the clustering number parameter. Two thousand sixty-eight differentially expressed genes (listed in Supplemental Table S4) were used in clustering analysis.