**Benchmark**

Supplementary material for:

**Directional high-throughput sequencing of RNAs without gene-specific primers**



**Supplementary Figure S1. M13-RNA concentration changes in ligation reaction had effect on profiles of ligation product.** Tape Station HS RNA comparison analysis shows size distribution profiles of ligation products. M13-RNA concentration in ligation reaction was adjusted to 2 500-, 5 000- or 10 000-folded compared to 16S/18S rRNA concentration. Increasing M13-RNA concentration decreased self-ligation of the original 16S/18S rRNA sample. 25 nt peak refers to lower marker.



**Supplementary Figure S2. Comparison of abundances of species when analysis comprises only proportion of the sequencing reads.** When random priming was used in the cDNA synthesis, abundances of species fluctuated depending on the size fraction that was picked from the reads, due to non-continuous random priming. To avoid a possible bias, it is recommended to select a wide size fraction of the sequencing reads. Data analysis was performed using CLC Genomics Workbench 11 software ([www.qiagenbioinformatics.com](http://www.qiagenbioinformatics.com)).



**Supplementary Figure S3. Analysis of the length distribution of the random primed cDNAs.** All the cDNA products were amplified with Euk1A SSU rRNA gene specific forward primer and P1 reverse primer, and analysed using Tape Station HS D1000 (Agilent) agarose gel electrophoresis. (A) Amplification of cDNAs of *Apocalathium malmogiense*, *Monoraphidium* sp., and *Melosira arctica* shows non-continuous random priming in the reverse transcription for all the species. (B) Increasing or decreasing the number of degenerate (N) bases in the 3’-end of the P1 oligo did not prevent the pattern of non-continuous random priming in the reverse transcription, when *Monoraphidium* sp. cDNA was further amplified using the gene specific forward primer Euk1A and P1. (C) 10 % DMSO in the cDNA synthesis reactions of 18S rRNA of *Monoraphidium* sp. did not prevent the non-continuous random priming. (D) Comparison of random primed 18S rRNA (secondary structures) and luciferase protein coding RNA (linear) shows that random priming was non-continuous for both the rRNA and protein coding gene transcripts. (E) When using random oligos that were manufactured with hand-mixing (Integrated DNA Technologies, Germany), size distribution of the random amplified fragments was spread out more equally than using standard random oligos. 25 bp peak refers to the lower marker and 1500 bp peaks to the upper marker peak.