

## **Supplemental Text: DNA and RNA Extraction Methods**

### **Brazelton et al. “Metabolic strategies shared by basement residents of the Lost City hydrothermal field”**

*Note: The following text was accidentally removed from the published version of the manuscript.*

#### **Extraction and purification of DNA and RNA**

Extraction of DNA from all Sterivex filters (including *in situ*-filtered fluids and fluids collected in Kynar bags and filtered shipboard) was conducted as described previously (1, 2). The full protocol is available in the Zenodo-archived GitHub repository (DOI: 10.5281/zenodo.5798015) and on protocols.io (DOI: [dx.doi.org/10.17504/protocols.io.bykqpuvw](https://doi.org/10.17504/protocols.io.bykqpuvw)). Cell lysis was performed at 65 °C in a pH 10 extraction buffer followed by bead-beating with 0.1 mm glass beads. Lysates were purified with pH 8 phenol/chloroform/isoamyl alcohol extractions, precipitated overnight in ethanol, resuspended in low-EDTA TE, and further purified with magnetic beads (3).

Total RNA was extracted from the Sterivex filters with a modification of the DNA extraction protocol optimized for RNA. The full protocol is available in the Zenodo-archived GitHub repository (DOI: 10.5281/zenodo.5798015) and on protocols.io (DOI: [dx.doi.org/10.17504/protocols.io.bykspuwe](https://doi.org/10.17504/protocols.io.bykspuwe)). Cell lysis was performed at room temperature in a pH 7 extraction buffer, followed by bead-beating with 0.1 mm glass beads. Lysates were purified with pH 5.2 phenol/chloroform/isoamyl alcohol extractions, precipitated overnight in ethanol, resuspended in ultrapure water, and further purified with magnetic beads (3). First-strand synthesis of cDNA was performed with SuperScript IV Reverse Transcriptase and random hexamers (Thermo Fisher).

## References

1. Brazelton WJ, Thornton CN, Hyer A, Twing KI, Longino AA, Lang SQ, Lilley MD, Früh-Green GL, Schrenk MO. 2017. Metagenomic identification of active methanogens and methanotrophs in serpentinite springs of the Voltri Massif, Italy. *PeerJ* 5:e2945.
2. Thornton CN, Tanner WD, VanDerslice JA, Brazelton WJ. 2020. Localized effect of treated wastewater effluent on the resistome of an urban watershed. *GigaScience* 9:giaa125.
3. Rohland N, Reich D. 2012. Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Research* 22:939–946.