

## Sample preparation, RNA isolation, library preparation and RNA sequencing procedures for newly generated samples

Two specimens of the palpi-grade *Eukoenenia zariquieyi* (Condé, 1951) were manually captured by GMR, PBG, JLF and RF in the type locality: Coves del Salnitre, Montserrat massif, Barcelona, Spain (41°34'24.96" N, 1°50'4.81" E). Sampling permits were granted by the Fauna and Flora Service of the Generalitat de Catalunya (SF/0036/21). Specimens were immediately placed in tubes containing 50 µl lysis buffer with 1% 2-mercaptoethanol (β-ME) added and kept in cold storage for transportation to the laboratory where samples were flash-frozen and then stored at -80°C until the RNA extraction.

RNA isolation steps were performed in cold and RNase free conditions. We used the commercial HigherPurity™ Tissue Total RNA Extraction Kit (Canvax Biotech). We obtained an efficient RNA extraction from a single whole specimen of *E. zariquieyi*. Total RNA yield was quantified in a fluorometer using the Qubit® RNA HS Assay Kit (Thermo Fisher Scientific) obtaining 4.76 ng/µl.

Total RNA was shipped in dry ice to the sequencing facility. The SMART-Seq™ v4 Ultra™ Low Input RNA Kit and the TruSeq RNA Sample Prep Kit v2 were used for a stranded mRNA library preparation. A quality control check was performed using Agilent Technologies 2100 Bioanalyzer using a DNA 1000 chip. The Illumina Novaseq 6000 platform was used in paired-end mode with a read length of 2x150bp. For the raw data generation, base calling was performed with the software Real Time Analyses (RTA) and the binary data was converted to FASTQ utilizing illumina package bcl2fastq. cDNA libraries and mRNA sequencing were performed at the sequencing facility (Macrogen, Seoul, Republic of Korea).

QC result of RNA:

#	Sample Name	Conc. (ng/ul)	Final Volume (ul)	Total Amount (ug)	RIN	rRNA Ratio	Result*	
1	Euk_Collbato	14.923	15	0.224	8.2	3.9	Pass	

Library QC Result of RNA:

#	Library Name	Library Type	Conc. (ng/ul)	Conc. (nM)	Size (bp)	Result*	
1	Euk_Collbato	SMARTer Ultra low input RNA library (PolyA)	15.9	58.5	380	Pass	

Raw data statistics:

Sample ID	Total read bases (bp)	Total reads	GC(%)	AT(%)	Q20(%)	Q30(%)
Euk_Collbato	13,036,635,736	86,335,336	43.83	56.17	96.6	92.19