DATA NOTE

Novel transcriptome resources for three scleractinian coral species from the Indo-Pacific

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Abstract

Transcriptomic resources for coral species can provide insight into coral evolutionary history and stress-response physiology. \textit{Goniopora columna}, \textit{Galaxea astreata}, and \textit{Galaxea acrhelia} are scleractinian corals of the Indo-Pacific, representing a diversity of morphologies and life-history traits. \textit{G. columna} and \textit{G. astreata} are common and cosmopolitan, while \textit{G. acrhelia} is largely restricted to the coral triangle and Great Barrier Reef. Reference transcriptomes for these species were assembled from replicate colony fragments exposed to elevated (31°C) and ambient (27°C) temperatures. Trinity was used to create de novo assemblies for each species from 92–102 million raw Illumina Hiseq 2 × 150 bp reads. Host-specific assemblies contained 65 460–72 405 contigs, representing 26 693–37 894 isogroups (~genes) with an average N50 of 2254. Gene name and/or gene ontology annotations were possible for 58% of isogroups on average. Transcriptomes contained 93.1–94.3% of EuKaryotic Orthologous Groups comprising the core eukaryotic gene set, and 89.98–91.92% of the single-copy metazoan core gene set orthologs were complete, indicating fairly comprehensive assemblies. This work expands the complement of transcriptomic resources available for scleractinian coral species, including the first reference for a representative of \textit{Goniopora} spp. as well as species with novel morphology.

Keywords: \textit{Galaxea astreata}; \textit{Galaxea acrhelia}; \textit{Goniopora columna}; thermal stress; functional genomics

Data Description

Background

A growing body of genomic information for reef-building corals has resolved phylogenetic relationships and helped reveal how this unique taxonomic group calcifies and responds to thermal stress [1–4]. Such information is critical for understanding the adaptive capacity of these ecologically important organisms, particularly in an era of global climate change [5]. Transcriptomic and/or genomic resources are currently available for 23 scleractinian species representing 14 genera and 11 families [1, 4, 6–16]. We assembled the transcriptomes of 3 scleractinian coral species: the congeners \textit{Galaxea astreata}, \textit{G. acrhelia}, and \textit{Goniopora columna}. This is the first sequence resource for \textit{Goniopora} spp. and extends the phenotypic diversity represented by coral transcriptomic resources to include submassive (\textit{G. astreata}) and columnar (\textit{G. columna}) morphologies [17], which should facilitate additional insight into the evolutionary history of this taxonomic order.

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Samples and sequencing

Samples of Galaxea astreata and Galaxea acrhelia were collected from Davies Reef (18°49.816′S, 147°37.888′E) on 8–11 April 2015, and samples of Goniopora columna were collected from Pandora Reef (18°48.778′S, 146°25.593′E) on 20–22 April 2015 under Great Barrier Reef Marine Authority permit G12/35 236.1 and G14/37 318.1.

To generate more comprehensive reference transcriptomes, 4–5 replicate cores of a single colony were subjected to a 2-week temperature stress experiment as described in Kenkel and Bay (2017) [18], and paired samples from control (27°C) and heat (31°C) treatments were snap-frozen in liquid nitrogen on day 2, day 4, and day 17 (Table 1; note for G. acrhelia, heat-treated fragments were only included for day 4 and day 17). Samples were crushed in liquid nitrogen, and total RNA was extracted using an Aurum Total RNA mini kit (Bio-Rad, Irvine, CA, USA). RNA quality and quantity were assessed using the NanoDrop ND-200 UV-Vis Spectrophotometer (Thermo Scientific, Waltham, MA, USA) and gel electrophoresis.

For transcriptome sequencing, RNA samples from replicate fragments were pooled in equal proportions, and ~1 µg was shipped on dry ice to the Oklahoma Medical Research Foundation NGS Core, where Illumina TruSeq Stranded libraries were prepared and sequenced on 1 lane of the Illumina Hiseq 3000/4000 to generate 2×106 reads per sample.

Transcriptome assembly and annotation

Sequencing yielded 92–102 million raw PE reads (Table 1). The fastx_toolkit [19] was used to discard reads <50 bp or having a homopolymer run of “A” ≥9 bases, retain reads with a PHRED quality of at least 20 over 80% of the read, and to trim TruSeq sequencing adaptors. Polymerase chain reaction duplicates were then removed using a custom perl script [20]. Remaining high-quality filtered reads (26–35 million paired reads, 4–6 million unpaired reads) (Table 1) were assembled using Trinity v.2.0.6 (Trinity, RRID:SCR_013048) [21] using the default parameters and an in silico read normalization step at the Texas Advanced Computing Center at the University of Texas at Austin.

Table 1: Assembly statistics for de novo transcriptomes by coral species

|               | Galaxea astreata | Galaxea acrhelia | Goniopora columna |
|---------------|-----------------|-----------------|------------------|
| N heat        | 3               | 2               | 3                |
| N ctrl        | 2               | 2               | 2                |
| N raw reads (<10⁸) | 92.8         | 96.0            | 102.8            |
| N qual filtered: PE, SE (<10⁸) | 35.0, 5.8        | 33.3, 6.0       | 26.9, 4.7        |
| N contigs holobiont | 173,883       | 164,996         | 185,625          |
| N contigs host only | 65,460        | 67,127          | 72,405           |
| Mean GC content host only | 42.3%       | 42.1%           | 42.2%            |
| N isogroups   | 29,145          | 26,693          | 37,894           |
| Mean contig length (bp) | 1754         | 1894            | 1492             |
| NS0 (bp)      | 2300            | 2480            | 1984             |
| Contiguity at 0.75 | 0.40            | 0.41            | 0.37             |
| % annotated   | 62.4            | 60.7            | 50.1             |
| % core KOGs   | 94.3            | 94.0            | 93.1             |

BUSCOs

|               | Galaxea astreata | Galaxea acrhelia | Goniopora columna |
|---------------|-----------------|-----------------|------------------|
| N complete (%) | 880 (89.98%)     | 899 (91.92%)     | 881 (90.08%)     |
| N partial (%)  | 36 (3.68%)       | 30 (3.07%)       | 31 (3.17%)       |
| N missing (%)  | 62 (6.34%)       | 49 (5.01%)       | 66 (6.75%)       |

Since corals are “holobionts” comprised of host, Symbiodinium, and other microbial components, resulting assemblies were filtered to identify the host component following the protocol described in Kitchen et al. (2015) [4], with one modification. Briefly, small clusters (= contigs, <400bp) were removed, and a hierarchical series of blast searches against potential contaminants was conducted. First, assemblies were compared to the most complete Cnidarian RNA database (SILVA: ABAV01023297, ABAV01023333) [22] using BLASTn [23], and good matches (bit-score >45) were removed. Next, transcriptomes were compared to a Cnidarian mitochondrial genome using BLASTn (Acropora tenuis, NCBI: NC_03522.1) [24], again discarding contigs with match bit-scores >45. The taxonomic origin of remaining contigs was identified using a series of BLASTx searches against the most complete coral and Symbiodinium gene models (coral: Acropora digitifera, adi_v1.01.prot, [14]; Symbiodinium: S. kawagutii, Symbiodinium_kawagutii.0819.final.gene.pep, [25]) and NCBI’s nonredundant (nr) protein database (downloaded 25 July 2016) [23]. For a contig to remain in the host-specific assembly, it had to both match (E value ≤ 10⁻⁵) a gene in the coral proteome more closely than the Symbiodinium proteome and match a metazoan sequence or have no match in the nr database. In addition, contigs with no match to either proteome were also retained if they exhibited a best match to a Cnidarian in the nr database search, a slightly less stringent criterion than that used by Kitchen et al. (2015) [4]. Annotation of host transcriptomes was performed following the protocols and scripts described in [26]. Host contigs were assigned putative gene names and gene ontologies using a BLASTx search (E value ≤ 10⁻⁴) against the UniProt Knowledgebase Swiss-Prot database [27]. Eukaryotic Orthologous Groups (KOG) annotations were assigned using a BLAST search against the core eukaryotic gene set from the CEGMA pipeline (CEGMA, RRID:SCR_015055) [28] and the WebMGA server (WebMGA, RRID:SCR_011951; [29]) [30] and Kyoto Encyclopedia of Genes and Genomes (KEGG) IDs using the KAAS server [31, 32]. The stats.sh command of the BBMap package [33] was used to calculate GC content of host transcriptomes. Transcriptome completeness was evaluated through comparison to the Benchmarking Universal Single-Copy Ortholog v. 2 (BUSCO, RRID:SCR_015008) [34] set for metazoans using the gVolante server [35, 36].
Evaluation of assemblies

The initial holobiont assemblies contained 164 996–185 625 contigs over 400 bp in length ($N_{50} = 1543–1848$). Of these, 34–94 were discarded as matching non-mRNAs (9–10 rRNA, 25–74 mitochondrial). Following screening for biological contamination, 64 249–68 968 contigs had a best match to the Acropora digitifera proteome, and of these, 59 875–65 367 matched either a metazoan or had no match in NCBI’s nr database. An additional 5585–7038 contigs matched neither proteome but exhibited a best hit to a Cnidarian in the nr database and were also retained. These host-specific assemblies represented 26 693–37 894 isogroups (~genes) with an average length of 1492–1894 bp and an N50 of 1984–2480 (Table 1). Mean GC content of host-specific assemblies was 42% (Table 1), which is consistent with other anthozoan transcriptomes where Symbiodinium reads have been effectively filtered [16]. Protein coverage exceeded 0.75 for 37–41% of contigs (Table 1). Gene name and/or gene ontology annotations were possible for 16 196–19 306 (50.1–62.4%) of these isogroups based on sequence homology comparisons to the Swiss-Prot database (Table 1) [27]. KEGG pathway annotation [32] resulted in 4488–4728 unique matches for 7105–8712 isogroups. Comparison of these assemblies to the core eukaryotic 248-gene set [28] revealed that 93.1–94.3% of KOGs were represented, and annotation of isogroups resulted in 23–24 unique KOG matches for 8700–10 025 isogroups (Table 1). Of the 978 core BUSCO gene sets for metazoans [34], 89.98–91.92% were found to be complete, while an additional 3.07–3.68% were partially assembled, indicating that assemblies are fairly comprehensive (Table 1).

Re-use potential

These coral host-specific assemblies are sufficient for use as transcriptome references for Tag-based RNAseq (TagSeq) [37], a cost-effective method that was recently shown to be more accurate at quantifying gene expression levels than traditional RNAseq [38]. The fasta files and associated annotation files have been formatted for direct use in the TagSeq read mapping [39] and GO-MWU analysis pipelines [40].

Data accessibility

Raw reads are archived at NCBI’s SRA under project numbers PRJNA350363: Goniopora columna; PRJNA352640: Galaxea archelia; PRJNA352641: Galaxea astreata. Transcriptomes, annotation files, and other supporting data are available via the Gigascience repository, GigaDB [41]. The assembled transcriptomes and associated annotation files can also be obtained from http://dornsife.usc.edu/labs/carllab/data/ or from the Australian Institute of Marine Science Data Centre at http://data.aims.gov.au/metadata/viewer/faces/view.xhtml?uuid=3c2d31c9-b921-491c-ae27-0d169fa98c94.

Abbreviations

KEGG: Kyoto Encyclopedia of Genes and Genomes; KOG: EuKaryotic Orthologous Groups; TagSeq: Tag-based RNAseq.

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Competing interests

The authors have no competing interests to declare.

Author contributions

C.D.K. conceived and designed the experiments; C.D.K. and L.K.B. performed the experiments; C.D.K. performed bioinformatics analyses and wrote the first draft. L.K.B. contributed to revisions and read and approved the final manuscript.

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