

LABORATORY OF GENOME INFORMATICS



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The accumulation of biological data has recently been accelerated by various high-throughput “omics” technologies including genomics, transcriptomics, and proteomics. The field of genome informatics is aimed at utilizing this data, or finding the principles behind it, to understand complex living systems by integrating the data with current biological knowledge via the use of various computational techniques. In this laboratory we focus on developing computational methods and tools for comparative genome analysis, which is a useful approach for finding functional or evolutionary clues to interpret the genomic information of various species.

The current focus of our research is the comparative analysis of microbial genomes, the number of which has been increasing rapidly. Since different types of information about biological functions and evolutionary processes can be extracted by comparing genomes at different evolutionary distances, we are developing methods to conduct comparative analyses not only of distantly related but also closely related genomes.

I. Microbial genome database for comparative analysis

The microbial genome database for comparative analysis (MBGD, <http://mbgd.genome.ad.jp/>) is a comprehensive platform for microbial comparative genomics. The central function of MBGD is to create orthologous groups among multiple genomes using the DomClust program combined with the DomRefine program. Through the application of these programs, MBGD not only provides comprehensive orthologous groups among the latest genomic data, but also allows users to create their own ortholog groups on the fly by using a specified set of organisms. MBGD also has pre-calculated ortholog tables for each major taxonomic group, and provides several viewing modes to display the entirety of each ortholog table. For some closely related taxa, MBGD provides conserved synteny information calculated using the CoreAligner program. MBGD additionally provides a ‘MyMBGD’ mode, which allows users to add their own genomes to MBGD.

MBGD now contains 6318 genomes, including 5861 bacteria, 254 archaea, and 203 eukaryota. These data sets are classified based on the hierarchical ortholog classification strategy described in the section below.

II. Hierarchical strategy for creating ortholog tables

MBGD previously calculated all-against-all similarities among the stored genomes and independently created two types of ortholog tables: the standard ortholog table containing one representative genome from each genus covering the entire taxonomic range, and the taxon specific ortholog tables containing the genomes belonging to each taxonomic

group (species, genus, family and so on). Although this approach enabled us to effectively calculate a set of ortholog tables for both closely related and distantly related genome comparisons, a considerable amount of information may be lost from the standard ortholog table, given that within-species and within-genus genomic diversity is generally large.

To address this problem, we developed a stepwise protocol to construct orthologous relationships. First, for each species with at least two genomes, all-against-all similarities among the genomes belonging to that species are calculated and a within-species ortholog table is created. The species-level pan-genome is then created by picking one representative gene from each orthologous group. Next, for each genus with at least two species, all-against-all similarities among the species-level pan-genomes are calculated and a within-genus ortholog table is created. The genus-level pan-genome is then created by picking one representative gene from each orthologous group. Finally, all-against-all similarities among the genus-level pan-genomes are calculated and the standard ortholog table covering the entire taxonomic range is created. To calculate within-species or within-genus all-against-all similarities, we used a faster, but less sensitive, similarity search program, UBLAST. Using this method, we can reduce the computation time required for all-against-all similarities.

III. Orthologous gene classification among multiple genomes at the domain level

As a core technology of our comparative genomics tools, we have developed a rapid automated method of ortholog grouping, named DomClust, which allows us to simultaneously compare numerous genomes. This method classifies genes based on a hierarchical clustering algorithm using all-against-all similarity data as an input, but it also detects domain fusion or fission events and splits clusters into domains when required. As a result, the procedure can split genes into the domains minimally required for ortholog grouping.

We have also developed a procedure to refine the DomClust classification based on multiple sequence alignments instead of pairwise sequence alignments. The method is based on a scoring scheme, the domain-specific sum-of-pairs (DSP) score, which evaluates domain-level classification using the sum total of domain-level alignment scores. On the basis of this idea, we have developed a refinement pipeline to improve domain-level clustering, DomRefine, by optimizing DSP scores. DomRefine is now used to construct the standard ortholog table covering all the representative genomes stored in MBGD.

Domain-level classification is a unique feature of our ortholog classification system. In fact, it is different from conventional domain databases like Pfam in that it is based on orthology instead of homology. In particular, this data is considered suitable for analyzing domain fusion events that have occurred during evolution. By analyzing the domain-level ortholog grouping data combined with taxonomic and functional information, we are now trying to elucidate when and in what kind of genes domain fusion events frequently occur.

IV. Development and application of a workbench for comparative genomics and transcriptomics

We have developed a comparative genomics workbench named RECOG (Research Environment for Comparative Genomics), which aims to extend the current MGD system by incorporating more advanced analysis functionalities. The central function of RECOG is to display and manipulate large-scale ortholog tables. The ortholog table viewer is a spreadsheet like viewer that can display an entire ortholog table containing more than a thousand genomes. In RECOG, several basic operations on the ortholog table are defined, which are classified into categories such as filtering, sorting, and coloring. By combining these basic operations, various comparative analyses can be performed. In addition, RECOG allows the user to input arbitrary gene properties and compare these properties among orthologs in various genomes.

We are continuing to develop the system and apply it to various genome comparison studies as part of various collaborative research projects. Among these, we applied RECOG to the comparative analyses of transcriptomic data of *Chattonella antiqua* and other harmful algae causing red tide in collaboration with Dr. Shikata (FRA). In this analysis, we compared two newly determined and three existing RNA-seq datasets of harmful algae and 10 existing genome sequences of various algae and a plant model organism, *Arabidopsis thaliana* using RECOG, and identified common sequence features among orthologous genes belonging to harmful algae that may be related to red tide outbreaks and their toxicity to fish.

V. Ortholog data representation using the Semantic Web technology to integrate various microbial databases

Orthology is a key to integrating knowledge of various organisms through comparative analysis. In order to integrate genomic data and various types of biological information with this idea, we have constructed an ortholog database using Semantic Web technology. To formalize the structure of the ortholog information in the Semantic Web, we developed an ortholog ontology (OrthO) and described the ortholog information in MGD in the form of the Resource Description Framework (RDF). OrthO is now superseded by the Orthology Ontology (ORTH) that integrates OrthO and other ontologies and is adopted by other orthology resources.

On the basis of this framework, we have integrated various kinds of microbial data using the ortholog information as a hub, as part of the MicrobeDB.jp project (<http://microbedb.jp/>) under the auspices of the National Bioscience Database Center.

VI. A novel approach for identification of genomic islands

Genomes of bacterial species can show great variation in their gene content, and thus systematic analysis of the entire gene repertoire, termed the “pan-genome”, is important for understanding bacterial intra-species diversity. As we have already developed a procedure (CoreAligner) to define the

core genome as the genes conserved among the genomes of the given species, characterizing the remaining part of the genomes (non-core genomes) should be important for understanding the species’ diversity. To this end, we are developing a method (FindIsland) to identify a set of non-core genes that have conserved gene order by using a modified version of the CoreAligner algorithm (Figure 1). We applied the method to the sets of genomes of prokaryotic species stored in MGD and found that in many cases the resulting conserved clusters correspond to known mobile genetic elements and/or have sequence features common to known genomic islands.

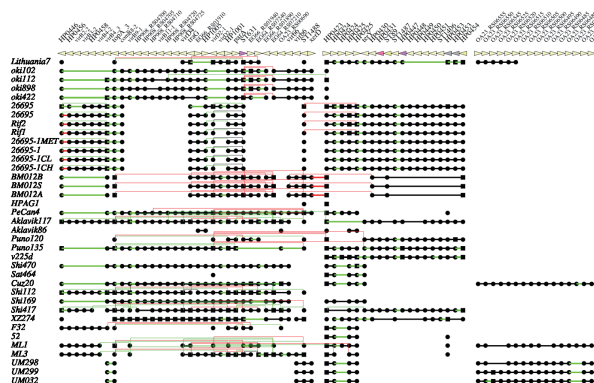


Figure 1. Visualization of the genomic islands identified by the FindIsland program.-

Publication List:

[Original papers]

- Matsunami, M., Suzuki, M., Haramoto, Y., Fukui, A., Inoue, T., Yamaguchi, K., Uchiyama, I., Mori, K., Tashiro, K., Ito, Y., Takeuchi, T., Suzuki, K.-I.T., Agata, K., Shigenobu, S., and Hayashi, T. (2019). A comprehensive reference transcriptome resource for the Iberian ribbed newt *Pleurodeles waltl*, an emerging model for developmental and regeneration biology. *DNA Res.* 26, 217-229. doi: 10.1093/dnares/dsz003
- Nagao, R., Kato, K., Suzuki, T., Ifuku, K., Uchiyama, I., Kashino, Y., Dohmae, N., Akimoto, S., Shen, J.-R., Miyazaki, N., and Akita, F. (2019). Structural basis for energy harvesting and dissipation in a diatom PSII-FCPII supercomplex. *Nat. Plants* 5, 890-901. doi: 10.1038/s41477-019-0477-x
- Shikata, T., Takahashi, F., Nishide, H., Shigenobu, S., Kamei, Y., Sakamoto, S., Yuasa, K., Nishiyama, Y., Yamasaki, Y., and Uchiyama, I. (2019). RNA-Seq analysis reveals genes related to photoreception, nutrient uptake, and toxicity in a noxious red-tide raphidophyte *Chattonella antiqua*. *Front. Microbiol.* 10, 1764. doi: 10.3389/fmicb.2019.01764
- Uchiyama, I., Mihara, M., Nishide, H., Chiba, H., and Kato, M. (2019). MGD update 2018: Microbial genome database based on hierarchical orthology relations covering closely related and distantly related comparisons. *Nucleic Acids Res.* 47, D382-D389. doi: 10.1093/nar/gky1054