

# ADAPTdb/ADAPT - A Framework for the Analysis of ARISA Data Sets

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## Introduction

A goal of microbial community profiling projects is to understand the influence of environmental changes on microbial communities. Here, we present a computational system, consisting of the database ADAPTdb and the program ADAPT, for the analysis of Automated Ribosomal Intergenic Spacer Analysis (ARISA) data sets. ARISA is a method for analyzing the composition of microbial communities that is both faster and cheaper than other community profiling techniques. The method relies on the analysis of intergenic regions called Internal Transcribed Spacers (ITS), which are located between the 16S and 23S rRNA genes. ARISA has been used to analyze several bacterial communities, including samples from freshwater, marine, and terrestrial environments. It is used for clinical studies to detect and identify microbes species.

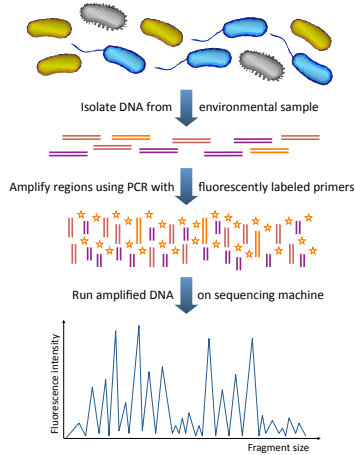


Figure 1. Basic approach of ARISA

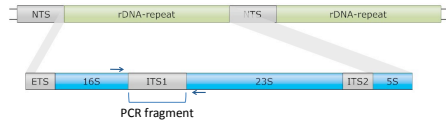


Figure 2. Arrangement of prokaryotic 16S, 23S and 5S rDNA in a tandem repetitive cluster. NTS stands for non-transcribed spacer, ETS for external transcribed spacer and ITS for internal transcribed spacer.

## ADAPTdb database

- ADAPTdb is a collection of annotated 16S-ITS-23S regions along with information about their source organisms using a MySQL database as data storage backend
- Content is based on data from NCBI resources, The SEED database, and literature
- Data currently stored in ADAPTdb includes: data source, accession and version information, organism name, taxonomy, trophic classification, pathogenesis, host description, ITS and flanking 16S/23S region sequence

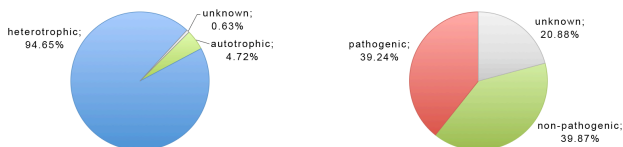


Figure 3. Trophic and pathogenic composition of organism entries in ADAPTdb.

Table 1. Overview of the taxonomic composition of ADAPTdb organism entries.

Kingdom	Phylum	# Organisms	# 16S-ITS-23S regions	Regions per organism
Archaea	Crenarchaeota	22 (2.31%)	24 (0.59%)	1.09
Archaea	Euryarchaeota	35 (3.67%)	93 (2.29%)	2.66
Archaea	Korarchaeota	1 (0.10%)	1 (0.02%)	1.00
Bacteria	Actinobacteria	85 (8.92%)	224 (5.51%)	2.64
Bacteria	Bacteroidetes/Chlorobi group	34 (3.56%)	106 (2.60%)	3.12
Bacteria	Chlamydiae/Verrucomicrobia group	17 (1.77%)	35 (0.85%)	2.06
Bacteria	Cyanobacteria	41 (4.30%)	97 (2.38%)	2.37
Bacteria	Firmicutes	199 (20.88%)	1435 (35.27%)	7.21
Bacteria	Proteobacteria	458 (48.06%)	1927 (47.36%)	4.21
Bacteria	Tenericutes	21 (2.20%)	51 (1.25%)	2.43
Bacteria	Rare bacterial spp.	40 (4.17%)	76 (1.84%)	1.70
<b>Total</b>		<b>953</b>	<b>4069</b>	<b>4.27</b>

Table 2. Number of regions and organisms matching different primer sets that were used for the ARISA analysis. The numbers are based on exact matches for the kingdom Bacteria.

Number of	Value for primer set		
	1406f / 23Sr	ITSf / ITSReub	S-D-Bact-1522-b-S-20 / L-D-Bact-132-a-A-18
Regions matching primer set	3441 (87 %)	1433 (36 %)	0
Regions not matching primer set	510 (13 %)	2518 (64 %)	3951 (100 %)
Organisms matching primer set with all regions	745 (78 %)	390 (41 %)	0
Organisms matching primer set with any regions	764 (80 %)	401 (42 %)	0

## ADAPT program

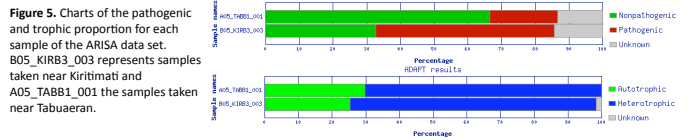
- Presents a web-based system for the analysis of ARISA data sets
- Taxonomically characterizes ARISA data sets using the ADAPTdb database
- Performs pathogenic and autotrophic/heterotrophic comparisons

Figure 4. Interactive web interface facilitates data input and parameter setup (left), as well as navigation through the output of the analysis results (right).

Try out ADAPT on the web at [edwards.sdsu.edu/adapt](http://edwards.sdsu.edu/adapt)

## Application example

Water samples were collected in 2005 at the coral reefs near the two Northern Line Islands Kiritimati and Tabuaeran. The islands lie within the same biogeographic region, but show a gradient of human disturbance. The population gradient (2005 census: Tabuaeran 2539, Kiritimati 5115) was used to investigate the influence of human activities on the microbes living in the coral reefs. Bacteria were isolated by filtering about 1 liter of seawater through a filter. The DNA was then isolated from the bacterial cells as described in Dinsdale *et al.* (2008), one sample per island processed using ARISA as described in Fisher *et al.* (1999), and analyzed using the ADAPT program.



The results identified that a range of potentially pathogenic bacterial species were present near the two islands:

### Kiritimati:

- Bdellovibrio bacteriovorus* HD100
- Coxiella burnetii* spp.
- Laribacter hongkongensis*
- Microcystis aeruginosa* NIES-843
- Pseudomonas syringae* pv. tomato str. DC3000
- Renibacterium salmoninarum* ATCC 33209
- Shigella flexneri* 5 str. 8401
- Streptococcus pneumoniae* SP14-BS69
- Xanthomonas campestris* pv. vesicatoria str. 85-10
- Xylella fastidiosa* spp

### Tabuaeran:

- Brucella abortus* S19
- Brucella abortus* bv. 1 str. 9-941
- Brucella ovis* ATCC 25840
- Brucella suis* 1330
- Granulibacter bethesdensis* CGDNIH1
- Lawsonia intracellularis* PHE/MN1-00
- Streptococcus pneumoniae* SP14-BS69

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