RiboaptDB: A Comprehensive Database of Ribozymes and Aptamers
Venkata Thodima, Mehdi Pirooznia and Youping Deng*

Address: Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, Mississippi 39406, USA
Email: Venkata Thodima - venkata.thodima@usm.edu; Mehdi Pirooznia - mehdi.pirooznia@usm.edu; Youping Deng* - youping.deng@usm.edu
* Corresponding author

Abstract

**Background:** Catalytic RNA molecules are called ribozymes. The aptamers are DNA or RNA molecules that have been selected from vast populations of random sequences, through a combinatorial approach known as SELEX. The selected oligo-nucleotide sequences (~200 bp in length) have the ability to recognize a broad range of specific ligands by forming binding pockets. These novel aptamer sequences can bind to nucleic acids, proteins or small organic and inorganic chemical compounds and have many potential uses in medicine and technology.

**Results:** The comprehensive sequence information on aptamers and ribozymes that have been generated by *in vitro* selection methods are included in this RiboaptDB database. Such types of unnatural data generated by *in vitro* methods are not available in the public ‘natural’ sequence databases such as GenBank and EMBL. The amount of sequence data generated by *in vitro* selection experiments has been accumulating exponentially. There are 370 artificial riboyme sequences and 3842 aptamer sequences in the total 4212 sequences from 423 citations in this RiboaptDB. We included general search feature, and individual feature wise search, user submission form for new data through online and also local BLAST search.

**Conclusion:** This database, besides serving as a storehouse of sequences that may have diagnostic or therapeutic utility in medicine, provides valuable information for computational and theoretical biologist. The RiboaptDB is extremely useful for garnering information about *in vitro* selection experiments as a whole and for better understanding the distribution of functional nucleic acids in sequence space. The database is updated regularly and is publicly available at http://mfgn.usm.edu/ebl/riboapt/.

Background

Until about 25 years ago, all known enzymes were proteins. But then it was discovered that some RNA molecules also have enzymatic property; that is, catalyze covalent changes in the structure of substrates (most of which are also RNA molecules) [1-3]. Catalytic RNA molecules are called ribozymes. Since the discovery of ribozymes that exist in living organisms, there has been a lot of interest in
the study of new synthetic ribozymes made in the laboratory. First Tang and Breaker [4] lab isolated self-cleaving RNAs originating from random-sequence RNAs by using *in vitro* selection method. A large number of self-cleaving RNAs have been produced that have good enzymatic activity [5-7]. Some of the synthetic ribozymes that were produced had novel structures, while some were similar to the naturally occurring hammerhead ribozyme [2,8].

The aptamers are DNA or RNA molecules, possessing desirable affinity, selected by SELEX – Systemic Evolution of Ligands by Exponential enrichment method. This SELEX method is an *in vitro* iterative process that isolates binding aptamers from the random pool and amplifies each sequence by the polymerase chain reaction after each round of isolation [9-16]. The selected oligo-nucleotide sequences (~200 bp in length) have the ability to recognize specific ligands by forming binding pockets and can bind to nucleic acids, proteins or small organic, inorganic chemical compounds and even small organisms like viruses [17-25].

Aptamers are a promising class of compounds, both for target validation and therapy. As designer drugs, they exhibit high specificity, high affinity, and modifiable bioavailability [26-30]. The ability to generate inhibitors with such properties against a variety of target proteins will be invaluable as the human genome and proteome are deciphered [12,31-37].

The RiboaptDB is not only extremely useful both for identifying available aptamers and artificial ribozymes. It is also useful for acquiring information about *in vitro* selection experiments like the type of the nucleic acid, type of the target and conditions of the experiment as a whole and for better understanding the distribution of functional nucleic acids in the given sequence space. Like other types of sequences, the amount of sequences generated by *in vitro* selection experiments has been accumulating exponentially [10,14]. The sheer number and diversity of selection experiments has risen to the point where it is now essential to gather all the sequence data into a comprehensive, continuously updated database. The general sequence databases like GenBank, EMBL and DDBJ do not maintain the complete collection of artificial nucleic acid sequences like aptamer and ribozyme. Another database, 'Aptamer database' also contains lot of information on this type of data but not regularly updating with new data [38,39].

**Construction and Content**

**Structure and implementation**

The design of the RiboaptDB database schema follows the three level schema architecture as shown in the Figure 1. The database has been implemented using open source relational database MySQL [40] server version 4.1. The user-friendly web interface has been developed by using PHP language [41]. The web server is an open source Apache web server version 2.0. The figure 2 shows the Entity-Relationship (ER) schema of the database.

The "sequence" table is the key table in the database to which all other tables are related directly or indirectly. This table contains the sequence ID and relates directly with its child tables, "aptamer" and "ribozyme", which contains the corresponding sequence information. The other important tables in the database are "publication" and "experiment" which store the citation information like title, journal name, authors, pubmed ID and experiment details like template type and experiment conditions respectively. The target specific information, the target name and its category (‘organic’, ‘inorganic’, ‘nucleic’, ‘peptide’, ‘protein’ and ‘other’) obtained from the "target" table. If any information about non-canonical base pair is available, it can be retrieved through the "non-canonical" table.

**Content**

RiboaptDB is relatively small database but is, nonetheless, essentially complete. The data was sourced from a previous compilation and exhaustive searching of the primary literature. The current size of the database 4212 sequences from 423 citations.

In this, there are 370 artificial ribozyme sequences and 3842 aptamer sequences in the total 4212 sequences. The database is updated every month as new literature comes
on aptamers and artificial ribozyme sequences. The initial collection of data is done through searching the NCBI-Pubmed for the literature with keywords like 'artificial ribozymes', 'ribozyme', 'aptamers', 'SELEX' etc. The usefulness of a database is governed by the accuracy of the data it contains. The data in this database is compiled manually from previous published, peer-reviewed articles, and verified.

Utility and Discussion
RiboaptDB provides users with an easy-to-use web interface with flexibility to select either ribozyme or aptamer sequences to browse the corresponding information. Beginning at the welcome page (Figure 3), the user can navigate via the top menu or the browse database tables on the side menu. A brief description about the navigation is given below.

Search
The general complete search option provides an interface for a variety number of queries to the database. It can be used to search the database for sequence, experiment, target, author, publication and non-canonical along with either ribozyme or aptamer or both and also either natural or artificial type of sequences.

Local Blast
The local Blast option can be used to do blast search against the local archived data to perform sequence-similarity searches using the BLAST family of programs (Figure 4). This will useful to user to know the most similar sequences to the submitted sequences and also useful to know further information about its target and experiment details (Figure 5).

Submit data
It facilitates online sequence submission to the database. It allows users to fill in a form containing new sequence information.

Figure 2
The schema and the dataflow of the RiboaptDB.
information along with user details (Figure 6). Related information can also be submitted through uploading a text file. The data which is then saved into a directory on the server side and an email is sent automatically to the curator who then checks the data to make sure there are no errors and then the information is loaded into the database automatically.

Alternate to the general search option on top menu, there is a search option on the side menu on the home page to search the whole database on a specific keyword. Also, specific table search is available on side menu of each related pages. The user can also retrieve the selected sequences into a text file for further studies.

The idea behind the combining of ribozymes and aptamers data into one database is, increasing the chance of generating ribozymes with modified and novel properties [26]. One example is combining both the ‘target identification’ of aptamer and ‘catalytic activity’ of ribozymes into a commercial ‘riboswitch’ application [42-45].

**Future Perspectives**

RiboaptDB project is young. With respect to future work, the database needs to be maintained and developed regularly, ensuring our links to external databases remain up to date and newly published data is added. Initially, as with all databases, random errors will have occurred due to human error during the data accumulation or will be extant within the original experimental data. The database will be assessed for errors and inconsistencies, thus maintaining, as far as possible, the overall veracity of our data.
Data Submission

Submit your new sequence data by using the form below:
(The data will be added to the database after verification)

Your Information:
- Name:
- Institution:
- Address:
- Phone:
- Email:

Data submission:
- Sequence class (Ribozyme / Aptamer)
- Target Information

Experiment details:
- Sequence:
- Any more information:

Citation Information:
- Any more information:

Attach information file (optional):
- Browse:

Submit | Reset

Figure 6
View of the data submission form by the user.

Conclusion
The goal of RibopartDB constructors was the collection of all ribozyme and aptamer sequences that have appeared to date and their detailed and correct annotation. The ease of access to the data is of great importance and the bespoke search system and the inclusion of a BLAST search greatly facilitates this. The better the organisation of the data, the easier the work will be for researchers dealing with aptamers and ribozymes.

Availability and Requirements
RibopartDB was created and is maintained in the Department of Biological Sciences at the University of Southern Mississippi. It is publicly available at the http://mfgn.usm.edu/ebl/riboapt/.

List of abbreviations
- SELEX: Systemic Evolution of Ligands by Exponential enrichment
- BLAST: Basic Local Alignment Search Tool
- EMBL: European Molecular Biology Laboratory
- DDBJ: DNA Data Bank of Japan

Authors’ contributions
VT and MP both participated in the design and implementation of the study of the database. VT conceived of the study, worked on identification of relevant data sources, comprehensive data annotation and drafted the manuscript. MP carried out the design and development of the user interface of the database. YD coordinated and directed the project and revised the manuscript. All authors have read and approved the final manuscript.

Acknowledgements
This work was supported by Dean’s Research Initiative award of the University of Southern Mississippi to Youping Deng and the Mississippi Functional Genomics Network (DHHS/NIH/NCRR Grant# 2P20RR016476-04).

References
1. Jaeger L: The New World of ribozymes. Curr Opin Struct Biol 1997, 7(3):324-335.
2. Kumar PK, Ellington AD: Artificial evolution and natural ribozymes. Faseb J 1995, 9(12):1183-1195.
3. Stull RA, Szoka FC Jr: Antigene, ribozyme and aptamer nucleic acid drugs: progress and prospects. Pharm Res 1995, 12(4):465-483.
4. Tang J, Breaker RR: Rational design of allosteric ribozymes. Chem Biol 1997, 4(6):453-459.
5. Murakami H, Suga H: [Artificial ribozymes: selection and application for nonnatural amino acid mutagenesis]. Tanpakushitsu Kakusan Koso 2003, 48(11 Suppl):1511-1518.
6. Atkins D, Gerlach WL: Artificial ribozyme and antisense gene expression in Saccharomyces cerevisiae. Antisense Res Dev 1994, 4(2):109-117.
7. Jaschke A: Artificial ribozymes and deoxyribozymes. Curr Opin Struct Biol 2001, 11(3):321-326.
8. Joseph S, Berzal-Herranz A, Chowira BM, Butcher SE, Burke JM: Substrate selection rules for the hairpin ribozyme determined by in vitro selection, mutation, and analysis of mismatched substrates. Genes Dev 1993, 7(1):130-138.
9. Brackett DM, Dieckmann T: Aptamer to Ribozyme: The Intrinsically Catalytic Potential of a Small RNA. ChemBioChem 2006, 7(5):839-843.
10. Nimjee SM, Rusconi CP, Sullenger BA: Aptamers: an emerging class of therapeutics. Annu Rev Med 2005, 56:535-583.
11. Sen D: Aptamer rivalry. Chem Biol 2002, 9(8):851-852.
12. White RR, Sullenger BA, Rusconi CP: Developing aptamers into therapeutics. J Clin Invest 2000, 106(8):929-934.
13. Brody EN, Gold L: Aptamers as therapeutic and diagnostic agents. J Biotechnol 2000, 74(5-13).
14. Kug SJ, Fumulok M: All you wanted to know about SELEX. Mol Biol Rep 1994, 20(2):97-107.
15. Eaton BE: The joys of in vitro selection: chemically dressing oligonucleotides to satiate protein targets. Curr Opin Chem Biol 1997, 1(1):10-16.
16. Stoltenburg R, Reinemann C, Strehlitz B: FluMag-SELEX as an advantageous method for DNA aptamer selection. Anal Bioanal Chem 2005, 383(1):83-91.
17. Huizenga DE, Szostak JW: A DNA aptamer that binds adenosine and ATP. Biochemistry 1995, 34(2):656-665.
18. Sazani PL, Larralde R, Szostak JW: A small aptamer with strong and specific recognition of the triphosphate of ATP. J Am Chem Soc 2004, 126(27):8370-8371.
19. Sekiya S, Noda K, Nishikawa F, Yokoyama T, Kumar PK, Nishikawa S: Characterization and Application of a Novel RNA Aptamer against the Mouse Prion Protein. J Biochem (Tokyo) 2006, 139(3):383-390.
20. Hale SP, Schimmel P: Protein synthesis editing by a DNA aptamer. Proc Natl Acad Sci U S A 1996, 93(7):2755-2758.
21. Yang Y, Kochoyan M, Burgstaller P, Westhoff E, Fumulok M: Structural basis of ligand discrimination by two related RNA aptamers resolved by NMR spectroscopy. Science 1996, 272(5266):1343-1347.
22. Mitani Y, Nakayama T, Harbers M, Hayashizaki Y: Aptamer-dependent full-length cDNA synthesis by overlap extension PCR. Biotechniques 2004, 37(1):128-129.

23. Meyers LA, Lee JF, Cowperthwaite M, Ellington AD: The robustness of naturally and artificially selected nucleic acid secondary structures. J Mol Evol 2004, 58(6):681-691.

24. Kumar RM, Joyce GF: A modular, bifunctional RNA that integrates itself into a target RNA. Proc Natl Acad Sci U S A 2003, 100(17):9738-9743.

25. Hamaguchi N, Ellington A, Stanton M: Aptamer beacons for the direct detection of proteins. Anal Biochem 2001, 294(2):126-131.

26. Burgstaller P, Jenne A, Blind M: Aptamers and aaptazymes: accelerating small molecule drug discovery. Curr Opin Drug Discov Devel 2002, 5(5):690-700.

27. Sanghvi YS, Shulite M: Therapeutic oligonucleotides: the state-of-the-art in purification technologies. Curr Opin Drug Discov Devel 2004, 7(6):765-776.

28. Ogawa A, Tomita N, Kikuchi N, Sando S, Aoyama Y: Aptamer selection for the inhibition of cell adhesion with fibronectin as target. Bioorg Med Chem Lett 2004, 14(15):4001-4004.

29. Becker RC, Rusconi C, Sullenger B: Nucleic acid aptamers in therapeutic anticoagulation. Technology, development and clinical application. Thromb Haemost 2005, 93(6):1014-1020.

30. Gokulrangan G, Unruh JR, Holub DF, Ingram B, Johnson CK, Wilson GS: DNA aptamer-based bioanalysis of IgE by fluorescence anisotropy. Anal Chem 2005, 77(7):1963-1970.

31. Lee JH, Canny MD, De Erkenez A, Krilleke D, Ng YS, Shima DT, Pardi A, Jucker F: A therapeutic aptamer inhibits angiogenesis by specifically targeting the heparin binding domain of VEGF165. Proc Natl Acad Sci U S A 2005, 102(52):18902-18907.

32. Akiyama H, Kachi S, RL ES, Umeda N, Hackett SF, McCauley D, McCauley T, Zoltoski A, Epstein DM, Campochiaro PA: Intraocular injection of an aptamer that binds PDGF-B: A potential treatment for proliferative retinopathies. J Cell Physiol 2006, 207(2):407-412.

33. Yamamoto-Fujita R, Kumar PK: Aptamer-derived nucleic acid oligos: applications to develop nucleic acid chips to analyze proteins and small ligands. Anal Chem 2005, 77(17):5460-5466.

34. Tombelli S, Suri AK, Jiang F, Jiang L, Fan P, Kumar RA, Nonin S: Structure, recognition and adaptive binding in RNA aptamer complexes. J Mol Biol 1997, 272(5):645-664.

35. Lee JF, Hesselberth JR, Meyers LA, Ellington AD: Aptamer database. Nucleic Acids Res 2004, 32(Database issue):D95-100.

36. Ponomarenko JV, Orlova GV, Frolov AS, Gelfand MS, Ponomarenko MP: SELEX DB: a database on in vitro selected oligomers adapted for recognizing natural sites and for analyzing both SNPs and site-directed mutagenesis data. Nucleic Acids Res 2002, 30(1):195-199.

37. Patil DJ, Suri AK, Jiang F, Jiang L, Fan P, Kumar RA, Nonin S: Structure, recognition and adaptive binding in RNA aptamer complexes. J Mol Biol 1997, 272(5):645-664.