Nucleic Acids Research, 2002, Vol. 30, No. 1 **203–204**

VirOligo: a database of virus-specific oligonucleotides

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Received September 5, 2001; Accepted September 24, 2001

ABSTRACT

VirOligo is a database of virus-specific oligonucleotides. The VirOligo database consists of two tables, Common data and Oligo data. The Oligo data table contains PCR primers and hybridization probes used for detection of viral nucleic acids and the Common data table contains the experimental conditions used in their detection. Each oligonucleotide entry contains links to PubMed, GenBank, NCBI Taxonomy databases and BLAST. As of July 2001, the VirOligo database contains a complete listing of oligonucleotides specific to viral agents associated with bovine respiratory disease that were published in English in peer-reviewed journals. The viruses are bovine herpes virus types 1, 3, 4 and 5, bovine viral diarrhea virus, bovine parainfluenza 3 virus, bovine respiratory syncytial virus, bovine adenovirus, bovine rhinovirus, bovine coronavirus, bovine reovirus, bovine enterovirus and alcelaphine herpesvirus-1. The VirOligo database is being expanded to other viruses and can be accessed through the Internet at http://viroligo.okstate.edu/.

INTRODUCTION

Oligonucleotides have become indispensable tools for virus identification and detection through their use as PCR primers, real-time PCR probes, Southern blotting probes and microarray probes (1). Oligonucleotide design for virus detection and identification is time-consuming work. Oligonucleotides that are conserved among all isolates of the group of viruses which they are meant to target need to be identified. They need also to be chosen so that they will not react with other sequences that possibly could contaminate the sample to be analyzed. In addition, for PCR primers, oligonucleotides that form hairpins or primer dimers must be avoided. Furthermore, for PCR reactions, experiments are required to identify optimum times and temperatures for each PCR cycle step and optimum $MgCl_2$ concentrations, ionic strengths and pH values for the reaction. Finally, specificity and selectivity of the oligonucleotides must be evaluated experimentally since there is no guarantee that the oligonucleotide is useable in real PCR or hybridization experiments.

More than 8000 articles reporting virus-specific PCR were published after the invention of PCR. Many of them contain valuable information about virus-specific oligonucleotides. These articles are scattered in a large variety of journals. We initiated the VirOligo database project to collect this information and make it easily accessible for two reasons. First, many journals containing virus oligonucleotide information are relatively inaccessible, despite recent advances in electronic availability. Accumulation of such information in one publicly available database should facilitate more rapid choice of oligonucleotides and/or PCR assays for laboratories beginning new projects and diagnostic laboratories around the world. Secondly, the potential use of viral oligonucleotides for multivirus detection and identification has increased tremendously with recent development of GeneChip® and microarray technologies. For example, we have recently examined the possibilities of PCR on arrays of immobilized primer pairs and hybridization to arrays of virus-specific oligonucleotides (2). In these preliminary studies, we successfully detected bovine herpesvirus (BHV) and bovine viral diarrhea virus (BVDV) in a multiplex manner (3). To expand such detection methods to a wider range of viruses, virus-specific oligonucleotide sequences need to be collected into a single database. We initiated the VirOligo database project for development and expansion of PCR and microarray-based detection methods.

Three oligonucleotide databases are publicly available through the Internet. The UK Human Genome Mapping Project Resource Centre offered the Primer Bank until 7 July 1999, but it was withdrawn and no data are shown currently. The European Bioinformatics Institute (EBI) has hosted the PCR primers database since 1996 (4), containing a total of 60 primer pairs as of December 2000. The Molecular Probe Database (MPDB) has acquired 4300 oligonucleotide sequences from the date it was first published in 1992 to December 2000 (5). The Oligonucleotide Probe Database (OPD) contains 96 PCR primers and probes and experimental conditions along with brief results and references for 16S-18S-like SSU rRNA, 23S-28S-like LSU rRNA and ATPase8 (6). None of these databases was updated in the past 4 years and they are not specific to viruses.

VirOligo DATABASE

The VirOligo database is a web-interfaced relational database constructed using MySQL and PHP (Hypertext Preprocessor) programming. It consists of two MySQL tables, oligonucleotide information (Oligo data) and PCR and hybridization conditions (Common data), and a PHP query retrieval Common Gateway Interface (CGI). Each Oligo data entry consists of oligonucleotide sequence, target region, name of the oligonucleotide, type of usage (PCR primer, PCR probe, hybridization or other), note and direction of the PCR oligonucleotide (forward or reverse). Degeneracy, length and dissociation temperature (T_d) of oligonucleotides are calculated automatically. Oligonucleotide

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sequences have direct links to BLAST (NCBI) so that users can check up-to-date knowledge about the specificity of the oligonucleotide by one click without typing the oligonucleotide sequence themselves. Each Common data entry contains publication date of the reference, its PMID (PubMed unique identifier), GI (from GenBank) of the target virus sequence, virus name, virus taxonomy ID (from NCBI), PCR cycle or hybridization temperature, buffer, dNTPs and $MgCl_2$ concentrations, polymerase, product size, note and type of hybridization or PCR (i.e. nested PCR). The note field contains additional information, such as reverse transcription conditions or unique features of each entry. PMID and GI are directly linked to the NCBI databases, so that users can find citations or reference sequences used in each entry easily. A nested PCR is separated into two or more entries for user-convenient oligonucleotide selection when PCR conditions are different among steps, and these nested PCR entries are linked to one another.

An oligonucleotide selection method was developed to provide user-friendly access to the oligonucleotide data. Users needing a common PCR cycle to detect several viruses can select oligonucleotides based on viral specificity and T_d. Users can compare oligonucleotides from a variety of fields, such as the target gene of viruses and oligonucleotide length. Search queries can be initiated with virus name, T_d, length of oligonucleotide, PMID or the VirOligo ID number. A summary of the query results is presented with virus name, oligonucleotide name and use, type of experiment (type of PCR), PCR cycle, T_d, publication date and PMID with the VirOligo ID number. By clicking the VirOligo ID number for each result, users can see the complete information for the identified entry.

As of July 2001, the VirOligo database contained more than 1637 oligonucleotides. The strategy adopted to fill the database was to obtain as near complete coverage as possible of one group of viruses at a time. For each new group, articles are identified whose PubMed abstract contains the virus names and 'PCR' or 'oligonucleotide'. Our first group, for which virtually complete coverage has been achieved, was bovine respiratory disease (BRD) associated viral agents. Although it has been extensively investigated in recent years, BRD remains a major cause of economic loss in cattle. An estimated $3 billion is spent annually for prevention and treatment (7,8). BRD viruses are BHV-1, -3, -4 and -5, BVDV, bovine parainfluenza 3 virus, bovine respiratory syncytial virus, bovine adenovirus, bovine rhinovirus, bovine coronavirus, bovine reovirus, bovine enterovirus and alcelaphine herpesvirus-1 (7,9–13). To obtain complete coverage of BRD-associated viruses, we used PubMed (http://www.ncbi.nlm.nih.gov/) to identify all articles in which one of the BRD associated viruses appeared in the abstract and entered all oligonucleotide data in these articles, regardless of virus. Thus, the VirOligo database stores more than BRD-associated virus oligonucleotides.

Entry of oligonucleotides of BRD-related viruses was used as a pilot project to devise efficient data identification and entry strategies. Lessons learned in this pilot project allow substantial acceleration of the speed of entries and should thus permit expansion of VirOligo to cover all viruses. Oligonucleotides used in influenza virus research are currently being entered. Announcement of completions of new entries are made on the site's home page. Along with the expansion of virus groups, the VirOligo is continually updated for already entered virus groups.

AVAILABILITY

VirOligo database provides free public access to valuable oligonucleotides and experimental conditions through the web at http://viroligo.okstate.edu/. We invite and encourage oligonucleotide entries from users. An oligonucleotide entry form and instructions can be found at http://viroligo.okstate.edu/entry.html or from a link on the main page. All entries are subject to the curator's verification before addition to the VirOligo database.

ACKNOWLEDGEMENTS

The work at VirOligo database was supported by the Samuel Roberts Noble Foundation (Ardmore, OK), R. J. Sirny Professorship and the Oklahoma Agricultural Experiment Station.

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