ELISA-BASE: an integrated bioinformatics tool for analyzing and tracking ELISA microarray data

Amanda M. White1,∗, James R. Collett1, Shannon L. Seurynck-Servoss2, Don S. Daly1 and Richard C. Zangar1

1Pacific Northwest National Laboratory, PO Box 999, Richland, WA 99352 and 2Department of Chemical Engineering, University of Arkansas, 3202 Bell Engineering Center, Fayetteville, AR 72701, USA

Received on December 12, 2008; revised on March 26, 2009; accepted on March 28, 2009
Advance Access publication April 3, 2009

ABSTRACT

Summary: ELISA-BASE is an open source database for capturing, organizing and analyzing enzyme-linked immunosorbent assay (ELISA) microarray data. ELISA-BASE is an extension of the BioArray Software Environment (BASE) database system.

Contact: amanda.white@pnl.gov

1 INTRODUCTION

Enzyme-linked immunosorbent assay (ELISA) microarrays have several advantages over other tools for validating candidate biomarkers, including the fact that they utilize extremely small amounts of reagents and samples. We have been developing a high-throughput ELISA microarray platform for biomarker validation (Zangar et al., 2006). As part of this effort, we created ProMAT (Protein Microarray Analysis Tool). ProMAT is the only freeware program specifically designed for ELISA microarray data analysis (Daly et al., 2008; White et al., 2006). ProMAT is a statistically sound program for creating standard curves and estimating protein concentrations; however, it lacks the ability to track metadata or compare results across studies. Such capabilities are beneficial when comparing data quality and reproducibility over time. Therefore, as described in this article, we integrated ProMAT with the BioArray Software Environment (BASE) (Saal et al., 2002). BASE (http://base.thep.lu.se) is an open source, web-enabled database system designed for DNA gene expression microarray data and experimental workflow management.

2 APPROACH

There are many differences between DNA microarray and sandwich ELISA microarray studies (Zangar et al., 2006) that must be taken into account when integrating ProMAT and BASE. Although it is possible to modify the BASE programming to make it more suitable for ELISA microarray data, this is not desirable because BASE is regularly upgraded and our custom modifications would not be included in future updates. Therefore, we created plugins and annotations to adapt BASE for ELISA microarray data, which should be readily transferrable to newer BASE versions.

∗To whom correspondence should be addressed.
stored in an Annotation. The antigen mixture and detection mixture are pooled to form a new Extract, and then the Extract is associated with a Label to create the Labeled Extract. This Labeled Extract now points back to all its constituent parts and their metadata. The case for chips that are treated with a biological sample is very similar to that for the purified antigen mixture, where the antigen mixture hierarchy in Figure 1 is replaced by the biological Sample and its diluted Extract.

2.2 Experimental data
A typical DNA microarray slide has one chip, and thus one sample, per slide. Each scan of a slide is represented as one Raw Bioassay in BASE. This design is not ideal for ELISA microarray experiments since multiple chips may be printed on each slide, and each chip will be treated with a different sample. Working with this underlying structure of BASE is feasible, but does create some minor redundancy in data items, particularly Scan objects.

In ELISA-BASE each chip is stored as a Raw Bioassay and each Raw Bioassay is attached to a Scan object which has Annotations containing scanner settings (e.g. laser power) that are needed when creating standard curves. The Scan is linked to a Hybridization1 object, which is connected to the biomaterials hierarchy described above via a Labeled Extract object. A set of chips where some may be treated with antigen mixtures and some with biological samples, are organized into an Experiment.

3 RESULTS
We have created BASE plugins to import, organize and analyze ELISA microarray data. The plugins are written in Java and R and are available along with source code and documentation at http://www.pnl.gov/statistics/ProMAT/ELISA-BASE.stm. R is a free, open source, cross-platform statistical programming language. The implementation of the ELISA-BASE tools is broken down into the following parts.

3.1 ELISA Experiment Importer Plugin
The ELISA Experiment Import Plugin imports raw data files and associated experimental metadata (e.g. sample names, antigen and detection mixtures and dilutions) into BASE. This plugin creates the necessary BASE objects and annotations so that the data are stored as described in the Approach section. The importer also adds all the Raw Bioassays to the selected Experiment, and creates a new Bioassay Set in that Experiment. The data are organized in a way to be able to run the ProMAT plugin immediately, although the user may still choose to transform or filter the spot intensities if desired.

To use the plugin, the user provides the image analysis results and a metadata file which lists the slide ID, sample ID, scanner settings and dilution for each chip.

3.2 ProMAT Plugin
A new version of our ProMAT tool has been developed that is a plugin to BASE and is designed to work with the ELISA Experiment Importer. ProMAT calculates standard curves from the data derived from the chips treated with antigen mixture, and uses these curves to calculate protein concentrations for the biological samples. It also provides uncertainty estimates on the protein concentrations. ProMAT provides options for different curve types, log transforming data and graphical output.

3.3 Antigen/Detection Mix Concentration Importer Plugin
The Antigen/Detection Mix Concentration Importer Plugin is a convenience tool to allow researchers to easily create in BASE the pooled samples of antigens or detection antibodies that are used in the ELISA microarray experiment. While it is possible to create these files directly in BASE, we find this tool to be quicker in many cases. The plugin allows the user to upload a file containing the IDs and concentrations of either the antigens or the detection antibodies and the concentration values are stored as Annotations.

4 DISCUSSION
Much of the early DNA microarray work suffered from lack of reproducibility and accuracy (Shi et al., 2004). Recognition of this problem led to standardization of protocols and the development of bioinformatics tools such as BASE to track and record experimental data, thereby improving the ability to replicate and understand published data. At present, many of the problems that plagued early DNA microarray studies have not been addressed for antibody microarray technology. ELISA-BASE represents a significant step forward in that it is the first tool to allow for the systematic tracking of all data and data processing steps from an ELISA microarray experiment. ELISA-BASE also provides data analysis capabilities that are necessary for ELISA microarray experiments, such as standard curve generation. Our tools make the workflow of ELISA microarray data management and analysis easier for the researcher, thus facilitating high-throughput experimentation.

Funding: National Institute of Biomedical Imaging & Bioengineering (R01 EB006177); National Cancer Institute (U01 CA117378).

Conflict of Interest: none declared.

REFERENCES