

Biomarker Discovery Using SELDI Technology

A Guide to Successful Study and Experimental Design

BIO-RAD

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About This Guide

The goal of clinical proteomics research is to discover protein markers (biomarkers) and use them to improve the diagnostic, prognostic, or therapeutic outcome for patients or to assist in the development of novel drug candidates. For many of these applications, the use of various proteomics technologies and the recent emphasis on protein biomarkers have yielded a large number of candidate biomarkers; however, the small size and poor design of many studies have made validating these biomarkers challenging.

Biomarker attrition can be reduced with appropriate study designs that screen larger numbers of patients and take into account both preanalytical and analytical biases. The goal of this guide is to provide a series of recommendations for effective study design. General guidelines are provided that apply to the use of virtually any proteomics technology, and specific recommendations are also given for use of the ProteinChip® surface-enhanced laser desorption/ionization (SELDI) system.

A content map and summary of the main recommendations are provided at left.

- Part I offers an overview of the biomarker discovery process and the factors to consider when designing any biomarker discovery project, regardless of the technology used
- Part II discusses the process and recommendations to follow during study design, with an emphasis on factors that most influence the quality and reproducibility of data generated
- Part III offers specific recommendations and considerations for using the ProteinChip SELDI system; methods and protocols that have been optimized by Bio-Rad's Biomarker Research Centers for use with specific cell types are provided in Appendix A

Part I The Biomarker Discovery Process

Study Design

Define the clinical question, samples, and workflow

Discovery

Detect multiple biomarker candidates

Validation

Select biomarkers with highest predictive value

Identification

Purify and identify biomarkers

Diagnostic Assay Implementation

Design and implement biomarker-based clinical assay

Part II Study Design

Clinical Question

- Ask a clear question that addresses a clinical need
- Determine the type of study
- Define success criteria

Sample Selection

- Select the model system and sample type
- Determine the appropriate sampling size
- Select appropriate controls
- Stratify sample populations
- Determine inclusion and exclusion criteria
- Compile detailed sample annotations

Sample Collection, Handling, and Storage

- Implement standard methods for sample collection and handling
- Avoid systematic bias associated with collection site
- Freeze samples uniformly and avoid repeated freeze-thaw cycles

Experimental Design

- Understand the unique requirements of each study phase
- Define the general workflow for each phase
- Define the timing of the phases

Part III SELDI Experimental Design

Assay Design

- Define the workflow
- Select the samples, controls, and standards
- Select appropriate ProteinChip SELDI array chemistries
- Select methods of data analysis

Sample Preparation

- Use consistent and appropriate liquid-handling techniques
- Define protocols for initial processing of samples
- Use fractionation and depletion techniques to improve resolution of low-abundance proteins

Array Processing

- Determine the sample layout for each array
- Optimize sample dilution and buffer composition
- Standardize methods for matrix application

Data Collection

- Perform regular instrument maintenance and calibration
- Optimize acquisition protocols
- Acquire data using default spectrum processing parameters

Data Analysis

- Ensure proper annotation of spectra
- Process spectral data
- Group spectra into folders
- Detect, label, and cluster peaks within one condition
- Evaluate the quality of the data
- Perform univariate statistical analyses
- Perform multivariate statistical analyses

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The SELDI process is covered by U.S. patents 5,719,060, 6,225,047, 6,579,719, and 6,818,411 and other issued patents and pending applications in the U.S. and other jurisdictions.

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Part I: The Biomarker Discovery Process

The goal of biomarker or clinical proteomics research is to discover protein markers and use them to improve the diagnostic, prognostic, or therapeutic outcome for patients or to assist in the development of novel drug candidates. This section details the various phases of a biomarker discovery project and offers general recommendations that should be followed regardless of the proteomics technologies used.

Overview

Biomarkers are generally discovered through differential expression analysis, the determination of protein expression levels as influenced by disease state, therapy, or other differences between sample cohorts. Once differentially expressed proteins are identified, their expression levels can be used to classify organisms, individuals, disease states, metabolic conditions, or phenotypic responses to environmental or chemical challenges.

Five phases compose the path from study design to clinical application, and each phase presents different goals and requires unique experimental approaches (Table 1):

- Study design — In this initial phase, the objective is to detail the clinical question being asked and the types and number of samples, experimental workflow, and technologies that will be used. A successful biomarker research program begins with careful study design and implementation
- Discovery — In this phase, the objective is to find candidate biomarker proteins. For this purpose, screen a large number of conditions to detect the maximum number of proteins and enrich low-abundance proteins. Choose samples carefully and in sufficient quantities to provide statistical significance. Proteins exhibiting

The Biomarker Discovery Process

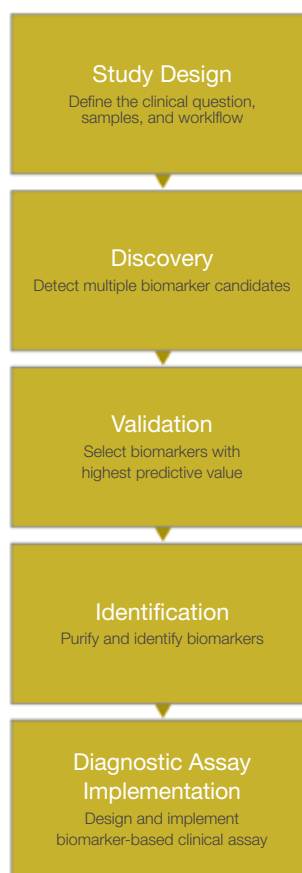


Table 1. Phases of a biomarker research project.

Phase	Goal	Number of Samples and Proteins Monitored
Study design	Define the clinical question, select and collect the appropriate samples and controls to address the clinical question, and select the proteomics platform(s) that will be used in each phase of the project	—
Discovery	Find candidate biomarker proteins by screening a well-defined sample population using a large number of experimental conditions for the reproducible detection of the maximum number of proteins	10s of samples 1,000s of proteins
Validation	Assess the validity of candidate biomarkers against a larger, more heterogeneous population using a reduced set of experimental conditions	100s of samples 100s of proteins
Identification	Purify and positively identify candidate biomarkers	Varies
Diagnostic assay implementation	Develop and apply chromatographic or antibody-based assays that are optimized to provide robust, sensitive, and quantitative protein biomarker assays	1,000s of samples 10s of proteins

statistically significant group- or time-dependent differences are described as candidate biomarkers and can be used alone (univariate analysis) or in combination (multivariate analysis) to generate predictive models

- Validation — The objective of this phase is to assess the validity of a marker against a larger, more heterogeneous population. The robustness of the candidate markers is tested against a level of biological variability that more accurately reflects the variability in the target population. This phase can either repeat and confirm the findings from the discovery phase on a larger sample set, or it may explore different variables that may affect the validity of the markers for a large population and, ultimately, the clinical utility of the biomarkers themselves
- Identification — In this phase, the most promising markers are enriched and purified; purified proteins are subsequently positively identified by peptide mapping or sequence analysis
- Diagnostic assay implementation — This phase can be performed at multiple points in a study. Assays can

be either chromatographic or antibody-based, both of which can be optimized to provide a robust, sensitive, and quantitative assay

The order in which the five phases are completed depends on multiple factors, including the identity of the protein, availability of antibodies and their appropriate samples for validation, and the drug development time line. While the discovery phase is ideally followed by a comprehensive validation study, design and completion of subsequent clinical trials often require extensive periods of time. It is, therefore, common to proceed directly to the identification phase following discovery. Identification facilitates the development of analyte-specific assays and provides insight into the biological process. The ProteinChip SELDI system, however, allows rapid and efficient validation of candidate biomarkers from large numbers of samples, increasing the statistical significance of any potential biomarker before identification.

Regardless of the final order of the validation and purification steps, a successful biomarker research program always begins with careful study design.