



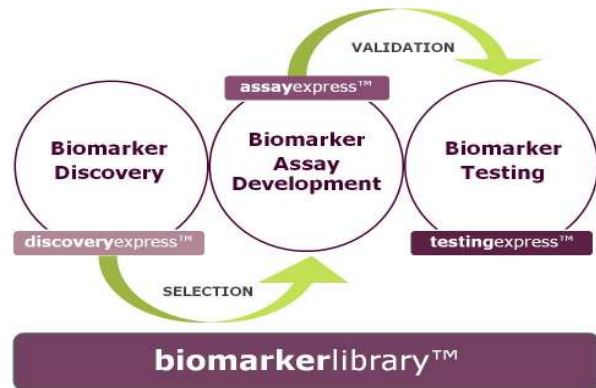
biomarkerexpress™

biomarkerexpress™ is a suite of biomarker services for research and development that utilizes a proprietary platform to significantly decrease timelines and increase the success rate of biomarker development.

Human Plasma

biomarkerexpress™ 6 month pipeline

Designed for rapid progression
from biomarker discovery
through assay development
to biomarker testing

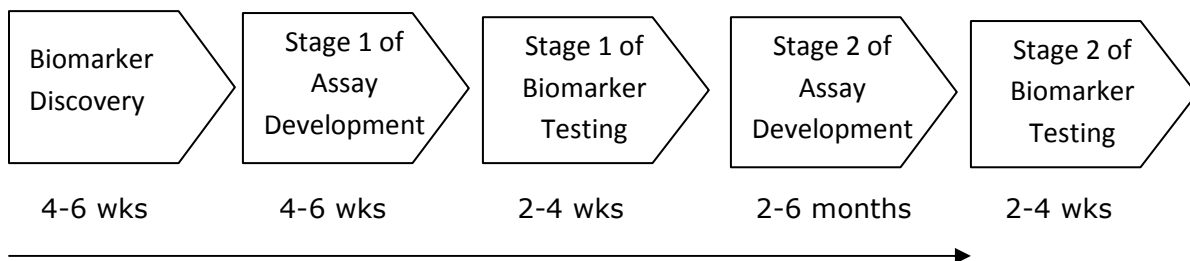


Problem: *The ability to routinely test protein biomarker levels is the goal for researchers and clinicians.* However, assay development has been a major bottleneck to reaching this goal. In many cases substantial resource has been expended to generate a list of putative, biologically relevant protein biomarkers, however assay development has always been a rate limiting step to the validation and acceptance of these molecules. The excitement of new biomarkers often fades as the limitations of ELISA/sandwich assays restrict the number of biomarkers that can move through development.

Solution: NextGen Sciences has developed a mass spectrometry based strategy to rapidly progress all of your biomarkers to the validation phase without a requirement for expensive and time consuming antibodies.

Strategy: The biomarker discovery approach taken by NextGen Sciences generates information which is crucial to the development of accurate, precise and robust assays. This accelerates the development of multiplexed assays to weeks and allows the validation of all the putative protein biomarkers in record time.

NextGen Sciences Biomarker Workflow



Overall Timeline for Biomarker Development

biomarkerexpress™ services workflow and timelines from biomarker discovery through biomarker assay development to biomarker testing based on a model experiment described in the right-hand column.

<p>biomarkerlibrary™: 1 day</p> <p>Examine NextGen’s protein indexes (www.nextgensciences.com) to determine if proteins of interest have already been detected by NextGen. Protein in the indexes can be readily developed into assays.</p>	<p>10 putative biomarkers</p>
<p>Biomarker Discovery - <u>New biomarker discovery: 4-6 weeks</u></p> <p><u>Option 1. GeLC-MS platform</u> – This platform performs in-depth analysis of the protein composition of samples because either 24 or 40 fractions of the samples are analyzed by mass spectrometry. In some cases to reduce complexity, individual samples within a treatment group are pooled into 1 sample. If pooling is used, NGS recommends processing two samples per group(1 pooled + 1 individual) in order to assess biological variation. Typically 3,000 proteins are detected in cells and tissues. Proteins are identified and relative quantitation data is done with by spectral counts (number of peptide observations per protein).</p> <p><u>Option 2. Differential MS platform</u>- This platform allows for more in-depth quantitative analysis (amenable to rigorous statistical analysis) across samples. Each sample is processed individually. Proteins differentially expressed are identified and relative quantitation data by integrated peak areas (area under the curve value) is reported.</p>	<p>Input: 50 total plasma samples; 25 control, 25 disease relevant</p> <p>Deliverable:</p> <ol style="list-style-type: none"> 1) ~500 proteins detected; 15 proteins with greater than 2 fold change in spectral counts 2) ~350 proteins detected; 15 proteins with statistical significant changes.
<ul style="list-style-type: none"> ○ Assay Development <u>Stage 1 of Assay Development: 4-6 weeks</u> Assay development using peptide Multiple Reaction Monitoring (pMRM) – relative quantification of protein biomarkers Using the biomarker list generated from the prior 2 steps, peptides to be used as surrogate markers for proteins are selected. Typically 2 peptides per protein are chosen and 2-7 product ions for each peptide are used for identification and quantitation. The pMRM assay is optimized for multiplexing simultaneous measurement of all 25 proteins, short run times and lowest sample requirement. The reproducibility of the assay (each peptide) is assessed (analytical, technical and biological variation). 	<p>Input: Biomarker List 1 has 25 proteins</p> <p>Deliverable: Methodology for an assay to monitor relative levels of 25 proteins. Ready for the testing phase.</p>
<p>Biomarker Testing <u>Stage 1 Biomarker Testing: 2-4 weeks</u></p> <ul style="list-style-type: none"> ○ Using the Stage 1 assay, relative quantitation data is obtained for all 25 proteins in all samples ○ Data goes through a rigorous statistical analysis to determine which of the putative biomarkers are confirmed. Typically less than half of the putative biomarkers turn into confirmed biomarkers after the sample set is expanded to large numbers. <p>The data accumulated to this point could be presented to the FDA as part of their Voluntary Exploratory Data Submission (VXDS) program.</p>	<p>Input: The original 50 plasma samples plus a 2nd experiment with 150 plasma samples; 50 controls + 100 disease relevant.</p> <p>Deliverable: Relative quantitation confirms 12 of the 25 biomarkers across a larger experimental dataset</p>

Biomarker Validation is an iterative process of study design and biomarker testing. The US FDA has designed a two stage process for establishing biomarkers as validated for a specific purpose. Stage 1 establishes biomarkers as “probable known biomarkers” and Stage 2 establishes biomarkers as “known biomarkers”. Additional samples will be required for these stages.

<p>Assay Development: Stage 2 of Assay Development: 2-6 months</p> <p>Assay development for absolute quantification using the Stage 1 assay as the starting point:</p> <ul style="list-style-type: none"> ○ Peptides or protein standards are made in order to generate the calibration curves that provides the conversion to absolute quantitation ○ A calibration curve is developed to provide a working range for determining protein concentrations in samples (4-6 point curves). ○ Peptides or protein standards are spiked into the samples to be analyzed and the assay is checked to ensure data for all 12 proteins is obtained. ○ Assay validation to establish LOD, LOQ, reproducibility, etc. 	<p>Input: Biomarker List 2 includes 12 proteins.</p> <p>Deliverable:</p> <p>One assay for 12 proteins that provides absolute quantification of proteins (protein concentration). Assay has been validated for LOD, LOQ, reproducibility and data range.</p>
<p>Biomarker Testing: Stage 2 of Biomarker Testing: 2-4 weeks</p> <ul style="list-style-type: none"> ○ Using the Stage 2 assay, protein concentration data (absolute quantitation) is obtained for all 12 proteins in all samples ○ Data goes through a rigorous statistical analysis ○ Testing can be done to validate biomarkers and also for routine testing of samples in pre-clinical and clinical studies. <p>The Stage 2 assay is used many times.</p>	<p>Input: 500 plasma samples for testing</p> <p>Deliverable:</p> <p>Protein concentration changes are monitored in all samples to provide the investigator with sufficient quantity and quality of data to validate these 12 biomarkers.</p>

The above workflow is for proteins that are present at an abundance level (in the tissue or biofluid provided) that do not require protein specific enrichment procedures.

Skipping the biomarker discovery step. Assay development can also be done from a client list of biomarkers. These might be proteins identified from the literature or from previous studies (pharmacogenomics, transcriptomics (DNA arrays) or proteomic technologies).

The workflow in these cases starts with Assay Development. A critical step is the selection of peptides for the assay. This step can be accomplished by selecting peptides in silico and then testing using mass spectrometry or a representative sample can be run on GeLC-MS to generate lists of peptides for all proteins in the sample.

In some cases the protein is present in too low an abundance level to detect by mass spectrometry without a pre-fractionation step to enrich for the proteins of interest. Pre-fractionation strategies include:

- Use antibodies for immunoprecipitation to enrich for the protein(s) of interest and pool immunoprecipitations prior to mass spec. Note – affinity reagents are not used to quantify the protein.
- Produce peptide antibodies to enrich for the peptides that will provide the data for quantification (method is called SISCAPA, Leigh Anderson, Plasma Proteome Institute)
- Perform fractionation methods to fractionate, enrich and re-pool the fractions containing the peptides/proteins of interest.

Pricing.

NextGen Sciences provides custom quotes for all biomarker projects. Please call or email with a pricing inquiry or for a quote for a specific project.

Phone: 734-973-7914 (USA toll free 866-973-7914) or +44 (0)1462 685 788 (Europe) and ask to talk to someone about Biomarker Services.

Email: biomarkers@nextgensciences.com.

Project specific timelines will be estimated with a quote.

Director of Biomarkers and Proteomics

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