Institutional Profile

The Emory Biomarker Service Center

The Emory Biomarker Service Center (EBSC) provides state-of-the-art analytical technologies to impact biomarker discovery, development and clinical translation of biomarker research in a spectrum of disease and therapeutic areas. EBSC technologies are primarily concentrated in medical genomics, microarray expression profiling and data analysis. The EBSC provides state-of-the-art services in nucleic acid extraction, genomic sequencing, custom and whole-genome genotyping, cytosine-phosphate-guanine dinucleotide (CpG) methylation, mRNA and miRNA expression analyses. The EBSC typically processes tens of thousands of samples per year, utilizing instrumentation and automation from Tecan, Qiagen, Applied Biosystems, Eppendorf and Beckman Coulter, which are supported by the ThermoFisher Nautilus Laboratory Information Management System data-management architecture. The EBSC provides services and platforms for biomarker profiling of fresh, frozen or formalin-fixed paraffin-embedded tissues using both Affymetrix, Inc. and Illumina, Inc. platforms. It provides quality-control and data analysis in a high-throughput manner in a Clinical Laboratory Improvement Amendments certified laboratory. Bioinformatics support is provided to users by a team of analysts using a variety of standard and custom software tools.

Recently, the Winship Cancer Institute (WCI) at Emory University (FIGURE 1) earned designation as a National Cancer Institute (NCI) Designated Cancer Center. The Emory Biomarker Service Center (EBSC), a critical component of the NCI Cancer Center Support Grant to the WCI, was established to provide Emory and WCI researchers access to state-of-the-art analytical tools, technologies and platforms that span genome-wide analyses of RNA and DNA samples. The EBSC enables investigators (both clinical and basic) at different levels of sophistication and intellectual and technological involvement to perform cutting-edge genomic research: from proper sample preparation and storage, to whole-genome analysis at the DNA/RNA level, to collaborative studies aimed at novel biomarker discovery, development and validation. In addition, the EBSC interactively helps researchers with experimental design, data acquisition and

data analysis. Importantly, and quite uniquely, it operates its facilities in both research and clinical environments. The EBSC is located on the 4th floor of the WCI Clinic B building, in 3000 sq. ft of newly renovated laboratory space [101], and is the result of a merger of two previously established facilities, the Center for Medical Genomics (CMG) and the WCI Microarray Core (WCIMC).

The rationale for integrating these facilities was to enhance an excellent sample-management system and standard operating procedures (SOPs) to store, manage and process large numbers of samples and to leverage shared instrumentation between both facilities. The consolidation of these key core facilities and infrastructure at Emory provides a 'critical mass' of capabilities housed in one contiguous space, and has additional advantages of allowing a seamless 'one stop shop' giving a brand name and appropriate visibility, as well as facilitating staff

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Figure 1. The Winship Cancer Institute.

cross-training and leveraging the benefits of ‘economies of scale’. The move to consolidate and expand core facilities in this manner (with a quality regulatory aspect, such as Clinical Laboratory Improvement Amendments [CLIA]) is likely to continue to be an emerging trend for academic institutes across the USA. The genetic sample management SOPs for the EBSC are transparent, easily accessible and supported by the ThermoFisher Nautilus laboratory information management system (LIMS) and, importantly, operated in a CLIA-certified laboratory. All samples are bar-coded upon drop-off, and entered and tracked using our LIMS to ensure proper sample management. Standardized sample management with highly annotated and quality controlled samples whose SOPs take into account all systems-based downstream analysis instrumentation form the basis of all experiments, from genomics to epigenomics, to expression profiling, to data analysis.

History

The CMG was initially formed in 2002 to support projects for the Department of Human Genetics and, since 2005, in order to meet the increasing needs for the Emory community, the CMG grew under the directorship of Mark Bouzyk (FIGURE 2), so as to support the whole of Emory University and beyond, including an increasing portfolio for WCI investigators. This center offered a wide variety of services that included genetic sample management (e.g., storage and extraction of DNA), DNA sequencing, microsatellite genotyping, single-nucleotide polymorphism (SNP) genotyping and methylation analysis supported by a state-of-the-art LIMS. The overarching goal of

the CMG was to link human genetics to health and disease by providing a ‘center of excellence for experimental studies in genetic variation’ – from disease susceptibility genetic-association studies to diagnostic development.

The CMG made a strategic decision in 2005 to operate in a CLIA environment. CLIA accreditation positioned the facility to generate genotypic and other molecular data that can be used directly in medical care. The CMG developed 24 genetic tests for rare disorders, which are now being used in the clinic and, thus, took the first steps in operating high-throughput research projects in a clinical regulatory setting, bridging the translational gap and, thereby, benefiting clinical investigators by quickly developing genetic tests. The CMG also supported candidate gene- and genomic-association studies in common disorders to identify susceptibility genes and pharmacogenetic studies.

The WCIMC was established by Carlos MORENO (FIGURE 2) in June 2006. Microarray services included RNA preparation from formalin-fixed paraffin-embedded (FFPE) tissues, RNA quality control, whole-genome expression analysis for human, mouse and rat genomes and data validation by quantitative TaqMan® real-time quantitative PCR (Q-PCR). The WCI Microarray Core served a variety of investigators at Emory that are members and nonmembers of WCI, as well as outside users across GA (USA) and around the world.

The CMG and WCIMC merged in 2008 to form the EBSC to enable the sharing of instrumentation, LIMS software sample management and to provide a single integrated resource for all Emory investigators interested in high-throughput genomic, epigenomic and expression analyses. The 3000 sq. ft of laboratory space in the WCI enables separate dedicated rooms for pre-PCR RNA, pre-PCR DNA and post-PCR workflows.

Services & capabilities

The EBSC tracks all its samples from receipt and throughout the life of the experiment using its LIMS architecture. Processes and protocols for genetic sample management are readily available.

DNA extraction is performed using a KingFisher MagMAX™ instrument and a Qiagen M48 automated extractor. DNA sequencing, microsatellite genotyping and fragment analysis currently utilize Applied Biosystems' 3100 genetic analyzer. Alternatively, Pyrosequencing™ technology has been used for DNA sequencing of short fragments, SNP detection or for quantitative methylation analysis. SNP genotyping is carried out using either Applied Biosystems' TaqMan or Beckman's SNPStream® platform, which enables multiplex genotyping of up to 48 SNPs per sample in one assay. In addition, the Illumina BeadStation 500 instrument enables whole-genome SNP genotyping and GoldenGate SNP assays, copy-number variation assays, as well as custom or whole-genome methylation studies. Liquid handling is automated utilizing robotics instrumentation, including a Tecan's EVO® and Beckman Coulter's Biomek® NX. The automation equipment can be used to perform routine liquid transfers, such as the transfer of nucleic acids from individual vials into 96- or 384-well storage and amplification plates required by downstream applications. The instrumentation can be utilized to prepare a plate in order to determine the concentration of the extracted nucleic acid and, using the concentrations obtained, to create a normalized plate of samples to be used for amplification and genetic analysis. The automation can also create mother–daughter replica plates, transfer individual samples into a new plate or vial and prepare and dispense the necessary reagents for amplification. The NX is reserved for all aspects of liquid handling in post-PCR space. All staff members are trained to work according to specific SOPs appropriate for processes and all equipment. All instrumentation and procedures are rigorously quality controlled, including the daily logging of equipment functionality. The EBSC balances its fee for service work and collaborations with a healthy mix of grant and contract awards of its own. The EBSC provides high-throughput genotyping capabilities for candidate gene and SNP studies on both breast and prostate cancer with the American Cancer Society. The EBSC also provides the genomics

capabilities for the recently funded NIH Atlanta Clinical & Translational Science Institute (CTSA) initiative [102], as well as Emory's strategic Predictive Health Initiative through funded institutional Woodruff grants in collaboration with clinical investigators. In addition, the EBSC serves as the genomics core for a funded National Institute for Neurological Diseases and Stroke (NINDS) program and an NIH-funded Digestive Disease Research Center. These awards really reflect the diversity of projects and disease areas the EBSC is now called on to support. Moreover, the EBSC has regular 'portfolio' meetings to appropriately schedule a constellation of up to 50 projects of differing size it manages at any one time.

For expression-profiling analysis, the EBSC currently offers services on both the Illumina and Affymetrix expression platforms. The Illumina BeadStation 500 instrument enables high-quality whole-genome expression profiling of protein-coding and miRNA genes. It also enables genome-wide analysis of SNPs using both GoldenGate and Infinium technologies. The Affymetrix GeneChip® microfluidics station enables a variety of whole-genome expression-profiling applications, including Gene ST and Exon ST GeneChip analysis. In addition, the platform can be used for comparative genomic hybridization, resequencing, chromatin immunoprecipitation followed by microarray hybridization (ChIP–chip) and SNP genotyping studies. The EBSC can prepare DNA or total RNA (including miRNAs) from FFPE tissues manually, or in 96-well format using a KingFisher MagMAX 96

Figure 2. Mark Bouzyk (left) and Carlos S Moreno (right) are co-Directors of the Emory Biomarker Service Center.

instrument, which was recently acquired to enable high-throughput, automated, standardized RNA preparation protocols. We have optimized these protocols for DNA, total RNA and miRNA yields [1,2]. The EBSC also provides RNA quantity- and quality-control analysis using NanoDrop™ spectrophotometry, Agilent 2100 bioanalyzer analysis and *RPL13a* TaqMan assays. Other services offered by EBSC include:

- RNA preparation from FFPE tissues;
- Illumina cDNA-mediated annealing, selection, extension and ligation (DASL) assays for expression from FFPE tissues and high-throughput cytosine–phosphate–guanine dinucleotide (CpG) methylation assays;
- TaqMan low-density arrays;
- TaqMan Q-PCR data validation.

Microarray services were recently expanded to enable miRNA TaqMan assays and TaqMan low-density arrays. The EBSC also can perform RNA amplification for very low quantities of RNA samples. In addition, the EBSC offers support for microarray data analysis for core users.

The EBSC provides a variety of levels of data analysis, depending upon the end user's needs. Analyses can range for as little as output of raw or normalized data, to development of scripts to automate data pipelines, to creation of biomarker predictor gene sets and cross-validation analyses. A team of bioinformatics specialists at the EBSC provides data analysis using multiple standard and custom software tools, including custom software for analysis of conserved transcription factor-binding sites that regulate coordinated changes in gene expression developed by Moreno's laboratory [3,103]. Analyses methods provided to customers include significance analysis of microarrays, Conserved Transcription Factor-Binding Site (CONFAC) analysis, ingenuity pathway analysis, gene set enrichment analysis and predictive gene set cross-validation with GenePattern software. We are currently implementing caArray for data storage and analysis to enable integration with Cancer Bioinformatics Grid (caBIG) software architecture.

Strategic importance & benefits

The EBSC integrates state-of-the-art genomic, microarray and Q-PCR platforms, and concentrates a critical mass of know-how in a single unit; this would be virtually impossible for an individual laboratory to duplicate. To gain access to such expertise, enabling technologies and products, local investigators would otherwise have to purchase them at higher prices from outside vendors. By concentrating these facilities into a central location, investigators reap the benefits of complex technology without incurring the heavy equipment and training expenses generally associated with these systems. In addition, synergies are achieved by providing the one-stop-shop approach for seamless and regulated data management using common LIMS. In turn, this approach considerably improves the timeframe of data delivery that is a substantial benefit to increasing the pace of NIH-funded research at Emory.

CLIA accreditation has positioned the EBSC to generate molecular data that can be used directly in medical care. While such capacity is currently useful only in a relatively small set of situations, we anticipate that rapid advances in cancer genetics and other areas of molecular diagnostics enable many researchers to provide clinically useful data, thus providing growing numbers of opportunities to pursue bench-to-bed-side translational research goals. Thus, the proposed realignment of high-throughput instrumentation for gene expression and genotype determination, and its installation and operation under CLIA standards, will meet increasing demands for the generation of clinically useable molecular data. Our explicit emphasis on the facility as a CLIA translational-research laboratory distinguishes it in important and forward-looking ways compared with most other core research laboratories.

Future perspective

We anticipate that future research in biomarkers will rely heavily on large-scale studies that analyze genome-wide data sets. In addition, many of these studies will leverage archived clinical FFPE samples from clinical trials. Much of the future research

at the EBSC will utilize these resources in collaboration with large, international cooperative groups, to identify biomarkers (in our case, these could be SNPs, microsatellites, variable-number tandem repeats or fragment-length polymorphisms, copy-number variations, methylation signatures, mRNA signatures and miRNA signatures) that can improve the quality of care for patients. These new biomarkers will enable more personalized therapies that will benefit patients through more effective therapies and, ultimately, reduce healthcare costs by the identification of these therapeutic regimens through biomarker analysis.

The EBSC receives, processes and tracks tens of thousands of samples per year using automated liquid-handling instruments and a LIMS.

The EBSC supports small-scale and large-scale projects from investigators throughout Emory University, the State of Georgia and beyond, with competitively priced, high-quality data and analysis.

The EBSC operates in a CLIA environment to ensure high-quality data generation and enable the development of clinical tests for translational research.

The EBSC has developed genetic tests and continues to work towards the development of genetic, epigenetic and pharmacogenetic tests for a variety of investigators locally, nationally and internationally.

The EBSC also processes large numbers of FFPE samples from clinical trials to help establish and validate biomarkers.

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Websites