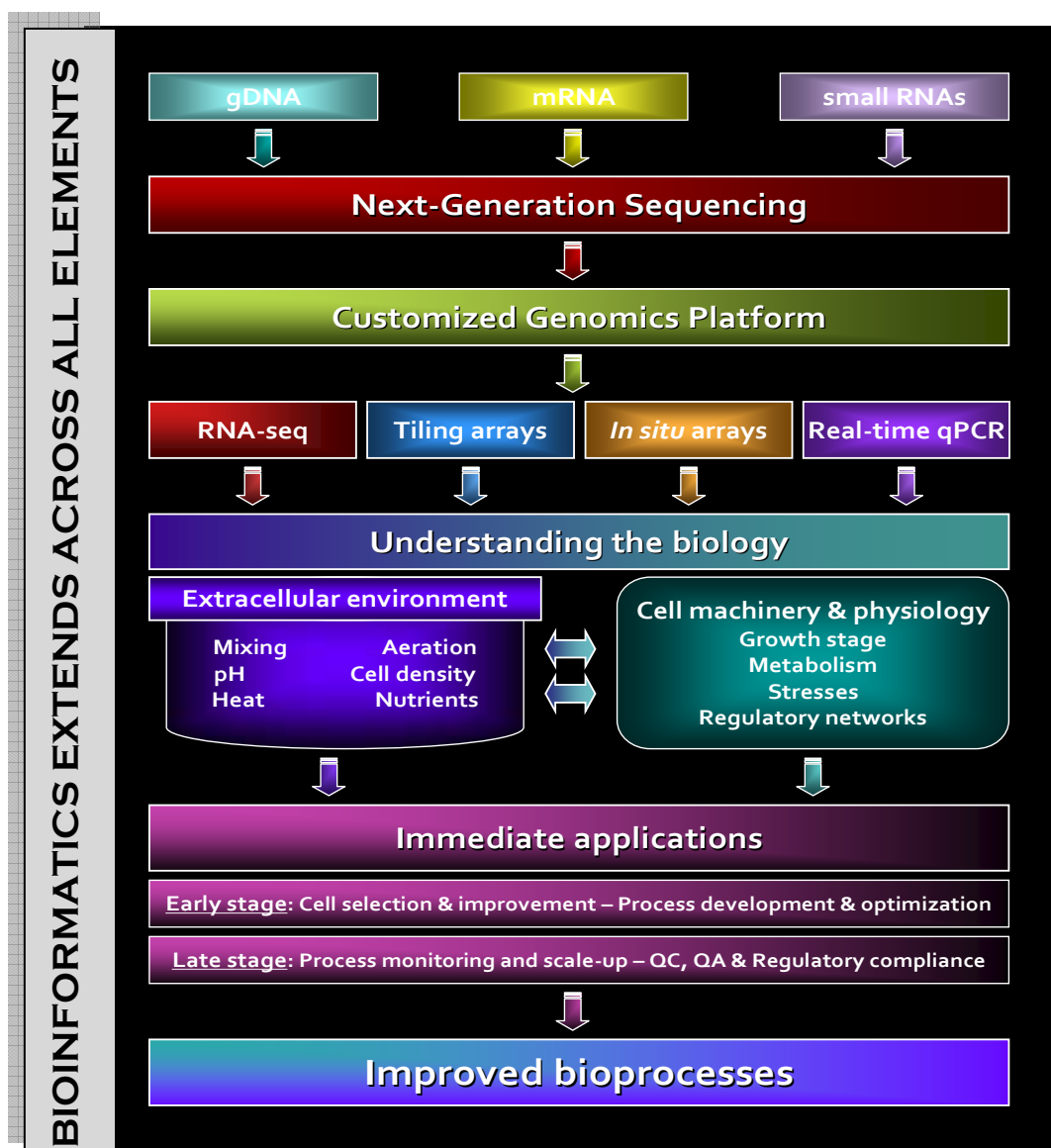




Overview of Next Generation Sequencing (NGS) Applications in Bioprocess Development, Scale-Up, and Commercial Biomanufacturing

ArrayXpress provides research and development services based on first-in-class NGS and bioinformatics to increase the performance, speed, and cost effectiveness of bioprocess development and biomanufacturing. The flow of applications and techniques we provide to partners are presented in the figure below. NGS technologies provide an eagle's-eye view into cellular metabolism and physiology, under changing growth and production conditions, applicable from the selection of a specific cell or microorganism, through bioprocess development/scale-up, and into commercial manufacturing.





ArrayXpress has developed a number of applications that lead to effective, evidence-based bioprocess optimization. Three key applications in the biotechnology sector are: (i) strain/cell line development and improvement; (ii) process optimization, and (iii) commercial manufacturing. Bioinformatics plays a key role in the analysis and mining of large amounts of data.

(1) Sequencing genomic DNA, mRNA (cDNA) and/or microRNAs from production strains or cell lines provides reference genomic information that has a direct impact on understanding the overall biology of an organism. Specifically, it enables the understanding of gene regulation and regulatory networks (e.g., the role of non-coding regulatory RNA elements in gene regulation) and genome structure and dynamics (chromosomal rearrangements and alternative splicing). Understanding the genetic architecture of a production cell/organism lays the foundation for biology-guided process development and phenotype improvement.

(2) To develop functional gene-based markers, RNA-seq of contrasting production conditions or among strains/cell lines can be used to identify candidate genes involved in the metabolism of the production phenotype. Such genetic markers can then be used as molecular fingerprints to assist with the strain/cell line selection and guide the earlier and later stages of bioprocess development, optimization, scale-up and manufacturing. Active monitoring throughout the process life cycle maximizes product yield and quality while minimizing inputs and costs.

(3) Coupled to pathway analysis, RNA-seq of production strains/cell lines in different process and manufacturing conditions can identify key metabolic pathways and the regulation thereof and to identify negative activities that decrease production. The ability to zero in on the control points governing metabolic flow towards increased production allows for process manipulation (for instance, media composition, growth conditions, and feed strategy) based on focused empirical knowledge instead of large designs of experiments and brute force methods. This provides critical knowledge for targeted cell improvement, in a systems metabolic engineering strategy.

(4) Coupled to global transcription machinery engineering, RNA-seq provides a complete, quantitative view of the transcriptome and allows for the mechanistic understanding of the affected transcriptional regulatory networks. The knowledge harvested can be applied to future rounds of cell improvement towards desired production phenotypes.

(5) Better bioprocess characterization may also assist with (a) regulatory compliance, particularly related to FDA biopharmaceutical initiatives on Quality by Design (QbD) and Process Analytical Technology (PAT) and (b) contribute to the protection of valuable intellectual property.

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Biological Process Optimization

A CASE STUDY

Accelerating Media and Fermentation Development

Using Microarray and RNA-Seq Technology

"Gene expression analysis allows researchers to see exactly how an organism responds to environmental stimuli, media components, and changes in growth phases, which in turn can lead to research discoveries that couldn't be found through traditional analytical techniques. The challenge is that a huge amount of data is generated, and finding these discoveries is looking for a needle in a haystack. The right tools and techniques can make it much easier." Sr. Technology Engineer, Pfizer

SUMMARY: ArrayXpress is collaborating with Pfizer with the objective to increase, by at least two-fold, the production efficiency of a key metabolite component of a vaccine in a proprietary bacterium. Analysis of gene expression data mapped onto inter-converting metabolic pathways led to optimizing an amino acid concentration in the growth media that resulted in a remarkable three-fold increase of the metabolite to date.

METHODS: Microarray technology is being used in combination with cutting-edge bioinformatics and data mining techniques to better understand the biology and metabolism of the bacterium used for production. The bacterium produces a secondary metabolite that is a key component of the final vaccine product. Research efforts at Pfizer were underway to increase productivity in the fermentation process by testing new chemically-defined growth media. To enhance the production of the secondary metabolite, it was necessary to more fully understand aspects of the biology of the bacterium under changes in the media and other environmental conditions. Hypothesis-driven studies using a custom-designed microarray was undertaken to reveal changes in the transcriptome, followed by detailed metabolic pathway reconstruction, and bioinformatic analyses under different growth conditions.

RESULTS: A three-fold increase in the targeted product was achieved and a custom, full transcriptome microarray was generated for use in continuing studies to further enhance the production of the product from carefully designed, hypothesis-driven experiments in which further environmental factors will be probed. The cost reductions in the bioprocess are already substantial.