Simplifying Genomic Analysis

With Microfluidics Technology

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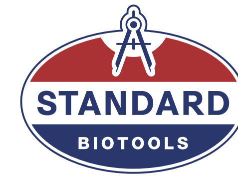

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INTRODUCING THE NEW
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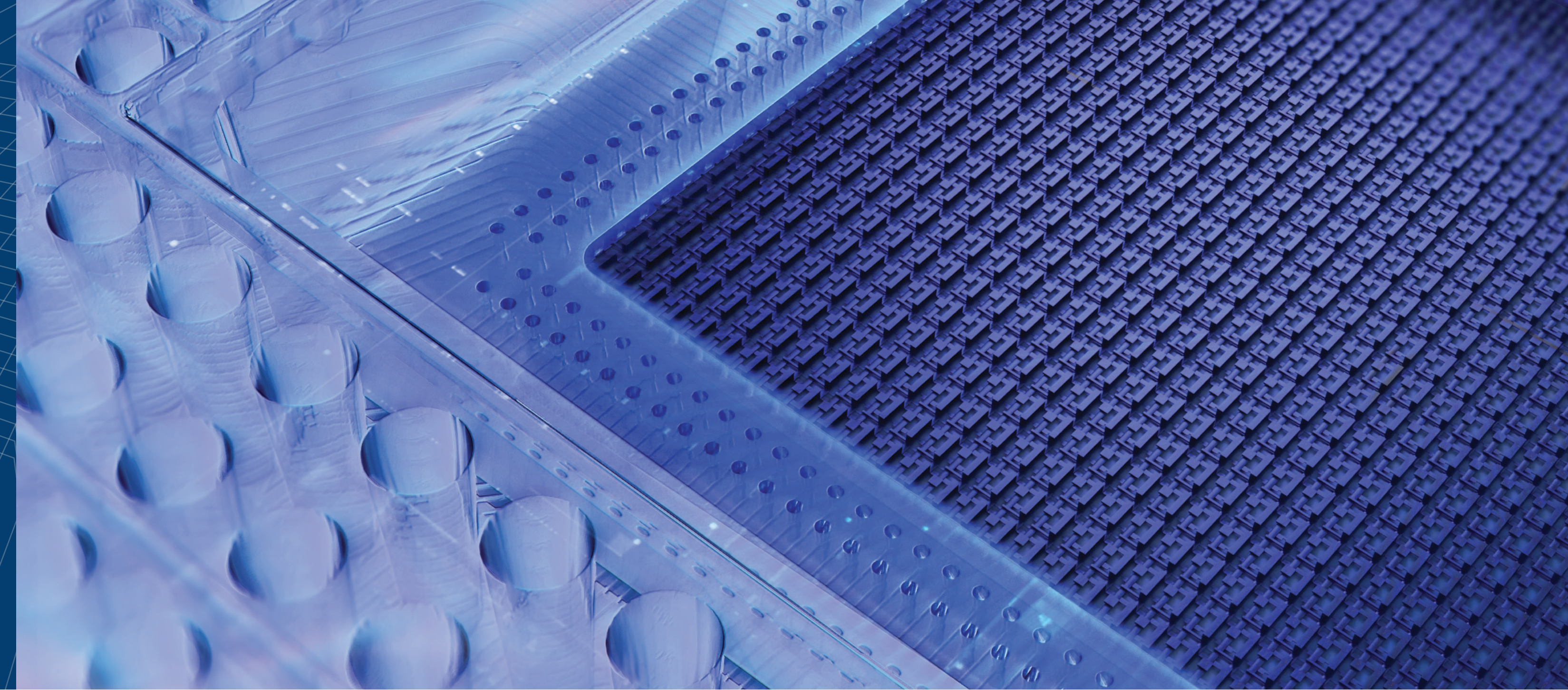
CHAPTER 1

The Benefits of Microfluidics-Based PCR

Microfluidics-based PCR is revolutionizing how labs approach genomics analysis.



Pharmacogenomics research is rapidly becoming a critical component of precision medicine and individualized drug therapy.[1] Precision medicine is built on individual variation in responses to therapeutics and side effects, which can impact and inform clinical decisions regarding treatment strategies. Detecting changes in gene expression that impact drug reactions at the molecular level – pharmacogenomics – will be required to implement such individualized drug therapy approaches in the future.

Due to its sensitivity, classification accuracy, ease of operation, and quick processing time, real-time PCR (RT-PCR) is the most popular approach for pharmacogenomics research.[1] Despite these advantages, there remain some challenges to using RT-PCR. Perhaps the biggest in the context of pharmacogenomics research is scale-up and the associated costs of prep time and reagent use, which are significant already with small-scale operations. Additional challenges include modifying assays to include newly discovered biomarkers, improving data, and standardizing processes.

Fortunately, there is a way to address these challenges: microfluidics. Due to the small size of the reactions they facilitate, microfluidics-based protocols provide significant cost savings on reagents and consumables. This capability to run numerous nanoliter reactions at once allows for more information to be collected from less precious sample material. Microfluidics protocols are also easier to standardize, improve reproducibility, and require less human intervention, saving personnel time. In this eBook, we explore how microfluidics-based PCR is revolutionizing how labs approach genomics analysis.

What is microfluidics?

Microfluidics is, essentially, the manipulation of small volumes of fluids (nanoliters or less) within networks of channels that are tens to hundreds of microns in diameter. Microfluidic instruments have low sample and reagent volume requirements and short analysis times and can facilitate the type of miniaturization that has led to the rise of lab-on-a-chip technologies.[2] Microfluidic platforms are used across a broad range of application areas including pharmacogenomics, sample identification, and agricultural genomics and are revolutionizing the way research in these areas is carried out.

The benefits of microfluidics for RT-PCR

Automated microfluidics-based protocols yield several benefits for RT-PCR and NGS library preparation, particularly when using them for research applications that require scalability, flexibility, and accuracy, such as pharmacogenomics:

- Scalability (For example, the Standard BioTools™ microfluidics-based RT-PCR system requires only about 2 µL of sample for 384 targets, facilitating over 9,000 reactions at once.)
- Flexible, easy-to-adjust protocols and experimental plans
- Small sample and reagent requirements, preserving samples and reducing reagent costs
- Multiple integrated fluidic circuit (IFC) formats to accommodate different combinations of samples or assays with the ability to quickly modify or add to an assay
- Ease of use and standardization capabilities
- Closed system that is reliable, eliminates many individual pipetting steps, and has low contamination risk

These benefits together increase the power of real-time PCR based on microfluidics for applications such as pharmacogenomics by yielding more data and deeper insights with less effort. In this eBook, we'll dig deeper into some of the critical advantages made possible through microfluidics technology.

Less effort

Automation is a critical aspect of flexible, scalable, and accurate microfluidics-based technologies. Contrary to popular opinion, automation isn't just for labs processing thousands of samples regularly. It yields several advantages for any lab, regardless of size, such as improved reproducibility with, and because of, less manual effort. In Chapter 2 of this eBook, we discuss the benefits automation provides to any lab, whether small- or large-scale, and provide real-world examples of benefits experienced by research groups utilizing Standard BioTools microfluidics technology. Less time and effort needed from personnel leads to significant cost savings, which we also explore.

More data and deeper insights

A major benefit provided by automated microfluidics-based technologies is the deeper insights they enable. Multiplexing has long been used to extract more data per sample from experiments, but it suffers from uneven amplification across different targets, an issue that is addressed using singleplex reactions. With microfluidics technology, samples and reagents can be loaded separately and then automatically combined in a pairwise manner within the closed system to create individual singleplex reactions in a multiplex format. In Chapter 3 of this eBook, we dig deeper into the benefits provided by singleplex simplicity at multiplex throughput.

Concluding remarks

Pharmacogenomics research is a critical research area enabling precision medicine and personalized therapeutics. Doing it at scale is the only way to identify the unique interactions between host genetics and environment that impact how individuals respond (or don't) to specific therapeutic interventions and schedules. But scaling RT-PCR, the major biomolecular tool enabling pharmacogenomics, has been a challenge.

In this introduction, we provided a small sampling of the efficiency, time and personnel savings, scalability, and flexibility that microfluidics technology can bring to RT-PCR. Download and read the entire eBook to learn more about how microfluidics technology can make your RT-PCR protocols work harder and better for you, facilitating safety and efficacy studies for improved disease management.

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CHAPTER 2

When and Why to Automate

Benefits include improving lab efficiencies and cost savings for years to come.

By **Steve Kain**

To automate or not to automate

Over the past several years, I've worked with researchers in a variety of lab environments. These include both individual labs and genomics core labs, both in academia and commercial settings.

When considering automation systems for workflows such as genotyping or gene expression by real-time PCR, the most common driver for adoption is sample number. Researchers often feel that unless they are routinely processing hundreds to thousands of genomic samples, investments in an automation system do not make sense.

Consideration of sample volumes is important, and no doubt automation can save money at high volumes of samples, but these are not the most important reasons to consider automating your genomics workflows.

First and foremost, automation leads to more robust assay performance with improved reproducibility.

The length of many genomics workflows requires several small-volume pipetting steps, mixing, incubations, and transfer steps. Each of these steps can introduce considerable variance, leading to questionable results and the need for assay replicates to minimize variable findings. When automation systems are employed, such processing steps are standardized and lead to less variability.

For example, one of the more commonly used assays with Standard BioTools microfluidics technology is genotyping performed on the Biomark™ X system with a 96.96 Dynamic Array™. This system can generate 9,216 datapoints from 96 samples and 96 individual PCR reactions run in parallel. The equivalent experiment using conventional systems requires preparing 96 x 96-well plates.

Imagine the variability that could be introduced between reactions due to manual processing at this scale, not to mention the additional time and costs incurred. In contrast, the Dynamic Array is a closed system that automatically runs singleplex reactions at the same time.

Many of the processing steps are fully automated on Biomark X™, including thermocycling and data acquisition. Because of the exacting standards for the design and manufacture of the Biomark X system components, you can be assured of great reproducibility between samples, runs, and laboratory operators. Definitely consider sample volume when you think about automating genomic workflows, but understand that the most important reason is to improve the reproducibility of your assays and hence the reliability of your experimental results.

Invest in automation to save money in the long run

For core lab directors, your most precious and expensive resource likely leaves the lab at the end of every day – your staff.

In order to reduce overhead and save money, core labs should focus on a new metric popular in startup companies. It's known as revenue per employee. If your average revenue per employee is lower than your expense, then you can focus on maximizing ways to increase that revenue by implementing processes that make your employees more productive. One such example is to invest in automation solutions to get the most out of your labor costs.

As one example, customers who have implemented the Advanta™ RNA-Seq NGS Library Prep Kit together with the Juno™ system can perform cost-efficient NGS library prep due to reduced reagent expenses and lowered labor costs.

In comparison to standard manual processing, the automated library prep method can save almost $200,000 USD for the preparation of 5,000 RNA-seq libraries. In addition, the intuitive interface and streamlined operations of Juno mean labs are able to minimize time spent by staff in preparing libraries.

Because the majority of the processing steps are automated, the hands-on time for library preparation is significantly reduced and requires less-skilled operators. This latter benefit also helps to ease the burden of training new people when lab staff turns over. Lastly, by investing in quality instrumentation such as Juno, you will have lab automation that is built to last, thereby improving lab efficiencies and cost savings for years to come.

About the author

Steve Kain, PhD, combines his scientific knowledge with commercial experience in genomics, cell biology, drug discovery, next-generation DNA sequencing, and botanical science and testing services.

CHAPTER 3

To Singleplex or Multiplex

What's the difference?

Several key factors must be considered when designing a real-time PCR experiment, including how many targets you intend to detect. Many researchers opt for multiplex detection chemistries due to its ability to provide more data per sample with minimal added cost, but you need enough targets for it to make sense. When getting ready to start your real-time PCR experiment, one of the first key decisions is assay design, and you need to decide whether singleplex or multiplex detection is the best fit. These two detection methods involve different assay design and setup, each with its own set of advantages and disadvantages.

Why multiplex

In multiplex PCR, multiple target sequences are amplified in a single reaction. In real-time PCR, these target sequences are commonly detected using probes that have different dye labels. Multiplex assays are attractive because they can help you save time, cost, and most importantly, sample. However, multiplexing has its drawbacks. Primer design plays a crucial role in multiplex success, and often multiple rounds of optimization are needed to determine an appropriate primer concentration. Primers and unintended PCR products or artifacts may compete in amplification, resulting in uneven amplification for different targets. In fact, it's common practice to perform singleplex PCR in order to amplify loci that multiplexing has failed to amplify.

Once optimized, multiplex assays can also be difficult to modify. Given the time and effort required to design a successful assay with a particular combination of compatible primer pairs, redesigning easily becomes a headache. Changes in routine assays are inevitable to advance any system, whether for screening or testing, but the ability to adapt quickly to these changes is not afforded with multiplexed reactions.

Why singleplex

Singleplex PCR is easier than multiplexing. Only one target is amplified per reaction, so your assay is easier to design and implement due to the absence of potential competition during PCR. However, many researchers find singleplexing limiting, since using several reactions to detect multiple targets can mean higher costs in materials and labor and more sample used per reaction.

The ability to scale an assay is also challenging when using singleplex reactions. Since each reaction must be isolated in an individual well, you can only scale to the number of reactions often done in 96- or 384-well plates that can be completed in a day. And when you consider the need for more reagents every time you add more reactions, cost can quickly become an issue.

Multiplex with singleplex simplicity with Standard BioTools microfluidics technology

Standard BioTools microfluidics technology assembles your PCR reactions for you in an automated, miniaturized fashion. The integrated fluidic circuits (IFCs) have nanoscale reaction volumes, so you use less of your precious sample and reagent. Samples and assays are loaded into the IFC separately and then automatically combined in a pairwise manner within the closed system of the IFC to create individual singleplex reactions, with up to 192 singleplex reactions per sample (depending on IFC format). You can add, remove, or replace assays on demand and scale throughput without changing technologies, allowing up to 96 individual samples and controls to be interrogated with up to 96 individual assays with the same dye label, for a total of up to 9,216 individual reactions per run. Think of it as multiplex throughput with singleplex simplicity.

Learn more about microfluidics technology >

CHAPTER 4

Considerations for Cost-Effective Analysis in Genomics-Driven Fields

Microfluidics technology empowers the generation of timely and actionable answers that can be transformative.

The end result of any genomic investigation is discovering the role that genetics plays in health, disease, and potential treatment. In order for basic, translational, and clinical researchers to learn about differences and changes in an individual's genetic makeup, they must identify, measure, and compare genomic features or regulatory and functional elements within the genome.

While there are a variety of technologies and tools used in genomic analysis, progress and improvement in high-throughput and cost-effective methods are inconsistent depending on the application and the accuracy of data needed.

Get big answers with micro-sized technology

Comprehensive and versatile genomic analysis with microfluidics technology empowers the generation of timely and actionable answers that can transform all genomics studies. Proven to enable high-complexity analysis with unprecedented proficiency, microfluidics-based solutions streamline workflows for applications demanding sensitivity and broad range, including genotyping, gene expression, sample identification, copy number variation, and NGS library preparation.

Simplifying workflows through nanoscale automation maximizes efficiency and provides the flexibility to scale projects with increased data output. This allows you to adjust your experimental plan to match your needs and interests with microfluidics-based PCR and NGS library preparation.

Here are just some of the areas in which the life sciences community is using microfluidics technology to accelerate and advance genomics knowledge.

The promise of personalized medicine with pharmacogenomics

Drug development and efficacy testing can be improved by more comprehensively understanding the effects of drugs and their mechanism of action. Pharmacogenomic research and pharmacodynamic studies are a key element of personalized and precision medicine, significant in understanding response to medications.

Research has shown that a pharmacogenomic profile can be used to guide care and reduce the incidence of adverse drug reactions. These studies can be accelerated using microfluidics-based real-time PCR to test multiple targets with multiple interactions at once. This provides a more complete overview of how a target molecule functions when binding to an active site on an enzyme or interacting with cell surface signaling proteins that can disrupt downstream signaling.

Microfluidics technology facilitates pharmacogenomics studies with a single high-throughput workflow that consolidates several assays into one experiment without the need for multiplexing. This allows labs to customize content, avoiding the limitation of fixed-format panels, and cut sample and reagent use by 100x.

The Advanta Pharmacogenomics Assay:

- Supports extraction-free and extracted buccal swabs
- Uses a single workflow for SNPs and CNVs
- Contains a core panel of actionable targets with flexibility to add custom content
- Combines analysis software with interpreted results

Case study

In a 2021 paper, a group from the University of Minnesota describes developing and implementing a cost-effective in-house pharmacogenomic testing research program at a major academic health system. Pharmacogenomics testing in clinical research presents a major opportunity for improving therapeutic outcomes and could become a significant part of precision medicine. However, this value is limited by successful implementation of actionable testing.

This work outlines the development and technical validation of an in-house SNP targeting multiplex PCR-based assay on the 96.96 Dynamic Array used with a Standard BioTools IFC Controller HX, FC1™ cycler, and Biomark HD.

Principles and methods of sample identification to support integrative genomic analysis

Single-nucleotide polymorphism (SNP) genotyping methods are broadly used for sample identification and quality control in a biobank facility, core lab, or sequencing center. Genotyping with a dedicated set of SNP assays for genetic sex determination, DNA quality assessment, or a SNP fingerprint can confirm sample integrity and origin.

Biobanks and genomics centers must overcome several challenges to ensure that they provide the highest-quality, correct samples for research projects. Sample mix-ups, contamination, or handling errors can occur before or after samples enter the molecular laboratory or storage facility. Processing misidentified, poor-quality, or contaminated samples may lead to incorrect interpretation of results.

Implementing a standard genotyping workflow to confirm the identity and quality of each sample before analysis represents an ideal solution to maximize the integrity of study results.

Microfluidics-based PCR offers the quality needed for confidence in sample identification data. The Advanta Sample ID Genotyping Panel is a 96-SNP assay enabling laboratories to generate a sample-specific genetic fingerprint and quality assessment from research specimens throughout the sample journey.

What is molecular sample identification?

This proactive sample identification system is also known as sample ID or DNA fingerprinting. Panels of DNA markers are analyzed for each sample, generating a unique genotype or fingerprint. The genotype obtained is an indelible, nontransferable identifier for the sample. Carefully selected markers can provide additional information, such as assessment of sample quality, genetic gender identity, and population prediction.

How is it used?

DNA samples can be fingerprinted (identified) prior to distribution.

Sample identity can be confirmed.

- Compare genetic sex to reported sex.
- Compare genotypes from distribution sample to accession genotypes.

Low-quality DNA issues can be identified.

- Low genotype calling rate
- Sample contamination

Cancer genomics for precision oncology

Personalized approaches for cancer treatments are challenged by the individual nature of cancer itself, presenting differently in each patient. The identification of predictive biomarkers and signatures could help direct which patients would benefit from a therapy or accurately assess the risk of recurrence and progression.

Microfluidics-based gene-expression assays can aid in the research for therapy decision-making by identifying suitable biomarker candidates. The capability to scale this kind of discovery while reducing the volume of reactions supports the active search for effective biomarkers without the barriers of cost and time.

Case study

In a 2022 paper in OncoImmunology, "Innate lymphoid cells: NK and cytotoxic ILC3 subsets infiltrate metastatic breast cancer lymph nodes," Institut de Recherche Saint-Louis scientists investigated the impact of the local tumor environment on innate immune response in the lymph nodes of breast cancer patients. Innate lymphoid cells (ILCs), including cytotoxic natural killer (NK) cells and helper-type ILCs, are important regulators of tissue immune homeostasis.

Through flow cytometry, cytokine release assays, and qPCR (using Juno and Biomark HD), they characterized the ILC populations that were infiltrating lymph nodes. Data showed that the local tumor microenvironment inhibited NK cell functions, but cytokine stimulation restored their functionality. Results suggest a consideration of combination immunotherapies for improved efficacy.

As emerging therapies reveal new biomarkers and expand the need for samples, the costs and labor required to complete this important research work also rise. The Advanta IO Gene Expression Assay, used with the Biomark systems, was developed in collaboration with leading researchers in the biopharmaceutical industry to provide the right balance of biomarker breadth, assay flexibility, and workflow efficiency, providing a reliable, sensitive, and cost-effective toolset for identifying gene-expression signatures from immune and cancer cells.

The panel consists of 170 genes, including markers for:

- Immune cell identification
- Immune and cancer cell function
- Immune regulation and cell fate
- Checkpoint therapy response

Optimized for formalin-fixed, paraffin-embedded and fresh frozen tumor samples, the assay uses TaqMan® chemistry to sensitively measure gene expression, with five reference genes serving as analysis controls. Each reaction is miniaturized to nanoliter volume and controlled using precise automation to empower accurate and cost-effective qPCR analysis across a large dynamic range. Since assay introduction into the IFC is under user control, researchers also have the flexibility to add up to 17 new assays or exchange gene assays within the panel to achieve experimental goals – all without affecting the original panel content, protocol, or workflow.

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CHAPTER 5

Challenges and Perspectives in Evaluating Immune Function and Mechanisms of Cell Differentiation

When designing a study, many factors must be considered that, when put together, empower the generation of valuable data that can be turned into actionable insights.

A well-designed study can provide the framework to ensure scientific integrity and credibility of data. We've talked with a group of experts in the fields of infectious disease, immunology, and molecular genetics about their philosophies on how to approach a research question, what tools make the most sense, and why they chose what they did.

When you thought about designing your research study on the immune response to infection, how were you able to test so many samples within a set timeframe and budget?

Leisha McGrath, a PhD student at the Marine and Freshwater Research Centre in Ireland, focused her graduate research on the presence of antimicrobial peptides in Atlantic salmon. Knowledge of the initial immune response in fish infected with amoebic gill disease could potentially offer a method to detect onset as climate change increases risk of disease.

The system we used was very beneficial for us, and the setup was ideal – very straightforward, very easy to follow up, and much faster than we had been expecting. We actually started setting up an overwhelming number of qPCR reactions manually in tubes, working out the number of tissues we needed to test, how many replicates would be ideal for this type of experiment, and what the cost would be in running so many reactions.

The center had just installed a Biomark HD system, and the team was told they would be able to do all their reactions on one microfluidic array instead of multiples of tubes. This was very appealing. We thought that would be a much better and simpler approach. Plus, we didn't have to go back and do multiple rounds of qPCR, which in itself would introduce more variation and more issues.

I suppose it's difficult to visualize it before you see the system work for yourself. But once you perform the experiment and get the readout, you understand that compared to a standard reaction volume or standard tube, it is such an advantage to be able to stretch everything out a bit further.

I think what we're showing with this study is that the Biomark system enables simple setup across a range of experiments, empowering you to screen bigger populations detection, and study different infections or even support vaccine research or understanding response to treatments. You could do it all.

How did you choose the right approach to RNA analysis in your hypoxia-on-a-chip research, given the small size of your experimental platform and minimal cellular material obtained?

Scott Magness, PhD, is an Associate Professor and the Founder and Director of the Center for Gastrointestinal Biology and Disease Advanced Analytics Core at the University of North Carolina School of Medicine and is interested in understanding the genetic mechanisms that control stem cell maintenance and differentiation.

For us, the Advanta RNA-seq workflow was vital to our studies because we were challenged with only being able to retrieve a small number of cells from our model for subsequent analysis. The culture chamber in the gut-on-a-chip system is very small – the entire five-well platform is smaller than a microscope slide – and we only get about 50,000 cells that generate a confluent monolayer. This translates to small amounts of cellular material and small amounts of harvested total RNA to use in the library prep protocol.

Conventional RNA-seq systems simply cannot achieve what we need on this system. Also, in order to perform all technical and biological replicates necessary for these experiments, conventional systems become very expensive. The Advanta system was a game changer, enabling us to use these microdevices and characterize as many transcriptional responses as we can while managing costs.

Our data quality from our samples with low cell numbers was excellent, providing a really nice overview of what was happening to these stem cells during a hypoxic event. For example, we obtained >30 million reads per sample, 98.5% aligned to the human genome reference and 80.2% aligned to the transcriptome reference. This level of clean data helped us immensely in pulling out differential gene-expression patterns that we could use to generate hypotheses for how the cells were responding to hypoxia over time.

Read Scott's interview to see how he developed a hypoxia-on-a-chip environment.

Why is scalability and cost-efficiency so important when DNA fingerprinting samples at the genetics biorepository?

Kelly Nudelman, PhD, Assistant Research Professor, and Tae-Hwi (Linus) Schwantes-An, PhD, Assistant Research Professor, highlight the importance of sample identification and quality control at the Indiana University Genetics Biobank.

One of our goals is to ensure that all of our samples have been quality-controlled to the highest standard. Using DNA fingerprinting to assess samples prior to accession and distribution can save time and resources. Researchers who receive samples from the biorepository need to have confidence that the samples they receive have been correctly identified from the sample source and will yield interpretable data from their planned downstream analyses.

The Standard BioTools workflow was chosen because of its scalability and cost-efficiency and the small quantity of DNA needed to test samples.

We found that many alternative workflows are either high-throughput/high-cost or low-throughput/low-cost. What makes the Standard BioTools microfluidics-based workflow unique is that it is high-throughput at a cost that is scalable to our process. It saves our customers and our lab time and resources.

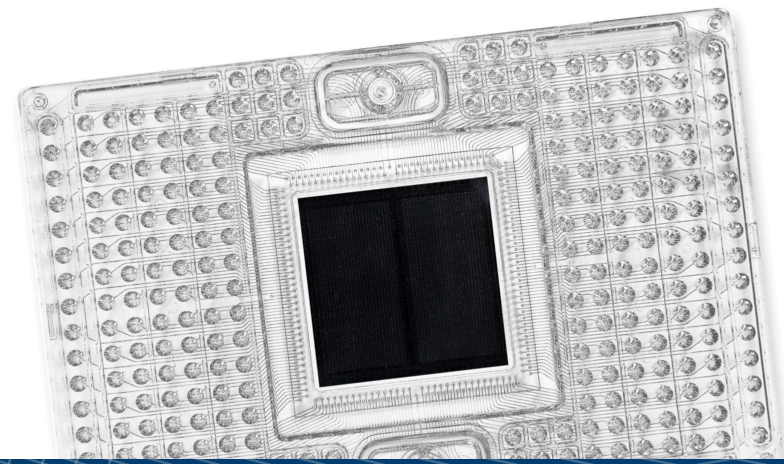
For example, as the field of neurodegenerative disease grows, new targets of interest will be identified. Being able to customize the assay allows us to adapt to how the field is changing, and this is something we can do using the Standard BioTools workflow. Let's say a new variant is discovered, and everyone wants to know the status for that particular variant in her or his sample, we'll be able to easily make a modification to our panel, adding the new variant quickly.

What can you reveal with single-cell versus bulk analysis and how can you obtain the level of sensitivity needed to detect a wider range of gene expression?

Christophe Lancrin, PhD, Research Group Leader at the European Molecular Biology Laboratory, is focused on discovering an intermediate cell population with endothelial and hematopoietic characteristics co-expressing seven essential transcription factors at the single-cell level using C1™ and Biomark HD.

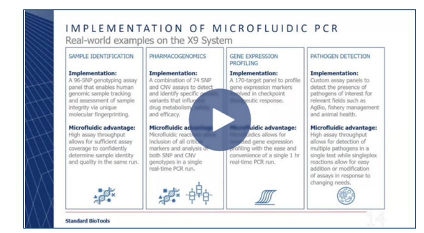
Single-cell analysis is powerful. When you work in bulk, you miss information. Working with single cells is definitely a big plus but you need to use the right technology for the right question. Although bulk transcriptomics can reveal crucial overall gene correlations between semi-stable cellular states, it cannot resolve subtler gene interactions occurring in complex transitional states. In addition, using a bulk approach makes it difficult to infer the direct consequences on the transcriptional landscape upon which these transcription factors are acting. These limitations can be overcome by the use of single-cell approaches.

We needed the C1 full-length mRNA sequencing technology because of its sensitivity. Not only were we detecting transcription factors, but we also had to detect a range of gene-expression levels. We required higher-quality sequencing data to perform these challenging analyses.



CHAPTER 6

Resources



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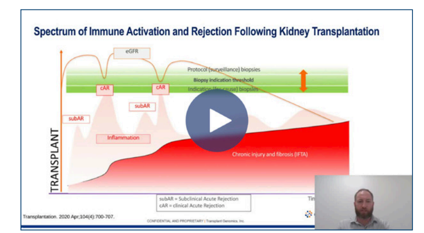
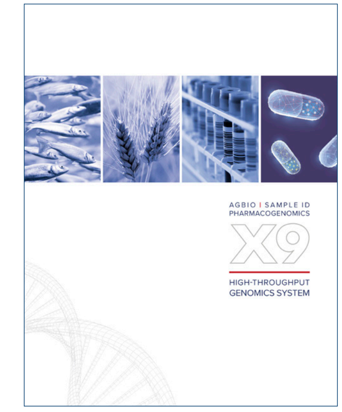
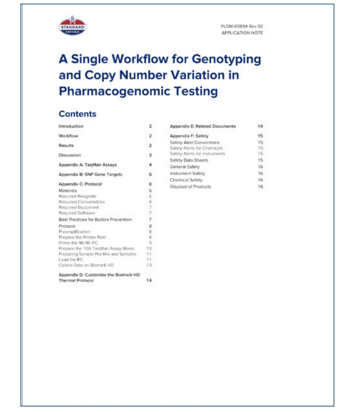


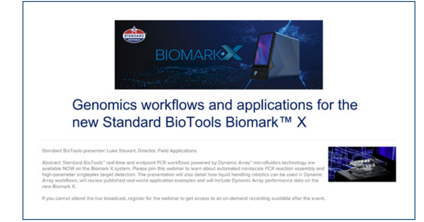
The PCR Advantage: How the Biomark HD System Enhances the TruGraf Gene-Expression Test for Kidney Rejection



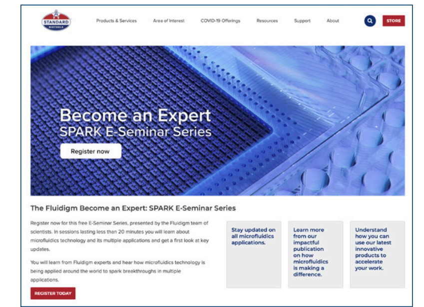
Brochure: X9 High-Throughput Genomics System



A Single Workflow for Genotyping and Copy Number Variation in Pharmacogenomic Testing Application Note



Genomics workflows and applications for the new Standard BioTools Biomark X



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