

Contents

Personalized Medicine: Genomics to Proteomics

p 1

Tools for medical research

FTA® Elute: A Tool for Pharmacogenomics Research

p 3

Medium for DNA collection and preparation

Protein Microarrays on FAST Slides as Tools to Study Cellular Signal Transduction

p 7

Tools for studying Protein-Protein Interactions

Contributing Authors:

Dr. Breck Parker**Dr. Betsy Moran****Dr. Michael Harvey****Lisa M. L. D'Anzi****Dr. Michael Walther**

Editors:

Senior Editor:

Betsy A. Moran, Ph.D.

Managing Editor:

Lisa M. L. D'Anzi

SOLUTIONS ENABLING PERSONALIZED MEDICINE

FROM GENOMICS TO PROTEOMICS

The development of new technologies in the areas of Genomics and Proteomics is advancing our understanding of biological systems and their functionality. Genomics technologies provide researchers and clinicians with the ability to identify genetic differences between individuals and correlate them to disease states, drug treatments, dietary requirements and decisions on courses of medical treatments. Proteomics technologies allow for the identification and detection of proteins indicative of disease onset and progression. The combination of advances in genomics and proteomics is paving the way for whole systems biology as a process representing critical guide-wires that lead to improvements in medical treatments for humans as well as animals.

Genomics applications begin with sample collection and isolation of pure, high quality nucleic acids. The identification of novel markers for disease prognosis requires large numbers of samples and can take many forms. For example, samples may be in the form of whole blood, buffy coats, buccal swabs, urine, and saliva. FTA® Elute is uniquely suited as a matrix to collect, store and process DNA from a variety of sample types. FTA Elute is a chemically impregnated matrix that lyses cells, irreversibly binds proteins to the matrix, and releases Genomic DNA using a simple heat and water elution protocol. The eluted DNA is of sufficient quantity and quality to make it well suited as a substrate for real time PCR based assays, allelic discrimination assays, and whole genome amplification to name a few of the genomics applications that need no additional sample processing. FTA Elute provides a matrix that stabilizes



nucleic acids for long term storage inexpensively at room temperature, and the bacteriocidal and virucidal chemistry renders the sample safe for mailing.

Many genomics applications require input DNA at approximately 1 ng. This is important for processes that multiplex the reaction, including STR analysis, allelic discrimination assays, and related real time PCR applications. The DNA concentration obtained from whole blood spotted onto FTA Elute can range from 0.2 ng/ μ L to 2.0 ng/ μ L when eluted from two 3mm punches in 60 μ L of water. The variability in the range of DNA concentrations may be due to the natural variability observed between different blood donors, i.e., differences in hematocrit, leukocyte counts, platelet levels, sex, age, race and state of health of the donor. Total yields from two 3 mm punches can reach as high as 200 ng of high quality, amplifiable genomic DNA ready for genomics based applications.



Several proteomics programs have been built on the strength of FAST® Slides, a 3-dimensional nitrocellulose glass slide based surface for the immobilization of proteins in ordered arrays. Multiplexed, slide-based protein analysis has become a significant technology within the discipline of proteomics. This field of protein arrays is emerging as an important tool for basic and clinical research as well as diagnostics. Whatman's protein array product portfolio enables researchers to carry out large numbers of protein analysis experiments in a highly parallel fashion. The FAST Quant® line of kits and services enables the screening of many sample types (serum, plasma, lysates, CSF, wound exudates, etc.) to determine the concentrations of ten or more proteins with high accuracy and sensitivity in a multiplexed format. The Serum Biomarker Chip (SBC) can screen clinical samples to discover potential cancer biomarkers. This approach can be a valuable tool in identifying drug targets to reduce the cost of the drug development process. The CombiChip

AutoImmune kit is an array of 14 antigens which are accepted markers to diagnose collagenosis and vasculitis-type autoimmune diseases; this product was recently launched as a CE-marked diagnostic in Europe. Researchers throughout the field of proteomics have adopted the FAST Slides array platform and associated reagents and components for a variety of applications. This includes their use in reverse arrays, which involves arraying up to thousands of samples onto FAST Slides and screening them in parallel for the presence of protein targets. The continued implementation of protein arrays will enable further scientific discovery, cost reduction, and removal of logistical bottlenecks for both basic research and drug development.



FTA ELUTE: A TOOL FOR PHARMACOGENOMICS RESEARCH

MEDIUM FOR DNA COLLECTION AND PREPARATION

The National Center for Biotechnology Information determined that in 1994 there were over 2.2 million cases of adverse drug reactions in the United States. Adverse drug reactions, which are rooted in individuals' variable responses to medication, are a major cause of hospitalizations. Medications and doses, which are developed around the average response of a population, may affect each individual very differently.

The study of pharmacogenomics can provide a solution to this problem. Pharmacogenomics refers to the study of the numerous genes in an individual that determine his or her reaction to drugs. Each drug acts on a particular metabolic



Figure 1 To prepare DNA, remove a 3 mm disk from the sample area on FTA Elute, wash the disk with water then elute the DNA by heating the disk in water to 95°C

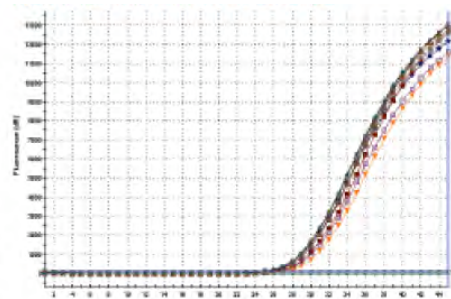
pathway in the human body. Each pathway involves many proteins, and therefore many genes.

A person's genetic make up can have a profound influence on how he or she will respond to a drug. This discovery has led to the study of pharmacogenetics, which refers to the relationship between an individual's genetic profile and his or her corresponding drug metabolism and response. Genetic tests, which analyze an individual's genomic pattern of Single Nucleotide Polymorphisms (SNPs), can reliably predict his or her likely response to a given drug. The successful pharmacogenetic analysis of an individual will, ideally, lead to a doctor prescribing the best drug at a dose that will maximize benefit while minimizing risk.

Genetic testing facilities currently use several methods of genotyping, from traditional DNA sequencing to mass spectrometry to microarray technology. The first bottleneck of pharmacogenetics, however, is sample collection and DNA preparation. The dominant method of sample collection involves the use of traditional vacutainer-based blood samples, which has a number of drawbacks. First, individuals participating in a study or drug trial must have their blood drawn by skilled personnel at a centralized location, and the drawn samples necessitate refrigeration. Shipping the blood, which is designated as a biohazardous material, requires ice and insulated packaging. Extracting DNA from the blood samples, a task performed at a genotyping laboratory, requires labor-intensive and time-consuming methods. Finally, there is the drawback that the extracted DNA samples must be stored in -70°C freezers, which are both expensive and susceptible to electrical power outages. A common technique used to mitigate this risk involves storing portions of each sample in several locations, thus requiring multiplied resources of redundant freezer units, labor, and electricity.



The pressing need for a method and device to rapidly collect, ship, store, and purify DNA has led to the development of FTA Elute matrix devices. FTA Elute is a chemically coated matrix which reversibly traps DNA from multiple types of biological samples. The chemicals within the matrix preserve DNA such that the samples are stable at room temperature for extended periods of time, simplifying sample storage and shipping. Potentially harmful viruses and pathogens are inactivated by the matrix coating so that the samples are safe to handle and free of biohazardous material. Just a few drops of blood from a finger-prick (approximately 10 – 40 µl) provide enough DNA for many genetic analyses. When used in combination with Whole Genome Amplification (WGA) technologies, FTA Elute provides virtually unlimited supplies of DNA for a wide number of tests. Valuable biological samples can be archived or banked at ambient temperature, replacing expensive and space-consuming freezer banks.



Ten individual samples of DNA purified using FTA Elute were prepared for real time PCR of an in-house reference DNA fragment. For this assay 2.5 µl of the extracted DNA was mixed with the DNA binding dye Yo-Pro 1 (Invitrogen) to monitor real-time PCR Amplification. A no template control was included in the assay and appears as the flat line at the bottom of the profile.

Figure 2 Real-time PCR of 10 individual blood samples

FTA Elute matrix devices are a simple and rapid method for purifying DNA and can easily be automated for high throughput genotyping laboratories, saving time and money. To purify DNA from FTA Elute, a small (3mm) punch of the FTA card is placed in a microcentrifuge tube or multiwell plate and rinsed with water (Figure 1). The wash is removed, fresh elution water is added and the disk heated for 30 minutes at 95°C—this step releases DNA from the matrix while proteins, impurities, and inhibitors remain bound to the matrix. The DNA is then ready for use in a number of genotyping methodologies.

Real Time PCR

DNA eluted from the FTA Elute matrix device was subjected to quantitative PCR to demonstrate the quality of the purified DNA. Blood samples were collected from 10 separate individuals onto the FTA Elute matrix. Disks (3.0mm) were

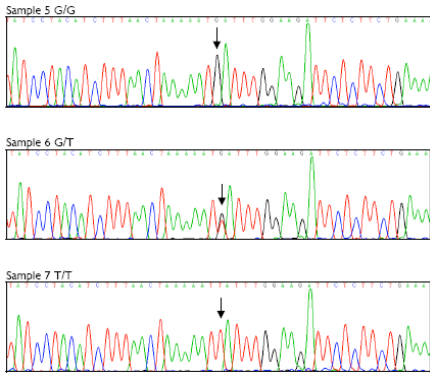


Figure 3 SNP genotyping by sequencing of DNA from FTA Elute

extracted according to the water extraction protocol in a final volume of 100µl. Approximately 2.5µl of the purified DNA was added to the real time PCR mixture and amplified using the Yo-Pro 1 DNA binding dye (Invitrogen). Figure 2 shows a very tight grouping of curves with an average Ct of 26.58 which equals 22.14 ng of DNA in the 100µl final volume. The yield of DNA from FTA Elute is sample dependent. For blood and buccal samples, the yields are 55-70% and 60-75%, respectively.

DNA Sequencing

DNA sequencing is the “gold standard” for detecting polymorphisms in gene sequences. DNA purified with FTA Elute was tested as a template for sequencing a 1.05 kb fragment from exon 1 of the 2B15 variant of the UDP-glucuronosyltransferase (UGT) gene. The G/T polymorphism at the D85Y locus was the target SNP. Figure 3 shows an example of three sequences from individuals showing polymorphisms in the sequence. This profile indicates that the DNA extracted with FTA Elute yields excellent sequencing data.

Multiplex Gene Deletion Assay

Many labs perform multiplex gene deletion assays using end-point PCR and agarose gel electrophoresis as a means of genotyping. Figure 4 shows a multiplex PCR performed with DNA purified with FTA Elute detecting a deletion in the UCT2B17 gene. The wild type and mutant genes are demonstrated by bands of 316 bp and 884 bp, respectively. As can be seen, the individual in lane 7 is homozygous for the mutant form of the gene. The ability to multiplex with multiple primers requires high quality DNA to prevent mis-priming and non-specific banding patterns. DNA purified by FTA Elute is robust and less than 1 ng is required to yield clean strong bands in the multiplex PCR.

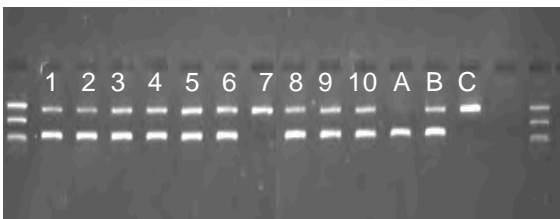


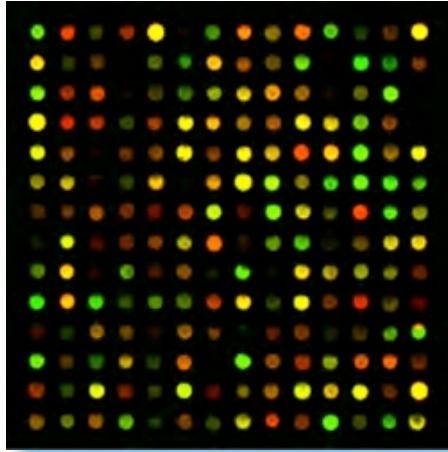
Figure 4 Multiplex Gene Deletion Assay
Lanes 1-10: experimental
Lane A: wt/wt
Lane B: wt/mutant
Lane C: mutant/mutant

FTA Elute is a unique and dynamic product positioned to serve the pharmacogenomics and bio-banking markets. FTA Elute implements a simple, one step method to collect and stabilize DNA for genotyping and SNP analysis. A quick hot water elution method yields high quality DNA for a variety of genotyping methods such as Amplification Refractory Mutation Systems (ARMS™) Scorpion™, and Allele-Specific Oligonucleotide (ASO) genotyping. DNA from FTA Elute can also serve as a template for Whole Genome Amplification (WGA), thus supplying a nearly unlimited amount of DNA for extended genetic studies. The archiving ability of the FTA Elute matrix is beneficial in “look back” studies and for archiving samples for future genetic analysis.

PROTEIN MICROARRAYS ON FAST[®] SLIDES: TOOLS TO STUDY CELLULAR SIGNAL TRANSDUCTION

PART 2: STUDYING PROTEIN-PROTEIN INTERACTIONS

Proteins interact in a multitude of different ways. When a protein kinase phosphorylates a target molecule, these two proteins certainly interact; however, the term protein-protein interaction usually describes binding of a noncatalytic domain of one protein to a specific motif in a second protein. Most interaction domains are small, globular entities which range in size from about 35 to 150 amino acids. Their binding partners are usually very small motifs of less than 10 amino acids. Protein domains are modular entities embedded in larger protein sequences. Their function is to tether together multiprotein complexes. Recruitment of



proteins into signal transduction cascades via protein interaction domains is a general principle found in many receptor mediated pathways.

In many cases, binding is regulated by posttranslational modification of the motif, such as acetylation or phosphorylation. In fact, one of the most important functions of protein phosphorylation is to generate or abolish binding sites. Protein domains can be grouped by sequence homologies into families such as SH2 and SH3 (Src-Homology 2 and 3) or WW (a domain with two conserved tryptophans). These families can be grouped by their target motifs into phospho-Tyr binding domains (SH2, PTB), phospho-Ser/Thr binding (e.g. WW, 14-3-3), proline-rich motif binding (e.g. SH3), etc.

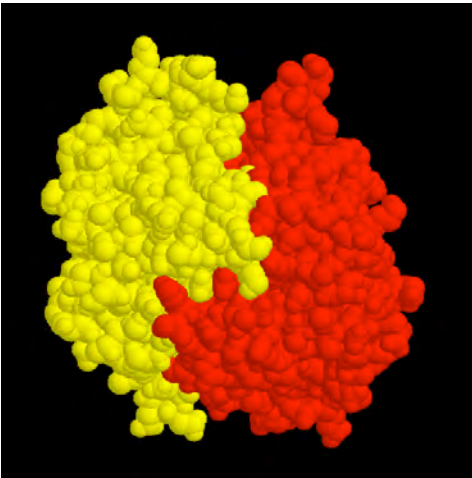
A widely used method to screen for protein-protein interaction is the yeast two-hybrid system. Tandem affinity purification (tagging) of protein complexes followed by identification of the proteins by Mass Spectrometry (MS) is an alternative approach. Currently, lack of consistency between these methods as well as within the same method is a serious drawback.

PROTEIN-PROTEIN INTERACTION DATA OBTAINED FROM MICROARRAY EXPERIMENTS ON FAST SLIDES IS RELIABLE

Several of the well-known interactions of calmodulin were missed in the large-scale yeast two-hybrid and/or affinity purification-MS studies, including the interaction of calmodulin with calmodulin kinase (Schweitzer et al. 2003). In order to demonstrate that a protein array can identify reciprocal interaction of proteins, Schweitzer et al. (2003) probed identical yeast whole-proteome arrays with biotinylated calmodulin and biotinylated calmodulin kinase, respectively. Detection was

made with Cy5-labelled streptavidin. The interaction was found irrespective of which protein was present in solution and which was immobilized on the FAST Slide surface.

Seitz et al. (2006) used protein microarrays on FAST Slides and Biacore technology as two independent methods to verify putative interaction partners of two members of the S100 protein family, S100A6 (Calcyclin) and S100B. S100 family proteins are characterized by two EFh (EF-hand) Ca^{2+} binding domains, which undergo a major conformational change upon binding of a divalent cation. This conformational change exposes a hydrophobic target binding site which can interact with effector proteins, thus eliciting a physiological response. 80 recombinant human proteins (RGS-His₆-tagged) derived from a proteome-wide expression library were arrayed. The two S100 proteins, S100A6 and S100B, were expressed and purified as GST fusion proteins. Their affinity to the arrayed “prey proteins” was assayed in the presence of calcium ions and, as a control, in the presence of EDTA without Ca^{2+} . An anti-GST-Cy5 conjugate was used for detection. The same set of proteins was analyzed using Biacore technology. For S100A6, 9 and 11 interactions were found using microarray and Biacore, respectively. 6 of these were identical with both methods. Interestingly, there was less overlap in the Biacore analysis when the reverse experiments were performed (i.e. probing of immobilized S100 proteins with the “prey protein” in the solution phase).



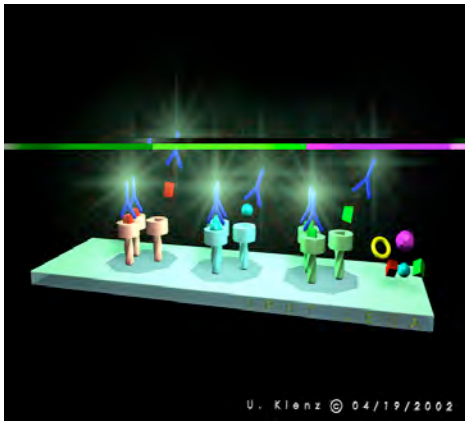
PROTEIN-DOMAIN MICROARRAYS IDENTIFY NOVEL PROTEIN-PROTEIN INTERACTIONS

A microarray displaying protein interaction domains is a valuable tool to study the protein-protein interaction network of the cell. Espejo et al. (2002) expressed 145 proteins containing interaction domains as GST fusion proteins (33 WW, 23 SH3, 17 SH2, 23 PH, 23 PDZ, seven 14-3-3, 5 PTB, 4 FHA, 8 FF and 2 KH). These, plus 67 proteins “of general interest” but without

canonical protein interaction domains, were arrayed on FAST Slides. As a test for specificity of this chip, several binding motifs were synthesized as biotinylated peptides. Motifs included the proline-rich P3 motif of Sam68 (which binds SH3 and WW domains) and the proline/glycine/methionine (PGM) motif of the ribonucleoprotein SmB' (which binds to a specific subfamily of WW domains). These peptides, pre-bound to streptavidin-Cy3 or streptavidin-Cy5, were subsequently used to probe the array. Unique binding profiles were found for each of the peptides. Interaction with the arrayed proteins was as predicted from the presence of domains.

Arginine-methylation is known to reduce binding of the P3 motif of Sam68 to SH3 but not to WW domains. To test this, a mixture of unmethylated peptide conjugated to streptavidin-Cy3 and methylated peptide conjugated to streptavidin-Cy5 was used to probe one chip. The unmethylated peptide was found to bind two WW domains and six SH3 domains. The methylated P3 bound the same two WW but only three SH3 domains. The next step was to analyze the interaction of Sam68 (Src-associated during mitosis 68) and the splicing factor SmB' from cell lysates with the arrayed domains. The proline-rich motifs (P3 and PGM) are different and as expected, the binding patterns on the chip were distinct, with some overlap. Binding patterns of both proteins were very similar to those observed with the synthetic binding motifs.

The same protein domain array was used to study the function of the tumor suppressor protein tuberin (Liu et al. 2002). Tuberin is a target for phosphorylation by Akt. It plays a role in receptor tyrosine kinase signalling by negatively regulating phosphatidylinositol 3' kinase (PI3K) downstream of Akt. An *in-silico* analysis of tuberin indicated several high-stringency 14-3-3 binding motifs which overlap with Akt phosphorylation sites. Short peptides containing predicted 14-3-3 binding motifs were



UPCOMING TOPICS

FTA and FTA Elute

Two chemistries for diverse applications

ELISA (Enzyme-Linked Immunosorbent Assay)

Advancements in assay speed and sensitivity

High-throughput Processing of Protein Microarrays

Processing arrays using robotics

synthesized and fluorophore-tagged. One peptide harbouring the Ser⁹³⁹ residue was found to have no specific binding to 14-3-3 domains on the array in its unphosphorylated form. However, the phosphorylated form of the peptide did bind to 14-3-3 α and, to a lesser extent, to 14-3-3 ζ domains. To confirm this finding *in vivo*, all 14-3-3 isoforms were expressed as GST fusion proteins and incubated with lysates from serum-stimulated NIH3T3 cells. GST pull-down experiments were performed, and tuberin was found to co-precipitate with all 14-3-3 isoforms. In the presence of the phosphorylated Ser⁹³⁹ peptide this tuberin co-precipitation was greatly reduced. These results corroborate the domain array data. A very comprehensive description of generating and processing protein interaction domain arrays can be found in Espejo and Bedford (2003).

REFERENCES

- Espejo et al. 2002. A protein-domain microarray identifies novel protein-protein interactions. *Biochem J.* 367(3): 697-702.
- Espejo and Bedford 2004. Protein-domain microarrays. *Methods in Molecular Biology.* Vol 264: 173-181.
- Liu et al. 2002. 14-3-3 Interacts with the Tumor Suppressor Tuberin at Akt Phosphorylation Site(s). *Cancer Research* 62: 6475-6480.
- Schweitzer et al. 2003. Microarrays to characterize protein interactions on a whole-proteome scale. *Proteomics* 3: 2190-2199.
- Seitz et al. 2006. Differential binding studies applying functional protein microarrays and surface plasmon resonance. *Proteomics* 6: 5132-5139.

UPCOMING EVENTS

➤ **International Society for Biological and Environmental Repositories (ISBER)**

30 May - 2 Jun 2007 Singapore

Workshop

Title: Room Temperature DNA Storage in Biorepositories

Presenter: Breck O. Parker, Ph.D., Development Manager, Molecular Biology Research and Development, Whatman Inc.

Round Table Discussions

Title: Room Temperature Storage of DNA Samples for Cost Effective Secondary Repository Storage

Presenter: Breck O. Parker, Ph.D., Development Manager, Molecular Biology Research and Development, Whatman Inc.

Visit <http://www.isber.org/> for further information.

➤ **European Neonatal Screening Meeting**

10 - 12 Jun 2007 Reykjavik, Iceland

Visit <http://www.hi.is/nam/laek/enbs> for further information.

➤ **Biodetection Technologies 2007**

14 – 15 Jun, 2007 Atlanta, GA, USA

Visit <http://www.knowledgepress.com/events/12191356.htm> for more information

➤ **European Human Genetics Conference (ESHG 2007)**

16 – 19 Jun, 2007 Nice, France

Poster presentation

Title: Collection, storage and Processing of Clinical Samples for Molecular Analysis

Presenter: Michael Harvey, Ph.D., Director of Development, Microarrays, Molecular Biology, Whatman, Inc.

Visit <http://www.eshg.org/eshg2007/index1.htm> for further information

➤ **American Association for Clinical Chemistry (AACC)**

15 - 19 Jul 2007 San Diego, CA, USA

Visit http://www.aacc.org/AACC/events/ann_meet/annual2007/ for further information.

➤ **Discovery2Diagnostics/Chips to Hits®**

17 - 19 Sep 2007 Philadelphia, PA, USA

Visit <http://www.biochipnet.com/node/3202> for further information