

MicroLab Diagnostics, Inc. Scientists Recognized by AAAS, Nature, Science, and Analytical Chemistry Journal for Integrated Biochip Advances

MicroLab Diagnostics, Inc. has achieved a breakthrough in microchip technology for DNA and related biotechnology, medical, forensics and homeland security applications that has recently been published in the Proceedings of the National Academy of Science. This accomplishment has been cited and recognized by The American Association for the Advancement of Science (AAAS), and the prestigious magazines **Science**, **Nature**, and **Analytical Chemistry** as one of the most significant recent scientific advancements.

MicroLab's scientists, led by Professor James P. Landers at the University of Virginia, one of **MicroLab's** founders, have achieved for the first time a totally integrated "Biochip" that can take a raw biological sample and deliver an exact DNA analysis completely onboard the chip in a matter of minutes.

This accomplishment opens the possibility of rapid, low cost DNA analyses in settings outside of the laboratory where it is presently only possible to perform these tests. The many settings where these analyses would be very useful are in doctor's offices, crime scenes, military field use, Bio-Defense, infectious disease detection, WMD detection, DNA data banking, personalized medicine, pharmaceutical companies, and many others.

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GENETICS

Generating Varieties

Paralogs are the result of a gene duplication event arising after speciation. By examining expressed sequence tags (ESTs) in the B73 strain of maize, Emrich *et al.* identified gene copies with 98% or more similarity, which they have labeled as nearly identical paralogs (NIPs). Approximately 1% of all genes in maize (*Zea mays* L.) have a NIP, a significantly higher rate than found in *Arabidopsis*. Many of these NIPs demonstrate linkage, suggesting that they originated via tandem gene duplication. Among NIP families it was found that both gene copies were often expressed (~80%) and that the expression of an individual gene often differed from that of its paralog. These data suggest that paralogs may be a means by which organisms generate variation and, in the case of maize, may have been important in providing varieties for selection and domestication by humans. — LMZ

Genetics 10.1534/genetics.106.064006 (2006).



PSYCHOLOGY

Morality on the Web

Inconsistencies—for instance, between what is observed and what is reported—can be fecund ground for researchers to till, and a topic of current interest is the incongruence between the moral judgments that people make and the reasons that people proffer as a basis for those judgments. At one side are the proponents of conscious or deliberative thought as the means for making choices when confronted with moral dilemmas, whereas another view favors intuitions arrived at via automatic or inaccessible processes as the motivation for their responses.

Cushman *et al.* have elicited “ought versus ought not” judgments and postjudgment rationales from more than 500 people by using a Web-based script. Participants read carefully constructed scenarios and registered their judgments; they were then presented with their choices in pairs of the scenarios that differed in only one of three dimensions and asked for a justification. In situations where action (or inaction) was involved, participants were consistent in their judgments and generally had no difficulty in articulating a reasoned argument for how they had decided which behavior was morally better. In contrast, when intentional (or unintentional) harm

was the issue, the pattern of judgment was just as clear as in the action scenarios, but most participants could not explain why they had chosen as they did. Hence, there may be more than one way to reach a decision on morality. — GJC

Psychol. Sci. 17, 1082 (2006).

CHEMISTRY

From Soup to Nuts

The promise of microfluidic systems, in which very small volumes of liquids are manipulated, processed, and interrogated, is that it may be possible to develop low-cost diagnostic systems, particularly for use under challenging field conditions. Although there has been tremendous progress in developing microfluidic components, creating an integrated system that can analyze an unpurified sample has remained a goal.

Easley *et al.* describe a microfluidic system with three distinct functional domains. The first two are for sample preparation, consisting of solid-phase extraction (SPE) to pull out sample DNA from a crude specimen and for subsequent PCR amplification.

After this, the amplified products are then injected along with a DNA standard into an electrophoretic detection domain. One key

aspect of the device (3 x 6 cm) is a series of valves that are used to isolate each unit, thus keeping SPE reagents from reaching the PCR domain; these valves are also used in a diaphragm-like fashion to pump the amplified DNA into the analytical chamber. The authors demonstrate the detection of *Bacillus anthracis* in 750 nL of whole blood taken from infected but asymptomatic mice, and they also are able to measure *Bordetella pertussis* in 1 µL of nasal aspirate taken from a patient suspected of having whooping cough. — MSL

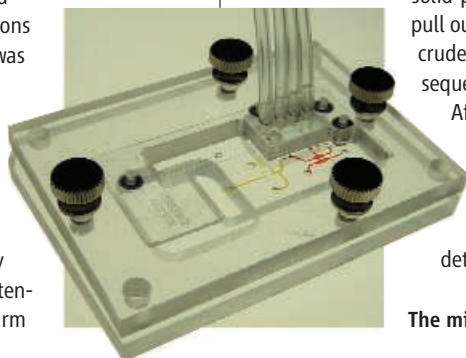
Proc. Natl. Acad. Sci. U.S.A. 103, 19272 (2006).

IMMUNOLOGY

A Loss of Intestinal Fortitude

The large-scale and rapid depletion of CD4⁺ T cells in the weeks after HIV infection occurs predominantly in the gastrointestinal tract. Accompanying this loss is a sustained whole-scale activation of the immune system, which corresponds directly with the eventual progression to AIDS.

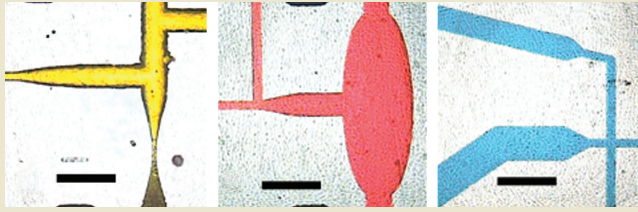
Brenchley *et al.* propose that the two processes are tightly coupled, with impaired intestinal integrity leading to the translocation of gut microbes, or some of their constituent components, which overstimulate the immune system. Circulating levels of bacterial lipopolysaccharide (LPS), which was used as a marker for microbial translocation, were markedly elevated in the sera of chronically infected HIV individuals and in macaques experimentally infected with the simian immunodeficiency virus (SIV). This increase corresponded directly with footprints of immune activation.



The microfluidic lab bench.

CREDITS (TOP TO BOTTOM): MISSOURI BOTANICAL GARDEN/HTTP://WWW.BOTANICUS.ORG; EASLEY ET AL., PROC. NATL. ACAD. SCI. U.S.A. 103, 19272 (2006)

Sample-to-readout on a chip



A fully integrated microfluidic system has been developed that can detect a pathogen in whole blood and other bodily fluids in less than 30 minutes. Combining recent advances in microfluidic technologies, Easley and colleagues have created a system that successfully integrates three distinct functions: solid-phase extraction of DNA from complex samples, PCR amplification, and electrophoretic separation of the PCR product for size analysis. Their design overcomes the incompatibility between reagents used in solid-phase DNA extraction and those used in PCR through the clever manipulation of differential flow resistances, elastomeric valves and laminar flows. The authors demonstrated the chip's utility by identifying the presence of *Bacillus anthracis* in 0.75 μl of whole blood from infected mice and of *Bordetella pertussis*, the causative agent of whooping cough, in 1 μl of human nasal aspirate. The integrated design substantially reduces the turnaround time for sample processing and genetic analysis, representing another step toward personalized medicine at lower cost. (*Proc. Natl. Acad. Sci. USA*, published online 11 December 2006, doi:10.1073/pnas.0604663103) AL

Boosting chemotherapeutic immunogenicity

Although the majority of anticancer agents kill tumor cells via nonimmunogenic programmed cell death, the anthracyclins are capable of both killing cells and eliciting an immune response. Obeid *et al.* now show that anthracyclins' enhanced killing power is associated with the rapid and specific exposure on the cell surface of calreticulin, a protein known to induce dendritic cell-dependent phagocytosis when it is translocated from the cytoplasm to the cell surface. Although surface exposure of calreticulin is insufficient to elicit apoptosis, it appears to determine the immunogenicity of apoptosis, because RNA interference (RNAi)-mediated calreticulin knockdown inhibits *in vivo* anticancer vaccination by anthracyclins. The authors show that anthracyclins and protein phosphatase 1 (PP1)/GADD34 act on the same calreticulin translocation pathway, as calreticulin exposure triggered by RNAi-mediated depletion of PP1 or GADD34 is not enhanced by anthracyclins. They then apply this knowledge to potentiate the anticancer activity of two nonimmunogenic apoptosis inducers, etoposide or mitomycin C, showing that their combination with recombinant calreticulin or inhibitors of PP1/GADD34 is capable of complete tumor xenograft regression in immunocompetent mice, whereas monotherapy is not. As the majority of apoptosis-inducing drugs tested in this study appear to be nonimmunogenic, this combination treatment strategy might boost the effectiveness of currently approved anticancer agents. (*Nat. Med.* 13, 52–59, 2007) JWT

Research Highlights written by Kathy Aschheim, Ania Levinson, Gaspar Taroncher-Oldenburg & Jan-Willem Theunissen.

Antiprogesterones in breast cancer

Mutations in the breast cancer susceptibility gene *BRCA1* can trigger breast and ovarian cancer. Previous studies have shown that *BRCA1* suppresses tumorigenesis by regulating estrogen receptor α and two isoforms of the progesterone receptor. To determine how these estrogen and progesterone receptors contribute to *BRCA1*-mediated cancers, Poole *et al.* study mice with mammary epithelial cells lacking both *Brca1* and *p53*, two genes that are also mutated in most human *BRCA1*-associated breast tumors. The authors show that mice lacking *Brca1/p53* exhibit increased estradiol- and progesterone-dependent mammary gland cell proliferation, branching and alveoli formation. *Brca1* deficiency most likely increases the mitogenic effect of estradiol and progesterone by increasing progesterone receptor isoform protein levels—due to decreased ubiquitin-dependent proteasome degradation—without affecting estrogen receptor α protein levels. Indeed, when the authors treated *Brca1/p53* deficient mice with the progesterone antagonist mifepristone, mammary carcinogenesis was prevented, suggesting that antiprogesterones might prove effective prophylactics and therapeutics for women who carry *BRCA1* mutations. (*Science* 314, 1467–1470, 2006) JWT

Parsing the heart

All blood cells arise from a single cell type, the hematopoietic stem cell. In the heart, the origin of the three main lineages—cardiac, endothelial and vascular smooth muscle cells—is still unclear, with some evidence supporting the existence of a common precursor cell and other data pointing to distinct precursors. Using genetic lineage tracing in mice, Moretti *et al.* show that a subset of cells within the “second cardiogenic progenitor field,” defined by expression of the LIM-homeobox transcription factor islet-1 (*isl1*), gives rise to the heart's three main cell types. The authors also worked out conditions for recapitulating this developmental process *in vitro*. Embryonic stem cells expressing reporter genes knocked in to the *isl1* locus were used to follow the appearance of *isl1*⁺ cells in embryoid bodies and to optimize differentiation conditions. The authors conclude that the heart's three lineages arise from progenitor cells expressing *isl1*, *flk1* and *Nkx2.5*. A similar cell derived from human embryonic stem cells may be useful for heart-regeneration therapies. (*Cell* 127, 1151–1165, 2006) KA

Phagosome network digest

Phagosomes, specialized vesicles for sequestering particles ingested by the cell, represent the first line of defense against many pathogens. Ezekowitz and colleagues have applied a system-wide approach to untangling some of the mechanisms involved in phagosome function in the fruit fly, *Drosophila melanogaster*. Within a phagosome, the intruding microbes are killed and digested for antigen presentation. Using a combination of SDS PAGE, tandem mass spectrometry, protein-protein interaction screens, computational analysis and RNA interference-based validation, the authors define a set of 617 proteins unique to the *D. melanogaster* phagosome and analyze the interactions among them as well as with other secondary players linking them to the cell-wide protein-protein interaction network. Besides generating a detailed model of the phagosome, the authors identify several novel regulators of phagocytosis and previously unknown molecules/pathways potentially involved in host defense. Because ~70% of mammalian phagosome proteins have counterparts in *D. melanogaster*, this and other system-wide studies on the fly phagosome will likely inform studies on the human organelle. (*Nature* published online December 6, doi:10.1038/nature05380) GTO



Gene analysis on a single chip

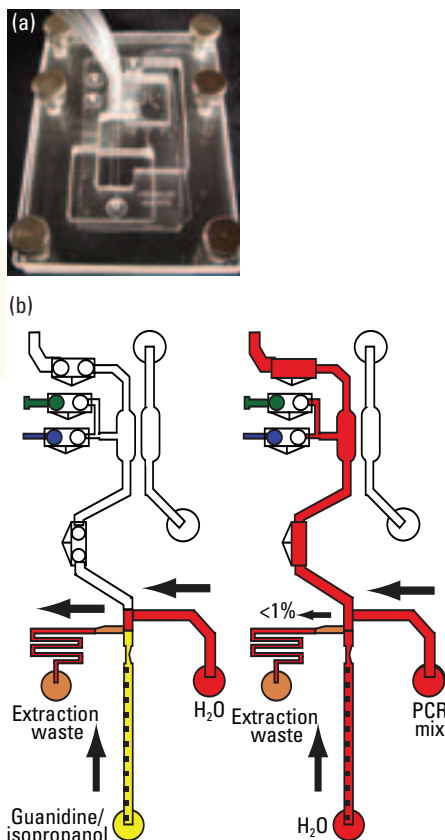
Integrated microfluidic analysis of blood identifies pathogens and cancer in <1 hour.

A major goal for microfluidics researchers is to develop a single, easy-to-manufacture device that takes in a blood sample at one end and yields diagnostic results at the other quickly and inexpensively. James Landers and colleagues at the University of Virginia and the U.S. Food and Drug Administration have built just such a device and used it to create a genetic analysis system capable of diagnosing infectious diseases and cancer in less than an hour from unprocessed clinical samples (*Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 19,272–19,277).

Any fully integrated microfluidic analysis system must include subsystems capable of sample pretreatment, separation, and analysis. Researchers have developed components capable of purifying samples or conducting PCR amplification, but they have struggled to assemble the components onto one chip. “The real challenge in integrating sample prep,” says Landers, “is that the fluid stream exiting one component is often incompatible with the chemistry in the next part of the chip.” For example, extraction of nucleic acids from whole blood requires guanidine and isopropanol, but the reagents are incompatible with PCR in the next step.

The investigators solved this problem with laminar flow and vacuum-activated PDMS valves invented by Richard Mathies and colleagues at the University of California, Berkeley. Whole blood mixed with guanidine and proteinase K was loaded into a microfluidic channel packed with silica beads for solid-phase extraction (SPE) and washed for 5 min with 80% isopropanol.

During this DNA purification step, a valve separating the SPE chamber and PCR subsystem remained closed while water was pumped through a perpendicular channel past the outlet end of the SPE channel. Because of the laminar flow properties of the fluids and the geometries of the intersecting channels,



(a) Genetic analysis device loaded into its manifold. (b) Schematics show how fluid flowing out of the nucleic acid extraction subsystem can be forced into a waste collector (left) or allowed to enter the PCR chamber (right). (Adapted with permission. Copyright 2006 National Academy of Sciences, U.S.A.)

the water stream drew the extraction reagents away from that valve and into a waste channel.

DNA was eluted from the silica beads and transferred to the PCR chamber when the valve was opened, and water was run through the purification bed while PCR reagents were brought in through a side channel. Once the PCR chamber was filled, an IR light source took the mixture through 30 20-s thermal cycles. After amplification was complete, another valve opened and the

reaction mixture was injected into the electrophoresis subsystem for the separation and detection of nucleic acids.

To show the device’s capabilities, Landers and colleagues performed several rapid assays with it. The entire process, from injection to data generation, for detecting anthrax in blood took <30 min, as did a second test for *Bacillus pertussis*, the pathogen that causes whooping cough, in human nasal aspirate samples. An assay for T-cell lymphoma took just less than an hour.

Landers says that making the etched-in-glass microfluidic device was straightforward, thanks to help from Mathies, who provided detailed instructions on how to fabricate the PDMS valves. All of the computer-controlled vacuum pumps, the IR light for thermal cycling, and the confocal laser detection system were interfaced through an external manifold that clamped around the glass slide. “Our goal was to build a device that you use once and throw away. So, to keep the per-use cost low, we off-loaded as much of the hardware as we could from the chip,” says Landers. He adds that his group is now collaborating with engineers to refine the manifold for eventual clinical validation.

As it stands now, however, Landers’s system is getting good reviews. “What’s great about it,” says Mark Burns at the University of Michigan, “is that it can handle different samples, such as whole blood and nasal aspirate, and deliver clinically useful data. I can see this system being useful for quickly diagnosing infectious diseases or any disease where you have a gene to identify.” For Piotr Grodzinski at the U.S. National Cancer Institute, the potential low cost of this relatively simple system is a key attraction. “This could be the type of black-box, low-cost-per-use device that could move cancer diagnosis into the doctor’s office,” he says. ▀

—Joe Alper