

Introduction to BioArray Solutions' BeadChip Technology

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BioArray Solutions is a relative newcomer to the field of DNA-based testing, and has been the subject of interest and discussion in the fields of HLA and Transfusion medicine. Their product platform, the BeadChip, is a novel technology in which several straightforward technologies have been combined to perform DNA typing.

How does it work? The following simplified explanation illustrates how it works for DNA testing:

To manufacture a DNA-based BeadChip, one would obtain a set of small polystyrene beads of various colors. DNA oligonucleotides are then attached to the beads, one sequence per bead (see Image 1). At this point, there is a library of colored beads such that each color of bead has a specific DNA oligonucleotide attached. From this library, different colored beads are selected and attached to a chip in such a way that they form a closely packed single layer of thousands of randomly distributed beads (Image 2). A digital photomicrograph is taken of the beads on the chip, called a "decoder" image. At this point, computerized image analysis of the color of the beads makes it possible to determine the locations of each of the primers on the chip.

When the chip is used, prepared DNA fragments are exposed to the surface of the chip, so, when there is a match, DNA binds to matching beads. The unbound sample DNA is then washed away, and bound DNA is elongated with fluorescence-labeled dNTPs. Following elongation, a photomicrograph is taken of the fluorescence (Image 3). By comparing the locations of bead fluorescence with the decoder image, the system software is able to determine what beads have associated fluorescence. By knowing which beads fluoresced, the computer is able to determine what primers have annealed to DNA, and, thus, what DNA sequences were present in the sample. Although one might imagine a few of the beads could be defective, the presence of multiple copies of each color of bead limits false negative reactions. After measuring the fluorescent signal at each of the marker locations, the computer can perform sophisticated statistical analysis to quantify the signal strength.

Current product lines include HLA A & B typing, red blood cell antigen typing (marketed as Human Erythrocyte Antigen or HEA typing), and Human Platelet Antigen typing. As with other DNA typing technologies, the BeadChip system can be applied in a variety of sequence identification applications. Although the FDA has cleared the system in principle, the

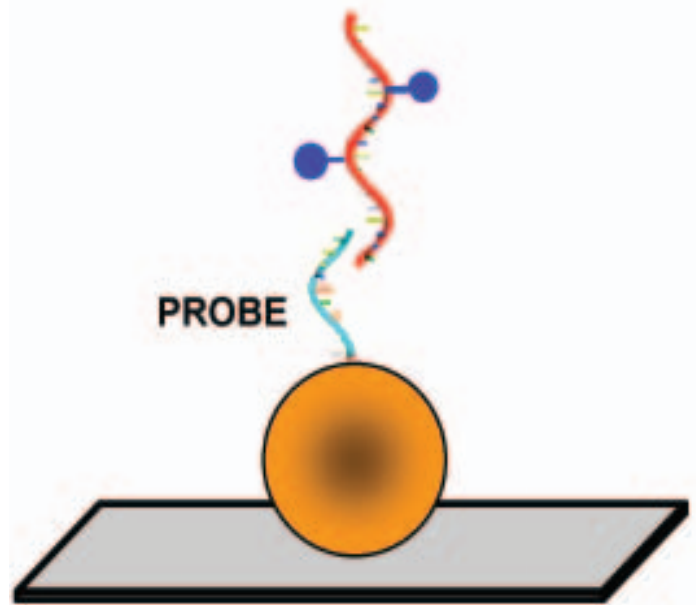


Image 1 Schematic of polystyrene bead with attached oligonucleotide.

HLA, HEA, and HPA antigen typing kits are all currently available as "Research Use Only" products.

The application of the BeadChip system to HLA technologies is thus far limited to HLA-A and HLA-B typing. There has been some work on class II. The limitation to HLA-A and -B typing prevents the platform from being ideal for most transplant applications at this time. Ambiguous results need to be resolved using alternative methodologies, so the BeadChip is not yet a good choice as the primary typing methodology for most labs.

There are, however, two applications for which class I typing using the BeadChip system may be very useful.

First, some laboratories perform class I HLA screen typing in support of a blood bank to develop a catalogue of typed platelet donors for matching with alloimmunized patients. These laboratories may find the current configuration particularly well suited for their needs. Since the class I kits are currently available as groups of eight tests, it is convenient that sample (prod-

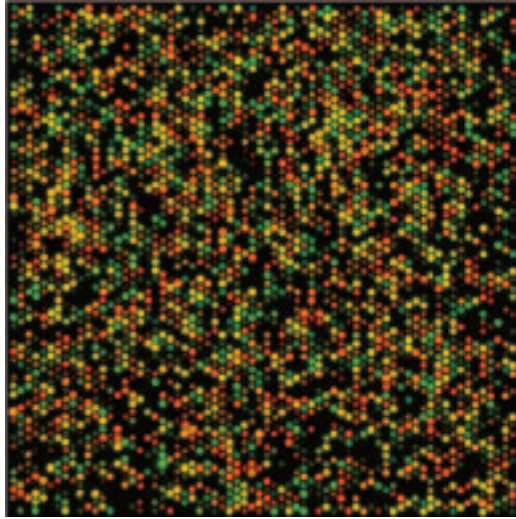


Image 2 Photomicrograph of BeadChip for location of colored polystyrene beads.

uct) stability permits delaying a run until a full batch can be performed.

Second, the BeadChip system also has potential for engraftment monitoring in hematopoietic progenitor cell transplantation settings. Since the BeadChip can assay multiple PCR reactions in a single test, multiple SNPs are assessed simultaneously. By comparing donor and recipient samples using the standard array of beads, informative SNPs can be selected and used for post-transplant chimerism monitoring. A poster presentation demonstrated results consistent with measured means from other labs for all samples, graded as “good.”

More importantly, BioArray Solutions demonstrated a 4 percent sensitivity in measuring ratios of laboratory-mixed donor

and recipient DNA test samples. It will be interesting to see if that confidence level is borne out in the hands of end-users, as many laboratories reported sensitivity levels in the 10-20 percent range for the same assays. Of course, as this is an HLA test, this assay is currently useful only in non-identical HLA transplantation. An alternative BeadChip can be imagined that might incorporate SNP primers for multiple chromosomes, making it a single, simple solution for engraftment monitoring for all allogeneic HPC transplants.

The BeadChips for Red Blood Cell Antigens include Human Erythrocyte Antigen (HEA) for typing for minor blood group antigens, and Rh-CE and Rh-D for typing for Rh antigens. The HEA chip, in particular, has been of significant interest in transfusion literature, as it has the potential to transform erythrocyte antigen typing. Current standard practices check for the presence of antigens on RBCs by mixing RBCs with serum containing specific antibodies, with an agglutination response demonstrating a reaction between the RBC and the antibody. This approach essentially requires anti-sera for each antigen to be typed. Unfortunately, some anti-sera are rare (and expensive). Validation of the BeadChip system was first published in *Transfusion* in May 2005, titled “A Flexible Array Format for Large-scale, Rapid Blood Group DNA Typing,” which demonstrated that results of the BeadChip HEA genotyping were consistent with serologic and PCR-based typing methods. As might be expected with DNA-based screening, new alleles were discovered as a result of their study. The Blood Bank of San Bernardino and Riverside Counties, a regional blood supplier in Southern California, reports they have validated the HEA assay against serologic techniques and found no discrepancies.

One of the challenges in transfusion medicine is the problem of approaching transfusion in the alloimmunized patient. As patients develop antibodies, locating compatible (antigen neg-

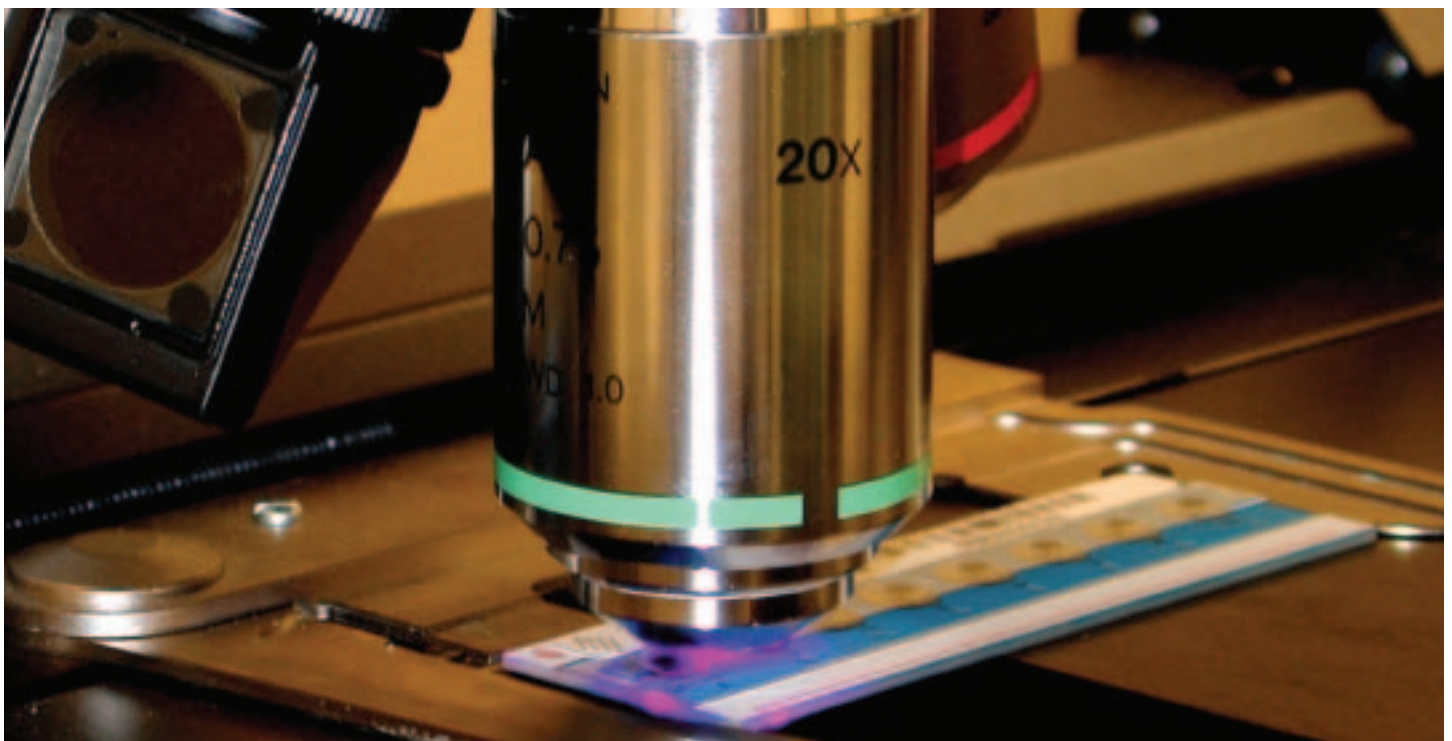


Image 3 Photomicrography of fluorescent chip.

ative) blood becomes more and more difficult. Most blood centers have extensively phenotyped only a handful of their donors, at least partially because of the limited availability of anti-sera, which limits the availability of multiple-antigen negative blood. The cost and limited availability of anti-sera make serologic phenotyping almost prohibitively expensive. The HEA BeadChip is particularly helpful in blood banking for one-time screening of blood donors, as it makes donor phenotyping feasible in broader settings.

Prospectively providing phenotypically matched RBCs may be effective in improving outcomes for repeatedly transfused patients, such as those with Sickle Cell Disease. One big question is whether it is cost-effective to do so. Utilizing cost-effective phenotyping, the blood banking industry may be able to develop a more extensive library of phenotypically-matched blood for patients, thereby reducing the likelihood of antibody development, and better meeting the transfusion needs of those who have already formed multiple antibodies.

The Human Platelet Antigen BeadChip is similarly of interest in blood banking for patient and donor applications. The rapid

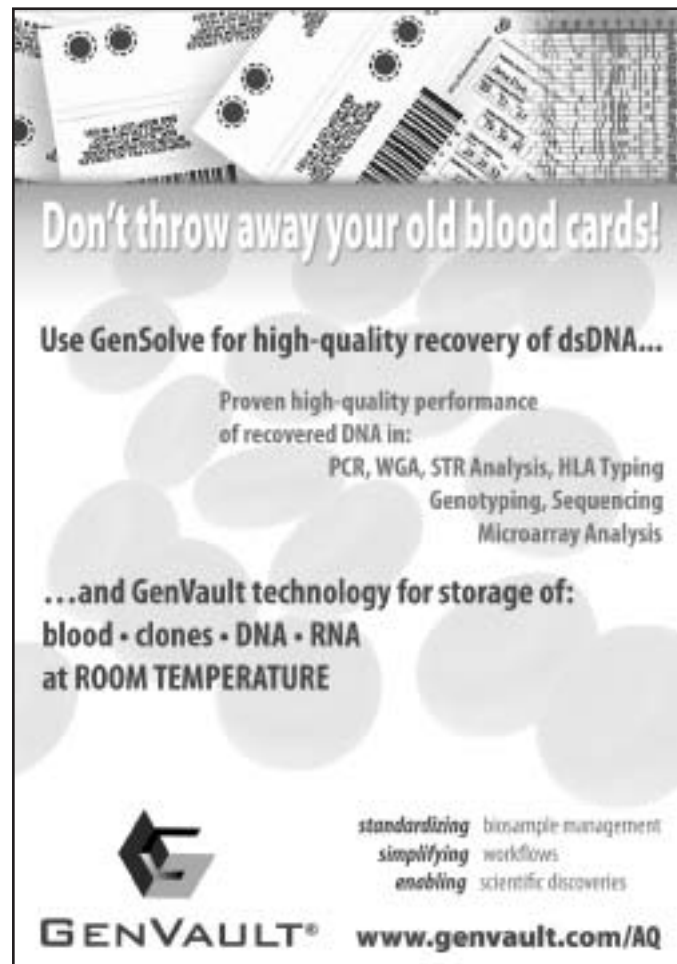
confirmation of HPA status can be important in confirming diagnoses of neonatal alloimmune thrombocytopenia and post-transfusion purpura. The HEA BeadChip will also be useful for one-time screening of platelet donors for donor centers wishing to develop a list of possible donors for those patients who rapidly need antigen-negative platelets.

The BioArray Solutions BeadChip platform is a novel and flexible technology allowing for a variety of DNA-based testing. The first BeadChips to market, HLA, HEA, and HPA typing, all represent new tools for performing DNA genotyping. This is an evolving technology with promise of additional kits of interest to the Immunogenetics community.

BeadChip is a trademark of BioArray solutions.

Dr. Stevens has served as a consultant in the past to BioArray Solutions.

Dr. Iwaki is on the Board of Directors of BioArray Solutions.




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