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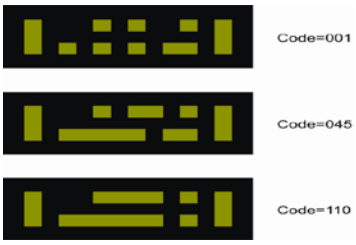

Figure 1: Examples of 128-plex barcoded magnetic beads

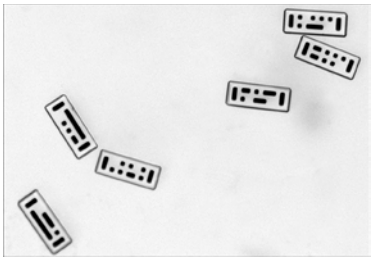

Figure 2: Actual 128-plex barcoded magnetic bead dimension: 25 x 70 x 6 um

Chemistry Applications

The surface of the Barcoded Magnetic Beads is functionality modified with carboxyl, streptavidin, or passive hydrophobic absorption for probe immobilization. Carboxyl beads permit probes or specific primers to bind the bead surface covalently via NH_2-modified 5' termini (Figure 3). This is very simple procedure but is preformed in large batches under rigorous manufacturing controls. The shelf- life of Barcoded Magnetic Bead is two years in room temperature environment.

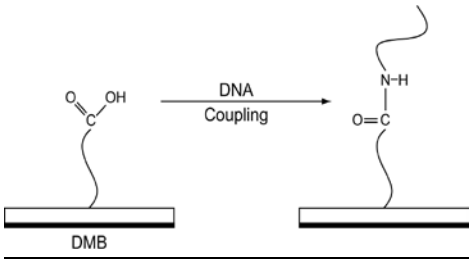

Figure 3: Reaction for functionally modifying Barcoded Magnetic Beads

Target probes within an assay are then assigned to a unique Barcoded Magnetic Bead and hybridized to the functional group on that set of beads. A fluorescent dye is attached to the reporter complementary target and when the target is present in the sample, the dye fluoresces (Figure 4). The amount of fluorescence given off by each set of unique barcodes is used to identify a positive or negative reaction, or to quantify the amount of each target in the sample. Different dyes can also be used by simply switch the filter set. For example, the phycoerythrin dye is excited at 530nm and read at 575nm.

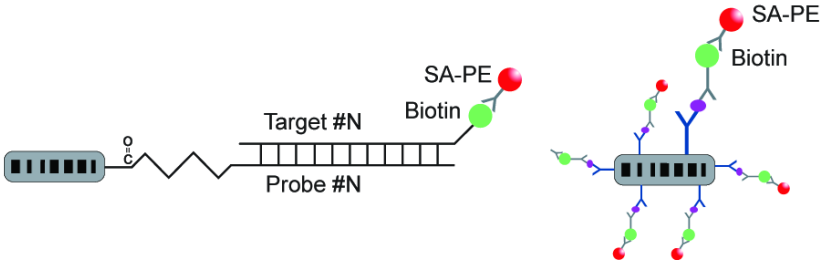

Figure 4: Fluorescent dye complex for nucleic acid and protein probes.

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Barcoded Magnetic Bead Testing Protocol

The protocol for running multiplexed chemistries is very straight forward. Individual target probes are attached to each Barcoded Magnetic Bead. Approximately 25-50 BMB beads are used for each plex type within an assay. The Barcoded Magnetic Beads are pooled into a stock mix and hybridized with buffer to produce a Working Bead Mix. Patient sample and Working Bead Mix are combined in the microplate well and hybridized. The streptavidin phycoeyrthrin (SA-PE) label is added and then the sample is washed. The detection buffer is added and the sample microplate is moved to the BioCode 1000A for detection. Figure 5 illustrates this process.

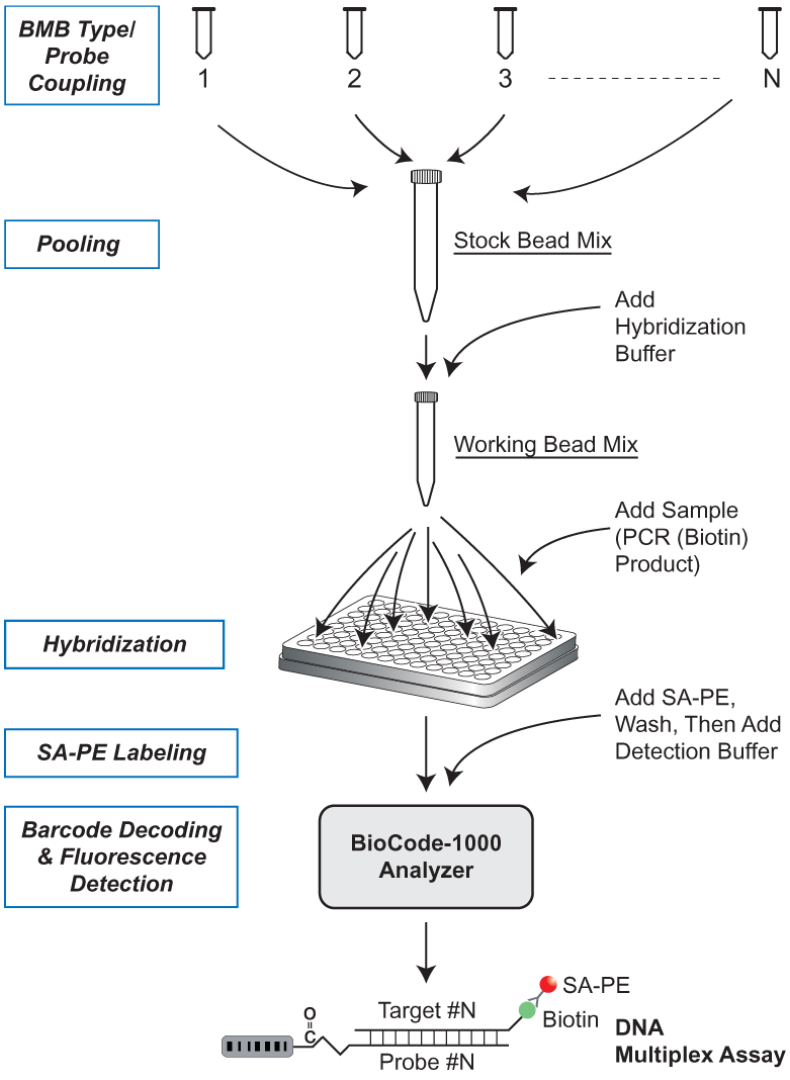

Figure 5: Barcoded Magnetic Bead Test protocol

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BioCode Detection Systems

There are two different detection systems for decoding the Barcoded Magnetic Beads. One system for the low to moderate (128) plex assays and one for the moderate to high (4,096) plex assays. The first system, the BioCode 1000A, is a small bench top system that is used for the detection of low to moderate plex assays in 96 microwells. The system is used to test up to a 128 plex assay (up to ~4,000 beads per microwell) where it identifies the barcode on each Barcoded Magnetic Bead in an assay and simultaneously measures the fluorescence signal associated with each bead as shown in Figures 6 and 7. The system has been available and is in operation at a number of clinical laboratories for the evaluation and/or development of molecular and immuno diagnostic assays. The second product, the BioCode HP, is currently under development and will be utilized for assays with up to 4,096 plex. This product is currently under development.

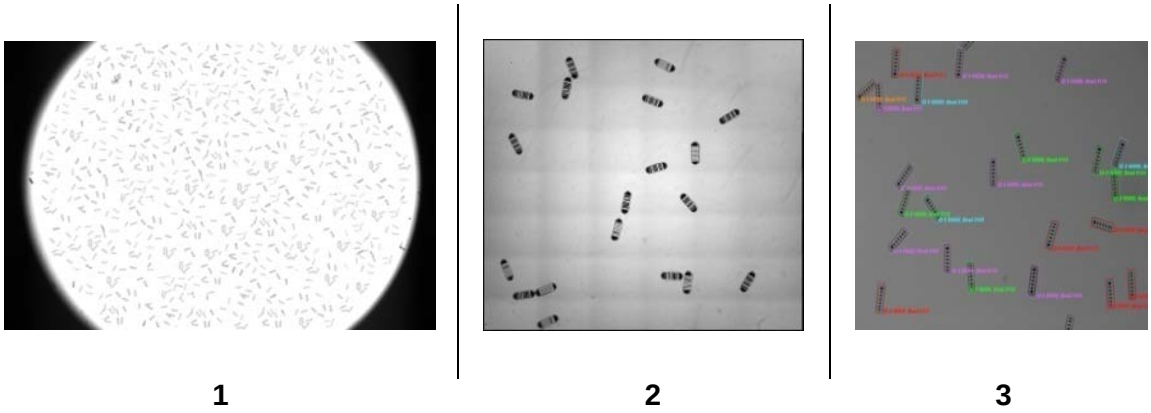

Figure 6 (1) Photo shows BMB beads in bottom of microplate well. (2) Close up photo of BMB's on the bottom of microplate well. (3) Computer reading barcode assigning and fluorescence intensity associating of every individual bead in the well.

Figure 7. The photos show different fluorescence intensities of (Carboxyl BMB) – (GAPDH Probe) – (GAPDH Target)–Biotin) – (SA–PE) associating of every individual bead in the well.

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Figure 8. (1) Biocode 1000A System, (2, 3) Manual loading of Microplate and Automated Loading Tray

The operator, or a robotic system, loads the microplate onto the stage (Figure 8) of the BioCode 1000A. Pictures are taken with a CCD camera of the bottom of the microplate well. A LED light is used to illuminate the barcodes and a lamp is used to generate fluorescence. The BioCode 1000A microprocessor matches each barcode with the appropriate level of fluorescence. A final report is generated by the system identifying each BMB population with fluorescent intensity. Total detection time for reading all barcodes and determining fluorescent intensity for 128-plex assay is 40 seconds per sample test (~ 12,288 data points/hour). In addition to the ease of use, low cost, and multiplex capabilities of the Biocode 1000A system, it also has the ability to rerun samples. The BMB beads are not consumed during the detection process. The fluorescence has been demonstrated to remain for several weeks at refrigerate temperatures.

Sample Results of Barcoded Magnetic Bead Testing

Many different types of assays have already been performed on the BioCode 1000A system to demonstrate the utility of the system. These include diagnostic assays for Respiratory Virus Panel, Hospital Acquired Infection Panel for MRSA, Blood Group Genotyping, Thrombo SNP Genotyping, Infectious Diseases, Prenatal Testing, and testing for various Cancer markers. A few examples of the results of these assays types can be found in the following examples.

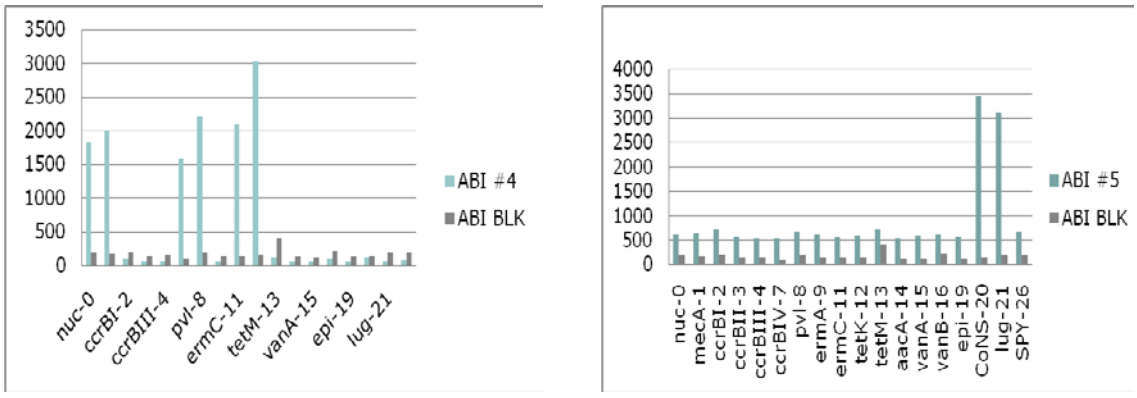

Figure 9. Respiratory Virus Panel – 18-plex MRSA Panel: (Left) Sample - Positive Targets: 0; 1; 7; 8; 11; and 12. (Right) Sample - Positive Targets: 20; and 21

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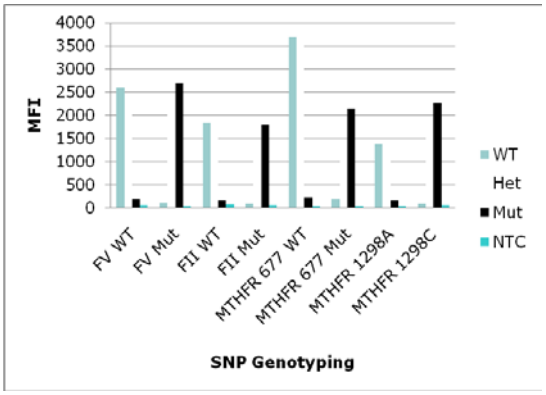

Figure 10. Coagulation Panel- Thrombo SNP Genotyping

Barcode Magnetic Bead Manufacturing

The Barcoded Magnetic Beads are produced by photo lithography so they are inexpensive to manufacture and are produced with a very high degree of repeatability. Leveraging technology from the computer industry, the Barcoded Magnetic Beads can be manufactured in large consistent lots with extremely low lot to lot variation. Photolithography is the foundation of the semiconductor industry (Figure 11). The unprecedented growth of the global chip industry in the past few decades is the perfect proof of the robustness and scalability of this fabrication process. Barcoded Magnetic Beads are composed of an encapsulated layer of chemically inert polymer, making the beads highly stable under many conditions, unlike most of the fluorescent multiplexed beads on the market, which are very sensitive to light.

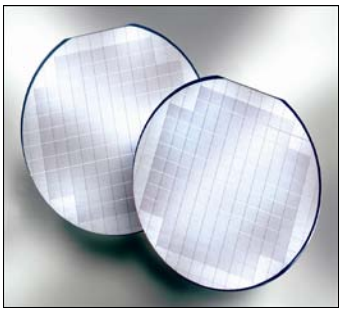

Figure 11a. Silicon Wafers with Barcode Magnetic Beads.

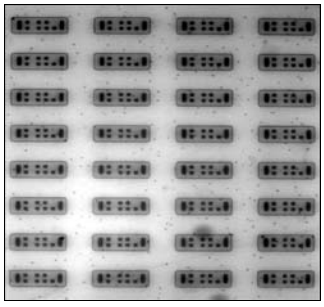

Figure 11b. Actual magnified 128-plex BMBs on Silicon Wafer

Conclusion

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Advantages of the BMB Digital decoding over Analog (fluorescence) decoding are:

- **Open-ended Barcode Number** - Digital multiplexing offers unlimited barcodes by simply adding more digits.
- **High Decoding Accuracy** - High contrast optical barcode enables accurate decoding; no ambiguous barcode classification.
- **No Bead Photon-bleaching and No Fluorescence Interference –** Permanent barcode on BMB, ambient light stable, and without fluorescence interference from impregnated dyes.
- **Fewer Lot-to-Lot Variations –** Semiconductor manufacturing process is highly reproducible. All beads are created equal. Only the barcode varies.
- **Ease of Operation –** Biocode 1000A for 128-plex Analyzer is similar to ELISA reader, all processes can be performed in a 96-well microplate without bead loss. BMB's paramagnetic properties make them well suited for automation.
- **Robust & Easy to Maintain –** No complicated lasers, no flow cytometer, and no waste bottles.