SNP Assay Multiplexing Using Barcoded Magnetic Beads in Array Tape™

Terry Rusch, Nathan Westad, Andrew Haug – Douglas Scientific, LLC

Overview:

High throughput Single Nucleotide Polymorphism (SNP) screening has been a standard method used in identifying traits in plants and other applications. Douglas Scientific provides the Array Tape™ platform for processing millions of SNP data points at a low volume of 800nL for DNA and assay mix. For ultra high throughput assays, or when fixed sets of SNP markers are required, a means to multiplex the assays in one well is desired. The proposed concept was to use barcoded magnetic beads (BMB) from Applied Biocode (http://www.apbiocode.com/DigiMagBeads.htm) to isolate a single assay to a unique barcoded bead. By dispensing several unique barcode assays in one well, a multiplex assay could be performed. The following describes the proof of concept work completed to demonstrate a seven multiplex BMB multiplex assay.

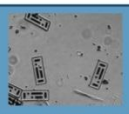

Figure 1: 25µm x 72µm Back illuminated barcoded magnetic bead

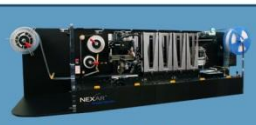

Figure 2: Nexar liquid handling and assay processing

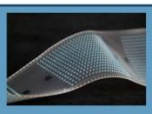

Figure 3: Array Tape™ standard well and volume format

Introduction:

There is an increasing need to maximize the throughput for SNP detection. In the agriculture market, trait identification is critical in developing the next generation of crops. Multiplexing assays in a single well is one method to accomplish this goal. The protocol selected for this proof of concept is established by Applied Biocode for multiplexing assays using barcoded magnetic beads (BMB) [Figure 1]. The BMB multiplex technique has proven effective in the clinical test market. The Douglas Scientific Nexar platform [Figure 2] is an in-line automated liquid handling and assay processing system based on Array Tape™ [Figure 3]. Array Tape™ has transformed the high throughput market by reducing reagent volumes and cost per data point while increasing throughput. The next logical step is to combine the Douglas Scientific and Applied Biocode approaches to further increase throughput. The initial step in the process was to determine if the merge between Array Tape™ and BMB's is feasible. The following required steps are the basis for this proof of concept work.

- Dispense barcoded magnetic beads (BMB) into Array Tape™ and seal
- Scan BMB in Array Tape™ with the seal as the viewing surface
- Process the scanned BMB images
  - Locate the BMB's
  - Decode the BMB barcode
  - Assign fluorescence intensity values to each dye image
  - Score BMB by unique barcode and determine dye tags representative of traits

Figure 4: Alternate Array Tape™ configuration for multiplexing

Figure 5: Prototype optics reader head

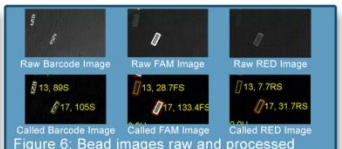

Figure 6: Bead images raw and processed
Text: particle # with barcode or fluorescence intensity. Red border is an identified particle. #13 is an unlabeled bead. #17 is a FAM labeled bead.

Methods:

The process for testing the dye labeled BMB's is as follows:

- Dual dye labeled beads were provided:
  - Hologic® (FAM and RED)
  - Applied Biocode (Hex and Redmond Red)
- Modified Array TapeTM and seal provided by Douglas Scientific
- 2-4uL bead solution and 2uL RO water (DNA substitute) were dispensed using either
  - Adapted Innovadyne™ dispenser from IDEX Health & Science
  - Adapted nanoQuad dispenser from Douglas Scientific
- Sealed Array Tape™ was scanned inverted on custom developed scanner
  - Three cameras, one for barcode reading and one for each dye
  - Three excitation sources, LED for barcode illuminated and lasers for dye excitation
- Image processing was performed using custom software
- Post processing software was used to format data into readable format
  - .txt files for Excel analysis and statistics
  - .txt/.csv files for input into AcuSNP software for plotting and scoring analysis

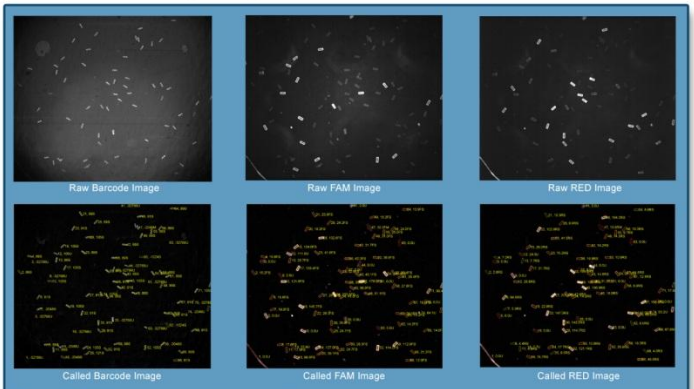

Figure 7: Well images raw and processed
Text indicates particle #, with barcode or fluorescence intensity. An S on the text indicates single bead and U is unknown. Red border is identified particle. Yellow rectangle is analysis area.

Discussion and Results:

The proof of concept required the development of custom Array Tape™ format [Figure 4]. A square well format was selected to maximize viewing area and camera image utilization. A custom read head Was developed [Figure 5]. Custom software was developed to control the scanning system and process the captured images. The scanned beads were processed by custom software [Figure 6]. There are three cameras and captured images:

- Barcode image: used to decode beads
- FAM image: used to locate beads and assign a FAM fluorescence intensity
- RED image: used to locate beads and assign a RED fluorescence intensity

Discussion and Results (continued):
The processed images were captured and labeled with:

- Particle number common across all three images
- Decoded barcode value or fluorescence intensity [Figure 6 and 7]
- Red outline is the particle outlines which were identified
- Yellow squares are software assigned areas for decoding bead and assigning fluorescence intensity values

A sample well showing the raw and processed images is shown [Figure 7]. Comparing images, the different fluorescence labeled or unlabeled beads can be identified. A negative barcode number indicates a non-bead or beads that failed to be fully processed.
The process software accumulates a confidence value. If the confidence is below a specified value, a negative value is assigned to the bead code. The negative bead code also indicates the reason the particle failed the decoding process. The best way to show the data and processing quality is using a scatter plot [Figure 8]. Each bead type was scored according to the dye label attached. The data shows good separation between FAM labeled beads (blue dots), RED labeled beads (red dots) and unlabeled beads (black dots). The work remaining includes optimizing the process for 100% call rate. Additional bead dispensing and scanning is required to validate the proof of concept.

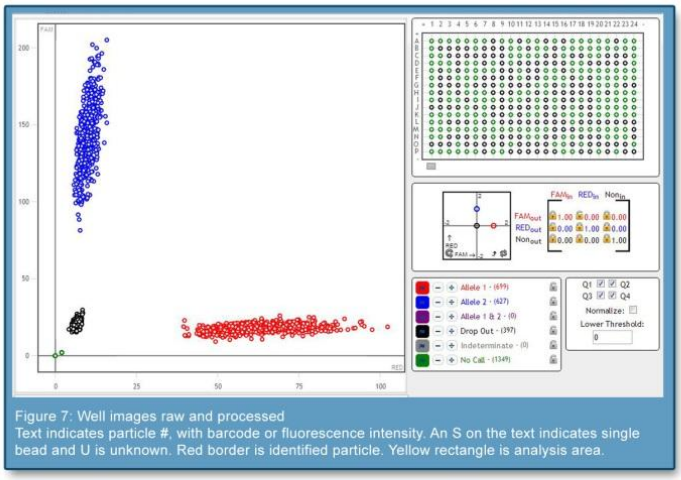

Figure 7: Well images raw and processed
Text indicates particle #, with barcode or fluorescence intensity. An S on the text indicates single bead and U is unknown. Red border is identified particle. Yellow rectangle is analysis area.

Conclusion:
The process and hardware developed for the multiplexing bead assay demonstrates the feasibility of performing a single well multiplex assay. The following items were demonstrated:

- Dispensing of dye labeled beads performed well with a consistent number of beads per well.
- Scanning of BMB was performed using the seal as the imaging surface. This method appears adequate for a seven multiplex assay. Improvements can be anticipated with improved seal materials, altered chemistry properties, or improved bead manipulation.
- Processed BMB images were generated for an eight multiplex assay per well. By further improving the image processing a higher multiplex is expected.

The data summary and processing using AcuSNP software works well and can be used to validate full chemistry tests on known samples. Multiple high quality array dispenses and reads still need to be performed. Overall the route to performing a seven multiplex assay appears feasible based on the test results obtained to date.

Acknowledgements: Darren Cook, Paul Wagner, Eric Johnson – Douglas Scientific; Winston Ho – Applied Biocode; Robert Roeven - Hologic SLAS 2013 2nd Annual Conference, January 14, 2013