Project FY22-BA-013: A Low-Cost Genotyping Platform to Develop FHB-Resistant Barley

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1. What are the major goals and objectives of the research project?

The objectives of the research project are to:
1) Characterize population sub-structure and diversity of US breeding germplasm.
2) Identify a set of evenly spaced markers for a low-cost assay
3) Evaluate dual-hybridization mode of Infinium multi-species array

2. What was accomplished under these goals or objectives? *(For each major goal/objective, address these three items below.)*

Objective 1:

What were the major activities?
Analysis of 50K and 9K genotyping of ~12,000 breeding lines from the US.

What were the significant results?
We identified nine distinct, but related sub-groups overlapping with geographic growing area.

List key outcomes or other achievements.
NA

Objective 2:

What were the major activities?
The identification of 3,089 SNPs for barley that represent the variation is as many geographic areas as possible. Pilot studies of all the Barley CP PIs with several hundred of their breeding lines were run (totaling 2,304 samples). The fine-tuning of cluster-files for genotype calling and development of pipelines to make processing at the Fargo Genotyping Lab semi-automatic. Users have been evaluating their own pilot data and providing feedback on usefulness. Imputation evaluation has also been conducted. Since the inception of the array, we have genotyped 5,136 barley lines with this array

What were the significant results?
We now provide ready-to-use genotyping files for the Barley CP PIs without the need for additional analysis (unless desired). Additionally, the 3K set of SNPs provide enough information to accurately impute the higher-density 50K array (99.9% median taxa accuracy on 17,000 markers with maf >= 0.05)

List key outcomes or other achievements.
Barley CP members that have used the 3K array are happy with the results and have shown that genomic selection models built with it are equivalent to models built with higher density marker data.

Objective 3:

What were the major activities?
A dual -hybridization pilot of 384 Barley/Wheat, 768 Barley/Oat, 96 Barley/Durum and 384 Barley/Oat/Wheat mixed samples were run on the 3K array and evaluated. Custom calling scripts were developed to aid in high-throughput clustering. In production, 1,680 barley samples were run in dual mode during this time period.

What were the significant results?
Clusters can migrate compared to single-mode requires an orthologous technique to determine the proper cluster positions and genotype call. In some cases, the clusters merge into one another when the sample pair hybridizes with the probe. However, in most cases, the anticipated target outcompetes the other crop. Approximately 5-10% of markers cannot be used in dual/multi mode.

List key outcomes or other achievements.
A pipeline was developed to assess single-vs dual mode for any set of germplasm and provide cluster call thresholds for accurate genotyping. Every multi-project in the future can be "trained" with this pipeline to determine the most accurate call at the lowest price.

3. **What opportunities for training and professional development has the project provided?**
Nothing to Report

4. **How have the results been disseminated to communities of interest?**
This array is commercially available directly from Illumina. Most customers know about this array through direct communication. We have established a collaboration with the genotyping company SGS (formally TraitGenetics) where they have been exploring running this array for their large commercial partners.

5. **What do you plan to do during the next reporting period to accomplish the goals and objectives?**
The 3K array is useful for its intended application of supporting genomic selection and fingerprinting. However, this use-case is not required for many Bar-CP PIs, so more 50K high-density arrays will used in place of 3K arrays when appropriate.