applied biosystems

Same day, low marker density, high throughput genotyping

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ABSTRACT – P0138

Genetic gain effectively relates four core factors that influence breeding progress: the degree of phenotypic variation present in a population, the probability that a trait will be transmitted from parent to offspring, the proportion of the population selected as parents for the next generation and the length of time necessary to complete a cycle of selection. The length of time is not only how many generations are required to complete a selection cycle, but also how quickly the generations can be completed. This includes the time taken to obtain genotypes from samples.

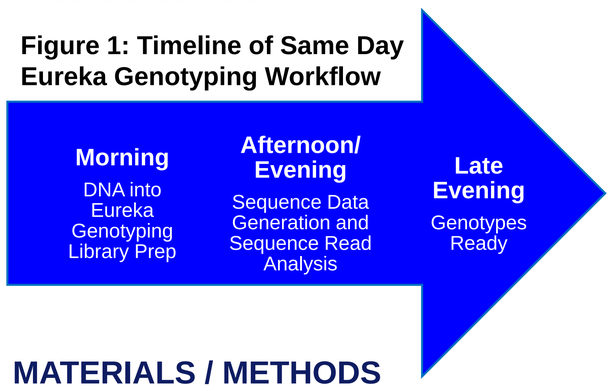
Targeted genotyping by sequencing is emerging as a valuable tool for high throughput, low cost single nucleotide polymorphism (SNP) detection in both plant and animal genomics. Plant breeding has a long history of integrating the latest innovations in biology and genetics to enhance crop improvement. Using Eureka Genotyping Panels developed for various plants we have demonstrated genotypes in less than 12 hours.

Aquaculture breeding programs need rapid, high throughput genotyping to develop genetically improved stocks for cost-effective production. Older fish (more growing days), are often the larger fish. Thus, in aquaculture breeding the age of the fish can obscure genetic potential. Genotyping fish the same day they are spawned would increase the efficiency of the breeding program. Using a 500-plex Eureka Genotyping Panel developed for *Oncorhynchus keta* (chum salmon, a residual tetraploid), we have demonstrated genotypes in less than 12 hours.

INTRODUCTION

A new workflow for Applied Biosystems™ Eureka™ Genotyping allows high throughput same day determination of SNP and Indel (even within homo-polymers) genotypes (**Figure 1**). This new workflow goes from DNA to genotypes in less than 12 hours.

Figure 1: Timeline of Same Day Eureka Genotyping Workflow



MATERIALS / METHODS

To demonstrate the feasibility of the same day genotyping workflow we tested Eureka genotyping panels from three species:

- chum salmon (350 SNPs)
- wheat (180 SNPs)
- bovine (120 SNPs)

The workflow presented here (**Figure 1**) differs from the standard commercially available workflow in terms of reagent formulas, steps, reactions times, probe composition, applications architecture and sequencer integration.

The Eureka Genotyping same day workflow **(Figures 1**) ends with quantitated libraries. The quantitated libraries are combined and sequence data is generated. Sequence data QC, library QC and genotype generation automatically proceed without user intervention once the sequence run is completed.
All sequence data was re-sampled down to an average depth of 200X for each library. Data was analyzed using Applied Biosystems Eureka Analysis Suite software.

The presented same day workflow allows genotypes to be ready less than 12 hours after libraries are started. We provide sufficient barcodes for genotypes from 4,224 samples in a single sequencing run.

RESULTS

Figures 2-5 show example cluster plots obtained with the Eureka Genotyping new same day workflow (left) and the standard workflow from the Eureka Genotyping User guide (right) using various panels and DNA sources. Each plot is a single locus and each sample is a single point in the plot. The Y-axis is the magnitude of the signal. The X-axis is the contrast between the signal of the A-allele and the signal of the B-allele. The BB (blue), AB (yellow), or AA (red) genotype of a sample is shown.

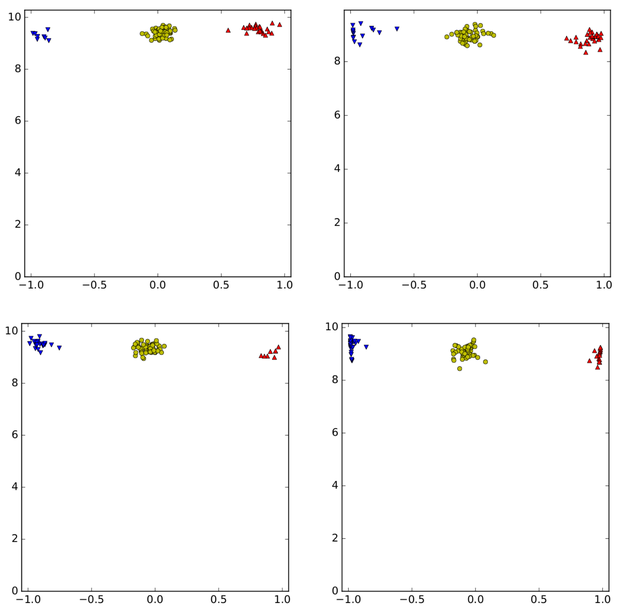


Figure 2: Cluster plots for two loci (top and bottom) in a 120–plex bovine genotyping panel and bovine DNA obtained with Eureka Genotyping same day workflow (left) and standard workflow (right).

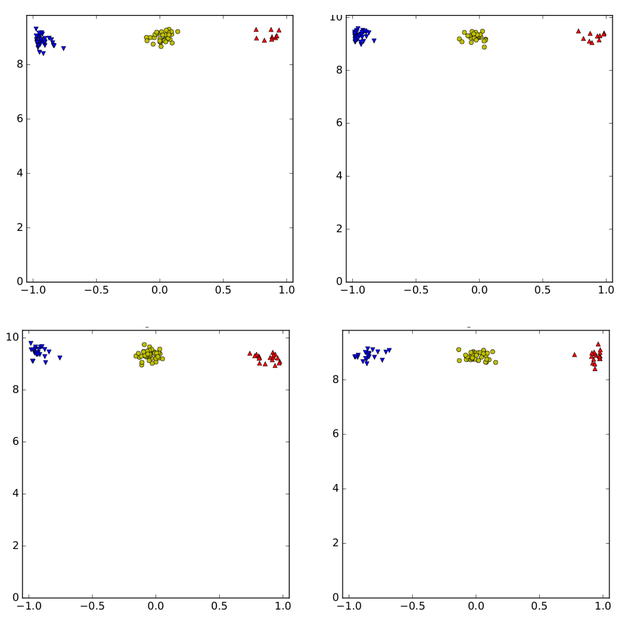


Figure 3: Cluster plots for two loci (top and bottom) in a 350–plex chum salmon genotyping panel and chum salmon DNA (a residual tetraploid) obtained with Eureka Genotyping same day workflow (left) and standard workflow (right).

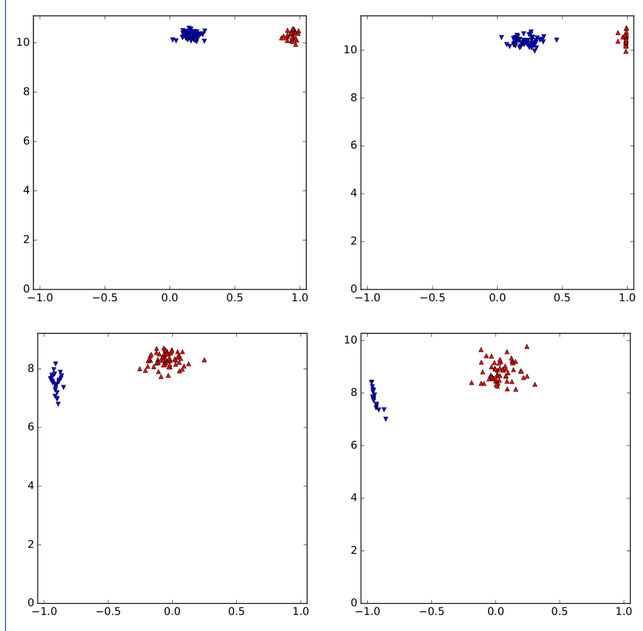


Figure 4: Cluster plots for two loci (top and bottom) in a 180–plex wheat genotyping panel and wheat DNA (a hexaploid) obtained with Eureka Genotyping same day workflow (left) and standard workflow (right).

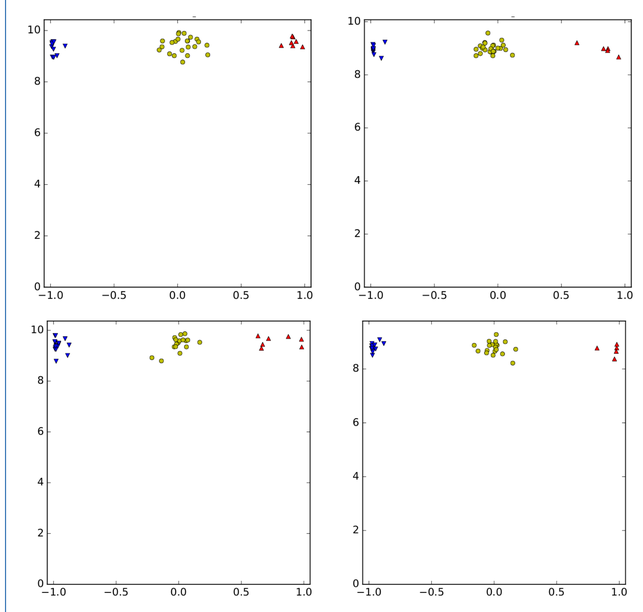


Figure 5: Cluster plots for two loci (top and bottom) in a 120–plex bovine genotyping panel and crude lysates from bovine hair follicles obtained with Eureka Genotyping same day workflow (left) and standard workflow (right).

Amount of DNA Input	Call Rate	Total Relative Concordance
50 ng	99.5	99.36
20 ng	99.25	99.36
10 ng	98.28	98.89
5 ng	97.5	97.55

Table 1: **Impact of total amount of input DNA used in the same day Eureka Genotyping workflow.** The standard commercially available workflow specifies 150 ng initial DNA input. With this input call rate ≥ 99% and total relative concordance ≥ 99% are routinely achieved. The same day workflow is robust and produces high call rate and total relative concordance with much lower input DNA. Different initial total input of bovine genomic DNA were used in the same day workflow and a 120-plex panel. Call rate and total relative concordance comparable to those obtained with the standard workflow were obtained with 20 ng total DNA input.

CONCLUSIONS

The Eureka Genotyping workflow presented in this poster provides a streamlined, effective method for GBS library prep. **The new workflow allows DNA to genotypes 12 hours.** All wet lab hands on time occurs in the first 4 hours. Eleven sets of 384 samples indices are available. Samples on all eleven 384-well plates could be processed in a single day using the same day workflow. Up to 4,224 samples can be put on a single sequence data generation run.

The same day workflow has been demonstrated with three panels and plant and animal genomic DNA, animal crude lysate, DNA from a residual tetraploid (chum salmon), and DNA from a hexaploid (wheat). In conclusion, Same day Eureka Genotyping is a robust and efficient library prep and genotype generation workflow.

	Same Day	Standard
384 well plates	2 x 4 = 8	5 x 4 = 20
DNA to pooled libraries total time	2 ½ hr	24 hr
DNA to pooled libraries hands on time	30 min	200 min
Sequence data generation	6.5 hr	6.5 hr
Auto-start sequence data to genotypes	30 min	Wait till human starts

Table 2: Consumables and time required for the Eureka Genotyping same day workflow and the standard workflow. A significant reduction in consumables, total time and hands on time occurs with the same day assay workflow compared to the standard commercial workflow.

In summary the advantages of the Eureka Genotyping same day workflow are:

- Fewer consumables
- Less hands on time
- Less time on equipment
- Integration of sequencer to analysis
- Less total time
- Reduced amount of input DNA

FURTHER INFORMATION

- Visit us at Booth 111 to learn more
- Attend our workshop: Diverse applications using smarter genomics, Monday, January 15th 12:50 – 3:00, Pacific Salon 3
- Visit Poster 307: Measuring Comparative Performance of the Applied Biosystems™ Axiom™ Microbiome Array with Respect to 16S rRNA and Shotgun Metagenomic Sequencing for a UK Biobank Pilot Cohort
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