

# High Throughput Agrigenomic SNP Genotyping using the Applied Biosystems™ Axiom™ Genotyping Solution

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## ABSTRACT

High density DNA microarrays play an important role in agrigenomic research as climate change, population growth, and urbanization threaten the ability of farmers to meet the world's food demands. Microarrays enable accurate, cost-effective genotyping of variants that include single nucleotide, insertion/deletion and multiallelic polymorphisms. Applications include genomic selection, marker-assisted selection (MAS) and marker-assisted breeding (MAB), parentage and characterization of genetically modified organisms (GMOs). Here an overview of recent advances related to scalable, high throughput workflows using the Axiom solution is presented.

The Axiom solution currently enables complete automation of DNA target preparation on several liquid handling instruments. A new high throughput workflow that scales to 20,000 samples per week with a focus on cost-effective lab configurations has been developed. This workflow emphasizes smaller, simpler devices coupled with bulk reagent packaging. Customizable content can be used for genotyping of any species, genome size, or ploidy level in a 96-array layout (up to 650,000 variants) or 384-array layout (up to 50,000 variants). Additionally, a protocol enabling 48 hour turnaround time from sample to answer is described.

In summary, new workflows to the Axiom solution will drive expansion of microarrays into research on complex genetic traits in plants or animals. Scalable lab automation extends the platform capabilities to genotype SNPs in a single assay with a sample throughput consistent with the needs of both breeders and farmers who are employing new genomic strategies in order to use fewer environmental resources, antibiotics and pesticides to develop higher producing livestock, poultry, and crops.

## INTRODUCTION

### Axiom Genotyping Solution for agrigenomics

- Powerful**
  - Genotype any species, genome size, and ploidy, including complex mosaic genomes
  - Include markers for multiple species on a single array with no restrictions on number of markers per species
- Robust**
  - Highly specific, ligation-based, two-color enzymatic assay that enables reliable design and detection of complex markers
  - Robust and 100% reproducible manufacturing process that helps to ensure no loss of any SNPs

- Scalable**
  - Freedom from huge capital investment for liquid handlers
  - Flexibility towards cost-effective scale-up and to maximization of resources
  - Start with smaller sample volume and scale exponentially as demand grows to 1M samples per year and beyond
  - Bulk reagent packaging for increased operational efficiency
  - Easy replacement of smaller equipment reduces workflow disruption and increases lab uptime

Figure 1. High Density Genotyping Application Focus



Figure 2. Axiom Workflow Evolution

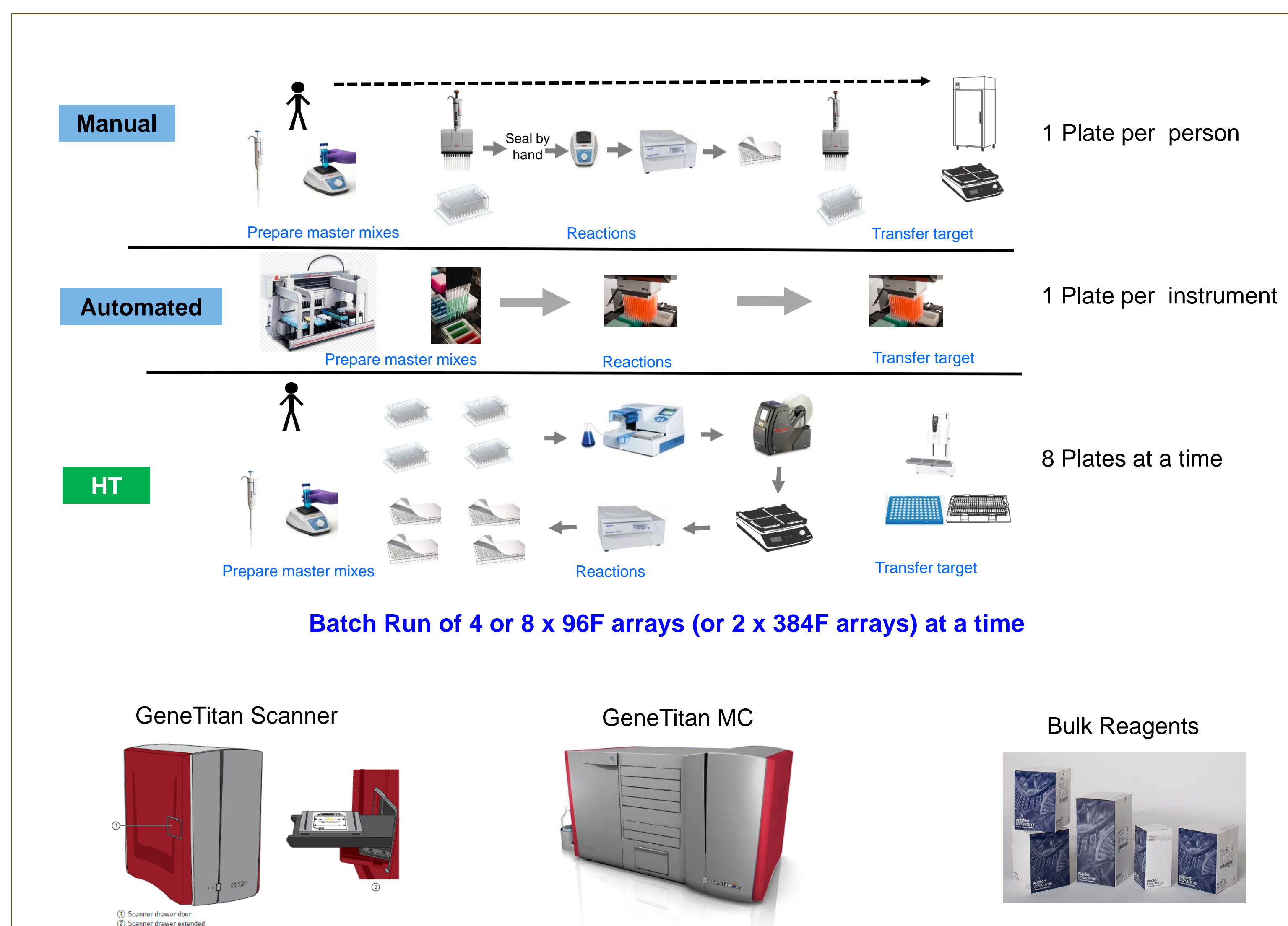


Figure 3. DNA target preps from 100 plates of genomic DNA samples. Each plate contained 96 DNA samples and were processed using the Axiom HT workflow. Yields of amplified DNA were measured using OD 260 and all plates show a median >1200ug.

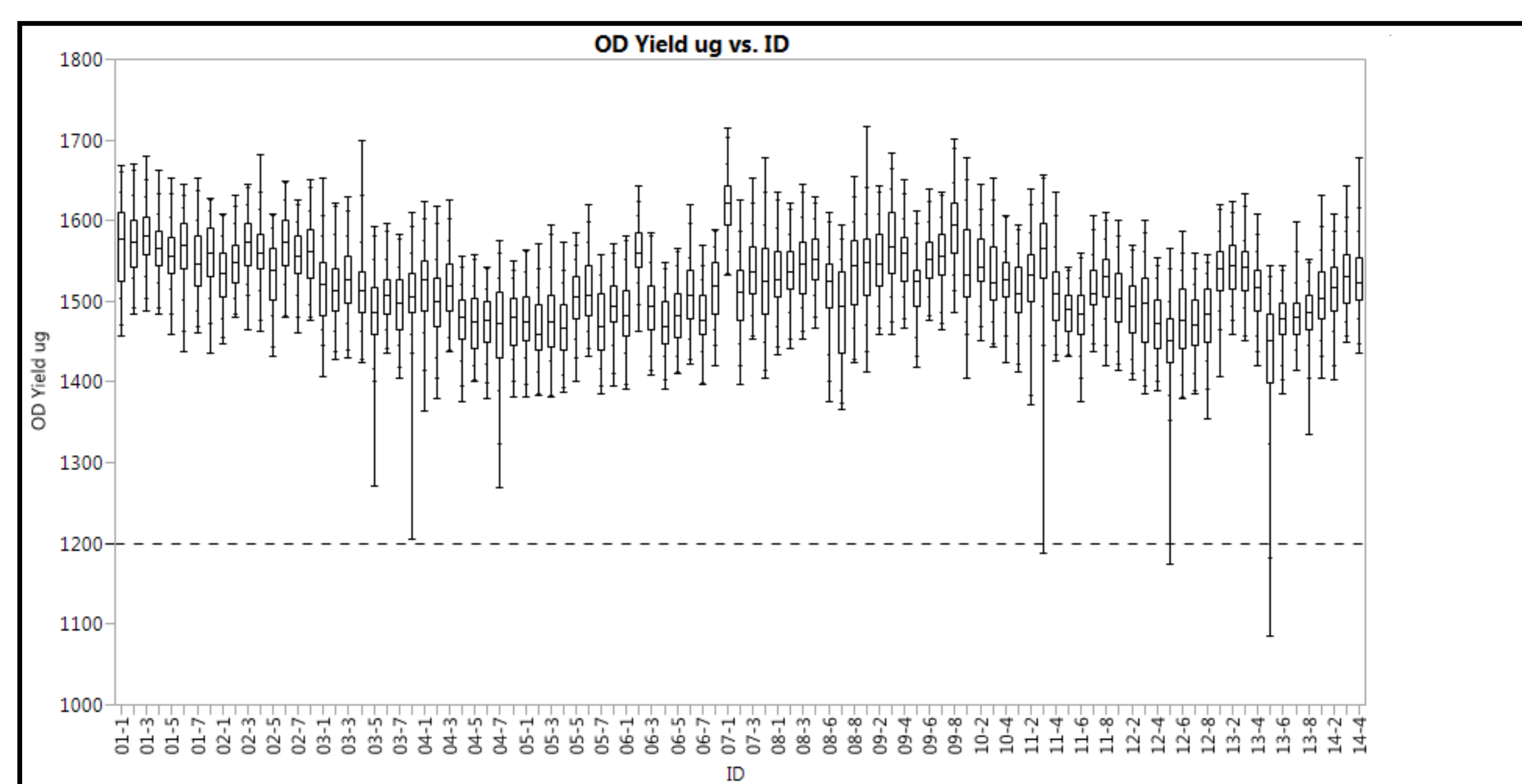


Figure 4. Axiom genotyping performance of 12 array plates using the HT workflow. All plates pass standard genotyping criteria. The blue horizontal line in each plot indicates the acceptance value.

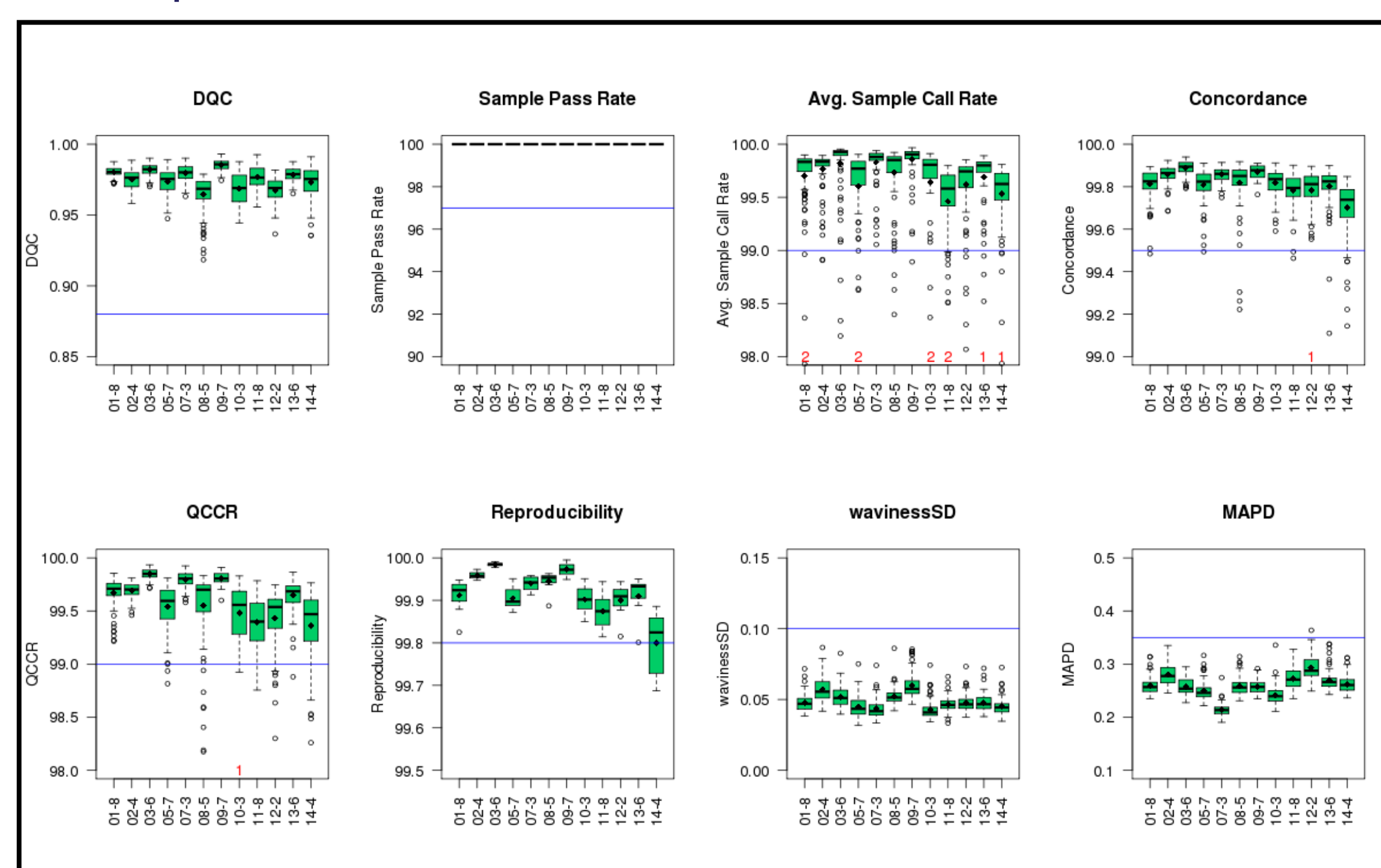
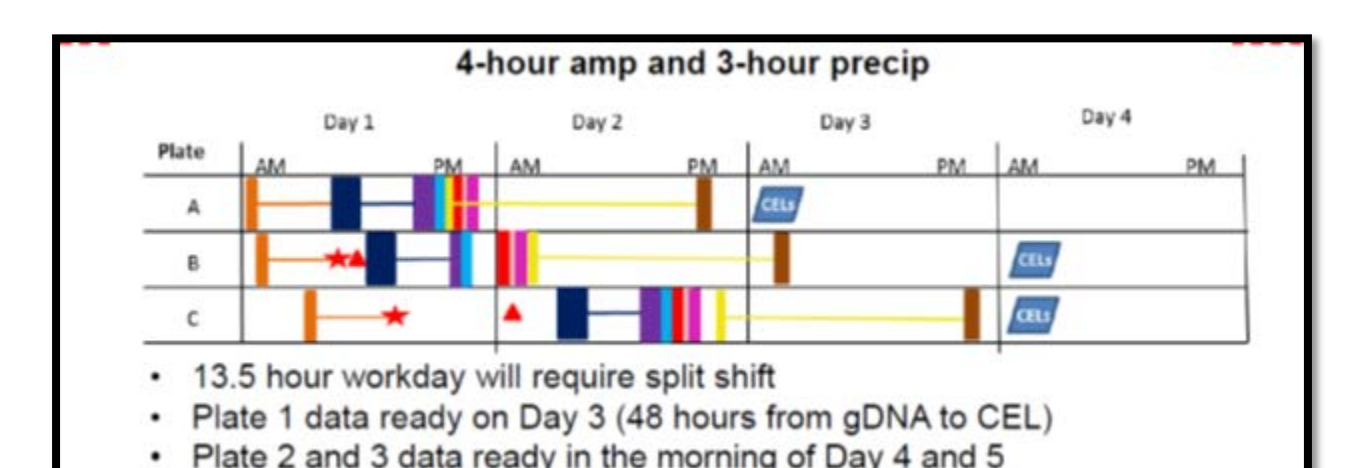
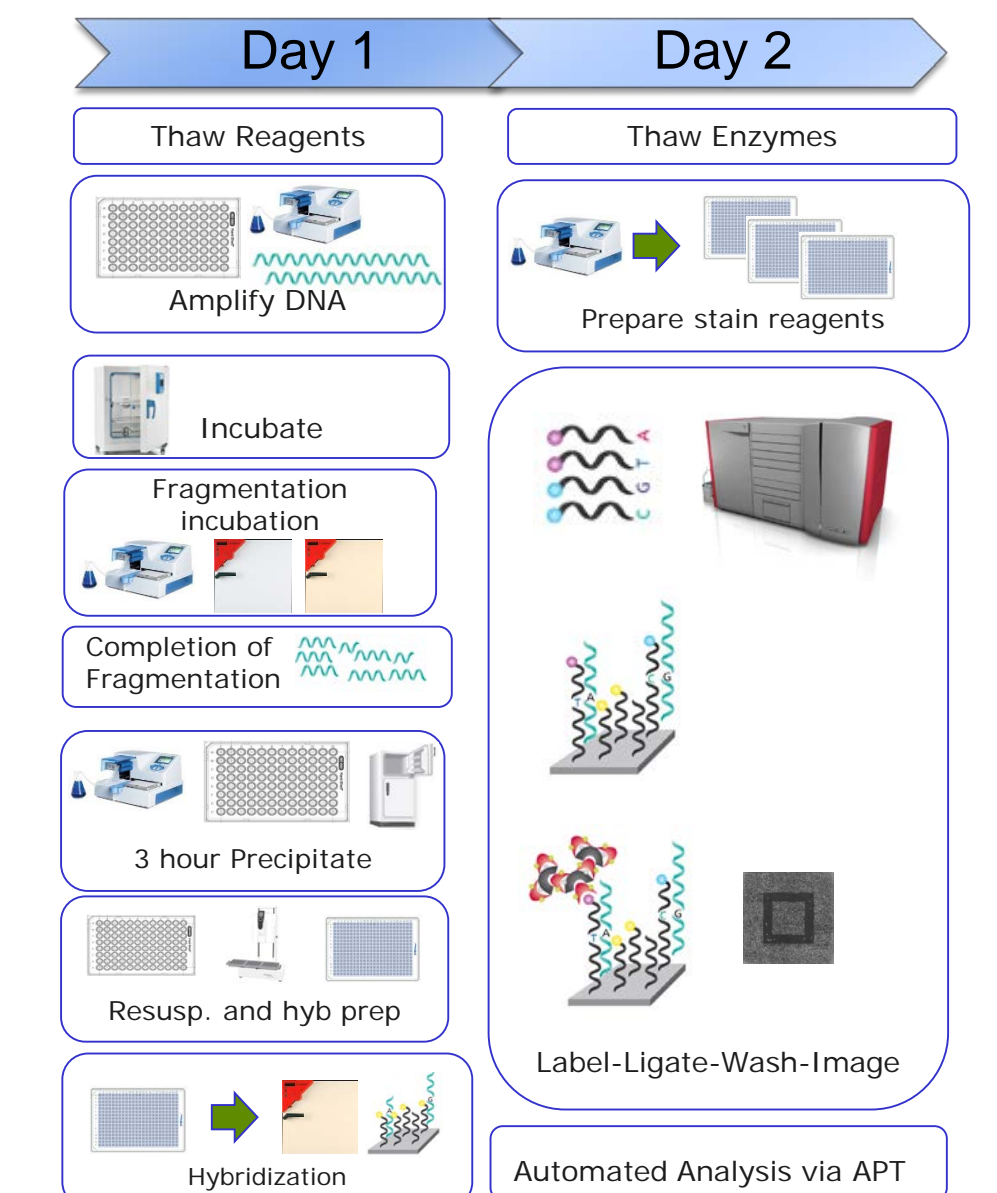


Figure 5. 48 hour Axiom Workflow.



- Obtain scan data from gDNA to CEL within 48 hours
- Expedite priority samples
- Make timely breeding decisions

Table 1: Axiom Configurator

- Axiom Configurator** is a tool to help customers evaluate different use case scenarios to select the best option
- Step 1:** Technical Sales Specialist (TSS) inputs customer requirements for sample volume, work schedule details
  - Step 2:** Configurator provides list of required equipment and consumables
  - Step 3:** Output is used to discuss best option for customer and order accordingly

## CONCLUSIONS

- The new Axiom HT workflow breaks the dependency on large liquid handling instruments with fixed capacity and instead leverages cost-effective lab equipment scales and maximizes resources as demand grows
- Bulk reagent packaging increases operational efficiency
- New workflow for 384F arrays increases the turnaround time from sample to answer from 4 days to 2 days which allows fast results for time-sensitive breeding decisions and urgent results on priority samples
- Stand alone Gene Titan Scanner supplements GeneTitan MC and allows rescans of urgent samples without disrupting production cycle and shorter scan times (2 hrs savings for 96F array and 15 min savings for 384F array)

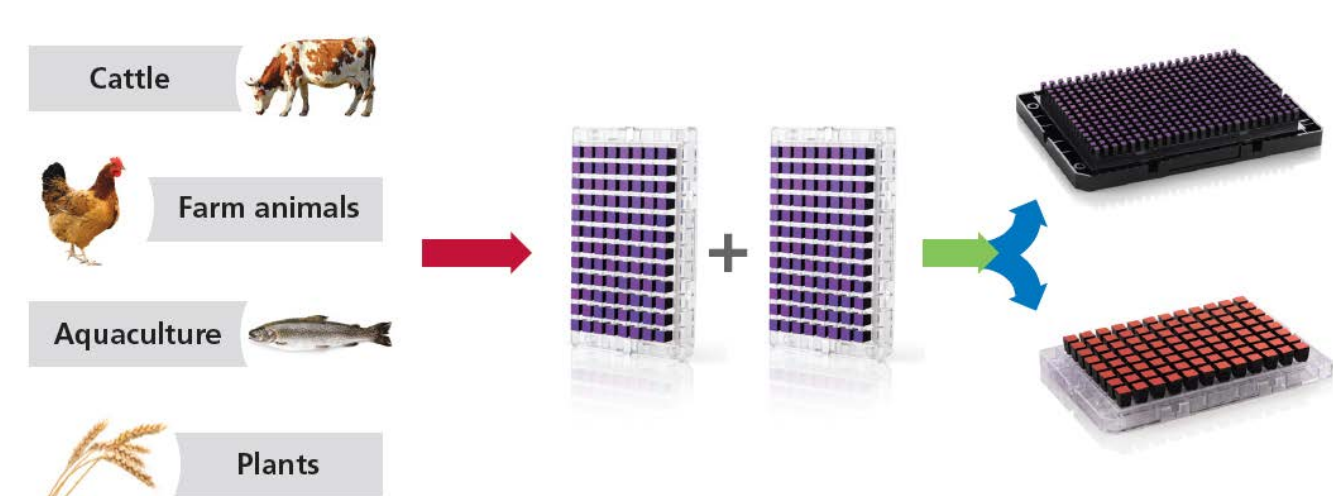
## ACKNOWLEDGEMENTS

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## TRADEMARKS/LICENSING

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## NGS Verification Genomic selection and Routine Use



- Selection of markers using *in silico* design
- Verify millions of SNPs across hundreds of samples
- Sub-select markers
- Add new markers