

Efficient genotyping of diploid apple leaves using whole cell lysates and SwiftArrayStudio Microarray Analyzer

In this application note, we show:

- Applied Biosystems™ Axiom™ Apple Microarray and Applied Biosystems™ SwiftArrayStudio™ Microarray Analyzer enables robust genotyping from apple samples
- Thermo Scientific™ KingFisher™ Flex Purification System and Applied Biosystems™ MagMAX™ Plant DNA Purification kit can be used for automated genomic DNA extraction from apple leaf samples
- Applied Biosystems™ AgriSeq™ Genomic DNA extraction kit is a crudel lysate option for collecting apple genotypes
- Purified genomic DNA and crude lysates provide highly correlative genotyping data from apple leaf samples

Introduction

Genetic testing is becoming an increasingly important tool in agricultural management. Researchers and commercial apple growers have found genotyping to be valuable for cultivar identification, germplasm management, pedigree validation, trait mapping, and marker-assisted and genomic selection. Tools developed by these researchers have helped verify cultivar authenticity, identify redundant or mislabeled accessions, assess parental compatibility, and support selection for desirable traits [1-4]. Reliable marker selection and validation remain critical, however, because genomic complexity can affect genotyping accuracy [5]. Advances in SNP microarray technology, culminating in high-density platforms such as the Applied Biosystems™ Axiom™ Apple array [6], have greatly expanded the resolution and utility of apple genotyping (for example, [7]). Together, these tools and applications demonstrate the value of robust genotyping solutions for improving apple breeding and germplasm management.

The practical implementation of crossing strategies and selection for desired traits depend on fast and efficient methods for genotyping genomic DNA. To address the growing demand for ease of use and speed in microarray analysis, Thermo Fisher Scientific developed the Applied Biosystems™ SwiftArrayStudio™ Microarray Analyzer and Applied Biosystems™ SwiftArray™ Assay. This solution combines a DNA target preparation workflow that can be completed in a single workday with an instrument that automates hybridization, washing, and scanning without in-process user intervention. Following overnight hybridization, the SwiftArrayStudio Microarray Analyzer processes and scans Applied Biosystems™ Axiom™ array plates. This assay and streamlined workflow reduce sample-to-answer time from five days to two days, allowing researchers to rapidly generate and interpret genetic data.



Figure 1. Workflow used in this study for collecting DNA and analyzing apple leaf samples using SwiftArray solution. In this application note, we show how both purified DNA and crude whole cell lysates, without DNA purification, can be analyzed. Note that samples taken from fruit did not yield enough DNA for analysis.

Description of experiments

In this application note, we demonstrate the use of the SwiftArrayStudio solution for apple genotyping. Leaves and fruit from a variety of apple cultivars were collected from local nurseries and markets. Approximately 10 mm^2 replicate pieces were cut from each leaf sample and processed as described below. Purified genomic DNA (gDNA) or crude whole-cell lysate (WCL) samples were prepared for hybridization using the SwiftArray Assay Kit according to the user guide. Targets were analyzed using the Axiom Apple microarray, which queries more than 400,000 variants identified across more than 60 cultivars, providing extensive information on polymorphisms throughout the apple genome. Hybridization, washing, and scanning were performed on the SwiftArrayStudio Microarray Analyzer using a 16-hour hybridization protocol. Subsequent analyses were performed using Axiom Analysis Suite 6.0 and best-practice analysis parameters.

Sample extraction

To facilitate high-throughput sample preparation, we evaluated two methods with reducedl hands-on time. First, apple samples (fruit or leaf) were homogenized in 100 µL of 100 mM citrate buffer, pH 5.0, using a microcentrifuge tube homogenizer and blue pestles. Cellulase and pectinase were each added to a final concentration of 1 ng/µL, and the homogenates were incubated at 37°C for 1 hour with shaking. Next, 100 µL of proteinase K buffer (Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA, and 0.1% SDS) and proteinase K to a final concentration of 0.5 ng/µL were added to the homogenates. Samples were incubated at 65°C for 1 hour with shaking. Debris was removed by centrifugation, and genomic DNA was purified from the supernatant using a KingFisher™ Flex instrument and MagMAX™ Plant DNA Purification Kit according to the recommended protocol. Final DNA concentrations were determined using an Invitrogen™ Qubit™ fluorometer. Insufficient gDNA was recovered from fruit samples; therefore, these samples were not processed further. The gDNA concentration from leaf samples averaged 17.7 ± 3.0 ng/µL.

We also prepared crude whole cell lysates for genotyping analysis using the Applied Biosystems™ AgriSeq™ Genomic DNA Extraction Kit. Approximately 10 mm^2 pieces of the same tissues were homogenized in 250 µL of the supplied lysis solution using blue pestles. Homogenates were heated to 95°C for 10 minutes and then allowed to cool at room temperature for approximately 5 minutes. An equal volume of stabilization solution was added, the samples were rehomogenized, and debris was removed by centrifugation. The resulting supernatant was transferred to a new tube.

Results of microarray analysis

After DNA extraction, samples were processed using the SwiftArray Assay workflow. For purified gDNA samples, approximately 100 ng of gDNA (5 µL of recovered DNA) was used. For matched whole-cell lysates, either 5 µL or 25 µL of supernatant was used (Figure 2A). Following amplification, fragmentation, and precipitation, the recovered DNA was analyzed using the recommended quality-control assays, including agarose gel electrophoresis (not shown) and quantification by absorbance at 260 nm (Figure 2B). An average of 854 ± 170 µg was produced from samples containing purified genomic DNA. Whole-cell lysates generated an average of 839 ± 174 µg with a 5 µL input and 894 ± 124 µg with a 25 µL input.

Samples were then loaded onto the SwiftArrayStudio Microarray Analyzer for automated hybridization, washing, and scanning. QC metrics were generated using Axiom Analysis Suite and the default best-practice analysis parameters. One of these metrics, Dish QC (DQC), measures the separation between signal distributions generated by non-polymorphic probes, with higher values indicating better sample quality. Most samples, whether prepared as crude WCL or purified gDNA, passed the default DQC threshold (Figure 2C). However, two samples (leaf 5 and leaf 6) failed in every gDNA replicate but not in the corresponding WCL samples, suggesting a sample preparation–specific issue.

Another quality metric generated by Axiom Analysis Suite is QC call rate, which represents the percentage of QC SNPs for which a genotype call is assigned rather than a NoCall. All genomic DNA samples that passed DQC also passed the QC call-rate threshold (Figure 2D). Among the crude WCL samples, leaf 3 and leaf 4 fell below the passing threshold but still produced recoverable genotyping data. Interestingly, the amount of input lysate had little effect on the QC metrics overall; 5 µL and 25 µL inputs produced data of similar quality.

Correlation of genotyping data

To compare data quality between purified gDNA and whole-cell lysate samples, we analyzed genotype clustering quality; two representative examples are shown in Figure 3. The vast majority of genotype calls obtained from purified gDNA and crude lysates were concordant. To further assess agreement, we compared genotypes at each of approximately 400,000 loci across replicates prepared from either crude WCL or purified gDNA. Across all calls, the average concordance between crude WCL and gDNA samples was 99.2%. When loci for which a replicate failed to produce a genotype call (NoCall) were excluded, average concordance increased to 99.8%. These results demonstrate that whole-cell lysates can generate genotyping data highly concordant with those obtained from purified genomic DNA.

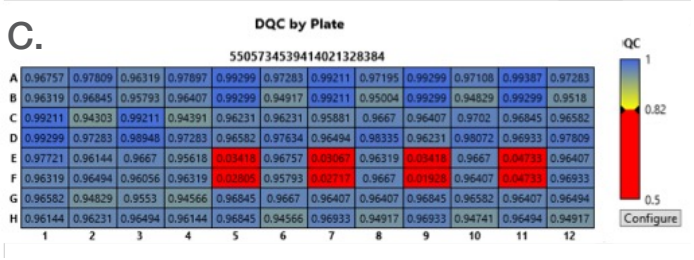
A.



B.

	1	2	3	4	5	6	7	8	9	10	11	12
A	962.4	1117	947.7	1011	711.7	594.4	561.5	665.0	705.2	722.4	684.3	740.4
B	683.8	549.4	835.9	661.0	703.3	1010	660.3	1100	712.1	1157	821.6	1160
C	654.8	871.5	410.7	975.5	882.0	590.1	919.1	591.0	921.5	673.6	942.7	737.8
D	632.9	629.8	345.0	711.2	882.0	1060	789.2	1044	915.1	1052	966.2	1144
E	1014	936.7	983.6	973.6	641.5	917.5	686.4	928.6	718.6	942.2	802.8	994.1
F	1009	874.6	1019	917.2	672.4	936.0	738.8	906.8	753.5	922.7	873.2	980.3
G	911.8	914.1	901.0	954.6	916.3	706.7	875.8	701.4	906.0	541.1	902.2	664.1
H	995.7	669.8	953.9	673.8	972.4	1089	1060	1013	1012	953.2	1048	1101

C.



D.

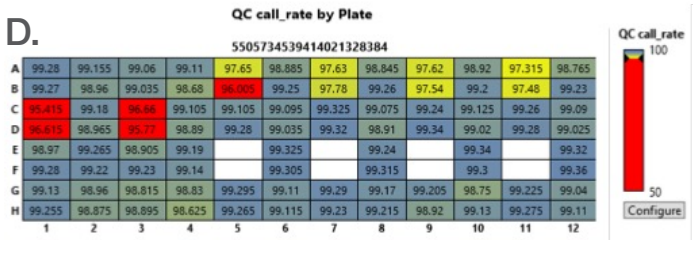


Figure 2. QC metrics of apple samples analyzed. A. Layout of samples used. B. Amount of DNA recovered after whole genome amplification, fragmentation, and precipitation using SwiftArray Assay workflow. Amounts are in micrograms; shading is from highest amount (green) to lowest amount (red). Note that leaf3 and leaf4 did not have 25µl lysate available; only about 2µl was left for use. C. Dish QC values obtained from the samples, shaded from best (blue) to failing (red). D. QC Call rate for the samples. Note that samples that failed to meet minimal DQC values (0.82) were not evaluated by the Axiom Analysis Suite software (empty boxes).A

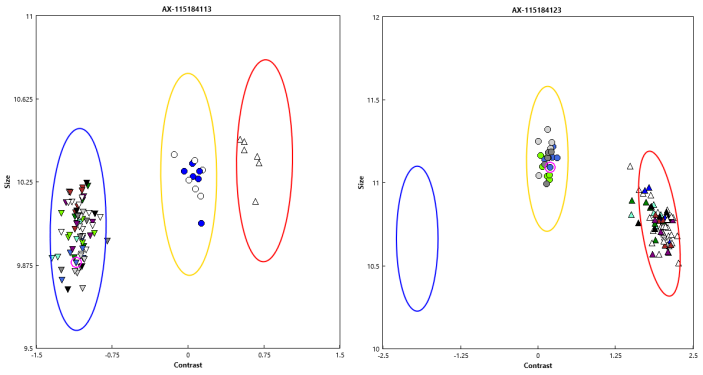


Figure 3. Cluster plots of genotypes from leaf samples obtained with gDNA and crude WCL. An example cluster plot from a PolyHigRes (left) and NoMinHom (right) locus is shown. Markers colored the same indicate samples from the same leaf, including whole cell lysates and purified genomic DNA. Shapes are genotype call classes (AA, AB or BB). Note that not every different leaf sample could be assigned a color by the software; those that could not be assigned were left white.

Summary

In this application note, we demonstrate how the SwiftArray solution for microarray analysis can be used for genotyping apple samples. We show that an automated sample preparation workflow can be used to extract genomic DNA from apple leaves. As an alternative approach requiring less sample processing, we demonstrate the use of crude whole-cell lysates for genotyping apple leaf samples. We also demonstrate the use of an Axiom apple genotyping array with the efficient SwiftArrayStudio Microarray Analyzer. Finally, genotyping results obtained from whole-cell lysates showed 99.8% concordance with those obtained from purified genomic DNA when NoCalls were excluded. Together, these workflows provide efficient options for generating high-quality apple genotyping data to support breeding, cultivar identification, and other agricultural applications.

Ordering information

Description	Cat. No
SwiftArray Reagents and Systems	
Axiom™ Apple Genotyping Array, 96F	Contact us
SwiftArrayStudio™ Microarray Analyzer	00-0471
Axiom™ SwiftArrayStudio™ Consumables Kit	952674
KingFisher Reagents and Systems	
KingFisher™ Flex Purification System with 96 DW head	5400630
Thermo Scientific™ Cellulase	J64019.03
Thermo Scientific™ Pectinase	J63408.MD
MagMAX™ Plant DNA Purification Kit	A32580
Crude Lysate Preparation Reagents	
AgriSeq™ Genomic DNA Extraction Kit	A66428
Fisherbrand™ RNase-Free Disposable Pellet Pestles	12-141-364

Table 1. Analysis of 16 apple leaf samples using SwiftArrayStudio. The Axiom Apple microarray has approximately 400,000 genotyping probes; concordance of calls at each probe were made for replicate leaves (n). Percentage identity was calculated whether they were analyzed by gDNA or crude whole cell lysate (WCL). Percentages are the average of call concordance on two separate arrays.

	n=	% identical, including NoCalls	% identical, excluding NoCalls
Leaf1	5 DNA, 5 WCL	99.858	99.957
Leaf2	5 DNA, 5 WCL	99.561	99.821
Leaf3	5 DNA, 4 WCL	95.744	99.127
Leaf4	5 DNA, 4 WCL	96.699	99.262
Leaf5	1 DNA, 4 WCL	99.813	99.961
Leaf6	1 DNA, 4 WCL	99.956	99.989
Leaf7	5 DNA, 5 WCL	99.723	99.937
Leaf8	5 DNA, 5 WCL	99.340	99.702
Leaf9	5 DNA, 4 WCL	99.315	99.865
Leaf10	5 DNA, 4 WCL	99.236	99.774
Leaf11	5 DNA, 5 WCL	99.708	99.932
Leaf12	5 DNA, 4 WCL	99.061	99.777
Leaf13	5 DNA, 4 WCL	99.840	99.946
Leaf14	5 DNA, 4 WCL	99.871	99.953
Leaf15	5 DNA, 4 WCL	99.627	99.929
Leaf16	5 DNA, 4 WCL	99.708	99.795

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