APPLICATION NOTE

TaqMan SNP Genotyping Assays

Plant SNP genotyping by real-time PCR

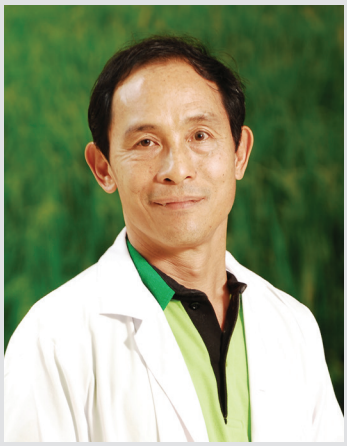
A streamlined sample-to-results workflow with a wide range of throughput options

Abstract

Real-time PCR can offer a fast and scalable approach for single-nucleotide polymorphism (SNP) genotyping in plants. Using a streamlined sample-to-results workflow with the Applied Biosystems™ TaqMan™ Sample-to-SNP™ Kit and a range of Custom TaqMan™ SNP Assays, genotyping was performed on maize samples, demonstrating medium- and high-throughput utility. High concordance was seen between results from 384-well plates used on the Applied Biosystems™ ViiA™ 7 Real-Time PCR System and those from OpenArray™ plates on the Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System. High-quality genotyping results were obtained on both platforms using the direct lysis approach for sample input, with preamplification on the OpenArray™ platform further improving reporter signals and cluster separation. The versatility of plant genotyping solutions from Thermo Fisher Scientific is demonstrated by successful use of marker-assisted selection and gene pyramid approaches in Thai jasmine rice samples from a collaborator at the Rice Gene Discovery and Rice Science Center, Kasetsart University, Thailand.

Dr. Apichart Vanavichit
Rice Gene Discovery and Rice Science Center
Kasetsart University, Thailand

Dr. Vanavichit is moving away from simple sequence length polymorphisms (SSLPs) and simple sequence repeats (SSRs) towards SNP genotyping, which he sees as “the answer for functional markers” in plants. He now screens his jasmine rice populations with Custom TaqMan SNP Assays designed for a range of functional markers and disease resistance traits. The high-throughput capacity of OpenArray plates on the QuantStudio 12K Flex Real-Time PCR System supports Dr. Vanavichit in his work associating SNPs with selected mutants for starch qualities, iron content, and iron toxicity, and with selected progeny from gene pyramiding programs.



Introduction

Plant breeding, or the purposeful manipulation of plant species, has been in practice for thousands of years, and approaches continue to develop with scientific advances. Insight into the molecular genetics of plant species allows for the linking of traits to the underlying genetic code, often in the form of single-nucleotide polymorphisms (SNPs). Techniques that detect or characterize SNPs can be applied to the successful management of plant genomes, including marker-assisted selection, genomic selection, and gene pyramid approaches. The continued advancement and adoption of real-time PCR practices has led to the development of simplified and cost-effective solutions for SNP genotyping. We offer a streamlined and scalable plant SNP genotyping workflow, combining minimal hands-on time with industry-standard assays and instrumentation capable of generating up to 100,000 data points in a single 8-hour day (Figure 1).

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Integral to our sample-to-results workflow is the TaqMan Sample-to-SNP Kit (Figure 2), which provides all necessary reagents for a simple, 5-minute direct lysis procedure suitable for leaf or seed samples. The Applied Biosystems™ TaqMan™ GTXpress™ Master Mix is also supplied, specifically designed for use with TaqMan SNP and custom SNP assays for robust SNP genotyping. We will design custom assays for any SNP in any organism, based on the input target sequence. Custom assays ordered through the Applied Biosystems™ Custom TaqMan™ Assay Design Tool (CADT) are secure and confidential, and tested for synthesis accuracy and formulation completeness. We offer a range of sample throughput options, from the 48-well Applied Biosystems™ StepOne™

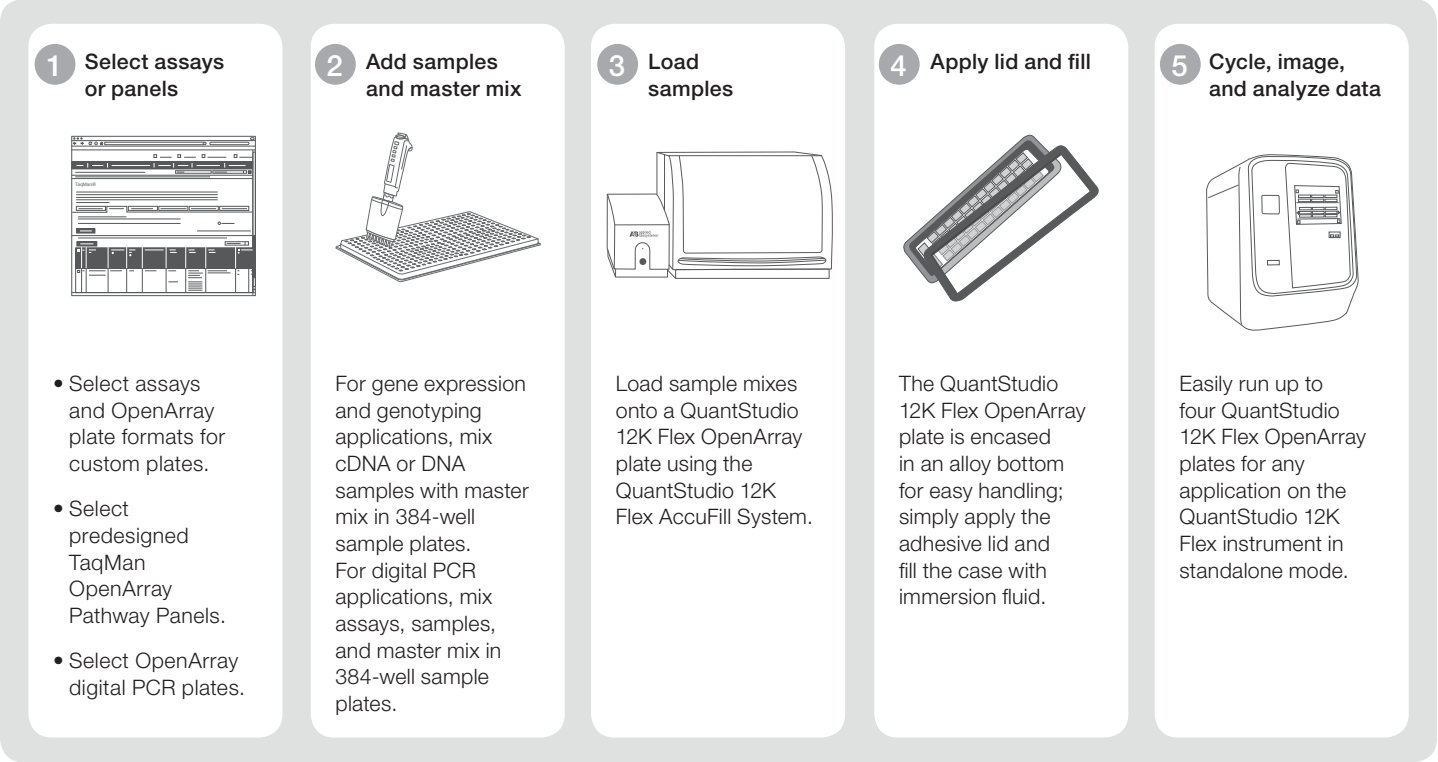


Figure 1. SNP genotyping workflow with OpenArray plates and the QuantStudio 12K Flex System. This workflow offers the convenience of high-throughput flexibility in conjunction with Custom TaqMan SNP Genotyping Assays. With each through-hole of the OpenArray plate having a capacity of 33 nL, reagent costs are minimized while maximizing throughput and retaining quality results.

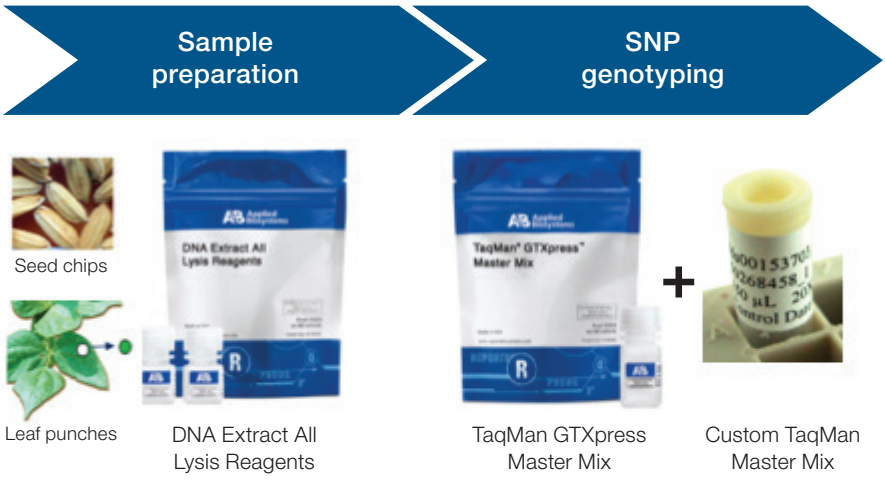


Figure 2. The TaqMan Sample-to-SNP Kit workflow. This workflow includes a 5-minute process for the preparation of lysates from a wide range of sample types, including plant tissues such as seed chips and leaf punches. Subsequent SNP genotyping reactions are performed using the TaqMan GTXpress Master Mix (supplied with the kit) and Fast cycling protocols on a range of Thermo Fisher Scientific real-time PCR platforms.

and 96-well StepOnePlus™ systems to the versatile ViiA 7 and QuantStudio 12K Flex systems. With the ViiA 7 and QuantStudio 12K Flex systems, assay format options include 96-well, 384-well, and Applied Biosystems™ TaqMan™ array card. In addition, the QuantStudio 12K Flex system is compatible with OpenArray plates.

OpenArray technology uses a microscope slide–sized plate with 3,072 through-holes with a capacity of 33 nL each. Reagents are retained in the through-holes via surface tension, and one OpenArray plate can hold as many samples as eight traditional 384-well plates—greatly increasing throughput while minimizing reagent costs. A variety of custom formats (preloaded with Applied Biosystems TaqMan Assays) are available to support a broad range of study sizes.

Here we demonstrate the ease of use and scalability of the plant SNP genotyping workflow using the TaqMan Sample-to-SNP Kit with Custom TaqMan Assays designed for maize samples. Results generated with 384-well plates on the ViiA 7 Real-Time PCR System are compared to results with OpenArray plates run on the QuantStudio 12K Flex Real-Time PCR System. In addition, we discuss the use of preamplification techniques and look at the effect of these approaches on the SNP genotyping results.

This workflow is versatile and applicable to a wide range of plant genotyping needs, as demonstrated in the adoption of this protocol by Dr. Apichart Vanavichit from the Rice Gene Discovery and Rice Science Center, Kasetsart University, Thailand, who is currently switching from microsatellite markers in his Thai jasmine rice studies to what he calls “the future of next-generation plant breeding.”

Materials and methods

Sample preparation was performed by following the protocol supplied with the TaqMan Sample-to-SNP Kit. Seed chips (2–3 mm) from 19 maize samples were added to 75 µL of lysis solution and incubated at 95°C for 3 minutes, after which 75 µL of stabilizing solution was added. The crude lysates were used directly for subsequent real-time PCR (lysates can be stored at 4°C or –20°C until needed).

Custom TaqMan SNP Genotyping Assays were designed for 12 different markers using the CADT (found at **thermofisher.com/snpcadt**). SNP genotyping reactions were assembled using the TaqMan GTXpress Master Mix following the protocol supplied with the TaqMan Sample-to-SNP Kit.

Ten-microliter reactions were run using a 384-well plate format on the ViiA 7 Real-Time PCR System with a universal Fast cycling protocol: 60°C for 30 sec (preread), 95°C for 20 sec (hold), 95°C for 1 sec and then 60°C for 20 sec (40 cycles), and 60°C for 30 sec (postread). The same 19 maize crude lysate samples were screened using a custom OpenArray Plate prespotted with 12 TaqMan Assays (using subarrays with 64 through-holes in an 8 x 8 pattern). Five microliters of reaction mix for each sample was transferred into each well of a 384-well sample plate (corresponding to one subarray on the OpenArray Plate) and loaded onto the OpenArray Plate using the automation process of the Applied Biosystems™ QuantStudio™ 12K Flex AccuFill™ System (refer to protocol P/N 4470935, Rev. B).

Preamplification of the maize crude lysate samples was performed using Applied Biosystems™ TaqMan™ PreAmp Master Mix following the protocol supplied. The QuantStudio 12K Flex AccuFill System was used to add diluted, preamplified sample to OpenArray plates in the same configuration as described above. Analysis of results was performed using the genotyping analysis software supplied with the real-time PCR instruments.

Results

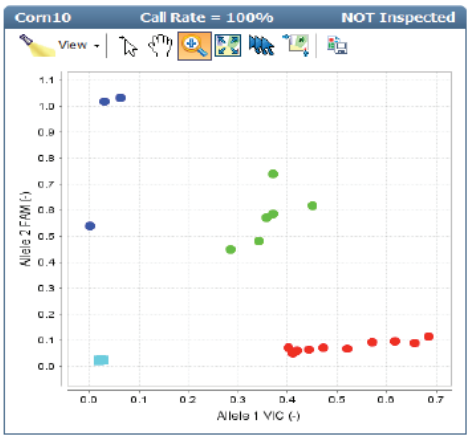
Signal intensity was consistent across all samples and platforms without the need to normalize sample input. The crude lysates from the 19 maize samples exhibited consistent, tight, and well-separated clusters for all 12 Custom TaqMan SNP Genotyping Assays on the ViiA 7 system and for 11 assays on the QuantStudio 12K Flex system (see Figure 3 for representative data). Preamplification was shown to increase signal intensity and subsequent cluster separation for all assays without affecting genotyping calls.

Concordance in genotyping calls was high between the systems, with 11 assays showing 100% concordance (Table 1). A single sample on the OpenArray plate did not amplify, reducing concordance to 94.7% for the remaining assay. Three instances of nonamplification occurred for the OpenArray plate containing preamplified samples.

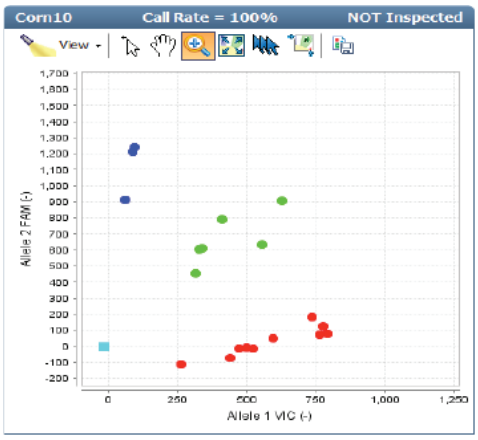
Table 1. Concordance of genotyping calls from the ViiA 7 system compared with OpenArray plates run on the QuantStudio 12K Flex system with and without preamplification.

Assay	OpenArray plates, no preamplification	OpenArray plates, preamplified samples
01	94.7%	94.7%
02	100%	94.7%
03	100%	100%
04	100%	94.7%
05	100%	100%
06	100%	100%
07	100%	100%
08	100%	100%
09	100%	100%
10	100%	100%
11	100%	100%
12	100%	100%
Total average	**99.59%**	**98.67%**

ViiA 7 system



QuantStudio 12K Flex system



QuantStudio 12K Flex system with preamplified samples

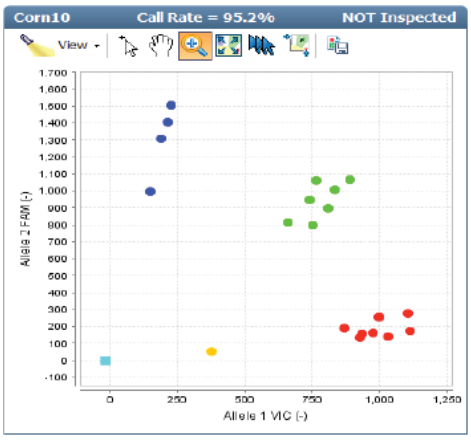


Figure 3. SNP genotyping results. Genotyping plots from a single Custom TaqMan SNP Genotyping Assay, showing concordance of results between the ViiA 7 Real-Time PCR System, QuantStudio 12K Flex Real-Time PCR System, and preamplified samples run on the QuantStudio 12K Flex Real-Time PCR System. The orange dot on the far right plot indicates nonamplification for one of the samples.

The integrated genotyping software analysis packages of both the ViiA 7 and QuantStudio 12K Flex systems offer tools to trace the real-time signal of each data point (Figure 4). This tracing tool is useful in situations where the position of the data point in the genotyping plot makes for a potentially ambiguous call. By tracing the path of fluorescence from the reaction starting point, genotyping calls can be more clearly identified.

Discussion

The TaqMan Sample-to-SNP Kit and real-time PCR instrumentation from us provide a fast and robust solution for a range of plant genotyping needs. The high concordance in results between the ViiA 7 Real-Time PCR System and the QuantStudio 12K Flex system demonstrates the scalability of the workflow for a wide range of throughput requirements, from running single samples on 96-well plates to large sample numbers on OpenArray plates. The direct lysis approach, applicable to a wide range of sample input amounts, is an easy 5-minute procedure that streamlines front-end processing, providing ample starting material for real-time PCR with no normalization required. Samples can be stored long-term for continued use and testing.

The QuantStudio 12K Flex Real-Time PCR System with OpenArray plates provides a stable, high-throughput platform with demonstrated success with our maize samples as well as with Thai jasmine rice samples from Dr. Vanavichit (data not shown). TaqMan PreAmp Master Mix, in conjunction with the OpenArray workflow, can be a useful tool to improve cluster separation and signal intensity while keeping confidence in the concordance of genotyping results, and the troubleshooting real-time tracing tool offers further confidence in call accuracy.

The flexibility and versatility of this plant genotyping workflow are demonstrated by practical application in high-value crops such as rice. Dr. Vanavichit, in conjunction with the Rice Gene Discovery and Rice Science Center at Kasetsart University, is developing TaqMan Assays for rice SNP markers to screen and select for important traits such as disease resistance and aromatic properties.

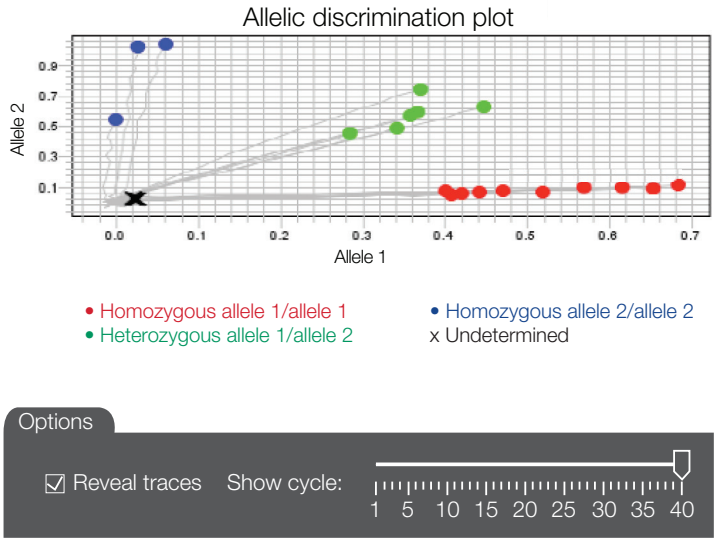


Figure 4. Real-time trace tool for ambiguous calls. This tool acts as a helpful troubleshooting aid, showing the source and path of the fluorescent signal that leads to the endpoint reading. This troubleshooting tool is available on both ViiA 7 and QuantStudio 12K Flex systems, allowing potentially ambiguous calls to be more clearly identified.

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Ordering information

Product	Quantity	Cat. No.
TaqMan Sample-to-SNP Kit	200 mL lysis reagents, 10 mL PCR master mix	4403081
	20 mL lysis reagents, 10 mL PCR master mix	4403083
	20 mL lysis reagents, 50 mL PCR master mix	4403087
Custom TaqMan SNP Genotyping Assays—nonhuman	1,500 reactions* (40X)	4332077
	5,000 reactions* (40X)	4332075
	12,000 reactions* (80X)	4332076
ViiA 7 Real-Time PCR System	Go to **thermofisher.com/viia7**	
QuantStudio 12K Flex Real-Time PCR System	Go to **thermofisher.com/quantstudio**	

* Reactions calculated at 5 µL volume for 384-well plate format.

Find out more at **thermofisher.com/plant-genotyping**

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