TaqMan® Sample-to-SNP™ Kit

One hour. One kit. Any sample.

The TaqMan® Sample-to-SNP™ Kit is a uniquely formulated product designed to streamline the real-time PCR–based genotyping workflow. It has the following benefits:

- **Fast**—raw biological samples to SNP genotype results, typically in less than one hour
  - Single-protocol extraction of genomic DNA from many sample types in as little as 5 minutes
  - Genotyping PCR in 50 minutes or less
- **Simple**—a brief protocol with few pipetting steps
  - A colored dye in the TaqMan® GTXpress™ Master Mix for easy tracking
- **Robust**—universal protocol for all sample types
  - Get accurate genotyping results without DNA quantitation
- **Flexible**—validated with all types of TaqMan® Genotyping Assays available from Life Technologies
  - Extended PCR cycling possible for poorly amplified or sample-limited reactions

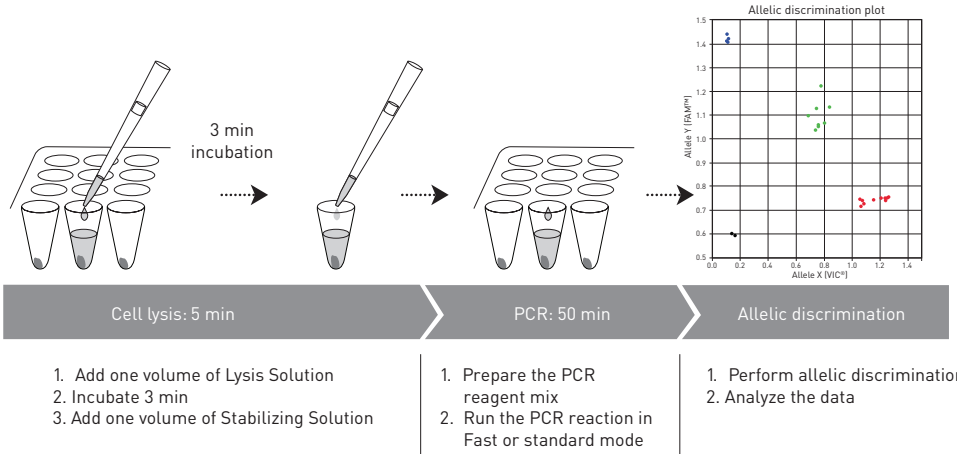

Figure 1. The TaqMan® Sample-to-SNP™ Kit workflow. The DNA Extract All Lysis Reagents require only 5 minutes to release DNA into a lysate solution that is compatible with TaqMan® GTXpress™ Master Mix. TaqMan® GTXpress™ Master Mix enables robust PCR amplification in 50 minutes or less.

Introduction

SNP genotyping workflows using TaqMan® chemistry are fairly straightforward and robust if the starting material is purified genomic DNA. However, getting from the biological sample to the purified DNA is often a time-consuming and laborious process. It can take up to 16 hours to purify DNA from some difficult samples such as mouse tail or formalin-fixed, paraffin-embedded (FFPE) tissues.

The TaqMan® Sample-to-SNP™ Kit addresses these critical needs. The kit consists of two parts: DNA Extract All Lysis Reagents and TaqMan® GTXpress™ Master Mix. The DNA Extract All Lysis Reagents reduce the time for release of real-time PCR–ready DNA to a 5-minute protocol. These reagents can process a wide variety of samples ranging from blood to buccal swabs to plant tissues. TaqMan® GTXpress™ Master Mix enables robust PCR amplification in 50 minutes or less. The TaqMan® Sample-to-SNP™ Kit provides a simple and quick workflow (Figure 1).

Ease of use

Compared to other reagent kits, the TaqMan® Sample-to-SNP™ Kit provides superior speed and ease of use for the genotyping workflow (Table 1). With the TaqMan® Sample-to-SNP™ Kit, there is only one kit and a streamlined protocol regardless of the sample type. Its robustness and flexibility provides universal-sample capability, thus enabling a process that is less error-prone. Sample-specific reagents and protocols are not needed. The simple protocol makes it easy for new users to adopt, and easy for high-throughput customers who need to scale up to thousands of samples.

Table 1. A comparison of vendors' solutions for rapid DNA preparation for PCR genotyping.

Vendor	Product/brand	Number of sample prep kits	Time	
			Extraction	PCR
Life Technologies	TaqMan® Sample-to-SNP™ Kit	• 1 kit for all sample types	5 min	50 min
Sigma-Aldrich	Extract-N-Amp™	• 1 kit for blood • 1 kit for seeds • 1 kit for tissue	5 min	2 hr 33 min
Epicentre Biotechnologies	QuickExtract™	• 1 kit for some sample types • 1 kit for FFPE • 1 kit for buccal swabs • 1 kit for plants	3–8 min	1 hr 47 min
Molecular Research Center, Inc.	DNAzol® Direct	• 1 kit	10–15 min	NA
BioVentures, Inc.	GeneReleaser™	• 1 kit (using microwave)	11–16 mi	NA

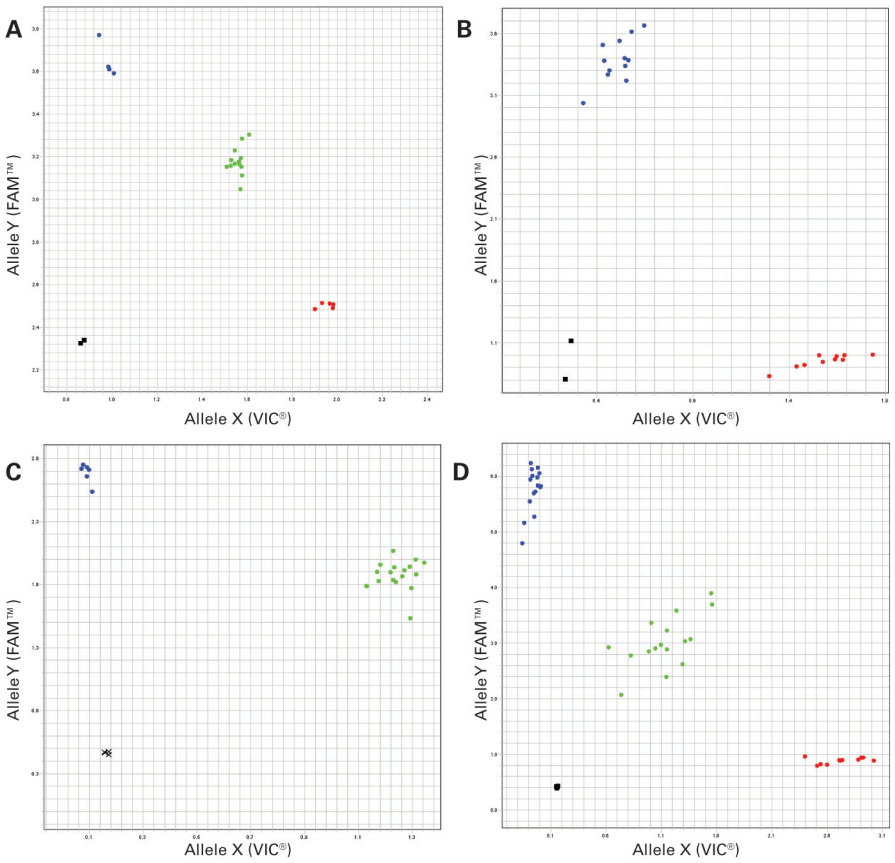

Figure 2. Allelic discrimination plots from various samples. Buccal swab **(A)**, mouse tail **(B)**, corn leaf **(C)**, and FFPE **(D)** samples were processed with the TaqMan® Sample-to-SNP™ Kit, and genotyping results were obtained following the recommended user protocol. All amplifications and allelic discriminations were performed on a 7900HT Fast Real-Time PCR System with a 384-well block.

Universal sample preparation

The versatility of the TaqMan® Sample-to-SNP™ Kit enables the use of a single protocol and a single set of reagents for virtually any sample type. The sample types that have been tested for compatibility in SNP genotyping assays with this reagent system include:

- Blood (freshly drawn, EDTA, citrate, heparin)
- Blood on blood cards or FTA paper
- Cell suspension
- Buccal swab
- Rat or mouse tail
- Tissue
- Hair
- Leaf punch
- Seed chip
- FFPE tissue

Many of these sample types contain a wide range of compounds that may inhibit PCR. However, the specially formulated DNA Extract All Lysis Reagents and TaqMan® GTXpress™ Master Mix work together to overcome the effects of these inhibitors and provide excellent amplification without purifying DNA. Figure 2 shows SNP genotyping data collected using the TaqMan® Sample-to-SNP™ Kit on various sample types.

Speed without compromise

The TaqMan® Sample-to-SNP™ Kit enables Fast PCR cycling and cuts the overall genotyping PCR reaction time by half (to 50 minutes or less). Including sample preparation with the kit, SNP genotyping results can be obtained from biological samples in less than one hour without compromising data quality. Figure 3 compares the performance of the TaqMan® Sample-to-SNP™ Kit with a similar product from another vendor. In addition to the superior performance, the TaqMan® Sample-to-SNP™ Kit delivers the fastest time-to-results.

Robust reagent system

Many master mix products can perform reasonably well with highly purified DNA. However, even purified DNA from inhibitory samples such as blood or FFPE tissue can pose challenges for these mixes. TaqMan® GTXpress™ Master Mix is formulated to handle a broad spectrum of inhibitors contained in samples as varied as blood and cotton.

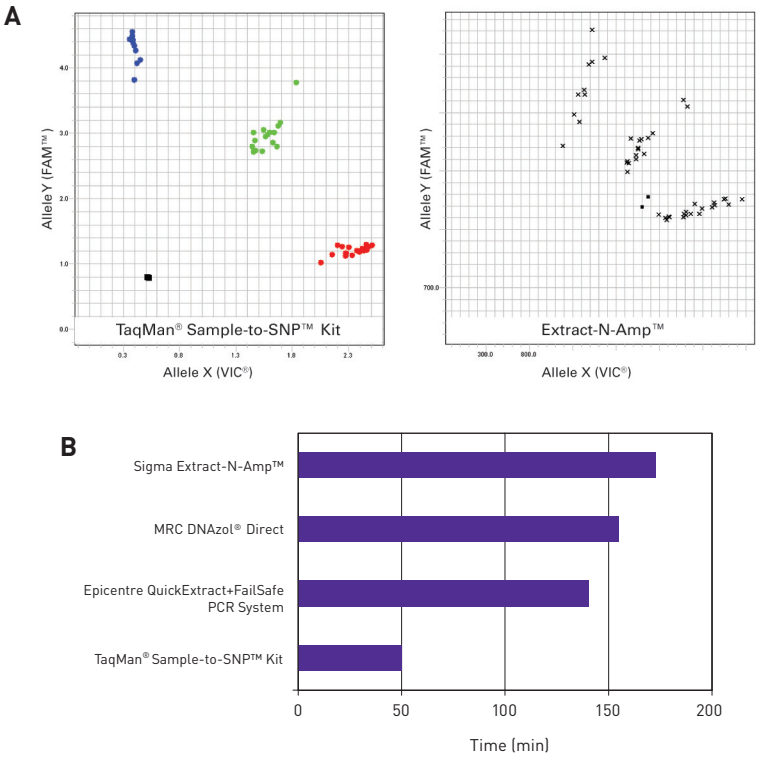

Figure 3. Performance and speed of the TaqMan® Sample-to-SNP™ Kit compared with other reagent kits. (A) Allelic discrimination plots show the tightly clustered genotyping results from the TaqMan® Sample-to-SNP™ Kit compared to the results from another vendor's reagents. **(B)** The total time-to-results, including sample preparation, PCR hold time, and ramp time, for various kits shows that the TaqMan® Sample-to-SNP™ Kit generated high-quality results in the shortest amount of time. DNA from whole blood was extracted and amplified with the kit, and then compared with DNA extracted with other vendors' kits followed by amplification with their master mixes. All experiments were performed on a 7900HT Fast Real-Time PCR System according to recommended protocols.

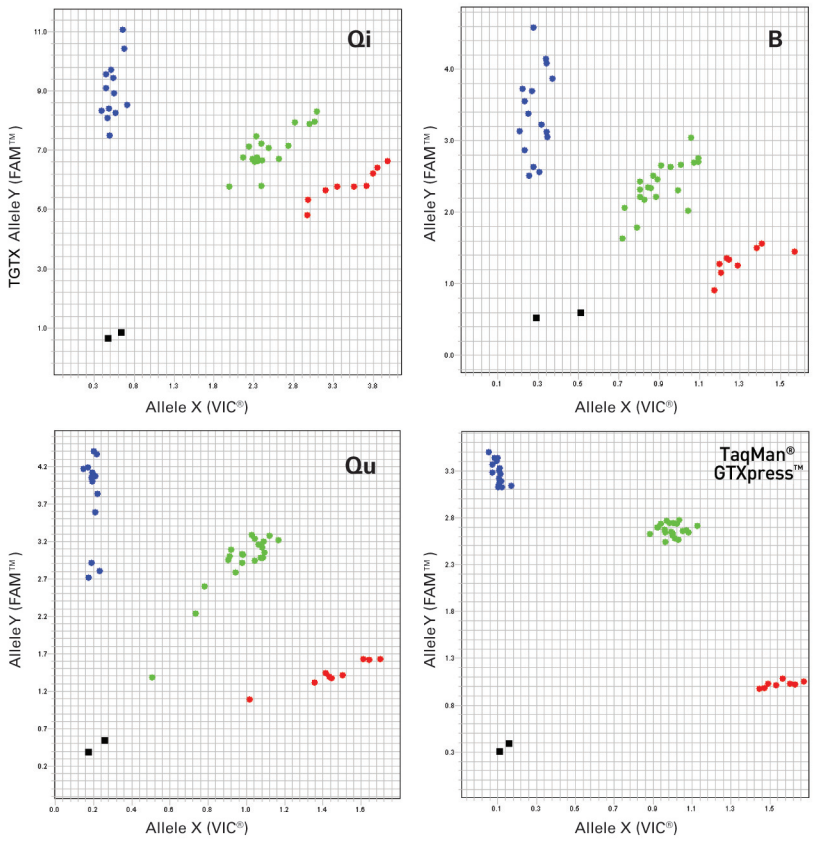

Figure 4. Performance comparison of TaqMan® GTXpress™ Master Mix and other Fast master mixes (Qi, B, Qu). DNA was extracted from blood samples using the DNA Extract All Lysis buffer and amplified with TaqMan® GTXpress™ Master Mix or other vendors' master mixes for assay C___ 1318101_10 on a 7900HT Fast Real-Time PCR System.

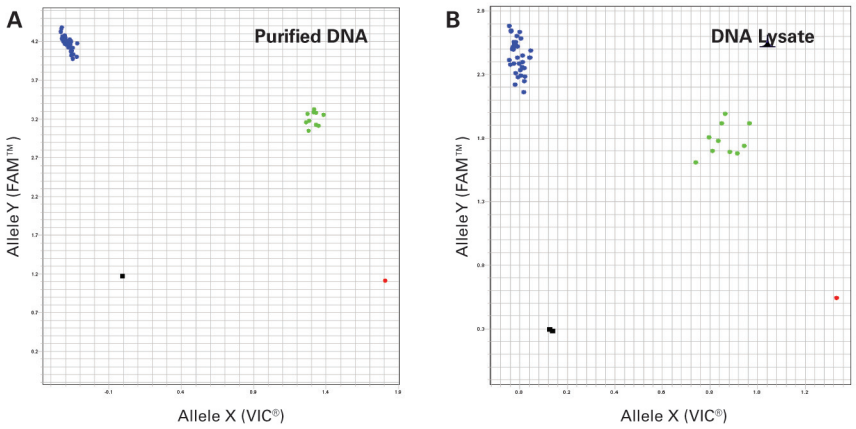

Figure 5. Example of genotyping cluster plots using purified blood DNA or a blood DNA lysate obtained with the TaqMan® Sample-to-SNP™ Kit. Purified DNA (A) or lysate (B) was mixed with TaqMan® GTXpress™ Master Mix for assay C__8814556_30 and amplified on a Dual 384-Well GeneAmp® PCR System 9700. Allelic discrimination was performed on a 7900HT Fast Real-Time PCR System with a 384-well block.

Figure 4 shows a comparison of the results obtained with other vendors' master mixes and TaqMan® GTXpress™ Master Mix using unpurified DNA samples. The results show better clustering with the TaqMan® GTXpress™ Master Mix.

Another convenient feature of this kit is that DNA quantitation prior to PCR amplification is not necessary. Commonly used reagents for genotyping require that all DNA samples be within a range of concentration, typically 1–10 ng. This step is extremely time-consuming, particularly for processing large numbers of samples. Using standard protocols for TaqMan® Assay–based genotyping, each sample must be quantitated and diluted to the appropriate range. The TaqMan® Sample-to-SNP™ Kit, by contrast, does not require prior quantification to obtain reliable genotyping calls. All data presented here were obtained using the DNA lysate without quantifying the DNA.

Concordance with purified DNA

The TaqMan® Sample-to-SNP™ Kit streamlines the SNP genotyping workflow without compromising the call rate. This kit was tested extensively for call concordance between the DNA lysate and purified DNA. As shown in Figure 5, the DNA lysate provides excellent cluster separation when compared to purified DNA. While the actual clustering is assay-dependent, a broad study comparing the genotyping call concordance between purified DNA and the DNA lysate across 46 EDTA-treated blood samples and two no-template controls showed excellent agreement. These 46 samples were genotyped with a panel of 200 predesigned TaqMan® SNP

Genotyping Assays, 100 TaqMan® Drug Metabolism Genotyping Assays, and 100 Custom TaqMan® SNP Genotyping Assays.

As shown in Table 2, there is excellent concordance between purified DNA and the DNA lysates prepared with the TaqMan® Sample-to-SNP™ Kit. The kit clearly provides speed and convenience without compromising data quality.

Reagent stability

TaqMan® GTXpress™ Master Mix is a fast-activating enzyme system. Fast enzyme systems often have poor or no room temperature stability, which is highly inconvenient for setting up large numbers of plates. TaqMan® GTXpress™ Master Mix, unlike products from other vendors, has at least 6 hours of pre-PCR stability at room temperature with all reaction components assembled, and at least 24 hours of post-PCR stability (Figure 6). In many cases, the stability of the assembled pre-PCR reaction is significantly longer than 6 hours. The enhanced stability of TaqMan® GTXpress™ Master Mix enables high-throughput users to use automated plate-loading systems to perform the allelic discrimination reads.

Summary

The TaqMan® Sample-to-SNP™ Kit provides a streamlined protocol for performing genotyping from any sample with a single kit. It is fast, simple, and robust. In addition, TaqMan® GTXpress™ Master Mix offers flexibility and speed for fast genotyping whether the starting material is raw biological sample or purified DNA. The TaqMan® Sample-to-SNP™ Kit makes SNP genotyping easy.

Table 2. Call rates and concordance between purified DNA and DNA lysate. Experiments were done as described in Figure 5 using 200 predesigned TaqMan® SNP Genotyping Assays, 100 TaqMan® Drug Metabolism Genotyping Assays, and 100 Custom TaqMan® SNP Genotyping Assays.

Assay type	Purified DNA call rate (%)	DNA lysate call rate (%)	Concordance (%)
Predesigned TaqMan® Genotyping Assays	100.00	100.00	99.8%
TaqMan® Drug Metabolism Genotyping Assays	100.00	100.00	99.7%
Custom TaqMan® SNP Genotyping Assays	100.00	100.00	99.9%

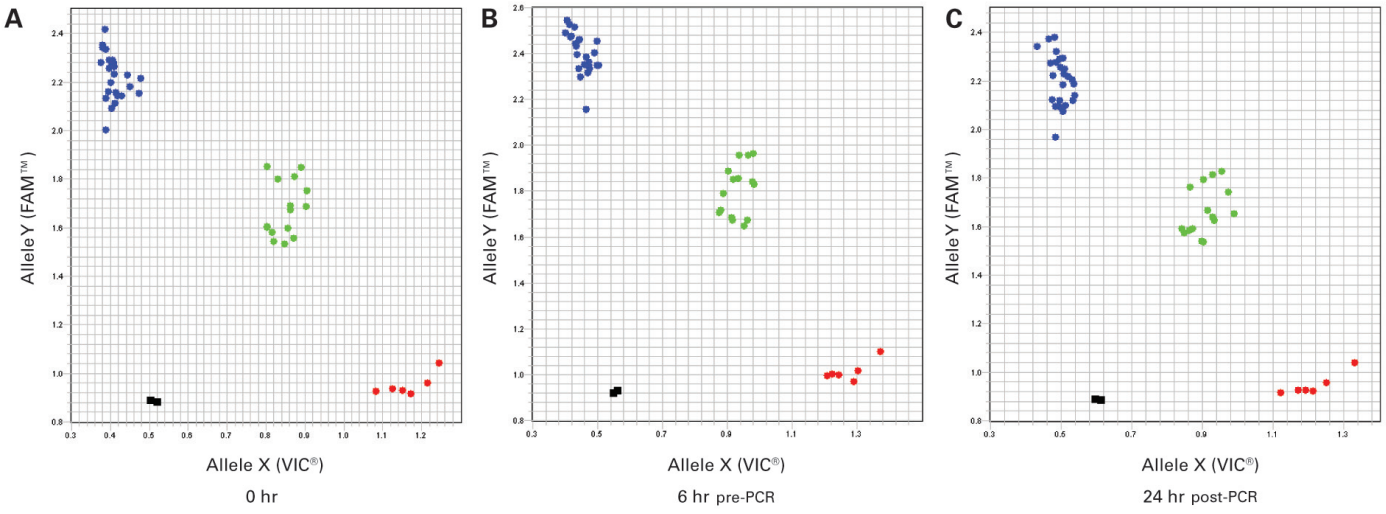

Figure 6. Stability of PCR reactions before and after PCR. Blood lysates extracted with the DNA Extract All Lysis Reagents were amplified with TaqMan® GTXpress™ Master Mix. **(A)** The PCR reactions were assembled and run immediately. **(B)** The assembled reactions were stored at room temperature for 6 hours before cycling on a PCR instrument. **(C)** The reactions were run and the PCR products were stored at room temperature for 24 hours prior to performing the allelic discrimination analysis.

TaqMan® Sample-to-SNP™ Kit

Ordering information

Product	Quantity	Cat. No.
TaqMan® Sample-to-SNP™ Kit		
5 mL sample prep, 1 mL PCR	100 rxns sample prep, 400 rxns PCR*	4403313
20 mL sample prep, 10 mL PCR	400 rxns sample prep, 4,000 rxns PCR*	4403083
20 mL sample prep, 50 mL PCR	400 rxns sample prep, 20,000 rxns PCR*	4403087
200 mL sample prep, 10 mL PCR	4,000 rxns sample prep, 4,000 rxns PCR*	4403081
Quick Reference Card	1 card	4402745
Protocol	1 protocol	4402136
TaqMan® GTXpress™ Master Mix		
1 mL	400 rxns PCR	4403311
10 mL	4,000 rxns PCR	4401892
50 mL	20,000 rxns PCR	4401890
100 mL (2 x 50 mL)	40,000 rxns PCR	4401857
Protocol	1 protocol	4402746
Related products		
TaqMan® PreAmp Master Mix	40 rxns	4391128
Foam Buccal Swabs	100 swabs	4402088

*Based on 50 µL sample prep reaction and 5 µL PCR reaction. For other configurations, please contact your sales representative.

Please note: DNA Extract All Lysis Reagents are not available for individual sale. They are only available as a part of the TaqMan® Sample-to-SNP™ Kit.

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