TaqMan® Sample-to-SNP™ Kit

Protocol

Applied Biosystems

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Preface

This preface covers:

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Safety information

Note: For general safety information, see this Preface and Appendix C, Safety on page 35. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" Appendix for the complete alert on the chemical or instrument.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see "Obtaining MSDSs" on page 38.

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.

How to use this guide

Purpose of this guide

The *Applied Biosystems* TaqMan® Sample-to-SNP™ Kit Protocol provides all the information you need to perform fast DNA extraction on biological samples then fast genotyping with the resulting sample lysates.

Audience

This guide is intended for users who have had some experience performing PCR.

Assumptions

This guide assumes that your real-time PCR system and/or your thermal cycler has been installed by an Applied Biosystems technical representative and that the real-time PCR system is capable of running allelic discrimination software.

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:
  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:
  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:
  Select **File ▸ Open ▸ Spot Set**.
  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User attention words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

TaqMan® Sample-to-SNP™ Kit Protocol

This chapter covers:

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Product information

Purpose of the product

TaqMan® Sample-to-SNP™ Kit streamlines genotyping, significantly reducing the time typically required to process samples and run standard genotyping protocols. You use the TaqMan® Sample-to-SNP™ Kit (excluding primers, probes, template, and water) to conveniently release DNA from samples such as tissues and cells before you genotype for single nucleotide polymorphisms (SNPs). With the TaqMan® Sample-to-SNP™ Kit, there is no need to quantitate the DNA for fast genotyping.

The TaqMan® Sample-to-SNP™ Kit can be used with unprocessed biological sample of your choice and a TaqMan® genotyping assay, including:

–TaqMan® SNP Genotyping Assays

–Custom TaqMan® SNP Genotyping Assays

–TaqMan® Drug Metabolism Genotyping Assays

–TaqMan® Pre-Designed Assay Reagents for Allelic Discrimination

Compatible instruments

You can perform PCR amplification and plate read analysis for any TaqMan genotyping assay using any of the following systems:

- Applied Biosystems 7300/7500 Real-Time PCR Systems
- Applied Biosystems 7500 Fast/7900HT Fast Real-Time PCR Systems (fast or standard)
- StepOne™ and StepOne Plus™ thermal cyclers (fast or standard)

You can perform PCR amplification without plate-read analysis using the:

- GeneAmp® PCR System 9700 Thermal Cycler or
- Applied Biosystems Veriti™ Thermal Cycler

After PCR amplification with a stand-alone thermal cycler, you can use any Applied Biosystems real-time PCR system that supports the plate format that you use for allelic discrimination.

Available kit and master mix packaging

The TaqMan® Sample-to-SNP™ Kit is supplied by Applied Biosystems in the packaging sizes described below. The Kit contains DNA Extract All, which is used to extract DNA from biological samples, and TaqMan® GTXpress™ Master Mix, which is used for fast-genotyping reactions.

Contents	Part Number
TaqMan® Sample-to-SNP™ Kit: • DNA Extract All Reagents Kit (5 mL) • TaqMan® GTXpress™ Master Mix (1 mL)	4403313
TaqMan® Sample-to-SNP™ Kit: • DNA Extract All Reagents Kit (200 mL) • TaqMan® GTXpress™ Master Mix (10 mL)	4403081
TaqMan® Sample-to-SNP™ Kit: • DNA Extract All Reagents Kit (20 mL) • TaqMan® GTXpress™ Master Mix (10 mL)	4403083
TaqMan® Sample-to-SNP™ Kit: • DNA Extract All Reagents Kit (200 mL) • TaqMan® GTXpress™ Master Mix (250 mL)	4403085
TaqMan® Sample-to-SNP™ Kit: • DNA Extract All Reagents Kit (20 mL) • TaqMan® GTXpress™ Master Mix (50 mL)	4403087

The TaqMan® GTXpress™ Master Mix may be purchased separately:

Contents‡	Part Number
TaqMan® GTXpress™ Master Mix, 1 mL (400 reactions)	4403311
TaqMan® GTXpress™ Master Mix, 10 mL (4000 reactions)	4401892
TaqMan® GTXpress™ Master Mix, 50 mL (20,000 reactions)	4401890
TaqMan® GTXpress™ Master Mix, 50 mL × 2 (20,000 × 2 reactions)	4401857
TaqMan® GTXpress™ Master Mix, 250 mL (100,000 reactions)	4401888

‡ Based on a 5-µL reaction size.

The TaqMan® PreAmp Master Mix may be purchased separately:

Contents‡	Part Number
TaqMan® PreAmp Master Mix	4391128

‡ For 40 reactions of 50-µL reaction size.

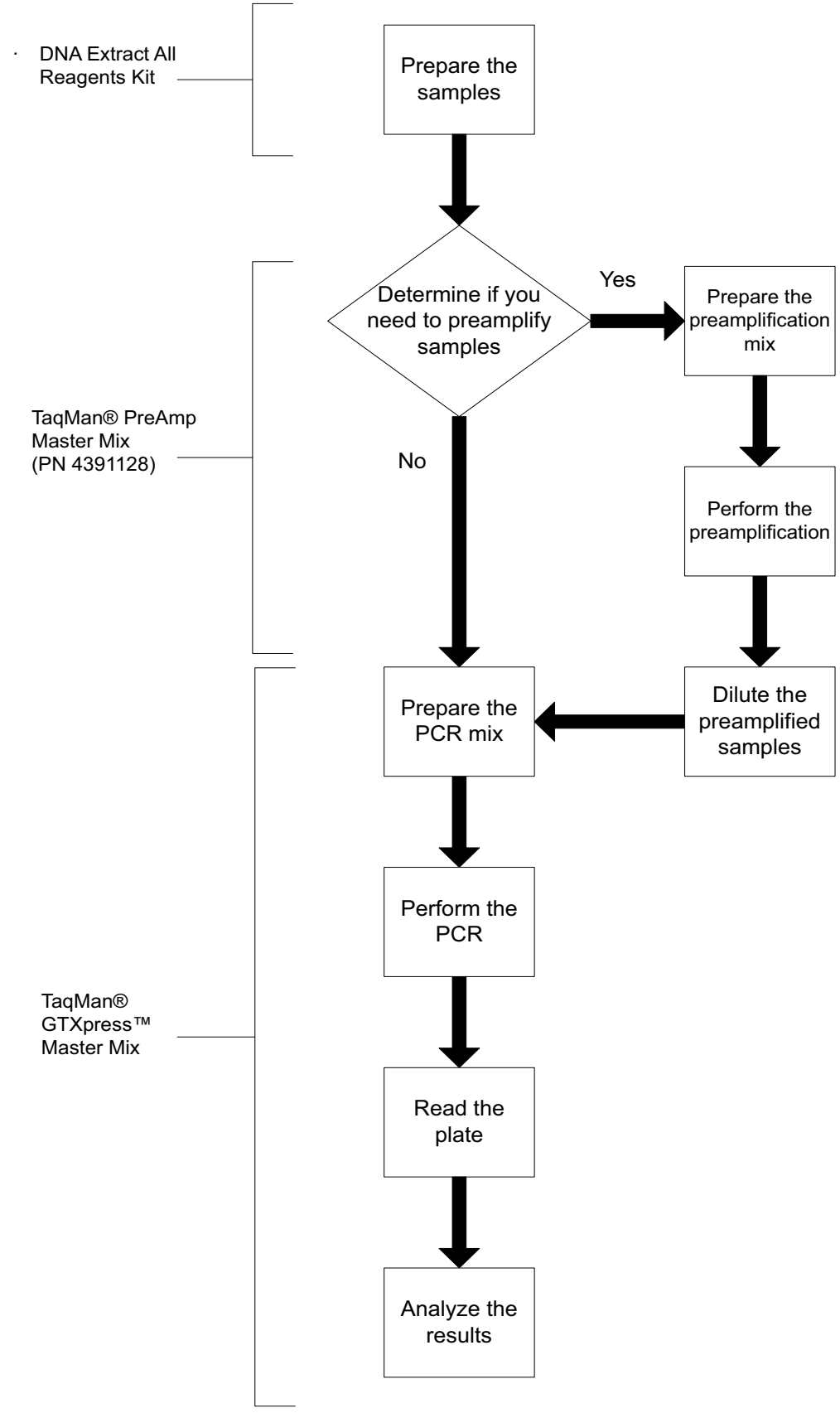
Storage

Store the TaqMan® Sample-to-SNP™ Kit at 2 to 8 °C. The Kit is stable through the date on the package and bottle label when stored at 2 to 8 °C. Applied Biosystems does not recommend using TaqMan® Sample-to-SNP™ Kit after the date printed on the package and bottle label.

For more information

To learn from customers who use the TaqMan® Sample-to-SNP™ Kit and the TaqMan® GTXpress™ Master Mix for sample preparation and fast genotyping, go to: **www.appliedbiosystems.com/sampletosnp**.

Workflow


DNA Extract All Reagents Kit
Prepare the samples
Determine if you need to preamplify samples
Yes
Prepare the preamplification mix
TaqMan® PreAmp Master Mix (PN 4391128)
No
Perform the preamplification
Prepare the PCR mix
Dilute the preamplified samples
Perform the PCR
TaqMan® GTXpress™ Master Mix
Read the plate
Analyze the results

Prepare the samples

For the following hazards, see the complete safety alert descriptions in "Chemical alerts" on page 41:

⚠ **DANGER! CHEMICAL HAZARD. Lysis Solution.**

⚠ **WARNING! CHEMICAL HAZARD. DNA Stabilizing Solution.**

IMPORTANT! For larger samples, Lysis Solution and DNA Stabilizing Solution volumes can be scaled up.

Lyse the samples

1. Obtain the samples for lysis according to Table 1 on page 7.
2. Thoroughly mix the Lysis Solution.
3. Add one volume of Lysis Solution to each 1.5-mL microcentrifuge tube or well of the plate that contains the sample. Refer to Table 1 on page 7 for volumes based on sample type and sample quantity.
4. Pipette up and down to mix the Lysis Solution and the sample in the tube or well on the plate.
5. Seal the plate with an adhesive cover, or cap the tubes, then centrifuge the plate or tubes briefly.

Incubate the samples

Incubate the samples according to sample type as shown in Table 1 on page 7. For samples incubated at 95 °C, cool at room temperature for 30 seconds before stabilizing the DNA.

Stabilize the DNA

1. Thoroughly mix the DNA Stabilizing Solution.
2. Open the tube or uncover the plate.
3. Add one volume of DNA Stabilizing Solution to each tube or well of the plate that contains sample. See Table 1 on page 7 for volumes based on sample type.
4. Pipette up and down to mix the solutions on the plate or in the tube.
5. Seal the plate with an adhesive cover, or cap the tubes, then centrifuge the plate or tubes briefly.

(Optional) Store the sample lysates

You can store the sample lysate at 4 °C. For longer storage, you can store the sample lysate at –20 °C. Before use, mix the sample lysate.

Table 1 Preparation of sample lysate according to sample type

Sample type	Sample input	Volume of Lysis Solution (µL)	Incubation Temperature for 3 minutes (°C)	Volume of DNA Stabilizing Solution (µL)	Notes
Blood (freshly drawn, EDTA, citrate, heparin)	2 µL	20	Room temperature	20	—
Blood, cells, saliva (blood cards, FTA paper)	3-mm punch	50	95	50	—
Cell culture suspension	2 µL	20	Room temperature	20	—
Buccal swab	1	400	95	400	1. Twist the swab from the cap. 2. Rotate and firmly brush the swab using 20 strokes throughout the inside cheek.‡ 3. Use a 1.5-mL screw-capped tube and immerse the swab into the Lysis Solution. 4. Rotate the swab 5 times. 5. Lift the swab above the Lysis Solution, then press the swab against the side of the tube to squeeze out its contents. 6. Dispose of the swab. 7. Continue preparing the sample (see "Incubate the samples" on page 6).
Rat or mouse tail	1 to 2 mm	50	95	50	—
Tissue	1 to 2 mm	50	95	50	—
Hair with follicle	2 to 3 follicles	50	95	50	Ensure that the hair and follicles are immersed in Lysis Solution.
Leaf punch or needle	3-mm leaf punch or 2- to 3-mm needle	50	95	50	—
Seed chip	2- to 3-mm seed chip or 2 to 5 mg pulverized seed	50	95	50	—
Formalin-fixed paraffin-embedded tissue (FFPE)	2 to 3 pieces of a 10-µm section	200	95	200	• Before the lysis step, you can deparafinize the FFPE tissue using a standard protocol. • Ensure that the FFPE is immersed in Lysis Solution.

‡ The swab may be air-dried, re-capped, then stored at room temperature.

Preamplify the samples or perform fast genotyping

If you:

- Need to preamplify the samples, go to "Preamplify the samples" on page 9.
- Do *not* need to preamplify the samples, go to "Before you perform fast genotyping" on page 10.
- Need to decide on preamplification, go to "Determine if you need to preamplify samples" on page 9.

Determine if you need to preamplify samples

If the amount of sample is limited, preamplification may be necessary. Applied Biosystems recommends a test study without preamplification to determine if the fluorescence signal is sufficient for good allelic discrimination.

If you:

- Need to preamplify the samples, go to "Preamplify the samples" on page 9.
- Do *not* need to preamplify the samples, go to "Before you perform fast genotyping" on page 10.

Preamplify the samples

Prepare the preamplification mix

1. Thoroughly mix the TaqMan® PreAmp Master Mix (PN 4391128) by swirling the bottle.
2. Thaw any frozen TaqMan assay reagents by placing them on ice. Vortex then centrifuge the tubes briefly.
3. Combine then dilute all 20× TaqMan® SNP Genotyping assays of interest to a final concentration of 0.2×:
   a. Combine equal volumes of 20× TaqMan® SNP Genotyping assays of up to 100 assays. If you choose to aliquot 10 µL from each assay (you can choose another volume, and you choose 50 assays, then), the total volume of the combined assays is 500 µL, and the concentration of the combined assays is 0.4× (10 µL × 20×/500 µL = 0.4×).
   b. Dilute the combined assays in 1× TE buffer to a final concentration of 0.2×. For example, add 500 µL of 1× TE buffer to 500 µL of the combined assays at 0.4× concentration. The final volume of the diluted combined assays is 1 mL and the final concentration is 0.2×.
4. For each sample, combine in a PCR tube the components as shown in Table 2. Multiply the volume for one reaction component (Table 2) by the total number of reactions, then add that volume to the tube.

Table 2 Preamplification of sample

Component for preamplification	Volume for one 10-µL reaction (µL)	Volume for one 50-µL reaction (µL)
TaqMan® PreAmp Master Mix, 2×	5	25
0.2× Pooled assay mix	2.5	12.5
Sample lysate	1.2	6
DNase-free water	1.3	6.5
Total	10	50

Set up the run method

Set up the run method using the following conditions:

- Thermal-cycling conditions:

Stage	Step	Temp	Time
Holding	DNA polymerase activation	95 °C	10 min
Cycling (14 cycles)	Denature	95 °C	15 sec
	Anneal/Extend	60 °C	4 min

- Run speed: **9600 emulation** or **standard**
- Reaction volume: **10** µL or **50** µL

Load and run the plate

Load the reaction plate into the thermal cycler, then start the run.

Dilute the preamplified samples

After the run, dilute the preamplified products 1 to 20 in 1× TE Buffer.

(Optional) Stopping point

For long-term storage, store the preamplified sample at –20 °C. When you are ready to perform fast genotyping, go to "Before you perform fast genotyping".

Before you perform fast genotyping

Prevent contamination

Review "PCR good laboratory practices" on page 34.

Select an instrument and reaction plate

IMPORTANT! You can use TaqMan® GTXpress™ Master Mix with *Fast* or *Standard* mode thermal cycling conditions.

You can perform PCR amplification with any of the instruments and compatible plates listed in Appendix A on page 27. Alternatively, if you use a thermal cycler for PCR amplification, you must subsequently perform the endpoint plate read separately on a real-time PCR system.

Determine the number of required reactions

Determine the number of reactions to perform for each assay. Include extra reactions (approximately one extra reaction for every 10 required reactions) to compensate for the volume loss that occurs during reagent transfers. For example, for a 96-well plate, prepare enough volume of each PCR component for approximately 110 reactions. Include at least two no-template controls (NTCs) and (if needed) at least one genomic DNA control of known genotype on each plate to ensure accurate genotype calling.

IMPORTANT! You can run multiple genotyping assays on one reaction plate. Include controls for each assay that you run on a plate.

Perform fast genotyping

For the following hazards, see the complete safety alert descriptions in "Chemical alerts" on page 41:

⚠WARNING! CHEMICAL HAZARD. TaqMan® GTXpress™ Master Mix.

The first step in a genotyping assay is PCR amplification, which requires you to:

- Prepare the PCR mix (page 11)
- Perform the PCR (page 13)
- Read the plate (page 14)
- Analyze the results (page 14)

Prepare the PCR mix

IMPORTANT! Keep all TaqMan reagents protected from light until you are ready to use them. Excessive exposure to light may affect the fluorescent probes. Minimize freeze-thaw cycles. Prepare the PCR reaction mix for each assay before transferring it to the optical reaction plate for thermal cycling and fluorescence analysis.

Note: The TaqMan® GTXpress™ Master Mix contains a purple tracking dye that allows you to see if the plate wells are filled uniformly.

1. Thoroughly mix the TaqMan® GTXpress™ Master Mix by swirling the bottle. Avoid creating bubbles.
2. Thaw any frozen TaqMan assay reagents by placing them on ice. Vortex then centrifuge the tubes briefly.
3. Thaw any frozen genomic DNA or sample lysates by placing them on ice. After the samples thaw, mix them if needed by vortexing, then centrifuge the tubes briefly.
4. In an appropriate tube, combine the reaction mix components shown in Table 3 on page 12:
    a. Determine the reaction volume appropriate to the instrument and plate (see Table 4 on page 12).

b. Multiply the volume for one reaction component (see Table 2 on page 9) by the total number of reactions,.

c. Add the volume calculated from step 4b for each component to the tube.

Table 3 PCR reaction mix volume (µL/well)

Component	Volume for 5-µL PCR reaction	Volume for 10-µL PCR reaction	Volume for 25-µL PCR reaction
TaqMan® GTXpress™ Master Mix (2X)	2.50	5.0	12.50
TaqMan genotyping assay mix (20X)‡ §	0.25	0.5	1.25
DNase-free water	1.25	2.5	6.25
Total	4.0	8.0	20.0

‡ For ease of use, dilute 40X and 80X Assay Mixes to 20X working solutions with 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Use DNase-free water.

§ If you use Custom TaqMan Probes and Sequence Detection Primers rather than a TaqMan genotyping assay, Applied Biosystems recommends 900 nM for primers and 200 nM for probes.

Table 4 Recommended volumes according to instrument

Applied Biosystems instruments	Plate well volume	Reaction volume per well (µL)
7900HT Real-Time PCR System (384 block)	384 wells, 0.02 mL	5 to 20
• Applied Biosystems 7300/7500 Real-Time PCR Systems • 7900HT Real-Time PCR System	96 wells, 0.2 mL	20 to 50
• 7500 Fast Real-Time PCR System • 7900HT Fast Real-Time PCR System	96 wells, 0.1 mL	10 to 30
StepOne™	48 wells, 0.1 mL	10 to 30

5. Cap the tube(s).
6. Vortex the tube(s) briefly to mix the solutions.
7. Centrifuge the tube(s) briefly to spin down the contents and to eliminate air bubbles from the solution.
8. Into each well of a reaction plate, pipette the PCR reaction mix volume (4, 8, or 20 µL) appropriate to your plate.
9. Observe the purple tracking dye in each well to ensure uniform filling.
10. Seal the plate with a MicroAmp™ clear adhesive film.

11. Centrifuge the plate briefly to spin down the contents and eliminate any air bubbles.
12. Remove the clear adhesive film from the plate, then pipette one control or diluted DNA sample into the appropriate well(s).
13. Add sample lysate, diluted preamplification product, or DNA control to each well according to the volume of the PCR reaction:

Volume of sample lysate, diluted preamplification product, or DNA control (µL/PCR reaction)		
5-µL reaction	**10-µL reaction**	**25-µL reaction**
1.0	2.0	5.0

14. Cover the plate with MicroAmp™ Optical Adhesive Film or MicroAmp™ Optical Caps.
15. Centrifuge the plate briefly to spin down the contents and eliminate air bubbles.
16. Use a MicroAmp™ Optical Film Compression Pad when you use a MicroAmp Optical Adhesive Film. Ensure that the gray, nonreflective side of the pad faces down on the plate. Also use a compression pad with a MicroAmp™ Optical 96-well plate on the 7900HT Real-Time PCR System.

Perform the PCR

1. Set up the following run conditions:

IMPORTANT! These conditions are optimized for use only with TaqMan® genotyping assays on the PCR systems specified in the table below and with the instruments and reaction plates specified in Appendix A on page 27.

Stage	Step	Temp	Time (StepOne™, StepOne Plus™,7900)	Time (Fast 7500)	Time (7300, 7500)
Holding	DNA polymerase activation	95 °C	20 sec	20 sec	20 sec
Cycling (40 cycles)	Denature	95 °C	3 sec	3 sec	15 sec
	Anneal/Extend	60 °C	20 sec‡	30 sec	60 sec

‡ Use the minimum extension time available on your instrument but no less than 20 seconds.

- Run speed: **Fast** or **Standard**
- Reaction volume: **5**, **10**, or **25** µL

2. Load the reaction plate into the thermal cycler, then start the run.

Read the plate

After PCR amplification, you perform an endpoint plate read on a real-time PCR instrument.

IMPORTANT! For all real-time PCR instruments, regardless of default temperature, use a post-read temperature of 25 °C when using the TaqMan® GTXpress™ Master Mix.

The SDS software uses the fluorescence measurements from each well made during the plate read, then plots R_n (signal) values. The software determines which alleles are in each sample for later allelic discrimination analysis. Refer to the allelic discrimination section of the appropriate instrument user guide for instructions on how to use the system software to perform the plate read and analysis.

Analyze the results

The SDS software records the results of the allelic discrimination run on a scatter plot of Allele 1 versus Allele 2. Each well of the 96-well or 384-well reaction plate is represented as an individual point on the plot (for example, see Figure 1).

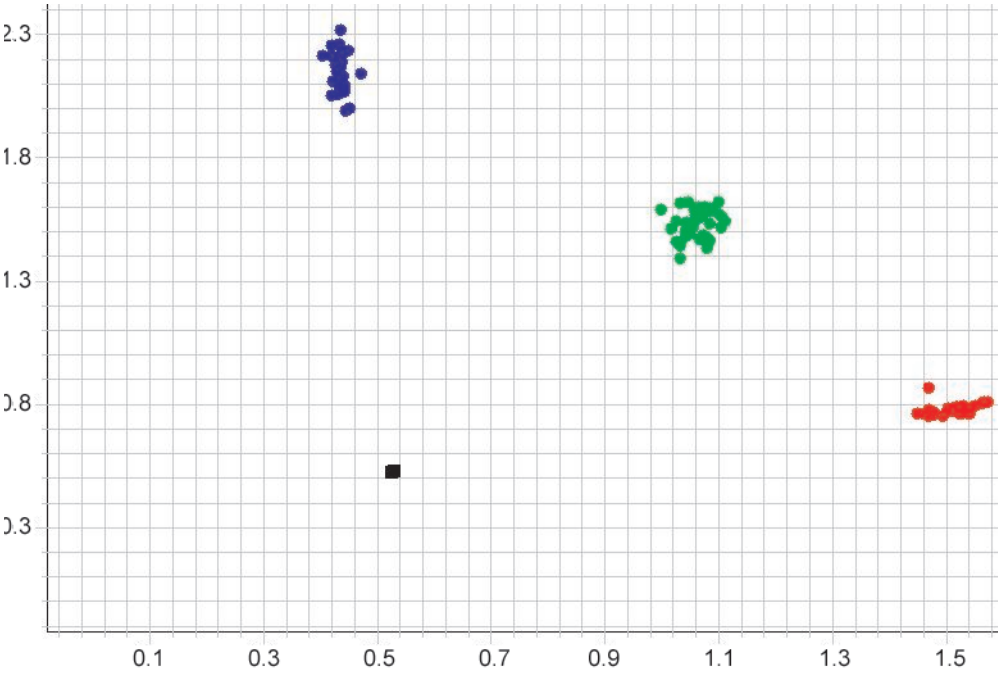


Figure 1 The clusters in the allelic discrimination plot show the three genotypes of one SNP.

(Optional) Repeat fast genotyping

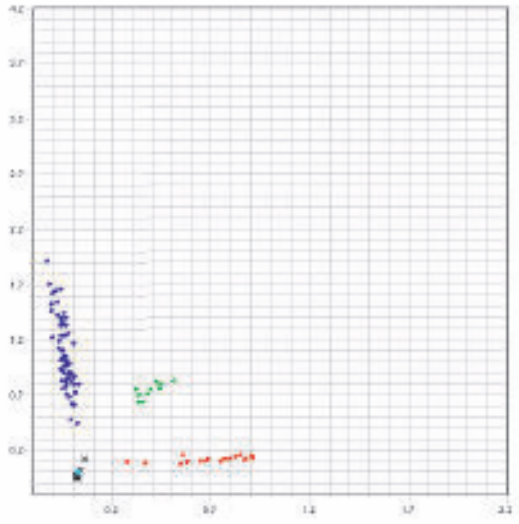
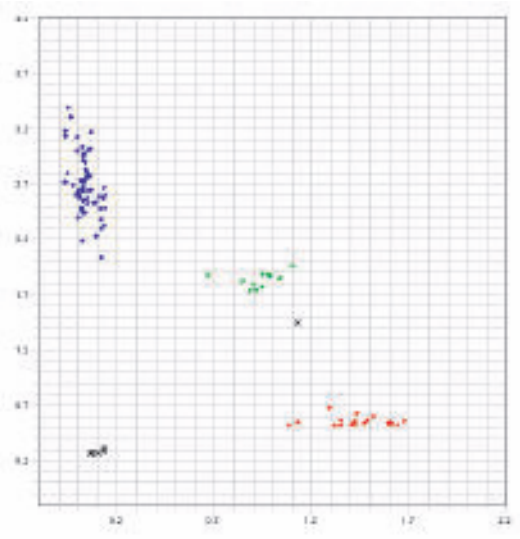
1. If allelic discrimination is not possible because of low fluorescence, return the plate to the thermal cycler, then perform another 10 PCR cycles using the following thermal-cycling conditions change according to run conditions in previous table:

Stage	Step	Temp	Time (StepOne™, StepOne Plus™,7900)	Time (Fast 7500)	Time (7300, 7500)
Cycling 10 cycles	Denature	95 °C	3 sec	3 sec	15 sec
	Anneal/Extend	60 °C	20 sec‡	30 sec	60 sec

‡ Use the minimum extension time available on your instrument but no less than 20 seconds.

2. Perform allelic discrimination analysis again to see if the results improve. For optimal results, never exceed a total of 50 cycles (see Table 5).

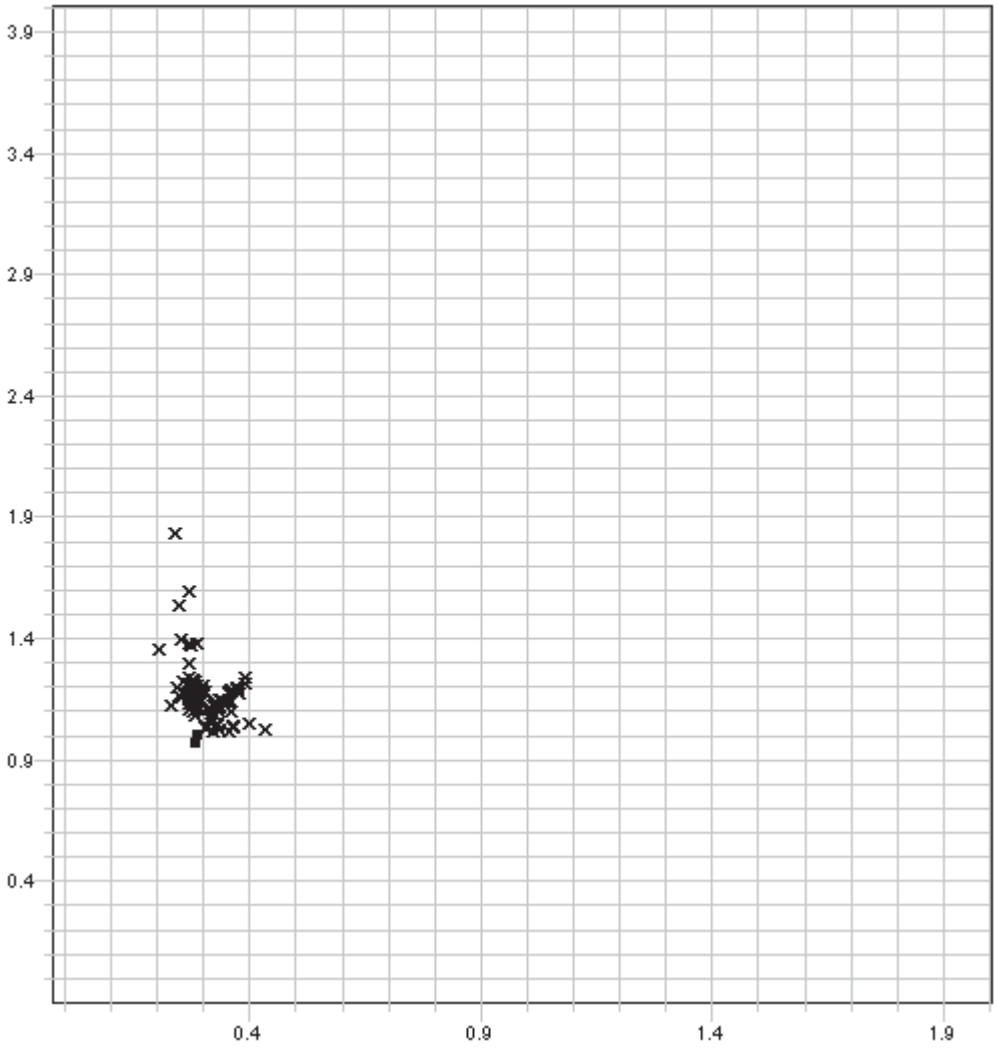
Table 5 Optimizing allelic discrimination within a limited number of cycles

	
40 Cycles	**40+10 Cycles**

Troubleshooting

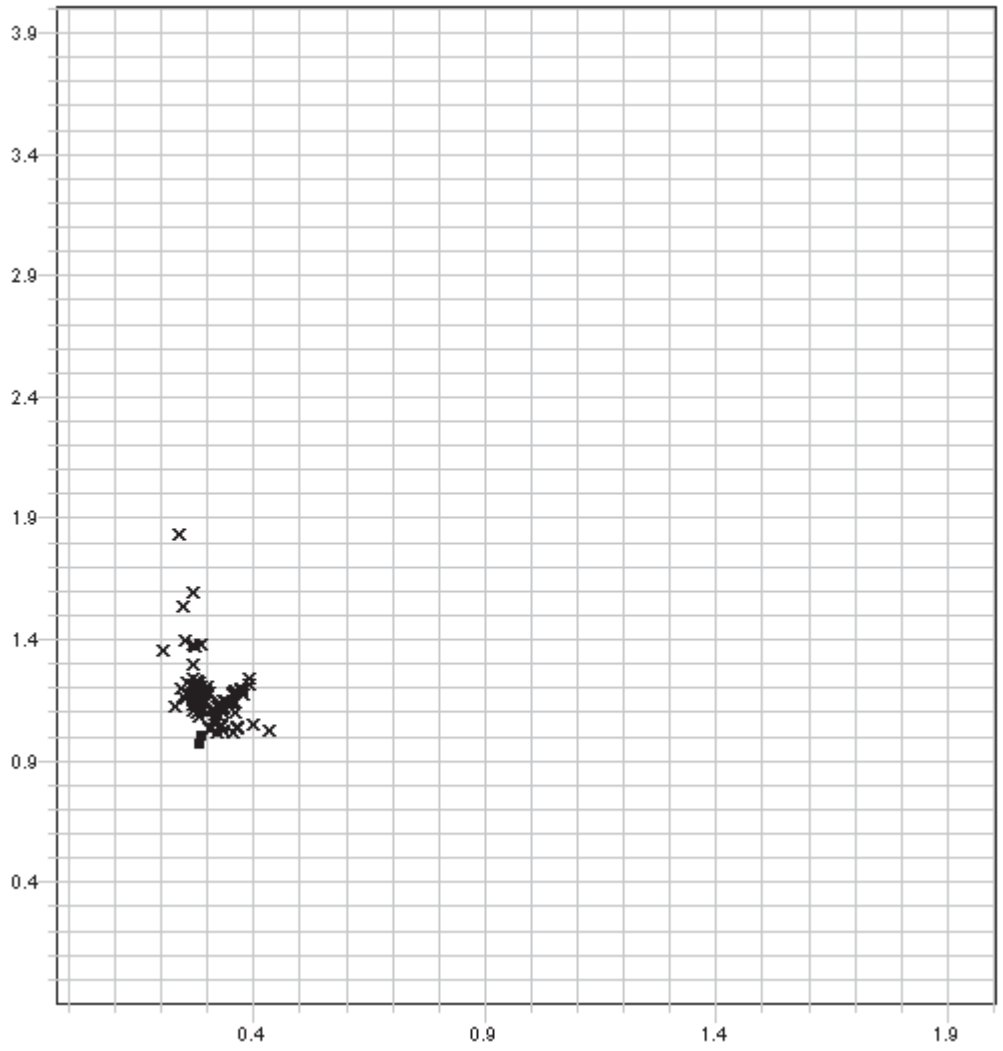
Match your allelic discrimination plot with one of the observations below. Find the "Possible cause," then follow the "Recommendation."

Observation 1: No or low amplification



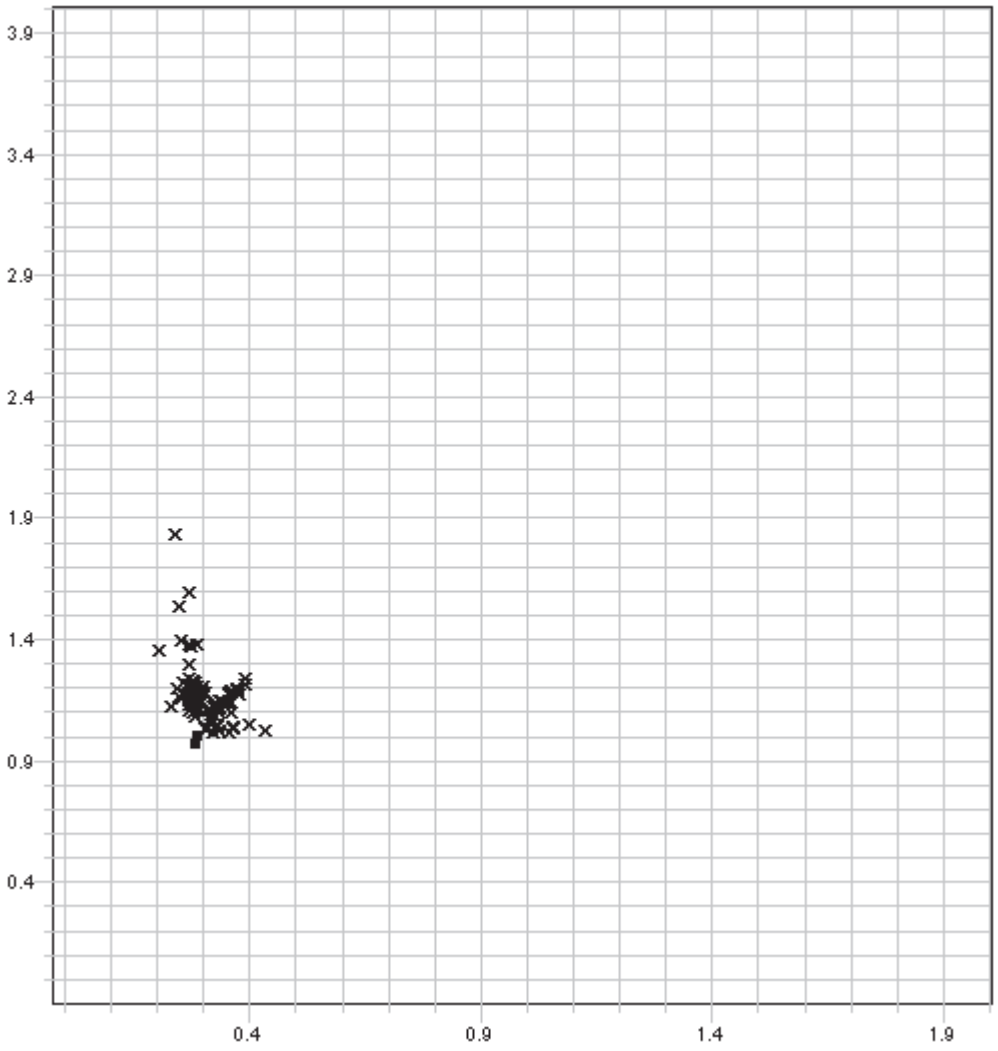
Possible cause	Recommendation
Samples	
Sample degradation	Run an agarose gel to verify that DNA is degraded.
Incorrect DNA quantitation (genomic only)	Perform concentration measurements.
PCR inhibitors	Dilute the DNA sample.
Too much or too little starting material	Titrate sample input for the DNA extraction step.

Observation 1: No or low amplification



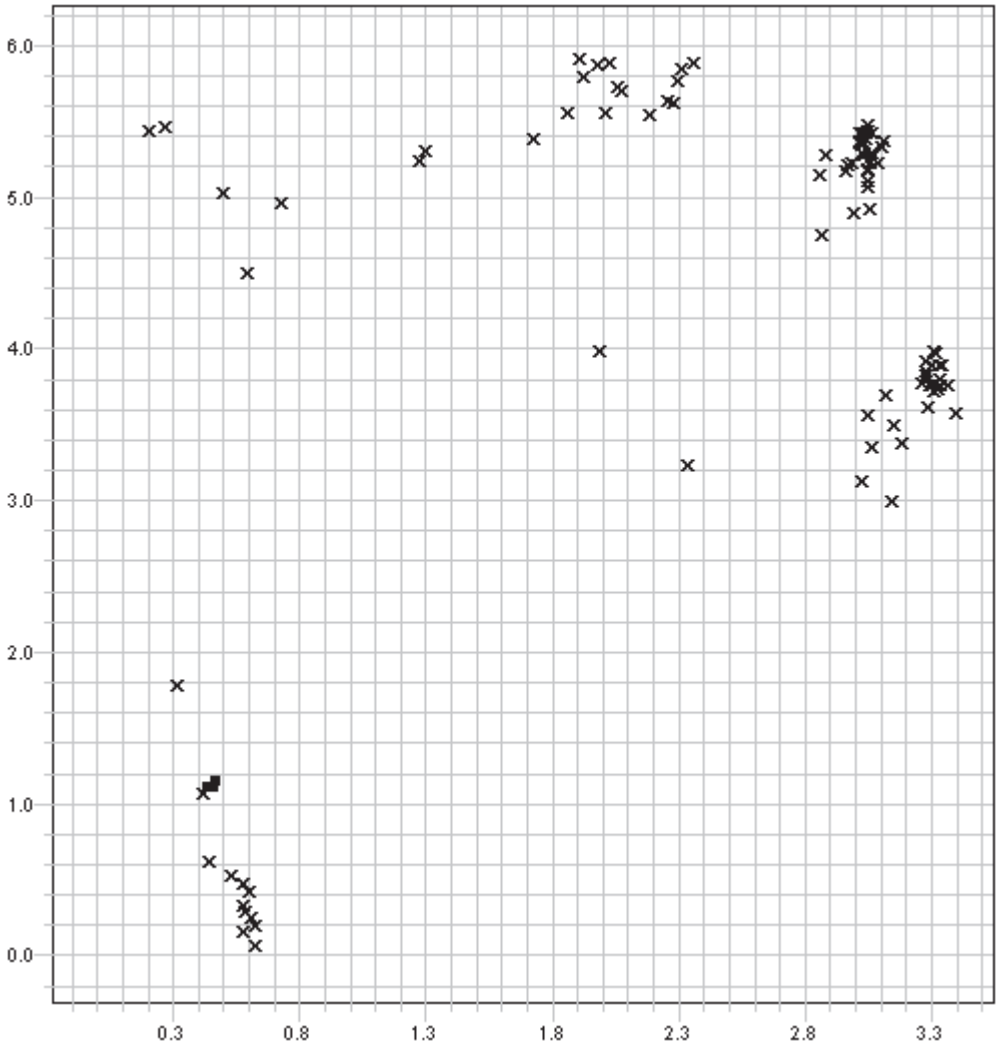
Possible cause	Recommendation
Samples (continued)	
Too little DNA was used for PCR	Perform another 10 PCR cycles, increase the DNA input for PCR, or perform preamplification reactions.
Reagents	
Reagents expired or mishandled	Perform the assay again with newly prepared reagents. Ensure that storage conditions are correct.
Reagents not added to a well	Visually inspect the well.
Evaporation	Ensure that the reaction plate is sealed properly. Use a compression pad if recommended.
Bubbles in the wells	Ensure that the reaction plate is spun down before thermal cycling.

Observation 1: No or low amplification



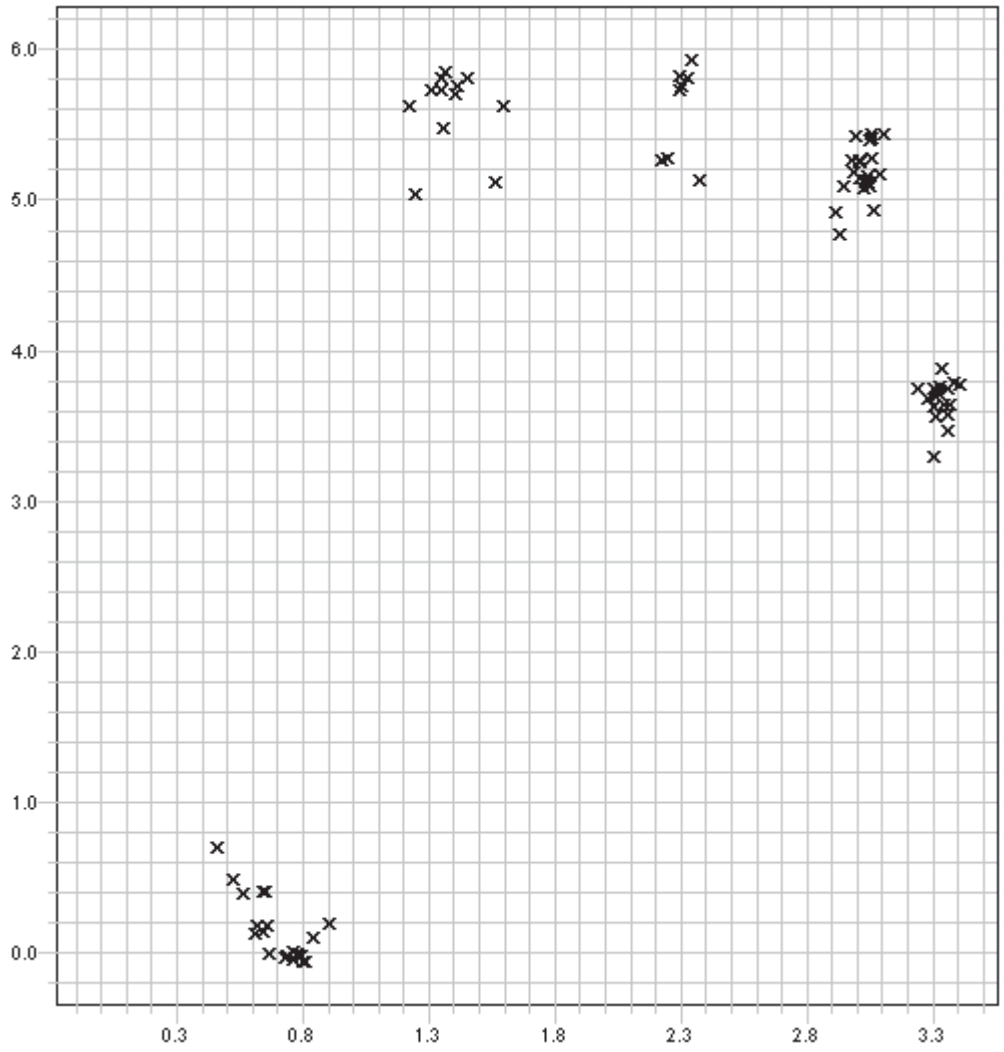
Possible cause	Recommendation
Reagents (continued)	
SNP is embedded in primer designs	Perform BLAST to verify that no SNP is in the primer region. If necessary, redesign the primer to avoid the SNP region.
Instrument	
Wrong reporter dyes were chosen	Verify the dye settings and reanalyze the plate read
Thermal cycler is poorly calibrated	Check thermal-cycling conditions and make sure the thermal cycler is correctly calibrated

Observation 2: No clusters



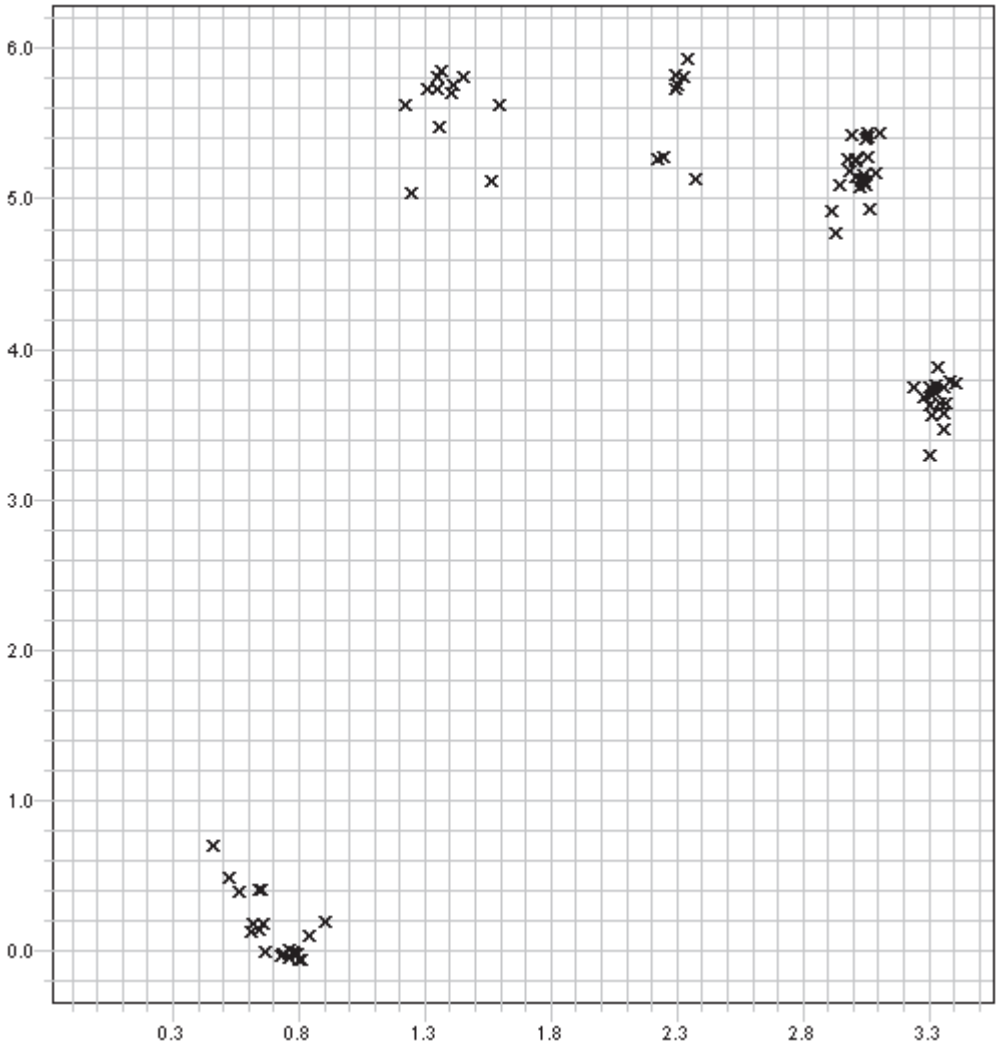
Possible cause	Recommendation
Samples	
PCR inhibitors	Dilute the DNA sample
Too little DNA used for PCR	Perform another 10 PCR cycles, increase the DNA input for PCR, or perform preamplification reactions.
Instrument	
Wrong reporter dyes chosen	Verify the dye settings and reanalyze the plate read.
ROX™ dye is not selected	Ensure that the proper passive reference is selected.

Observation 3: Too many clusters



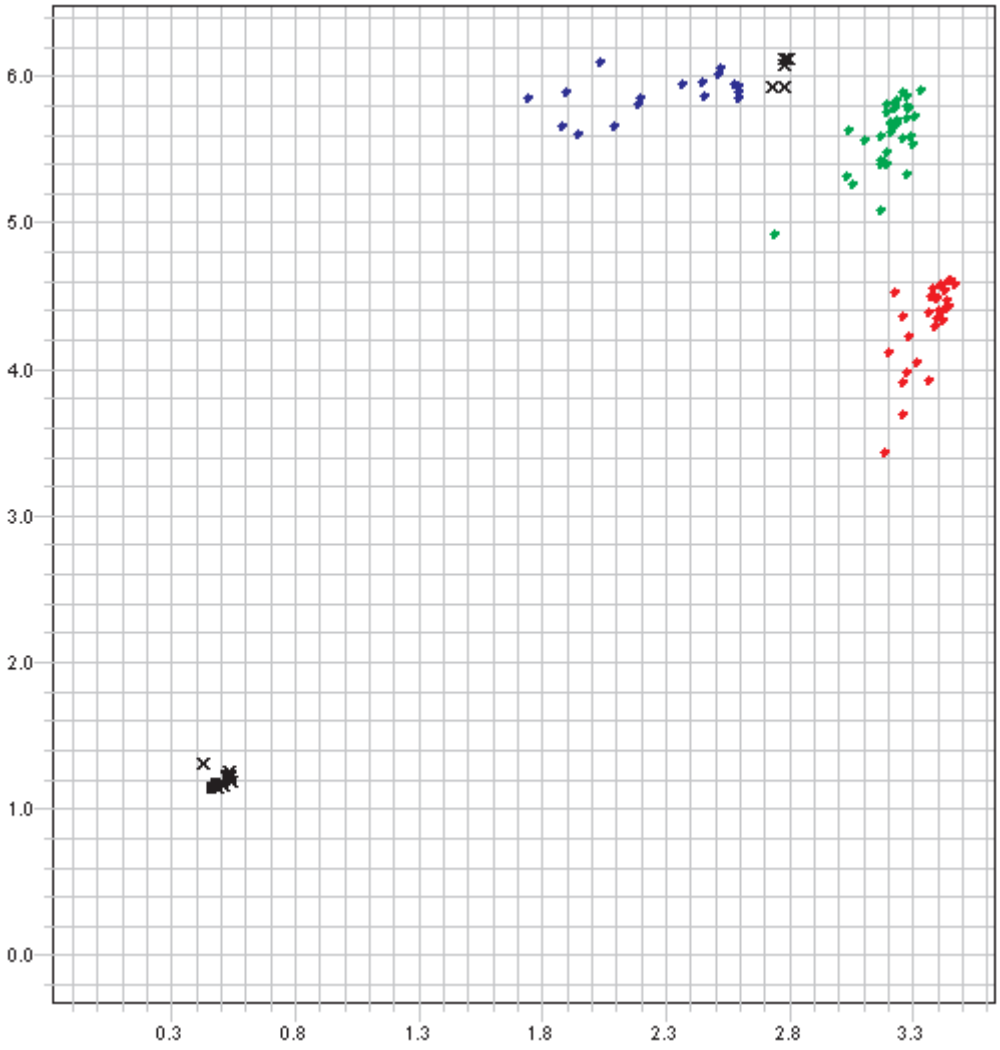
Possible cause	Recommendation
Genetics	
The probe sequence may contain a second SNP	Check the SNP database to see if an additional SNP has been discovered.
Copy number: There are more than two copies of the SNP.	Perform a copy number assay to determine the copy number. Perform comparative sequencing.
SNP is multi-allelic.	Perform comparative sequencing to verify the presence of more than two alleles. Repeat the experiment to verify that the performance is consistent.

Observation 3: Too many clusters



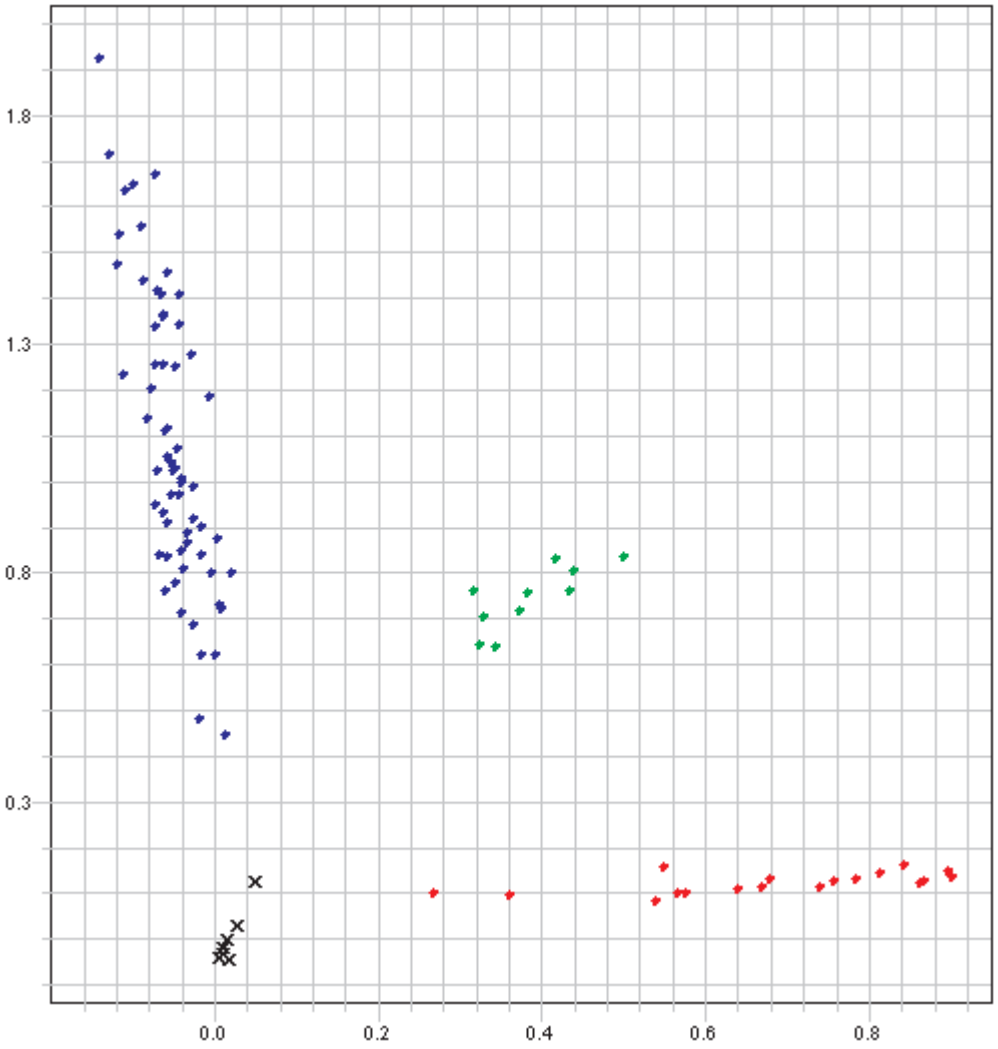
Possible cause	Recommendation
Samples	
Sample contamination	Check the performance of the samples in other assays to rule out problems caused by contamination or degradation.
Instrument	
One marker is assigned to multiple assays	Ensure that you use only one marker per assay.

Observation 4: Clusters too close



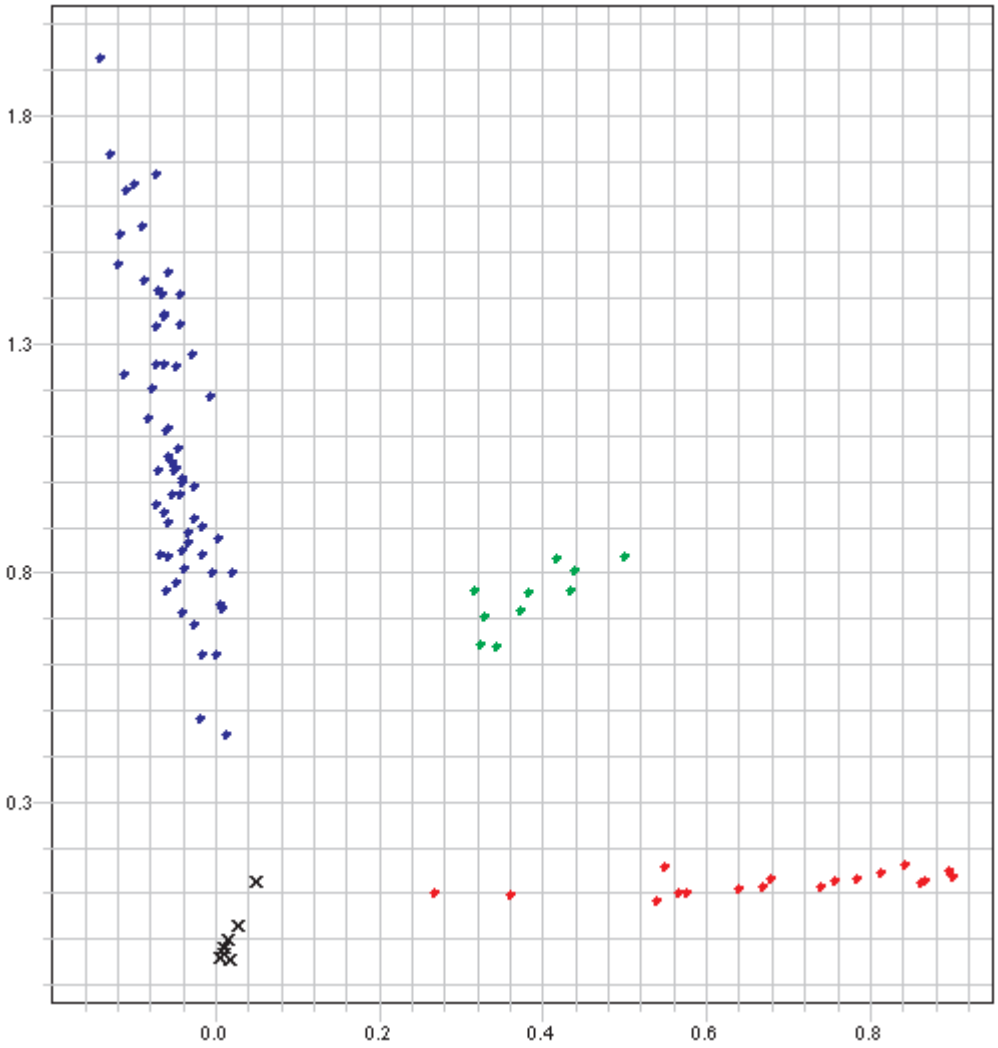
Possible cause	Recommendation
Samples	
Sample degradation	Run an agarose gel to verify if DNA is degraded.
Reagents	
Probe degradation	Perform the assay again with newly prepared reagents. Ensure that the reagents are stored correctly.
Assay design	Verify that the probe designs are within good T_m range.
Instrument	
Too many cycles run	If the reaction has been thermocycled for more than 40 cycles, rerun the PCR with fewer cycles.

Observation 5: "Chicken-feet" clusters



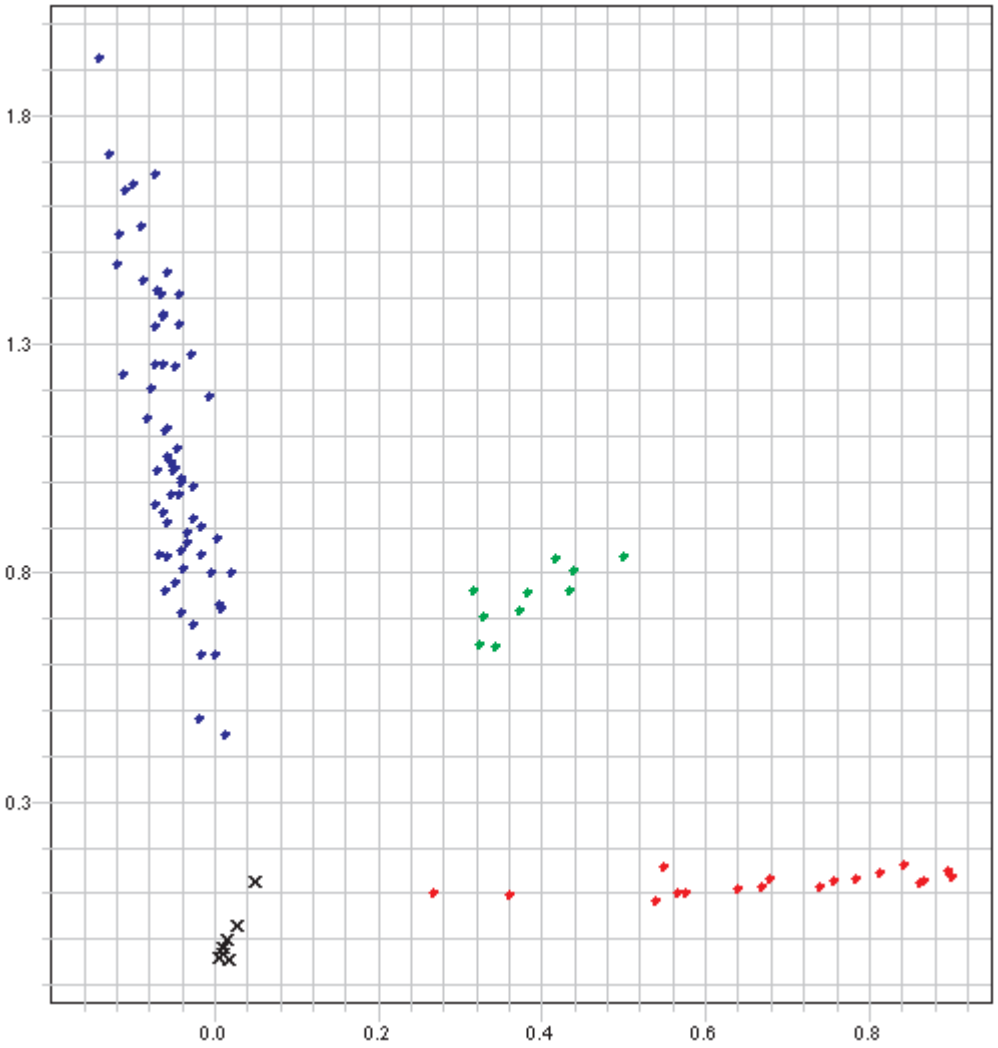
Possible cause	Recommendation
Samples	
Sample degradation	Run an agarose gel to verify that the DNA is degraded.
Incorrect DNA quantitation	Perform concentration measurements.
PCR inhibitors	Dilute the DNA sample.
Variable sample input	Check the performance of the samples in other assays. Requantitate the DNA if applicable, or ensure that the sample input for DNA extraction is within the recommended range.

Observation 5: "Chicken-feet" clusters



Possible cause	Recommendation
Reagents	
Reagents expired or mishandled	Perform the assay again with newly prepared reagents. Ensure that the reagents are stored correctly.
Reagents not added to the well	Visually inspect the well.
Evaporation	Ensure that the reaction plate is sealed properly. Use a compression pad, if recommended.
ROX™ dye is not in the Master Mix.	Use the TaqMan® GTXpress™ Master Mix or the TaqMan® Genotyping Master Mix.
Insufficient mixing of reagents	Ensure that the reagents are mixed properly, then rerun the reaction.

Observation 5: "Chicken-feet" clusters



Possible cause	Recommendation
Instrument	
Thermal cycler is poorly calibrated	Check the thermal-cycling conditions and make sure that the thermal cycler is correctly calibrated.
ROX™ dye is not selected	Ensure that the proper passive reference is selected.

Appendix A Ordering Information

This appendix covers:

Materials and equipment not included 28
Recommended thermal cyclers 28
Reagents 28
Consumables and equipment 30

Materials and equipment not included

Recommended thermal cyclers

Instrument‡	Source
Applied Biosystems 7300 Real-Time PCR System	Contact your Applied Biosystems sales representative.
Applied Biosystems 7500 Real-Time PCR System	
Applied Biosystems 7500 Fast Real-Time PCR System	
Applied Biosystems 7900HT Fast Real-Time PCR System	
GeneAmp® PCR System 9700 thermal cycler	
Applied Biosystems 9800 Fast Thermal Cycler	
Applied Biosystems Veriti™ Thermal Cycler	
Applied Biosystems StepOne™ Real-Time PCR System	
Applied Biosystems StepOne Plus™ Real-Time PCR System	

‡ The TaqMan® Sample-to-SNP™ Kit is also compatible with equivalent thermal cyclers that are not on the list. Ensure that the thermal cycler is calibrated.

Reagents

Item	Applied Biosystems part number
Sequence Detection Primers	
• 10,000 pmol	4304970
• 80,000 pmol	4304971
• 130,000 pmol	4304972
TaqMan® MGB Probe	
• 6000 pmol	4316034
• 20,000 pmol	4316033
• 50,000 pmol	4316032
Custom TaqMan® SNP Genotyping Assays:	
• Small-Scale, human 40X concentration (1000 × 5-µL reactions)	4331349
• Small-Scale, non-human 40X concentration (1000 × 5-µL reactions)	4332077
• Medium-Scale, human 40X concentration (3000 × 5-µL reactions)	4332072
• Medium-Scale, non-human 40X concentration (3,000 × 5-µL reactions)	4332075
• Large-Scale, human 80X concentration (12,000 × 5-µL reactions)	4332073
• Large-Scale, non-human 80X concentration (12,000 × 5-µL reactions)	4332076

Item	Applied Biosystems part number
TaqMan® Pre-Designed SNP Genotyping Assays: • Small-Scale, 40× concentration (1500 × 5-µL reactions) • Medium-Scale, 40× concentration (5000 × 5-µL reactions) • Large-Scale, 80× concentration (12,000 × 5-µL reactions)	 4351379 4351376 4351374
TaqMan® Validated and Coding Genotyping Assays: Small-Scale, 20× concentration (750 × 5-µL reactions)	 4331183
TaqMan® Pre-Developed Assay Reagents for Allelic Discrimination: • CYP2C19*2, (400 reactions) • CYP2C9*2, (400 reactions) • CYP2C9*3, (400 reactions) • CYP2D6*3, (400 reactions) • CYP2D6*4, (400 reactions • CYP2D6*6, (400 reactions) • CYP2D6*7, (400 reactions) • CYP2D6*8, (400 reactions)	 •4312561 •4312559 •4312560 •4312554 •4312555 •4312556 •4312557 •4312558
TaqMan® Drug Metabolism Genotyping Assays Includes CD with protocol, Assay Information File (AIF), DME Assay Index, and Troubleshooting Guide.	Go to: **www.appliedbiosystems.com**, then search: **TaqMan Drug Metabolism Assay**
Tris-EDTA (TE) buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0, made using DNase-free, RNase-free sterile-filtered water)	AM9849
DNAZap™ Solution, two, 250-mL bottles	AM9890
RT-PCR Grade Water, ten, 1.75-mL bottles	AM9935
DNase-free water	AM9914G

Consumables and equipment

Item	Applied Biosystems part number
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 500 plates	4326659
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 20 plates	4306737
MicroAmp™ Optical 384-Well Reaction Plate with Barcode, 50 plates	4309849
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1-mL, 20 plates	4346906
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 200 plates	4366932
MicroAmp™ Optical 96-Well Reaction Plate without barcode, 10	N8010560
MicroAmp™ Optical 96-Well Reaction Plate, 500 plates (without barcode)	4316813
MicroAmp™ Optical 96-Well Reaction Plate, 1000 plates (without barcode)	4343370
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL without barcode,10	4346907
MicroAmp™ Fast Optical 96-Well Reaction Plate, 20 plates, (without barcode; for StepOne™)	4375816
MicroAmp™ Optical 8-Tube Strip, 0.2-mL, 1000 tubes in strips of 8	4316567
MicroAmp™ Optical 8-Cap Strip, 300 strips	4323032
MicroAmp™ Optical Adhesive Film, 100 optical adhesive covers	4311971
MicroAmp™ Optical Adhesive Film Kit	4313663
MicroAmp™ Optical Adhesive Film, 25 optical adhesive covers	4360954
MicroAmp™ Clear Adhesive Films, 100 films	4306311
MicroAmp™ Optical Film Compression Pad‡.	4312639
MicroAmp™ Snap-On Optical Film Compression Pad‡	4333292
MicroAmp™ Multi Removal Tool	4313950

Item	Applied Biosystems part number
Centrifuge with plate adapter	Major Laboratory Supplier (MLS)§
Disposable gloves	MLS
Microcentrifuge	MLS
Microsoft Excel® software or equivalent spreadsheet and analysis software	Software suppliers
Heat block or waterbath or thermal cycler to 95 °C	MLS
1.5-mL microcentrifuge tubes	AM12400
Barrier (Filter) Tips, 10 µL size - Pipetman™ (Ten 8 × 12 racks)	AM12640
Barrier (Filter) Tips, 10 µL size - Eppendorf® (Ten 8 × 12 racks)	AM12635
Barrier (Filter) Tips, 20 µL size (Ten 8 × 12 racks)	AM12645
Barrier (Filter) Tips, 1000 µL size (Ten 100 ct racks)	AM12665
Barrier (Filter) Tips, 200 µL size (Ten 8 × 12 racks)	AM12655

‡ See instrument manual for compatibility.

§ For the MSDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

Appendix B PCR Good Laboratory Practices

This appendix covers:

PCR good laboratory practices 34

PCR good laboratory practices

When preparing samples for PCR amplification:

- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Centrifuge tubes before opening. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution. Use DNA*Zap*™ Solution (PN AM9890).

Appendix C Safety

This appendix covers:

Chemical hazard warnings 36
Chemical safety guidelines 37
MSDSs. 38
Chemical waste hazards 38
Chemical waste safety guidelines 39
Waste disposal 39
Biological hazard safety 40
Chemical alerts 41

Chemical hazard warnings

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page 38.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

MSDSs

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **MSDS**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard warning

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**)
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Chemical alerts

General alerts for all chemicals

Avoid contact with (skin, eyes, and/or clothing). Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Specific chemical alerts

DANGER! CHEMICAL HAZARD. Lysis Buffer contains guanidine thiocyanate. When guanidine thiocyanate comes in contact with acids or bleach, it liberates a very toxic gas. Do not add acids or bleaches to any liquid wastes containing Lysis Buffer.

WARNING! CHEMICAL HAZARD. DNA Stabilizing Solution may cause eye and skin irritation.

WARNING! CHEMICAL HAZARD. TaqMan® GTXpress™ Master Mix may cause eye and skin irritation. Exposure may cause discomfort if swallowed or inhaled.

Documentation and Support

This chapter covers:

Related documentation 44
How to obtain support 45

Related documentation

You can download these and other documents from:
http://docs.appliedbiosystems.com/search.taf

Document	Applied Biosystems part number
Applied Biosystems TaqMan® Sample-to-SNP™ Quick Reference Card	4402745
Applied Biosystems TaqMan® GTXpress Master™ Mix Protocol	4402746
TaqMan® SNP Genotyping Assays Protocol	4332856
Real-Time PCR Systems Chemistry Guide	4348358
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Allelic Discrimination Getting Started Guide	4347822
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4347828
Applied Biosystems 7900HT Fast Real-Time PCR System and SDS Enterprise Database User Guide	4351684
GeneAmp® PCR System 9700 thermal cycler User's Manuals: *Base Module* *96-Well Sample Block Module* *Dual 384-Well Sample Block Module* *0.5-mL Sample Block Module* *Auto-Lid Dual 96 Sample Block Module and Dual 96 Sample Block Module* *Auto-Lid Dual 384 Sample Block Module*	 4303481 4316011 4304215 4307808 4343363 4310838
Applied Biosystems 9800 Fast Thermal Cycler User Guide	4350087
Applied Biosystems Veriti™ Thermal Cycler User Guide	4375799
Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide	4376786

Note: For additional documentation, see "How to obtain support" on page 45.

How to obtain support

For the latest services and support information for all locations, go to **www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

Send us your comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is for submitting comments and suggestions relating only to documentation. To order documents, download PDF files, or for help with a technical question, see "How to obtain support" above.

Worldwide Sales and Support

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our Web site at **www.appliedbiosystems.com**.

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