June 2011

Type-it® Fast SNP Probe PCR Handbook

For 5'-nuclease probe-based SNP detection with reliably high call rates

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QIAGEN Sample and Assay Technologies

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Contents

Kit Contents

Type-it Fast SNP Probe PCR Kit	(100)	(800)	(4000)
Catalog no.	**206042**	**206045**	**206047**
Number of 25 µl reactions	**100**	**800**	**4000**
Number of 10 µl reactions	**250**	**2000**	**10000**
Number of 5 µl reactions	**500**	**4000**	**20000**
Type-it Fast SNP Probe PCR Master Mix, 2x*	1.7 ml	6 x 1.7 ml	2 x 25 ml
RNase-Free Water	1.9 ml	6 x 1.9 ml	2 x 20 ml
Q-Solution®, 5x	2 ml	2 ml	1 x 10 ml
Handbook	1	1	1

* Contains HotStarTaq® *Plus* DNA Polymerase, Type-it Fast SNP Probe PCR Buffer, dNTP Mix (dATP, dCTP, dGTP, dTTP), and ROX™ passive reference dye.

Shipping and Storage

The Type-it Fast SNP Probe PCR Kit is shipped on dry ice. It should be stored immediately upon receipt at –20°C in a constant-temperature freezer. When stored under these conditions and handled correctly, the product can be kept at least until the expiration date (see the inside of the kit lid) without showing any reduction in performance. The 2x Type-it Fast SNP Probe PCR Master Mix can be stored at 2–8°C for up to 2 months without showing any reduction in performance.

Product Use Limitations

The Type-it Fast SNP Probe PCR Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the Type-it Fast SNP Probe PCR Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the Type-it Fast SNP Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Product Specifications

Type-it Fast SNP Probe PCR Master Mix, 2x:

HotStarTaq *Plus* DNA Polymerase:	HotStarTaq *Plus* DNA Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*, cloned into *E. coli*. (Deoxynucleoside-triphosphate: DNA deoxynucleotidyl-transferase, EC 2.7.7.7). The enzyme is activated by a 5-minute, 95°C incubation step.
Type-it Fast SNP Probe PCR Buffer:	Novel fast-cycling PCR buffer including Q-Bond® for probe-based SNP genotyping providing wide allele cluster resolution for unambiguous SNP allelic discrimination.
dNTP mix:	10 mM each of dATP, dCTP, dGTP, and dTTP; ultrapure quality
Passive reference fluorescent dye:	ROX dye
Q-Solution:	5x Q-Solution for successful cluster resolution with difficult SNP loci
RNase-free water:	Ultrapure quality; PCR-grade

Introduction

Accurate genotyping analysis often requires extensive optimization of experimental parameters. Sample materials may be limiting in genotyping studies, for example, when large numbers of SNPs need to be analyzed or when working with sample materials such as biopsies or formalin-fixed, paraffin embedded (FFPE) tissue. Some studies require analysis of a large number of different mutations of a certain gene related to a disease (e.g., deletions, translocations, or SNPs). Including the necessary internal controls, a large number of PCR reactions are required, leading to increases in both costs and analysis time. QIAGEN recognizes these different challenges and has developed the Type-it PCR Kits — a new PCR-based product line dedicated for different genotyping applications, ranging from analysis of SNPs to detection of mutations and identification of microsatellite loci.

A single nucleotide polymorphism (SNP) — a single base pair difference — is the most commonly occurring variation in DNA. Because of the ubiquity of SNPs, this particular type of variant is valuable to understanding the correlation between genetic variation and its effect on the observed phenotype. Consequently, SNPs are a focus in pharmacogenomic and biomedical research.

The Type-it Fast SNP Probe PCR Kit is available in a convenient master mix format consisting of highly specific HotStarTaq *Plus* DNA Polymerase and a newly developed SNP genotyping buffer system designed for fast and efficient amplification (even of difficult SNP loci) as well as reliable SNP genotyping with low template amounts. Both unique components result in tight allele clusters in addition to high specificity and accuracy for reproducible results.

The Type-it Fast SNP Probe PCR Kit is functionally verified with commercially available SNP genotyping assays and compatible with TaqMan MGB™ Probes as well as user-developed probe-based assays consisting of TaqMan MGB, TaqMan®, or other dual-labeled probes.

High call rates even for low amounts of template

Sample materials may be limiting in genotyping studies, for example, when analyzing large numbers of SNPs or when working with biopsies or FFPE tissue. The Type-it Fast SNP Probe PCR Kit overcomes these limitations, and optimal results are achieved even when using low amounts of template DNA. Dense clusters are obtained — even with 1 ng of template DNA. The innovative Type-it Fast SNP Probe PCR Kit contains chemically modified HotStarTaq *Plus* DNA Polymerase and the unique Type-it Fast SNP Probe PCR Buffer and is a powerful system for the amplification of even the most challenging genomic regions.

HotStarTaq *Plus* DNA Polymerase

HotStarTaq *Plus* DNA Polymerase is a chemically modified form of QIAGEN *Taq* DNA Polymerase. HotStarTaq *Plus* DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient temperatures. This prevents the formation of misprimed products and primer–dimers during reaction setup and the first denaturation step. Competition for reactants by PCR artifacts is therefore avoided, enabling high PCR specificity and accurate genotyping. The enzyme is easily activated by a 5-minute, 95°C incubation step, which is easily incorporated into existing thermal cycling programs.

Type-it Fast SNP Probe PCR Buffer

The Type-it Fast SNP PCR Probe Buffer is specifically designed for fast-cycling SNP genotyping using sequence-specific 5'-nuclease probes. The unique composition of the Type-it Fast SNP PCR Buffer provides highly stringent and specific binding of the allele-specific probe (match probe). This is due to the altered melting behaviour of the probes resulting in a narrower probe melting temperature window. Compared to other commercially available SNP genotyping master mix chemistries, wider and clearer separation of allele clusters is obtained. Additives in the Type-it Fast SNP Probe PCR Buffer such as Q-Solution provide reaction conditions for amplification of difficult genomic regions and difficult SNP loci, while innovative Q-Bond technology for fast cycling means SNP genotyping results can be achieved faster.

Q-Solution

A defined concentration of Q-Solution is already included in the 2x master mix and is also provided as a separate kit component for greater flexibility. Q-Solution is an innovative PCR additive that improves amplification of difficult templates by modifying the melting behavior of DNA. This unique reagent will often enable or improve a suboptimal PCR caused by templates that have a high degree of secondary structure or that are GC-rich, noticeable by a higher plateau and tighter clusters. Unlike other commonly used PCR additives such as DMSO, Q-Solution is used at just one working concentration and is optimized for the requirements of SNP genotyping of difficult gene loci. It is nontoxic and ensures PCR purity. For further information, read Appendix D on page 35.

ROX passive reference dye

For certain real-time thermal cyclers, the presence of ROX passive reference dye compensates for non-PCR–related variations in fluorescence detection. The use of ROX dye is necessary for instruments from Applied Biosystems.

The master mix supplied with the Type-it Fast SNP Probe PCR Kit contains ROX dye at a concentration compatible with SNP genotyping on all instruments from Applied Biosystems. However, ROX dye is also compatible with other instruments not requiring ROX as passive reference (see Table 1, page 12).

Fast cycling

The Type-it Fast SNP Probe PCR Master Mix provides reaction conditions for fast-cycling PCR using sequence-specific probes. A novel additive in the buffer, Q-Bond, allows short cycling times on standard cyclers and on fast cyclers with rapid ramping rates. Q-Bond increases the affinity of *Taq* DNA polymerases for short single-stranded DNA, reducing the time required for primer–probe annealing to a few seconds. This allows a combined annealing/extension step of only 30 seconds. Denaturation and extension times are also reduced due to the unique buffer composition, which supports the melting behavior of DNA.

Recommended cyclers

The Type-it Fast SNP Probe PCR Kit has been optimized for amplification in SNP PCR assays on any suitable standard or fast ramping cycler compatible with the optical PCR plates fitting into real-time PCR instruments used for fluorescence plate read analysis. The fluorescence plate read analysis for allelic discrimination is suited to all instruments from Applied Biosystems (models *7000*, *7300*, *7500*, *7500* Fast, *7700*, *7900* Fast, *7900* HT, StepOne™, and StepOnePlus™). SNP genotyping experiments can also be performed on instruments from other suppliers allowing allelic discrimination, mostly requiring PCR and post-PCR fluorescence read to be carried out on the same instrument (see Table 1, page 12)

TaqMan MGB and TaqMan 5'-nuclease probes

TaqMan probes are sequence-specific oligonucleotides with a fluorophore and a quencher. TaqMan MGB probes additionally harbor a minor groove binder (MGB) moiety, which helps to increase allelic discrimination using two probes that only differ by one nucleotide. During PCR, the PCR primers anneal to the target sequence during the PCR annealing step. Two TaqMan probes, each specific for one of the available alleles (e.g., C or T) are present in the reaction, specifically annealing to the target region between the two primers. The proximity of the fluorophore with the quencher results in efficient quenching of fluorescence from the fluorophore. The *Taq* DNA polymerase extends the primer. When the enzyme reaches the TaqMan probe, its 5'—3' exonuclease activity degrades the probe, resulting in physical separation of the fluorophore from the quencher. Increased fluorescence associated with the measured Rn values from the released fluorophore indicates which alleles are present in the sample.

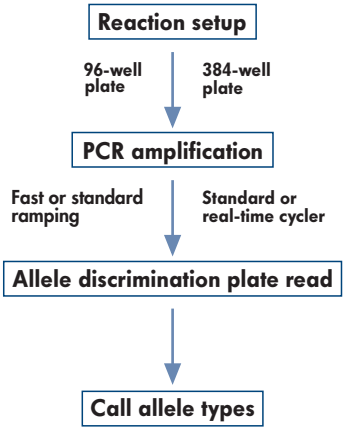

Figure 1. The Type-it Fast SNP Probe PCR Kit enables fast and convenient genotyping.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- Primers and 5'-nuclease probes
- Ready-to-use, functionally validated TaqMan SNP Genotyping Assays from Applied Biosystems
- Ready-to-use assays from Applied Biosystems as well as TaqMan MGB probes. Primers and probes according to your own design can also be used. Use appropriate software for design of SNP assays (e.g., Primer Express®, Applied Biosystems).
- Lyophilized primers and probes should be dissolved in TE buffer to provide a stock solution of 100 µM; concentration should be checked by spectrophotometry. Primer and probe stock solutions should be stored in aliquots at –20°C. Probe stock solutions should be protected from exposure to light. Avoid repeated freeze/thaw cycles of primers and probes. Prepare working solutions in small aliquots for single use.
- Nuclease-free (DNase-free) consumables: special care should be taken to avoid nuclease contamination of all reagents and consumables used to set up PCR.
- Real-time thermal cycler, optional end-point thermal cycler (see Table 1, page 12)
- Optical PCR tubes or plates (use thin-walled PCR tubes or plates recommended by the manufacturer of your thermal cycler) and optical caps/seals
- **Optional**: Trizma® base and EDTA for preparing TE buffer for storing primers and probes (see Appendix A, page 30). Use nuclease-free water and plastic consumables to prepare TE buffer.

Important Notes

Cycler selection

The following instruments are recommended when using the Type-it Fast SNP Probe PCR Master Mix.

Note: Use reaction plates that fit properly in the cycler used for PCR. All the protocols provided in this handbook are suitable for each of the listed cyclers.

Table1. Recommended instruments

			Allelic discrimination analysis	
Supplier	**Cycler**	**Reaction volume (µl)**	**Real-time PCR + post-PCR plate read**	**Separate post plate read possible***
QIAGEN				
Rotor-Gene® Q (and Rotor-Gene 6000 and 3000)	Real-time cycler	25 (36 position rotor) 10 (72 position rotor)	Yes	Yes
Applied Biosystems				
7900 Fast Real-Time PCR System	Real-time cycler	10	Yes	Yes
7900HT Real-Time PCR System (384-well)	Real-time cycler	5	Yes	Yes
7900HT Real-Time PCR System (96-well)	Real-time cycler	25	Yes	Yes
7500 Real-Time PCR System	Real-time cycler	25	Yes	Yes
7500 Fast Real-Time PCR System	Real-time cycler	10	Yes	Yes
7300 Real-Time PCR System	Real-time cycler	25	Yes	Yes

* The post-PCR plate read for allelic discrimination analysis enables use of any thermal cycler to perform the PCR, using the maximum heating and cooling rates for the fast-cycling protocol. A real-time cycler is only required for allelic discrimination plate read and analysis.

Supplier	Cycler	Reaction volume (µl)	Allelic discrimination analysis	
			Real-time PCR + post-PCR plate read	Separate post plate read possible*
Applied Biosystems				
ABIPRISM 7700 Sequence Detection System	Real-time cycler	25	Yes	Yes
ABIPRISM 7000 Sequence Detection System	Real-time cycler	25	Yes	Yes
StepOne and StepOnePlus Real-Time PCR Systems	Real-time cycler	10	Yes	Yes
Instruments from other suppliers				
Stratagene Mx3000P® and Mx3005P™QPCR Systems	Real-time cycler	25	Yes	Yes
iCycler iQ™ Real-Time PCR Detection System (Bio-rad)	Real-time cycler	25	Yes	No
Roche LightCycler 480 (384-well) †	Real-time cycler	5	Yes	No
Roche LightCycler 480 (96-well)	Real-time cycler	10	Yes	No
Standard PCR cycling instruments				
GeneAmp® PCR System 9700 thermal cycler	Thermal cycler	25	No	No
Applied Biosystems 9800 Fast Thermal Cycler (in standard or fast mode)	Thermal cycler	10	No	No
Applied Biosystems Veriti™ Thermal Cycler (all models)	Thermal cycler	10	No	No
Other PCR instruments	Thermal cycler	Depends on compatible plate type	No	No

* The post-PCR plate read for allelic discrimination analysis enables use of any thermal cycler to perform the PCR, using the maximum heating and cooling rates for the fast-cycling protocol. A real-time cycler is only required for allelic discrimination plate read and analysis.

Table 2. Fluorescence signal correlation

Fluorescence Increase	Indication
VIC® dye fluorescence only	Homozygosity for allele 1
FAM™ dye fluorescence only	Homozygosity for allele 2
Fluorescence signals for both dyes	Heterozygosity for allele 1and allele 2

Genomic template DNA

The optimal amount of template genomic DNA to use with the Type-it Fast SNP Probe PCR Kit is given in Table 3 and Table 6, pages 17 and 21, respectively. Ensure that the DNA used for allelic discrimination is of sufficient quantity and quality, not degraded, and free of impurities. For sample preparation, we recommend QIAGEN's silicia–membrane–based kits (e.g., QIAamp® and QIAprep® Kits) for reliable PCR results. Alternatively, whole genome amplification kits, which amplify very small amounts of genomic DNA without introducing sequence bias, can be used (e.g., REPLI-g® Kits). Visit www.qiagen.com to see our complete range of DNA purification products, all of which provide pure DNA from a wide variety of sample types and ensure successful downstream analysis, including accurate PCR results.

Guidelines for effective SNP genotyping assays

The Type-it Fast SNP Probe PCR Kit is designed for TaqMan MGB and standard TaqMan probe-based assays that have been designed using standard design methods, including the different formats of the TaqMan SNP Genotyping Assay from Applied Biosystems, and is also compatible with other probe systems.

However, for optimal performance of a probe system in SNP genotyping PCR, some considerations need to be made, including the choice of a compatible combination of reporter dyes (i.e., the fluorophores on the probes) and the quality of the primers and probes. Please read the following guidelines before starting.

- Choose compatible reporter dyes and quenchers. Refer to the instructions of the manufacturer of the real-time PCR instrument for fluorescence readout to identify suitable dyes.
- Always start with the cycling conditions specified in the respective protocols in this handbook. It is not recommended to shorten the denaturation step to less than 15 seconds.
- For existing or commercially available assays, use the preoptimized primer and probe concentrations in combination with the cycling protocol provided in this handbook.

- It is important to use the 5-minute hot-start step for activation of HotStarTaq *Plus* DNA Polymerase.
- For self-designed primers and probes: check the concentration and integrity of primers and probes before starting. For details, see Appendix A, page 30.
- Some real-time cyclers require a calibration procedure to be performed for each reporter dye. Check whether the reporter dyes you have selected for your SNP genotyping assay are part of the standard set of dyes already calibrated on your instrument. If they are not, perform a calibration procedure for each dye before using them for the first time.
- If testing your SNP assay by real-time PCR, checking optimal analysis settings (i.e., baseline settings and threshold values) for each reporter dye is a prerequisite for accurate data. For details, follow the instructions from the manufacturer of your real-time cycler.

Controls

No template control (NTC)

At least two NTC reactions should be included in each PCR run, containing all the components of the reaction except for the template. This is required to determine the background in fluorescence plate read analysis.

Positive control

Include at least one genomic DNA control of known genotype for each assay tested in the experiment on the plate to ensure accurate genotype calling.

Choosing the right protocol

This handbook contains 3 protocols.

SNP Genotyping Using the Type-it Fast SNP Probe PCR Master Mix (Addition of Template Directly to PCR Reaction)

See page 16. Choose this protocol if adding the template DNA solution directly to each PCR reaction.

SNP Genotyping Using the Type-it Fast SNP Probe PCR Master Mix (Drying Out the Template DNA)

See page 19. Choose this protocol if the template DNA is added to the wells before PCR setup and dried down in each well before addition of the PCR master mix.

Allelic Discrimination Plate Read and Analysis

See page 23. Choose this protocol when performing allelic discrimination plate read on a real-time PCR instrument following PCR amplification.

Protocol: SNP Genotyping Using the Type-it Fast SNP Probe PCR Master Mix (Addition of Template Directly to PCR Reaction)

This protocol should be used if adding the template DNA directly to each PCR reaction. If adding the template DNA to the wells and drying it out before PCR setup, please refer to the protocol "SNP Genotyping Using the Type-it Fast SNP Probe PCR Master Mix (Drying Out the Template DNA)" on page 19.

Important points before starting

- This protocol is optimized for use with TaqMan probes (TaqMan MGB or conventional TaqMan probes) and real-time cyclers from Applied Biosystems as well as other instruments compatible with probe-based SNP genotyping (see Table 1, page 12).
- **Always start with the cycling conditions specified in this protocol.**
- Use commercially available, ready-to-use TaqMan-based SNP genotyping assays (primer–probe mixes) at a 1x concentration in combination with the cycling conditions specified in this protocol.
- It is optional to use Q-Solution, which may improve amplification of difficult target sequences (e.g., highly GC-rich sequences). Please refer to Appendix D, page 35 for details on the function of Q-Solution in SNP genotyping.
- **Optional: If running PCR on a real-time PCR instrument in order to evaluate assay performance based on C_T values, optimal analysis settings are a prerequisite for accurate real-time data.** For data analysis, you should always readjust the analysis settings (i.e., baseline settings and threshold values) for analysis of every reporter dye channel in every run.

Things to do before starting

- For ease of use, preparing a 20x primer–probe mix for each target containing target-specific primers and probes is recommended. See Appendix A, page 30.

Procedure

1. **Thaw the Type-it Fast SNP Probe PCR Master Mix, primer and probe solutions, RNase-free water, control genomic DNAs, and template DNAs.**

   IMPORTANT: Mix the solutions completely before use to avoid localized concentrations of salt.

   Note: If using Q-Solution, thaw it as well.

2. **Prepare a reaction mix according to Table 3.**

   Note: It is recommended to prepare a volume of reaction mix 10% greater than that required for the total number of reactions to be performed.

   Typically, reaction setup can be done at room temperature (15–25°C). However, it is recommended to keep the individual reagents, samples, and controls on ice.

Table 3. Reaction composition using Type-it Fast SNP Probe PCR Master Mix

	Volume/reaction			
Component	**96-well standard plate**	**96-well fast plate**	**384-well block**	**Final concentration**
Reaction mix				
Type-it Fast SNP Probe PCR Master Mix, 2x	12.5 µl	5 µl	2.5 µl	1x
20x primer–probe mix*	1.25 µl	0.5 µl	0.25 µl	1x 0.9 µM both primers 0.2 µM both probes
Optional:				
Q-Solution, 5x	2.5 µl	1 µl	0.5 µl	0.5x
RNase-free water	Variable	Variable	Variable	–
Template DNA	Variable	Variable	Variable	1–100 ng
Total volume per reaction	**25 µl**	**10 µl**	**5 µl**	**–**

* For ease of use, we recommend preparing a 20x primer–probe mix for each of your targets containing target-specific primers and probes. See Appendix A, page 30.

3. **Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes or the wells of a PCR plate.**
4. **Add template DNAs to the individual PCR tubes or wells and mix thoroughly.**

   Note: The maximum volume for template DNA is 45% of the final reaction volume. If your template DNA is of a low concentration and a larger volume of template is required, the protocol on page 19 is recommended.

   Note: Ensure that the reaction mix and template are thoroughly mixed.
5. **Program the cycler according to Table 4.**

Table 4. Preoptimized cycling protocol

Step	Time	Temperature	Additional comments
Initial PCR activation step	**5 min**	**95°C**	HotStarTaq *Plus* DNA Polymerase is activated by this heating step.
2-step cycling:*			**Important: Optimal performance is only assured using these cycling conditions.**
Denaturation	**15 s**	**95°C**	
Annealing/extension	**30 s**	**60°C**	For PCR products up to 300 bp.
Number of cycles	40		For 1–100 ng
	45		For <1 ng

* The protocol is compatible with maximum heating and cooling rates of the cycling block available on recent fast cyclers.

6. **Centrifuge the PCR tube/plate briefly to spin down the contents and eliminate air bubbles from the solution.**
7. **Place the PCR tubes or plate in the cycler and start the PCR cycling program.**

   Note: You may run the PCR later and store the prepared plates for up to 3 days at 2–8°C protected from light.
8. **After PCR amplification, perform an end-point plate read on a real-time PCR instrument, as described on page 23.**

   Note: If the plate read is performed up to 3 days later, it is recommended to store the plates at room temperature or at 2–8°C, protected from light. For long-term storage, place at –20°C.

   Optional: Before the post-PCR plate-read analysis, spin down the plate to remove liquid on the cover or air bubbles.

Protocol: SNP Genotyping Using the Type-it Fast SNP Probe PCR Master Mix (Drying Out the Template DNA)

This protocol should be used if the template DNA is added to the wells before PCR setup and dried down in each well before the addition of the PCR master mix. If you intend to add your template DNA as solution directly during PCR setup, please refer to the protocol "SNP Genotyping Using the Type-it Fast SNP Probe PCR Master Mix (Addition of Template Directly to PCR Reaction)" on page 16.

Important points before starting

- This protocol is optimized for use with TaqMan probes (TaqMan MGB or conventional TaqMan probes) and real-time cyclers from Applied Biosystems as well as other instruments compatible with probe-based SNP genotyping (see Table 1, pages 12).
- **Always start with the cycling conditions specified in this protocol.**
- Use commercially available, ready-to-use TaqMan-based SNP genotyping assays (primer–probe mixes) at a 1x concentration in combination with the cycling conditions specified in this protocol.
- This protocol contains the optional use of Q-Solution, which may improve amplification of difficult target sequences (e.g., sequences that are highly GC-rich). Please refer to Appendix D, page 35 for details on the function of Q-Solution in SNP genotyping.
- **Optional: If running PCR on a real-time PCR instrument in order to evaluate assay performance based on C_T values, optimal analysis settings are a prerequisite for accurate real-time PCR data.** For data analysis, you should always readjust the analysis settings (i.e., baseline settings and threshold values) for analysis of every reporter dye channel in every run.

Things to do before starting

- For ease of use, we recommend preparing a 20x primer–probe mix for each of your targets containing target-specific primers and probes. See Appendix A, page 30.

Procedure

1. **Thaw the template DNAs and control genomic DNAs. Mix the individual samples by vortexing.**

   IMPORTANT: Mix the solutions completely before use to avoid localized concentrations of salt.

2. **Dispense the respective volume of each DNA sample to the bottom of individual PCR tubes or the wells of a PCR plate.**

   Use 0.1–100 ng of genomic DNA per reaction.

   Optimal amount: 1–20 ng.

   Note: We recommend using equal volumes of each DNA sample to prevent over-drying of samples delivered in a low volume. In order to minimize drying time, the recommended volume for template DNA is 2–5 µl.

3. **Dry down the DNA samples. DNA samples may be dried down by evaporating the liquid at room temperature or by placing the PCR tubes or plate in a PCR thermal cycler set to a constant temperature of 37°C with the lid of the cycler open.**

   Note: Avoid contamination with PCR amplicons that may affect the SNP assay. Turn off the heated lid of the thermal cycler to avoid accidental burning of hands.

Table 5. Dry down conditions for template DNA*

	Approximate drying time at 37°C	
Volume	**96-well**	**384-well**
2 µl	**1 h**	**1 h 20 min**
5 µl	**2 h 15 min**	**2 h 30 min**

* Drying time may strongly vary depending on lab temperature and humidity.

4. **Thaw the Type-it Fast SNP Probe PCR Master Mix, primer and probe solutions, RNase-free water, and (optional) Q-Solution. Mix the individual solutions.**

   IMPORTANT: Mix the solutions completely before use to avoid localized concentrations of salt.

5. **Prepare a reaction mix according to Table 6, page 21.**

   It is recommended to prepare a volume of reaction mix 10% greater than that required for the total number of reactions to be performed.

   Typically, reaction setup can be done at room temperature (15–25°C). However, it is recommended to keep the primers and probes on ice.

Table 6. Reaction composition using the Type-it Fast SNP Probe PCR Master Mix

Component	Volume/reaction			Final concentration
	96-well standard plate	96-well fast plate	384-well block	
Reaction mix				
Type-it Fast SNP Probe PCR Master Mix, 2x	12.5 µl	5 µl	2.5 µl	1x
20x primer–probe mix*	1.25 µl	0.5 µl	0.25 µl	1x 0.9 µM both primers 0.2 µM both probes
Optional:				
Q-Solution, 5x	2.5 µl	1 µl	0.5 µl	0.5x
RNase-free water	Variable	Variable	Variable	–
Template DNA	Variable	Variable	Variable	1–100 ng
Total volume per reaction	**25 µl**	**10 µl**	**5 µl**	**–**

* For ease of use, we recommend preparing a 20x primer–probe mix for each of your targets containing target-specific primers and probes. See Appendix A, page 30.

6. **Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes or the wells of a PCR plate.**
7. **Program the cycler according to Table 7, page 22.**

Table 7. Preoptimized cycling protocol

Step	Time	Temperature	Additional comments
Initial PCR activation step	**5 min**	**95°C**	HotStarTaq *Plus* DNA Polymerase is activated by this heating step.
2-step cycling:*			**Important: Optimal performance is only assured using these cycling conditions.**
Denaturation	**15 s**	**95°C**	
Annealing/extension	**30 s**	**60°C**	For PCR products up to 300 bp.
Number of cycles	40		For 1–100 ng
	45		For <1 ng

* The protocol is compatible with maximum heating and cooling rates of the cycling block available on recent fast cyclers.

8. **Centrifuge the PCR tubes/plate briefly to spin down the contents and eliminate air bubbles from the solution.**
9. **Place the PCR tubes or plate in the cycler and start the PCR cycling program.**

   Note: You may run the PCR later and store the prepared plates for up to 3 days at 2–8°C protected from light.
10. **After PCR amplification, perform an end-point plate read on a real-time PCR instrument, as described on page 23.**

    Note: If the plate read is performed up to 3 days later, it is recommended to store the plates at room temperature or at 2–8°C, protected from light. For long term storage, place at –20°C.

    Optional: Spin down the plate to remove liquid on the cover or air bubbles before the post-PCR plate-read analysis.

Protocol: Allelic Discrimination Plate Read and Analysis

Following PCR amplification, end-point plate read is performed on a real-time PCR instrument. Using the fluorescence measurements made during the plate read, the software of the real-time PCR instrument plots R_n values based on the fluorescence signals from each well, and then determines which alleles are in each sample.

The general process for analyzing data for genotyping involves:

- Creating and setting up an allelic discrimination plate read document
- Performing an allelic discrimination plate read on a real-time PCR instrument.

  Note: If using Applied Biosystems *7500* and *7500 Fast* PCR Systems, please note that due to the optimized formulation of the ROX passive reference dye in the Type-it Fast SNP Probe PCR Kit, a variation in the scale of R_n values occurs in the resulting scatter plot. It is expected to be between 0.1 and 1 and may differ from results obtained with other master mixes. It has no influence on the accuracy of the allele calling experiment.
- Analysis of the plate read document
- Making automatic or manual allele calls
- Verification of allele types

  Note: 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.

The software of the real-time PCR instrument plots 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.

Figure 2 shows variation in clustering due to the genotype of the target allele. VIC fluorescence is shown on the x-axis and FAM fluorescence is shown on the y-axis. Squares in the lower left represent No Template Controls (NTC), circles represent samples homozygous for the allele detected by VIC, diamonds represent samples homozygous for the allele detected by FAM, and triangles represent heterozygous samples (FAM and VIC positive).

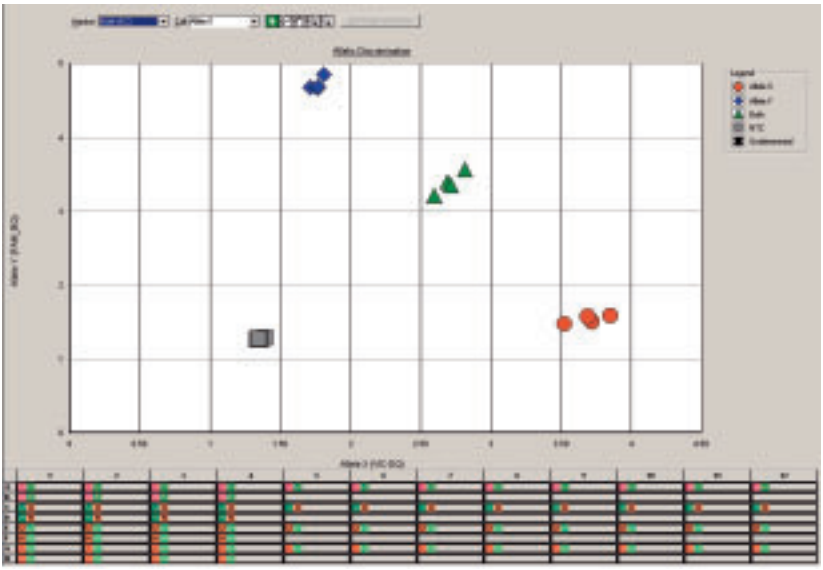

Figure 2. Variation in clustering due to the genotype of the target gene.

Supplementary information on data analysis: Data analysis varies depending on the instrument. Refer to the instrument handbook for more information about analyzing your genotyping data.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

		Comments and suggestions
No signal, poor Rn value, or signal detected late in PCR		
a)	Wrong cycling conditions	Always start with the optimized cycling conditions specified in the protocols.
b)	HotStarTaq *Plus* DNA Polymerase not activated	Ensure that the cycling program includes the HotStarTaq *Plus* DNA Polymerase activation step (5 min at 95°C) as described in the protocols.
c)	Pipetting error or missing reagent	Check the concentrations and storage conditions of the reagents, including primers, probes, and template nucleic acid. See Appendix A, page 30 for details on evaluating the concentration of primers and probes. Repeat the assay.
d)	Wrong or no detection step in real-time analysis	For real-time analysis, ensure that fluorescence detection takes place during the combined annealing/extension step when using TaqMan probes.
e)	Poor PCR efficiency	Use the primer and probe concentrations given in the protocol. If using self-designed primers and probes, check the concentration. See Appendix A, page 30 for details on evaluating the concentration of primers and probes. Avoid repeated freezing and thawing of primers and probes. Prepare small aliquots and only thaw a few times. For PCR amplicons >300 bp, increase annealing/extension time to 60 seconds; optionally, also increase the number of cycles to 50.

		Comments and suggestions
f)	Problems with starting template DNA	Check the concentration, storage conditions, and quality of the template and control DNA. If necessary, make new serial dilutions of the template DNA from the stock solutions. Repeat the assay using the new dilutions. Efficient removal of PCR inhibitors is essential for optimal results. Purify nucleic acids from your sample using an appropriate purification method. Ensure that all reagents, buffers, and solutions used for isolating and dilution of template nucleic acids are free of nucleases (RNases/DNases).
g)	Insufficient or degraded template DNA	Check if template amount is in the range specified in the protocol (Tables 3 and 6, pages 17 and 21, respectively). Increase the amount of template if possible. Use 40 cycles for 10–100 ng of template genomic DNA. Use 45 cycles for 0.1–9 ng of template genomic DNA. A further increase to 50 cycles may be helpful in rare cases for minute and/or degraded starting template. Use a different PCR assay to retest the respective DNA sample for amplifiable DNA.
h)	Difficult genomic locus	Amplification of difficult genomic loci (secondary structure or GC-rich) may be improved using Q-Solution. Run the reaction with and without Q-Solution.
i)	Probe design not optimal	If the amplification reaction was successful, there may be a problem with the probe. Review the probe design guidelines. See Appendix A, page 30.
j)	Wrong detection channel/filter chosen	Ensure that the correct detection channel is activated or the correct filter set is chosen for each reporter dye. Check whether the chosen combination of reporter dyes is compatible with the selected detection channels or filter sets.
k)	Fluorescence crosstalk	Check that the reporter dyes used in your assay are suitable for SNP genotyping analysis on your instrument. Run appropriate controls to estimate potential crosstalk effects.

		Comments and suggestions
Increased fluorescence or C_T value for "No Template Control" (NTC)		
a)	Contamination of reagents	Discard all the components of the SNP assay (e.g., master mix, primers, and probes). Repeat the SNP assay using new components and decontaminated pipettes and consumables.
b)	Contamination of real-time cycler	Decontaminate the real-time cycler according to the manufacturer's instructions.
c)	Real-time cycler no longer calibrated	Recalibrate the real-time cycler according to the manufacturer's instructions.
Distinct clusters are not observed or a sample does not cluster with one specific allele type, or varying fluorescence		
a)	Reporter dye inappropriately assigned or improperly selected quencher dye	Verify dye settings and reanalyze data.
b)	Problem with template genomic DNA	Recheck the DNA concentrations of the samples. Ensure that comparable amounts of template genomic DNA are used for all samples. Retest the samples to ensure that the clusters are now distinct. Use a different genotyping assay to test integrity of the genomic DNA in the samples. Note that a rare allelic variant, sequence duplications, or mixed samples (containing multiplex alleles) may generate a signal outside of the expected genotyping clusters.

		Comments and suggestions
c)	Inaccurate reagent delivery or evaporation occurred	Check each well for a variation in volume, then redo any assay that did not contain the proper volume. Use compression pads when using optical adhesive film with a: ■ Standard 96-well plate on the ABI PRISM 7000 Sequence Detection System ■ Standard 96-well plate on the Applied Biosystems 7900HT Fast Real-Time PCR System ■ Standard 96-well plate or 384-well plate on the GeneAmp PCR System 9700 Thermal Cycler ■ Fast 96-well plate on the Applied Biosystems 9800 Fast Thermal Cycler
d)	Bubbles in the wells	Spin down plates to remove air bubbles and remove any liquid form the plate cover. Repeat the post-PCR plate read. If your instrument does not allow repeated post-PCR plate read, prepare a new reaction plate, making sure the plate is centrifuged before performing PCR.
e)	Inaccurate ROX signal	On all instruments from Applied Biosystems, select ROX dye as the passive reference when setting up the plate document. Too low a reaction volume may generate an inaccurate ROX signal. Use the recommended reaction volume. Check instrument hardware for problems and check if the instrument is well calibrated.

		Comments and suggestions
f)	Samples on plate edges fail to be called	Check the PCR plate for uniform thermal contact with the heating block. On Applied Biosystems instruments, use compression pads when using optical adhesive film with a: ■ Standard 96-well plate on the ABI PRISM 7000 Sequence Detection System ■ Standard 96-well plate on the Applied Biosystems 7900HT Fast Real-Time PCR System ■ Standard 96-well plate or 384-well plate on the GeneAmp PCR System 9700 Thermal Cycler ■ Fast 96-well plate on the Applied Biosystems 9800 Fast Thermal Cycler
g)	Reaction components improperly mixed	Follow mixing procedures in the protocol.
h)	Contamination of real-time cycler	Decontaminate the real-time cycler according to the manufacturer's instructions.
i)	Real-time cycler no longer calibrated	Recalibrate the real-time cycler according to the manufacturer's instructions.

Special hints for instruments from Applied Biosystems: Scale (x and y axis values) missing on allelic discrimination plot

a)	ROX dye was not selected as the passive reference when the plate document was set up	Select ROX dye as the passive reference when the plate document is set up.
b)	Applied Biosystems 7500 and 7500 Fast PCR System: Different scale of x and y axis in scatter plot	Rn values on these instruments can be expected to be between 0.1 and 1, resulting in a different scale on the axis of the scatter plot. This is due to the different formulation of the ROX passive reference dye
c)	ABI PRISM 7000 SDS: Uneven curves or high standard deviations	Do not use reaction volumes smaller than 25 µl and always use optical adhesive covers to seal plates. In some cases, increasing the reaction volume to 50 µl may improve results.

Appendix A: Preparing a 20x Primer–Probe Mix

For ease of use, we recommend preparing a 20x primer–probe mix.

Table 8. A 20x primer–probe mix

Component	Concentration (20x)	Final concentration
Forward primer	18 µM	0.9 µM*
Reverse primer	18 µM	0.9 µM*
Probe Allele 1	4 µM	0.2 µM†
Probe Allele 2	4 µM	0.2 µM†
TE buffer	–	–

* A final primer concentration of 0.9 µM is optimal. Before adapting primer concentration, verify the concentration of your primer solutions.

† A final probe concentration of 0.2 µM gives satisfactory results in most cases. Depending on the synthesis quality and purification method used, the optimal concentration may be between 0.1 µM and 0.4 µM.

Appendix B: Starting Template

Both the quality and quantity of nucleic acid starting template affect PCR, in particular the sensitivity and efficiency of amplification.‡

Quality of starting template

Since PCR consists of multiple rounds of enzymatic reactions, it is more sensitive to impurities such as proteins, phenol/chloroform, salts, ethanol, EDTA, and other chemical solvents than single-step enzyme-catalyzed processes. QIAGEN offers a complete range of nucleic acid preparation systems, ensuring the highest-quality templates for PCR. These include the QIAprep system for rapid plasmid purification, the QIAamp and DNeasy® systems for rapid purification of genomic DNA and viral nucleic acids, and the RNeasy® system for RNA preparation from a variety of sources. For more information about QIAprep, QIAamp, DNeasy, and RNeasy products, contact one of our Technical Service Departments (see back cover) or visit www.qiagen.com/productfinder.

‡ For further information see our guide *Critical Factors for Successful PCR*. To obtain a copy, visit the QIAGEN Web site at www.qiagen.com or call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Quantity of starting template

The annealing efficiency of primer to template is an important factor in PCR. Owing to the thermodynamic nature of the reaction, the primer to template ratio strongly influences the specificity and efficiency of PCR and should be optimized empirically. If too little template is used, primers may not be able to find their complementary sequences. Too much template may lead to an increase in mispriming events. As an initial guide, spectrophotometric and molar conversion values for different nucleic acid templates are listed in Tables 9 and 10, respectively.

Table 9. Spectrophotometric conversions for nucleic acid templates

1 A_{260} unit*	Concentration (µg/ml)
Double-stranded DNA	50
Single-stranded DNA	33
Single-stranded RNA	40

* Absorbance at 260 nm = 1

Table 10. Molar conversions for nucleic acid templates

Nucleic acid	Size	pmol/µg	Molecules/µg
1 kb DNA	1000 bp	1.52	9.1×10^{11}
pUC19 DNA	2686 bp	0.57	3.4×10^{11}
pTZ18R DNA	2870 bp	0.54	3.2×10^{11}
pBluescript® II DNA	2961 bp	0.52	3.1×10^{11}
Lambda DNA	48,502 bp	0.03	1.8×10^{10}
Average mRNA	1930 nt	1.67	1.0×10^{12}
Genomic DNA			
Escherichia coli	4.7×10^{6}†	3.0×10^{-4}	1.8×10^{8}‡
Drosophila melanogaster	1.4×10^{8}†	1.1×10^{-5}	6.6×10^{5}‡
Mus musculus (mouse)	2.7×10^{9}†	5.7×10^{-7}	3.4×10^{5}‡
Homo sapiens (human)	3.3×10^{9}†	4.7×10^{-7}	2.8×10^{5}‡

† Base pairs in haploid genome.

‡ For single-copy genes.

Appendix C: Assay Design and Handling Primers and Probes

Important factors for successful SNP genotyping PCR include the design of optimal primer pairs and probes, the use of appropriate primer and probe concentrations, and the correct storage of primers and probes.

Assay design

Guidelines for the optimal design of primers and probes are given below. It is particularly important to minimize nonspecific annealing of primers and probes. This can be achieved through careful assay design.

T_m of primers for TaqMan assays

- Use specialized design software (e.g., Primer Express Software) to design primers and probes.
- T_m of all primers should be 58–62°C and within 2°C of each other.
- T_m of probes should be 8–10°C higher than the T_m of the primers.
- SNP should be in the middle third of each probe.
- Avoid a guanidine at the 5' end of probes, next to the reporter, since this causes quenching.
- Avoid runs of 4 or more of the same nucleotide, especially of guanidine.
- Choose the binding strand so that the probe has more C than G bases.
- All assays should be designed using the same settings to ensure that they will work optimally under the same cycling conditions (60°C annealing/extension).

Primer sequence

- Length: 18–30 nucleotides.
- GC content: 30–70%.
- Always check the specificity of primers by performing a BLAST® search (www.ncbi.nlm.nih.gov/blast). Ensure that primer sequences are unique for your template sequence.
- Check that primers and probes are not complementary to each other.
- Try to avoid highly repetitive sequences.
- Avoid complementarity of 2 or 3 bases at the 3' ends of primer pairs to minimize primer–dimer formation.
- Avoid mismatches between the 3' end of primers and the template sequence.
- Avoid runs of 3 or more Gs and/or Cs at the 3' end.
- Avoid complementary sequences within a primer sequence and between the primer pair.

Product size

Ensure that the length of PCR products is 60–300 bp.

Handling and storing primers and probes

Guidelines for handling and storing primers and probes are given below. For optimal results, we recommend only combining primers of comparable quality.

Storage buffer

Lyophilized primers and probes should be dissolved in a small volume of low-salt buffer to give a concentrated stock solution (e.g., 100 µM). We recommend using TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0) for standard primers and probes labeled with most fluorescent dyes.

However, probes labeled with fluorescent dyes such as Cy®3, Cy3.5, Cy5, and Cy5.5 should be stored in TE, pH 7.0, since they tend to degrade at higher pH.

Storage

Primers should be stored in sterile, nuclease-free TE buffer in small aliquots at –20°C. Standard primers are stable under these conditions for at least 1 year. Fluorescently labeled probes are usually stable under these conditions for at least 6–9 months. Repeated freeze–thaw cycles should be avoided, since they may lead to degradation.

For easy and reproducible handling of primer–probe sets used in SNP genotyping assays, we recommend preparing 20x primer–probe mixes, each containing the 2 primers and the 2 probes for a particular SNP at the suggested concentrations (see protocols).

Dissolving primers and probes

Before opening a tube containing lyophilized primer or probe, spin the tube briefly to collect all material at the bottom of the tube. To dissolve the primer or the probe, add the required volume of sterile, nuclease-free TE buffer, mix, and leave for 20 minutes to allow the primer or probe to completely dissolve. Mix again and determine the concentration by spectrophotometry as described below.

We do not recommend dissolving primers and probes in water. They are less stable in water than in TE buffer and some may not dissolve easily in water.

Concentration

Spectrophotometric conversion for primers and probes:

1 A_{260} unit = 20–30 µg/ml

To check primer concentration, the molar extinction coefficient (ε_{260}) can be used:

$A_{260} = \varepsilon_{260}$ x molar concentration of primer or probe

If the ε_{260} value is not given on the data sheet supplied with the primers or probes, it can be calculated from the primer sequence using the following formula:

$\varepsilon_{260} = 0.89 \times [(A \times 15{,}480) + (C \times 7340) + (G \times 11{,}760) + (T \times 8850)]$

Example

Concentration of diluted primer: 1 µM = 1 x 10^{-6} M
Primer length: 24 nucleotides with 6 each of A, C, G, and T bases

Calculation of expected A_{260}: 0.89 x [(6 x 15,480) + (6 x 7340) + (6 x 11,760) + (6 x 8850)] x (1 x 10^{-6}) = 0.232

The measured A_{260} should be within ± 30% of the theoretical value. If the measured A_{260} is very different to the theoretical value, we recommend recalculating the concentration of the primers or probes, or having the primers or probes resynthesized. For probes, the fluorescent dye does not significantly affect the A_{260} value.

Primer and probe quality

The quality of 18–30mers can be checked on a 15% denaturing polyacrylamide gel;* a single band should be seen. Please contact QIAGEN Technical Services or your local distributor (see back cover) or visit www.qiagen.com for a protocol.

Probe quality

The quality of the fluorescent label and the purity of TaqMan probes can be determined by comparing fluorescence before and after DNase digestion. Incubate probes with or without 5 units DNase at 37°C for 1 hour. A significant difference in fluorescence following DNase treatment should be detectable.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Appendix D: Use of Q-Solution in SNP Genotyping

Q-Solution may be used to improve amplification of target sequences that do not amplify well under standard conditions and has been specifically optimized for SNP genotyping PCR using probes.

- Q-Solution changes the melting behavior of DNA and will often improve a suboptimal PCR caused by templates that have a high degree of secondary structure or that are GC-rich (≥65% GC content of the PCR product).
- It also improves allelic discrimination on difficult SNPs, including those located in GC-rich genomic regions. It may also be helpful in other cases.

When using Q-Solution for the first time, always perform parallel reactions with and without Q-Solution. This recommendation should also be followed if another PCR additive (such as DMSO) was previously used for a particular primer–template pair.

When using Q-Solution, the following effects may be observed depending on the individual SNP genotyping assay:

Case A: Q-Solution enables allelic discrimination of a genomic locus that previously failed.

Case B: Q-Solution increases PCR specificity in certain primer–probe–template systems and enables a more accurate allelic discrimination.

Case C: Q-Solution has no effect on PCR performance and allelic discrimination R_n values.

Case D: Q-Solution causes reduced efficiency or failure of a previously successful amplification reaction. In this case, addition of Q-Solution disturbs the previously optimal primer–probe–template annealing. Therefore, when using Q-Solution for the first time with a particular SNP genotyping PCR assay, always perform reactions with and without Q-Solution.

Appendix E: Customized Pipetting Scheme for Separate Primer and Probe Solutions

For ease of use, we recommend preparing a 20x primer–probe mix for each of your targets containing target-specific primers and probes. However, in some cases, it may be preferable to prepare the reaction mix with separate primer and probe solutions. If you commonly set up reactions this way, it may be helpful to copy and fill in Table 11 with the calculated volumes of each primer to use.

Table 11. Reaction mix for SNP genotyping analysis using separate primer and probe solutions

	Volume per reaction		
Component	**25 µl**	**10 µl**	**Other: ____ µl**
Reaction mix			
2x Type-it Fast SNP Probe PCR Master Mix	12.5 µl	5 µl	_____ µl
Forward primer ☐ 0.9 µM ☐ ____	_____ µl	_____ µl	_____ µl
Reverse primer ☐ 0.9 µM ☐ ____	_____ µl	_____ µl	_____ µl
Probe Allele 1 (0.2 µM)	_____ µl	_____ µl	_____ µl
Probe Allele 2 (0.2 µM)	_____ µl	_____ µl	_____ µl
Optional: Q-Solution, 5x	2.5 µl	1 µl	_____ µl
RNase-free water	_____ µl	_____ µl	_____ µl
Template DNA	_____ µl	_____ µl	_____ µl
Total volume	**25 µl**	**10 µl**	**_____ µl**

Ordering Information

Product	Contents	Cat. no.
Type-it Fast SNP Probe PCR Kit (100)	For 100 x 25 µl reactions: 2x Type-it Fast SNP Probe PCR Master Mix,* 5x Q-Solution, RNase-Free Water	206042
Type-it Fast SNP Probe PCR Kit (800)	For 800 x 25 µl reactions: 5.1 ml of 2x Type-it Fast SNP Probe PCR Master Mix,* 5x Q-Solution, RNase-Free Water	206045
Type-it Fast SNP Probe PCR Kit (4000)	For 4000 x 25 µl reactions: 40 ml of 2x Type-it Fast SNP Probe PCR Master Mix,*† 5x Q-Solution, RNase-Free Water	206047
Related products		
Type-it HRM® PCR Kit — for accurate genotyping by High-Resolution Melting (HRM) analysis		
Type-it HRM PCR Kit (100)‡	For 100 x 25 µl reactions: 1 x 1.3 ml of 2x HRM PCR Master Mix§, and RNase-Free Water	206542
Type-it Mutation Detect PCR Kit — for reliable detection of mutations by multiplex PCR		
Type-it Mutation Detect PCR Kit (70)‡	For 70 x 25 µl reactions: Type-it Multiplex PCR Master Mix,¶ 5x Q-Solution, RNase-Free Water, and 10x CoralLoad® Dye	206341
Type-it Microsatellite PCR Kit — for reliable microsatellite analysis by multiplex PCR		
Type-it Microsatellite PCR Kit (70)‡	For 70 x 25 µl reactions: Type-it Multiplex PCR Master Mix,¶ 5x Q-Solution, and RNase-Free Water	206241

* Contains HotStarTaq *Plus* DNA Polymerase, ROX dye, and dNTPs with optimized concentration of $MgCl_2$ and Q-Solution.

† Master mix supplied in 2 individual tubes.

‡ Larger kit sizes available; see www.qiagen.com.

§ Contains HotStarTaq *Plus* DNA Polymerase, EvaGreen® dye, optimized concentration of Q-Solution, dNTPs, and $MgCl_2$.

¶ Contains HotStarTaq *Plus* DNA Polymerase, optimized $MgCl_2$ concentration, and 200 µM each dNTP.

Ordering Information

Product	Contents	Cat. no.
HotStarTaq *Plus* DNA Polymerase — for highly specific hot-start PCR without optimization		
HotStarTaq *Plus* DNA Polymerase (250 U)*	250 units HotStarTaq *Plus* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer, 5x Q-Solution, 25 mM $MgCl_2$	203603
HotStarTaq *Plus* Master Mix Kit — for fast and highly specific amplification		
HotStarTaq *Plus* Master Mix Kit (250)*	3 x 0.85 ml HotStarTaq *Plus* Master Mix,‡ containing 250 units of HotStarTaq *Plus* DNA Polymerase total, 1 x 0.55 ml CoralLoad Concentrate, 2 x 1.9 ml RNase-Free Water for 250 x 20 µl reactions	203643
QIAamp DNA Kits — for purification of genomic, mitochondrial, bacterial, parasite, or viral DNA		
QIAamp DNA Mini Kit (50)*	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
DNeasy Blood & Tissue Kits — for purification of total DNA from animal blood and tissues, and from cells, yeast, bacteria, or viruses		
DNeasy Blood & Tissue Kit (50)*	50 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

* Larger kit sizes available; see www.qiagen.com.

† Contains 15 mM $MgCl_2$.

‡ Contains 3 mM $MgCl_2$ and 400 µM each dNTP.

www.qiagen.com

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Austria ▪ Orders 0800-28-10-10 ▪ Fax 0800/28-10-19 ▪ Technical 0800-28-10-11

Belgium ▪ Orders 0800-79612 ▪ Fax 0800-79611 ▪ Technical 0800-79556

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Canada ▪ Orders 800-572-9613 ▪ Fax 800-713-5951 ▪ Technical 800-DNA-PREP (800-362-7737)

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Ireland ▪ Orders 1800 555 049 ▪ Fax 1800 555 048 ▪ Technical 1800 555 061

Italy ▪ Orders 800-789-544 ▪ Fax 02-334304-826 ▪ Technical 800-787980

Japan ▪ Telephone 03-6890-7300 ▪ Fax 03-5547-0818 ▪ Technical 03-6890-7300

Korea (South) ▪ Orders 080-000-7146 ▪ Fax 02-2626-5703 ▪ Technical 080-000-7145

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Switzerland ▪ Orders 055-254-22-11 ▪ Fax 055-254-22-13 ▪ Technical 055-254-22-12

UK ▪ Orders 01293-422-911 ▪ Fax 01293-422-922 ▪ Technical 01293-422-999

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