August 2016

ipsogen® JAK2 MutaQ*uant*® Kit Handbook

Σ 12 (catalog no. 673522)

Σ 24 (catalog no. 673523)

Version 1

IVD

Quantitative in vitro diagnostics

For use with Rotor-Gene® Q, ABI PRISM® 7900HT SDS, Applied Biosystems® 7500 Real-Time PCR System, and LightCycler® instruments

CE

REF 673522, 673523

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Contents

Intended Use

The *ipsogen* JAK2 Muta*Quant* Kit is an in vitro quantitative test intended for the detection and quantification of JAK2 V617F/G1849T allele in genomic DNA extracted from peripheral blood of subjects with suspected myeloproliferative neoplasm (MPN).

The absence of the JAK2 V617F/G1849T mutation does not exclude the presence of other JAK2 mutations. The test can report false negative results in case of additional mutations located in nucleotides 88504 to 88622 (1).

Note: The kit should be used following the instructions given in this manual, in combination with validated reagents and instruments. Any off-label use of this product and/or modification of the components will void QIAGEN's liability.

Summary and Explanation

A recurrent somatic mutation, V617F, affecting the Janus tyrosine kinase 2 (JAK2) gene, has been identified in 2005 (2–5), leading to a major breakthrough in the understanding, classification, and diagnosis of myeloproliferative neoplasms (MPN). JAK2 is a critical intracellular signaling molecule for a number of cytokines, including erythropoietin.

The JAK2 V617F mutation is detected in >95% of patients with polycythemia vera (PV), 50–60% of patients with essential thrombocythemia (ET), and in 50% of patients with primary myelofibrosis (PMF). JAK2 V617F has been also detected in some rare cases of chronic myelomonocytic leukemia, myelodysplastic syndrome, systemic mastocytosis, and chronic neutrophilic leukemia, but in 0% of CML (6).

The mutation corresponds to a single-nucleotide change of JAK2 nucleotide 1849 in exon 14, resulting in a unique valine (V) to phenylalanine (F) substitution at position 617 of the protein (JH2 domain). It leads to constitutive activation of JAK2, hematopoietic transformation in vitro, and erythropoietin-independent erythroid colony (EEC) growth in all patients with PV and a large proportion of ET and PMF patients (7). JAK2 V617F represents a key driver in the transformation of hematopoietic cells in MPN, but the exact pathological mechanisms leading, with the same unique mutation, to such different clinical and biological entities remain to be fully elucidated.

Traditionally, the diagnosis of MPNs was based on clinical, bone marrow histology and cytogenetic criteria. The discovery of a disease-specific molecular marker resulted in both simplification of the process and increased diagnostic accuracy. Detection of the JAK2 V617F mutation is now part of the reference WHO 2008 criteria for the diagnosis of BCR-ABL negative MPN (Table 1), and presence of this mutation is a major criterion for diagnostic confirmation.

Table 1. WHO criteria for the diagnosis of MPN (adapted from reference 8)

Criteria for a diagnosis of polycythemia vera (PV)	
Major	1. Hemoglobin (Hgb) >18.5 g.dl^{-1} (men) or >16.5 g.dl^{-1} (women) or Hgb or hematocrit (Hct) >99th percentile of reference range for age, sex, or altitude of residence or Hgb >17 g.dl^{-1} (men) or >15 g.dl^{-1} (women) if associated with sustained increase of ≥2 g.dl^{-1} from baseline that cannot be attributed to correction of iron deficiency or Elevated red cell mass >25% above mean normal predicted value 2. Presence of *JAK2V617F* or similar mutation
Minor	1. Bone marrow trilineage myeloproliferation 2. Subnormal serum erythropoietin level 3. Endogenous erythroid colony (EEC) growth
Criteria for a diagnosis of essential thrombocythemia (ET)	
Major	1. Platelet count ≥450 x 10^{9} l^{-1} 2. Megakaryocyte proliferation with large and mature morphology. No or little granulocyte or erythroid proliferation 3. Not meeting WHO criteria for chronic myeloid leukemia (CML), PV, primary myelofibrosis (PMF), myelodysplastic syndrome (MDS), or other myeloid neoplasm 4. Demonstration of *JAK2V617F* or other clonal marker or No evidence of reactive thrombocytosis
Minor	-
Criteria for a diagnosis of primary myelofibrosis (PMF)	
Major	1. Megakaryocyte proliferation and atypia accompanied by either reticulin and/or collagen fibrosis or In the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation and often decreased erythropoiesis (i.e. prefibrotic PMF) 2. Not meeting WHO criteria for (CML), PV, MDS, or other myeloid neoplasm 3. Demonstration of *JAK2V617F* or other clonal marker or No evidence of reactive marrow fibrosis
Minor	1. Leukoerythroblastosis 2. Increased serum lactate dehydrogenase (LDH) 3. Anemia 4. Palpable splenomegaly

Recently, international experts have proposed criteria for therapeutic trials in PV and ET. Based on data on allograft, alpha-interferon, or hydroxyurea, JAK2V617F quantification has been incorporated as a potentially useful tool to monitor treatment response (9). A decrease in JAK2 V617F burden has been

observed in response to some of the new anti-JAK2 targeted drugs in clinical development (10).

Principle of the Procedure

Several different techniques have been proposed to quantitatively determine the proportion of single nucleotide polymorphisms (SNPs) in DNA samples. Of these, methods based on real-time quantitative polymerase chain reaction (qPCR) are preferred because of their higher sensitivity allowing monitoring of allele burden in a longitudinal fashion. Many of these techniques have a moderate sensitivity of 1–10%, for example, TaqMan® allelic discrimination, Pyrosequencing®, melting curve assay, and direct sequencing. Some, such as melting curve and sequencing, are only semiquantitative while others, such as Pyrosequencing, require post-PCR processing or need instrumentation that is not readily available or has prohibitively high setup costs for routine laboratory testing. A highly sensitive approach with a sensitivity <0.1% requires the use of a SNP specific primer that permits the selective amplification of the mutant or wild type allele that is easily detectable on a real-time qPCR instrument. The *ipsogen* JAK2 Muta*Quant* Kit is based on this technique.

The use of qPCR permits the accurate quantification of PCR products during the exponential phase of the PCR amplification process. Quantitative PCR data can be rapidly obtained, without post-PCR processing, by real-time detection of fluorescent signals during and/or subsequent to PCR cycling, thereby drastically reducing the risk of PCR product contamination. At present, 3 main types of qPCR techniques are available: qPCR analysis using SYBR® Green I Dye, qPCR analysis using hydrolysis probes, and qPCR analysis using hybridization probes.

This assay exploits the qPCR double-dye oligonucleotide hydrolysis principle. During PCR, forward and reverse primers hybridize to a specific sequence. A double-dye oligonucleotide is contained in the same mix. This probe, which consists of an oligonucleotide labeled with a 5' reporter dye and a downstream, 3'quencher dye, hybridizes to a target sequence within the PCR product. qPCR analysis with hydrolysis probes exploits the 5'→3' exonuclease activity of the *Thermus aquaticus* (*Taq*) DNA polymerase. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence primarily by Förster-type energy transfer.

During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites. The 5'→3' exonuclease activity of the DNA polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are then displaced from the target, and polymerization of the strand continues. The 3' end of the probe is blocked to prevent extension of the probe during PCR (Figure 1). This process occurs in every cycle and does not interfere with the exponential accumulation of product.

The increase in fluorescence signal is detected only if the target sequence is complementary to the probe and hence amplified during PCR. Because of these requirements, nonspecific amplification is not detected. Thus, the increase in fluorescence is directly proportional to the target amplification during PCR.

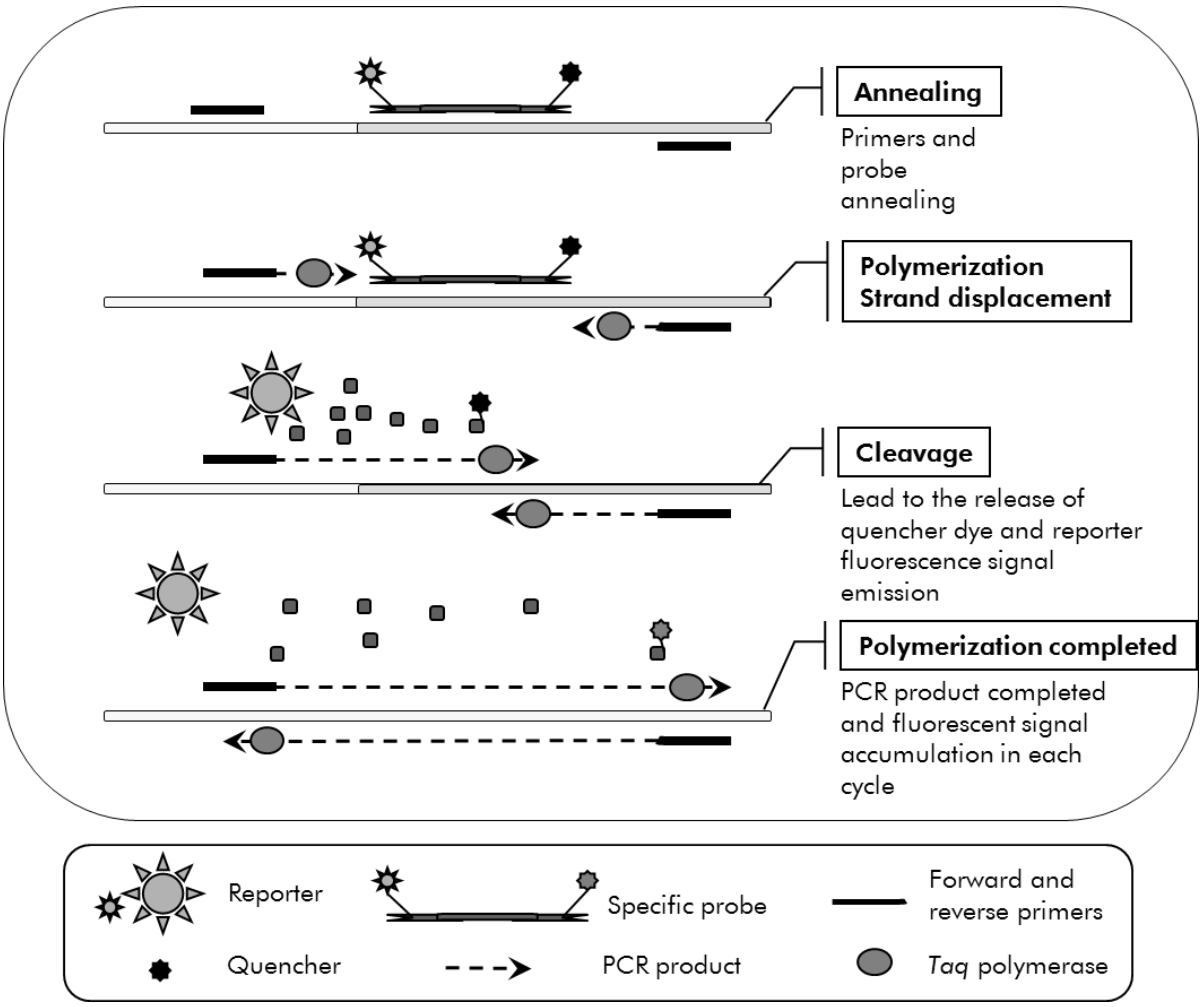

Figure 1. Reaction principle.

The quantitative allele specific PCR technology used in this assay kit allows a sensitive, accurate, and highly reproducible detection of SNPs. This technique is based on the use of specific forward primers, for the wild-type and the V617F allele (11). Only perfect match between primer and target DNA allows extension and amplification in the PCR (Figure 2).

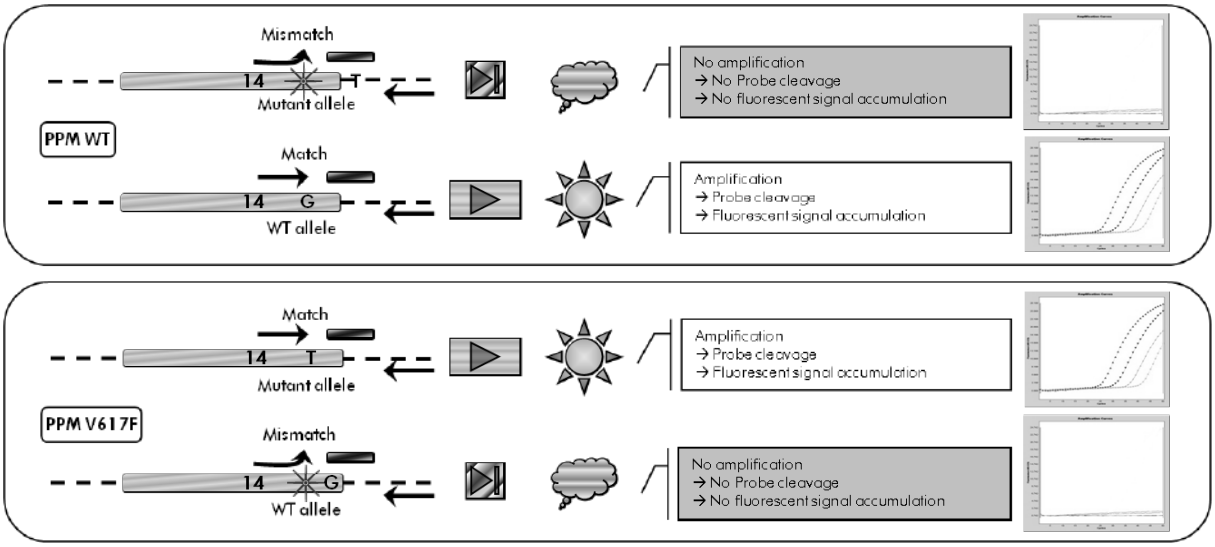

Figure 2. Allele specific PCR. Use of wild-type or the V617F primers and probe mix allows the specific detection of the wild-type or mutated allele in two separate reactions conducted using the same sample. Results can be expressed as percentage of VF copies among total JAK2 copies.

Materials Provided

Kit contents

***ipsogen* JAK2 MutaQ*uant* Kit**		**(12)**	**(24)**
Catalog no.		**673522**	**673523**
Number of reactions		**12**	**24**
V617F positive control (100% V617F allele)	PC-VF-JAK2 PC-VF-JAK2 Mini	40 µl	60 µl
V617F negative control (100% wild-type allele)	NC-VF-JAK2 NC-VF-JAK2 Mini	40 µl	60 µl
M1-VF Standard Dilution, 50 copies (5 x 10^1 V617F copies/5 µl)	M1-VF M1-VF Mini	20 µl	30 µl
M2-VF Standard Dilution, 500 copies (5 x 10^2 V617F copies/5 µl)	M2-VF M2-VF Mini	20 µl	30 µl
M3-VF Standard Dilution, 5000 copies (5 x 10^3 V617F copies/5 µl)	M3-VF M3-VF Mini	20 µl	30 µl
M4-VF Standard Dilution, 50,000 copies (5 x 10^4 V617F copies/5 µl)	M4-VF M4-VF Mini	20 µl	30 µl
WT-1 Standard Dilution, 50 copies (5 x 10^1 wild-type copies/5 µl)	WT-1 WT-1 Mini	20 µl	30 µl
WT-2 Standard Dilution, 500 copies (5 x 10^2 wild-type copies/5 µl)	WT-2 WT-2 Mini	20 µl	30 µl
WT-3 Standard Dilution, 5000 copies (5 x 10^3 wild-type copies/5 µl)	WT-3 WT-3 Mini	20 µl	30 µl
WT-4 Standard Dilution, 50,000 copies (5 x 10^4 wild-type copies/5 µl)	WT-4 WT-4 Mini	20 µl	30 µl
Primers and Probe Mix JAK2 WT*	PPM-JAK2 WT 25x PPM-JAK2 WT Mini 25x	52 µl	95 µl
Primers and Probe Mix JAK2 V617F†	PPM-JAK2 V617F 25x PPM-JAK2 V617F Mini 25x	52 µl	95 µl
ipsogen *JAK2 Muta*Quant *Kit Handbook* (English)		1	1

* Mix of specific reverse and forward primers for the wild-type JAK2 control gene plus a specific FAM™–TAMRA™ probe.

† Mix of specific reverse and forward primers for the JAK2 V617F mutation plus a specific FAM–TAMRA probe.

Note: Vortex and briefly centrifuge the standard dilutions and the primers and probe mixes before use.

Materials Required but Not Provided

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

Reagents

- Nuclease-free PCR grade water
- Buffer and *Taq* DNA polymerase: The validated reagents are TaqMan Universal PCR Master Mix (Master Mix PCR 2x) (Thermo Fisher Scientific, cat. no. 4304437) and LightCycler TaqMan Master (Master Mix PCR 5x) (Roche, cat. no. 04535286001) or LightCycler FastStart DNA Master^{PLUS} HybProbe® (Master Mix 5x) (Roche, cat. No. 03515567001)

Consumables

- Nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters
- 0.5 ml or 1.5 ml nuclease-free PCR tubes
- Ice

Equipment

- Microliter pipet* dedicated for PCR (1–10 µl; 10–100 µl; 100–1000 µl)
- Benchtop centrifuge* with rotor for 0.5 ml/1.5 ml reaction tubes and microplates (capable of attaining 13,000–14,000 rpm)
- Real-time PCR instrument:* Rotor-Gene Q 5plex HRM or other Rotor-Gene; LightCycler 1.2, or 480; ABI PRISM 7900HT SDS; Applied Biosystems 7500 Real-Time PCR System; and associated specific material
- Biophotometer

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Warnings and Precautions

For in vitro diagnostic use

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

Discard sample and assay waste according to your local safety regulations.

General precautions

Use of qPCR tests require good laboratory practices, including maintenance of equipment, that are dedicated to molecular biology and is compliant with applicable regulations and relevant standards.

This kit is intended for in vitro diagnostic use. Reagents and instructions supplied in this kit have been validated for optimal performance. Further dilution of the reagents or alteration of incubation times and temperatures may result in erroneous or discordant data. PPM-WT and PPM-VF reagents may be altered if exposed to light. All reagents are formulated specifically for use with this test. For optimal performance of the test, no substitutions should be made.

Use extreme caution to prevent:

- DNase contamination that might cause degradation of the template DNA
- DNA or PCR carryover contamination resulting in false positive signal

We therefore recommend the following.

- Use nuclease-free labware (e.g., pipets, pipet tips, reaction vials) and wear gloves when performing the assay.
- Use fresh aerosol-resistant pipet tips for all pipetting steps to avoid cross-contamination of the samples and reagents.
- Prepare pre-PCR master mix with dedicated material (pipets, tips, etc.) in a dedicated area where no DNA matrixes (DNA, plasmid, or PCR products) are introduced. Add template in a separate zone (preferably in a separate room) with specific material (pipets, tips, etc.).

Reagent Storage and Handling

The kits are shipped on dry ice and must be stored at –15°C to –30°C upon receipt.

- Minimize exposure to light of the primers and probe mixes (PPM-WT and PPM-VF tubes).

- Gently mix and centrifuge the tubes before opening.
- Store all kit components in original containers.

These storage conditions apply to both opened and unopened components. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

Expiration dates for each reagent are indicated on the individual component labels. Under correct storage conditions, the product will maintain performance until the expiration date printed on the label.

There are no obvious signs to indicate instability of this product. However, positive and negative controls should be run simultaneously with unknown specimens.

Procedure

Sample DNA preparation

Genomic DNA should be obtained either from whole blood, purified peripheral blood lymphocytes of whole blood, polynuclear cells, or granulocytes. For comparable results it is recommended that the same cellular fraction and DNA extraction method are used. DNA extraction can be performed using a home-brew method or a commercially available kit.

DNA quantity should be determined by measuring the optical density (OD) of the sample at 260 nm and DNA quality can be determined either by spectrophotometry or gel* electrophoresis.

- The OD_{260}/OD_{280} ratio should be 1.7–1.9 and smaller ratios than this may indicate protein contamination or the presence of organic chemicals.
- Electrophoretic analysis on a 0.8–1.0% agarose gel* should allow the visualization of the isolated DNA as a distinct band of approximately 20 kb (a slight smear will give acceptable results).

The resultant DNA will need to be diluted to a concentration of 5 ng/µl in 1x TE buffer* at pH 8.0 and then stored at +4 to +8°C for 1 week or at –20°C if longer term storage is required.

The qPCR reaction is optimized for DNA samples containing 25 ng purified genomic DNA.

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

Protocol: qPCR on Rotor-Gene Q MDx 5plex HRM or Rotor-Gene Q 5plex HRM instruments with 72-tube rotor

Using this instrument, we recommend performing all measurements in duplicate, as indicated in Table 2.

Table 2. Number of reactions for Rotor-Gene Q instruments with 72-tube rotor

Samples	Reactions
With the JAK2 V617F primers and probe mix (PPM-VF)	
4 M-VF standards	8 reactions, each one tested in duplicate
n DNA samples	n x 2 reactions
2 DNA controls	4 reactions: positive control (PC-VF) and negative control (NC-VF), each one tested in duplicate
Water control	2 reactions
With the JAK2 wild-type primers and probe mix (PPM-WT)	
4 wild-type standards	8 reactions, each one tested in duplicate
n DNA samples	n x 2 reactions
2 DNA controls	4 reactions: PC-VF and NC-VF, each one tested in duplicate
Water control	2 reactions

Sample processing on Rotor-Gene Q instruments with 72-tube rotor

We recommend testing at least eight DNA samples with the 24 reaction kit (cat. no. 673523) and at least six DNA samples with the 12 reaction kit (cat. no. 673522) in the same experiment to optimize the use of the standards and the primers and probe mixes.

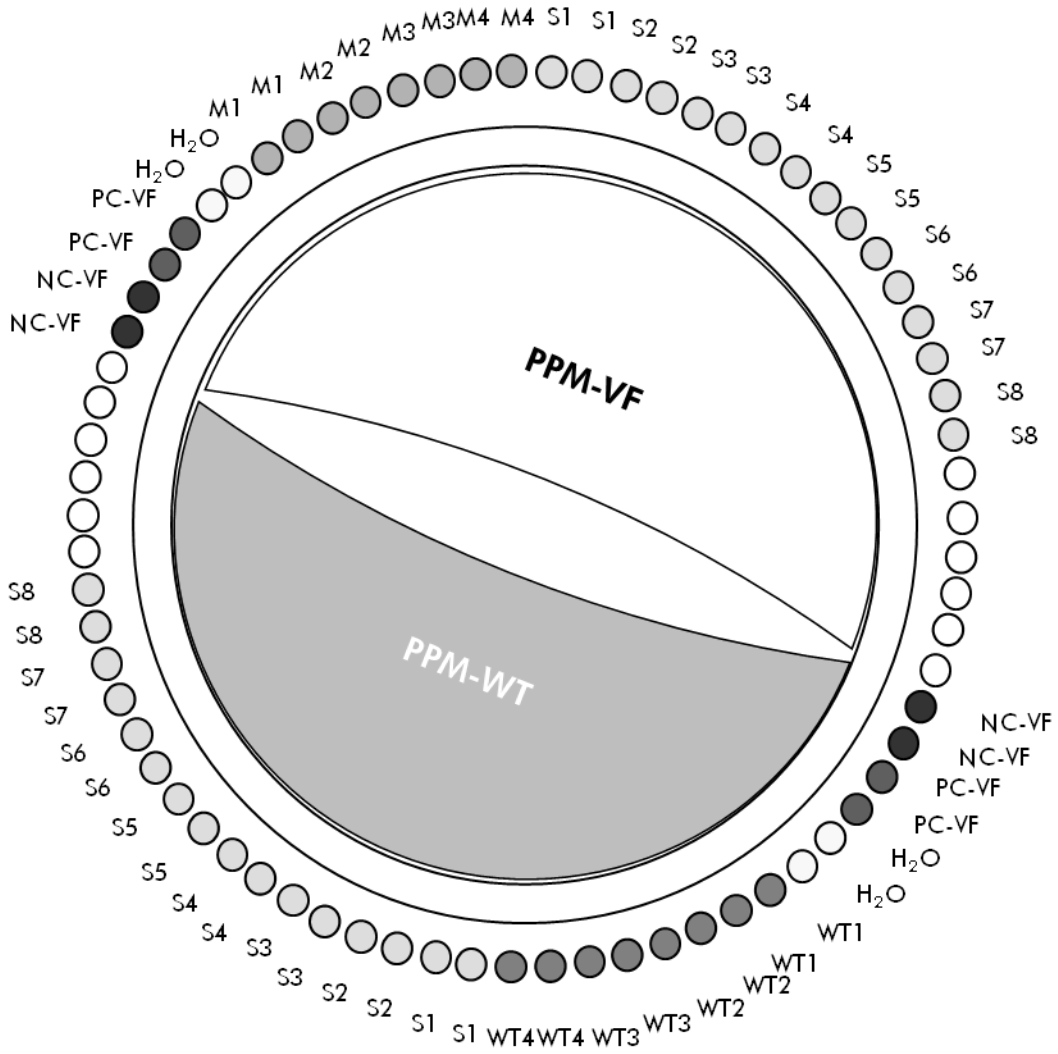

Figure 3. Suggested rotor setup for each experiment with the *ipsogen* 24 sample JAK2 *MutaQuant* Kit. PC-VF: V617F positive control; **NC-VF**: V617F negative control; **M-VF**: V617F standards; **M-WT**: wild-type standards; **S**: DNA sample; **H_2O**: water control.

Note: Take care to always place a sample to be tested in position 1 of the rotor. Otherwise, during the calibration step, the instrument will not perform calibration, and incorrect fluorescence data will be acquired.

Fill all other positions with empty tubes.

qPCR on Rotor-Gene Q instruments with 72-tube rotor

Note: Perform all steps on ice.

Procedure

1. **Thaw all necessary components and place them on ice.**
2. **Prepare the following qPCR mix according to the number of samples being processed.**

   All concentrations are for the final volume of the reaction.

Table 3 and 4 describe the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 µl. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix (either PPM-VF or PPM-WT). Extra volumes are included to compensate for pipetting error.

Table 3. Preparation of qPCR mix

Component	1 reaction (µl)	V617F pre-mix 30 + 1 reactions (µl)	Final concentration
TaqMan Universal PCR Master Mix, 2x	12.5	387.5	1x
Primers and probe mix, PPM-VF 25x	1.0	31	1x
Nuclease-free PCR-grade water	6.5	201.5	–
Sample (to be added at step 4)	5.0	5 each	–
Total volume	25.0	25 each	–

Table 4. Preparation of qPCR mix

Component	1 reaction (µl)	WT pre-mix 30 + 1 reactions (µl)	Final concentration
TaqMan Universal PCR Master Mix, 2x	12.5	387.5	1x
Primers and probe mix, PPM-WT 25x	1.0	31	1x
Nuclease-free PCR-grade water	6.5	201.5	–
Sample (to be added at step 4)	5.0	5 each	–
Total volume	25.0	25 each	–

3. **Dispense 20 µl of the qPCR pre-mix (VF or WT) per tube.**
4. **Add 5 µl of the material to be quantified (25 ng sample genomic DNA or control) in the corresponding tube (total volume 25 µl).**
5. **Mix gently, by pipetting up and down.**
6. **Place the tubes in the thermal cycler according to the manufacturer recommendations.**
7. **Program the Rotor-Gene Q instrument with the thermal cycling program as indicated in Table 5.**

Table 5. Temperature profile

Mode of analysis	Quantitation
Hold	Temperature: 50 deg Time: 2 mins
Hold 2	Temperature: 95°C Time: 10 mins
Cycling	50 times 95°C for 15 secs 62°C for 1 min with acquisition of FAM fluorescence in channel Green: Single

8. **For Rotor-Gene Q instruments, select "Slope Correct" for the analysis. We recommend setting the threshold at 0.03. Start the thermal cycling program, as indicated in Table 5.**

Protocol: qPCR on ABI PRISM 7900HT SDS, Applied Biosystems 7500 Real-Time PCR System, and LightCycler 480 instrument

Using 96-well-plate qPCR equipment, we recommend performing all measurements in duplicate, as indicated in Table 6.

Table 6. Number of reactions using 96-well-plate qPCR equipment

Samples	Reactions
With the JAK2 V617F primers and probe mix (PPM-VF)	
4 M-VF standards	8 reactions, each one tested in duplicate
n DNA samples	n x 2 reactions
2 DNA controls	4 reactions: PC-VF and NC-VF, each one tested in duplicate
Water control	2 reactions
With the JAK2 wild-type primers and probe mix (PPM-WT)	
4 wild-type standards	8 reactions, each one tested in duplicate
n DNA samples	n x 2 reactions
2 DNA controls	4 reactions: PC-VF and NC-VF, each one tested in duplicate
Water control	2 reactions

Sample processing on ABI PRISM 7900HT SDS, Applied Biosystems 7500 Real-Time PCR System, and LightCycler 480 instrument

We recommend testing eight DNA samples with the 24 reaction kit (cat. no. 673523) and at least six DNA samples with the 12 reaction kit (cat. no. 673522) in the same experiment to optimize the use of the standards and the primers and probe mixes.

The plate scheme in Figure 4 shows an example of such an experiment using the 24 reaction kit (cat. no. 673523), and Figure 5 shows an example of such an experiment using the 12 reaction kit (cat. no. 673522).

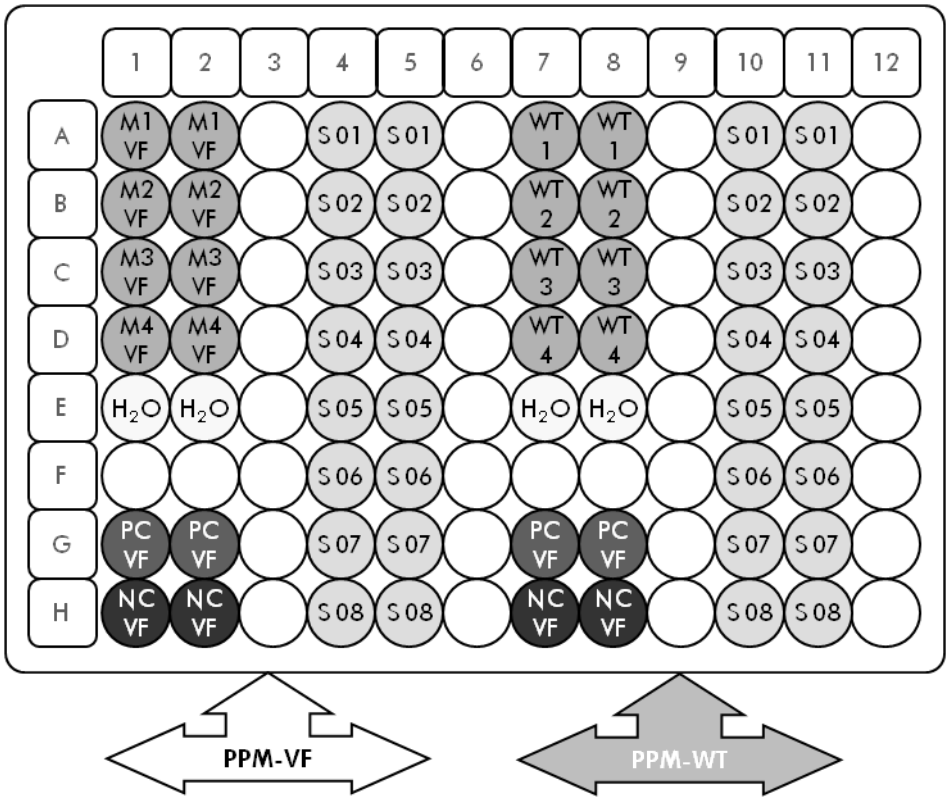

Figure 4. Suggested plate setup for one experiment using the 24 reaction kit (catalog no. 673523). PC-VF: V617F positive control; **NC-VF**: V617F negative control; **M-VF**: V617F standards; **M-WT**: wild-type standards; **S**: DNA sample; **H_2O**: water control

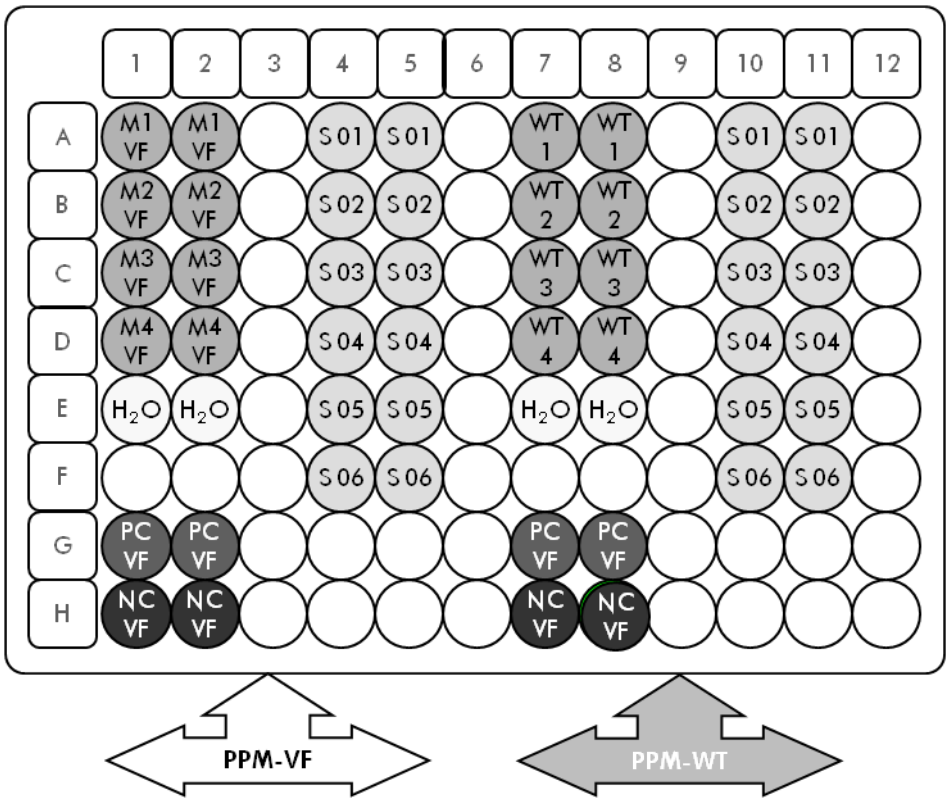

Figure 5. Suggested plate setup for one experiment using the 12 reaction kit (catalog no. 673522). PC-VF: V617F positive control; **NC-VF**: V617F negative control; **M-VF**: V617F standards; **M-WT**: wild-type standards; **S**: DNA sample; **H_2O**: water control

qPCR on ABI PRISM 7900HT SDS, Applied Biosystems 7500 Real-Time PCR System, and LightCycler 480 instrument

Note: Perform all steps on ice.

Procedure

1. **Thaw all necessary components and place them on ice.**
2. **Prepare the following qPCR mix according to the number of samples being processed.**

   All concentrations are for the final volume of the reaction.

   Table 7 and 8 describe the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 µl. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix (either PPM-VF or PPM-WT). Extra volumes are included to compensate for pipetting error.

Table 7. Preparation of qPCR mix

	V617F pre-mix			
Component	**1 reaction (µl)**	**26 + 1 reactions (µl)**	**30 + 1 reactions (µl)**	**Final concentration**
TaqMan Universal PCR Master Mix, 2x	12.5	337.5	387.5	1x
Primers and probe mix, PPM-VF 25x	1.0	27	31	1x
Nuclease-free PCR-grade water	6.5	175.5	201.5	–
Sample (to be added at step 4)	5.0	5 each	5 each	–
Total volume	25.0	25 each	25 each	–

Table 8. Preparation of qPCR mix

	WT pre-mix			
Component	**1 reaction (µl)**	**26 + 1 reactions (µl)**	**30 + 1 reactions (µl)**	**Final concentration**
TaqMan Universal PCR Master Mix, 2x	12.5	337.5	387.5	1x
Primers and probe mix, PPM-WT 25x	1.0	27	31	1x
Nuclease-free PCR-grade water	6.5	175.5	201.5	–
Sample (to be added at step 4)	5.0	5 each	5 each	–
Total volume	25.0	25 each	25 each	–

3. **Dispense 20 µl of the qPCR pre-mix (VF or WT) per well.**
4. **Add 5 µl of the material to be quantified (25 ng sample genomic DNA or control) in the corresponding well (total volume 25 µl).**
5. **Mix gently, by pipetting up and down.**
6. **Close the plate and briefly centrifuge (300 x *g*, approximately 10 seconds).**
7. **Place the plate in the thermal cycler according to the manufacturer recommendations.**
8. **Program the thermal cycler with the thermal cycling program and set the instrument for the acquisition of dual labeled FAM fluorescent probe as indicated in Table 9 for ABI PRISM 7900HT SDS and Applied Biosystems 7500 Real-Time PCR System, or Table 10 for the LightCycler 480 instrument.**

Table 9. Temperature profile for ABI PRISM 7900HT SDS and Applied Biosystems 7500 Real-Time PCR System

Mode of analysis	Standard Curve – Absolute Quantitation
Hold	Temperature: 50°C Time: 2 minutes
Hold 2	Temperature: 95°C Time: 10 minutes
Cycling	50 times 95°C for 15 seconds 63°C for 1 minute 30 seconds with acquisition of FAM fluorescence; quencher: TAMRA

Table 10. Temperature profile for LightCycler 480 instrument

Mode of analysis	Absolute Quantification ("Abs Quant")
Detection formats	Select "Simple Probe" in the Detection formats window
Hold	Temperature: 50°C Time: 2 minutes
Hold 2	Temperature: 95°C Time: 10 minutes
Cycling	50 times 95°C for 15 seconds 63°C for 1 minute 30 seconds with acquisition of FAM fluorescence corresponding to (483–533 nm) for LC version 01 and (465–510 nm) for LC version 02

9. For the ABI PRISM 7900HT and Applied Biosystems 7500 Real-Time PCR System, follow step 8a. For the LightCycler 480 instrument, follow step 8b.

9a. ABI PRISM 7900HT and Applied Biosystems 7500 Real-Time PCR System: We recommend a threshold set at 0.1. Start the cycling program, as indicated in Table 9.

9b. LightCycler 480: We recommend a Fit point analysis mode with background at 2.0 and threshold at 2.0. Start the thermal cycling program, as indicated in Table 10.

Protocol: qPCR on LightCycler 1.2 instrument

Using capillary instruments, we recommend measuring samples in duplicate and controls only once, as indicated in Table 11.

Table 11. Number of reactions for LightCycler 1.2 instrument

Samples	Reactions
With the JAK2 V617F primers and probe mix (PPM-VF)	
4 M-VF standards	4 reactions, each tested once
n DNA samples	n x 2 reactions
2 DNA controls	2 reactions: PC-VF and NC-VF, each tested once
Water control	1 reaction
With the JAK2 wild-type primers and probe mix (PPM-WT)	
4 wild-type standards	4 reactions, each one tested once
n DNA samples	n x 2 reactions
2 DNA controls	2 reactions: PC-VF and NC-VF, each one tested once
Water control	1 reaction

Sample processing on LightCycler 1.2 instrument

We recommend testing four DNA samples in the same experiment to optimize the use of the standards and primers and probe mixes. The capillary scheme in Figure 6 shows an example of an experiment.

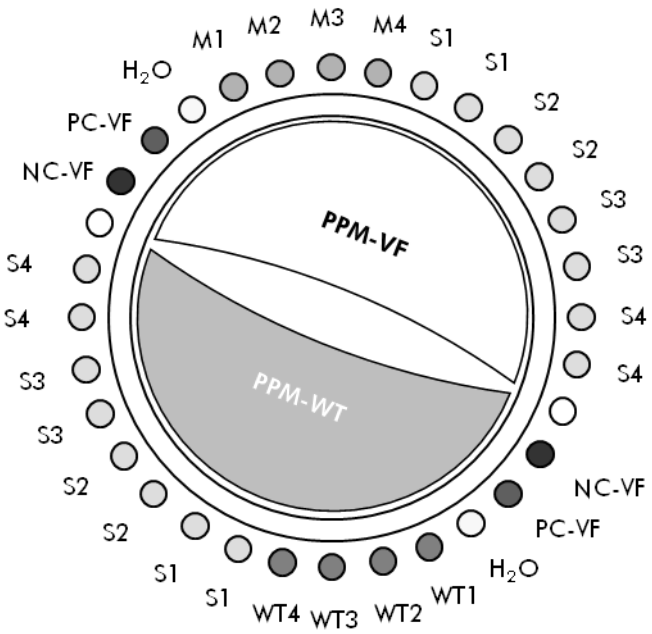

Figure 6. Suggested rotor setup for each experiment with the *ipsogen* JAK2 MutaQ*uant* Kit. PC-VF: V617F positive control; **NC-VF**: V617F negative control; **M-VF**: V617F standards; **M-WT**: wild-type standards; **S**: DNA sample; **H_2O**: water control**.**

qPCR on LightCycler 1.2 instrument

Note: Because of particular technological requirements, LightCycler experiments must be performed using specific reagents. We recommend the use of LightCycler FastStart DNA Master^{PLUS} HybProbe and following the manufacturer's instructions to prepare the Master Mix 5x.

Note: Perform all steps on ice.

Procedure

1. **Thaw all necessary components and place them on ice.**
2. **Prepare the following qPCR mix according to the number of samples being processed.**

   All concentrations are for the final volume of the reaction.

   Table 12 and 13 describe the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 20 µl. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix (either PPM-VF or PPM-WT). Extra volumes are included to compensate for pipetting error.

Table 12. Preparation of qPCR mix

Component	1 reaction (µl)	V617F pre-mix 15 + 1 reactions (µl)	Final concentration
Freshly prepared LightCycler FastStart DNA Master^{PLUS} HybProbe Mix, 5x	4.0	64.0	1x
Primers and probe mix, PPM-VF 25x	0.8	12.8	1x
Nuclease-free PCR grade water	10.2	163.2	–
Sample (to be added at step 4)	5.0	5 each	–
Total volume	20.0	20 each	–

Table 13. Preparation of qPCR mix

Component	1 reaction (µl)	WT pre-mix 15 + 1 reactions (µl)	Final concentration
Freshly prepared LightCycler FastStart DNA Master^{PLUS} HybProbe Mix, 5x	4.0	64.0	1x
Primers and probe mix, PPM-WT 25x	0.8	12.8	1x
Nuclease-free PCR grade water	10.2	163.2	–
Sample (to be added at step 4)	5.0	5 each	–
Total volume	20.0	20 each	–

3. Dispense 15 µl of the qPCR pre-mix (VF or WT) per capillary.

4. **Add 5 µl of the material to be quantified (25 ng sample genomic DNA or control) in the corresponding tube (total volume 20 µl).**
5. **Mix gently, by pipetting up and down.**
6. **Place the capillaries in the adapters provided with the apparatus, and briefly centrifuge (700 x *g*, approximately 10 seconds).**
7. **Load the capillaries into the thermal cycler according to the manufacturer recommendations.**
8. **Program the LightCycler 1.2 instrument with the thermal cycling program as indicated in Table 14.**

Table 14. Temperature profile

Mode of analysis	Quantification
Hold 1	Temperature: 55°C Time: 2 minutes Ramp: 20
Hold 2	Temperature: 95°C Time: 10 minutes Ramp: 20
Cycling	50 times 95°C for 15 seconds; ramp: 20 66°C for 1 minute; ramp: 20; with acquisition of FAM fluorescence: Single

9. **For the LightCycler 1.2, the F1/F2 and "2nd derivative analysis" mode is recommended. Start the thermal cycling program, as indicated in Table 14.**

Interpretation of Results

Data analysis principle

Data for the threshold cycle (C_T) and crossing point (C_P) values can be exported from the qPCR instrument and pasted into an Excel® file for analysis. These values can then be used to calculate the mean value for C_P and C_T and the standard mean C_T values can be plotted to obtain a standard curve for both the wild-type and V617F standards using the following equation and Table 15.

y = Mean C_P; x = $\log_{10}$ CN where CN= gene copy number in the 5 µl sample

Table 15. Quantitative data for the wild-type and V617F standards

Standard	Copy number (CN)	$\log_{10}$ CN
M1-VF	5×10^1 VF	1.7
M2-VF	5×10^2 VF	2.7
M3-VF	5×10^3 VF	3.7
M4-VF	5×10^4 VF	4.7
WT-1	5×10^1 WT	1.7
WT-2	5×10^2 WT	2.7
WT-3	5×10^3 WT	3.7
WT-4	5×10^4 WT	4.7

Note: Each user should measure their own reproducibility in their laboratory.

Standard curve and quality criteria

Figures 7 and 9 show examples of results obtained with *ipsogen* JAK2 Muta*Quant* Kit and Figures 8 and 10 show an example of the theoretical curve calculated on four standard dilutions.

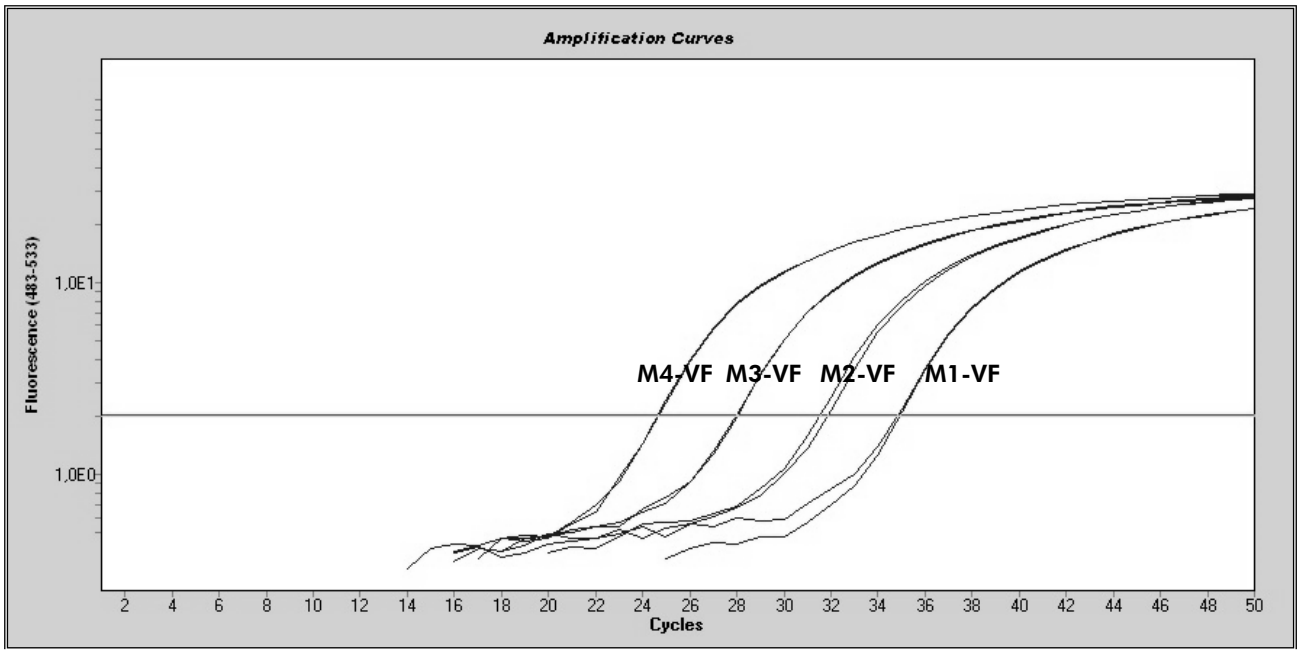

Figure 7. Amplification plot of 5 x 10^1, 5 x 10^2, 5 x 10^3, and 5 x 10^4 copies of the JAK2 V617F plasmid (controls M1-VF, M2-VF, M3-VF, M4-VF, respectively).

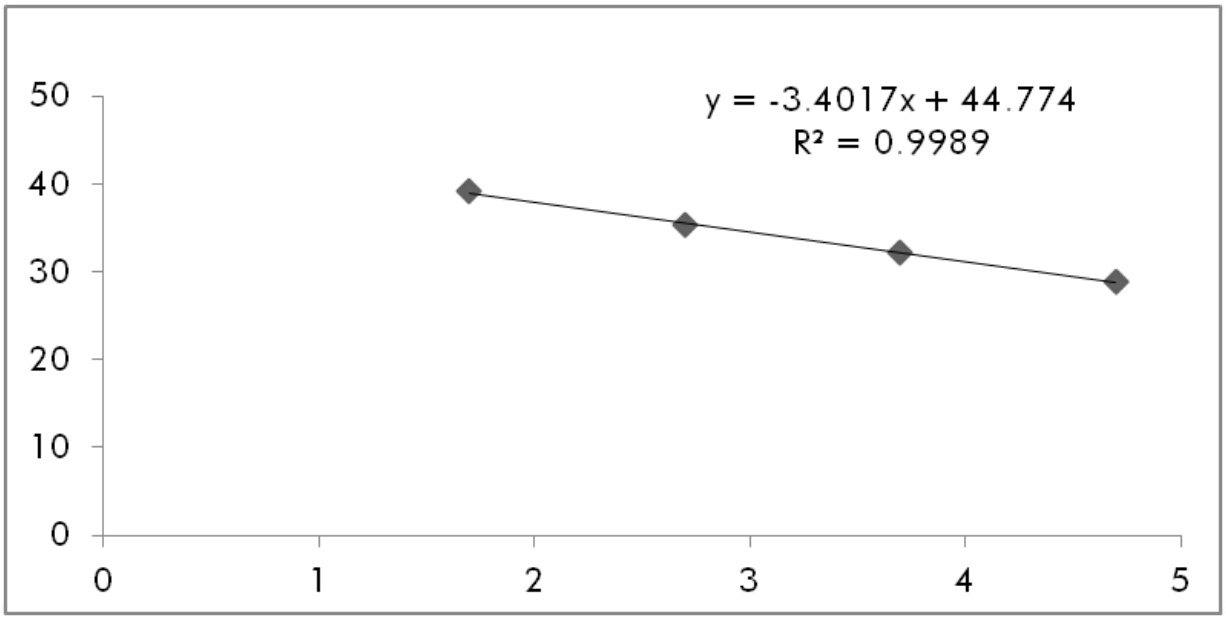

Figure 8. Standard curve for JAK2 V617F.

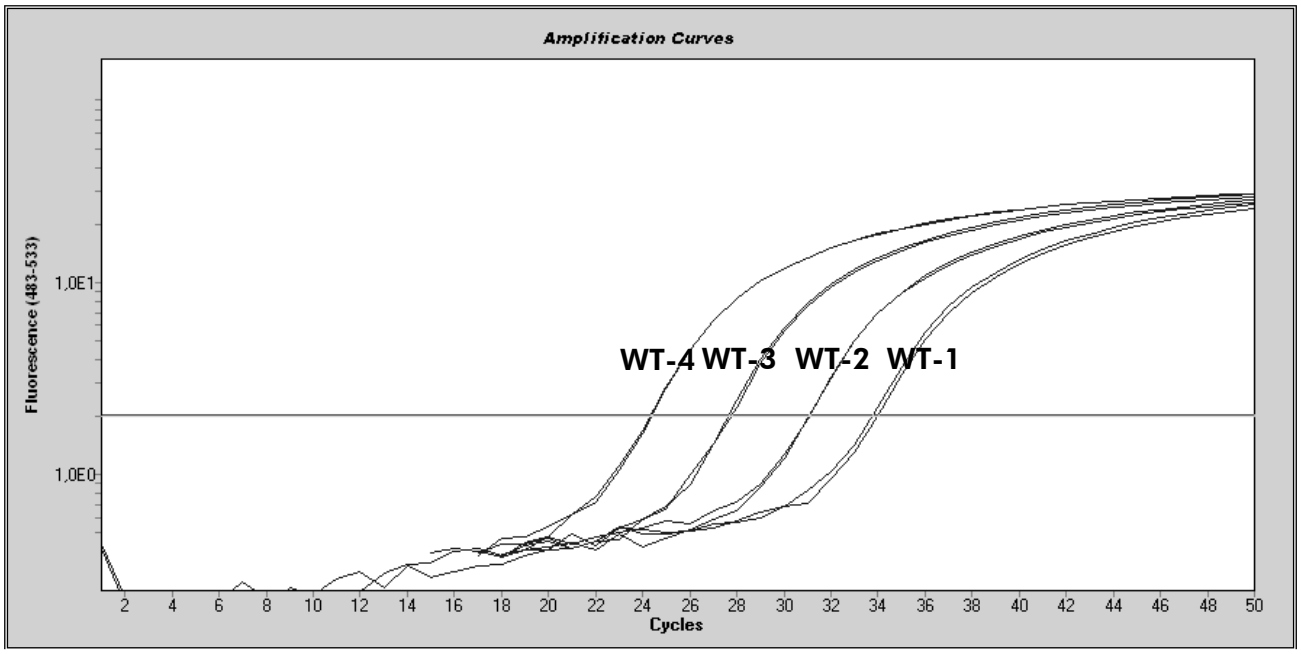

Figure 9. Amplification plot of 5 x 10^1, 5 x 10^2, 5 x 10^3, and 5 x 10^4 copies of the JAK2 wild-type plasmid (controls WT-1, WT-2, WT-3, and WT-4, respectively).

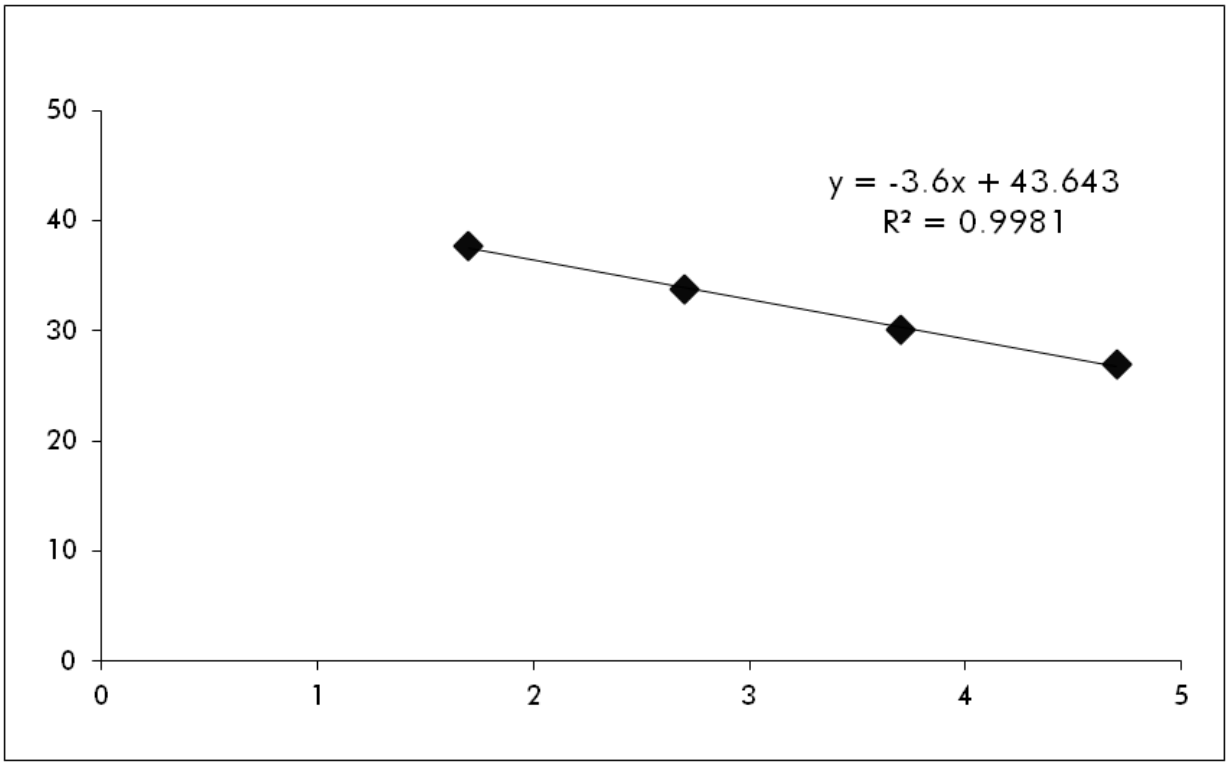

Figure 10. Standard curve for JAK2 wild-type.

As standards are 10-fold dilutions, the theoretical slope of the curve is –3.32. A slope between –3.0 and –3.9 is acceptable as long as R^2 is >0.95 (12). However, a value for R^2 >0.98 is desirable for precise results (13).

The standard curve equations can then be used to calculate V617F and WT $\log_{10}$ copy numbers in the unknown samples.
The V617F standard curve equation should be used to transform raw C_P/ C_T value means (obtained with PPM-VF) for the unknown and control samples, into JAK2 V617F copy numbers (CN_{V617F}).

$$\log_{10} CN_{V617F} = \frac{(\text{Mean } C_{pV617F} - \text{Standard curve intercept}_{V617F})}{\text{Standard curve slope}_{V617F}}$$

The wild-type standard curve equation should be used to transform raw mean C_P/C_T value (obtained with PPM-WT) for the unknown and control samples, into JAK2 wild-type copy numbers (CN_{WT}).

$$\log_{10} CN_{WT} = \frac{(\text{Mean } C_{pWT} - \text{Standard curve intercept}_{WT})}{\text{Standard curve slope}_{WT}}$$

Expression of the results

Results are relative to 25 ng of total genomic DNA and should be expressed as the percentage of JAK2 V617F as follows.

$$\text{JAK2 V617F \%} = \frac{CN_{V617F}}{(CN_{V617F} + CN_{WT})} \times 100$$

Reproducibility between replicates

The data obtained should be consistent between duplicates.

Positive and negative controls

The positive control or PC-VF should give a JAK2 V617F percentage that is higher than 99.9%.

The negative control or NC-VF should give a JAK2 V617F percentage that is lower than 0.1%.

If these controls fail to function correctly, please see the "Troubleshooting guide", page 32, to find a solution.

Water controls

Negative controls should give zero CN for both the JAK2 V617F and JAK2 wild-type detection.

A positive water control results from a cross-contamination. See "Troubleshooting guide", below, to find a solution.

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 protocol in this handbook or sample and assay technologies (for contact information, see "Contact Information", page 41).

	Comments and suggestions
Standard curve for wild-type or V617F are not linear	
Vial inversion, inversion during distribution, cross-contamination, partial degradation of the standard, RQPCR reagent, non-specific amplification, or PCR program error	Check pipetting scheme and the setup of the reaction. Store the *ipsogen* JAK2 Muta*Quant* Kit at –15 to –30°C and keep primers and probe mixes) protected from light. See "Reagent Storage and Handling", page 11. Avoid repeated freezing and thawing.
No or low signal for one standard	
Standard not distributed, or use of same PPM mix	Check pipetting scheme and the setup of the reaction. Repeat the PCR run.

	Comments and suggestions
Negative (H_2O) control is positive	
Cross-contamination, reagent contamination, instrument error, well or capillary inversion, or probe degradation	Replace all critical reagents. Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carry over contamination. Keep primers and probe mixes protected from light. Check for false positives on fluorescence curves.
No signal, even in standard control	
a) Incorrect detection channel has been chosen	Set channel to F1/F2 or 530 nm/640 nm.
b) Pipetting error or omitted reagents	Check pipetting scheme and the setup of the reaction. Repeat the PCR run.
c) No data acquisition program	Check the cycle program. Select acquisition mode "Single" at the end of each annealing segment of the PCR program.
Absent or low signal in samples but standard controls okay	
Inhibitory effects of sample material caused by insufficient purification	Always check the DNA quality (OD_{260}/OD_{280}) and concentration before starting. Repeat DNA preparation.
Fluorescence intensity too low	
a) Inappropriate storage of kit components	Aliquot reagents for storage. Store the *ipsogen* JAK2 MutaQ*uant* Kit at –15 to –30°C and keep primers and probe mixes protected from light. See "Reagent Storage and Handling", page 11. Avoid repeated freezing and thawing.

	Comments and suggestions
b) Very low initial amount of target DNA	Check the amount of the sample DNA. **Note**: Depending of the chosen method of DNA preparation, inhibitory effects may occur.
Negative controls are positive	
Carry over contamination	Replace all critical reagents. Repeat the experiment with new aliquots of all reagents. Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carry-over contamination.
Fluorescence intensity varies	
a) Pipetting error	Vortex and spin all reagents after thawing. LightCycler variability caused by so-called "pipetting error" can be reduced by analyzing data in the F1/F2 or 530 nm/640 nm mode.
b) Insufficient centrifugation of the plate, tubes, or capillaries, or the prepared PCR mix may still be in the upper vessel of the capillary, or an air bubble could be trapped in the capillary tip	Always centrifuge capillaries loaded with the reaction mix as described in the specific operating manual of the apparatus.
c) Outer surface of the capillary tip dirty	Always wear gloves when handling the capillaries.

	Comments and suggestions
Wild-type or V617F positive controls signal using the reciprocal PPM	
Cross-contamination, reagent contamination, or well or capillary inversion	Replace all critical reagents. Repeat the experiment with new aliquots of all reagents. Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carry-over contamination. Check pipetting scheme and setup of the reaction.
Inverted detection of positive control	
Distributed inversion of PPM in well or capillary or in premix	Check pipetting scheme and setup of the reaction.
No signal for one positive control or both	
PPM or control DNA omitted	Check pipetting scheme and setup of the reaction.
High background	
Fluorophore bleaching	Store and handle probe protected from light.
Poor reproducibility for the duplicate samples	
Pipetting error or cross-contamination	Check pipetting scheme and the setup of the reaction.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *ipsogen* JAK2 MutaQ*uant* Kit is tested against predetermined specifications to ensure consistent product quality. Certificates of analysis are available on request at **www.qiagen.com/support/**.

Limitations

The users must be trained and familiar with this technology prior the use of this device. This kit should be used following the instructions given in this manual, in combination with a validated instrument mentioned in "Materials Required but Not Provided", page 10.

Any diagnostic results generated must be interpreted in conjunction with other clinical or laboratory findings. It is the user's responsibility to validate system performance for any procedures used in their laboratory that are not covered by the QIAGEN performance studies.

Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.

Performance Characteristics

Nonclinical studies

Precision

A precision study was conducted using 12 DNA samples extracted from cell lines that corresponded to different JAK2 V617F allele burdens. A total of 80 measurements were performed on each sample, using 3 different batches of the *ipsogen* JAK2 MutaQ*uant* Kit. This precision study utilized an Applied Biosystems 7500 Real-Time PCR System.

Analytical data are summarized in Table 15.

Table 15. Precision data DNA samples

Sample	Theoretical JAK2 V617F (%)	n*	Mean (%)	CV (%)	Percentile 5	Percentile 95
A	0	73	0.004	117.5	0.000	0.015
B	0.05	80	0.101	89.2	0.003	0.284
C	0.5	79	0.449	61.6	0.161	0.950
D	1	68	1.169	41.6	0.611	1.998
E	2	80	2.046	33.5	1.168	3.185
F	4	80	3.733	30.6	2.120	5.560
G	5	77	5.246	22.4	3.647	7.309
H	12.5	80	16.633	16.6	12.792	22.335
I	31	80	28.602	14.8	22.705	34.773
J	50	76	56.181	6.6	50.024	63.724
K	78	80	80.153	3.8	75.352	85.551
L	100	70	99.998	0.003	99.992	100.000

* Outlying values were excluded. These were defined as values smaller than the lower quartile minus 3 times the interquartile range or larger than the upper quartile plus 3 times the interquartile range on a Box and Whisker plot.

n = number of validated samples; CV = global coefficient of variation.

Limit of blank and limit of detection

The background level or level of blank (LOB) was determined on negative samples (8 samples, 76 measurements). This was found to be 0.014%.

The limit of detection (LOD) was determined using samples that were known to be positive but with low expression (7 samples, 68 measurements). This was found to be 0.061%, with a 90% confidence interval upper bound at 0.091%.

This optimal sensitivity can be obtained on specimens containing at least 10,000 copies of the JAK2 gene (wild-type or V617F mutation).

Quantification data should be reported as follows.

- JAK2 V617F ≤0.014% can be interpreted as the JAK2 V617F mutation was not detected.
- JAK2 V617F is >0.014% but <0.091% can be interpreted as an inconclusive result.
- JAK2 V617F ≥0.091% can be interpreted as a positive result and that the JAK2 V617F mutation has been detected.

Linearity

Linearity studies were performed on 12 samples, with each one obtained from a different mix of DNA extracted from cell lines that were positive and negative for the JAK2 V617F mutation. Each sample was tested 5 times. Data from this study showed that the *ipsogen* JAK2 Muta*Quant* Kit gave linear results across the dynamic range.

Clinical studies

DNA from blood or bone marrow were extracted from 87 patient samples and analyzed using the *ipsogen* JAK2 Muta*Quant* Kit. In addition, the percentage of JAK2 V617F mutations were quantified and compared with screening test results obtained with the *ipsogen* JAK2 Muta*Screen* EZ Kit (cat. no. 673223). Data obtained are shown in Table 16.

Table 16. Contingency table showing the agreement between results obtained with the *ipsogen* JAK2 MutaQ*uant* Kit and the *ipsogen* JAK2 MutaS*creen* EZ Kit

		Results from *ipsogen* JAK2 MutaS*creen* EZ Kit			
		Mutation detected	Inconclusive result	Mutation undetected	n
Results from *ipsogen* JAK2 MutaQ*uant* Kit	Mutation detected	40	2	7	49
	Inconclusive result	0	0	21	21
	Mutation undetected	0	0	17	17
	n	40	2	45	87
Positive agreement	100% (95% confidence interval: 91%, 100%)				
Negative agreement	71% (95% confidence interval: 51%, 85%)				
Overall agreement	89% (95% confidence interval: 79%, 95%)				

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Symbols

The following symbols may appear on the packaging and labeling:

	Contains reagents sufficient for <N> reactions
	Use by
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Material number
	Global Trade Item Number
	Temperature limitation
	Manufacturer
	Consult instructions for use

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
ipsogen JAK2 MutaQ*uant* Kit (12)	For 12 reactions: Wild-type JAK2 Gene Control, JAK2 V617F Control Gene, Primers and Probe Mix PPM-WT, Primers and Probe Mix PPM-VF	673522
ipsogen JAK2 MutaQ*uant* Kit (24)	For 24 reactions: Wild-type JAK2 Gene Control, JAK2 V617F Control Gene, Primers and Probe Mix PPM-WT, Primers and Probe Mix PPM-VF	673523
Rotor-Gene Q MDx – for IVD-validated real-time PCR analysis in clinical applications		
Rotor-Gene Q MDx 5plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9002032
Rotor-Gene Q MDx 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9002033

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.

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Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the *ipsogen* JAK2 MutaQ*uant* Kit to the following terms:

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January 2020

ipsogen® MPL W515L/K MutaScreen Handbook

For research use only. Not for use in diagnostic procedures.

For use with Rotor-Gene® Q 5plex HRM, ABI PRISM® 7000, 7300, 7700 and 7900HT SDS, Applied Biosystems® 7500 Real-Time PCR System, LightCycler® 480 and 2.0 instruments

676413

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

Contents

Intended Use

The *ipsogen* MPL W515L/K Muta*Screen* Kit is for research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed of recombinant DNA experiments, or to other applicable guidelines.

Principle of the Procedure

Two probes are used in a multiplexed assay as part of an allelic discrimination experiment. One is a perfect match to the allele 1 sequence (e.g., the wild-type allele) while the other one is a perfect match to the allele 2 (e.g., the allele with a mutation). Each probe is labeled with a distinctive fluorescent dye at its 5' end such as FAM™ or VIC® forming a reporter. The probe also contains a non-fluorescent quencher at the 3' end. The inclusion of a minor grove binder (MGB™) in the probe sequence permits the use of shorter probes with greater stability and thereby a more accurate allelic discrimination.

During the extension phase of the PCR, the perfectly matched probe is cleaved by the 5'→3' exonuclease activity of *Thermus aquaticus* (*Taq*) polymerase. This separates the reporter dye from the quencher and thus releases detectable fluorescence. If the probe is not a perfect match, it will be displaced rather than cleaved by the *Taq* polymerase and no reporter dye will be released. The fluorescence signal (VIC or FAM) generated is collected at the end of the PCR and immediately indicates the presence of the targeted sequence(s) in the sample (wild-type allele, mutated allele, or both). This negates the need for long and laborious post-

PCR steps, which may also increase the contamination risk. The *ipsogen* MPL W515L/K Muta*Screen* Kit uses this technology as illustrated in Figure 1.

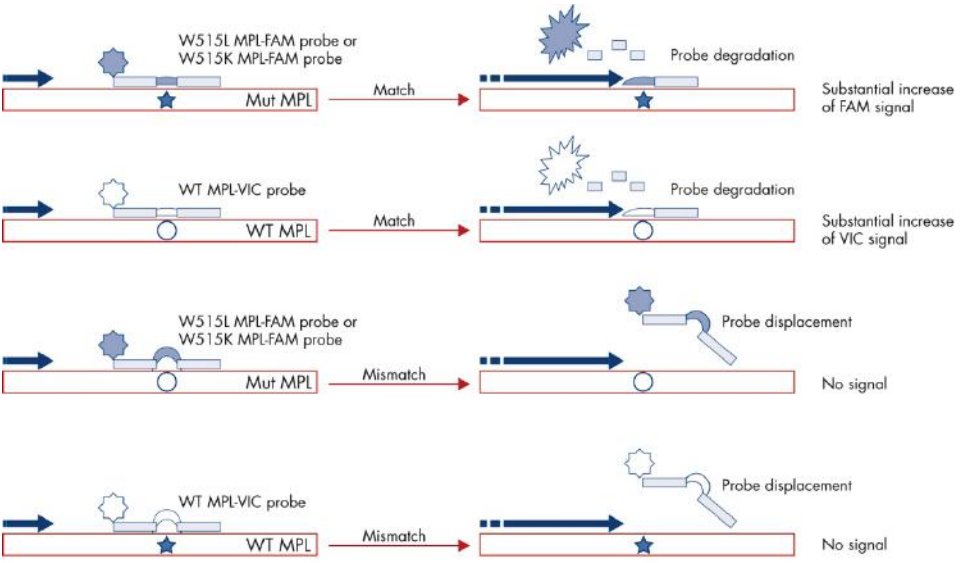


Figure 1. Reaction principle of the *ipsogen* MPL W515L/K Muta*Screen* Kit allelic discrimination assay.

Materials Provided

Kit contents

***ipsogen* MPL W515L/K Muta*Screen* Kit**		**(24)**
Catalog no.		**676413**
Number of reactions		**24**
W515L positive control	PC-515L	30 µl
W515K positive control	PC-515K	30 µl
MPL negative control	NC-MPL	60 µl
MPL W515L cut-off sample containing 1.5% W515L/wild-type allele mix	COS-515L	30 µl
MPL W515K cut-off sample containing 1.5% W515K/wild-type allele mix	COS-515K	30 µl
Primers and Probe Mix PPM-MPL W515L*	PPM-MPL W515L 10x	145 µl
Primers and Probe Mix PPM-MPL-W515K†	PPM-MPL W515K 10x	145 µl

* Mix of specific reverse and forward primers for the MPL gene plus a specific W515L FAM probe and a wild-type VIC probe.

† Mix of specific reverse and forward primers for the MPL gene plus a specific W515K FAM probe and a wild-type VIC probe.

Note: Vortex and briefly centrifuge the tubes before use.

Materials Required but Not Provided

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

Reagents

- Nuclease-free, PCR-grade water
- Buffer and *Taq* DNA polymerase: The recommended reagents are TaqMan® Universal PCR Master Mix (Master Mix PCR 2x) (Thermo Fisher Scientific Inc., cat. no. 4304437) and LightCycler TaqMan Master (Master Mix PCR 5x) (Roche, cat. no. 04535286001)

Consumables

- Nuclease-free, aerosol-resistant sterile PCR pipet tips with hydrophobic filters
- 0.5 ml or 0.2 ml nuclease-free PCR tubes
- 0.1 ml strip tubes and caps, if using the Rotor-Gene Q 5plex HRM instrument
- Ice

Equipment

- Microtiter pipets dedicated for PCR (1–10 µl; 10–100 µl; 100–1000 µl)
- Benchtop centrifuge* with rotor for 0.2 ml/0.5 ml reaction tubes (capable of attaining 13,000 to 14,000 rpm)

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Real-time PCR instrument:* Rotor-Gene Q 5plex HRM; ABI PRISM 7000, 7300, 7700, or 7900HT SDS; Applied Biosystems 7500 Real-Time PCR System, LightCycler 480 or 2.0; and associated specific material

Warnings and Precautions

Safety information

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

Discard sample and assay waste according to your local safety regulations.

General precautions

Use of quantitative PCR (qPCR) requires good laboratory practices, including maintenance of equipment, that are dedicated to molecular biology and are compliant with applicable regulations and relevant standards.

This kit is intended for research use. Reagents and instructions supplied in this kit have been tested for optimal performance. Further dilution of the reagents or alteration of incubation times and temperatures may result in erroneous or discordant data. PPM-MPL reagents may be altered if exposed to light. All reagents are formulated specifically for use with this kit. For optimal performance of the kit, no substitutions should be made.

Use extreme caution to prevent:

- DNase contamination, which might cause degradation of the template DNA
- DNA or PCR carryover contamination resulting in false positive signal

We therefore recommend the following:

- Use nuclease-free labware (e.g., pipets, pipet tips, reaction vials) and wear gloves when performing the assay.
- Use fresh aerosol-resistant pipet tips for all pipetting steps to avoid cross-contamination of the samples and reagents.
- Prepare pre-PCR master mix with dedicated material (pipets, tips, etc.) in a dedicated area where no DNA matrixes (cDNA, DNA, plasmid) are introduced. Add template in a separate zone (preferably in a separate room) with specific material (pipets, tips, etc.).

Reagent Storage and Handling

The kits are shipped on dry ice and must be stored at –30 to –15°C upon receipt.

- Minimize exposure to light of the primers and probe mixes (PPM-MPL tubes).
- Gently mix and centrifuge the tubes before opening.
- Store all kit components in original containers.

These storage conditions apply to both opened and unopened components. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

Expiration dates for each reagent are indicated on the individual component labels. Under correct storage conditions, the product will maintain performance until the expiration date printed on the label.

There are no obvious signs to indicate instability of this product. However, positive and negative controls should be run simultaneously with unknown specimens.

Procedure

Sample DNA preparation

Genomic DNA should be obtained either from whole blood, purified blood lymphocytes of whole blood, polynuclear cells, or granulocytes. The same cellular fraction and DNA extraction method should be used for all samples to allow results to be compared. DNA extraction should be performed by any commercial or home brew method.

- DNA quantity is determined by measuring the absorbance at 260 nm.
- DNA quality should be assessed either by spectrophotometry or by gel electrophoresis.
- The ratio of absorbance at 260 nm to 280 nm (A_{260}/A_{280}) should be 1.7–1.9. Smaller ratios usually indicate contamination by protein or organic chemicals.

Electrophoretic analysis on a 0.8–1.0% agarose gel should allow visualization of the isolated DNA as a distinct band of about 20 kb. A slight smear is acceptable.

The DNA obtained in the extraction should be diluted to a concentration of 5 ng/µl in TE pH 8.0 buffer. The DNA sample can be stored at 4–8°C for a week or at –20°C if longer-term storage is required.

The qPCR is optimized for 25 ng of purified gDNA.

The quality of the assay is largely dependent on the quality of input DNA. We therefore recommend the QIAamp® DNA Blood Maxi Kit (cat. no. 51194).

Protocol: qPCR on Rotor-Gene Q 5plex HRM Instruments

Using this instrument, we recommend performing all measurements in duplicate, as indicated in Table 1 or Table 2.

Table 1. Number of reactions for a MPL W515L experiment using Rotor-Gene Q instruments with a 72-tube rotor with 0.1 ml strip tubes and caps

Samples	Reactions
With the PPM-MPL W515L/WT primers and probe mix	
n DNA samples	n x 2 reactions
3 controls	2 x 3 reactions (PC-515L, NC-MPL and COS-515L, each one tested in duplicate)
Water control	2 reactions

Table 2. Number of reactions for a MPL W515K experiment using Rotor-Gene Q instruments with a 72-tube rotor with 0.1 ml strip tubes and caps

Samples	Reactions
With the PPM-MPL W515K/WT primers and probe mix	
n DNA samples	n x 2 reactions
3 controls	2 x 3 reactions (PC-515K, NC-MPL and COS-515K, each one tested in duplicate)
Water control	2 reactions

Sample processing on Rotor-Gene Q instruments with 72-tube rotor with 0.1 ml strip tubes and caps

We recommend testing at least 24 genomic DNA samples in duplicate in one experiment, 10 samples in duplicate in two experiments, or five samples in duplicate in three experiments to optimize the use of the controls, and the primers and probe mixes. The rotor scheme in Figure 2 shows an example of such an experiment.

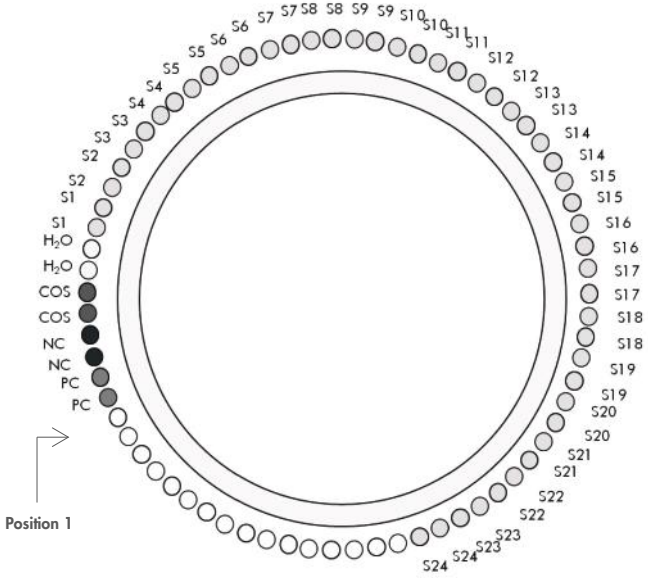


Figure 2. Suggested rotor setup for each experiment with the *ipsogen* MPL W515L/K Muta*Screen* Kit. **PC**: Positive control; **NC**: Negative control; **COS**: Cut-off sample (either 1.5% W5151L/wild-type allele mix or 1.5% W515K/wild-type allele mix); **H_2O**: Water control; **S**: Unknown DNA sample.

Important: Take care to always place the Positive Control in position 1 of the rotor. Otherwise, during the calibration step, the instrument will not perform calibration, and incorrect fluorescence data will be acquired. Fill all other positions with empty tubes.

qPCR on Rotor-Gene Q instruments with 72-tube rotor with 0.1 ml strip tubes and caps

Note: Perform all steps on ice.

Procedure

1. Thaw all necessary components and place them on ice.
2. Prepare the following qPCR mix according to the number of samples being processed.

   All concentrations are for the final volume of the reaction.

   Table 3 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 µl. A pre-mix can be prepared, according to the number of reactions, using the same primers and probe mix (either PPM-MPL W515L or PPM-MPL W515K). Extra volumes are included to compensate for pipetting error.

Table 3. Preparation of qPCR mix

Component	1 rxn (µl)	18+1 rxns (µl)	28+1 rxns (µl)	56+1 rxns (µl)	Final conc.
TaqMan Universal PCR Master Mix, 2x	12.5	237.5	362.5	712.5	1x
Primers and probe mix (PPM-MPL W515L or PPM-MPL W515K), 10x	2.5	47.5	72.5	142.5	1x
Nuclease-free, PCR-grade water	5.0	95.0	145.0	285	–
Sample (to be added at step 4)	5	5 each	5 each	5 each	–
Total volume	**25**	**25 each**	**25 each**	**25 each**	**–**

Conc.: concentration; rxn(s): reaction(s).

3. Dispense 20 µl of the qPCR pre-mix per tube.

4. Add 5 µl of the material to be tested (25 ng sample genomic DNA or controls) in the corresponding tube (total volume 25 µl).
5. Mix gently by pipetting up and down.
6. Place the tubes in the thermal cycler according to the manufacturer recommendations.
7. Program the Rotor-Gene Q instrument with the thermal cycling program as indicated in Table 4.

Table 4. Temperature profile

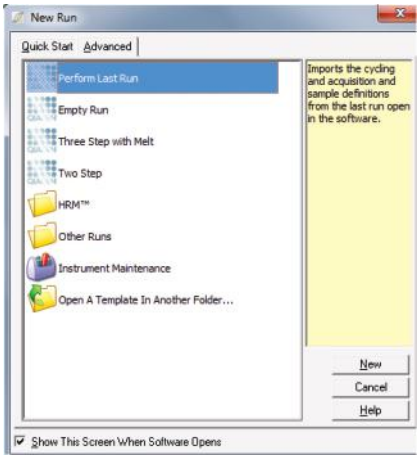
Parameter	
Mode of analysis	Quantitation
Hold 1	Temperature: 50°C Time: 2 min
Hold 2	Temperature: 95°C Time: 10 min
Cycling	50 times 92°C for 15 s 60°C for 1 min with acquisition of FAM fluorescence in channel Green and VIC fluorescence in channel Yellow: Single

8. Start the thermal cycling program, as indicated in Table 4.

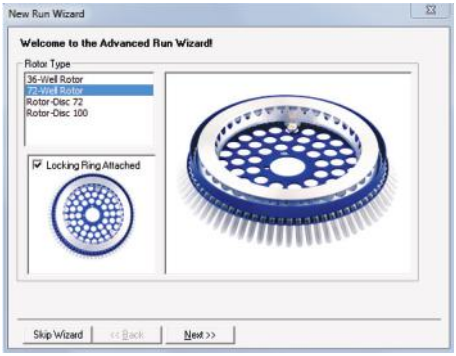
Detailed procedure for the Rotor-Gene Q instrument

Note: Instrument settings could slightly differ between instruments. Refer to your user manual for more details.

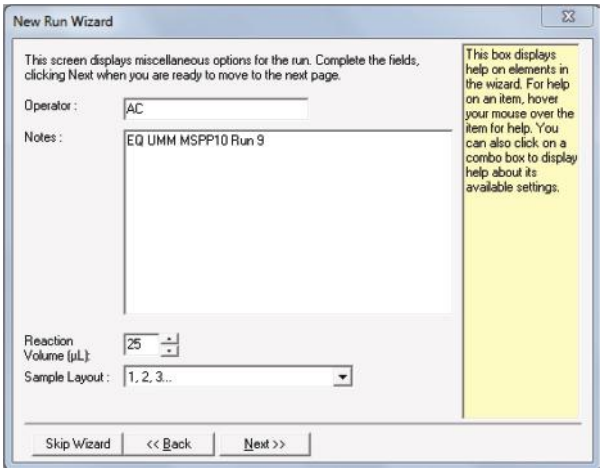
1. Start the Rotor-Gene software. In the New Run pop-up window, select the Advanced tab and click **New**.



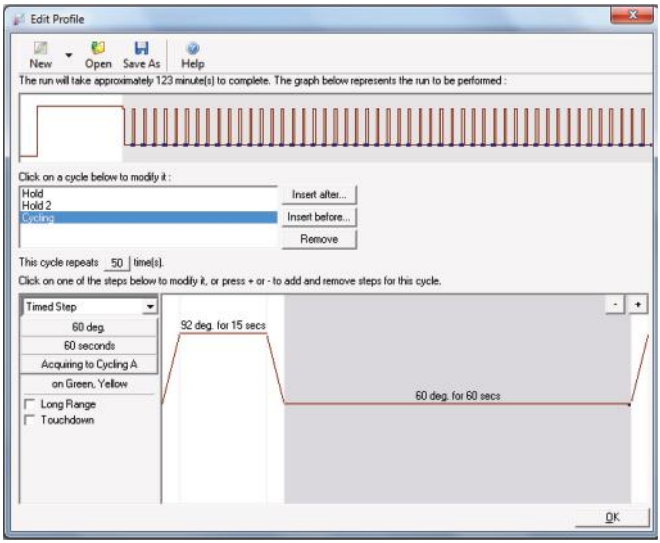
2. In the New Run Wizard dialog box, select **72-Well Rotor** from the drop-down list. Check the **Locking Ring Attached** box, then click **Next**.



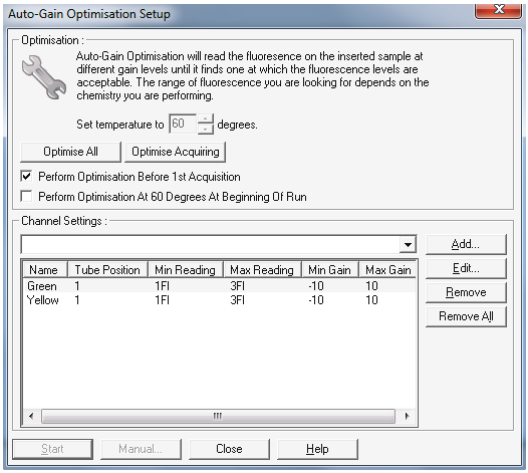
3. On the next window, set the reaction volume field to **25 µl** and click **Next**.



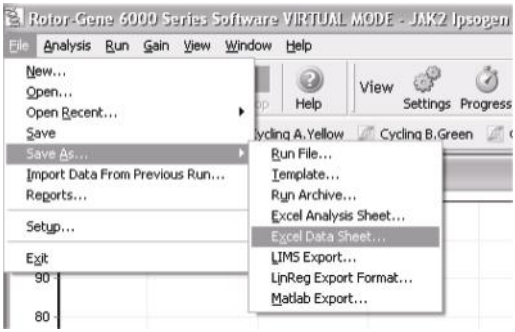
4. In the next window, click the **Edit Profile** to create the PCR program according to Table 4.



5. Click **Gain Optimisation** in the New Run Wizard dialog box to open the Auto-Gain Optimisation Setup dialog box. Set the range for the Green and Yellow channels from **1 Fl** in the Min Reading column to **3 Fl** in the Max Reading column and the acceptable Gain range from **–10** to **10**.



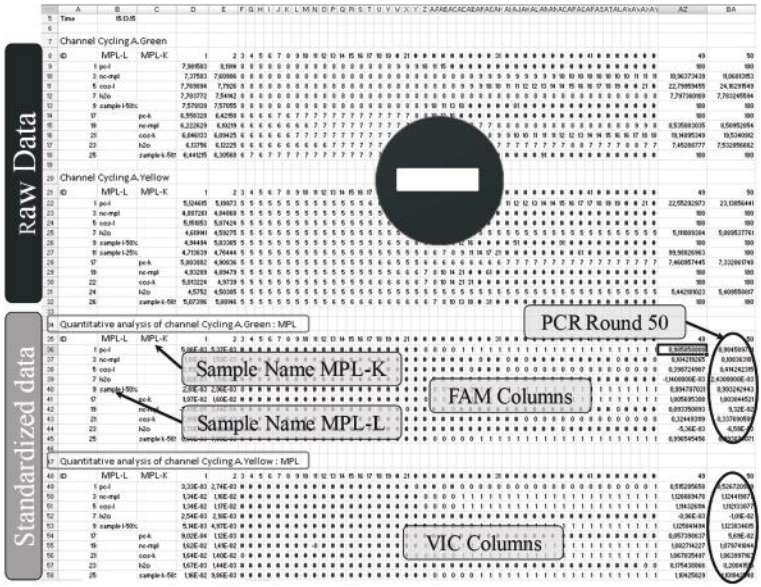
6. Check the **Perform Optimisation Before 1st Acquisition** box, then click **Close** to close the Auto-Gain Optimisation Setup dialog box.
7. Start the thermal cycling program.
8. After the cycling program has ended, click **Analysis** then double-click **Cycling A. Green**.
9. Select **Dynamic Tube** and **Slope Correct**.
10. Repeat steps 8–9 for **Cycling A. Yellow**.
11. To export the data, navigate to **File** > **Save As** > **Excel Data Sheet**.



12. After adding a name for the datasheet, a pop-up window appears as follows. Click **OK** to leave the option disabled.

13. Open the .csv file using Excel®.

    An example of the output obtained is as follows:



The file contains both raw data and standardized data, but only the standardized should be considered. These data are given in the "Quantitative analysis of channel Cycling A Green" and "Quantitative analysis of channel Cycling A Yellow" sections of the table. The data that need to be interpreted are values acquired at the 50th PCR cycle, as shown by the circle in the above figure.

Protocol: qPCR on ABI PRISM 7000, 7300, 7700 and 7900HT SDS Instruments, the Applied Biosystems 7500 Real-Time PCR System, and the LightCycler 480

Using 96-well-plate qPCR equipment, we recommend performing all measurements in duplicate, as indicated in Table 5 or Table 6.

Table 5. Number of reactions for a MPL W515L experiment using 96 well-plate qPCR equipment

Samples	Reactions
With the PPM-MPL W515L/WT primers and probe mix	
n DNA samples	n x 2 reactions
3 controls	2 x 3 reactions (PC-515L, NC-MPL and COS-515L, each one tested in duplicate)
Water control	2 reactions

Table 6. Number of reactions for a MPL W515K experiment using 96 well-plate qPCR equipment

Samples	Reactions
With the PPM-MPL W515K/WT primers and probe mix	
n DNA samples	n x 2 reactions
3 controls	2 x 3 reactions (PC-515K, NC-MPL and COS-515K, each one tested in duplicate)
Water control	2 reactions

Sample processing on ABI PRISM 7000, 7300, 7700, and 7900 SDS instruments, the Applied Biosystems 7500 Real-Time PCR System, and the LightCycler 480

We recommend testing at least 24 genomic DNA samples in duplicate in one experiment, 10 samples in duplicate in two experiments, or five samples in duplicate in three experiments to optimize the use of the controls, and the primers and probe mixes. The plate scheme in Figure 3 shows an example of such an experiment.

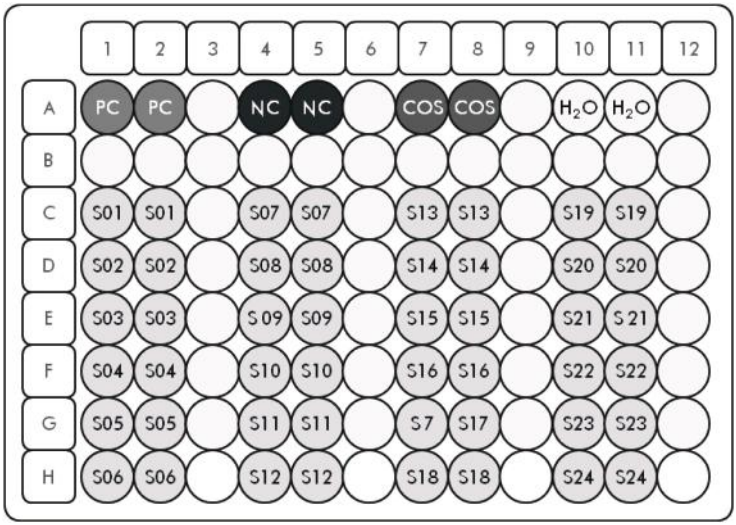


Figure 3. Suggested plate setup for one experiment with the *ipsogen* MPL W515L/K Muta*Screen* Kit. PC: Positive control; **NC**: Negative control; **COS**: Cut-off sample; **H_2O**: Water control; **S**: DNA sample.

qPCR on ABI PRISM 7000, 7300, 7700 and 7900 SDS instruments, the Applied Biosystems 7500 Real-Time PCR System, and the LightCycler 480

Note: Perform all steps on ice.

Procedure

1. Thaw all necessary components and place them on ice.
2. Prepare the following qPCR mix according to the number of samples being processed. If using 96-well-plate qPCR equipment, we recommend performing all measurements in duplicate.

   Note: All concentrations are for the final volume of the reaction.

   Table 7 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 µl. A pre-mix can be prepared, according to the number of reactions, using the same primers and probe mix (either PPM-MPL W515L or PPM-MPL W515K). Extra volumes are included to compensate for pipetting error.

Table 7. Preparation of qPCR mix

Component	1 rxn (µl)	18+1 rxns (µl)	28+1 rxns (µl)	56+1 rxns (µl)	Final conc.
TaqMan Universal PCR Master Mix, 2x	12.5	237.5	362.5	712.5	1x
Primers and probe mix (PPM-MPL W515L or PPM-MPL W515K), 10x	2.5	47.5	72.5	142.5	1x
Nuclease-free, PCR-grade water	5.0	95.0	145.0	285	–
Sample (to be added at step 4)	5	5 each	5 each	5 each	–
Total volume	**25**	**25 each**	**25 each**	**25 each**	**–**

Conc.: concentration; rxn(s): reaction(s).

3. Dispense 20 µl of the qPCR pre-mix per well.
4. Add 5 µl of the material to be tested (25 ng sample genomic DNA or controls) in the corresponding well (total volume 25 µl).
5. Mix gently by pipetting up and down.
6. Close the plate and briefly centrifuge (300 x *g* for approximately 10 s).
7. Load the plate in the thermal cycler apparatus according to the manufacturer's recommendations.
8. Program the thermal cycler with the thermal cycling program as specified in Table 8 or 9 depending on the instrument being used.

Detailed procedure for the ABI PRISM 7000, 7300, 7700 and 7900HT SDS instruments, and the Applied Biosystems 7500 Real-Time PCR System

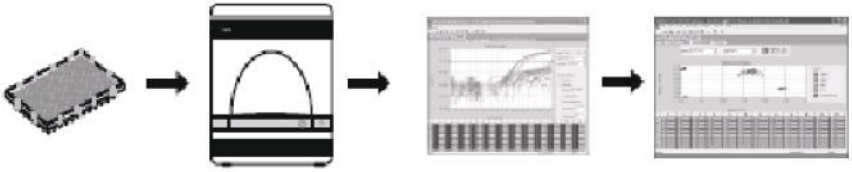
The thermal cycling program is shown in Table 8.

Table 8. Temperature profile for ABI PRISM 7000, 7300, 7700 and 7900HT SDS instruments, and the Applied Biosystems 7500 Real-Time PCR System

Parameters	
Mode of analysis	Standard Curve – Absolute Quantitation
Hold	Temperature: 50°C Time: 2 min
Hold 2	Temperature: 95°C Time: 10 min
Cycling	50 times 92°C for 15 s 60°C for 1 min with acquisition of FAM and VIC fluorescence; quencher: MGB

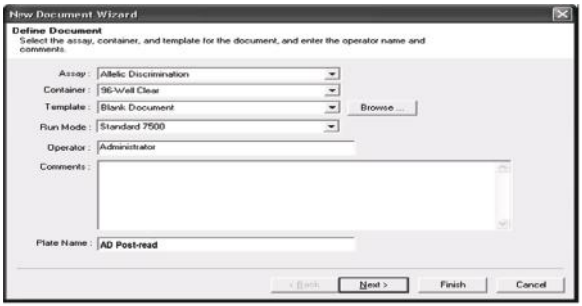
Detailed procedure for the ABI PRISM 7000, 7300, 7700 and 7900HT SDS, and the Applied Biosystems 7500 Real-Time PCR System instrument setting of post-read run analysis

- Users who are familiar with the use of the function “allelic discrimination” should go directly to step 6.
- Instrument setting could slightly differ between instruments. Refer to your user manual for more details.

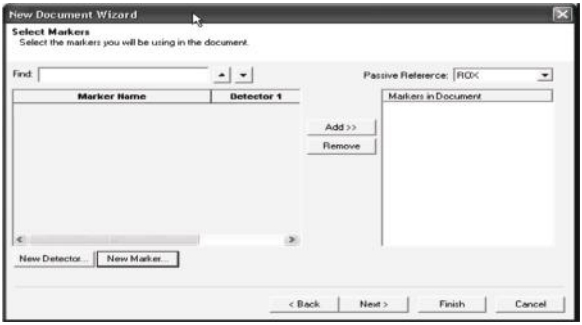


Reaction plate System Amplification run Post-read run

1. Navigate to **Start** > **Programs.**
2. Select “File” and then “New”.
3. In the new document wizard:
   - 3a. From the Assay drop-down list, select **Allelic Discrimination**.
   - 3b. Accept the default settings for the **Container** and **Template** fields (96-well clear and blank document).
   - 3c. In the **Plate Name** field, enter **AD post-read**.



4. Click **Next** to access the "Select Markers" page. If the marker list on this page contains a marker suitable for your application, skip to step 6.



5. If the marker list does not contain a marker suitable for your application, as above, follow these steps to create detectors and marker:

   5a. Click **New Detector**.

   5b. In the New Detector dialog box, enter **Allele A** in the **Name** field.

   5c. Leave the **Reporter Dye** field set to **FAM**.

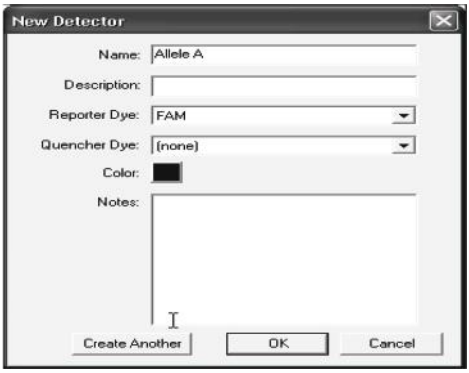
   5d. Select a color you prefer, and then click **OK**.

   5e. Click **Create Another**.

   5f. In the **Name** field, enter **Allele B**.

   5g. Select **VIC** in the Reporter Dye field.

5h. Select a color you prefer, then click **OK**.



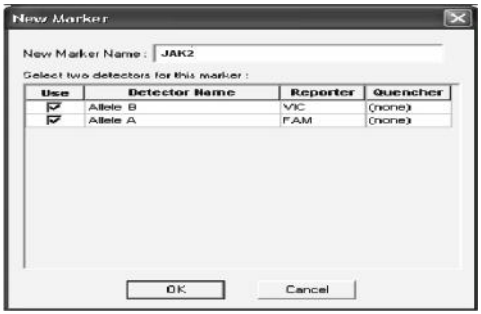
6. In the select markers window:

6a. Click **New Marker**.

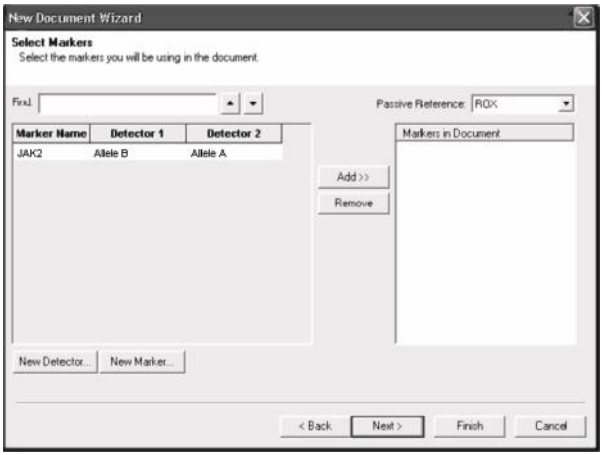
6b. In the New Marker dialog box, enter **MPL** in the New Marker Name field.

6c. In the Detector Name field, select the **Allele A** and **Allele B** detectors you created in the step 5.

6d. Click **OK**.



7. In the Select Markers window, select either the **MPL** marker you created in step 5 or a suitable marker, then click **Add**.

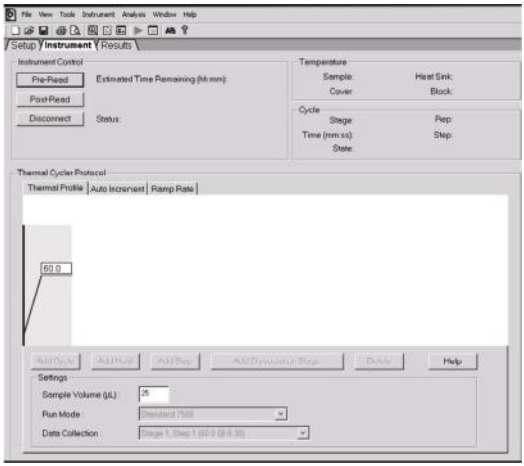


Note: To remove a marker, select it, and then click **Remove**.

8. Click **Next**.
9. In the Setup Sample Plate page, select the marker for wells. Click-drag to select wells that contain samples.
10. Click **Finish**.
11. Select the Instrument tab and set the value in the Sample Volume field to **25 µL**.
12. Click **File** > **Save** > **Save** to retain the name you assigned when you created the plate document.
13. Load the reaction plate into the instrument.
14. Start the post-read run.
15. Click **Post-Read**.

    The instrument will perform a run of 1 cycle of 60 s at 60°C.

    Note: During this post-read run, the instrument collects FAM and VIC fluorescence in each well.



To export the results, click **File** then **Export**.Click **Results**.

Analyze export file. An example of the output is shown below:

Comments:							VIC Fluorescence	FAM Fluorescence			
SDS v1.2											
Well	Sample Name	Marker	Task	Passive Ref	Allele X	Allele Y	Allele X Rn	Allele Y Rn	Call	Quality Value	Method
A1	PC-WK	VIC	Unknown		MPL 515K-VIC	MPL 515 K-FAM	9.367	29.565	Undetermined	100.00	Manual Call
A2	PC-WK	VIC	Unknown		MPL 515K-VIC	MPL 515 K-FAM	9.733	29.988	Undetermined	100.00	Manual Call
A3		VIC	Unknown		MPL 515K-VIC	MPL 515 K-FAM			Undetermined	100.00	Manual Call
A4	NC-MPL	VIC	Unknown		MPL 515K-VIC	MPL 515 K-FAM	14.476	4.686	Undetermined	100.00	Manual Call
A5	NC-MPL	VIC	Unknown		MPL 515K-VIC	MPL 515 K-FAM	14.034	4.596	Undetermined	100.00	Manual Call
A6		VIC	Unknown		MPL 515K-VIC	MPL 515 K-FAM			Undetermined	100.00	Manual Call
A7	RS-WK	VIC	Unknown		MPL 515K-VIC	MPL 515 K-FAM	15.488	7.79	Undetermined	100.00	Manual Call
A8	RS-WK	VIC	Unknown		MPL 515K-VIC	MPL 515 K-FAM	14.267	7.422	Undetermined	100.00	Manual Call
A9		VIC	Unknown		MPL 515K-VIC	MPL 515 K-FAM			Undetermined	100.00	Manual Call
A10	H2O	VIC	Unknown		MPL 515K-VIC	MPL 515 K-FAM	0.18	0.168	Undetermined	100.00	Manual Call
A11	H2O	VIC	Unknown		MPL 515K-VIC	MPL 515 K-FAM	0.177	0.165	Undetermined	100.00	Manual Call

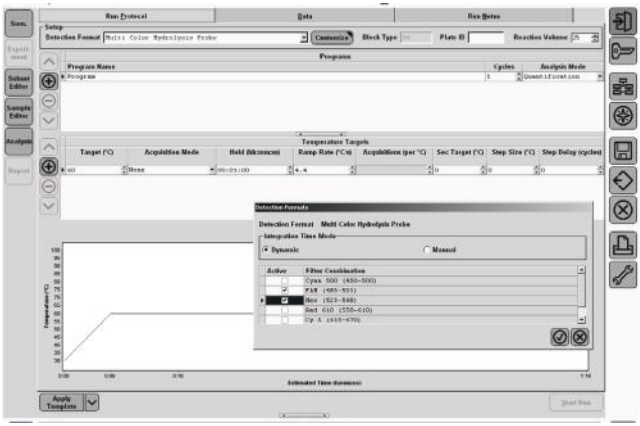
Detailed procedure for the LightCycler 480 instrument

1. On the home page, click **New Experiment**.
2. Depending on the instrument used (LightCycler 480 I or II), perform either step 2a for the LightCycler 480 I or step 2b for the LightCycler 480 II.

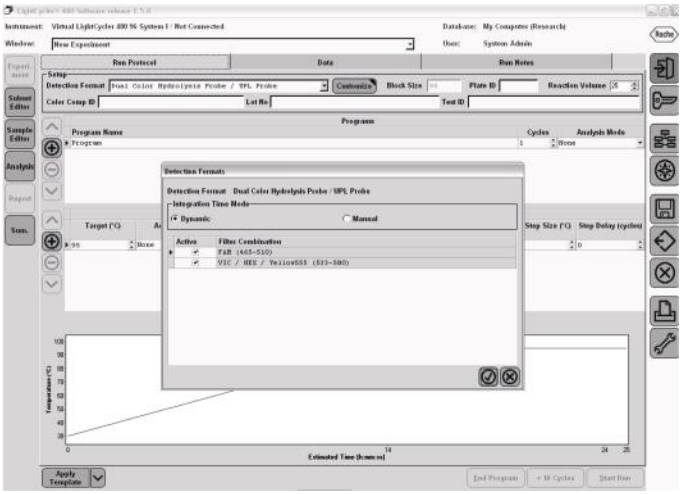
   2a. For the LightCycler 480 I: Select **Multi Color Hydrolysis Probe** in the **Detection Format** field, then click **Customize**. Verify that the channels **FAM (483-533)** and **VIC/Hex (523-568)** are selected in the **Detection Format** field.

   2b. For the LightCycler 480 II: Select **Dual Color Hydrolysis Probe** in the **Detection Format** field, then click **Customize**. Verify that the channels **FAM (465-510)** and **VIC/Hex (533-580)** are selected in the **Detection Format** field.

   A screenshot from the LightCycler 480 I instrument is shown below.



A screenshot from the LightCycler 480 II instrument is shown below.



Note: When describing the plate set up on the instrument, click **Endpt Geno** and in step 1, select **Workflow** section.

3. Run the PCR program, which is shown in Table 9.

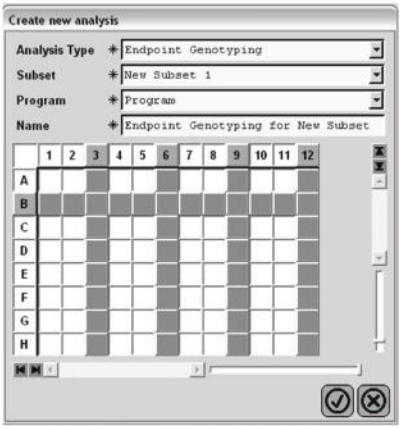
Table 9. Temperature profile for the LightCycler 480 instrument

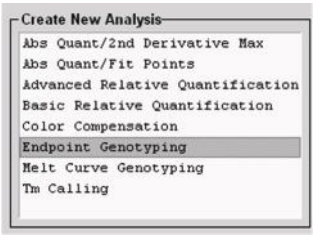
Parameters	
Hold 1	Temperature: 50°C Time: 2 min
Hold 2	Temperature: 95°C Time: 10 min
Cycling	50 times 92°C for 15 s 60°C for 1 min with acquisition of FAM fluorescence corresponding to 483–533 nm and VIC fluorescence corresponding to 523–568 nm for LC480 version 01 and acquisition of FAM fluorescence corresponding to 465–510 nm and VIC fluorescence corresponding to 533–580 nm for LC480 version 02
Hold 3	Temperature: 60°C Time: 1 min with acquisition of FAM fluorescence corresponding to 483–533 nm and VIC fluorescence corresponding to 523–568 nm for LC480 version 01 and acquisition of FAM fluorescence corresponding to 465–510 nm and VIC fluorescence corresponding to 533–580 nm for LC480 version 02

Detailed procedure for LightCycler 480 instrument setting of end point analysis

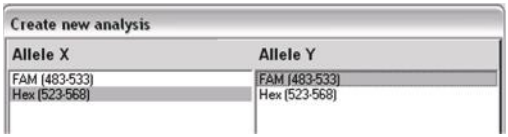
Users who are familiar with the use of the function "Allelic Discrimination" can go directly to step 6.

1. At the end of PCR, click **Analysis**.
2. In the Create New Analysis dialog box, select **Endpoint Genotyping** in the **Analysis Type** field, then select the subset to analyze in the **Subset** field.

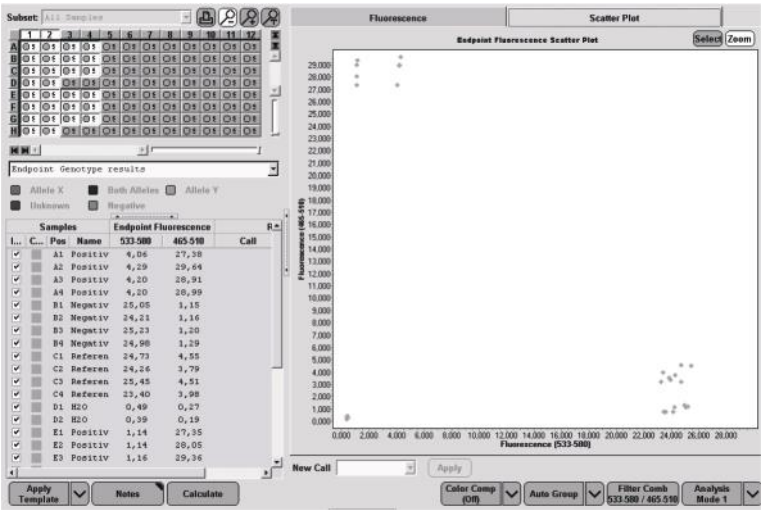




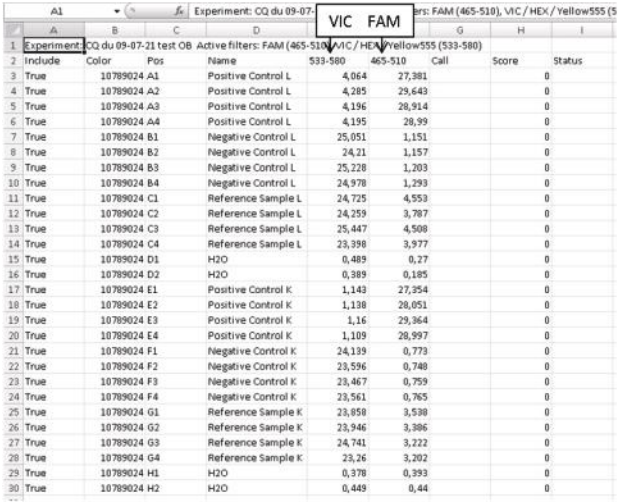
3. In the next window, select **VIC/HEX** fluorescence for the X allele column and **FAM** fluorescence for the Y allele column.



The following window appears. In this window, the upper left segment shows the plate set up while the bottom left shows the fluorescence results for each sample. On the right of the screen, a scatter plot is shown that gives the allelic discrimination based on FAM and VIC fluorescence measured at the 50th PCR cycle.



4. To export data, right-click thesamples results template, then select **Export Table**. The file will be saved in a text (*.txt) format.
5. To view and analyze results, open the file using Excel.



A1 | Experiment: CQ du 09-07- ... ers: FAM (465-510), VIC / HEX / Yellow555 (5

VIC FAM

	A	B	C	D	E	F	G	H	I
1	Experiment:	CQ du 09-07-21 test OB		Active filters: FAM (465-510), VIC / HEX / Yellow555 (533-580)					
2	Include	Color	Pos	Name	533-580	465-510	Call	Score	Status
3	True	10789024	A1	Positive Control L	4,064	27,381		0	
4	True	10789024	A2	Positive Control L	4,285	29,643		0	
5	True	10789024	A3	Positive Control L	4,196	28,914		0	
6	True	10789024	A4	Positive Control L	4,195	28,99		0	
7	True	10789024	B1	Negative Control L	25,051	1,151		0	
8	True	10789024	B2	Negative Control L	24,21	1,157		0	
9	True	10789024	B3	Negative Control L	25,228	1,203		0	
10	True	10789024	B4	Negative Control L	24,978	1,293		0	
11	True	10789024	C1	Reference Sample L	24,725	4,553		0	
12	True	10789024	C2	Reference Sample L	24,259	3,787		0	
13	True	10789024	C3	Reference Sample L	25,447	4,508		0	
14	True	10789024	C4	Reference Sample L	23,398	3,977		0	
15	True	10789024	D1	H2O	0,489	0,27		0	
16	True	10789024	D2	H2O	0,389	0,185		0	
17	True	10789024	E1	Positive Control K	1,143	27,354		0	
18	True	10789024	E2	Positive Control K	1,138	28,051		0	
19	True	10789024	E3	Positive Control K	1,16	29,364		0	
20	True	10789024	E4	Positive Control K	1,109	28,997		0	
21	True	10789024	F1	Negative Control K	24,139	0,773		0	
22	True	10789024	F2	Negative Control K	23,596	0,748		0	
23	True	10789024	F3	Negative Control K	23,467	0,759		0	
24	True	10789024	F4	Negative Control K	23,561	0,765		0	
25	True	10789024	G1	Reference Sample K	23,858	3,538		0	
26	True	10789024	G2	Reference Sample K	23,946	3,386		0	
27	True	10789024	G3	Reference Sample K	24,741	3,222		0	
28	True	10789024	G4	Reference Sample K	23,26	3,202		0	
29	True	10789024	H1	H2O	0,378	0,393		0	
30	True	10789024	H2	H2O	0,449	0,44		0	

Protocol: qPCR on LightCycler 2.0 Instruments

Using capillary instruments, we recommend measuring samples and controls in duplicate, as indicated in Table 10 or Table 11.

Table 10. Number of reactions for a MPL W515L experiment using LightCycler 2.0 instruments

Samples	Reactions
With the PPM-MPL W515L/WT primers and probe mix	
n DNA samples	n x 2 reactions
Plasmid controls	2 x 3 reactions (PC-515L, NC-MPL and COS-515L, each one tested in duplicate)
Water control	2 reactions

Table 11. Number of reactions for a MPL W515K experiment using LightCycler 2.0 instruments

Samples	Reactions
With the PPM-MPL W515K/WT primers and probe mix	
n DNA samples	n x 2 reactions
controls	2 x 3 reactions (PC-515K, NC-MPL and COS-515K, each one tested in duplicate)
Water control	2 reactions

Sample processing on LightCycler 2.0 instrument

We recommend testing at least 12 DNA samples in the same experiment to optimize the use of the controls, and primers and probe mixes. The capillary scheme in Figure 4 shows an example of an experiment.

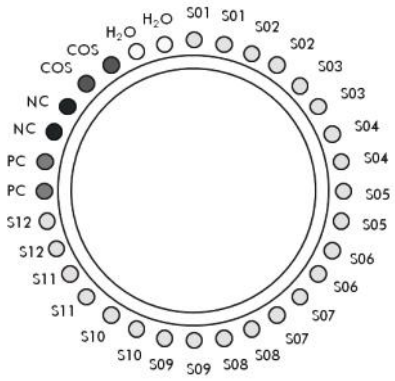


Figure 4. Suggested rotor setup for each experiment with the *ipsogen* MPL W515L/K Muta*Screen* Kit. PC: Positive control; **NC**: Negative control; **H_2O**: Water control; **S**: Unknown DNA sample to be analyzed.

qPCR on LightCycler 2.0 instruments

Note: Because of particular technological requirements, LightCycler 2.0 experiments must be performed using specific reagents. We recommend to use the LightCycler TaqMan Master and to follow the manufacturer's instructions to prepare the Master Mix 5x.

Note: Perform all steps on ice.

Procedure

1. Thaw all necessary components and place them on ice.
2. Prepare the following qPCR mix according to the number of samples being processed.

   Note: All concentrations are for the final volume of the reaction.

   Table 12 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 20 µl. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix (either PPM-MPL W515L or PPM-MPL W515K). Extra volumes are included to compensate for pipetting error.

Table 12. Preparation of qPCR mix

Component	1 rxn (µl)	32+1 rxns (µl)	Final conc.
LightCycler TaqMan Master Mix, 5x	4	132	1x
Primers and probe mix (PPM-MPL W515L or PPM-MPL W515K), 10x	2.0	66.0	1x
Nuclease-free, PCR-grade water	9	297.0	–
Sample (to be added at step 4)	5	5 each	–
Total volume	**20**	**20 each**	**–**

Conc.: concentration; rxn(s): reaction(s).

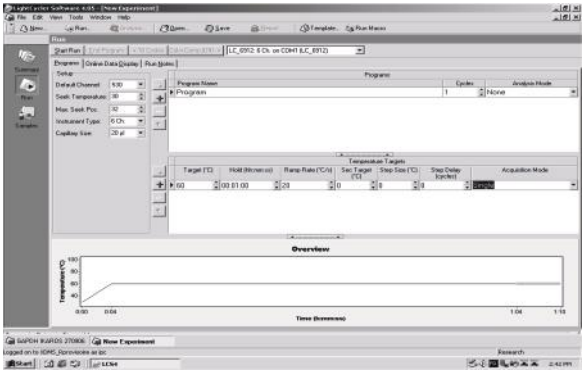
3. Dispense 15 µl of the qPCR pre-mix per capillary.
4. Add 5 µl of the material to be tested (25 ng sample genomic DNA) in the corresponding capillary (total volume 20 µl).
5. Mix gently by pipetting up and down.
6. Place the capillaries in the adapters provided with the instrument, and briefly centrifuge (700 x *g*, approximately 10 s).
7. Load the capillaries into the thermal cycler according to the manufacturer recommendations.
8. Program the LightCycler 2.0 instrument with the thermal cycling program as indicated in Table 13.

Table 13. Temperature profile

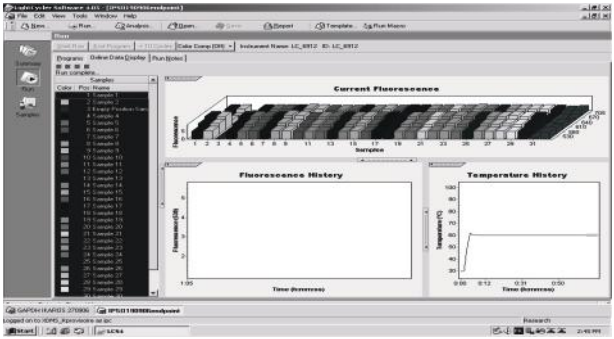
Parameters	
Mode of analysis	Quantitation
Hold 1	Temperature: 55°C Time: 2 min Ramp: 20
Hold 2	Temperature: 95°C Time: 10 min Ramp: 20
Cycling	50 times 92°C for 15 s; ramp: 20 60°C for 1 min; ramp: 20; with acquisition of FAM fluorescence: Single acquisition of VIC fluorescence: Single

Detailed procedure for the LightCycler 2.0 instrument setting of post-read run analysis

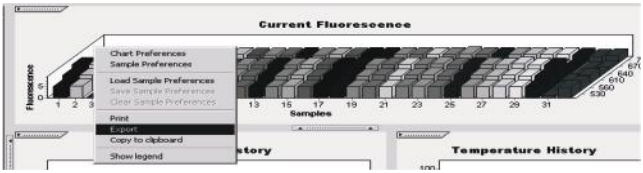
1. At the end of the amplification run, select a new LightCycler experiment.
2. Click **Post read** and run a PCR program of 1 cycle of 1 min at 60°C, a ramp of 20, and FAM and VIC single acquisition.



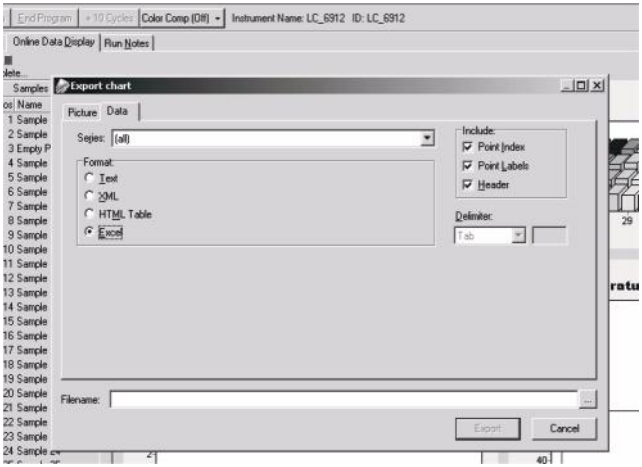
3. In the window Online Data Display, right-click near the **Current Data Fluorescent** graph as shown in the following screenshot.



4. Select **Export**.



5. In the Export Chart dialog box, select **Excel** from the **Format** list.



6. In the **Filename** field, browse the location to export your result file to.
7. Click **Export**.
8. Analyze the export file. An example of the output is shown in the following screenshot.

I	J	K	L	M	N	O	P	Q	R	S	T	U
X	Bar	Text	X	Bar	Text	X	Bar	Text	X	Bar		
1	2,9709	1: Sample 1 (610)	1	8,2734	1: Sample 1 (560)	1	6,6361	1: Sample 1 (530)	1	4,9943		
2	3,0182	2: Sample 2 (610)	2	8,4428	2: Sample 2 (560)	2	6,7659	2: Sample 2 (530)	2	5,0767		
3	2,9496	3: Sample 3 (610)			3: Sample 3 (560)	3	6,5568	3: Sample 3 (530)	3	4,9699		
4	2,9526	4: Sample 4 (610)	4	8,2887	4: Sample 4 (560)	4	6,6163	4: Sample 4 (530)	4	4,9119		
5	2,9450	5: Sample 5 (610)	5	8,2689	5: Sample 5 (560)	5	6,6209	5: Sample 5 (530)	5	4,9638		
6	2,9969	6: Sample 6 (610)	6	8,4184	6: Sample 6 (560)	6	6,7674	6: Sample 6 (530)	6	5,1209		
7	3,0045	7: Sample 7 (610)	7	8,4520	7: Sample 7 (560)	7	6,7506	7: Sample 7 (530)	7	5,0507		
8	3,2822	8: Sample 8 (610)	8	9,1936	8: Sample 8 (560)	8	7,3960	8: Sample 8 (530)	8	5,5314		
9	3,0274	9: Sample 9 (610)	9	8,5557	9: Sample 9 (560)	9	6,8437	9: Sample 9 (530)	9	5,0843		
10	2,8336	10: Sample 10 (610)	10	7,9713	10: Sample 10 (560)	10	6,3905	10: Sample 10 (530)	10	4,7883		
11	2,8275	11: Sample 11 (610)	11	7,9774	11: Sample 11 (560)	11	6,3874	11: Sample 11 (530)	11	4,7669		
12	2,8351	12: Sample 12 (610)	12	8,0171	12: Sample 12 (560)	12	6,4118	12: Sample 12 (530)	12	4,7944		
13	2,9511	13: Sample 13 (610)	13	8,3726	13: Sample 13 (560)	13	6,6957	13: Sample 13 (530)	13	4,9699		
14	2,8367	14: Sample 14 (610)	14	8,0217	14: Sample 14 (560)	14	6,4439	14: Sample 14 (530)	14	4,7654		
15	2,9908	15: Sample 15 (610)	15	8,4337	15: Sample 15 (560)	15	6,7445	15: Sample 15 (530)	15	5,0523		
16	2,8885	16: Sample 16 (610)	16	8,1498	16: Sample 16 (560)	16	6,5568	16: Sample 16 (530)	16	4,9577		
17	3,0152	17: Sample 17 (610)	17	8,4901	17: Sample 17 (560)	17	6,8193	17: Sample 17 (530)	17	5,1225		

VIC data **FAM data**

Results

FAM/VIC ratio calculation and genotyping

Regardless of the instrument used, an analysis export file will be created and this can be used to extract the data and process it as follows.

Procedure

1. For each test, calculate the FAM/VIC ratio.
2. If the fluorescence data are consistent between duplicates, calculate the mean ratio for each sample including the control samples.
3. Compare the mean ratio value obtained for each unknown sample (Ratio Sample) with the cut-off sample mean ratio (Ratio COS-WL– Ratio COS-WK, detection limit) as shown in Table 14.

   If the ratio of the sample is greater than or equal to the ratio for COS-WL or COS-WK, then the sample is either MPL W515L positive or MPL W515K depending on which probe mix was used.

   If the ratio of the sample is strictly less than the ratio for COS-WL or COS-WK, then either the MPL W515L mutation was not detected or the MPL W515K mutation was not detected depending on which probe mix was used.

Table 14. FAM/VIC ratio calculation and genotyping using MPL W515K as an example

Name	VIC	FAM	Ratio FAM/VIC	Mean ratio	Genotype
NC-MPL*	14.965 14.974	4.065 4.267	0.272 0.285	0.278	–
COS-WK*	14.661 13.938	6.023 5.782	0.411 0.415	0.413	–
PC-WK*	9.091 10.368	21.594 24.663	2.375 2.379	2.377	–
H_20*	0.195 0.225	0.258 0.304	1.323 1.351	X	NA
Sample 1*	14.429 14.058	5.571 5.382	0.386 0.383	0.384	Wild-type
Sample 2*	13.339 13.887	6.417 6.657	0.481 0.479	0.480	Mutant
Sample 3*	12.047 12.328	3.819 3.86	0.317 0.313	0.315	Wild-type
Sample 4*	12.141 12.145	3.362 3.493	0.277 0.288	0.282	Wild-type
Sample 5*	11.256 11.424	3.481 3.483	0.309 0.305	0.307	Wild-type
Sample 6*	12.643 11.847	3.459 3.365	0.274 0.284	0.279	Wild-type
Sample 7*	11.364 11.421	3.439 3.474	0.303 0.304	0.303	Wild-type

NA: not applicable.

* Assay performed in duplicate.

Graphical representation and quality control criteria

Figure 5 shows an example of the results allelic discrimination pattern that can be obtained.

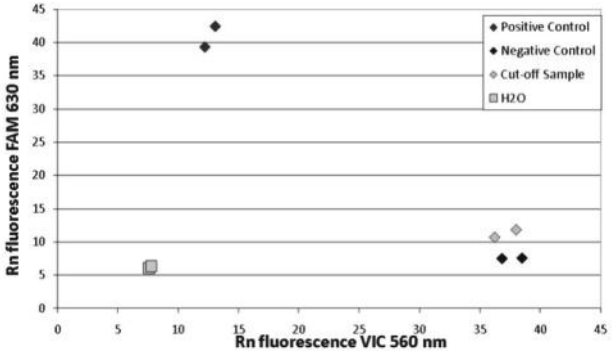


Figure 5. Allelic discrimination. Scatter plot with fluorescence data; VIC values are plotted on the x-axis, FAM values are plotted on the y-axis.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *ipsogen* MPL W515L/K Muta*Screen* Kit is tested against predetermined specifications to ensure consistent product quality. Certificates of analysis are available on request at **www.qiagen.com/support/**.

Symbols

The following symbols may appear on the packaging and labeling:

Symbol	Symbol definition
 <N>	Contains reagents sufficient for <N> reactions
	Use by
	Catalog number
	Lot number
	Material number
	Global Trade Item Number
	Temperature limitation
	Manufacturer
	Consult instructions for use

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN

Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
ipsogen MPL W515L/K Muta*Screen* Kit (24)	Kit for the detection of MPL W515L and W515K mutations in human genomic DNA.	676413
Rotor-Gene Q for outstanding performance in real-time PCR		
Rotor-Gene Q 5plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included.	9001580

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.

Document Revision History

Date	Changes
January 2020	Corrected typo error throughout the document: “Standards” is replaced with “controls”; “to be quantified” is replaced with “to be tested”
	Corrected typo error in Table 3 from “RT mix” to “qPCR mix”
	Minor revision to Protocol: qPCR on Rotor-Gene Q 5plex HRM Instruments section to align Note description with Figure 2 layout
	Updated the Detailed procedure for Rotor-Gene Q instrument section to include additional steps in the procedure, add an analysis of parameters, and improve the data export process
	Removed Mode of analysis from Table 9
	Corrected typo error to qPCR on LightCycler 2.0 instrument and removed 1.2 from the section title of Sample processing on LightCycler 2.0 instruments
	Added VIC single acquisition in Table 13
	Added FAM and VIC single acquisition in Detailed procedure for the LightCycler 2.0 instrument setting of post-read analysis section
	Corrected title of Table 14 to FAM/VIC ratio calculation and genotyping using MPL W515K as an example

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Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific
GeneJET FFPE DNA Purification Kit
#K0881, #K0882

Lot __

Expiry Date __

Read Storage information (p. 4) before the first use!

www.thermoscientific.com/onebio

CERTIFICATE OF ANALYSIS

The purity of isolated DNA from two sections (10 µm) of FFPE tissue is evaluated spectrophotometrically. The purified DNA has an A260/A280 ratio of 1.8–2.3. The functional quality of purified DNA is evaluated by PCR analysis.

Quality authorized by:  Jurgita Zilinskiene

Rev.1 71

CONTENTS page

COMPONENTS OF THE KIT

GeneJET FFPE DNA Purification Kit	#K0881 50 preps	#K0882 250 preps
Proteinase K Solution	1.2 mL	4 × 1.3 mL
RNase A Solution	0.7 mL	3 × 1 mL
Digestion Buffer for GeneJET FFPE DNA Purification Kit	11 mL	55 mL
Binding Buffer for GeneJET FFPE DNA Purification Kit	11 mL	55 mL
Wash Buffer 1 (conc.) for GeneJET FFPE DNA Purification Kit	10 mL	40 mL
Wash Buffer 2 (conc.) for GeneJET FFPE DNA Purification Kit	10 mL	40 mL
Elution Buffer for GeneJET FFPE DNA Purification Kit	10 mL	40 mL
GeneJET DNA Purification Columns & Collection Tubes	50	250
Collection Tubes	50	250

STORAGE

The unopened vials of Proteinase K and RNase A solutions are stable at room temperature. Once the vial is opened, it should be stored at -20 °C. Other components of the kit should be stored at room temperature (15-25 °C).

DESCRIPTION

Formalin fixed and paraffin embedded (FFPE) tissue samples are routinely prepared from human surgical tissue samples. GeneJET DNA Purification Kit for FFPE samples is designed for fast and convenient purification of DNA from various amounts of FFPE tissue. Up to 8 sections (10 µm thickness) of FFPE samples can be used for genomic DNA extraction without overnight incubation. Elimination of toxic reagents commonly used for deparaffinization allows an environmentally-friendly procedure. Each preparation recovers up to 8 µg of genomic DNA from one section that can be eluted in 20 µL to 80 µL of Elution Buffer. High quality eluted DNA can be directly used in downstream applications such as qPCR, PCR, NGS library preparation, or stored at -20 °C.

PRINCIPLE

Sections of FFPE samples are subjected to enzymatic digestion and lysis to liberate genomic DNA. The released DNA is decrosslinked by heat incubation. Subsequently, the resulting solution is centrifuged and the supernatant containing DNA is mixed with Binding Buffer. After addition of ethanol, the lysate is loaded onto the purification column. The adsorbed DNA is washed to remove contaminants and then eluted with the Elution Buffer.

IMPORTANT NOTES

- DNA yield and quality from FFPE tissue may vary considerably depending on the tissue source, the thickness of the slice, the age of the sample, post-sampling delay before fixation, fixation time, etc.
- Paraffin sections can be stored at or below 4 °C for 1 year without observable effects on DNA yield and usability. Longer-term storage of FFPE sections may have negative effect on the DNA due to oxidation.
- To minimize DNA degradation, avoid repeated freeze/thaw cycles of the samples. For short–term, DNA solution may be stored at 0-4 °C, and for long-term at -20 °C.
- Add the indicated volume of ethanol (96-100%) to **Wash Buffer 1** (concentrated) and **Wash Buffer 2** (concentrated) prior to the first use:

	#K0881 50 preps		**#K0882** 250 preps	
	Wash Buffer 1	**Wash Buffer 2**	**Wash Buffer 1**	**Wash Buffer 2**
Concentrated wash solution	10 mL	10 mL	40 mL	40 mL
Ethanol (96-100%)	**30 mL**	**30 mL**	**120 mL**	**120 mL**
Total volume:	40 mL	40 mL	160 mL	160 mL

After ethanol has been added, mark the check box on the bottle's cap to indicate the completed step.

- Check the **Digestion Buffer** and **Binding Buffer** for salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37 °C, then cool it back down to 25 °C before use.
- Set two thermal heating-blocks or waterbaths, one at 65 °C and one at 90 °C.
- It is recommended to use microcentrifuge tubes with screw caps in the steps 1 to 4 (see the Genomic DNA purification from FFPE samples protocol, p.6).
- Wear gloves when handling the **Binding Buffer, Wash Buffer 1 and Proteinase K Solution** as these reagents contain irritants (*see* p.8 for SAFETY INFORMATION).

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipets and pipet tips
- Vortex mixer
- Ethanol (96-100%)
- 1.5 mL microcentrifuge tubes (for sample lysis and DNA elution)
- Centrifuge for 1.5 mL microcentrifuge tubes (≥ 12,000 × g)
- Thermal heating-blocks or waterbath (adjustable to 65 °C and 90 °C)
- Disposable gloves

PROTOCOL OF GENOMIC DNA PURIFICATION FROM FFPE SAMPLES

- This protocol describes how to extract DNA from one to eight sections of FFPE tissue (when each section is up to 10 µm thick).

Step	Procedure
1	Add 200 µL of Digestion Buffer to a microcentrifuge tube (not provided) containing one or more sections (up to eight) of FFPE tissue. Incubate for 3 min at 90 °C. During the incubation mix the sample a few times by gently shaking the tube. Make sure the tissue sections stay submerged in the solution. After incubation, mix thoroughly with a vortex mixer to completely dissolve the paraffin. Cool the sample down to room temperature. If necessary, spin down briefly to clear the lid. **Note. It is not necessary to cut off the excess paraffin. Use a microcentrifuge tube with a screw cap. Incubation time should be prolonged to 6 min if more than one section of FFPE tissue is used.**
2	Add 20 µL of Proteinase K solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension. Spin down briefly to clear the lid.
3	Incubate the sample at 65 °C for 50 min in a thermoshaker or a water bath with occasional vortexing (300-400 rpm). **Note. Lysis time varies on the type and amount of FFPE sample processed. In some cases incubation time should be prolonged to 2 hours. Yield of DNA typically increases with extended lysis time**.
4	Transfer the samples to the heat block set to 90 °C and heat for 40 min. **Note. Prevent samples from being heated above 90 °C for a prolonged period of time.**
5	Centrifuge hot samples at 6000 × g for 1 min and transfer 200 µL of the digested lysate to a new 1.5 mL microcentrifuge tube (not provided). **Note. Transfer the entire liquid layer to a new tube leaving behind any wax particulates. Small amounts of debris will not affect the DNA yield. When using eight sections of FFPE tissue (each 10 µm thick), the digested lysate volume is 160-180 µL.**
6	Add 10 µL of RNase A solution and mix thoroughly by vortexing. Spin down briefly to clear the lid. Leave at room temperature for 10 min.
7	Add 200 µL of Binding Buffer. Vortex thoroughly for 10 seconds until homogeneous mixture is obtained. Spin down briefly to clear the lid.
8	Add 400 µL of ethanol (96-100%) to the sample. Vortex thoroughly for 10 seconds until homogeneous mixture is obtained. Spin down briefly to clear the lid.
9	Transfer the lysate to a GeneJET DNA Purification Column inserted into collection tube. Centrifuge for 1 min at 6000 × g. Discard the collection tube with the flow-through and place the column in a new collection tube (provided).
10	Add 500 µL of Wash Buffer 1 (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
11	Add 500 µL of Wash Buffer 2 (with ethanol added). Centrifuge for 3 min at maximum speed (≥ 12000 × g). Empty the collection tube, place the purification column back into the collection tube and re-spin the column for 1 min at maximum speed to dry the membrane. Discard the collection tube containing the flow-through solution and transfer the GeneJET DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not provided).

12	Add 60 µL of Elution Buffer directly to the center of the purification column membrane. Leave for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note. For maximum DNA yield, repeat the elution step with additional 60 µL of Elution Buffer. (perform the second elution using different tube).** **If more concentrated DNA is required or DNA is isolated from a small amount of starting material (e.g., one section of FFPE sample) the volume of the Elution Buffer added to the column can be reduced to 20 µL. Elution volumes in the range of 20-80 µL are recommended, the default volume is 60 µL.**
13	Discard the column. Use the purified DNA immediately in downstream applications or store at -20 °C.

TROUBLESHOOTING

Problem	Possible cause and solution
Low yield of purified DNA	**Excess sample used during lysate preparation.** Reduce the amount of starting material. Do not use more tissue than indicated in lysis protocols. **Starting material was not completely digested.** If the suspension does not clarify during Proteinase K digestion, this could indicate that it is oxidized. Extend the Proteinase K digestion at 65 °C until complete lysis occurs and no particles remain. **Ethanol was not added to the lysate.** Make sure that the ethanol was added to the lysate before applying the sample to the Purification Column. **Ethanol was not mixed with the lysate.** After the addition of ethanol to the lysate mix the sample by vortexing or pipetting. **Ethanol was not added to Wash Buffers.** Make sure that ethanol was added to Wash Buffer 1 and Wash Buffer 2 before use. Follow the instructions for Wash Buffer preparation on p.5. **Poor sample quality.** Sample fixation, embedding and storage have a significant impact on quality and amount of the DNA in FFPE tissue samples.
RNA contamination	**RNase A treatment was not carried out.** Carry out RNase A treatment step described in the purification procedure.
Column becomes clogged during purification	**Excess sample was used during lysate preparation.** Too much starting material was used. Overloading may lead to a decrease in DNA yield. **Tissue was not completely digested.** Insufficient disruption and / or homogenization of starting material. Extend the Proteinase K digestion at 65 °C until complete lysis occurs and no particles remain.
Inhibition of downstream enzymatic reactions	**Purified DNA contains residual ethanol.** Do not let the flow-through touch the column outlet after the second wash with Wash Buffer 2. Always re-spin the column for an additional 1 min. at maximum speed (≥ 12000 × g) after the second wash. **Purified DNA contains residual salt.** Use the correct order for the Washing Buffers. Always wash the purification column with Wash Buffer 1 first and then proceed to wash with Wash Buffer 2.

SAFETY INFORMATION

Proteinase K

Danger
Hazard statements:
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Precautionary statements:
P285 In case of inadequate ventilation wear respiratory protection.
P261 Avoid breathing dust/fume/gas/mist/vapours/spray.
P342+P311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
P304+P341 IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Binding Buffer

Warning
Hazard statements:
H302 Harmful if swallowed.
H315 Causes skin irritation
H319 Causes serious eye irritation.
Precautionary statements:
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P321 Specific treatment (see on this label).
P362 Take off contaminated clothing and wash before reuse.
P301+P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Wash Buffer 1

Warning
Hazard statements:
H302 Harmful if swallowed.
H315 Causes skin irritation
H319 Causes serious eye irritation.
Precautionary statements:
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P321 Specific treatment (see on this label).
P362 Take off contaminated clothing and wash before reuse.
P301+P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

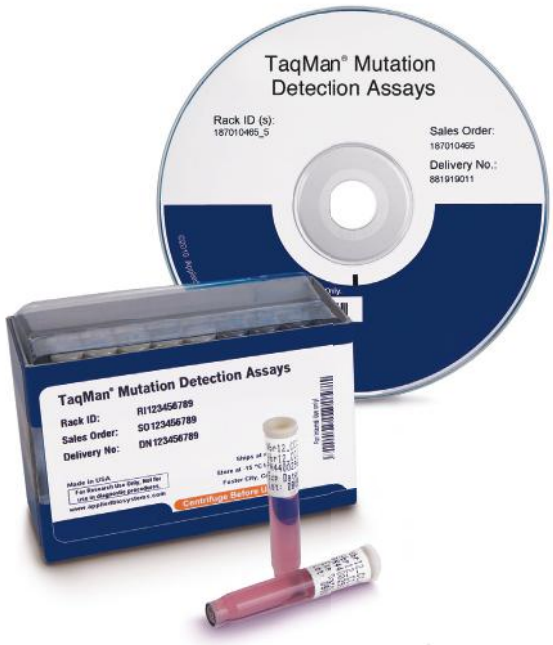
PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

Accurate and sensitive somatic mutation detection powered by castPCR™ technology

TaqMan® Mutation Detection Assays

- High specificity—mutant allele detection is based on an allele-specific primer, while wild type background is suppressed by the proprietary MGB blocker oligonucleotide
- High sensitivity—assays can detect down to 0.1% mutation in a background of wild type DNA, as demonstrated in spiking experiments
- Wide dynamic range and excellent PCR efficiency—assays demonstrate at least 4 logs of dynamic range and an average PCR efficiency of 100% ± 10%
- Fast, simple workflow—like other TaqMan® Assays, typically requires 3 hours from sample to results, with minimum hands-on time



Cancer research samples often contain rare somatic mutations within a high background of normal wild type DNA. Many mutation detection methods compatible with tumor specimens, including gene sequencing and real-time PCR, have been reported in the literature and are commercially available. However, commercially available kits have various limitations in terms of sensitivity, specificity, cost, workflow, and turnaround time. We have developed sensitive and easy-to-use TaqMan® Mutation Detection Assays to accurately assess mutation status. TaqMan® Mutation Detection Assays were designed based on the novel competitive allele-specific TaqMan® PCR (castPCR™) technology, which combines allele-specific TaqMan® qPCR with allele-specific MGB blocker oligonucleotides that effectively suppress nonspecific amplification from the off-target allele.

Currently, the assay portfolio covers key somatic mutations identified in various cancer genes including, but not limited to, *KRAS*, *BRAF*, *HRAS*, *NRAS*, *EGFR*, *PIK3CA*, *KIT*, *PTEN*, and *TP53* genes, which have been implicated in many types of cancer. These mutations were selected from the comprehensive Sanger COSMIC database for somatic mutations. The target selection was based on frequency of occurrence and input from leading cancer researchers. We will continually add more mutation assays to cover additional cancer gene mutations. For the most updated list of available assays, refer to the TaqMan® Mutation Detection Assay index file at **lifetechnologies.com/castpcr**.

life technologies

About the assays

TaqMan® Mutation Detection Assays contain mutant allele assays, which specifically detect one or more mutant alleles, and corresponding gene reference assays, which detect mutation-free regions of the genes in which the target mutations reside (Figure 1). The validated assay set additionally includes corresponding wild type allele assays (not described here; refer to the TaqMan® Mutation Detection Assay protocol for further information).

Two experiment types

Two types of experiments are required for mutation detection analysis:

1. Detection ΔC_t cutoff determination

A mutant allele assay and corresponding gene reference assay are run on three or more wild type gDNA samples that are from the same sample type as the test samples (e.g., gDNA from FFPE tissue samples, Figure 2). ΔC_t values are calculated for each sample run with a mutant allele assay/gene reference assay pair. The average ΔC_t value for all samples is then calculated and is used to derive the detection ΔC_t cutoff value for the mutant allele assay.

2. Mutation detection

A test sample is run with one or more mutant allele assays and a corresponding gene reference assay (Figure 2). The ΔC_t value for the mutant allele assay/gene reference assay pair is calculated, and this value is compared to the previously determined detection ΔC_t cutoff value to determine the sample mutation status.

Optional use of internal positive control (IPC)

You can duplex the IPC reagents with any TaqMan® Mutation Detection Assay to distinguish true target negatives from PCR failure or inhibition (Figure 3).

Figure 1. TaqMan® Mutation Detection Assay types.

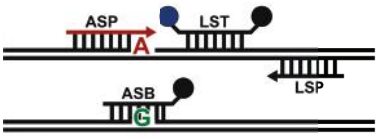
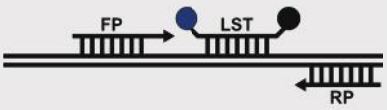
Assay type	Description	Schematic
Mutant allele assay	• Detects specific or multiple mutant alleles • An allele-specific primer detects the mutant allele • An MGB blocker oligonucleotide suppresses the wild type allele	 ASP = Allele-specific primer ASB = Allele-specific blocker (MGB) LST = Locus-specific TaqMan® probe LSP = Locus-specific primer
Gene reference assay	• Detects the gene within which the target mutations reside • A locus-specific pair of forward and reverse primers amplifies a mutation-free region of the target gene	 FP = Forward primer RP = Reverse primer LST = Locus-specific TaqMan® probe

Figure 2. Gene reference and mutant allele assays are run with a genomic DNA sample to determine the mutation status of each target mutation within the cancer gene.

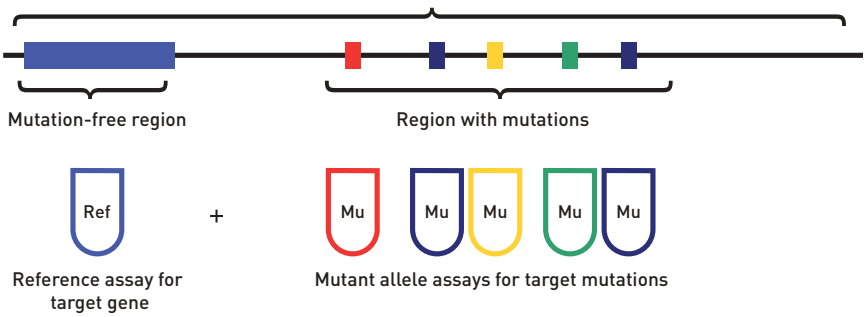
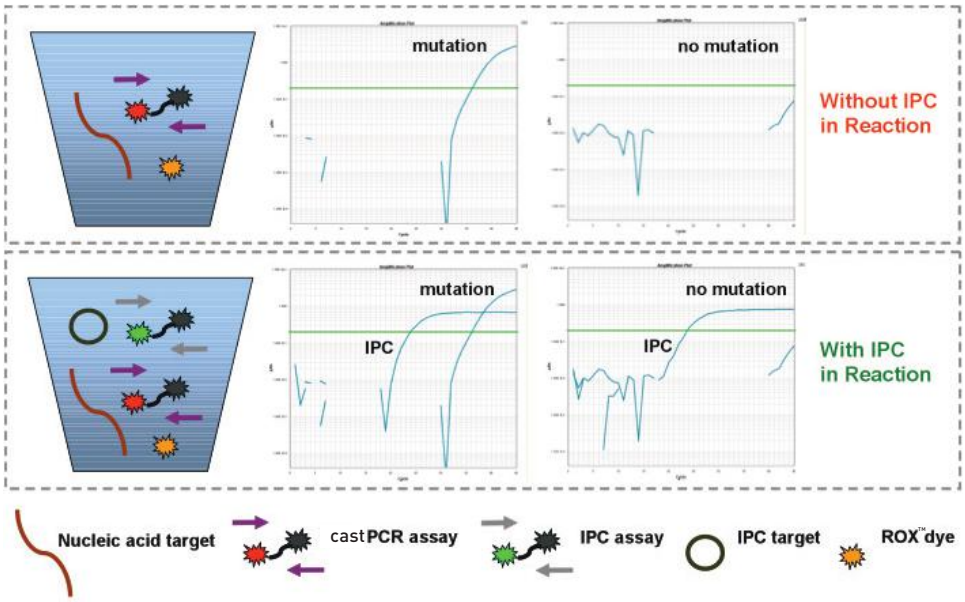


Figure 3. Internal positive controls. The TaqMan® Mutation Detection IPC Reagent Kit is a set of optional internal positive control reagents that can be duplexed with any TaqMan® Mutation Detection Assay to provide a positive PCR control result. The IPC reagents can distinguish a mutation target negative result from a PCR failure result.



Procedure

Purified gDNA, extracted from a sample with an unknown mutation status, is run with one or more mutant allele assays and the corresponding gene reference assay. For each real-time PCR reaction, the gDNA is combined with:

- **A TaqMan® Mutation Detection Assay**—contains two primers and a FAM™ dye–labeled MGB probe to detect a mutant allele or reference gene target. Mutant allele assays also contain an MGB oligonucleotide blocker.
- **TaqMan® Genotyping Master Mix**—contains AmpliTaq Gold® DNA Polymerase UP (Ultra Pure), dNTPs, and buffer
- **(Optional) TaqMan® Mutation Detection IPC Reagent Kit**—contains an internal positive control (IPC) template, two primers, and a VIC® dye–labeled TAMRA™ probe. It can be used to distinguish true target negatives from PCR failure or inhibition.

Reactions are run on a real-time PCR system, using a universal mutation detection thermal cycling protocol. After the run, the real-time PCR system's analysis software determines the C_t values for each TaqMan® Mutation Detection Assay and (optional) IPC reagent reactions. Real-time results export files can be opened in the free Mutation Detector™ Software for post-PCR data analysis. The C_t difference between each mutant allele assay and reference assay is calculated. This ΔC_t value, which represents the quantity of a specific mutant allele detected in a sample, is used to determine sample mutation status by comparison to a previously determined detection ΔC_t cutoff value. You can search for, or download a list of, currently available TaqMan® Mutation Detection Assays at **lifetechnologies.com/castpcr**.

Note: All TaqMan® Mutation Detection Assays have undergone extensive testing to ensure high sensitivity and specificity. The first set of released assays, covering 14 *KRAS*, 29 *EGFR*, and the *BRAF* V600E mutations, underwent additional testing, including determination of: the inherent amplification efficiency difference between mutant allele assays and corresponding reference assays, to enable quantitative analysis of percent mutation in a sample; and assay detection ΔC_t cutoff values using spiked cell line gDNA samples.

Assay performance

Specificity

Mutant allele detection is based on an allele-specific primer, while the wild type allele background is suppressed by the proprietary MGB blocker oligonucleotide. Assays can detect down to 0.1% mutant allele in the presence of a wild type allele background (Figure 4).

High sensitivity

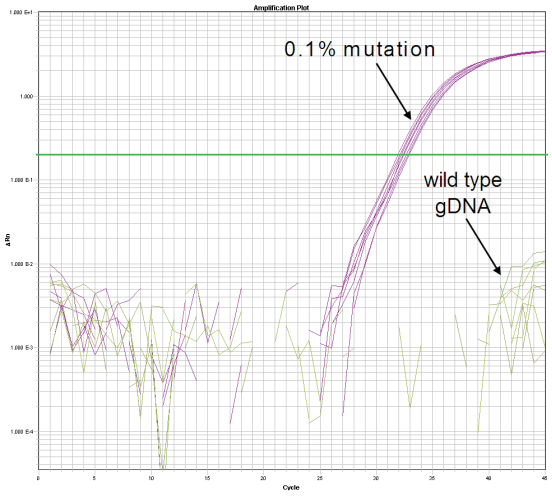
TaqMan® Mutation Detection Assays can detect as few as 1–5 mutant copies in up to one million copies of wild type background. Assay sensitivity is demonstrated using synthetic template spiking experiments (Figure 5 and 6).

Wide dynamic range and excellent PCR efficiency

Assays demonstrate up to 7 logs of dynamic range and an average PCR efficiency of 100% ± 10% (Figure 6).

Figure 4. C_t difference between 0.1% mutation samples and wild type gDNA. For each assay, 0.1% mutant allele samples were obtained by spiking 10 copies of mutant allele synthetic templates into 10,000 copies of cell line wild type gDNA. **(A)** Example of amplification plot for KRAS_522_mu assay on 0.1% mutant allele sample and wild type gDNA. **(B)** There is a significant difference in amplification C_t values between the 0.1% mutant allele sample and wild type gDNA (*P* value < 0.05 for 46 out of 48 assays in the example graph).

A



B

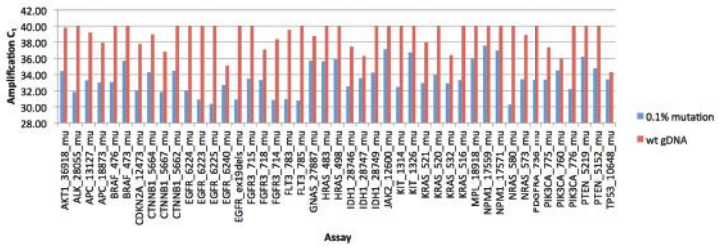


Figure 5. Assay sensitivity and selectivity. For every single assay, the sensitivity and selectivity were analyzed through synthetic template spiking experiments. 10 copies to 10^5 copies of mutant allele synthetic template were spiked into a constant background of 10^5 copies of wild type cell line genomic DNA. For a subset of the assays, 1 copy to 10^6 copies of mutant allele synthetic template were spiked into a constant background of 10^6 copies of wild type allele synthetic template. In the example shown, the BRAF_476_mu assay can detect 1 copy of mutant allele in a background of 10^6 copies of wild type allele.

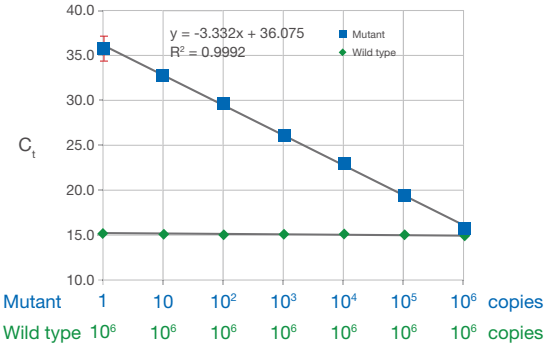
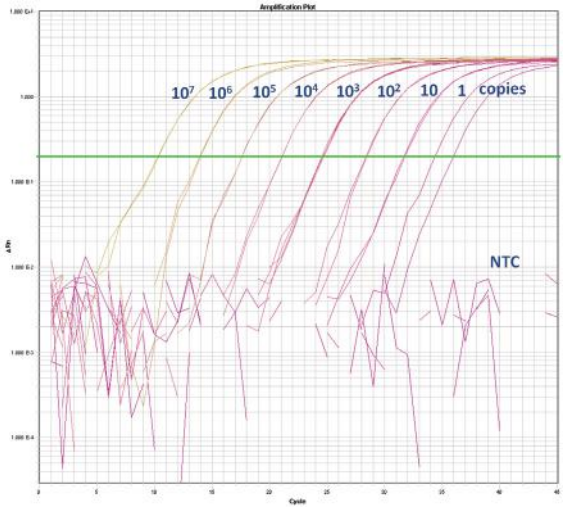


Figure 6. Assay dynamic range. Each assay was tested with 10^5 copies to 10 copies of synthetic template within a constant background of 10^5 copies of wild type genomic DNA. A subset of the assays was tested with 10^7 copies to 1 copy of synthetic template within a constant background of 10^7 copies of wild type allele synthetic template. In the example shown, the KRAS_532_mu assay has 7 logs of dynamic range, with an average PCR efficiency of 100% ± 10%.



Accuracy and reproducibility

Assays demonstrate excellent reproducibility and accurate quantification (Table 1).

Sample type compatibility

The assays can be used with gDNA samples extracted from FFPE tissues, fresh frozen tissues, and cell lines.

Data analysis software

For data analysis, Mutation Detector™ Software allows users to determine the mutation status and quantify the % mutation of their samples from TaqMan® Mutation Detection Assay data collected on the Applied Biosystems® ViiA™ 7, 7900HT, 7500, 7500 Fast, and StepOnePlus™ Real-Time PCR Systems (Table 2).

Table 1. Accuracy and reproducibility. Selected assays were tested in gDNA spiking experiments. In the example shown, G12C mutant cell line gDNA was spiked into wild type cell line gDNA at percentages ranging from 100% to 0.1%. The measured percent mutation was averaged from three experiment runs. The measured percent mutation is highly concordant with the expected percent mutation (R^2 = 0.9997). Accurate and precise quantification (CV < 20%) is obtained among the replicate runs when the target allele copy number is >30.

Copy number, target mutant allele	Expected (%)	Measured (%)	CV (%)
3,000	100.0	100.0	0.0
1,500	50.0	48.9	2.2
750	25.0	23.3	3.8
375	12.5	11.2	7.8
188	6.3	5.7	7.5
90	3.0	2.6	9.0
30	1.0	0.8	17.0
15	0.5	0.4	26.0
3	0.1	0.1	23.0

Table 2. Instrument compatibility.

Applied Biosystems® real-time PCR system	Block module	Software version
StepOnePlus™ system	Fast 96-Well Block Module	StepOne™ Software v2.X
7500 system	Standard 96-Well Block Module	SDS v1.X and v2.X
7500 Fast system	Fast 96-Well Block Module	SDS v1.X and v2.X
7900HT Fast system	Standard 96-Well Block Module, Fast 96-Well Block Module, 384-Well Block Module	SDS v2.X
ViiA™ 7 system	Standard 96-Well Block Module, Fast 96-Well Block Module, 384-Well Block Module	ViiA™ 7 Software v1.X
QuantStudio® 12K Flex system	Standard 96-Well Block Module, Fast 96-Well Block Module, 384-Well Block Module	QuantStudio® Software v1.0

Ordering information

Product	Quantity	Cat. No.
TaqMan® Mutation Detection Assays	150 µL, 10X	4465804
TaqMan® Mutation Detection Reference Assays	150 µL, 10X	4465807
TaqMan® EGFR Exon 19 Deletions Assay	150 µL, 10X	4465805
TaqMan® Mutation Detection IPC Reagent Kit	1 kit	4467538

For more information and full terms of the TaqMan® Assays QPCR Guarantee, go to **lifetechnologies.com/taqmanguarantee**

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A Thermo Fisher Scientific Brand

applied biosystems

TaqMan Assays for genetic variation research

Superior performance—reliable, robust solutions

ThermoFisher SCIENTIFIC

Genetic variation: decoding the blueprint for biodiversity

Research on genetic variation in animals and plants has expanded our understanding of evolution and human diseases, accelerated the pace of drug development, and helped identify and breed agricultural traits to improve the world's food and fuel supply. Researchers are looking to uncover the association between genetic makeup and phenotypes in studies focusing on single nucleotide polymorphisms (SNPs), copy number variants (CNVs), insertion/deletions (indels), and somatic mutations. A genomics revolution, fueled by advances in biotechnology tools, has significantly increased the rate at which we are able to obtain and analyze data to better understand biodiversity.

We're at the forefront of this revolution, and our reagents, Applied Biosystems™ TaqMan™ Assays, and Applied Biosystems™ platforms for genetic variation analysis, are the preeminent real-time PCR tools for variation research.

Coupled with Applied Biosystems™ capillary electrophoresis, and Ion Torrent™ DNA sequencing systems, we offer a complete solution for genetic analysis research, from discovery to confirmation.

TaqMan Assays for analyzing genetic variation

TaqMan Assays comprise preoptimized PCR primer pairs and one or two probes (depending on product family) for allelic discrimination or quantitative real-time PCR (qPCR). Each assay contains:

- An unlabeled PCR primer pair
- An Applied Biosystems™ TaqMan™ probe with a FAM™ or VIC™ dye label on the 5′ end, and a minor groove binder (MGB) and nonfluorescent quencher (NFQ) on the 3′ end

TaqMan Assays are used to amplify and detect specific variants in target genomic DNA (gDNA). Figure 1 depicts the Applied Biosystems™ TaqMan™ SNP Genotyping Assay process. Real-time PCR using TaqMan Assays is based on the 5′ nuclease activity of *Taq* DNA polymerase.

Here's how it works:

1. TaqMan probes hybridize to the target DNA between the two unlabeled PCR primers. Signal from the fluorescent dye on the 5′ end of a TaqMan probe is quenched by the NFQ on its 3′ end through fluorescence resonance energy transfer (FRET).
2. During PCR, *Taq* polymerase extends the unlabeled primers using the template strand as a guide.
3. When the polymerase reaches the TaqMan probe, it cleaves the molecule, separating the dye from the quencher. The qPCR instrument detects fluorescence from the unquenched FAM or VIC dye.

With each cycle of PCR, more dye molecules are released, resulting in an increase in fluorescence intensity proportional to the amount of amplicon synthesized.

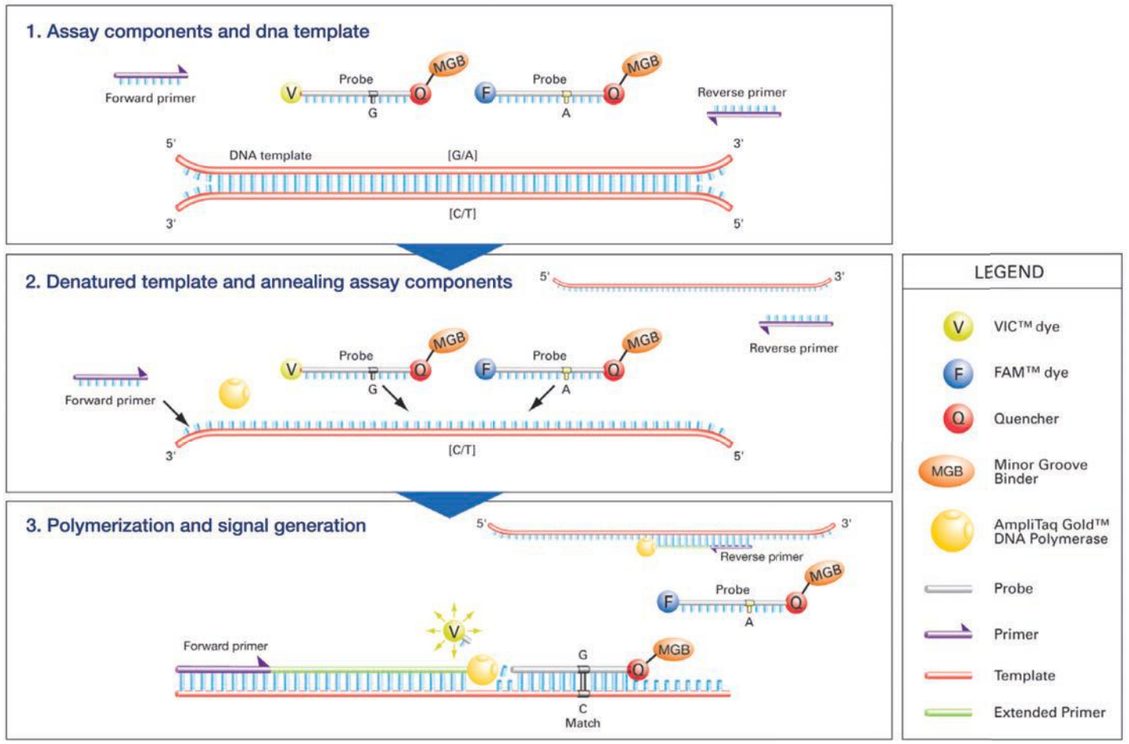


Figure 1. The TaqMan SNP Genotyping Assay. (1) The four TaqMan SNP Genotyping Assay components and the target DNA template with the SNP alleles (in brackets). **(2)** The denatured DNA target and annealing of the assay components. **(3)** Signal generation leading to specific allele detection.

TaqMan SNP Genotyping Assays

- **Better allelic discrimination**—TaqMan probes incorporate 3´ MGB technology to stabilize the probe-template complex
- **Minimize failures**—TaqMan SNP Genotyping Assays are subject to a robust design pipeline, and functional QC testing for human assays on 20 gDNA samples
- **Full-coverage assay pool**—over 7 million human SNP assays (including 160,000 validated assays tested on four ethnic populations of 45 gDNAs each) and over 10,000 mouse SNP assays
- **Simplicity**—all probes and primers are contained in a single tube: no need to optimize probe, primer, salt concentrations, or temperature; all assays use universal PCR conditions
- **Integrated run and analysis solutions**—Applied Biosystems™ instruments and associated software help you move easily from run to results

SNPs are heritable single-base pair variations that occur throughout an organism's genome. SNPs comprise the most common form of genetic variation, with some estimates of SNPs in a given human genome numbering more than 10 million. SNP genotyping plays a central role in characterizing individuals and populations, studying disease traits in humans and other organisms, and identifying genes responsible for advantageous crop traits.

TaqMan SNP Genotyping Assays provide a highly flexible technology for detection of polymorphisms within any genome. TaqMan Assays have a simple workflow and provide a quick way to generate genotyping data (Figure 2). Based on powerful TaqMan chemistry and robust probe and primer designs, and coupled to dependable Applied Biosystems instruments and software, these made-to-order assays produce high-confidence results. TaqMan Assays are ideal for genotyping applications including association studies, candidate region or gene analysis, and fine-mapping studies.

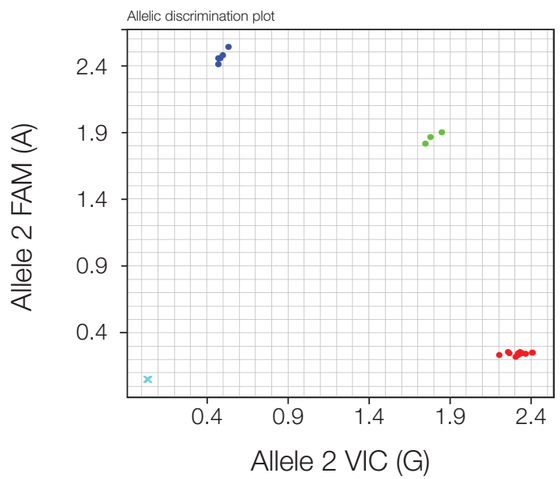


Figure 2. A three-cluster allelic discrimination plot generated with TaqMan SNP Genotyping Assay, C___1202883_20 (rs1801133) for *MTHFR* gene.

Easy online ordering

Predesigned TaqMan SNP Genotyping Assays
Find predesigned assays using our new TaqMan Assay search tool at **thermofisher.com/ordertaqman**

- Easy-to-use interface with a powerful, logical search engine
- Search by keyword (gene, SNP ID) or genomic location
- Filter by SNP type (e.g., missense mutation, intronic, UTR)
- View results on a genome alignment map for easy selection

Custom TaqMan SNP Genotyping Assays
Can't find your assay in our predesigned assay collection? Try designing a custom assay using our Applied Biosystems™ Custom TaqMan™ Assay Design Tool at **thermofisher.com/snpcadt**

- Manually enter your own custom target sequences or upload a file for batch design
- Enter custom primers and probes you have already designed to have us manufacture a ready-to-use assay for you

Simple workflow for quick results
TaqMan SNP Genotyping Assays constitute the simplest SNP genotyping technology available. We deliver your ready-to-use SNP genotyping assay in your choice of format: single-tube, 96- or 384-well plate (custom plating service), or Applied Biosystems™ TaqMan™ OpenArray™ plate (Figure 3). The rest is easy. Just combine the assay with Applied Biosystems™ TaqMan™ Genotyping Master Mix or TaqMan™ Universal PCR Master Mix and your purified DNA sample. There is no need to optimize probe, primer, salt concentrations, or temperature, because all assays use universal reagent concentrations and thermal cycling conditions. After generating an endpoint read using a thermal cycler or real-time PCR instrument, no transfers, washes, or additional reagents are required, and the plate remains sealed; just read the plate and analyze the genotypes. This helps reduce the chance of contamination, sample mix-ups, and sample loss. The simplicity of the chemistry allows you to easily automate the reaction for massively parallel genotyping studies, readily increasing the number of assays, number of samples, or both. Additionally, the analysis software allows you to auto-call genotypes, minimizing manual effort.

Simple data analysis
Applied Biosystems™ TaqMan™ Genotyper Software is a great resource for fast and accurate genotype calling. It is a free SNP genotyping data analysis tool for use with TaqMan SNP Genotyping Assays performed in 48-, 96-, or 384-well microtiter plates or OpenArray plates.

TaqMan Genotyper Software can be downloaded at **thermofisher.com/taqmangenotyper**

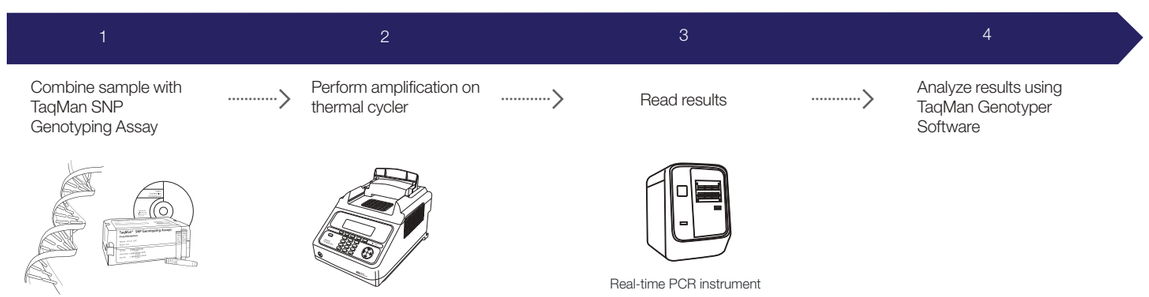


Figure 3. Workflow for TaqMan SNP Genotyping Assays.

Predesigned TaqMan SNP Genotyping Assays
Compatible Applied Biosystems™ TaqMan™ Master Mix and sample prep reagents have been developed to work in conjunction with TaqMan SNP Genotyping Assays to ensure high-quality results.

- TaqMan Genotyping Master Mix
- Applied Biosystems™ TaqMan™ Sample-to-SNP™ Kit
- Applied Biosystems™ TaqMan™ GTXpress™ Master Mix
- Applied Biosystems™ TaqMan™ Universal Master Mix II

The choice of which master mix to use depends on your sample type (tissue, blood, plant, etc.), sample preparation method (purified DNA or crude lysate), and use of fast or standard PCR cycling. For more information, go to **thermofisher.com/taqmansnp**

Ordering information

	Number of SNPs	Number of 5 µL rxns (384-well plate)	Number of 25 µL rxns (96-well plate)	Assay mix formulation	Assay type	Human assays (Cat. No.)	Nonhuman assays (Cat. No.)
Predesigned TaqMan SNP Genotyping Assays for Human and Mouse							
Small-scale	>7 million	1,500	300	40X	Made-to-order	4351379	4351384*
Medium-scale	>7 million	5,000	1,000	40X	Made-to-order	4351376	4351382*
Large-scale	>7 million	12,000	2,400	80X	Made-to-order	4351374	4351380*
Custom TaqMan SNP Genotyping Assays							
Small-scale	∞	1,500	300	40X	Made-to-order	4331349	4332077
Medium-scale	∞	5,000	1,000	40X	Made-to-order	4332072	4332075
Large-scale	∞	12,000	2,400	80X		4332073	4332076
TaqMan Drug Metabolism Genotyping Assays							
Small-scale	2,700	750	150	20X	Inventoried	4362691	N/A

***Over 10,000 mouse assays available.**

All assays are quality-control tested using a mass spectrometer to verify sequence and yield. In addition, all human (predesigned and custom) TaqMan SNP Genotyping Assays receive a genomic functional test on first synthesis. The subsequent syntheses of already-tested human assays and all nonhuman assays receive a fill volume check and mass spectrometry. All assays have a VIC dye–labeled probe, a FAM dye–labeled probe, and two target-specific primers.

Go to **thermofisher.com/taqmansnp** to order.

TaqMan Drug Metabolism Genotyping Assays

- **Excellent ADME panel coverage**—target polymorphisms in 221 genes encoding drug metabolism enzymes and associated transport proteins
- **Simple protocol**—all assays in the collection are run under the same PCR conditions, and specific allele detection is achieved with the Applied Biosystems™ TaqMan™ 5´ nuclease chemistry
- **Detects multiple polymorphisms**—detect SNPs, insertion/deletions (indels), and multinucleotide polymorphisms (MNPs)
- **Rapid receipt of order**—performance-tested assays are already in inventory, ready to ship to you.
- **Assays match databases**—assays are aligned with allele nomenclature from public allele nomenclature sites

Pharmacogenetics is the study of how a person's genetic makeup affects how he or she responds to drugs. This research offers the promise of providing information that will not only allow current drugs to be dosed and delivered more effectively but also allow the development of drugs that are specifically tailored to treat an individual.

We offer 2,700 unique Applied Biosystems™ TaqMan™ Drug Metabolism Genotyping Assays for detecting polymorphisms in 221 genes that code for various drug metabolism enzymes (DMEs) and associated transport proteins. Polymorphisms associated with these genes may influence the rate of drug metabolism within individuals, potentially affecting drug efficacy and the occurrence of side effects (Figure 4). The complex nature of these genes have had limited research conducted because few technologies and products could effectively characterize these polymorphisms. All of the assays in this collection target potentially causative polymorphisms, including those within regulatory elements, coding regions, and associated splice junctions.

TaqMan SNP Genotyping Assay technology delivers superior specificity
Each TaqMan Drug Metabolism Genotyping Assay contains two allele-specific probes and a primer pair to detect the specific SNP target. Both the probes and primers uniquely align within the genome, enabling the TaqMan genotyping technology to provide superior specificity. It is this specificity that allows these assays to detect targets residing in highly homologous gene families that may include pseudogenes.

TaqMan Drug Metabolism Genotyping Assays were developed using a high level of bioinformatics and wet-lab stringency. The assays were designed with information from several public SNP databases, including recognized public allele nomenclature sites. All assays have passed performance tests involving 180 unique DNA samples from four different populations.

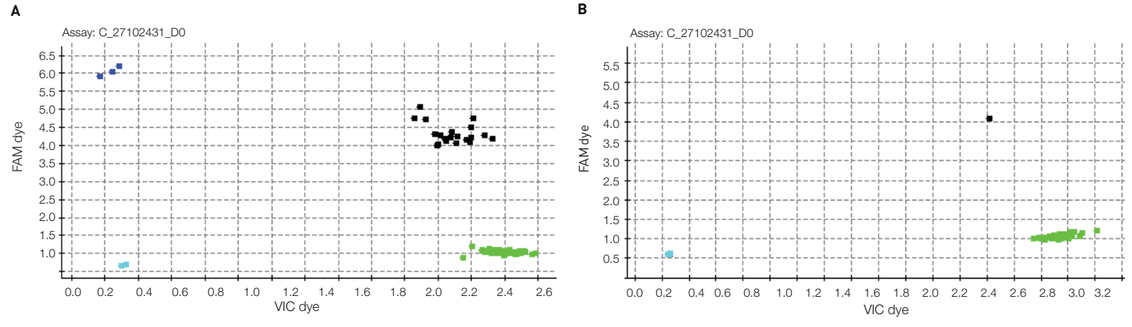


Figure 4. Allelic discrimination plots for the C__27102431_D0 assay run on (A) 45 each African-American and Caucasian, and (B) 45 each Chinese and Japanese gDNA samples. C__27102431_D0 targets the CYP2D6*4,g.1846G>A polymorphism, which encodes an mRNA splicing defect that results in a nonfunctional CYP2D6 protein. If an individual carries two nonfunctional CYP2D6 alleles, they will have the poor metabolizer (PM) phenotype and the metabolism of numerous drugs will be impacted.

Markers relevant for drug metabolism

The Applied Biosystems™ TaqMan™ DME Assay PharmaADME Core Marker Set contains a predefined group of TaqMan Drug Metabolism Genotyping and Applied Biosystems™ TaqMan™ Copy Number Assays, providing over 95% coverage of core markers in 33 ADME genes identified by the PharmaADME consortium.

This assay set greatly simplifies the study of these key putative functional genetic ADME variants and consists of:

- 164 DME assays for SNP and indel polymorphisms
- 14 copy number assays for copy number and hybrid gene variants

Assay sets are delivered in individual tubes, providing the flexibility to select a subset of assays or the entire PharmaADME Core Marker Set.

DME Assay Index

A DME Assay Index is also available with all drug metabolism assays. This file lists each assay along with context sequence, location on the NCBI assembly, the refSNP number (from dbSNP), and the common allele nomenclature from a public allele nomenclature site, when available.

Quick delivery, convenient format

For fast delivery, all assays in this collection have been manufactured and placed into inventory and are ready to ship at ambient temperature. Like other TaqMan SNP Genotyping Assays, these single-tube products consist of two allele-specific TaqMan MGB probes (labeled with either VIC or FAM dye) and two locus-specific primers. TaqMan Drug Metabolism Genotyping Assays are supplied as single tubes and in 96- and 384-well plates (custom plating service). Additionally, all products are formulated for the small-scale reaction size: a 20X single-tube assay, supporting 750 reactions at a 5 µL reaction size.

Optimized supporting reagents

Compatible TaqMan Master Mix and sample preparation reagents have been developed to work in conjunction with TaqMan Drug Metabolism Genotyping Assays to ensure high-quality results:

- TaqMan Genotyping Master Mix
- TaqMan Universal Master Mix II

Additional information about TaqMan Drug Metabolism Genotyping Assays, including links to the PharmaADME Core Marker Set and the DME Assay Index, can be found at **thermofisher.com/taqmandme**

TaqMan Copy Number Assays

- **Gold standard technology**—extraordinary accuracy and reliability; performance guaranteed for all predesigned assays**
- **Results in hours**—simplest method available to study CNV
- **Scalable solution**—automated workflow offers optimum platform for high-throughput validation of copy number changes
- **Comprehensive assay collection**—predesigned assays for human, mouse, and common vector marker/reporter genes
- **Option for custom assays**—Custom Plus and Custom TaqMan Assays for user-defined targets of interest

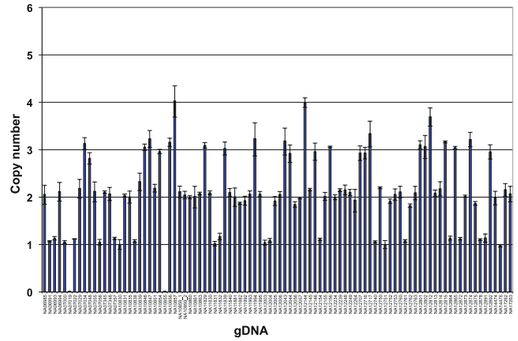
CNV, initially defined as variation in copy number of segments of DNA ≥1 kb in size, between individuals, is found in all humans as well as other animals and plants.

CNV affects a significant portion of the genome (approximately 12% of the human genome) and includes deletions, duplications, and other complex genotyping patterns. These CNVs can influence gene expression and be associated with specific phenotypes and diseases, as observed in microdeletion and microduplication syndromes.

Superior chemistry and streamlined methods offer reliable results

TaqMan Copy Number Assays combine Applied Biosystems™ TaqMan™ Assay chemistry with Applied Biosystems™ real-time PCR instruments to form a method for obtaining specific, reproducible, and easy-to-interpret copy number results (Figure 5). TaqMan Copy Number Assays are an ideal validation tool for microarray or next-generation sequencing follow-up studies and can be used to find specific targets. The workflow can be automated so that several hundred to thousands of samples can be processed in a single day.

A



B

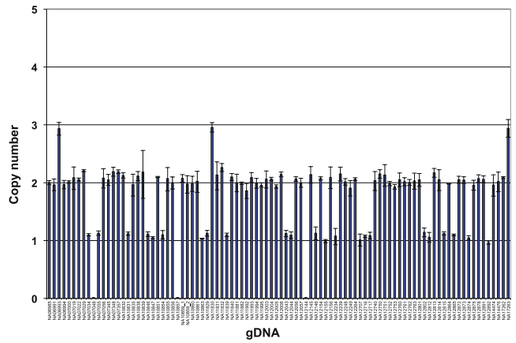


Figure 5. High specificity of TaqMan Copy Number Assays. C4A and C4B represent two isoforms of the C4 gene family. The sequences for these two genes differ in only 5 bases, but the encoded C4A and C4B proteins are functionally different. Differential detection of **(A)** C4A and **(B)** C4B is very challenging. Shown are TaqMan Copy Number Assays for C4A and C4B with the HAPMAP CEU sample set. Distinct copy number changes are observed. (JPT/CHB and YRI data not shown.)

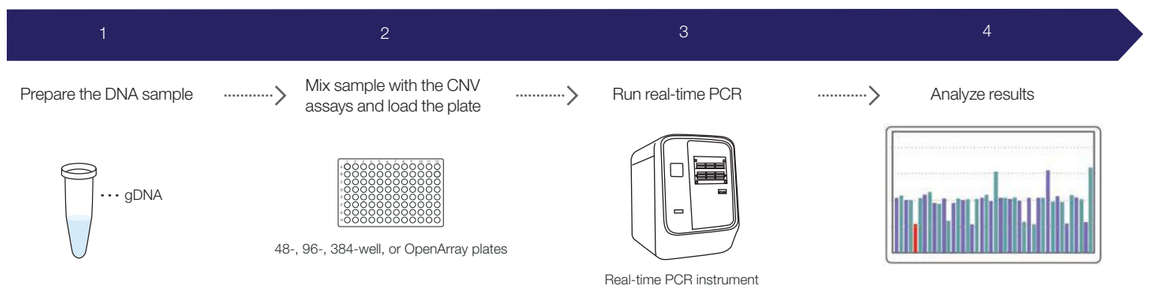


Figure 6. Workflow for TaqMan Copy Number Assays.

TaqMan Copy Number Assays

TaqMan Copy Number Assays include predesigned collections for both human and mouse genomes. The human collection includes more than 1.6 million assays for genome-wide coverage. The mouse predesigned collection includes more than 180,000 assays targeting gene exons. Predesigned assays to common vector marker and reporter genes are also available for transgenic studies.

Find predesigned assays using our online TaqMan Assay search tool at **thermofisher.com/cnv**

Applied Biosystems™ Custom Plus TaqMan™ Copy Number Assays are an optimal solution for studying variation in human and mouse genomic regions of interest for which a predesigned assay is not available. Custom Plus assays use the same bioinformatics pipeline used to manufacture predesigned TaqMan Copy Number Assays (which includes premasking of SNPs and repetitive sequences and assay genome uniqueness checks) and can be generated for high-quality genomic targets of interest using the online Applied Biosystems™ GeneAssist™ Copy Number Assay Tool. Standard Custom TaqMan Copy Number Assays are an option for additional targets of interest. Unlike Custom Plus assays, standard Custom assay designs do not go through premasking or genome quality checks, but can be compared with the human or mouse reference assays for compatibility in duplex reactions.

Two Applied Biosystems™ TaqMan™ Copy Number Reference Assays are available for copy number analysis in both human and mouse species. Note that the reference assays are species-specific.

Feature	Predesigned TaqMan Copy Number Assay	Custom Plus TaqMan Copy Number Assay	Custom TaqMan Copy Number Assay
Designed using copy number–specific algorithm optimized for performance	✓	✓	✓
Availability limited to human and mouse assays	✓	✓	
Contains TaqMan FAM dye–labeled MGB probes and two unlabeled PCR primers	✓	✓	✓
Targets undergo SNP and repetitive sequence masking	✓	✓	
Genome specificity check	✓	✓	
Reference assay compatibility check	✓	✓ (optional)	✓
Assay sequences provided			✓
Assay context sequences and genome location provided	✓	✓	

A simple CNV analysis workflow

TaqMan Copy Number Assays have one of the simplest workflows of all currently available CNV analysis methods (Figure 6). The test assay (FAM dye–labeled), the reference assay (VIC dye–labeled), your sample DNA, and TaqMan Master Mix (TaqMan Genotyping Master Mix is recommended, with TaqMan Universal Master Mix II and Applied Biosystems™ TaqMan™ Gene Expression Master Mix also being compatible) are combined and then run on an Applied Biosystems real-time PCR system using standard TaqMan Assay PCR conditions. On average, setup to primary analysis takes only 3–4 hours (including a ~2 hour PCR run).

Analysis tools and methods

TaqMan Copy Number Assays are supplied in single tubes, or the assays can be custom-plated in 96- and 384-well plates. The assay reactions are run on a real-time PCR instrument, and the data are analyzed using Applied Biosystems™ CopyCaller™ Software.

Additional information on TaqMan Copy Number Assays, as well as links to CopyCaller Software and the GeneAssist Copy Number Assay Tool, can be found at **thermofisher.com/cnv**

	Number of 10 µL rxns (384-well plate)	Number of 20 µL rxns (96-well plate)	Assay mix formulation	Assay type	Cat. No.
Predesigned TaqMan Copy Number Assays					
Small-scale	720	360	20X	Made-to-order	4400291
Medium-scale	1,500	750	20X	Made-to-order	4400292
Large-scale	5,800	2,900	60X	Made-to-order	4400293
Custom Plus TaqMan Copy Number Assays					
Small-scale	720	360	20X	Made-to-order	4442487
Medium-scale	1,500	750	20X	Made-to-order	4442520
Large-scale	5,800	2,900	60X	Made-to-order	4442488
Custom TaqMan Copy Number Assays					
Small-scale	720	360	20X	Made-to-order	4400294
Medium-scale	1,500	750	20X	Made-to-order	4400295
Large-scale	5,800	2,900	60X	Made-to-order	4400296
TaqMan Copy Number Reference Assays (Human)					
RNase P	1,500	750	20X (1 tube)	Inventoried	4403326
RNase P	6,000	3,000	20X (4 tubes)	Inventoried	4403328
TERT	1,500	750	20X (1 tube)	Inventoried	4403316
TERT	6,000	3,000	20X (4 tubes)	Inventoried	4403315
TaqMan Copy Number Reference Assays (Mouse)					
Tfrc	1,500	750	20X (1 tube)	Inventoried	4458366
Tfrc	6,000	3,000	20X (4 tubes)	Inventoried	4458367
Tert	1,500	750	20X (1 tube)	Inventoried	4458368
Tert	6,000	3,000	20X (4 tubes)	Inventoried	4458369

Looking for a different formulation, scale, or label? The TaqMan Custom Assay and Oligo Service can accommodate special requests. To learn more, email specialoligos@thermofisher.com or contact your local sales representative.

Go to **thermofisher.com/cnv** to order.

TaqMan Mutation Detection Assays for somatic mutation detection

- **High specificity**—mutant allele detection is based on an allele-specific primer, while wild type background is suppressed by the proprietary MGB blocker oligonucleotide
- **High sensitivity**—assays can detect down to 0.1% mutant molecules in a background of wild type DNA, as demonstrated in spiking experiments (Figure 8)
- **Detect multiple types of mutations**—detect single- and multiple-nucleotide mutations and insertion/deletions (indels)
- **Wide dynamic range and excellent PCR efficiency**—assays demonstrate at least 4 logs of dynamic range and an average efficiency of 100% ± 10%
- **Fast, simple workflow**—like other TaqMan Assays, typically require 3 hours from sample to results, with minimum hands-on time

Somatic mutations can be present at low levels against a high background of wild type sequences, and methods used to detect and characterize these mutations in tumor specimens need to be highly sensitive and accurate. Methods that are commonly used include gene sequencing (including pyrosequencing and traditional Sanger sequencing) and real-time PCR.

Applied Biosystems™ TaqMan™ Mutation Detection Assays were designed based on a novel competitive allele-specific Applied Biosystems™ TaqMan™ (castPCR™) technology (Figure 7), which combines allele-specific TaqMan qPCR with an allele-specific MGB blocker oligonucleotide to effectively suppress nonspecific amplification of the off-target allele. These assays target mutations in 45 genes implicated in a number of cancer models:

ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11, TP53, VHL

TaqMan Mutation Detection Assays

TaqMan Mutation Detection Assays contain mutant allele assays, which specifically detect one or more mutant alleles, and corresponding gene reference assays, which detect mutation-free regions of the genes in which the target mutations reside.

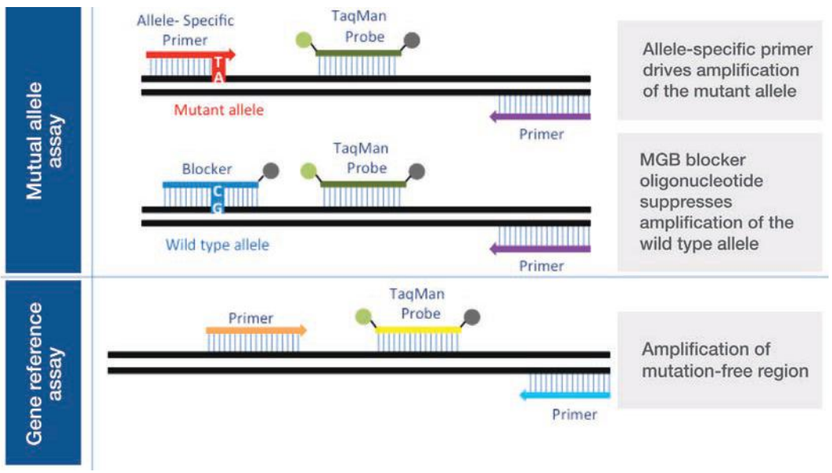


Figure 7. TaqMan Mutation Detection Assay types.

Two experiment types

Mutation detection analysis requires two types of experiments:

Detection ΔC_t cutoff determination

A mutant allele assay and corresponding gene reference assay are run on three or more wild type gDNA samples that are from the same sample type as the test samples (e.g., gDNA from FFPE tissue samples). The ΔC_t value is calculated for the mutant allele assay/gene reference assay pair, for each sample. The average ΔC_t for all samples is then calculated and is used to derive the detection ΔC_t cutoff value for the mutant allele assay.

Mutation detection

A test sample is run with one or more mutant allele assays and a corresponding gene reference assay. The ΔC_t for the mutant allele assay/gene reference assay pair is calculated, and this value is compared to the previously determined detection ΔC_t cutoff value to determine the sample's mutation status.

Simple workflow

Purified gDNA, extracted from a sample of unknown mutation status, is run with one or more mutant allele assays and corresponding gene reference assays. For each real-time PCR, the gDNA is combined with:

- A TaqMan Mutation Detection Assay
- TaqMan Genotyping Master Mix
- (Optional) Applied Biosystems™ TaqMan Mutation Detection IPC Reagent Kit—to distinguish true target negatives from PCR failure or inhibition

Reactions are run on a real-time PCR system using a universal thermal cycling protocol for mutation detection. After the run, the real-time PCR system analysis software determines the C_t for each TaqMan Mutation Detection Assay and (optional) IPC reagent reactions. Real-time results can be exported as files that can be opened in free Applied Biosystems™ Mutation Detector™ Software.

Ordering information

Product	Size	Assay type	Cat. No.
TaqMan Mutation Detection Assays	150 µL, 10X	Inventoried	4465804
TaqMan Mutation Detection Reference Assays	150 µL, 10X	Inventoried	4465807
TaqMan EGFR Exon 19 Deletions Assay	150 µL, 10X	Inventoried	4465805
TaqMan Mutation Detection IPC Reagent Kit	1 kit	Inventoried	4467538

New assays for other cancer gene mutation targets will continually be released.

Go to **thermofisher.com/castpcr** for the most current list.

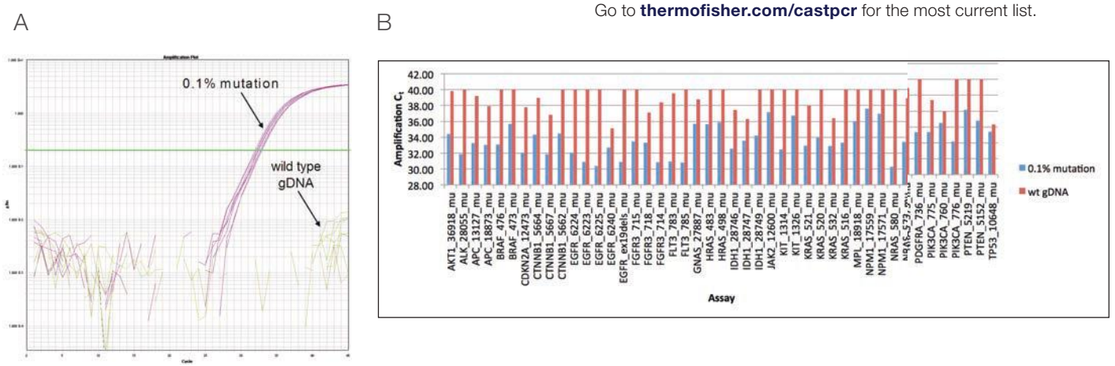


Figure 8. C_t differences between 0.1% mutation samples and wild type gDNA in TaqMan Mutation Detection Assays. For each mutant allele assay, 0.1% mutant allele samples were obtained by spiking cell line wild type gDNA (30 ng, ~10,000 copies) with 10 copies of mutant allele synthetic templates. **(A)** Example of amplification plot for KRAS_522_mu assay run on a 0.1% mutation sample and a wild type gDNA sample (30 ng gDNA). **(B)** For a majority of the assays, there is a significant difference in amplification C_t values between the 0.1% mutant allele sample and wild type gDNA (*P* value < 0.05).

TaqMan genotyping reagents for optimal performances

TaqMan Sample-to-SNP Kit
The TaqMan Sample-to-SNP Kit takes you from biological sample to results typically in less than an hour, without isolating DNA. The kit consists of two parts: the Applied Biosystems™ DNA Extract All Reagents and the TaqMan GTXpress Master Mix. The DNA All Lysis Reagents reduce prolonged procedures for the release of real-time PCR–ready DNA to a 5-minute protocol. They are compatible with a wide variety of samples ranging from blood to buccal swabs to animal and plant tissues. DNA extracted with DNA Extract All Reagents can be used with TaqMan SNP Genotyping Assays (not recommended for other TaqMan Assays).

TaqMan master mixes
TaqMan master mixes contain buffer, dNTPs, passive reference dye, thermostable hot-start DNA polymerase, and other components, and are provided in a convenient single-vial format. They are formulated to provide optimal results for TaqMan Assays.

- **TaqMan Genotyping Master Mix**—the TaqMan Genotyping Master Mix is optimized for end-point fluorescence detection in SNP genotyping applications in standard mode; the TaqMan Genotyping Master Mix provides excellent pre- and post-PCR stability for high-throughput setup and analysis
- **TaqMan GTXpress Master Mix**—the TaqMan GTXpress Master Mix is designed to deliver accurate genotyping results with robust performance in less than 50 minutes; the TaqMan GTXpress Master Mix is also available as part of the TaqMan Sample-to-SNP Kit

Ordering information and assay compatibility

	TaqMan Genotyping Master Mix	TaqMan GTXpress Master Mix
Cat. No. (size)	4371355 (10 mL)†	4401892 (10 mL)
TaqMan SNP Genotyping Assays	††	††
TaqMan Drug Metabolism Genotyping Assays	††	+
TaqMan Copy Number Assays	††	–
TaqMan Mutation Detection Assays for somatic mutation detection	††	–

†Other pack sizes are available.

††Thermo Fisher Scientific validated: We have performed extensive testing and optimization.
+Thermo Fisher Scientific demonstrated: Limited testing has been performed. We cannot guarantee optimal performance for all TaqMan Assays.
–Not recommended.

Quality service and support at every step of your workflow

From manufacturing to follow-up—consistent reliability

TaqMan Assays are designed, manufactured, packaged, tested, and shipped using the highest-quality materials and methods. Furthermore, they are backed by our worldwide technical support teams.

Quality manufacturing and stringent quality control
TaqMan Assays are manufactured in-house at our ISO 13485–certified manufacturing facilities and are never outsourced.

Comprehensive worldwide support
Whether you need help finding a TaqMan Assay for your target, deciding which format best suits your needs, placing your order through our online ordering system, or setting up your reactions, our sales and technical support staff are here to help.

Sales support
Your sales representative can help you find Web and print resources to help you choose the right TaqMan Assay products for your genetic variation research. For more demanding projects, she or he can also involve our technical sales specialists, who have more in-depth knowledge of TaqMan Assay technology and our relevant supporting reagents and instruments.

Technical support
If you have questions about how to use TaqMan Assays or how to analyze results, go to **thermofisher.com/support** to contact our technical support specialists. These agents are skilled in experimental planning and design, are expert troubleshooters, and are familiar with a wide variety of applications that use TaqMan Assays.

Rapid delivery
We continually strive to minimize delivery time on TaqMan Assay products. To that end, we have implemented streamlined order processing systems that interface with our new manufacturing facilities to help reduce delivery times.

TaqMan Assay type	Estimated delivery time (business days/weeks)
Inventoried (in stock)	1–4 days
Made-to-order/Custom TaqMan Assays (manufactured when order is placed)	5–12 days
TaqMan Custom Plating Service (configure 96- or 384-well plates with any TaqMan assays)	2–5 weeks

**The TaqMan Assays QPCR Guarantee
We stand behind every predesigned TaqMan Assay you buy. We're committed to helping you achieve your research goals and believe our predesigned TaqMan Assays establish the benchmark for high-quality and easy-to-use real-time PCR products. If you are not satisfied with the performance of a predesigned TaqMan Assay, we'll replace it at no cost or credit your account. For more information, and full terms and conditions of the guarantee, go to **thermofisher.com/taqmanguarantee**

applied biosystems

Find out more at **thermofisher.com/taqman**

ThermoFisher SCIENTIFIC

TaqMan® Genotyping Master Mix

Catalog Numbers 4371353, 4371355, 4381656, 4371357, and 4381657

Pub. No. 4374656 **Rev.** D

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TaqMan® Genotyping Master Mix Protocol* (Pub. No. 4371131). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Contents and storage

Cat. No.	Number of reactions	Contents	Storage
4371353	40	1 mL	2–8°C for up to one year
4371355	400	10 mL	
4381656	800	2 × 10 mL	
4371357	2,000	50 mL	
4381657	4,000	2 × 50 mL	

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Manufacturer: Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania

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Revision history: Pub. No. 4374656

Revision	Date	Description
D	04 September 2018	Updated manufacturing address, branding, licensing, trademarks, general style and format.
C	September 2011	Baseline for this revision.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Real-time PCR master mixes and instrument compatibility

	TaqMan® and TaqPath real-time PCR master mixes								One-step real-time RT-PCR master mixes					
Applications	**Gene expression/ small RNA**	**Genotyping/ CNV**	**Gene expression/ genotyping**	**Gene expression/ genotyping**	**Gene expression**	**Genotyping/ SNP**	**Genotyping/ SNP**	**Gene expression**	**Gene expression/ pathogen detection**	**Gene expression/ pathogen detection**	**Gene expression/ pathogen detection**	**Gene expression for 1-step and multiplex**	**Gene expression/ pathogen detection**	**Gene expression/ pathogen detection**
	TaqMan Fast Advanced Master Mix	TaqPath ProAmp Master Mix	TaqMan Universal PCR Master Mix	TaqMan Universal Master Mix II	TaqMan Gene Expression Master Mix	TaqMan Genotyping Master Mix	TaqMan GTXpress Master Mix	TaqMan Fast Universal PCR Master Mix	TaqMan RNA-to-C_T *1-Step* Kit	*Power* SYBR Green RNA-to-C_T *1-Step* Kit	TaqMan Fast Virus 1-Step Master Mix	TaqPath 1-Step Multiplex Master Mix	SuperScript III Platinum One-Step qRT-PCR Kit	SuperScript III Platinum One-Step qRT-PCR Kit w/ ROX
Instruments	4444556 4444557 4444963 4444964 4444965 4444558	A32704, A30865, A30866, A30871, A30867, A30872, A32705, A30868, A30869, A30873, A30870, A30874	4304437, 4305719, 4318157, 4326708, 4364338, 4364340, 4324018, 4324020, 4326614, 4364341, 4364343	4440043, 4440040, 4440047, 4440048, 4440049, 4440041, 4440042, 4440038, 4440044, 4440045, 4440046, 4440039	4370048 4369016 4369514 4369510 4369542 4370074	4371353 4371355 4381656 4371357 4381657	4403311 4401892 4401890 4401857	4352042 4364103 4366072 4366073 4367846	4392653 4392938 4392656	4391178 4389986	4444432 4444434 4444436	A28521, A28522, A28523, A28525, A28526, A28527	11732020 11732088	11745500 11745100
Analytik Jena qTOWER	√	√	√	√	√	√	√	√	√	√	√	√	√	
Applied Biosystems 7500	√	√	√	√	√	√	√	√	√	√	√	√	√	
Applied Biosystems 7500 Fast	√	√	√	√	√	√	√	√	√	√	√	√	√	
Applied Biosystems 7300	√	√	√	√	√	√	√	√	√	√	√	√*	√	√
Applied Biosystems 7900HT	√	√	√	√	√	√	√	√	√	√	√	√*	√	√
Applied Biosystems QuantStudio 12K Flex	√	√	√	√	√	√	√	√	√	√	√	√	√	
Applied Biosystems QuantStudio 6	√	√	√	√	√	√	√	√	√	√	√	√	√	
Applied Biosystems QuantStudio 3/5	√	√*	√	√	√	√	√	√	√	√	√	√*	√	
Applied Biosystems QuantStudio 7	√	√	√	√	√	√	√	√	√	√	√	√	√	
Applied Biosystems StepOne	√	*	√	√	√	√	√	√	√	√	√	*	√	√
Applied Biosystems StepOnePlus	√	*	√	√	√	√	√	√	√	√	√	*	√	√
Applied Biosystems ViiA 7	√	√	√	√	√	√	√	√	√	√	√	√	√	
Bio-Rad CFX384	√	√	√	√	√	√	√	√	√	√	√	√	√	
Bio-Rad CFX96	√	√	√	√	√	√	√	√	√	√	√	√	√	
Bio-Rad iQ5	√	√	√	√	√	√	√	√	√	√	√	√	√	
Bio-Rad MiniOpticon	√	√	√	√	√	√	√	√	√	√	√	√	√	
Bio-Rad/MJ Chromo4	√	√	√	√	√	√	√	√	√	√	√	√	√	
Bio-Rad/MJ Opticon	√	√	√	√	√	√	√	√	√	√	√	√	√	
Bio-Rad/MJ Opticon 2	√	√	√	√	√	√	√	√	√	√	√	√	√	
Eppendorf Mastercycler ep realplex	√	√	√	√	√	√	√	√	√	√	√	√	√	
Qiagen/Corbett Rotor-Gene 6000	√	√	√	√	√	√	√	√	√	√	√	√	√	
Qiagen/Corbett Rotor-Gene Q	√	√	√	√	√	√	√	√	√	√	√	√	√	
Qiagen/Corbett Rotor-Gene 3000	√	√	√	√	√	√	√	√	√	√	√	√	√	
Roche LightCycler 480	√	√	√	√	√	√	√	√	√	√	√	√	√	
Roche LightCycler Nano	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	
Agilent/Stratagene MX3000P	√	√	√	√	√	√	√	√	√	√	√	√	√	
Agilent/Stratagene MX3005P	√	√	√	√	√	√	√	√	√	√	√	√	√	
Agilent/Stratagene MX4000	√	√	√	√	√	√	√	√	√	√	√	√	√	
TaKaRa T800	◊	◊	◊	◊	◊	◊	◊	◊	◊	√	◊	◊	◊	

√ – Indicates preferred kit and tested on this instrument.
◊ – Indicates kit should work, but has not been tested on this instrument.
* – Not compatible with multiplexing. The QuantStudio 3 system can be used for limited multiplexing; it is not compatible with Mustang Purple or Cy®5 dyes. The QuantStudio 5 system is compatible with multiplexing.

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Real-time PCR master mixes and instrument compatibility

	SYBR Green real-time PCR master mixes					
Applications	**Gene expression**	**Gene expression**	**Gene expression**	**Gene expression**	**Gene expression**	**Gene expression**
	SYBR Select Master Mix	Fast SYBR Green Master Mix	*Power* SYBR Green PCR Master Mix	SYBR Green PCR Master Mix	SYBR GreenER qPCR SuperMix Universal	PowerUp SYBR Green Master Mix
Instruments	4472903, 4472908, 4472913, 4472918, 4472919, 4472920	4385610, 4385612, 4385616, 4385617, 4385618, 4385614	4367659, 4367660, 4368577, 4368702, 4368706, 4368708	4344463, 4309155, 4364344, 4364346, 4334973, 4312704	1176202K, 11762100, 11762500	A25742, A25743, A25776, A25777, A25778, A25779, A25780, A25918
Applied Biosystems 7500	√	√	√	√	√	√
Applied Biosystems 7500 Fast	√	√	√	√	√	√
Applied Biosystems 7300		√	√	√	√	√
Applied Biosystems 7900HT		√	√	√	√	√
Applied Biosystems QuantStudio 12K Flex	√	√	√	√	√	√
Applied Biosystems QuantStudio 6	√	√	√	√	√	√
Applied Biosystems QuantStudio 3/5	√	√	√	√	√	√
Applied Biosystems QuantStudio 7	√	√	√	√	√	√
Applied Biosystems StepOne	√*	√	√	√	√	√
Applied Biosystems StepOnePlus	√*	√	√	√	√	√
Applied Biosystems ViiA 7	√	√	√	√	√	√
Bio-Rad CFX384	√	√	√	◊	◊	√
Bio-Rad CFX96	√	√	√	◊	◊	√
Bio-Rad iQ5	√	√	√	√		√
Bio-Rad MiniOpticon	√	√	√	√	√	√
Bio-Rad/MJ Chromo4	√	√	√	√	√	√
Bio-Rad/MJ Opticon	√	√	√	√	√	√
Bio-Rad/MJ Opticon 2	√	√	√	√	√	√
Eppendorf Mastercycler ep realplex		√	√	√		√
Qiagen/Corbett Rotor-Gene 6000	√*	√	√	√	√	√
Qiagen/Corbett Rotor-Gene Q	√*	√	√	√	◊	√
Qiagen/Corbett Rotor-Gene 3000	√*	√	√	√	◊	√
Roche LightCycler 480	√	√	√	√		√
Roche LightCycler Nano		◊	◊	◊		◊
Agilent/Stratagene MX3000P	√	√	√	√	√	√
Agilent/Stratagene MX3005P	√	√	√	√	√	√
Agilent/Stratagene MX4000	√	√	√	√	√	√
TaKaRa T800		√	√	√		√

√ – Indicates preferred kit and tested on this instrument.
√* – Ensure primer concentration guide is followed (<200 nM for standard mode and 300–400 nM for fast mode). For StepOne/StepOnePlus System, install software version 2.3 or higher update.
◊ – Indicates kit should work, but has not been tested, on this instrument.

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WHITE PAPER

Modifications to TaqMan® Universal PCR Master Mix have no effect on functional performance or stability

Abstract

Beginning in April 2014, minor modifications will be made to the TaqMan® Universal PCR Master Mix and TaqMan® Universal PCR Master Mix, No AmpErase® UNG. These modifications include transfer of manufacturing from Branchburg, NJ to Warrington, UK and changes in source/vendor for some raw material components. In recognition of the importance of these products in existing protocols, we have conducted extensive studies to demonstrate functional equivalency. These changes have been shown to have no effect on the overall functional characteristics of the master mixes. This paper describes the testing process and test results for dynamic range, sensitivity, specificity, discrimination, C_t and ΔRn comparisons, and pre-PCR stability. The results show no differences between the current TaqMan® Universal PCR Master Mix manufactured in Branchburg, NJ and validation lots manufactured in Warrington, UK with the described changes.

Introduction

As part of the ongoing efforts at Life Technologies to provide the highest quality qPCR master mixes, modifications will be made to the TaqMan® Universal PCR Master Mix and TaqMan® Universal PCR Master Mix, No AmpErase® UNG, beginning in April 2014. The impacted catalog numbers are listed in the Appendix (Table 4). These modifications include the transfer of manufacturing from Branchburg, NJ (Roche Molecular Systems, third party manufacturer) to Warrington, UK (Thermo Fisher Scientific Inc., formerly Life Technologies). The formulation remains unchanged; however, there is a change in source/vendor for some raw material components such as dNTPs and AmpliTaq Gold® DNA Polymerase.

The execution of the transfer is tightly controlled in a vigilant manner to minimize disruption to the many laboratories that rely on these reagents every day. The most important aspect of this process is to maintain consistent performance and reliability. This paper describes the testing process, involving multiple lots, with a number of gene expression assays, to assess dynamic range, sensitivity, specificity, discrimination, and pre-PCR stability. Results show no functional difference between current TaqMan® Universal PCR Master Mix manufactured in Branchburg, NJ and validation lots manufactured in Warrington, UK with the implemented changes. A separate document is available describing testing and results for TaqMan® Universal PCR Master Mix, No AmpErase® UNG.

Materials and methods

Material lots tested

For performance comparisons, four unique lots of TaqMan® Universal PCR Master Mix made in Branchburg, NJ (designated R1, R2, R3, and R4) were ordered in 50 mL kits directly from the Life Technologies website.

life technologies™

Three validation lots (designated L1, L2, and L3) were formulated in full-scale volumes at the Warrington, UK manufacturing site.

Validation material QC

Validation lots passed analytical QC specifications set for Mg^{2+} concentration (HPIC), dNTP concentrations (HPLC), DNase/RNase activity, *E. coli* contamination, and pH. They also passed functional tests with RNase P and ß-actin gene expression assays.

Functional performance

Dynamic range

Four TaqMan® Gene Expression Assays (FAM™-MGB, 20X) were tested across a 5-log concentration dynamic range: FN1 (Hs00277509_m1), PGK1 (Hs99999906_m1), RPLP0 (Hs99999902_m1), and B2M (Hs00187842_m1). The serial dilution spanned final concentrations of 100 ng to 1 pg of cDNA per reaction. The cDNA template was synthesized from Universal Human RNA (Stratagene) and the SuperScript® VILO™ cDNA Synthesis Kit (Cat. No. 11754250). Additionally, duplex performance was tested with B2M and an exogenous internal positive control (IPC) (VIC®-TAMRA™ probe; Cat. No. 4308323). Reactions were run in the 384-well format on a ViiA™ 7 Real-Time PCR System using universal cycling conditions (50°C, 2 min; 95°C, 10 min; 95°C, 15 sec; and 60°C, 1 min for 40 cycles). Each reaction was tested with six technical replicates and was further repeated across three PCR runs. ViiA™ 7 software v1.2.2 was used to generate amplification plots and determine C_t values (analysis settings: auto-baseline; threshold set at 0.1).

Gene expression panel

138 TaqMan® Gene Expression Assays (FAM™-MGB, 20X) and six TaqMan® endogenous controls were functionally tested. Refer to Table 3 in the Appendix for assay information. The six endogenous controls were primer-limited (150 nM instead of 900 nM) and had VIC®-TAMRA™ probes. Reactions followed the standard product protocol for a 10 µL reaction volume. 1 ng of cDNA synthesized from Universal Human RNA (Stratagene) and the SuperScript® VILO™ cDNA Synthesis Kit was used as the final template amount for all reactions. Reactions were run in the 384-well format on a ViiA™ 7 Real-Time PCR System using universal cycling conditions (50°C, 2 min; 95°C, 10 min; 95°C, 15 sec; and 60°C, 1 min for 40 cycles). Each reaction was tested with six technical replicates. ViiA™ 7 software v1.2.2 was used to generate amplification plots and determine C_t values (analysis settings: auto-baseline; threshold: set at 0.1).

Pre-PCR stability

The 144-assay gene expression panel was also tested for pre-PCR stability of 24 and 72 hours. Reactions were assembled as described above and the sealed 384-well plates were stored on the benchtop at room temperature, exposed to intermittent light before being run on the ViiA™ 7 Real-Time PCR System. Thermal cycling conditions and analysis settings were identical to those previously described.

Specificity

The 144-assay gene expression panel was also tested for specificity by running "no-template control" (NTC) reactions. Reactions were assembled as described above, except that water replaced the sample volume. Thermal cycling conditions and analysis settings were identical to those previously described.

Sensitivity

The RNase P assay from the TaqMan® RNase P Detection Reagents Kit (Cat. No. 4316831) was tested with CEPH gDNA (Cat. No. 403062) diluted to a final concentration of 2 copies per 20 µL reaction. Reactions were run in the 384-well format on a ViiA™ 7 Real-Time PCR System using universal cycling conditions (50°C, 2 min; 95°C, 10 min; 95°C, 15 sec; and 60°C, 1 min for 40 cycles). Each reaction was tested with 20 technical replicates to overcome sampling error at the low concentration. NTC reactions were tested with six technical replicates. Each set of replicates was further repeated across three PCR runs. ViiA™ 7 software v1.2.2 was used to determine C_t values (analysis settings: auto-baseline; threshold set at 0.1).

Discrimination

The RNase P assay from the TaqMan® RNase P Detection Reagents Kit (Cat. No. 4316831) was tested with CEPH gDNA (Cat. No. 403062) diluted to final concentrations of either 1,600 or 800 copies per 20 µL reaction. Reactions were run in the 384-well format on a ViiA™ 7 Real-Time PCR

System using universal cycling conditions (50°C, 2 min; 95°C, 10 min; 95°C, 15 sec; and 60°C, 1 min for 40 cycles). Each reaction was tested with 10 technical replicates. NTC reactions were tested with six technical replicates. Each set of replicates was further repeated across three PCR runs. ViiA™ 7 software v1.2.2 was used to determine C_t values (analysis settings: auto-baseline; threshold set at 0.1).

Results

Dynamic range

With each of the four TaqMan® Gene Expression Assays—FN1, PGK1, RPLP0, and B2M—no difference was observed between master mix lots for the mean C_t values calculated from the 18 data points at each dilution point. Three validation lots (UMM L1–L3) were compared against four lots of the current product (UMM R1–R4).

The following graphs depict the mean C_t vs. log concentration for the four tested assays (Figures 1A–4A) and a representation of the amplification plots for each assay (Figures 1B–4B), showing strong linearity (R^2 values ≥ 0.998), tight clustering across the series, and clean amplification curves.

Figures 5A and 5B depict the dilution plot and amplification curve, respectively, of the duplex B2M assay with IPC, once again displaying strong linearity (R^2 ≥ 0.999) and amplification.

PCR efficiency values were determined for the three tested lots using the slope of the mean C_t values for each of the four assays and the duplex assay (see Figure 6). Efficiencies across lots were consistent (< 2% difference) for each tested assay.

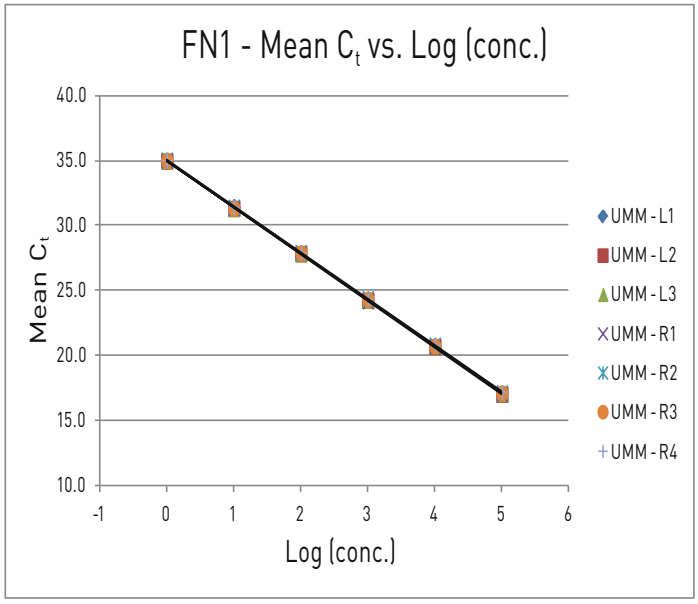


Figure 1A. FN1 (Hs00277509_m1) mean C_t values plotted across 5 logs of cDNA sample for all seven lots of Universal PCR Master Mix.

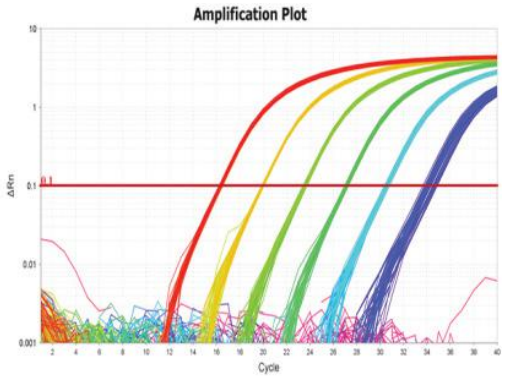


Figure 1B. PCR amplification plot overlaying technical replicates from all seven master mix lots at each concentration point.

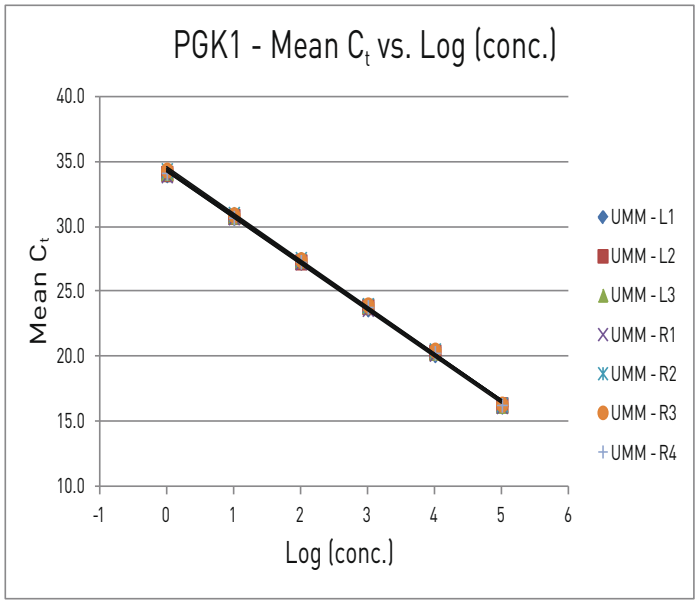


Figure 2A. PGK1 (Hs99999906_m1) mean C_t values plotted across 5 logs of cDNA sample for all seven lots of Universal PCR Master Mix.

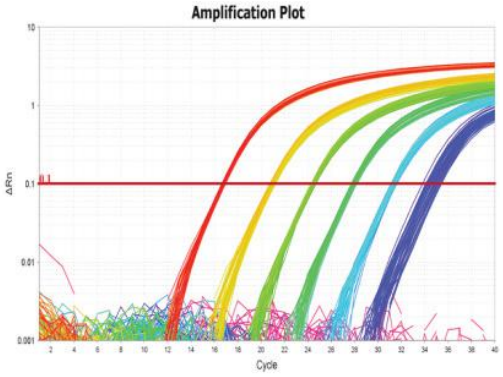


Figure 2B. PCR amplification plot overlaying technical replicates from all seven master mix lots at each concentration point.

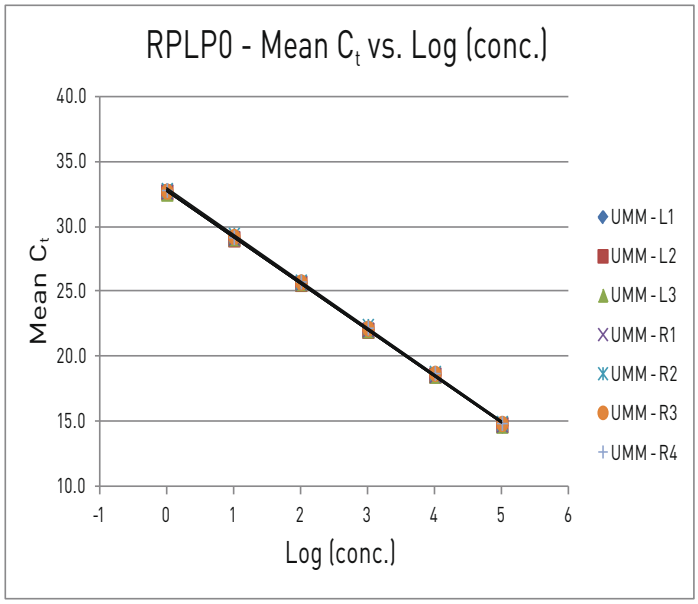


Figure 3A. RPLP0 (Hs99999902_m1) mean C_t values plotted across 5 logs of cDNA sample for all seven lots of Universal PCR Master Mix.

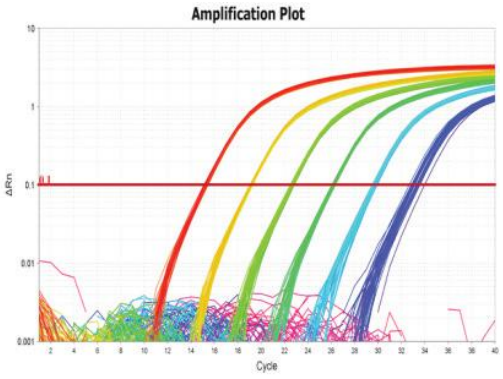


Figure 3B. PCR amplification plot overlaying technical replicates from all seven master mix lots at each concentration point.

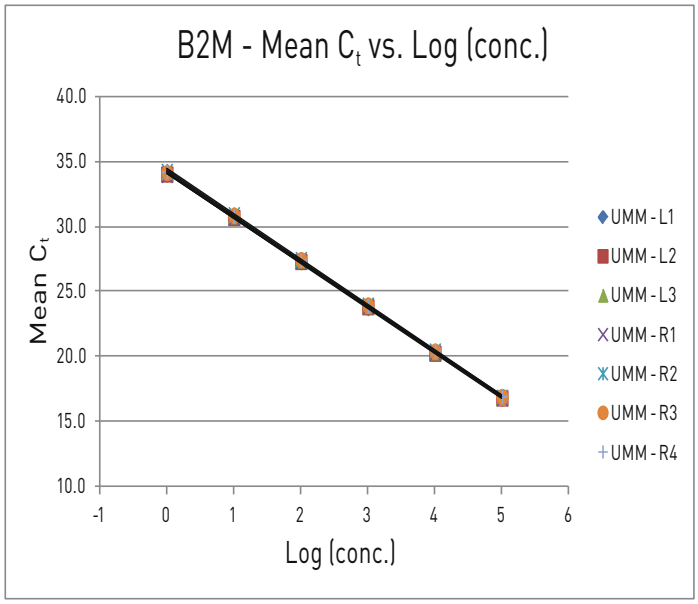


Figure 4A. B2M (Hs00363670_m1) mean C_t values plotted across 5 logs of cDNA sample for all seven lots of Universal Master Mix.

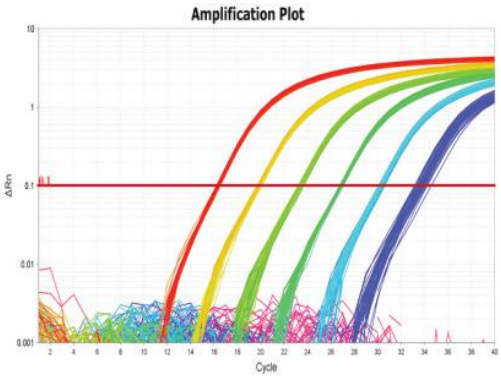


Figure 4B. PCR amplification plot overlaying technical replicates from all seven master mix lots at each concentration point.

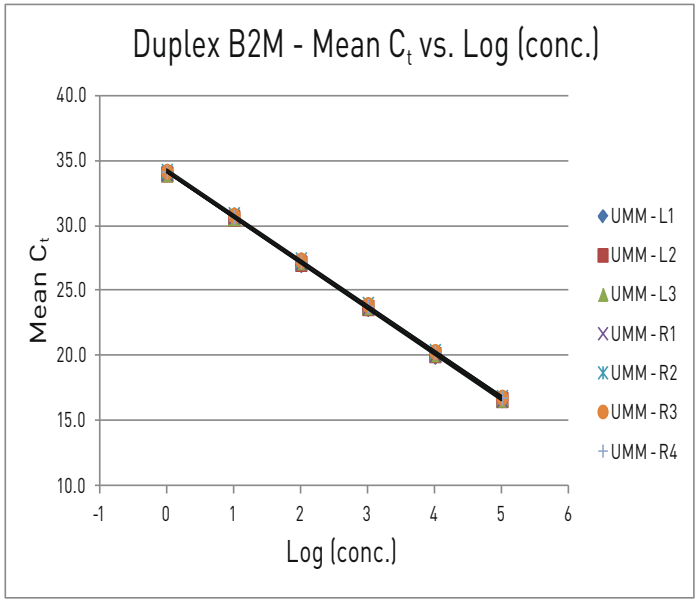


Figure 5A. B2M (Hs00363670_m1) mean C_t values when duplexed with an exogenous IPC (4308323); plotted across 5 logs of cDNA sample for all seven lots of Universal PCR Master Mix.

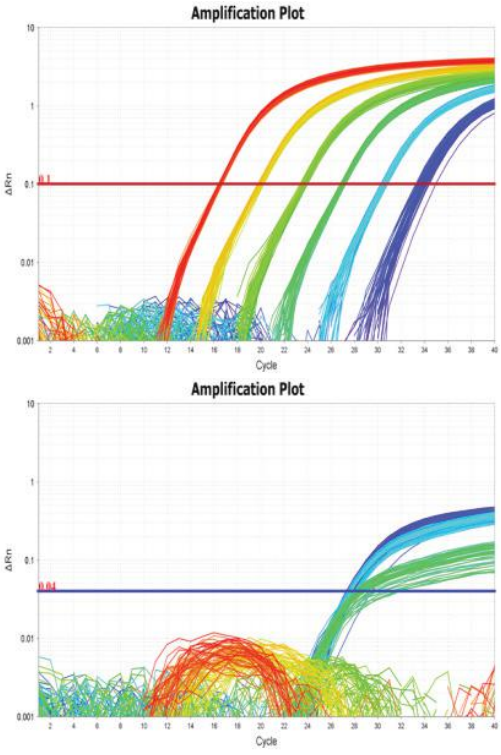


Figure 5B. PCR amplification plot overlaying technical replicates from all seven master mix lots at each concentration point and for the exogenous IPC (bottom). Note that at high concentrations the B2M assay out-competes the exogenous IPC, accounting for the observed variability in the IPC amplification plot.

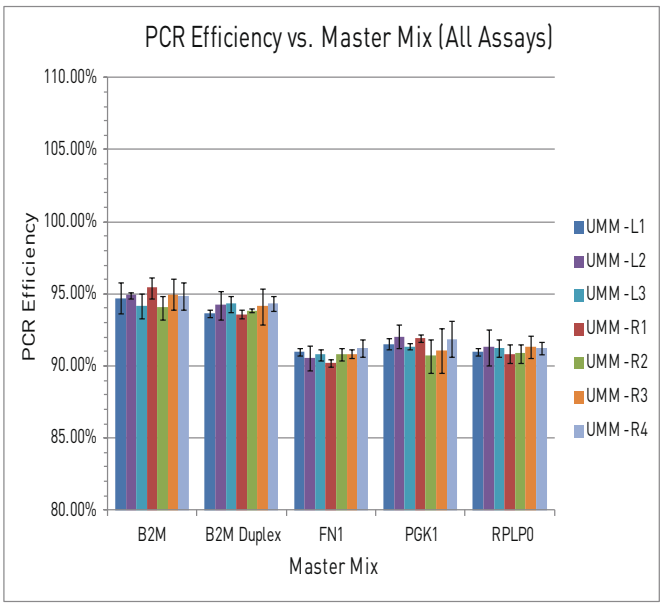
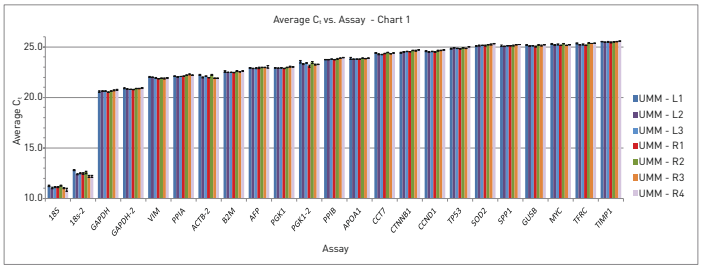


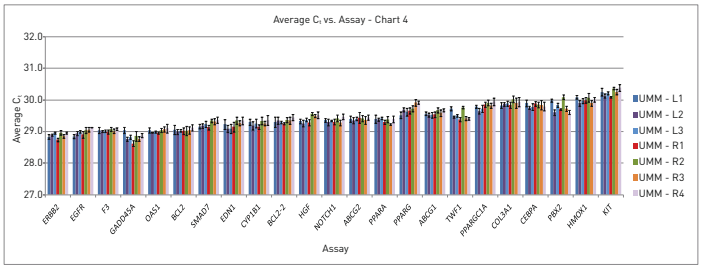
Figure 6. PCR efficiency values for each gene expression assay, calculated from the slope of the mean C_t vs. log(conc.) plot. Each assay exhibited consistent efficiencies between lots of Universal PCR Master Mix (differences <2%).

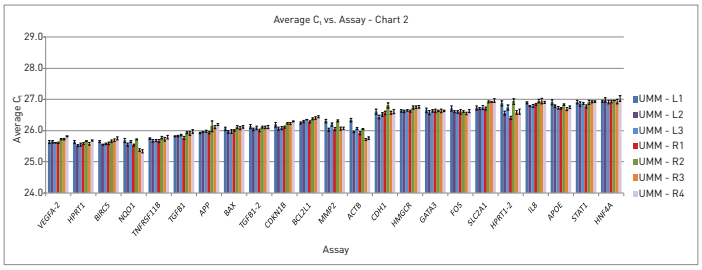
Gene expression panel

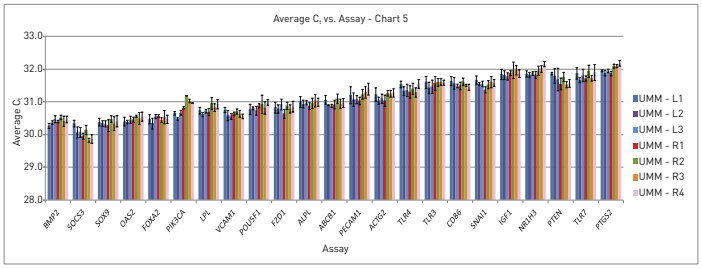
Performance in gene expression assays was tested across the seven lots using 138 TaqMan® Gene Expression Assays and six endogenous control assays with average C_t values calculated from six technical replicates. The plots in Figure 7 show clear consistency of C_t values (average difference: <0.4%) within each assay across the seven lots from low-, medium-, and high-expressing genes.

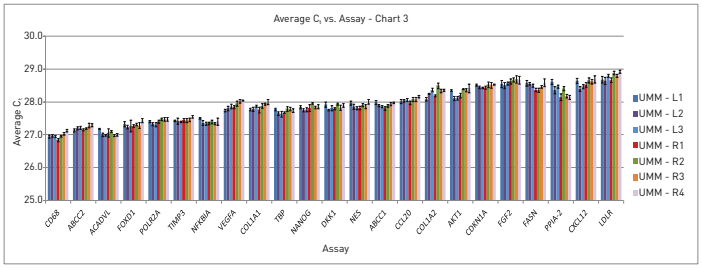
Figure 8 depicts the mean ΔRn values for the same 144 TaqMan® Gene Expression Assays. No real differences are observed, with 99% of assays showing between-lot differences of <20% (calculated with the mean ΔRn of each population for each assay).











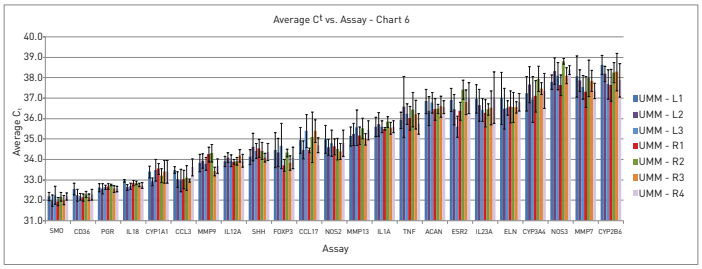


Figure 7. Mean C_t values obtained from each master mix (L1–L3 of Universal Master Mix and current lots R1–R4 Universal Master Mix) across all 138 TaqMan® Gene Expression Assays and 6 endogenous control assays. Six assays were excluded because of no amplification with any lot groups: ADIPOQ, CYP2C9, IL12B, IL2, FABP4, and FASLG. Six technical replicates per assay were run with each master mix on the same PCR plate (n = 6; error bars are ±1σ).

Figure 8. Mean ΔRn values obtained from each master mix (L1–L3 of Universal Master Mix and current lots R1–R4 Universal Master Mix) across all 138 TaqMan® Gene Expression Assays and 6 endogenous control assays. Six assays were excluded because of no amplification with any lot groups: ADIPOQ, CYP2C9, IL12B, IL2, FABP4, and FASLG. Six technical replicates per assay were run with each master mix on the same PCR plate (n = 6; error bars are ±1σ).

Pre-PCR stability

Extensive stability testing was performed on all 144 assays used for the performance testing above. Assembled reaction plates were sealed and left at room temperature for 24 and 72 hours, and all results calculated and collated. Figure 9 is a representation from a single validation lot (left) and current lot (right), showing mean C_t values for each assay with clear correlation across time points (Pearson's value correlation scores were ≥0.998 for validation lots and current lots). Similarly, Figure 10 shows data for mean ΔRn values for the same two lots (Pearson's r-value correlation scores were ≥ 0.98 for validation lots and ≥0.97 for current lots).

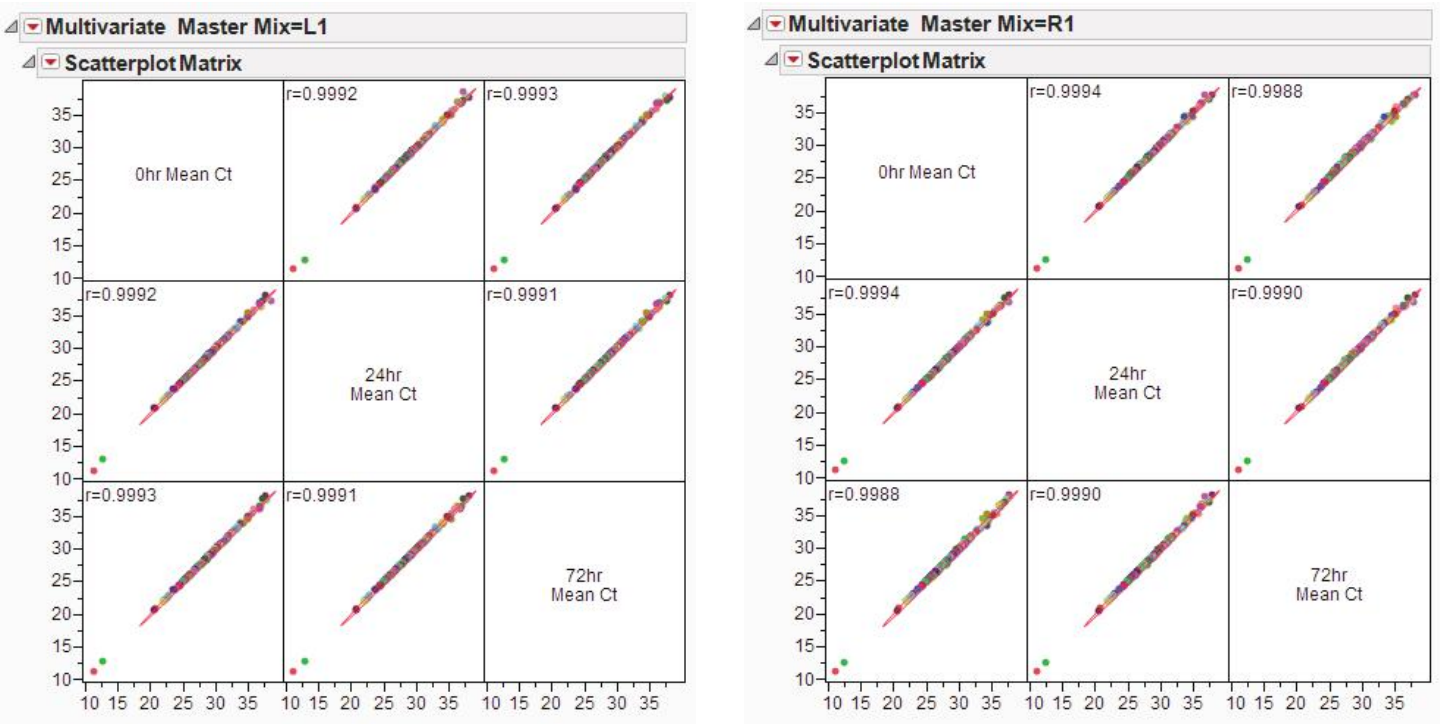


Figure 9. Mean C_t values compared between benchtop stability time points of 0, 24, and 72 hours (JMP 10, SAS Inc.). Each point represents a single assay out of the 144 gene expression assay panel. Data are displayed for validation lot L1 (left) and a representative lot of current UMM (right). Correlation values were ≥0.998, with red oval boundaries representing the 95% confidence curves (α = 0.05).

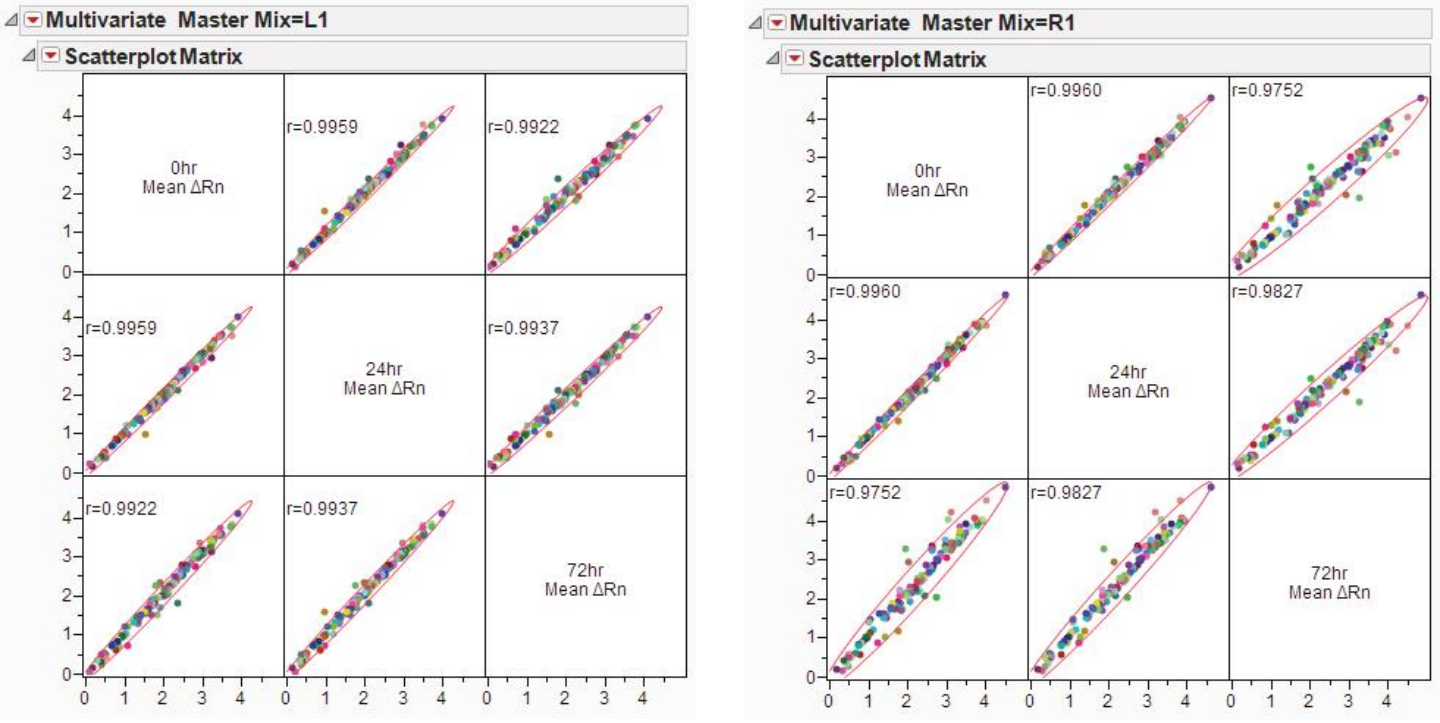


Figure 10. Mean ΔRn values compared between benchtop stability time points of 0, 24, and 72 hours (JMP 10, SAS Inc.). Each point represents a single assay out of the 144 gene expression assay panel. Data are displayed for validation lot L1 (left) and a representative lot of Branchburg, NJ-made UMM (right). Correlation values were ≥0.97, with red oval boundaries representing the 95% confidence curves (α = 0.05).

Specificity

To test performance with regards to nonspecific amplification, primer-dimer formation, and other specificity concerns, the same 144 TaqMan® Gene Expression Assays were run using water in place of template. Greater than 98% of NTC reactions across all assays exhibited no amplification. Table 1 shows the NTC amplification percentage statistics (C_t values <40) for the current and validation lots, with no statistical difference (p = 0.781) observed between the two populations.

Sensitivity

Sensitivity performance was tested using the TaqMan® RNase P assay on CEPH gDNA diluted to a 2-copy final dilution. Figure 11 shows distribution graphs for a total of 60 data points (collected across three runs of 20 replicates) for a single, representative master mix lot. Fitted normal distribution curves are shown in red.

NTC Amplification Percentage.

	Mean	St. Dev.	95% Conf.	Interval
Validation	1.33%	0.10%	1.22%	1.44%
Current	1.39%	0.32%	1.08%	1.70%

Table 1. Percentage of NTC reactions containing positive amplification (nonspecific amplification) vs. master mix (three validation lots (n = 3) and four current lots (n = 4)). All 144 gene expression assays were run with four technical replicates each (total n = 576), and the percentage was calculated for each master mix lot. Positive amplifications were largely from both 18S assays (Hs99999901_s1; 4310893E).

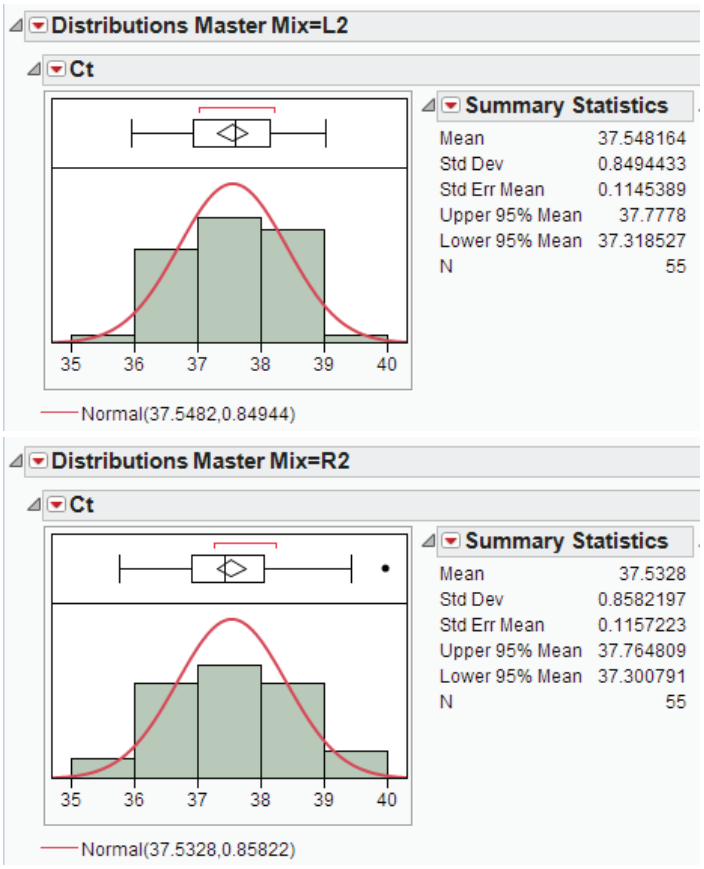


Figure 11. Distribution of RNase P C_t values obtained from 2 copies/rxn of CEPH gDNA. Data from three PCR setups were combined (20 technical replicates each, total n = 60). Data are displayed using the JMP 10 Statistical Software package (SAS Institute Inc.) for a single, representative master mix lot. Reactions that did not amplify were considered to have 0 copies of CEPH gDNA, a result of sampling error near the digital concentration range.

Discrimination

Two-fold discrimination performance was tested using the TaqMan® RNase P assay on CEPH gDNA diluted to either 1,600 or 800 copies per reaction. Figure 12 shows distribution graphs for a total of 30 data points (collected across three runs of 10 replicates) for a single, representative master mix lot. Fitted normal distribution curves are shown in red for each mean copy number. Mean C_t values for the two concentrations were separated by >6 SD, providing sufficient separation for 2-fold copy number resolution.

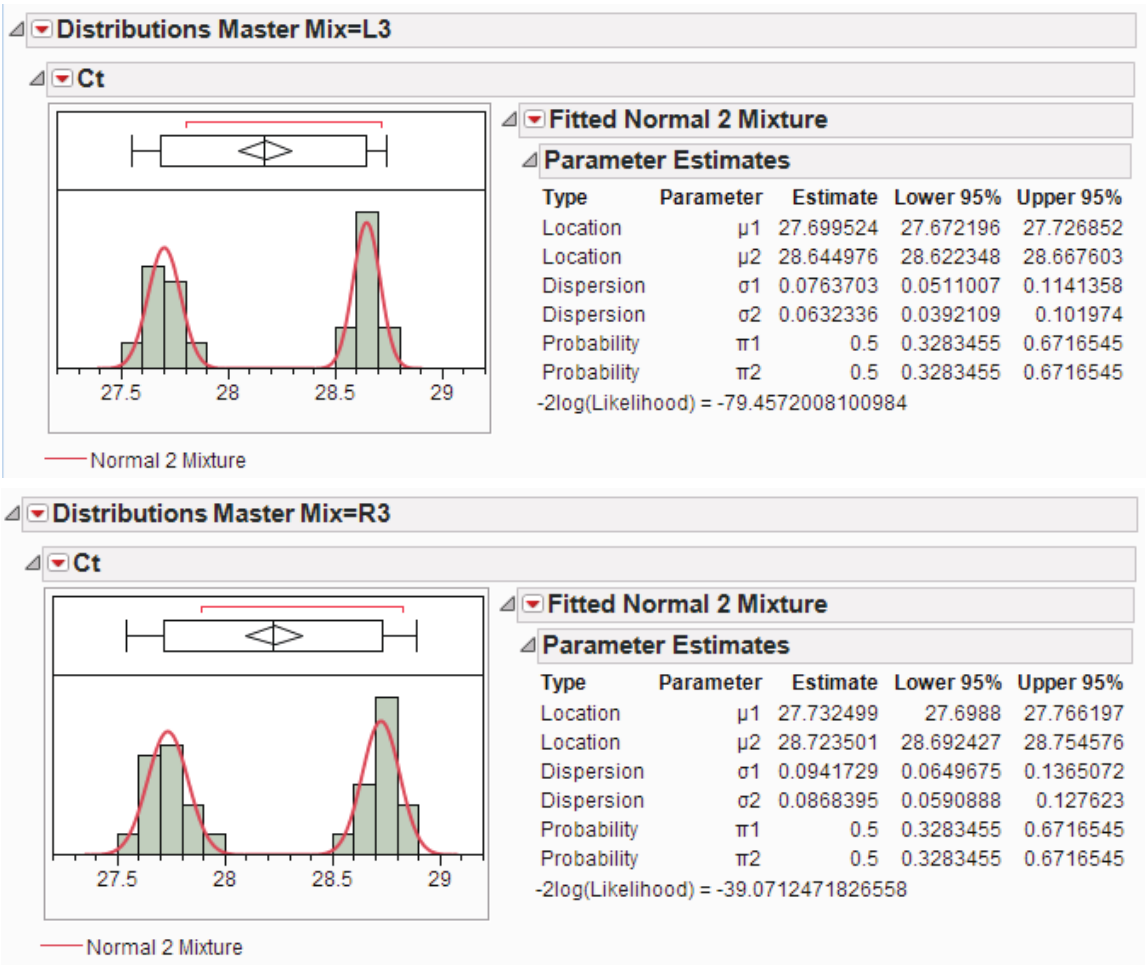


Figure 12. Distributions of RNase P C_t values obtained from 1,600 and 800 copies/rxn of CEPH gDNA. Data from three PCR setups were combined (10 technical replicates for each concentration, total n = 30). Data are displayed using the JMP 10 Statistical Software package (SAS Institute Inc.) for a single, representative master mix lot.

Conclusion

Validation lots incorporating the manufacturing changes were extensively tested against current lots of TaqMan® Universal PCR Master Mix to ascertain whether the changes would impact the functionality of the reagents. Tests showed no discernible differences in dynamic range, sensitivity, specificity, discrimination, and pre-PCR stability as well as general assay performance for gene expression assays selected to cover a representative range.

We are confident that the TaqMan® Universal PCR Master Mix offered after April 2014 will continue to perform with the same quality, integrity, and functional performance as exists today.

Appendix

Table 2. Linear dynamic range assays.

Number	Assay ID	Gene symbol
1	Hs00277509_m1	FN1
2	Hs99999906_m1	PGK1
3	Hs99999902_m1	RPLP0
4	Hs00187842_m1	B2M
Duplex	4308323	Exo IPC

Table 3. Gene expression assays.

Number	Assay ID	Gene symbol
1	Hs99999901_s1	18S
2	Hs00184500_m1	ABCB1
3	Hs00219905_m1	ABCC1
4	Hs00245154_m1	ABCG1
5	Hs00153936_m1	ACAN
6	Hs99999903_m1	ACTB
7	Hs00242273_m1	ACTG2
8	Hs00173490_m1	AFP
9	Hs00178289_m1	AKT1
10	Hs00163641_m1	APOA1
11	Hs00171168_m1	APOE
12	Hs99999907_m1	B2M
13	Hs00180269_m1	BAX
14	Hs00153353_m1	BIRC5
15	Hs00277039_m1	CCND1
16	Hs00154355_m1	CD68
17	Hs00170423_m1	CDH1
18	Hs00355782_m1	CDKN1A
19	Hs00153277_m1	CDKN1B
20	Hs00269972_s1	CEBPA
21	Hs00164004_m1	COL1A1
22	Hs00171022_m1	CXCL12
23	Hs00164383_m1	CYP1B1
24	Hs00604506_m1	CYP3A4
25	Hs00183740_m1	DKK1
26	Hs00174961_m1	EDN1
27	Hs00230957_m1	ESR2
28	Hs00266645_m1	FGF2
29	Hs00170630_m1	FOS
30	Hs00232764_m1	FOXA2
31	Hs00203958_m1	FOXP3
32	Hs00268943_s1	FZD1
33	Hs00169255_m1	GADD45A
34	Hs99999908_m1	GUSB
35	Hs00168352_m1	HMGCR
36	Hs00157965_m1	HMOX1
37	Hs00168405_m1	IL12A

Gene expression assays, *continued*

Number	Assay ID	Gene symbol
38	Hs00155517_m1	IL18
39	Hs00174092_m1	IL1A
40	Hs00174103_m1	IL8
41	Hs00174029_m1	KIT
42	Hs00234422_m1	MMP2
43	Hs00159163_m1	MMP7
44	Hs02387400_g1	NANOG
45	Hs00707120_s1	NES
46	Hs00167166_m1	NOS3
47	Hs00242943_m1	OAS1
48	Hs00855025_s1	PBX2
49	Hs99999906_m1	PGK1
50	Hs00172183_m1	PGR
51	Hs00180679_m1	PIK3CA
52	Hs00172187_m1	POLR2A
53	Hs00742896_s1	POU5F1
54	Hs00173304_m1	PPARGC1A
55	Hs99999904_m1	PPIA
56	Hs00168719_m1	PPIB
57	Hs00197884_m1	SLC2A1
58	Hs00170665_m1	SMO
59	Hs00269575_s1	SOCS3
60	Hs00167093_m1	SPP1
61	Hs00234829_m1	STAT1
62	Hs00427620_m1	TBP
63	Hs99999911_m1	TFRC
64	Hs00171257_m1	TGFB1
65	Hs99999918_m1	TGFB1
66	Hs00171558_m1	TIMP1
67	Hs00174128_m1	TNF
68	Hs00171068_m1	TNFRSF11B
69	Hs00173626_m1	VEGFA
70*	4310893E	18s-2
71*	4310881E	ACTB-2
72*	4310884E	GAPDH-2
73	Hs00166123_m1	ABCC2
74	Hs00184979_m1	ABCG2
75	Hs00817723_g1	ACADVL
76	Hs00605917_m1	ADIPOQ
77	Hs00758162_m1	ALPL
78	Hs00169098_m1	APP
79	Hs00153350_m1	BCL2
80	Hs00608023_m1	BCL2
81	Hs00236329_m1	BCL2L1
82	Hs00154192_m1	BMP2
83	Hs00171074_m1	CCL17
84	Hs00171125_m1	CCL20
85	Hs00234142_m1	CCL3

Gene expression assays, *continued*

Number	Assay ID	Gene symbol
86	Hs00362446_m1	CCT7
87	Hs00169627_m1	CD36
88	Hs00199349_m1	CD86
89	Hs00164099_m1	COL1A2
90	Hs00164103_m1	COL3A1
91	Hs00170025_m1	CTNNB1
92	Hs00153120_m1	CYP1A1
93	Hs00167937_g1	CYP2B6
94	Hs00426397_m1	CYP2C9
95	Hs00193306_m1	EGFR
96	Hs00355783_m1	ELN
97	Hs00170433_m1	ERBB2
98	Hs00175225_m1	F3
99	Hs00609791_m1	FABP4
100	Hs00181225_m1	FASLG
101	Hs00188012_m1	FASN
102	Hs00270117_s1	FOXD1
103	Hs02758991_g1	GAPDH
104	Hs00231122_m1	GATA3
105	Hs00300159_m1	HGF
106	Hs00230853_m1	HNF4A
107	Hs99999909_m1	HPRT1
108	Hs00153126_m1	IGF1
109	Hs00233688_m1	IL12B
110	Hs00174114_m1	IL2
111	Hs00372324_m1	IL23A
112	Hs00181192_m1	LDLR
113	Hs00173425_m1	LPL
114	Hs00233992_m1	MMP13
115	Hs00234579_m1	MMP9
116	Hs00153408_m1	MYC
117	Hs00153283_m1	NFKBIA
118	Hs00167248_m1	NOS2
119	Hs00413187_m1	NOTCH1
120	Hs00168547_m1	NQO1
121	Hs00172885_m1	NR1H3
122	Hs00159719_m1	OAS2
123	Hs00169777_m1	PECAM1
124	Hs00231882_m1	PPARA
125	Hs00234592_m1	PPARG
126	Hs00829813_s1	PTEN
127	Hs00153133_m1	PTGS2
128	Hs00179843_m1	SHH
129	Hs00178696_m1	SMAD7
130	Hs00195591_m1	SNAI1

Gene expression assays, *continued*

Number	Assay ID	Gene symbol
131	Hs00167309_m1	SOD2
132	Hs00165814_m1	SOX9
133	Hs00165949_m1	TIMP3
134	Hs00152933_m1	TLR3
135	Hs00152939_m1	TLR4
136	Hs00152971_m1	TLR7
137	Hs00153340_m1	TP53
138	Hs00702289_s1	TWF1
139	Hs00365486_m1	VCAM1
140	Hs00900054_m1	VEGFA
141	Hs00185584_m1	VIM
142*	4310890E	HPRT1-2
143*	4310885E	PGK1-2
144*	4310883E	PPIA-2

* TaqMan® endogenous control assays; primer-limited (150 nM instead of 900 nM) with VIC-TAMRA probes.

Table 4. Affected catalog numbers.

List of all affected catalog numbers of TaqMan® Universal PCR Master Mix and TaqMan® Universal Master Mix, No AmpErase® UNG impacted by the manufacturing site change.

Product name	Cat. No.
TaqMan® Universal PCR Master Mix, 1-Pack (1 x 5 mL)	4304437
TaqMan® Universal PCR Master Mix, 10-Pack (10 x 5 mL)	4305719
TaqMan® Universal PCR Master Mix, 10 Unit Pack (10 x 5 mL)	4318157
TaqMan® Universal PCR Master Mix, 1 Bulk Pack (1 x 50 mL)	4326708
TaqMan® Universal PCR Master Mix, 2-Pack (2 x 5 mL)	4364338
TaqMan® Universal PCR Master Mix, 5-Pack (5 x 5 mL)	4364340
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 1-Pack (1 x 5 mL)	4324018
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 10 Unit Pack (10 x 5 mL)	4324020
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 1 Bulk Pack (1 x 50 mL)	4326614
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 2-Pack (2 x 5 mL)	4364341
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 5-Pack (5 x 5 mL)	4364343

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Company code 122351387, VAT code LT223513811

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CERTIFICATE OF ANALYSIS

K0722 GeneJET Genomic DNA Purification Kit

Packaging Lot: **2935530**
Expiry Date: 20.02.2026 (DD.MM.YYYY)
Storage: at 5±3°C
Note: IMPORTANT Check Individual Components for Storage Conditions

Filling lots for components in package:

Lot	Quantity	Description
2903820	5 × 1.2 mL	Proteinase K Solution, 20mg/ml
2897386	5 × 1 mL	RNase A Solution, 10 mg/ml
2919848	150 mL	Elution Buffer
2927229	120 mL	Lysis solution
2870689	40 mL	Wash Solution I (concentrated)
2916574	40 mL	Wash Solution II (concentrated)
2906653	55 mL	Digestion Solution
2934240	5 × 1 pack	GeneJET DNA Purification Columns & collection Tubes
2934507	5 × 1 pack	Collection Tubes 2 ml

QUALITY CONTROL

Parameter	Method	Requirement	Result
Specific activity (RNase A)	One unit is the amount of the enzyme which produces an increase in soluble reaction products by an OD of 1.0 at A260 nm using yeast RNA as substrate in 15 minutes at 37 °C.	≥ 5000 U/mg	Conforms
Activity (Proteinase K)	The unit activity of a solution of Proteinase K is determined. One unit liberates 1 µmol of Folin-positive amino acids, measured as tyrosine, at 37°C, pH 7.5, using denatured bovine hemoglobin as the substrate.	Within range of predetermined specifications	Conforms
pH (Relevant kit components)	Measured using a pH meter.	Within range of predetermined specifications	Conforms
Density (Relevant kit components)	Measured using a densitometer.	Within range of predetermined specifications	Conforms
Refractive Index (Relevant kit components)	Measured using a refractometer.	Within range of predetermined specifications	Conforms
Conductivity (Relevant kit components)	Measured using a conductometer.	Within range of predetermined specifications	Conforms

ISO CERTIFICATION
Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

Quality authorized by QC: J. Žilinskienė 

thermo scientific

PRODUCT INFORMATION

Thermo Scientific GeneJET Genomic DNA Purification Kit #K0721, #K0722

Pub. No. MAN0012663
Rev. Date 12 October 2016 (Rev. B.00)


Read Storage information (p. 2) before first use!

www.thermofisher.com

For Research Use Only. Not for use in diagnostic procedures.

#_
Lot _
Exp. _

CERTIFICATE OF ANALYSIS

Thermo Scientific GeneJET Genomic DNA Purification Kit is qualified by isolating genomic DNA from 200 µL of blood and 5 mg of mammalian tissue following described protocols. The purified genomic DNA has an $A_{260/280}$ ratio of ≥1.7. A single band of more than 30 kb is seen after agarose gel electrophoresis and ethidium bromide staining. Functional quality of genomic DNA is evaluated by PCR amplification of a single-copy gene and digestion with restriction enzymes.

Quality authorized by:  Jurgita Zilinskiene

CONTENTS page

COMPONENTS OF THE KIT

GeneJET Genomic DNA Purification Kit	#K0721 50 preps	#K0722 250 preps
Proteinase K Solution	1.2 mL	5 × 1.2 mL
RNase A Solution	1 mL	5 × 1 mL
Digestion Solution	11 mL	55 mL
Lysis Solution	24 mL	2 × 60 mL
Wash Buffer I (concentrated)	10 mL	40 mL
Wash Buffer II (concentrated)	10 mL	40 mL
Elution Buffer (10 mM Tris-Cl, pH 9.0, 0.1 mM EDTA)	30 mL	150 mL
GeneJET Genomic DNA Purification Columns pre-assembled with Collection Tubes	50	250
Collection Tubes	50	250

STORAGE

Proteinase K and RNase A solutions are stable at room temperature as long as not opened. After being opened they should be stored at -20 °C. Other components of the kit should be stored at room temperature (15-25 °C).

Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!

DESCRIPTION

The GeneJET™ Genomic DNA Purification Kit is designed for rapid and efficient purification of high quality genomic DNA from various mammalian cell culture and tissue samples, whole blood, bacteria and yeast. The kit utilizes silica-based membrane technology in the form of a convenient spin column, eliminating the need for expensive resins, toxic phenol-chloroform extractions, or time-consuming alcohol precipitation. The standard procedure takes less than 20 minutes following cell lysis and yields purified DNA of more than 30 kb in size. Isolated DNA can be used directly in PCR, Southern blotting and enzymatic reactions. *See* Table 1 for typical genomic DNA yields from various sources.

PRINCIPLE

Depending on the starting material, samples are digested with Proteinase K in either the supplied Digestion or Lysis Solution. RNA is removed by treating the samples with RNase A. The lysate is then mixed with ethanol and loaded on the purification column where the DNA binds to the silica membrane. Impurities are effectively removed by washing the column with the prepared wash buffers. Genomic DNA is then eluted under low ionic strength conditions with the Elution Buffer.

Table 1. Typical genomic DNA yields from various sources.

Source	Quantity	Yield, µg
Mammalian blood	200 µL	4-6
Mouse heart	10 mg	10-15
Mouse tail	0.5 cm	8-10
Rat liver	10 mg	10-20
Rat spleen	5 mg	20-30
Rat kidney	10 mg	25-30
Rabbit ear	20 mg	5-10
Bacillus pumilis cells	2×10^9 cells	10-15
Escherichia coli cells	2×10^9 cells	10-15
HeLa cells	2×10^6 cells	15-20
Jurkat cells	5×10^6 cells	25-30
Saccharomyces cerevisiae cells	1×10^8 cells	3-5

IMPORTANT NOTES

- To minimize DNA degradation, avoid repeated freeze/thaw cycles of the samples and perform extractions from fresh material or material that has been immediately frozen and stored at -20 °C or -70 °C.
- Add the indicated volume of ethanol (96-100%) to **Wash Buffer I** (concentrated) and **Wash Buffer II** (concentrated) prior to first use:

	#K0721 50 preps		**#K0722** 250 preps	
	Wash Buffer I	**Wash Buffer II**	**Wash Buffer I**	**Wash Buffer II**
Concentrated wash solution	10 mL	10 mL	40 mL	40 mL
Ethanol (96-100%)	**30 mL**	**30 mL**	**120 mL**	**120 mL**
Total volume:	40 mL	40 mL	160 mL	160 mL

After the ethanol has been added, mark the check box on the bottle's cap to indicate the completed step.

- Check the **Digestion Solution** and **Lysis Solution** for salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37 °C, then cool back down to 25 °C before use.
- Wear gloves when handling the **Lysis Solution and Wash Buffer I** as these reagents contain irritants.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipets and pipet tips
- Vortex
- Ethanol (96-100%)
- 1.5 mL microcentrifuge tubes
- Microcentrifuge
- Thermomixer, shaking water bath or rocking platform capable of heating up to 56 °C
- Disposable gloves

Buffers

For mammalian cell lysate preparation:

- PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4)
- TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)

For gram-positive bacteria lysate preparation

- Gram-positive bacteria lysis buffer (20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 1.2% Triton X-100, add lysozyme to 20 mg/mL immediately before use)

For yeast lysate preparation:

- Yeast lysis buffer (5 mg/mL zymolyase 20T, 1 M sorbitol, 0.1 M EDTA)

GENOMIC DNA PURIFICATION PROTOCOLS

Protocols for genomic DNA purification from mammalian tissue and rodent tail, cultured mammalian cells, mammalian blood, gram-negative, gram-positive bacteria, yeast and buccal swabs are described on p.4-11.

A. Mammalian Tissue and Rodent Tail Genomic DNA Purification Protocol

<table>
<tr><th>Step</th><th>Procedure</th></tr>
<tr><td>1</td><td>Grind up to 20 mg of mammalian tissue (use up to 10 mg of spleen tissue), 0.6 cm (rat) or 0.5 cm (mouse) tail clip in liquid nitrogen using a mortar and pestle. Alternatively, cut the tissue into small pieces or disrupt it using a homogenizer.</td></tr>
<tr><td>2</td><td>Collect the material into a 1.5 mL microcentrifuge tube (not provided) and resuspend in 180 µL of Digestion Solution. Add 20 µL of Proteinase K Solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension.</td></tr>
<tr><td>3</td><td>Incubate the sample at 56 °C until the tissue is completely lysed and no particles remain. During incubation vortex the vial occasionally or use a shaking water bath, rocking platform or thermomixer.
Suggested incubation times:
<table>
<tr><th>Quantity</th><th>Suggested incubation time</th></tr>
<tr><td>5 mg of tissue (except spleen)</td><td>1 hour</td></tr>
<tr><td>10 mg of tissue (except spleen)</td><td>2 hours</td></tr>
<tr><td>20 mg of tissue (except spleen)</td><td>3 hours</td></tr>
<tr><td>5 mg of spleen tissue</td><td>2 hours</td></tr>
<tr><td>10 mg of spleen tissue</td><td>3 hours</td></tr>
<tr><td>Mouse tail (0.5 cm), rat tail (0.6 cm)</td><td>6 hours</td></tr>
</table>
Note. Lysis time varies on the type and amount of tissue processed. In some cases incubation time should be prolonged to 6-8 hours or overnight (for rodent tail) until complete lysis occurs.</td></tr>
<tr><td>4</td><td>Add 20 µL of RNase A Solution, mix by vortexing then incubate for 10 min at room temperature.</td></tr>
<tr><td>5</td><td>Add 200 µL of Lysis Solution. Mix thoroughly by vortexing for 15 s until a homogeneous mixture is obtained.</td></tr>
<tr><td>6</td><td>Add 400 µL of 50% ethanol and mix by pipetting or vortexing.</td></tr>
<tr><td>7</td><td>Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included).
Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!</td></tr>
<tr><td>8</td><td>Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.</td></tr>
</table>

Step	Procedure
9	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
10	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note** • For maximum DNA yield, repeat the elution step with additional 200 µL of Elution Buffer. • If more concentrated DNA is required or DNA is isolated from a small amount of starting material (e.g., <5 mg of tissue) the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
11	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

B. Cultured Mammalian Cells Genomic DNA Purification Protocol

Step	Procedure
1	a) Suspension cells Collect up to 5×10^6 cells in a centrifuge tube. Pellet cells by centrifugation for 5 min at 250 × g. Discard the supernatant. Rinse cells once with PBS to remove residual medium and repeat the centrifugation step. Discard the supernatant. b) Adherent cells Remove the growth medium from a culture plate containing up to 2×10^6 cells. Rinse cells once with PBS to remove residual medium. Discard PBS. Detach the cells from the culture plate by scraping in an appropriate volume of PBS or by trypsinization. Transfer the cells to a microcentrifuge tube and pellet them by centrifugation for 5 minutes at 250 × g. Discard supernatant.
2	Resuspend the cells collected in step 1a or 1b in 200 µL of TE buffer or PBS. Add 200 µL of Lysis Solution and 20 µL of Proteinase K Solution to the cell pellet. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
3	Incubate the sample at 56 °C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (10 min).
4	Add 20 µL of RNase A Solution, mix by vortexing and incubate the mixture for 10 min at room temperature.
5	Add 400 µL of 50% ethanol and mix by pipetting or vortexing.
6	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
7	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
8	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
9	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note** • For maximum DNA yield, repeat the elution step with additional 200 µL of Elution Buffer. • If more concentrated DNA is required or DNA is isolated from a small amount of starting material (e.g., $\leq1\times10^6$ of cultured mammalian cells) the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
10	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

C. Mammalian Blood Genomic DNA Purification Protocol

Step	Procedure
1	Add 400 µL of Lysis Solution and 20 µL of Proteinase K Solution to 200 µL of whole blood, mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
2	Incubate the sample at 56 °C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (10 min).
3	Add 200 µL of ethanol (96-100%) and mix by pipetting or vortexing.
4	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
5	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
6	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
7	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note** • For maximum DNA yield, repeat the elution step with additional 200 µL of Elution Buffer. • If more concentrated DNA is required or DNA is isolated from a small amount of starting material (e.g., 50 µL) the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
8	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

D. Gram-Negative Bacteria Genomic DNA Purification Protocol

Step	Procedure
1	Harvest up to 2×10^9 bacterial cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation for 10 min at 5000 × g. Discard the supernatant.
2	Resuspend the pellet in 180 µL of Digestion Solution. Add 20 µL of Proteinase K Solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
3	Incubate the sample at 56 °C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (~30 min).
4	Add 20 µL of RNase A Solution, mix by vortexing and incubate the mixture for 10 min at room temperature.
5	Add 200 µL of Lysis Solution to the sample. Mix thoroughly by vortexing for about 15 s until a homogeneous mixture is obtained.
6	Add 400 µL of 50% ethanol and mix by pipetting or vortexing.
7	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
8	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
9	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
10	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note** • For maximum DNA yield, repeat the elution step with additional 200 µL of Elution Buffer. • If more concentrated DNA is required or DNA is isolated from a small amount of starting material the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
11	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

E. Gram-Positive Bacteria Genomic DNA Purification Protocol

Before starting

Prepare Gram-positive bacteria lysis buffer: 20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 1.2% Triton X-100, add lysozyme to 20 mg/mL immediately before use.

Step	Procedure
1	Harvest up to 2×10^9 bacterial cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation for 10 min at 5000 × g. Discard the supernatant.
2	Resuspend the pellet in 180 µL of Gram-positive bacteria lysis buffer. Incubate for 30 min at 37 °C.
3	Add 200 µL of Lysis Solution and 20 µL of Proteinase K. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
4	Incubate the sample at 56 °C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (~30 min).
5	Add 20 µL of RNase A Solution, mix by vortexing and incubate the mixture for 10 min at room temperature.
6	Add 400 µL of 50% ethanol and mix by pipetting or vortexing.
7	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
8	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
9	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
10	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note** • For maximum DNA yield, repeat the elution step with additional 200 µL of Elution Buffer. • If more concentrated DNA is required or DNA is isolated from a small amount of starting material the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
11	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

F. Yeast Genomic DNA Purification Protocol

Before starting

Prepare Yeast lysis buffer: 5 mg/mL zymolyase 20T, 1 M sorbitol, 0.1 M EDTA.

Step	Procedure
1	Harvest up to 1×10^8 yeast cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation for 5-10 s at maximum speed ≥12000 × g. Discard the supernatant.
2	Resuspend the pellet in 500 µL of Yeast lysis buffer. Incubate for 1 hour at 37 °C.
3	Centrifuge cells for 10 min at 3000 × g. Discard the supernatant.
4	Resuspend the pellet in 180 µL of Digestion Solution. Add 20 µL of Proteinase K Solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
5	Incubate the sample at 56 °C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (~45 min).
6	Add 20 µL of RNase A Solution, mix by vortexing and incubate the mixture for 10 min at room temperature.
7	Add 200 µL of Lysis Solution. Mix thoroughly by vortexing for 15 s until a homogeneous mixture is obtained.
8	Add 400 µL of 50% ethanol and mix by pipetting or vortexing.
9	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
10	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
11	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
12	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note** • For maximum DNA yield, repeat the elution step with additional 200 µL of Elution Buffer. • If more concentrated DNA is required or DNA is isolated from a small amount of starting material the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
13	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

G. DNA Purification from Buccal Swabs

Step	Procedure
1	To collect a sample, scrape the swab 5-6 times against the inside cheek.
2	Swirl the swab for 30-60 s in 200 µL of 1 × PBS.
3	Go to step 1 of the standard Mammalian Blood Genomic DNA Purification Protocol (p. 7).

TROUBLESHOOTING

Problem	Possible cause and solution
Low yield of purified DNA	**Excess sample used during lysate preparation.** Reduce the amount of starting material. Do not use more tissue or cells than indicated in lysis protocols. **Starting material was not completely digested.** Extend the Proteinase K digestion at 56 °C until complete lysis occurs and no particles remain. **Ethanol was not added to the lysate.** Make sure that the ethanol was added to the lysate before applying the sample to the Purification Column. **Ethanol was not mixed with the lysate.** After the addition of ethanol to the lysate mix the sample by vortexing or pipetting. **Ethanol was not added to Wash Buffers.** Make sure that ethanol was added to Wash Buffer I and Wash Buffer II before use. Follow the instructions for Wash Buffer preparation on p.3.
Purified DNA is degraded	**Sample was frozen and thawed repeatedly.** Avoid repeated freeze / thaw cycles of the samples. Use a new sample for DNA isolation. Perform extractions from fresh material when possible. **Inappropriate sample storage conditions.** Store mammalian tissues at -70 °C and bacteria at -20 °C until use. Whole blood can be stored at 4 °C for no longer than 1-2 days. For long term storage blood samples should be aliquoted in 200 µL portions and stored at -20 °C.
RNA contamination	**RNase A treatment was not carried out.** Carry out RNase A treatment step described in the purification procedure.
Column becomes clogged during purification	**Excess sample was used during lysate preparation.** Reduce the amount of starting material. A maximum of 2×10^9 of bacteria cells, $5x10^6$ of suspension cells and 20 mg of mammalian tissue is recommended for lysate preparation. **Tissue was not completely digested.** Extend the Proteinase K digestion at 56 °C until complete lysis occurs and no particles remain.
Inhibition of downstream enzymatic reactions	**Purified DNA contains residual ethanol.** If residual solution is seen in the purification column after washing the column with Wash Buffer II, empty the collection tube and re-spin the column for an additional 1 min. at maximum speed (≥12000 × g). **Purified DNA contains residual salt.** Use the correct order for the Washing Buffers. Always wash the purification column with Wash Buffer I first and then proceed to washing with Wash Buffer II.

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TaqMan® Universal PCR Master Mix

Pub. no. 4480831 **Rev.** A

Catalog No.	Quantity	Storage condition
4304437, 1-Pack	1 × 5 mL	Store at 2°C to 8°C.
4364338, 2-Pack	2 × 5 mL	
4364340, 5-Pack	5 × 5 mL	
4305719, 10-Pack	10 × 5 mL	
4318157, 10 Unit Pack	10 × 5 mL	
4326708, 1 Bulk Pack	1 × 50 mL	

Product Use

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Procedure for use

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