November 2017

ipsogen® PML-RARA bcr1 Kit Handbook

24

Version 1

Quantitative in vitro diagnostics

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

IVD

CE

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Sample to Insight

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Contents

Intended Use

The *ipsogen* PML-RARA bcr1 Kit is intended for the quantification of PML-RARA type bcr1 fusion transcripts in bone marrow or peripheral blood samples in a subgroup of acute myeloid leukemia (AML) patients diagnosed with M3 cytomorphology and t(15;17)(q22;q21) translocation, with a breakpoint into PML intron 6. The results obtained are intended to be used as an aid to monitor efficacy of treatment in patients undergoing therapy, and for minimal residual disease (MRD) follow-up to monitor disease relapse.

Summary and Explanation

The PML-RARA fusion gene (FG) transcripts, which are the molecular result of the t(15;17)(q22;q21) translocation, are associated with the majority of acute progranulocytic leukemia (APL) cases (>90%), a distinct AML subset with M3 cytomorphology that accounts for 10–15% of all cases of AML. The balanced reciprocal translocation t(15;17) leads to the fusion of the promyelocytic leukemia (PML) gene to the retinoic acid receptor alpha (RARA) to generate the PML-RARA fusion protein. The chimeric PML-RARA protein is a transcriptional repressor. Its expression is associated with impaired myeloid differentiation, due to increased affinity for the nuclear repressor protein complex (NcoR), alteration of chromatin structure by histone deacetylase (HDAC) and inhibition of transcription. Treatment with all-trans retinoic acid (ATRA) is highly effective in APL and acts as a differentiating agent by promoting release of the NCoR/HDAC complex, thereby restoring normal transcription.

RARA breakpoints always occur in intron 2. Depending on the location of breakpoints within the PML site, intron 6, exon 6 and intron 3, the respective PML-RARA transcript subtypes referred to as long (L or bcr1), variant (V or bcr2), and short (S or bcr3), may be formed (Figure 1). These transcript subtypes represent 55%, 5%, and 40% of the cases, respectively.

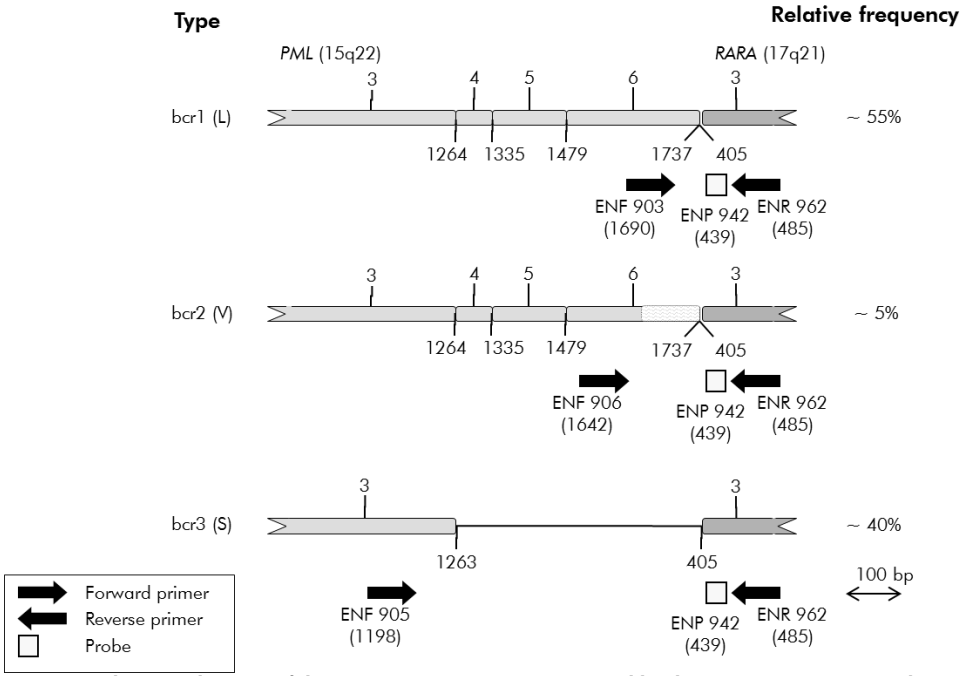

Figure 1. Schematic diagram of the PML-RARA FG transcript covered by the EAC qPCR primers and probe set. For type bcr1 (L): ENF903–ENP942–ENR962. For type bcr2 (V): ENF906–ENP942–ENR962. For type bcr3 (S): ENF905–ENP942–ENR962. The number under the primers and probe refers to their nucleotide position in the normal gene transcript. Relative frequency refers to the proportion of each type of FG transcripts among PML-RARA variants.

Combined treatment with anthracycline-based chemotherapy and ATRA is highly successful in APL, providing long-lasting remissions and probable cure in up to 70% of newly diagnosed patients. However, relapse and low survival rates are still seen in 15–25% of patients. The detection of the unique PML-RARA fusion gene by conventional qualitative reverse transcription polymerase chain reaction (RT-PCR) has been widely used for rapid diagnosis and prediction of response to therapies. However, this technique presents drawbacks and its sensitivity is relatively low.

Quantification of PML-RARA copy number by real-time quantitative PCR (qPCR) presents several advantages. It is a highly sensitive and reproducible technique that also allows an assessment of kinetics. The analysis of the prognostic value of a well-established standardized qPCR protocol (EAC Program) in APL patients during different phases of treatment has indicated that this approach is a robust alternative for assessing MRD, and that relapse-risk stratification can be established based on PML-RARA normalized copy number. During post-consolidation analysis, positive qPCR assay is a strong predictor of subsequent hematologic relapse. During maintenance therapy, and beyond the end of treatment, a positive qPCR test is associated with a higher relapse risk and shorter survival. The relapse-risk stratification based on quantification of PML-RARA normalized copy number (NCN) divides patients into 3 groups: those at high risk of relapse, those with an intermediate risk, and those with a low risk of relapse (1). PML-RARA monitoring through sensitive detection of the transcript is regarded as an integral part of the overall treatment strategy in APL (see references 2 and 3 for details), whereby the treatment type and intensity are modulated in patients at different risks of relapse during follow-up.

Standardization and validation of the MRD quantification method have been established in a multicenter project conducted by the EAC and published in 2003 (4, 5). The *ipsogen* PML-RARA bcr1 Kit is based on this technique.

Principle of the Procedure

The technique of qPCR permits the accurate quantification of PCR products during the exponential phase of the PCR amplification process. In addition, qPCR 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 2). 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.

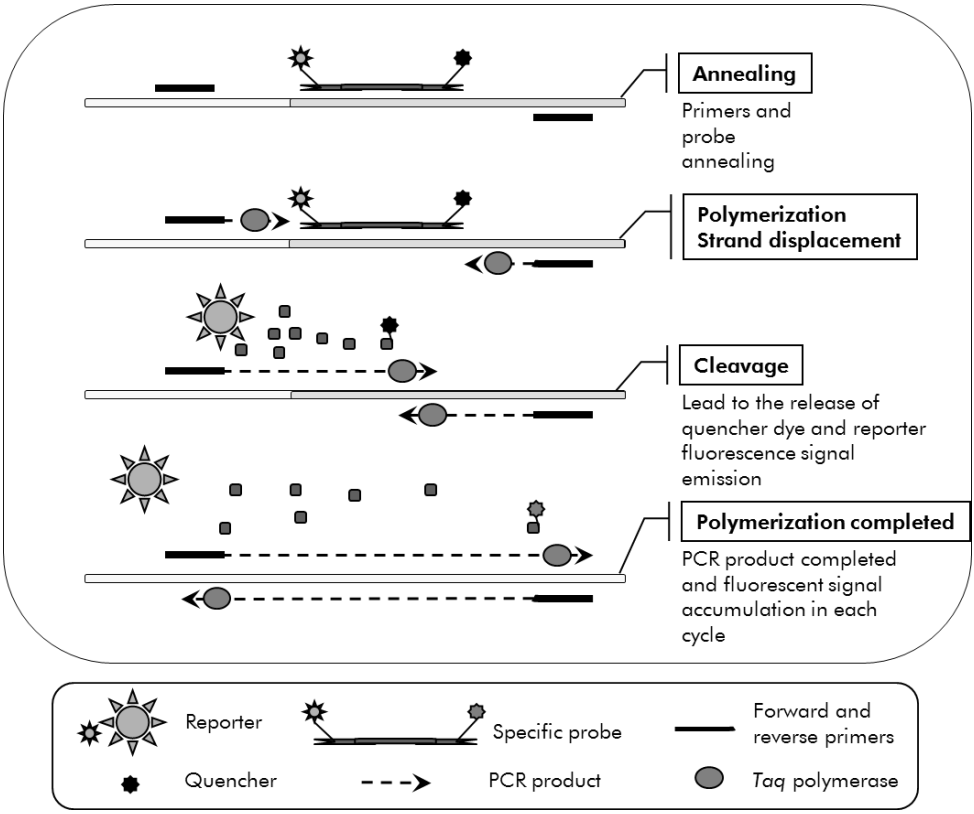

Figure 2. Reaction principle. Total RNA is reverse-transcribed, and the generated cDNA is amplified by PCR using a pair of specific primers and a specific internal double-dye probe (FAM™–TAMRA™). The probe binds to the amplicon during each annealing step of the PCR. When the Taq extends from the primer bound to the amplicon, it displaces the 5′ end of the probe, which is then degraded by the 5′→3′ exonuclease activity of the Taq DNA polymerase. Cleavage continues until the remaining probe melts off the amplicon. This process releases the fluorophore and quencher into solution, spatially separating them and leading to an increase in fluorescence from the FAM and a decrease in fluorescence from the TAMRA.

Materials Provided

Kit contents

***ipsogen* PML-RARA bcr1 Kit**		**(24)**
Catalog number		**672123**
Number of reactions		**24**
Component	**Name**	**Amount**
ABL Control Gene Standard Dilution (10^3 copies/5 µl)	C1-ABL	50 µl
ABL Control Gene Standard Dilution (10^4 copies/5 µl)	C2-ABL	50 µl
ABL Control Gene Standard Dilution (10^5 copies/5 µl)	C3-ABL	50 µl
PML-RARA bcr1 Fusion Gene Standard Dilution (10^1 copies/5 µl)	F1-PML-RARA bcr1	50 µl
PML-RARA bcr1 Fusion Gene Standard Dilution (10^2 copies/5 µl)	F2-PML-RARA bcr1	50 µl
PML-RARA bcr1 Fusion Gene Standard Dilution (10^3 copies/5 µl)	F3-PML-RARA bcr1	50 µl
PML-RARA bcr1 Fusion Gene Standard Dilution (10^5 copies/5 µl)	F4-PML-RARA bcr1	50 µl
PML-RARA bcr1 Fusion Gene Standard Dilution (10^6 copies/5 µl)	F5-PML-RARA bcr1	50 µl
Primers and Probe Mix ABL*	PPC-ABL 25x	90 µl
Primers and Probe Mix PML-RARA bcr1 Fusion Gene†	PPF-PML-RARA bcr1 25x	110 µl
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* Mix of specific reverse and forward primers for the ABL control gene plus a specific FAM–TAMRA probe.
† Mix of specific reverse and forward primers for the PML-RARA bcr1 fusion gene plus a specific FAM–TAMRA probe.

Note: 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.

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

Reagents

- Nuclease-free PCR grade water
- Reagents for reverse transcription: The validated reagent is Superscript® II (or Superscript) Reverse Transcriptase, includes 5x first-strand buffer, 100 mM DTT (Life Technologies, cat. no. 18064-022)
- RNase inhibitor: The validated reagent is RNaseOUT™ (Life Technologies, cat. no. 10777-019)
- Set of dNTPs, PCR grade
- Random hexamer
- $MgCl_2$
- Buffer and Taq DNA polymerase: The validated reagents are TaqMan® Universal PCR Master Mix (Master Mix PCR 2x) (Life Technologies, 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 RNase- and DNase-free PCR tubes
- Ice

Equipment

- Microliter 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 (with a maximum speed of 13,000/14,000 rpm)
- Real-time PCR instrument: Rotor-Gene Q MDx 5plex HRM or other Rotor Gene Q instruments; LightCycler 1.2, 2.0, or 480; ABI PRISM 7000, 7700 or 7900HT SDS; Applied Biosystems 7500 Real-Time PCR System; or SmartCycler; and associated specific material
- Thermal cycler or water bath (reverse transcription step)

Complementary reagents

- *ipsogen* PML-RARA bcr1 Controls Kit (cat. no. 672091) for research use only, consisting of cell lines with negative, high and low positive expression of the PML-RARA bcr1 fusion gene for the qualitative validation of the RNA extraction and the reverse transcription

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 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, which are dedicated to molecular biology and 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. PPC and PPF 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.

Determining transcript levels using qPCR requires both the reverse transcription of the mRNA and the amplification of the generated cDNA by PCR. Therefore, the entire assay procedure must be performed under RNase-/DNase-free conditions.

Use extreme caution to prevent:

- RNase/DNase contamination, which might cause degradation of the template mRNA and the generated cDNA
- mRNA 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.).
- Handle the standard dilutions (C1–3 and F1–5) in a separate room.

Reagent Storage and Handling

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

- Minimize exposure to light of the primers and probe mixes (PPC and PPF 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 RNA preparation

RNA preparation from patient samples (blood or bone marrow) must have been performed using a validated procedure. The quality of the assay is largely dependent on the quality of input RNA. We therefore recommend qualifying the purified RNA by agarose* gel electrophoresis or by using Agilent® Bioanalyzer® prior to analysis.

Protocol: Recommended standardized EAC reverse transcription

Things to do before starting

- Prepare dNTPs, 10 mM each. Store at –20°C in aliquots.
- Prepare random hexamer, 100 µM. Store at –20°C in aliquots.
- Prepare $MgCl_2$, 50 mM. Store at –20°C in aliquots.

Procedure

1. Thaw all necessary components and place them on ice.
2. Incubate 1 µg of RNA (1–4 µl) for 10 minutes at 70°C and immediately cool on ice for 5 minutes.
3. Centrifuge briefly (approximately 10 seconds, 10,000 rpm) to collect the liquid in the bottom of the tube. Then keep on ice.
4. Prepare the following RT mix according to the number of samples being processed (Table 1).

* When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.

Table 1. Preparation of RT mix

Component	Volume per sample (µl)	Final concentration
First-Strand Buffer (supplied with Superscript II Reverse Transcriptase), 5x	4.0	1x
MgCl2 (50 mM)	2.0	5 mM
dNTPs (10 mM each, to be prepared previously and stored at –20°C in aliquots)	2.0	1 mM
DTT (100 mM, supplied with Superscript II Reverse Transcriptase)	2.0	10 mM
RNase inhibitor (40 U/µl)	0.5	1 U/µl
RNase inhibitor (40 U/µl)	0.5	1 U/µl
Random hexamer (100 µM)	5.0	25 µM
Superscript II or Superscript Reverse Transcriptase (200 U/µl)	0.5	5 U/µl
Heated RNA sample (to be added in step 5)	1.0–4.0	50 ng/µl
Nuclease-free PCR grade water (to be added in step 5)	0.0–3.0	–
Final volume	20.0	–

5. Pipet 16 µl of RT mix into each PCR tube. Then add 1–4 µl (1 µg) RNA (from step 3), and adjust the volume to 20 µl with nuclease-free PCR grade water (see Table 2).

Table 2. Preparation of reverse transcription reaction

Component	Volume (µl)
RT mix	16
Heated sample RNA (1 µg)	1–4
Nuclease-free PCR grade water	0–3
Final volume	20

6. Mix well and centrifuge briefly (approximately 10 seconds, 10,000 rpm) to collect the liquid in the bottom of the tube.
7. Incubate at 20°C for 10 minutes.
8. Incubate at 42°C on a thermal cycler for 45 minutes, then immediately at 99°C for 3 minutes.
9. Cool on ice (to stop the reaction) for 5 minutes.
10. Briefly centrifuge (approximately 10 seconds, 10,000 rpm) to collect the liquid in the bottom of the tube. Then keep on ice.
11. Dilute the final cDNA with 30 µl of nuclease-free PCR grade water so that the final volume is 50 µl.
12. Carry out PCR according to the following protocols, according to your qPCR instrument.

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 3.

Table 3. Number of reactions for Rotor-Gene Q instruments with 72 tube rotor

Samples	Reactions
With the ABL primers and probe mix (PPC-ABL)	
n cDNA samples	n x 2 reactions
ABL standard	2 x 3 reactions (3 dilutions, each one tested in duplicate)
Water control	2 reactions
With the PML-RARA bcr1 primers and probe mix (PPF-PML-RARA bcr1)	
n cDNA samples	n x 2 reactions
PML-RARA standard	2 x 5 reactions (5 dilutions, 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 8 cDNA samples in the same experiment to optimize the use of the standards and the primers and probe mixes.

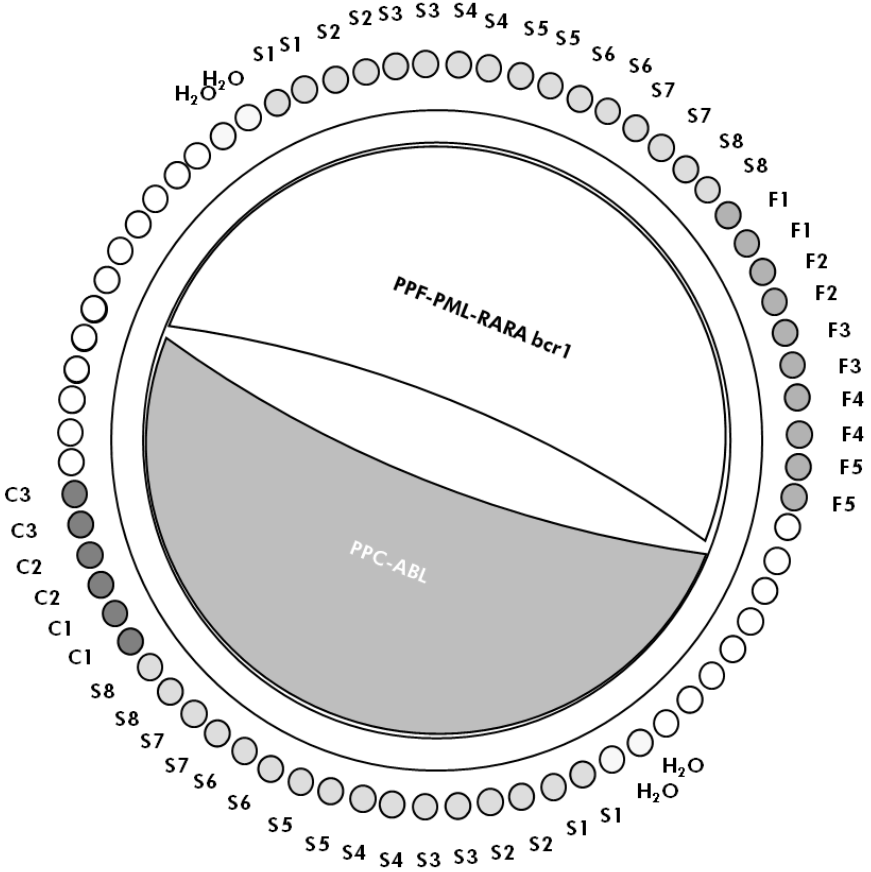

Figure 3. Suggested rotor setup for each experiment with the *ipsogen* PML-RARA bcr1 Kit.
F1–5: PML-RARA bcr1 standards; C1–3: ABL standards; S: cDNA sample; H2O: water control.

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 4 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 PPC-ABL or PPF-PML-RARA bcr1). Extra volumes are included to compensate for pipetting error.

Table 4. Preparation of qPCR mix

Component	1 reaction (µl)	ABL: 24 + 1 reactions (µl)	PML-RARA bcr1: 28 + 1 reactions (µl)	Final concentration
TaqMan Universal PCR Master Mix, 2x	12.5	312.5	362.5	1x
Primers and probe mix, 25x	1	25	29	1x
Nuclease-free PCR grade water	6.5	162.5	188.5	–
Sample (to be added at step 4)	5	5 each	5 each	–
Total volume	25	25 each	25 each	–

3. Dispense 20 µl of the qPCR pre-mix per tube.
4. Add 5 µl of the RT product (cDNA, 100 ng RNA equivalent) obtained in the reverse transcription (see "Protocol: Recommended standardized EAC reverse transcription", page 14) 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's 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 deg Time: 10 mins
Cycling	50 times 95 deg for 15 secs 60 deg for 1 min with acquisition of FAM fluorescence in channel Green: Single

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

Protocol: qPCR on ABI PRISM 7000, 7700 and 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 ABL primers and probe mix (PPC-ABL)	
n cDNA samples	n x 2 reactions
ABL standard	2 x 3 reactions (3 dilutions, each one tested in duplicate)
Water control	2 reactions
With the PML-RARA bcr1 primers and probe mix (PPF-PML-RARA bcr1)	
n cDNA samples	n x 2 reactions
PML-RARA standard	2 x 5 reactions (5 dilutions, each one tested in duplicate)
Water control	2 reactions

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

We recommend testing at least 8 cDNA samples 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.

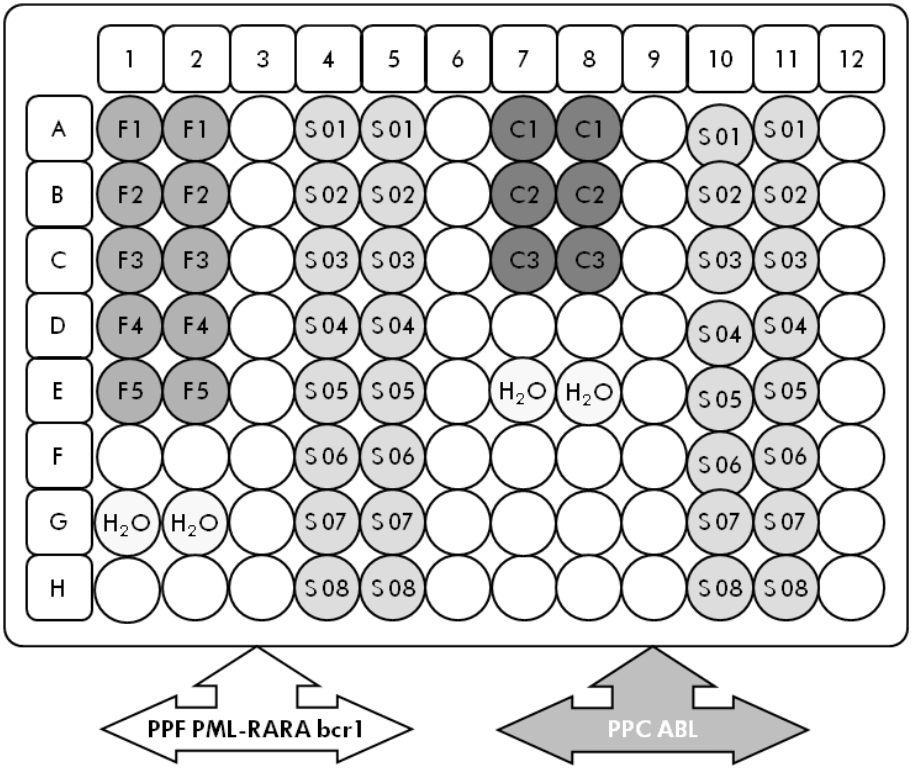

Figure 4. Suggested plate setup for one experiment. S: cDNA sample; F1–5: PML-RARA bcr1 standards; C1–3: ABL standards; H2O: water control.

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

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 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 PPC-ABL or PPF-PML-RARA bcr1). Extra volumes are included to compensate for pipetting error.

Table 7. Preparation of qPCR mix

Component	1 reaction (µl)	ABL: 24 + 1 reactions (µl)	PML-RARA bcr1: 28 + 1 reactions (µl)	Final concentration
TaqMan Universal PCR Master Mix, 2x	12.5	312.5	362.5	1x
Primers and probe mix, 25x	1	25	29	1x
Nuclease-free PCR grade water	6.5	162.5	188.5	–
Sample (to be added at step 4)	5	5 each	5 each	–
Total volume	25	25 each	25 each	–

3. Dispense 20 µl of the qPCR pre-mix per well.
4. Add 5 µl of the RT product (cDNA, 100 ng RNA equivalent) obtained in the reverse transcription (see "Protocol: Recommended standardized EAC reverse transcription", page 14) 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's recommendations.

Program the thermal cycler with the thermal cycling program as indicated in Table 8 for ABI PRISM 7000, 7700 and 7900HT SDS, and Applied Biosystems 7500 Real-Time PCR System, or Table 9 for the LightCycler 480 instrument.

Table 8. Temperature profile for ABI PRISM 7000, 7700 and 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 60°C for 1 minute with acquisition of FAM fluorescence; quencher: TAMRA

Table 9. 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 60°C for 1 minute with acquisition of FAM fluorescence corresponding to (483–533 nm) for LC version 01 and (465–510 nm) for LC version 02

8. For the ABI PRISM 7000, 7700 and 7900HT SDS, and Applied Biosystems 7500 Real-Time PCR System follow step 8a. For the LightCycler 480, follow step 8b.
   8a. ABI PRISM 7000, 7700 and 7900HT SDS, and Applied Biosystems 7500 Real-Time PCR System: We recommend a threshold set at 0.1 as described in the EAC protocol in the analysis step and a baseline set between cycles 3 and 15. Start the cycling program, as indicated in Table 8.
   8b. 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 9.

Protocol: qPCR on LightCycler 1.2 and 2.0 instruments

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

Table 10. Number of reactions for LightCycler 1.2 and 2.0 instruments

Samples	Reactions
With the ABL primers and probe mix (PPC-ABL)	
n cDNA samples	n x 2 reactions
ABL standard	1 x 3 reactions (3 standard dilutions, each one tested once)
Water control	1 reaction
With the PML-RARA bcr1 primers and probe mix (PPF-PML-RARA bcr1)	
n cDNA samples	n x 2 reactions
PML-RARA standard	1 x 5 reactions (5 standard dilutions, each one tested once)
Water control	1 reaction

Sample processing on LightCycler 1.2 and 2.0 instruments

We recommend testing at least 5 cDNA samples in the same experiment to optimize the use of the standards and primers and probe mixes. The capillary scheme in Figure 5 shows an example of an experiment.

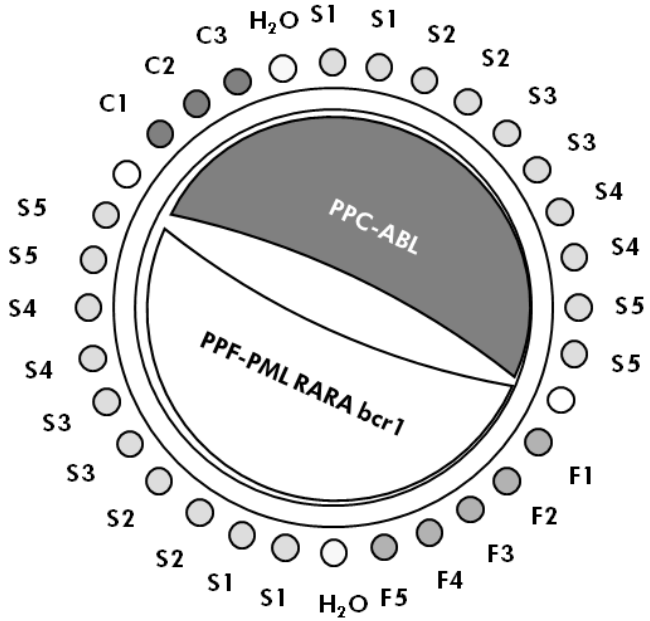

Figure 5. Suggested rotor setup for each experiment with the *ipsogen* PML-RARA bcr1 Kit.
F1–5: PML-RARA bcr1 standards; C1–3: ABL standards; S: unknown DNA sample to be analyzed; H2O: water control.

qPCR on LightCycler 1.2 and 2.0 instruments

Note: Because of particular technological requirements, LightCycler 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.

   All concentrations are for the final volume of the reaction.

   Table 11 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 primers and probe mix (either PPC-ABL or PPF-PML-RARA bcr1). Extra volumes are included to compensate for pipetting error.

Table 11. Preparation of qPCR mix

Component	1 reaction (µl)	ABL: 14 + 1 reactions (µl)	PML-RARA bcr1: 16 + 1 reactions (µl)	Final concentration
Freshly prepared LightCycler TaqMan Master Mix, 5x	4.0	60.0	68.0	1x
Primers and probe mix, 25x	0.8	12.0	13.6	1x
Nuclease-free PCR grade water	10.2	153.0	173.4	–
Sample (to be added at step 4)	5	5 each	5 each	–
Total volume	20	20 each	20 each	–

3. Dispense 15 µl of the qPCR pre-mix per capillary.
4. Add 5 µl of the RT product (cDNA, 100 ng RNA equivalent) obtained in the reverse transcription (see "Protocol: Recommended standardized EAC reverse transcription", page 14) 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's recommendations.
8. Program the LightCycler 1.2 or 2.0 instruments with the thermal cycling program as indicated in Table 12.

Table 12. Temperature profile

Mode of analysis	Quantification
Hold	Temperature: 95°C Time: 10 minutes Ramp: 20
Cycling	50 times 95°C for 10 seconds; ramp: 20 60°C for 1 minute; ramp: 20; with acquisition of FAM fluorescence: Single
Hold 2	45°C for 1 minute; ramp: 20

9. For the LightCycler 1.2, follow step 9a. For the LightCycler 2.0, follow step 9b.
   9a. LightCycler 1.2: The F1/F2 and "2nd derivative analysis" mode is recommended. Start the thermal cycling program, as indicated in Table 12.
   9b. LightCycler 2.0: We recommend using Automated (F''max) analysis on LightCycler 2.0 Software version 4.0 to obtain reproducible results. Start the thermal cycling program, as indicated in Table 12.

Protocol: qPCR on the SmartCycler instrument

Using this instrument, we recommend measuring samples in duplicate and controls only once, as indicated in Table 13.

Table 13. Number of reactions for the SmartCycler instrument

Samples	Reactions
With the ABL primers and probe mix (PPC-ABL)	
n cDNA samples	n x 2 reactions
ABL standard	1 x 3 reactions (3 standard dilutions, each one tested once)
Water control	1 reaction
With the PML-RARA bcr1 primers and probe mix (PPF-PML-RARA bcr1)	
n cDNA samples	n x 2 reactions
PML-RARA standard	1 x 5 reactions (5 standard dilutions, each one tested once)
Water control	1 reaction

Sample processing on the SmartCycler instrument

We recommend testing at least 5 cDNA samples in the same experiment to optimize the use of the standards and primers and probe mixes. The two-block scheme in Figure 6 shows an example.

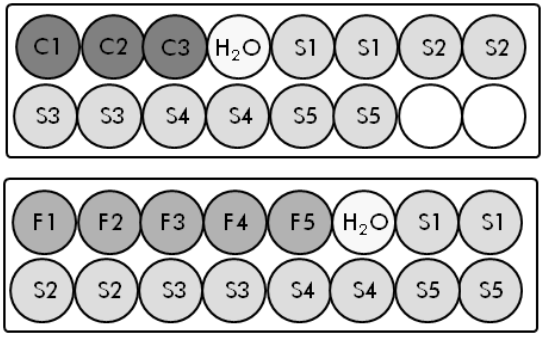

Figure 6. Suggested plate setup for one experiment. S: cDNA sample; F1–5: PML-RARA bcr1 standards; C1–3: ABL standards; H2O: water control.

qPCR on the SmartCycler 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 14 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 PPC-ABL or PPF-PML-RARA bcr1). Extra volumes are included to compensate for pipetting error.

Table 14. Preparation of qPCR mix

Component	1 reaction (µl)	ABL: 14 + 1 reactions (µl)	PML-RARA bcr1: 16 + 1 reactions (µl)	Final concentration
TaqMan Universal PCR Master Mix, 2x	12.5	187.5	212.5	1x
Primers and probe mix, 25x	1	15	17	1x
Nuclease-free PCR grade water	6.5	97.5	110.5	–
Sample (to be added at step 4)	5	5 each	5 each	–
Total volume	25	25 each	25 each	–

3. Dispense 20 µl of the qPCR pre-mix per well.
4. Add 5 µl of the RT product (cDNA, 100 ng RNA equivalent) obtained in the reverse transcription (see "Protocol: Recommended standardized EAC reverse transcription", page 14) in the corresponding tube (total volume 25 µl).
5. Mix gently, by pipetting up and down.
6. Load the samples into the thermal cycler according to the manufacturer's recommendations.
7. Program the SmartCycler instrument with the thermal cycling program as indicated in Table 15.

Table 15. Temperature profile

Hold	Temperature 50°C Time: 2 minutes
Hold 2	Temperature: 95°C Time: 10 minutes
Cycling	50 times 95°C for 15 seconds 60°C for 1 minute with acquisition: Single

8. We recommend a threshold set at 30. Start the thermal cycling program, as indicated in Table 15.

Interpretation of Results

Data analysis principle

Using TaqMan technology, the number of PCR cycles necessary to detect a signal above the threshold is called the threshold cycle (C_T) and is directly proportional to the amount of target present at the beginning of the reaction.

Using standards with a known number of molecules, one can establish a standard curve and determine the precise amount of target present in the test sample. The *ipsogen* standard curves are plasmid-based; we use 3 plasmid standard dilutions for the ABL control gene (CG), and 5 standard dilutions for the fusion gene (PML-RARA bcr1) to ensure accurate standard curves. Figures 7 and 8 show an example of TaqMan amplification curves obtained with the *ipsogen* PML-RARA bcr1 Kit.

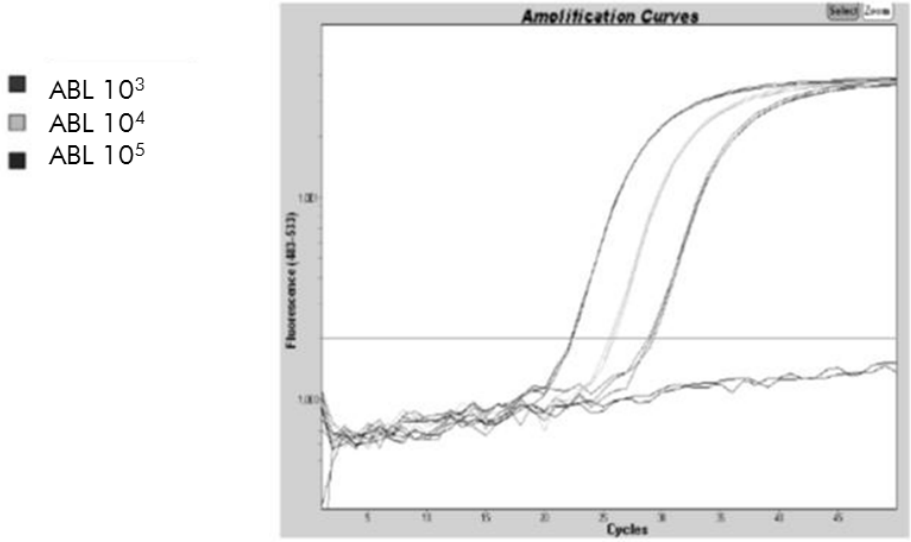

Figure 7. Detection of ABL standards (C1, C2, C3). 10^3, 10^4 and 10^5 copies/5 µl.

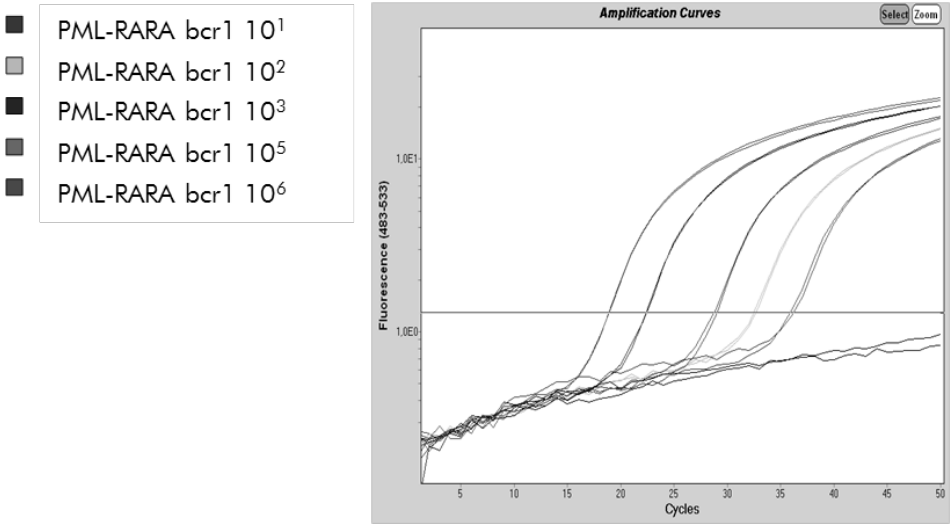

Figure 8. Detection of PML-RARA bcr1 standards detection (F1–F5). 10^1, 10^2, 10^3, 10^5 and 10^6 copies/5 µl.

Results

Standard curve and quality criteria

Raw data can be pasted into an Excel® file for analysis.

For each gene (ABL and PML-RARA), raw C_T values obtained from plasmid standard dilutions are plotted according to the log copy number (3, 4 and 5 for C1, C2 and C3; 1, 2, 3, 5 and 6 for F1, F2, F3, F4 and F5). Figure 9 shows an example of the theoretical curve calculated on 5 standard dilutions.

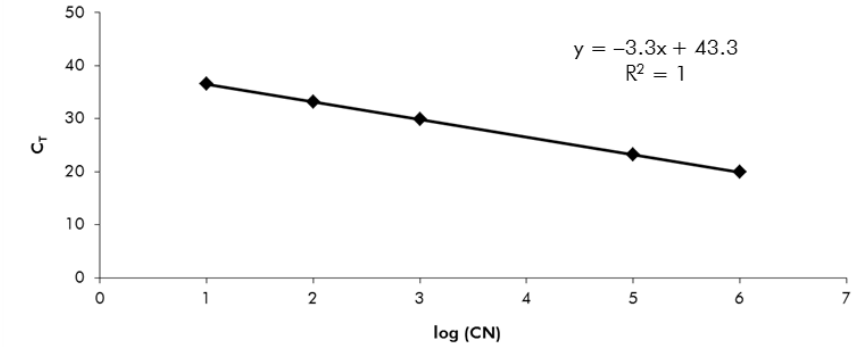

Figure 9. Theoretical curve calculated from the 5 standard dilutions. A linear regression curve (y = ax + b) is calculated for each gene (ABL and PML-RARA), where a is the slope of the line and b is the y-intercept, which is the y-coordinate of the point where the line crosses the y axis. Its equation and coefficient of determination (R^2) are printed on the graph.

As standards are ten-fold dilutions, the theoretical slope of the curve is –3.3. A slope between –3.0 and –3.9 is acceptable as long as R^2 is >0.95 (6). However, a value for R^2 >0.98 is desirable for precise results (7).

Normalized copy number (NCN)

The ABL standard curve equation should be used to transform raw C_T values (obtained with PPC-ABL) for the unknown samples into ABL copy numbers (ABL_{CN}).

The PML-RARA standard curve equation should be used to transform raw C_T values (obtained with PPF-PML-RARA) for the unknown samples, into PML-RARA copy numbers ($PML\text{-}RARA_{CN}$).

The ratio of these CN values gives the normalized copy number (NCN):

$$NCN = \frac{PML\text{-}RARA_{CN}}{ABL_{CN}}$$

MRD value

The minimal residual disease (MRD) value is the ratio between the CG normalized expression of the FG in follow-up $(FG_{CN}/CG_{CN})_{FUP}$ and diagnostic samples $(FG_{CN}/CG_{CN})_{DX}$.

$$\text{MRD Value } (MRD_V) = \frac{(FG_{CN}/CG_{CN})_{FUP}}{(FG_{CN}/CG_{CN})_{DX}}$$

Sensitivity

The sensitivity ($SENS_V$) is calculated according to the relative expression of the FG at diagnosis $(FG_{CN}/CG_{CN})_{DX}$ and CG expression ($CG_{CN,FUP}$) in the follow-up sample.

$$\text{Sensitivity } (SENS_V) = \frac{CG_{CN,DX}}{CG_{CN,FUP} \times FG_{CN,DX}}$$

Quality control on ABL values

Poor quality of the RNA or problems during the qPCR steps result in low ABL_{CN}. We recommend discarding results from samples giving ABL_{CN} <1318 (lower value of the 95% CI from patient samples in the EAC study, reference 5).

Reproducibility between replicates

The variation in C_T values between replicates should be <2, corresponding to a 4-fold change in copy number values.

Variation in C_T values between replicates is generally <1.5 if the mean C_T value of the replicates is <36 (6).

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

Water controls

Negative controls should give zero CN.

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, consult your clinical coordinator, or visit **www.qiagen.com**.

	Comments and suggestions
Negative result for the control gene (ABL) and PML-RARA bcr1 in all the samples — standard okay	
a) Poor RNA quality	Always check the RNA quality and concentration before starting. Run a cell line RNA positive control (*ipsogen* PML-RARA bcr1 Controls Kit, cat. no. 672091*) in parallel.
b) Failure of reverse transcription step	Always check the RNA quality and concentration before starting. Run a cell line RNA positive control (*ipsogen* PML-RARA bcr1 Controls Kit, cat. no. 672091*) in parallel.
Negative result for the control gene (ABL) in the samples — standard okay	
a) Poor RNA quality	Always check the RNA quality and concentration before starting. Run a cell line RNA positive control (*ipsogen* PML-RARA bcr1 Controls Kit, cat. no. 672091*) in parallel.
b) Failure of reverse transcription step	Always check the RNA quality and concentration before starting. Run a cell line RNA positive control (*ipsogen* PML-RARA bcr1 Controls Kit, cat. no. 672091*) in parallel.
Standard signal negative	
a) Pipetting error	Check pipetting scheme and the setup of the reaction. Repeat the PCR run.

		Comments and suggestions
b)	Inappropriate storage of kit components	Store the *ipsogen* PML-RARA bcr1 Kit at –15 to –30°C and keep primers and probe mixes (PPC and PPF) protected from light. See "Reagent Storage and Handling", page 13. Avoid repeated freezing and thawing. Aliquot reagents for storage.
Negative controls are positive		
	Cross-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.
No signal, even in standard controls		
a)	Pipetting error or omitted reagents	Check pipetting scheme and the setup of the reaction. Repeat the PCR run.
b)	Inhibitory effects of the sample material, caused by insufficient purification	Repeat the RNA preparation.
c)	LightCycler: Incorrect detection channel chosen	Set Channel Setting to F1/F2 or 530 nm/640 nm.
d)	LightCycler: No data acquisition programmed	Check the cycle programs. 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		
a)	Poor RNA quality or low concentration	Always check the RNA quality and concentration before starting. Run a cell line RNA positive control (*ipsogen* PML-RARA bcr1 Controls Kit, cat. no. 672091*) in parallel.

		Comments and suggestions
b)	Failure of reverse transcription step	Always check the RNA quality and concentration before starting. Run a cell line RNA positive control (*ipsogen* PML-RARA bcr1 Controls Kit, cat. no. 672091*) in parallel.
Fluorescence intensity too low		
a)	Inappropriate storage of kit components	Store the *ipsogen* PML-RARA bcr1 Kit at –15 to –30°C and keep primers and probe mixes (PPC and PPF) protected from light. See "Reagent Storage and Handling", page 13. Avoid repeated freezing and thawing. Aliquot reagents for storage.
b)	Very low initial amount of target RNA	Increase the amount of sample RNA. **Note**: Depending of the chosen method of RNA preparation, inhibitory effects may occur.
LightCycler: Fluorescence intensity varies		
a)	Pipetting error	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 capillaries	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.
LightCycler: Error of the standard curve		
	Pipetting error	Variability caused by so-called "pipetting error" can be reduced by analyzing data in the F1/F2 or 530 nm/640 nm mode.

*__Note__: The *ipsogen* PML-RARA bcr1 Controls Kit, cat. no. 672091, 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.

Quality Control

Quality control of the complete kit has been performed on a LightCycler 480 instrument. This kit is manufactured according to ISO 13485:2003 standard. 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 which 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.

Note: The kit has been designed according to the "Europe Against Cancer" (EAC) studies (4, 5). It 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.

Performance Characteristics

Nonclinical studies

Materials and methods

Performance evaluation was performed on an ABI PRISM 7700 SDS, in combination with reagents listed in "Materials Required but Not Provided", page 10. Equivalence studies validated its use on the following instruments: ABI PRISM 7000 and 7900HT SDS, LightCycler 1.2 and 480, Rotor-Gene 3000 and SmartCycler.

Nonclinical studies were conducted to establish the analytical performance of the *ipsogen* PML-RARA bcr1 Kit. These nonclinical laboratory studies were performed on total RNA from the NB4 cell line diluted in a constant final amount of MV4-11 cell line total RNA.

To determine the repeatability of the assay, 5 different concentrations of NB4 total RNA (5 ng, 500 pg, 50 pg, 5 pg and 0.5 pg) diluted in MV4-11 total RNA, in a constant final total amount of 200 ng, were analyzed in 5 replicates per run and in 4 different runs. The samples with 5 pg and 0.5 pg of NB4 RNA in MV4-11 RNA were too low to give results (Figure 10).

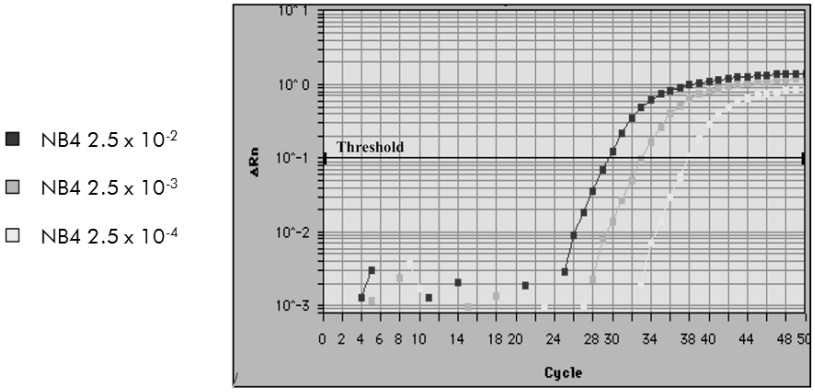

Figure 10. Amplification plots of 2.5 x 10^{-2} (5 ng), 2.5 x 10^{-3} (0.5 ng) and 2.5 x 10^{-4} (0.05 ng) dilutions of NB4 total RNA in MV4-11 negative total RNA.

Analytical data

Tables 16–19 show the inter-assay analyses with the mean threshold cycle (C_T), standard deviation (SD), number of samples (n), coefficient of variation (CV), mean copy number (CN) and mean normalized copy number (NCN).

Table 16. Inter- and intra-assay analysis — cell lines PML-RARA and ABL

		Inter-assay analysis				Intra-assay analysis	
Cell line	**Dilution**	**Mean C_T**	**SD**	**n**	**CV (%)**	**Mean CV**	**Max CV**
PML-RARA	5 ng	29.86	0.29	20	0.98	0.32	1.42
	0.5 ng	33.70	0.48	20	1.42	0.56	2.16
	0.05 ng	37.03	0.37	18	1.01	1.07	2.03
ABL	–	24.06	0.22	100	0.92	0.15	2.31

Table 17. Inter-assay analysis — plasmids

Gene	**Plasmid**	**Mean C_T**	**SD**	**n**	**CV (%)**
PML-RARA	F1 (10^1 copies)	35.95	0.29	8	0.79
	F2 (10^2 copies)	32.25	0.59	8	1.84
	F3 (10^3 copies)	28.71	0.55	8	1.90
	F4 (10^5 copies)	22.14	0.49	7	2.23
	F5 (10^6 copies)	18.64	0.72	8	3.84
ABL	C1 (10^3 copies)	28.85	0.76	7	2.62
	C2 (10^4 copies)	25.25	0.71	8	2.82
	C3 (10^5 copies)	21.74	0.81	8	3.74

Table 18. Inter-assay analysis — cell lines PML-RARA bcr1 and ABL (mean CN)

Cell line	Dilution	Mean CN	SD	n	CV (%)
PML-RARA bcr1	2.5×10^{-2} (5 ng/200 µg)	583.95	149.19	20	25.55
	2.5×10^{-3} (0.5 ng/200 ng)	44.98	12.25	20	27.23
	2.5×10^{-4} (0.05 ng/200 ng)	4.91	1.55	19	31.52
ABL	–	35,171.47	22,448.3	99	63.83

Table 19. Inter-assay analysis — cell line PML-RARA bcr1 (mean NCN)

Cell line	Dilution	Mean NCN*	SD	n	CV (%)
PML-RARA bcr1	2.5×10^{-2} (5 ng/200 µg)	271.4	150.00	20	55.56
	2.5×10^{-3} (0.5 ng/200 ng)	15.35	8.12	20	52.87
	2.5×10^{-4} (0.05 ng/200 ng)	1.66	0.91	18	55.14

* For these study results only, the NCN is given as $\frac{\text{PML-RARA bcr1}_{CN}}{\text{ABL}_{CN}} \times 10{,}000$

Clinical studies

Performance evaluation was performed on a ABI PRISM 7700 SDS, in combination with reagents listed in "Materials Required but Not Provided", page 10. Equivalence studies validated its use on the following instruments: ABI PRISM 7000 and 7900HT SDS, LightCycler 1.2 and 480, Rotor-Gene 3000 and SmartCycler.

A group of 26 laboratories, in 10 European countries, organized in a EAC concerted action, used plasmids provided by *ipsogen* to establish a standardized protocol for qPCR analysis of the major leukemia-associated fusion genes in the clinical setting. The PML-RARA bcr1 transcript was one of the fusion genes (FG) included in the study. We present here a summary of this validation study; full results have been published in 2003 (4, 5).

Inter-laboratory reproducibility for CG and FG plasmid standards

A total of 11 laboratories performed an inter-laboratory reproducibility experiment to assess variability in the measurement of CG and FG plasmid standard dilutions. Dilutions were performed in duplicate at each facility. Table 20 reports the mean, standard deviation and CV (%) for each dilution.

Table 20. Inter-laboratory reproducibility for CG and FG plasmid standards

Gene	Dilution	Mean	C_T SD	CV (%)
ABL control gene	C1	29.26	0.69	2.31
	C2	25.79	0.65	2.53
	C3	22.40	0.61	2.70
PML-RARA bcr1 fusion gene	F1	35.84	0.79	2.21
	F2	32.47	0.49	1.50
	F3	28.91	0.34	1.17
	F4	21.82	0.30	1.40
	F5	18.47	0.29	1.55

Expression values of the PML-RARA bcr1 FG transcript

Tables 21 and 22 show the expression values of the PML-RARA bcr1 FG transcript and ABL CG, for the NB4 cell line, APL patients at diagnosis, and for negative control patients.

Table 21. Expression values of the PML-RARA bcr1 FG transcript and ABL CG — CT values

	C_T values (95% range)	
	PML-RARA bcr1	**ABL**
NB4 cell line	24.7	23.7
APL patient samples		
Bone marrow (n = 14)	25.6 (23.1–27.5)	24.5 (21.7–28.5)
Peripheral blood (n = 9)	25.7 (23.7–29.4)	24.6 (22.0–27.4)
Negative patent samples		
Bone marrow (n = 26)	–	25.35 (24.68–26.02)
Peripheral blood (n = 74)	–	25.15 (24.83–25.48)

ABL C_T values did not differ significantly between normal and leukemic samples, nor between samples types (PB or BM) or leukemia samples from patients diagnosed with APL.

Table 22. Expression values of the PML-RARA bcr1 FG transcript and ABL CG — CN and NCN values

	CN values (95% range)		NCN values (95% range)
	PML-RARA bcr1	**ABL**	**CN bcr1/ CN ABL**
Patient samples			
Bone marrow (n = 14)	5129 (1480–25,704)	1538.7 (133.2–46,781.28)	0.30 (0.09–1.82)
Peripheral blood (n = 9)	3891 (475–14,454)	1400.76 (50.27–11,274)	0.36 (0.11–0.78)
Negative patient samples			
Bone marrow (n = 26)	–	19,201 (12,922–25,480)	–
Peripheral blood (n = 74)	–	21,136 (17,834–24,437)	–

False positive and false negative rates

False negative and false positive rates were computed using the following controls.

- Positive controls: NB4 cells, a cell line well known for its positivity for PML-RARA bcr1 FG; patients' samples already assessed for PML-RARA bcr1 positivity
- Negative controls: Negative RNA samples, no amplification controls (NAC) made of *E. coli* RNA instead of human RNA to check for PCR contamination, and no template controls (NTC), which contained water instead of human RNA

Amplification on RNA samples of the FG was performed in triplicate and in duplicate for the CG.

A false-negative sample was defined as a positive RNA sample with less than 50% of positive wells (0/2, 0/3 or 1/3).

A false-positive sample was defined as a negative sample with at least 50% of positive wells (1/2, 2/3 or 3/3).

Table 23 shows the number and percentage of false negative and false positive samples.

Table 23. False negative and false positive samples

False negativity		False positivity	
10^{-3}	10^{-4}	FG negative control	NAC/NTC
0% (0/29)	0% (0/28)	11% (5/45)	5% (5/100)

References

1. Santamarie, C. et al. (2007) Using quantification of the PML-RARalpha transcript to stratify the risk of relapse in patients with acute promyelocytic leukemia. Haematologica **92**, 315.
2. Kern, W. et al. (2004) Monitoring of minimal residual disease in acute myeloid leukemia. Atlas Genet. Cytogenet. Oncol. Haematol. **112**, 4.
3. Lo-Coco, F. and Ammantuna, E. (2006) The biology of acute promyelocytic leukemia and its impact on diagnosis and treatment. Hematology ASH Educ. Program **514**, 156.
4. Beillard, E. et al. (2003) Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) - a Europe against cancer program. Leukemia **17**, 2474.
5. Gabert, J. et al. (2003) Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia — a Europe Against Cancer program. Leukemia **17**, 2318.
6. van der Velden, V.H. et al. (2003) Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. Leukemia **17**, 1013.
7. Branford, S. et al. (2006) Rationale for the recommendations for harmonizing current methodology for detecting BCR-ABL transcripts in patients with chronic myeloid leukemia. Leukemia **20**, 1925.

Symbols

The following symbols may appear on the packaging and labeling:

 <N>	Contains reagents sufficient for <N> reactions
	Use by
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Material number (i.e., component labeling)
	Global Trade Item Number
	Temperature limitation
	Manufacturer
	Consult instructions for use

Document revision history	
R5, November 2017	Notes added that *ipsogen* PML-RARA bcr1 Controls Kit, cat. no. 672091, is for Research Use Only; minor typing errors corrected.

Ordering Information

Product	Contents	Cat. no.
ipsogen PML-RARA bcr1 Kit (24)	For 24 reactions: ABL Control Gene Standards, PML-RARA bcr1 Fusion Gene Standards, Primers and Probe Mix ABL, Primers and Probe Mix PML-RARA bcr1 Fusion Gene	672123
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

***ipsogen* PML-RARA bcr1 Controls Kit — for qualitative validation of RNA extraction and reverse transcription of the PML-RARA bcr1 fusion gene**		
ipsogen PML-RARA bcr1 Controls Kit	Cell lines with negative, high, and low positive expression of the PML-RARA bcr1 fusion gene	672091*

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