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ScienceDirect
Leukemia Research 32 (2008) 579–585
Leukemia Research
www.elsevier.com/locate/leukres

# A multiplex PCR for improved detection of typical and atypical *BCR–ABL* fusion transcripts

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Received 4 July 2007; received in revised form 19 August 2007; accepted 20 August 2007
Available online 24 October 2007

**Abstract**

RT-PCR is the method of choice for detecting *BCR–ABL* in CML and ALL. The three predominant mRNA transcripts found are e1a2 (in ALL), e13a2, and e14a2 (in CML and ALL). However, a number of "atypical" *BCR–ABL* transcripts (e1a3, e13a3, e14a3, e19a2, e6a2, e8a2, etc.) resulting from chromosomal breakpoints outside *ABL* intron 1 or *BCR* intron 1, 13 or 14, respectively, have been reported. These atypical transcripts may escape detection when using methods that are optimized to detect just the typical ones. We present here a novel, fast, and reliable multiplex PCR for improved detection of typical and atypical *BCR–ABL* transcripts.


*Keywords:* *BCR–ABL* fusion proteins; Myeloproliferative disorders; Chronic myeloid leukemia; Acute lymphoblastic leukemia; Polymerase chain reaction

## 1. Introduction

The Philadelphia chromosome was the first recurrent chromosomal aberration to be identified in a hematologic disease [1]. After the identification of the two genes involved, *BCR* on 22q11, and *ABL* on 9q34 [2,3] and the advent of PCR techniques in hematology RT-PCR became the diagnostic gold standard for detecting the *BCR–ABL* fusion gene [4].

Several different PCR methods have been published [5,6, and others] and proposals for a standardization of the conventional [7,8] and real-time quantitative PCR diagnostics [9] as well as recommendations for follow-up investigations [10,11] have been issued. During the past 15 years a number of different *BCR–ABL* mRNA transcripts have been experimentally identified, according to the chromosomal breakpoint sites in the two genes involved. The vast majority of transcripts are made up of the three variants e1a2, e13a2, or e14a2, resulting from chromosomal breakpoints in *ABL* intron 1 and *BCR* introns 1, 13 or 14, respectively. More than 95% of *BCR–ABL* transcripts in CML are either e13a2 or e14a2 ("major breakpoint cluster region" or "M-bcr"), while about 70% of transcripts found in *BCR–ABL*-positive ALL are e1a2 ("minor breakpoint cluster region" or "m-bcr") and more than 25% e13a2 or e14a2.

"Atypical" transcripts are sometimes observed. These include transcripts with a breakpoint in *ABL* intron 1 and *BCR* intron 6 (e6a2 [12,13]) or intron 19 (e19a2 [14,15,16,17]). Moreover, transcripts have been reported with a breakpoint in *ABL* intron 2 and *BCR* intron 1 (e1a3 [18,19]), *BCR* intron 13 (e13a3 [20]), and *BCR* intron 14 (e14a3 [21,22]). Transcripts have also occasionally been observed with fusion of *out-of-frame* exons and interposed intronic sequences or breakpoints within exons (e8a2 [23], and others).

Most current RT-PCR methods for detecting *BCR–ABL* are designed and optimized for detecting the M-bcr and m-bcr transcripts. Other transcripts may also be detected by these methods but very large PCR products are sometimes involved. We hypothesize that some of these atypical transcripts may therefore escape detection, as has also been reported [24]. Damaj and coworkers [25] recently used a novel multiplex PCR to better detect atypical *BCR–ABL* transcripts for investigating essential thrombocythemia but provided no sensitivity or specificity data or gel images. In

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doi:10.1016/j.leukres.2007.08.017

an attempt to establish a reliable RT-PCR for detecting all known *BCR–ABL* transcripts in a single-round multiplex format we cloned the eight most prevalent *BCR–ABL* transcripts (e1a2, e1a3, e6a2, e13a2, e13a3, e14a2, e14a3, e19a2) and developed a novel multiplex PCR. We tested this PCR on the cloned transcripts and numerous patient samples and found it to be reliable, fast, and capable of detecting all currently known *BCR–ABL* transcripts.

## 2. Materials and methods

### 2.1. RNA preparation and reverse transcription

Total RNA was routinely isolated from fresh or archived material using the *Trizol* method, but other methods also yielded good results. The RNA isolation step always included DNA digestion. Various reverse transcription methods were applied and yielded good results. Sensitivity was assayed using the *Ready-To-Go* kit (Amersham Biosciences, Freiburg, Germany) with random hexamers reverse transcription of 1 μg of total RNA in a total volume of 33 μl for 60 min according to the manufacturer's recommendations. The PCR was done using 5 μl cDNA, i.e. the equivalent of 150 ng RNA.

### 2.2. PCR method

Primer sequences are given in Table 1. All primers were obtained from *Metabion Inc.* (Martinsried, Germany), HPLC-purified and stored at −20 °C. Each primer had a concentration of 100 nM in the 50 μl reaction mix. The HotStarTaq Master Mix (QIAGEN, Hilden, Germany) was used for all PCR experiments, including those evaluating PCR sensitivity in *BCR–ABL*-positive cell line dilutions. The following program was used in a Perkin-Elmer 2400 thermal cycler: 15 min at 95 °C to activate the hot start polymerase according to the manufacturer's recommendations, 40 cycles: 95 °C for 10 s, 63 °C for 20 s, and 72 °C for 30 s. This PCR took 2 h on the Perkin-Elmer 2400 cycler.

### 2.3. Gel electrophoresis

Aliquots of the PCR reaction mix were electrophoretically separated in a 1.5% agarose gel in TBE buffer. The ϕX174/HaeIII digest was used as the size standard. The electrophoresis runtime was optimized to best distinguish fragments between 50 bp and 700 bp.

### 2.4. Cloning BCR–ABL transcripts

Our laboratory has already cloned e1a2, e13a2, and e14a2 earlier [8]. The following *BCR–ABL* transcripts were additionally cloned from patient material in the *pCR2.1-TOPO* vector (Invitrogen, Karlsruhe, Germany): e1a3, e13a3, e14a3, e6a2, and e19a2. Patient cDNA was kindly supplied by Philippe Jonveaux, CHU Nancy-Brabois, France (e6a2), Cedrik Haškovec, ÚHKT, Prague, Czech Republic (e14a3), Joaquín Martínez-López, Hospital Universitario 12 de Octubre Madrid, Spain (e19a2), and Hideo Tanaka, Hiroshima University, Japan (e19a2). Our laboratory detected e1a3- and e13a3-positive samples. The following primers (5′–3′) were used to generate a PCR product for cloning: ACTTGTCGTAGTTGGGGGACACACCA in combination with GCGAACAAGGGCAGCAAGGCTACG for e13a2, e13a3, e14a2, e14a3, and e19a2 or in combination with GTACCAGCCCTACCAGAGCATCTACG for e1a2, e1a3, and e6a2. The cloned transcripts were sequenced by standard techniques and the sequences of the plasmid inserts have been submitted to the EMBL/Genbank nucleotide database: AF113911 (e1a2), HSA131467 (e13a2), AJ131466 (e14a2), AM491359 (e13a3), AM491360 (e14a3), AM491361 (e1a3), AM491362 (e6a2), AM491363 (e19a2).

## 3. Results

### 3.1. Principles and rationale of primer design

Our PCR was designed according to the following principles: (1) it should detect the three most prevalent transcripts e13a2, e14a2, and e1a2 at least as reliably as previous methods, (2) all published transcript variants should be covered, (3) "Atypical" transcripts should be easily recognizable as such from their product size, as far as possible, (4) an internal control reaction should be included to correctly interpret negative results, and (5) all *BCR–ABL* PCR products should be smaller than the internal control product.

### 3.2. ABL primer

The *ABL* primer was located in *ABL* exon 3 to enable the detection of fusions with either *ABL* exon 2 (a2) or exon 3 (a3).

Table 1
Primer sequences

| Primer | Sequence (5′ → 3′) | Location | Orientation |
|---|---|---|---|
| *ABL*-3 | CCATTGTGATTATAGCCTAAGACCCGGAG | *ABL* exon 3 | − |
| *BCR*-1 | CTCCAGCGAGGAGGACTTCTCCT | *BCR* exon 1 | + |
| *BCR*-6 | CCTGAGAGCCAGAAGCAACAAAGATGCC | *BCR* exon 6 | + |
| *BCR*-12 | AGAACATCCGGGAGCAGCAGAAGAA | *BCR* exon 12 | + |
| *BCR*-19 | ACTGAAGGCAGCCTTCGACGTC | *BCR* exon 19 | + |
| *BCR*-R | ATGTCCGTGGCCACACCGGACAC | *BCR* exon 19 | − |