

Size Fractionation of BCR/ABL1 Quant (RUO)* PCR Products by Capillary Electrophoresis to Determine e1a2, b2a2, and b3a2 Fusion Transcripts Identity

Introduction

Asuragen's BCR/ABL1 Quant (RUO)* is a research tool for simultaneous amplification and detection of BCR/ABL1 fusion transcripts, ABL1 transcripts (endogenous control), and BCR/ABL1Quant Norm (exogenous process control) using total RNA extracted from human blood, bone marrow, or cultured cells. The assay uses multiplex reverse transcription-PCR (RT-PCR) in combination with real-time TaqMan[®] technology. The 3 most common BCR/ABL1 fusion transcripts, e1a2, b2a2 and b3a2, are simultaneously amplified, detected, and quantified using a TaqMan[®] probe that is visualized in the FAM channel. The resulting PCR products are also compatible with capillary electrophoresis (CE) for subsequent determination of the fusion transcripts identity (e1a2, b2a2, or b3a2) via size fractionation. Here, we provide a protocol and general recommendations for CE analysis of BCR/ABL1 Quant (RUO) PCR products.

Materials and Methods

Following RT-PCR as per BCR/ABL1 Quant (RUO)* protocol (Asuragen Catalog Number 46080), the PCR products were diluted in water, mixed with GeneScan[™] 500 ROX[™] Size Standard (Applied Biosystems Part Number 401734) and Hi-Di[™] Formamide (Applied Biosystems Part Number 4311320), and run on an Applied Biosystems 3130xl Genetic Analyzer. Dye Set D spectral calibration of the 3130xl was performed with the DS-30 Matrix Standard Kit (Applied Biosystems Part Number 4345837). For a 36 cm capillary (Applied Biosystems Part Number 4315831) with POP-4[™] Polymer (Applied Biosystems Part Number 4352755) or POP-7[™] Polymer (Applied Biosystems Part Number 4352759), the following protocol was used:

1. Dilute the PCR products 1:50 in nuclease-free water (1uL of sample + 49 uL of nuclease-free water).
2. Prepare ABI Prism Genescan-500 ROX / Hi-Di Formamide mix for all samples. Per sample, mix 0.5 µL of ABI Genescan-500 ROX + 13.5 uL of Hi-Di[™] Formamide
3. Add 1 µL of diluted PCR products to individual wells of a 96-well plate (Applied Biosystems Part Number 4306737 or equivalent).
4. Add 14 µL of Genescan-500 ROX / Hi-Di Formamide mix to each well.
5. Cover the plate with adhesive film (ABgene AB-0558 or equivalent) and heat at 95°C for 2 min followed by transfer to ice or cold block.
6. Briefly centrifuge the plate (~400 xg) to collect all liquid at the bottom of the wells.
7. Load samples on the Genetic Analyzer for electrophoresis.

Instrument setup for POP-4

Pre Run: 15 kV for 180 s
Temperature: 60 °C
Injection: 1.2 kV for 18 sec injection.

Run: 15 kV for 25 min
 Other settings = default
 Instrument setup for POP-7
 Pre Run: 15 kV for 180 s
 Temperature: 60 °C
 Injection: 1.2 kV for 23 sec injection.
 Run: 15 kV for 30 min
 Other settings = default

Raw data (.fsa file) were analyzed with the GeneMapper® Software V4.0

Results and Discussion

Total RNA specimens isolated from human blood, bone marrow or cultured cells dilutions were successfully evaluated with the BCR/ABL1 Quant (RUO) kit in this research study. For each specimen positive by real time PCR (BCR/ABL1 Ct < 38-39) and containing about 50 to 1×10^7 copies of target, the expected e1a2, b2a2, or b3a2 peak could be detected by CE analysis in the FAM channel. Representative examples with cell line RNA dilutions using 36 cm capillaries with POP-4 or POP-7 separation matrix on a 3130xl Genetic Analyzer are shown in Figures 1 and 2*.

The theoretical PCR product sizes are 90, 119 and 160 base pairs (bp) for b2a2, e1a2 and b3a2 targets, respectively. However, the observed PCR product size can vary by a few base-pairs according to the CE instrument, the capillary size, the separation matrix and/or the sizing standard used. It is therefore recommended to first analysis known positive and negative specimens to determine the true expected peak size for each BCR/ABL1 target. Observed peak sizes obtained with GeneScan 500 ROX Size Standard using 36 cm capillaries with POP-4 or POP-7 separation matrix on a 3130xl Genetic Analyzer are shown in Table 1.

Table1. Expected and observed amplicon sizes

Fusion Transcript	Calculated Size (bp)	Observed Size with POP-4 (bp)	Observed Size with POP-7 (bp)
b2a2	90	87	88
e1a2	119	115	116
b3a2	160	164	166

In general, the PCR products have to be diluted 1:50 in water prior to CE analysis. For specimens with high target copy number (BCR/ABL1 Ct < 25), the excess of PCR product may interfere with correct size fractionation resulting in aberrant peak size. In this case, the PCR products may have to be further diluted (1:100 to 1:500). The Calibrator Set provided with the kit contains four vials with four different levels of BCR/ABL1 fusion transcripts (e1a2 at 1×10^2 , 1×10^3 , 1×10^4 or 1×10^6 copies per RT when following the kit protocol). Running these PCR products together with a negative control on the same plate as the tested specimens can be helpful to optimize the dilution factor and other CE analysis parameters. Representative examples obtained with Calibrator 1 and 3 are shown in Figure 3.

* For Research Use Only. Not For Use in Diagnostic Procedures. The performance characteristics of this product have not been established.

Conclusions

The capillary electrophoresis protocol described here allows size fractionation and identification of e1a2, b2a2 and b3a2 PCR products generated with Asuragen's BCR/ABL1 Quant (RUO) kit. This protocol may require further optimization when using different instruments or materials. For assistance with BCR/ABL1 Quant (RUO) kit and downstream CE analysis please contact Asuragen technical support at 877.777.1874 or 512.681.5200 or by email at techservdx@asuragen.com.

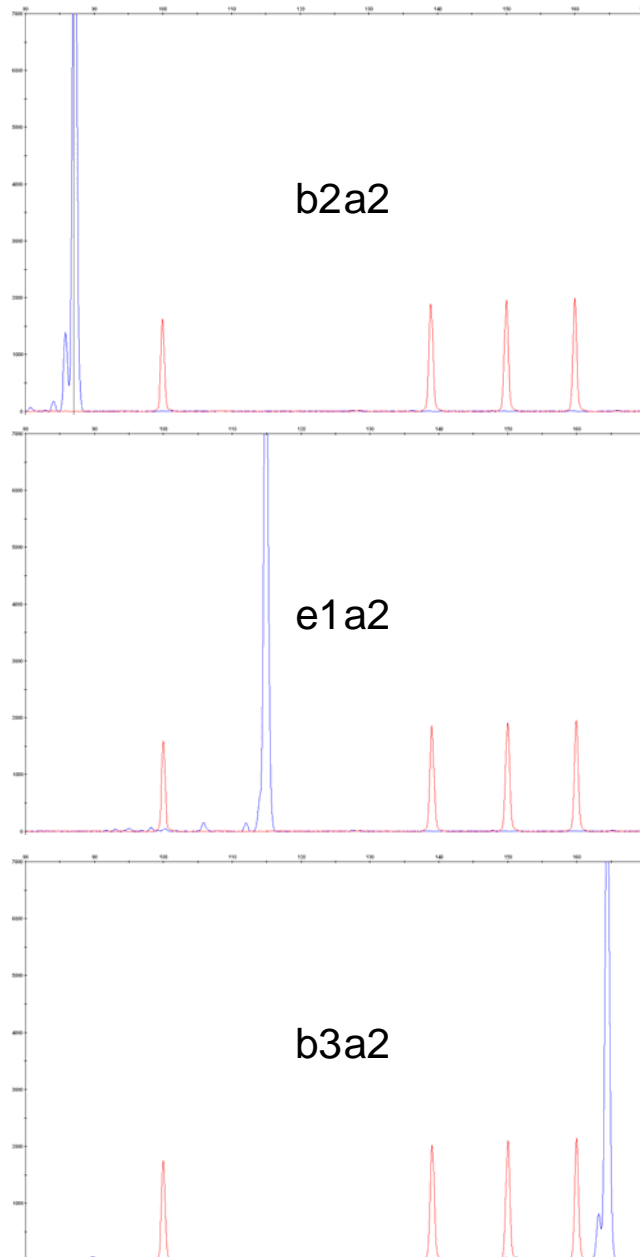


Figure 1: Representative examples with POP-4 separation matrix. Samples were prepared by diluting total RNA purified from cell lines expressing e1a2, b2a2, or b3a2 fusion transcripts in total RNA purified from a translocation negative cell line (HL-60). Following real-time PCR, the amplification products were analyzed on an ABI 3130xl Genetic Analyzer as described in Materials and Methods. Fusion transcript-specific peaks are shown in blue, size standard are shown in red.

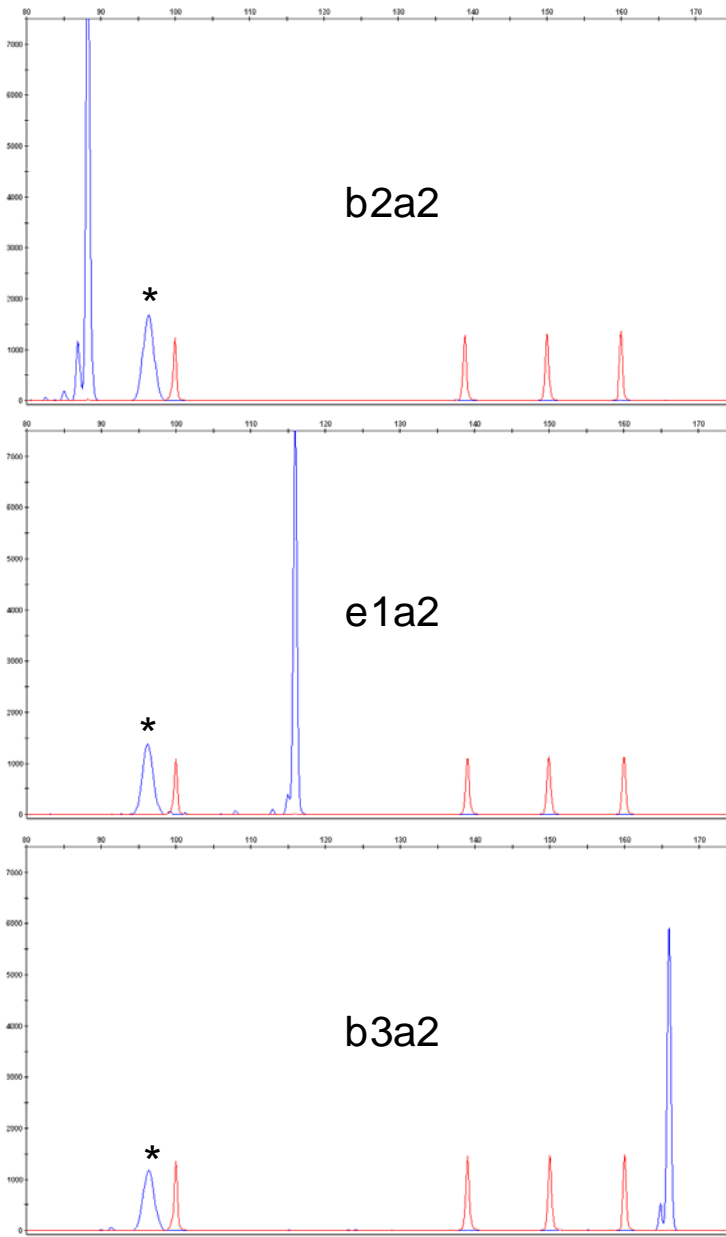


Figure 2: Representative examples with POP-7 separation matrix. The same PCR products as in Figure 1 were analyzed. * indicates a non-specific peak observed only when using POP-7 polymer.

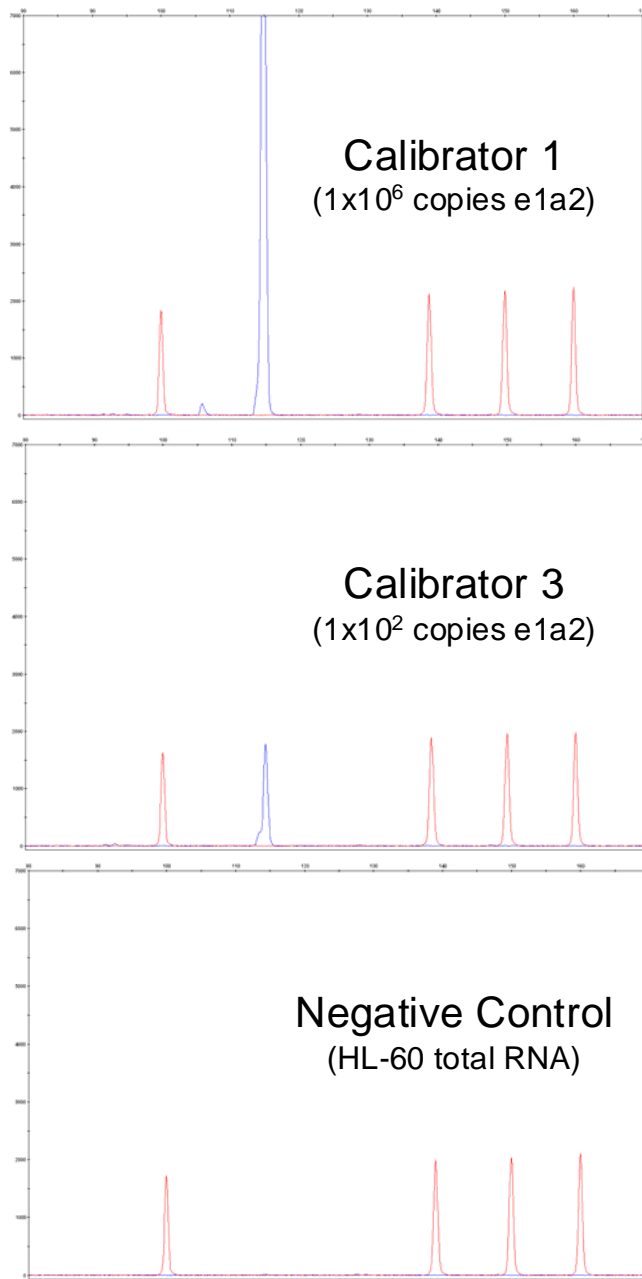


Figure 3: Representative examples with BCR/ABL1 Calibrators 1 and 3. The PCR products were analyzed with POP-4 separation matrix as described in Materials and Methods. The negative control consisted of total RNA purified from a translocation negative cell line (HL-60).