Quantifying Major BCR-ABL1 (e13a2, e14a2) Transcripts Using Optolane's Digital Realtime PCR System

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APPLICATION NOTE

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The presence of BCR-ABL1 or Philadelphia chromosome is associated with Chronic Myeloid Leukemia (CML). Over 90% of CML patients carries major forms (e13a2/b2a2 and e14a2/b3a2) of BCR-ABL1 fusion transcripts which has the fusion breakpoints in the exon 13 of BCR gene (Figure 1). Patients with BCR-ABL1 transcripts are preferably treated with targeted therapy i.e. tyrosine kinase inhibitors (TKIs) such as nilotinib. Thus, timely and highly sensitive detection of BCR-ABL1 transcripts is crucial for effective diagnosis, efficient treatments and tracking of recurrence in the CML patients. This application note demonstrates the use of Optolane's digital realtime PCR (Dr. PCR) system as an exciting new tool for rapid, ultra-sensitive BCR-ABL1 transcript analysis.

Optolane's Dr. PCR as a New Tool in BCR-ABL1 Analysis

The use of RT-qPCR to measure BCR-ABL1 fusion transcripts has limitations when there is low amount of template DNA or when there are in a high background of non-fusion DNA. Digital PCR is a newly developed technology that allows separation of individual target molecules into compartments for digital counting with qPCR primers and probes. Its rather simple workflow (e.g. 1-2 hours for LOAA system) also makes digital PCR a highly desirable for cancer diagnosis and minimum residual disease (MRD) monitoring. However, there are also concerns of false positive in the end-point PCR especially when copy number of targets are very low. Optolane's Dr. PCR systems uniquely provides powerful, highly accurate realtime measurement of transcripts with the final realtime amplification curves. The realtime curves allow easy discrimination of false positive from the real positive data points. Optolane's Dr. PCR also provides simplified workflow without the need of droplet generations (Figure 2).

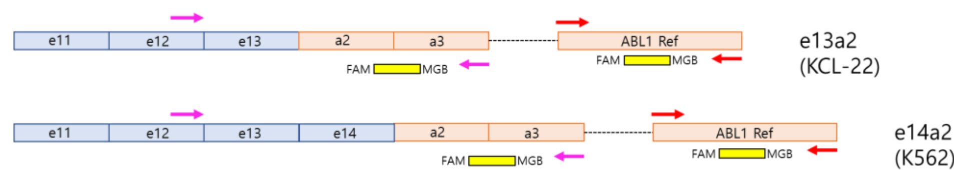

Figure1 Schematic of primers and probes for detection of BCR-ABL1 fusion transcripts (e13a2 and e14a2)

Measuring Major BCR-ABL1 Transcripts with Optolane's Dr. PCR System

Primers and TaqMan minor-groove binder (MGB^{TM}) probes were designed to specifically target major variants of BCR-ABL1 fusion gene (e13a2 and e14a2) and the non-fusion site of ABL1 gene (Figure 1). We used cDNA generated from leukemia cell lines known to carry each variant of major BCR-ABL1 fusion transcripts (K562 and KCL-22) and negative cell line (A549). Specificity of primers and TaqMan MGB probes were extensively tested with the conventional qPCR system (Data not shown).

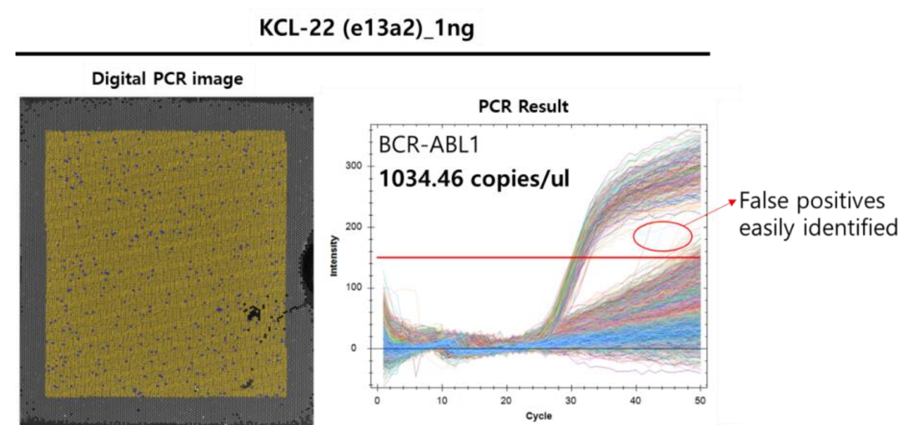

Figure3 Absolute quantification of BCR-ABL1 e13a2 transcripts using Optolane's Dr. PCR system

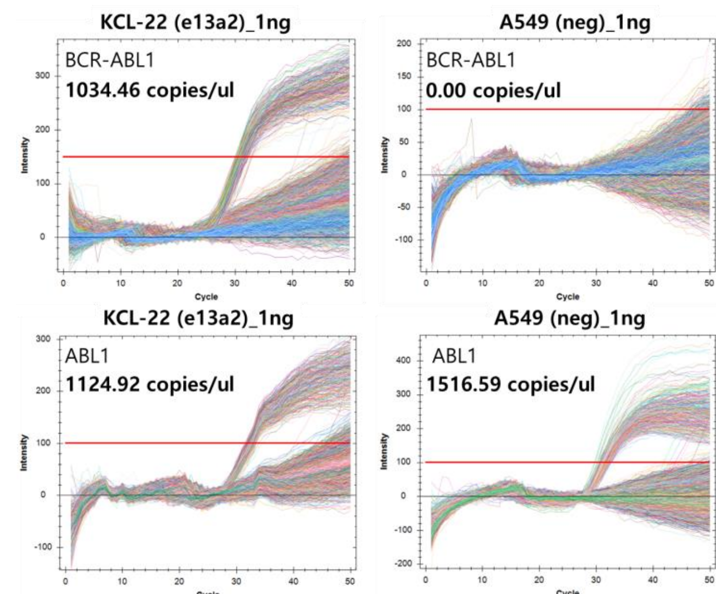

Figure4 Specificity of BCR-ABL1 transcripts detection using Optolane's Dr. PCR system

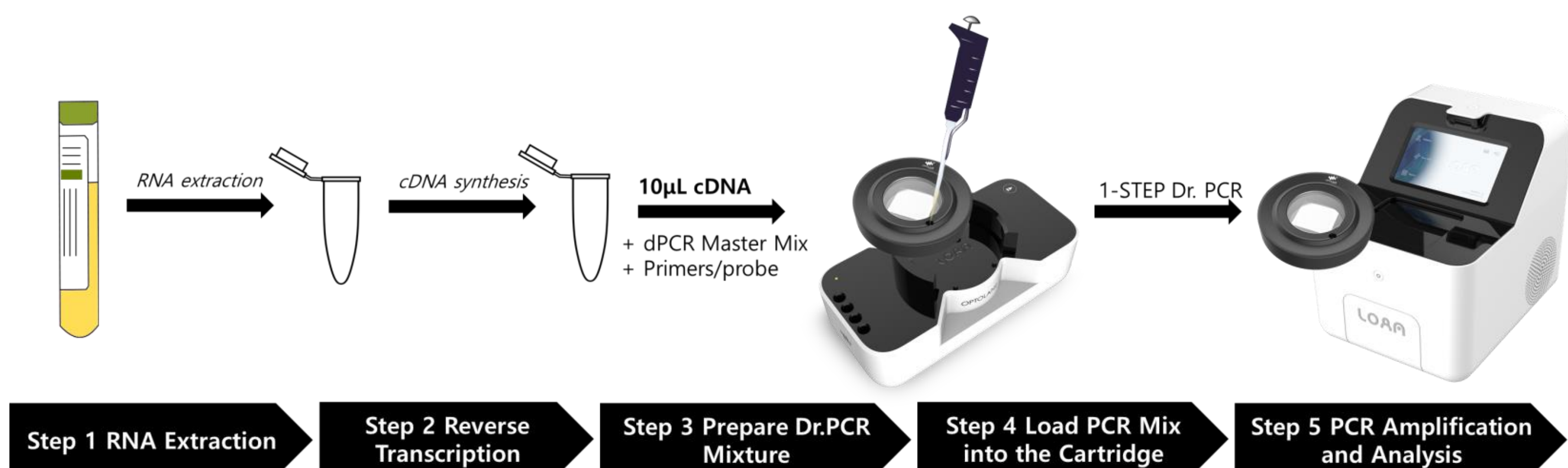

Figure2 Workflow of BCR-ABL1 transcript quantification using 2-step digital realtime PCR system by Optolane Technologies

Superior Accuracy with Visually Identifiable False Positives for Detecting Major BCR-ABL1 Transcripts

Figure 2 illustrates a simple workflow of Optolane's Dr. PCR system. Firstly, RNA was extracted from respective cancer cell lines using commercially available kits, such as NucleoSpin RNA (Machery Nagel). In this application note, 1µg of RNA was used for cDNA synthesis using High capacity cDNA reverse transcription kit with RNAse inhibitor (Applied Biosystems). cDNA was then diluted and loaded at 1ng equivalent of RNA per reaction. For each Dr. PCR reaction, cDNA was mixed with digital PCR master mix, 500nM of specific primers and 500nM TaqMan FAM/MGB probes designed as illustrated in figure1. For each reaction, 30µL of PCR mix was loaded onto Optolane's Dr. PCR cartridge and run using standard "hot-start' qPCR method (10 min at 95°C for hot start and 30sec at 95°C and 1min at 60°C for a total of 50 cycles). After amplification was completed, outliers and/or false positive wells were easily identified and excluded from calculation (Figure 3). Absolute BCR-ABL1 transcript number per microliter was automatically calculated using Poisson statistics by Optolane's Dr. PCR analysis software (Figure 4). As shown in figure 4, the designed primers and probed work effectively and specifically in detecting BCR-ABL1 in the positive cell line (KCL-22) and not in the negative cell (A549). To confirm that both KCL-22 (positive cells) and A549 (negative cells) express the non-fusion part of ABL1 gene, primers and probes specific for the ABL1 were used (Figure 4).

Ultra-sensitive Detection of Rare BCR-ABL1 Transcripts

To evaluate Optolane's Dr. PCR system performance in detecting various concentration of BCR-ABL1 transcripts. cDNA of BCR-ABL1 positive cells (KCL-22) were diluted in non-fusion cDNA over 6-fold concentrations to demonstrate the sensitivity of the Dr. PCR system. Figure 5 reveals detection of the fusion transcripts across the range of concentrations within the 95% confidence intervals (represented by dotted lines) with R^2 of 0.9838. The data are compared with the standard method of RT-qPCR which produces R^2 of 0.9885. Importantly, when plotting the correlation of our Dr.PCR and standard RT-qPCR, both datasets are highly correlated with R^2 of 0.9973. These results provide an evidence that Optolane's Dr. PCR system can be used to accurately determine the copy number of BCR-ABL1 transcripts and the percentage of BCR-ABL1 harboring cells (Figure 6)

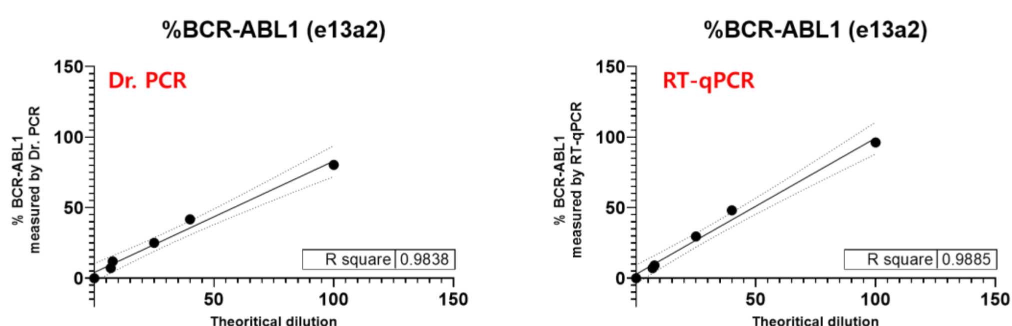

Figure 5 Detection of BCR-ABL1 transcripts across dynamic range of %International Standard (%BCR-ABL1 compared to ABL1 reference) using Optolane's Dr. PCR and traditional RT-qPCR systems

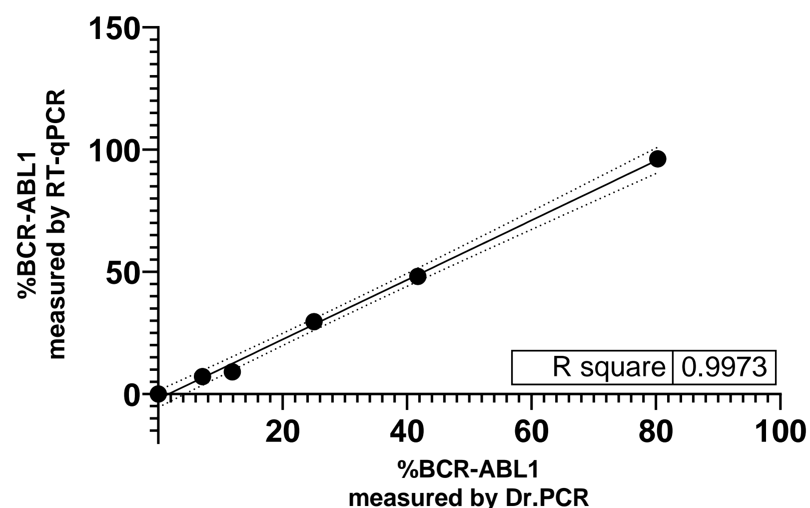

Figure 6 Linear regression of the Optolane's Dr. PCR vs traditional RT-qPCR systems using cell line cDNA samples.

Conclusion

- Optolane's Digital Realtime PCR (Dr. PCR) is a robust and versatile system to measure BCR-ABL1 fusion transcript
- Unlike other digital PCR systems, Optolane's Dr. PCR provides realtime curve for every individual well, allowing easy false positive identification
- Low level of BCR-ABL1 fusion transcripts can be detected in a high background of non-fusion DNA
- The percentage of BCR-ABL1 transcripts measured by both Optolane's Dr. PCR system and traditional RT-qPCR are highly correlated

Optolane's Dr. PCR system is for Research Use Only; not for the use in diagnostic procedure