LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

LLNL-TR-520096

Detection of BCR-ABL Fusion mRNA Using Reverse Transcriptase Loop-mediated Isothermal Amplification

L. C. Dugan, S. Hall, A. Kohlgruber, S. Urbin, C. Torres, P. Wilson

December 12, 2011

Disclaimer

This document was prepared as an account of work sponsored by an agency of the United States government. Neither the United States government nor Lawrence Livermore National Security, LLC, nor any of their employees makes any warranty, expressed or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States government or Lawrence Livermore National Security, LLC. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States government or Lawrence Livermore National Security, LLC, and shall not be used for advertising or product endorsement purposes.

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Detection of BCR-ABL Fusion mRNA Using Reverse Transcriptase Loop-mediated Isothermal Amplification

Lawrence C. Dugan[1*], Sally Hall[1], Ayano Kohlgruber[1], Salustra Urbin[1], Clinton Torres[2] and Paul Wilson[3]

LLNL-TR-520096

1. Biology and Biotechnology Division, Lawrence Livermore National Laboratory. P.O. Box 808. Livermore, CA, 94551
2. Computations Directorate, Lawrence Livermore National Laboratory. P.O. Box 808. Livermore, CA, 94551
3. Brookhaven National Laboratory, Upton, NY, 11973

*Please address correspondence to: Lawrence C. Dugan, Ph.D.

Pharmacology and Toxicology Group

Biology and Biotechnology Division

Lawrence Livermore National Laboratory

7000 East Avenue PO Box 808, L-174

Livermore, CA. 94551

Phone: (925) 423-5539

Fax: (925) 423-6437

E-mail: dugan3@llnl.gov

Abstract

RT-PCR is commonly used for the detection of *Bcr-Abl* fusion transcripts in patients diagnosed with chronic myelogenous leukemia, CML. Two fusion transcripts predominate in CML, *Br-Abl* e13a2 and e14a2. We have developed reverse transcriptase isothermal loop-mediated amplification (RT-LAMP) assays to detect these two fusion transcripts along with the normal *Bcr* transcript.

Introduction

The fusion of the breakpoint cluster region (*Bcr*) on chromosome 22q11 with the Abelson (*Abl*) proto-oncogene on chromosome 9q34 results in a reciprocal translocation known as the Philadelphia chromosome (Ph) (Nowell, 1960; Rowley, 1973). Several breakpoints have been identified in *Bcr* and the fusion of these different breakpoints to *Abl* results in chimeric proteins of different length. These chimeric proteins have elevated tyrosine kinase activity and are associated with distinct forms of leukemia. The most common *Bcr*-*Abl* fusions found in human leukemia involve fusion of *Bcr* exon 1 and *Abl* exon 2 (e1a2, p190), found in approximately 70% of acute lymphocytic leukemia (ALL), *Bcr* exon 13 and *Abl* exon 2 (e13a2, p210) and *Bcr* exon 14 and *Abl* exon 2 (e14a2, p210). Combined, e13a2 and e14a2 have been found in greater than 95% of chronic myelogenous leukemia (CML) and 25% of ALL (Burmeister, 2008). The *BCR* and *Abl* breakpoint regions and fusion transcripts are shown in **Figure 1** taken from Burmeister and Reinhardt (Burmeister, 2008).

Cytogenetic studies can be performed on bone marrow of patients diagnosed with CML to detect the Philadelphia chromosome. Additionally, molecular diagnostic tests for detection of *Bcr*-*Abl* fusion transcripts, including real-time revere transcriptase polymerase chain reaction (rt RT-PCR) have been developed (Tefferi, 2005). Laboratory use of these molecular tests has led to efforts to develop standardized tests to ensure quality control and reduce lab-to-lab variability (Gabert, 2003). Recently, researchers

have developed multiplex PCR assays for detection of the most common *Bcr-Abl* fusion transcripts along with minor variants that have been identified (Burmeister, 2008).

Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification technique that was first described by Notomi and coworkers in 2000. This technique originally utilized four primers targeting six distinct sequences on the template nucleic acid. The addition of reverse transcriptase into the reaction, termed reverse-transcription LAMP or RT-LAMP, allows for the detection of RNA templates under the same conditions (Notomi, 2000). The addition of loop primers was later shown to increase the rate of the reaction and reduced amplification times significantly (Nagamine, 2002). The utility of LAMP and RT-LAMP has been reported for numerous human and animal bacterial, protozoan and viral pathogens including: *Salmonella enterica* (Ohtsuka, 2005), African trypanosomes (Kuboki, 2003) and Foot and Mouth disease virus (Dukes, 2006), amongst many others. Several recent reports demonstrate the feasibility of using RT-LAMP in the detection of metastatic gastric (Horibe, 2007; Muto, 2011), lung (Maeda, 2009) and breast (Visser, 2008) cancer.

We have developed real-time RT-LAMP assays for the detection of *Bcr* mRNA along with e13a2 and e14a2 *Bcr-Abl* fusion transcripts. Assays were tested on a synthetic control template consisting of the *Bcr* e13 and e14 exons and *Abl* a2 and a3 exons. Assays were then down-selected based on these results and tested on RNA from apparently normal human fibroblasts and CML cell lines.

Materials and Methods

Cell lines and cell culture

Cell lines used in this work are listed in **Table 1**. All cell lines, except Kasumi-4, were maintained at 37 °C with 5% CO_2 in RPMI media (Gibco, BRL Life Technologies, Grand Island, NY) supplemented with 15% fetal bovine serum (Gibco), GlutaMAX-I supplement, Penicillin/Streptomycin, Vitamins solution, NEAA solution, amino acid solution and NaOH. Kasumi-4 cells were cultured in the above media plus 10 ng/ml GM-CSF, 10 mM HEPES, 1 mM sodium pyruvate and 20% fetal bovine serum.

Primer design

LAMP signatures were designed with the LAVA software (Torres, 2011), based on the sequences of the typical and atypical BCR-ABL fusion transcripts. The specific fusion transcripts which are targeted are described in Burmeister et al. (Burmeister, 2008). Whenever possible, multiple transcript sequences were used to create a consensus sequence for signature design, which helps increase assay sensitivity. The goal with each individual signature candidate design was to achieve exclusive amplification of only one fusion transcript. In some cases, such as with the e13 variants, exclusive amplification doesn't appear to be possible with every primer region combination because primers targeting e13 variants are likely to detect e14 variants as well. To help minimize problems like this, of the possible signature candidates for a transcript, candidates were preferred which had primers that lied across, or as near to, the fusion site as possible. Primers used in this work are listed in **Table 2** (RT-PCR) and **Table 3** (RT-LAMP).

Loop-mediated isothermal amplification

HPLC-purified primers were obtained from Biosearch Technologies (Biosearch Technologies, Novato, CA). LAMP reactions contained 1.6 μM each FIP and BIP primers, 0.2 μM each F3 and B3 primers, 0.8 μM each LF and LB primers, 320 nM Picogreen dye (Invitrogen), 1x ThermoPol reaction buffer (NEB, Ipswich, MA), 1.4 mM each dNTP (NEB), 0.8 M Betaine (Sigma), 8 mM $MgSO_4$, 8 units *Bst* polymerase large fragment (NEB) and 5 μl sample brought up in a 25 μl final volume with nuclease-free water (NEB). Reverse transcriptase-LAMP (RT-LAMP) reactions contained the same reagents plus 1 unit ThermoScript reverse transcriptase (Invitrogen). Reactions were amplified on either a MyIQ or CFX96 real-time instrument (BioRad, Hercules, CA) with constant heating to 63 °C and fluorescence detection measurements taken every minute for 100 min.

PCR

Primers were obtained from Biosearch Technologies. PCR reactions contained 0.4 μM each forward and reverse primers, 1x Taq PCR buffer (Invitrogen), 320 nM Picogreen dye (Invitrogen), 0.2 mM each dNTP (NEB), 3.5 mM $MgCl_2$, 1.25 units Platinum *Taq* polymerase (Invitrogen) and 5 μl sample brought up in a 25 μl final volume with nuclease-free water (NEB). Reactions were amplified on a MyIQ real-time instrument (BioRad) using a heating profile of 95 °C for 5 min followed by 40 cycles of 95 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min, followed by a 3 min extension at 72 °C. Fluorescence detection measurements were taken every cycle.

RNA generated from synthetic template

A synthetic expression template comprising *BCR* exons 13 and 14 and *Abl* exons 2 and 3 from NCBI sequence AJ131466 was generated (Genscript, Piscataway, NJ). The nucleotide sequence was cloned into pUC57 and pGS21a plasmids for DNA and RNA template, respectively. RNA was generated from the pGS21a construct using T7 polymerase amplification kit (BioLine, Tauton, MA) using manufacturer's instructions. Resulting RNA was treated with DNase I to degrade pGS21a construct to prevent false amplification of DNA template instead of RNA template. RNA was then aliquoted and stored at -80 °C until needed.

Results and Discussion

Detection of wild-type *BCR*

A LAMP primer signature, bcrw_8, targeting the *BCR* mRNA transcript was designed and tested against total RNA extracted from several Ph^+ and Ph^- cell lines. All of the cell lines tested should be positive for *BCR*. Results shown in **Figure 2** indicate that signature bcrw_8 can detect wild-type *BCR* mRNA transcripts. Average threshold times (Tt), the time at which the fluorescence signal becomes significantly greater than the background signal, ranged from <25 min to >50 min, with no false positive reactions. This variation in average Tt existed between cell lines and RNA isolations, suggesting either sequence variation exists within the *BCR* coding regions or there was inconsistently generated RNA template available for these experiments.

Detection of *BCR-Abl* fusion transcripts

LAMP primer signatures targeting the e1a2 *BCR-Abl* mRNA fusion transcript failed to successfully amplify total RNA from the e1a3$^+$ cell line SUP-B1, therefore work on this assay was suspended. Two LAMP primer signatures targeting the e13a2 BCR-Abl mRNA fusion transcript were then tested. Signature e13a3_16 successfully amplified target mRNA in e13a2$^+$ cell lines MEG-01 and Kasumi-4 along with e14a2$^+$ cell lines KU-812 and K-562. As shown in Figure 1, these two e14a2$^+$ cell lines contain *BCR-Abl* mRNA fusion transcript that include *BCR* exon 13 and therefore should be positive in reactions targeting e13a2 fusions. Signature e13a3_7 failed to amplify target mRNA in e13a2$^+$ cell lines MEG-01 and Kasumi-4 along with e14a2$^+$ cell lines KU-812 and K-562 and was removed from future studies. Two LAMP primer signatures targeting the e14a2

BCR-Abl mRNA fusion transcript were then tested. Signature e14a3_124 and e14a3_201 both failed to amplify target mRNA in e14a2^{+} cell lines KU-812 and K-562.

PCR detection of *BCR-Abl* mRNA fusion transcripts

Due to the sub-par performance of the LAMP assays on extracted RNA, we generated cDNA from the cell line-derived RNA. Previously published PCR primers (Burmeister, *Leukemia Research* 2008;32:579, see Table 2) were then tested on these cDNAs. Results of these experiments are shown in **Figure 3 A-E**. PCR primers BCR1/ABL3 should detect e1a2 fusion transcripts and successfully detected them in the e1a2^{+} cell line SUPB15, with no false positives in control cell lines TK6 and Nalm-6 (Figure 3 A). PCR with these primers should be negative in e13a2^{+} cell line MEG01 and e14a2^{+} cell lines KU812 and K562 due to very large product size. PCR primers BCR6/ABL3 should detect e13a2 and e14a2 fusion transcripts and successfully detected them in the e13a2^{+} cell line MEG01 and e14a2^{+} cell lines KU812 and K562, with no false positives in control cell lines TK6 and Nalm-6 or the e1a2^{+} cell line SUPB15 (Figure 3 B). PCR primers BCR12/ABL3 should detect e13a2 and e14a2 fusion transcripts and successfully detected them in the e13a2^{+} cell line MEG01 and e14a2^{+} cell lines KU812 and K562, with no false positives in control cell lines TK6 and Nalm-6 (Figure 3 C). However, an unexpected secondary band was found in all three replicates of SUPB15 cDNA amplified with this primer set. PCR primers BCR19/ABL3 should not detect e1a2, e13a2 or e14a2 fusion transcripts and was negative in all cell lines tested (Figure 3 D). Finally, PCR primers BCR12/BCR-R should detect wild-type BCR transcripts and successfully detected them in in control cell lines TK6 and Nalm-6 (Figure 3 E). Positive reactions in the e1a2^{+} cell

line SUPB15 and the e14a2$^+$ cell line K562 suggest that these aneuploid cell lines contain at least one copy of the wild-type *BCR* gene.

Additional published PCR primers (Gabert *Leukemia* 2003;17:2318, see Table 2) were then tested. Wild-type Abl transcripts were successfully detected using ENF1003/ENR1063 in cDNA generated from RNA extracted from Ph$^-$ control cell lines and Ph$^+$ cell lines. Primer set ENF402/ENR561 successfully detected e1a2 transcripts in the e1a2$^+$ cell line SUPB15, but produced some false positive reactions in the control cell line Nalm-6 and the e14a2$^+$ cell line K562. Primer set ENF501/ENR561 successfully detected e14a2 transcripts in the e14a2$^+$ cell lines K562 and Ku812, without false positives.

Development of a synthetic target for LAMP primer development

Due to the sub-par performance of the LAMP assays on extracted RNA and the results of PCR amplification on cDNA generated from the extracted RNA, a synthetic template comprised of the *BCR* exons 13 and 14 and *Abl* exons 2 and 3 from NCBI sequence information, AJ131466, was generated by Genscript (Piscataway, NJ). The 661 nucleotide sequence was inserted into pUC57 and pGS21a plasmids for DNA and RNA template, respectively. **Figure 4** shows a nucleotide alignment map of this insert against accession number AJ131466, the *BCR*-*Abl* e14a2 fusion transcript. This synthetic target allows us to test PCR primers for wild-type *Abl* along with PCR and LAMP primers for the two most common CML fusions, e13a2 and e14a2.

Conclusions

We designed and tested loop-mediated isothermal amplification primer signatures to detect the presence of wild-type BCR, BCR-Abl e13a2 and BCR-Abl e14a2 transcripts in Ph^+ cell lines. Our results indicate that we can detect BCR-Abl e13a2 and BCR-Abl e14a2 transcripts, however, significant work still needs to be performed in order to fully understand the properties of these signatures.

Acknowledgments

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344 with support from the LLNL Laboratory Directed Research and Development program.

References

Burmeister T., and R. Reinhardt, 2008, A multiplex PCR for improved detection of typical and atypical BCR-ABL fusion transcripts. *Leukemia Research*, **32**:579

Torres C., Vitalis A., Baker R., Gardner N., Torres W., and J. Dzenitis, 2011, LAVA: An Open-Source Approach To Designing LAMP (Loop-Mediated Isothermal Amplification) DNA Signatures. *BMC Bioinformatics*, **12**:240

J.D. Rowley, 1973, A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and giemsa staining. *Nature*, **243**:290

Nowell, P. and D. Hungerford, 1960, A minute chromosome in human chronic graulocytic leukemia. *Science*, **132**:1479

Tefferi, A., Dewald, G.W., Litzow, M.L., et al., 2005, Chronic myelogenous leukemia: Current applications of cytogenetics and molecular testing for diagnosis and treatment. *Mayo Clinic Proceedings*, **80**(3):390

Gabert, J. Beillard, E., van der Velden, V.H.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

Notomi, T., H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino, and T. Hase. 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, **28**:E63

Nagamine, K., T. Hase, and T. Notomi. 2002. Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Molecular and Cellular Probes*, **16**:223

Ohtsuka, K., K. Yanagawa, K. Takatori, and Y. Hara-Kudo. 2005. Detection of *Salmonella enterica* in naturally contaminated liquid eggs by loop-mediated isothermal amplification, and characterization of *Salmonella* isolates. *Applied Environmental Microbiology*, **71**:6730

Kuboki, N. N. Inoue, T. Sakurai, F. Di Cello, D.J. Grab, H. Suzuki, C. Sugimoto, and I. Igarashi. Loop-mediated isothermal amplification for detection of African trypanosomes. *Journal of Clinical Microbiology*, **41**:5517

Dukes, J.P., D.P. King, and S. Alexanderson. 2006. Novel reverse transcription loop-mediated isothermal amplification for rapid detection of foot-and-mouth disease virus. *Archives of Virology*, **151**:1093

Horibe, D., Ochiai, T., Shimada, H., et al., 2006. Rapid detection of metastasis of gastric cancer using reverse transcription loop-mediated isothermal amplification. *International Journal of Cancer*, **120**:1063

Muto, Y., Matubara, H., Tanizawa, T., et al., 2011. Rapid diagnosis of micrometastasis of gastric cancer using reverse transcription loop-mediated isothermal amplification. *Oncology Reports*, **26**:789

Maeda, J., Inoue, M., Nakabayashi, K., et al., 2009. Rapid diagnosis of lymph node metastasis in lung cancer with loop-mediated isothermal amplification using carcinoembryonic antigen-mRNA. *Lung Cancer*, **65**:324

Visser, M. Jiwa, M., Hortsman, A., et al., 2008. Intra-operative rapid diagnostic method based on CK19 mRNA expression for the detection of lymph node metastases in breast cancer. *International Journal of Cancer*, **122**:2562

Figures

Figure 1. Burmeister, T and Reinhardt, R, *Leukemia Research* 2008;32:579-585.

BCR and *Abl* breakpoint regions and resulting fusion transcripts.

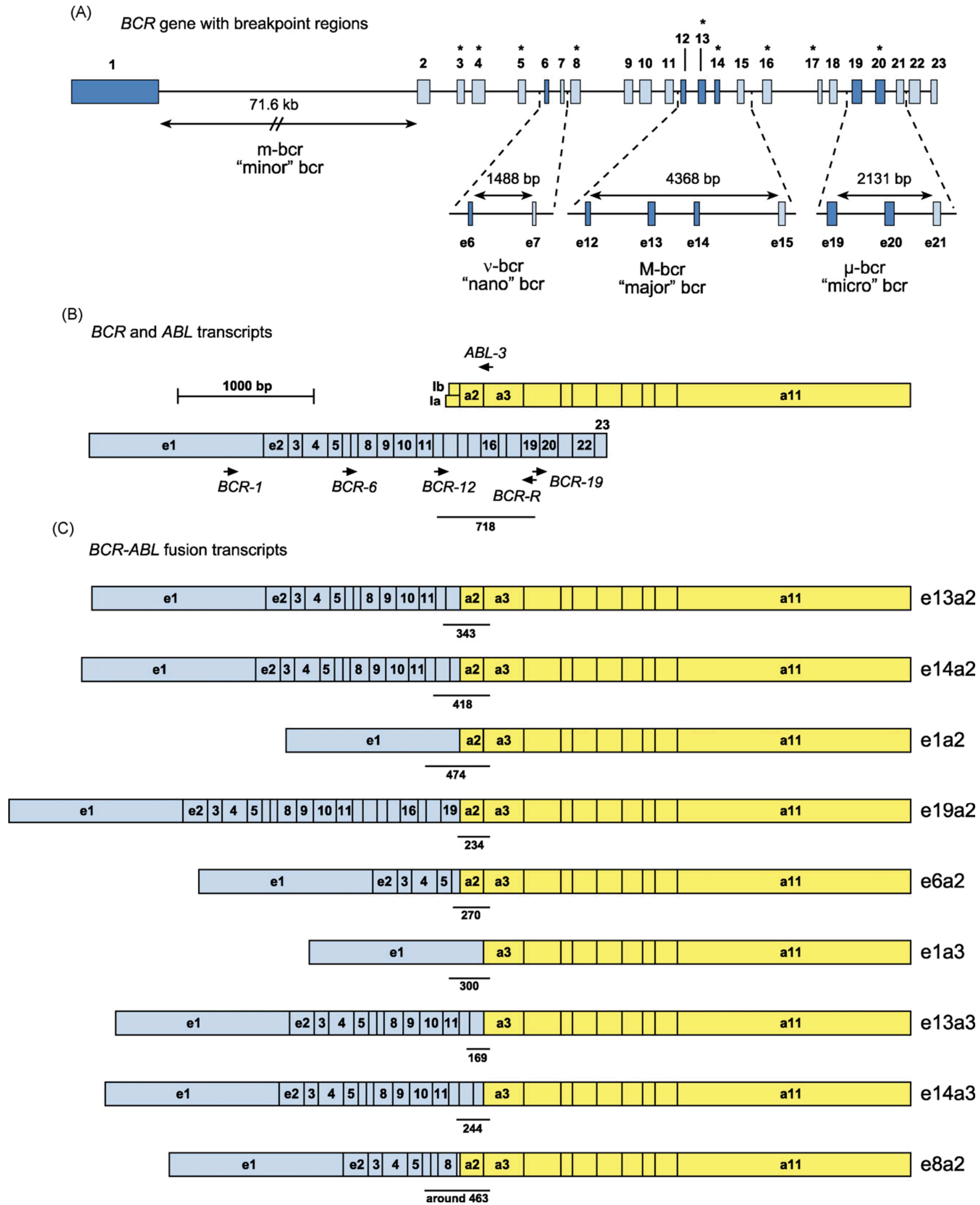


Figure 2. Detection of *BCR* using RT-LAMP. mRNA isolated from listed cell lines was amplified using LAMP primer set bcrw_8 targeting the *BCR* of chromosome 22. Dates shown in legend are dates of RNA extraction. Dates shown on X-axis are dates RT-LAMP reactions were performed. Values are averages of three wells per sample. Error bars are +/- one standard deviation.

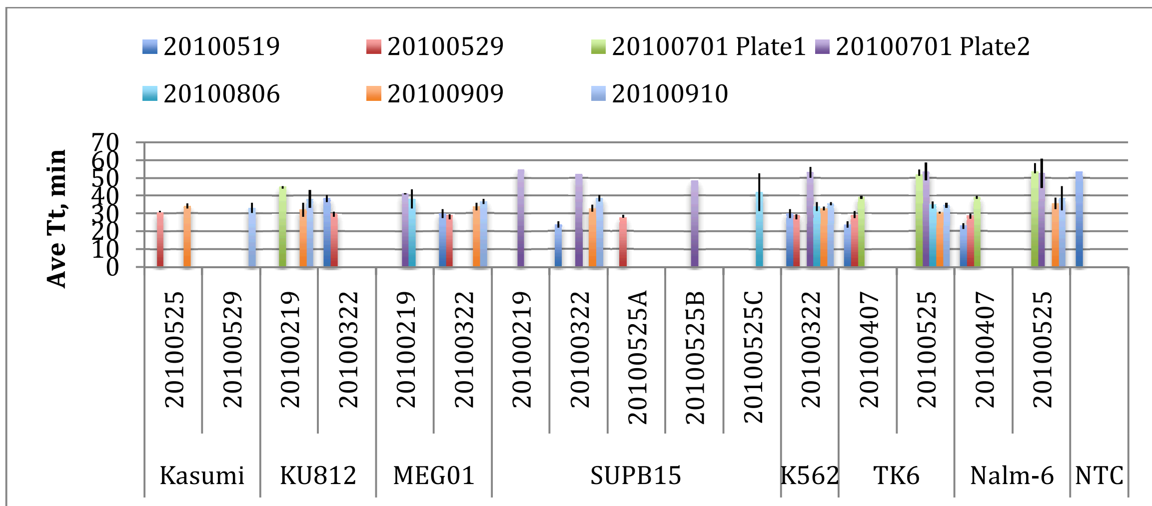
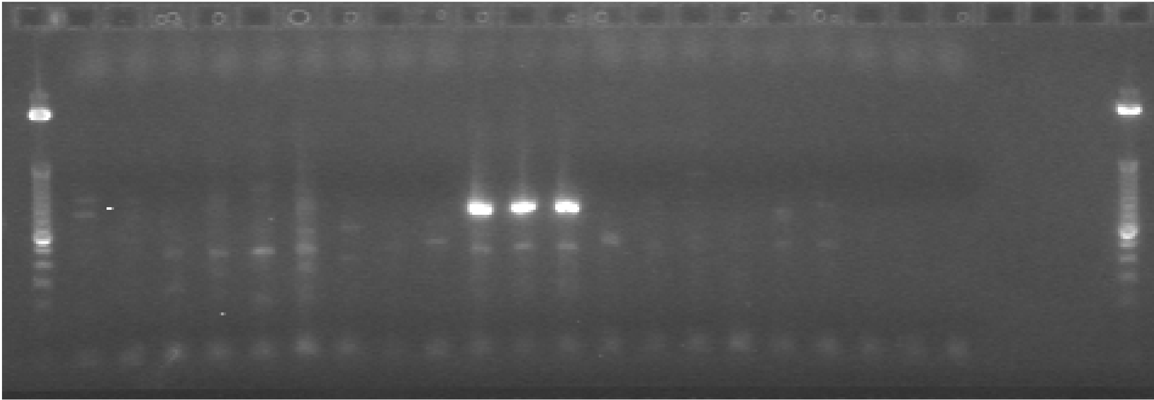


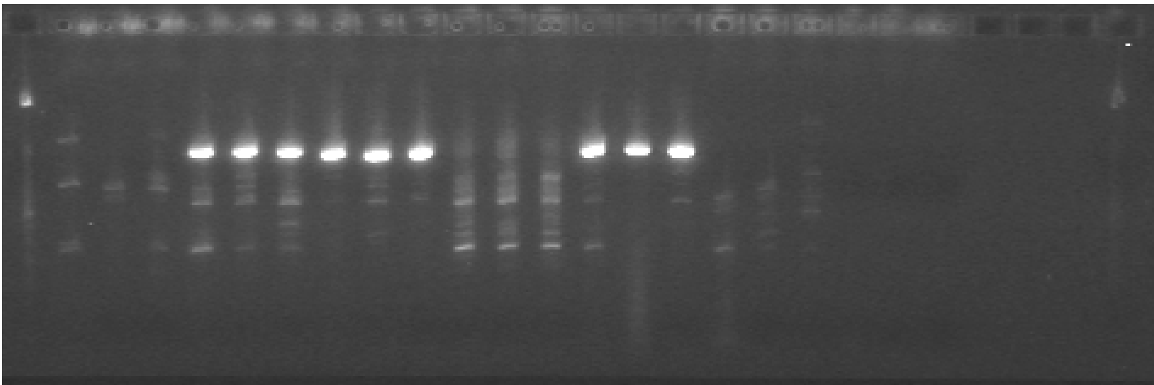
Figure 3. PCR Amplification of cDNA from Ph⁺ and Ph⁻ cell lines.

A. BCR1/ABL3

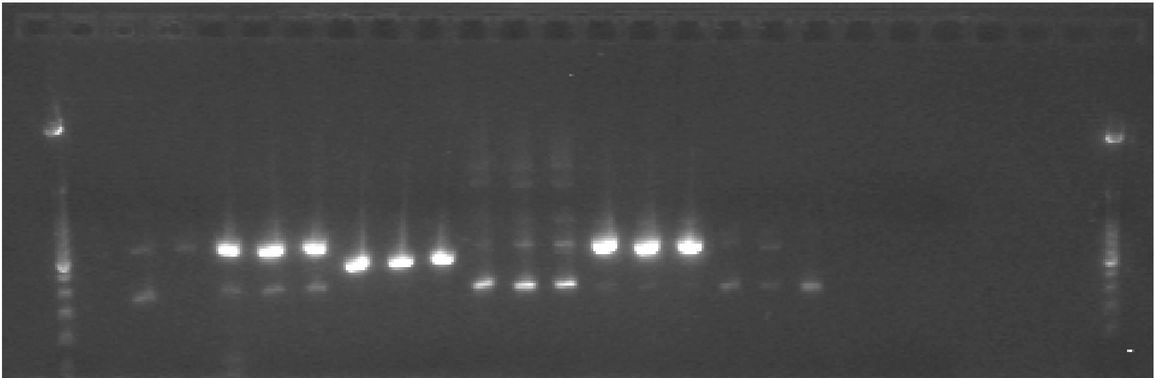
M TK6 KU812 MEG01 SUPB15 K562 Nalm-6 NTC M



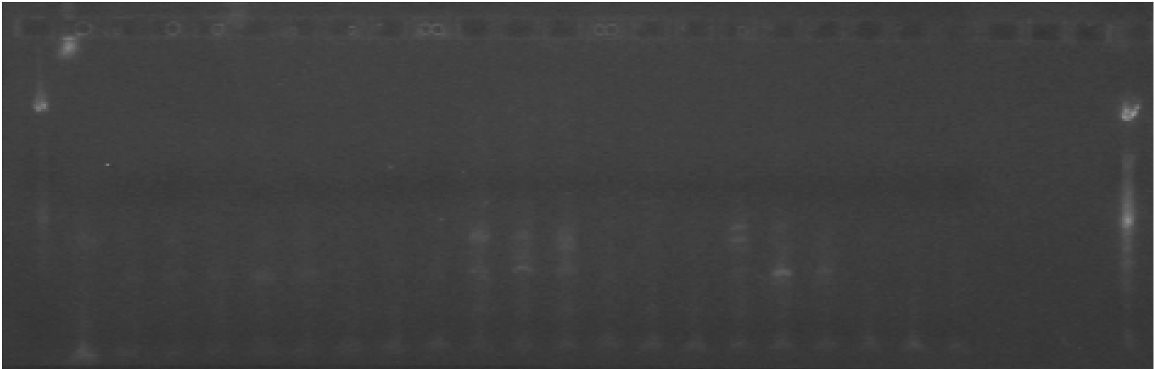
B. BCR6/ABL3



C. BCR12/ABL3



D. BCR19/ABL3



E. BCR12/BCR-R

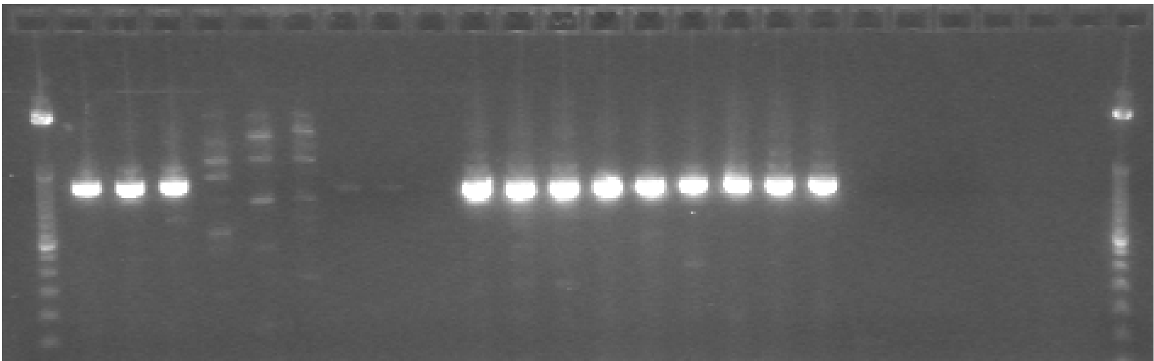
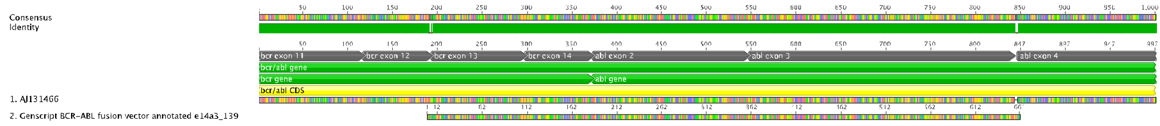


Figure 4. Synthetic target template representing the *BCR-Abl* e13a2 fusion transcript.



Tables

Table 1. Cell lines used in this study.

Cell Line	Cell Type	Patient Age/Sex	Ploidy	No. of Ph chr.	*BCR-Abl* fusion
K-562 (CCL-243)	Erythrocytic	53 F	Triploid	0	e14-a2
Kasumi-4 (CRL-2726)	Myelocytic	6 F	Diploid	1	e13-a2
KU-812 (CRL-2099)	Myelocytic	38 M	Triploid	2	e14-a2
MEG-01 (CRL-2021)	Megakaryocytic	55 M	Diploid	1	e13-a2
SUP-B15 (CRL-1929)	B-cell precursor	9 M	Diploid	1	e1-a2
SD-1 (DSMZ ACC 366)	B lymphoblastoid cell (ALL)	F	near-tetraploid	?	e1-a2
TOM-1 (DSMZ ACC 578)	B-cell precursor	54 F	hyperdiploid, 7% polyploidy	?	e1-a2
IM9 (GM04680)	B-lymphocyte	F	Tetraploid	0	-
MOLT-4 (CRL-1582)	Lymphoblastoid	19 M	Hypertetraploid	0	-
NALM-6 (DSMZ ACC 128)	B-cell ALL	19 M	Diploid	0	-
TK-6 (CLR-8015)	T-cell Lymphoblastoid	5 M	Diploid	0	-

Table 2. PCR primers used in this study.

Primer	Sequence, 5'-3'	Gene Target or location	Ref.
ENF1003	TGGAGATAACACTCTAAGCATAACTAAAGGT	*ABL*	Gabert
ENR1063	GATGTAGTTGCTTGGGACCCA	*ABL*	Gabert
			Gabert
ENF402	CTGGCCCAACGATGGCGA	e1a2	Gabert
ENR561	CACTCAGACCCTGAGGCTCAA	e1a2, e13a2, e14a2	Gabert
ENF501	TCCGCTGACCATCAATAAGGA	e13a2, e14a2	Gabert
ABL-3	CCATTGTGATTATAGCCTAAGACCCGGAG	*ABL* exon 3	Burmeister
BCR-1	CTCCAGCGAGGAGGACTTCTCCT	*BCR* exon 1	Burmeister
BCR-6	CCTGAGAGCCAGAAGCAACAAAGATGCC	*BCR* exon 6	Burmeister
BCR-12	AGAACATCCGGGAGCAGCAGAAGAA	*BCR* exon 12	Burmeister
BCR-19	ACTGAAGGCAGCCTTCGACGTC	*BCR* exon 19	Burmeister
BCR-R	ATGTCCGTGGCCACACCGGACAC	*BCR* exon 19	Burmeister

Table 3. RT-LAMP primers used in this study.

A. ***BCR*** **target**

Identifier	Primer
bcrw_8_F3	GCCTGGCTTTCTCTTGGTAA
bcrw_8_B3	CTGATTGAGCACTGAGGCCT
bcrw_8_FIP	TTTTAATGAAAGTGTCGGCCACGCTGGTGTCTTAGCAGCTGCA
bcrw_8_BIP	ACTTGCGTGCATGTTGACTTCATTATCACTGCACCTGCTGCTT
bcrw_8_FL	GGTGTGTAGGTGGCTGAGCT
bcrw_8_BL	TGGGAAGAACCCCTGAGC

B1. ***BCR-Abl*** **e13a2 fusion target, initial**

Identifier	Primer
e13a3_16_F3	CCTGACATCCGTGGAGCT
e13a3_16_B3	TTTCTCCAGACTGTTGGCTG
e13a3_16_FIP	GGAGCTTTTCACCTTCCTTGTTGGATGCTGACCAACTCGTGTG
e13a3_16_BIP	TAATCACAATGGGGAATGGTGTGCGTGATGTAGTTGCTTGGGA
e13a3_16_FL	TGCTGTGGACAGTCTGGAGT
e13a3_16_BL	AAACCAAAAATGGCCAAGG
e13a3_7_F3	TGGAGCTGCAGATGCTGA
e13a3_7_B3	TTTCTCCAGACTGTTGGCTG
e13a3_7_FIP	CTAAGACCCGGAGCTTTTCACCTCAACTCGTGTGTGAAACTCCA
e13a3_7_BIP	TAATCACAATGGGGAATGGTGTGCGTGATGTAGTTGCTTGGGA
e13a3_7_FL	TGTTGATGGTCAGCGGAAT
e13a3_7_BL	AAACCAAAAATGGCCAAGG
e13a3_38_F3	GATGCTGACCAACTCGTGTG
e13a3_38_B3	GGACACAGGCCCATGGTA
e13a3_38_FIP	TGATTATAGCCTAAGACCCGGAGCACTCCAGACTGTCCACAGCA
e13a3_38_BIP	GTGTGAAGCCCAAACCAAAAATGTTTCTCCAGACTGTTGGCTG
e13a3_38_FL	TCACCTTCCTTGTTGATGGTC
e13a3_38_BL	TCCCAAGCAACTACATCACG
e13a3_39_F3	TGTGTGAAACTCCAGACTGTCC
e13a3_39_B3	GGACACAGGCCCATGGTA
e13a3_39_FIP	CACACCATTCCCCATTGTGATTAATTCCGCTGACCATCAACA
e13a3_39_BIP	AAACCAAAAATGGCCAAGGCTAGGAGTGTTTCTCCAGACTGTTG
e13a3_39_FL	CCTAAGACCCGGAGCTTTTC
e13a3_39_BL	TCCCAAGCAACTACATCACG

B2. ***BCR-Abl*** **e13a2 fusion target, modified**

Identifier	Primer
e13a3_16_F3	CCTGACATCCGTGGAGCT
e13a3_16_B3_MOD	TTTCTCCAGACTGTTG**A**CTG
e13a3_16_FIP_MOD	GGAGCTTTTCACCTTT**A**GTT**A**GATGCTGACCAACTCGTGTG
e13a3_16_BIP	TAATCACAATGGGGAATGGTGTGCGTGATGTAGTTGCTTGGGA
e13a3_16_FL	TGCTGTGGACAGTCTGGAGT
e13a3_16_BL	AAACCAAAAATGGCCAAGG
e13a3_7_F3	TGGAGCTGCAGATGCTGA
e13a3_7_B3_MOD	TTTCTCCAGACTGTTG**A**CTG
e13a3_7_FIP	CTAAGACCCGGAGCTTTTCACCTCAACTCGTGTGTGAAACTCCA
e13a3_7_BIP	TAATCACAATGGGGAATGGTGTGCGTGATGTAGTTGCTTGGGA
e13a3_7_FL_MOD	T**A**TTGATGGTCAGCGGAAT
e13a3_7_BL	AAACCAAAAATGGCCAAGG
e13a3_38_F3	GATGCTGACCAACTCGTGTG
e13a3_38_B3	GGACACAGGCCCATGGTA
e13a3_38_FIP	TGATTATAGCCTAAGACCCGGAGCACTCCAGACTGTCCACAGCA
e13a3_38_BIP MOD	GTGTGAAGCCCAAACCAAAAATGTTTCTCCAGACTGTTG**A**CTG
e13a3_38_FL MOD	TCA**T**CTTCCTT**A**TTGATGGTC
e13a3_38_BL	TCCCAAGCAACTACATCACG
e13a3_39_F3	TGTGTGAAACTCCAGACTGTCC
e13a3_39_B3	GGACACAGGCCCATGGTA
e13a3_39_FIP MOD	CACACCATTCCCCATTGTGATTAATTCCGCTGACCATCAA**T**A
e13a3_39_BIP	AAACCAAAAATGGCCAAGGCTAGGAGTGTTTCTCCAGACTGTTG
e13a3_39_FL	CCTAAGACCCGGAGCTTTTC
e13a3_39_BL	TCCCAAGCAACTACATCACG

C1. *BCR-Abl* e14a2 fusion target, initial

Identifier	Primer
e14a3_124_F3	GGGCTCTATGGGTTTCTGAA
e14a3_124_B3	GGACACAGGCCCATGGTA
e14a3_124_FIP	CATTCCCCATTGTGATTATAGCCTGTCCACTCAGCCACTGGATT
e14a3_124_BIP	AAACCAAAAATGGCCAAGGCTAGGAGTGTTTCTCCAGACTGTTG
e14a3_124_FL	CCGGAGCTTTTCACTTGAAC
e14a3_124_BL	TCCCAAGCAACTACATCACG
e14a3_201_F3	ATGAGTCTCCGGGGCTCTAT
e14a3_201_B3	GGACACAGGCCCATGGTA
e14a3_201_FIP	CCCATTGTGATTATAGCCTAAGACCCTGAATGTCATCGTCCACTCAG
e14a3_201_BIP	GTGTGAAGCCCAAACCAAAAATGTTCTCCAGACTGTTGACTGGC
e14a3_201_FL	CACTTGAACTCTGCTTAAATCCA
e14a3_201_BL	CTGGGTCCCAAGCAACTACA
e14a3_139_F3	TGGGTTTCTGAATGTCATCG
e14a3_139_B3	AGCAGATACTCAGCGGCATT
e14a3_139_FIP	CACACCATTCCCCATTGTGATTACAGCCACTGGATTTAAGCAGA
e14a3_139_BIP	AAAATGGCCAAGGCTGGGTCCCCATGGTACCAGGAGTGTT
e14a3_139_FL	CCTAAGACCCGGAGCTTTTC
e14a3_139_BL	CATCACGCCAGTCAACAGTC
e14a3_11_F3	GAAGATGATGAGTCTCCGGG
e14a3_11_B3	ACCAGGAGTGTTTCTCCAGACT
e14a3_11_FIP	TAAGACCCGGAGCTTTTCACTTGTGGGTTTCTGAATGTCATCG
e14a3_11_BIP	TAATCACAATGGGGAATGGTGTGCGTGATGTAGTTGCTTGGGA
e14a3_11_FL	TGCTTAAATCCAGTGGCTGA
e14a3_11_BL	AAACCAAAAATGGCCAAGG

C2. *BCR-Abl* e14a2 fusion target, modified

Identifier	**Primer**
e14a3_124_F3	GGGCTCTATGGGTTTCTGAA
e14a3_124_B3	GGACACAGGCCCATGGTA
e14a3_124_FIP	CATTCCCCATTGTGATTATAGCCTGTCCACTCAGCCACTGGATT
e14a3_124_BIP	AAACCAAAAATGGCCAAGGCTAGGAGTGTTTCTCCAGACTGTTG
e14a3_124_FL_MOD	CCGGAGCTTTTCAC**C**T**TT**A**G**
e14a3_124_BL	TCCCAAGCAACTACATCACG
e14a3_201_F3	ATGAGTCTCCGGGGCTCTAT
e14a3_201_B3	GGACACAGGCCCATGGTA
e14a3_201_FIP	CCCATTGTGATTATAGCCTAAGACCCTGAATGTCATCGTCCACTCAG
e14a3_201_BIP	GTGTGAAGCCCAAACCAAAAATGTTCTCCAGACTGTTGACTGGC
e14a3_201_FL_MOD	C**TT**TTGAACTCTGCTTAAATCCA
e14a3_201_BL	CTGGGTCCCAAGCAACTACA
e14a3_139_F3	TGGGTTTCTGAATGTCATCG
e14a3_139_B3	AGCAGATACTCAGCGGCATT
e14a3_139_FIP	CACACCATTCCCCATTGTGATTACAGCCACTGGATTTAAGCAGA
e14a3_139_BIP	AAAATGGCCAAGGCTGGGTCCCCATGGTACCAGGAGTGTT
e14a3_139_FL	CCTAAGACCCGGAGCTTTTC
e14a3_139_BL	CATCACGCCAGTCAACAGTC
e14a3_11_F3	GAAGATGATGAGTCTCCGGG
e14a3_11_B3	ACCAGGAGTGTTTCTCCAGACT
e14a3_11_FIP mod	TAAGACCCGGAGCTTTTCAC**C**T**A**TGGGTTTCTGAATGTCATCG
e14a3_11_BIP	TAATCACAATGGGGAATGGTGTGCGTGATGTAGTTGCTTGGGA
e14a3_11_FL	TGCTTAAATCCAGTGGCTGA
e14a3_11_BL	AAACCAAAAATGGCCAAGG